Safety evaluation of *Lactobacillus reuteri* PNW1 and *Lactobacillus acidophil*us PNW3 as probiotics and purification of their bacteriocins

KA Alayande

orcid.org 0000-0001-8446-1733

Thesis accepted in fulfilment of the requirements for the degree

Doctor of Philosophy in Science with Biology

at the North-West University

Promoter: Prof CN Ateba

Co-promoter: Dr OA Aiyegoro

Graduation ceremony: July 2020

Student number: 29800455

DEDICATION

This study is dedicated to my late mother, Mrs B. Alayande. I pray to Almight Allah to widen her grave, cleanse her of her shortcomings, give her abode better than her home and household better than those she left behind and a company better than her previous company. I also pray for the Lord to admit her into paradise and protect her from the punishment of the grave and that of hell-fire.

ACKNOWLEDGEMENT

All praises are due to Allah, the Lord of the cosmos.

I wish to acknowledge of my promoter, Prof. C. N. Ateba and co-promoter, Dr A. O. Aiyegoro, for their professional and moral support, which made this study a reality. I also acknowledge the Agricultural Research Council and the North-West University, South Africa, for providing a convenient plartform and an enabling environment during the course of this journey.

I wish to express sincere gratitude to my colleagues within and outside our research group, expecially Dr A. S. Ayangbenro and Mr M. Nengwekhulu, for their support and encouragement during my studies. You are all sincerely appreciated.

I profoundly gratitude to my wife, Mrs A. S. Alayande and children (Faozan, Haneefah and Hafsoh), for their patience and understanding during the course of this study.

Table of contents

List of tables

List of figures

Abstract

Background: Probiotics are live microorganisms that confer health benefit on the host when administered in adequate dose. Strains with probiotic potential are to be carefully selected based on their functionality, safety and genome stability.

Objectives: This study enumerates important probiotic features harboured by two identified lactic acid bacteria as probiotic candidates and evaluate possible undesirable traits in both organisms. It further purifies and assesses the antimicrobial efficacy of the bioactive peptides produced by these isolates.

Methodology: Identification of the isolates was confirmed via PCR amplification of the 16S rRNA region while the genomic DNA of the isolate was extracted and the entire genome was sequenced using illuminal Miseq instrument. The draft assemblies for both *Lactobacillus reuteri* PNW1 and *Lactobacillus acidophilus* PNW3 were annotated with Prokaryotic Genome Annotation Pipeline (PGAP) and Rapid Annotations using Subsystems Technology (RAST). Further genome-based down stream analyses were carried out using a number of bioinformatic tools which includes antiSMASH, PathogenFinder, ResFinder, Comprehensive Antibiotic Resistance Database (CARD), Phage Search Tool Enhanced Release (PHASTER), ISfinder search tool, Insertion Sequence Semi-Automatic Genome Annotation (ISsaga), Optimized Annotation System for Insertion Sequences (OASIS) and CRISPRCasFinder among others. Efficacy of the bioactive peptides produced by the isolates against pathogenic *Escherichia coli* O177 was assessed using agar well diffusion method. The bioactive peptides were thereafter precipitated with 80% saturated ammonium sulphate and further purified using HPLC.

Results: Among all known genes which may be responsible for production of toxic biochemicals, arginine deiminase (EC3.5.3.6) and Ornithine decarboxylase (EC 4.1.1.17)

were spotted harbouring within the genome of *L. reuteri* PNW1 and *L. acidophilus* PNW3, respectively. Resistance genes against lincosamide (*lnuC*) and tetracycline (*tetW*) were found present in both isolates; only the *lnuC* is flanked by a passenger gene found within the genome of *L. reuteri* PNW1. Other mobile genetic elements found within the genome are not in association with the indentified resistance genes. There are plethora of probiotic important genes found within the genome of both isolates and no hit was found for the virulent determinants. Five putative coding sequences were also identified for the CRISPR in *L*. *reuteri* PNW1 genome and only one was found in the *L. acidophilus* PNW3 genome; each of CRISPR is associated with Cas genes. This trait, thus denotes genome stability for both isolates. The maximum zone of inhibition exhibited by the bacteriocin produced by *L. reuteri* PNW1 is 20.0±1.00 mm (crude) and 23.3±1.15 mm (at 0.25 mg/ml) after partial purification. While on the other hand the maximum for the *L. acidophilus* PNW3 is 21.7±0.58 mm (crude) and 24.3±1.15 mm after partially purified and tested at a concentration of 0.25 mg/ml.

Conclussion: Both isolates possess desired trait for a typical viable and safe probiotic, though further *in vivo* assessments are required before developed into functional products for application in animal husbandry within the nearest future.

Keywords: Gut microbiome; Lactic acid bacteria; Bioactive peptides; In-feed probiotics; Antimicrobial resistance; Virulence determinants; Mobile genetic elements; CRISPR-Cas system; Complete-genome

CHAPTER 1 − Introduction

1.1. Rationale for the study

The increasing ban or reduction in the administration of in-feed antibiotics in animal husbandry across the globe have given birth to increased research findings on safe and promising alternatives. Conventional antibiotics have been in use for ages as growth promoters to enhance the performance of farm animals and prevent the occurrence of infectious diseases among flock. Consequentially, the practice is a major role player in the emergence of antibiotic resistant microbes; a germane setback to public health. A number of frontline clinically important antibacterial drugs have been rendered useless due to the development of multiple resistant genes by pathogens. For instance, the emergence of mobile colistin resistant gene *mcr-1* was traced to have its origin from animals before spreading to the human population (Xia *et al.*, 2019). Probiotic is among the identified viable alternatives which has received intensive research focus in recent time.

Probiotics are live organisms, most often, bacteria, with beneficial effect on health besides the usual nutritional advantage when consumed in sufficient amounts (Anadon *et al.*, 2006). Probiotics are widely in use in the prevention and treatment of several kinds of infectious diseases, with substantial scientific evidence supporting their potency in clinical applications (Boyle *et al.*, 2006). The effectiveness of probiotics are strain specific and cannot be generalised (Pandey *et al.*, 2015) and the same applies to their safety characteristics. Despite the wide acceptance for the application of probiotics, it is imperative to subject each and every novel strain of probiotic value to safety evaluation. Though the most commonly used microbial species as probiotics have attained a Generally Regarded As Safe status, but just as in other species, a probiotic strain may possess undesirable trait such as acquired drug resistant genes and production of toxic metabolites, among others. This study is, therefore, designed to enumerate undesired

traits and characteristics which could be inherent in the two identified promising probiotic strains isolated from weaned piglets of the indigenous South African windsnyer pig breed. This is to ensure that the global campaign on food safety and security is a success.

1.2. Introduction

Gastrointestinal tract infections, such as the inflammatory bowel disease, have been identified as important health disorders ravaging dairy farms and human well-being.In the same vein, porcine neonatal and post-weaning diarrhoea, caused by enterotoxigenic *Escherichia coli,* is an economical important disease, resulting in significant morbidity and mortality in pigs (Koh *et al.*, 2008). Manipulation of the intestinal microbiota through the direct feeding of beneficial microorganisms in the form of probiotics may attenuate the enteric health challenge (Celiberto *et al.*, 2017).

Evidence from scientific reports have proved that probiotics have a beneficial effect in the gastrointestinal environment through modulation of the immune and certain physiological systems, thus reducing the incidence of diseases (Chen *et al.*, 2017). Probiotics have been argued to fall into the class of the most popular bioactive and health functional foods (Bosnea *et al.*, 2017). These potentials are enhanced by the inherent tolerance of probiotics for the bile components and gastric juice, adhesion to intestinal mucosa and epithelial cells and improvement of intestinal micro-flora balance (Saxami *et al.*, 2012).

Several species belonging to the genera *Lactobacillus*, *Streptococcus*, *Lactococcus* and *Bifidobacterium*, have been used as probiotics over the years (Oliveira *et al.*, 2017). These organisms are lactic acid bacteria (LAB), with the exception of the genus Bifidobacterium, which belongs to Actinobacteria, but shares many metabolic properties with LAB, namely; Gram-positive, fermentative and production of lactate (Vankerckhoven *et al.*, 2008). *Lactobacilli* and *Bifidobacteria* species are the most commonly used probiotics due to their

extremely rare cases of infections, and their non-pathogenicity extends across all categories (Borriello *et al.*, 2003).

The medicinal potentials of these strains are mostly associated with their ability to antagonise infesting pathogens, reduce symptoms of lactose intolerance, enhance immune system and anti-carcinogenic activity (de LeBlanc and LeBlanc, 2014; Plessas *et al.*, 2017). Studies have revealed that bioactive secondary metabolites produced by many probiotic agents, have implications on bacterial community interaction and, consequently, attenuate the virulent markers on a number of pathogens (Nordeste *et al.*, 2017). For instance, Lactic acids produced by LAB, hinder the survival of neighbouring pathogens and inactivates human immune virus by decreasing the pH of the surrounding environment (Chetwin *et al.*, 2019). Likewise bacteriocin, a bioactive peptide produced against competitive pathogens (Lan *et al.*, 2017).

Probiotic strains are to be carefully selected based on their functionality, safety and shelf life (Oliveira *et al.*, 2017). Probiotics are considered among the most popular bioactive agents in formulating health functional products (Bosnea *et al.*, 2017). They have been used in the treatment and prevention of infectious and inflammatory diseases as well as alleviating allergic symptoms. These medicinal benefits provide opportunities for the development of health functional animal feeds, fermented food products, cosmetics and medications using probiotics as additives (Isolauri *et al.*, 2004; Hwang *et al.*, 2013).

Probiotics have been successfully applied in industries and healthcare sectors across various categories. For instance, in food industries, probiotics have been used in preventing spoilage and eliminating pathogens (Borriello *et al.*, 2003). The use of probiotic strains in fish farming against bacterial and viral infections is a common practice in aquaculture industries. Olive flounder fish was reportedly fed with adequate dose of Lactobacil (commercial lactic acid bacteria) and resulted in significant increase in the

survival rate against lymphocystis disease virus (Harikrishnan *et al.*, 2010), the same way *Bacillus subtilis* E20 was used as a feed supplement and effectively reduced the mortality rate against Iridovirus infected grouper fishes (Liu *et al.*, 2012).

Moreover, mixed probiotic purple non-sulfur bacteria have also showcased significant potentials in the improvement of water quality and prevention of acute hepatopancreatic necrosis disease in the cultivation of white shrimp (Chumpol *et al.*, 2017). A combination of yeast and lactic acid bacteria,as a probiotic, reportedly, results in the production of folate and phytases, which are known to increase the nutritional quality of fermented foods and, in turn, confers health benefits on mammalian hosts, which, by default, cannot synthesise the folate (Greppi *et al.*, 2017).

Cellular and transcriptomic treatment with *Vibrio lentus* probiotics have been reported with significant responses in the modification of gene expressions, related not only to cell proliferation and cell death, but also cell adhesion, reactive oxygen species metabolism, iron transport and immune systems (Schaeck *et al.*, 2017). Several research outputs on microbiota composition, regarding health and disease management, are pointers towards new potential applications of probiotics in the areas of psychotropic activity through gut-brain axis, anti-mutagenic activities and metabolic syndrome such as obesity, diabetes and cardiovascular diseases (Zoumpopoulou *et al.*, 2017).

Though most probiotic strains have acquired the ‘Generally Recognised As Safe’ (GRAS) status (Plessas *et al.*, 2017), however, on rare occasions, infectious diseases such asendocarditis, bacteraemia, pneumoniae, meningitis and septic arthritis associated with certain *Lactobacillus* and *Enterococci* strains have been reported mostly in immunocompromised patients (Vankerckhoven *et al.*, 2008). Over a long period of time, the probiotic *Lactobacillus rhamnosis* GG has been administered to patients with chronic inflammatory disease such as bowel disease, crohn’s disease and juvenile rheumatoid

arthritis as well as to patients with HIV infection and no record of significant adverse effect has been reported. In quantitative terms, the risk of bacteremia, which is the most commonly reported of all these infections, is less than one case per million individuals. Thus, it is significantly improbable to propose a risk of death because of infections involving *Lactobacilli,* with underlying clinical conditions (Borriello *et al.*, 2003; Suresh *et al.*, 2013). However, despite the insignificant risks of detrimental infections, it is imperative upon every probiotic investigator to adequately assess the safety status of individual novel strains.

1.3. Scope and objectives of this work

The development and application of probiotics is meant to offer an alternative to antibiotics in some aspects of prevention and treatment of infectious diseases. This is with a view to reducing the alarming rate of bacterial resistance to multiple conventional antibiotics. Proliferation of multidrug resistant organisms extensively impedes success both in clinical and veterinary practices. This often happens due to irregular or prolonged use of antibiotics, such as in-feed or sub-therapeuthic antibiotics, in the treatment or prevention of infectious diseases. This unfortunate occurrence has, undoubtedly, rendered the wide array of first-line antimicrobial drugs useless so quickly that global public health disaster is imminent.

Lactic acid bacteria (LAB) are the most common strains in use as probiotics, both in humans and animals, due to their safe history of non-virulence. Seldom involvement of this group of bacteria in opportunistic infections, however, should not be downplayed. Hence, every strain, with probiotic potential, requires adequate screening for safety assurance.

This study is, therefore, designed to investigate safety properties of the two promising probiotic candidates belonging to LAB isolated from weaned piglets of the Indigenous

South African Windsnyer Pig Breed and to purify the bioactive peptide produced by these isolates.

The specific objectives of this study were to:

i. Identify the presence of genes putatively encoding for lactic acid production;
ii. Identify the presence of genes putatively involved in bioactive peptide production;
iii. Identify the presence of genes putatively involved in adhesion to epithelial cells and mucus layers;
iv. Determine the possible presence of genes encoding for some extracellular digestive enzymes;
v. Determine the possible presence of genes putatively involved in stress resistance;
vi. Determine the possible presence of genes putatively involved in active metabolism in the host;
vii. Assay and identify genes putatively involved in the production of biogenic amines namely; histidine decarboxylase, tyrosine decarboxylase, ornithine decarboxilase and agmatine deiminase pathway;
viii. Determine the possible presence of virulence determinants such as sex pheromones, gelatinase, cytolysin hyaluronidase, aggregation substance, enterococcal surface protein, endocarditis antigen, adhesine of collagen and integration factors;
ix. Investigate antimicrobial resistance profiling of isolates through the determination of acquired resistant genes;
x. Investigate the bioactive effectiveness of bacteriocin produced by the isolates; and
xi. Purify the bacteriocins produced by the isolates.

References

Anadon, A., Martinez-Larranaga, M. R., Aranzazu Martinez, M. 2006. Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regul Toxicol Pharmacol,* 45, 91–95.

Borriello, S. P., Hammes, W. P., Holzapfel, W., Marteau, P., Schrezenmeir, J., Vaara, M., Valtonen, V. 2003. Safety of Probiotics that contain *Lactobacilli* or *Bifidobacteria*. *Clin Infect Dis,* 36, 775–780.

Bosnea, L. A., Moschakis, T., Nigam, P. S., Biliaderis, C. G. 2017. Growth adaptation of probiotics in biopolymer-based coacervate structures to enhance cell viability. *Lwt-*Food *Sci Technol,* 77, 282–289.

Boyle, R., Robins-Browne, R. M., Tang, M. L. 2006. Probiotic use in clinical practice: what are the risks? Review Article. *Am J Clin Nutr,* 83, 1256–1264.

Celiberto, L. S., Bedani, R., Rossi, E. A., Cavallini, D. C. 2017. Probiotics: The scientific evidence in the context of inflammatory bowel disease. *Crit Rev Food Sci Nutr,* 57, 1759–1768.

Chen, J., Wang, Q., Liu, C. M., Gong, J. 2017. Issues deserve attention in encapsulating probiotics: Critical review of existing literature. *Crit Rev Food Sci Nutr,* 57, 1228–1238.

Chetwin, E., Manhanzva, M. T., Abrahams, A. G., Froissart, R., Gamieldien, H., Jaspan, H., Jaumdally, S. Z., Barnabas, S. L., Dabee, S., Happel, A. U., Bowers, D., Davids, L., Passmore, J. S., Masson, L. 2019. Antimicrobial and inflammatory properties of South African clinical *Lactobacillus* isolates and vaginal probiotics. *Sci Rep,* 9, 1917.

Chumpol, S., Kantachote, D., Nitoda, T., Kanzaki, H. 2017. The roles of probiotic purple nonsulfur bacteria to control water quality and prevent acute hepatopancreatic

necrosis disease (AHPND) for enhancement growth with higher survival in white shrimp (Litopenaeus vannamei) during cultivation. *Aquaculture,* 473, 327–336.

De Leblanc, A. D. M., Leblanc, J. G. 2014. Effect of probiotic administration on the intestinal microbiota, current knowledge and potential applications. *World J Gastroenterol* 20, 16518–16528.

Greppi, A., Saubade, F., Botta, C., Humblot, C., Guyot, J. P., Cocolin, L. 2017. Potential probiotic *Pichia kudriavzevii* strains and their ability to enhance folate content of traditional cereal-based African fermented food. *Food Microbiol,* 62, 169–177.

Harikrishnan, R., Balasundaramb, C., Heo, M.-S. 2010. Effect of probiotics enriched diet on Paralichthys olivaceus infected with lymphocystis disease virus (LCDV). *Fish Shellfish Immun* 29, 868–874.

Hwang, C. E., Seo, W. T., Cho, K. M. 2013. Enhanced antioxidant effect of black soybean by cheonggukjang with potential probiotic *Bacillus subtilis* CSY191. *The Korean J Microbiol,* 49, 391–397.

Isolauri, E., Salminen, S., Ouwehand, A. C. 2004. Probiotics. *Best pract res Clin gastroenterol,* 18, 299–313.

Koh, S. Y., George, S., Bro¨Zel, V., Moxley, R., Francis, D., Kaushik, R. S. 2008. Porcine intestinal epithelial cell lines as a new in vitro model for studying adherence and pathogenesis of enterotoxigenic *Escherichia coli*. *Vet Microbiol,* 130, 191–197.

Lan, R., Tran, H., Kim, I. 2017. Effects of probiotic supplementation in different nutrient density diets on growth performance, nutrient digestibility, blood profiles, fecal microflora and noxious gas emission in weaning pig. *J Sci Food Agric,* 97, 1335–1341.

Liu, C.-H., Chiu, C.-H., Wang, S.-W., Cheng, W. 2012. Dietary administration of the probiotic, *Bacillus subtilis* E20, enhances the growth, innate immune responses,

and disease resistance of the grouper, *Epinephelus coioides*. *Fish Shellfish Immun,* 33, 699–706.

Nordeste, R., Tessema, A., Sharma, S., Kovac, Z., Wang, C., Morales, R., Griffiths, M. W. 2017. Molecules produced by probiotics prevent enteric colibacillosis in pigs. *BMC Vet Res,* 13, 335.

Oliveira, D., Vidal, L., Ares, G., Walter, E. H. M., Rosenthal, A., Deliza, R. 2017. Sensory, microbiological and physicochemical screening of probiotic cultures for the development of non-fermented probiotic milk. *LWT - Food Sci Technol,* 79, 234–241.

Pandey, K. R., Naik, S. R., Vakil, B. V. 2015. Probiotics, prebiotics and synbiotics- a review. *J Food Sci Technol,* 52, 7577–7587.

Plessas, S., Nouska, C., Karapetsas, A., Kazakos, S., Alexopoulos, A., Mantzourani, I., Chondrou, P., Fournomiti, M., Galanis, A., Bezirtzoglou, E. 2017. Isolation, characterization and evaluation of the probiotic potential of a novel *Lactobacillus* strain isolated from Feta-type cheese. *Food Chem,* 226, 102–108.

Saxami, G., Ypsilantis, P., Sidira, M., Simopoulos, C., Kourkoutas, Y., Galanis, A. 2012. Distinct adhesion of probiotic strain *Lactobacillus casei* ATCC 393 to rat intestinal mucosa. *Anaerobe,* 18 417–420.

Schaeck, M., Reyes-Lopez, F. E., Vallejos-Vidal, E., Van Cleemput, J., Duchateau, L., Van Den Broeck, W., Tort, L., Decostere, A. 2017. Cellular and transcriptomic response to treatment with the probiotic candidate *Vibrio lentus* in gnotobiotic sea bass (Dicentrarchus labrax) larvae. *Fish Shellfish Immunol,* 63**,** 147–156.

Suresh, K., Srinath, K., Pravesh, B. 2013. Safety concerns of Probiotic use: A review. *IOSR J Dental Medical Sci,* 12, 56–60.

Vankerckhoven, V., Huys, G., Vancanneyt, M., Vael, C., Klare, I., Romond, M.-B., Entenza, J. M., Moreillon, P., Wind, R. D., Knol, J., Wiertz, E., Pot, B., Vaughan, E.

E., Kahlmeter, G., Goossens, H. 2008. Biosafety assessment of probiotics used for human consumption: recommendations from the EU-PROSAFE project. *Trends in Food Sci Technol,* 19, 102–114.

Xia, X., Wang, Z., Fu, Y., Du, X.-D., Gao, B., Zhou, Y., He, J., Wang, Y., Shen, J., Jiang, H., Wu, Y. 2019. Association of colistin residues and manure treatment with the abundance of mcr-1 gene in swine feedlots. *Environ Int*, 127, 361–370.

Zoumpopoulou, G., Pot, B., Tsakalidou, E., Papadimitriou, K. 2017. Dairy probiotics: Beyond the role of promoting gut and immune health. *Int Dairy J*, 67, 46–60.

CHAPTER 2 − Review of literature

(This chapter has been published in *Sustainability*, 2020, 12: 1087. doi: 10.3390/su12031087)

Abstract

Probiotics have become an emerging, safe and viable alternative to antibiotics, for increasing performance in livestock. They have been applied as prophylaxes and therapeutics, both in clinical and veterinary practices. Besides improved immunomodulatory potential, in-feed probiotics have shown a drastic reduction in the invasion of pathogens in the gastrointestinal tracts of animals. Although most probiotic organisms have acquired the “Generally Recognised As Safe” (GRAS) status, however, every novel strain of probiotic cannot be assumed to share the historical safety status with conventional strains. For instance, potential risk due to horizontal transfer of resistant genes within the microbiota of the host gastrointestinal environment is a possibility. Hence, it has been recommended that any probiotic strain not belonging to the wildtype distribution of relevant antimicrobials and/or harbours (virulent determinants), should not be developed further as functional products for consumption. The mode of identification of strains and transmigration potential of such strains across the gastrointestinal barrier, which could result in invasive opportunistic infections, must be scrutinised. Among other potential risk factors to be put under the spotlight, are the possibility of promoting deleterious metabolic effects, excessive immune stimulation, purity of the product and genetic stability of strains over times. The adverse effects of probiotics could be strain-specific, depending on the prevailing immunological and physiological condition of the host. Unfortunately, this is poorly documented. Moreover, peculiarity of functions of a probiotic is more important than the source of the isolate. The most crucial concern is the potential of a probiotic agent to remain viable over a considerable period of time at the target site. The possibility of probiotics used in animal feed entering the human food chain cannot be downplayed. Though there is limited information on the risk of human food due

to contamination from in-feed probiotics, established safety measures in the development of probiotics must be strictly adhered to, in order to ensure this falls in line with the global campaigns on food safety and security.

Keywords: Adverse effects, antimicrobial resistance, in-feed probiotics, immuno-compromised host, virulence factors

2.1. Probiotics in animal husbandry: Applicability and associated risk factors

2.1.1. Introduction

Probiotics have been widely studied because of their ability to modulate gut microbiota and immunological systems in both humans and livestock (Celiberto *et al*., 2017; Chen *et al*., 2017) where they serve as prophylaxes and for therapeutic purposes in clinical and veterinary practices (de Llano *et al*., 2016; Abushelaibi *et al*., 2017; Srinivas *et al*., 2017). Thus, probiotics are considered as an emerging, safe and viable alternative to antibiotics, for increasing the performance of farm animals. The addition of probiotics to animal feed improves growth performance and nutrient digestibility, reduces serum cholesterol and decreases incidence of diarrhoea in dairy animals (Cavalheiro *et al*., 2015; Zhao and Kim, 2015; Lan *et al*., 2017). Probiotics, in addition, have also demonstrated improved aerobic conditions in a gastrointestinal environment through the depletion of oxygen-scavenging compounds such as nitrates. They have shown the ability to secrete hydrolytic enzymes against bacterial toxins and even to inactivate toxin receptors, thus limiting the occurrence of toxin-mediated infections in livestock animals (Hossain *et al*., 2017).

Undoubtedly, animal feed is crucial in livestock farming and thus has attracted several studies seeking to improve its potency through feed additives. Since the ban of in-feed antibiotics by the European legislation in 2006, the resultant heavy decline in the use of antibiotics paved the way for significant reductions in the prevalence of resistance genes

among the gut microflora of pigs from Europe (Xiao *et al*., 2016). Now that the use of antibiotics as a growth enhancer in livestock diets is being faced with widespread bans across many countries (Abd El-Tawab *et al*., 2016), the development of various health functional animal feeds and fermented food products using probiotics as additives has received unprecedented attention across the continents (Isolauri *et al*., 2004; Hwang *et al*., 2013).

Probiotics are live microorganisms that confer health benefits on the host when administered in adequate dosage. Several species belonging to the genera of *Lactobacillus*, *Streptococcus*, *Lactococcus* and *Bifidobacterium* remain the most popular probiotic agents to date (Oliveira *et al*., 2017). These beneficial microbial agents are, at a regulatory level, classified as zootechnical additives (Bernardeau and Vernoux, 2009). It is required of a probiotic candidate to demonstrate a minimum of one performance feature before being certified for a particular target animal (Bernardeau and Vernoux, 2013). The desired characteristics of a candidate probiotic may include modulation of immune and certain physiological systems of the host, attenuation of virulent markers on a number of pathogens, treatment and prevention of infectious and inflammatory disease conditions, acting as a biocontrol agent in preventing spoilage, etc. (Celiberto *et al*., 2017; Hossain *et al*., 2017; Diaz-vergara *et al*., 2017). This review, therefore, serves to highlight the significance of the applications of probiotics in animal husbandry, and the importance of intensive safety analyses of every probiotic candidate before further development into health functional products and their release for public consumption.

2.1.2 Significance of probiotics in animal health

The improvement of growth performance due to probiotics was confirmed through the increased production of volatile fatty acids, nutrient digestibility, feed conversion rate and the stimulation of lactic acid-dependent protozoa (Abd El-Tawab *et al*., 2016). Probiotics have been used to increase the efficiency of the utilisation of feed, to increase milk

production and to reduce diarrhoea both in pigs and cattle, and to control the colonisation of the intestinal tract by *Salmonella* in chickens (Bernardeau and Vernoux, 2013). Besides its improved immunomodulatory potential, the commercially available in-feed probiotic, Lavipan, drastically reduces the invasion of *Campylobacter* spp. in the gastrointestinal tract of poultry birds, thus suppressing pathogenic contaminants and improving hygiene in the poultry environment (Smialek *et al*., 2018).

Roselli *et al*. (2017) observed that probiotics fed to weaned piglets and sows yielded positive results by improving gut health through balanced microbiota, improving immunological and physiological processes, and preventing gastrointestinal disorders. The major responses were observed as prompt changes in the gastrointestinal microbial ecosystem, through antagonizing the survival of the neighbouring pathogens coupled with the production of favourable fermentation products. This was affirmed through a related study by Hanczakowska and colleagues (2016), who found that *Enterococcus faecium*, fed to piglets as feed supplement, exhibited an inhibitory effect against *Clostridium perfringens*. The microflora within the gastrointestinal environment of animals can be considered an active metabolic organ due to its biodiversity. Therefore, it is important to maintain effective gut microflora in the battle against the invasion of pathogens among livestock with high population density (Gaggia *et al*., 2010). In general, lactic acid bacteria with probiotic potential secrete organic acids which increase the acidity of the gastrointestinal tract environment, and therefore lower the risk of pathogen infestation while at the same time regulating the microbial ecosystem within the gut habitat (Servin *et al*., 2004).

A cocktail of probiotic supplements containing strains of *Lactobacillus* significantly reduces *Salmonella* and *Shigella* in the faecal samples of goats (Apás *et al*., 2010). Likewise, a complex mixture of lactobacilli, isolated from the guts of piglets, reportedly increased the

density of beneficial microbes and reduced that of enteric pathogens such as *Escherichia coli* in the gastrointestinal tract (Chiang *et al*., 2015). Weaned piglets fed with lactic acid bacteria supplements in their basal diet showed significant improvements in terms of growth performance, digestion rate, faecal microbial count, intestinal morphology, diarrhoea control and maintenance of pH in the gastrointestinal tract (Giang *et al*., 2010; Dowarah *et al*., 2017). Dietary inclusion of lactobacilli has shown increased egg-laying performance in chickens, and improved body weight on a daily basis in turkeys (Gadde *et al*., 2017).

Specifically, *Lactobacillus johnsonii* FI9785 was reported to have successfully ameliorated necrotic enteritis due to *Clostridium perfringens* upon its administration to poultry (La Ragione *et al*., 2004). Likewise, *Lactobacillus salivarius* SMXD51 showed effective prevention of gut colonization by *Campylobacter jejuni* in broiler chickens when administered via oral gavage (Saint-Cyr *et al*., 2017). *Lactobacillus plantarum* PCA 236, when used as a feed supplement for goats, repressed their *Clostridium* gut colonization (Maragkoudakis *et al*., 2009). *Lactobacillus fermentum* I5007, when orally administered to four-day-old piglets as a post-weaning supplement, resulted in improved intestinal health, increased the height of jejunum villi, increased the concentrations of butyrate and branched chain fatty acids and reduced potential colon pathogens (Liu *et al*., 2014).

Moreover, bifidobacteria constitute an important component in the gut microflora of chickens and have proven records of positive effects when administered to piglets and other mammals. A commercial strain of *Bifidobacterium bifidum* (InstitutRosell Inc. Montreal, QC, Canada) was effective in the treatment of cellulitis-infected broiler chickens, and *B. longum* PCB 133 significantly reduced *Campylobacter jejuni* concentration in poultry faeces when administered to chickens (Santini *et al*., 2010; Estrada *et al*., 2001). *B. adolescentis* Z25 equally exhibited significant potential in the treatment of blood sugar

imbalance, lipid metabolism disorders, tissue damage and gut microbiota dysbiosis (Zhu *et al*., 2018). Several species of *Bifidobacterium* have also demonstrated great potential to increase production of the enzyme β-galactosidase, therefore reducing lactose intolerance. *B. longum* LC67 and *L. plantarum* LC27 synergistically remedied 2, 4, 6-trinitrobenzesulfonic acid-induced colitis and liver injury in mice, via readjustment of the gut ecosystem imbalance and inhibition of inflammatory responses (Jang *et al*., 2018). The activities of the probiotics *B. adolescentis* Z25 and *L. plantarum* LC27 mentioned above are the output of laboratory research based on mouse models. This might only be relevant to mammalian livestock.

Several probiotic agents have been traditionally applied as bioprotectors on meat products (de Llano *et al*., 2016; Chaillou *et al*., 2014). They have been reportedly secreting exopolysaccharides that are capable of inhibiting biofilm formation by pathogenic contaminants (Kim *et al*., 2009). Strains of *Lactobacillus* have also yielded commendable results on raw chicken meat in protection against *Listeria monocytogenes* and *Salmonella enteriditis* (Maragkoudakis *et al*., 2010).

In addition, mycotoxins are often found contaminating animal feed, thereby exposing livestock to serious health risks, with a tendency to cross-contaminate the human food chain through meat and other dairy products (Gajecka *et al*., 2004; Anfossi *et al*., 2016). Ochratoxin A, a nephrotoxic, carcinogenic and immunotoxic mycotoxin, was detoxified to a greater extent in chickens after the administration of a lactobacilli-based probiotic preparation (Markowiak *et al*., 2019). In another study by Chlebicz and Śliżewska (2019), monocultures of different strains of *Lactobacillus* spp. were tested for detoxification potentials against a number of mycotoxins directly used to contaminate animal feed. After 6 h of incubation, the concentration of fumonisin B1 and B2, aflatoxin B1, T-2 toxin and zearalenone were significantly reduced, by 77%, 60%, 61% and 57%, respectively.

Several scientific reports have indicated that lactic acid bacteria are capable of detoxifying different forms of mycotoxin. When compared to physical and chemical decontamination methods, biological detoxification is more efficient, specific and environmentally friendly (Zhu *et al*., 2017; Milani *et al*., 2017). The two main mechanisms by which mycotoxins are detoxified by probiotics involve adsorption of toxins by the microbial cell wall and biotransformation. Additionally, combined use of a consortium of probiotics and mycotoxin-degrading enzymes is yet another growing strategy for mycotoxin decontamination (Milani *et al*., 2017; Wang *et al*., 2019).

2.1.3. Probiotics as a viable alternative to in-feed antibiotics

Antibiotics have been extensively used over decades as prophylactic and growth-promoting agents in the livestock sector. This has contributed a great deal to the uncontrollable increase in the emergence of multidrug-resistant (MDR) pathogens. This has consequently reduced therapeutic options both in human and veterinary clinics, leading to reduced clinical success on previously curable infections, and in some cases, can result in a prolonged stay in hospital. The MDR pathogens constitute a major setback, hampering progress in public health both in humans and farm animals. Concerted efforts have been made by major stakeholders towards global awareness on the shared consequences of the indiscriminate and irresponsible use of antibiotics. Despite years of relentless campaigning, MDR pathogens continue to emerge.

In-feed antimicrobials are the most common route of drug administration in Europe, especially in pig farming (Sarrazin *et al*., 2019). This form of drug administration predisposes healthy animals to unnecessary antimicrobials while feeding alongside the infected ones, thus increasing the risk of selecting resistant bacteria. Increasing trends in the practices that led to the evolution of antimicrobial resistance genes prompted the United States Animal Agriculture Sector to prohibit the use of subtherapeutic antibiotic growth promoters (AGPs) in early 2017, through implementation of the Veterinary Feed

Directive (VFD) (Helm *et al*., 2019; Johnson *et al*., 2019). This highlights an urgent need for more efforts to discover alternative growth promoters.

Moreover, the consumption of antimicrobials by livestock was estimated to be above 240,000 metric tons annually across continents. However, some countries are now experiencing a substantial decline in the sales of antimicrobials for food-producing animals (Vieco-Saiz *et al*., 2019). Resistance associated with the use of antibiotics in agricultural practices, and the potential transfer of MDR pathogens from food-producing animals to humans, is a disturbing health concern (Cattaneo *et al*., 2009). Antibiotics administered to farm animals are often excreted in urine and egested in faeces into the nearby environment, thus potentially selecting the microorganisms in such an environment for the development of multiple resistance genes in a bid for survival (Qiao *et al*., 2018). The use of antibiotics in food-producing animals is under intense scrutiny because of the perceived risk of zoonotic transfer of the resistant pathogens into the human populace (Coyne *et al*., 2018).

For instance, β-lactam antibiotics such as penicillin, cephalosporin and carbapenem are no longer relevant in human clinical therapy (Temkin *et al*., 2014). Despite the high degree of effectiveness of colistin against carbapenemase-producing Enterobacteriaceae, plasmid-mediated *mcr-1*reportedly emerged in 2015 and, subsequently, was identified in over fifty different countries, along with additional seven *mcr-1* gene variants (Wang *et al*., 2017; Sun *et al*., 2018; Wang *et al*., 2018). Colistin has been in use for several years in the livestock industries in China, and research findings suggest *mcr-1* originated from animals before spreading to the human population. The agricultural usage of colistin as a growth promoter was consequently banned by the Chinese authorities in 2017 (Xia *et al*., 2019).

Furthermore, the emergence of vancomycin-resistant enterococci in European countries was implicated in the illegal use of glycopeptide antibiotics such as avoparcin in animal

feeds (Song *et al*., 2019). In 2016, the Animal Health Authority in France passed a decree regulating the use of critically important antibiotics, such as 3rd- and 4th-generation cephalosporins, fluoroquinolones and macrolides. This was intended to promote the rational use of antibiotics, and to foster the use of alternatives in the veterinary clinical system (Bourély *et al*., 2018). Additionally, in the United Kingdom, there was a shared desire to seek an alternative approach to prophylactic measures other than the conventional antimicrobials, and many farmers showed interest in reducing the use of antibiotics. Hence, the guidelines on promoting prudent prescriptions, and providing advice on alternative methods to treat and prevent diseases in pigs, were put together by the Responsible Use of Medicines in Agriculture Alliance (RUMA) in 2013 and the Pig Veterinary Society (PVS) in 2014 (Wang *et al*., 2019).

In a real-world longitudinal study by Burow and colleagues (Burow *et al*., 2019) on the assessment of dynamics of the risk of resistance in *Escherichia coli* to clinically important antimicrobials in pigs, it was concluded that reduction in antibiotic resistance in pigs could lead to a lower level of beta-lactam-or macrolide-resistant *E. coli* among their progeny. In another study by Wang et al. (2017), Pacific white shrimp feed was supplemented with Ciprofloxacin and Sulfonamide to investigate the microbial community targeting the V4 region of 16S rRNA genes. Four days into the experiment, a significant increase in the abundance of the Ciprofloxacin- and Sulfonamide-resistant genes (*qnrB*, *qnrD*, *qnrS* and *sul1*, *sul2*, *sul3*, respectively) was observed. On a more general note, the clinical preservation and maintenance of treatment options against infectious diseases require the restriction of antibiotic use to unavoidable cases.

Campaigns for the removal of in-feed antibiotics in animal husbandry are increasingly gaining momentum across the globe. This has prompted several studies on developing and promoting alternative additives such as probiotics, prebiotics, plant secondary

metabolites, acidifiers, enzymes, bacteriocins and bacteriophages (Seal *et al*., 2018; Cowieson *et al*., 2019). Combined probiotics and exogenous enzymes, when used as feed additives, have demonstrated beneficial impacts on growth performance and on the weight-gains-to-feed ratio in calves (Ponce *et al*., 2011; Kocyigit *et al*., 2016).

The effects of two commercially available feed additives containing lactic acid bacteria, *Lactobacillus*fermentation products and plant-sourced enzymes, in comparison to in-feed antibiotics, were extensively evaluated based on growth performance, carcass characteristics and blood metabolites of steers by Ran and colleagues (Ran *et al*., 2019). The study established that the supplements improved average daily weight gains and feed efficiency during the early portion of the growing phase. Additionally, steers supplemented with these products required fewer therapeutic antimicrobials compared to the control groups in the experiment, thus confirming probiotic additives as potential alternatives to AGPs in growing steers.

Moreover, a diet of *Astyanax bimaculatus* supplemented with *Lactobacillus* spp. resulted in increased amounts of leucocytes in the circulatory system, thus conferring greater resistance to gut pathogens and, consequently, higher survival rates, in addition to improved feed efficiency (De Moraes *et al*., 2018). In another study conducted by Vase-Khavari et al. (2019), poultry bird feed supplemented with probiotics (superzist) revealed significant influences on the growth performance of the broilers, with laudable responses on total cholesterol, triglyceride levels and immunological parameters, and a notable reduction in colon pathogens. Administration of *Lactococcus lactis* subspecies *lactis* 2 probiotic to broilers reduced the cholesterol level and fat content in the breast and thigh meat more prominently when compared to the effects of zinc bacitracin antibiotics, which were used as a control (Mujnisa *et al*., 2018).

Askelson and colleagues (Askelson *et al*., 2013) demonstrated the potential of administering a phytate-degrading probiotic culture, in place of feed enzymes, to improve performance in livestock animals. In their study, recombinant *Lactobacillus gallinarum* and *L. gasseri* were cloned to express *Bacillus subtilis* phytase (pTRK882), which improved the weight gain of broiler chickens up to 10-fold and 18-fold, respectively. This could eventually combine the performance benefits of feed enzymes with that of animal health and food safety traditionally associated with probiotics. Moreover, a poultry basal diet supplemented with colonies of *Lactobacillus plantarum* 16 and *Paenibacillus polymyxa* 10 led to the maintenance of intestinal barrier integrity and the adequate expression of barrier functional genes, a reduction in the level of malondialdehyde in the jejunal mucosa and serum, an increment in the activities of hepatic glutathione peroxidase, a reduction in the rate of cell proliferation and apoptosis, and a significant reduction in nitric oxide and in the expression of cyclooxygenase-2 enzyme (Wu *et al*., 2019).

Direct feeding with a probiotic culture preparation containing *Lactobacillus reuteri*, *L. salivarius* and *Streptococcus salivarius* significantly improved the growth performance, blood parameters and IgG stimulation in weaned piglets (Dlamini *et al*., 2017). *Lactobacillus salivarius* LS6 demonstrates promising probiotic traits for potential use as a feed additive for pigs, through preventing disruption of the epithelial cells in the small intestine via inhibiting the colonisation by enterotoxigenic *Escherichia coli* K88 (Yeo *et al*., 2016). Oral administration of *Lactobacillus delbrueckii* to suckling porcine that were denied access to in-feed antibiotics showed tremendously improved antioxidant capacity and an intestinal immune response with long-lasting effects (Li *et al*., 2019). Tasfaye and Hailu (2019) recently compiled several scientific studies to indicate how directly feeding probiotic to dairy cows significantly improves milk production and quality.

2.1.4. Established risk assessment protocol for the probiotics

Notwithstanding the clinical efficacy of probiotics, the safety of the microorganism involved must be assured. This suggests that the evaluation of the risk factors of a specific strain must receive adequate attention. Most probiotic strains have acquired the "Generally Recognized As Safe" (GRAS) status due to their long history of use as probiotics (Tsai *et al*., 2014; Plessas *et al*., 2017); however, every novel probiotic cannot be assumed to share historical safety with the conventional strains (Donohue, 2006). Just as in other organisms, probiotics may possess undesirable properties such as virulence factors, transferable antimicrobial resistance, haemolytic potential and production of toxic biochemicals (Lee *et al*., 2017).

For instance, the potential risk of a horizontal transfer of antimicrobial-resistant genes in a probiotic strain to other bacteria in the microbiota of the host's gastrointestinal environment has been emphasized (Plessas *et al*., 2017). This is in addition to rare cases of infectious diseases like endocarditis, bacteraemia, pneumoniae, meningitis and septic arthritis associated with certain *Lactobacillus* and *Enterococcus* strains which have also been reported mostly in immuno-compromised patients (Vankerckhoven *et al*., 2008).

During the 2006 two-day workshop organised by academic and industrial scientists on biosafety evaluation of lactic acid bacteria as probiotics, at the University of Antwerp, Belgium, it was recommended that any potential probiotic strain not belonging to the wild-type distributions of relevant antimicrobials, or harbouring a known virulence determinants, should be avoided and not developed as future functional products for human or animal consumption (Vankerckhoven *et al*., 2008). In a joint report of the Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Consultation in Rome (2006), *Enterococcus* was mentioned as one of the bacteria with a high potential for virulence features, although from lactic acid bacteria. Participants thus discouraged the use of

Enterococcus as a probiotic, on the grounds that the genus commonly expresses a high level of vancomycin-resistant genes. Such resistance can be transferred to neighbouring pathogens, which may, in the end, enhance their pathogenicity. This is coupled with the fact that some strains of vancomycin-resistant enterococci are frequently associated with nosocomial infections.

Moreover, the assessment of the strain identity, coupled with the mode of identification of the strains, deserves adequate attention. The transmigration potential of the strain across the gastrointestinal barrier, which may result in invasive opportunistic infection, should be scrutinized as well (Sanders *et al*., 2010; Huys *et al*., 2013; Suresh *et al*., 2013). Among other potential risk factors, to be put under the spotlight are the ability to transfer acquired antimicrobial resistance, the possibility of promoting deleterious metabolic effects, excessive immune stimulation, virulence determinants and toxigenicity of the specific strain, level of purity of the product, and colonisation and genetic stability of the strain over time (Vankerckhoven *et al.*, 2008; Sanders *et al.*, 2010; Suresh *et al.*, 2013; Sornplang and Piyadeatsoontorn, 2016).

In addition, the FAO working group report (2002), equally recommends that potential probiotic strains should be screened for undesirable secondary medical effects in end users, virulence in animal models with compromised immunity and impending adverse effects on end users. Kim *et al*. (2018) also suggest guidelines for the safety assessment and regulation of probiotics, as endorsed by the European Union Scientific Committee on Animal Nutrition, as follows: taxonomical definition of the strains, collection of substantial information revealing data such ashistory of use, industrial applications, ecological niche, human intervention, exclusion of pathogenicity and description of end users. A chat summarizing combined established safety assessment protocols for a typical probiotic candidate is shown in the figure 1.

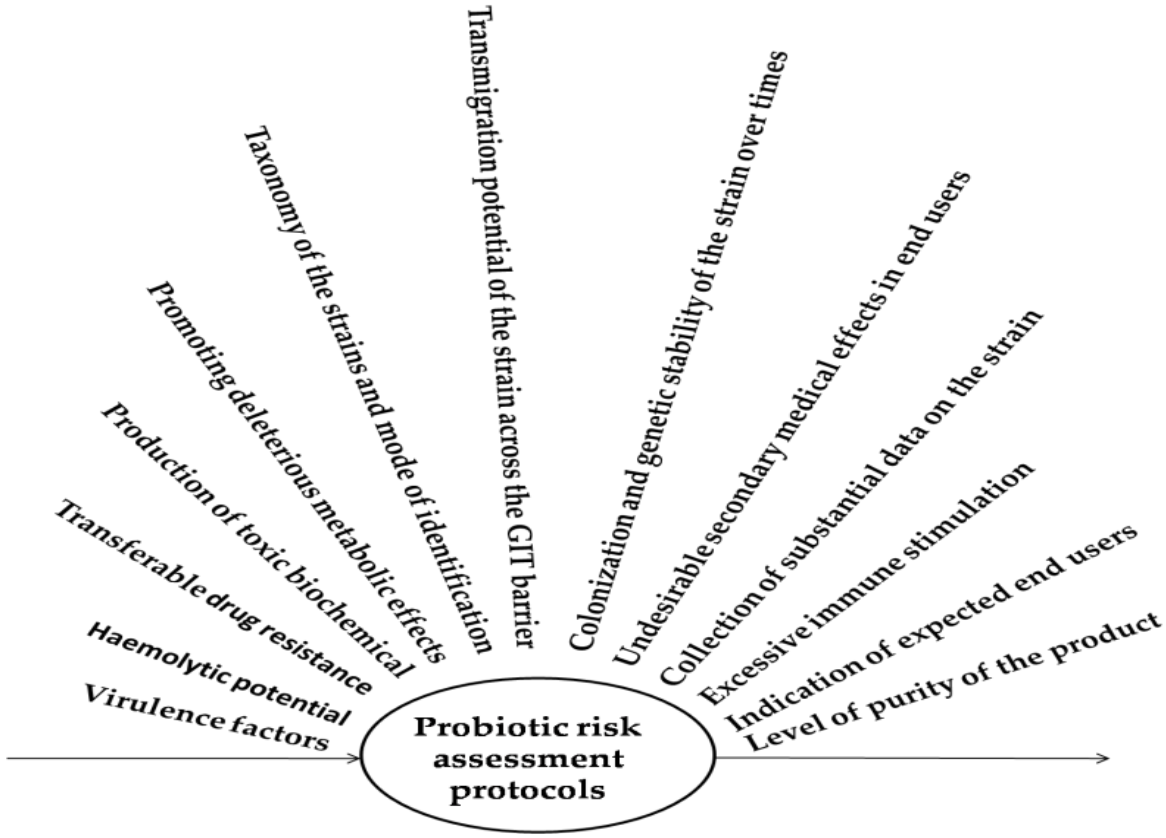

Figure 1. Established safety assessment protocols for a probiotic candidate.

2.1.5. Adverse effects due to application of probiotics

The safety of probiotics is best discussed in general as it applies to both human and farm animals. The peculiar functions of a probiotic are more important than the source of the isolate. It is crucial that the potential of a probiotic agent remains viable over a considerable period of time at the site of action. It is very difficult to ascertain the source of a microorganism in the gastrointestinal tract since the origin of the intestinal microbiota has not been well founded. Moreover, the possibility of probiotics used in animal feed cross-contaminating the human food chain cannot be downplayed. Although there is very little available information on the risk to human food due to contamination from in-feed probiotics (FAO/WHO, 2006; Bajagai *et al*., 2016), the adverse effects of probiotics could be strain specific, depending on the prevailing immunological and physiological condition of the host. In a systematic study on published data and information on the safety of

probiotics, credited to the Agency for Healthcare Research and Quality under the US Department of Health and Human Services, it was concluded that there is a lack of assessment and systematic reporting on adverse effects due to probiotic intervention, and that interventions are poorly documented (Hempel *et al*., 2011).

2.1.6. Conclusions

Considering the detrimental effects of in-feed antibiotics, and irresponsible administration of antibiotics in veterinary practice, probiotics stand a good chance as a viable alternative prophylactic and therapeutic agent in animal husbandry. Although the events of probiotics turning into opportunistic infections are not well documented, a few available case reports reveal that the likelihood is more pronounced in immunocompromised hosts, and there are few or no reports on affected livestock. The risk of human food contamination from in-feed probiotics is a possibility, although less investigated. Several health agencies and academics across continents have established and documented adequate safety protocols for probiotics development. As tremendous as the benefits of probiotics are, safety measures for every strain must be strictly adhered to at all levels in order to make the ongoing global campaign on food safety and security a reality.

Reference

Abd El-Tawab, M.M., Youssef, I.M., Bakr, H.A., Fthenakis, G.C., Giadinis, N.D. 2016. Role of probiotics in nutrition and health of small ruminants. *Pol J Vet Sci*, 19, 893–906.

Abushelaibi, A., Al-mahadin, S., El-tarabily, K., Shah, N.P., Ayyash, M. 2017. Characterization of potential probiotic lactic acid bacteria isolated from camel milk. *LWT-Food Sci Technol*, 79, 316–325.

Anfossi, L., Giovannoli, C., Baggiani, C. 2016. Mycotoxin detection. *Curr Opin Biotechnol*, 37, 120–126.

Apás, A.L., Dupraz, J., Ross, R., González, S.N., Arena, M.E. 2010. Probiotic administration effect on fecal mutagenicity and microflora in the goat's gut *J Biosci Bioeng*, 110, 537–540.

Askelson, T.E., Campasino, A., Lee, J.T., Duong, T. 2013. Evaluation of Phytate-Degrading *Lactobacillus* culture administration to broiler chickens. *Appl Environ Microbiol*, 80, 943–950.

Bajagai,Y.S., Klieve, A.V., Dart, P.J., Bryden, W.L. 2016. Probiotics in Animal Nutrition–Production, Impact and Regulationby FAO Animal Production and Health Paper *No. 179*; Harinder, P.S., Ed.; FAO: Rome, Italy, ISBN 978-92-5-109333-7.

Bernardeau, M., Vernoux, J. Overview of the use of probiotics in the feed/food chain. Probiotics: Production, evaluation and uses in animal feed. *Kerala India Biotechnol. Appl. Biochem.* 2009, 2009, 15–45.

Bernardeau, M., Vernoux, J.P. 2013. Overview of differences between microbial feed additives and probiotics for food regarding regulation, growth promotion effects and health properties and consequences for extrapolation of farm animal results to humans. *Clin. Microbiol Infect*, *19*, 321–330.

Bourély, C., Fortanéd, N., Calavas, D., Leblond, A., Gay, É. 2018. Why do veterinarians ask for antimicrobial susceptibility testing? A qualitative study exploring

determinants and evaluating the impact of antibiotic reduction policy. *Prev Vet Med*, 159, 123–134.

Burow, E., Rostalski, A., Harlizius, J., Gangl, A., Simoneit, C., Grobbel, M., Kollas, C., Tenhagen, B.-A., Käsbohrer, A. 2019. Antibiotic resistance in *Escherichia coli* from pigs from birth to slaughter and its association with antibiotic treatment. *Prev Vet Med* 2019, 165, 52–62.

Cattaneo, A.A., Wilson, R., Doohan, D., Lejeune, J.T. 2009. Bovine veterinarians' knowledge, beliefs, and practices regarding antibiotic resistance on Ohio dairy farms *J Dairy Sci*, 92, 3494–3502.

Cavalheiro, C.P. Ruiz-Capillas, C., Herrero, A.M., Jiménez-Colmenero, F., Ragagnin, M.C., Martins, F.L.L. 2015. Application of probiotic delivery systems in meat products. *Trends Food Sci Technol*, 46, 120–131.

Celiberto, L.S., Bedani, R., Rossi, E.A., Cavallini, D.C. 2017. Probiotics: The scientific evidence in the context of inflammatory bowel disease. *Crit Rev Food Sci Nutr* 2017, 57, 1759–1768.

Chaillou, S., Christieans, S., Rivollier, M., Lucquin, I., Champomier-Verges, M.-C., Zagorec, M. 2014. Quantification and efficiency of *Lactobacillus sakei* strain mixtures used as protective cultures in ground beef. *Meat Sci*, *97*, 332–338.

Chen, J., Wang, Q., Liu, C.M., Gong, J. 2017. Issues deserve attention in encapsulating probiotics: Critical review of existing literature. *Crit Rev Food Sci Nutr*, *57*, 1228–1238.

Chiang, M.L., Chen, H.C., Chen, K.N., Lin, Y.C., Lin, Y.T., Chen, M.-J. 2015. Optimizing production of two potential probiotic lactobacilli strains isolated from piglet feces as feed additives for weaned piglets. *Asian Australas J Anim Sci*, *28*, 1163.

Chlebicz, A., Śliżewska, K. 2019. In Vitro Detoxification of Aflatoxin B1, Deoxynivalenol, Fumonisins, T-2 Toxin and Zearalenone by Probiotic Bacteria from Genus

Lactobacillus and *Saccharomyces cerevisiae* Yeast. *Probiotics Antimicrob Proteins*, doi:10.1007/s12602-018-9512-x.

Cowieson, A.J., Kluenter, A.M. 2019. Contribution of exogenous enzymes to potentiate the removal of antibiotic growth promoters in poultry production. *Anim Feed Sci Technol*, 250, 81–92.

Coyne, L.A., Latham, S.M., Dawson, S., Donald, I.J., Pearson, R.B., Smith, R.F., Williams, N.J., Pinchbeck, G.L. 2018. Antimicrobial use practices, attitudes and responsibilities in UK farm animal veterinary surgeons. *Prev Vet Med*, 161, 115–126.

de Llano, D.G., Gil-sánchez, I., Esteban-fernández, A., Ramos, A.M., Fernández-díaz, M., Cueva, C., Moreno-arribas, M.V., Bartolomé, B. 2016. Reciprocal beneficial effects between wine polyphenols and probiotics: An exploratory study. *Eur Food Res Technol*, 243, 531–538.

De Moraes, A.V., Pereira, M.D.O., Moraes, K.N., Rodrigues-Soares, J.P., Jesus, G.F. 2018. Autochthonous probiotic as growth promoter and immunomodulator for Astyanax bimaculatus cultured in water recirculation system. *Aquac Res*, 49, 2808–2814.

Diaz-vergara, L., Pereyra, C.M., Montenegro, M., Pena, G.A., Aminahuel, C.A., Cavaglieri, L.R. 2017. Encapsulated whey-native yeast *Kluyveromyces marxianus* as a feed additive for animal production. *Food Addit Contam Part A*, 34, 750–759.

Dlamini, Z.C., Langa, R.L.S., Aiyegoro, O.A., Okoh, A.I. 2017. Effects of probiotics on growth performance, blood parameters, and antibody stimulation in piglets. *S Afr J Anim Sci*, 47, doi:10.4314/sajas.v47i6.4.

Donohue, D.C. 2006. Safety of probiotics. *Asia Pac J Clin Nutr*, *15*, 563–569.

Dowarah, R., Verma, A.K., Agarwal, N., Patel, B.H.M., Singh, P. 2017. Effect of swine based probiotic on performance, diarrhoea scores, intestinal microbiota and gut health of grower-finisher crossbred pigs. *Livest Sci*, *195*, 74–79.

Estrada, A., Wilkins, D.C., Drew, M. 2001. Administration of *Bifidobacterium bifidum* to chicken broilers reduces the number of carcass condemnations for cellulitis at the abattoir. *J Appl Poult Res*, *10*, 329–334.

FAO/WHO (Joint Report) Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live. In *Health and Nutrition Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria*; FAO Food and Nutrition Paper 85; FAO: Rome, Italy, 2006; ISSN 0254-4725.

FAO/WHO (Joint Report) Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food; London, Ontario, Canada, 30 April and 1 May 2002. https://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf (accessed on 06/04/2019).

Gadde, U., Kim, W.H., Oh, S.T., Lillehoj, H.S. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. *Anim Health Res Rev*, *18*, 26–45.

Gaggia, F., Mattarelli, P., Biavati, B. 2010. Probiotics and prebiotics in animal feeding for safe food production. *Int J Food Microbiol*, 141, S15–S28.

Gajecka, M., Jakimiuk, E., Skorska-Wyszynska, E., Zielonka, Ł., Polak, M., Paluszewski, A., Rybarczyk, L., Gajecki, M. 2004. Influence of zearalenone micotoxicosis on selected immunological, haematological and biochemical index of blood plasma in bitches. *Pol J Vet Sci*, 7, 175–180.

Giang, H.H., Viet, T.Q., Ogle, B., Lindberg, J.E. 2010. Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. *Livest Sci*, 129, 95–103.

Hanczakowska, E., Świątkiewicz, M., Natonek-Wiśniewska, M., Okoń, K. 2016. Medium chain fatty acids (MCFA) and/or probiotic *Enterococcus faecium* as a feed supplement for piglets. *Livest Sci*, 192, 1–7.

Helm, E.T., Curry, S., Trachsel, J.M., Schroyen, M., Gabler, N.K. 2019. Evaluating nursery pig responses to in-feed sub-therapeutic antibiotics. *PLoS ONE*, 14, e0216070, doi:10.1371/journal.pone.0216070.

Hempel, S., Newberry, S., Ruelaz, A., Wang, Z., Miles, J. 2011. *Safety of Probiotics to Reduce Risk and Prevent or Treat Disease. Evidence Report/Technology Assessment No. 200*; (Prepared by the Southern California Evidence-Based Practice Center under Contract No. 290-2007-10062-I.) AHRQ Publication No. 11-E007; Agency for Healthcare Research and Quality: Rockville, MD, USA. Available online: http://www.ahrq. gov/clinic/tp/probiotictp.htm (accessed on 09/04/2019).

Hossain, M.I., Sadekuzzaman, M., Ha, S.D. 2017. Probiotics as potential alternative biocontrol agents in the agriculture and food industries: A review. *Food Res Int*, 100, 63–73.

Huys, G., Botteldoorn, N., Delvigne, F., De Vuyst, L., Heyndrickx, M., Pot, B., Dubois, J.J., Daube, G. 2013. Microbial characterization of probiotics-advisory report of the working group “8651 probiotics” of the Belgian Superior Health Council (SHC). *Mol Nutr Food Res*, 57, 1479–1504.

Hwang, C.E., Seo, W.T., Cho, K.M. 2013. Enhanced antioxidant effect of black soybean by cheonggukjang with potential probiotic *Bacillus subtilis* CSY191. *Korean J Microbiol* 2013, 49, 391–397.

Isolauri, E., Salminen, S., Ouwehand, A.C. 2004. Probiotics. *Best Pract Res Clin Gastroenterol*, 18, 299–313.

Jang, S.E., Jeong, J.J., Kim, J.K., Han, M.J., Kim, D.H. 2018. Simultaneous amelioratation of colitis and liver injury in mice by *Bifidobacterium longum* LC67 and *Lactobacillus plantarum* LC27. *Sci Rep*, 8, 7500, doi:10.1038/s41598-018-25775-0.

Johnson, T.A., Sylte, M.J., Looft, T. 2019. In-feed bacitracin methylene disalicylate modulates the turkey microbiota and metabolome in a dose-dependent manner. *Sci Rep*, 9, 8212, doi:10.1038/s41598-019-44338-5.

Kim, M.J., Ku, S., Kim, S.Y., Lee, H.H., Jin, H., Kang, S., Li, R., Johnston, T.V., Park, M.S., Ji, G.E. 2018. Safety evaluations of *Bifidobacterium bifidum* BGN4 and *Bifidobacterium longum* BORI. *Int J Mol Sci*, 19, 1422, doi:10.3390/ijms19051422.

Kim, Y., Kim, S.H. 2009. Released exopolysaccharide (r-EPS) produced from probiotic bacteria reduce biofilm formation of enterohemorrhagic *Escherichia coli* O157: H7. *Biochem. Biophys Res Commu*, 379, 324–329.

Kocyigit, R., Aydin, R., Yanar, M., Diler, A., Avci, M., Ozyurek, S. 2016. The effect of direct-fed microbials plus exogenous feed enzyme supplements on the growth, feed efficiency ratio and some behavioural traits of brown swiss x eastern anatolian red F1 calves. *Pak J Zool*, 48, 1389–1393.

La Ragione, R., Narbad, A., Gasson, M., Woodward, M.J. 2004. In vivo characterization of *Lactobacillus johnsonii* FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry. *Lett Appl Microbiol*, 38, 197–205.

Lan, R., Tran, H., Kim, I. 2017. Effects of probiotic supplementation in different nutrient density diets on growth performance, nutrient digestibility, blood profiles, fecal microflora and noxious gas emission in weaning pig. *J Sci Food Agric*, 97, 1335–1341.

Lee, S., Lee, J., Jin, Y.-I., Jeong, J.-C., Chang, Y.H., Lee, Y., Jeong, Y., Kim, M. 2017. Probiotic characteristics of *Bacillus* strains isolated from Korean traditional soy sauce. *LWT-Food Sci Technol*, 79, 518–524.

Li, Y., Hou, S., Peng, W., Lin, Q., Chen, F., Yang, L., Li, F., Huang, X. 2019. Oral Administration of *Lactobacillus delbrueckii* during the suckling phase improves antioxidant activities and immune responses after the weaning event in a piglet Model. *Oxidative Med Cell Longev*, 6919803, doi:10.1155/2019/6919803.

Liu, H., Zhang, J., Zhang, S., Yang, F., Thacker, P.A., Zhang, G., Qiao, S., Ma, X. 2014. Oral administration of *Lactobacillus fermentum* I5007 favors intestinal development and alters the intestinal microbiota in formula-fed piglets. *J Agric Food Chem*, 62, 860–866.

Maragkoudakis, P.A., Mountzouris, K.C., Psyrras, D., Cremonese, S., Fischer, J., Dalaka, E., Hadjipetrou, A., Theofanous, G., Strozzi, G.P., Carlini, N. 2009. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. *Int J Food Microbiol*, 130, 219–226.

Maragkoudakis, P.A., Mountzouris, K.C., Rosu, C., Zoumpopoulou, G., Papadimitriou, K., Dalaka, E., Hadjipetrou, A., Theofanous, G., Strozzi, G.P., Carlini, N. 2010. Feed supplementation of *Lactobacillus plantarum* PCA 236 modulates gut microbiota and milk fatty acid composition in dairy goats—A preliminary study. *Int J Food Microbiol*, 141, S109–S116.

Markowiak, P., S.´lizewska, K., Nowak, A., Chlebicz, A., Zbikowski, A., Pawłowski, K., Szeleszczuk, P. 2019. Probiotic microorganisms detoxify ochratoxin A in both a chicken liver cell line and chickens *J Sci Food Agric*, 99, 4309–4318.

Milani, J., Heidari, S. 2017. Stability of ochratoxin A during bread making process. *J Food Saf*, 37, 1–6. doi:10.1111/jfs.12283.

Mujnisa, A., Gustina, L., Natsir, A., Hasan, S. 2018. Dosage Effects of *Lactococcuslactis* ssp. lactis 2 as a probiotic on the percentage of carcass, abdominal fat content and cholesterol level in broilers. *Int J Poult Sci*, 17, 100–105.

Oliveira, D., Vidal, L., Ares, G., Walter, E.H.M., Rosenthal, A., Deliza, R.S. 2017. Microbiological and physicochemical screening of probiotic cultures for the development of non-fermented probiotic milk. *LWT-Food Sci Technol*, 79, 234–241.

Plessas, S., Nouska, C., Karapetsas, A., Kazakos, S., Alexopoulos, A., Mantzourani, I., Chondrou, P., Fournomiti, M., Galanis, A., Bezirtzoglou, E. 2017. Isolation, characterization and evaluation of the probiotic potential of a novel *Lactobacillus* strain isolated from Feta-type cheese. *Food Chem*, 226, 102–108.

Ponce, C.H., Dilorenzo, N., Quinn, M.J., Smith, D.R., May, M.L., Galyean, M.L. 2011. Case study: Effects of a directfed microbial on finishing beef cattle performance, carcass characteristics, and in vitro fermentation. *Prof Anim Sci*, 27, 276–281.

Qiao, M., Ying, G.-G., Singer, A.C., Zhu, Y-G. 2018. Review of antibiotic resistance in China and its environment. *Environ Int*, 110, 160–172.

Ran, T., Gomaa, W.M.S., Shen, Y.Z., Saleem, A.M., Yang, W.Z., McAllister, T.A. 2019. Use of naturally sourced feed additives (lactobacillus fermentation products and enzymes) in growing and finishing steers: Effects on performance, carcass characteristics and blood metabolites. *Anim Feed Sci Technol*, 254, 114190, doi:10.1016/j.anifeedsci.2019.05.013.

Roselli, M., Pieper, R., Rogel-Gaillard, C., De Vries, H., Bailey, M., Smidt, H., Lauridsen, C. 2017. Immunomodulating effects of probiotics for microbiota modulation, gut health and disease resistance in pigs. *Anim Feed Sci Technol*, 233, 104–119.

Saint-Cyr, M.J., Haddad, N., Taminiau, B., Poezevara, T., Quesne, S., Amelot, M., Daube, G., Chemaly, M., Dousset, X., Guyard-Nicodeme, M. 2017. Use of the potential

probiotic strain Lactobacillus salivarius SMXD51 to control Campylobacter jejuni in broilers. *Int J Food Microbiol*, 247, 9–17.

Sanders, M. E., Akkermans, L. M., Haller, D., Hammerman, C., Heimbach, J., Hormannsperger, G., Huys, G., Levy, D. D., Lutgendorff, F., Mack, D., Phothirath, P., Solano-Aguilar, G., Vaughan, E. 2010. 2010. Safety assessment of probiotics for human use. *Gut Microbes* 1, 164–185.

Santini, C., Baffoni, L., Gaggia, F., Granata, M., Gasbarri, R., Di Gioia, D., Biavati, B. 2010. Characterization of probiotic strains: An application as feed additives in poultry against Campylobacter jejuni. *Int J Food Microbiol*, 141, S98–S108.

Sarrazin, S., Joosten, P., Gompel, L.V., Luiken, R.E.C., Mevius, D.J., Wagenaar, J.A., Heederik, D.J.J. 2019. Quantitative and qualitative analysis of antimicrobial usage patterns in 180 selected farrow-to-finish pig farms from nine European countries based on single batch and purchase data. *J Antimicrob Chemother*, *74*, 807–816.

Seal, B.S., Drider, D., Oakley, B.B., Brüssow, H., Bikard, D., Rich, J.O., Miller, S., Devillard, E., Kwan, J., Bertin, G., Reeves, S., Swift, S. M., Raicek, M., Gay, C. G. 2018. Microbial-derived products as potential new antimicrobials. *Vet Res* 2018, 49, 66.

Servin, A.L. 2004. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiol Rev*, 28, 405–440, ISSN: 1574–6976.

Smialek, M., Burchardt, S., Koncicki, A. 2018. The influence of probiotic supplementation in broiler chickens on population and carcass contamination with Campylobacter spp-Field study. *Res Vet Sci*, 118, 312–316.

Song, X., Huang, Q., Zhang, Y., Zhang, M., Xie, J., He, L. 2019. Rapid multiresidue analysis of authorized/banned cyclopolypeptide antibiotics in feed by liquid chromatography–tandem mass spectrometry based on dispersive solid-phase extraction. *J Pharm Biomed*, 170, 234–242.

Sornplang, P.; Piyadeatsoontorn, S. Probiotic isolates from unconventional sources: a review. *J. Anim. Sci. Technol.* 2016, *58*, 26, doi:10.1186/s40781-016-0108-2.

Srinivas, B., Rani, G.S., Kumar, B.K., Chandrasekhar, B., Krishna, K.V., Devi, T.A., Bhima, B. 2017. Evaluating the probiotic and therapeutic potentials of *Saccharomyces cerevisiae* strain (OBS2) isolated from fermented nectar of toddy palm. *AMB Express*, 7, 2, doi:10.1186/s13568-016-0301-1.

Sun, J., Zhang, H., Liu, Y.-H., Feng, Y. 2018. Towards understanding MCR-like colistin resistance. *Trends Microbiol*, 26, 794–808.

Suresh, K., Srinath, K., Pravesh, B. 2013. Safety concerns of probiotic use: a review. IOSR *J Dent Med Sci* 12, 56–60.

Temkin, E., Adler, A., Lerner, A., Carmel, Y. 2014. Carbapenem-resistant Enterobacteriaceae: biology, epidemiology, and management. *Ann N Y Acad Sci*, 1323, 22–42.

Tesfaye, A., Hailu, Y. 2019. The effects of probiotics supplementation on milk yield and composition of lactating dairy cows. *J Phytopharmacol*, 8, 12–17.

Tsai, C.C., Leu, S.F., Huang, Q.R., Chou, L.C., Huang, C.C. 2014. Safety evaluation of multiple strains of *Lactobacillus plantarum* and *Pediococcus pentosaceus* in Wistar rats based on the Ames test and a 28-day feeding study. *Sci World J*, 928652, doi:10.1155/2014/928652.

Vankerckhoven, V., Huys, G., Vancanneyt, M., Vael, C., Klare, I., Romond, M.-B., Entenza, J. M., Moreillon, P., Wind, R. D., Knol, J., Wiertz, E., Pot, B., Vaughan, E. E., Kahlmeter, G., Goossens, H. 2008. Biosafety assessment of probiotics used for human consumption: Recommendations from the EU-PROSAFE project. *Trends Food Sci Technol*, 19, 102–114.

Vase-Khavari, K., Mortezavi, S.-H., Rasouli, B., Khusro, A., Salem, A.Z.M. 2019. The effect of three tropical medicinal plants and superzist probiotic on growth

performance, carcass characteristics, blood constitutes, immune response, and gut microflora of broiler. *Trop Anim Health Prod*, 51, 33–42.

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., Drider, D. 2019. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Front Microbiol*, 10, 57, doi:10.3389/fmicb.2019.00057.

Wang, N., Wu, W., Pan, J., Long, M. 2019. Detoxification strategies for zearalenone using microorganisms: A review. *Microorganisms* 2019, 7, 208, doi:10.3390/microorganisms7070208.

Wang, Q., Huang, S.Q., Li, C.Q., Xu, Q., Zeng, Q.P. 2017. *Akkermansia muciniphila* may determine chondroitin sulfate ameliorating or aggravating osteoarthritis. *Front Microbiol* 2017, 8, 1955, doi:10.3389/fmicb.2017.01955.

Wang, X., Wang, Y., Zhou, Y., Li, J., Yin, W., Wang, S., Zhang, S., Shen, J., Shen, Z., Wang, Y. 2018. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. *Emerg Microbes Infect*, 7, 1–9, doi:10.1038/s41426-018-0124-z.

Wu, Y., Wang, B., Zeng, Z., Liu, R., Tang, L., Gong, L., LI, W. 2019. Effects of probiotics Lactobacillus plantarum 16 and Paenibacilluspolymyxa 10 on intestinal barrier function, antioxidative capacity, apoptosis, immune response, and biochemical parameters in broilers. *Poult Sci*, 98, 5028–5039.

Xia, X., Wang, Z., Fu, Y., Du, X.-D., Gao, B., Zhou, Y., He, J., Wang, Y., Shen, J., Jiang, H., Wu, Y. 2019. Association of colistin residues and manure treatment with the abundance of mcr-1 gene in swine feedlots. *Environ Int*, 127, 361–370.

Xiao, L., Estellé, J., Kiilerich, P., Ramayo-Caldas, Y., Xia, Z., Feng, Q.S., Pedersen, A.Ø., Kjeldsen, N.J., Liu, C. A 2016. Reference gene catalogue of the pig gut microbiome. *Nat Microbiol*, 1, 16161, doi:10.1038/nmicrobiol.2016.161.

Yeo, S., Lee, S., Park, H., Shin, H., Holzapfel, W. 2016. Development of putative probiotics as feed additives: Validation in a porcine-specific gastrointestinal tract model. *Appl Microbiol Biotechnol*, 100, 10043–10054.

Zhao, P., Kim, I. 2015. Effect of direct-fed microbial on growth performance, nutrient digestibility, fecal noxious gas emission, fecal microbial flora and diarrhea score in weanling pigs. *Anim Feed Sci Technol* 2015, 200, 86–92.

Zhu, G., Ma, F., Wang, G., Wang, Y., Zhao, J., Zhang, H., Chen, W. 2018. Bifidobacteria attenuate the development of metabolic disorders, with inter-and intra-species differences. *Food Funct*, 9, 3509–3522.

Zhu, Y., Hassan, Y.I., Lepp, D., Shao, S., Zhou, T. 2017. Strategies and methodologies for developing microbial detoxification systems to mitigate mycotoxins. *Toxins*, 9, 130, doi:10.3390/toxins9040130.

CHAPTER 3 − Complete genomic analysis of *Lactobacillus reuteri* PNW1

(Part of this chapter has been published in the *Journal of Microbiology Resource Announcement,* 2019,8(8): e00034-19, https://doi.org/10.1128/MRA.00034-19)

Abstract

This study focuses on the whole-genome sequence of *Lactobacillus reuteri* PNW1. The draft genome of *L. reuteri* PNW1 was assessed in order to identify important genes supporting its probiotic potential. The genomic DNA of the isolate was extracted and sequenced using illumina Miseq instrument. The sequenced genomic data was assessed for quality reads using FastQC before assembling with SPAdes. The draft assembly was annotated with Prokaryotic Genome Annotation Pipeline (PGAP) and Rapid Annotations using Subsystems Technology (RAST). Rapid *in silico* analysis of secondary metabolite biosynthesis gene clusters was assessed using antiSMASH. PathogenFinder and ResFinder were used to determine the pathogenicity of *L. reuteri* PNW1 towards human hosts and the presence of acquired antimicrobial resistance genes respectively. A total of 5.2 GB data comprising 8,209,104 paired-end reads were generated. The assembled genome was 2,430,215 bp long in 420 contigs with 39% G+C content. The number of protein-coding sequences and structural RNAs were 2,581 and 79, respectively. Among all known genes which could be responsible for the production of toxic biochemicals, only arginine deiminase (EC3.5.3.6) was spotted. Four coding sequences (CDS) putative for D-lactate dehydrogenase (EC1.1.1.28) of different sizes were found at different locations within the assembly genome, while CDS putatively encode for L-lactate dehydrogenase (EC1.1.1.27) were also found at different locations across the contigis. Lipase/esterase and protease are found among the extracellular digestive enzymes. Members of EPS cluster for exopolysaccharide biosynthesis are among the genes identified to be putatively involved in adhesion. Some coding sequences predictably involved in secretion of

bioactive secondary metabolites, improve metabolism in the host while those required for stress tolerance, are located in different loci with the genome.

3.1. Introduction

Probiotics have been considered to fall into the class of most popular bioactive and health functional foods (Bosnea *et al.*, 2017). Lactic acid bacteria (LAB) have been reported to be the most commonly used bacterial strains as probiotics. Among many other direct beneficial effects to the host system, LAB have the ability to inhibit the growth of pathogens, reduce symptoms of lactose intolerance, enhance the immune system and demonstrate anti-carcinogenic activity (de LeBlanc and LeBlanc, 2014; Plessas *et al.*, 2017).

Lactobacillus reuteri is a known and appropriate model in studies that involve microbial ecological and evolutionary mechanisms of vertebrates gut symbionts. The organism is found in the GIT of most mammals such as humans, pigs, mice, rats, dogs, sheep, cattle and birds (Oh *et al.*, 2010; Walter *et al.*, 2011). Though its abundance in human is much lower and sometimes occasionally found (Walter, 2008), intensive studies by different experts have been conducted over the past decades unveiling several beneficial features of *L*. *reuteri* due to its popular use as probiotics. Different strains of *L. reuteri* have been phylogenetically identified to have a stable evolutionary relationship with the host, thus inferring a predictable potential for viable mutualism between the organism and the host (Foster and Wenseleers, 2006; Dethlefsen *et al.*, 2007; Douglas, 2008).

Lactobacillus reuteri, as a probiotic agent, has been described with adaptation to nutrient availability, gut environmental conditions, enhanced adhesion mechanisms, secretion of bioactive principles and immunomodulatory potential through secretion of exopolysaccharides (Saulnier *et al*., 2011). Gut resident microbes are playing more important roles in the immunomodulatory mechanisms of the hosts than was previously

expected. In a study conducted by Lee *et al.* (2016), aged mice were supplied with anti-inflammatory *L*. *reuteri* BM36301 through drinking water alongside regular diet for a period of 5 months. The bacterial strains evoked different gender-based consequences in mice. Males mice experienced less weight gain, higher testosterone levels, development of higher serum levels of TNF-α and insulin, while female mice maintained lower serum TNF-α, healthy skin with active folliculogenesis and hair growth.

Moreover, various strains of *L. reuteri* produce array of bioactive principles against different pathogens (Spinler *et al.*, 2008). Reuterin is a combination of different beta-hydroxypropionaldehyde with broad spectrum inhibitory effect against pathogens. High degree secretion of reuterin makes *L. reuteri* unique in the reduction of glycerol. Increased elimination of the parasitic intestinal *Cryptosporidium parvum* by *L. reuteri* was observed in a murine model with AIDS condition. Administration of *L. reuteri* was reported to reduce mortality in chickens and turkeys upon infection with Salmonella (Walter *et al.*, 2011) and confirmed reducing duration and severity of diarrhoea caused by rotavirus in children (Weizman *et al.*, 2005).

This study is therefore conceptualized to assess the entire genome of *Lactobacillus reuteri* PNW1 in order to dertimine genome-based probiotics features which maybe present in the strain.

3.2. Materials and methods

3.2.1. Extraction of genomic DNA

The candidate probiotic bacterial strain, isolated from the gastrointestinal tracts of compassionately sacrificed weaned piglets of the indigenous South African Windsnyer pig breed, was collected from the Agricultural Research Council, Animal Production Institute, Gastrointestinal Microbiology and Biotechnology Division, Irene, South Africa (APIEC13/008). The organism was cultured in de Man-Rogosa-Sharpe broth (Oxoid, UK),

under strict anaerobic conditions and incubated at 37 °C for 24 hours in an anaerobic jar provided with an AnaeroGen system (Thermofisher, UK). The culture was later washed twice in phosphate buffer saline (PBS) and centrifuge at 6000 rpm for 5 minutes each time. Bacterial genomic DNA was extracted with a DNA extraction kit (Zymo Research, USA), in accordance with the manufacturer's instructions. The degree of purity and the concentration of the extracted genomic DNA was determined using a nanodrop spectrophotometer (NanoDrop 2000, ThermoFisher). The genomic DNA material was kept at −20 ^{o}C for further use.

3.2.2. 16S rRNA identification of the isolates

Identification of isolates was confirmed through PCR amplification of the 16S rRNA region. Genomic DNA was used as the template (16S rDNA) with universal primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-AAGGAGGTGATCCAGCC-3'). The PCR conditions included a first step of 95 ^{o}C for 2 min and 40 cycles of 95 ^{o}C for 30 sec (denaturation), followed by 45 ^{o}C for 30 sec (annealing) and 72 ^{o}C for 30 sec (extension) with a final extension step of 72 ^{o}C for 7 min (Hwanhlem *et al.*, 2017). Amplicon was confirmed by gel-electrophoresis for 45 minutes at 60 V in 1% (w/v) agarose gel with ethidium bromide (0.5 mg/ml) in 0.5TAE (Tris-Acetate-EDTA) buffer (pH 8.0) and then observed under UV light.

The PCR amplicon was sequenced at the Agricultural Research Council, Pretoria, South Africa. The sequenced nucleotide was aligned with the National Centre of Biotechnology Information (NCBI) database, using the Basic Local Alignment Search Tool (BLAST) algorithm. The partial sequenced data (1247 bp) was later submitted to the GenBank data base and accession number (MK123483) received for the isolates (https://www.ncbi.nlm.nih.gov/nuccore/MK123483.1/).

3.2.3. Whole genome sequence (WGS) of the isolates

The genome was prepared using an Illumina Nextera DNA Flex library prep kit, and the run performed on an Illumina MiSeq Platform1 system at the Agricultural Research Council, Biotechnology Platform (Pretoria, South Africa). A total of 5.2 GB data comprising 8,209,104 paired-end reads was generated, with a maximum read length of 2 × 300 bp. The data was filtered for low-quality reads and adapter regions using Trimmomatic version 0.32 (Bolger *et al.*, 2014), with the minimum quality score of 15 and minimum sequence length of 70, the 5' and 3' minimum quality score required was 14. The adapter sequences were clipped using a mismatch value of 2, a palindrome clip threshold of 30, and a simple clip threshold of 15. The quality of the trimmed reads was assessed using FastQC version 0.11.5. A draft genome assembly was constructed using SPAdes version 3.7.1 (Bankevich *et al.*, 2012). Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v. 4.6 (Tatusova *et al.*, 2016; Haft *et al.*, 2018) and Rapid Annotations using Subsystems Technology (RAST) (Overbeek *et al.*, 2014). Rapid *in silico* analysis of secondary metabolite biosynthesis gene clusters was assessed using antiSMASH v. 4.2.0 (Blin *et al.*, 2017). PathogenFinder v. 1.1(Cosentino *et al.*, 2013) and ResFinder v. 3.1 (Zankari *et al.*, 2012) were used to determine the pathogenicity of *L. reuteri* PNW1 towards human hosts and the possible presence of antimicrobial resistance genes. Default parameters were used for all the software employed in the analysis.

3.3. Results

3.3.1. Summary of the entire genome of *Lactobacillus reuteri* PNW1

The assembled genome was 2,430,215 bp long in 420 contigs (with protein-encoding genes [PEGs]) and final coverage of 1,248x. The *N*50 value was 28,048 bp, the number of contigs that were ≥*N*50 was 24, while the average G+C content was 39%. The numbers of protein-coding sequences and structural RNAs were 2,581 and 79, respectively. The number of tRNAs was 68 and 3 for non-coding RNAs. The total number of pseudo genes

was 132. Out of this number, one pseudo gene was the result of ambiguous residues, 41 genes resulted from frameshift, 73 were the outcome of incomplete nucleotides, 30 were due to internal stop while 11 pseudo genes were the result of multiple problems. The circular representation of the entire genome of the *Lactobacillus reuteri* PNW1 is presented in Figure 3.1 and the graphical distribution of the subsystem features within the genome is presented in Figure 3.2.

In silico analyses of secondary metabolites of the genome sequence revealed seven different secondary metabolites on nine gene clusters. Bacteriocin, an important bioactive peptide, was found in gene cluster 7 at c00087_NODE_87 and located between 1 - 5663 nucleotides (Fig 3.3). *Lactobacillus reuteri* PNW1 was found harbouring acquired resistant gene *lnuC*, which confers resistance to Lincosamide and also *tetW*, which confers resistance against Tetracycline. The pathogenFinder revealed the isolate as a non-human pathogen. No virulent determinant or other genes with possible haemolytic potentials were detected. Among all known genes which may be responsible for the production of toxic biochemicals, only arginine deiminase (EC3.5.3.6) was spotted at a locus with Contig Identity NODE_63_length_11058_cov_736.490989 and the CDS is 1233 bp in length. All software was employed with default parameters.

Availability of data: This whole-genome shotgun project was deposited at DDBJ/ENA/GenBank under accession number RJWE00000000. The version described in this study is RJWE01000000 with SRA accession number PRJNA504734.

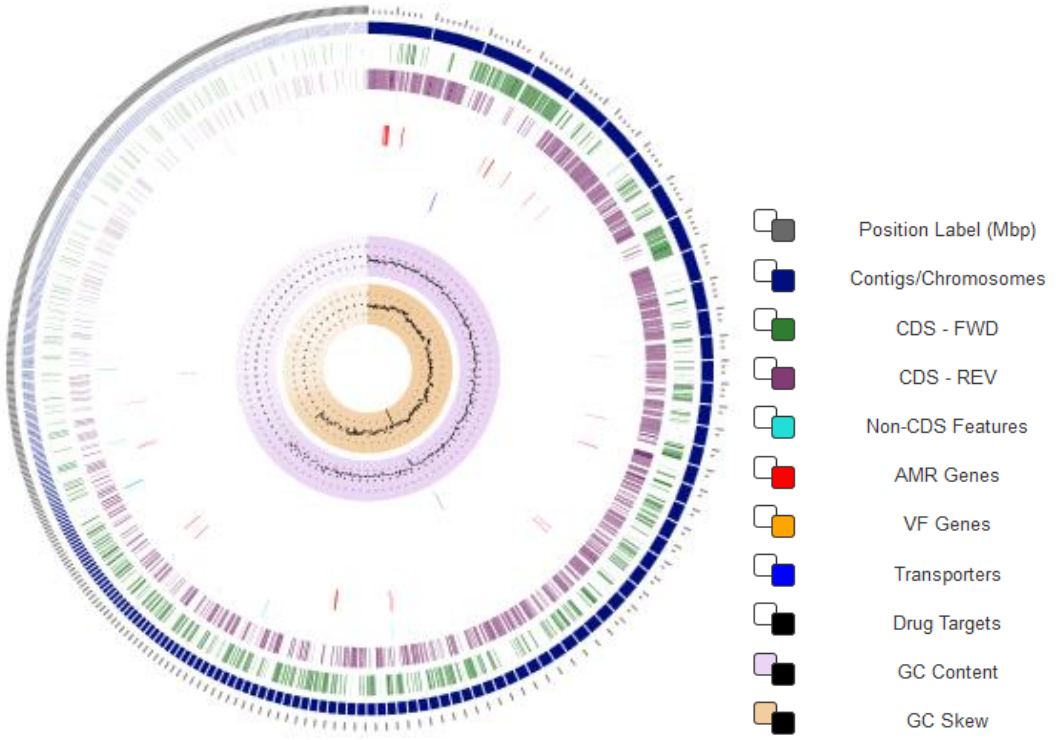

Figure 3.1: Circular genome map of the *L. reuteri* PNW1. The circular genome was generated with PATRIC sever **3.5.43**

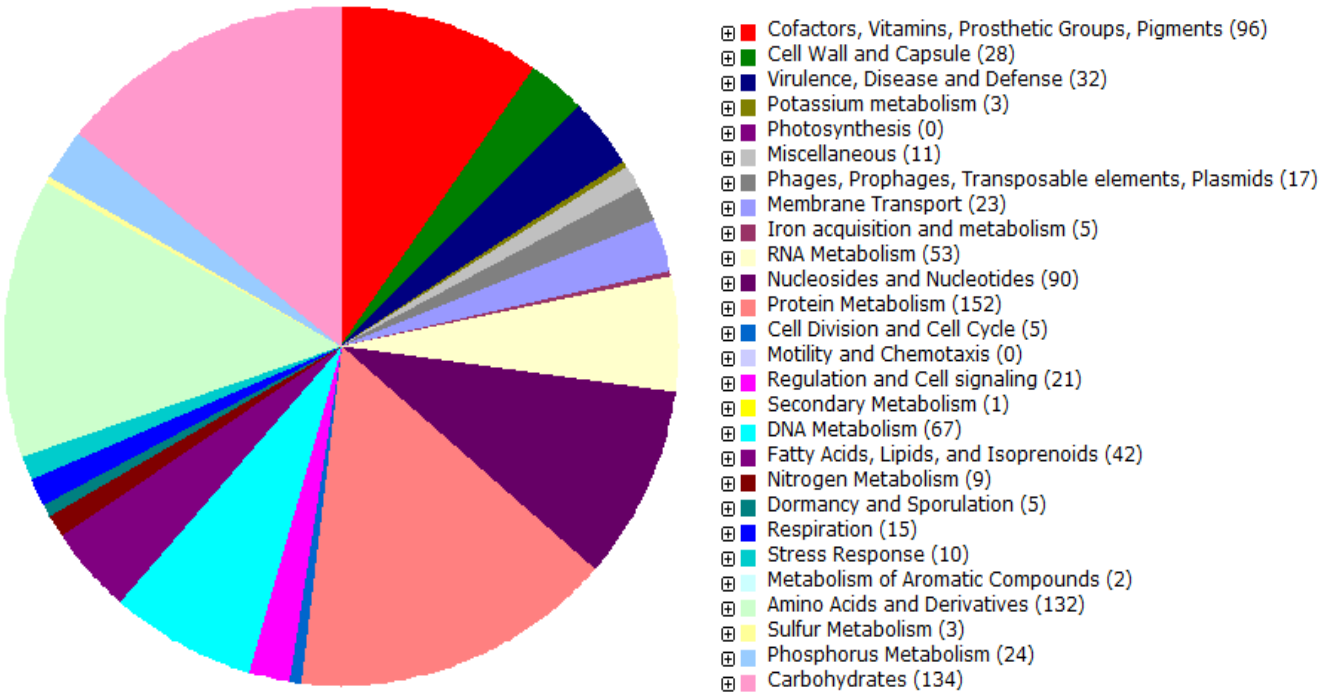

Figure 3.2: Distribution of subsystem features within the *L. reuteri* PNW1 genome. The distribution was generated on RAST sever with SEED viewer v.2.0

Figure 3.3: Gene cluster showing the position of biosynthetic bacteriocin.Core biosynthetic (bacteriocin) genes ( ), other genes ( ), TTA codon ( )

3.3.3. Overview of the functional importance of probiotic genes in the draft genome assembly

3.3.3.1. Coding sequence putatively involved in lactic acids production

D-lactate dehydrogenase

The coding sequences predictably encoding for D-lactate dehydrogenase (EC 1.1.1.28) were found at four (4) different locations with Contigs Identities as follows: NODE_121_length_2039_cov_707.017259, 992 base pairs long on the positive strand (Figure 3.4); NODE_34_length_20428_cov_693.602778, 1004 bp long also on the positive strand (Figure 3.5); NODE_84_length_6364_cov_694.430335, 992 bp long and stretching along the negative strand (Figure 3.6); and NODE_9_length_59183_cov_692.886159; 995 bp long also on the negative strand (Figure 3.7).

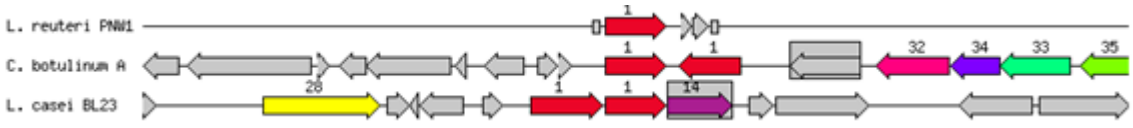

Figure 3.4: Annotation diagram showing the D-lactate dehydrogenase (EC 1.1.1.28) ( ) found at node 121 and 992 bp long on the +ve strand

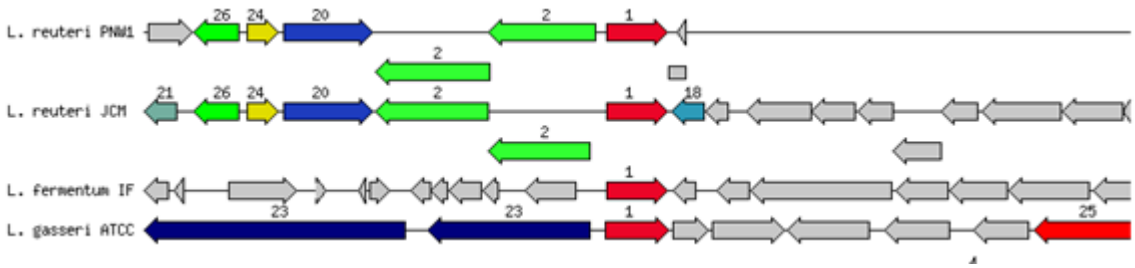

Figure 3.5: Annotation diagram showing the D-lactate dehydrogenase (EC 1.1.1.28) ( ) found at node 34 and 1004 bp long on the +ve strand

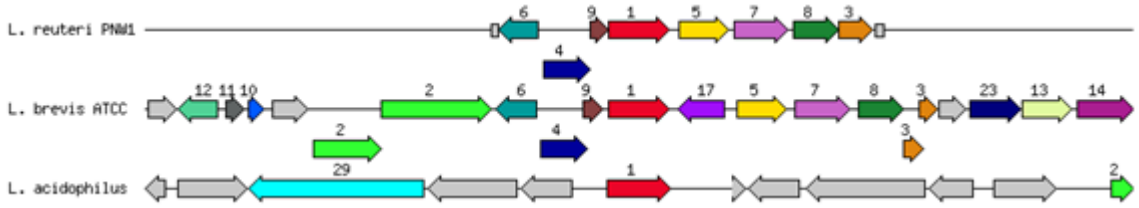

Figure 3.6: Annotation diagram showing the D-lactate dehydrogenase (EC 1.1.1.28) ( ) found at node 84 and 992 bp long on the –ve strand

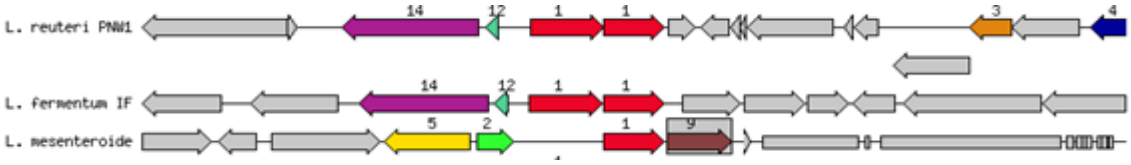

Figure 3.7: Annotation diagram showing the D-lactate dehydrogenase (EC 1.1.1.28) ( ) found at node 9 and 995 bp long on the –ve strand

L-lactate dehydrogenase

The coding sequences predicted for L-lactate dehydrogenase (EC 1.1.1.27) were found on eight (8) different loci, occuring both on the positive and negative strands, with Contigs Identities as follows: NODE_1_length_112835_cov_728.170920, 931 bp long, occuring on the negative strand (Figure 3.8); and NODE_3_length_89934_cov_728.092832, 974 bp in length along the positive strand (Figure 3.9). Two of the coding sequences are found on the same Contig Identity NODE_5_length_72796_cov_657.017518, one of them was 704 bp long (Figure 3.10) and the other 272 bp long (Figure 3.11), both occuring along the positive strand. Others included NODE_7_length_63720_cov_661.990109,932 bp long, occuring on the negative strand (Figure 3.12), NODE_8_length_60149_cov_679.692879, 959 bp long, occuring on the positive strand (Figure 3.13), NODE_16_length_34715_cov_674.706950, 923 bp long, also found on the negative strand (Figure 3.14) and NODE_38_length_17401_cov_672.160067, 950 bp in length, occuring on the negative strand (Figure 3.15).

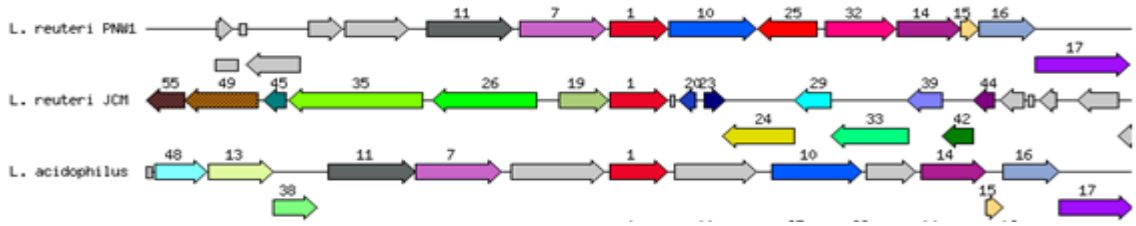

Figure 3.8: Annotation diagram showing the location of *L-lactate dehydrogenase* (EC 1.1.1.27) ( ) found at node 1 and 931 bp long on the –ve strand

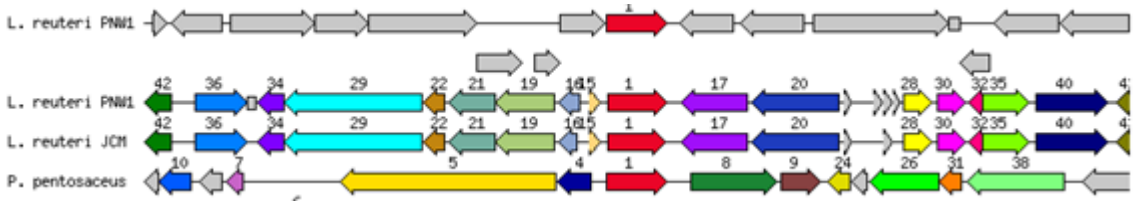

Figure 3.9: Annotation diagram the showing position of *L-lactate dehydrogenase* (EC 1.1.1.27) ( ) found at node 3 and 974 bp long on the +ve strand

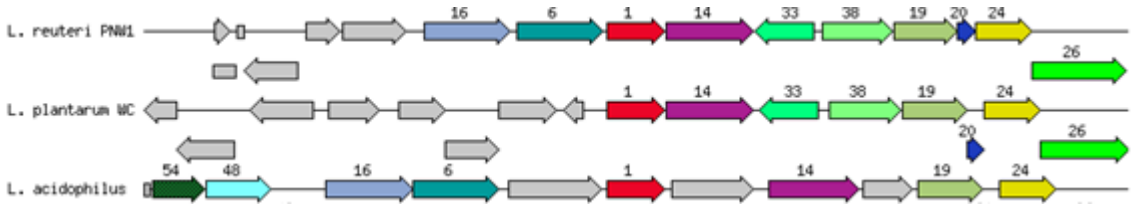

Figure 3.10: Annotation diagram showing the location of *L-lactate dehydrogenase* (EC 1.1.1.27) ( ) found at node 5 and 704 bp long on the +ve strand

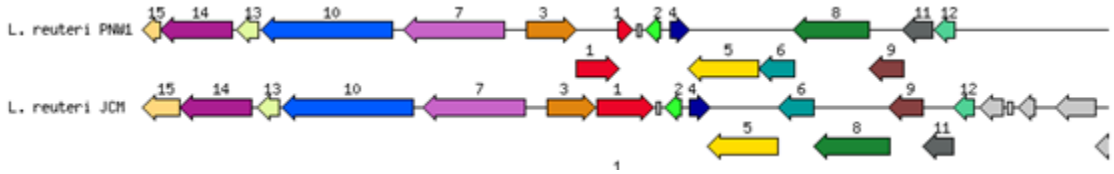

Figure 3.11: Annotation diagram showing the location of *L-lactate dehydrogenase* (EC 1.1.1.27) ( ) found at node 5 and 272 bp long on the +ve strand

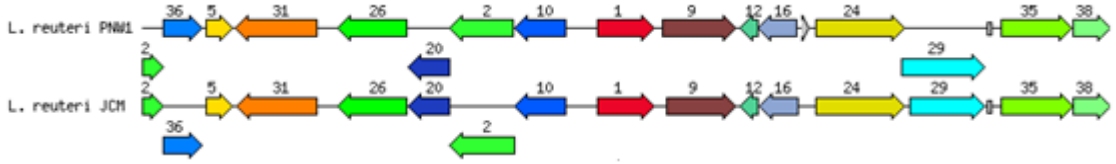

Figure 3.12: Annotation diagram showing the location of *L-lactate dehydrogenase* (EC 1.1.1.27) ( ) found at node 7 and 932 bp long on the –ve strand

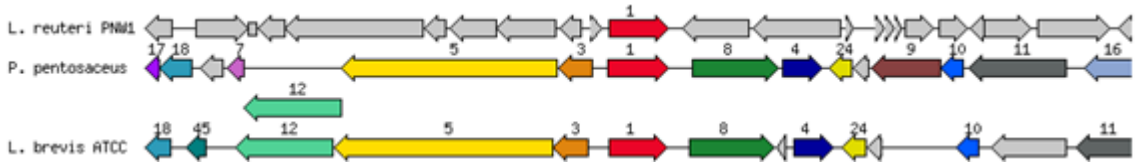

Figure 3.13: Annotation diagram showing the location of *L-lactate dehydrogenase* (EC 1.1.1.27) ( ) found at node 8 and 959 bp long on the +ve strand

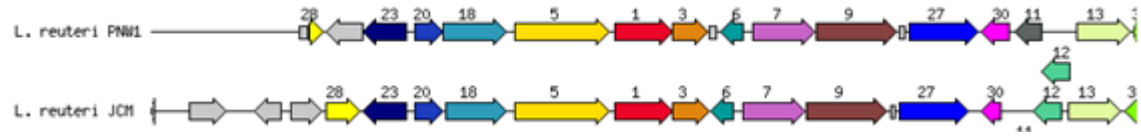

Figure 3.14: Annotation diagram showing the location of *L-lactate dehydrogenase* (EC 1.1.1.27) ( ) found at node 16 and 923 bp long on the –ve strand

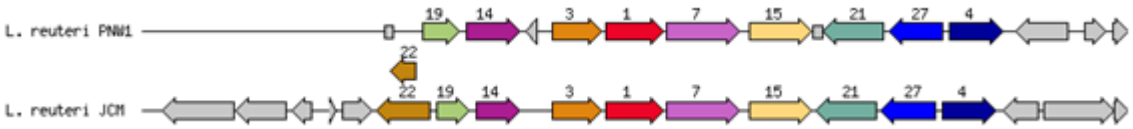

Figure 3.15: Annotation diagram showing the location of *L-lactate dehydrogenase* (EC 1.1.1.27) ( ) found at node 38 and 950 bp long on the –ve strand

NB. The sequence of nucleotide and amino acids of identified genes putatively involved in production of lactic acids are shown in Appendix A.

3.3.3.2. Coding sequence putatively involved in bioactive peptide production

The coding sequence for a bioactive peptide predicted to be bacteriocin helveticin J was found on the locus, with Contig Identity NODE_430_length_501_cov_0.737968. The entire fragment comprised 318 bp, starting from 183 nucleotide position and stretching to 500 along the positive strand (Figure 3.16). Another CDS putative for the function of probiotic, S-ribosylhomocysteine lyase (EC 4.4.1.21) @Autoinducer-2 production protein LuxS was found on a locus, with Contig Identity NODE_14_length_35644_cov_752.147873. The fragment is 477 bp long and stretches from 7515 to 7039 nucleotide position along the negative strand (Figure 3.17).

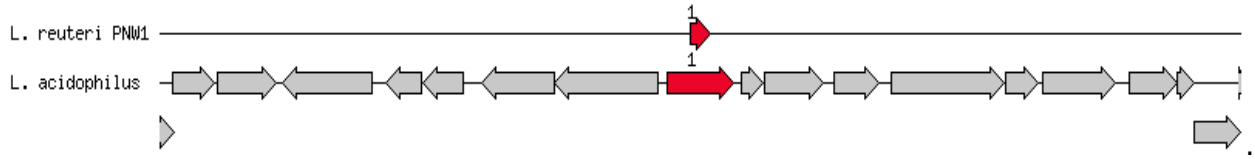

Figure 3.16: Annotation diagram showing the location of bacteriocin helveticin J ( ) found at node 430 and 318 bp long on the +ve strand

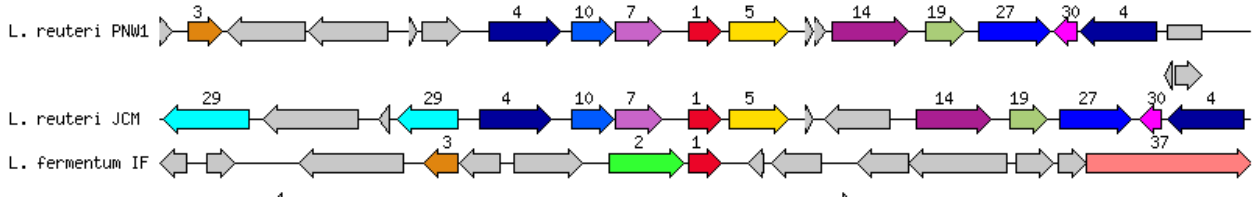

Figure 3.17: Annotation diagram showing the location of S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS ( ) found at node 14 and 477 bp long on the –ve strand

NB. The sequence of nucleotides and amino acids of identified genes putatively involved in production of bioactive peptide is shown in Appendix B

3.3.3.3. Coding sequence putatively involved in adhesion

A coding sequence predicted for Antiadhesin PIs, binding to squamous nasal epithelial cells was found at Contig Identity NODE_14_length_35644_cov_752.147873. The fragment was 1070 bp long, stretching between the nucleotide position at 16625 and 15555 along the negative strand (Figure 3.18). Another 705 base pairs long putatively involved in adhesion was Sortase A, LPXTG specific. It was equally predicted to have subsystem features as heme, hemin uptake and utilised systems in Gram-positive Sortase. The CDS was found on the locus with Contig Identity NODE_19_length_31967_cov_707.251162, starting from nucleotide position 21517 and stoping at 20813 along the negative strand (Figure 3.19).

Moreover, coding sequences predicted for the exopolysaccharides (EPS) cluster were also found on different loci within the draft genome assembly.Tyrosine-protein kinase transmembrane modulator EpsC, with subsystem feature predicted as Exopolysaccharide Biosynthesis was located on two different loci. The CDS, beginning from nucleotide position 981 and ending at 352 along the negative strand, 630 bp long and located on

Contig Identity NODE_16_length_34715_cov_674.706950 (Fig. 3.20). CDS located on Contig Identity NODE_40_length_16809_cov_641.784678 was 876 bp long, stretching between 3327 and 2452 nucleotide position along the negative strand (Figure 3.21). Another member of the EPS cluster, Tyrosine-protein kinase EpsD (EC 2.7.10.2), with Exopolysaccharide Biosynthesis, as predicted subsystem feature, was found on Contig Identity NODE_40_length_16809_cov_641.784678. The fragment was 747 bp long, running between 2439 and 1693 nucleotide position along the negative strand (Figure 3.22).

Other members of the EPS cluster also located in the genome included ATP synthase epsilon chain (EC 3.6.3.14) (with predicted subsystem features as F0F1-type ATP synthase) and DNA polymerase III, epsilon subunit related 3'-5' exonuclease. Both were found at Contig Identity NODE_27_length_25847_cov_752.015708 and NODE_49_length_14453_cov_753.679185 respectively. The ATP synthase epsilon chain (EC 3.6.3.14) was 432 bp long, stretching from 5543 to 5112 nucleotide position along the negative strand (Figure 3.23) while the DNA polymerase III, epsilon subunit related 3'-5' exonuclease, measure 540 bp long, occupied the space between 12311 and 11772 nucleotide position, also on the negative strand (Figure 3.24).

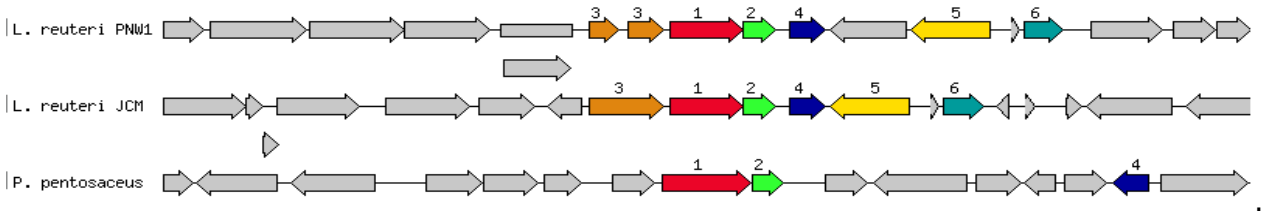

Figure 3.18: Annotation diagram showing the location of Antiadhesin Pls ( ) found at node 14 and 1070 bp long on the –ve strand

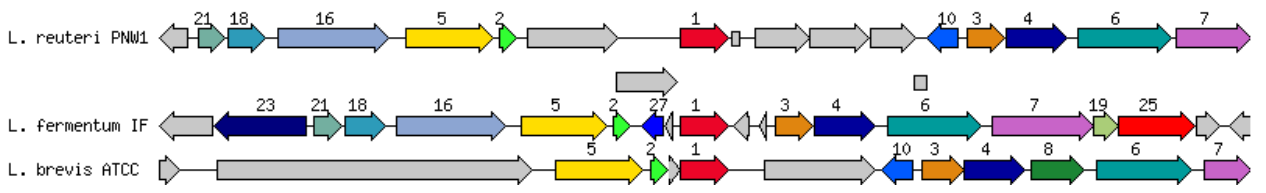

Figure 3.19: Annotation diagram showing the location of Sortase A, LPXTG ( ) found at node 19 and 705 bp long on the –ve strand

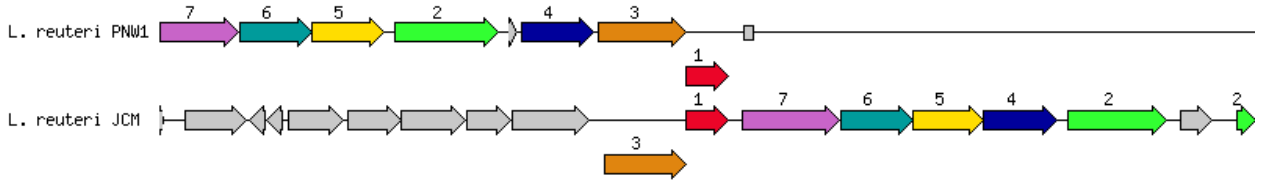

Figure 3.20: Annotation diagram showing the location of Tyrosine-protein kinase transmembrane modulator EpsC ( ) found at node 16 and 630 bp long on the –ve strand

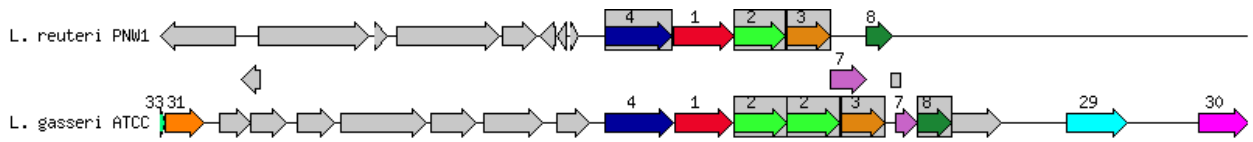

Figure 3.21: Annotation diagram showing the location of Tyrosine-protein kinase transmembrane modulator EpsC ( ) found at node 40 and 876 bp long on the –ve strand

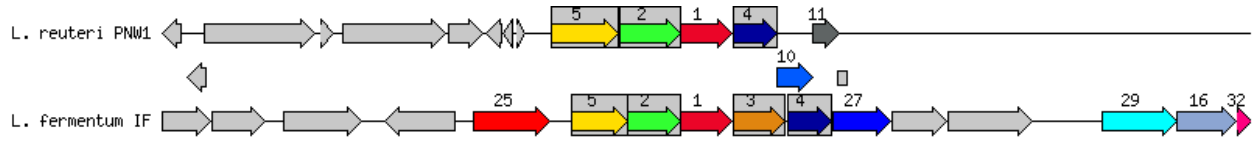

Figure 3.22: Annotation diagram showing the location of Tyrosine-protein kinase EpsD (EC 2.7.10.2) ( ) found at node 40 and 747 bp long on the –ve strand

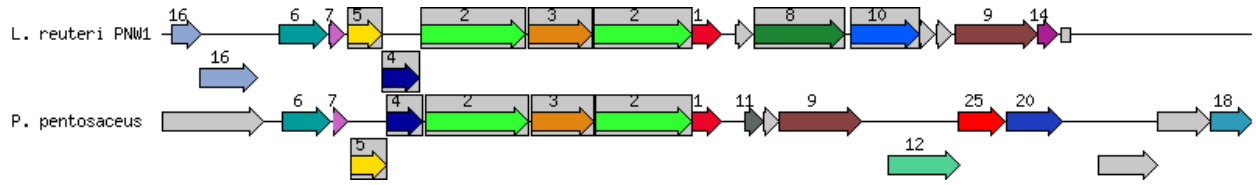

Figure 3.23: Annotation diagram showing the location of ATP synthase epsilon chain (EC 3.6.3.14) ( ) found at node 27 and 432 bp long on the –ve strand

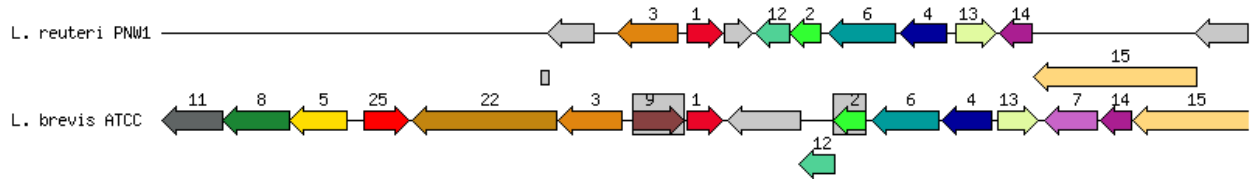

Figure 3.24: Annotation diagram showing the location of DNA polymerase III, epsilon subunit related 3'-5' exonuclease ( ) found at node 49 and 540 bp long on the –ve strand

NB. The sequences of nucleotides and amino acids of identified genes putatively involved in adhesion are shown in Appendix C.

3.3.3.4. Coding sequence putatively involved in the production of extracellular enzymes

Lipase

The CDS encoding for esterase/lipase-like protein was found at a locus on Contig Identity NODE_12_length_42516_cov_677.763948. The sequenced fragment began from the nucleotide position 21236 and stopped at 20370 along the negative strand with a total length of 867 bp (Fig. 3.25). A multifunctional Lipase/Acylhydrolase with GDSL-such as motif was also found at a location with Contig Identity NODE_13_length_38492_cov_730.424345. The coding sequence stretched from 21615 to 20689 nucleotide position on the negative strand, and was 927 bp long (Figuer 3.26). A CDS, measuring 984 bp long, predicted for ester hydrolase, Esterase/lipase (EC 3.1.1.-) was also located on Contig Identity NODE_1_length_112835_cov_728.170920. The fragment stretched from 90840 and extended to 89857 nucleotide position along the negative strand (Figure 3.27). A predicted Esterase/lipase/thioesterase is another lipase found on the Contig Identity NODE_46_length_15521_cov_666.702481. Its coding sequence starts from 14455 and extends over 860 nucleotides along the positive strand (Figure 3.28).

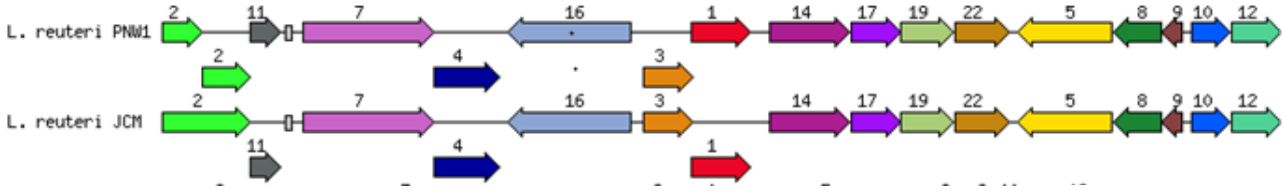

Figure 3.25: Annotation diagram showing the location of Esterase/lipase-like protein ( ) found at node 12 and 867 bp long on the –ve strand

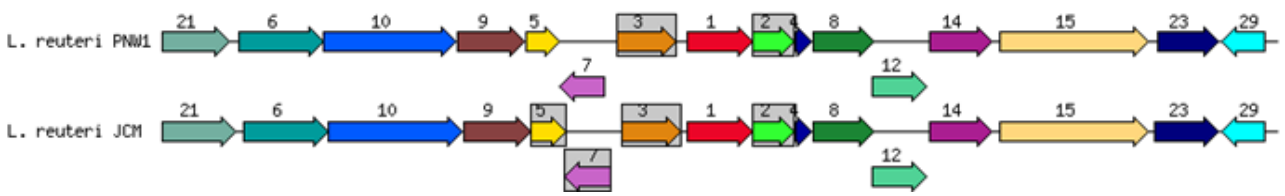

Figure 3.26: Annotation diagram showing the location of Lipase/Acylhydrolase with GDSL-like motif ( ) found at node 13 and 927 bp long on the –ve strand

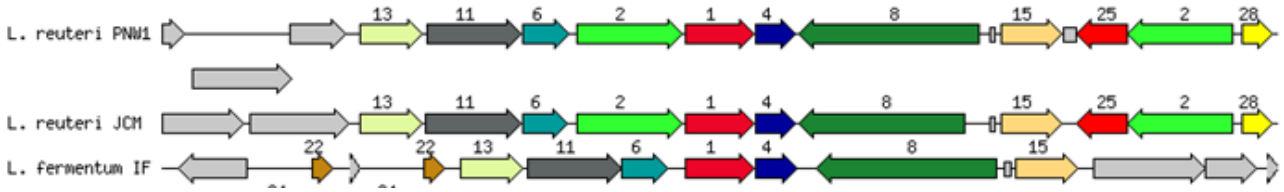

Figure 3.27: Annotation diagram showing the location of Esterase/lipase (EC 3.1.1.-) ( ) found at node 1 and 984 bp long on the –ve strand

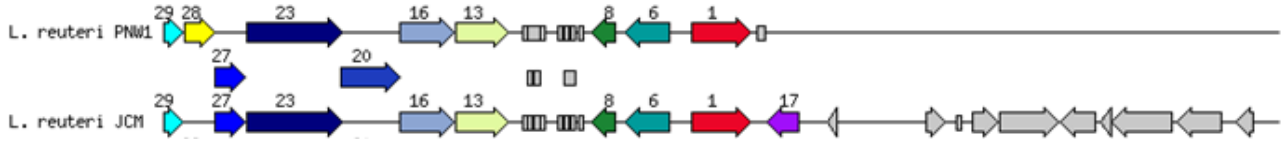

Figure 3.28: Annotation diagram showing the location of Esterase/lipase/thioesterase (EC 3.1.1.-) ( ) found at node 46 and 861 long on the +ve strand

Protease

A number of coding sequences predictably encoding for different types of protease, were found in the genome assembly. A Coding sequence putative for SOS-response repressor and protease LexA (EC 3.4.21.88), was found on Contig Identity NODE_102_length_3863_cov_652.891863. The fragment, which was 627 bp long, stretched from 1117 to 1743 nucleotides position along the positive strand (Figure 3.29). The two other similar CDS, for the same protease (EC 3.4.21.88), with their coding sequences measuring 144 and 129 bp in length, were also found co-existing on the same locus with Contig Identity NODE_8_length_60149_cov_679.692879.

A coding sequence putative for proteolytic enzyme, ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92) was aslo found on Contig Identity NODE_11_length_48244_cov_705.716961. The CDS, measuring 594 bp in length, stretched from 7693 nucleotide position along the negative strand and stopped at 7100 (Figure 3.30). Another proteolytic enzyme, ATP-dependent Clp protease, ATP-binding subunit ClpC, running from 55978 nucleotides position to 53486 along the negative strand, was also located on Contig Identity NODE_1_length_112835_cov_728.170920. The length of the entire fragment was 2493 bp (Figure 3.31). Two other closely related proteolytic

enzymes, ATP-dependent Clp protease ATP-binding subunit ClpX (1251 bp) and ATP-dependent Clp protease, ATP-binding subunit ClpE (2205 bp) were also located on different loci.

A putative Zn-dependent protease, measuring 582 bp, occupied the space between nucleotide position 5132 and 4551 along the negative strand. The CDS was found on Contig Identity NODE_31_length_22919_cov_653.947306 (Figure 3.32). An 867 bp long Membrane protease family protein BA0301 was found on Contig Identity NODE_3_length_89934_cov_728.092832, from 10656 to 11522 nucleotide position (Figure 3.33). A predicted Prophage Clp protease-like protein attributed to cyclic adenosine monophosphate (cAMP) signaling, was found on Contig Identity NODE_3_length_89934_cov_728.092832. The CDS fragment, measuring 777 bp, was in-between 67414 and 68190 nucleotide positions along the positive strand (Figure 3.34). Lon-like protease with PDZ domain and 1053 base pairs, was found at a locus with Contig Identity NODE_4_length_78326_cov_682.922812. Its CDS fragment was in-between 63466 and 64518 nucleotide position along the positive strand (Figure 3.35).

Moreover, a CDS encoding for Peptidyl-prolyl cis-trans isomerase containing cluster, FIG056164: rhomboid family serine protease was equally found at a locus with Contig Identity NODE_5_length_72796_cov_657.017518. The encoding gene was 660 pb long, stretching along the negative strand between the nucleotide at postions 3872 and 3213 (Figure 3.36). A coding sequence for FIG001621: Zinc protease was also found at Contig Identity NODE_6_length_70679_cov_675.485642. The CDS was 1248 bp long along the negative strand, starting from 46795 nucleotide position in the entire assembly and ending at 45548 (Figure 3.37). Lastly, a putative Serine protease, DegP/HtrA, do-like (EC 3.4.21.), measuring 1275 bp was found on Contig Identity NODE_9_length_59183_cov_692.886159. The CDS occupied the space between 8877 and 10151 positions of nucleotides along the positive strand (Figure 3.38).

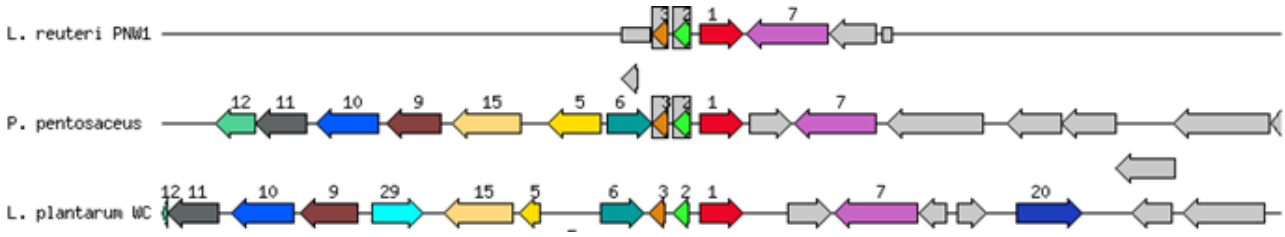

Figure 3.29: Annotation diagram showing the location of SOS-response repressor and protease LexA (EC 3.4.21.88) ( ) found at node 102 and 627 bp long on the +ve strand

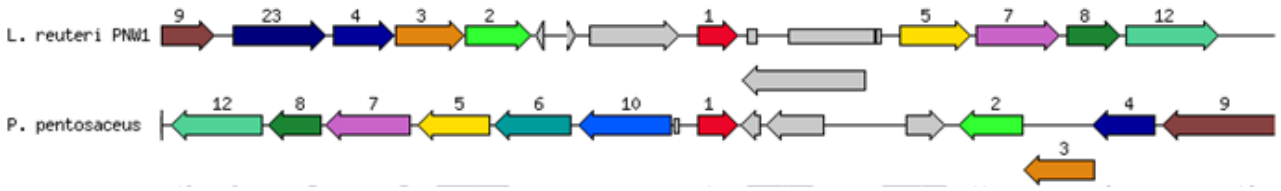

Figure 3.30: Annotation diagram showing the location of ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92) ( ) found at node 11 and 594 bp long on the –ve strand

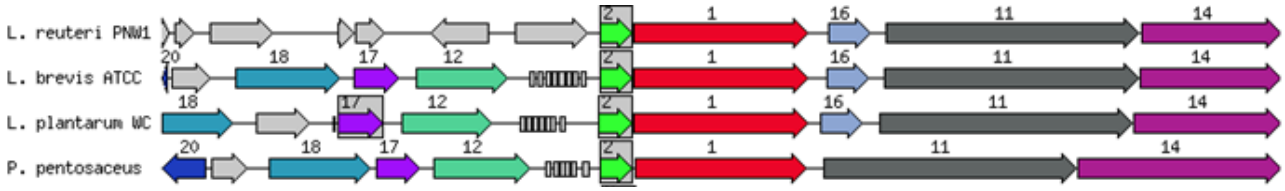

Figure 3.31: Annotation diagram showing the location of ATP-dependent Clp protease, ATP-binding subunit ClpC ( ) found at node 1 and 2493 bp long on the –ve strand

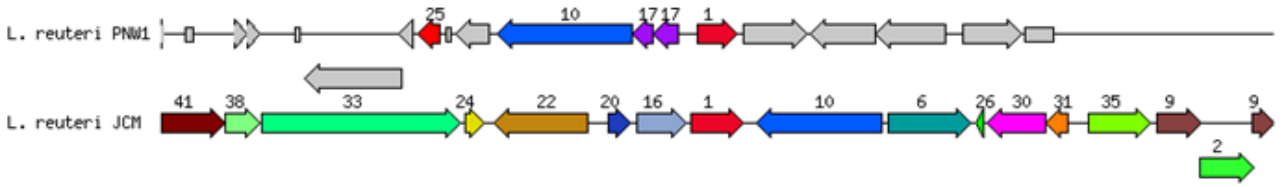

Figure 3.32: Annotation diagram showing the location of A putative Zn-dependent protease ( ) found at node 31 and 582 bp long on the –ve strand

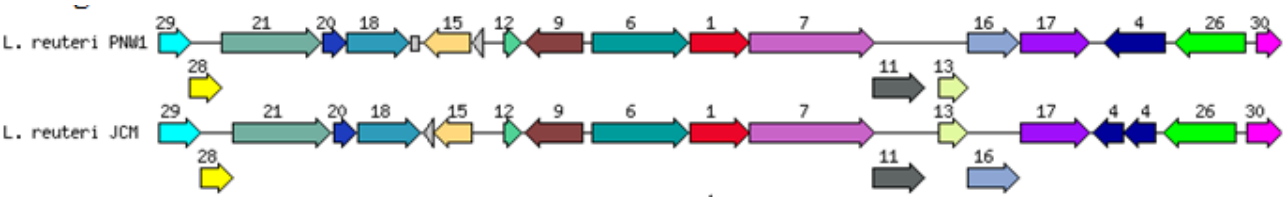

Figure 3.33: Annotation diagram showing the location of Membrane protease family protein BA0301 ( ) found at node 3 and 867 bp long on the –ve strand

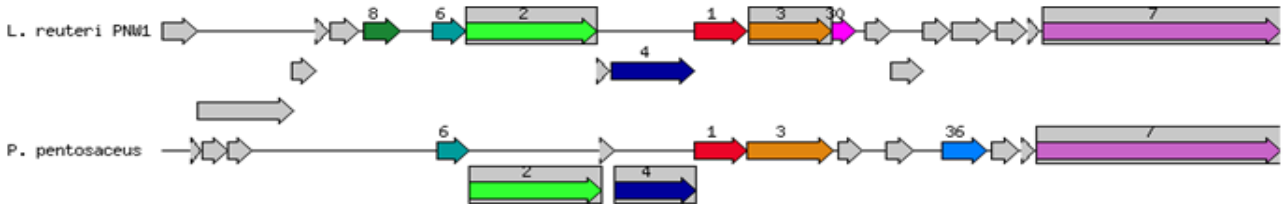

Figure 3.34: Annotation diagram showing the location of Prophage Clp protease-like protein ( ) found at node 3 and 777 bp long on the +ve strand

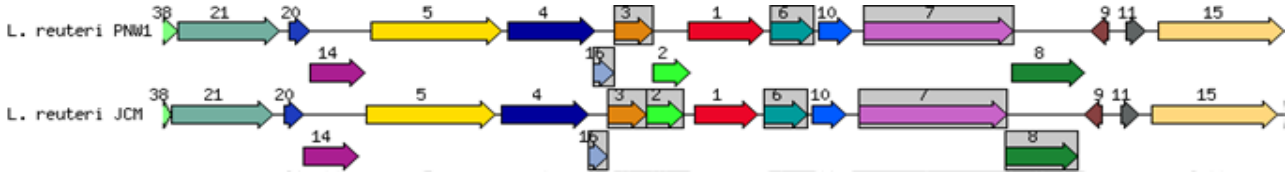

Figure 3.35: Annotation diagram showing the location of Lon-like protease with PDZ domain ( ) found at node 4 and 1053 b plong on the +ve strand

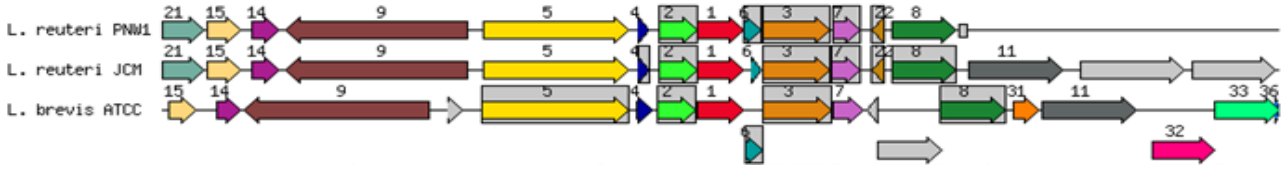

Figure 3.36: Annotation diagram showing the location of FIG056164: rhomboid family serine protease ( ) found at node 5 and 600 bp long on the +ve strand

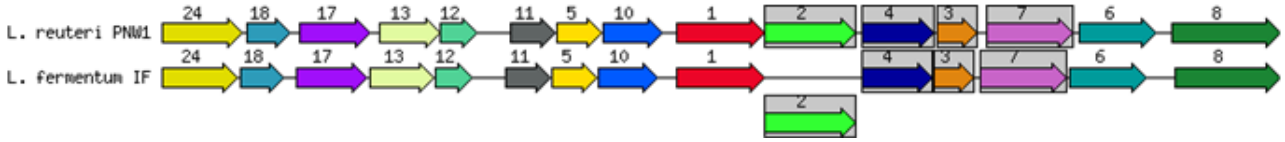

Figure 3.37: Annotation diagram showing the location of Zinc protease ( ) found at node 6 and 1248 bp long on the –ve strand

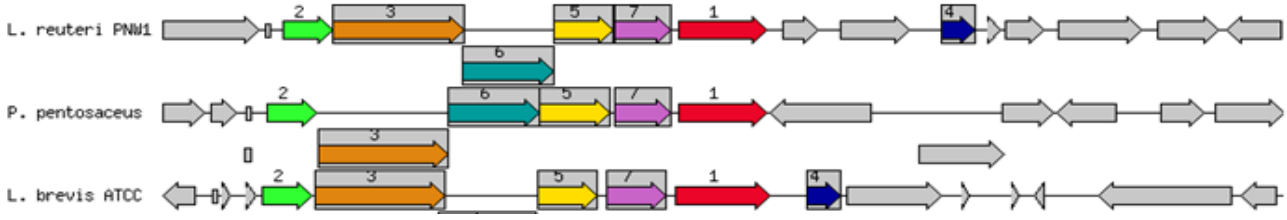

Figure 3.38: Annotation diagram showing the location of Serine protease, DegP/HtrA, do-like (EC 3.4.21.-) ( ) found at node 9 and 1275 long on the +ve strand

NB. The sequences of nucleotides and amino acids of identified genes putatively involved in production of extracellular enzymes are shown in Appendix D.

3.3.3.5. Coding sequence putatively involved in stress resistance

Coding sequence predictably encoding for DNA protection during starvation protein was found at two different loci within the genome assembly. CDS was located on Contigs Identity NODE_21_length_31034_cov_693.120005, measuring 468 bp, stretching from nucleotide position 22336 to 22803 along the positive strand (Figure 3.39). The CDS for the same DNA protection protein found on another locus had the following Contig Identity: NODE_53_length_13546_cov_697.963634. The fragment stretched from 5681 to 6226 along the positive strand, measuring 546 bp (Figure 3.40). Another stress resistant gene putatively encoding for Phosphate starvation-inducible protein PhoH, predicted ATPase, with CBSS-56780.10.peg.1536 showing subsystem feature Glycyl-tRNA synthetase with a cluster. Phosphate metabolism was detected on the locus with Contig Identity NODE_8_length_60149_cov_679.692879. The fragment was 1008 bp long located between 10248 and 9241 nucleotide position along the negative strand (Figure 3.41).

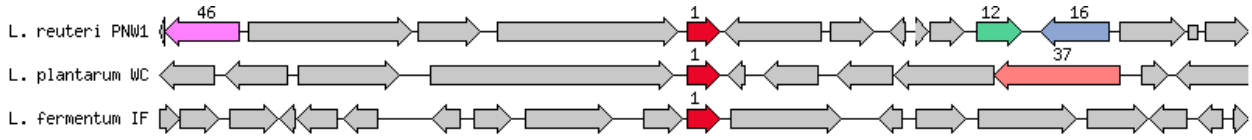

Figure 3.39: Annotation diagram showing the location of DNA protection during starvation protein ( ) found at node 21 and 468 bp long on the +ve strand

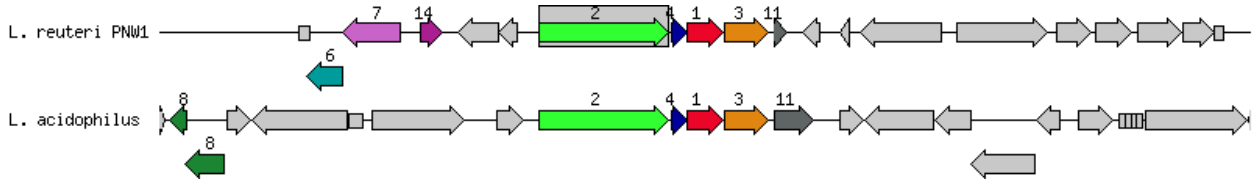

Figure 3.40: Annotation diagram showing the location of DNA protection during starvation protein ( ) found at node 53 and 546 bp long on the +ve strand

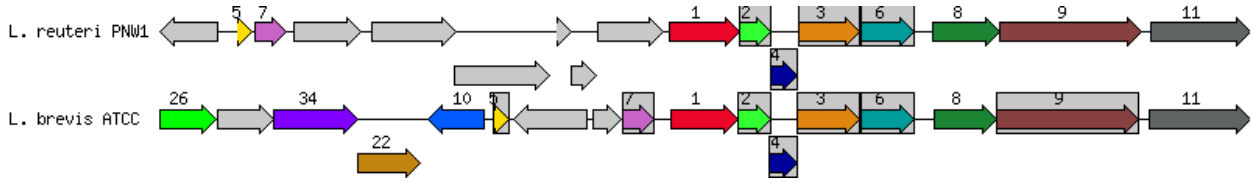

Figure 3.41: Annotation diagram showing the location of Phosphate starvation-inducible protein PhoH, predicted ATPase ( ) found at node 8 and 1008 bp long on the –ve strand

NB. The sequences of nucleotides and amino acids of identified genes putatively involved in stress resistance are shown in Appendix E.

3.3.3.6. Coding sequence putatively involved in active metabolism in the host

Some of the coding sequences involved in improving the host metabolism were also found in the draft assembly. Xylose isomerase domain protein TIM barrel was located at Contig Identity NODE_103_length_3774_cov_639.310392. The fragment runs from nucleotide position 1669 to 2508, measuring 840 bp and situated along the positive strand (Figure 3.42). Poly (glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52) is another CDS predicted to be involved in the host active metabolism. It is located on Contig Identity NODE_5_length_72796_cov_657.017518 and stretches from nucleotide position 47264 to 48787 along the positive strand, measuring 1524 bp (Figure 3.43). Two other CDS predicted for the same enzyme (EC 2.4.1.52) were found on the same locus, with Contig Identity NODE_72_length_9092_cov_690.969994. One of the fragments, measuring 1503 bp, stretched from 6862 to 5360 nucleotides position (Figure 3.44), while the other, measuring 1542 bp, stretched from 8405 to 6864, both along the negative strand (Figure 3.45). A CDS predicted for Beta-1, 3-glucosyltransferase, stretching from 3365 to 4378, was located at Contig Identity NODE_95_length_4914_cov_619.611657. The sequence was 1014 bp long and located on the positive strand (Figure 3.46).

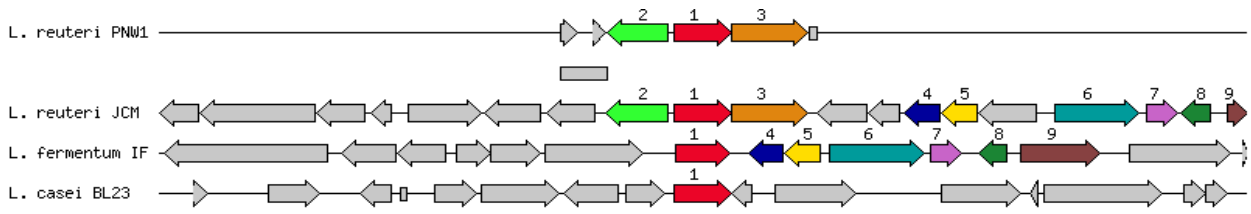

Figure 3.42: Annotation diagram showing the location of Xylose isomerase domain protein TIM barrel ( ) found at node 103 and 840 bp long on the +ve strand

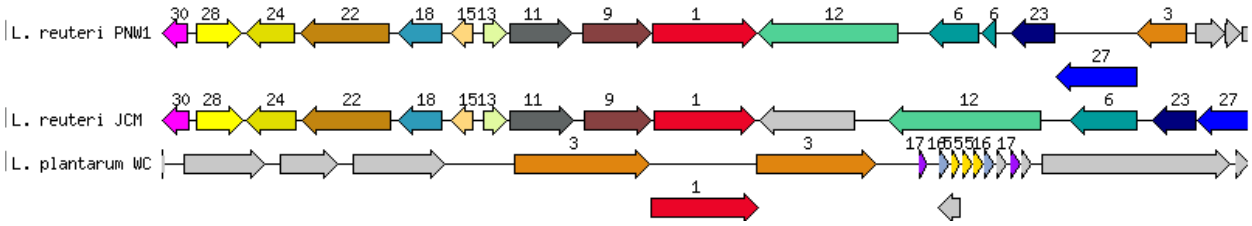

Figure 3.43: Annotation diagram showing the location of Poly (glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52) ( ) found at node 5 and 1524 bp long on the +ve strand

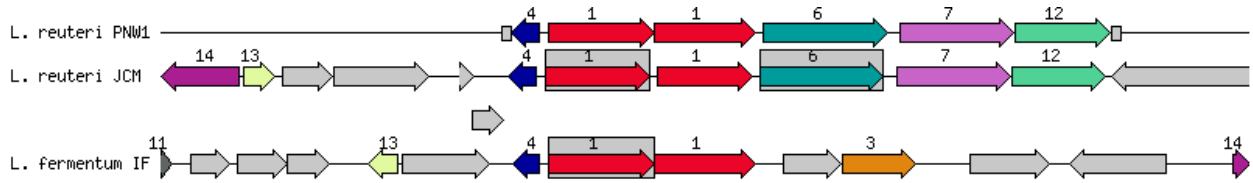

Figure 3.44: Annotation diagram showing the location of Poly (glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52) ( ) found at node 72 and 1504 bp long on the –ve strand

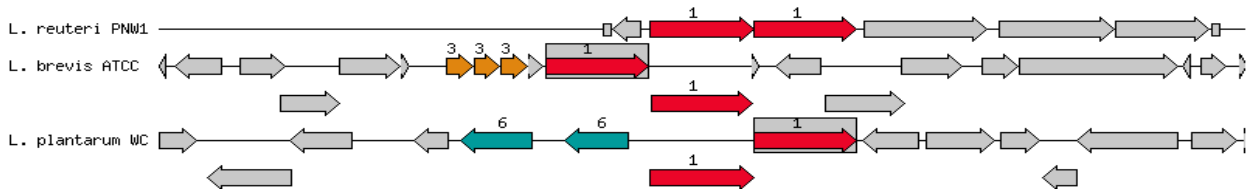

Figure 3.45: Annotation diagram showing the location of Poly (glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52) ( ) found at node 72 and 1542 bp long on –ve strand

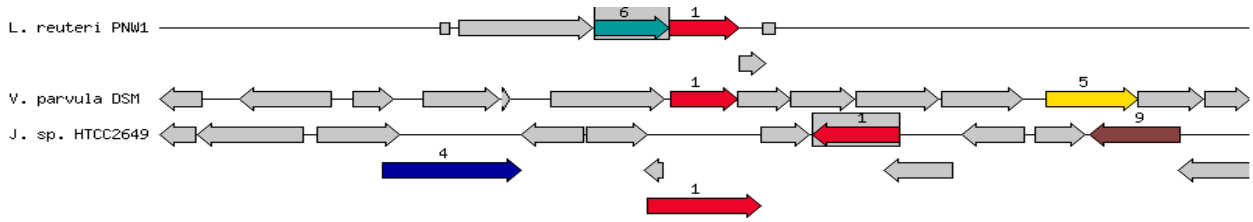

Figure 3.46: Annotation diagram showing the location of Beta-1, 3-glucosyltransferase ( ) found at node 95 and 1542 bp long on the –ve strand

NB. The sequences of nucleotides and amino acids of identified genes putatively involved in active metabolism in the host are shown in Appendix F.

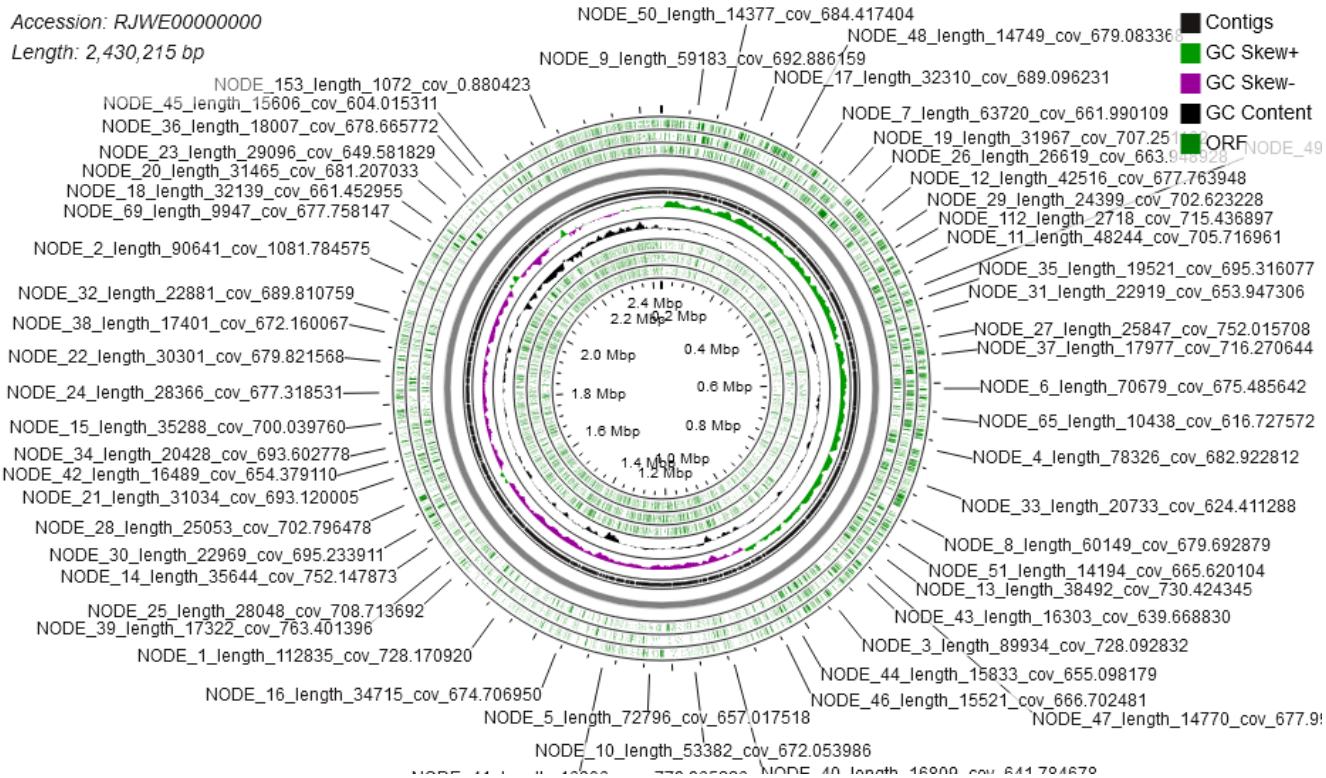

Figure 3.47: Circular genome mapping showing position of each Contig within the *L. reuteri* PNW1 genome. Mapping was generated using CGView (Grant and Stothard, 2008)

3.4. Discussion and conclusion

Genomic analysis and functional annotation of the entire genome of *Lactobacillus reuteri* PNW1 revealed the presence of several genes within the genome assembly, which are, in support of its probiotic efficacy. Several scientific reports have indicated that bioactive secondary metabolites produced by many probiotic agents, have implications on bacterial community interaction and, consequently, attenuate virulent markers on a number of pathogens (Choi *et al*., 2015; Oliveira *et al*., 2017; Sandes *et al.,* 2017; Dlamini *et al*., 2018). Lactic acids produced by Lactic acid bacteria (LAB), hinder the survival of neighbouring pathogens and inactivates human immune virus by increasing acidity of the surrounding environment (Nordeste *et al*., 2017). A total of 12 coding sequences were found in the genome of *L. reuteri* PNW1, predictably encoding for the production of lactic acids, four of which were putative for D-lactate dehydrogenase (EC 1.1.1.28) and eight for L-lactate dehydrogenase (EC 1.1.1.27).

A vast majority of diverse microorganisms are found in the gastrointestinal tract of humans and animals. A promising probiotic candidate with inherent ability for producing bioactive peptides and hydrogen peroxide would be an added advantage in antagonising neighbouring pathogens within the gut ecosystem (Kapse *et al*., 2019). Bacteriocin is one of the secondary metabolites secreted by certain beneficial microbes and characterised with significant bioactive potential against intestinal pathogens (Cotter *et al*., 2013). The *in silico* analysis of the entire genome for putative secondary metabolites, coupled with the functional annotation of the coding sequences within the *L. reuteri* PNW1 genome assembly, revealed the occurrence of CDS for bacteriocin helveticin J and S-ribosyl homocysteine lyase (EC 4.4.1.21) @Autoinducer-2 production protein LuxS, which is also a protein predictably involved in the secretion of bioactive peptides.

Adhesion of a probiotic candidate to the epithelia cells and mucus layer is among the desired trait for a probiotic. Adhesion provides ample opportunity for stability of the strain and effectively prolongs the antagonistic effects against unwanted gut residents, thus aiding in effective colonisation of the gut environment and exclusion of pathogens (Salas-Jara*et al*., 2016; Granato *et al*., 2004). The functional annotation of the *L. reuteri* PNW1 predicted seven different encoding genes within the genomic assembly, putative for adhesive characteristics in the isolate as follows: Antiadhesin Pls; binding to squamous nasal epithelial cells; Sortase A; LPXTG specific; and five different members of exopolysaccharide (EPS) cluster.

Among the most important traits expected in a probiotic candidate, is EPS biosynthesis (Rasmussen *et al*., 2008). Exopolysaccharides are produced by most lactic acid bacteria, including *L. reuteri* and are generally regarded as a food-grade. Exopolysaccharides contribute a great deal to the probiotic-host interactions within the intestinal mucosa and epithelial cells, thus impacting on the strain specificity of probiotic candidates (Zeng *et al*.,

2019). Many of the EPSs have been attributed to certain essential probiotic features such as biofilm formation, immunomodulation, aggregation, antioxidant and antimicrobial potentials (Wu *et al*., 2014).

Antiadhesive molecules specifically interact with the adhesin markers on infecting pathogens, thus preventing colonisation (Rafsanjany *et al*., 2013). Presence of the encoding gene putative for the Antiadhesin Plsin the genome of *L. reuteri* PNW1 improves the clinical functionality of the isolate as a prophylactic agent against infectious diseases.Sortase dependent proteins are an important group of cell surface proteins in *Lactobacillus* species and are responsible for sorting the largest number and various kinds of cell surface proteins, thus playing an important role in adhesion (Boekhorst *et al*., 2005; Marraffini *et al*., 2006). The TLPXTG specific Sortase A enzyme located in the *L. reuteri* PNW1 genome, is predicted to covalently anchor surface protein precursor to the cell wall, after the precursor has been transferred to the cell membrane (Zeng *et al*., 2019).

Some of the genes required by a microorganism in order to survive harsh environmental conditions are among the pool of beneficial genes identified in the draft assembly of *L. reuteri* PNW1. Two different CDS putative for protecting DNA during starvation protein are found on two different loci. These are important for the probiotic organism as a form of stress resistant mechanism. The genome also contains Phosphate starvation-inducible protein PhoH, predicted ATPase in its defence against harsh environments. The CDS is predicted to be involved in Glycyl-tRNA synthetase containg cluster and phosphate metabolisms. The Phosphate starvation-inducible protein PhoH induces the PhoH gene belonging to phosphate regulon and sB-dependent gene belonging to general stress regulon. The sB-dependent general stress proteins (Gsps) are predicted to provide cells with several kinds of nonspecific stress tolerance. It is also putatively involved in the protection of DNA, membranes and other proteins against oxidative stress and assists

cells to survive extreme conditions, such as heat, osmotic pressure and irregular pH in the environment (Antelmann *et al*., 2000).

An improved metabolic activity is one the important features expected of a probiotic organism to confer on a host. The genome of the *L. reuteri* PNW1 harbours a number of genes predicted to be directly involved in improving the metabolism. Xylose isomerase domain protein TIM barrel is one of the genes identified in this capacity. The protein xylose isomerase is responsible for the isomerisation of glucose and pentose sugars in microbial cells (Shahbaaz *et al*., 2015). Beta-1,3-glucosyltransferase and Poly (glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52) are also among the genes predicted for the same purpose. Four different coding sequences encoding for Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52) are found within the genome. β 1-3-glucosyl transferase catalyzes glucose attached to O-linked fucose through β 1–3 glycosidic linkage on thrombospondin type1 repeats (Weh *et al*., 2017). Considering the plethora of probiotic important genes harboured in the genome of *L. reuteri* PNW1, the isolate will presumably make a promising candidate for a probiotic supplement. Further analysis is underway to ellucidate on the safety features required of a typical probiotic agent as part of the risk assessment protocol for the *L. reuteri* PNW1.

References

Antelmann, H., Scharf, C., Hecker, M. 2000. Phosphate Starvation-Inducible Proteins of *Bacillus subtilis*: Proteomics and Transcriptional Analysis. *J Bacteriol*, 182(16) 4478–4490.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A., Dvorkin, M., Kulikov, A., Lesin, V., Nikolenko, S., Pham, S., Prjibelski, A., Pyshkin, A., Sirotkin, A., Vyahhi, N., Tesler, G., Alekseyev, M., Pevzner, P. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*, 19**,** 455–477.

Blin, K., Wolf, T., Chevrette, M., Lu, X., Schwalen, C., Kautsar, S., Suarez Duran, H., De Los Santos, E., Kim, H., Nave, M., Dickschat, J., Mitchell, D., Shelest, E., Breitling, R., Takano, E., Lee, S., Weber, T., Medema, M. 2017. antiSMASH 4.0: improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res*, 45**,** 36–41.

Boekhorst, J., De Been, M. Kleerebezem, M., Siezen, R.J. 2005. Genome-wide detection and analysis of cell wall-bound proteins with LPxTG-like sorting motifs. *J Bacteriol*, 187: 4928–4934.

Bolger, A., Lohse, M., Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinform,* 30**,** 2114 –2120.

Bosnea, L. A., Moschakis, T., Nigam, P. S., Biliaderis, C. G. 2017. Growth adaptation of probiotics in biopolymer-based coacervate structures to enhance cell viability. *LWT-Food Sci Technol,* 77**,** 282-289.

Cosentino, S., Voldby, L. M., Møller, A. F., Lund, O. 2013. PathogenFinder - Distinguishing friend from foe using bacterial whole genome sequence data. *PLoS ONE,* 8**,** e77302.

Cotter, P.D., Ross, R.P., Hill, C. 2013. Bacteriocins - a viable alternative to antibiotics? Nat Rev Microbiol, 11 (2), 95 - 105

De Leblanc, A. D. M., Leblanc, J. G. 2014. Effect of probiotic administration on the intestinal microbiota, current knowledge and potential applications. *World J Gastroenterol*, 20**,** 16518-16528.

Dethlefsen, L., Mcfall-Ngai, M., Relman, D. 2007. An ecological and evolutionary perspective on human-microbe mutualism and disease *Nat*, 449**,** 811–818.

Dlamini ZC, Langa RLS, Aiyegoro OA, Okoh AI. 2018. Safety evaluation and colonisation abilities of four lactic acid bacteria as future probiotics. *Probiotics Antimicrob Proteins*, https://doi.org/10.1007/s12602-018-9430-y

Douglas, A. 2008. Conflict, cheats and the persistence of symbioses. *New Phytol,* 177**,** 849–858.

Foster, K., Wenseleers, T. 2006. A general model for the evolution of mutualisms. *J Evol Biol*, 19**,** 1283–1293.

Granato, D., Bergonzelli, G.E., Pridmore, R.D., Marvin, L., Rouvet, M., Corthésy- Theulaz, I.E. 2004. Cell surface-associated elongation factor Tu mediates the attachment of *Lactobacillus johnsonii* NCC533 (La1) to human intestinal cells and mucins, *Infect Immun*, 72 (4), 2160–2169.

Grant, J. R., Stothard, P. 2008. The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Res,* 36**,** W181–W184.

Haft, D., Dicuccio, M., Badretdin, A., Brover, V., Chetvernin, V., O'neill, K., Li, W., Chitsaz, F., Derbyshire, M., Gonzales, N., Gwadz, M., Lu, F., Marchler, G., Song, J., Thanki, N., Yamashita, R., Zheng, C., Thibaud-Nissen, F., Geer, L., Marchler-Bauer, A., Pruitt, K. 2018. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Res,* 4**,** D851-D860.

Hak-Jong Choi, Na-Kyoung Lee, and Hyun-Dong Paik. 2015. Health Benefits of Lactic Acid Bacteria Isolated from Kimchi, with Respect to Immunomodulatory Effects *Food Sci Biotechnol*, 24(3), 783-789.

Hwanhlem, N., Ivanova, T., Biscola, V., Choiset, Y., Haertle, T. 2017. Bacteriocin producing *Enterococcus faecalis* isolated from chicken gastrointestinal tract originating from Phitsanulok, Thailand: Isolation, screening, safety evaluation and probiotic properties. *Food Control*, 78, 187-195.

Lee, J., Yang, W., Hostetler, A., Schultz, N., Suckow, M. A., Stewart, K. L., Kim, D. D., Kim, H. S. 2016. Characterization of the anti-inflammatory *Lactobacillus reuteri* BM36301 and its probiotic benefits on aged mice. *BMC Microbiol*, 16**,** 69.

Marraffini, L.A., Dedent, A.C., Schneewind, O. 2006. Sortases and the art of anchoring proteins to the envelopes of gram-positive bacteria. *Microbiol Mol Biol Rev*, 70, 192–221.

Nordeste R, Tessema A, Sharma S, Kovač Z, Wang C, Morales R, Griffiths MW. 2017. Molecules produced by probiotics prevent enteric colibacillosis in pigs. *BMC Vet Res*, 13:335 DOI 10.1186/s12917-017-1246-6.

Oh, P. L., Benson, A. K., Peterson, D. A., Patil, P. B., Moriyama, E. N., Roos, S., Walter, J. 2010. Diversification of the gut symbiont *Lactobacillus reuteri* as a result of host-driven evolution. *The ISME Journal,* 4**,** 377–387.

Oliveira, L.C, Saraiva, T.D.L., Silva, W.M., Pereira, U.P., Campos, B.C., Benevides, L.J., Rocha, F.S., Figueiredo, H.C.P., Azevedo, V., Soares, S.C. 2017. Analyses of the probiotic property and stress resistance-related genes of *Lactococcus lactis* subsp. *lactis* NCDO 2118 through comparative genomics and *in vitro* assays. *PLoS ONE* 12(4), e0175116

Overbeek, R., Olson, R., Pusch, G., Olsen, G., Davis, J., Disz, T., Edwards, R., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A., Xia, F., Stevens, R. 2014. The

SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res*, 42**,** 206 –214.

Plessas, S., Nouska, C., Karapetsas, A., Kazakos, S., Alexopoulos, A., Mantzourani, I., Chondrou, P., Fournomiti, M., Galanis, A., Bezirtzoglou, E. 2017. Isolation, characterization and evaluation of the probiotic potential of a novel *Lactobacillus* strain isolated from Feta-type cheese. *Food Chem,* 226**,** 102-108.

Rafsanjany, N., Lechtenberg, M., Petereit, F., Hensel, A. 2013. Antiadhesion as a functional concept for protection against uropathogenic *Escherichia coli*: *In vitro* studies with traditionally used plants with antiadhesive activity against uropathognic *Escherichia coli*. *J Ethnopharmacol*, 145, 591–597.

Rasmussen, T.B., Danielsen, M., Valina, O., Garrigues, C., Johansen, E., Pedersen, M.B. 2008. *Streptococcus thermophilus* core genome: comparative genome hybridization study of 47 strains. *Appl Environ Microbiol*, 74 (15), 4703–4710.

Salas-Jara, M.J., Ilabaca, A., Vega, M., García, A. 2016. Biofilm forming *Lactobacillus*: new challenges for the development of probiotics, *Microorganisms* 4 (3), 35.

Sandes, S., Alvim, L., Silva, B., Acurcio, L., Santos, C., Campos, M., Santos, C., Nicoli, J., Neumann, E., Nunes, A. 2017. Selection of new lactic acid bacteria strains bearing probiotic features from mucosal microbiota of healthy calves: Looking for immunobiotics through *in vitro* and *in vivo* approaches for immunoprophylaxis applications. *Microbiol Res*, 200, 1–13.

Saulnier, D., Santos, F., Roos, S., Mistretta, T., Spinler, J., Molenaar, D. 2011. Exploring metabolic pathway reconstruction and genome-wide expression profiling in *Lactobacillus reuteri* to define functional probiotic features. *PLoS ONE*, 6, 18783.

Shahbaaz, M., Ahmad F., Hassan, M.I. 2005. Structure-based function analysis of putative conserved proteins with isomerase activity from *Haemophilus influenza*. *3 Biotech* 5, 741–763.

Spinler, J. K., Taweechotipatr, M., Rognerud, C. L., Ou, C. N., Tumwasorn, S., Versalovic, J. 2008. Human-Derived probiotic *Lactobacillus reuteri* demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. *Anaerobe,* 14**,** 166–171.

Tatusova, T., Dicuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E., Zaslavsky, L., Lomsadze, A., Pruitt, K., Borodovsky, M., Ostell, J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res,* 44**,** 6614-6624.

Walter, J. 2008. Ecological role of lactobacilli in the gastrointestinal tract: Implications for fundamental and biomedical research. *Appl Environ Microbiol*, 74**,** 4985–4996.

Walter, J., Britton, R. A., Roos, S. 2011. Host-microbial symbiosis in the vertebrate gastrointestinal tract and the *Lactobacillus reuteri* paradigm. *PNAS,* 108**,** 4645–4652.

Weizman, Z., Asli, G., Alsheikh, A. 2005. Effect of a probiotic infant formula on infections in child care centers: Comparison of two probiotic agents. *Pediatrics* 115**,** 5–9.

Wu, Q., Tun, H.M., Leung, F.C.-C., Shah, N.P. 2014. Genomic insights into high exopolysaccharide-producing dairy starter bacterium *Streptococcus thermophilus* ASCC 1275. Sci Rep, 4, 4974. doi:10.1038/srep04974.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F., Larsen, M. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemotherapy,* 67**,** 2640–2644.

Zeng Z, Zuo F, Marcotte H. 2019. Putative adhesion factors in vaginal *Lactobacillus gasseri* DSM 14869: functional characterization. Appl Environ Microbiol, 85:e00800-19.

CHAPTER 4 − Complete genome analysis of *Lactobacillus acidophilus* PNW3

(Part of this chapter has been published in the journal of *microbiology resource announcement*, 2019, 8(25): e00362-19, https://doi.org/10.1128/MRA.00362-19)

Abstract

A draft genome sequence of *Lactobacillus acidophilus* PNW3 was assessed in order to identify important genes in support of its probiotic potential. The genomic DNA of the isolate was extracted and the entire genome sequenced using illuminal Miseq instrument at 2 × 300 read length. The sequenced genomic data was assessed for quality control, using FastQC before being assembled with SPAdes. Prior to further downstream analysis, the draft assembly was annotated with Prokaryotic Genome Annotation Pipeline (PGAP) and Rapid Annotations using Subsystems Technology (RAST). The genome assembly was 1,857,655 bp long in 25 contigs, an N50 value of 230,557 bp and a G+C content of 34.6%. The total number of predicted protein-coding genes was 1,776, with 58 predicted RNAs and 42 predicted pseudogenes. A bioactive protein predicted to be helveticin J was identified as one of the likely secondary metabolites. Quick analysis of the entire assembly on the pathogenicity predicted the isolate not to be a human pathogen. Resistance genes against Lincosamide and Tetracycline were spotted as the only resistance genes harboured by *L. acidophilus* PNW3. The plethora of encoding genes putative for a number of traits, important to the probiotic functionality of the isolate were among the CDS identified and located at different loci within the genome assembly. These included encoding genes putative for lactic acids and production of bioactive peptides, adhesion to the epithelia mucus layer, resistance to the harsh gut environmental conditions and general improvement of metabolic activities in the host.

4.1. Introduction

Lactic acid bacteria (LAB) are widely used as probiotics across host species (Dlamini *et al.*, 2019). *Lactobacillus acidophilus* is a lactic acid bacterium commonly used in dairy industries as starter culture in the production of high quality health functional foods such as yoghurt, cheese and beverages (Yang *et al.*, 2016). Complete genome sequence have contributed a great deal in elucidation of the probiotic potentials exerted by lactic acid bacteria (Oliveira *et al*., 2017). Several strains of *L*. *acidophilus* have been extensively characterised and their probiotic features have been judiciously documented (Stahl and Barrangou, 2013). Studies have indicated that bioactive secondary metabolites produced by many probiotic agents affect bacterial community interactions and potentially attenuate disease symptoms caused by pathogens (Nordeste *et al.*, 2017; Chetwin *et al.*, 2019).

Probiotic yogurt containing *L. acidophilus* improved total cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations in both male and female patients suffering from type2 diabetes. Thus, suggesting a great potential towards ameliorating risk factors associated with cardiovascular diseases (Ejtahed *et al.*, 2011). Surface layer proteins of the probiotic *L. acidophilus* NCFM was revealed with a potential to protect intestinal epithelial barrier function against TNF-α-elicited inflammation through regulation of tight junction protein expression, prevention of loose intestinal permeability, blockage of cell apoptosis and obstruction of NF-κB signaling pathway (Wang *et al.*, 2019b). This promising bioactive peptide supports the use *L*. *acidophilus* NCFM in the development of functional foods and a potential prophylactic agent for inflammatory bowel diseases.The surface layer protein extracted from *L. acidophilus* NCFM also induces formation of ROS, which, in turn, results in induced autophagic death in HCT116 colon cancer cells through suppression of the mammalian target of rapamycin (mTOR) activity and activation the c-Jun N-terminal kinase signaling pathway (Wang *et al.*, 2019a). In a study conducted by Guo *et al.* (2018), using a rat model, it was also concluded that administration of *L.*

acidophilus CICC6074 reduced levels of inflammatory cytokines TNF-α and IL-6, intestinal apoptosis and symptoms of Neonatal necrotising enterocolitis.

Gut microbiota plays a crucial role in the wellbeing of the host and protection against pathogens. The mutualism between host species and their gut microbial ecosystem begins at birth and stabilises in human beings at around 3 years of age (Yatsunenko *et al.*, 2012). *Lactobacillus acidophilus* was also reported to be efficacious in readjustment of the impaired microbiota and inflammation induced in Th1 (C57BL/6) and Th2 (BALB/c) biased mouse after being challenged with *Salmonella* Typhimurium at infective dose levels (Pradhan *et al.*, 2019).

This study is therefore conceptualized to assess the entire genome of *Lactobacillus acidophilus* PNW3 in order to dertimine genome-based probiotics features which maybe present in the strain.

4.2. Materials and methods

4.2.1. Extraction of genomic DNA

Extraction of the genomic DNA performed as explained in the previous Chapter (see 2.2.1).

4.2.2. 16S rRNA identification of the isolate

The 16S rRNA identification of *Lactobacillus acidophilus* PNW3 was performed as described in Chapter (see 2.2.2). The partial sequenced data (1,552 bp) was, thereafter, submitted to the GenBank data base and accession number (MK123485) received for the isolate (https://www.ncbi.nlm.nih.gov/nuccore/MK123485.1/).

4.2.3. Whole genome sequence (WGS) of the isolates

Genomic DNA was prepared with an Illumina Nextera DNA flex library prep kit. This library was sequenced on an Illumina MiSeq instrument at the Agricultural Research Council Biotechnology Platform (Pretoria, South Africa), generating 3.05 GB of data.

A total of 4,944,578 reads were collected from the run with 2× 300 bp paired-end read lengths. The data was filtered for low-quality reads and adapter regions using Trimmomatic v. 0.32 (Bolger *et al.*, 2014) with the minimum quality score and sequence length of 15 and 70 respectively. The 5' and 3' minimum quality score required was 14 and the adaptor sequences were clipped using a mismatch value of 2, palindrome clip threshold of 30 and simple clip threshold of 15. The quality of the trimmed reads was assessed using FastQC version 0.11.5. A draft genome was assembled using SPAdes v. 3.12.0 (Bankevich*et al*., 2013) through the KBase platform (Arkin *et al*., 2018) with a final coverage of 377.0x.

The draft assembly was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v. 4.7 (Tatusova *et al.*, 2016; Haft *et al.*, 2018) and Rapid Annotations using Subsystems Technology (RAST) with SEED viewer v. 2.0 (Brettin *et al.*, 2015). Rapid *in silico* analysis of secondary metabolite biosynthesis gene clusters was assessed using antiSMASH v. 5.0.0beta1 (Blin *et al.*, 2017). PathogenFinder v. 1.1 (Cosentino *et al.*, 2013) and ResFinder v. 3.1 (Zankari *et al.*, 2012) were used to determine the pathogenicity of *L. acidophilus* PNW3 towards human hosts and the possible presence of antimicrobial resistant genes. Default parameters were used for all the software employed in the analysis.

4.3. Results

4.3.1. Summary of the entire genome of *Lactobacillus acidophilus* PNW1

The genome assembly size of *L. acidophilus* PNW3 is 1,857,655 bp, consisted of 25 contigs with a G+C content of 34.6%. Half of the sequence in the entire draft assembly was covered by the contigs of ≥230,557 bp. There were 1,776 annotated protein-coding genes with 55 predicted tRNAs, 3 predicted rRNAs, and 42 predicted pseudogenes. Among the pseudogenes, 22 were due to frameshift, 16 were due to incomplete

nucleotides while those resulting from premature internal stop codon and multiple problems were 12 and 7 respectively. Though the isolate was predicted not to be a human pathogen, Lincosamide (*lnuC*) and Tetracycline (*tetW*) resistant genes were spotted as the only resistant genes harboured by *L. acidophilus* PNW3.

A predicted bioactive protein (Fig. 4.1) was identified as one of the likely secondary metabolites. This was found at c00002_NODE_3, located between 53,258 – 66,302 nucleotides positions and comprising 13,045 nucleotides in total. A coding sequence for Ornithine decarboxylase (EC 4.1.1.17) was found at NODE_2_length_675994_cov_165.027269, measuring 2094 bp. Circular representation of the entire *L. acidophilus* PNW3 genome is presented in Figure 4.2 and the graphical distribution of the subsystems within the genome is presented in Figure 4.3 below.

Availabilityof data: This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank with accession number SMLT00000000. The version described in this study is SMLT01000000. The SRA accession number is SRX5395058, the Bio-Project number is PRJNA504734, and the BioSample number is SAMN10979321.

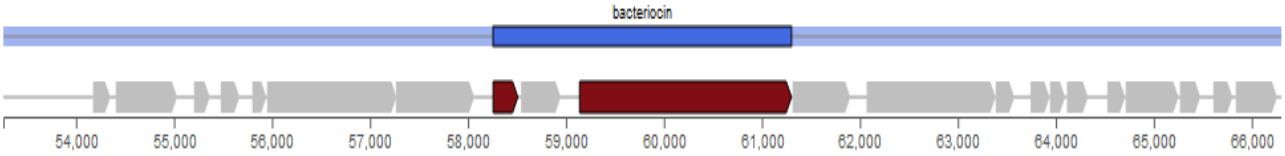

Figure 4.1: Gene cluster showing the position of biosynthetic bacteriacin. Core biosynthetic (bacteriocin) genes (■), other genes (■)

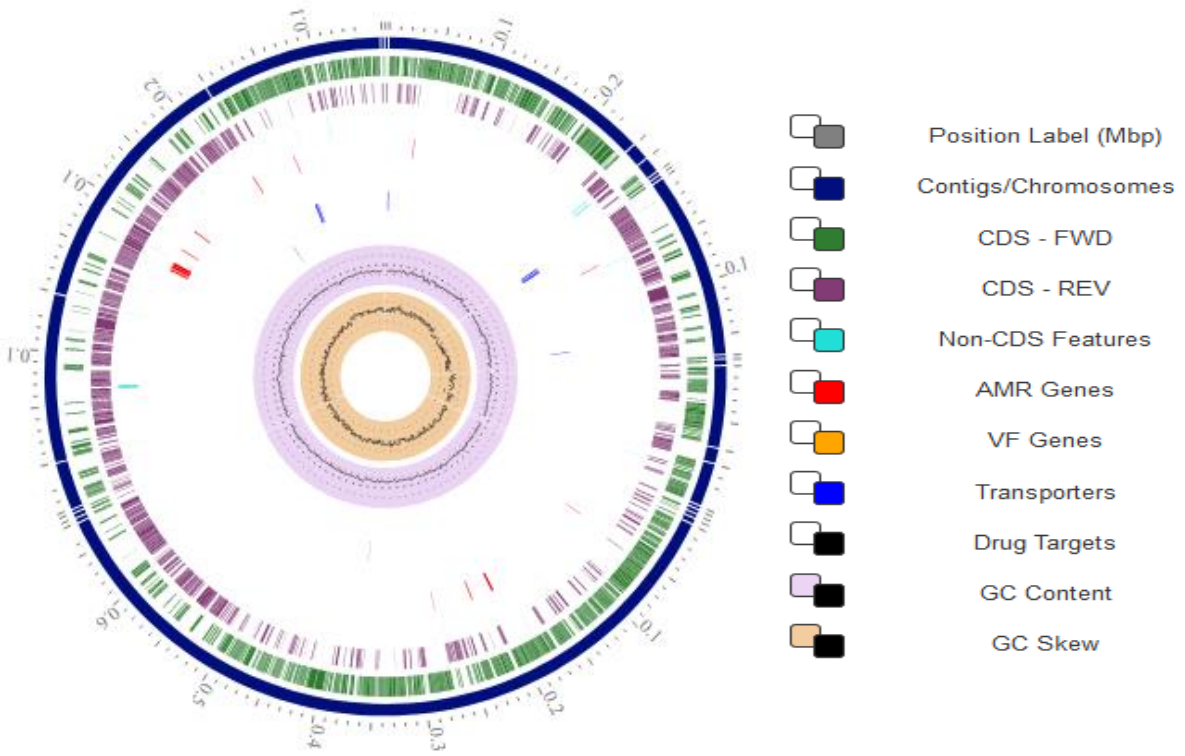

Figure 4.2: Circular genome mapping of the *L. acidophilus* PNW3. The circular genome was generated usingPATRIC sever 3.5.43

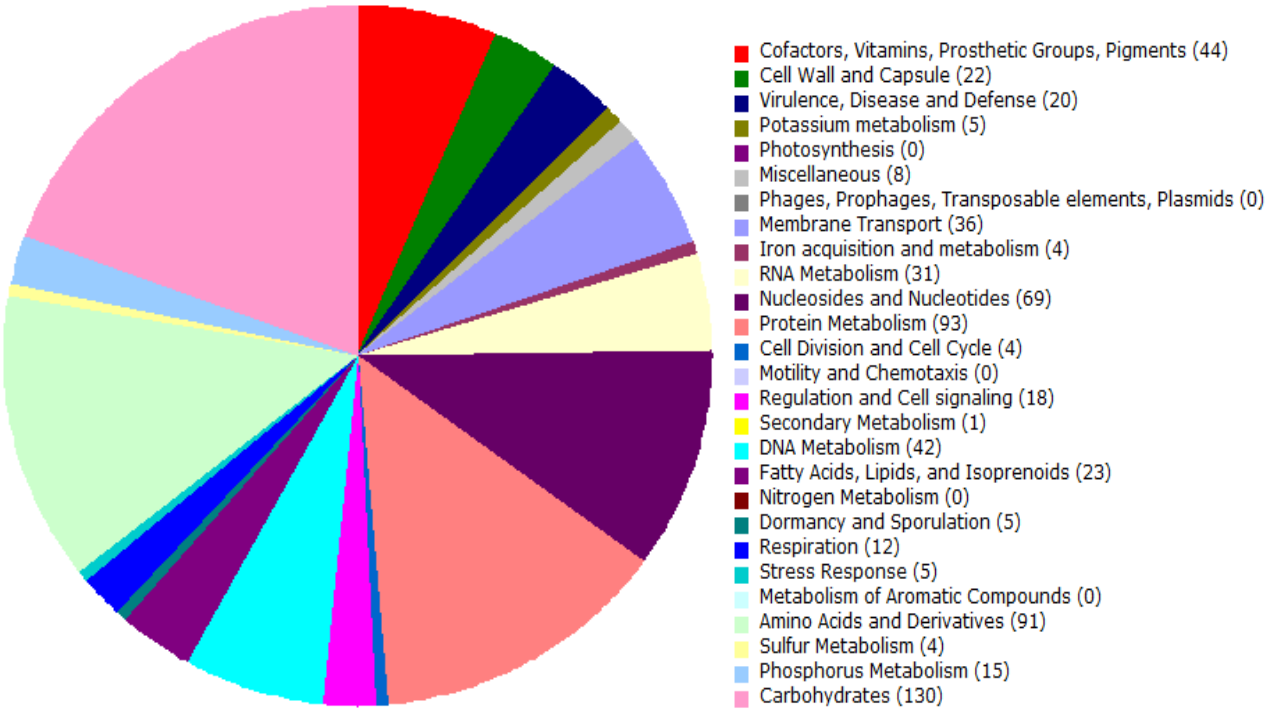

Figure 4.3: Distribution of subsystem features within the *L. acidophilus* PNW3 genome. The distribution was generated usingRAST sever with SEED viewer v.2.0

4.3.2. Overview of important probiotic genes in the draft genome assembly

4.3.2.1. Coding sequence putatively involved in the production of lactic acids

The genome of *L. acidophilus* PNW3 contains three coding sequences (CDS), predictably encoding for L-lactate dehydrogenase (EC 1.1.1.27). The protein putatively functions in the fermentation of lactate and mixed acids. The CDS found in the locus, with Contig Identity NODE_10_length_151230_cov_215.460261, was 915 bp long, stretching from 35179 nucleotide position to 34265 along the negative strand (Figure 4.4). Coding sequences found on the two other loci located on Contig Identities NODE_2_length_675994_cov_165.027269 & NODE_4_length_230557_cov_252.939857 were 927 bp (Fig. 4.5) and 972 bp (Figure 4.6) long respectively and situated along the positive strand.

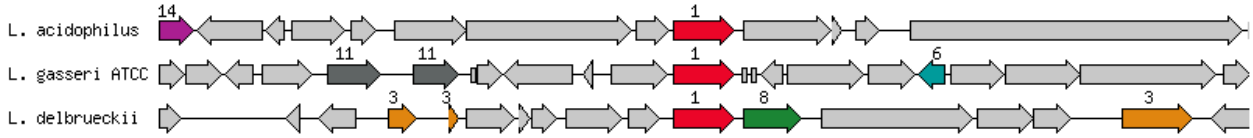

Figure 4.4: Annotation diagram showing location of theL-lactate dehydrogenase (EC 1.1.1.27) ( ) found at node 10 and 915 bp long on the –ve strand

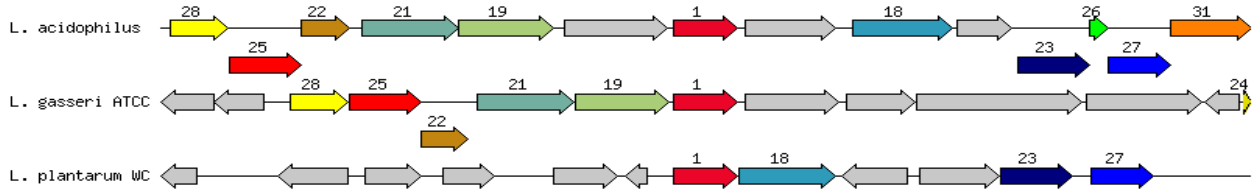

Figure 4.5: Annotation diagram showing location of the L-lactate dehydrogenase (EC 1.1.1.27) ( ) found at node 2 and 927 bp long on the +ve strand

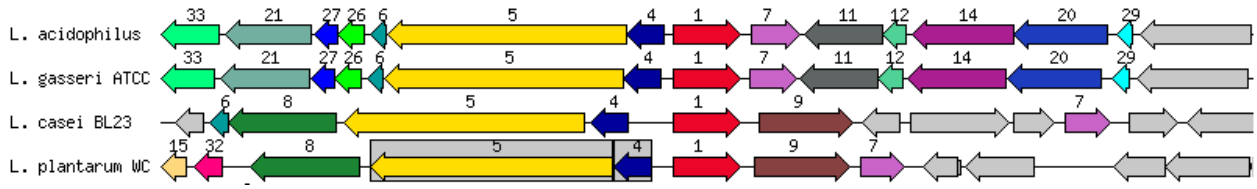

Figure 4.6: Annotation diagram showing location of the L-lactate dehydrogenase (EC 1.1.1.27) ( ) found at node 4 and 972 bp long on the +ve strand

NB: The sequence of nucleotides and amino acids of identified genes putatively involved in the production of lactic acids is shown in Appendix G.

4.3.2.2. Coding sequence putatively involved in bioactive peptide production

Six different coding sequences putatively involved in the synthesis of bacteriocin were situated within the genome of *L. acidophilus* PNW3. The CDS predicted for bacteriocin helveticin J was found on Contig Identity NODE_10_length_151230_cov_215.460261, stretching from 83235 to 82258 nucleotide position. The fragment was 978 bp long, located on the negative strand (Figure 4.7). Two protein-encoding sequences putative for Bacteriocin pre-peptide or inducing factor for bacteriocin synthesis were found on the same locus with Contig Identity NODE_3_length_242459_cov_251.537351. The fragment between 55204 and 55365 nucleotide positions was 162 bp long and situated on the positive strand (Figure 4.8) while the other was 192 bp, between 55478 and 55669 nucleotide positions and also on the positive strand (Figure 4.9).

CDS predicted for three-component quorum-sensing regulatory system, inducing peptide for bacteriocin biosynthesis, also located on Contig Identity NODE_3_length_242459_cov_251.537351. The 144 bp long sequenced fragment stretched from 55800 nucleotide position to 55943 along the positive strand (Figure 4.10). Two additional coding sequences putative for Bacteriocin ABC-transporter, ATP-binding and permease component shared the same Contig Identity with the three-component quorum-sensing regulatory system, one of the CDS (2163 bp) was in-between 59140 and 61302 nucleotide position (Figure 4.11) while the other one (591 bp) was between 61313 and 61903 nucleotide position (Figure 4.12).

Moreover, the two coding sequences putative for S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS were also found on the same Contig Identity NODE_2_length_675994_cov_165.027269 with subsystems methionine biosynthesis and methionine degradation. The fragment between 460026 and 460499

nucleotide positions was 474 base pairs in length (Figure 4. 13) while the other fragment on the same locus was 156 bp long, stretching between 467389 and 467544 nucleotide positions (Figure 4.14). Both fragments exist on the positive strand. In addition, CDS putatively involved in the synthesis of type 1 capsular polysaccharide biosynthesis protein (EC:2.4.1.-) (Figure 4.15) and mannosyltransferase involved in polysaccharide biosynthesis (Figure 4.16) were also found on Contig Identity NODE_2_length_675994_cov_165.027269 and NODE_9_length_161393_cov_196.936481, respectively. The lengths of the fragments were 702 bp along the negative strand and 699 bp along the positive strand respectively.

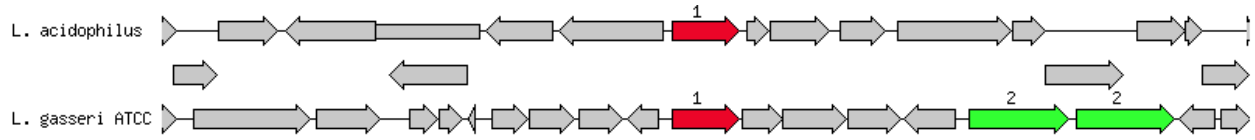

Figure 4.7: Annotation diagram showing location of the bacteriocin helveticin J ( ) found at node 10 and 978 bp long on the –ve strand

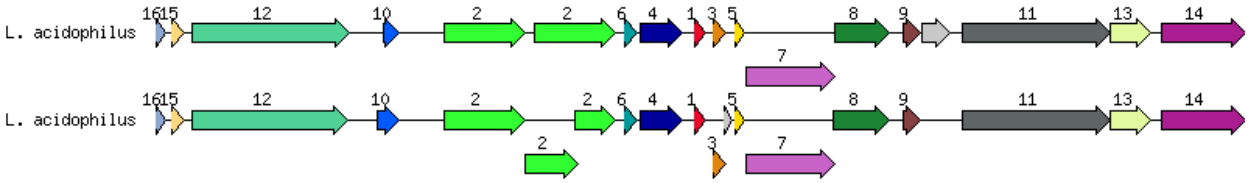

Figure 4.8: Annotation diagram showing location of the Bacteriocin prepeptide or inducing factor for bacteriocin synthesis ( ) found at node 3 and 168 bp long on the +ve strand

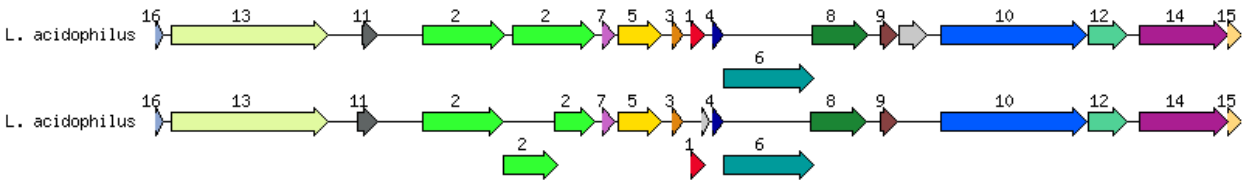

Figure 4.9: Annotation diagram showing location of the Bacteriocin prepeptide or inducing factor for bacteriocin synthesis ( ) found at node 3 and 192 bp long on the +ve strand

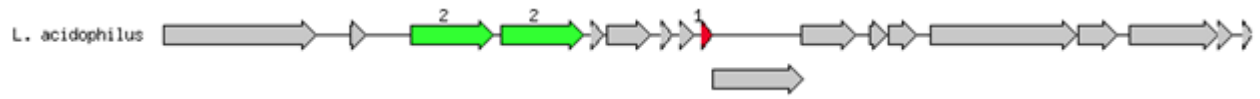

Figure 4.10: Annotation diagram showing location of the Three-component quorum-sensing regulatory system, inducing peptide for bacteriocin biosynthesis ( ) found at node 3 and 144 bp long on the +ve strand

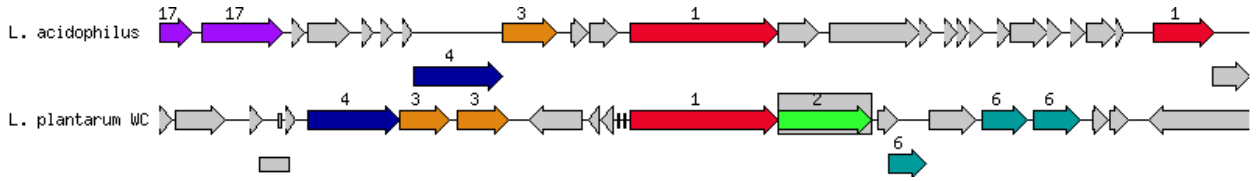

Figure 4.11: Annotation diagram showing location of the Bacteriocin ABC-transporter, ATP-binding and permease ( ) found at node 3 and 2163 bp long on the +ve strand

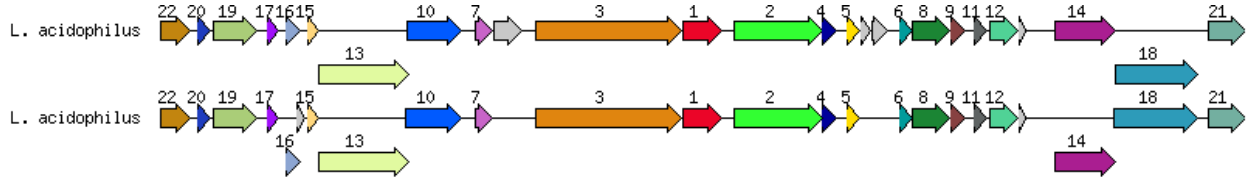

Figure 4.12: Annotation diagram showing location of the Bacteriocin ABC-transporter, auxillary protein ( ) found at node 3 and 591 bp long on the +ve strand

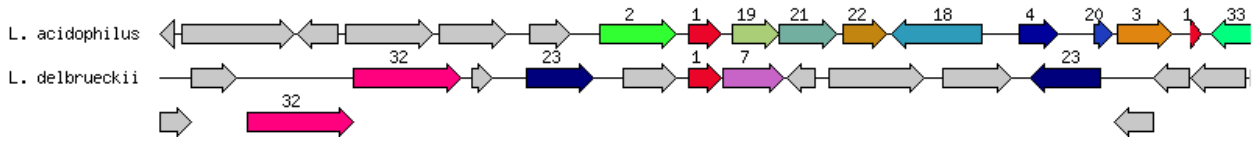

Figure 4.13: Annotation diagram showing location of the S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS ( ) found at node 2 and 474 bp long on the +ve strand

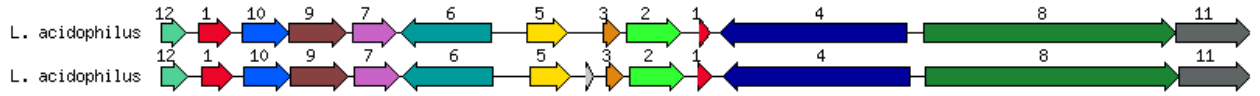

Figure 4.14: Annotation diagram showing location of the S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS ( ) found at node 2 and 156 bp long on the +ve strand

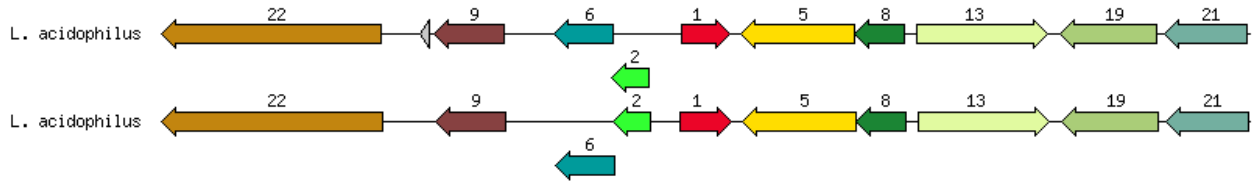

Figure 4.15: Annotation diagram showing location of the type 1 capsular polysaccharide biosynthesis protein (EC:2.4.1.-) ( ) found at node 2 and 702 bp long on the –ve strand

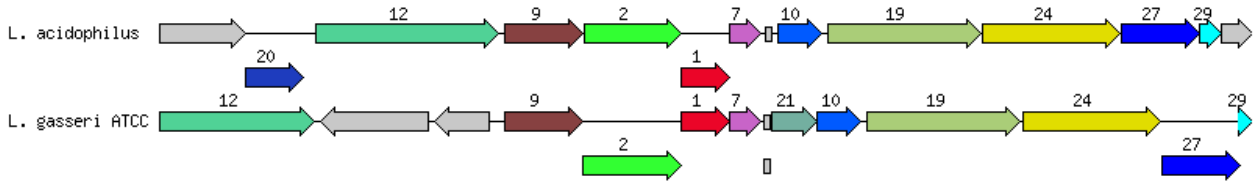

Figure 4.16: Annotation diagram showing location of the type 1 capsular polysaccharide biosynthesis protein (EC:2.4.1.-) ( ) found at node 2 and 699 bp long on the +ve strand

NB: The sequence of nucleotide and amino acids of the identified gene putatively involved in the production of bioactive peptide is presentedin Appendix H.

4.3.2.3. Coding sequence putatively involved in adhesion

The coding sequence putative for Sortase A, LPXTG specific with subsystem heme, hemin uptake and utilisation systems in Gram positives and as well as Sortase was located on Contig Identity NODE_2_length_675994_cov_165.027269. The fragment, which was 690 bp long, laid on the negative strand in-between 619228 and 618539 nucleotide position (Figure 4.17). Five different coding sequences putative for different categories of cell surface proteins, functioning as adhesives, were also determined within the *L. acidophilus* PNW3 genome assembly. The two protein-encoding sequences predicted for the cell surface protein, ErfK family were found on two different loci, with Contig Identity NODE_10_length_151230_cov_215.460261& NODE_2_length_675994_cov_165.027269. The nucleotides fragment located on the negative strand was 690 bp long (Fig. 4.18) while the other one, located on the positive strand, was 609 bp long (Figure 4.19) respectively. Other predictably secreted cell surface proteins included the following: cell surface protein precursor (1932 bp) (Figure 4.20); cell surface protein (1020 bp) (Figure 4.21); and Fibronectin/fibrinogen-binding protein (1692 bp) (Figure 4.22). The responsible encoding genes were located respectively on Contig Identities NODE_10_length_151230_cov_215.460261, NODE_7_length_167441_cov_180.361088 and NODE_2_length_675994_cov_165.027266.

Furthermore, 1500 base pairs long CDS predicted for S-layer protein precursor (Fig. 4.23) was also found along the positive strand at Contig Identity NODE_9_length_161393_cov_196.936481. The fragment stretched from 59807 to 61306 nucleotide position. Four members of the Exopolysaccharides (EPS) clusters were found as part of the entire genome. The coding sequence, which was 441 bp long and putative for ATP synthase epsilon chain (EC 3.6.3.14), with subsystem F0F1-type ATP synthase (Figure 4.24), was located on Contig Identity NODE_2_length_675994_cov_165.027269 along the positive strand. The CDS for Tyrosine-protein kinase transmembrane modulator EpsC (Fig. 4.25) and Tyrosine-protein kinase EpsD (EC 2.7.10.2) (Figure 4.26), both with subsystem Exopolysaccharide Biosynthesis, coexisted at Contig Identity NODE_3_length_242459_cov_251.537351. Both fragments occured on the positive strand and were respectively 876 and 783 bp long.

Another coding sequence was predicted for "COG1887: Putative glycosyl/glycerophosphate transferases involved in teichoic acid biosynthesis TagF/TagB/EpsJ/RodC / Putative polyribitolphosphotransferase / CDP-ribitol: poly (ribitol phosphate) ribitol phosphotransferase / CDP-glycerol: poly (glycerophosphate) glycerophosphotransferase (EC 2.7.8.12) / CDP-glycerol: N-acetyl-beta-D-mannosaminyl-1,4-N-acetyl-D-glucosaminyldiphosphoundecaprenyl glycerophosphotransferase". The fragment was 1155 bp long, stretching between 71466 and 72620 nucleotide positions along the positive strand (Figure 4.27).

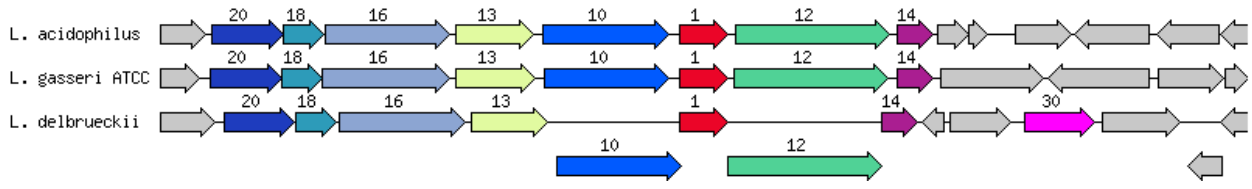

Figure 4.17: Annotation diagram showing location of the Sortase A, LPXTG specific ( ) found at node 2 and 699 bp long on the –ve strand

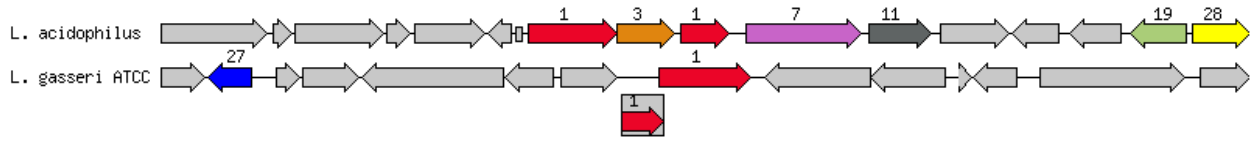

Figure 4.18: Annotation diagram showing location of the cell surface protein, ErfK family ( ) found at node 10 and 690 bp long on the –ve strand

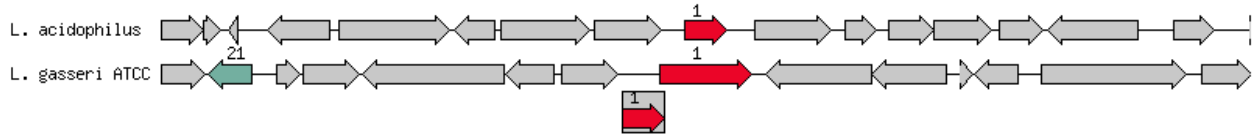

Figure 4.19: Annotation diagram showing location of the cell surface protein, ErfK family ( ) found at node 2 and 609 bp long on the +ve strand

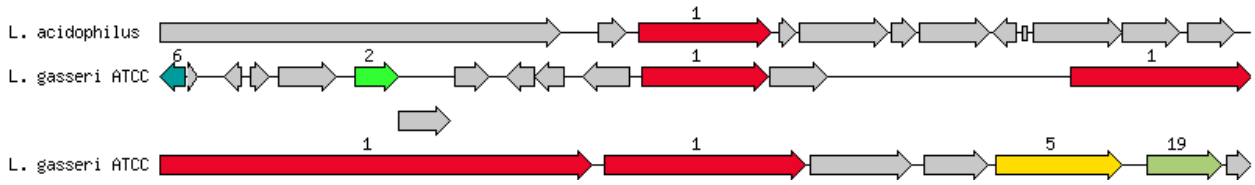

Figure 4.20: Annotation diagram showing location of the cell surface protein precursor ( ) found at node 10 and 1932 bp long on the –ve strand

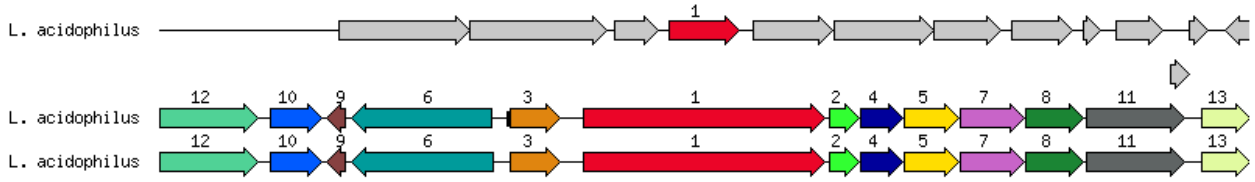

Figure 4.21: Annotation diagram showing location of the cell surface protein ( ) found at node 7 and 1020 bp long on the –ve strand

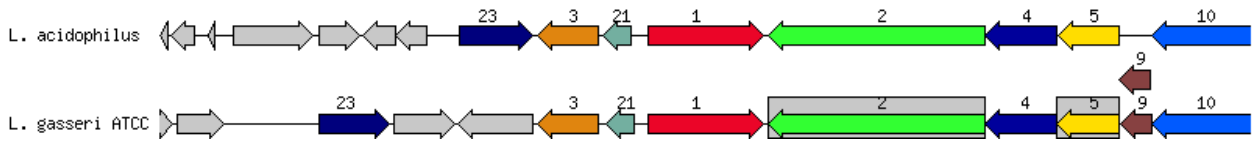

Figure 4.22: Annotation diagram showing location of the Fibronectin/fibrinogen-binding protein ( ) found at node 2 and 1692 bp long on the +ve strand

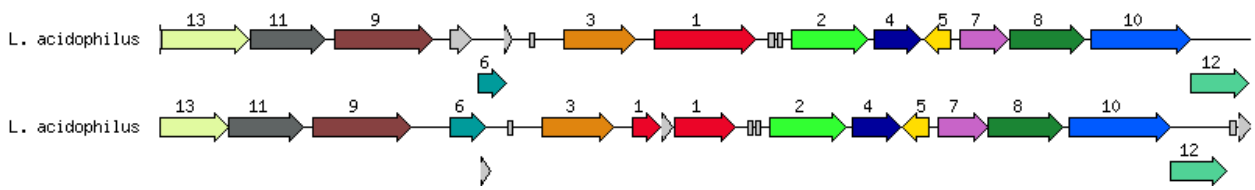

Figure 4.23: Annotation diagram showing location of the S-layer protein precursor ( ) found at node 9 and 1500 bp long on the +ve strand

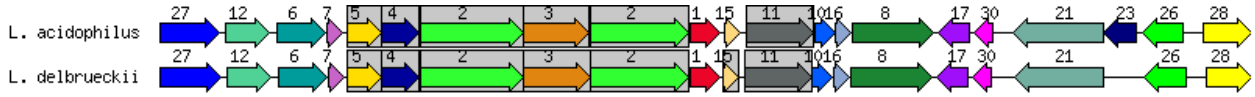

Figure 4.24: Annotation diagram showing location of the ATP synthase epsilon chain (EC 3.6.3.14) ( ) found at node 2 and 441 bp long on the +ve strand

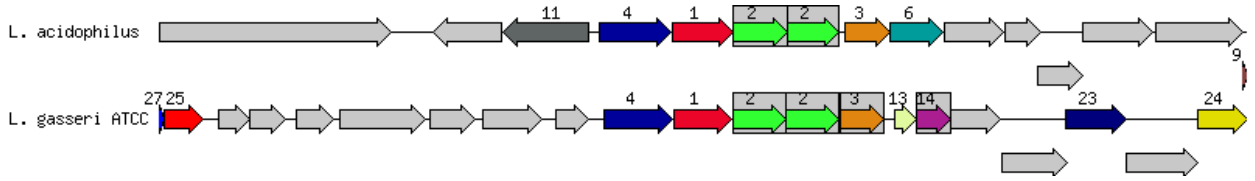

Figure 4.25: Annotation diagram showing location of the Tyrosine-protein kinase transmembrane modulator EpsC ( ) found at node 2 and 876 bp long on the +ve strand

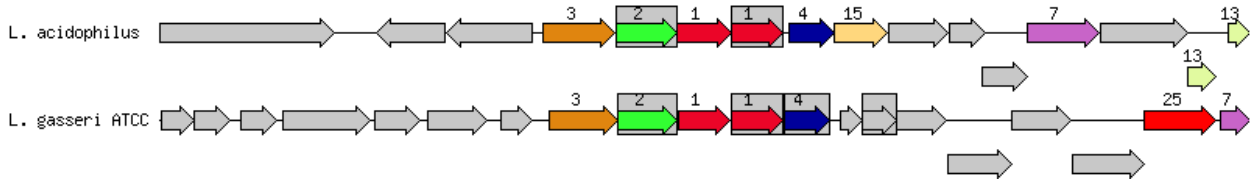

Figure 4.26: Annotation diagram showing location of the Tyrosine-protein kinase EpsD (EC 2.7.10.2) ( ) found at node 2 and 783 bp long on the +ve strand

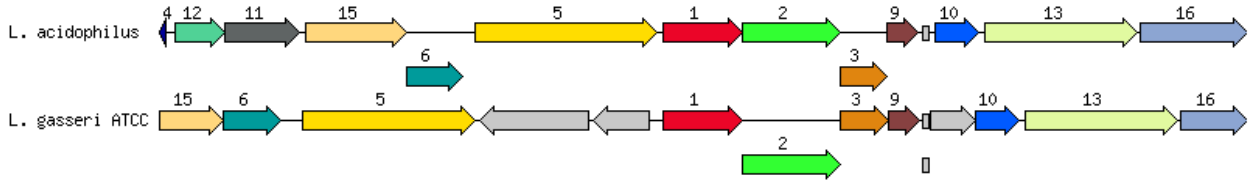

Figure 4.27: Annotation diagram showing location of the COG1887: Putative glycosyl/glycerophosphate transferases involved in teichoic acid biosynthesis TagF/TagB/EpsJ/RodC / Putative polyribitolphosphotransferase / CDP-ribitol:poly(ribitol phosphate) ribitol phosphotransferase / CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase (EC 2.7.8.12) / CDP-glycerol: N-acetyl-beta-D-mannosaminyl-1,4-N-acetyl-D-glucosaminyldiphosphoundecaprenyl glycerophosphotransferase (EC 2.7.10.2) ( ) found at node 9 and 1155 bp long on the +ve strand

NB. The sequence of nucleotide and amino acids of the identified genes putatively involved in adhesion is presentedin Appendix I.

4.3.2.4. Coding sequence putatively involved in the production of extracellular enzymes

Lipase

Coding sequence putative for FIG006988: Lipase/Acylhydrolase with GDSL-like motif was found on Contig Identity NODE_38_length_2064_cov_2.239423 (Figure 4.28). The 390 bp long CDS stretched from 391 along the negative strand and stopped at the second

nucloetide position within the genome. Three different CDS putative for Esterase/lipase were also found on different loci within the genome assemply. CDS measuring 795 bp long were located on Contig Identity NODE_3_length_242459_cov_251.537351 (Figure 4.29) while the other two were located on NODE_4_length_230557_cov_252.939857 (Figure 4.30) and NODE_7_length_167441_cov_180.361088 (Figure 4.31). CDS were respectively 822 bp long on the negative strand and 897 bp long on the positive strand.

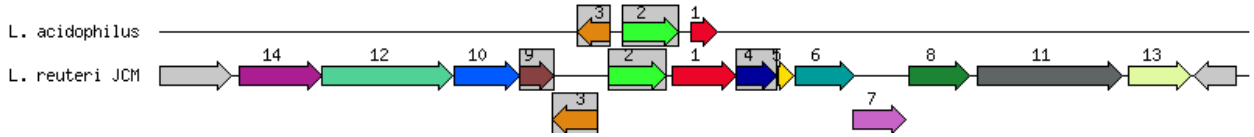

Figure 4.28: Annotation diagram showing location of the FIG006988: Lipase/Acylhydrolase with GDSL-like motif ( ) found at node 2 and 390 bp long on –ve strand

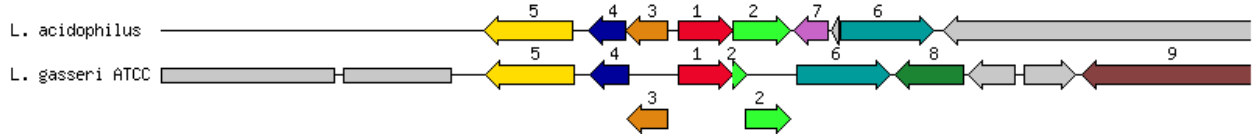

Figure 4.29: Annotation diagram showing the location of Lipase/esterase ( ) found at node 3 and 795 bp long on the –ve strand

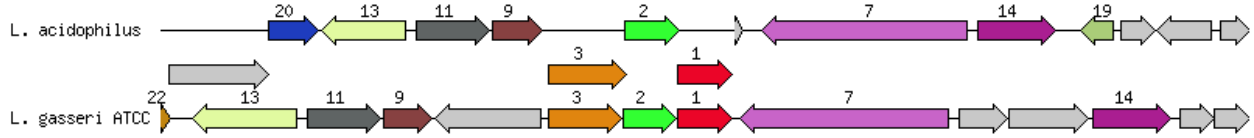

Figure 4.30: Annotation diagram showing the location of Lipase/esterase ( ) found at node 4 and 822 bp long on the –ve strand

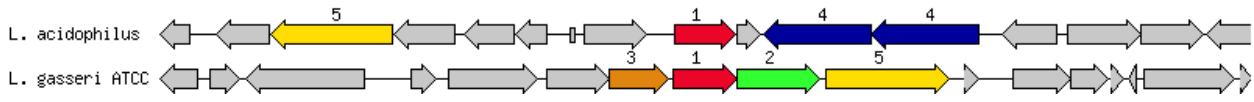

Figure 4.31: Annotation diagram showing the location of Lipase/esterase ( ) found at node 7 and 897 bp long on the +ve strand

Protease

A 681 base pairs long coding sequence putative for FIG056164: rhomboid family serine protease with Peptidyl-prolyl cis-trans isomerase containing a cluster as subsystem feature was found at Contig Identity NODE_10_length_151230_cov_215.460261. The CDS

stretched from 20039 to 19359 nucleotide position along the negative starnd (Figure 4.32). Moreover, anotHer CDS predicted for Serine protease, DegP/HtrA, do-like (EC 3.4.21.-) was found on Contig Identity NODE_17_length_42073_cov_277.775810, measuring 1239 bp with the fragment stretching in-bwteen 35811 and 34573 along the negative strand (Figure 4.33).

The CDS predicted for FIG001621: Zinc protease was located on Contig Identity NODE_2_length_675994_cov_165.027269. The fragment was 1215 bp long and laid in-between 44526 and 45740 on the positive strand (Figure 4.34). The ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92) (Figure 4.35) was also found on the same Contig Identity, with subsystem features such as Proteolysis in bacteria and ATP-dependent cAMP signaling as bacteria among the predicted protease within the genome. The fragment, measuring 588 bp, laid between 86016 and 85429 nucleotide position along the negative strand. Lon-like protease with PDZ domain (Figure 4.36), measuring 1041 bp, was also found on the same Contig Identity as Zinc protease. The fragment stretched from 216785 to 217825 nucleotide position. Other predicted enzymes found on this Contig Identity included the following: ATP-dependent Clp protease ATP-binding subunit ClpX (1263 bp) (Figure 4.37); ATP-dependent protease subunit HslV (EC 3.4.25.2) (525 bp) (Figure 4.38); Intramembrane protease RasP/YluC, implicated in cell division based on FtsL cleavage (subsystem; periplasmic stress response) (1257 bp) (Figure 4.39); and SOS-response repressor and protease LexA (EC 3.4.21.88) (627 bp) (Figure 4.40).

Furthermore, CDS putative for late competence protein ComC, processing protease (Figure 4.41) was located on Contige Identity NODE_4_length_230557_cov_252.939857, measuring 690 bp and lying between 66212 and 66901 nucleotide positions on the positive strand. The coding sequence putative for Predicted Zn-dependent protease (Figure 4.42) and Predicted metal-dependent membrane protease (Figure 4.43) were also

found located at Contig Identity NODE_7_length_167441_cov_180.361088 and NODE_9_length_161393_cov_196.936481 respectively. Predicted Zn-dependent protease was 669 bp long, stretching from 91711 to 92379 nucleotide postions while the predicted metal-dependent membrane protease was 759 bp long stretching from 102433 to 101677 nucleotide position.

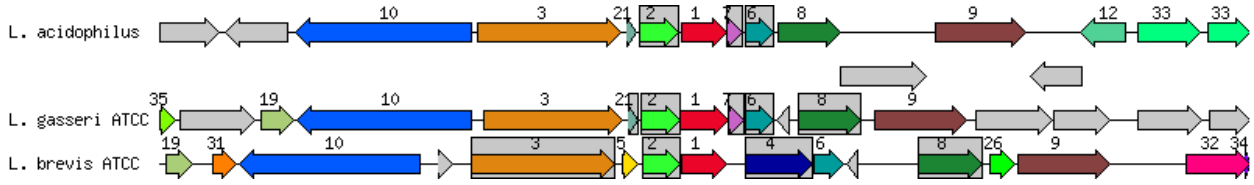

Figure 4.32: Annotation diagram showing location of the FIG056164: rhomboid family serine protease () found at node 10 and 681 bp long on the –ve strand

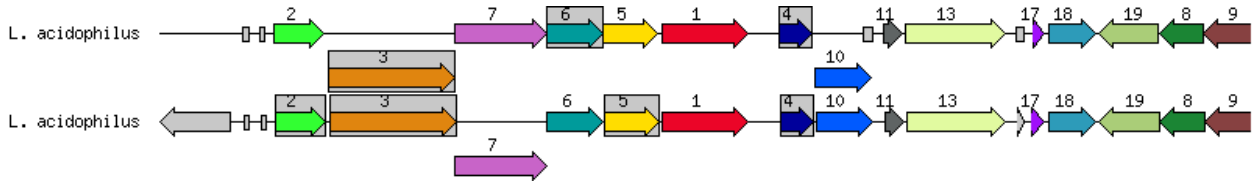

Figure 4.33: Annotation diagram showing location of the FIG056164: rhomboid family serine protease () found at node 17 and 1239 bp long on the –ve strand

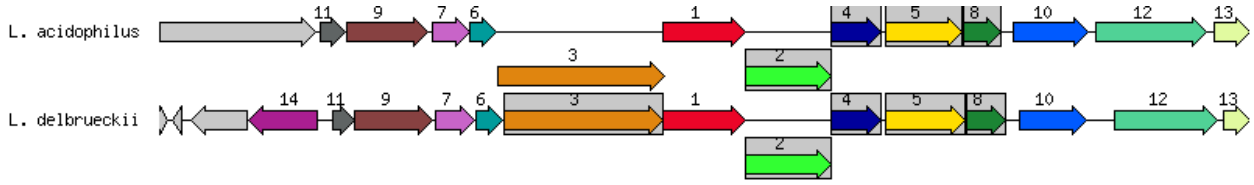

Figure 4.34: Annotation diagram showing location of the FIG001621: Zinc protease () found at node 2 and 1215 bp long on the +ve strand

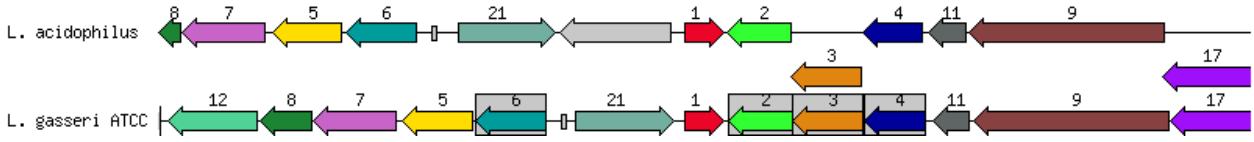

Figure 4.35: Annotation diagram showing location of the ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92) () found at node 2 and 1215 bp long on the –ve strand

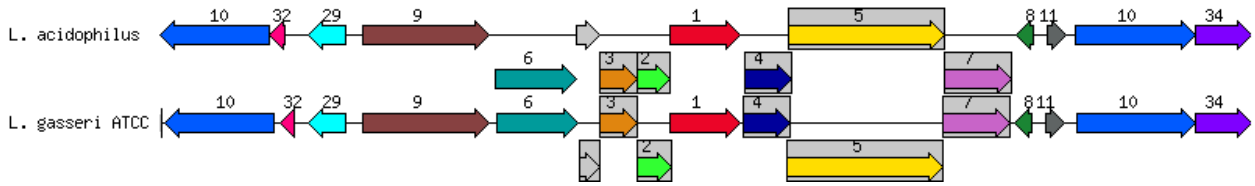

Figure 4.36: Annotation diagram showing location of the Lon-like protease with PDZ domain ( ) found at node 2 and 1041 bp long on the +ve strand

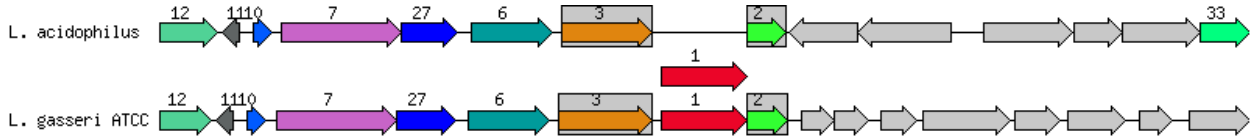

Figure 4.37: Annotation diagram showing location of the ATP-dependent Clp protease ATP-binding subunit ClpX ( ) found at node 2 and 1263 bp long on the +ve strand

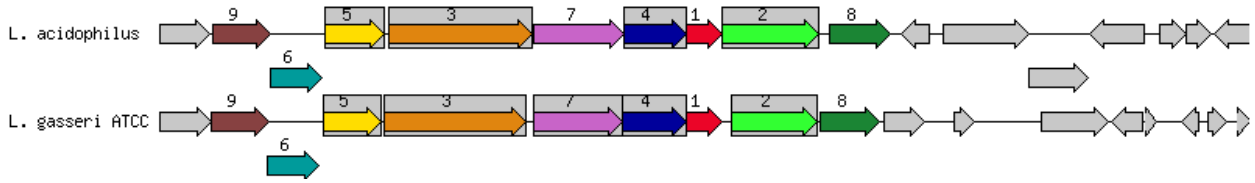

Figure 4.38: Annotation diagram showing location of the ATP-dependent protease subunit HslV (EC 3.4.25.2) ( ) found at node 2 and 525 bp long on the +ve strand

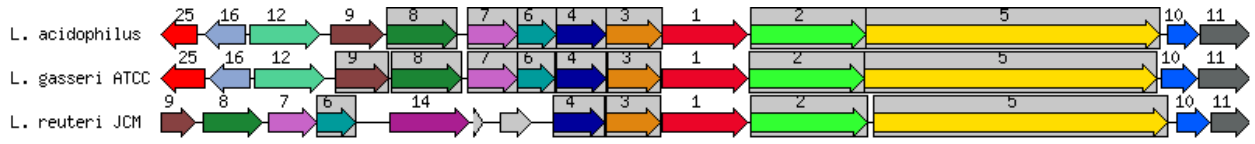

Figure 4.39: Annotation diagram showing location of the Intramembrane protease RasP/YluC, implicated in cell division based on FtsL cleavage ( ) found at node 2 and 1257 bp long on the –ve strand

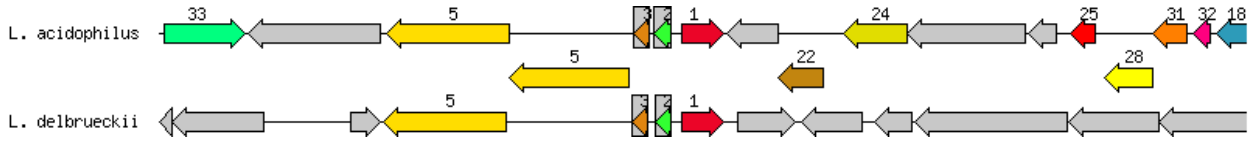

Figure 4.40: Annotation diagram showing location of the SOS-response repressor and protease LexA (EC 3.4.21.88) ( ) found at node 2 and 627 bp long on the –ve strand

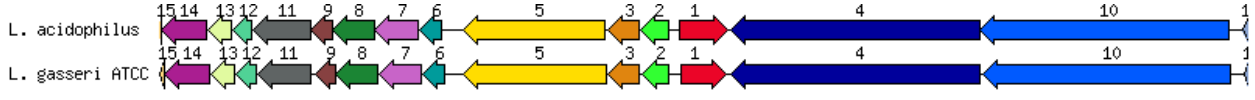

Figure 4.41: Annotation diagram showing location of the Late competence protein ComC, processing protease ( ) found at node 4 and 690 bp long on the +ve strand

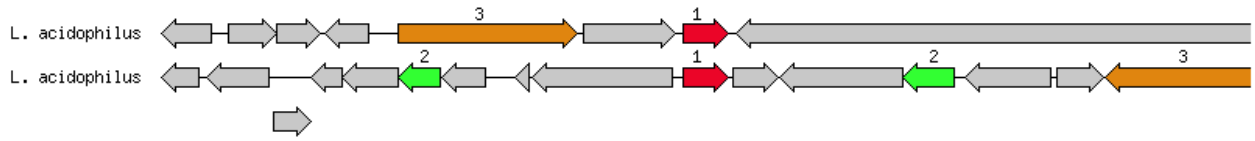

Figure 4.42: Annotation diagram showing the location of Predicted Zn-dependent protease ( ) found at node 7 and 669 bp long on the +ve strand

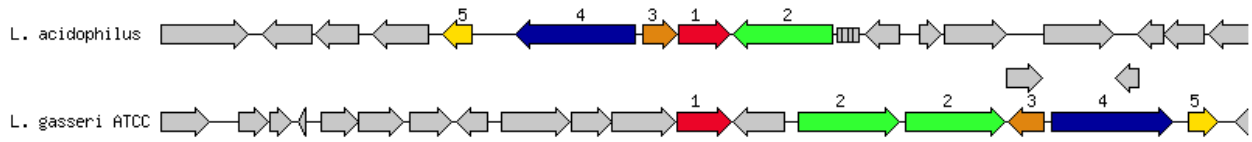

Figure 4.43: Annotation diagram showing the location of Predicted metal-dependent membrane protease ( ) found at node 9 and 759 bp long on the –ve strand

NB. Sequences of nucleotide and amino acids of the identified genes putatively involved in the production of extracellular enzymes are shown in Appendix J.

4.3.2.5. Coding sequence putatively involved in stress resistance

Three different coding sequences putative for Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2 were found within the entire genome assemply. Two of them were located on the same Contig Identity NODE_3_length_242459_cov_251.537351. One of them was 1275 bp long, stretching from 83070 to 84344 nucleotide position on the positive strand (Figure 4.44). The second CDS was 1056 bp long, stretching between 105749 and 106804 nucleotide positions along the positive strand (Figure 4.45). The other was found on a separate Contig Identity NODE_4_length_230557_cov_252.939857. The fragment was 1104 bp long, lying in-between 172603 and 171500 nucleotide positions along the negative strand (Figure 4.46).

The coding sequence putative for ATP-dependent Clp protease, ATP-binding subunit ClpE (Fig. 4.47) was found on Contig Identity NODE_2_length_675994_cov_165.027269. The fragment was 2187 bp long, stretching between 24138 and 21952 nucleotide positions along the negative strand. Another CDS predicted for ATP-dependent Clp protease, ATP-binding subunit ClpC, was also located on NODE_4_length_230557_cov_252.939857. The CDS was 2478 bp (Figure 4.48), stretching from 76960 to 74483 nucleotide positions on the negative strand. In addition, Peptide-methionine (R)-S-oxide reductase MsrB (EC

1.8.4.12) predictably encoded for by a CDS, was located on Contig Identity NODE_7_length_167441_cov_180.361088 as a stress resistant gene within the genome. The CDS was 438 bp long, stretching from 70733 to 70296 nucleotide positions along the negative strand (Figure 4.49).

Two different CDS putative for S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS with Methionine Biosynthesis subsystem were also found on the same Contig Identity NODE_2_length_675994_cov_165.027269. The fragment, measuring 474 bp, stretched between 460026 and 460499 nucleotide positions on the positive strand (Figure 4.50), while the fragment, measuring156 bp, laid between 467389 and 467544 on the positive strand (Figur 4.51).

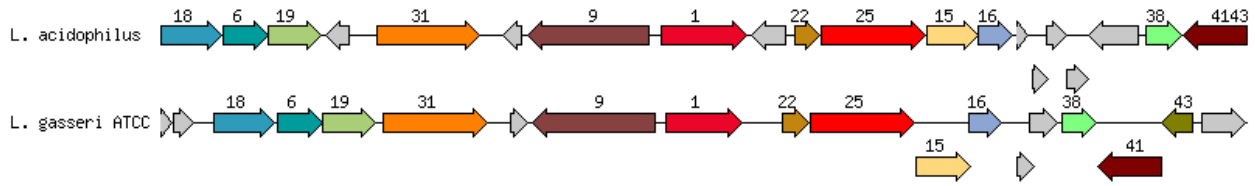

Figure 4.44: Annotation diagram showing the location of Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2 ( ) found at node 3 and 1275 bp long on the +ve strand

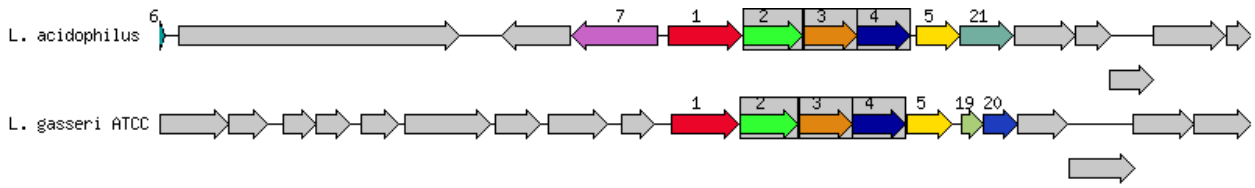

Figure 4.45: Annotation diagram showing the location of Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2 ( ) found at node 3 and 1056 bp long on the +ve strand

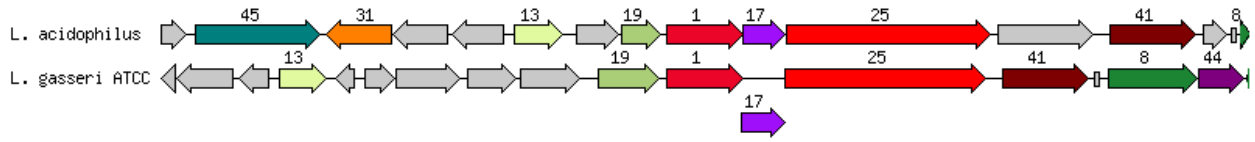

Figure 4.46: Annotation diagram showing the location of Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2 ( ) found at node 3 and 1104 bp long on the –ve strand

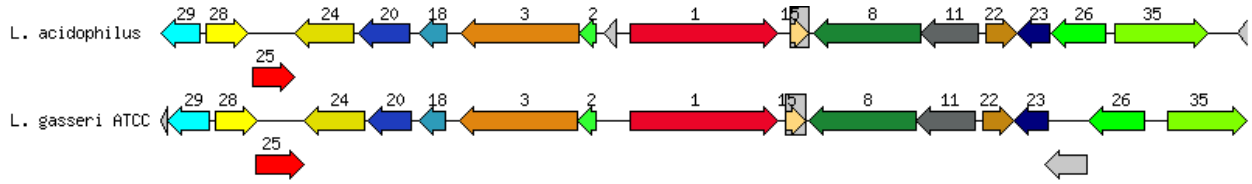

Figure 4.47: Annotation diagram showing the location of ATP-dependent Clp protease, ATP-binding subunit ClpE ( ) found at node 2 and 2187 bp long on the –ve strand

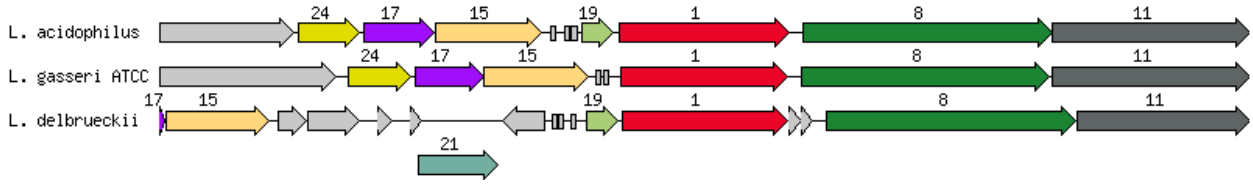

Figure 4.48: Annotation diagram showing the location of ATP-dependent Clp protease, ATP-binding subunit ClpC ( ) found at node 4 and 2478 bp long on the –ve strand

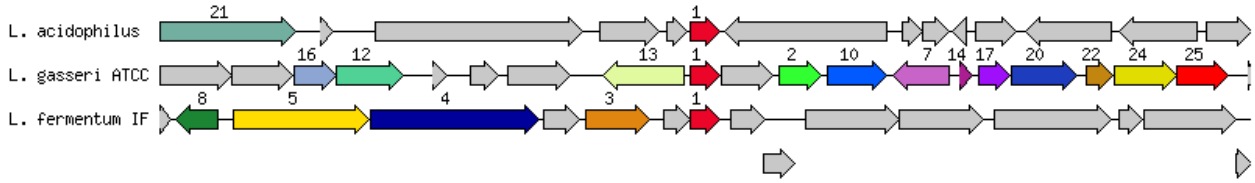

Figure 4.49: Annotation diagram showing the location of Peptide-methionine (R)-S-oxide reductase MsrB (EC 1.8.4.12) ( ) found at node 7 and 438 bp long on the –ve strand

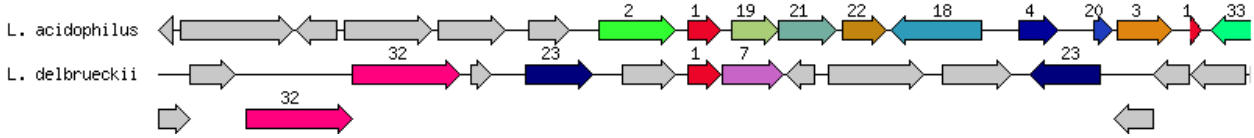

Figure 4.50: Annotation diagram showing the location of S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS ( ) found at node 2 and 474 bp long on the +ve strand

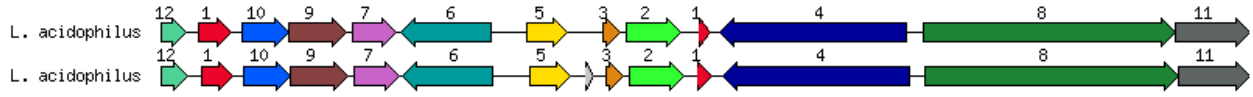

Figure 4.51: Annotation diagram showing the location of S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS ( ) found at node 2 and 156 bp long on the +ve strand

NB. The sequences of nucleotide and amino acids of the identified genes putatively involved in stress resistance are shown in Appendix K.

4.3.2.6. Coding sequence putatively involved in active metabolism in the host

Among the functional categories, one carbohydrate metabolism, two diacylglycerol and three glucosyltransferase (EC 2.4.1.337) predictably encoded for the coding sequence located on Contig Identity NODE_20_length_14896_cov_167.363722. The fragment was

1164 bp long and laid in-between 8667 and 7504 nucleotide position on the negative strand (Figure 4.52). Another CDS putative for Poly (glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52), an enzyme within the same functional category, with subsystem Teichoic and lipoteichoic acids biosynthesis, was also found on Contig Identity NODE_9_length_161393_cov_196.936481. The sequence fragment was 1092 bp long, stretching from 65023 to 66114 nucleotide position along the positive strand (Figure 4.53).

The CDS predicted for cellobiose phosphor transferase system celC was found on Contig Identity NODE_22_length_12729_cov_198.662143. The fragment, measuring 447 bp, stretched between 534 and 88 nucleotide positions (Figure 4.54). Seven different coding sequences putative for PTS system, cellobiose-specific IIC component were located on different loci within the entire genome of the isolates. The first of them, which was 1326 bp long, was located at Contig Identity NODE_2_length_675994_cov_165.027269. The nucleotide fragment stretched from 3413 to 4738 nucleotide position along the positive strand (Figure 4.55). The length of other CDS putative for similar functions were 1428, 1269 and 1062 bp respectively and situated on the positive strand while those measuring 1326, 1254 and 1338 bp were situated on the negative strand.

Furthermore, CDS putative for PTS system, cellobiose-specific IIB component (EC 2.7.1.205) (Figure 4.56) was also located on Contig Identity NODE_2_length_675994_cov_165.027269. The coding sequence, stretching from 253392 to 253727 nucleotide position, was 336 bp long and situated on the positive strand. Another predicted CDS for outer surface protein of unknown function, cellobiose operon (Fig. 4.57), was also located on Contig Identity NODE_3_length_242459_cov_251.537351. The sequenced fragment measuring 1062 bp, stretched between 148410 and 149471 nucleotide position along the positive strand. Lastly, from the functional category for protein metabolism, Methionine synthase II

(cobalamin-independent) (Figure 4.58) was identified as predictably encoded for by the codig sequence at Contig Identity NODE_2_length_675994_cov_165.027269. The fragment measuring 1119 bp, stretched from the 458718 nocleotide position to 459836.

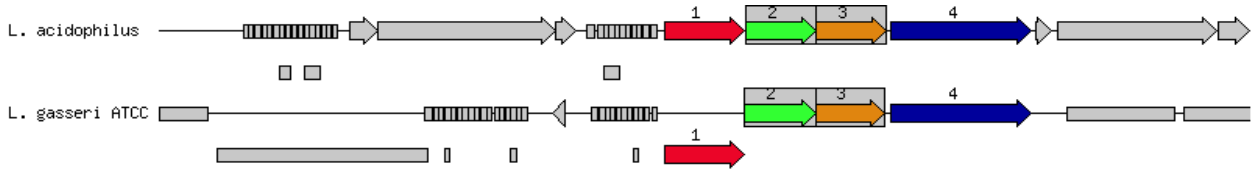

Figure 4.52: Annotation diagram showing the location of 1, 2-diacylglycerol 3-glucosyltransferase (EC 2.4.1.337) ( ) found at node 20 and 1164 bp long on the –ve strand

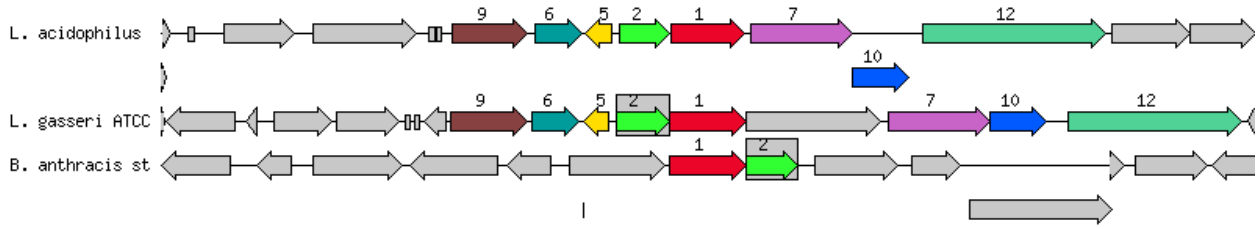

Figure 4.53: Annotation diagram showing the location of Poly (glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52) ( ) found at node 9 and 1092 bp long on the +ve strand

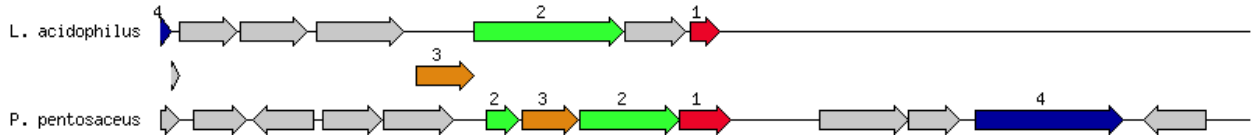

Figure 4.54: Annotation diagram showing the location of cellobiose phosphotransferase system celC ( ) found at node 22 and 447 bp long on the –ve strand

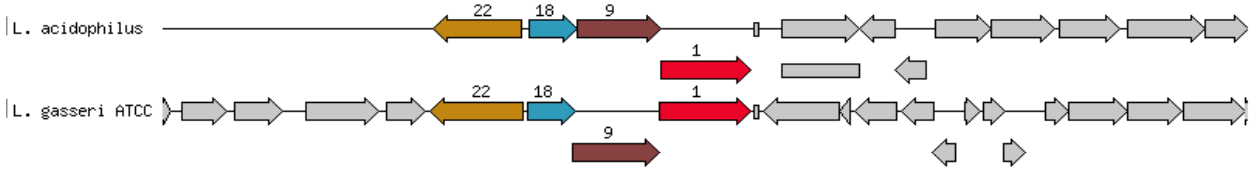

Figure 4.55: Annotation diagram showing the location of PTS system, cellobiose-specific IIC component ( ) found at node 2 and 1326 bp long on the +ve strand

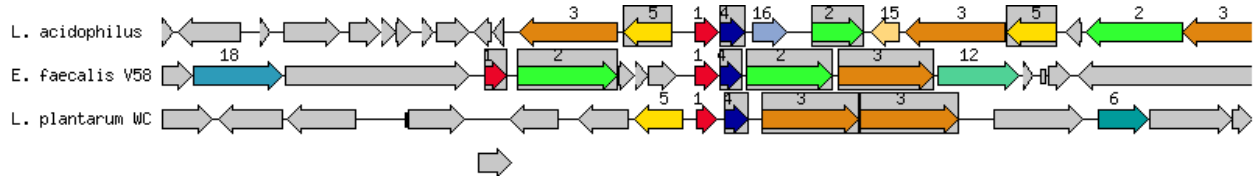

Figure 4.56: Annotation diagram showing the location of PTS system, cellobiose-specific IIC component ( ) found at node 2 and 336 bp long on the +ve strand

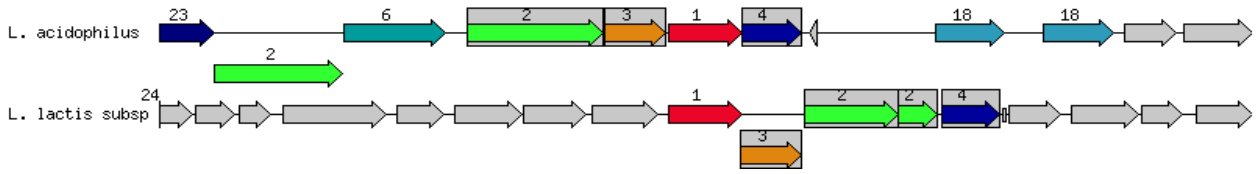

Figure 4.57: Annotation diagram showing the location of Outer surface protein of unknown function, cellobiose operon ( ) found at node 3 and 1062 bp long on the +ve strand

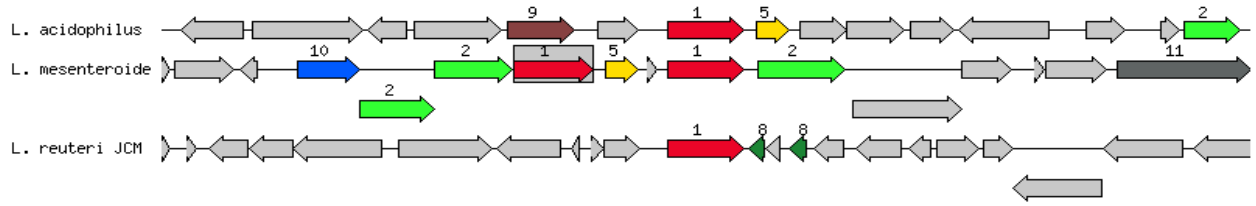

Figure 4.58: Annotation diagram showing the location of Methionine synthase II (cobalamin-independent) ( ) found at node 2 and 1119 bp long on the +ve strand

NB. The sequences of nucleotide and amino acids of the identified genes putatively involved in active metabolism in the host are shown in Appendix L.

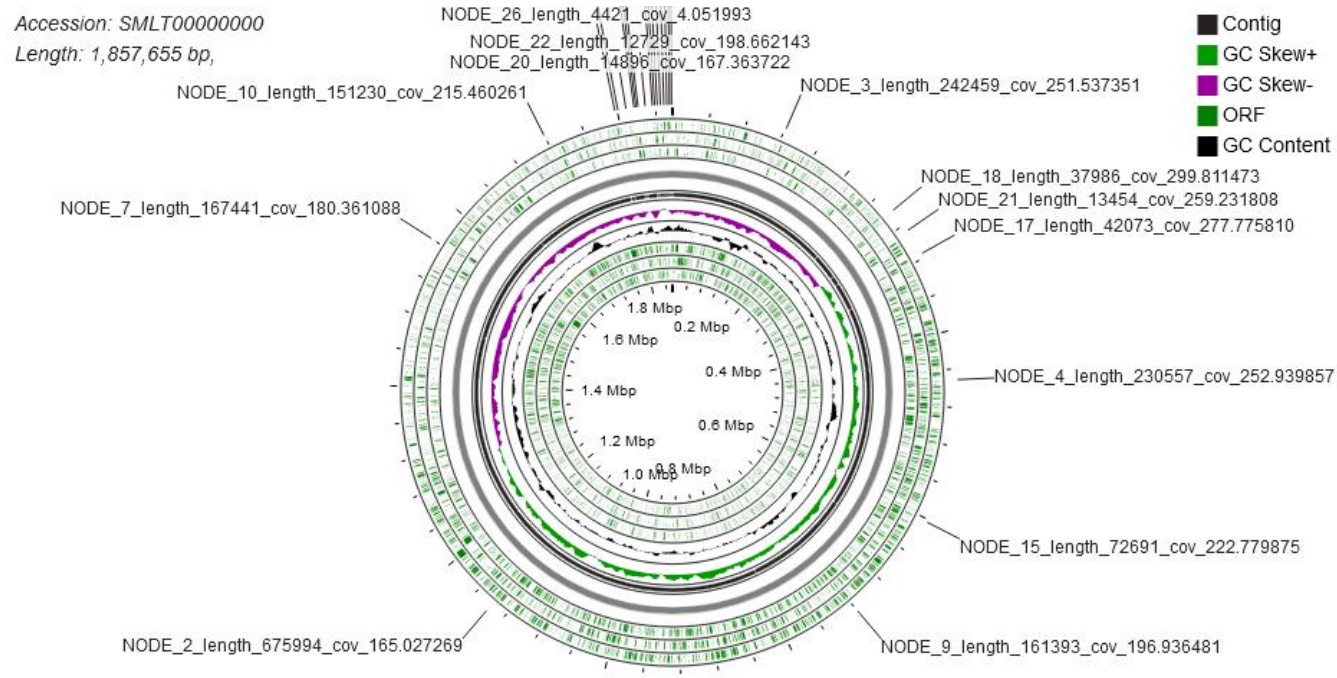

Figure 4.59: Circular genome mapping showing the position of each Contig within the *L. acidophilus* PNW3 genome. The mapping was generated using CGView (Grant and Stothard, 2008)

4.4. Discussion and conclusion

This study is an overhaul of the entire genome of *Lactobacillus acidophilus* PNW3, in order to identify functional coding sequeneces (CDSs) related to the probiotic potential of the isolate. Functional annotation through NCBI Prokaryotic Genome Annotation Pipeline and Rapid Annotations using Subsystems Technology with SEED viewer platforms revealed several inherent genes putative for enzymes and secondary metabolites in support of the medicinal importance of a typical probiotic candidate.

Three protein-encoding genes were determined, within the genome, putative for L-lactate dehydrogenase (EC 1.1.1.27), identified as an important enzyme required in the fermentation of lactate and mixed acids. In *in vitro* and *in vivo* studies conducted by Yang *et al*. (2012), lactic acid was confirmed as an inducer of rapid de-phosphorylation of Epidermal growth factor receptor. It also exhibited inhibitory effects on the production of IL-8 induced by IL-1β and colony formation by HT29 cells. Furthermore, lactic acid commendably increases the rate of survival when administered through oral route to ApcMin mice (Apc/multiple intestinal neoplasia) with advanced stage malignant tumor. In another independent study, L-lactate dehydrogenase was found to possess the ability to stimulate bioenergetics of the mitochondria in the heart, muscle and liver, just as effective as pyruvate. It can as well stimulate production of reactive oxygen species in the mitochondrion in the same rate as pyruvate induces production of hydrogen peroxide (Young *et al*., 2020).

Among the probiotic important plethora of genes harboured within the genome of *L. acidophilus* PNW3, were genes putative for bioactive peptides. The coding sequences for bacteriocin, Autoinducer-2 production protein, type 1 capsular polysaccharide biosynthesis protein and mannosyltransferase involved in polysaccharide biosynthesis were revealed. Bioactive peptides, generated as secondary metabolites by the lactic acid bacteria,

provided a source of alternative bioactive compounds of natural origin to synthetic antimicrobials. Several studies have revealed the potency of active compounds secreted by LAB against clinically important pathogens and malignant cells (Axel *et al*., 2016; Wang *et al*., 2018; Muhialdin *et al*., 2020).

The effectiveness of a probiotic is largely enhanced by its ability to adhere and competitively colonise the gut epithelia cells. Sortase A, LPXTG specific, identified with heme, hemin uptake and utilisation systems in Gram Positives Sortase, is among the important adhesion player in the *L*. *acidophilus* PNW3. Sortase A is an enzyme used by Gram-positive bacteria to coat the cell surface with functional proteins and pili assemblage that enable interactions between the cells and their environment. The enzyme is highly important in the cell physiology and defence, and is made up of protein domains connected with adhesion of cells to host cells and extracellular matrix proteins (Jacobitz *et al.,* 2017; Stanborough *et al*., 2018). Other important adhesive encoding genes identified include the following: cell surface and S-layer proteins; fibronectin/fibrinogen-binding protein; and members of the EPS cluster such as EpsC, EpsD, EpsJ and ATP synthase epsilon chain.

Gut microbiota vested with diverse enzymatic potentials plays a crucial role in the improvement of the host metabolism, repression of pathogens, contributing additional nutrients and improvement on nutrient digestibility and absorption (Sumathi *et al*., 2011; Nora-Azirah *et al*., 2016). Extracellular enzymes, such as lipase, protease and amylase, among others, produced by most lactic acid bacteria, play an important role in the gut of animals and human beings, including infants (Tallapragada *et al*., 2018). Four coding sequences putative for different kinds of lipases and twelve codoing sequences putative for different proteases were among the probiotic supportive genes located in the genome of *L*. *acidophilus* PNW3. This, therefore, supports the envisaging potential of the isolate

with regard to improving nutrient digestibility in the host as a probiotic candidate. This may directly translate into growth performance improvement in the target farm animal.

Functional annotation of the entire genome assembly of the *L*. *acidophilus* PNW3 revealed several coding genes putative for stress tolerance within the gut environment in relation to bile salts and gastric pH. These include ATP synthase epsilon chain, PTS system, cellobiose-specific IIC component, ATP-dependent Clp protease ATP-binding subunit and L-lactate dehydrogenase, all responsible for the maintenance of acid stress resistance (Oliveira *et al*., 2017). Autoinducer-2 production protein LuxS, which is among the putative stress resistant CDS identified in the genome, has been identified as part of proteins involved in both acid and bile salt stress tolerance (Jia *et al*., 2018). Another CDS identified along the Cell Envelope subcategory is the Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2. The hydrophobic nature of the cell envelope of microorganism, assists with adhesion of organisms to the host epithelia cell, thus conferring competitive edge for colonisation of the gastrointestinal tract (Vinderola *et al*., 2003).

Considering the foregoing, the genome of *L. acidophilus* PNW3 is vested with enough protein-encoding genes in support of its probiotic efficacy. Thus, the isolate could be considered for further analysis required to certify the strain as a viable and safe probiotic agent for application in animal husbandry.

References

Arkin, A. P, Cottingham, R. W., Henry, C. S., Harris, N. L., Stevens, R. L., Maslov, S., Dehal, P., Ware, D., Perez, F., Canon, S., Sneddon, M. W., Henderson, M. L., Riehl, W. J., Murphy-Olson, D., Chan, S. Y., Kamimura, R. T., Kumari, S., Drake, M. M., Brettin, T. S., Glass, E. M., Chivian, D., Gunter, D., Weston, D. J., Allen, B. H., Baumohl, J., Best, A. A., Bowen, B., Brenner, S. E., Bun, C. C., Chandonia, J-M., Chia, J-M., Colasanti, R., Conrad, N., Davis, J. J., Davison, B. H., DeJongh, M., Devoid, S., Dietrich, E., Dubchak, I., Edirisinghe, J. N., Fang, G., Faria, J.P., Frybarger, P.M., Gerlach, W., Gerstein, M., Greiner, A., Gurtowski, J., Haun, H. L., He, F., Jain, R. et al. 2018. KBase: the United States Department of Energy Systems Biology Knowledgebase. *Nat Biotechnol*, 36, 566 –569.

Axel, C., Brosnan, B., Zannini, E., Furey, A., Coffey, A., Arendt, EK. 2016. Antifungal sourdough lactic acid bacteria as biopreservation tool in quinoa and rice bread. *Int J Food Microbiol,* 239, 86–94.

Blin, K., Wolf, T., Chevrette, M., Lu, X., Schwalen, C., Kautsar, S., Duran, H., Santos, E., Kim, H., Nave, M., Dickschat, J., Mitchell, D., Shelest, E., Breitling, R., Akano, E., Lee, S., Weber, T., Medema, M. 2017. Improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res,* 45, W36–W41.

Bolger, A., Lohse, M., Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinform*, 30**,** 2114 –2120.

Brettin, T., Davis, J., Disz, T., Edwards, R., Gerdes, S., Olsen, G., Olson, R., Overbeek, R., Parrello, B., Pusch, G., Shukla, M., Thomason, J., Stevens, R., Vonstein, V., Wattam, A., Xia, F. 2015. RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep,* 5**,** 8365.

Chetwin, E., Manhanzva, M. T., Abrahams, A. G., Froissart, R., Gamieldien, H., Jaspan, H., Jaumdally, S. Z., Barnabas, S. L., Dabee, S., Happel, A. U., Bowers, D., Davids, L., Passmore, J. S., Masson, L. 2019. Antimicrobial and inflammatory properties of South African clinical *Lactobacillus* isolates and vaginal probiotics. *Sci Rep,* 9**,** 1917.

Cosentino, S., Voldby, L. M., Møller, A. F., Lund, O. 2013. PathogenFinder - Distinguishing friend from foe using bacterial whole genome sequence data. *PLoS ONE,* 8**,** e77302.

Dlamini, Z. C., Langa, R. L., Aiyegoro, O. A., Okoh, A. I. 2019. Safety evaluation and colonisation abilities of four lactic acid bacteria as future probiotics. *Probiotics Antimicrob Proteins,* 11**,** 397–402.

Ejtahed, H., Mohtadi-Nia, J., Homayouni-Rad, A., Niafar, M., Asghari-Jafarabadi, M., Mofid, V., Akbarian-Moghari, A. 2011. Effect of probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* on lipid profile in individuals with type 2 diabetes mellitus. *J Dairy Sci* 94**,** 3288–3294.

Guo, Y., Jiang, X., Yang, Y., Zhang, J., Zeng, X., Wu, Z., Sun, Y., Pan, D. 2018. Prevention of necrotizing enterocolitis through surface layer protein of *Lactobacillus acidophilus* CICC6074 reducing intestinal epithelial apoptosis. *J Funct Foods,* 47**,** 91–99.

Haft, D., Dicuccio, M., Badretdin, A., Brover, V., Chetvernin, V., O'neill, K., Li, W., Chitsaz, F., Derbyshire, M., Gonzales, N., Gwadz, M., Lu, F., Marchler, G., Song, J., Thanki, N., Yamashita, R., Zheng, C., Thibaud-Nissen, F., Geer, L., Marchler-Bauer, A., Pruitt, K. 2018. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Res,* 4**,** D851-D860.

Jacobitz, A. W., Kattke, M. D., Wereszczynski, J., Clubb, R. T. 2017. Sortase transpeptidases: structural biology and catalytic mechanism. *Adv Protein Chem Struct Biol*, 109, 223–64.

Jia, F-F., Zheng, H-Q., Sun, S-R., Pang, X-H., Liang, Y Shang, J-C., Zhu, Z-T., Meng, X-C. 2018. Role of *luxS* in Stress Tolerance and Adhesion Ability in *Lactobacillus plantarum* KLDS1.0391. *BioMed Res Int,* Article ID 4506829, doi:org/10.1155/2018/4506829.

Muhialdin, B. J., Algboory, H.L., Kadum, H, Mohammed, NK, Saari, N, Hassan, Z, Hussin, ASM. 2020. Antifungal activity determination for the peptides generated by *Lactobacillus plantarum* TE10 against *Aspergillus flavus* in maize seeds. *Food Control*, 109, 106898.

Nora-Azirah, M. Z., Marini, I., Murni, K., Harmin, S. A., Ina-Salwany, M. Y. 2016. Extracellular Enzyme Production of Probiotic *Bacillus* JAQ04 and *Micrococcus* JAQ07 isolated from tiger grouper (*Epinephelus fuscoguttatus*) *Int J Chem Environ Biol* Sci, 4(1), ISSN 2320–4087.

Nordeste, R., Tessema, A., Sharma, S., Kovac, Z., Wang, C., Morales, R., Griffiths, M. W. 2017. Molecules produced by probiotics prevent enteric colibacillosis in pigs. *BMC Vet Res,* 13**,** 335.

Nurk S., Bankevich A., Antipov D., Gurevich A., Korobeynikov A., Lapidus A., Prjibelsky A., Pyshkin A., Sirotkin A., Sirotkin Y., Stepanauskas R., McLean J., Lasken R., Clingenpeel S. R., Woyke T., Tesler G., Alekseyev M. A., Pevzner P. A. 2013. Assembling genomes and mini-metagenomes from highly chimeric reads, p 158–170. *In* Deng M, Jiang R, Sun F, Zhang X (eds), Research in computational molecular biology. Lecture notes in computer science, vol 7821. Springer, Berlin, Germany.

Oliveira, L.C., Saraiva. T.D.L., Silva, W.M., Pereira, U.P., Campos, B.C., Benevides, L. J., Rocha, F. S., Figueiredo, H. C. P., Azevedo, V., Soares, S. C. 2017. Analyses of the probiotic property and stress resistance-related genes of *Lactococcus lactis* subsp. *lactis* NCDO 2118 through comparative genomics and *in vitro* assays. PLoS ONE 12(4): e0175116. doi:10.1371/journal.pone.0175116.

Pradhan, B., Guha, D., Naik, A. K., Banerjee, A., Tambat, S., Chawla, S., Senapati, S., Aich, P. 2019. Probiotics *L. acidophilus* and *B. clausii* Modulate Gut Microbiota in Th1and Th2-Biased Mice to Ameliorate *Salmonella Typhimurium*-Induced Diarrhea. *Probiotics Antimicrob Proteins*, 11, 887–904.

Stahl, B., Barrangou, R. 2013. Complete Genome Sequence of Probiotic Strain *Lactobacillus acidophilus* La-14. *Genome Announc,* 1, e00376-13.

Stanborough, T., Suryadinata R, Fegan N, Powell S. M., Tamplin M., Nuttall, S.D., Chandry, P.S. 2018. Characterisation of the *Brochothrix thermosphacta* sortase A enzyme. *FEMS Microbiol Lett*, 365, fny184. doi: 10.1093/femsle/fny184.

Sumathi, C., Priya, D. M., Babu, V. D., Sekaran, G. 2011. Microbial & biochemical technology analysis of enzyme activities of the gut bacterial communities in *Labeo rohita* fed differentially. *Treated Animal Fleshing Diets*, 3(5), 112–120.

Tallapragada, P., Rayavarapu, B., Rao, P. P., Ranganath, N. N., Veerabhadrappa, P. P. 2018. Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. *J Genetic Engineering Biotechnol*, 16, 357–362.

Tatusova, T., Dicuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E., Zaslavsky, L., Lomsadze, A., Pruitt, K., Borodovsky, M., Ostell, J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res*, 44, 6614-6624.

Vinderola, C., Reinheimer, J. 2003. Lactic acid starter and probiotic bacteria: A comparative "*in vitro*" study of probiotic characteristics and biological barrier resistance. *Food Res Int*, 36, 895–904.

Wang Y, Wang X, Jiang W, Wang K, Luo J, Li W, Zhou X, Zhang l. 2018. Antimicrobial peptide GH12 suppresses cariogenic virulence factors of *Streptococcus mutans*, J Oral Microbiol, 10:1. DOI:10.1080/20002297.2018.1442089

Wang, H., Cheng, X., Zhang, L., Xu, S., Zhanga, Q., Lu, R. 2019a. A surface-layer protein from *Lactobacillus acidophilus* NCFM induces autophagic death in HCT116 cells requiring ROS-mediated modulation of mTOR and JNK signaling pathways. *Food Funct*, 10, 4102–4112.

Wang, H., Zhang, Q., Niu, Y., Zhang, X., Lu, R. 2019b. Surface-layer protein from *Lactobacillus acidophilus* NCFM attenuates tumor necrosis factor-α-induced intestinal barrier dysfunction and inflammation. *Int Bioll Macromol*, 136, 27–34.

Yang, G., Xu, H., Kelly, C.P., CHEN, X. 2012, Lactic acids mediates anti-cancer function of probiotic yeast (188), *Gastroenterol*, 142(5), S-46.

Yang, L., Chen, Y., Li, Z., Shi, Y., Li, Z., Zhao, X. 2016. Complete genome sequence of *Lactobacillus acidophilus* MN-BM-F01. *Genome Announc*, 4**,** e01699-15.

Yatsunenko, T., Rey, F., Manary, M., Trehan, I., Dominguez-Bello, M., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R., Anokhin, A., Heath, A., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J., Lozupone, C., Lauber, C., Clemente, J., Knights, D., Knight, R., Gordon, J. 2012. Human gut microbiome viewed across age and geography. *Nat,* 486**,** 222–227.

Younga A., Oldforda C., Mailloux R. J. 2020 Lactate dehydrogenase supports lactate oxidation in mitochondria isolated from different mouse tissues. Redox Biol, 28, 101339.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F., Larsen, M. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemotherapy,* 67, 2640–2644.

CHAPTER 5 − Integrated assessment of safety for *Lactobacillus reuteri* PNW1 and *Lactobacillus acidophilus* PNW3

Abstract

This study focuses on the safety features of the *L*. *reuteri* PNW1 and *L*. *acidophilus* PNW3 as potential probiotic candidates. The presence of acquired antimicrobial resistant genes was assessed using ResFinder, Comprehensive Antibiotic Resistance Database (CARD) and Rapid Annotations using Subsystems Technology (RAST). VirulenceFinder and RAST were used to detect the presence of virulence factors and PathogenFinder was employed to determine the pathogenicity of both isolates. Phage Search Tool (PHAST), Phage Search Tool Enhanced Release (PHASTER), ISfinder search tool, Insertion Sequence Semi-Automatic Genome Annotation (ISsaga) and Optimised Annotation System for Insertion Sequences (OASIS) were used to locate the positions of the Mobile Genetic Elements within the genomes, while the CRISPRCasFinder was used to determine CRISPR-Cas sequence within the genome. Manual searching through functional annotation data was used to determine protein-encoding sequences putative for toxic biochemical. Production of biogenic amines was biochemically confirmed through HPLC analysis. The evolutionary trend of the isolates and similarity between the two genomes were determined through phylogeny and comparative genomic analysis. Both isolates only harbour resistant genes putative for Lincosamide (*lnuC*) and Tetracycline resistant genes (*tetW*). There was no hit found for virulence factors and the calculated matched pathogenic families for both isolates were zero. Two intact prophage regions were detected within the genome of *L. reuteri* PNW1 while the only prophage region identified within the genome of the *L. acidophilus* PNW3 was designated as incomplete prophage type. Within the *L. reuteri* PNW1 genome, 9 CDS were identified for IS by OASIS and distributed into seven different families, while in the *L. acidophilus* PNW3, 3 CDS were identified for IS and

attributed to 3 different families. Five putative CDS were identified for the CRISPR in *L. reuteri* PNW1 genome while only one was found in the *L. acidophilus* PNW3 genome and each of them associated with Cas genes. CDS putative for arginine deiminase and Ornithine decarboxylase, among the notable enzyme with toxic biochemicals production potential, were found in *L. reuteri* PNW1 and *L. acidophilus* PNW3 respectively. With the two isolates predicted as non-human pathogens, coupled with some other indentified factors, the *L. reuteri* PNW1 and *L. acidophilus* PNW3 stands a chance of making good and safe candidates for probiotic product, though further *in vivo* investigations are still necessary.

5.1. Introduction

Awareness on the role of health functional food in the gut microbial ecosystem (Saarela, 2019) and its impact on the well-being of humans and animals is fast spreading among consumers. In many countries aroud the world, probiotic is among the fastest-growing health functional food (Byakika *et al.*, 2019). The general and increasing interest in probiotics is responsible for concerted efforts the towards development of probiotic-based products (Banwo *et al.*, 2013; Borah *et al.*, 2016). Hence, a number of efficacy and safety assessment protocols have been recommended by experts for a putative probiotic candidate before confirmation and acceptance for public consumption.

Although majority of commonly used organisms as probiotics are regarded as safe, however, the continuous emergence of new strains, partly due to environmental pressure, makes proper and thorough safety screening for every novel strain inevitable. No new strain should be assumed of sharing the same documented safety history with preexisting ones (Pradhan *et al.*, 2020), coupled with the fact that the health functional benefit of a probiotic is strain-specific. In addition, among the associated potential side-effects of a

probiotic, systemic opportunistic infections are the most important cause for concern (Vankerckhoven *et al.*, 2008).

Many traditional pathogens have been characterised with a number of virulent determinants, with few or none of these determinants reported in any case of *Lactobacillus* species. Nevertheless, the ability of any viable microorganism to cause infectious diseases cannot be candidly excluded (Pradhan *et al.*, 2020). The possibility of the microorganism gaining entrance into the blood stream through passive movement, direct invasion, compromised intestinal barrier and inflammatory conditions could result in disease conditions, especially in immuno-compromised individuals. Taking into consideration the usual high dosage recommendation for probiotics, any associated virulent determinant in such occasion could be fatal (Bunesova *et al.*, 2015). Meanwhile, it was established in the *in vivo* study conducted by Pradhan *et al.* (2019) that both short and long-term administration of high dosage of probiotics cells did not alter any of the general health parameters or cause a specific disorder in any organ in instances of functional immune system.

5.2. Materials and methods

5.2.1. Identification of antimicrobial resistance genes

A quick identification of protein-encoding sequences putative for antimicrobial resistance genes acquired within the genome of *Lactobacillus reuteri* PNW1 and *Lactobacillus acidophilus* PNW3 was performed using ResFinder v. 3.1 (Zankari *et al.*, 2012) and Comprehensive Antibiotic Resistance Database (CARD) v. RGI 5.1.0, CARD 3.0.7(Alcock *et al.*, 2020). Data from the functional annotation through Rapid Annotations, using Subsystems Technology (RAST) (Overbeek *et al.*, 2014), were manually searched for resistance against a number of clinically important antimicrobials.

5.2.2. Identification of virulent determinant genes

Putative virulence determinance and pathogenicity of isolates were determined using VirulenceFinder v. 2.0 (Joensen *et al*., 2014) and PathogenFinder v. 1.1 (Cosentino *et al.*, 2013) respectively. In addition, manual searches for protein-encoding genes related to virulence in bacteria were also employed based on the functional annotation from the RAST platform (Overbeek *et al.*, 2014). Factors considered included the following: sex pheromones; gelatinase; cytolysin; hyaluronidase; aggregation substance; enterococcal surface protein; endocarditis antigen; adhesine of collagen; and integration factors.

5.2.3. Identification of prophage, transposase and other insertion sequences (IS) within the genome

Rapid identification and annotation of prophage sequences within the genome of the *L*. *reuteri* PNW1 and *L*. *acidophilus* PNW3 was determined using the Phage Search Tool (PHAST) (Zhou *et al.*, 2011) and the Phage Search Tool Enhanced Release (PHASTER) (Arndt *et al.*, 2016), a significant upgrade search tool to the former. The protein-encoding gene for transposase was manually searched within the functional annotation data generated from the NCBI PGAP (Tatusova *et al.*, 2016; Haft *et al.*, 2018) and RAST platform (Overbeek *et al.*, 2014). Genome was searched for insertion sequences (IS) using ISfinder search tool, Insertion Sequence Semi-Automatic Genome Annotation (ISsaga V. 2.0) (Siguier *et al.*, 2006) and Optimised Annotation System for Insertion Sequences (OASIS) (Robinson *et al.*, 2012).

5.2.4. Identification of CRISPR−Cas sequences within the genome

Coding sequences for Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated genes (Cas) were indentified using CRISPRCasFinder v. 1.1.2 - I2BC(Grissa *et al.*, 2007) and manual searches through the functional annotation generated from RAST database (Overbeek *et al*., 2014).

5.2.5. Determination of toxic biochemicals and associated genes

5.2.5.1. Genomic-based

Protein-encoding sequences putative for enzymes directly related to the production of biogenic amines, such as histidine decarboxylase, tyrosine decarboxylase, ornithine decarboxylase, agmatine dehydrolase, L-lysine decarboxylase and agmatine deiminase pathway, were manually investigated through functional annotation from NCBI PGAP (Tatusova *et al.*, 2016; Haft *et al.*, 2018) and RAST platform (Overbeek *et al.*, 2014). Further genes involved in the production of toxins, such as haemolysin and cytotoxin K, those involved in the production of lipopeptides, such as fengygin, surfactins and lychenisin, were also investigated from the same database (Salvetti *et al.*, 2016).

5.2.5.2. Biochemical extraction and determination of biogenic amines

Bacterial cells were sub-cultured in de Man Rogosa Sharpe broth supplemented with amino acid supplements, which included L-histidine mono-hydrochloride (2.5 g/L), L-tyrosine disodium salt (2.5 g/L), L-ornithine mono-hydrochloride (2.5 g/L), L-lysine mono-hydrochloride (2.5 g/L) and agmatine sulfate salt (1g/L) (Sigma-Aldrich, Germany). The cultures were incubated, under strict anaerobic conditions, in an anaerobic jar provided with anaerocult A (Merk, Germany), at 37 ^{0}C for 3 days without agitation (Salvetti *et al.*, 2016; Elsanhoty and Ramadan, 2016).

Extraction and determination of histamine, tyramine, putrescine, cadaverin and agmatine were performed as previously described by Singracha *et al.* (2017). Bacterial culture (50 ml) was centrifuged at 8000 *g* for 10 minutes at 10 ^{0}C and the supernatant (5 ml) extracted in 25 ml of 0.4 M perchloric acid and then transferred into a screw-capped bottle. Then, 1 ml of crude extract was added with 10 µl of the internal standard (1, 7- diaminoheptane), 200 µl of 2 M sodium hydroxide (NaOH), 300 µl of saturated sodium bicarbonate ($NaHCO_3$) and 1000 µl of dansyl chloride (10 mg/ml in acetone) and then vortexed. The mixture was incubated at 70 ^{0}C for 30 minutes and excess dansyl chloride precipitated

with 100 µl of ammonium hydroxide (NH_4OH, 30%). The supernatant was adjusted to 50 ml with acetonitrile and filtered through 0.45 µm Polytetrafluoroethylene membrane filter and the supernatant kept at -28 ^{0}C prior to HPLC analysis.

Determination of biogenic amines from the extract was carried out using High Performance Liquid Chromatography (PerkinElmer Altus, A-10 PDA Detector) with a C18 RP-HPLC column (Analytical C18 Column 100 x 4.6 mm, 5 µm particle size). The gradient elution system used for the HPLC analysis was deionized water and acetonitrile at the flow rate of 0.5 ml/min. Elution gradient of 65% acetonitrile was used at 0 minute, increased to 70% at 5 minutes and 100% at 20 minutes and then 65% at 25 minutes. Histamine, tyramine, putrescine, L-agmatine and cadaverine (Sigma-Aldrich, Germany) were used as standards.

5.2.6. Multiple proteins based phylogenetic and comparative genome analyses of *L. reuteri* PNW1 and *L. acidophilus* PNW3

Evolutionary relationship between the *L*. *reuteri* PNW1 and *L. acidophilus* PNW3 was assessed through phylogenetic and comparative genomic analyses. The phylogenetic tree was built based on concatenation of multiple proteins shared within the comparing genomes, using PATRIC's tree building pipeline v. 3.5.43 (Davis *et al.*, 2016; Wattam *et al.*, 2017). The pipeline is a conglomerate of the following specific components: Basic Local Alignment Search Tool (BLAST) (Boratyn *et al.*, 2013); MCL, MUSCLE (Edgar, 2004); Hidden Markov Model (HMM) hmmbuild/hmmsearch (Potter *et al.*, 2018), Gblocks (Talavera and Castresana, 2007); FastTree (Price *et al.*, 2010); and RAxML v.8 (Stamatakis, 2014). The two genomes under study, were compared with eight other related species carefully selected from the public database (GenBank) and the generated phylogeny was opened and refined in FigTree v.1.4.4 (Rambaut, 2018).

The comparative genomic was assessed using Artemis Comparison Tool (ACT) (Carver *et al.*, 2005). The ACT consists of blocks connecting regions of similarities or high identities

between the two genomes. The alignment file for the *L*. *reuteri* PNW1 and *L. acidophilus* PNW3 was generated with BLASTn through the NCBI platform.

5.3. Results

5.3.1. Putative genes for antimicrobial resistance

Separate searches through the Centre for Genomic Epidemiology database ResFinder and Comprehensive Antibiotic Resistance Database (CARD) for acquired antimicrobial resistance encoding genes revealed that *Lactocbacillus reuteri* PNW1 and *L. acidophilus* PNW3 only harbour resistant genes putative for lincosamide nucleotidyltransferase (*lnuC*), confering resistance to Lincosamide and Tetracycline-resistant ribosomal protection protein (*tetW*), which confers resistance against Tetracycline. Manual searches through the functional annotation data derived from RAST platform also revealed the same result, even though the manual search through the *L. acidophilus* PNW3 genome revealed the harbouring resistant gene as only multidrug resistant protein with assisting heterodimetric efflux ABC transporter.

In the genome of *Lactobacillus reuteri* PNW1, CDS putative for Lincosamide nucleotidyltransferase (*lnuC*) was located on Contig Idendity NODE_126_length_1725_cov_2232.632040. The fragment, measuring 495 bp, stretched from nucleotide position 1150 to 1644 along the positive strand (Figure 5.1). Two coding sequences of different sizes putative for Tetracycline resistance and Ribosomal protection type (*TetW*) were located on the same locus, Contig Identity NODE_89_length_5548_cov_623.805571. The CDS stretching from 1027 to 368 nucleotide position was 660 bp long (Figure 5.2) while the other one stretching from 2289 to 1048 nucleotide position contained 1242 base pairs (Figure 4.3). Both fragments were foind along the negative strand. Another CDS putative for Ribosome protection-type Tetracycline resistance-related proteins, group 2, was located on Contig Identity

NODE_34_length_20428_cov_693.602778. The fragment, measuring 1929 bp, in-between 1887 and 3815 nucleotide position, was along the positive strand (Figure 5.4).

On the other hand, manual searches through the genome of *L. acidophilus* PNW3 revealed the presence of three coding sequences putatively involved in multidrug resistance. These included a 549 bp long CDS predicted for Multidrug resistant protein, occurring at Contig Identity NODE_4_length_230557_cov_252.939857. The region position occupied by the fragment was between 107273 and 106725 along the negative strand (Figure 5.5). The two other CDS putative for Heterodimeric efflux ABC transporter, multidrug resistance => LmrC subunit of LmrCDwere found on the same locus, at Contig Identity NODE_9_length_161393_cov_196.936481. One of the fragments was 1731 bp long, in-between 120729 and 122459 nucleotide positions along the positive strand (Figure 5.6) while the other was 1770 bp long, stretching from 122459 to 124228 nucleotide position (Figure 5.7).

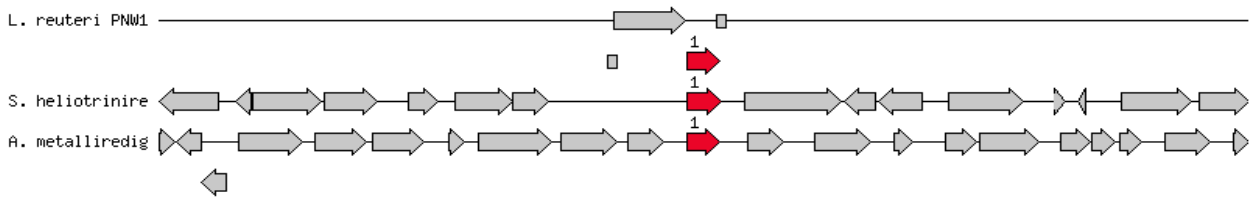

Figure 5.1 Annotation diagram showing the location of Lincosamide nucleotidyltransferase (*lnuC*) ( ) found at node 126 and 495 bp long on the +ve strand

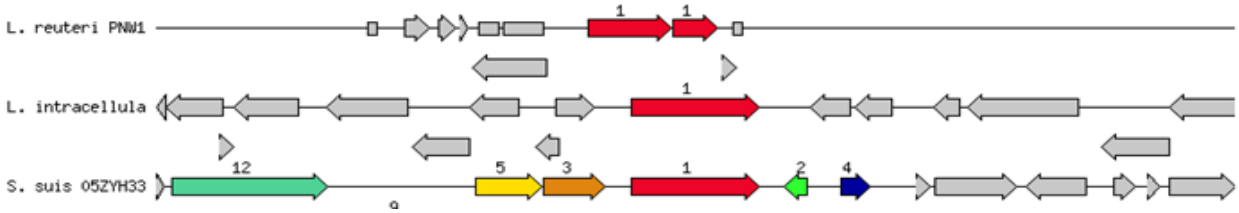

Figure 5.2 Annotation diagram showing the location of Tetracycline resistance, ribosomal protection type (*TetW*) ( ) found at node 89 and 660 bp long on the –ve strand

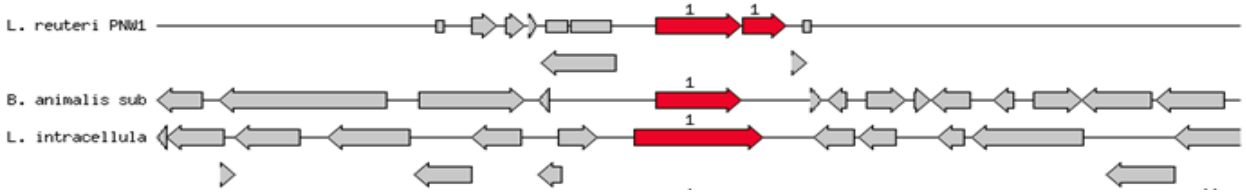

Figure 5.3 Annotation diagram showing the location of Tetracycline resistance, ribosomal protection type (*TetW*) ( ) found at node 1242 and bp long on the –ve strand

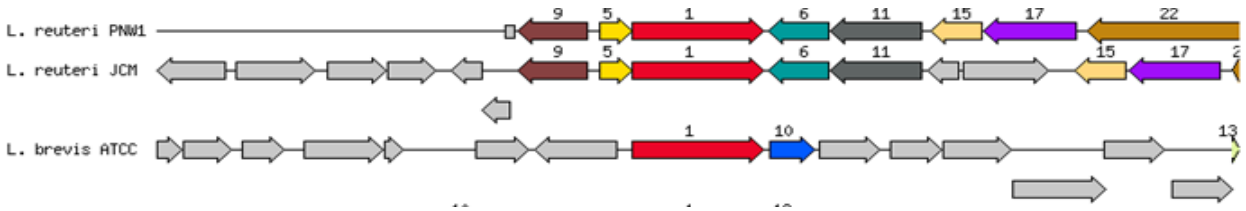

Figure 5.4 Annotation diagram showing the location of Ribosome protection-type tetracycline resistance related proteins, group 2 ( ) found at node 34 and 1929 bp long on the –ve strand

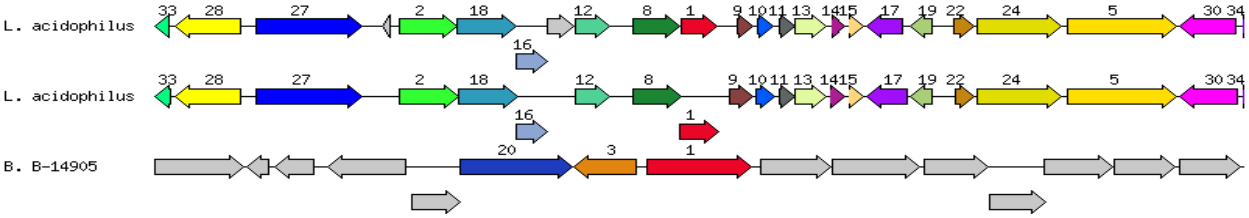

Figure 5.5 Annotation diagram showing the location of Multidrug resistance protein ( ) found at node 4 and 549 bp long on –ve strand

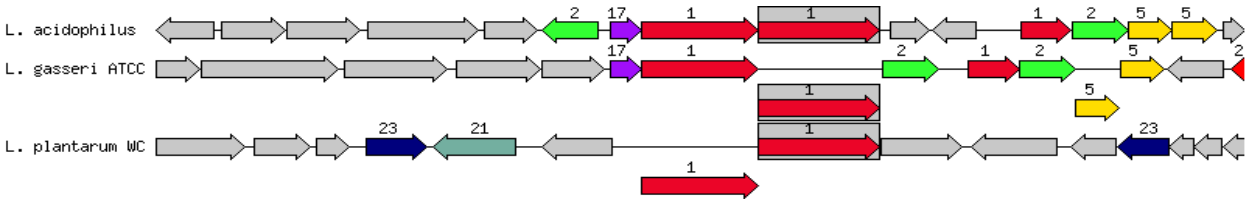

Figure 5.6 Annotation diagram showing the location of Heterodimeric efflux ABC transporter, multidrug resistance => LmrC subunit of LmrCD ( ) found at node 9 and 1731 bp long on the +ve strand

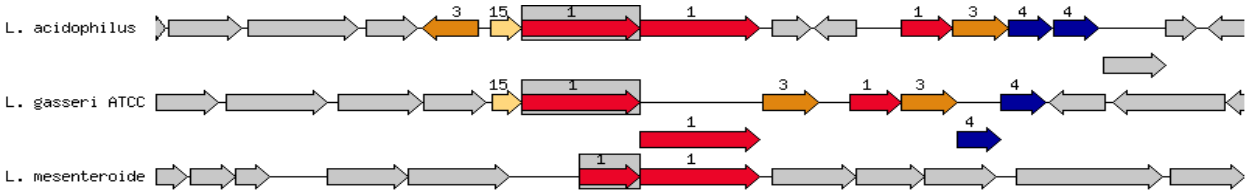

Figure 5.7 Annotation diagram showing the location of Heterodimeric efflux ABC transporter, multidrug resistance => LmrC subunit of LmrCD ( ) found at node 9 and 1770 bp long on the +ve strand

NB. The sequences of nucleotide and amino acids of identified genes putatively involved in antimicrobial resistance are shown in Appendix M.

5.3.2. Putative genes for virulence determinants

In order to determine the pathogenicity of the *L. reuteri* PNW1 and *L. acidophilus* PNW3, the search was carried out using PathogenFinder tool host by the Centre for Genomic Epidemiology. The calculated Matched Pathogenic Families for *L. reuteri* PNW1 was 0, the Matched Not Pathogenic Families was 343 and the Probability of being a human pathogen

was calculated to be 0.217. Thus, *L. reuteri* PNW1 was predicted as a non-human pathogen. On the other hand, the calculated Matched Pathogenic Families for *L. acidophilus* PNW3 was 0, the Matched Not Pathogenic Families was 597 and the probability of being a human pathogen was calculated to be 0.2. The isolate was equally predicted as a non-human pathogen.

VirulenceFinder was also used to determine the presence of virulence factors possibly harboured within the genome of the isolates, and there was no hit for virulence factors using the search tool. The entire gemone assembly was futher searched manually, through the funtional annotation data generated from RAST. Nine notable virulence determinants were manually searched and none of them was found within the genome of the two isolates.

5.3.3. Putative genes for mobile genetic elements within the genome

A number of Mobile Genetic Elements (MGE), such as prophage, tranposease and other insertion sequences were identified within the genomes of the *Lactobacillus reuteri* PNW1 and *Lactobacillus acidophilus* PNW3.

Prophage

Seven predicted prophage regions were identified within the genome of the *L. reuteri* PNW1 (Figures 5.8 and 5.9). Two among the regions were considered to be intact while four were designated as incomplete and the remaining one region was questionable. Only one prophage region was identified within the entire genome of the *L. acidophilus* PNW3 and the coding sequence was designated as incomplete prophage type (Figures 5.10 and 5.11).

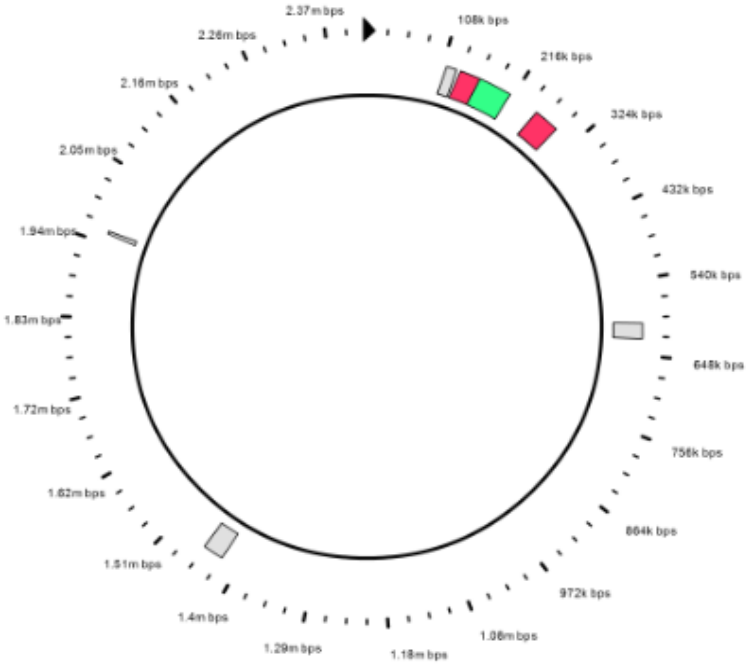

Figure 5.8: Circular genome of the *L. reuteri* PNW1 showing the distribution of types of prophage indentified as intact ( ), incomplete ( ) and questionable ( )

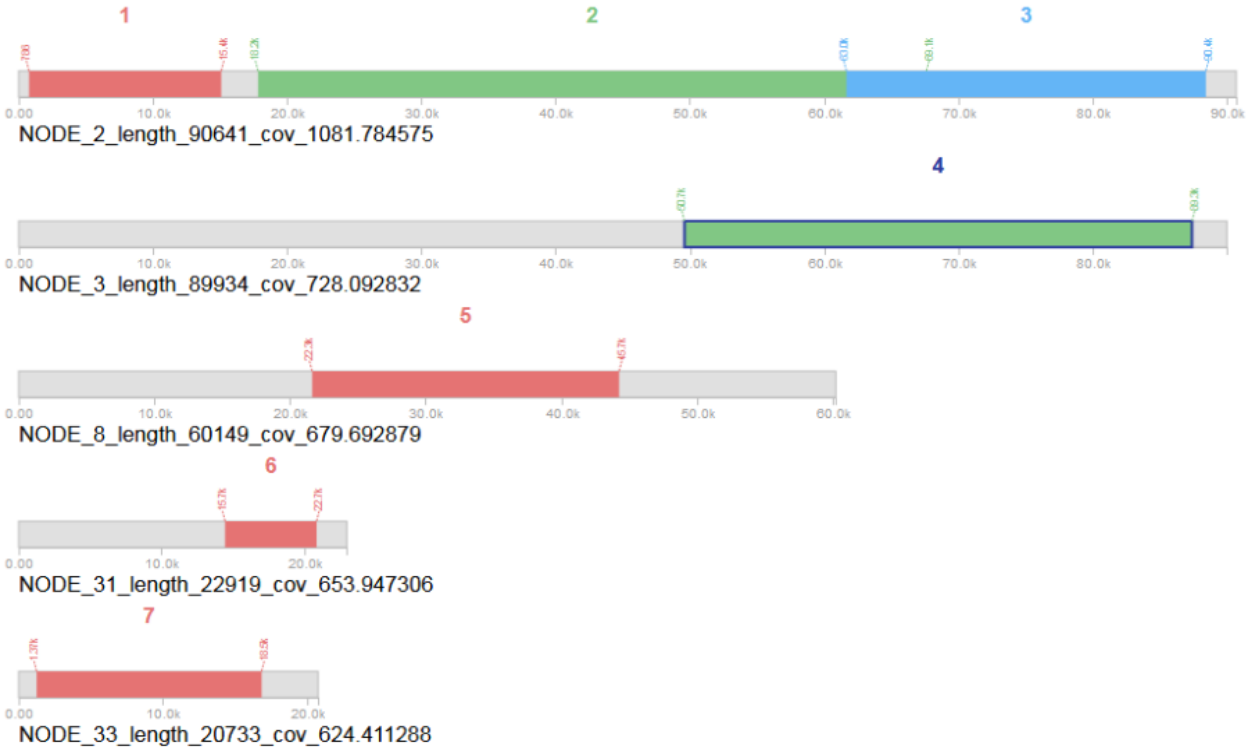

Figure 5.9: Genome mapping of the *L. reuteri* PNW1 showing the region and positions occupied by the different types of prophage identified as intact ( ), incomplete ( ) and questionable ( )

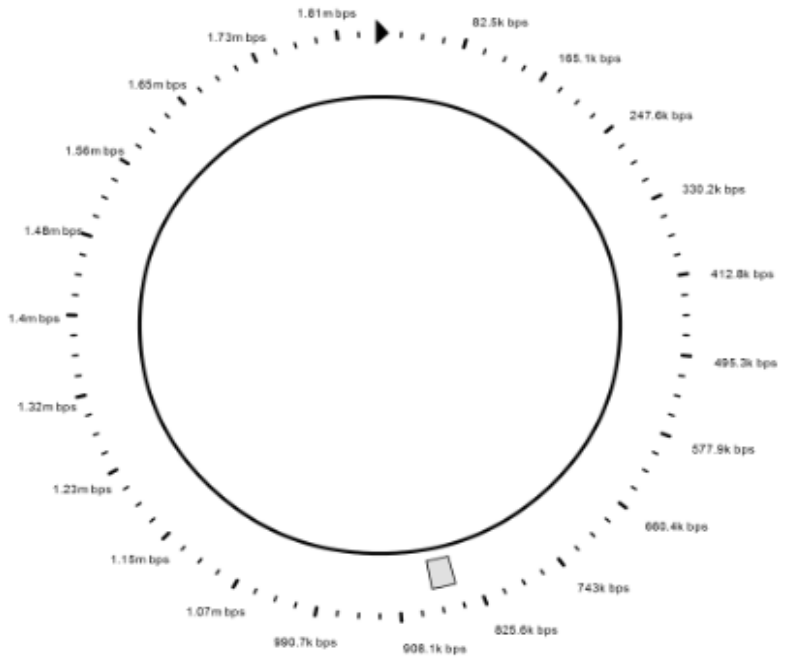

Figure 5.10: Circular genome of the *L. acidophilus* PNW3 showing the distribution of the types of prophage indentified as incomplete ( )

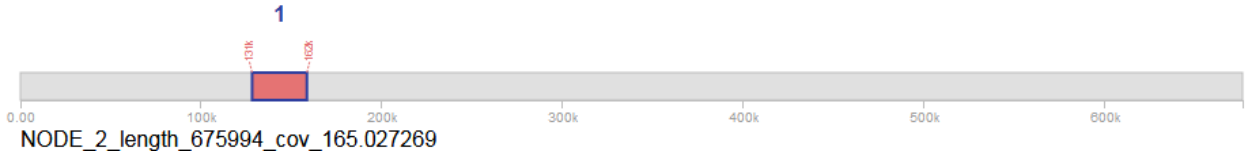

Figure 5.11: Genome mapping of the *L. acidophilus* PNW3 showing the region and positions occupied by the types of prophage identified as incomplete ( )

Tranposase and other insertion sequences

Detection and classification of transposase and other putative insertion sequences and associated proteins were performed using the Optimised Annotation System for Insertion Sequences search tool (OASIS). Within the *L. reuteri* PNW1 genome, the total number of coding sequences predicted for IS in accordance with OASIS was 14. Out of this number, nine different insertion sequences were identified and distributed into seven different families. The IS families identified were as follows: IS1182; IS1595; IS200--IS605; IS21; IS30;IS3; and ISLre2. On the other hand, 21 putative complete ORF were roughly identified as IS and distributed into 12 different IS families in accordance with the ISsaga search tools (Figure 5.12). Out of this number, only 5 were completely annotated and

provided with details of the sources, functions and insertion sites in accordance with the ISfinder (Table 5.1).

Moreover, within the genome of the *L. acidophilus* PNW3, the Optimised Annotation System for Insertion Sequences search tool predicted 5 coding sequences for insertion sequences with 3 different identified IS distributed into 3 different families. The associated IS families were: S200--IS605; IS30; and IS66. On the other hand, 10 putative complete open reading frames (ORF) putative for IS were identified using the ISsaga search tool and distributed into 10 different families (Figure 5.13). Only one among the roughly identified IS were completely annotated by the ISfinder and provided with details of the sources, functions and insertion sites (Table 5.1*****).

Table 5.1: Semi-automatic complete annotation of the IS found within *L. reuteri* PNW1 genome using the ISfinder search tool

IS Name	IS Family	IS Origin	Length (bp)	ORF Function
ISLre2*****	ISLre2	*Lactobacillus reuteri*	1570 bp	Transposase
ISLac1	IS1182	*Lactobacillus acidophilus*	1833 bp	Transposase
ISCco2	IS1595	*Campylobacter coli*	7852 bp	1. Transposase 2. Passenger Gene (Streptothricin Adenyltransferase, Streptothricin acetyltransferase)
ISSag10	IS1595	*Streptococcus agalactiae*	1724 bp	1. Transposase 2. Passenger Gene (O-lincosamide nucleotidyltransferase)
IS2001	ISL3	*Bifidobacterium lactis*	1406 bp	Transposase

Key: ORF = open reading frame, ***** = annotation shared by both the *L. reuteri* PNW1 and *L. acidophilus* PNW3

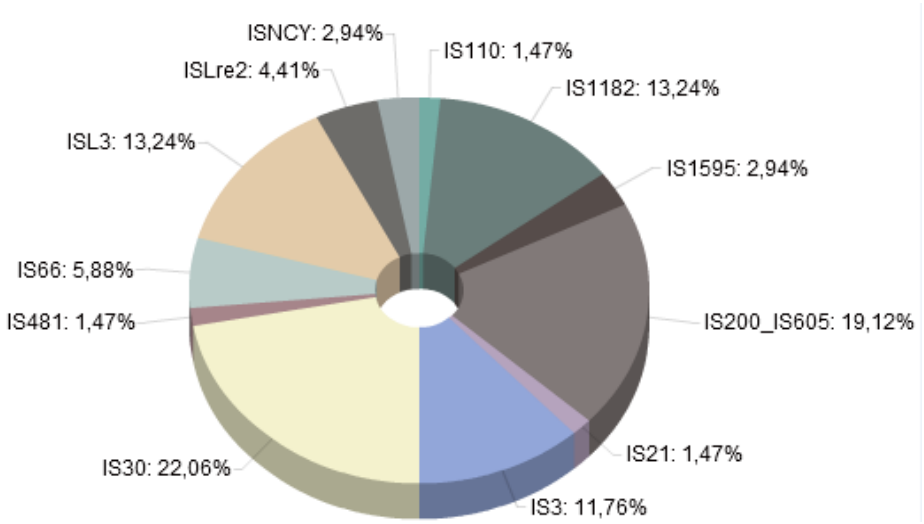

Figure 5.12: Genome mapping of the *L. reuteri* PNW1 showing thedistribution of roughly predicted IS family within the genome using the ISsaga v 2.0

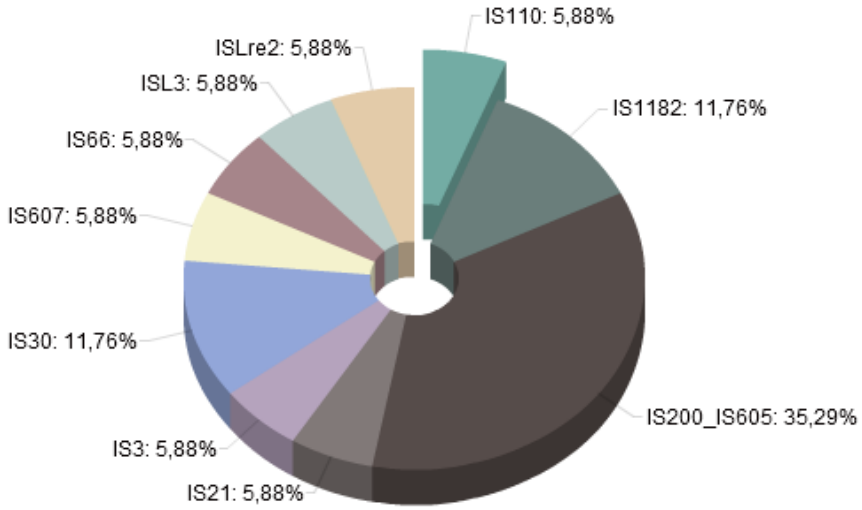

Figure 5.13: Genome mapping of the *L. acidophilus* PNW3 showing the distribution of roughly predicted IS family within the genome usingy the ISsaga v. 2.0

5.3.4. Putative coding sequences for CRISPR−Cas sequences within the genome

Five putative coding sequences for the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) were detected by the CRISPRCasFinder within the *Lactobacillus reuteri* PNW1 genome. Each of the coding sequences contained single associated cas-gene or repeat consensus and single spacer gene (Table 5.2). On the other hand, a search using the *L. acidophilus* PNW3 genome revealed only one CDS putative for CRISPR sequence. The fragment occurred on Contig Identity

NODE_10_length_151230_cov_215_460261_1 and occupied the region between 64572 and 66430. The identified CRISPR sequence contained 30 spacer genes and a total length of 28 bp repeat consensus *(*GGATCACCTCCACATACGTGGAGAAAAT).

Table 5.2: Putative CRISPR-Cas sequences found within *L. reuteri* PNW1 genome

Contigs	start	Stop	Spacers count	Repeat consensus/cas-genes
56	1	133	1	ACTGCAGATAGTGGTCAGCCCAACAACGCTCAAACCAAAC
56	12460	12586	1	GGTCAGCCCAACAACGCTCAAACCAAACCTGGTA
136	1173	1253	1	GCCGAAGACATGAGACAACTTATTT
169	*134*	*224*	1	ATCGATTCCGCGCGTGACATGGGCGCCAA
410	*31*	114	1	GCCCCTTCTGCGTAAAAAGAGAC

5.3.5. Putative genes associated with toxic biochemicals

Functional annotation of the entire genome of *Lactobacillus reuteri* PNW1 revealed that the organism does not possess any protein-encoding gene involved in the production of biogenic amines, which are products of decarboxylation of specific free amino acids, with the exception of CDS putative for arginine deiminase. Seven different coding sequences related to arginine deiminase pathway with functional ornithine degradation were found in the genome of *L. reuteri* PNWI.

The CDS predicted for Arginine deiminase (EC 3.5.3.6) was found on Contig Identity NODE_63_length_11058_cov_736.490989. The fragment, measuring 1233 bp,was found between 4969 and 3737 on the negative strand (Figure 5.14). Another protein-encoding sequence putative for Ornithine carbamoyltransferase (EC 2.1.3.3), invloved in arginine deiminase pathway was foundon Contig Identity NODE_31_length_22919_cov_653.947306. The CDS was 1008 bp long, stretching from

nucleotide position 1553 to 2560 along the positive strand (Figure 5.15). The other five coding sequences were putative for Arginine/Ornithine antiporter ArcD, two of them were found on the same locus, on Contig Identity NODE_63_length_11058_cov_736.490989. One of the CDS, measuring 1422 bp, stetched from 3144 to 1723 nucleotide position along the negative strand (Figure 5.16) while the other, measuring 1398 bp, stretched from 1665 to 268 nucleotide position (Figure 5.17). The remaing three, measuring 1518 (Figure 5.18), 1554 (Figure 5.19) and 1422 bp (Figure 5.20), were found on Contig Identities NODE_25_length_28048_cov_708.713692, NODE_57_length_12548_cov_694.778198 and NODE_16_length_34715_cov_674.706950 respectively.

On the other hand, only one protein-encoding sequence related to the production of biogenic amine was found within the entire genome of the *L. acidophilus* PNW3. The CDS was putative for Ornithine decarboxylase (EC 4.1.1.17) and and located on Contig Identity NODE_2_length_675994_cov_165.027269. The sequenced fragment, measuring 2094 bp, stetched between 368158 and 370251 along the possitive strand (Figure 5.21). None of the manually searched enteroxins and lipopeptides was detected within the genome of both organisms.

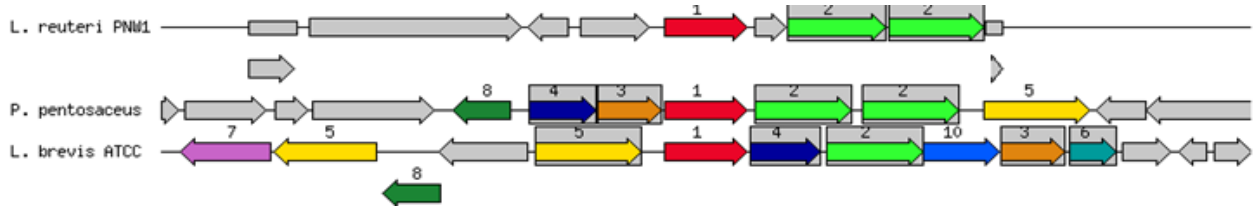

Figure 5.14: Annotation diagram showing the location of Arginine deiminase (EC 3.5.3.6) ( ) found at node 63 and 1233 bp long on the –ve strand

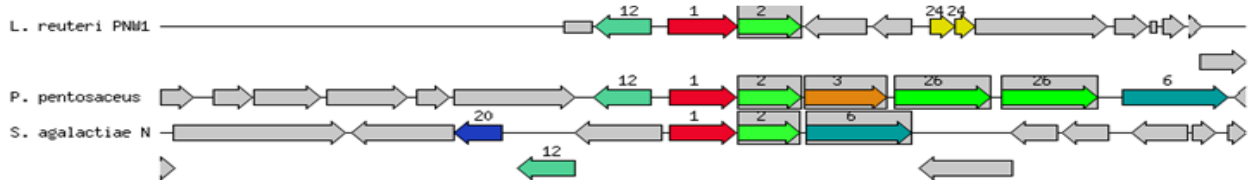

Figure 5.15: Annotation diagram showing the location of Ornithine carbamoyltransferase (EC 2.1.3.3) ( ) found at node 31 and 1008 bp long on the +ve strand

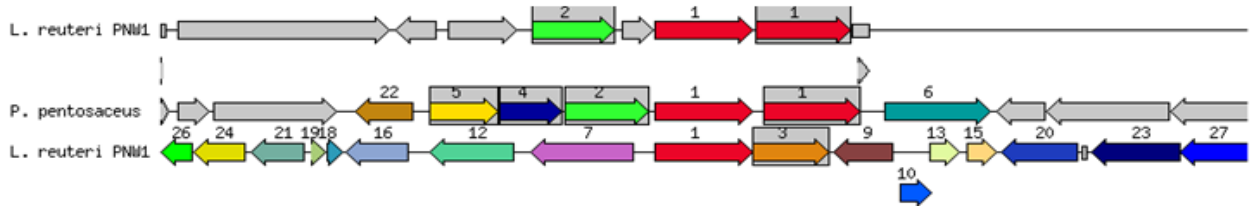

Figure 5.16: Annotation diagram showing the location of Arginine/ornithine antiporter ArcD ( ) found at node 63 and 1422 bp long on the –ve strand

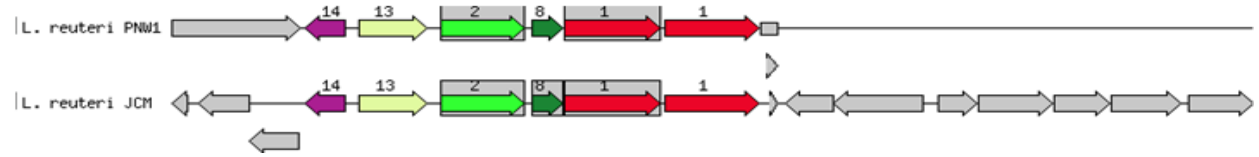

Figure 5.17: Annotation diagram showing the location of Arginine/ornithine antiporter ArcD ( ) found at node 63 and 1398 bp long on the –ve strand

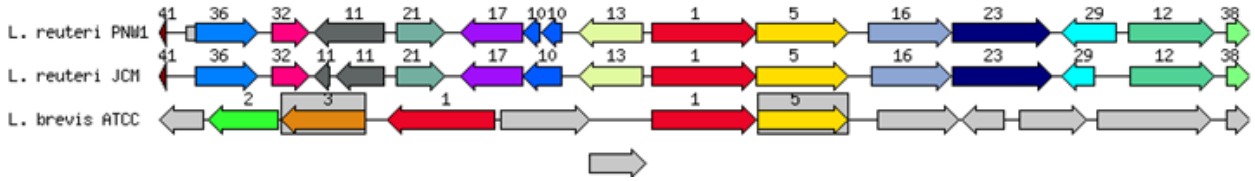

Figure 5.18: Annotation diagram showing the location of Arginine/ornithine antiporter ArcD ( ) found at node 25 and 1518 bp long on the –ve strand

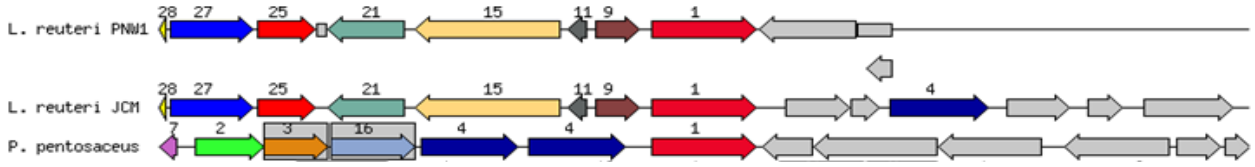

Figure 5.19: Annotation diagram showing the location of Arginine/ornithine antiporter ArcD ( ) found at node 57 and 1554 bp long on the +ve strand

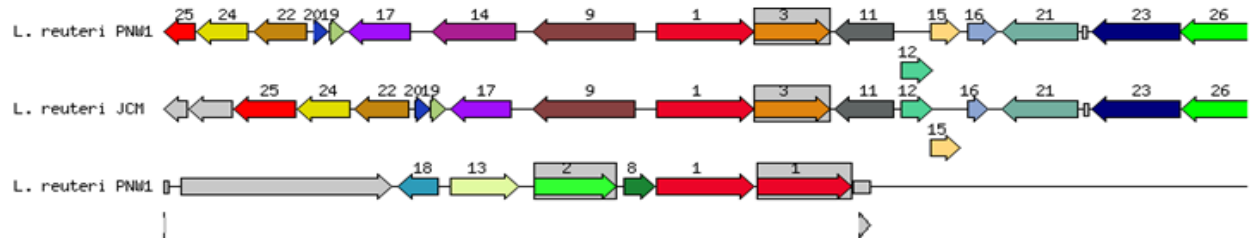

Figure 5.20: Annotation diagram showing the location of Arginine/ornithine antiporter ArcD ( ) found at node 16 and 1422 bp long on the +ve strand

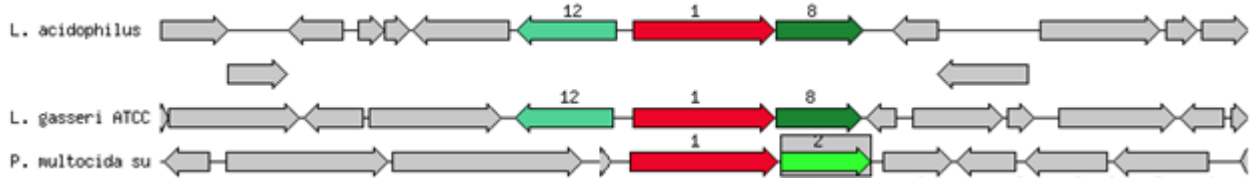

Figure 5.21: Annotation diagram showing the location of Ornithine decarboxylase (EC 4.1.1.17) ( ) found at node 2 and 2094 bp long on the +ve strand

NB. The sequences of nucleotides and amino acids of putative genes associated with toxic biochemicals are shown in Appendix N.

5.3.6. HPLC analysis of the biogenic amines

Analysis of the HPLC carried out on the extracted biogenic amines produced by both isolates revealed the presence of putrescine as the only biogenic amine produced. The putrescine produced by *L. reuteri* PNW1 was eluted within a retention time of 2. 334 min at a flow rate of 0.5 ml/min while the putrescine produced by the *L. acidophilus* PNW3 was eluted within 2.337 min retention time. When compared, the putrescine used as standard reference eluted at a retention time of 2.436 min under the same flow rate of 0.5 ml/min. The 1, 7- diaminoheptane used as internal standard for the biogenic amine, was identified with a pick common to both samples and eluted at the same retention time of 11.366 min.

5.3.7. Multiple proteins based phylogenetic and comparative genome analyses of *L. reuteri* PNW1 and *L. acidophilus* PNW3

All proteins shared by the genomes were compared in order to trace the evolutionary trend and relationship between the two organisms (Figure 5.22). The isolate *L. acidophilus* PNW3 clustered closely with two other strains of the same species and clustered a bit distantly with the *L. acidophilus* NCTC 13720. The reason for the fairly distant relationship could be that *L. acidophilus* PNW3 and two other strains could have undergone mutation over a period of time. *L. acidophilus* NCTC 13720 was isolated from a human source (female vagina) in London, United Kingdom in 1999 while the two other strains, closely clustered, were isolated lately between 2011 and 2016 from swine intestine and human commercial dietary supplements respectively. All the three strains of the same species, with the *L. reuteri* PNW1, clustered closely. Comparison of the entire genomic contents of *L. reuteri* PNW1 and *L. acidophilus* PNW3 in the Artemis Comparison Tool (ACT) revealed that both organisms did not share a greater part of the entire genomic contents in common (Figures 5.23 and 5.24).

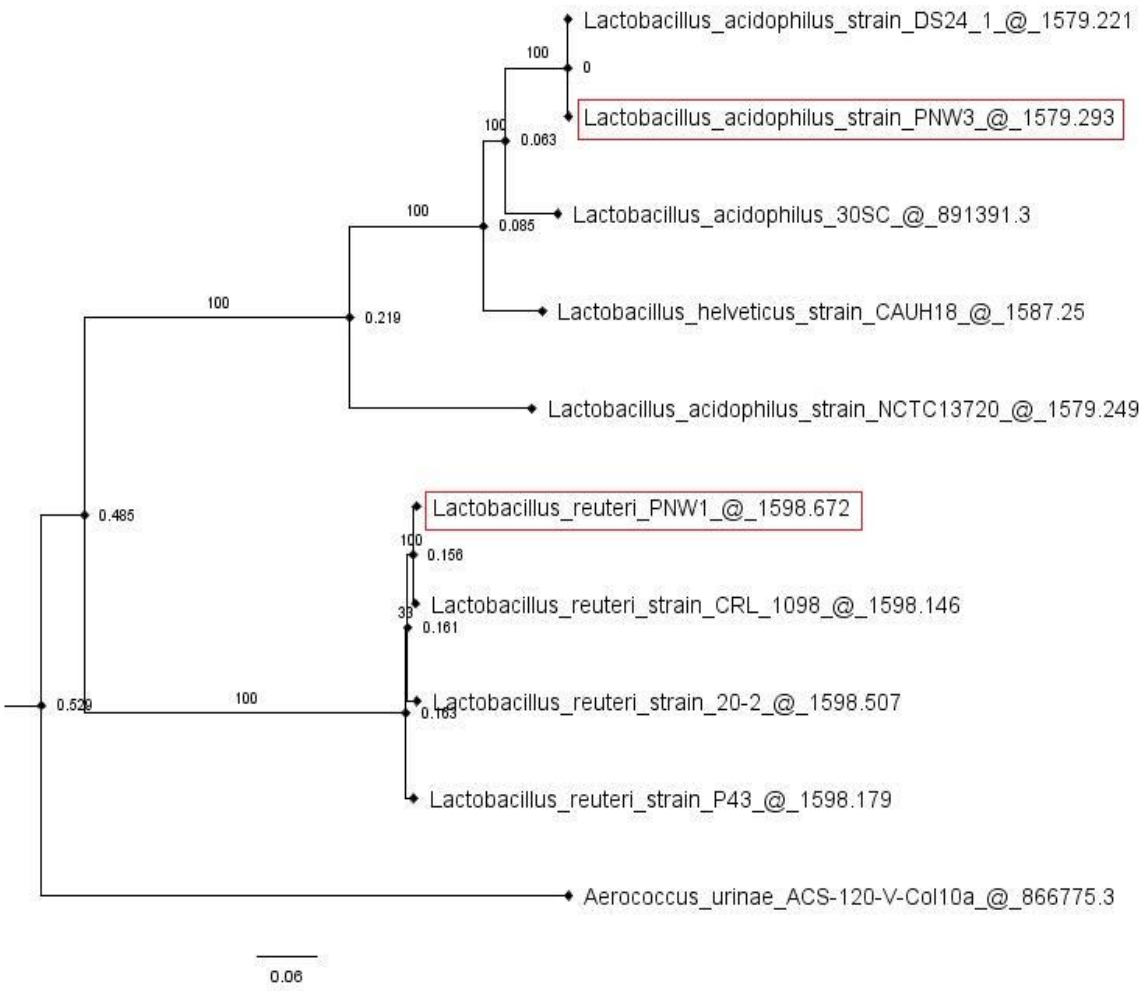

Figure 5.22: Phylogenetic analysis of *L. reuteri* PNW1 and *L. acidophilus* PNW3. The tree was developed using PATRIC's tree building pipeline v. 3.5.43 using the whole genome sequence approach by comparing all shared proteins among the isolates.

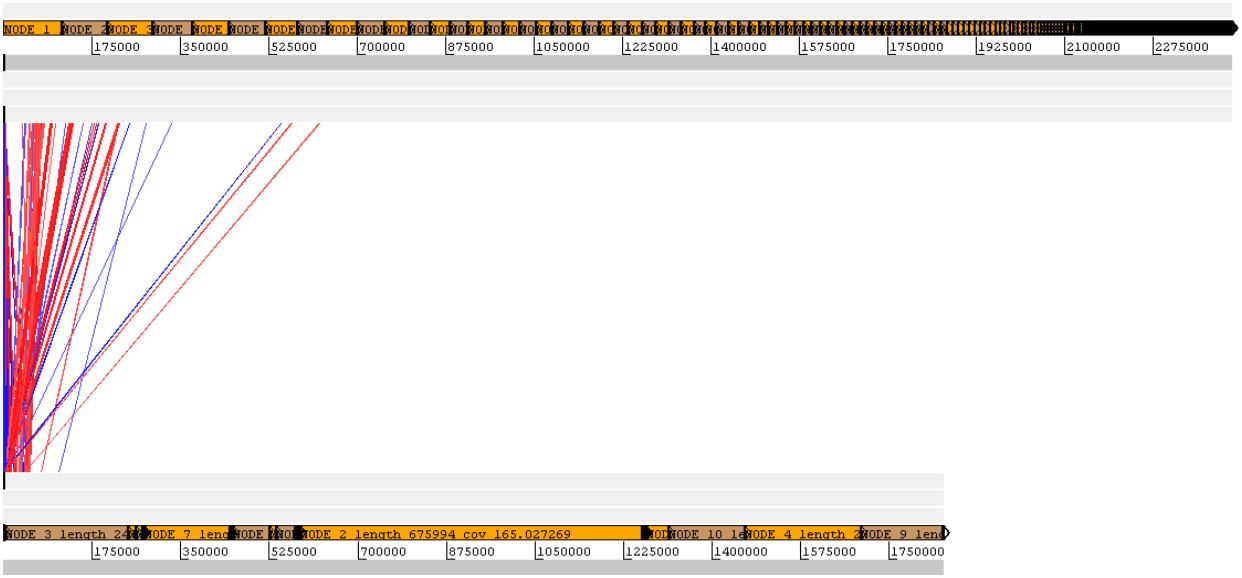

Figure 5.23: Virtual similarity within the entire genomes of the *L. reuteri* PNW1 (upper layer) and *L. acidophilus* PNW3 (lower layer). The red connecting blocks indicate regions of high level similarities between the two genomes while the blue blocks indicate inversion sequences. The mapping was generated using ACT v3.

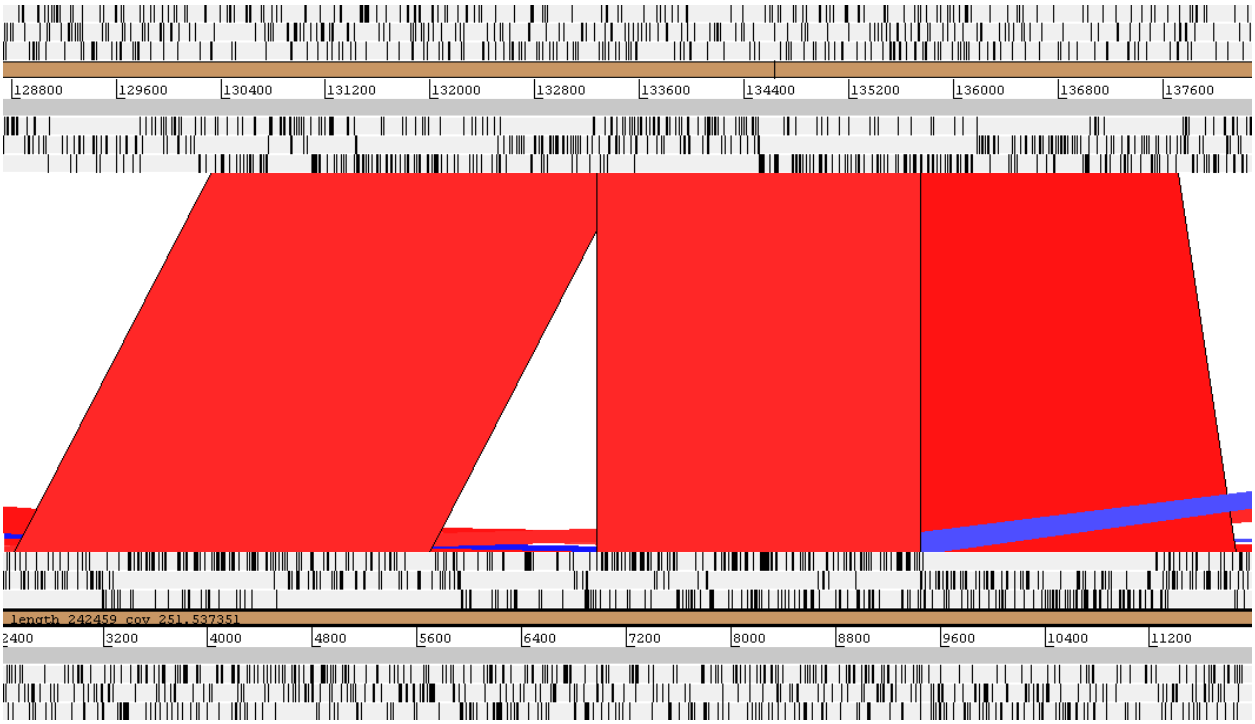

Figure 5.24: A section indicating regions of high degree of similarity between genomes of the *L. reuteri* PNW1 (upper layer) and *L. acidophilus* PNW3 (lower layer). The red connecting blocks indicate regions of similar coding sequences between the two genomes. The mapping was generated using ACT v3.

5.4. Discussion and Conclusion

Quick searches through different databases and manual search through functional annotation revealed that both *L. reuteri* PNW1 and *L. acidophilus* PNW3 harbour resistant genes against Lincosamide and Tetracycline. Functional annotation also revealed the presence of heterodimeric efflux ABC transporter, multidrug resistance in the genome of *L. acidophilus* PNW3. Though several heterodimeric ABC transporters have been reportedly involved in multidrug efflux in Gram-positive organisms, however, it is worthy to note that most of the annotated multidrug efflux transporters are not capable of transporting drugs but rather get involved in other physiological functions within the cell (Hürlimann *et al*., 2016). Moreover, heterodimeric efflux ABC transporter is an intrinsic form of resistance, which has little or no chances of being transferred to other bacteria (Das *et al*., 2019).

The only prophage region found within the *L. acidophilus* PNW3 genome was designated as incomplete prophage type, an indication of its non-functionality. None of the

transposase identified and other insertion sequences flanked the resistance genes, further limiting the transferability of these antimicrobial resistance genes. On the other hand, the passenger gene, which was identified among the insertion sequences in the *L. reuteri* PNW1 genome, was found in association with O-lincosamide nucleotidyltransferase (Table 5.1). This could suggest the possibility of increased chances of desemination, however, the co-existing Tetracycline-resistant ribosomal protection protein (*tetW*) was not flanked by any of the annotated insertion sequences.

Furthermore, the genomes of both isolates harboured the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), with each of the coding sequences associated with Cas-gene and spacer. The presence of CRISPR region within a genome limits the spread of antimicrobial resistant genes through obstruction of multiple pathways of horizontal gene transfer (Maraffini and Sontheimer, 2008). CRISPR regions in the genome equip the bacteria with sequence specific defence-line against plasmids, insertion sequences and phages with about 45% of bacterial strains possessing effective CRISPR region within their genomes (Grissa *et al*., 2007; Palmer and Gilmore, 2010). CRISPR locus, with associated Cas-genes, provides the host strain with the potential to defend itself against any incoming extra-chromosomal DNA molecules (Palmer and Gilmore, 2010). This is an indication of stability of the genome and a very low possibility of such strain to acquire antimicrobial resistant genes since resistance genes are mostly introduced by mobile genetic elements such as plasmids and transposon, among others.

Among all the protein-encoding sequences related to the production of toxic biochemical, only CDS predicted for arginine deiminase with functional ornithine degradation was found within the genome of the *L. reuteri* PNW1 and ornithine decarboxylase (EC 4.1.1.17), which is also the only encoding sequence with the potential for producing biogenic amine compound in the *L. acidophilus* PNW3. The protein arginine deiminase is a post translational modification enzyme that catalyses hydrolysis of peptidyl-arginine to

uncharged non-standard peptidyl-citrulline on relevant proteins such as histones and fibrinogen. This is referred to as citrullination or deamination, that is, post-translational modification of histones, which is the essential building block of chromatin (Mohanan *et al*., 2012; Bicker and Thompson, 2014). Upregulation and overexpression of the arginine deiminase activity, most importantly, class IV, has been observed in several disease conditions such as rheumatoid arthritis, Alzheimer's disease, multiple sclerosis and cancer (Jones*et al*., 2009). It is equally important to note that protein arginine deiminase class IV is the only isozyme that has been confirmed to be involved in the deimination of histone (Bicker and Thompson, 2014). Protein arginine deiminase are now being identified with potential involvement in tumor progression (Mohanan *et al*., 2012).

Decarboxylation of amino acid ornithine by the enzyme ornithine decarboxylase, leads to the formation of putrescine, which is a biochemical that has been confirmed to down-regulate activities of macrophages in response to injury due to microbial infection (Hardbower *et al*., 2017). Production of putrescine by both isolates was practically confirmed through HPLC analysis. Ornithine decarboxylase also limits the rate of polyamine biosynthesis thus, increasing the potential for oncogenesis (Shukla-Dave *et al*., 2016). Increased activity of the enzyme has been reportedly observed in the rapid proliferation of normal and neoplastic cellls. Although the activity of Ornithine decarboxylase is under strict regulation at transcriptional and posttranslational stages, due to its impact on cellular processes (Kaprio *et al*., 2019), Ornithine degradation is regulated by a protein known as antizyme in response to the density of polyamine and is also one of the targets of the Myc/Max transcription factor (Pegg, 2006).

The all shared-proteins concept phylogeny revealed a level of distance evolutionary relationship between the *L. reuteri* PNW1 and *L. acidophilus* PNW3. This is equally evidenced from the overview of the virtual comparative genomic analysed in Artemis

Comparison Tool (ACT), where only a small fragment of the entire genome in both isolates are shown to have regions of high level similarities in protein-encoding sequences.

In general, with both isolates predicted as non-human pathogens, coupled with the presence of the CRISPR-Cas regions, which serves to protect and ensure stability of the genomes, together with some other indentified factors, the *L. reuteri* PNW1 and *L. acidophilus* PNW3 stand a chance of making good and safe candidates for future development of probiotic. However, further *in vivo* investigations in the animal model and human efficacy trials are still required in order to effectively assess the level of expression and, possibly, suppression mechanism for the single identified gene putative for the production of biogemic amine in each of the isolates.

References

Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A.-L. V., Cheng, A. A., Liu, S., Sally Y. Min, Miroshnichenko, A., Tran, H.-K., Werfalli, R. E., Nasir, J. A., Oloni, M., Speicher, D. J., Florescu, A., Singh, B., Faltyn, M., Hernandez-Koutoucheva, A., Sharma, A. N., Bordeleau, E., Pawlowski, A. C., Zubyk, H. L., Dooley, D., Griffiths, E., Maguire, F., Winsor, G. L., Beiko, R. G., Brinkman, F. S. L., Hsiao, W. W. L., Domselaar, G. V., Mcarthur, A. G. 2020. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res*, 1–9.

Arndt, D., Grant, J., Marcu, A., Sajed, T., Pon, A., Liang, Y., Wishart, D. S. 2016. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res,* 44, W16–W21.

Banwo, K., Sanni, A., Tan, H. 2013. Technological properties and probiotic potential of *Enterococcus faecum* strains isolated from cow milk. *J Appl Microbiol,* 114, 229–241.

Borah, D., Gogoi, O., Adhikari, C., Kakoti, B. B. 2016. Isolation and characterization of the new indigenous *Staphylococcus* sp. DBOCP06 as a probiotic bacterium from traditionally fermented fish and meat products of Assam state. *Egyptian J Basic Appl Sci,* 3, 232–240.

Boratyn, G., Camacho, C., Cooper, P. 2013. BLAST: a more efficient report with usability improvements. *Nucleic Acids Res,* 41, W29-W33.

Bunesova, V., Musilova, S., Geigerova, M., Pechar, R., Rada, V. 2015. Comparison of mupirocin-based media for selective enumeration of bifidobacteria in probiotic supplements. . *J Microbiol Meth,* 109, 106–109.

Byakika, S., Mukisa, I. M., Byaruhanga, Y. B., Muyanja, C. 2019. A review of criteria and methods for evaluating the probiotic potential of microorganisms. *Food Rev Inte,* 35, 427–466.

Carver, T. J., Rutherford, K. M., Berriman, M., Rajandream, M.-A., Barrell, B. G., Parkhill, J. 2005. ACT: the Artemis comparison tool. 21, 3422–3423.

Cosentino, S., Voldby, L. M., Møller, A. F., Lund, O. 2013. PathogenFinder - Distinguishing friend from foe using bacterial whole genome sequence data. *PLoS ONE,* 8, e77302.

Das, Devika J., Shankar, Aparna, Johnson, John B., Thomas, Sabu. 2019. Critical insights into antibiotic resistance transferability in probiotic *Lactobacillus*. *Nutr,* 69, 110567.

Davis, J., Gerdes, S., Olsen, G., Olson, R., Pusch, G., Shukla, M., Vonstein, V., Wattam, A., Yoo, H. 2016. PATtyFams: Protein families for the microbial genomes in the PATRIC database. *Front Microbiol* 7, 118.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res,* 32, 1792–1797.

Elsanhoty, R. M., Ramadan, M. F. 2016. Genetic screening of biogenic amines production capacity from some lactic acid bacteria strains. *Food Control,* 68**,** 220-228.

Grissa, I., Vergnaud, G., Pourcel, C. 2007. CRISPRFinder: A web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Res,* 35, W52–W57.

Grissa, I., Vergnaud, G., Pourcel, C. 2007. The CRISPRdb database and tools to display CRISPRs and to generate dictionaries of spacers and repeats. *BMC Bioinform*, 8,172.

Haft, D., Dicuccio, M., Badretdin, A., Brover, V., Chetvernin, V., O'neill, K., Li, W., Chitsaz, F., Derbyshire, M., Gonzales, N., Gwadz, M., Lu, F., Marchler, G., Song, J., Thanki, N., Yamashita, R., Zheng, C., Thibaud-Nissen, F., Geer, L., Marchler-Bauer, A.,

Pruitt, K. 2018. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Res,* 4, D851–D860.

Hardbower, Dana M., Asim, Mohammad, Luis, Paula B., Singh, Kshipra, Barry, Daniel P., Yang, Chunying, Steeves, Meredith A., Cleveland, John L., Schneider, Claus, Piazuelo, M. Blanca, Gobert, Alain P., Wilson, Keith T. 2017. Ornithine decarboxylase regulates M1 macrophage activation and mucosal inflammation via histone modifications. *PNAS*, E751–E760.

Hürlimann, L.M., Corradi, V., Hohl, M., Bloemberg, G.V., Tieleman, D.P., Seeger, M.A. 2016. The heterodimeric ABC transporter EfrCD mediates multidrug efflux in *Enterococcus faecalis*. *Antimicrob Agents Chemotherapy*, 60, 5400 –5411.

Joensen, K., Scheutz, F., Lund, O., Hasman, H., Kaas, R., Nielsen, E., Aarestrup, F. 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. *J Clin Micobiol,* 52, 1501–1510.

Jones J.E., Causey CP, Knuckley B, Slack-Noyes JL, Thompson PR. 2009. Protein arginine deiminase 4 (PAD4): Current understanding and future therapeutic potential. *Curr Opin Drug Disc Devel*, 12, 616–627.

Kaprio, T., Rasila, T., Hagstro, ¨m J., Mustonen, H., Lankila, P., Haglund, C., Andersson, L. C. 2019. Ornithine decarboxylase antizyme inhibitor 2 (AZIN2) is a signature of secretory phenotype and independent predictor of adverse prognosis in colorectal cancer. *PLoS ONE* 14(2), e0211564.

Maraffini, L.A., Sontheimer, E.J. 2008. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. *Sci*, 322, 1843–1845.

Mohanan, S., Cherrington, B. D., Horibata, S., McElwee, J. L., Thompson, P. R., Coonrod, S. A. 2012. Potential role of peptidylarginine deiminase enzymes and protein citrullination in cancer pathogenesis, *Biochem Res Inte*, ID 895343, 11 pages.

Overbeek, R., Olson, R., Pusch, G., Olsen, G., Davis, J., Disz, T., Edwards, R., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A., Xia, F., Stevens, R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res,* 42, 206–214.

Palmer, K. L., M. S. Gilmore. 2010. Multidrug-resistant enterococci lack CRISPR-cas. *mBio* 1(4): e00227–10.

Pegg, A. E. 2006. Regulation of ornithine decarboxylase. *J Biol Chem,* 281(21), 14529–14532.

Potter, S. C., Luciani, A. E., Eddy, S. R., Park, Y., Lopez, R., Finn, R. D. 2018. HMMER web server: 2018 update. *Nucleic Acids Res,* 46, W200–W204.

Pradhan, D., Mallappa, R. H., Sunita, G. 2020. Comprehensive approaches for assessing the safety of probiotic bacteria. *Food Control*, 108, 106872.

Pradhan, D., Singh, R., Tyagi, A., Rashmi, H. M., Batish, V. K., Grover, S. 2019. Assessing safety of *Lactobacillus plantarum* MTCC 5690 and *Lactobacillus fermentum* MTCC 5689 using *in vitro* approaches and an *in vivo* murine model. *Regul Toxicol Pharmacol*, 101, 1-11.

Price, M., Dehal, P., Arkin, A. 2010. FastTree 2â€“approximately maximum-likelihood trees for large alignments. *PLoS One*, 5, e9490.

Rambaut, A. FigTree v1. 3.1: Tree figure drawing tool. 2019.

Robinson, D. G., Lee, M.-C., Marx, C. J. 2012. OASIS: an automated program for global investigation of bacterial and archaeal insertion sequences. *Nucleic Acids Res,* 40**,** e174.

Saarela, M. H. 2019. Safety aspects of next generation probiotics. *Current Opinion in Food Sci,* 30, 8–13.

Salvetti, E., Orrù, L., Capozzi, V., Martina, A., Lamontanara, A., Keller, D., Cash, H., Felis, G. E., Cattivelli, L., Torriani, S., Spano, G. 2016. Integrate genome-based

assessment of safety for probiotic strains: *Bacillus coagulans* GBI-30, 6086 as a case study. *Appl Microbiol Biotechnol*, 100, 4595–4605.

Shukla-Dave, A., Castillo-Martin, M., Chen, M., Lobo, J., Gladoun, N., Collazo-Lorduy, A., Khan, F. M., Ponomarev, V., Yi, Z., Zhang, W., Pandolfi, P. P., Hricak, H., Cordon-Cardo, C. 2016. Ornithine decarboxylase is sufficient for prostate tumorigenesis via androgen receptor signaling. *Am J Pathol*, 186(12), 3131−3145.

Siguier, P., Perochon, J., Lestrade, L., Mahillon, J., Chandler, M. 2006. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res,* 34, D32–D36.

Singracha, P., Niamsiri, N., Visessanguan, W., Lertsiri, S., Assavanig, A. 2017. Application of lactic acid bacteria and yeasts as starter cultures for reduced-salt soy sauce (moromi) fermentation. *LWT - Food Sci Technol,* 78**,** 181-188.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinform,* 30**,** 1312–1313.

Talavera, G., Castresana, J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst Biol,* 56, 564–577.

Tatusova, T., Dicuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E., Zaslavsky, L., Lomsadze, A., Pruitt, K., Borodovsky, M., Ostell, J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res,* 44**,** 6614–6624.

Vankerckhoven, V., Huys, G., Vancanneyt, M., Vael, C., Klare, I., Romond, M.-B., Entenza, J. M., Moreillon, P., Wind, R. D., Knol, J., Wiertz, E., Pot, B., Vaughan, E. E., Kahlmeter, G., Goossens, H. 2008. Biosafety assessment of probiotics used for human consumption: recommendations from the EU-PROSAFE project. *Trends Food Sci Technol,* 19**,** 102–114.

Wattam, A. R., Davis, J. J., Assaf, R., Boisvert, S. E., Brettin, T., Bun, C., Conrad, N., Dietrich, E. M., Disz, T., Jgabbard, O. L., Gerdes, S., Henry, C. S., Kenyon, R. W., Machi, D., Mao, C., Nordberg, E. K., Olsen, G. J., Murphy-Olson, D. E., Olson, R., Overbeek, R., Parrello, B., Pusch, G. D., Shukla, M., Vonstein, V., Warren, A., Xia, F., Yoo, H., Stevens, R. L. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Res,* 45**,** D535–D542.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F., Larsen, M. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemotherapy,* 67, 2640–2644.

Zhou, Y., Liang, Y., Lynch, K., Dennis, J. J., Wishart, D. S. 2011. PHAST: A fast phage search tool. *Nucleic Acids Res*, 39, W347–W352.

CHAPTER 6 − Purification of bacteriocins produced by *L. reuteri* PNW1 and *L. acidophilus* PNW3 and evaluation of their effectiveness against *E. coli* O177

Abstract

Bacteriocins are ribosomaly secreted antimicrobial peptides produced by bacteria; most of them are active against organisms with closely related phylogeny while some are of broad spectrum. This study was an evaluation of antimicrobial capability of bioactive peptides produced by *L. reuteri* PNW1 and *L. acidophilus* PNW3, both crude and partially purified. Agar well diffusion method was used to assess the susceptibility of the test pathogens (*E. coli* O177) towards bioactive peptides. The maximum zone of inhibition exhibited by the bacteriocin produced by *L. reuteri* PNW1 is 20.0±1.00 mm (crude) and 23.3±1.15 mm (at 0.25 mg/ml) after being partially purified. On the other hand, the maximum zone of inhibition exhibited by the *L. acidophilus* PNW3 was 21.7±0.58 mm (crude) and 24.3±1.15 mm after being partially purified and tested at a concentration of 0.25 mg/ml. The partially purified peptide pellets, which were re-constituted in PBS and applied to the RP-HPLC C18 columns, were ran for 40 min at 0.2 ml/min flow rate. The active fraction appeared at 1 min in 34% acetonitrile.

6.1. Introduction

The capability of a probiotic candidate to antagonize the growth of neighbouring microorganisms may further appreciate, to an extent, the complete competitive repression of entire gastrointestinal microbes and pathogens (Nagpal *et al*., 2012). In order for a microbial candidate to be classified as probiotic, it has to possess a number of criteria. One of which is the potential to produce ribosomally synthesized compounds with antimicrobial properties (Kazuñ, 2018). This amounts to why majority of professional nutritionists and gastroenterologists have considered probiotics as effective and safe gastrointestinal symptoms management therapy (Valdovinos-García*et al*., 2018).

Bacteriocins have been widely investigated globally in relation to their desirable impact on the health of human beings and animals (Svetoch and Stern, 2010). They are small heterogeneous bioactive peptides synthesized by probiotic bacteria against neighbouring competitive pathogenic organisms (Cotter *et al*., 2005). Most bacteriocins are highly specific in their clinical effectiveness. They are commonly operate distinct mechanisms of action away from those exhibiting by the conventional synthetic antimicrobials; and are susceptible to genetic manipulation aimed at a desired traits due to their peptide nature (Cotter *et al*., 2013). The specificity of bacteriocins allows room for controlled target against pathogens with little or no undesirable effects on the gut microbial ecosystem. Although there are other strains that secrete broad spectrum bacteriocins, such can be applied in the situation of infections with difficult-to-identify causative agents (Rea *et al*., 2010; Boakes *et al*., 2012).

Among the numerous naturally produced bioactive compounds, lactic acid bacteria (LAB) synthesised bacteriocins are given higher priority among scientists because of the generally recognised as safe status of the LAB, and their significant presence in the gut microbiota, both in human beings and animals. Some of the bacteriocins, such as nisin, are currently in use with public approval as food additive in about 50 countries to improve the quality and health functionality of food (Hu *et al*., 2017; Ekblad and Kristiansen, 2019; de Castilho *et al*., 2020). Many of the bacteriocins also offer a natural alternative to synthetic additives as well as improved organoleptic characteristics of food (Kondrotiene *et al*., 2018).

Bacteriocins have been broadly classified into two major groups as follows: the lanthionine-containing group (e.g. nicin, bovicin HJ50 and mersacidin); and non-lanthionine-containing group (e.g.pediocin PA-1carnobacteriocins and lactococcins G). The non-lanthionine-containing group is further subdivided into pediocin-like bacteriocins,

two-peptide bacteriocins, cyclic bacteriocins and non-pediocin-like one-peptide bacteriocins (Oppegård *et al*. 2007; Nissen-Meyer *et al*., 2009; Garsa *et al*., 2019).

Moreover, besides the bactericidal effect against pathogens, a number of bacteriocins have been established to have capability to significantly reduce the amount of methane (CH_4) produced by ruminant animals, during nutrient digestion, through depopulation of their intestinal methanogens (Gill *et al*., 2010; Kumar *et al*., 2014). Therefore, mitigating the effect of methane, as a green house gas. This account for 18% of the total world emission on global warming and, at the same time, prevent loss of metabolic energy from the rumen (Garsa *et al*., 2019).

The aim of this study, therefore, was to purify and evalute the bioactive efficacy of bacteriocins produced by *Lactobacillus reuteri* PNW1 and *Lactobacillus acidophilus* PNW3 against pathogenic *Escherichia coli* O177.

6.2. Materials and methods

6.2.1. Cultivation of bacteriocin produced by *L. reuteri* PNW1 and *L. acidophilus* PNW3

The probiotic bacterial isolate was sub-cultured into De Man Rogosa Sharpe (MRS) broth under complete anaerobic condition and incubated at 37 ^{0}C for 24 hours in an anaerobic jar supplemented with AnaeroGen system (Thermofisher, UK). Thereafter, the culture was centrifuged at 10,000 rpm for 10 minutes and the pH of the supernatant adjusted to 6.5 using a pH meter. After which the supernatant was filtered through 0.22 µm syringe filter (Millex syringe filters, Sigma Aldrich) and the filtrate was kept in the refrigerator for further analysis (Zommiti *et al.*, 2016).

6.2.2. Determination of the antimicrobial potential of cultivated bacteriocin produced by *L. reuteri* PNW1 and *L. acidophilus* PNW3

Agar well diffusion method was used to determine the antimicrobial potential of bacteriocin produced by probiotic bacterial isolates against pathogenic *Escherichia coli* O177 (Goh and Philip, 2015). Two different strains of pathogenic *E. coli* O177 were collected from culture collection of the Molecular Microbiology Research Laboratory, North-West University Mafikeng Campus, South Africa. An 18 – 24 hour old nutrient broth culture of each of the test isolates was standardised following 0.5 McFarland standard. Then 100 µl of the standard inoculums was inoculated into molten (50 ^{0}C) sterile freshly prepared Muller Hinton agar, gently mixed and then poured into a sterile petri dish. The molten agar medium was allowed to set before wells were bored into it, using 6 mm cock borer. The wells were carefully filled with the bacteriocin extract and not allowed to spill. The plates were left on the laboratory bench for 2 hours to allow proper diffusion of the bacteriocin extract into the agar metrix and incubated at 37 ^{0}C for 24 hours. After this process, the clear zone of inhibition was measured. The experiment was carried out in three replicates.

6.2.3. Purification and HPLC analysis of bacteriocins produced by the *L. reuteri* PNW1 and *L. acidophilus* PNW3

Purification of crude bacteriocin was done as previously described (Wannun *et al.*, 2016; Zommiti *et al.*, 2016). One litre of crude bacteriocin was partially purified by precipitation with 80% saturated ammonium sulphate and left overnight at 4 ^{0}C with regular stirring. The precipitates were collected and then re-suspended in 0.1 M phosphate buffer solution (pH 7). This was, thereafter, extracted in a mixture of chloroform and methanol (2:1, v/v).

The bacteriocin was further purified with high performance liquid chromatography (PerkinElmer Altus, A-10 PDA Detector) using a C18 RP-HPLC column (Analytical C18 Column 100 x 4.6 mm, 5 µm particle size). The sample was applied to the C18 RP-HPLC column and eluted with 34% acetonitrile to produce a pure peptide. The fraction with a

retention time of 1 minute and absorption at 240 nm, was identified as the purified bacteriocin.

6.3. Results

The bioactive peptides (bacteriocins) extracted from the *Lactobacillus reuteri* PNW1 and *Lactobacillus acidophilus* PNW3 were tested against 2 identified strains of pathogenic *E. coli* O177. Both test strains were susceptible to bacteriocins produced by the two probiotic organisms. The zone of inhibition exhibited by the crude bacteriocin extracts ranged between 18.7±0.58 and 21.7±0.58 mm. After the crude bacteriocins were partially purified and tested at a concentration of 0.25 mg/ml, the zones of inhibition ranged between 22.0±0.00 and 24.3±1.15 mm (Table 6.1).

The partially purified bioactive peptide (pellet) was re-constituted in phosphate buffer saline (PBS) and applied to the RP-HPLC C18 columns. The flow-rate of the elution was 0.2 ml/min with a linear gradient of 0 to 60% run over 40 minutes. The active fraction appeared at 1 minute in 34% acetonitrile (Figures 6.1 and 6.2).

Table 6.1: Susceptibility test of crude and partially purified bacteriocins produced *L. reuteri* PNW1 and *L. acidophilus* PNW3

Organisms	**Zones of inhibition (mm)***			
	BP1	**BP1_PR** (0.25 mg/ml)	**BP3**	**BP3_PR** (0.25 mg/ml)
E. coli C1	18.7±0.58	23.3±1.15	21.7±0.58	24.3±1.15
E. coli C2	20.0±1.00	22.0±0.00	19.3±0.58	24.0±1.00

Key: BP1 - BP1 - Crude bioactive peptide by *L. reuteri* PNW1, BP3 - Crude bioactive peptide by *L. acidophilus* PNW3, BP1_PR – Partially purified bioactive peptide by *L. reuteri* PNW1, BP3_PR – Partially purified bioactive peptide by *L. acidophilus* PNW3, * - Mean values of three replicates

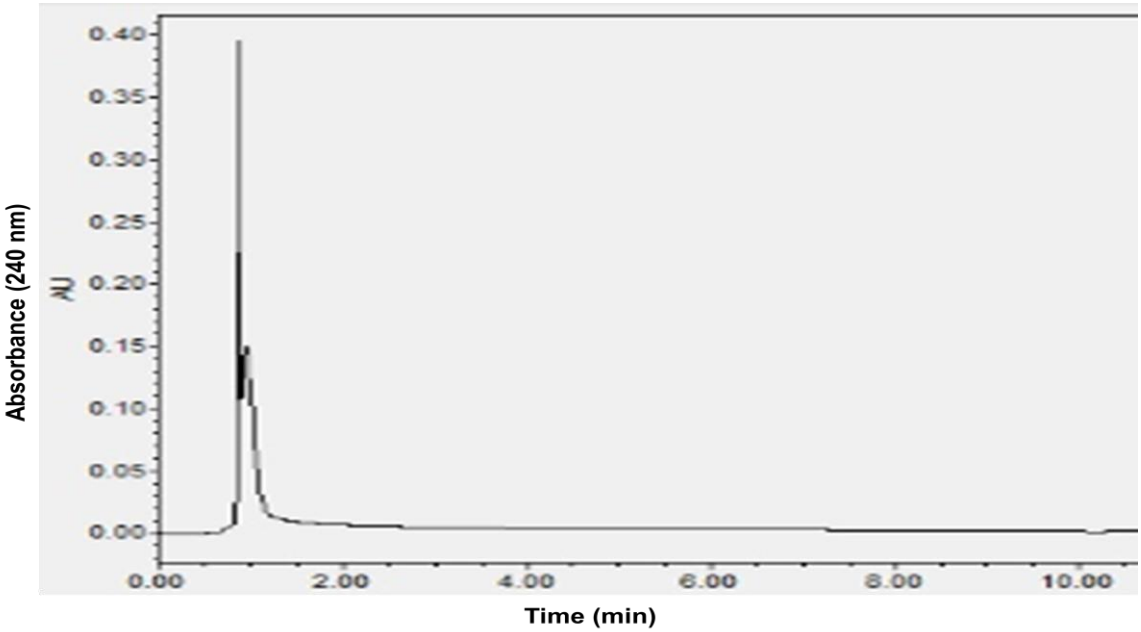

Figure 6.1: HPLC analysis of bacteriocin produced by *L. reuteri* PNW1 using analytical reverse phase C18 column. The flow rate for the elution was 0.2 ml/min with a linear gradient from 0 to 60% over a period of 40 minutes.

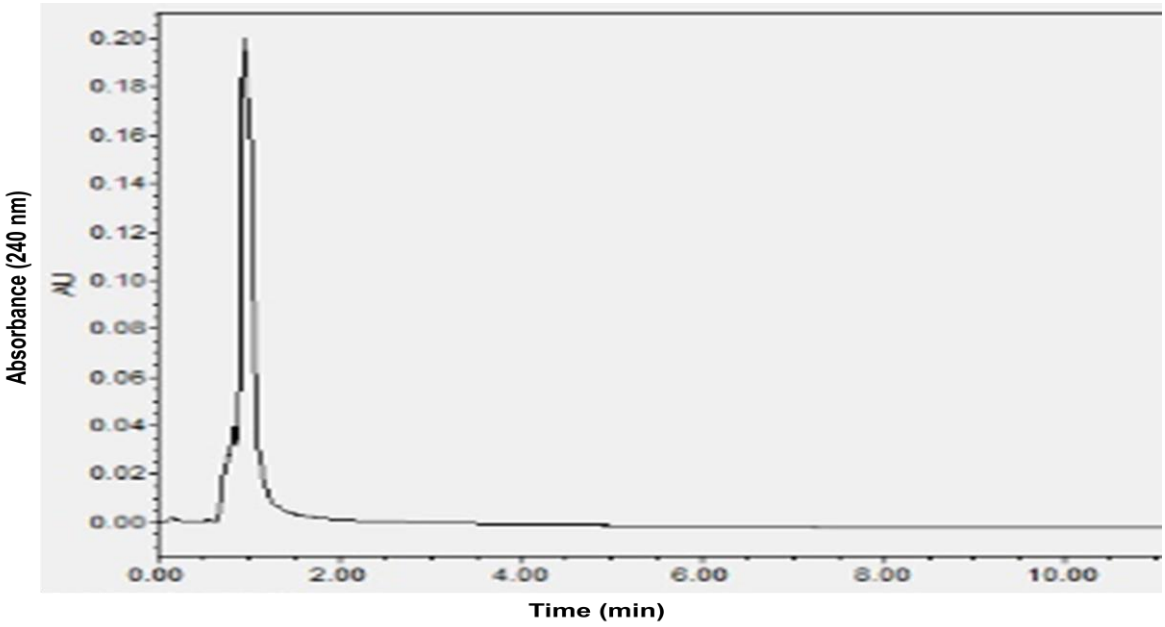

Figure 6.2: HPLC analysis of bacteriocin produced by *L. acidophilus* PNW3 using analytical reverse phase C18 column. The flow rate for the elution was 0.2 ml/min with a linear gradient from 0 to 60% over a period of 40 minutes.

6.4. Discussion and conclusion

Analysis of complete genome comfirmed the presence of protein-encoding sequences putative for the production of bacteriocins within the genome of *L. reuteri* PNWI (Figure 3.3) and *L. acidophilus* PNW3 (Figure 4.1). The functional annotation of the entire genome

of *L. reuteri* PNW1 and *L. acidophilus* PNW3 predicted the bacteriocin identified for both organisms as bacteriocin helveticin J (Figure 3.16). The relationship between the two protein-coding sequences from each isolate was further investigated through SmartBlast of the sequence of the amino acids of the relative bacteriocin helveticin J. There was 100% similarity between with the bacteriocin helveticin J sequenced from *L. reuteri* PNW1 and another protein found in *L. acidophilus* within the searched database (Figure 6.3). This further butresses the possibility of both organisms containing similar bacteriocins as previously predicted from the funtional annotation coupled with the close similarity between the two spectra of the purified bacteriocins as revealed by the HPLC (Figures 5.1 and 5.2). The protein encoding sequence for bacteriocin helveticin J in *L. acidophilus* PNW3 showed 90.15% similarity with another protein found in *Lactobacillus kitasatonis* within the searched database (Figure 6.4).

Lastly, the susceptibility test of bacteriocins against shiga toxin producing *E. coli* O177 revealed the efficacy of bacteriocins produced by both organisms (Table 6.1). This could be a pointer towards the use of these organisms as probiotic agents with a focus on controlling post-weaning diarrhoea, which is a major challenge in piggery and dairy farming, coupled with several other physiological benefits of a probiotic agent.

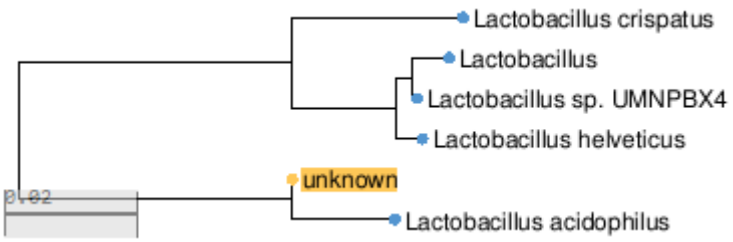

Figure 6.3: Protein-based relationship of the sequence of amino acids (106 aas) of bacteriocin helveticin J from *Lactobacillus reuteri* PNW1 (unknown). The tree was generated using NCBI SmartBlast.

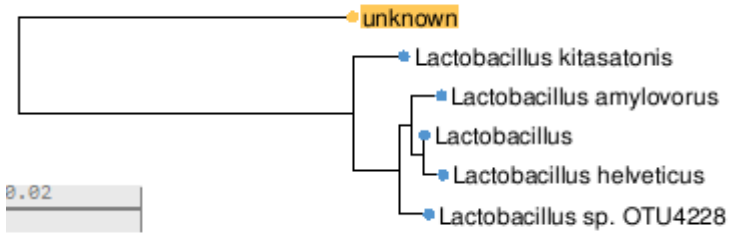

Figure 6.4: Sequence of protein-based amino acids (325 aas) of bacteriocin helveticin J from Lactobacillus acidophilus PNW3 (unknown). The tree was generated using NCBI SmartBlast.

References

Boakes, S., Ayala, T., Herman, M., Appleyard, A. N., Dawson, M J., Cortés, J. 2012. Generation of an actagardine A variant library through saturation mutagenesis. *Appl Microbiol Biotechnol*, 95, 1509–1517.

Cotter, P. D., Hill, C., Ross, R. P. 2005. Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol*, 3, 777–788.

Cotter, P.D., R. Paul Ross, P. P., Hill, C. 2013. Bacteriocins — A viable alternative to antibiotics? *Nat Rev Microbiol*, 11, 95 – 105.

de Castilho, N. P. A., Todorov, S. D., Oliveira, L. L., Bersot, L. S., Nero, L. A. 2020. Inhibition of Listeria monocytogenes in fresh sausage by bacteriocinogenic *Lactobacillus curvatus* UFV-NPAC1 and its semi-purified bacteriocin *LWT - Food Sci Technol*, 118*,* 108757.

Ekblad, B., Kristiansen, P. E. 2019. NMR structures and mutational analysis of the two peptides constituting the bacteriocin plantaricin S. *Nat Sci Rep*, 9, 2333.

Garsa, A. K., Choudhury, P. K., Puniya, A. K., Dhewa, T., Malik, R. K., Kumar, S., Tomar, S. K. 2019. Bovicins: The bacteriocins of *Streptococci* and their potential in methane mitigation. *Probiotics Antimicrob Proteins*, 11, 1403–1413.

Gill, M., Smit, P., Wilkinson, J. M. 2010. Mitigating climate change: the role of domestic livestock. Animal, 4, 323–333.

Goh, H. F., Philip, K. 2015. Purification and characterization of bacteriocin produced by weissella confusa A3 of dairy origin. *PLoS One,* 10**,** e0140434.

Hu, Y., Liu, X., Shan, C., Xia, X., Wang, Y., Dong, M., Zhou, J. 2017. Novel bacteriocin produced by Lactobacillus alimentarius FM-MM4 from a traditional Chinese fermented meat Nanx Wudl: Purification, identification and antimicrobial characteristics. *Food Control*, 77, 290-297.

Kazuñ, B., Kazuñ, K., Siwicki, A.K., ¯yliñska, J. 2018. *In vitro* study of *Lactobacillus plantarum* properties as a potential probiotic strain and an alternative method to antibiotic treatment of fish. *Fish Aquat Life*, 26, 47-55.

Kondrotiene, K., Kasnauskyte, N., Serniene, L., G€olz, G., Alter, T., Kaskoniene, V., Maruska, A. S., Malakauskas, M. 2018. Characterization and application of newly isolated nisin producing *Lactococcus lactis* strains for control of Listeria monocytogenes growth in fresh cheese LWT - Food Sci Technol, 87, 507-514.

Kumar, S., Dagar, S.S., Hadi, S., Ebrahimi, M. R. K., Upadhyay, R. C., Puniya, A. K. 2014. Prospective use of bacteriocinogenic *Pediococcus pentosaceus* as direct-fed microbial having methane reducing potential. *J Integ Agric*, 14(3), 561–566.

Nagpal, R, Kumar, A., Kumar, M., Behare, P.V., Jain, S., Yadav, H. 2012. *FEMS Microbiol Lett*, 334, 1.

Nissen-Meyer, J., Rogne, P., Oppegard, C., Haugen, H. S., Kristiansen, P. E. 2009. Structure-function relationships of the nonlanthionine- containing peptide (class II) bacteriocins produced by gram-positive bacteria. *Curr Pharm Biotechnol*, 10, 19–37.

Oppegård, C., Rogne, P., Emanuelsen, L., Kristiansen, P.E., Fimland, G., Nissen-Meyer, J. 2007.The two-peptide class II bacteriocins: Structure, production, and mode of action. *J Mol Microbiol Biotechnol*, 13, 210–219.

Rea, M. C., Sit, C. S., Clayton, E., O'Connor, P. M., Whittal, R. M., Zheng, J., Vederas, J. C., Ross, R. P., Hill, C. 2010. Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile. PNAS*, 107(20), 9352–9357.

Svetoch, E. A., Stern, N. J. 2010. Bacteriocins to control *Campylobacter* spp. in poultry—a review. *Poult Sci*, 89, 1763–1768.

Valdovinos-García, L.R., Abreu, A.T., Valdovinos-Díaz, M.A. 2018. Uso de probióticos en la prácticaclínica: resultados de una encuesta nacional a gastroenterólogos y nutriólogos. *Rev Gastroenterol Méx*, 84(3), 303–309.

Wannun, P., Piwat, S., Teanpaisan, R. 2016. Purification, characterization, and optimum conditions of fermencin SD11, a bacteriocin produced by human orally *Lactobacillus fermentum* SD11. *Appl Biochem Biotechnol*, 179, 572–582.

Zommiti, M., Almohammed, H., Ferchichi, M. 2016. Purification and characterization of a novel anti-campylobacter bacteriocin produced by *Lactobacillus curvatus* DN317. *Probiotics Antimicrob Proteins,* 8, 191–201.

CHAPTER 7 − General discussion and conclusion

Availability of the whole-genome sequence technology creates adequate room for in-depth characterization of a potential probiotic strain and substantial evaluation of the safety features of probiotic bacteria. Complete genome analysis and functional annotation of the entire genome of *Lactobacillus reuteri* PNW1 and *Lactobacillus acidophilus* PNW3 revealed a plethora of genes within the draft assembly in support of their probiotic candidacy. Besides a mandatory test of safety for every novel strain of probiotics, each probiotic agent is expected to possess certain desired traits such as modulation of immune and physiological systems of the host, deactivation or attenuation of virulent markers on neighbouring pathogens, prevention of infectious and inflammatory disease conditions; and also improved stability of the genome, ability to withstand adverse gut conditions and potential to adhere to the epithelia cells and/or mucosal layer (Saxami *et al.*, 2012; Celiberto *et al.*, 2017; Chen *et al.*, 2017).

Both *L. reuteri* PNW1 and *L*. *acidophilus* PNW3 were confirmed at the genomic level to have shown potentials for producing lactic acids. These lactic acids have been proven to be active in antagonizing survival of neighbouring pathogens and simultaneously inactivates human immune virus (Nordeste *et al*., 2017). They have also been confirmed having inhibitory effects on the production of IL-8 induced by IL-1β and colony formation by HT29 cells, thus preventing colorectal associated malignancy (Yang *et al*. 2012).

Bacteriocin which is a secondary metabolite characterized with significant bioactive potential against intestinal pathogens (Cotter *et al*., 2013) was found present within the genome of both isolates. This was biochemically extracted from the isolates to allow *in vitro* test of efficacy against pathogenic *Escherichia coli* O177. The extracted bacteriocins showed a commendable inhibitory potential both in the crude and partially purified forms (Table 6.1). The Bacteriocin secreted as secondary metabolites by these two lactic acid

bacteria could provide a source of alternative bioactive compounds of natural origin to synthetic antimicrobials. Thereby mitigate the incessant occurrence of multidrug resistant pathogens due to misuse of conventional antimicrobials. To further buttress this point, several scientific reports have revealed the potency of active compounds secreted by LAB against clinically important pathogens as well as malignant cells (Axel *et al*., 2016; Wang *et al*., 2018; Muhialdin *et al*., 2020). Functional annotation of the entire genome predicted the bacteriocin secreted by *L. reuteri* PNW1 and *L. acidophilus* PNW3 as bacteriocin helveticin J. The relationship between the two protein-coding sequences from each isolate was further confirmed through NCBI SmartBlast (Figure 6.3 and 6.4) and further supported by the close similarity in the HPLC spectra.

Performance of probiotic agent is largely enhanced by ability to adhere and competitively colonise gut epithelia cells and mucosal layer. This will provides ample opportunity for stability of the strain and effectively prolongs the antagonistic effects against unwanted gut microbial residents (Salas-Jara *et al*., 2016). Sortase A, LPXTG specific and members of exopolysaccharide (EPS) cluster are among important adhesion players common to both *L. reuteri* PNW1 and *L*. *acidophilus* PNW3. Sortase A is an important enzyme involves in the cell physiology and defence. It is made up of functional proteins and pili assemblage that facilitate adhesion of probiotic cells to host cells, thus enable stable interactions between the cells and their environment (Jacobitz *et al.,* 2017; Stanborough *et al*., 2018). The EPSs contribute major impact on the strain specificity of probiotic candidates (Zeng *et al*., 2019) several of its members such as EpsC, EpsD, EpsJ have been directly linked to some important probiotic features such as biofilm formation, immunomodulation, aggregation, antioxidant and antimicrobial potentials (Wu *et al*., 2014).

Moreover, Extracellular digestive enzymes produced by most lactic acid bacteria play important role in the gastrointestinal tract of animals (Tallapragada *et al*., 2018). The

protein-encoding sequence predicted for lipase and protease which were commonly found in both isolates supports the envisaging potential of the isolates with regard to improving nutrient digestibility in the host as a probiotic candidate; this in turn may directly translate into growth performance improvement in the target farm animal. In addition, the lower pH of the gastric juice and higher concentration of the bile salt predispose gut resident microbes to adverse condition. Some of the coding sequences required by a microorganism in order to survive harsh environmental conditions are on the list of genes found within the genome of both isolates. For instance, Autoinducer-2 production protein LuxS and sB-dependent general stress proteins (Gsps) are among the proteins involved in acid and bile salt stress tolerance; and several kinds of nonspecific stress tolerance respectively (Antelmann *et al*., 2000; Jia *et al*., 2018).

Investigation towards pathogenicity and occurrence of virulent determinants revealed that *L. reuteri* PNW1 and *L. acidophilus* PNW3 have zero matches for pathogenic family and there was not hit found for virulent determinants. Therefore identifies both isolates as non pathogenic for both human and animals. Although coding sequences putative for antimicrobial resistance genes against lincosamide (*lnuC*) and tetracycline (*TetW*) was found harbouring by both isolates. Only the *lnuC* acquired by the *L. reuteri* PNW1 is flanked by a mobile genetic element (MGE) while none of the resistance genes found within the *L. acidophilus* PNW3 is flanked by any MGE (Table 5.1). Having known that most resistance genes are acquired through the MGE such as; plasmids, transposons and passenger genes, therefore, possibility of lateral transfer of resistance genes from the isolates is minimal.

Furthermore, occurrence of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) region within microbial genome limits the spread of antimicrobial resistant genes by obstructing multiple pathways of horizontal gene transfer (Maraffini and Sontheimer,

2008). Besides, CRISPR region equips microbes with sequence specific defense-line against plasmids, insertion sequences and phages. CRISPR locus, with associated Cas-genes, provides the host organism with self-defense mechanism against intruding extra-chromosomal genetic molecules (Palmer and Gilmore, 2010). Presence of the CRISPR-Cas region within the genome of *L. reuteri* PNW1 and *L. acidophilus* PNW3, therefore, suggest genomic stability which is an important trait required of a probiotic candidate.

Lastly, functional annotation of the draft assembly revealed the presence of protein-encoding sequence predicted for arginine deiminase within the genome of the *L. reuteri* PNW1 and ornithine decarboxylase (EC 4.1.1.17) within that of *L. acidophilus* PNW3. Arginine deiminase is an enzyme that is involve in post translational modification of the protein histone, an essential building block of chromatin (Mohanan *et al*., 2012; Bicker and Thompson, 2014). Although It is worthy to note that arginine deiminase class IV is the only isozyme of this protein that has been confirmed to be clearly involved in the citrullination i.e. deimination of histone (Bicker and Thompson, 2014); and has been identified with potential involvement in tumor progression (Mohanan *et al*., 2012). This suggests that further study is required to characterize the class of the protein arginine deiminase which may be produce by *L. retueri* PNW1.

Likewise the ornithine decarboxylase found in *L. acidophilus* PNW3 which is responsible for the formation of putrescine, a biogenic amine capable of down-regulating activities of macrophages during inflammatory response (Hardbower *et al*., 2017). Ornithine decarboxylase limits the rate of polyamine biosynthesis, therefore, increasing the potential for oncogenesis (Shukla-Dave *et al*., 2016). Although activity of ornithine decarboxylase is under strict regulation at transcriptional and post translational stages, due to its impact on cellular processes (Kaprio *et al*., 2019). This therefore makes the threat due to putrescine less pronounced.

In conclusion, considering abundance of important genes, putative for several desired traits in a typical probiotic candidate, embeded within the genome of *L. reuteri* PNW1 and *L. acidophilus* PNW3; both isolates could make a good and safe choice for future development into functional probiotics with a target on farm animals. Further studies are required to elucidate on the potency and repression technique for the ascribed biogenic amine confirmed produced by both isolates and citrullination potential of the argenine deiminase found harboured in *L. reuteri* PNW1.

References

Antelmann, H., Scharf, C., Hecker, M. 2000. Phosphate starvation-inducible proteins of *Bacillus subtilis*: Proteomics and transcriptional analysis. *J Bacteriol* 182(16), 4478–4490.

Axel, C., Brosnan, B., Zannini, E., Furey, A., Coffey, A., & Arendt, EK. 2016. Antifungal sourdough lactic acid bacteria as biopreservation tool in quinoa and rice bread. *Int J Food Microbiol*, 239, 86–94.

Bicker K. L., Thompson, P. R. 2014. The protein arginine deiminases (PADs): Structure, function, inhibition, and disease. *Biopolymers*, 99(2), 155–163.

Celiberto, L. S., Bedani, R., Rossi, E. A., Cavallini, D. C. 2017. Probiotics: The scientific evidence in the context of inflammatory bowel disease. *Crit Rev Food Sci Nutr,* 57, 1759-1768.

Chen, J., Wang, Q., Liu, C. M., Gong, J. 2017. Issues deserve attention in encapsulating probiotics: Critical review of existing literature. *Crit Rev Food Sci Nutr,* 57, 1228-1238.

Cotter, P.D., R. Paul Ross, P. P., Hill, C. 2013. Bacteriocins — a viable alternative to antibiotics? *Nat Rev Microbiol*, 11, 95 – 105.

Hardbower, Dana M., Asim, Mohammad, Luis, Paula B., Singh, Kshipra, Barry, Daniel P., Yang, Chunying, Steeves, Meredith A., Cleveland, John L., Schneider, Claus, Piazuelo, M. Blanca, Gobert, Alain P., Wilson, Keith T. 2017. Ornithine decarboxylase regulates M1 macrophage activation and mucosal inflammation via histone modifications. *PNAS*, E751–E760

Jacobitz, A. W., Kattke, M. D., Wereszczynski, J., Clubb, R. T. 2017. Sortase transpeptidases: Structural biology and catalytic mechanism. *Adv Protein Chem Struct Biol*, 109, 223–64.

Jia, F-F., Zheng, H-Q., Sun, S-R., Pang, X-H., Liang, Y Shang, J-C., Zhu, Z-T., Meng, X-C. 2018. Role of *luxS* in Stress Tolerance and Adhesion Ability in *Lactobacillus plantarum* KLDS1.0391. *BioMed Res Int,* Article ID 4506829, doi:org/10.1155/2018/4506829.

Kaprio, T., Rasila, T., Hagstro, ¨m J., Mustonen, H., Lankila, P., Haglund, C., Andersson, L. C. 2019. Ornithine decarboxylase antizyme inhibitor 2 (AZIN2) is a signature of secretory phenotype and independent predictor of adverse prognosis in colorectal cancer. *PLoS ONE* 14(2): e0211564.

Maraffini, L.A., Sontheimer, E.J. 2008. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. *Sci*, 322, 1843–1845.

Mohanan, S., Cherrington, B. D., Horibata, S., McElwee, J. L., Thompson, P. R., Coonrod, S. A. 2012. Potential role of peptidylarginine deiminase enzymes and protein citrullination in cancer pathogenesis. *Biochem Res Int*, ID 895343, 11 pages.

Muhialdin, B. J., Algboory, H.L., Kadum, H, Mohammed, NK, Saari, N, Hassan, Z, Hussin, ASM. 2020. Antifungal activity determination for the peptides generated by *Lactobacillus plantarum* TE10 against *Aspergillus flavus* in maize seeds. *Food Control*, 109, 106898.

Nordeste R, Tessema A, Sharma S, Kovač Z, Wang C, Morales R, Griffiths MW. 2017. Molecules produced by probiotics prevent enteric colibacillosis in pigs. *BMC Vet Res* 13:335 DOI 10.1186/s12917-017-1246-6.

Palmer, K. L., M. S. Gilmore. 2010. Multidrug-resistant enterococci lack CRISPR-cas. *mBio* 1(4), e00227-10.

Salas-Jara, M.J., Ilabaca, A., Vega, M., García, A. 2016. Biofilm forming Lactobacillus: new challenges for the development of probiotics, Microorganisms 4 (3), 35.

Saxami, G., Ypsilantis, P., Sidira, M., Simopoulos, C., Kourkoutas, Y., Galanis, A. 2012. Distinct adhesion of probiotic strain *Lactobacillus casei* ATCC 393 to rat intestinal mucosa. *Anaerobe,* 18, 417-420.

Shukla-Dave, A., Castillo-Martin, M., Chen, M., Lobo, J., Gladoun, N., Collazo-Lorduy, A., Khan, F. M., Ponomarev, V., Yi, Z., Zhang, W., Pandolfi, P. P., Hricak, H., Cordon-Cardo, C. 2016. Ornithine decarboxylase is sufficient for prostate tumorigenesis via androgen receptor signaling. *Am J Pathol*, 186(12), 3131−3145.

Stanborough, T., Suryadinata R, Fegan N, Powell S. M., Tamplin M., Nuttall, S.D., Chandry, P.S. 2018. Characterisation of the *Brochothrix thermosphacta* sortase A enzyme. *FEMS Microbiol Lett*, 365, fny184. doi: 10.1093/femsle/fny184

Tallapragada, P., Rayavarapu, B., Rao, P. P., Ranganath, N. N., Veerabhadrappa, P. P. 2018. Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. *J Genetic Engineering Biotechnol*, 16, 357–362.

Wang Y, Wang X, Jiang W, Wang K, Luo J, Li W, Zhou X, Zhang l. 2018. Antimicrobial peptide GH12 suppresses cariogenic virulence factors of *Streptococcus mutans*, *J Oral Microbiol*, 10:1. DOI:10.1080/20002297.2018.1442089.

Wu, Q., Tun, H.M., Leung, F.C.-C., Shah, N.P. 2014. Genomic insights into high exopolysaccharide-producing dairy starter bacterium *Streptococcus thermophilus* ASCC 1275. *Sci Rep*, 4, 4974. doi:10.1038/srep04974 (2014).

Yang, G., Xu, H., Kelly, C.P., CHEN, X. 2012, Lactic acids mediates anti-cancer function of probiotic yeast (188), *Gastroenterol*, 142(5), S-46.

Zeng Z, Zuo F, Marcotte H. 2019. Putative adhesion factors in vaginal *Lactobacillus gasseri* DSM 14869: functional characterization. *Appl Environ Microbiol*, 85, e00800-19.

APPENDICES

Appendix A

The sequence of nucleotide and amino acids of identified genes putatively involved in lactic acids production

D-lactate dehydrogenase (EC 1.1.1.28)

>fig|1598.593.peg.171 **D-lactate dehydrogenase (EC 1.1.1.28)** [Lactobacillus reuteri PNW1]
atgaaattaattatgtatagcgttcgtgacgctgaaaagccttatattgaagcatgggaa
aagaagacaggcaatgacgtcaagatggttactgaaccattaaatgctgatacagttaag
cttgcagagggatatgatggaatttccttacagcaaacaacaaaacttggggatgaacaa
ctgtatgagcaattagcagctatgggaataaagcaattagctgctcggatggttggtgtt
gatatttttgatttagatgcatgtaaagcaaatggcatcattgtaacgaatgtccctatt
tattcaccaagggcaatcgcagaaatgggcgttactcaggcgatgtacttgttacgacgg
attggtgaattcgaacaacgaatgagccatggtgattttcggtggagcgatgacttgatc
agtaatgaaatttacaatttaaccgtgggaattgttggccttggtaatattggtggggcc
actgctcagatttataaagcattaggcgcaaaagttcttgcatatgatccatcgtacaat
gtcgaatatgaaccatacgttgaatataccgatttcgatactgttattaagaatgcggat
attttgtcattgcatacaccgttgttaccaagtaccgaaaacatgattgcagcaccacaa
tttaagatgatgaaagacaatgcttacttgattaacatggctcgtggaaaactggtaaat
accgcggacttaatttcagcattggaaaataaagaaattgcgggtgctggcctagataca
ttagctgacgaaacttctttctttgggaagcaagttacatcagatcaaattccagatgat
tacaagaaattagcggcaatgccaaatgttcttgttacaccccacgttgcgttcctaacc
gaaacatctattcgtaatatggtcgagattagtttgaatgatgctgttacgattttagaa
ggcaagcattcacgaaatgaaataagaatgtaa

>fig|1598.593.peg.171 **D-lactate dehydrogenase (EC 1.1.1.28)** [Lactobacillus reuteri PNW1]
MKLIMYSVRDAEKPYIEAWEKKTGNDVKMVTEPLNADTVKLAEGYDGISLQQTTKLGDEQ
LYEQLAAMGIKQLAARMVGVDIFDLDACKANGIIVTNVPIYSPRAIAEMGVTQAMYLLRR
IGEFEQRMSHGDFRWSDDLISNEIYNLTVGIVGLGNIGGATAQIYKALGAKVLAYDPSYN
VEYEPYVEYTDFDTVIKNADILSLHTPLLPSTENMIAAPQFKMMKDNAYLINMARGKLVN
TADLISALENKEIAGAGLDTLADETSFFGKQVTSDQIPDDYKKLAAMPNVLVTPHVAFLT
ETSIRNMVEISLNDAVTILEGKHSRNEIRM

>fig|1598.593.peg.1321 **D-lactate dehydrogenase (EC 1.1.1.28)** [Lactobacillus reuteri PNW1]
atggttaaaaaaatttttgcttttagtatccgaaaagatgaagaaccttatgttaaagaa
tgggctaaggatcatcccgaagtagaagtagaatatacggatgaactcttaactcctgaa
actgccgcaaaagctaaaggagctgacggggtagtcgtttaccaacaactcgattacaca
ccagaaacgctacaagcactggctgacgaaggtattacaaagatgtcactacgtaatgtt
ggtgttgataacattgatatggctaaggcaaaagaacttggctttgaaattaccaatgtt
cctgtttactcaccaaacgcaattgccgaacatgctgctattcaaacagcccgaattctt
cgccaaaccaaggccttagatgaaaagattgctaatggcgatttacgttgggccccaaca
attggtcgagaagtgcgtgatcaaactgttggtgttatcggaactggtcatatcggacgt
gtttatatgcaaattatggaaggcttcggtgctaaggtaattgcatatgacccatttgaa
aatccagaattaaagaagcaaggatattacgttgacagccttgatgatctatatgctcaa
gctgatgtggtttctcttcacgttccggctaccaaagagaacttccatatgattgataaa
gacgcaattgctaaaatgaaagacaatgttgtaattgttaactgttcacgaggcgcatta
gttgatactgatgctgtaatcgaaggacttgatagtggaaagatctttggctttattatg
gacacttacgaagatgaagttggaatctttaatgaagattggcgtggcaaggagttccct
gacaagcgacttaaagaccttattgatcgttcaaacgttcttgttactccacatacagct
ttctatactactcatgctgtccgcaacatggttcttaatgcctttgacaataaccttaaa
ttaattaatggtgaagaagctgacacaccggttaaggttggctaa

>fig|1598.593.peg.1321 **D-lactate dehydrogenase (EC 1.1.1.28)** [Lactobacillus reuteri PNW1]
MVKKIFAFSIRKDEEPYVKEWAKDHPEVEVEYTDELLTPETAAKAKGADGVVVYQQLDYT
PETLQALADEGITKMSLRNVGVDNIDMAKAKELGFEITNVPVYSPNAIAEHAAIQTARIL
RQTKALDEKIANGDLRWAPTIGREVRDQTVGVIGTGHIGRVYMQIMEGFGAKVIAYDPFE
NPELKKQGYYVDSLDDLYAQADVVSLHVPATKENFHMIDKDAIAKMKDNVVIVNCSRGAL
VDTDAVIEGLDSGKIFGFIMDTYEDEVGIFNEDWRGKEFPDKRLKDLIDRSNVLVTPHTA
FYTTHAVRNMVLNAFDNNLKLINGEEADTPVKVG

>fig|1598.593.peg.2392 **D-lactate dehydrogenase (EC 1.1.1.28)** [Lactobacillus reuteri PNW1]
atgaagatttatatgtatggtgtttatcaggatgaagttccttacattaaggaatggcaa
gaggaacatcctgaagtaactgtagactcaacaacgaaacttttagacgaaagcacagtt
aacttgtctaaaggaagcgatggagtagtagtattccagcaaaagccttattcagacgag
gccttacgtcaattagcacttaatggtattacaaagatgtcattgcgaaatgttggggtt
gataatcttaaccatgaattagtacaagaacttggttttcaaattaccaatgtaccggtt
tattccccagctgcgattgctgaattttcagttactcaagcacttaacctttacgtcga
acaaaagaattttatttgaagttagcaaaaggagattataactgggcaccgcatattgct
aaggaaatgaataagcaggttgttgggatagttggaacaggaaacattggatctacggcg
gctaaaatctttgctggttttggtgcaaaagtgattgcatatagccgtcaccaaaataag
gaactagagggaattgttgaatacgttagccttgatgaattatataaacgggcaacaatt
atttcactttatttacctcatgttccagcgacagataaaatgcttaatgaaaaaacattt
gcaatgatgcaggacggggtcttactggttaacactgcacgtggaccattagtcgatgaa
aaggcattgattgaagcattgaatagcggtaaagtcggaggagctgctctagacgtaatg
actggtgaaaccaagatttttaaccggcaaattaatttccaagaagttgattatgacgaa
tttaaagatttagttgaccggccaaatgttttaatcacgccacatattgctttttatact
gatcaagcaatcaaaaatatggttaagatgagtttgtctgccaatctagatttgattaaa
actggcacctcagataaactagttaaattttaa

>fig|1598.593.peg.2392 **D-lactate dehydrogenase (EC 1.1.1.28)** [Lactobacillus reuteri PNW1]
MKIYMYGVYQDEVPYIKEWQEEHPEVTVDSTTKLLDESTVNLSKGSDGVVVFQQKPYSDE
ALRQLALNGITKMSLRNVGVDNLNHELVQELGFQITNVPVYSPAAIAEFSVTQALNLLRR
TKEFYLKLAKGDYNWAPHIAKEMNKQVVGIVGTGNIGSTAAKIFAGFGAKVIAYSRHQNK
ELEGIVEYVSLDELYKRATIISLYLPHVPATDKMLNEKTFAMMQDGVLLVNTARGPLVDE
KALIEALNSGKVGGAALDVMTGETKIFNRQINFQEVDYDEFKDLVDRPNVLITPHIAFYT
DQAIKNMVKMSLSANLDLIKTGTSDKLVKF

>fig|1598.593.peg.2561 **D-lactate dehydrogenase (EC 1.1.1.28)** [Lactobacillus reuteri PNW1]
atgacgaaaatcttgatgactagtatgcgggacgatgaacaaaccgccattaacgaatat
gcgaaagaacataatatcgaaatcatcacaacgccgaaattgattgacgatgctgttgaa
ttaacggccgatgttgatggactcgtgattcagcaacgtagtaaagttccggctgatatt
tatgaaaagctacatgccaatggtctgaaacaaattgctactcggaccgcgggatttgac
atggtcgacatcaaaaaggctaacgaaaatgacttagtcgtcaccaatgtaccagcatat
tcaccgcggagtgtcgccgagtttgcattaatgcagattttccggttgcttcggaaaact
taccgctttgaccaccaagtcgccgaaaatgacttccgctggtttagcgatgaacagtca
accgaaattcacacggcaaggattggaattattggggtcgggcgtatcggcggaacactt
gctaaacttcttaatgccctcggtgccactgttcttggttatgacacgaatccgcgcact
gacctcgatggaattgtccagttcgtttctaaagaagaattactaaagcaagccgatgtt
gttagtctccacgttgatctaaacccaacttctaaaggtttgctgaccgctaacgacttt
gtaatgatgaaaccaactgctggtttggtcaatgctagtcgtggtccagtcgttaataca
gctgacctcgtcacagcattaaaatctggtgaaatcgccgcggccaccctcgataccttt
gaaggtgaagaaacagttgcagctgctgatcgccgagaaaaagggcttgatgatcagccc
cttgttaaagaattacacgaaatggataatgtcattcttactccccatattgccttcttt
actaatttagcagtcaaaaacatggtggatatttcacttgatgatgtgatgacaatcctt
aatagcgaaaaatcaccacatgaaatcaccttataa

>fig|1598.593.peg.2561 D-lactate dehydrogenase (EC 1.1.1.28) [Lactobacillus reuteri PNW1]
MTKILMTSMRDDEQTAINEYAKEHNIEIITTPKLIDDAVELTADVDGLVIQQRSKVPADI
YEKLHANGLKQIATRTAGFDMVDIKKANENDLVVTNVPAYSPRSVAEFALMQIFRLLRKT
YRFDHQVAENDFRWFSDEQSTEIHTARIGIIGVGRIGGTLAKLLNALGATVLGYDTNPRT
DLDGIVQFVSKEELLKQADVVSLHVDLNPTSKGLLTANDFVMMKPTAGLVNASRGPVVNT
ADLVTALKSGEIAAATLDTFEGEETVAAADRREKGLDDQPLVKELHEMDNVILTPHIAFF
TNLAVKNMVDISLDDVMTILNSEKSPHEITL

L-lactate dehydrogenase (EC 1.1.1.27)

>fig|1598.593.peg.419 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
atgagaaagattggaattattggtctcggtcatgtgggtgaaatgttagccaaccagttg
gtaatgaacggaaaagttgacgaattagttttgattgatgacaaagatcaactagcaatt
gcaattcaggccgatttaaatgatgcgcaggcggtcttggcgacccatacaaaaataatt
attcaagattatgctgccttagccgatgcggatgttcttattacagcctttggaaagagt
gctttgatgaagcaacaaccaatggctgagcttgagaccagttatcagcaggccttacaa
gttggtaataagatttttaagagtgattttagtgggatcctaattaaccttactaatcct
aatgagtcaattactgctgttctccagcaaaaagttggcttaccgcaaaaacaagtaatt
gggattggcacagttgttgaaaccgctcgcctttatcgggcaattgcagaaaccgcaaag
gtcgctgctgctaatgtaactggctttgtctatggtcagcatgatggccatcaagtattc
gcttggtcaactgttcgcgtaaatggccagcccttaaccgcggcaatcaatggccaccac
ttagatcaaagtcagcttaaaattcaggcgaaccttagtaattggtacactcttgatggg
ttagggtataatgttagtgctgttactgcctggactctccgaattattacggcgattttc
gcggatgaacaattagcgttgccagttgcaatttatcagccgcaatattccacttacatc
agttttccagcgttaattggtcgtcaaggaattggcaaccttctgctcttaaaactttat
ccattggaagaagaagagattaagagtgcggcagcaacaattaagcatcaattagattct
ctaaaaaatattaaaggggagtag

>fig|1598.593.peg.419 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
MRKIGIIGLGHVGEMLANQLVMNGKVDELVLIDDKDQLAIAIQADLNDAQAVLATHTKII
IQDYAALADADVLITAFGKSALMKQQPMAELETSYQQALQVGNKIFKSDFSGILINLTNP
NESITAVLQQKVGLPQKQVIGIGTVVETARLYRAIAETAKVAAANVTGFVYGQHDGHQVF
AWSTVRVNGQPLTAAINGHHLDQSQLKIQANLSNWYTLDGLGYNVSAVTAWTLRIITAIF
ADEQLALPVAIYQPQYSTYISFPALIGRQGIGNLLLLKLYPLEEEEIKSAAATIKHQLDS
LKNIKGE

>fig|1598.593.peg.644 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
atgttaggacatcatcataaagttgtgcttgttggtgatggggcagtaggatcatcattt
gctttctcgctccttcaaacaacacaagaaattgatgaattagttattgttgacctaaag
aaagataaggcgactggagagtcattggatttacaagatattactcccctaactagtcca
gtcaacattcacgcgggagattattctgatgctactgatgctgatgttgtagttattact
gctggagttccacgaaaaccgggagagactcgtttagatcttgttgccaagaatacaaaa
attctttcaactattgttaacccaattgttgaaagcggatttagtggtatttttgttgta
tcaagcaatcctgttgatattctaacaacagtaacgcaacaactttctggtttccctaaa
catcgggttattggaaccggtacttcactagatacagcacgattggatgtattattatca
caaaaattaaatattccagtaaatgaaatagatgctttagttttaggagaacatggtgat
acatcatttggtgcatttgatgaagcaactattaatggtaagccattaaaagaagcaact
aatttaactgttgaagattatgatgaacttgaaaaagcagttaaagaacgtgggggcaag
attatagctgggaaaggagctactttctatggggtagctaaatatcttgcttatattgta
aaagcgattattgaaaatcgtaatattatgttaccgatatctgctccattaacaggtcaa
tacgggattgataatttatatcttggaatgccagctattattaatcgtactggggtagca
aaggtcattgattatgacttgtcagatgcagagacagaaaaactaaaaaattcagccgct
aaaatgaaagaagttttagatggcgtgaaaatttaa

>fig|1598.593.peg.644 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
MLGHHHKVVLVGDGAVGSSFAFSLLQTTQEIDELVIVDLKKDKATGESLDLQDITPLTSP
VNIHAGDYSDATDADVVVITAGVPRKPGETRLDLVAKNTKILSTIVNPIVESGFSGIFVV
SSNPVDILTTVTQQLSGFPKHRVIGTGTSLDTARLDVLLSQKLNIPVNEIDALVLGEHGD
TSFGAFDEATINGKPLKEATNLTVEDYDELEKAVKERGGKIIAGKGATFYGVAKYLAYIV
KAIIENRNIMLPISAPLTGQYGIDNLYLGMPAIINRTGVAKVIDYDLSDAETEKLKNSAA
KMKEVLDGVKI

>fig|1598.593.peg.1417 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
atgacgagaaaagtagcagtagtcggaatgggtcacgttggggcaacggtagcgcactat
ttagttgctggtggctttgtcgatgacctcgcattatttgatactaatgaagctaaggtg
caagcggatgctttggacttacgagatgcaatggccaatttgccttaccatactaacctg
actgttaatgatgaaagccaattgcgtgattgtgatgtggtcgtgtcagccctagggaaa
tcaaagttggttgatacgccagatcatgatcgctttgctgaatttaagtttacgcggacc
caagtgcccttggtagcgaagatgttagtagacaatggatttcacggtaagttagttgat
gttactaacccttgtgatgtgattacctcaatgtaccaaaaattaacgggattaccaaaa
gaacacgtgatggggacggggaccttactcgactctgcacggatgcgggcccgggttggt
gaagcgttgaaagttgattcacgctctgttgttggttttaatcttggggaacatggcaat
tcgcaatttacggcatggtcgacagtccgcgtcctggggaaaacggtcactgaactagca
aaagaacggggattaaagctttctgagctagaagaacgtgctcgtcaaggaggctacctc
gtatatcaagggaagaaatacactaattatggtgttgccacggcagcagtccggttaacc
aacgcattattgaatgatgcgcgaactgaaatgccagtttctaattatcgtgaagaatac
ggcacgtacctttcatatccagctgtcgttggccgcgacggggtagtggagcaactgcat
ctggacttgacgccggaagaagaagcaaaactagcaacatccgcaacatacatcaaagaa
cgattgaaaaaagaagaagagcgagcaaaccagctagctcaggcagaatag

>fig|1598.593.peg.1417 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
MTRKVAVVGMGHVGATVAHYLVAGGFVDDLALFDTNEAKVQADALDLRDAMANLPYHTNL
TVNDESQLRDCDVVVSALGKSKLVDTPDHDRFAEFKFTRTQVPLVAKMLVDNGFHGKLVD
VTNPCDVITSMYQKLTGLPKEHVMGTGTLLDSARMRARVGEALKVDSRSVVGFNLGEHGN
SQFTAWSTVRVLGKTVTELAKERGLKLSELEERARQGGYLVYQGKKYTNYGVATAAVRLT
NALLNDARTEMPVSNYREEYGTYLSYPAVVGRDGVVEQLHLDLTPEEEAKLATSATYIKE
RLKKEEERANQLAQAE

>fig|1598.593.peg.1472 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
atggcgacagaagaacatcaaaaggttgttttaattggtgatggagttgtggggtccgct
tatgcttttagcgtgatccagcaaggtttagctgaggaattagttattataaataagagt
aatgagcgaagcgttggggatgcactagatttggaagacgcaacgccatttactgcccca
gtaaaggtcaaggctggtagctatcaagattgtaaagatgctgatattattactatttgt
gcaggcgctgcgcaaaagccaggtgaaacacggcttaaattagttgaacgcaacttaaag
attatgaaggaaatcgttcaagaagtagttaataccggatttaatgggatcttcttaatt
gctgccaacccagtcgatatcttgacatatgctgttcaaaaaatttctggctttccagca
cataaagttatcggatcgggaacttctcttgattcagcgcgcttaagagtagcgattggt
aaaaagctggctattgatccccgagatgttcatgttgatatgttagcggagcatggagat
tcagaatttgctgcttattcttgtgggacaatcggtggaacgcctttaatggattatgtt
ttagctaatggattaacaaagcaagaactgttaaaactagaagaagaagtacgtaataag
gcgtatgagattattaatcgaaaaggcgcaacatattatggagtagcgactgctttggca
agaataacgaaagcaattttatatgaccaaaatacagtattacccgtgagtgcatattta
gatggtgaatatggagaaaaggatatttatctcgggacaccggctgtaattgataaagac
ggtattcaaaaagtagtagaattaccattagattcacgtgaacaagaggctttaattaat
tcagcatcagttttgcgtgaaacgacagcaaatgggatgactaaagcggggctgttgaac
tatctttggaaataa

>fig|1598.593.peg.1472 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
MATEEHQKVVLIGDGVVGSAYAFSVIQQGLAEELVIINKSNERSVGDALDLEDATPFTAP
VKVKAGSYQDCKDADIITICAGAAQKPGETRLKLVERNLKIMKEIVQEVVNTGFNGIFLI
AANPVDILTYAVQKISGFPAHKVIGSGTSLDSARLRVAIGKKLAIDPRDVHVDMLAEHGD
SEFAAYSCGTIGGTPLMDYVLANGLTKQELLKLEEEVRNKAYEIINRKGATYYGVATALA
RITKAILYDQNTVLPVSAYLDGEYGEKDIYLGTPAVIDKDGIQKVVELPLDSREQEALIN
SASVLRETTANGMTKAGLLNYLWK

>fig|1598.593.peg.2022 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
atgcttgatacgtataggcacaaagttgtattaataggtgacggggctgtcggctcatca
tttgcattttcattattgcagtcaacaaatgaggtcgatgaattggtactggtggatcgt
acaaggtcaaaagcagttggggatgcggctgatcttgctgatattacacccctgacaaac
ccagtaaagatttatgcgggaacctatgaagatgctgctgatgctgacgtagttgttatt
acggctgggattccccgtaaacctggtgaaactcgtttagaccttgttaataaaaacact
acgattcttaagtcaattattgaaccaattgtcaaaagcggattcactggtgttttcgtt
atctcgagcaatcctgttgatatccttacaacaattgcgcagcgaatcagcgggtttcct
aaagaacgtgttattgggaccggaacttctcttgattcaatgcggctacgggtcctctta
agcaagaaattacacctatccgtcaatgtcatcgatgccttaatgcttggcgaacatggt
gatacttcttttgcggcctttaatgaaatcacaatcggtggaaaggccctcaatacaatt
actgccctttcaaatactgataaaagtgaaattgaaaaagcagttcacgaagctggcagt
caaataattgccaataaaggggctactttctacggaattgctaa

>fig|1598.593.peg.2022 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
MLDTYRHKVVLIGDGAVGSSFAFSLLQSTNEVDELVLVDRTRSKAVGDAADLADITPLTN
PVKIYAGTYEDAADADVVVITAGIPRKPGETRLDLVNKNTTILKSIIEPIVKSGFTGVFV
ISSNPVDILTTIAQRISGFPKERVIGTGTSLDSMRLRVLLSKKLHLSVNVIDALMLGEHG
DTSFAAFNEITIGGKALNTITALSNTDKSEIEKAVHEAGSQIIANKGGYFLRNC

>fig|1598.593.peg.2023 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
ttgccaataaaggggctactttctacggaattgctaaatgcctctcgtatattacacgg
gcaatcatcgaaaaccgtagtcttgtcctaccgatttcggctccgcttgatggacagtac
ggaattaagggcctgtacttaggcacgcctgccatcattaatagtcaaggaatcggccag
gtcgttgaatatccattaacatcagatgaagttaaaaagatgcaacagtctgctgaagcg
atgcatcaagttttagctaagattgagatctaa

>fig|1598.593.peg.2023 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
MPIKGATFYGIAKCLSYITRAIIENRSLVLPISAPLDGQYGIKGLYLGTPAIINSQGIGQ
VVEYPLTSDEVKKMQQSAEAMHQVLAKIEI

>fig|1598.593.peg.2310 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
atgacacgaaaagttggagttatcggaatgggtaatgtggggtcaacagttgcccactat
attgtggcaatgggttttgcagatgacctagttctgatcgacaaaaacgaagcaaaggtt
aaggcggatgcgcttgattttgaagatgcgatggctaatttgcctttccataccaatatt
actgttaatgattacagtgcgttgaaggatacggatgtaattgtatccgcgttaggaaat
atcaaacttcaagataatcctaatgctgatcgttttgcggaacttccatttacccgccaa
gcagtaaaagaggttgcacaaaagattaaggaaagtggctttaaaggtaagattgtggct
attactaacccggttgatgttattacctcgctctaccaaaaaataactggccttccgaag
aaccatgtattaggaacagggaccttgcttgactctgcacgaatgaagcgggcagtggct
gaacggcttaatttagatccgcgttctgtagatggttataatcttggagagcatggtaat
tcgcaatttacagcatggtctactgttcgcgttcttggccgtcctttaactgaattagca
gataagcgtggattagacttagaagagcttgataaggaagctaagatgggtggctggact

gtctttcaagggaaaaagtatactaattatggggttgcaacggcggctgttaagcttgtc
aatgcaatttttatccgattcattgactgaattaccggtatcgaacttccgtgaagaatac
ggagtctacttgtcttacccagcggtcgttggtcgtgatggagttgtagagcaagcacaa
cttgacttgacagaagaagaactgcaaaagctgcaaacatcagccgatttcattaaggaa
aagtatcaagaaagtttgcaagcaaaagattaa

>fig|1598.593.peg.2310 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
MTRKVGVIGMGNVGSTVAHYIVAMGFADDLVLIDKNEAKVKADALDFEDAMANLPFHTNI
TVNDYSALKDTDVIVSALGNIKLQDNPNADRFAELPFTRQAVKEVAQKIKESGFKGKIVA
ITNPVDVITSLYQKITGLPKNHVLGTGTLLDSARMKRAVAERLNLDPRSVDGYNLGEHGN
SQFTAWSTVRVLGRPLTELADKRGLDLEELDKEAKMGGWTVFQGKKYTNYGVATAAVKLV
NAILSDSLTELPVSNFREEYGVYLSYPAVVGRDGVVEQAQLDLTEEELQKLQTSADFIKE
KYQESLQAKD

>fig|1598.593.peg.2455 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
atgtctaagaatcatcaaaaagttgttcttgttggtgacggtgctgtcggttcaagttac
gccttcgcaatggctcaacaaggaattgctgaagaatttgctattgttgatattatcaag
gaacggactgaaggggacgcaatggaccttgaagacgctactgctttcaccgctcctaag
aacatctactcagcagattacgacacttgcaaagatgctgatttagtagttattactgcc
ggtgctccacaaaagcctggtgaaactcgtcttcaattagttgataagaacttgaagatc
attaagtccgttgtagaaccaatcgttaagtctggttttgacggaatcttcttagttgca
gctaacccagttgacatccttacctatgctgttcaaaagctttctggcttccctaagaac
aaggttgttggttcaggtacttcacttgattccgcacgtcttcgggttgctcttggtaag
aagcttcatgttgaccctcgtgatgttattgctaacattatgggtgaacatggtgattcc
gaatttgctgcttactcaagtgctactgttggtggcaagccattacttgacatcgctaag
gatgaaggtatttcagaagatgaattacttaagattgaagatgatgttcgtaacaaagca
tacgaaatcattaaccgcaaaggtgctaccttctatggtgttgccactgctttaatgcgg
atttcaaaggctattcttcgtgatgaaaattctgttctccctatcggtgccccaatgaac
ggtgaatacggactcaacgacctttacatcggtactccagctgttgttaatgcttctggt
gttgcaaaggttattgaagttccacttaacgaccgtgaaaagaaggctatggccgactct
gctaagcaattggaagaagttgctaagaacggtatggcaaagttacaaggtaacaactaa

>fig|1598.593.peg.2455 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus reuteri PNW1]
MSKNHQKVVLVGDGAVGSSYAFAMAQQGIAEEFAIVDIIKERTEGDAMDLEDATAFTAPK
NIYSADYDTCKDADLVVITAGAPQKPGETRLQLVDKNLKIIKSVVEPIVKSGFDGIFLVA
ANPVDILTYAVQKLSGFPKNKVVGSGTSLDSARLRVALGKKLHVDPRDVIANIMGEHGDS
EFAAYSSATVGGKPLLDIAKDEGISEDELLKIEDDVRNKAYEIINRKGATFYGVATALMR
ISKAILRDENSVLPIGAPMNGEYGLNDLYIGTPAVVNASGVAKVIEVPLNDREKKAMADS
AKQLEEVAKNGMAKLQGNN

Appendix B

The sequence of nucleotide and amino acids of identified genes putatively involved in bioactive peptide production

>fig|1598.592.peg.1620 **bacteriocin helveticin J** [Lactobacillus reuteri PNWU1]
atggtcggaagtattacacctaaattggtttatcgcttgaatgggatgcaccatgtagta
gcacaagttggtgcagtaaatggtgatcatgtttttgctttgcaactgcttcacagcgcg
catgatgtattagtttatagaaagcataagggactgaccaaggatattaattatactaat
cctcacttagtaatgaccggctttggtcatacacaaacctgggttccagcaaatgataac
gatgaatatttcgttggtgctaaacctaattctggtaactggactacacaaattgcacgt
gtaaaatatccaagatta

>fig|1598.592.peg.1620 **bacteriocin helveticin J** [Lactobacillus reuteri PNWU1]
MVGSITPKLVYRLNGMHHVVAQVGAVNGDHVFALQLLHSAHDVLVYRKHKGLTKDINYTN
PHLVMTGFGHTQTWVPANDNDEYFVGAKPNSGNWTTQIARVKYPRL

>fig|1598.593.peg.298 **S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS** [Lactobacillus reuteri PNW1]
atggctaaagttgaaagttttacattagatcacacaaaggttaaggcgccttacgttcgt
ttaattaccgttgaagaagggccaaagggagacaagattagtaactatgatttacggtta
gttcaaccaaatgaaaatgcaattccaactgctggtttgcatacgattgaacacttgctt
gctggcttgcttcgtgaccgtttggatggcgtaattgattgctctccatttggttgccga
actggtttccacttaattacatggggtgaacactcaactactgaagttgccaaggccttg
aagtcttcacttgaagaaattgcctacaagactaagtgggaagatgtacaaggaacaacg
attgaatcttgcggtaactaccgtgatcattcattattctctgctaaggaatggtcaaag
aagattcttgatgaaggtatttcagataagccatttgaacgccacgtggttgactaa

>fig|1598.593.peg.298 **S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS** [Lactobacillus reuteri PNW1]
MAKVESFTLDHTKVKAPYVRLITVEEGPKGDKISNYDLRLVQPNENAIPTAGLHTIEHLL
AGLLRDRLDGVIDCSPFGCRTGFHLITWGEHSTTEVAKALKSSLEEIAYKTKWEDVQGTT
IESCGNYRDHSLFSAKEWSKKILDEGISDKPFERHVVD

Appendix C

The sequence of nucleotide and amino acids of identified genes putatively involved in adhesion

>fig|1598.593.peg.308 **Antiadhesin Pls, binding to squamous nasal epithelial cells** [Lactobacillus reuteri PNW1]
atgaagttaagtaagaagcaactagcagctgctttgatgactgctgttttagccggctcg
gtaataattggctcggtagcgattgcggaggcggataatgccagtggatcaccagaagag
caggctgatattactaattggattgccaataccccgaacaaatttctaataatatggcg
atgcaacacattaataccaataaccttaatggcactcgttatattattcaatggggtgat
actttatcggggatctcagccgcaactggaatctctgtggctaaactttgctacgataac
aacattcaaaatgctaatttaatttttgctggggatgttcttatcttaaaccgtgaagga
agcgttcctgctggttatgatcccaacgtaaatccgaatgttgttgcccagacaaagatt
acaatcaataatggcccaacaacagtgaatattaccgttcaaccacaatctgttgttaag
aattatgataattccgacaattctgatcattcgacaacaatttacaaggcaggcgcggta
aattcatttaacaacaacgacaatgataatgaacaaagtgctagtcaatccagctcaact
aagtctgctaagcatcagaagcatacttcatcagtcagtgcttctgacattgtgagcggc
ttgaatgatgccaataataatgacaaactgtcctttgacgaaggggatgggggtagtgac
gctgatgaacttaatgttgattcagcagctatcttaaaggatgctaagaagaatgactat
gatgcgacccttagtgaaatcgaaagtgccctaggttcaaaagccaagaaggatacgaca
gtttacattgaaaaagatggcaacgaaatccatgtctacgctagtgttaatgatgacgct
gataacagcaatagtagcagcactaaggatgatgaagatagcgatagcagcagtagtagt
agcgaaagtgaaagcagcgatgacagcagtcaaagcagcagtgatgatgaggatagcaac
agtcaagctactacaagtgaccacgacaccgacacttctgacgatgagtaa

>fig|1598.593.peg.308 **Antiadhesin Pls, binding to squamous nasal epithelial cells** [Lactobacillus reuteri PNW1]
MKLSKKQLAAALMTAVLAGSVIIGSVAIAEADNASGSPEEQADITNWIANTPEQISNNMA
MQHINTNNLNGTRYIIQWGDTLSGISAATGISVAKLCYDNNIQNANLIFAGDVLILNREG
SVPAGYDPNVNPNVVAQTKITINNGPTTVNITVQPQSVVKNYDNSDNSDHSTTIYKAGAV
NSFNNNDNDNEQSASQSSSTKSAKHQKHTSSVSASDIVSGLNDANNNDKLSFDEGDGGSD
ADELNVDSAAILKDAKKNDYDATLSEIESALGSKAKKDTTVYIEKDGNEIHVYASVNDDA
DNSNSSSTKDDEDSDSSSSSSESESSDDSSQSSSDDEDSNSQATTSDHDTDTSDDE

>fig|1598.593.peg.520 **Sortase A, LPXTG specific** [Lactobacillus reuteri PNW1]
gtgaaaaaagataaaaagcgatcatttgaatggttacggtggacagcggttgttgtactg
ttactggtatcagttgtcttaatttttaaccaacagattaagtcttacttagtagggagt
tataaacctgagattactcggcaaacggttcaaagcaaccaaaagaaaaaagcaacctat
gattttcaaagtgtcaaagatcttaacttgcaaacagctgccaaggctcgtgcaaataag
caatcgattaataccattggagcaattacggttccggctattaatatgacgattccaatt
gctaatggagttgataatacaactcttgcgttagcagcaggaacccttcgtccagacatg
aagatgggggaaggcaactatgcgttagctggtcataatatggcccatgggagcaaaatc
ctcttttctccattgtactatcatgctaaggtagggcagatgatttatatcaccaacatg
gatcgcgtttatgaatataagatttatcaacgtgaattcattgcggcaacccgggttgat
gtggtagacaatacgccggaaaagattataactttgattacttgtgacgctaccggggcc
aatcggttgatgatccgcggtaaatttgttaaatcagagccatttacgaaagcaccacaa
aatgtgcaaaagaattttagcgaaaaatatacgacaggtcgttaa

>fig|1598.593.peg.520 **Sortase A, LPXTG specific** [Lactobacillus reuteri PNW1]
MKKDKKRSFEWLRWTAVVVLLLVSVVLIFNQQIKSYLVGSYKPEITRQTVQSNQKKKATY
DFQSVKDLNLQTAAKARANKQSINTIGAITVPAINMTIPIANGVDNTTLALAAGTLRPDM
KMGEGNYALAGHNMAHGSKILFSPLYYHAKVGQMIYITNMDRVYEYKIYQREFIAATRVD
VVDNTPEKIITLITCDATGANRLMIRGKFVKSEPFTKAPQNVQKNFSEKYTTGR
>fig|1598.593.peg.390 **Tyrosine-protein kinase transmembrane modulator EpsC** [Lactobacillus reuteri PNW1]
atgaataaaaaagatttcagtataaaagatatttcgaaaattgtttggataaacgtatgt
attattttggttgcgacaatactatttggattaggcgggaatttatatgcaaaacataag
cagcatacaacttatgaatctgttaggaatattatgactacacgatcatatgatggtgct
gcagctaatgaagaacttcaagctgatttaagtcttggaaatacatatgcggaaatagta
gaaagcaatgatactgctaaggctgctcgcaagtatttacctaaaaatttaagaaagaaa
tacagttcagagcaaatttcatcaatgataaatgctgaaccaataatgcaaactactatt

gttaaggtaagtgcaaaagcaaataccgcaaaggattctgcagctatcgttaacgcagtt
gctaaggcatccgctaataagatacctcagaaagtaacttctgctggcaaaatttctctt
ttctctaaagctgtagctagcgaagcgaagagtaatacttcaccatcaactaaaaagctt
actttattaggagcggctattggatttttattaggccttgtgttatctttttcagctact
acttgggtttacttaattaagcggaaatag

>fig|1598.593.peg.390 **Tyrosine-protein kinase transmembrane modulator EpsC** [Lactobacillus reuteri PNW1]
MNKKDFSIKDISKIVWINVCIILVATILFGLGGNLYAKHKQHTTYESVRNIMTTRSYDGA
AANEELQADLSLGNTYAEIVESNDTAKAARKYLPKNLRKKYSSEQISSMINAEPIMQTTI
VKVSAKANTAKDSAAIVNAVAKASANKIPQKVTSAGKISLFSKAVASEAKSNTSPSTKKL
TLLGAAIGFLLGLVLSFSATTWVYLIKRK

>fig|1598.593.peg.1563 **Tyrosine-protein kinase transmembrane modulator EpsC** [Lactobacillus reuteri PNW1]
gtgaatagttcacaacaaacagcgaataatacaattgatttgcatcggttaatgatgctc
tgtcgtaaacatattaagatgttaattatctggacattacttgccggagtgctcgggtat
gtcgtcgcccagttcgtcgtggtaccaaagtatacggcgacgaccgaaatcttggttaac
caaaagcatgaaaataacgataatgggcaagcatataataatcaacaagctgatatccaa
atgatcaacacctataaggacatcattactaaccaagtgattctgagtaaggcaagtaag
caacttaagaatccagttcgtgttattaaacctgcacaaaaagcagtttatcggactaat
gcagatggtacgcggaagttgattaaggaagcccaaccggcagtcgttgaacgcggtggt
aagagctataatctttcaactgccgaattaaaagaggccatcagtgtccaaacccaacaa
aattcacaggtcttttctcttcaagttaaaactgatgatccacaagaatcagcagtagtg
gctaatgcagttgccaatgtcttcaagcaacaaattaagaagatcatgagtgtgaataat
gtaacaatcgtttcacgggcaagtacgccagatgaaccatcattcccgaataagaagtta
tttgccttagctggtgctgtattaggcttgattcttagtttcttatacatcctaatcggc
gacttaatggatactagtgtgcacgatgatgattaccttactaatgaattaggattaact
aatctggggcatgtgaaccacattgagatgagtcgggactttaaagttaataatcaagaa
agtcggcgccaaaataacggtaatcgacgggtttaa

>fig|1598.593.peg.1563 **Tyrosine-protein kinase transmembrane modulator EpsC** [Lactobacillus reuteri PNW1]
MNSSQQTANNTIDLHRLMMLCRKHIKMLIIWTLLAGVLGYVVAQFVVVPKYTATTEILVN
QKHENNDNGQAYNNQQADIQMINTYKDIITNQVILSKASKQLKNPVRVIKPAQKAVYRTN
ADGTRKLIKEAQPAVVERGGKSYNLSTAELKEAISVQTQQNSQVFSLQVKTDDPQESAVV
ANAVANVFKQQIKKIMSVNNVTIVSRASTPDEPSFPNKKLFALAGAVLGLILSFLYILIG
DLMDTSVHDDDYLTNELGLTNLGHVNHIEMSRDFKVNNQESRRQNNGNRRV

>fig|1598.593.peg.935 **ATP synthase epsilon chain (EC 3.6.3.14)** [Lactobacillus reuteri PNW1]
atggctgaaaatacttttaaggtcactattattactccagatggcaccgtctatgataat
gataaggttaccatgctcgttatgaacactgctggtggacaaatgggaatcatggctaac
cacgtaccattaattgctgcacttgaaatcagtacagttcgaattaaacattctgaaggc
actgatgaagttgcagcggttaacggtgggattattcaatttgatggtcaaaatgctaca
atcgcagctgatagtgctgaaatgcctgaagcaattgacgttgagcgggcacagagagct
aaaaagcgttctgagtctgcaatcgcagaagcaaagaagaagcataaccaaagcgactta
tcacgtgcagaagttcacctcaagcgtgcaattaaccgtttgaatgctgcttctaagcaa
cgcaatatctaa

>fig|1598.593.peg.935 **ATP synthase epsilon chain (EC 3.6.3.14)** [Lactobacillus reuteri PNW1]
MAENTFKVTIITPDGTVYDNDKVTMLVMNTAGGQMGIMANHVPLIAALEISTVRIKHSEG
TDEVAAVNGGIIQFDGQNATIAADSAEMPEAIDVERAQRAKKRSESAIAEAKKKHNQSDL
SRAEVHLKRAINRLNAASKQRNI

>fig|1598.593.peg.1562 **Tyrosine-protein kinase EpsD** (EC 2.7.10.2) [Lactobacillus reuteri PNW1]
atgtcattatttcatcgaacacaacaacaatcaacggataccatggataatggggcaaaa
ttgattactgttgcccatccgaagagcccaatttctgaacagttccggacgattcggacc
aatattaactttatggcaatcgataagccgattaagactttagcaatgacctctgccaat
gtaagtgaaggaaagtcaaccgtcaccgataatgtggcagttgtttgggcacaaactggt
caaaaagtattgttaattgattcggatttacgacgcccaactcttcacgcaacgtttagc
aagagtaatcaacacgggttaacgacgattctaattagtggtactaattccgttgattta
cgcgaaatcattcaacctagtggtgttgataaccttgatgtcttaaccgcagggcccatt
ccacctaatcccgcggaattattaaactcacaacggatgaaaacattactggatacggtt
aaaggtatttacgatatggtcattgtggatgtgccaccaatgttagaagttactgataca
caaattctttcgcgtcacctggatgcggttgtcttagttgtaaagcaagggcaaacccaa
aagttagccgttaagcgggccgttgaattattaaacttagcacatgctaatttacttggt
tatataatgaatgacgtcaatgctgatggcgatgctgcttatggttatggatatgggtat
ggctacggagaagataatggtaaatag

>fig|1598.593.peg.1562 **Tyrosine-protein kinase EpsD** (EC 2.7.10.2) [Lactobacillus reuteri PNW1]
MSLFHRTQQQSTDTMDNGAKLITVAHPKSPISEQFRTIRTNINFMAIDKPIKTLAMTSAN
VSEGKSTVTDNVAVVWAQTGQKVLLIDSDLRRPTLHATFSKSNQHGLTTILISGTNSVDL
REIIQPSGVDNLDVLTAGPIPPNPAELLNSQRMKTLLDTVKGIYDMVIVDVPPMLEVTDT
QILSRHLDAVVLVVKQGQTQKLAVKRAVELLNLAHANLLGYIMNDVNADGDAAYGYGYGY
GYGEDNGK

>fig|1598.593.peg.1731 **DNA polymerase III, epsilon subunit related 3'-5' exonuclease** [Lactobacillus reuteri PNW1]
atgatgaattttatagcaatggactttgagactgctaatggcaagcgttacagtgcatgt
tcacttgcactcacaatcgttcggaatggacaaattgccgacgaattttacaccctaatt
aatcctcatactaaattcttttggcgtaatactcagatccacggtattcatgagcgtgac
gtccaaaatgcgcctgattttccagaagtatgggaacatatcaaccaattctacactcct
gataaactggttattgcccataataatcggtttgataatagcgttttaagaaatacgtta
gagcattacaatatcgaagtccccgcctaccagacactcgatactgttgcttcaagccgc
cagttgattccaggactaacaaattataaacttaatactgtttgcgacgcgttaaatatt
gaccttcatcaccaccataatgctcttgatgatgcccaggcttgcgctaacattctgctt
tatcaaagtaagcattttaccccgcaacaaattcagccctttattaatttaatcggctaa

>fig|1598.593.peg.1731 **DNA polymerase III, epsilon subunit related 3'-5' exonuclease** [Lactobacillus reuteri PNW1]
MMNFIAMDFETANGKRYSACSLALTIVRNGQIADEFYTLINPHTKFFWRNTQIHGIHERD
VQNAPDFPEVWEHINQFYTPDKLVIAHNNRFDNSVLRNTLEHYNIEVPAYQTLDTVASSR
QLIPGLTNYKLNTVCDALNIDLHHHHNALDDAQACANILLYQSKHFTPQQIQPFINLIG

Appendix D

The sequences of nucleotide and amino acids of identified genes putatively involved in production of extracellular enzymes

Lipase

>fig|1598.593.peg.211 **Esterase/lipase-like protein** [Lactobacillus reuteri PNW1]
atgacaatacacgaattagcaaataacccaacgttaagcggccaagtacgcttgattgaa
aatattgtttatggtgcgatggatggtgaggcattacatatgtcgatcttagcaccgtgg
acgcaacgtttcccgaaacaatatcaaactgaacctcgaccattgattgtctttgttcaa
ggaagctcgtggcgaacaccaaaaatgggagaagaaattccacaactggttcaatttgtt
cgggccggttatattgtagcgactgttcaacaccgtagttcaattgatagccacccattt
cctgccttttgcaagatgttaagactgccattcgtttcttacgggccaatgcgcaaaaa
tatgcaattgatccgcaacaggttgcaatttgggggacttcctctggagccaatgcggca
atgctagtcggcttaacgggtgatgatccgcgctataaagttgacctttatcaagacgaa
tcggatgcagtagatgctgtggttagttgttttgccccaatggacgtggagaagacgttt
gagtatgatgctaatgttccaggaaataagttactgcaatattgcttattagggcctgat
gtatcaaagtggccagaaattgaaaagcaaatgagtcccttatatcaagtcaaagatggg
caaaactatccaccattcttattgttccacggagatgctgataaagttgttccatatgaa
cagatggaaaaaatgtatatgcggttgaaggataatggaaattctgttgaagcgtaccgg
gttaagggtgcgaaccatgaacgagatttctggagtccaacaatttataatattgtgcag
aagtttcttgacgatcaatttaaataa

>fig|1598.593.peg.211 **Esterase/lipase-like protein** [Lactobacillus reuteri PNW1]
MTIHELANNPTLSGQVRLIENIVYGAMDGEALHMSILAPWTQRFPKQYQTEPRPLIVFVQ
GSSWRTPKMGEEIPQLVQFVRAGYIVATVQHRSSIDSHPFPAFLQDVKTAIRFLRANAQK
YAIDPQQVAIWGTSSGANAAMLVGLTGDDPRYKVDLYQDESDAVDAVVSCFAPMDVEKTF
EYDANVPGNKLLQYCLLGPDVSKWPEIEKQMSPLYQVKDGQNYPPFLLFHGDADKVVPYE
QMEKMYMRLKDNGNSVEAYRVKGANHERDFWSPTIYNIVQKFLDDQFK

>fig|1598.593.peg.261 FIG006988: **Lipase/Acylhydrolase with GDSL-like motif** [Lactobacillus reuteri PNW1]
atgaaaaagtggactaaatggctattattatcgcttttggctattatgattattggtgga
ggatggtatactgttaaccactttacaaatttaactagtaatagttcaaaagttgttaaa
ccaaaatatgttgaaaagaaaaatgtaaagcttgttgcactaggtgattcccttactcac
ggtcaaggggatgaaactaataatagtgggtacgttggcgtaattaagggaaaaatcgaa
catcgttaccaccaaactaaggtgacaacagtcaattacggggtaacaggggaccgatcc
gaccagattcttgaccggttaaatcagcaatctcaattacgtagtgacttacggagcgcg
gatgtgattacgatgactgttggtggaaatgatctgatgcaaatcctggaaaaaaacgta
atgggctctgaacggaaagttaccagtagtgttgaaagtggcgaaaagacttatcaacaa
aaattaattaagctatttgatgcagttcgaaaggaaaatcctaaggctcctatttttgta
atgagtatttataatcctttctatacctatttcccagatgtaaaaattatcaacaactca
attaatcaatggaaccaaactacgcaagatacaatgaataattataagtcaatgtatttt
gtgaacattaataagttgatgtcatatggtcaatatcagactaagagtcagcaacaacag
ctgatcaaggaagaagagaaggctaaccaagggcaggtcagtcaaaagcgggtcattgaa
attatgaatcataaggataagaaccttaataaatatatttcaacagaagataatttccat
cctaatcatacgggatacgtcaaaattgctgaccaattatttaaggtaatgcaaaaacat
gatagctgggaattcacacggaggtaa

>fig|1598.593.peg.261 FIG006988: **Lipase/Acylhydrolase with GDSL-like motif** [Lactobacillus reuteri PNW1]
MKKWTKWLLLSLLAIMIIGGGWYTVNHFTNLTSNSSKVVKPKYVEKKNVKLVALGDSLTH
GQGDETNNSGYVGVIKGKIEHRYHQTKVTTVNYGVTGDRSDQILDRLNQQSQLRSDLRSA
DVITMTVGGNDLMQILEKNVMGSERKVTSSVESGEKTYQQKLIKLFDAVRKENPKAPIFV
MSIYNPFYTYFPDVKIINNSINQWNQTTQDTMNNYKSMYFVNINKLMSYGQYQTKSQQQQ
LIKEEEKANQGQVSQKRVIEIMNHKDKNLNKYISTEDNFHPNHTGYVKIADQLFKVMQKH
DSWEFTRR

>fig|1598.593.peg.630 **Esterase/lipase (EC 3.1.1.-)** [Lactobacillus reuteri PNW1]
gtggagaaaataaagaaatttaatactcaaattgctagtcatttggctaataatcttgag
aatagtttgcgagagttacgacgatgtggtgctcatcgttctagtaaagcaacctttgct
gaaatgggaattcaattaagcggatttaaaaacgatcttgcagaaaataataagtttaat
gccttagtggggaacgcatggattgacaacgaggctgattactacttgcctgctgctgta
gtgacggttgatgttcaacgtcaagaacaggaagtaacaacatatgtcttaaatgatcat
ggagataatcaacgagtaatcatgtacctaaccgggggagcctatattcagcgccccgat
aagactcattggcaatatttaaatcggttggcaatcgccacggatgcgaagatttatgtc
ccgatttattcgttggttccgcatgctacttaccgtaccgcctaccaagaaattgctagt
ctttatagtaaaatttatacccttatgcctgctagtaaagtaactattatgggcgattca
gctggtgggggattagcagctggcttttgtgaatatcttggtaaaaaggggcttccacag
cctggtcacctcatcttatttctccttggctcgattttgaccttacaaatcctctcatt
agcaaatatgaggataaagatgttacactagcagtgaacggtttgcgaaaaattggtacg
atgtgggccggtgatacggatcatcaagactatcgcttaagtcctttatatgggaatctt
gatcagttgcgcgatgtgactgtttttgtagggacaagggaaataatgtatcctgatgtg
accttgtttgttcaaaaattgcgtgacgctgggatcactgtaaactcatatacgggtcgt
ggactgtttcatatttacccgctttatcaaattccagaagcaaagggtgtaatgaaacgg
gtagttgcaataatcaataattga

>fig|1598.593.peg.630 **Esterase/lipase (EC 3.1.1.-)** [Lactobacillus reuteri PNW1]
MEKIKKFNTQIASHLANNLENSLRELRRCGAHRSSKATFAEMGIQLSGFKNDLAENNKFN
ALVGNAWIDNEADYYLPAAVVTVDVQRQEQEVTTYVLNDHGDNQRVIMYLTGGAYIQRPD
KTHWQYLNRLAIATDAKIYVPIYSLVPHATYRTAYQEIASLYSKIYTLMPASKVTIMGDS
AGGGLAAGFCEYLGKKGLPQPGHLILFSPWLDFDLTNPLISKYEDKDVTLAVNGLRKIGT
MWAGDTDHQDYRLSPLYGNLDQLRDVTVFVGTREIMYPDVTLFVQKLRDAGITVNSYTGR
GLFHIYPLYQIPEAKGVMKRVVAIINN

>fig|1598.593.peg.871 **lipase/esterase (putative)** [Lactobacillus reuteri PNW1]
atggaaattaaaagtgttaacttagatcaaccatattcgtctctagatatttatcatagt
aatactgataaagctttgcccggtcttgttattttaccaggaggcagttataaccagatc
atggagcgagattctgaacgggtggcattaacgtttgcaacccatgcatggcaaacattt
gttgtacgatatccggtagttgagcataagaattatgaagaagccaaaatagcggttcac
caagcatttgaatatatcgtcaaccatgcagctgaattagatgttgacgctgatcggttg
gggattattggcttttctgcaggaggccaaattgccgctgcatatagtaatgaaaaacta
acacacgctagattcgccgcattaggatatcctgttattcaacccttgattgatgaacgt
atgggggttacaacagagaatgtagcgaaattagtaaatccgcaaacaccaccaaccttt
atgtggggatcggcaaaagatgaactgactccctttgttgatcaccttcaagtatatgca
gatgcgttaattaagaatgatattccatatgaattacatgagtttggcactgggggacat
ggaatcgcgttagctaacgaatatactggtattgttaataatgatcgggtagataatcat
atgggaaagtggttcccgctatttcttgagtggttaactgaactgaatttaatttag

>fig|1598.593.peg.871 **lipase/esterase (putative)** [Lactobacillus reuteri PNW1]
MEIKSVNLDQPYSSLDIYHSNTDKALPGLVILPGGSYNQIMERDSERVALTFATHAWQTF
VVRYPVVEHKNYEEAKIAVHQAFEYIVNHAAELDVDADRLGIIGFSAGGQIAAAYSNEKL
THARFAALGYPVIQPLIDERMGVTTENVAKLVNPQTPPTFMWGSAKDELTPFVDHLQVYA
DALIKNDIPYELHEFGTGGHGIALANEYTGIVNNDRVDNHMGKWFPLFLEWLTELNLI

>fig|1598.593.peg.1687 **Esterase/lipase/thioesterase** [Lactobacillus reuteri PNW1]
atgaaacatacgcttaaagttgatcaagtacgtgacggtttatggctagattcagatatt
acgtatacgcaagttcctggatggcttggtaatacaacgcgagatttgaagctttcagtc
attcgacattttcaaactaatgatgatacacgttatccagtaatttttggtttgctggt
ggcggctggatggatactgaccacaatgttcatctgccgaatttggttgattttgctcgg
catggttacattgttgtcggcgtcgaatatcgtgatagcaacaaagttcagtttcctggg
caattagaagatgctaaggctgctattcgttatatgagagctaaggccaagcgcttccaa
gctgatcctaatcggtttattgtgatgggagaatcggccggtggacatatggcaagtatg
ctaggtgttactaacggccttaaccaatttgacaaaggtgctaatttagattactccagt
gatgttcaagtagcagttccttttatggtgtggttgatcccttaaccgctaaaacagga
agtgcatcaaacgattttgattttgtttaccgtaacttgcttggtgctgagcctgaaaac
gctcctgagcttgattctgccgcaaatcccctcacctatgtaaattctaattctacgccc

tttcttatctttcatgggacagaagatgtcgttgttccaattaaagatagtgaaaagctt
tatgatgcattagttgaaaacaacgttcctgctgaattatacgaaatcgaaggcgcaagt
cacatggatgtgaaattcctccaaccacaggtatttaagattgtgatggacttttagat
aagtatttaactcggtcatag

>fig|1598.593.peg.1687 **Esterase/lipase/thioesterase** [Lactobacillus reuteri PNW1]
MKHTLKVDQVRDGLWLDSDITYTQVPGWLGNTTRDLKLSVIRHFQTNDDTRYPVIFWFAG
GGWMDTDHNVHLPNLVDFARHGYIVVGVEYRDSNKVQFPGQLEDAKAAIRYMRAKAKRFQ
ADPNRFIVMGESAGGHMASMLGVTNGLNQFDKGANLDYSSDVQVAVPFYGVVDPLTAKTG
SASNDFDFVYRNLLGAEPENAPELDSAANPLTYVNSNSTPFLIFHGTEDVVVPIKDSEKL
YDALVENNVPAELYEIEGASHMDVKFLQPQVFKIVMDFLDKYLTRS

Protease

>fig|1598.593.peg.12 **SOS-response repressor and protease LexA (EC 3.4.21.88)** [Lactobacillus reuteri PNW1]
atggcaaagctagcaaaaaataaacaaatggccgtcctaaattatattcacaaacaagtt
gaagatcatggctatccaccaactgttcgtgaaatttgtagtgctgttggcctgtcttca
acttcaacagttcatggacacatttcccgcttaattgaacaaggattcttacaaaaagac
ccttctaagcctcgagcacttgagattacacccaagggacttgatattttaggtgtaaaa
ccgattcaaaaagaaattccaatgcttggtgttgttacggctggacaaccaattttagca
gtcgaaaatgctactgagtttttcccgatacctccttctattcaagataataatgatttg
tttatgcttaccattcgcggaactagtatgattaaagcagggatttttaatggcgaccaa
gtaatcgtgcgtaaacaatccactgctaaaaatggtgatatcgttattgcaatgaacgat
gataatgaagctacttgtaaacgattctataaagaaaaaacacgttttcgtttacagcca
gaaaatgatacgatggagccaatcttcttagacaatgttaaaatccttggtaaagtagtg
ggactatttcgtgatcatattttctaa

>fig|1598.593.peg.12 SOS**-response repressor and protease LexA (EC 3.4.21.88)** [Lactobacillus reuteri PNW1]
MAKLAKNKQMAVLNYIHKQVEDHGYPPTVREICSAVGLSSTSTVHGHISRLIEQGFLQKD
PSKPRALEITPKGLDILGVKPIQKEIPMLGVVTAGQPILAVENATEFFPIPPSIQDNNDL
FMLTIRGTSMIKAGIFNGDQVIVRKQSTAKNGDIVIAMNDDNEATCKRFYKEKTRFRLQP
ENDTMEPIFLDNVKILGKVVGLFRDHIF

>fig|1598.593.peg.129 **ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)** [Lactobacillus reuteri PNW1]
atgaatttagtacctactgtcattgagcaatcatctcgtggtgaacgtgcttatgacatc
tactcacggcttctgaaggaccgtattattatgctctctggtccaattgaagacgaaatg
gcaaattcaattgttgcgcaacttttattccttgatgcgcaagattcaactaaagatatt
tacttatacatcaattcacctggtggggttgttacctcaggaatggcaatttatgacact
atgaactttattaaggctgatgttcaaactattgtaatcggaatggctgcttctatggcc
agtgtattagtatcatctggtgctaagggtaagcgttttggattaccacactcacaagtt
ttgattcaccaaccatctggtggagctcaaggtcaacagactgaaatcgaaattgctgct
actgaaattttgaaaacacggaagatgcttaacggcattcttgctaagaattctggtcaa
cctatcgaaaagatccaggctgatactgaacgtgatcattacttgacagcacaagaagca
gtagattacggcttacttgatggtgtaatggaaaataattcaaaattaaagtaa

>fig|1598.593.peg.129 **ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)** [Lactobacillus reuteri PNW1]
MNLVPTVIEQSSRGERAYDIYSRLLKDRIIMLSGPIEDEMANSIVAQLLFLDAQDSTKDI
YLYINSPGGVVTSGMAIYDTMNFIKADVQTIVIGMAASMASVLVSSGAKGKRFGLPHSQV
LIHQPSGGAQGQQTEIEIAATEILKTRKMLNGILAKNSGQPIEKIQADTERDHYLTAQEA
VDYGLLDGVMENNSKLK

>fig|1598.593.peg.593 **ATP-dependent Clp protease, ATP-binding subunit ClpC**
[Lactobacillus reuteri PNW1]
atggataatatgttcacgccaagtgcaaagcatgttctagcaattgcacaagaacaagca
aaatactttaaacaccaagcagtagggactgaacaccttctgcttgccctctcaatggat
aaagatgggattgctaataaaatatttgaacaattttctattacaaacgatgatattcgt
gaagaaattgaacgcttaattggctatggaacgatggaaaatctgggagcttcagattat
ctcccatactcaccaaaagcaaagcaagtattatcgttagctggtcgagaagcccaacaa
atgcatgcattgaagattggcactgaacacttattattagccttaatcgctgatgaaagt
gtcctatcttcacggattttatatagtctagatgttgtgccacgacagatgagaaaagta
attttacggcggttagggattgctgatagtcagcaacgcaatcctaatcgtcaatcatct
cgtcgacggattcagcaaactggtacaccaacattggataaactagctcgtgatatgaca
gaacttgctcggaatggccagcttgatcctgtaattggtcgaaataaggaagttaaacgt
gtggagcagattcttagtcgtcgtaccaagaacaatcctgtgctgatcggcgaacctggt
gtagggaagacggcgattgctgaagggcttgctcagcggatggttgatggtaaggttcca
gctgaattggctaataaacgtttgatgatgcttgatatgggatcattagttgcgggtacc
aagtatcgaggagaatttgaagatcgccttaagaaagtgattgatgagattcaaaacgac
gggcatgtgattctttttattgatgaactccataccttaattggtgctggtggcgctgaa
ggtgcaattgatgcttccaatattttgaaaccggccttagcgcgaggcgagttgcaaaca
attggtgctactacccttgacgaatatcagaaatatatagaatctgatgcagcacttgaa
cgacgctttgctactgttcaagttgatgagccaactactgaccaaaccctgcaaatcctg
cgaggactacgaccgaagtatgaagaacaccaccatgctaagattactgatgaagcgttg
gaagaagcggttaaattgtcggatcgttatatttcagatcgtttcttgccggataaagca
attgatcttattgatgaatcggcagctatggttcggattgatgctgaagataagaaaaat
catcagccttcattagaaagtcagttagaagatttgcgaacccaaaaagaagaagcaatt
gataatcaagactttgatcgtgcggctactcttcgtcaacaagaattagcactaaaagat
aagattgatcgcaaaaagcaacgtactcaacaaaaggattctcataactataaattgaaa
gtaactggtgaaaacattgcacaagttgttgctgaatggacaggagttcctttaactcaa
ttgaagaagagcgagagtgaacgattggttaacttggaaaaggttctgcaccaacgagta
attggtcaagatgaagcagttaccgtagtcgctaaggcaattcggcgtgcacgaagcggg
cttaaggatcctagtcggccgattggttcctttatgtttttaggaccgactggggtagga
aaaacagagcttgccaaggcattatccgctgcaatgtttggctcggaagataatatgatc
cggattgatatgtcagaatatatggaaaaatatagtactagtcgcttgattggtgctgct
ccaggatatgtcggctatgacgagggtggccaattaactgaaaaagtacggcaacatcca
tactcagttgtcttattagatgaagctgaaaaggcacatccggatgtatttaatttatta
cttcaagttttagatgatggttacttaactgatgcaaaaggtcggcgcgttgattttaga
aataccattattattatgacttctaaccttggagcaactcagcttcaggatgagaaagag
gttggttttggggcaaaagatatgtcacaagactataatgcgatggcggcggcgattaag
caacaaatgaggttatacttccgcccagaatttcttaatcggattgatgaaacgattatc
ttccattcattacaaaagaaagaacttcatcaaattgttaaactcatggttaatgattta
aataagcgggtaagcgagcaaggtattaacttgaaagttacgcctgctgcaattgatgtg
atcgcaaagcttggttacaatcccgcttatggagctcgtccgcttcgtcgtgctttacaa
gatcatgttgaggatgatttgagtactggactgcttagtggtgaaatcaatgtgggggat
gacgtaacagtaggtgcgcatcaaggtaaaattacttttaaagtaaagaagccggatgaa
gataaagctgttgaattaaaattgaataaataa

>fig|1598.593.peg.593 **ATP-dependent Clp protease, ATP-binding subunit ClpC**
[Lactobacillus reuteri PNW1]
MDNMFTPSAKHVLAIAQEQAKYFKHQAVGTEHLLLALSMDKDGIANKIFEQFSITNDDIR
EEIERLIGYGTMENLGASDYLPYSPKAKQVLSLAGREAQQMHALKIGTEHLLLALIADES
VLSSRILYSLDVVPRQMRKVILRRLGIADSQQRNPNRQSSRRRIQQTGTPTLDKLARDMT
ELARNGQLDPVIGRNKEVKRVEQILSRRTKNNPVLIGEPGVGKTAIAEGLAQRMVDGKVP
AELANKRLMMLDMGSLVAGTKYRGEFEDRLKKVIDEIQNDGHVILFIDELHTLIGAGGAE
GAIDASNILKPALARGELQTIGATTLDEYQKYIESDAALERRFATVQVDEPTTDQTLQIL
RGLRPKYEEHHHAKITDEALEEAVKLSDRYISDRFLPDKAIDLIDESAAMVRIDAEDKKN
HQPSLESQLEDLRTQKEEAIDNQDFDRAATLRQQELALKDKIDRKKQRTQQKDSHNYKLK
VTGENIAQVVAEWTGVPLTQLKKSESERLVNLEKVLHQRVIGQDEAVTVVAKAIRRARSG
LKDPSRPIGSFMFLGPTGVGKTELAKALSAAMFGSEDNMIRIDMSEYMEKYSTSRLIGAA
PGYVGYDEGGQLTEKVRQHPYSVVLLDEAEKAHPDVFNLLLQVLDDGYLTDAKGRRVDFR
NTIIIMTSNLGATQLQDEKEVGFGAKDMSQDYNAMAAAIKQQMRLYFRPEFLNRIDETII
FHSLQKKELHQIVKLMVNDLNKRVSEQGINLKVTPAAIDVIAKLGYNPAYGARPLRRALQ
DHVEDDLSTGLLSGEINVGDDVTVGAHQGKITFKVKKPDEDKAVELKLNK

>fig|1598.593.peg.1200 **putative Zn-dependent protease** [Lactobacillus reuteri PNW1]
gtgcttactcaatcgtggctacaccgaataaccggtaaagaagttgcaaccccaactaaa
caaaacgctgaccaacaaactggggcacaagatacacccgctgatgcacgctgggaacaa
aactctgcaacgatctatgttaatatcagtaatccagtattaaagaatgcaacagaaacc
gcaattgcccaatggaataacacgaaggcattcacatttaaaatcgttaatgataagaat
gcaaatattagtgtttccgctgttaatgatccaaataacggggcagcgggacttaccaat
acaagcatgaattccgcaactggatattatctgcacgcaacagtagagctaaattcttat
tacctattaaatcctgcctttggctactcacaagaacgaatcgtaaatactgctgagcat
gaattagggcatgcaattggtctacagcacacgaacaagatttctgtgatgcagccagct
ggatcatattatcccattcaatcacgagacatagaggctgtgaaggctttatattcacga
actccacaaccaatcatcgccgaaaacaattccaacagatag

>fig|1598.593.peg.1200 **putative Zn-dependent protease** [Lactobacillus reuteri PNW1]
MLTQSWLHRITGKEVATPTKQNADQQTGAQDTPADARWEQNSATIYVNISNPVLKNATET
AIAQWNNTKAFTFKIVNDKNANISVSAVNDPNNGAAGLTNTSMNSATGYYLHATVELNSY
YLLNPAFGYSQERIVNTAEHELGHAIGLQHTNKISVMQPAGSYYPIQSRDIEAVKALYSR
TPQPIIAENNSNR

>fig|1598.593.peg.1467 **Membrane protease family protein BA0301** [Lactobacillus reuteri PNW1]
atgaaagaaaaagcagcctttcacgttaacggctacctcgggttactcggggcgattatt
atcggtttaattagtttgtggctgatgtttgttggttttacgcgtaattttccggtgatt
ctgacaattggaattatttattcttgattgtgattctttttagtacatcattgacaatt
attcagccaaatgaagccaaagttttaacttttttttggtaattatattgggactattcgt
gatgcgggcttatttatgacggtaccatttacaaataaggagactgtttctcttcgtgtc
tgcaattttaatagtcaaattttaaaggtcaatgattcgaaaggtaatccagtcgaaatt
gcggccgttattgtttacaaagttgtcgataccgcaaaagcactcttttcggttgatgat
tatgagcaatttgttcaaattcaaagtgagtctgcggttcgtcatgttgctagtgaatat
ccatatgatagttttgaagatcaagatgcaatcactcttcgcgggaatccaaccgaagta
tcagaacgtttaactgctgaactgcaagaacggttaaatgtcgcgggggtcaaaattatt
gaaacacggttgacccacttagcctacgcaactgaaatagcgagtgccatgttgcaaaaa
caacagtcatcagcaattctatcagcgcgcaagataatcgttgagggtgctgtttcaatt
actgaagaagcgattgaacgactaagcaaggaagctaaccttgatttaacagatgagcaa
cgtttgcaaatcatcaataatatcatggtagcaattattagtgagcggggaacacaacca
gtaataaatacagggacacaaggataa

>fig|1598.593.peg.1467 **Membrane protease family protein BA0301** [Lactobacillus reuteri PNW1]
MKEKAAFHVNGYLGLLGAIIIGLISLWLMFVGFTRNFPVILTIGIILFLIVILFSTSLTI
IQPNEAKVLTFFGNYIGTIRDAGLFMTVPFTNKETVSLRVCNFNSQILKVNDSKGNPVEI
AAVIVYKVVDTAKALFSVDDYEQFVQIQSESAVRHVASEYPYDSFEDQDAITLRGNPTEV
SERLTAELQERLNVAGVKIIETRLTHLAYATEIASAMLQKQQSSAILSARKIIVEGAVSI
TEEAIERLSKEANLDLTDEQRLQIINNIMVAIISERGTQPVINTGTQG

>fig|1598.593.peg.1525 **Prophage Clp protease-like protein** [Lactobacillus reuteri PNW1]
gtgaagaagatgacgattaacggagatattgtcgacgacgataccgcatggatgtataac
ttctttggtatgagttgtacttcaccaaagtctgtcagagatgtgttacaagattctgac
gatgacgaaaccatccccgaagatttaattgttgatatttcttccggtggtggtgatgtc
tttgcaggtagtgaaatttacacgttactacgcaactataaaggaaaagttgttgttaac
gtttacggcatggccgcatctgctgcttctgtcattgcaatggcaggggatgaagtcaac
atgtcaccaactgctcaaatgatgattcataaagcgtggtctgtaaatcaaggcaacgct
gatgatcacgaacacgaagcaaaagtgttggattcaatcgaccaatcaattgttaatgca
tatgttgataagaccggtctaaagcgtgatgacattttacagatgatgcaaaatgaaacg
tggatgaccgctcaagaagccgttgataaaggctttgcaaatggcattatgttccacaat
gatgatgacgatgaagacgacacagacgatcacgaggatgctttacaggtcgccgctgct
actcacagcattcctaaaaaggacgctgttaagaagttcatgatgttactgcatagcgat

aaagctaaggaaactgtaaagaaacaacaaaataactcacaaaataagccaacttcttta
tatgacaagaagctggctttgcttttaaacaaaaacattactgaaaaggagaattaa

>fig|1598.593.peg.1525 **Prophage Clp protease-like protein** [Lactobacillus reuteri PNW1]
MKKMTINGDIVDDDTAWMYNFFGMSCTSPKSVRDVLQDSDDDETIPEDLIVDISSGGGDV
FAGSEIYTLLRNYKGKVVVNVYGMAASAASVIAMAGDEVNMSPTAQMMIHKAWSVNQGNA
DDHEHEAKVLDSIDQSIVNAYVDKTGLKRDDILQMMQNETWMTAQEAVDKGFANGIMFHN
DDDDEDDTDDHEDALQVAAATHSIPKKDAVKKFMMLLHSDKAKETVKKQQNNSQNKPTSL
YDKKLALLLNKNITEKEN

>fig|1598.593.peg.1800 **Lon-like protease with PDZ domain** [Lactobacillus reuteri PNW1]
atgaaaaagcaagataatgtaatattacgacgaataggaattattttagggcttgtcctt
ttactaggctgctttttgttctggcccctaaattcctatatcgaaagtccgggaacggca
gctgatttacagtcttttgttaaaataaagggacaccccgatcgatataaggggagtttt
atgttgacatcggtggcgattcaacgcgcccatcctgcaacatatttatatgccaaaatg
atgccctatatgtcaatcgagagtgcagaggatgtaactggcggtcaaaatagtgcaacg
tatgaccgtgtccaaaaatttttatatggatagctcaattaacgaggcaattgcagttgct
tacaatgccgctcaccaaaaagtaacgcgacgctatctaggaatttatgtattgcaagtt
cagcctaattctaaattcaaacatgatattcatgttggggatacaattacaaaagtagat
ggtcatcactttaataccgctcaaggctttcaaaaatatattggtgctaaaaaagttggc
actccgttagcggttacttatactcgagatgggaaaactaaaactgtcactcatccgctt
gttaaaattgctaatactaataagccgggaattgggatcatccttactaacaatatgcaa
gttaagactaagatcccagtaaaggttgatgctggtcaatacggtgggccatccggtggc
ttaatgttcagtttacaaatatatcaacaaattagtggaaaagatttgcaacggggacgt
aaaattgccggaactggaacgataaattcagacgggaccgttggagaaattggtggaatt
gacaagaaagtaatcgccgcgcaccgtgcaggagccactattttctttgccccttatata
aaaccaacgaaagagattcttaagtatgaagaaggtcatctgactaattatcaaatggct
aaaaaagcagctaaaaaatatgcaccgggaatgaaggttgtcccagtcacttcctttgat
gacgcggtgaaatatttacaaacgcataagtaa

>fig|1598.593.peg.1800 **Lon-like protease with PDZ domain** [Lactobacillus reuteri PNW1]
MKKQDNVILRRIGIILGLVLLLGCFLFWPLNSYIESPGTAADLQSFVKIKGHPDRYKGSF
MLTSVAIQRAHPATYLYAKMMPYMSIESAEDVTGGQNSATYDRVQKFYMDSSINEAIAVA
YNAAHQKVTRRYLGIYVLQVQPNSKFKHDIHVGDTITKVDGHHFNTAQGFQKYIGAKKVG
TPLAVTYTRDGKTKTVTHPLVKIANTNKPGIGIILTNNMQVKTKIPVKVDAGQYGGPSGG
LMFSLQIYQQISGKDLQRGRKIAGTGTINSDGTVGEIGGIDKKVIAAHRAGATIFFAPYI
KPTKEILKYEEGHLTNYQMAKKAAKKYAPGMKVVPVTSFDDAVKYLQTHK

>fig|1598.593.peg.1811 **ATP-dependent Clp protease ATP-binding subunit ClpX** [Lactobacillus reuteri PNW1]
atgtttgaagataccactggtatggattcagttcactgttcattttgtggaaagtcccaa
gatgaagtgaaaaaaattgttgctggtcctggcgtctatatttgtaacgaatgtgttgac
ctttgtaaacaaattattgatcaggaattagcagaagatgaagctaaaaaagctttccgt
gtgccaactccaggagagattgttaacgaactggatgactatgtaattggtcaaggtgat
gctaaaaaaacattagcagttgctgtttataaccactataagcgggttaatgcaatgatg
agtggtgataataatgatacagaacttcaaaagagcaacattgccgtaattggtccaact
ggttctgggaaaacctatcttgcccagtcacttgcccgcattctcaatgttccatttgcg
attgcagatgctacaactttaactgaagctggatacgttggtgaagatgttgagaatatt
atccttaaactacttcaggctgctgactttgatgtagaacgtgctgaaaaaggaatcatt
tacattgacgaaattgataaaatcgcaaagaagagtgaaaatgtatcaattacacgtgat
gtttctggagaaggggttcaacaagctcttttaaagattcttgaaggaacaattgctaat
gtaccgcctcagggaggacgtaagcatccacagcaagaatttatccaagtcgatacaaag
aatatcctctttattgtgggggagcctttgatgggatcgaaacaattgttaaggaacga
cttggtgataagacaattggatttggaacagactcacgagaagctgaagaggttacggat
aaaaatattttgcaacatgttattccagaagaccttcttaagtttggcttgattccagaa
tttatcggacgattacctgtgatgactgctcttgaaaaacttgatgaagatgaccttgtt

cgaatccttactgagccgaagaatgcattagttaagcaatatcaagagttaattcgcctt
gatggaagtcagttgacctttactgatggtgcattacgagcaatggcacaactagcaatc
aagcggaatacaggtgctcgtggtctccgttcgattattgaagatgtaatgcgggatgta
atgtttgacttgccaagtcgtaaagatgtagaaaaggtaattattgataaacgatgtgta
acgcaacatacagaacctcgttacgttttaaaggatgaaaaagcttcttaa

>fig|1598.593.peg.1811 **ATP-dependent Clp protease ATP-binding subunit ClpX**
[Lactobacillus reuteri PNW1]
MFEDTTGMDSVHCSFCGKSQDEVKKIVAGPGVYICNECVDLCKQIIDQELAEDEAKKAFR
VPTPGEIVNELDDYVIGQGDAKKTLAVAVYNHYKRVNAMMSGDNNDTELQKSNIAVIGPT
GSGKTYLAQSLARILNVPFAIADATTLTEAGYVGEDVENIILKLLQAADFDVERAEKGII
YIDEIDKIAKKSENVSITRDVSGEGVQQALLKILEGTIANVPPQGGRKHPQQEFIQVDTK
NILFIVGGAFDGIETIVKERLGDKTIGFGTDSREAEEVTDKNILQHVIPEDLLKFGLIPE
FIGRLPVMTALEKLDEDDLVRILTEPKNALVKQYQELIRLDGSQLTFTDGALRAMAQLAI
KRNTGARGLRSIIEDVMRDVMFDLPSRKDVEKVIIDKRCVTQHTEPRYVLKDEKAS

>fig|1598.593.peg.1891 **ATP-dependent Clp protease, ATP-binding subunit ClpE**
[Lactobacillus reuteri PNW1]
atgcaatgtcaatactgtcatcaaaatccagccacaattcatcttcaaatgaattttaat
ggacaacggattcaaatagacctctgccaaaattgttatcaaaaattacaaaatctacaa
acagatatgatgaatggaggtaacggaatgaataatttcggttttggaagcctcgaagac
ttcatgaacgcaatgaacaatatgcaaagtcaagctgccggtgctaatggtcaaaacatg
aacggccaagctcaacgacaaggtggcggccggaacggaaaaggaatccttggtcaatat
gggatcaaccttactgatcttgctcgccaaggtaaaattgacccagtcatcggacgcgac
aatgaaattaaacgagtaattgaaattttaaaccgtcgaaccaagaataacccagtcttg
attggtgaagctggggttggtaagaccgcggttgtcgaaggcctagctcaagcaatcgtt
agcggtcaagttcctgaaaagcttgctaacaaggaaattatccgactcgatgttgtttcc
ttagttcaaggtactggaattcggggacaatttgaaaaacggatgcaacaattaatggaa
gaagttcgtaagaataagaacatcattctatttattgatgaaatccacgaaatcatgggt
gcgggaaatgctgaaggcggcatggacgctggaaatgttcttaagcccgcccttgcccgt
ggtgacttccaattagttggtgccactactcttaatgaatatcgaaagatcgaaaaagat
gcagctcttgcacgacggttccaaccagttgaagttgatgaaccatccgttgaagaaaca
atccgcatcttaaacggaatcaaaaatcgttatcaagattaccaccatgttaaatacaca
gatgacgcaattgtagcagctgctaaactttctgaccgctacattcaagatcgttactta
cctgacaaagctatcgacttacttgatgaagcgggatcaaagaagaacttaacgcttaaa
aatgtggatccaaacgcaatcgaaaatgaaatccacacagctgaagcacacaagcaacaa
gcagctgataaccaagactatgaaaaagcagctttctaccgcgatcaagtcgctaaactt
gaaaaggcaaagaaagaagctgaagaaaaccatactgaagattctgcaactgttaccgtt
aaggatatgcagagaatcgttgaagaacggacaaatattccagttggtgacctgcaaaag
caagaagaaaatcaacttcgcgaccttgataagaagcttgatgaacacgttattggtcaa
acccaagcagtggataaggttgcccgtgccattcgtcgtaaccggatcggtttgaacaag
tctggtcggccaattggtagtttcctctttgttggtcctacgggggttggtaagactgaa
acggctaagcaacttgccctccaattgtttggttctaaggatgccatgattcggtttgat
atgtctgaatacatggacaagacctctacttctaaattaatcggggctgctcctggttat
gttggttacgaagaagctggccagttgactgaacaagtacggcggcacccttatagcttg
atcttacttgatgaagttgaaaaggctcacccagatgttatgcacatgttcttacaaatt
ctggatgatggtcgtttaactgattctcaaggacgcactgttagcttcaaggatacaatt
attatcatgacatctaatgccggaactggtgattcagaagcaagtgttggtttcggtgct
gagtctaatggtagtactcattccatcattgacaagttgacaaactacttcaagccagaa
ttcttaaaccggtttgatgacatcgttcaattcaatgccctctccaaggatgacttgatg
aagatcgttaacttaatgattgatgatgttaataacatgcttgctgataagaacttgcac
atcgaagtcactaacaacgttgaagaaaaattagttgacgtgggctttgatccaaagatg
ggtgctcgtcctcttcgtcgggtaatccaagaacaaatcgaagaccggattgctgattac
gttcttgatcacagtgacgctcataatttagttgctaaacttgatgataatggtgacatt
gttgtcgaagaaactgaagaagtaccaacaattgctaaaaaataa

>fig|1598.593.peg.1891 **ATP-dependent Clp protease, ATP-binding subunit ClpE**
[Lactobacillus reuteri PNW1]
MQCQYCHQNPATIHLQMNFNGQRIQIDLCQNCYQKLQNLQTDMMNGGNGMNNFGFGSLED
FMNAMNNMQSQAAGANGQNMNGQAQRQGGGRNGKGILGQYGINLTDLARQGKIDPVIGRD
NEIKRVIEILNRRTKNNPVLIGEAGVGKTAVVEGLAQAIVSGQVPEKLANKEIIRLDVVS

LVQGTGIRGQFEKRMQQLMEEVRKNKNIILFIDEIHEIMGAGNAEGGMDAGNVLKPALAR
GDFQLVGATTLNEYRKIEKDAALARRFQPVEVDEPSVEETIRILNGIKNRYQDYHHVKYT
DDAIVAAAKLSDRYIQDRYLPDKAIDLLDEAGSKKNLTLKNVDPNAIENEIHTAEAHKQQ
AADNQDYEKAAFYRDQVAKLEKAKKEAEENHTEDSATVTVKDMQRIVEERTNIPVGDLQK
QEENQLRDLDKKLDEHVIGQTQAVDKVARAIRRNRIGLNKSGRPIGSFLFVGPTGVGKTE
TAKQLALQLFGSKDAMIRFDMSEYMDKTSTSKLIGAAPGYVGYEEAGQLTEQVRRHPYSL
ILLDEVEKAHPDVMHMFLQILDDGRLTDSQGRTVSFKDTIIIMTSNAGTGDSEASVGFGA
ESNGSTHSIIDKLTNYFKPEFLNRFDDIVQFNALSKDDLMKIVNLMIDDVNNMLADKNLH
IEVTNNVEEKLVDVGFDPKMGARPLRRVIQEQIEDRIADYVLDHSDAHNLVAKLDDNGDI
VVEETEEVPTIAKK

>fig|1598.593.peg.1958 **FIG056164: rhomboid family serine protease** [Lactobacillus reuteri PNW1]
atgcggacgcagggactaagattagcgcccgtgacgcttaccttaataatctttcaggta
ttagtttattgctggttagtttatgcaggtggctcaactaatacagtaaccctcctgaat
atgggggctcgtagtacgcccttaatcagggaaggtgaatggtggcgcttagtatcacct
gtgttccttcatgttggtttatcacacttagtagtcaatagcgttacgcttttatatatc
ggccgttatattgaagaatttttcggtcattggcgaatggtagtcatatactttgtcagt
gcgctttttggtaactttaccagcgcggtcttcatgccatcgacaatttcagctggtgct
agtactgctatttttggattatttggcgcatttttaatgttaggcgtttgttttcgccat
aacgttattgttcgcgtcttaagtcgtactttttgttatttgttattattaatattgtg
atggatttttttcctgtcaggagttgacttgataggacatattggcggccttttggtggc
tttttcattgcatttattgttggtgctcccatgctaggaactgttgatcacctaaaacag
tttttaagtggagctgtattaacggtaagtttggtaattttgactctagaattgaagtga

>fig|1598.593.peg.1958 **FIG056164: rhomboid family serine protease** [Lactobacillus reuteri PNW1]
MRTQGLRLAPVTLTLIIFQVLVYCWLVYAGGSTNTVTLLNMGARSTPLIREGEWWRLVSP
VFLHVGLSHLVVNSVTLLYIGRYIEEFFGHWRMVVIYFVSALFGNFTSAVFMPSTISAGA
STAIFGLFGAFLMLGVCFRHNVIVRVLSRTFLLFVIINIVMDFFLSGVDLIGHIGGLFGG
FFIAFIVGAPMLGTVDHLKQFLSGAVLTVSLVILTLELK

>fig|1598.593.peg.2181 **Uncharacterized zinc protease YmfH** [Lactobacillus reuteri PNW1]
atggatagagaagtttatcaaaattttaaccagtccatttaccgtgaacaattatcaaat
ggtttgaaagtccagctcttaccaatggaaggctaccataagacatatgctattttaaca
gcagattttggttccattgataatcattttataccgtaccatcaaaagaaggcgattact
gttccagacggggttgcccacttccttgaacataaaatgtttgagaaaaaagatcatgat
gcctttgacttgttcggtaaactaggagctgattcaaatgcctttactagttttacccaa
acaagttatctttttttcgacgaccagtaatctccatgagaatttagatgttctattggat
tttgttcaagatccatactttactgcagaaacggtgaagaaagagcagggaattattggc
caagaaattcaaatgtatgaggatgacccgagttggcggctttacttaggtattttaggt
aatttgtaccccaaagatccaatgcggattgatattgcgggaacggttgaatcaatcagt
catattacaccagaaattctgatggatagttatcggactttctatcaaccaaccaatatg
aacctttttccttgtaggacgcttagatccagaagaaacaatggcatggatcaagcaaaat
caagaacaaaagattttcgctcccgcagagacaccccaacggttatttagcctgaacgat
ccaactgcgcatgatgtaattccattccgttcattaacgatggatattgttcgtcctaaa
gtgatggtaggcttacgagggaccaagcaatttgatgatggtaaagaacggctccactac
aaattagcaattgatcttttgttagatgttttgtttgacgatacttcagataactatttg
cgtttgtacaacaatgaaacattggatgacacgtttagctacaactttgaaatgcaacgg
ggctttcattttgcgtactttagctctgatactgatcagatggaacgctttgcagatgag
gttattgatattcttgaaagtgctgatcagcaaattgaagcggcgcggacaaggtttgag
ggaattaaaaaggcagaactagggcggttaattggcttattagattcgccagaagcaatt
gctaatcgttatgctggagacctctttgctggcgctagcttgatggatgagatagcgacc
ttggaaactattacgattgatgatctttatcaagttgcaaaagagtttattactccgcaa
ggtatttcggtataccaagttgttccgcaacatcaataa

>fig|1598.593.peg.2181 **Uncharacterized zinc protease YmfH** [Lactobacillus reuteri PNW1]
MDREVYQNFNQSIYREQLSNGLKVQLLPMEGYHKTYAILTADFGSIDNHFIPYHQKKAIT
VPDGVAHFLEHKMFEKKDHDAFDLFGKLGADSNAFTSFTQTSYLFSTTSNLHENLDVLLD
FVQDPYFTAETVKKEQGIIGQEIQMYEDDPSWRLYLGILGNLYPKDPMRIDIAGTVESIS
HITPEILMDSYRTFYQPTNMNLFLVGRLDPEETMAWIKQNQEQKIFAPAETPQRLFSLND
PTAHDVIPFRSLTMDIVRPKVMVGLRGTKQFDDGKERLHYKLAIDLLLDVLFDDTSDNYL
RLYNNETLDDTFSYNFEMQRGFHFAYFSSDTDQMERFADEVIDILESADQQIEAARTRFE
GIKKAELGRLIGLLDSPEAIANRYAGDLFAGASLMDEIATLETITIDDLYQVAKEFITPQ
GISVYQVVPQHQ

>fig|1598.593.peg.2182 **FIG001621: Zinc protease** [Lactobacillus reuteri PNW1]
gtggattttaatcttgctcgtggcgtggacttgcacattatacccacaaaacaatttaag
atgaatcacgtcttaatagattttgcgactccgcaaacaccaacgaatgctactgcacga
aatttattggcaaacctattagagaccagcacgcatcgctatccaacacagacggctttg
gctcgtcaattggcatctttatatggtgcctatgttaatttatatgttaaccggctgggg
acattgcatactgtccgattacgagcgagttttgtcaataatcgctttgttgatgaagat
ttatttgaaaagattagtggcttaataaatgagatcattttccatccgctaatcgatgac
ggggaatttgatggtcctacttttagaattcaatctaataatttaaaaagtgcgataaag
tctctttatgacgataaacaatttttggctaatcaacgtcttatggacttgtattaccaa
aatgattctgtaatgaaggtacctagtttcggtcaaattgctgatcttgagaacttaaat
gctaaaagtttagtttcaacgtatcattcaatgattaatgaagataaaattgatattttt
gtgttaggtgacattgatccgatgcgtgctcagcaagttattgctaagttgccttttgaa
gatcgcgatattgttaacaagtcacctttatatcatcaagcattgtatgatcaagttcag
agaaaaacagaatatcaacaagtaaatcaggcaaagttaaatttggcatattctttgcct
gtctattatcatgacgccgattactatgctggcttggtttttaatggacttttggtggc
acgccatattcaaagctttttacgaatgtccgtgaaaaagctagtttagcttattatgct
tctagccggcttcttccattcaatgggattgtaagtgttcaaacgggaattcaagcgagt
gatcaagaaaaagttcaaaatatgattcaggaacaattaatcgctttacaaaatggtgat
tttactactgagacgctaagtgaagttcaagatagtttgattaaccaataccgggcaggg
catgatttggcaagtaatgttttggaacaacaattagttaccaaattagttaatgaatcg
gataagaactttattaccgaaataaaaaaagtaaccgtagctgacgttatgcgtgttgct
aaacagatgaagcttcaggcagtttatttattaagtggtgagaaataa

>fig|1598.593.peg.2182 **FIG001621: Zinc protease** [Lactobacillus reuteri PNW1]
MDFNLARGVDLHIIPTKQFKMNHVLIDFATPQTPTNATARNLLANLLETSTHRYPTQTAL
ARQLASLYGAYVNLYVNRLGTLHTVRLRASFVNNRFVDEDLFEKISGLINEIIFHPLIDD
GEFDGPTFRIQSNNLKSAIKSLYDDKQFLANQRLMDLYYQNDSVMKVPSFGQIADLENLN
AKSLVSTYHSMINEDKIDIFVLGDIDPMRAQQVIAKLPFEDRDIVNKSPLYHQALYDQVQ
RKTEYQQVNQAKLNLAYSLPVYYHDADYYAGLVFNGLFGGTPYSKLFTNVREKASLAYYA
SSRLLPFNGIVSVQTGIQASDQEKVQNMIQEQLIALQNGDFTTETLSEVQDSLINQYRAG
HDLASNVLEQQLVTKLVNESDKNFITEIKKVTVADVMRVAKQMKLQAVYLLSGEK

>fig|1598.593.peg.2327 **putative Zn-dependent protease** [Lactobacillus reuteri PNW1]
gtgaacgagataaatttcatttttcgactgcttaagcggatatttataataggactttta
gtaggtggaggatggctttatttcaacgatgcacgggttcaagcaacggcgaaccaaact
gcatggaacgttcgtgaccgtattgctaagttaattggcagggacgatacaaattcaaac
gataattctaatctgcacttaaacgatgcaaataatggttcttccaaaaacgaacaagga
ccaacgacagaacaacaaacaagtacacaaacttcaattccatctactgggcgatgggcg
actaatcaagcaacagtctatgtaaatacaaataatgctcaacttgacgcagctactaat
accgcaatccaaaactggaatcaaacgggggcatttacatttaaaccagtaaacaatcaa
agtaaagctgatattgttgtaacgacgatgaaccgttctgattcaaatgctgctggatta
acgaagacatcctctaattcattgacaagaagatttatgcatgcgacggtgtacttaaat
acatattatttaactgacccaagttatggctatagtcaggaacgaattgttaatacagcc
gaacacgaactgggccacgcaattggactcgaccataccaatgctgtttcggtaatgcag
cctgctggatcattctatacgatccaaccagatgatgtgcaagcagtccaaaaactatat
gctaataataaataa

>fig|1598.593.peg.2327 **putative Zn-dependent protease** [Lactobacillus reuteri PNW1]
MNEINFIFRLLKRIFIIGLLVGGGWLYFNDARVQATANQTAWNVRDRIAKLIGRDDTNSN
DNSNLHLNDANNGSSKNEQGPTTEQQTSTQTSIPSTGRWATNQATVYVNTNNAQLDAATN
TAIQNWNQTGAFTFKPVNNQSKADIVVTTMNRSDSNAAGLTKTSSNSLTRRFMHATVYLN
TYYLTDPSYGYSQERIVNTAEHELGHAIGLDHTNAVSVMQPAGSFYTIQPDDVQAVQKLY
ANNK

>fig|1598.593.peg.2459 **SOS-response repressor and protease LexA (EC 3.4.21.88)** [Lactobacillus reuteri PNW1]
gtgccaactaatataatttatccattaggagataaatttcagcatgttagcatccccttc
attggcgaaattgcctgtggggatcctatcacagctgaagagaatattgaaggttacgtt
gaagagatttttcccaaaaggtaa

>fig|1598.593.peg.2459 **SOS-response repressor and protease LexA (EC 3.4.21.88)** [Lactobacillus reuteri PNW1]
MPTNIIYPLGDKFQHVSIPFIGEIACGDPITAEENIEGYVEEIFPKR

>fig|1598.593.peg.2460 **SOS-response repressor and protease LexA (EC 3.4.21.88)** [Lactobacillus reuteri PNW1]
atggaaccaaccattcacgacggttcaattgtaaccattagagaacagcccacagtggaa
gatggtgaaatagctgctgctctcgttgatggtgataatgaagcgacactgaaacgtgtt
aagcactaa

>fig|1598.593.peg.2460 **SOS-response repressor and protease LexA (EC 3.4.21.88)** [Lactobacillus reuteri PNW1]
MEPTIHDGSIVTIREQPTVEDGEIAAALVDGDNEATLKRVKH

>fig|1598.593.peg.2483 **Membrane-associated zinc metalloprotease** [Lactobacillus reuteri PNW1]
ttgataataacaattattacatttattattgtttttggaatccttgtccttgttcatgaa
tatggtcattattattttgctaaacgagcaggaattctggttcgtgaattttcaatagga
atgggtccaaaaatttggtggagacgaaaaaatggtactacctatactattcgaattttg
cccctcggtggttatgttcgtttagctggggctgatgatgaggaccaagatgagttaaag
ccaggtaccccgctaacgattcaactaaatgatcaagataaagttattagtattaatgct
agtgataaaacaaccttatttcaggggatccccccttcaactagttgcttgtgatttagaa
gatggcttatggataaaaggatatgtcaatggggacgaagatcagctaaaggtatatagt
gttgaccatgatgcaatgattattgagcgagacggaaccgaggtacagattgctccacgt
gatgtgcaatttcgttcagcaagtctgcctgcacggatgatgaccaattttgctggtccg
atgaataatttcattttgtccttagtggtatttatcattcttggcttcaccttaacgggc
gtaccaactaatagcaatcagttagggcaagtgaatgccggttccgtagctgccaaagcg
ggcttaaaagcaaacgatcggattgttaaggttaataaccaaaaaataaataattggact
gacctttcaacgaatatttcaaataagcctaataagacagtgtcagtaacgtatgagcgt
ggtaataaaacttatcatactaagctaacaccaaaagcggttgagcgaggtcatcaaaaa
gttggccaaattggaattgttgaaaagcaagaaaagagtttagctgcgcgtctgaagttt
ggttggcagcaatttatccaagcaggaacattaatctttagtgttcttggccacatggtt
actcatgggtttagcttaaatgaccttggtggaccggtagcaatttatgctgggacatcg
caagccacctcattgggaatcaatggagttttaaatttcttagccctcttatcaatcaac
ttaggaattgttaacttattaccaattccagcattggatggcgggaaattgttattgaat
attgttgaagcgatcattcggcgaccaattcctgaaaaagctgagggaattatcacaatg
attggtttcctaatcttactaacacttatggttctagtaacatggaacgatattcaaaga
tattttattcggtaa

>fig|1598.593.peg.2483 **Membrane-associated zinc metalloprotease** [Lactobacillus reuteri PNW1]
MIITIITFIIVFGILVLVHEYGHYYFAKRAGILVREFSIGMGPKIWWRRKNGTTYTIRIL
PLGGYVRLAGADDEDQDELKPGTPLTIQLNDQDKVISINASDKTTLFQGIPLQLVACDLE
DGLWIKGYVNGDEDQLKVYSVDHDAMIIERDGTEVQIAPRDVQFRSASLPARMMTNFAGP
MNNFILSLVVFIILGFTLTGVPTNSNQLGQVNAGSVAAKAGLKANDRIVKVNNQKINNWT
DLSTNISNKPNKTVSVTYERGNKTYHTKLTPKAVERGHQKVGQIGIVEKQEKSLAARLKF
GWQQFIQAGTLIFSVLGHMVTHGFSLNDLGGPVAIYAGTSQATSLGINGVLNFLALLSIN
LGIVNLLPIPALDGGKLLLNIVEAIIRRPIPEKAEGIITMIGFLILLTLMVLVTWNDIQR
YFIR

>fig|1598.593.peg.2540 **Serine protease, DegP/HtrA, do-like (EC 3.4.21.-)** [Lactobacillus reuteri PNW1]
atgaataggataaaacaagcttttaaagatcggttgtggttaggcgtaattattgtcggc
ttatttgctggtcttattggtggtggaatcgccttaggaattaataacctggtacaacat
catgaagaagtaacaagtacacgagtcccagctggctctaataagtctggtggtacgaag
gttaataagaataaggcggacttaaacggggaagcgtcccaagcttataagtccgtccaa
ggagccgttgttagtgttattaataaacaaaaggttcagcaatcaagtgggacgctagga
atttttggttatggcaacagtagtaacagcagcagtgattcttctagcgataataaatta
gaaaccgctagtgaagggtcaggagttatttataagaagagtggcaattcggcttatgtt
gtaactaataaccacgttgtcaaaggttcaaacgcgctccaagtaattttaagcaatggt
aaaaaagttaatgctgatttagttggtgctgattctgcaaccgacttagctgtattgaag
attaacgctacaaatgttaagacagttgcatcctttggtaactctaattcgattgttcct
ggtcaggatgtgctagcaatcggttcaccaatgggaagtgaatatgctaatacagtaacg
aaaggaattatttctgccaaagatcggacattaaaagccggtactgatggaactttgaca
tcggttatccaaacagatgccgctattaactctggtaattcaggtggtccattaattaat
atggcaggtcaagttatcggaattaattcaatgaaacttgcttctgatacacaaggctct
tcagttgaaggtattggttttgccattccaagtaatgaagttgtgacaattattaaccag
ttaattaagaatggtaaaataactcgtccatcactcggaattagtatggtggatcttagt
aatgttacttctgatcaacagcaatcagtcttaaaactaccaacaagcgtaagcaaaggg
gtagtgattatagatgtgaatagtggttcagttgctgatactgctggattgaaaaaatat
gatgtcattacaaaacttggtgacactcaagttacagatgcgggttcattaaaggcagcc
ttatataaatacaaggttggacaaaacgctaaagtcacttattaccgtgatggtcaacgg
cacaccgcaactttgcatttaacaaagagtgctgatacaacatcaactgatgactcacaa
caagataataattaa

>fig|1598.593.peg.2540 **Serine protease, DegP/HtrA, do-like (EC 3.4.21.-)** [Lactobacillus reuteri PNW1]
MNRIKQAFKDRLWLGVIIVGLFAGLIGGGIALGINNLVQHHEEVTSTRVPAGSNKSGGTK
VNKNKADLNGEASQAYKSVQGAVVSVINKQKVQQSSGTLGIFGYGNSSNSSSDSSSDNKL
ETASEGSGVIYKKSGNSAYVVTNNHVVKGSNALQVILSNGKKVNADLVGADSATDLAVLK
INATNVKTVASFGNSNSIVPGQDVLAIGSPMGSEYANTVTKGIISAKDRTLKAGTDGTLT
SVIQTDAAINSGNSGGPLINMAGQVIGINSMKLASDTQGSSVEGIGFAIPSNEVVTIINQ
LIKNGKITRPSLGISMVDLSNVTSDQQQSVLKLPTSVSKGVVIIDVNSGSVADTAGLKKY
DVITKLGDTQVTDAGSLKAALYKYKVGQNAKVTYYRDGQRHTATLHLTKSADTTSTDDSQ
QDNN

Appendix E

The sequences of nucleotide and amino acids of identified genes putatively involved in stress resistance

>fig|1598.593.peg.708 **DNA protection during starvation protein** [Lactobacillus reuteri PNW1]
atgaaataccaagaaaccaaagaaacacttaatcaattagttgctgatctgagtcaaatg
gcaatgataattcatcaaactcactggtatatgcgtggcactaactttctaaagcttcac
ccattaatggaccaatttatggatgaaatcaacgatcaacttgatgtaatatctgaacga
ttattaactctcgacggtgaaccttattcgacccttaaagaaatcgcaacccatacgaaa
attaaagactggccaggaaactttgataaaacaacaccagaacgactcgcacatttagtc
gctggttatcgttatcttgaggacctttatcagcatggaattgaagtgactgatattgaa
aaagattattcgacccaagatatcttcattggttttaagacggcaatcgaaaagaagatc
tggatgctgcaagcagaattagatcaggcaccagaaattgataattaa

>fig|1598.593.peg.708 **DNA protection during starvation protein** [Lactobacillus reuteri PNW1]
MKYQETKETLNQLVADLSQMAMIIHQTHWYMRGTNFLKLHPLMDQFMDEINDQLDVISER
LLTLDGEPYSTLKEIATHTKIKDWPGNFDKTTPERLAHLVAGYRYLEDLYQHGIEVTDIE
KDYSTQDIFIGFKTAIEKKIWMLQAELDQAPEIDN

>fig|1598.593.peg.1861 **DNA protection during starvation protein** [Lactobacillus reuteri PNW1]
atgaatgcagaagaaaaataccaggcggaattgaagcaaagcgaccttgatcaccatcag
ccaactgcagctgccatgactggtcacattatttctaacttgttaattcattctttaaag
attaaccaagctaacttatttgctaaaggatctactagtctttttttagccgaaaaagct
gctggttggattgcttatgaacgtcaggaatttgaccaattgaatcacttattggttaat
aatggtgaaagtattccaacaattacagctcaatttaaggaatatactatgctagaagaa
gatggcagtagtaaatatttaacaggtgataagcaattgtttgctttggtaaaagatttt
gatacgcaaacgctcttcattactaaggcaattgctttagctaacaatgaaagttggcta
gaattatctgctaatttaactaatttaatggcttggattaaggagcaaattcgggtaact
caagacttcttaggtcatgaactaaaagaaggattgtatgtcgaagacgacgatgatgac
ttttaa

>fig|1598.593.peg.1861 **DNA protection during starvation protein** [Lactobacillus reuteri PNW1]
MNAEEKYQAELKQSDLDHHQPTAAAMTGHIISNLLIHSLKINQANLFAKGSTSLFLAEKA
AGWIAYERQEFDQLNHLLVNNGESIPTITAQFKEYTMLEEDGSSKYLTGDKQLFALVKDF
DTQTLFITKAIALANNESWLELSANLTNLMAWIKEQIRVTQDFLGHELKEGLYVEDDDDD
F

>fig|1598.593.peg.2435 **Phosphate starvation-inducible protein PhoH, predicted ATPase** [Lactobacillus reuteri PNW1]
gtgggagaacaaaaaacgattgaacaaacttttcaaattgaaagtccagaaatagaagtt
ggcttactaggaacacaggataagtttgtgagcctgattgaacaaggaatggatgttgaa
atccgaccattcggtgaaaatcttaaagtaagtggacaagaagaagacgttaaacttacg
atcgatgtatttcgtgcccttatttcgttgctaaatcaaggaatccgcatccacagtaca
gatattgttagtgcgatgaagatggcgcatcgtggaacactggaatattttgctgacctt
tacagtgaaacgattattaaagatcgccggggaagagccattcgggtaaaaaattttggt
cagcggcagtatgttaacgcgatgaagcataatgatattacgtttgggattggccctgcc
ggaacagggaaaacttacctagcagtagcaatggccgtggcatccttaaagcgtggcgaa
gtggaacggattatcttgacgcgaccagcagttgaggctggtgaaagtttaggattctta
cccggtgaccttcgtgaaaaagtagatccttatttacgaccaatttatgatgccttaaat
gatatttttggggctgatcatacgcaacggttaattgatcggggaattatcgaaattgca
ccccttgcttatatgcgtggacgaactcttgacggagcatttgtaattttagatgaagcc

caaaatacgactaatgcccaaatgaagatgttccttacccgtttaggctttggctcaaaa
atggttattaatggggatgtttcacaaatcgatcttcctcatgggactcgcagtggtctt
gtaaacgcgcaacgaattctgaataatattaagtcaattaagtttgttaaatttagtgca
gaagatgttgttcgtcatccggttgtagcacggattattactgcttatgaggaccaaaca
tcaaaacaattagcagaaaaagataaagagaaaaaagaggaaaaatga

>fig|1598.593.peg.2435 **Phosphate starvation-inducible protein PhoH, predicted ATPase** [Lactobacillus reuteri PNW1]
MGEQKTIEQTFQIESPEIEVGLLGTQDKFVSLIEQGMDVEIRPFGENLKVSGQEEDVKLT
IDVFRALISLLNQGIRIHSTDIVSAMKMAHRGTLEYFADLYSETIIKDRRGRAIRVKNFG
QRQYVNAMKHNDITFGIGPAGTGKTYLAVAMAVASLKRGEVERIILTRPAVEAGESLGFL
PGDLREKVDPYLRPIYDALNDIFGADHTQRLIDRGIIEIAPLAYMRGRTLDGAFVILDEA
QNTTNAQMKMFLTRLGFGSKMVINGDVSQIDLPHGTRSGLVNAQRILNNIKSIKFVKFSA
EDVVRHPVVARIITAYEDQTSKQLAEKDKEKKEEK

Appendix F

The sequences of nucleotide and amino acids of identified genes putatively involved in active metabolism in the host

>fig|1598.593.peg.18 **Xylose isomerase domain protein TIM barrel** [Lactobacillus reuteri PNW1]
atgtttttaccacaattaggattaaaaggaagcagtcaactagaccaaattaatgatcgc
ctacaatataatcctattgcttatgaattttatactgatgctaatgattttactgatgaa
ggctaccaacgcctctatgatgcaattcaatacgttaaagatgctggaattaaaaatatt
attctacatcacccaatgaagtttcaggatcatcattcagaagtagtggcccccgaaaaa
cagtaccccgacctatatcgctttatcgaatttacaactgaaaagctccttttatttagcc
gatgaccttgatgttcaagttttggttcatggtggttatgccggtcctgaatcacgttac
tttgtttcactttatcccagtgtagaagttgcccgagatgctgtctatcagcggttggac
cgttttgcagatgctggtggagagcatatcatgtttgaaaattcaattgcaccagtcttt
gcttacggcgatcctcttcaagaagatgaaattcttgcacataattatcgacttgccttt
gatacctcccattgttttattgagcttcatgggaataaccagaaattaacaacatcccta
gaacacttaaaagatcgtatcgttcactatcacctagttgattcaatgggcaaaactcat
gatagtcttactctcggcaccggtaaaattgactgggctaacgttttgcctcacttaaat
ccagctgctactagtatttatgaaattaacttacacgatcaaaataattgtcgtgaacaa
attgacagtcataattatttagttaaactttaccaaaagcttaataatagaggagtttaa

>fig|1598.593.peg.18 **Xylose isomerase domain protein TIM barrel** [Lactobacillus reuteri PNW1]
MFLPQLGLKGSSQLDQINDRLQYNPIAYEFYTDANDFTDEGYQRLYDAIQYVKDAGIKNI
ILHHPMKFQDHHSEVVAPEKQYPDLYRFIEFTTEKLLYLADDLDVQVLVHGGYAGPESRY
FVSLYPSVEVARDAVYQRLDRFADAGGEHIMFENSIAPVFAYGDPLQEDEILAHNYRLAF
DTSHCFIELHGNNQKLTTSLEHLKDRIVHYHLVDSMGKTHDSLTLGTGKIDWANVLPHLN
PAATSIYEINLHDQNNCREQIDSHNYLVKLYQKLNNRGV

>fig|1598.593.peg.2006 **Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52)** [Lactobacillus reuteri PNW1]
atgaattatttcatcactagccggcaagatttacacacctcggccatcgaacttgcccaa
gtcaaacggctacgaattttcgatcatctaaatgtccccgcgatcattgtcacgatgctg
tataacttcgaccatcaagctgttgaagaaaagcttaaagttaaaggtcgcatccttaac
atctaccagtattatcaacagcttccttatcgtgatgatcccacagttgatcaagccatt
attaagcaggtattgactgtccctggctgccaagttaaggacaattgtgccctacgcaat
ggcaaagtgcgggtccgcgtgaacttccgcaacggacggttatactatatcgactacctt
gatcagtacggctttaccaatcgacgggacttttacgatcaaggatggcggacatatact
gactattttgaagataagggacggttaattgcccgccagtattacaatcatgatggacaa
gtaaagataatctatcattatcgtggtggcgaaggtaatgtccccattcttaccctcatc
cagctagttgatcaagggcatgaatggcaatttgataataaaatcgaatttcgggcgcac
tttttagatgagcttgttggagatgatccccaatcaatccttatcagcgatcgttctaat
atcgcgctaaaggcgtttacgctaatgaaaaagccggccagacggtatcaggtattccac
tcggcctttaccgacgatggtcaaagcaacggccccatttcggcaatctaccagccaatc
gcaacaatgctcgcaaaaggacagctaagcgggctgatctctgccacgcaacgggaagct
caagacgctgcgaaacggttccaaactaaccagagctatggcatccccgtgacttatttg
acgggtgcagagctcacaaaagaaatccccgttagccaacgccagacaggaaaattcatt
gcggttgcccggctttccaaaatcaaacaacttgatcatattatcaatgcgataattcgt
ctccaccaagactttccgcaagtaacccttgatatttatggttatagcgatagttggaat
aaaaaccaaaccgctaatgctctgaagtcattggtaaaggaaaataaagccgagagctat
attaatttcgttggttattgtgatgaccttagtgctgcctatgaacatgctcaagcagaa
attctaacgagctattacgaaggctttgcaatggcagtcctagaagcacagggtcatggt
tgcccagttgttagctatgacatcaattatggaccagcagatataattgatgaccagcag
agtggtaaactgattccgccgaatgatcaagaggccctgtatcaacaactgcgaaaacta
ttagcagatcccgccctcgcaaaaaagtacgcccaccatgcacaaaaagcggcccaaaaa
tacaactttgatcatgtcgccgaaaagtggcaaaagttaattaataaagaaaaaaggagt
aatcctcacagccaagatggctaa

>fig|1598.593.peg.2006 **Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52)** [Lactobacillus reuteri PNW1]
MNYFITSRQDLHTSAIELAQVKRLRIFDHLNVPAIIVTMLYNFDHQAVEEKLKVKGRILN
IYQYYQQLPYRDDPTVDQAIIKQVLTVPGCQVKDNCALRNGKVRVRVNFRNGRLYYIDYL
DQYGFTNRRDFYDQGWRTYTDYFEDKGRLIARQYYNHDGQVKIIYHYRGGEGNVPILTLI
QLVDQGHEWQFDNKIEFRAHFLDELVGDDPQSILISDRSNIALKAFTLMKKPARRYQVFH
SAFTDDGQSNGPISAIYQPIATMLAKGQLSGLISATQREAQDAAKRFQTNQSYGIPVTYL
TGAELTKEIPVSQRQTGKFIAVARLSKIKQLDHIINAIIRLHQDFPQVTLDIYGYSDSWN
KNQTANALKSLVKENKAESYINFVGYCDDLSAAYEHAQAEILTSYYEGFAMAVLEAQGHG
CPVVSYDINYGPADIIDDQQSGKLIPPNDQEALYQQLRKLLADPALAKKYAHHAQKAAQK
YNFDHVAEKWQKLINKEKRSNPHSQDG

>fig|1598.593.peg.2225 **Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52)** [Lactobacillus reuteri PNW1]
gtgtacttttttgtaaatcaatacttattaagtagcaattccagtgttgaacacgctgaa
ttaaaacgactagccctatttaaaaagcatgggaggaaagctaaattggtgacccgtgac
tttgacctcgttcttcatgatacaatcaaaaaatttggcttaactaacgaccagattgtt
aatatgtatgatttttttgcaaatacgactgattaccaaggcgaaaagctccatactgag
gatttgcctttaccaattgactatcaagtaggaactggaaataactatcgcgaagtaaaa
gacggtgaccgactcgtttgcgaagttcactttgcggctggaacgattgggcaagttaac
catgttgattactttgacattgctggtaacatgactcttcgccaacaatatgatattcgt
ggctttaaggcagctgacgaattctttggtgaagatggacaagaatattacgcgcgttat
tatcgcccagatggagaaacgtatcttgaacggtattttgtaaaatcggttgaaaatacc
cccattaattcgctgaatgtcttacgtaattatcaggggcaagatcgctttttttgattct
ttagaagacctctttatcttctttttagatgaattgaataaggcgaatggcgagaacaat
gtgtttattgctgaccgaccagctgttgcgattaaaccggttcaatcaatgatgacaaaa
gcaaagaaatttttgtggcttccgatgaaccagattaacgatggtcagaacttaataaat
ggtcccttaaatccgatgttgcaaggtcctgttactacggatgcggacaagtgggatggt
gtaatcgtcatgactgctaaacaagcggagattctccagcaacaaattcacaatactgtg
ccaatctatgtaattaatgggagccccgttcttgctaactatgcacggattaatgaagct
gatcggacaccgggtcaattgatttacgttggaagattagcagaagataagcaaactagt
caactaatcaatatgtttgcgcagattcatcagcaagtacctgaaagtaagctaactttc
tatgggtatggcactggtgaagatatggataactataaaaaacaggttaaaagccttgga
ttagacgaggcaattgagtttgccggttaccaaatatcattagatgaggcatatgatcaa
gctcaattatttgttgatgcaagtcgaatcgatgcacagccattagcgatgggcgaggcc
ttaaaccatggggtaccagtggtttcttatgattacctttatggaccacgtgaaatggta
acttcaggtaaaaatggcgagatcattcctttgaataaccagggacagttcattcaaacc
gttgtcaaattattacaagaccaagataaactgcaaagcttgagtactggtgcctatgac
aatctagatgaattggcagaagaaaaaacttggaagcaatggcaacaactatcagctatt
tag

>fig|1598.593.peg.2225 **Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52)** [Lactobacillus reuteri PNW1]
MYFFVNQYLLSSNSSVEHAELKRLALFKKHGRKAKLVTRDFDLVLHDTIKKFGLTNDQIV
NMYDFFANTTDYQGEKLHTEDLPLPIDYQVGTGNNYREVKDGDRLVCEVHFAAGTIGQVN
HVDYFDIAGNMTLRQQYDIRGFKAADEFFGEDGQEYYARYYRPDGETYLERYFVKSVENT
PINSLNVLRNYQGQDRFFDSLEDLFIFFLDELNKANGENNVFIADRPAVAIKPVQSMMTK
AKKFLWLPMNQINDGQNLINGPLNPMLQGPVTTDADKWDGVIVMTAKQAEILQQQIHNTV
PIYVINGSPVLANYARINEADRTPGQLIYVGRLAEDKQTSQLINMFAQIHQQVPESKLTF
YGYGTGEDMDNYKKQVKSLGLDEAIEFAGYQISLDEAYDQAQLFVDASRIDAQPLAMGEA
LNHGVPVVSYDYLYGPREMVTSGKNGEIIPLNNQGQFIQTVVKLLQDQDKLQSLSTGAYD
NLDELAEEKTWKQWQQLSAI

>fig|1598.593.peg.2226 **Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52)** [Lactobacillus reuteri PNW1]
atgctttttttcctaaacgataatattcaacgaaacaaatcaggaattgagcatgcgcaa
attaagcgtctgcatctgtttgaaaaatatcatcaaccagctaagattgttacccggcaa
tattcgaatgaattacacttggtaacagcagaagcagggattgatgatcgtaactttatt
aacttgtttgattattttcaagaggcttgtgaggtcccgcaaaagaatattcggattaaa
gatattccggttaacccccattgggagcgcaaagctgatgggattaattataattattat

caaaatggcaaacgtattctctatattcgtcggcgcagtgatactgatcggcgggtaatt
aatattcaatattttgatcattatggaaagcttctcaaagttagttggtttgacagccgc
ggttttatctcggtcgaacaactttatgactgggatggaaaaatggcaacggaaaactat
taccgtccagatggaaccttagcaatccagttagcccaccagcaggataagcggggaaat
gaaattaaaacttatcatctctttaattatcatggtcatgactaccagtttagtggattc
gaccagttaacccggttcttccttgatgagctggttgctgataaacaaatctgtggtgaa
ggtccgattggctttatcgttgaccgggtttatgaattaggctgggcagttctgcacatg
aaacatcatgtttttcgggttctccaattgcataatgaccatgtcaataatcctgatgat
atgcttcattctccccttaattataactatgattggggacttaagcacctccaagattgg
gatggggtcattgccttaactccgcaacaacaagaagatcttcaagaccggtttggcaag
tttggtgtaaagatctaccggattcctggtccgattgttccggcggcagttattgataag
cgccatgttccatttaaaaaacgaacgaagaaacaagttgtcatggtcgcccgcttgtct
cctgaaaaacagcaagatcacctattaaaagcatggccacaagtactagcagcagttcca
gacgccaaacttgatttttggggttatgccaatgatgactttgacaagacactcaataaa
attgtaaaagaggaagggattaatagttctgtgaccttccatgggtatacagatgacgtc
aattcggtttacgaagatgcgcaattacttattttgccaagtcgagctgaagggttacca
ctatctctagttgaggcccaatcccatggtttgccgattattgctaatgacataaaatat
ggtccttcagatgtcgttattgatcgacaggatggattgctgaccaagaacggtgatatt
gatggactggctcaagcgatcattcgcttattgcaaaatcaagagcagttagcgcagatg
agtgaaaatgcgtatgctgatagtgagcgttattctgaaccaaacgtgatgaagctatgg
aatgaactagttaatgacatgaaggaaaagggagaagagtaa

>fig|1598.593.peg.2226 **Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52)** [Lactobacillus reuteri PNW1]
MLFFLNDNIQRNKSGIEHAQIKRLHLFEKYHQPAKIVTRQYSNELHLVTAEAGIDDRNFI
NLFDYFQEACEVPQKNIRIKDIPVNPHWERKADGINYNYYQNGKRILYIRRRSDTDRRVI
NIQYFDHYGKLLKVSWFDSRGFISVEQLYDWDGKMATENYYRPDGTLAIQLAHQQDKRGN
EIKTYHLFNYHGHDYQFSGFDQLTRFFLDELVADKQICGEGPIGFIVDRVYELGWAVLHM
KHHVFRVLQLHNDHVNNPDDMLHSPLNYNYDWGLKHLQDWDGVIALTPQQQEDLQDRFGK
FGVKIYRIPGPIVPAAVIDKRHVPFKKRTKKQVVMVARLSPEKQQDHLLKAWPQVLAAVP
DAKLDFWGYANDDFDKTLNKIVKEEGINSSVTFHGYTDDVNSVYEDAQLLILPSRAEGLP
LSLVEAQSHGLPIIANDIKYGPSDVVIDRQDGLLTKNGDIDGLAQAIIRLLQNQEQLAQM
SENAYADSERYSEPNVMKLWNELVNDMKEKGEE

>fig|1598.593.peg.2514 **Beta-1,3-glucosyltransferase** [Lactobacillus reuteri PNW1]
atggaaaaaataagcataattgttccaatatataatgttaagtcttatttgcaaagatgt
gtacagagtatttctagacaaacatatagagatttagaaataattttgatagatgacggg
tcgacagatggtagtggagaactttgtgatagttttaaagataagcgtatcaaagttctt
cataaaaaaaatgagggcttggggttatcaagaaatgtaggaataaaggaagcaactggc
aagtatgtaatgtttgtagatagtgacgattatatagataagactatggtagaaaatcta
tataaggatttaattagtaataaagcagatacatgtattggtggatataagcgagtattg
aaagataaaactattaaaaatattaatccgctaagtggaaaggtatttgaatcttcacaa
attataaaagatgtacttgcaaaaatgatgggaagggcaagtaaaaatgatgatcatata
gaaatgtcagtttggaaagttttatttacactttctattattaaggaaaataatatagta
tttccatcagaaagaaagtttataagtgaagatattatttttgatacagagtattataca
aaatgtaaaagggtttgcatgagctctgatataggctataactactgcgataatagtaat
tcgttaactactaaatataatcccaatagatttaagcttcaaatcacactgtttgaagaa
ttaaaaaaaagagcttctaatttgaatatatatactttgtagatcaacgtttgtataat
aacttaatagcaaatacaaggtattgtataaaacttgaacaaaaatttaatcctaaaaat
gttgctttaaaaaatattaaagatatatgtagtaataaaaaactaaatgaagtatttagt
tattatttggatgatggacccttaaaatctaaagttattaataaggcaatacaaagaaag
cggtataacttttatggtggattatgtttgcaaaaaataaattgaacatatga

>fig|1598.593.peg.2514 **Beta-1,3-glucosyltransferase** [Lactobacillus reuteri PNW1]
MEKISIIVPIYNVKSYLQRCVQSISRQTYRDLEIILIDDGSTDGSGELCDSFKDKRIKVL
HKKNEGLGLSRNVGIKEATGKYVMFVDSDDYIDKTMVENLYKDLISNKADTCIGGYKRVL
KDKTIKNINPLSGKVFESSQIIKDVLAKMMGRASKNDDHIEMSVWKVLFTLSIIKENNIV
FPSERKFISEDIIFDTEYYTKCKRVCMSSDIGYNYCDNSNSLTTKYNPNRFKLQITLFEE
LKKRASNLNIYTFVDQRLYNNLIANTRYCIKLEQKFNPKNVALKNIKDICSNKKLNEVFS
YYLDDGPLKSKVINKAIQRKRYNFLWWIMFAKNKLNI

Lactobacillus acidophilus PNW3

Appendix G

The sequence of nucleotide and amino acids of identified gene putatively involved in lactic acids production

>fig|6666666.426754.peg.28 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus acidophilus PNW3]
atgagaaaagtaggtataatcggaatgggtcatgtaggtgctactgtagcatatactttg
tttactcatggaattgctgacgaattagttttaattgataaaaatgaagataaagttgct
gctgaatataatgacttacgtgattctttatcaagaaataattactacgttcgtgtaact
atgcaagattggcatgaattaaaagatgcggatattattgtaactgcttttggtgatatt
gcggctagtgtcaagactggcgatcgttttggtgaatttgaacttaacgctaagaatgca
aaagaagtcggtgaaaagattaagaacactggttttaagggtgtacttcttaatatctct
aatccatgtgatgctgttgcacaaatcttgcaagaaactactggattaagtaagaaccaa
gttttaggtactggtactttcttggatactgcaagaatgcaaagaattattggtgaaaag
ctaggtcaagatccaaaaaatgttgaaggttgggttttgggtgaacatggttcatcacaa
tttatcgcttggtccactgttcgtgttaacaacaagattgctattcaattatttagcgaa
aatgaacaaagaaagttaaataaggtaccaaacaagaattcatttgtggttgcaaatgga
aaaggctacactagttacgcaattgctacatgtgctgtaaggcttattcaagcaatcttt
tctgatgctcatttatatgctcctgtatcagtatacaatccagaatacaagacttatatt
ggctatcctgcaattattggtcgtgatggaattgaacaagtgattgaattgaaacttact
tcaaatgaacgtgaaaagcttcaagcagcagctgataagatcaaggaacacttggaacaa
ctgaagaacaagtaa

>fig|6666666.426754.peg.28 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus acidophilus PNW3]
MRKVGIIGMGHVGATVAYTLFTHGIADELVLIDKNEDKVAAEYNDLRDSLSRNNYYVRVT
MQDWHELKDADIIVTAFGDIAASVKTGDRFGEFELNAKNAKEVGEKIKNTGFKGVLLNIS
NPCDAVAQILQETTGLSKNQVLGTGTFLDTARMQRIIGEKLGQDPKNVEGWVLGEHGSSQ
FIAWSTVRVNNKIAIQLFSENEQRKLNKVPNKNSFVVANGKGYTSYAIATCAVRLIQAIF
SDAHLYAPVSVYNPEYKTYIGYPAIIGRDGIEQVIELKLTSNEREKLQAAADKIKEHLEQ
LKNK

>fig|6666666.426754.peg.621 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus acidophilus PNW3]
atgagtagaaaagtgtttcttgtaggtgatggtgctgttggttcaacttttgcaaatgac
ttattgcaaaatacaactgttgatgaattagcgatttttgatgttgctaaagatcgtcca
gttggtgattcaatggatttggaagatattactccatttacaggtcaaactaatattcat
ccagcagaatatagtgatgctaaagatgcagatgtgtgtgtaattactgctggtgttcct
cgtaaacctggtgaaactagacttgacttagttaataagaatgtaaagattttaaagact
attgttgatccggttgttgaatccggttttaagggtgtatttgttgtttcagctaacccg
gttgatattttaaccacattgactcaaaaaatatccggttttccaaaagatcgtgtaatt
ggtactggtacttcacttgattcaatgcgtcttcgcgttgaattggcaaagaaacttaat
gttccagtagctaaggttaactcaatggttcttggtgaacacggtgatactagttttgaa
aactttgacgaatcaactgttgacaataagccacttcgcgattactcagaaatcaatgat
aatgttttaagtgaaattgagtcagacgtccgtaaaaagggtggaaagatcatcactaac
aaaggagctacattctatggtgttgctatgatgcttactcaaattgttagtgctatttta
gataatcgttcaatttgtttgccattatcagccccaattaatggtgaatatggcattaag
catgatctttacttaggtactccaactataattaacggtaatggtattgaaaaagttatt
gaaactaaactttcagatgtagaaaaagctaagatgatcaattctgcagataagatgcaa
gaagttttatcaggtgttgaaatgtaa

>fig|6666666.426754.peg.621 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus acidophilus PNW3]
MSRKVFLVGDGAVGSTFANDLLQNTTVDELAIFDVAKDRPVGDSMDLEDITPFTGQTNIH
PAEYSDAKDADVCVITAGVPRKPGETRLDLVNKNVKILKTIVDPVVESGFKGVFVVSANP
VDILTTLTQKISGFPKDRVIGTGTSLDSMRLRVELAKKLNVPVAKVNSMVLGEHGDTSFE
NFDESTVDNKPLRDYSEINDNVLSEIESDVRKKGGKIITNKGATFYGVAMMLTQIVSAIL
DNRSICLPLSAPINGEYGIKHDLYLGTPTIINGNGIEKVIETKLSDVEKAKMINSADKMQ
EVLSGVEM

>fig|6666666.426754.peg.1358 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus acidophilus PNW3]
atggcaagagttgaaaaacctcgtaaagttattttagttggtgacggtgctgtaggttct
acctttgcattttcaatggtgcaacaaggtattgctgaagaattaggtatcattgatatt
gctaaggaacacgttgaaggtgacgcaatcgacttagcagatgctactccatggactttc
ccaaagaacatttacgcagctgactacgctgactgcaaggacgcagacttagtagttatt
actgctggtgctccacaaaagccaggtgaaactcgtcttgaccttgttaacaagaacttg
aagattttatcatcaatcgttgaaccagttgttgaatcaggctttgaaggtatcttctta
gtagttgctaacccagttgacatcttgactcacgcaacttggaagatttcaggcttccct
aaggatcgcgttattggttcaggtacttcacttgatactggtcgtcttcaaaaggttatc
ggtaagatggaacacgttgacccacgttcagttaatgcatacatgcttggtgaacacggt
gatactgaattcccagtatggagctacaacaatgttggtggcgtaaaggttagcgactgg
gttaaggctcacggtatggatgaatctaagcttgaagaaatccacaaggaagttgctgac
atggcttacgacattatcaacaagaagggtgctactttctacggtatcggtacagcttca
gcaatgatcgctaaggctatcttgaacgatgaacaccgtgtacttccactctcagttgca
atggatggtcaatacggtttacacgaccttcacattggtactcctgcagttgttggccgt
aacggtcttgaacaaattattgaaatgcctttaaccgctgatgaacaagctaagatggaa
gcttctgctaagcaattaaaggaagttatggacaaagcctttgaagaaactggcgttaag
gttcgtcaataa

>fig|6666666.426754.peg.1358 **L-lactate dehydrogenase (EC 1.1.1.27)** [Lactobacillus acidophilus PNW3]
MARVEKPRKVILVGDGAVGSTFAFSMVQQGIAEELGIIDIAKEHVEGDAIDLADATPWTF
PKNIYAADYADCKDADLVVITAGAPQKPGETRLDLVNKNLKILSSIVEPVVESGFEGIFL
VVANPVDILTHATWKISGFPKDRVIGSGTSLDTGRLQKVIGKMEHVDPRSVNAYMLGEHG
DTEFPVWSYNNVGGVKVSDWVKAHGMDESKLEEIHKEVADMAYDIINKKGATFYGIGTAS
AMIAKAILNDEHRVLPLSVAMDGQYGLHDLHIGTPAVVGRNGLEQIIEMPLTADEQAKME
ASAKQLKEVMDKAFEETGVKVRQ

Appendix H

The sequence of nucleotide and amino acids of identified gene putatively involved in bioactive peptide production

>fig|6666666.426754.peg.77 **bacteriocin helveticin J** [Lactobacillus acidophilus PNW3]
atggtcggaagtattacacctaaattggtttatcgcttgaatgggatgcaccatgtagta
gcacaagttggtgcagtaaatggtgatcatgtttttgctttgcaactgcttcacagcgcg
catgatgtattagtttatagaaagcataagggactgaccaaggatattaattatactaat
cctcacttagtaatgaccggctttggtcatacacaaacctgggttccagcaaatgataac
gatgaatatttcgttggtgctaaacctaattctggtaactggactacacaaattgcacgt
gtaaaatatccaagattactgtcagaaaattatacttcaaatacgcaattgccacgtttg
tcacatttaaaccgtgtaactgacgttccttatgatggtcacaatcatttgcacagagtt
gaagcttcagtttcaccaaacggtaaatattttatgattgcttcaatctggaataatggt
tctggtcactttggtttgtttgatctagatgaagtaaatcaaaaattagatgagaatggt
acaactaatacaccaattactgaccttcactgcttaagtgcatttcatattgataacttt
gataatccaagtgttgctcctgatgaagaagaaccaacaatgattgattcagtccagggt
tatgctattgataatgacaagaatatttatatatctaatcaattatcaccaaagattaac
catgaaactggtgaagtaaccacttgggcacgtaagattgttaagttcccatggggcgag
actgatagcaataattggcaagtagcaatgattgatggtattgatttacctgatcgctac
agcgaagtagaaagtattcatgttaatgctcccgacgatatttatttaacagttgcttac
caccaaaagattgttaagggcgatgaatatgctttaagaacattggaaaaccaaatcttt
catattgataatttataa

>fig|6666666.426754.peg.77 **bacteriocin helveticin J** [Lactobacillus acidophilus PNW3]
MVGSITPKLVYRLNGMHHVVAQVGAVNGDHVFALQLLHSAHDVLVYRKHKGLTKDINYTN
PHLVMTGFGHTQTWVPANDNDEYFVGAKPNSGNWTTQIARVKYPRLLSENYTSNTQLPRL
SHLNRVTDVPYDGHNHLHRVEASVSPNGKYFMIASIWNNGSGHFGLFDLDEVNQKLDENG
TTNTPITDLHCLSAFHIDNFDNPSVAPDEEEPTMIDSVQGYAIDNDKNIYISNQLSPKIN
HETGEVTTWARKIVKFPWGETDSNNWQVAMIDGIDLPDRYSEVESIHVNAPDDIYLTVAY
HQKIVKGDEYALRTLENQIFHIDNL

>fig|6666666.426754.peg.1077 **Bacteriocin prepeptide or inducing factor for bacteriocin synthesis** [Lactobacillus acidophilus PNW3]
atgcaggaatggaagaagactaccttaagtgacaatgagttgattgacgtaattggtggt
tcagcaaaaagctatattcgtagattaggacctgatggtggttatggcggtcgagagagt
aaattaatcgctatggcagacatgattagacgacgtatttaa

>fig|6666666.426754.peg.1077 **Bacteriocin prepeptide or inducing factor for bacteriocin synthesis** [Lactobacillus acidophilus PNW3]
MQEWKKTTLSDNELIDVIGGSAKSYIRRLGPDGGYGGRESKLIAMADMIRRRI

>fig|6666666.426754.peg.1078 **Bacteriocin prepeptide or inducing factor for bacteriocin synthesis** [Lactobacillus acidophilus PNW3]
atgaagcttagacaagaacaattgaatagaaaagaattaagtcaggttattggtggccga
agagatatgatcttggtagcgcttcctcatgctgtaggtccagatggtatgccaggtagc
ggtagaggtggtggtgctcaaatgagagccattggcagtattcctccatggcgtccaaat
tggtggaagtag

>fig|6666666.426754.peg.1078 **Bacteriocin prepeptide or inducing factor for bacteriocin synthesis** [Lactobacillus acidophilus PNW3]
MKLRQEQLNRKELSQVIGGRRDMILVALPHAVGPDGMPGSGRGGGAQMRAIGSIPPWRPN
WWK

>fig|6666666.426754.peg.1079 **Three-component quorum-sensing regulatory system, inducing peptide for bacteriocin biosynthesis** [Lactobacillus acidophilus PNW3]
atgaagaagaaagttgttaagaagactgttttgaaggaaaaagaattaactaaggttgtt
ggcgggaaaaaagcaccaatttctggttatgtaggtagaggactatgggaaaacctaagt
aatatatttaaacatcacaagtaa

>fig|6666666.426754.peg.1079 **Three-component quorum-sensing regulatory system, inducing peptide for bacteriocin biosynthesis** [Lactobacillus acidophilus PNW3]
MKKKVVKKTVLKEKELTKVVGGKKAPISGYVGRGLWENLSNIFKHHK

>fig|6666666.426754.peg.1084 **Bacteriocin ABC-transporter, ATP-binding and permease component** [Lactobacillus acidophilus PNW3]
atgctactacaatataaatcaatctatgtaccacaagtagacgaatcagattgcggtgtt
gcctgtttggctatgattcttaagaaatatcattctagagtatcattagcacatttgcgc
cattcagcacgtactaatttagaaggaactaccgcccttggcttagtcaaaacagcacaa
acatttaatttgaaaactgaagcggtcaaagctgatatgtccttgtttgatccagatacg
gatatccaatatccttttattgttcatgtcttaaaacaaggcgaattactccactattat
gttgtacttaaagcaactaaaaattatttagtaattgctgatccagatcctagcgttggt
ttaacgaaaatgtctaaagaaaaattctctcaagaatggactggtattgccctttttatg
gttcctaatgacgattttgagccagttaaggagaaaaaacgaaatctttggtcgttgttt
ccatatatgttcaagcaaaagaaattagttactaatattattttggccgccctttttaatg
acaattattagtatttgtagttcatatttcttacaaggtttaattgatacttatattcct
aatggcacatatcaaaccttatcaattctagccatcggtttattgatagcatatgtcttt
aattccatcttttcttatggtcaaaacttttttgttaaatatcttaggacaacgattaagt
attgatcttaatttacaatatattcgccatatttttgaattaccaatggaatttttttgtg
actcgtagaacaggtgaaatcacttctagattctctgatgctagtcgaattattgacgcc
ttagctagtacagttatttcattatttcttgacctttcaattgtaattgtaatgggaatt
gttttggcaattcaaaactctactttattcatgattactttgttagcactgccagtatac
gctgtagtgattcttagcttttctaagaaatttgaaaagctgaacaatgatcagatggaa
agtaatgcagttttaagttcttcagtaattgaggatattcaagggattgaaacgatcaag
gctttaaatagtgaacagacacggtaccgtaagattgatagtcaatttgtcgattatcta
aagaaatcctttcgttacagtaaaactgaatctttgcagtcagctctaaagactttcatt
caactgtctctcaatgtgattatcctttgggtaggagccaaagttgtaatgaacggtcag
atgagtatcggtcaattaatgacatttaatgcattgctatcatattttgtagatccactt
cagagtattattaatcttcagccaaccttacagtctgctaatgtagctcaaaatcgattg
aatgaagtatacatggttaagagcgagtttcagaaagatgtccagattagggatgcaaag
caattagtaggagatattgaataccataatgtcgattatcattatggttatggagttgat
gttttaaaggacgttaatttaaagattaagcagaatgataagttggcgatcgtaggaatg
agtggctcaggcaagtcaaccatggtaaagcttttagttgatttttctctccaagcaaa
ggcaagttaacttttaatggatttgattctactaaagtagataagcatgtcttacggtca
tacgtaaactatgttccccaaacaccatacatcttttcaggaacaatcaaagaaaatctg
ctcttaggtagtcgaccagatattacagaagaagatgtattgaaagcttgccagatagca
gagattgagtctgaaatcgagcaattaccattgcaatttgaaactaagatggatgaaaat
gccaagattttatccggtggacaaaagcaaaggttaactattgcacgtgcattattgtca
ccagctaaggttttgatttttgatgaagccacaagtggacttgatacgattacggagaaa
aaagtagtagataacttaatgaaattgaagaataaaactattatttttatcgcccatcgt
ttagcaattgcgcaaaggactaataatattgtggttgtagaccatggtcaaattgttgag
caaggaagccatgatgaattaatgcaaaaacatggattctattataacttagttgaaaat
tag

>fig|6666666.426754.peg.1084 **Bacteriocin ABC-transporter, ATP-binding and permease component** [Lactobacillus acidophilus PNW3]
MLLQYKSIYVPQVDESDCGVACLAMILKKYHSRVSLAHLRHSARTNLEGTTALGLVKTAQ
TFNLKTEAVKADMSLFDPDTDIQYPFIVHVLKQGELLHYYVVLKATKNYLVIADPDPSVG
LTKMSKEKFSQEWTGIALFMVPNDDFEPVKEKKRNLWSLFPYMFKQKKLVTNIILAALLM
TIISICSSYFLQGLIDTYIPNGTYQTLSILAIGLLIAYVFNSIFSYGQNFLLNILGQRLS
IDLNLQYIRHIFELPMEFFVTRRTGEITSRFSDASRIIDALASTVISLFLDLSIVIVMGI
VLAIQNSTLFMITLLALPVYAVVILSFSKKFEKLNNDQMESNAVLSSSVIEDIQGIETIK
ALNSEQTRYRKIDSQFVDYLKKSFRYSKTESLQSALKTFIQLSLNVIILWVGAKVVMNGQ
MSIGQLMTFNALLSYFVDPLQSIINLQPTLQSANVAQNRLNEVYMVKSEFQKDVQIRDAK
QLVGDIEYHNVDYHYGYGVDVLKDVNLKIKQNDKLAIVGMSGSGKSTMVKLLVDFFSPSK

GKLTFNGFDSTKVDKHVLRSYVNYVPQTPYIFSGTIKENLLLGSRPDITEEDVLKACQIA
EIESEIEQLPLQFETKMDENAKILSGGQKQRLTIARALLSPAKVLIFDEATSGLDTITEK
KVVDNLMKLKNKTIIFIAHRLAIAQRTNNIVVVDHGQIVEQGSHDELMQKHGFYYNLVEN

>fig|6666666.426754.peg.1085 **Bacteriocin ABC-transporter, auxillary protein** [Lactobacillus acidophilus PNW3]
atgaatagcaaagactatgaaagtactgaattctattcttataaatttaaaaatttttca
actatgattatcattcctatggcacttttagtatttatcttgataattggctctttcttt
gcaatcagacagagtacagttacatctactggaattgtagaaccacaaagtacacttgat
attgccaataaaaattatcatgagggacaaattattaaaagaaatagaagtaaatggatg
gttcatctagatgataaaaaagaaaatatagtgcatttattaccaataattaaagcaaaa
aaatcagttaatatcgttacatatttccctggtaataaaattggtgcaattaaaaaagga
caacccttacattttcaattatctaacgccaatggaacaacagatagactagttggggaa
gtgaaagaagtaggaatataccctgttaacttacacggaaacaatgtttacgaagtgatt
tgtaaagctaaattagataaagatgtgaagtatggaatggaaggcaatgcaacaattatt
acaggaaagagcacatattttgaatattttaaggataaaattcttaactaa

>fig|6666666.426754.peg.1085 **Bacteriocin ABC-transporter, auxillary protein** [Lactobacillus acidophilus PNW3]
MNSKDYESTEFYSYKFKNFSTMIIIPMALLVFILIIGSFFAIRQSTVTSTGIVEPQSTLD
IANKNYHEGQIIKRNRSKWMVHLDDKKENIVHLLPIIKAKKSVNIVTYFPGNKIGAIKKG
QPLHFQLSNANGTTDRLVGEVKEVGIYPVNLHGNNVYEVICKAKLDKDVKYGMEGNATII
TGKSTYFEYFKDKILN

>fig|6666666.426754.peg.784 **S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS** [Lactobacillus acidophilus PNW3]
atggcaaaagttgaaagttttacattagaccacactaaagttaaggcaccttacgttcgt
ttaattactgttgaagaaggtcctaaaggcgacaagatttctaactatgacttacgttta
gttcaaccgaacgaaaatgcaattcctaccggcggattgcatactattgaacacttactt
gccagcttacttcgtgaccgtcttgatggtgtaatcgattgttcaccatttggttgccga
acaggattccacctaatcgtttggggtgaacattcaactactgaagttgctaaagcattg
aagtcttcattagaggaaattcgtgacacaattacttgggaagatgtaccaggtacaact
attaagacttgtggtaactaccgtgatcactcattgttcaccgcaaaagaatggtgtcgt
gatattcttgaaaaaggaattagtgatgacccattcgaaagaaatgtgatttaa

>fig|6666666.426754.peg.784 **S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS** [Lactobacillus acidophilus PNW3]
MAKVESFTLDHTKVKAPYVRLITVEEGPKGDKISNYDLRLVQPNENAIPTGGLHTIEHLL
ASLLRDRLDGVIDCSPFGCRTGFHLIVWGEHSTTEVAKALKSSLEEIRDTITWEDVPGTT
IKTCGNYRDHSLFTAKEWCRDILEKGISDDPFERNVI

>fig|6666666.426754.peg.792 **S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS** [Lactobacillus acidophilus PNW3]
atgtggggtgaacattcaatgaccgaagtagctagggcattaaaatcatctcttgaagaa
attcgcgatatgattacttgggaagatgtgcctggaactacaattaagacttgtggtaac
tataaagatcattccttgttctctgcgaaagaatag

>fig|6666666.426754.peg.792 **S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS** [Lactobacillus acidophilus PNW3]
MWGEHSMTEVARALKSSLEEIRDMITWEDVPGTTIKTCGNYKDHSLFSAKE

>fig|6666666.426754.peg.718 **type 1 capsular polysaccharide biosynthesis protein(EC:2.4.1.-)** [Lactobacillus acidophilus PNW3]
atgactttcttcaaagtaaaatcatttaaaaattgtactgcattacaagttcctacacct
aaagtagctaaatggttaaagaaaaatcatttcaaacagaagctatttattgtttcaaac
ggtatcagcgaaaagtttattcaaaatccgcacaaagaaaaagttggacacccttcact
attttatgtatcggaagattttcacacgaaaaacgacaagaaactttattcaaagcaatg

cagctatccaaacatacttctgaaatccgtcttatttttgctggacaaggacctttagaa
aaggaatatgccaaattagctaaacagcttcctaagaaacctatcatgcgctattttgct
ccagcagatttgaaagaaattatgtttcagaccgatttggttgtgcactgtgctgatgta
gaaatagagggaatggcttgcatggaagctttttctagtggctgtgtatctgtaattgct
gatagttcgttatcttctactgttagctatgctttaagcgaaaataatcgtttctctgct
ggagacagtagagctcttgctcaaaaaattgattactggttcgagcatccctcagaatta
ataaaaatgagacaagaatatcgcagttacggtaaaacattaaatgttgcacgttcagcc
aaaatagccttaggtaatttagaaaatctgaccttaaagtaa

>fig|6666666.426754.peg.718 **type 1 capsular polysaccharide biosynthesis protein (EC:2.4.1.-)** [Lactobacillus acidophilus PNW3]
MTFFKVKSFKNCTALQVPTPKVAKWLKKNHFKQKLFIVSNGISEKFIQNPHKEKVGHPFT
ILCIGRFSHEKRQETLFKAMQLSKHTSEIRLIFAGQGPLEKEYAKLAKQLPKKPIMRYFA
PADLKEIMFQTDLVVHCADVEIEGMACMEAFSSGCVSVIADSSLSSTVSYALSENNRFSA
GDSRALAQKIDYWFEHPSELIKMRQEYRSYGKTLNVARSAKIALGNLENLTLK

>fig|6666666.426754.peg.1719 **Putative mannosyltransferase involved in polysaccharide biosynthesis** [Lactobacillus acidophilus PNW3]
atgattccaaaaatcatccattacgtttgggtaggtcataatccaaagtctaagttgatc
caagagtgtattgccacttggaaggaaaaattacctgattatcaatttattgagtggaat
gaagataattttgatatgcacgaaaataagtacattgagcaagcttataaggcaaaaaag
tgggcttttgtttcagattatattcgtgctaaagctatctatgaacaaggtgggatttat
ttagatacagatgtgcgtgtaattgctagtctgacgcctttgcttgataacaaggccttt
attggctttgaaaacaacaattatctttcggctgcaatttttggcgcagaaaaagggcat
ccatttatgcaggatatccttgattattataaagatcgtaattttgaatatgatgtaaat
aatcaaatggcaggcgtaaatagtgtttccgttactgatatgctgattgatcggtatggt
ttaaagattggtaacaaggaacaggaattaaaggaaggtattcatgtttatccagatggc
gttttatgtaatccgtctgttaactcactttcgattcacttatttacgggaacttggatg
aacggaaaacattcatttaaacataaacttgtcactttccttaagcgtcatattaataca
cctaaagaggcagggttatatgccaagtggattcgatga

>fig|6666666.426754.peg.1719 **Putative mannosyltransferase involved in polysaccharide biosynthesis** [Lactobacillus acidophilus PNW3]
MIPKIIHYVWVGHNPKSKLIQECIATWKEKLPDYQFIEWNEDNFDMHENKYIEQAYKAKK
WAFVSDYIRAKAIYEQGGIYLDTDVRVIASLTPLLDNKAFIGFENNNYLSAAIFGAEKGH
PFMQDILDYYKDRNFEYDVNNQMAGVNSVSVTDMLIDRYGLKIGNKEQELKEGIHVYPDG
VLCNPSVNSLSIHLFTGTWMNGKHSFKHKLVTFLKRHINTPKEAGLYAKWIR

Appendix I

The sequence of nucleotide and amino acids of identified genes putatively involved in adhesion

>fig|6666666.426754.peg.938 **Sortase A, LPXTG specific** [Lactobacillus acidophilus PNW3]
atggcaaagaataaacaaaagagtagtgttactacgattttagttagaattgttgcggtt
cttttgttagttataggtttggtccttatatttaataaacaaattagtaatcaaatgatc
aagcataatcaacagtcagctttgacaacactaactaaaaaacaagttgaagcaaatcaa
aagaaaaaaggtatgtatgactttagtaaggtgaagtcgatgaatatggggcaagctgca
cgatcacaggttaagaaaacttctggagcaattggtgcacttgcagtgcctgatgtaaat
atgtatttgccaatcatgcttggtttatcagatgatgcaatgtctacgggcggcggaacc
atgcgggcagatcaagtaatggggaaggggaattatccacttgcaggccattatatgact
gctaagggtattctttttcaccacttgaagatgtaaaaaagggacaaagaatctattta
actaatttaaagaaaatctatatttatcgtatttacatgaaaaagattgtagatccatct
gcagtttggttagttaataatactaagaaaaatattgtaactttaattacttgtgcggac
ggtggtactaatcgctgggctattcggggtaatttgattaaaactgaaaaagcaacagat
gaaaatttgaaggttttcaaattaaagtaa

>fig|6666666.426754.peg.938 **Sortase A, LPXTG specific** [Lactobacillus acidophilus PNW3]
MAKNKQKSSVTTILVRIVAVLLLVIGLVLIFNKQISNQMIKHNQQSALTTLTKKQVEANQ
KKKGMYDFSKVKSMNMGQAARSQVKKTSGAIGALAVPDVNMYLPIMLGLSDDAMSTGGGT
MRADQVMGKGNYPLAGHYMTAKGILFSPLEDVKKGQRIYLTNLKKIYIYRIYMKKIVDPS
AVWLVNNTKKNIVTLITCADGGTNRWAIRGNLIKTEKATDENLKVFKLK

>fig|6666666.426754.peg.109 **cell surface protein, ErfK family** [Lactobacillus acidophilus PNW3]
atgaagaaaaatatcgttataggagtaacttgcatcatcgttgcgtttggttcaattata
ggattagctaaagtgtgcgctcctaaatctgatactgcacaagaggcagtatctacaagt
gtaaaaaataaaccaaaaactagcggcaagaaaacaaatacagatcataaaaaagttaag
tcaaccggtgcagaatttcgtccttataaagatcctaaggatttaagaaaagaaggtact
tggactaaaaagagtgaaaccaagaagcatcctaagattaatcaaaatgaaacagattta
actttgcgtgtttcattaaaaggaaatcggacatacttactaagaaaaggcaaagtaatt
tatacaatgctttcaactggtggcatttataaaaagggaaaatcacttacgcctactggt
acatatagaattcaagcaggtcgtggagatagcttctttaattacaatctaaatgaagga
gctaataactggaccagctggagtccggacaatgtttatttgttccactctgtgcctact
aaaggggatggaaattataatttaaatgaagcagcaaaattgggcagaacacaaggttct
cacggctgtattcgtttgagtataccagattctaaatggattatggacaatatccccgat
ggaactaaggttgttattaaagatcgttaa

>fig|6666666.426754.peg.109 **cell surface protein, ErfK family** [Lactobacillus acidophilus PNW3]
MKKNIVIGVTCIIVAFGSIIGLAKVCAPKSDTAQEAVSTSVKNKPKTSGKKTNTDHKKVK
STGAEFRPYKDPKDLRKEGTWTKKSETKKHPKINQNETDLTLRVSLKGNRTYLLRKGKVI
YTMLSTGGIYKKGKSLTPTGTYRIQAGRGDSFFNYNLNEGANNWTSWSPDNVYLFHSVPT
KGDGNYNLNEAAKLGRTQGSHGCIRLSIPDSKWIMDNIPDGTKVVIKDR

>fig|6666666.426754.peg.117 **cell surface protein precursor** [Lactobacillus acidophilus PNW3]
atgtctctatttggtaaaaagaaaaaagctatcgttaattatatagatttggacaatgat
aaaaataatatcgctacatcaggtgaattgttaggtaatgttggtgaagagattggttac
agtagttcagatcagattgatgctttaaaaaagcaaggttatgttcttgtaaataatagt
tttgatccgagtgataaaccaacttttttcaaatgaagatgttcaaacttatactatttca
tttaaacatgatgtagaagcagttgataaacctaattctgattttggaattagtgaaagt
gatttacaaaaagtgggtacgcaaactgtccattatgaaggtgctgctactagaacacca
aaagataatgtgagtcaggttgtttttaaacgtagtgtagtctatgataaaatactaaaa

aaaatcattagtacttcaacatggatgcctgaaaagcaaagctttttattgatcgctaca
cctgaagtttcgggatatactactgttcaaactactgtgggtggtgaaacggtaactcct
gaagattgtgatcgtaattatgttgttgaatatgatattaatcatcaaccatctgtcgct
gatcaaaaagtagctgttaaatatgtcgatcaagatctagaaaataaagaaattactgaa
gatatattaactggtatgccaaattccttggtagattatgatcctaaagctactattgaa
agactcgaaagtgacgaaggatacgctttagtaaataatggttataatcctgcgggtgaa
gtacaattttatagcaacaatgacgattatgtgcctgtctttgttatgacaatgaagcac
actatcggtcaagtagatagtgaacatcctgatagtaaagtaaataagaacgaatatgat
aaagatgtcgcatttactattaattatgaaggagcagatgctgctactcctgttaacaat
gttcaaagatcacactggagtcgtagtttaaccgttgatcgtgttactggtgaaatttta
cctggaggtaaatatacaactgattggaaagtggatcgtgaaaagtatgatgatgttgat
gttccagtagttgatggatatcacaccgatgttaaaatgatcaaaggtgccgaggttaca
cgagaaaatattattagaacagtaaattatgttgctaatggccatattattccagtggat
tctgatggtaaagaaattgttggtgcaccacatccagtctttcaaactgatccaaatgat
ccaactcaagtgattactgatgaaccagtgccaaagattgatggatacaagtgtaatttg
gcaactattacaccgtataatccagcaaaagatatggaagttaagtataaggcagaagat
tctgatgttttagtaatttctgttggtgataagaaacccaagtcagaaactaaagcagtt
tcagtggaaaagccggttgttaaacctgaccctaaaccggaaaatactaattcagtaact
caacctgctgttactcaagatcaaaatcatgatcatgatcaagttgctataattaacttt
atagatcttgaccatgatggtaaacaactgacctcatctggtccactaactggtaagcca
ggtgaaagcattaacgatttgtatagtacagaattaccattaaaggcgatcgagcgtgca
ggttatcatgttgtctttaatggctttgatgataatggtacaattcaaagatttgacaat
aatgatttaatgacacaagtgtttacaattggtttgcgtaaaataagtgatgaacagcaa
acttcaattggacttgatgcattaaagaaacttgatcttcataataatgaggatgttgct
gcaattgcctttggagttgcttcaactatcattagtttaattggattaatcggtaataaa
aacgataagtaa

>fig|6666666.426754.peg.117 **cell surface protein precursor** [Lactobacillus acidophilus PNW3]
MSLFGKKKKAIVNYIDLDNDKNNIATSGELLGNVGEEIGYSSSDQIDALKKQGYVLVNNS
FDPSDKPTFSNEDVQTYTISFKHDVEAVDKPNSDFGISESDLQKVGTQTVHYEGAATRTP
KDNVSQVVFKRSVVYDKILKKIISTSTWMPEKQSFLLIATPEVSGYTTVQTTVGGETVTP
EDCDRNYVVEYDINHQPSVADQKVAVKYVDQDLENKEITEDILTGMPNSLVDYDPKATIE
RLESDEGYALVNNGYNPAGEVQFYSNNDDYVPVFVMTMKHTIGQVDSEHPDSKVNKNEYD
KDVAFTINYEGADAATPVNNVQRSHWSRSLTVDRVTGEILPGGKYTTDWKVDREKYDDVD
VPVVDGYHTDVKMIKGAEVTRENIIRTVNYVANGHIIPVDSDGKEIVGAPHPVFQTDPND
PTQVITDEPVPKIDGYKCNLATITPYNPAKDMEVKYKAEDSDVLVISVGDKKPKSETKAV
SVEKPVVKPDPKPENTNSVTQPAVTQDQNHDHDQVAIINFIDLDHDGKQLTSSGPLTGKP
GESINDLYSTELPLKAIERAGYHVVFNGFDDNGTIQRFDNNDLMTQVFTIGLRKISDEQQ
TSIGLDALKKLDLHNNEDVAAIAFGVASTIISLIGLIGNKNDK

>fig|6666666.426754.peg.782 **Cell surface protein, ErfK family** [Lactobacillus acidophilus PNW3]
atgaagacaaactttaagatggctttaatgactgctatattgctggcatttccgatggct
actaatgttgcacagccggttaatgccacaacaaaaacgacagtagtaaaattaaaaaaa
tcggactttcaaccatataatgatcctgctgatctacgcaagatgaccggaaactattgg
ttaaagtctagtgaaactaaaacagcatatcctaacttacgcaaagttaagaacttgaat
ttacgtgtttctattttgggtaaccgcacctatgttagaagtgggaagaaagtcctttat
actatgtacagttctgctggcaagattgtagatggtaaaagcttaacaccaactggtact
tatctcactaattcgtatcatccacatcgtttttcagaagctttatatccagtaggctgg
attggtcaattatatttattccactctgtaccaactcatatgtggtcaaatcaatttgtg
attagggaagccaataaattagggaagatgcccgctagtcacggatgtgttcgcctatca
gtaagggatgccaagtggcttcatgatcatgttccatataatacaaaagtaaatatctat
tataaataa

>fig|6666666.426754.peg.782 **Cell surface protein, ErfK family** [Lactobacillus acidophilus PNW3]
MKTNFKMALMTAILLAFPMATNVAQPVNATTKTTVVKLKKSDFQPYNDPADLRKMTGNYW
LKSSETKTAYPNLRKVKNLNLRVSILGNRTYVRSGKKVLYTMYSSAGKIVDGKSLTPTGT
YLTNSYHPHRFSEALYPVGWIGQLYLFHSVPTHMWSNQFVIREANKLGKMPASHGCVRLS
VRDAKWLHDHVPYNTKVNIYYK

>fig|6666666.426754.peg.1649 **Cell surface protein** [Lactobacillus acidophilus PNW3]
atgccacataaaaatacgaaatctaatgtacaaaaaattactgctagtgtagcttctgtg
attgtaagtactggaattgtaatgaattcaagtaatcgtagagttaaagcaactggtaaa
cgagaagatgaaattgttgaagttgaaagtttgaagagagctgatactgaaacgacgaag
gctgctgctgtgcaaactcctaaaataaaccaaactaaaaagatgagtcatcgtactcaa
aaggttacacagatgattcactatgtagatgaagatgaaagaccggtttatgatgattac
acggctagtttaatttttgaacaaacaggaattctggataatgttactggtaagcagaca
tggaatagcaattggttaccaaagactacggcaacttttagtgctgtagaacatcccgtg
gtaagaaatcatcatttagtaaatccagagataaatgaagtaagtgcgtatgatgttgaa
gtaacggatgatacttttaaaaatcctcttcttaaagttcataaagttgtatatgcacat
gatatagaaccaattaaacggacgcaggtagtaacgcaaactattcattatagatatgaa
gatggggcggttgcacatgatgatcatgttgtgtcattaatatttacacagtcaggtaag
agggatttgactaatggtaaagaaatttgggatagtaaatggtcgctgactcaaactttt
gaagctttgcctagcccagttattattggatatacagcagataagccgatggttggtcct
gatgaggttacggttgatagtaaaaatttccttgataaacaaaatagagaagaaaccgta
atttatagtgcgaatactattacgcaaaataagaaagatgggattagtgaagaaaagaat
gtaaataattctgttgctgtcgcgccactaaaaagtgctgtaatgattggcggagtaata
tctattttaaaatttaaaagattaatggttattataaaaaacaagaaggatcataaatag

>fig|6666666.426754.peg.1649 **Cell surface protein** [Lactobacillus acidophilus PNW3]
MPHKNTKSNVQKITASVASVIVSTGIVMNSSNRRVKATGKREDEIVEVESLKRADTETTK
AAAVQTPKINQTKKMSHRTQKVTQMIHYVDEDERPVYDDYTASLIFEQTGILDNVTGKQT
WNSNWLPKTTATFSAVEHPVVRNHHLVNPEINEVSAYDVEVTDDTFKNPLLKVHKVVYAH
DIEPIKRTQVVTQTIHYRYEDGAVAHDDHVVSLIFTQSGKRDLTNGKEIWDSKWSLTQTF
EALPSPVIIGYTADKPMVGPDEVTVDSKNFLDKQNREETVIYSANTITQNKKDGISEEKN
VNNSVAVAPLKSAVMIGGVISILKFKRLMVIIKNKKDHK

>fig|6666666.426754.peg.848 **Fibronectin/fibrinogen-binding protein** [Lactobacillus acidophilus PNW3]
atggcatttgacggattatttatccatagtttactacaagatttgacccctacattagta
gatggtcgattatcaaaaatttaccaaccatttgaacaagatctaatattaacttttaga
aaaaatagaaagaattatcaattattaatttcagccaacgctcaatatcctagaatgtat
ttaactgagcagactattgctaatccagacaaagcacctatttttgttatggttttaaga
aagtatttggaaggttcagtcttacaatctattgaacaaattggtgtgaaccgaattata
aaccttcatttcagtaatcgaaacgaattaggtgatcaagtaaaattagtattatccgtt
gaattaatgggacgacatagtaatgtaattctctatgatcaacagaatggtcatattatt
gatctattgaagcgaataaatcctgatgaaaatagagctcgtattttattacctaaggca
aaatatgagcttccccctcttaaacctggtataaatggtttagtagtaactgaaaatcaa
tttaaaaagttaagcaatgaaaaaagcattccagatttagtgaaatcaatggatggttta
gacagagacgatcgtgaagaattaactggttatcttgaagatgattttagctattcttca
tttaagactttctttgatcagtttaataatccaagagctttcgttttaaaaactccaaga
aataaacgtaagattttctgttatcttccctatcatttagatttagagaaagaaaattct
aatcctgatttaaataaaggtttagatgaattttatgaataccaagcaacacgtgattgg
gttaaacaacgtgctagtcaggttgaacgtgtcgtcaaaaacgaacagaacaaactaagt
aagaagattaagaaattagaaaagcaattaaacttagcagaaaattctgagggctatcgt
atcaagggtgaaatattaaatgctaatttaggacaagtaaaaccaggaatgactgaggtt
tccttacctaactattatgaaaataataaacctataagcattaaacttgatccagcttta
tctccggctcgaaatgcacaaaaatatttcactcgctataaaaaattacgtgattctatc
aaacacgttaacgaacaaattaaaatcgctaaagaaaatcttcgctattttgattctatc
caaacagctattgataatgctgatccacaagatattgatcaaataactgacgaattgatt
aatcaaggttatattagaaagcaacaaaaaaatagacgtaagaagaaaattactgaacgc
aatctaaatgaattccagctttcttctggaaaacatgtcttagtgggtaaaaataattac
caaaatgactggcttactctaaaaaaagctaataaattagattattggttccacgttaaa
aatatgcctggttcacatgttattttgcgagatgatcaacctaccgatcaagatattaaa
gaagctgcagagattgctgcatttttctctaaagccaaaaattctgcccacgttcaagtt
gactacgttcaagataagcgtgtaaagaagcctaatggggctaagccaggattcgtaatt
tacacaggacagaattcaatcgaagttacacctaaagaagacgaaattatggctaaaaaa
atcaataaataa

>fig|6666666.426754.peg.848 **Fibronectin/fibrinogen-binding protein** [Lactobacillus acidophilus PNW3]
MAFDGLFIHSLLQDLTPTLVDGRLSKIYQPFEQDLILTFRKNRKNYQLLISANAQYPRMY
LTEQTIANPDKAPIFVMVLRKYLEGSVLQSIEQIGVNRIINLHFSNRNELGDQVKLVLSV
ELMGRHSNVILYDQQNGHIIDLLKRINPDENRARILLPKAKYELPPLKPGINGLVVTENQ
FKKLSNEKSIPDLVKSMDGLDRDDREELTGYLEDDFSYSSFKTFFDQFNNPRAFVLKTPR
NKRKIFCYLPYHLDLEKENSNPDLNKGLDEFYEYQATRDWVKQRASQVERVVKNEQNKLS
KKIKKLEKQLNLAENSEGYRIKGEILNANLGQVKPGMTEVSLPNYYENNKPISIKLDPAL
SPARNAQKYFTRYKKLRDSIKHVNEQIKIAKENLRYFDSIQTAIDNADPQDIDQITDELI
NQGYIRKQQKNRRKKKITERNLNEFQLSSGKHVLVGKNNYQNDWLTLKKANKLDYWFHVK
NMPGSHVILRDDQPTDQDIKEAAEIAAFFSKAKNSAHVQVDYVQDKRVKKPNGAKPGFVI
YTGQNSIEVTPKEDEIMAKKINK

>fig|6666666.426754.peg.1708 **S-layer protein precursor** [Lactobacillus acidophilus PNW3]
atgaagaaaaatagaaaaatgttaggtttagccgctgctaccttgttagcagtcgcacct
gttgcaacttcagttgtaccagttcaagctgatactgccgtaaatgttggctcagcagca
ggcactggcgctaatactactaatacgaccacacaggcacctcaaaataaaccatatttc
acttataacaatgaaattattggtgaagctactcaaagtaatcctttaggtaatgtggta
cgtactactattagctttaagagtgatgataaggtttcagatttgattagtacaatttca
aaggctgtacaattccataaaaacaatagtgcaagtggtgaaaatgttactattaatgaa
aatgattttatcaaccaattaaaggctaatggcgtaacagttaagaccgttcagccttct
aataaaaacgaaaaagcatatgaagcaattgataaagtaccaagtacttcattcaacatt
actttaagcgcaactggtgataataatcaaactgcaaccattcaaattcctatggttcct
caaggtgcttcaacaccaacagatactacacaaaatccacaaattaattggactaagggt
ggtcaagcacaaagttcaagtttgaatggacaagtattccaagttgctgttggttcaaac
ttcaatccattgaactttactaacagtaacggtgaaaacatcattgtttctgctcaacaa
agtaagaacaacactacttttgcaagcattgaagcaacttcaaacccagttaatacttca
gaagcaggccgttactacaatgtaactttaactgcaactggtaacactggcaagaagact
acagctacttatactgttttaattacttcaagccaaaagcaaactttatacggtaatggt
gaaagtacgatttctacttacagtatttacggtaacaacgttttaagtaactcaaccact
tttaaagatggtgatcaagtttatgtttcagatcaaactaagactgttggtggagtttca
tactcacaagtttcacctaaatcaaagaatgatgctaactcaagtaacatttgggtaaag
acctcagcacttgtgaagccagcaggtgacaccaatgttaagacttacccagtaatggtt
gactcaagagcttacgacaagaacggtaactacttaggtcacatgtactatgcatatgac
aacattgacatcgttccaactgttgtaaccatcaacggcaagacttactacaaggttgct
aacaaggatgaatacgttcgtgtaactaacattactggtaaccaacgtaccttgaagcac
aacgcttacatttactggtcatcataccgtcgtactccaggtactggtaagatgtataga
ggtcaaactgtaactacttacggtccacaaatgaaattcaagaacggtaagaagtactac
agaattgaaggctgcagaaataacaacaagcgttacatcaaggctgtaaacttctattaa

>fig|6666666.426754.peg.1708 **S-layer protein precursor** [Lactobacillus acidophilus PNW3]
MKKNRKMLGLAAATLLAVAPVATSVVPVQADTAVNVGSAAGTGANTTNTTTQAPQNKPYF
TYNNEIIGEATQSNPLGNVVRTTISFKSDDKVSDLISTISKAVQFHKNNSASGENVTINE
NDFINQLKANGVTVKTVQPSNKNEKAYEAIDKVPSTSFNITLSATGDNNQTATIQIPMVP
QGASTPTDTTQNPQINWTKGGQAQSSSLNGQVFQVAVGSNFNPLNFTNSNGENIIVSAQQ
SKNNTTFASIEATSNPVNTSEAGRYYNVTLTATGNTGKKTTATYTVLITSSQKQTLYGNG
ESTISTYSIYGNNVLSNSTTFKDGDQVYVSDQTKTVGGVSYSQVSPKSKNDANSSNIWVK
TSALVKPAGDTNVKTYPVMVDSRAYDKNGNYLGHMYYAYDNIDIVPTVVTINGKTYYKVA
NKDEYVRVTNITGNQRTLKHNAYIYWSSYRRTPGTGKMYRGQTVTTYGPQMKFKNGKKYY
RIEGCRNNNKRYIKAVNFY

>fig|6666666.426754.peg.490 **ATP synthase epsilon chain** (EC 3.6.3.14) [Lactobacillus acidophilus PNW3]
atggcagatccagagaaacttttaaagtaattgttgtaactcctaatggaatgatttat
tctcatcgtggaagcattgttgatgtacgtgctattgatggtgaacgttcaatcttatat
aatcacattccaattttgactccacttgcaattagtgaagtcaaggttaaacgaagtcgt
gagatgggatctagaattgatcatatagccattagtggtggttatattgagttttccaat

aacgttgcaactattgttgctgatagtgctgaacgtgctagaaatattgatgtgtctcgt
gcacaagccgctaaggaacgtgccgagaaacgtttaagagaagcgcgtgagaagcatgat
gagcgtaatttggaacgagcacaagttgctttgaaacgagcaatgaaccgaattagtgtt
tacaacgctagaggtcattaa

>fig|6666666.426754.peg.490 **ATP synthase epsilon chain** (EC 3.6.3.14)
[Lactobacillus acidophilus PNW3]
MADPEKLFKVIVVTPNGMIYSHRGSIVDVRAIDGERSILYNHIPILTPLAISEVKVKRSR
EMGSRIDHIAISGGYIEFSNNVATIVADSAERARNIDVSRAQAAKERAEKRLREAREKHD
ERNLERAQVALKRAMNRISVYNARGH

>fig|6666666.426754.peg.1138 **Tyrosine-protein kinase transmembrane modulator EpsC** [Lactobacillus acidophilus PNW3]
atggaacaaaaacaagaacaagaaaatacaatcgatcttacccaattattacgcatttgc
cgtaagcatatctgggcattgattctttggagtgtaggtcttgccttagtgggctgggga
gtttcagaatttgtaatttctcctaaatatacttcaactgctcaattattagttaaccaa
aagagtcgtaacaatgatccaaacgcagcttatgcgactcaacaagctaacatgcagatg
gttactacttataaagacattgtaacaagtaacaagatcttaacagaagcttctaatcgt
ttggccaatccaactgttgttgtgaaaaaagcacaaaaagcagtgtacagaactgatgaa
aatggtagaagaagattagtaagaaaagctcaaccagctgtaattgaacgaagcggtaag
agttattcagtttctgcaagtgaacttgctaagagtatctcagtaggtacccaacaacaa
tcacaagtcttctcaatttcagcggaagccgatacaccagctaaggcgaaggcagaagta
aatgcagtagctgaaacgttccgtaaagaaattcctactattatgagcgttaataacgta
acgattgtggcaaatggtactaatggtgttcaatcctcacctaacgttaagttgttcact
ctagctggatttgtagttgggttagtattgagctttgctgtagttattattcgtgaaatg
agtaatactactgttcgtgacgatgaattcttaactcgtgaattaggcttaactaactta
ggtcaaattgctcacttccacttgtcatcttcatttactattaaaaagagtgcaaatatg
actaatcgtggacaagctaagagacgtagagtataa

>fig|6666666.426754.peg.1138 **Tyrosine-protein kinase transmembrane modulator EpsC** [Lactobacillus acidophilus PNW3]
MEQKQEQENTIDLTQLLRICRKHIWALILWSVGLALVGWGVSEFVISPKYTSTAQLLVNQ
KSRNNDPNAAYATQQANMQMVTTYKDIVTSNKILTEASNRLANPTVVVKKAQKAVYRTDE
NGRRRLVRKAQPAVIERSGKSYSVSASELAKSISVGTQQQSQVFSISAEADTPAKAKAEV
NAVAETFRKEIPTIMSVNNVTIVANGTNGVQSSPNVKLFTLAGFVVGLVLSFAVVIIREM
SNTTVRDDEFLTRELGLTNLGQIAHFHLSSSFTIKKSANMTNRGQAKRRRV

>fig|6666666.426754.peg.1139 **Tyrosine-protein kinase EpsD** (EC 2.7.10.2)
[Lactobacillus acidophilus PNW3]
atgccattatttaagaaaaagcgtggtacagatgaaactattaaacatggtgctaagtta
atcactgtagctaagccaaagagtccaatagctgaacaattccgtactgttcgtactaat
attaactttatggcagtggatcatgacattaagtctttagcatttacttctgctaatatt
agtgaaggtaaatctactgtggctgctaacgttgctgttacttatgctcaagcaggtcgt
aaagtcttattggtcgatgccgacttacgtagacctacagtgcactcaacctttaactta
agtaatcatgttggtcttagtacggttatctcttcaactgctaaggaagttgatcttgat
agcgtagtacaagagagcggcgtagataatctttacgtgttaacggctggtccgatgcca
cctaacccggcagaactgataggttctaagcgtatgcgtgactttgttaaacttactgaa
gaacactacgatttagttattatcgacttagctcctgttcttgaagtatctgatacacaa
gaacttgctagtcacttagatggggttgtcttagtagttcgccaagggaagacgcaaaag
atggccattaagcgtgctgttgaaatgcttgaatttgcaaaggcacgtatcttgggttac
atcatgaatgatgtaagttctgataatgcgggttatggctatggttacggctacggctat
ggttatggctacggagaagaagatacaaagaaaaaaggattgttctctaaatttaggaag
taa

>fig|6666666.426754.peg.1139 **Tyrosine-protein kinase EpsD** (EC 2.7.10.2)
[Lactobacillus acidophilus PNW3]
MPLFKKKRGTDETIKHGAKLITVAKPKSPIAEQFRTVRTNINFMAVDHDIKSLAFTSANI
SEGKSTVAANVAVTYAQAGRKVLLVDADLRRPTVHSTFNLSNHVGLSTVISSTAKEVDLD
SVVQESGVDNLYVLTAGPMPPNPAELIGSKRMRDFVKLTEEHYDLVIIDLAPVLEVSDTQ
ELASHLDGVVLVVRQGKTQKMAIKRAVEMLEFAKARILGYIMNDVSSDNAGYGYGYGYGY

GYGYGEEDTKKKGLFSKFRK

>fig|6666666.426754.peg.1717 COG1887: **Putative glycosyl/glycerophosphate transferases involved in teichoic acid biosynthesis TagF/TagB/EpsJ/RodC / Putative polyribitolphosphotransferase / CDP-ribitol:poly(ribitol phosphate) ribitol phosphotransferase / CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase (EC 2.7.8.12) / CDP-glycerol: N-acetyl-beta-D-mannosaminyl-1,4-N-acetyl-D-glucosaminyldiphosphoundecaprenyl glycerophosphotransferase** [Lactobacillus acidophilus PNW3]
atgaagagttttttatttcgtctttatttagcctggatgaaatttttaagtagatttacc
actatggaggacaataacgtagttgtcttaaatggatcaggtcgttcaggctctaatggt
tatgctttttataaatggctactaatcaaccatccagaatttaatgtgaccttagtcgaa
ccgtggccgtcatcacatcttaaatgggaaacttggcaaaagattggcgcagcgcgttac
gtgattacaacgcaccagccatttaaagtacgtaaacatcaaataaatgtgcaactatgg
catggcgttccattgaagagaatgggaatcatggctaacaatacgcggtataaagacaat
aaacgtaatgaaaaattatggcataagaatgctgatattgttgcttcaagttcagattta
tatgaaacattgatgagcgcctgcatggcaattgagtcaaagaaatatcaaaaattaggc
tttcctagattggatctcttgtccaaaccagttatttctaaaaaagaattgttaaaagac
ttatttgatcaagaagatgaacaagcagcaattggtatttatatgccaacttttagatat
gagcttgaagataaatcaattatgcaaaagattaaagaggggaacttttttgcttttgct
gattttgacggtgaaaagctaaataaagaactaaaaaaacgccatcagtatttaattgtt
aagttgcatccctatgaaatgcgcttgttcgataacttcaagagtcagtattccaatatt
tccttttttaaacaatgattacttgtttgaccacaattatgatttatatgaattattaggc
gatactgatttcttaatgactgatttttcatcaatttatttcgattacttgcatttaaat
aaaccaattgtatttgtaactaacttttttgaaacaatatgaaaaaacgcgtggtctttta
atggggccatatagtgaaattacgccgggcattagtgtaaattctgagcaagaattaatt
aataatcttgatcatttagataatcaacagatcgttaaccgtcgtatatactggttaaat
ttaactaatcaggttcatggcgattcatattgtgaaaatgtctttaaatacatgacacag
gaatataggggataa

>fig|6666666.426754.peg.1717 COG1887: **Putative glycosyl/glycerophosphate transferases involved in teichoic acid biosynthesis TagF/TagB/EpsJ/RodC / Putative polyribitolphosphotransferase / CDP-ribitol:poly(ribitol phosphate) ribitol phosphotransferase / CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase (EC 2.7.8.12) / CDP-glycerol: N-acetyl-beta-D-mannosaminyl-1,4-N-acetyl-D-glucosaminyldiphosphoundecaprenyl glycerophosphotransferase** [Lactobacillus acidophilus PNW3]
MKSFLFRLYLAWMKFLSRFTTMEDNNVVVLNGSGRSGSNGYAFYKWLLINHPEFNVTLVE
PWPSSHLKWETWQKIGAARYVITTHQPFKVRKHQINVQLWHGVPLKRMGIMANNTRYKDN
KRNEKLWHKNADIVASSSDLYETLMSACMAIESKKYQKLGFPRLDLLSKPVISKKELLKD
LFDQEDEQAAIGIYMPTFRYELEDKSIMQKIKEGNFFAFADFDGEKLNKELKKRHQYLIV
KLHPYEMRLFDNFKSQYSNISFLNNDYLFDHNYDLYELLGDTDFLMTDFSSIYFDYLHLN
KPIVFVTNFLKQYEKTRGLLMGPYSEITPGISVNSEQELINNLDHLDNQQIVNRRIYWLN
LTNQVHGDSYCENVFKYMTQEYRG

Appendix J

The sequences of nucleotide and amino acids of identified genes putatively involved in production of extracellular enzymes

Lipase

>fig|6666666.426754.peg.1011 **FIG006988: Lipase/Acylhydrolase with GDSL-like motif** [Lactobacillus acidophilus PNW3]
atgaaaaagtggactaaatggctattattatcgcttttggctattatgattattggtgga
ggatggtatactgttaaccactttacaaatttaactagtaatagttcaaaagttgttaaa
ccaaaatatgttgaaaagaaaaatgtaaagcttgttgcactaggtgattcccttactcac
ggtcaaggggatgaaactaataatagtgggtacgttggcgtaattaagggaaaaatcgaa
catcgttaccaccaaactaaggtgacaacagtcaattacggggtaacaggggaccgatcc
gaccagattcttgaccggttaaatcagcaatctcaattacgtagtgacttacggagcgcg
gatgtgattacgatgactgttggtggaaat

>fig|6666666.426754.peg.1011 **FIG006988: Lipase/Acylhydrolase with GDSL-like motif** [Lactobacillus acidophilus PNW3]
MKKWTKWLLLSLLAIMIIGGGWYTVNHFTNLTSNSSKVVKPKYVEKKNVKLVALGDSLTH
GQGDETNNSGYVGVIKGKIEHRYHQTKVTTVNYGVTGDRSDQILDRLNQQSQLRSDLRSA
DVITMTVGGN

>fig|6666666.426754.peg.1248 **Esterase/lipase** [Lactobacillus acidophilus PNW3]
atggttgtaattaaacgtaatattgtttatgacgaaaacaaagacttaagttctgatatc
tactatccaaatgatacaacttctaatactaaaatttttaatttttttggcatggcggaggt
tggttccgcggtaacaaagaaagtgctaaagaaataggtgttgccctagccaacgccggt
tttatgactttcatccccgattatagcttagcccctaaaaatatttttccagctgctcat
gatgatgcgctgcactttattgattggctattaaaatctgaatacactgaccaagatgat
cttaaaaatattgttcaaattggggcaagtgttggtggaaccttagctttatatgttgct
gggaaatatggtttttccaactgttacttggtcagctccagttgaatttttctaactggatt
agaaatcatcaaactacaaaagcatctaaagatgctaaaaatgaacttggaattagtgac
cctcagcaaattagagaagccttttataaatactttactctcacttatactggcactgac
aatgaaaaaatcttacaacaactagatgcatgttcctatgatctttctaaattaggaaaa
ctcatgatgatgaactccgctgatgaattaactccaattgcatcagttttaagtttcatt
cgctttttagctaataaaaacttaggtgttgaattgctagtcattccaggccatggacat
gcaatggactacggtagtgattatatcgatgaatcactagacttttatatcaaacgatt
aaaagacagaaataa

>fig|6666666.426754.peg.1248 **Esterase/lipase** [Lactobacillus acidophilus PNW3]
MVVIKRNIVYDENKDLSSDIYYPNDTTSNTKILIFWHGGGWFRGNKESAKEIGVALANAG
FMTFIPDYSLAPKNIFPAAHDDALHFIDWLLKSEYTDQDDLKNIVQIGASVGGTLALYVA
GKYGFPTVTWSAPVEFSNWIRNHQTTKASKDAKNELGISDPQQIREAFYKYFTLTYTGTD
NEKILQQLDACSYDLSKLGKLMMMNSADELTPIASVLSFIRFLANKNLGVELLVIPGHGH
AMDYGSDYIDESLDFLYQTIKRQK

>fig|6666666.426754.peg.1481 **Lipase/esterase** [Lactobacillus acidophilus PNW3]
atgaaaatagaaaatctgacactaactaactttaatgatcgccaatataaggtacatact
tatattttggagcaaaatccagagttggtagaacaaaaaaggcctttggctatcgtagtt
cctggtggcagttttgagcatttatctaaaagagaaggcgaaccagttgcattagctttt
aataatcaaggatttaacagtgttgtcatggaatataacttagtacatgatgaaggaaaa
atttatccagatgcgggtcttgatgttttaactacagtaaaatattttagagatcgtgca
gatgagtatcatttggatcctgaacgcattttaacgattggcttctcggctggtggacat
gtagttagtgtggctaacaatatggcgattgatccagaatatcaagaaaagtatggctta
aaaaaagatgaagtattacctaataaatcaattttgggctatccactgattaatattgag
aagattggatttcctattccaaaagatcaaatggataagatgcctagtgagaagaaaatt

cttgatagtgcattaggagttacacgtgaaacaccagacacatttgtttttcaagcttgg
gatgaccctgttgtcctgattggcaattcaattgaatatctggcagcgttaaataagaat
aaggtgtcagctgaggcacacttatttaatcacggttatcatggcttttctttagcacgc
cacaatgtacaaactaaggaacgcgaatggcaagaaaatccgcatgtagctcattggttt
aatttagcaatggaatggttgaagagtgaatggggcgaataa

>fig|6666666.426754.peg.1481 **Lipase/esterase** [Lactobacillus acidophilus PNW3]
MKIENLTLTNFNDRQYKVHTYILEQNPELVEQKRPLAIVVPGGSFEHLSKREGEPVALAF
NNQGFNSVVMEYNLVHDEGKIYPDAGLDVLTTVKYFRDRADEYHLDPERILTIGFSAGGH
VVSVANNMAIDPEYQEKYGLKKDEVLPNKSILGYPLINIEKIGFPIPKDQMDKMPSEKKI
LDSALGVTRETPDTFVFQAWDDPVVLIGNSIEYLAALNKNKVSAEAHLFNHGYHGFSLAR
HNVQTKEREWQENPHVAHWFNLAMEWLKSEWGE

>fig|6666666.426754.peg.1548 **Esterase/lipase** [Lactobacillus acidophilus PNW3]
atgaaacttacagataaaattaaacgagtagtagcagaatatcgctctggttgtaaaaaa
agtgatgactcacgtgacagcgatctaccacacgatatccctgaagtagaacgcattgat
aacctaccttacggcccagatgaaaaatggcatacattagatgtttaccttcctaaaaaa
acagataaacccttccctgtaattattaatatccacggtggtggctggatctatggcact
aaagaaacatatcaatattatggcatgagtttagctaaacgtggctttgcctttatcaat
cctaattaccgcctagcacctgaaaatgcggaatttcctgaagaattagatgacgttgat
ctttacatgcactgggttgacgaccacgctgaagaataccgtttggacagaaataatgtc
tttatcattggcgacagtgctggtggacaaatggctgaacaatatgtaactatcttgact
aatccagactatgccaaattattcccatataagcctcttaatctgaaatttagagctgca
ggacttaactgtgctgcctcattcattttgactcccaatactttgcaaggaattagcggc
ttatacttcaccaaagatgcagttaataagtatcatgaacaattagatgttgaaaaatac
atcaaccacaatttcttaccaacatttttaattacctcaaacaaggacttcttgcacgac
atacaatttaccctcttcggttttttacttggtcgcggtgttcatgctatctgtaagtca
tatggtgattcagacaacccacgtggtcatgttttctttgtcaatcaaaaagaccgtatc
gctgacattgctaatgatgaagaaatcgaattcttcagagaacatatgagtaaataa

>fig|6666666.426754.peg.1548 **Esterase/lipase** [Lactobacillus acidophilus PNW3]
MKLTDKIKRVVAEYRSGCKKSDDSRDSDLPHDIPEVERIDNLPYGPDEKWHTLDVYLPKK
TDKPFPVIINIHGGGWIYGTKETYQYYGMSLAKRGFAFINPNYRLAPENAEFPEELDDVD
LYMHWVDDHAEEYRLDRNNVFIIGDSAGGQMAEQYVTILTNPDYAKLFPYKPLNLKFRAA
GLNCAASFILTPNTLQGISGLYFTKDAVNKYHEQLDVEKYINHNFLPTFLITSNKDFLHD
IQFTLFGFLLGRGVHAICKSYGDSDNPRGHVFFVNQKDRIADIANDEEIEFFREHMSK

Protease

>fig|6666666.426754.peg.18 FIG056164: **rhomboid family serine protease**
[Lactobacillus acidophilus PNW3]
atgaatcgaaaaattaatttgtcacaatcatttgtgactttagggattttgattatttta
gtagtggtctttttagttgaaatgtttcttggcggatctgaaaatgtcaatgtattgatg
aaaatgggggccatgaataattttgctgtagtagcagggcatcaatggtggcgtttgttt
acagcacaatttctgcatattggggtaatgcaccttgtttctaatgcagtaattatttat
tacatgggtcaatatatggaaccaattatgggacatactagattcttagtgacatattta
ttagccggaattggtgggaacttgatgagtttagcctttagtgctgatagaggtttaagt
gctggtgcttctaccgctttgtttggtctctttggtgcaatgaccgcgatcggactacgt
aattttcgaaatccaatgatcagctatttgggtcgacaagctttggttttagctttaatt
aacttggcactggatatctttgttccaggaattgatatttggggacatattggtggctta
attgctggattcttactggcaattattctaggagatcgtgtcatgaagacttacaatcca
aaatggcgtgtcttagctgctgccgttttggttgtttatgtagtgtggactgttagaaca
ggaatggttatcaacttctaa

>fig|6666666.426754.peg.18 FIG056164: **rhomboid family serine protease**
[Lactobacillus acidophilus PNW3]
MNRKINLSQSFVTLGILIILVVVFLVEMFLGGSENVNVLMKMGAMNNFAVVAGHQWWRLF
TAQFLHIGVMHLVSNAVIIYYMGQYMEPIMGHTRFLVTYLLAGIGGNLMSLAFSADRGLS
AGASTALFGLFGAMTAIGLRNFRNPMISYLGRQALVLALINLALDIFVPGIDIWGHIGGL
IAGFLLAIILGDRVMKTYNPKWRVLAAAVLVVYVVWTVRTGMVINF

>fig|6666666.426754.peg.244 **Serine protease, DegP/HtrA, do-like (EC 3.4.21.-)** [Lactobacillus acidophilus PNW3]
atggtagaaaatcaaaataataatcaacgaccaagaaaaaatagtaatgcaaagatcatc
actactgcagctattgtaggtgtagttggtggtctgatcggcggtggcgtttcatattat
gcagctgatcaaatgaataacgctactgatactactacggcacaaactagtgtatcttca
aatagtagtaaggtatccgaaaaaagtgctaaaaccagtggtacgatgactactgcttat
aatgatgtaaaaggggctgtagtgtccgttattaacttaaagagacaatcatcctcaagt
agcgctaactctctttacagcagcttatttggggatgatagcgatagttcttcaggtaag
agcggcaagcttgagacttacagtgaaggttccggtgtagtttatatgaagtcaaatggt
aaaggctatattgtaactaataatcacgttatttcaggcagtgatgcagttcaagtgcaa
cttgctaatggcaagactgttagtgcaaaggttgttgggaaagatagtactactgactta
gctgttttatcaattgacgctaagtacgtaacacaaacagccgaatttggcgattctaag
agtcttcaagctggtcaaactgtaattgctgtaggttcaccacttggtagtgaatatgct
tctacggtaacgcaaggtattatatcagcaccggctagaactatctcaacttcatctggt
aatcagcaaacagttattcaaacagatgcagccattaacccaggtaactcaggtggtgca
ttggttaactcagctggtcaagttatcggtattaattctatgaagcttgctcaatcaagt
gatggtacttctgtagaaggtatgggatttgctattccttcgaatgaagttgtaactatc
gtaaatgaattggttaagaagggtaagattactcgtccacaacttggtgtaagagtagtt
gctcttgaaggtattcctgaagcatacagaagtcgcttaaagattaagtcaaaccttaag
agtggtatctatgttgcttcaattaataagaatagttcagctgcaaatgcaggcatgaag
agcggtgatgtcattactaaggtagatggcaagaaggttgatgatgtagcatcattacac
agtatcctttacagtcacaaggttggtgacactgtgaacataactattaatagaaatggt
agagatgtcaacttaaaggtaaaacttgaaggtaattaa

>fig|6666666.426754.peg.244 **Serine protease, DegP/HtrA, do-like (EC 3.4.21.-)** [Lactobacillus acidophilus PNW3]
MVENQNNNQRPRKNSNAKIITTAAIVGVVGGLIGGGVSYYAADQMNNATDTTTAQTSVSS
NSSKVSEKSAKTSGTMTTAYNDVKGAVVSVINLKRQSSSSSANSLYSSLFGDDSDSSSGK
SGKLETYSEGSGVVYMKSNGKGYIVTNNHVISGSDAVQVQLANGKTVSAKVVGKDSTTDL
AVLSIDAKYVTQTAEFGDSKSLQAGQTVIAVGSPLGSEYASTVTQGIISAPARTISTSSG
NQQTVIQTDAAINPGNSGGALVNSAGQVIGINSMKLAQSSDGTSVEGMGFAIPSNEVVTI
VNELVKKGKITRPQLGVRVVALEGIPEAYRSRLKIKSNLKSGIYVASINKNSSAANAGMK
SGDVITKVDGKKVDDVASLHSILYSHKVGDTVNITINRNGRDVNLKVKLEGN

>fig|6666666.426754.peg.379 **FIG001621: Zinc protease** [Lactobacillus acidophilus PNW3]
atgctaacaactaatataacaattagaaaaaataaaaaatttacaacagccggaataggc
tgtttttgcgtttaccgttaactaatcataatttagcttttgctagtttactttcgcga
ttgcaaatgaatactagtttgtcatatccaacaattgctgctcaacaaagaaagttagcc
caattatatgatttgcagcttgatattatgccgcaacttttggcaaccaaattattttg
atgtattatgctaattttgttgaaccgattgaagtattggatccagattatacttatgaa
gaaataatccaaactattagccaaattatcagatttccagcatatgataataatttattc
gattatgctaaaagacaacttgaagatgaatatcgtgaaattatggttcaaccttcaaat
tatgctctcgatcgctttttaaattatggtatgaagatcaaccagaatatgctgaaaac
tttatggggccaattgatgaaataaaaaatactacgattgttgagatgcgtgattttatt
gaaaatttgcgtgatataccaatggcggtaattggcatgggacgagacaatcaattaatg
actaaaatactcagaaatatttttaaaggggctggaattattaaaaaattccaagttagt
gatttagttattccagctaaaagaaaattaattgaaaaagttgatgagcaagacaatatt
caagctcaattattgatgggatttggttttaaacagagaattagttatcaagaacaagtt
gttggtttgcttttagaacaatatttagctggtgatcagtcttcaaaattatttagtcag
attagagaagagttaggtgcggcttatgatgttcaagcaagtgactttgctaataattct
ctctttttaattaatgctggaattgatcctcaaaaagtagaaccagccaaaagaattatt
cttaatgaaatgcaaaaattaatggatggtaatatagatgaagagctatttagaaaatcc
aaaaaggctgtatatcgaaacactaggattgggttagacaatcaaaattggcaattagga
caggccttgcgtgccgaattattaccagattatttagattttgatagagaagctgctata
aaaaaagcaacgccacatcaattaattaattttgttcaaaatttattctttaatgaaagt
tatattttaaaatga

>fig|6666666.426754.peg.379 **FIG001621: Zinc protease** [Lactobacillus acidophilus PNW3]
MLTTNITIRKNKKFTTAGIGCFLRLPLTNHNLAFASLLSRLQMNTSLSYPTIAAQQRKLA
QLYDLQLDIMPQLFGNQIILMYYANFVEPIEVLDPDYTYEEIIQTISQIIRFPAYDNNLF
DYAKRQLEDEYREIMVQPSNYALDRFFKLWYEDQPEYAENFMGPIDEIKNTTIVEMRDFI
ENLRDIPMAVIGMGRDNQLMTKILRNIFKGAGIIKKFQVSDLVIPAKRKLIEKVDEQDNI
QAQLLMGFGFKQRISYQEQVVGLLLEQYLAGDQSSKLFSQIREELGAAYDVQASDFANNS
LFLINAGIDPQKVEPAKRIILNEMQKLMDGNIDEELFRKSKKAVYRNTRIGLDNQNWQLG
QALRAELLPDYLDFDREAAIKKATPHQLINFVQNLFFNESYILK

>fig|6666666.426754.peg.411 **ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)** [Lactobacillus acidophilus PNW3]
atgctagtacctacagttattgaacaaactgctcgtggtgaacgtgcctatgatatttac
tcacgtttattaaaagacagaattattatgttgagtggcgaaattaatgaccaaatggcc
aactcaattatcgcccaactacttttcttggatgcccaagacaatactaaagatatttca
ttatatattaactcaccaggtggtgtaatcacttctggtcttgcaatcatggataccatg
aactttatcaagtcggatgtttctaccattgcaatcggtatggcagcatcaatggcttct
attcttttgacaagtggtacaaagggtaagcgttttgcacttcctaactcaacagttctt
attcaccaaccattaggtggtgcacaaggtcaacaaactgatattcaaattgcagctaat
gaaattttgaagagtcgtaagaagatcaacgaaatcttgcacgaaactactggtcaacct
ttggaaaagatccaaaaagatactgaacgtgataattacttaactgctgaagaagctaag
gaatatggcttaattgatgaaattatggtcaacaaaaaaagtaattaa

>fig|6666666.426754.peg.411 **ATP-dependent Clp protease proteolytic subunit (EC 3.4.21.92)** [Lactobacillus acidophilus PNW3]
MLVPTVIEQTARGERAYDIYSRLLKDRIIMLSGEINDQMANSIIAQLLFLDAQDNTKDIS
LYINSPGGVITSGLAIMDTMNFIKSDVSTIAIGMAASMASILLTSGTKGKRFALPNSTVL
IHQPLGGAQGQQTDIQIAANEILKSRKKINEILHETTGQPLEKIQKDTERDNYLTAEEAK
EYGLIDEIMVNKKSN

>fig|6666666.426754.peg.550 **Lon-like protease with PDZ domain** [Lactobacillus acidophilus PNW3]
atgaataatgaagttaaaaaacatcgtttacgtaattggctaattatagtaattgctatc
atagctgttggagttcttgctttttggccaactaattattatatcgagtctcctggtgaa
gtcgtacctgttaatcagttcattagatctaaaaataaaaagcctaataatttttatttg
gtgacagttagtgtaacgagtcagccagcgtctattttgcagtatttgtggagttatact
agaccatatgatgaacgagtaccaagtaaagaattactaggaggacaaactagttcgcaa
tataatgaattgcaaaattggtatatggagactagccagcagaacgccatttattacgca
gctaaaaaagcagggaaaaaacatagcttaaaatatttaggtgtctatgtaatggaagtg
cagaagaattctagctttaaaaataagctgcaaattggtgatacagttttaggtgctaat
ggacacaggtttcactcgactgaagagatgatgacttatttacgtaaacagaaaataaat
agtaaagtaaccatttctgtattaagaggtaaagagaaaaagaaatttaccggcaaaatt
gttaaggtaaagggtactgataaaccaggaataggtattcagttagtagaacatgtaacg
gttaaaacagaacctgaattaactattaatgctggtgagattggtggaccttcagctggc
ttgatgtttacattagaaagttatgaggtctttactaaacaaaatttaagccatggtcat
aaaattgcgggaaccggtacaatttctcctacagggaaagtcggaataattggtggagta
gataaaaaagtggtagcggcaagtcgcgagggagcagaagtctttttgcaccgactgat
tctactagcgtttctaaaaatcaaaccaattatgctgtagcgaagagaactgctaaaaaa
atacatactaagatgaaaattgtacctgtaagtactttcgatgatgcactaaattattta
aaatcacactataaaaattaa

>fig|6666666.426754.peg.550 **Lon-like protease with PDZ domain** [Lactobacillus acidophilus PNW3]
MNNEVKKHRLRNWLIIVIAIIAVGVLAFWPTNYYIESPGEVVPVNQFIRSKNKKPNNFYL
VTVSVTSQPASILQYLWSYTRPYDERVPSKELLGGQTSSQYNELQNWYMETSQQNAIYYA
AKKAGKKHSLKYLGVYVMEVQKNSSFKNKLQIGDTVLGANGHRFHSTEEMMTYLRKQKIN
SKVTISVLRGKEKKKFTGKIVKVKGTDKPGIGIQLVEHVTVKTEPELTINAGEIGGPSAG
LMFTLESYEVFTKQNLSHGHKIAGTGTISPTGKVGIIGGVDKKVVAASREGAEVFFAPTD
STSVSKNQTNYAVAKRTAKKIHTKMKIVPVSTFDDALNYLKSHYKN

>fig|6666666.426754.peg.560 **ATP-dependent Clp protease ATP-binding subunit ClpX** [Lactobacillus acidophilus PNW3]
atggcaaatcagtttacagatcaagaagagattaaatgtgctttttgtggtaaaactcaa
gaccaagttaaaaaaatgattgccggaaacggtgtatatatttgtaatgaatgtgttgac
ttggctaaaaaaattattgacgatgaattacgtgcagattcactaaaaactgcaagtgaa
ctgcctaaaccagttgaaattaaaaaacaacttgatcaatatgtaattggtcaagatcgt
gctaaaaaagtactatcagttgctgtttataatcactacaagagaatcagccaaatggat
gtagattcatcaacagaattacaaaagtctaatattgcaatgattggtcctactggatca
ggtaagacttatttagctcaaacacttgcacgtattttaaacgttccatttgcaattgct
gatgcaaccacgttaactgaagctggatatgttggtgaagatgtagaaaacattttgctt
aaattattacaaaatgctgattacgatcttgaacgagcacaacgtggaattatttatatt
gatgaaattgataagatttctaaaaaatctgaaaacgtatcaattacacgtgatgtttca
ggtgaaggtgtgcaacaatcgcttttgaagattttggaaggtactattgcttctgtgcct
cctcaaggtggacgaaagcatccgcaacaagaaatgataaagatggatactactaatatt
ctctttattgtaggtggagcatttgatggaattgaacagattgttaaaagccgtttaggt
aagaaaactattggttttggtgctgaaaacgaagttaacaaggtagatgctgatgattgg
actcgtcatttaactactgctgatttagttaaattcggtatgattccagaattcattggt
agaattccaattattaccacgcttgataaacttgataataaagatttagttcgcgtatta
actgagccaaagaatgctcttgttaagcaatataagaaacttctatcattagatggtgta
gaattgaagtttactgatggtgcattaaaagcaattgctgatttagctatccaaagaaat
atgggagctcgtggtttaagaacaatcattgaaaattcaattatggatattatgtatgaa
accccaagtgaagaagatattgaatcagtagaagtgactaaagacgtaataacccgtcac
gcacagcctcgcattactcgtaaaaatgctgaagaagttcaggtaaaagcaaatgataat
taa

>fig|6666666.426754.peg.560 **ATP-dependent Clp protease ATP-binding subunit ClpX** [Lactobacillus acidophilus PNW3]
MANQFTDQEEIKCAFCGKTQDQVKKMIAGNGVYICNECVDLAKKIIDDELRADSLKTASE
LPKPVEIKKQLDQYVIGQDRAKKVLSVAVYNHYKRISQMDVDSSTELQKSNIAMIGPTGS
GKTYLAQTLARILNVPFAIADATTLTEAGYVGEDVENILLKLLQNADYDLERAQRGIIYI
DEIDKISKKSENVSITRDVSGEGVQQSLLKILEGTIASVPPQGGRKHPQQEMIKMDTTNI
LFIVGGAFDGIEQIVKSRLGKKTIGFGAENEVNKVDADDWTRHLTTADLVKFGMIPEFIG
RIPIITTLDKLDNKDLVRVLTEPKNALVKQYKKLLSLDGVELKFTDGALKAIADLAIQRN
MGARGLRTIIENSIMDIMYETPSEEDIESVEVTKDVITRHAQPRITRKNAEEVQVKANDN

>fig|6666666.426754.peg.689 **ATP-dependent protease subunit HslV (EC 3.4.25.2)** [Lactobacillus acidophilus PNW3]
atgacaacaatttgttcagtaagatttaatggaaaaacagctattgctggagatggtcaa
gttactttaggtgaaaaagtaattgctaaggcaactgcaagaaaaattcgtcgtatttac
catgatcgtgtagttatcggctttgccggtggtgttgcggatgctgtaagtttacaagat
atgcttgaaggaaagcttgaaagttatagtggtgatttacgccgtgctgctgttgaaatg
gcacaagcatggcgtaaagatccaactttgcaaaaattaaacgcaatgctgatcgctttt
gatgataaagatcttcttttgatttcaggaaatggtgaggttcttgaacctgatgaaaat
gttgtagcgattggttcaggtggtaatttcgctcaagctgcagccattgcaatgacacgt
catgctaaagaaatgaaagccgatgagatcgcacatgaagcagttaagattgcgtcaggt
attgacgtgtttactgatgatcatattgttactgatgaaatttag

>fig|6666666.426754.peg.689 **ATP-dependent protease subunit HslV (EC 3.4.25.2)** [Lactobacillus acidophilus PNW3]
MTTICSVRFNGKTAIAGDGQVTLGEKVIAKATARKIRRIYHDRVVIGFAGGVADAVSLQD
MLEGKLESYSGDLRRAAVEMAQAWRKDPTLQKLNAMLIAFDDKDLLLISGNGEVLEPDEN
VVAIGSGGNFAQAAAIAMTRHAKEMKADEIAHEAVKIASGIDVFTDDHIVTDEI

>fig|6666666.426754.peg.956 **Intramembrane protease RasP/YluC, implicated in cell division based on FtsL cleavage** [Lactobacillus acidophilus PNW3]
ttgaagggtatactaatctttttagttgtctttggcttacttgtttttgttcatgaattt
ggacacttcattgtagctaaaaagtcaggaattcttgtgcgagaattctcaattggtatg
ggcccaaaactattccaaattagacgaaatcccaccacttataccattcgttggttacca
cttggtgggtatgttcgtttagcgggctctgatgatgaaagtaaattagatccgggtatg
actgtagtcttacagttaaatgataaaaatgaagtgattagaattgatgcttctgaatca
gatattccgatcgaggggattcccgttcaagtcactaaagctgatttagtggatgattta

ataatcgaaggttatgagaatggcgatgaaaatgatccagtaacttatcatgtaaatcat
gatgctaccattattgaaaaaaatggtactgaattaattatcgctccacgtgatacgcaa
tttaatcaggctaatgtctggcaaaaacttgcaactaattttgcaggacctttatgaat
attgtcttaggatttgtagtgttcttaatttggacttttacagttccaggtcctgcaact
acaactgtaggctctgtccaaacagattcgccagcgcgtagtgctaagatcgaatctggt
gatagaatagttgcaataaatgatcaaaacatcactaattttgatcaagtatcagaaaaa
ataaatcaaagtaagggtaagagcttacgttttaaattggaaaagaatggttctactaga
actatttctgttaagccaaaagcacataaagtgcaaaatcagactgtctatcaagtcggt
attgttgctaaaagtaacgaaaatgcaggcgtaaaattgaagagaggctgggacacagca
gtttctactactggtttaatttttaatgccgtaggtaatttatttagtcatttcagttta
aataagttatcaggtccagtgggaatttattctcaaacttctcaagtttctcagatgggc
tttacttatgtattagcattcttggggatgatttcaattaatctgggaattgtaaattta
attcctattccaggattagacggtggtaagcttttattgaacttgatcgaattaattcgc
ggtaaggcaatctctgaagaacacgaagcgattgttgaattaattggctttggtttatta
ttagtattaattattgcagttacaggtaacgatatttatcgctattttattaaataa

>fig|6666666.426754.peg.956 **Intramembrane protease RasP/YluC, implicated in cell division based on FtsL cleavage** [Lactobacillus acidophilus PNW3]
MKGILIFLVVFGLLVFVHEFGHFIVAKKSGILVREFSIGMGPKLFQIRRNPTTYTIRWLP
LGGYVRLAGSDDESKLDPGMTVVLQLNDKNEVIRIDASESDIPIEGIPVQVTKADLVDDL
IIEGYENGDENDPVTYHVNHDATIIEKNGTELIIAPRDTQFNQANVWQKLATNFAGPFMN
IVLGFVVFLIWTFTVPGPATTTVGSVQTDSPARSAKIESGDRIVAINDQNITNFDQVSEK
INQSKGKSLRFKLEKNGSTRTISVKPKAHKVQNQTVYQVGIVAKSNENAGVKLKRGWDTA
VSTTGLIFNAVGNLFSHFSLNKLSGPVGIYSQTSQVSQMGFTYVLAFLGMISINLGIVNL
IPIPGLDGGKLLLNLIELIRGKAISEEHEAIVELIGFGLLLVLIIAVTGNDIYRYFIK

>fig|6666666.426754.peg.971 **SOS-response repressor and protease LexA (EC 3.4.21.88)** [Lactobacillus acidophilus PNW3]
atgtctagaaaaaatagtgacaccaaacaactggaaattctacgctatatctacgataca
gtagaaaatcgtggatttcctccaacagttcgtgaaatttgtgctgcggttggcttatct
tccacttcaacagttcatggccatttatctcggcttgaacgcaagggtttttaatcaaa
gacgctactaagcctcgcgcccttgaaattacagctgaaggtaagactgaactgggtatt
aagccaaaagaaattcctgttgtcggagtagtcactgcaggtcaacctattttagcagtt
gaagacattagcgaatatttcccacttcctcctgatttagagagtgatgcaggcgaatta
ttcatgcttaaagtccacggtaacagtatgattaaagctggcattttaaatggtgacaat
gtaatcgtaagaaaacaaagtactgccaataacggagaaatagtagttgcaatgacagat
gaaaatgaggccacggttaaacgtttcttcaaagaagatgatcattatcgtcttcaacca
gaaaatgatacaatggcacctattattttgcaacaagtaagcattttaggtaaagtggtt
ggtctttaccgtaacaatattcaatag

>fig|6666666.426754.peg.971 **SOS-response repressor and protease LexA (EC 3.4.21.88)** [Lactobacillus acidophilus PNW3]
MSRKNSDTKQLEILRYIYDTVENRGFPPTVREICAAVGLSSTSTVHGHLSRLERKGFLIK
DATKPRALEITAEGKTELGIKPKEIPVVGVVTAGQPILAVEDISEYFPLPPDLESDAGEL
FMLKVHGNSMIKAGILNGDNVIVRKQSTANNGEIVVAMTDENEATVKRFFKEDDHYRLQP
ENDTMAPIILQQVSILGKVVGLYRNNIQ

>fig|6666666.426754.peg.1343 **Late competence protein ComC, processing protease** [Lactobacillus acidophilus PNW3]
ttgaactcaatatatttttttacttaatttttttcataggagcatgcttagcttcacatgca
aacgttatatacgaacgatgggatactcgtaactttatcttttctcggtcttattgtgat
aattgtaaaagtactctttcactattggacgaaattcctctattctcttatttacttttg
aaaggcaaatgtaagtattgccaaaaaaatattccgcgtgaattattctttttgaacta
gttggtggctttgctttttgtacaataaattttagtgataaaagtcaaattatcacttct
atctttatttttttctcttctttttaattgcaatctctgattattatcaaaatgaatttgat
ttaatttttatttttccagcaattattacttctatttttatttaatcgcatttacttattt
aactggattgaatggctatcttttctaccagttttgatagtccttagtatttattcattt
aaacaaaaaatgggattaggtgatttattaatctatgtcctaatctccacttatttttact
cccacttttgcaaacttaactttacttttttgctgctttaattttaataatcattcatttt
acagaaaataatttagcttcatataattatccatttattccattcatttttattggacta
attatttcaaaatttatttttgaacaataa

>fig|6666666.426754.peg.1343 **Late competence protein ComC, processing protease** [Lactobacillus acidophilus PNW3]
MNSIYFLLNFFIGACLASHANVIYERWDTRNFIFSRSYCDNCKSTLSLLDEIPLFSYLLL
KGKCKYCQKNIPRELFFFELVGGFAFCTINFSDKSQIITSIFIFSLLLIAISDYYQNEFD
LIFIFPAIITSILFNRIYLFNWIEWLSFLPVLIVLSIYSFKQKMGLGDLLIYVLISTYFT
PTFANLTLLFAALILIIIHFTENNLASYNYPFIPFIFIGLIISKFIFEQ

>fig|6666666.426754.peg.1582 **Predicted Zn-dependent protease** [Lactobacillus acidophilus PNW3]
atgaaaaaacataaattcttaaagaaaataatcattttaaccatgttactaggaggcttt
gccaatagcgcaccagtaattacttccagttcaacggcagaagctgcaactaaagttaaa
acgaaaaagaagacaaccaagaaatcaactcaaaagaaaactaagataaaagttgattct
aaccgaaaaaagaaaaatagagctgtcaaatttgtcaaaaagacaactaaaaaccaaatt
ccaactgccagcatctttatctatatcaaccagaatgatcctaattatcaaacagttcaa
gatgcaatcaaagcttggaatgccacaaaagtaatcaaatttaagcaagcttttaattat
caaaaagctcagatcatcgtaactgcacatgactacggagacacttcttgggcaggatta
acagaaatacctgacacaccacgtggttatctttacggctcagttgtttatttaaacaac
ttttaccttcgtcaatctactcctcaagttgcattatctgtagcagaacatgaactgggc
catgcaatcggcttgcaacacaatgatactcaaccttcagtaatgaattcttcagttact
gagcaaaatgcttacacgattcaaccatgcgatatcgcagccgttaaagcaatttataat
gaaaaataa

>fig|6666666.426754.peg.1582 **Predicted Zn-dependent protease** [Lactobacillus acidophilus PNW3]
MKKHKFLKKIIILTMLLGGFANSAPVITSSSTAEAATKVKTKKKTTKKSTQKKTKIKVDS
NRKKKNRAVKFVKKTTKNQIPTASIFIYINQNDPNYQTVQDAIKAWNATKVIKFKQAFNY
QKAQIIVTAHDYGDTSWAGLTEIPDTPRGYLYGSVVYLNNFYLRQSTPQVALSVAEHELG
HAIGLQHNDTQPSVMNSSVTEQNAYTIQPCDIAAVKAIYNEK

>fig|6666666.426754.peg.1748 **Predicted metal-dependent membrane protease** [Lactobacillus acidophilus PNW3]
atgattgaaaaacgaaatttcaagaaatcaattgctatctggatctgtttattattagtg
tatacattggctggtctagtactgcgaccaatcaataatttaaccttacgcttagcgatc
cgttgttttattgctctagttattacaggattctgtttttatttttatgcatggcagcaag
ctttattctaacgagcttaatttgcgacacattaaaatttataacactatttttattatt
attgttgctatcttctatttatttttttcgcctgccaatttggattgaattatttactact
caaagaagcggattaatcaattctttactgattgcagtttgtgcaggattttgtgaagaa
gccttgtttagaggaatgttgtttaatatctgcgctaattatttgaaaaaacatcggtat
atttggcttgaaacagctttagttacttcaattctttttggactaatgcactcagtcaat
ttgctttccagtgagccactaccctctgtaggtacacaagtttttacgcttttgccagc
ggattaatgtttgcatacctgcgtttaatgtctaaccatctttggccagctatcttggct
catgctgcctttgattttacaatcgtacctaaaaatgcggtatttgcaatcaacgctcaa
ggattatcactagtttacataatttttggtattcttaccgttatctatttattgttcatt
tggagcttcaacagattgtacaatgaaactaaagcctaa

>fig|6666666.426754.peg.1748 **Predicted metal-dependent membrane protease** [Lactobacillus acidophilus PNW3]
MIEKRNFKKSIAIWICLLLVYTLAGLVLRPINNLTLRLAIRCFIALVITGFCFYFMHGSK
LYSNELNLRHIKIYNTIFIIIVAIFYLFFRLPIWIELFTTQRSGLINSLLIAVCAGFCEE
ALFRGMLFNICANYLKKHRYIWLETALVTSILFGLMHSVNLLSSEPLPSVGTQVFYAFAS
GLMFAYLRLMSNHLWPAILAHAAFDFTIVPKNAVFAINAQGLSLVYIIFGILTVIYLLFI
WSFNRLYNETKA

Appendix K

The sequences of nucleotide and amino acids of identified genes putatively involved in stress resistance

>fig|6666666.426754.peg.1113 **Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2** [Lactobacillus acidophilus PNW3]
atggatccaaattccaaacgtagagaagattatcgcaaacattcatcattaaatcttcat
cgcaatcatgctttggcagcagattcttcatcttttaaagccggtaacatgtttgctcgt
tttattggggtgatcgcgttattaaccgtttgtttcggtgtggcatgggctgctcatatg
tatttcacaattcatagtgcagttgatggtaaaaataatggtaatgttgcgacatccgct
aaaatttcgactagacaaccagtttcagtattgattttgggagtcgatcaaggaattgaa
ggccgtcatgaccgaggtaactctgatactttaattttagcaacagctaatccgcaaaaa
aataaggcaacgatgacatctattccacgtgatacattggctgatattaaaggtgatcca
ggtgacaaatacttcatgtttagggttaactcagcttatgaaatcggtggtagtgaagca
agtatgaaaactgtctcaaatatgttaaacgtaccgataaactattatttagaagttaac
atgaaggcgctgcgcagtttagttaacgcagtgggcggcgttgatgtaaatgtgcctttc
gatttctcatatgattggtgtgacttccacaagggtaagcaacacttgaatggtcggcac
gcagttgcttatgtccgaatgagaaaagaagatccgcgaggtgactatggtagacaactc
cgtcagcgtcaagtaattgaagcaattgctcataaggctatgtcagttaatacaattagt
aactatcgtaaattaattgatatttttaacaaatatgtcaagactaacttaacttttaac
gatatgctcagtttagctcttaactaccgtggttgtatgggtaatttagatagtggctat
attcaaggtcatgatgcttggattgacggttcgtcaattcaagtagccccaactgcagaa
ttacaaaaaatttcaaataagctcagaaagaatctgaatttaccggctgaaacacttgat
aatgaagaaactcgtcaaaatgatttgaatgatcaaaataaccatgtaaaatgggatgat
cctcaggcatttactacttatcgtatttatgagcaaaatgcagataagccagcgggcggt
tcaaattcaggctatggtgaaaataaagataccaattcaggtagttcaaccacttcctct
agtatgtcgagttcatcggactcattaggtagctcatcttcgtctagtagtaagacttgg
aaatttcattggtaa

>fig|6666666.426754.peg.1113 **Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2** [Lactobacillus acidophilus PNW3]
MDPNSKRREDYRKHSSLNLHRNHALAADSSSFKAGNMFARFIGVIALLTVCFGVAWAAHM
YFTIHSAVDGKNNGNVATSAKISTRQPVSVLILGVDQGIEGRHDRGNSDTLILATANPQK
NKATMTSIPRDTLADIKGDPGDKYFMFRVNSAYEIGGSEASMKTVSNMLNVPINYYLEVN
MKALRSLVNAVGGVDVNVPFDFSYDWCDFHKGKQHLNGRHAVAYVRMRKEDPRGDYGRQL
RQRQVIEAIAHKAMSVNTISNYRKLIDIFNKYVKTNLTFNDMLSLALNYRGCMGNLDSGY
IQGHDAWIDGSSIQVAPTAELQKISNKLRKNLNLPAETLDNEETRQNDLNDQNNHVKWDD
PQAFTTYRIYEQNADKPAGGSNSGYGENKDTNSGSSTTSSSMSSSSDSLGSSSSSSSKTW
KFHW

>fig|6666666.426754.peg.1137 **Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2** [Lactobacillus acidophilus PNW3]
atggcagaaaataatcaaccaaataatgatgtacgtcgccatcaccaccatagacatcac
cgtcatcaccatcgtaagttttggcattggttctggattgtgatcggcgttattgtagtc
attgtacttttgtttgtggtatggtttataagaatttacgcgataccacgcaaaatatg
tatactcccgttgctaagacaactaagagcaacaagggacgtaatcttgataatttgcta
gcgcagaaaaaaccaatcaatattcttttgctcggtactgatactggagcaatgggacgt
agctggaaaggacgtactgataccatcatgatgatggcaattaatcctaagactaatagt
acgtcaattgtatctattccacgtgattcaaatgcaatcttcccagatttcccacaatat
ggagtaacgaagattaactctgcttatacactaggcggagttggtgaaacagttaagaca
ttggataaatactatagtgtgccaattgacggttacattatgatcaacatgggtggtctt
aagaaggctattgatcaagttggtggtattgatgtaacttcaccattgactttcgacaat
atgggataccacttccaagaaggtaagacctaccatatggatggtaagaaggcattagcc
tttgctcaacttagacatggggatccacgtcaagattatggtcgtcaagaccgtgatcgc
cgtgttgtcatggcacttcttaagaagtctatctcacctactacattacttaatactaaa
ttcttgaattcaatctcaagtgaaatgcaaactgacttgactatgaatcaaatgtacaag
atcgggatggattatagacatgcaacagataacttgtcacaagatcatgctcaaggtgta
agtaagcaaactcagaatcctaagtttggtactatggaaatcgaagtagtaagtagacaa

gaaaggcaaagagtgtctgataaattaagagcagcattaggtcttcctaaggtacgagtt
gctgctaatagtgccagctatgtcatgaatcaatag

>fig|6666666.426754.peg.1137 **Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2** [Lactobacillus acidophilus PNW3]
MAENNQPNNDVRRHHHHRHHRHHHRKFWHWFWIVIGVIVVIVLFVCGMVYKNLRDTTQNM
YTPVAKTTKSNKGRNLDNLLAQKKPINILLLGTDTGAMGRSWKGRTDTIMMMAINPKTNS
TSIVSIPRDSNAIFPDFPQYGVTKINSAYTLGGVGETVKTLDKYYSVPIDGYIMINMGGL
KKAIDQVGGIDVTSPLTFDNMGYHFQEGKTYHMDGKKALAFAQLRHGDPRQDYGRQDRDR
RVVMALLKKSISPTTLLNTKFLNSISSEMQTDLTMNQMYKIGMDYRHATDNLSQDHAQGV
SKQTQNPKFGTMEIEVVSRQERQRVSDKLRAALGLPKVRVAANSASYVMNQ

>fig|6666666.426754.peg.1440 **Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2** [Lactobacillus acidophilus PNW3]
atgaaggataacagagaaagagaaaatcacccgagcggtactcgagtgcaaagtcataaa
tataaaaatcgacatatttgggcgtgggctacagggataattttattagttgcagtcatt
gcggcagtaacatactttgcatctgtttatttttaaggcgaagaatgcagttgataaaacc
tatgatccagctacagcagtgaaaactacaggtgaatttaatggcaaaaagcgttttgcg
gttctattaatgggaacagacacaggggctttaaatagaactgagaaacgcggtagaact
gataccatgattttagctgtagttaaccctgctaaaaagcgttatacgttggtatctatt
ccgcgtgatacaatggcgcagatggttggctccagtagttttactactgaaaagatcaat
gctgcttatgaaattggcggagctcgaatgtcaatggacagcgtttctgctttgattaat
gtgccgattaagtattatgccgttgtcaacatgggtggaattatgaagatgattcgctat
gttggcgggatcaatattcgaccaacgcttagctttgagtatggcggctatgtctttaaa
aagggtaagttaacccatatgggtggtgccggtgcactggcttactcaagaatgcgttat
gatgatccgcgcggtgactatggtagacaagagcggcaaagacaagttattacaacgcta
attaaaaaagctgtttcagtaagttctttaactaatttagattcaattctgacttcagta
tcaagtaacgttagaactaatttgccatttagtgctttgcaacaaattgcgcttaattac
cgtggttgtgctaatagttcatcaagcgattatcttcatggctataatgcgatgatcgat
gatgccgcttatcaagttcaaccaacagaagaattgcagcggatttctgatttagtacgt
actgagttaggcttagagaaagaaaccattaataacaatgaaacttatcaaaataagcga
aatgaagaaaatggttttagttttaagagtactaagaatcaaacctaccatatttatgat
tacaccgaagaaggtgataattaa

>fig|6666666.426754.peg.1440 **Cell envelope-associated transcriptional attenuator LytR-CpsA-Psr, subfamily F2** [Lactobacillus acidophilus PNW3]
MKDNRERENHPSGTRVQSHKYKNRHIWAWATGIILLVAVIAAVTYFASVYFKAKNAVDKT
YDPATAVKTTGEFNGKKRFAVLLMGTDTGALNRTEKRGRTDTMILAVVNPAKKRYTLVSI
PRDTMAQMVGSSSFTTEKINAAYEIGGARMSMDSVSALINVPIKYYAVVNMGGIMKMIRY
VGGINIRPTLSFEYGGYVFKKGKLTHMGGAGALAYSRMRYDDPRGDYGRQERQRQVITTL
IKKAVSVSSLTNLDSILTSVSSNVRTNLPFSALQQIALNYRGCANSSSSDYLHGYNAMID
DAAYQVQPTEELQRISDLVRTELGLEKETINNNETYQNKRNEENGFSFKSTKNQTYHIYD
YTEEGDN

>fig|6666666.426754.peg.358 **ATP-dependent Clp protease, ATP-binding subunit ClpE** [Lactobacillus acidophilus PNW3]
ttgctttgccaaaattgtcatcaacggcctgccgctattcacctttttacaaaggtaaat
ggtcaaagccgtgaaattgatttatgtcaacaatgttatcaagaattaagaaatcaacaa
ggaaatctagaaaatatgaacaacaataacgaatttttggcgactttgatgatttattt
aacgcattaaacggaaataacaacaacgccgcaaataacaataataatatgaaaaataac
gacccaagaatgcaaatgggtggtggaaatggtggtcaaggtggtagaagcttacttgat
caatatggtactgatttaactgctcttgctaaaaaaggtaaaatcgatccagttatcggt
cgtgataaagaaatcgctcgcgtaattgaaattttaaacagaaggactaagaataatcca
gttttaattggtgaagccggtgttggtaagacagctgtagttgaaggccttgctcaagaa
attgtagatggttctgttccagctaaacttcagaataaacgtattatttcattaaatgtt
gtatcacttgttcaaggtactggcattcgtggtcaatttgaacaaagaatggaacaattg
attagagaattacaacaaaatgatgatatcatcctctttattgatgaaattcatgaaatt
gtaggcgccggaaatgccgaaggcggtatggacgcaggtaatattatcaaacctgcttta
gctcgtggtgaactccaattagttggtgctactactattaaagaatatcgtgatattgaa

aaagattcagctttagcacgtagattccaaccagttgaagtaaaagagccttcaattgat
gaaacgattcgcattttgaagggaatccaacaacgttatgaagactatcatcatgttcaa
tactccgatgattccattgagtctgctgttaaattatcagctagatacattcaagataga
ttcttacctgacaaggctattgatcttttagatgaagccggttcaagaatgaatttaact
attccttatattgataaaggaaaaatgcaagaacgtattaacgctgcagaacaattaaaa
gaggaatctttaaagaacgaagactacgaaaaagcagcttattatcgtgatcaaatcgaa
aaatatgaaaagatgaaggatcaaaaagttgatcctgataaatcaccaattattaccgat
aagattatgaacaagattgtcgaagaaaagacaggaattcctgttggtgatattcaaaag
caagaagaaaatcaattgcaaaacttggctagtgatttaaagtctaatgttattggtcaa
gacaaggcagtcgaaaaagttgctcgagctattcgacgtaacagaatcggtttcaataag
tcaggacgtccaattggttccttcctatttgttgggccaaccggtgttggtaagaccgaa
ttagctaaacaactagccaagcaaatgtttggttcagaagatgccatgattcgtttcgat
atgtcagaatacatggaacaatattctgtctccaaattaatcggctctgctccaggttac
gtaggttatgaagaagctggtcaattaactgaacaagttcgccataatccatatagcttg
attctacttgatgaaattgaaaaagctcatccagatgttttgaatcttttcttacaaatc
ttagacgacggccgcttaactgactctcaaggtagaaccgtttcatttaaggatactatt
attattatgacttctaacgcaggccaaggtatcaagaatgccagcgttggttttactgct
gaaaatgatgacgaatctagcgaatcagcaagaaataatatgagtcaattctttaaacca
gaatttttaaatcgtctagatgatgtaattgaattcaatgaattgactaagccagactta
ttggaaattgtagatcttatgcttcaaaacactaacaatatggttaaggatcaaggctta
catattgacgtaacctcagctgctaaaaataagcttgttgaagaaggctttaatcctgct
ttaggtgcccgtcctcttcgtcgtacaattcaagaagaaattgaagataaagttgcagat
tacaagcttgaccatactgaaagtaaaaacttaaaggctgacgtaattaatgatcaaatc
gtaatcagtgacgaaacagctcaataa

>fig|6666666.426754.peg.358 **ATP-dependent Clp protease, ATP-binding subunit ClpE** [Lactobacillus acidophilus PNW3]
MLCQNCHQRPAAIHLFTKVNGQSREIDLCQQCYQELRNQQGNLENMNNNNEFFGDFDDLF
NALNGNNNNAANNNNNMKNNDPRMQMGGGNGGQGGRSLLDQYGTDLTALAKKGKIDPVIG
RDKEIARVIEILNRRTKNNPVLIGEAGVGKTAVVEGLAQEIVDGSVPAKLQNKRIISLNV
VSLVQGTGIRGQFEQRMEQLIRELQQNDDIILFIDEIHEIVGAGNAEGGMDAGNIIKPAL
ARGELQLVGATTIKEYRDIEKDSALARRFQPVEVKEPSIDETIRILKGIQQRYEDYHHVQ
YSDDSIESAVKLSARYIQDRFLPDKAIDLLDEAGSRMNLTIPYIDKGKMQERINAAEQLK
EESLKNEDYEKAAYYRDQIEKYEKMKDQKVDPDKSPIITDKIMNKIVEEKTGIPVGDIQK
QEENQLQNLASDLKSNVIGQDKAVEKVARAIRRNRIGFNKSGRPIGSFLFVGPTGVGKTE
LAKQLAKQMFGSEDAMIRFDMSEYMEQYSVSKLIGSAPGYVGYEEAGQLTEQVRHNPYSL
ILLDEIEKAHPDVLNLFLQILDDGRLTDSQGRTVSFKDTIIIMTSNAGQGIKNASVGFTA
ENDDESSESARNNMSQFFKPEFLNRLDDVIEFNELTKPDLLEIVDLMLQNTNNMVKDQGL
HIDVTSAAKNKLVEEGFNPALGARPLRRTIQEEIEDKVADYKLDHTESKNLKADVINDQI
VISDETAQ

>fig|6666666.426754.peg.1346 **ATP-dependent Clp protease, ATP-binding subunit ClpC** [Lactobacillus acidophilus PNW3]
atggaaaactcatacagtaaaagtgtaaatcaagtgttggaaattgcgcgtgaacaagca
cagaatttccatcatcgtttaatcggaacagaacatattttattagccttagtaattgaa
actgatggcgaagctggaaaaattttacgttcatgggggctgacgcctaccgctatacgt
gaagaaattgaaagatatacaggatatggctcagctcccaaggctagctatatggaaatg
tcacctcgattgagcttggctttagattatgctaaaagacaagcagaacaaggtggatat
aaagaaattaaaaccaatcatgtgttattaggtataactgctagtgagcaagttttatct
gcaatgattttgaaaaacttaagcgtagatatcagtcgattacgtcaagatgctatcgac
agcttggaacaggatcaagattttaatgattctgctaattggttaggtaatagtgattct
aaccaaaagaaacgtaagaatagcactacgccaaccttggataaagtagcaattaacttg
aatcaacgtacacgtgaaggaaatattgatcctgttattggacgtgataaggaaattgaa
cgtgtaattcaaattttgtcacgtagaactaagaataatccggttttagtaggtgagcca
ggtgtaggtaagacggctgttgctgaagcaattgctactgaaattgttaaaaagagagtg
ccagaagatctgttagataagcgagttatggcattaaatatgggtaacttggttgctggt
actaaatatcgtggtgaattcgaagatcgaatgaagaagatcttggatgaaattgctaaa
gatggcaaagttatcttatttgttgatgaaatgcatactttaattggtgccggtggtgct
gaaggcgcaattgatgcttctaatattttaaagccatcacttgctcgtggtgatgttcaa
atgattggtgctactacatttgatgaataccaaaaatacattgaaaaagatcaagctttg
gcaagacgtttccaacaagtgcgcttaaatgaaccttcaaagaaagatacattagcaatt
ttggaaggtttaaaacccaagtatgaaaaattccatcatgttactattactgaagaaagt

ctagaagacgcagttgatttatctagtcgatatatttctgatcgctttttaccagataaa
gccattgacttggtagacgaagctagtgctgctgtaaaaatcaaaaataatgttaattct
gatgacgaattagtacagattaataacaagattaaaaatttaattgatcaaaaaaatgaa
gcagcagcaagtcaaaacttcgtaaaagctgctcaattacaagatgagcaaaataatttg
caagctcgtcgtgaaaaattagtaaatactttacatgaaaaaattagtgctaatgcgact
gttgagcccgaagatattgccaaagtagtatctgattggactggcgttcctgttacccaa
atgaaacgtaatgaaactcgtcaattagctaatttagaaggtattttacataagcgagtt
attggtcaagataaggctgtttctgcagttgctcgtgcaatccgtagaagtagaagcgga
attaaggatgaacgtcgtccaattggctcattcttgttcttaggcccaactggtgtgggt
aaaacagaattggctaaatctgttgctgcagcaatgtttggctcagaggataacctgata
cggcttgatatgtctgaatacatggatcaaatcgcttcaagtaaattgatcggttcagca
ccaggctatgtaggttatgaagaaggcggtcaactttcagaacaagttcgtcgccatcca
tattcagttgtgttgctcgatgaagtagaaaaagctcatcctgatgtatttaatctctta
cttcaggtcttggatgaaggatttatgactgattctaagggaaggaaagttgattttaga
aatacaatcattattatgacttctaaccttggttcacgttcattatttgatggcaatgcg
gttggattcaatgcggataaaattgatcaagcaaaagtaagacaagcgaaagttcaacaa
gctattaaacagttcttccgccctgaatttttaaacagaattgatgaaactattgtcttt
gatgaattaactaagaaacaacttcgtaacattgtaagtttacttactaataaattggta
gttcgtttacaaagaaaaggtattactttgaaactttcacgtgctgctttagataagatc
gtacaagatggttatgatccagaaaatggtgctcgtccacttaagagagctattcaaaat
gatgtggaagataaagttgccgaaatgttaattaatggggaagttaagagtggtgatact
cttaaaattggtagtcaacatggtaatttgaaattcgaagttgttgcacctaaaaaagaa
gtagaaaaagtcaagtag

>fig|6666666.426754.peg.1346 **ATP-dependent Clp protease, ATP-binding subunit ClpC** [Lactobacillus acidophilus PNW3]
MENSYSKSVNQVLEIAREQAQNFHHRLIGTEHILLALVIETDGEAGKILRSWGLTPTAIR
EEIERYTGYGSAPKASYMEMSPRLSLALDYAKRQAEQGGYKEIKTNHVLLGITASEQVLS
AMILKNLSVDISRLRQDAIDSLEQDQDFNDSANWLGNSDSNQKKRKNSTTPTLDKVAINL
NQRTREGNIDPVIGRDKEIERVIQILSRRTKNNPVLVGEPGVGKTAVAEAIATEIVKKRV
PEDLLDKRVMALNMGNLVAGTKYRGEFEDRMKKILDEIAKDGKVILFVDEMHTLIGAGGA
EGAIDASNILKPSLARGDVQMIGATTFDEYQKYIEKDQALARRFQQVRLNEPSKKDTLAI
LEGLKPKYEKFHHVTITEESLEDAVDLSSRYISDRFLPDKAIDLVDEASAAVKIKNNVNS
DDELVQINNKIKNLIDQKNEAAASQNFVKAAQLQDEQNNLQARREKLVNTLHEKISANAT
VEPEDIAKVVSDWTGVPVTQMKRNETRQLANLEGILHKRVIGQDKAVSAVARAIRRSRSG
IKDERRPIGSFLFLGPTGVGKTELAKSVAAAMFGSEDNLIRLDMSEYMDQIASSKLIGSA
PGYVGYEEGGQLSEQVRRHPYSVVLLDEVEKAHPDVFNLLLQVLDEGFMTDSKGRKVDFR
NTIIIMTSNLGSRSLFDGNAVGFNADKIDQAKVRQAKVQQAIKQFFRPEFLNRIDETIVF
DELTKKQLRNIVSLLTNKLVVRLQRKGITLKLSRAALDKIVQDGYDPENGARPLKRAIQN
DVEDKVAEMLINGEVKSGDTLKIGSQHGNLKFEVVAPKKEVEKVK

>fig|6666666.426754.peg.1566 **Peptide-methionine (R)-S-oxide reductase MsrB (EC 1.8.4.12)** [Lactobacillus acidophilus PNW3]
atggttttagatgaaagtaagaaaaaacaggcactgaagaagttaacccaagaagaatac
gatgtaacccagaacgctgccacggaatatccatttactggcaagtacgataatttttat
gaaaaaggcatctacgttgatgtggtgtctggtgagccgttgttttctagccaagataag
tatgatgcgggatgcggctggcctagttttaccaagccaattgaaaagctgcagtatcac
cgcgaccaatcgcatggcatggagcgtaccgaagtggtaagtccagaagcgcagtcgcat
ttagggcacgtttttaccgatggacctgttgatcgtggcggcttgcgttactgcattaat
tctgctgcattgaagtttattccatatgatcagctgaatgaagctggttatggtgagtat
aagaagttgtttaagtag

>fig|6666666.426754.peg.1566 **Peptide-methionine (R)-S-oxide reductase MsrB (EC 1.8.4.12)** [Lactobacillus acidophilus PNW3]
MVLDESKKKQALKKLTQEEYDVTQNAATEYPFTGKYDNFYEKGIYVDVVSGEPLFSSQDK
YDAGCGWPSFTKPIEKLQYHRDQSHGMERTEVVSPEAQSHLGHVFTDGPVDRGGLRYCIN
SAALKFIPYDQLNEAGYGEYKKLFK

>fig|6666666.426754.peg.784 **S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS** [Lactobacillus acidophilus PNW3]
atggcaaaagttgaaagttttacattagaccacactaaagttaaggcaccttacgttcgt
ttaattactgttgaagaaggtcctaaaggcgacaagatttctaactatgacttacgttta
gttcaaccgaacgaaaatgcaattcctaccggcggattgcatactattgaacacttactt
gccagcttacttcgtgaccgtcttgatggtgtaatcgattgttcaccatttggttgccga
acaggattccacctaatcgtttggggtgaacattcaactactgaagttgctaaagcattg
aagtcttcattagaggaaattcgtgacacaattacttgggaagatgtaccaggtacaact
attaagacttgtggtaactaccgtgatcactcattgttcaccgcaaaagaatggtgtcgt
gatattcttgaaaaaggaattagtgatgacccattcgaaagaaatgtgatttaa

>fig|6666666.426754.peg.784 **S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS** [Lactobacillus acidophilus PNW3]
MAKVESFTLDHTKVKAPYVRLITVEEGPKGDKISNYDLRLVQPNENAIPTGGLHTIEHLL
ASLLRDRLDGVIDCSPFGCRTGFHLIVWGEHSTTEVAKALKSSLEEIRDTITWEDVPGTT
IKTCGNYRDHSLFTAKEWCRDILEKGISDDPFERNVI

>fig|6666666.426754.peg.792 S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS [Lactobacillus acidophilus PNW3]
atgtggggtgaacattcaatgaccgaagtagctagggcattaaaatcatctcttgaagaa
attcgcgatatgattacttgggaagatgtgcctggaactacaattaagacttgtggtaac
tataaagatcattccttgttctctgcgaaagaatag

>fig|6666666.426754.peg.792 S-ribosylhomocysteine lyase (EC 4.4.1.21) @ Autoinducer-2 production protein LuxS [Lactobacillus acidophilus PNW3]
MWGEHSMTEVARALKSSLEEIRDMITWEDVPGTTIKTCGNYKDHSLFSAKE

Appendix L

The sequences of nucleotide and amino acids of identified genes putatively involved in active metabolism in the host

>fig|6666666.426754.peg.292 **1,2-diacylglycerol 3-glucosyltransferase (EC 2.4.1.337)** [Lactobacillus acidophilus PNW3]
atgaatattggtctttataccgatacatattttccccaaataagtggcgtagctacttct
attaggacgctaaaagatgcgcttgaaagacaggggcataatgtatttatttttacaact
acagatccaaatgtagaaaagggcactgttgagccaaatgtttttcgttttagcagtata
ccttttgtttcattcacagatcgtagaattgcatttagaggcttatttgaagcaactaag
gtagctaaggaagtaaatttggatattgtacatacacaaactgaatttgctttaggtaca
attggcaaatatgtagcccaccaattagatattcctgcaattcatacttatcacacaatg
tatgaagattatttgcattatattttaaatggtcacttattgcgaccatatcatgttaaa
caattcgtaaaaagctatttaaaaaatatggatggctgtattgccccaagtggacgtgta
gaagatttgttaaagcgatatggcgtgcaaattccaattagggtaattcctactggagta
gatttgcagggaatgaatggcgatgctgaacgtgatgtacgtcaggaattaggaatcgac
aaagatgctcctgtaattttaactttaagtagaattgcagcagaaaagaaaataaatcat
attcttaatgtgatgccagcaattgtagaagaatttccaaatattaaatttgtaattgcc
ggtgatggacctgatgttaaagtgctgaaagaacaagttgaacgtttaactttagaagat
tatgttttatttgtcggtaacgttgatcatggagatgtaggcaattattatcgaatggcc
gatcttttttgtttctgccagtgacactgaaacccaaggtcttacttatatagaagctttg
gctgcaggtacaccatgtgtagtttacgacactgattacactgaaaatatttttgataat
gatgtctttggacgtacttttgttacacagaaggaaatgttgcaagaaattattgaatta
ttgaaaaaaggacacaatagaattccacaagatcttttacaaaataaattgcagaagatt
tcatcggagcaatttgctacaaatgtccatgatttttataaatacgcgattgatcattat
caacctaaacatgaagaaatataa

>fig|6666666.426754.peg.292 **1,2-diacylglycerol 3-glucosyltransferase (EC 2.4.1.337)** [Lactobacillus acidophilus PNW3]
MNIGLYTDTYFPQISGVATSIRTLKDALERQGHNVFIFTTTDPNVEKGTVEPNVFRFSSI
PFVSFTDRRIAFRGLFEATKVAKEVNLDIVHTQTEFALGTIGKYVAHQLDIPAIHTYHTM
YEDYLHYILNGHLLRPYHVKQFVKSYLKNMDGCIAPSGRVEDLLKRYGVQIPIRVIPTGV
DLQGMNGDAERDVRQELGIDKDAPVILTLSRIAAEKKINHILNVMPAIVEEFPNIKFVIA
GDGPDVKVLKEQVERLTLEDYVLFVGNVDHGDVGNYYRMADLFVSASDTETQGLTYIEAL
AAGTPCVVYDTDYTENIFDNDVFGRTFVTQKEMLQEIIELLKKGHNRIPQDLLQNKLQKI
SSEQFATNVHDFYKYAIDHYQPKHEEI

>fig|6666666.426754.peg.1713 **Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52)** [Lactobacillus acidophilus PNW3]
atgaaggttttgcatgtaaatgctggtcttgaaaatggtggcgggttatcgcatattgta
aatttgcttactgaggctaaaagagaaaataaagattttgatttattaactttggctgat
ggtccagtggctgctgcagcaagagaacatggaattaatacttatgtacttggagcaaag
agtcgctacaatttggcaagtttaaaaaagttgatcaaatttattaatgatggacattat
gacatagtgcatacacatggtgcccgagctaatctttttctttctttaattcataagaga
atctcagctgtatggtgcgttactgtgcactctaacccatatcttgattttgaaggacgc
ggctttttaggcaaagttttcactaagtttaatttacgtgcattaaaaaaagcggactgt
atatttgcagtcactaagcgttttgccaatctgttagttaatcaaacacaactagataaa
aataaagtacatgtgatttataatgggatctttttccacaatgatagtgaaatccctgct
aaatatgagcatacttacttttaatgtaattaacgtagctcgtacagaaaaagtgaaaggg
caagaattgttacttaaagcagttaaaaagctcaacgatcaacatatccgtttacatatt
gcaggagacggcagtcaacttgaaccacttaaagctcttactcgccagctaaatatggca
ccccaagtaacttttcatggctttatgactcatcatcagttaagtggactttataaaaga
attgatctggctgttttaacatcatattcagaaagttttccccttgttttattagaagca
accgataatttaattcctatcttatctactgatgttggtgacatccataagatgattcca
ggacctaaatatggttttattgctaaaactggtgatattgattcaattgccaagcagctt
gaattagctgtcaataagactacaaagcagctacgtgaaatggcctatacagaaaaacgc
tatgctgaagaacacttttctgtgaagaatcaactagcagatattgaaaaagtttatagt
actttaatctaa

>fig|6666666.426754.peg.1713 **Poly(glycerol-phosphate) alpha-glucosyltransferase (EC 2.4.1.52)** [Lactobacillus acidophilus PNW3]
MKVLHVNAGLENGGGLSHIVNLLTEAKRENKDFDLLTLADGPVAAAAREHGINTYVLGAK
SRYNLASLKKLIKFINDGHYDIVHTHGARANLFLSLIHKRISAVWCVTVHSNPYLDFEGR
GFLGKVFTKFNLRALKKADCIFAVTKRFANLLVNQTQLDKNKVHVIYNGIFFHNDSEIPA
KYEHTYFNVINVARTEKVKGQELLLKAVKKLNDQHIRLHIAGDGSQLEPLKALTRQLNMA
PQVTFHGFMTHHQLSGLYKRIDLAVLTSYSESFPLVLLEATDNLIPILSTDVGDIHKMIP
GPKYGFIAKTGDIDSIAKQLELAVNKTTKQLREMAYTEKRYAEEHFSVKNQLADIEKVYS
TLI

>fig|6666666.426754.peg.311 **cellobiose phosphotransferase system celC** [Lactobacillus acidophilus PNW3]
atgaaaagattacttattagagcagatgatctaggatatgctagaagtgtgaattatggg
atatatgattcagttcatcaaggaattatcaataatgtaggtgttatggtgaatatgcct
gaaacacaaatgggtttagatttattgaaaaatgaaaatattgatttcggcttacatact
aatatttcaaatggagctcctattttgtcttctaatgaggtaccttcgttagtaggtgac
aatggtaattttagaagttcaaaggaatatcgtaaaaattatgttgacggtaaaaaagat
attatcgctttaaatgatgtagttgctgaaattgaagcacaatataaaaaatttattgaa
ttagttggtagaaagccagactattttgaaggacacgcagtaatgagtgataatttcgaa
aaaggactaaggattgtggtgtactga

>fig|6666666.426754.peg.311 **cellobiose phosphotransferase system celC** [Lactobacillus acidophilus PNW3]
MKRLLIRADDLGYARSVNYGIYDSVHQGIINNVGVMVNMPETQMGLDLLKNENIDFGLHT
NISNGAPILSSNEVPSLVGDNGNFRSSKEYRKNYVDGKKDIIALNDVVAEIEAQYKKFIE
LVGRKPDYFEGHAVMSDNFEKGLRIVVY

>fig|6666666.426754.peg.339 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
atgaatcgattagttagatggttagaagattatgttttacctatagctagtcggttggga
cggattagatggttggtagcattaagggatgcttttgtatctttgatgccaatcacgatc
gcggggtcattggccgttttaattaagagcttaatagaagcagctcaaactcatttgagc
tggactacttttgcttttgcaatgcagccattggtttcaattagtaatttagtatggcga
ggtacattttcgctatttgctttttttctttgcattagcgcttggctatcaattagctaaa
tcttttgaaggaaatcggctagcagcggcacttgtttcgctgtcttcatttgcattaagt
attgctagtgtagctaaaattaaatttcatggtgatagcgtcacaataaaaaatgcattt
gatattagccaattttcaactactggtctatttacagctattttatttggatcgattggt
tttgcaatatatttggcttgttataaggctagaataatgcttcatttatccgctaatatg
ccacatgcagagcaagccgcttttgattcactaattccagcaatgattgcaatttttggt
gtaggtggtgttaattatctgttccaaacttaacaggtgagtattttggcacttggtta
ctaaatagtattcaacttcctttggttaaatgggggcaaggctttggtactgtgcttttg
gttacgttgcttgttcaggtattttggttctttggaattaatggacttggagttttatca
ccaattcttgattcaatttggctaactgcacaaaacggtaatattactgcgattaagaat
ggtcatgttcccccttatgtttgggtacgtggctcatttgacgtatttgcatggtttgga
ggcgccggtggtacattaatgttgattgttgcaatattgatttttttcaaaaagaagtgac
taccgtacaattgccaaaattgccttagcgccaggaatttttaatattggagaaccaatt
ttgttgggcttacctgtagtgttaaatccagtttatttgattccatttttgttaacgcct
gtagttaacgtggcattttcatattgggtaagcatcatgggattggttaatccggttcaa
gtagccgtgccaagtgtaatgccgccaattattggaccgtttttagcatgtaattatgat
tggcgagcaattgttttaagtattataaatatgttaatagcacttgctatttggactcct
tttgtaattgcggctgataaaattgccaataataatcctaagaaatttttttatgactcaa
ttctaa

>fig|6666666.426754.peg.339 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
MNRLVRWLEDYVLPIASRLGRIRWLVALRDAFVSLMPITIAGSLAVLIKSLIEAAQTHLS
WTTFAFAMQPLVSISNLVWRGTFSLFAFFFALALGYQLAKSFEGNRLAAALVSLSSFALS
IASVAKIKFHGDSVTIKNAFDISQFSTTGLFTAILFGSIGFAIYLACYKARIMLHLSANM
PHAEQAAFDSLIPAMIAIFGVGGVNYLFQTLTGEYFGTWLLNSIQLPLVKWGQGFGTVLL

VTLLVQVFWFFGINGLGVLSPILDSIWLTAQNGNITAIKNGHVPPYVWVRGSFDVFAWFG
GAGGTLMLIVAILIFSKRSDYRTIAKIALAPGIFNIGEPILLGLPVVLNPVYLIPFLLTP
VVNVAFSYWVSIMGLVNPVQVAVPSVMPPIIGPFLACNYDWRAIVLSIINMLIALAIWTP
FVIAADKIANNNPKKFFMTQF

>fig|6666666.426754.peg.588 **PTS system, cellobiose-specific IIB component (EC 2.7.1.205)** [Lactobacillus acidophilus PNW3]
atggctgaacaaactattatgttaaattgtagtgctggtatgagtacttcacttttagta
actaagatgcaagctgctgctcaagaagaaggtattgacgctgaaatttttgcttgtcct
gcttcagaagctgatgacaaaatggcacaaaaacaaattgactgtgttcttcttggacct
caagtaagttacatgagaggtgaatttgaaaataaggttaagggcaagggcaaagacggc
aaggatattccattagatgtcattaacatgcaagactacggcatgatgaatggaaagaat
gttttggctcaagctgaaaagttaattaagggctaa

>fig|6666666.426754.peg.588 **PTS system, cellobiose-specific IIB component (EC 2.7.1.205)** [Lactobacillus acidophilus PNW3]
MAEQTIMLNCSAGMSTSLLVTKMQAAAQEEGIDAEIFACPASEADDKMAQKQIDCVLLGP
QVSYMRGEFENKVKGKGKDGKDIPLDVINMQDYGMMNGKNVLAQAEKLIKG

>fig|6666666.426754.peg.591 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
atgcctgcatcagttccatctggtgtttctaattcatttagtgccttaattcctggtttc
tgtattgcgttagtagttgctataattgaattgattttggtaactttaggaactgatatt
tttaaggtcctttacattccattttcattcattagtaatattgctgatacttggtgggga
ttcctaatcattatcttcttgatccacttgttatggtggtttggtatccatggtgcaaca
atcatgagttcattttatactcctattgttttggctaacatggctgctaacgtaaaaggg
gctacacacttctttgctggtgacccaatgaatgctttcgtaattatcggtggctcaggt
gctactttaggtatggctatttggttagcctttggttcacgttcagcccaattgaaagaa
attggtaaagttgaattagttccagccatcttcaacattaatgaaccgcttttgttcggg
ttgccaattgtttataatatcaatttattaattccattcatttgtgcgccacttgcttca
ggcattgtaggctatgtcgctgtatcgactcatcttgtacctaagattattgttcagcaa
ccatggccaacaccagttggtttgagtggttatcttgctactgtaagttggcaaggggca
gtactttctgtagtttgtgcgattgttgctttcttgatttggtttcctttcattaagcat
tacgataatgttcttcttaaaaaggaacaagcaggagctgctaagaactaa

>fig|6666666.426754.peg.591 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
MPASVPSGVSNSFSALIPGFCIALVVAIIELILVTLGTDIFKVLYIPFSFISNIADTWWG
FLIIIFLIHLLWWFGIHGATIMSSFYTPIVLANMAANVKGATHFFAGDPMNAFVIIGGSG
ATLGMAIWLAFGSRSAQLKEIGKVELVPAIFNINEPLLFGLPIVYNINLLIPFICAPLAS
GIVGYVAVSTHLVPKIIVQQPWPTPVGLSGYLATVSWQGAVLSVVCAIVAFLIWFPFIKH
YDNVLLKKEQAGAAKN

>fig|6666666.426754.peg.596 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
atggctgatcaaaaacaatcaggatttagcagttttgttaataacaaaattttgcctcct
gtaatgaaatttgttaacacgaaagctattactgcattacaaaatggtatgatttatact
ttaccatttattatcattggttctatttttcttaattttaggaaatattccaattcctgca
gtatcaaatgcgattaatgcttctggttggggagcaatctttaaccaagcttacacaaca
acattctcagtaatgtcactttgggcatcaattggtatcgcctatatttacgttaagaat
gagaatttggaaccattagcaccaggtcttacatcctgtgctgcattcttaatgcttcaa
actttatcaattgctagtcctgttaaaactgctttaagcagtggtattcctaatggtatg
aatgctaaagcatttactgctgctattagcgaattaccaaaagctgttcaaacttatatt
gaacaacctgttaccggtgtatttaacatcacttggcttggtggtgacggtatgatcgcc
gcaatcatcatcggtcttcttgttggttggatttacagtgctattgttaaaaaaggttgg
actattaagcttcctaagcaagttccacctgcagtttcaaaccaatttactgctatgatt
cctgcaggtattatcttaatcggttcaatgcttatctacgcatgcttcaacttatttgca
catactgacttcttgcaatggacttataacactattcaaactccattacaaggtatctca

gactcatttggtggtgctcttgctattggtttccttgttccattcttctggttctttggt
gttcacggtggtttgattgttggttcacttgctggtcctatgcttcaagctaactcattc
gataacgctcaattatacaaagctggtaagttaactattgctcaaggtgcacacgttgtt
accaacgaattctacaacaactttattaatttaactggttcaggtattactattggttta
attatcttcattcttgtcgctgccaaatcagctcaattgaagtcaattggtaaattggaa
ttagtaccaggtatctttaacattaatgaaccattcctcttcggtttaccaatcgttatg
aacccattccttgctgttccattcttcttaactccagtagttgttgcagcttcaacctac
ttagtaattagaactggtattattccaccacttaacggatttgctgctccttggacaact
ccagcaattatttccggtttcttaatcggtggttggaagatggctatctggcaagcatgt
actttggtaatttcaacacttatttactggccatttgctaagaagtacgataacgttctt
gttaaacgtgaagctgctaaaaaggctcaagaagacgctgccaagtaa

>fig|6666666.426754.peg.596 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
MADQKQSGFSSFVNNKILPPVMKFVNTKAITALQNGMIYTLPFIIIGSIFLILGNIPIPA
VSNAINASGWGAIFNQAYTTTFSVMSLWASIGIAYIYVKNENLEPLAPGLTSCAAFLMLQ
TLSIASPVKTALSSGIPNGMNAKAFTAAISELPKAVQTYIEQPVTGVFNITWLGGDGMIA
AIIIGLLVGWIYSAIVKKGWTIKLPKQVPPAVSNQFTAMIPAGIILIGSMLIYACFNLFA
HTDFLQWTYNTIQTPLQGISDSFGGALAIGFLVPFFWFFGVHGGLIVGSLAGPMLQANSF
DNAQLYKAGKLTIAQGAHVVTNEFYNNFINLTGSGITIGLIIFILVAAKSAQLKSIGKLE
LVPGIFNINEPFLFGLPIVMNPFLAVPFFLTPVVVAASTYLVIRTGIIPPLNGFAAPWTT
PAIISGFLIGGWKMAIWQACTLVISTLIYWPFAKKYDNVLVKREAAKKAQEDAAK

>fig|6666666.426754.peg.693 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
atgaatgaggataaaatagttagttggcttgttaagtacaaacaattaacttttataaaa
atcttacaacagacaatggtctcgctctttcctgttgctttgattggaacatattgttgg
acattggttaatagttgtttaacgacaaatagttttttggtcgtttaaccaatataacc
aaatggtttccagatcataatttgctagtgcaatagttaatgatgttagtttagctaca
acgggaatttttgcactttatgcggcatttgtcagtgcagaactaacaatgcgacattat
ggaaaagcttctagtattgtaggtatagcaagtgtttcgtcatatatattgatttttgtt
catactattcgcaatactcaacgagtggaaatgagttattatggtgcgacttggttcatt
tgcggtattattgtggggtattttgtaggattagtttttgctaaagtagggcatgatatt
ccgcgtattaaagatcgagatattattaaagaaatttttattaatattcgtcccatgtta
attgtgttagctgtggcattattgattcatttagcttttgcaacatatcggcaatatcaa
atggatcgtatagttgctcaaagtgtatcaactgtagctaatcaacatagtagttatggc
ctatcaattttgatatctttaattactgcctttactatgtggttaggatatgctggaaca
ttaaactttagtagtaatatttttggtagtgaatctattgctaatttgtcatatgcgtta
tcccataagacaccttggaatatcccgtatccttttaccttacaagcacttttttaatgga
tttggaaaaattggtggagttggcgctacactagctttagtcattgctttgattttactt
agtaatagaaagcaaaagcaaaaagttgcattagctagttctgtacctgtttttttttaac
gttaattttgctttagtattgggtataccaacaattttgaatccaatttatttaatacca
tttgttttaacgccaattgtgaatatgtttttaggtagtattgcaatcttacttaaaatg
tttccaccattagtttatccagtaccagatggtctaccaggtattttgggacctttaatg
ggaactggcggaaataatttagctttgatttatacaattttcattttaatagtagatgtt
tgtatctatctacctttttgttaaattagataataaagtcgctgataaatacgaggaaagt
ttaaaatga

>fig|6666666.426754.peg.693 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
MNEDKIVSWLVKYKQLTFIKILQQTMVSLFPVALIGTYCWTLVNSCLTTNSFFGRLTNIT
KWFPDHNFASAIVNDVSLATTGIFALYAAFVSAELTMRHYGKASSIVGIASVSSYILIFV
HTIRNTQRVEMSYYGATWFICGIIVGYFVGLVFAKVGHDIPRIKDRDIIKEIFINIRPML
IVLAVALLIHLAFATYRQYQMDRIVAQSVSTVANQHSSYGLSILISLITAFTMWLGYAGT
LNFSSNIFGSESIANLSYALSHKTPWNIPYPFTLQALFNGFGKIGGVGATLALVIALILL
SNRKQKQKVALASSVPVFFNVNFALVLGIPTILNPIYLIPFVLTPIVNMFLGSIAILLKM
FPPLVYPVPDGLPGILGPLMGTGGNNLALIYTIFILIVDVCIYLPFVKLDNKVADKYEES
LK
>fig|6666666.426754.peg.1173 **Outer surface protein of unknown function, cellobiose operon** [Lactobacillus acidophilus PNW3]
atgttaggattttcgatttatttaaataacgatattacttcagcaactgaacaatatatt
aatcgactaaagcaaaatgggtttgatgggatttttacctcagctcatattcctgaagaa

gatcctaagaaatatcaacaaagattattttctttaggaaatttagccaaaaataataat
ttaaaagtaatggttgatatagatcaagttagcttgaaaaaattagatatttcattaagt
agtttagattctttgaaaagaagaggtattactgggttaagaattgatgacaagttagcg
cctaagcagattgctgaattatcgcaaaaaattgaaattggaataaacggaagtacttttt
acagaagaagagttaaagcaattgggtactttcaatgcagaaatgaaaaatattcaggca
tggtttaatttttatccatgtcctgatacaggtattagtcgacaatttttagaaaacag
acgcaaatgtttaaacaatatgggctgaaggtacaagcatttttcgctggggatgaaaat
ctacgaggaccattacacgaaggattaccaactttggagtatcaaagaaattatgatccc
ttagctagtattttagaattaaaagaattaggagctgatttactttatttgggagatcca
ggaataagtgataaagctcttgatcagataaagaattatcaagaaacggacagtattatt
ctttatacgaaacaagcagaaaatatttctgaagaaatgtacaattatattctagggaaa
cacaaccagcgtcctgatcctgcagaacatgttgtccgttgtgaagattcacggatgcat
gctgcaccaaggattactccagaaaatacaattttaagagaagtggggagcattacttta
aacaattgtctttatgagcgatatgcgggagaaattcaactagttaaagatgctctcact
atgaataagaaggtaaatgtagttggaaaagtgactaatggctctcttcctttaatcgat
ttgattaaaccaagtcagaaattttatctatttaaagaatag

>fig|6666666.426754.peg.1173 **Outer surface protein of unknown function, cellobiose operon** [Lactobacillus acidophilus PNW3]
MLGFSIYLNNDITSATEQYINRLKQNGFDGIFTSAHIPEEDPKKYQQRLFSLGNLAKNNN
LKVMVDIDQVSLKKLDISLSSLDSLKRRGITGLRIDDKLAPKQIAELSQKIEIGINGSTF
TEEELKQLGTFNAEMKNIQAWFNFYPCPDTGISRQFFRKQTQMFKQYGLKVQAFFAGDEN
LRGPLHEGLPTLEYQRNYDPLASILELKELGADLLYLGDPGISDKALDQIKNYQETDSII
LYTKQAENISEEMYNYILGKHNQRPDPAEHVVRCEDSRMHAAPRITPENTILREVGSITL
NNCLYERYAGEIQLVKDALTMNKKVNVVGKVTNGSLPLIDLIKPSQKFYLFKE

>fig|6666666.426754.peg.1402 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
atgaaaaactttattaacacgcgcgtcttaccgccgataatgaaatttgttaacacaaga
ccaatcgttgcattaaaagatggtatggttatatctttgccatttattatgatcggttca
gtatttttacttcttgcttcatttccagttccagcagttgctaactggatggatgcaatg
gggttaactagattctggacgcaagcttataatgcttcatttggaattgtttcagtattt
gcagtaattggtattgcatatcaatgggcaaaaagtgaaaaagtagatccacttccatca
ggattaaccgcatttgttggctttttaattattatgaatccgactagcgctgtaatgaat
ggttcaaaaactattgtttcagctaatcaagcacctaatttattaagtggctatattgat
cgcgtttggcttggtggtcaaggtatgattgccgcaattattattggtttgattactgga
tggatttattcatggtttattaagaaaaagattaccattaagttaccagatcaagttcca
ccagcagtagcaggttcatttgttgctttgattccagcggcagttatttccacattatgg
ctatgtgtatacattttctttgaaaaagttgctaacactacattgactcaatggatctat
gctactatccaaacaccacttcaaggtatttcagattcattcggtggtatcttgattatg
actattttagtaccattcttttggttctttggtgttcatggttcaacacttgttggtggt
attgttggtccaatttacaagctaatgcattggaaaatgctgctatttttaagaaattt
ggctatgtagatgcagaacacggtggtcacattgttattcaggggttatttgatcaattt
tctactgtaactggtgcaggaatgactattggacttgttgtattcatggtcttctttgct
agatcacaacaacttaagagtattggtaaattaaccttggtgccaggtatctttaatatt
aatgagccggttttgttcggtgttcctattgttatgaacccaatgttggctattccattc
ttcatcatgccacctttatcagcaggttcaacttacttattaattaaagctggtattcta
ccataccttaacggtgtacaagtaccatggaccactccaccagttatttcaggattctta
attggtggatggaagatagctctttggcaggcaataattttagtaatttccttctttgtt
tattggccatttgctcgaagctatgacaagatgctctacaagcaggaacaacaagctaaa
aaataa

>fig|6666666.426754.peg.1402 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
MKNFINTRVLPPIMKFVNTRPIVALKDGMVISLPFIMIGSVFLLLASFPVPAVANWMDAM
GLTRFWTQAYNASFGIVSVFAVIGIAYQWAKSEKVDPLPSGLTAFVGFLIIMNPTSAVMN
GSKTIVSANQAPNLLSGYIDRVWLGGQGMIAAIIIGLITGWIYSWFIKKKITIKLPDQVP
PAVAGSFVALIPAAVISTLWLCVYIFFEKVANTTLTQWIYATIQTPLQGISDSFGGILIM
TILVPFFWFFGVHGSTLVGGIVGPILQANALENAAIFKKFGYVDAEHGGHIVIQGLFDQF
STVTGAGMTIGLVVFMVFFARSQQLKSIGKLTLVPGIFNINEPVLFGVPIVMNPMLAIPF
FIMPPLSAGSTYLLIKAGILPYLNGVQVPWTTPPVISGFLIGGWKIALWQAIILVISFFV
YWPFARSYDKMLYKQEQQAKK

>fig|6666666.426754.peg.1560 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
atgcaaaatagtaagttcatgaattggacgaggcaaaagttaatgcctgccttaggaaaa
atgggcgaaaacaaatatatggttgttttaagagatgggatgattatttcagttccattt
actatcttcggttctatctttatgatcattgccaacttgcctgtgccaggttggtcaaat
atcattaagccttatcttccagtattaaatgcgccaattaatatgtctactggtttgatt
ggtttaattattgcacttggtatttcatatactttagcccgtgaaaataagattgatcgt
ctatctgcatcagtaattggtgctattgcttttattatttgcgaattgaacaataaaatg
gtccttgatcctacaaacttaggagcttcaagtatgttcactgctatcgttgtttcactt
atcggtggcgaaatttataactggtttattaagcataaaattgttattaagttaccggat
tcagttccacctgcagtagctagttcatttgtctcattaattcctgcattagcaatttta
ggtggcgcttgggttattagatgtctgcttcatttagatatcaatgcctttattacctgg
atcttctcacctcttactgctactctgggtagtttctggggaatggaactgtttactttc
ttcacacttttgcttggactattggtatccatggtaataatactgttggtattgtagct
ggtcctgtttatggtaatttttttaatggacaatatgcataaagctatgaacggtggcgta
cccactcacatttgtgctgatggtttcttaacttttggtatggcaatgggtggtaccggt
gctgttctcggattagtaatttgtatgttatttgccaaaagtaagcgttataaacaatta
gctcacttaggtttcgttccatgtattttccaaatttctgagcctattatgtttggtgtc
cctgttgtattaaatcctacccttgcaattccatttattactattccttggtattacaa
gcaatctcttatttcttatttaaattccatatcttacatatgatcattgccagtgttcct
tggactactccaactttacttaatggtttcttgattactggtggtgattggaaagccgtt
gtatggcaagctatctgtgtattaatcgcagttgcaggttactggccattcttcaaaatt
ttggataaaaaagctttagcagttgaacaaggcgaaactgctcccgaagaataa

>fig|6666666.426754.peg.1560 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
MQNSKFMNWTRQKLMPALGKMGENKYMVVLRDGMIISVPFTIFGSIFMIIANLPVPGWSN
IIKPYLPVLNAPINMSTGLIGLIIALGISYTLARENKIDRLSASVIGAIAFIICELNNKM
VLDPTNLGASSMFTAIVVSLIGGEIYNWFIKHKIVIKLPDSVPPAVASSFVSLIPALAIL
GGAWVIRCLLHLDINAFITWIFSPLTATLGSFWGMELFTFFTLFAWTIGIHGNNTVGIVA
GPVYGNFLMDNMHKAMNGGVPTHICADGFLTFGMAMGGTGAVLGLVICMLFAKSKRYKQL
AHLGFVPCIFQISEPIMFGVPVVLNPTLAIPFITIPLVLQAISYFLFKFHILHMIIASVP
WTTPTLLNGFLITGGDWKAVVWQAICVLIAVAGYWPFFKILDKKALAVEQGETAPEE

>fig|6666666.426754.peg.1688 **PTS system, cellobiose-specific IIC component** [Lactobacillus acidophilus PNW3]
atggcaaacaaaaaaacaagtggaggtttaaatgcgttcgttaatgagcatattatgcca
gtggccgctaaaataggtaacttcaaacccttaattgctgttcgtgatggtattgcaatg
gcaatgcctttgatcattgtgggatcgttattcatgatcattaactccttcccagttcca
ggctggtctgactggctagctaaaacagcagtgcatggtgtgagtttatcccaaattatg
gctaaaattaccaatggatcatttgggattatggggctgattgctgcctttggtattgca
tggagttatgctaaccaacataaaaccgacggtgtatcagctggcattatttcagcatca
gtattctttattcttacaccaagtattatgagtggagacaaagttcctgtagaaggattc
ccttatacttatttaggtagtagaggtctatttgtagctattatttttggtcttttgagt
gcatggattttccaatggttcattaaccataacatccaaattaagatgccagaaactgta
ccacctgctgtagctaagagtttttcagctttaattccaggtgctgtagtaattgcaatt
gccggttgcgtttactggctatttgcttggactggctggggtaatattcatgatgtaatt
atgaatattttggctaagccgttaggtgccttgggtaatactttgattggtactttagta
tctattatattggtaagtttattctggtttgttggtatccatggcggtaatgttgttaac
acagctatgcaaccaatctggttaatgcaaactgatgctaatcgtgtattaaatcaagcg
ggtcatctagatttggcacacggtggtcacattattactcaaccatttattgataacttt
gtatacatgggtggtggtggtgccactatcggtttagtactttgcattggttacttagta
tggcgtaaacgtgcaagtaagcaaaatgaagttttggcaccattaacaattgttcctggc
ttatttaatattaatgaaccaacaatgtttggtttgccagttgttttaaacttaagtcta
ataattccatttattcttgcgccaatggttaatgcaattactacttatatcgctatgaat
ttaggcttagttccattatgtaacggtagtgttgttccttggactatgccaccaattatt
tctggatttttagcaaccggtagtattgcaggatcaattttgcaattaatcaatattgtt
ttagacatcttaatttacttgccattcatggcagcattaaataaacgtcagattgtagaa
gaaaatgaggcaaaataa

>fig|6666666.426754.peg.1688 **PTS system, cellobiose-specific IIC component**
[Lactobacillus acidophilus PNW3]
MANKKTSGGLNAFVNEHIMPVAAKIGNFKPLIAVRDGIAMAMPLIIVGSLFMIINSFPVP
GWSDWLAKTAVHGVSLSQIMAKITNGSFGIMGLIAAFGIAWSYANQHKTDGVSAGIISAS
VFFILTPSIMSGDKVPVEGFPYTYLGSRGLFVAIIFGLLSAWIFQWFINHNIQIKMPETV
PPAVAKSFSALIPGAVVIAIAGCVYWLFAWTGWGNIHDVIMNILAKPLGALGNTLIGTLV
SIILVSLFWFVGIHGGNVVNTAMQPIWLMQTDANRVLNQAGHLDLAHGGHIITQPFIDNF
VYMGGGGATIGLVLCIGYLVWRKRASKQNEVLAPLTIVPGLFNINEPTMFGLPVVLNLSL
IIPFILAPMVNAITTYIAMNLGLVPLCNGSVVPWTMPPIISGFLATGSIAGSILQLINIV
LDILIYLPFMAALNKRQIVEENEAK

>fig|6666666.426754.peg.783 **Methionine synthase II (cobalamin-independent)**
[Lactobacillus acidophilus PNW3]
atgagtaaaactttagtacattatgacattgttggtagtttcttaagaccagaagaattg
aaaaaggcacgtgctgattttgcagcgggtaacatttcaaaaactgacttgaaaaaggtt
gaagatgaagaaatcgctaaattagttaaaaaagaagaaaaagctggtttaaagattgta
actgatggtgaattcagaagaagttattggcaccttgatacttttggggcttcggtgga
attaagcacactactcaagaacatggctacttcttccacgatgaagaaactcgtaatgat
tctgctcaagttgagggaaagattaaatttacaggtgatcatccagatttagaagcattt
aagtttttgaagagtttaaccgatggcagtgatgtaactccacgtcaaagcattccttca
ccagcccaattttacgcagaactcgtccgtggcccagaaaatgttgcagcagtgaagaaa
gtttacgataccgaagacgaacttttaaacgatatttcaaaagcatattatgatttaatc
atcgcactttacaaagcaggttgtcgcgatgtgaaattggatgactgtacttggggaatg
gtcgtagatgatgatttctgggcaacaatggttaaacaaggttttgaccgtgacgaactt
caagaaaaataccttcgtgttaacaatggcgcacttaaagatctaccagctgatttaaga
acttcaactcatatttgtcgaggcaattaccactcaacttgggctgctaaaggtggttat
ggaccggttgccaaatacgtttttgcacaagaaaatgtcgatgcattctatcttgaattt
gataatgaaagatcaggtaacttcgatccaatcaaggaaattcctgctgataaagaagta
gtacttggtttagtaactagtaagaaacctgaattggaaaagccagaggatttgattgct
cgtatcaatgaagcaagtaagttccacgatttagccaacttagccttaagcactcaatgt
ggttttgcatcaaccgaggaaggaaaccaattgacagaagacgatgaatggaaaaagatt
ggcttagtaatcgatactgctaagcaagtttggaagtaa

>fig|6666666.426754.peg.783 **Methionine synthase II (cobalamin-independent)**
[Lactobacillus acidophilus PNW3]
MSKTLVHYDIVGSFLRPEELKKARADFAAGNISKTDLKKVEDEEIAKLVKKEEKAGLKIV
TDGEFRRSYWHLDTFWGFGGIKHTTQEHGYFFHDEETRNDSAQVEGKIKFTGDHPDLEAF
KFLKSLTDGSDVTPRQSIPSPAQFYAELVRGPENVAAVKKVYDTEDELLNDISKAYYDLI
IALYKAGCRDVKLDDCTWGMVVDDDFWATMVKQGFDRDELQEKYLRVNNGALKDLPADLR
TSTHICRGNYHSTWAAKGGYGPVAKYVFAQENVDAFYLEFDNERSGNFDPIKEIPADKEV
VLGLVTSKKPELEKPEDLIARINEASKFHDLANLALSTQCGFASTEEGNQLTEDDEWKKI
GLVIDTAKQVWK

Appendix M

The sequences of nucleotide and amino acids of identified genes putatively involved in antimicrobial resistance

Lactobacillus reuteri PNW1

>fig|1598.593.peg.1308 **Ribosome protection-type tetracycline resistance related proteins, group 2** [Lactobacillus reuteri PNW1]
gtgaaacaaattgttactggcatcgttgctcatgtcgatgctggaaaaacgactttaacc
gaagccctcatgtatgaaaccggcgctattcgaaagctaggccgcgtggataatggggat
gctttttagatcctaatacgctcgaaaagcaacgcggaatcactctttttacccatcaa
gctgaactaacctacaatgaccttgcgcttacccttctcgataccccaggacacgtcgat
tttgctagtcaaacagaacaagtcttgccagttcttgattatgcaattcttgtcgtttca
gccaccgatggtattcagggatacacgcgcacattatgggatcttcttgcacgttatgat
atcccgaccttcattttcattaacaaagaagacgtggttggtgctgatcctagcggtgtt
attgagcaactgcaaaaagaattttcgccggcttgccttgattttgctgatccattaact
gatgaagttcgtgaaacaattgccatgcaggatgacacagttttagaaaactacctcagt
actggcaatcttagtgatcaaacaattcaacaattgattaaacaacgtaaagtgttcccc
tgttatcgtggagctgccctaaaattaaccggaatcactaatttcttaaagggactggag
aaatggactgttacgcctgagttcgctgatgagtttgcggcgcaggtatttaagatttcc
catgatgacaagggcaacaggcttagttgggttcgcgtattcggtggcaaaattccagct
aaatcagttttactaaacgaagaaaaagtgaaccaaatccgaatatataacggggctaaa
tttactccgagtcaggttttaagcgcaggtcaagtcggtgctcttactggattaacttct
acatcccctggacttagccttggtaaaacaaagccactcccggcaccagtcataaaacca
gtcttaaattatgccgttaaagttgataaagaggaccttcatgcttgtctcaaagcctta
aaagagcttgaagatgaagatccacaactacagatctcttggcatcctcacatccaagag
attcacgttcaaattatgggagaagttcaacttcaaatcctgcaacaattgcttcaagaa
cgttaccaattggaagttacattcgatgagggaaatgttctctatcaagaaacgattact
aacaaagtcgaaggggtcggccactttgaaccattacgtcactatgccgaagcccacctt
cttttagaaccgggaaaacaaggctctggaattacaattagcagtgattgcagtcttgat
atccttgagcgcaattggcaacaccagatcttaactagtttacaggccaaagaacattta
ggggttcttacaggagcacccttaccgatgtcaaaatcaccctcattggtggtcgcggt
catattaaacatacagttggtggagattttcgtcaggctagttggcgagcggttcgccaa
ggattgatggaattgaagagacaaaacgaatgtcaattattagagccttggtatgctttc
caccttaaagtcccgaatgaccaagtaggacgtgcaattaatgatattcaacaaatgcat
ggtacgttcgagcttgatgaagctaatagtcaagacctaaccctgattacgggaaccact
cctgttgtgggcatgcgcgactacacccaaagcgttcgcgcctatactcacggccagggt
caactcgagcttgttgttgctggctattatccatgtcataatgctgaagatgttattaga
gatgttaattatgatccagttgctgacatagaaaacactcctgattccgtcttctgtgcg
catggtgctggtttccccgttccttggaatgaagtcccagcaatggcgcattatccgtat
agaaaatag

>fig|1598.593.peg.1308 **Ribosome protection-type tetracycline resistance related proteins, group 2** [Lactobacillus reuteri PNW1]
MKQIVTGIVAHVDAGKTTLTEALMYETGAIRKLGRVDNGDAFLDPNTLEKQRGITLFTHQ
AELTYNDLALTLLDTPGHVDFASQTEQVLPVLDYAILVVSATDGIQGYTRTLWDLLARYD
IPTFIFINKEDVVGADPSGVIEQLQKEFSPACLDFADPLTDEVRETIAMQDDTVLENYLS
TGNLSDQTIQQLIKQRKVFPCYRGAALKLTGITNFLKGLEKWTVTPEFADEFAAQVFKIS
HDDKGNRLSWVRVFGGKIPAKSVLLNEEKVNQIRIYNGAKFTPSQVLSAGQVGALTGLTS
TSPGLSLGKTKPLPAPVIKPVLNYAVKVDKEDLHACLKALKELEDEDPQLQISWHPHIQE
IHVQIMGEVQLQILQQLLQERYQLEVTFDEGNVLYQETITNKVEGVGHFEPLRHYAEAHL
LLEPGKQGSGITISSDCSLDILERNWQHQILTSLQAKEHLGVLTGAPLTDVKITLIGGRG
HIKHTVGGDFRQASWRAVRQGLMELKRQNECQLLEPWYAFHLKVPNDQVGRAINDIQQMH
GTFELDEANSQDLTLITGTTPVVGMRDYTQSVRAYTHGQGQLELVVAGYYPCHNAEDVIR
DVNYDPVADIENTPDSVFCAHGAGFPVPWNEVPAMAHYPYRK

>fig|1598.593.peg.2421 **Tetracycline resistance, ribosomal protection type => Tet(W)** [Lactobacillus reuteri PNW1]
atggagcggccgctcaaagcagccagccacaccatccatatcgaggtgccgcccaacccg
ttttgggcatccatcggactgtctgttacaccactcccgcttggctccggtgtacaatac
gagagccgggtttcgctgggatacttgaaccagagttttcaaaacgctgtcagggatggt
atccgttacgggctggagcagggcttgttcggctggaacgtaacggactgtaagatttgc
tttgaatacgggctttattacagtccggtcagcacgccggcggacttccgctcattggcc
ccgattgtattggaacaggcattgaaggaatcagggacgcaactgctggaaccttatctc
tccttcaccctctatgcgccccgggaatatctttccagggcttatcatgatgcaccgaaa
tactgtgccaccatcgaaacggtccaggtaaaaaaggatgaagttgtctttactggcgag
attcccgcccgctgtatacaggcataccgtactgatctggccttttacaccaacgggcag
agcgtatgccttacagaactgaaagggtatcaggccgctgtcggcaagccagtcatccag
ccccgccgtccaaacagccgcctggacaaggtgcgctatatgtttcagaagataatgtaa

>fig|1598.593.peg.2421 **Tetracycline resistance, ribosomal protection type => Tet(W)** [Lactobacillus reuteri PNW1]
MERPLKAASHTIHIEVPPNPFWASIGLSVTPLPLGSGVQYESRVSLGYLNQSFQNAVRDG
IRYGLEQGLFGWNVTDCKICFEYGLYYSPVSTPADFRSLAPIVLEQALKESGTQLLEPYL
SFTLYAPREYLSRAYHDAPKYCATIETVQVKKDEVVFTGEIPARCIQAYRTDLAFYTNGQ
SVCLTELKGYQAAVGKPVIQPRRPNSRLDKVRYMFQKIM

>fig|1598.593.peg.2422 **Tetracycline resistance, ribosomal protection type => Tet(W)** [Lactobacillus reuteri PNW1]
atgaaaataatcaatattggaattcttgcccatgtagacgctggaaagacgaccttgacg
gagagcctgctatatgccagcggagccatttcagaaccggggagcgtcgaaaaagggaca
acgaggacggacaccatgtttttggagcggcagcgtgggattaccattcaagcggcagtc
acttccttccagtggcacagatgtaaagtcaacattgtggatacgcccggccacatggat
tttttggcggaggtgtaccgctctttggctgttttagatggggccatcttggtgatctcc
gctaaagatggcgtgcaggcccagacccgtattctgttccatgccctgcggaaaatgaac
attcccaccgttatctttatcaacaagatcgaccaggctggcgttgatttgcagagcgtg
gttcagtctgttcgggataagctctccgccgatattatcatcaagcagacggtgtcgctg
tccccggaaatagtcctggaggaaaataccgacatagaagcatgggatgcggtcatcgaa
aataacgataaattattggaaaagtatatcgcaggagaaccaatcagccgggaaaaactt
gtgcgggaggaacagcggcgggttcaagacgcctccctgttcccggtctattatggcagc
gccaaaaagggccttggcattcaaccgttgatggatgcggtgacagggctgttccaaccg
attggggaacaggggagcgccgccctatgcggcagcgttttcaaggtggagtatacagat
tgcggccagcggcgtgtctatctacggctatacagcggaacgctgcgcctgcgggatacg
gtggccctggccgggagagaaaagctgaaaatcacagagatgcgtattccatccaaaggg
gaaattgttcggacagacaccgcttatccgggtgaaattgttatccttcccagcgacagc
gtgaggttaaacgatgtattaggggacccaacccggctccctcgtaaaaggtggcgtgag
gacccccctccccatgctgcggacgtcgattgcgccgaaaacggcagcgcaaagagaacgg
ctgctggacgctcttacgcaacttgcggatactgacccgcttttgcgctgcgaggtggat
tccatcacccatgagatcattctttcttttttgggccgggtgcagttggaggttgtttcc
gctttgctgtcggaaaaaaatacaagcttgaaacagtggtaa

>fig|1598.593.peg.2422 **Tetracycline resistance, ribosomal protection type => Tet(W)** [Lactobacillus reuteri PNW1]
MKIINIGILAHVDAGKTTLTESLLYASGAISEPGSVEKGTTRTDTMFLERQRGITIQAAV
TSFQWHRCKVNIVDTPGHMDFLAEVYRSLAVLDGAILVISAKDGVQAQTRILFHALRKMN
IPTVIFINKIDQAGVDLQSVVQSVRDKLSADIIIKQTVSLSPEIVLEENTDIEAWDAVIE
NNDKLLEKYIAGEPISREKLVREEQRRVQDASLFPVYYGSAKKGLGIQPLMDAVTGLFQP
IGEQGSAALCGSVFKVEYTDCGQRRVYLRLYSGTLRLRDTVALAGREKLKITEMRIPSKG
EIVRTDTAYPGEIVILPSDSVRLNDVLGDPTRLPRKRWREDPLPMLRTSIAPKTAAQRER
LLDALTQLADTDPLLRCEVDSITHEIILSFLGRVQLEVVSALLSEKNTSLKQW

Lactobacillus acidophilus PNW3

>fig|6666666.426754.peg.1378 **Multidrug resistance protein** [Lactobacillus acidophilus PNW3]
atggcagaaaaaagaaataaaaaaatcattctaatgatggcgacacttctgctggggagt
tttattacgactttagcagaaacgttaatgaacaatggcttgccgatgatcatggaagaa
acgcatattagtcagatgaatgcgcaatggcttaataccgggtatatgctggtggcaggg
atggttatgccactggccacattctttatgcaccgttttcctttgcgccatttgtttact
gcaacgatgagtatctttttattgggatcattggttgctacctttgcacctaatttttg
tttttgttgattggacgattggtgcaagcaatggtcgtcggaattaacatgccacttgta
accaatgttttaacgattattattccggctaaaaatagaggtttggcaatcggaatcgct
gggatcatcatcaatttaggaccggcgattgggccaactttatcaggagtaattcttgaa
ttttatagttggcgaatgttgtttatcattttgttgccatttacggttttgaccttgatt
tgtacgtaa

>fig|6666666.426754.peg.1378 **Multidrug resistance protein** [Lactobacillus acidophilus PNW3]
MAEKRNKKIILMMATLLLGSFITTLAETLMNNGLPMIMEETHISQMNAQWLNTGYMLVAG
MVMPLATFFMHRFPLRHLFTATMSIFLLGSLVATFAPNFLFLLIGRLVQAMVVGINMPLV
TNVLTIIIPAKNRGLAIGIAGIIINLGPAIGPTLSGVILEFYSWRMLFIILLPFTVLTLI
CT

>fig|6666666.426754.peg.1767 **Heterodimeric efflux ABC transporter, multidrug resistance => LmrC subunit of LmrCD** [Lactobacillus acidophilus PNW3]
atgcaaacttttaagattttaacaccatatttcaaacgctataaaaaagacgtttttgca
gcaatgattgcaattatagtttcagcttttgctacattatatcagccaaggctacttgaa
aatattcaacgagcaattatggctgaccaaagacaatctgtaattagagatggaattgtt
ctaattatttttaggaataattgcgatcattgctgggatttttaatgtttattatgcagca
agaatagcgcaaggtgtaacgagtgatttgcgtgaagatacttatgctaagatccaaagt
ttttcttttggtaatattgaaaaattttcggccggttcattaactacacgtttaattaac
gatatgaaccagatcatgaatatgatcatgcagctttttatgcaattacttagaatgccg
attttaattgttggttcgtttatttttttgtattgtgatcattccgcgcttttggtgggca
accgttgtgatggttatcttaatcatgggttttggtacgtatgtattaaaacaaatgaat
agcttgtttgaaaaatttcaacaaacaatgggcaaaatttcaggtcaagcccaagaaact
ttgcaaggtgttcgagtagttaaatcatttaatcaaggaccacaagagattaaaaaattt
gataaaacttcagatagattaaataactacaatattataatcggttattggttttcagca
atcatgccagccttccaattaattgcatacttagttattaccttagttgtttatttaatc
ggtcgcagcattagtcaacatcctaccgatgttgcagttgttagtccatttgttaattat
atattgacccttctttggacaattatgatcgctggtatgactatgatgcaatttgccaga
ggtaatatttcacttggtcgtattaaagaagttttggatacagagccagacgttaaattt
gttaaaaatggctcaactaagccactaaagggtagtgtggaatttgatcatgtttcattt
acttatcctgatggtaatgacgaaactcttaaggatatttcttttaaagtaaaacctgga
caaatggttggtattgttggggcaacgggatcaggtaaatctactttggctcaattaatt
gctagactatatgatccaactaaaggtgaaattaaaattggcggaaaaaatattaaagat
ataactgagactgccttgcgtaatactatttcttttgttttgcaacgtgcaattttattt
tcaggaacaattgcttctaacttacgtcaaggtaaggaacaagctaaacttcatgaattg
caaagagcagctaatatggcacaggcttcagaattcattaaccgctataatgattcgttt
gaacatgaagtagaagaacgatccgctaacttttcgggtggacaaaaacaaagattatct
attgctcgtggtttaatttctgagtctccaattttaattttggatgattcaacttcagcc
ttggatgctgaatcagaaaagaaagttcaacaagcactagaacatgaattatctgataca
actacatttattattgcagaaaagattgtttctgtagttaatgcagatacgattttagtt
ttagatgatggtaaacttgttgcacaaggtacacatgaagaattgttgaagacatcccct
gtctatcaagaaatctttaagacacaaaagggtaggaaaggagtggaataa

>fig|6666666.426754.peg.1767 **Heterodimeric efflux ABC transporter, multidrug resistance => LmrC subunit of LmrCD** [Lactobacillus acidophilus PNW3]
MQTFKILTPYFKRYKKDVFAAMIAIIVSAFATLYQPRLLENIQRAIMADQRQSVIRDGIV
LIILGIIAIIAGIFNVYYAARIAQGVTSDLREDTYAKIQSFSFGNIEKFSAGSLTTRLIN
DMNQIMNMIMQLFMQLLRMPILIVGSFIFCIVIIPRFWWATVVMVILIMGFGTYVLKQMN
SLFEKFQQTMGKISGQAQETLQGVRVVKSFNQGPQEIKKFDKTSDRLNNYNIIIGYWFSA
IMPAFQLIAYLVITLVVYLIGRSISQHPTDVAVVSPFVNYILTLLWTIMIAGMTMMQFAR

GNISLGRIKEVLDTEPDVKFVKNGSTKPLKGSVEFDHVSFTYPDGNDETLKDISFKVKPG
QMVGIVGATGSGKSTLAQLIARLYDPTKGEIKIGGKNIKDITETALRNTISFVLQRAILF
SGTIASNLRQGKEQAKLHELQRAANMAQASEFINRYNDSFEHEVEERSANFSGGQKQRLS
IARGLISESPILILDDSTSALDAESEKKVQQALEHELSDTTTFIIAEKIVSVVNADTILV
LDDGKLVAQGTHEELLKTSPVYQEIFKTQKGRKGVE

>fig|6666666.426754.peg.1768 **Heterodimeric efflux ABC transporter, multidrug resistance => LmrD subunit of LmrCD** [Lactobacillus acidophilus PNW3]
atgggtgatacacaaaaggcaatcaaatactttattaaatatttaaagccatattggaaa
ggaattattattgtagttgttctttccttagtttcaagtggtgcacaagttgttgctcct
gcattcttggggcaagctgtaacagacttaactacctatttatctgacttaagaaaaggt
acagctagtctatctacttttttatagtgcactgtggtcaatgctgttcttctatgtgtta
agtcaagtagctatcttcatcgcttggatggttatgtcaaaatttaacgcagattctact
aatgatatgagagaaaatcttttctctaaattccaaagaatgttagtgcgttattttgat
actcatcaagatggtaaactcctttcattatttacttctgatcttgataacattttcaat
gcaatgaacaacgctatttttgaaattatttcacagggaataatgttcattggtacgatt
atcattatgttcactgtaaatgcacaattagcatggactacgatagcaacaactccattg
attcttattgttagtgtatttattatgcgaaaagcaagaatttatcttgataaacaacaa
gatgaaattggtgatcttaacggttatattactgaacaaatcaatggtgaaaaggtaatt
attactaataatttacgtaaagaatcagttgatggcttcaaaatttacaacaatcgtgtt
agaaatgcgatgtttaaaggacaattttattcaggaattcttttcccattaatgaatggt
ttgaacttgcttaacttagcaattgttatcgcaatgggtgcttggatgattatttcaggt
caagtaactcaagcagtgggactgggattgattgtaactttcgttgaatattcccaaact
tacttccaacctttgactcaaattacttcaatttattcaatgattcagcttgccttaact
ggtgctagaagactagctacagttgaagaacaaaaggatgaaggtacagtacctaatggt
aaaattttggatggcttgaagaaatcagttaaattggaagacgttcattttggctatgac
aagaataaagaaattttacatggtgtaagtatttctgtagataaaggtaaatctgtagct
gtagtgggtccaactggttcaggtaaaactactatcatgaacttacttaaccgtttctat
gatgtaaattctggtaaagtaacgtttgatggtactgatgtacgtgatattaaattggaa
tcactgcgtaataatgtaggaattgttttgcaagattccattttatttagtggtacgatt
gcggataatattcgctatggtaaacctgatgcaagcatggatgaagtagtcagtgctgct
aagcaagcaaatattcatgattttattgaaagtttaccagatggctatgaaactcaagta
agcgatagtagttctgtcttttcaaccggacaaaaacaattggtttcaattgctagaact
attttgattaatccggatttcttaattttggatgaagcaacttctaacgtggataccgtt
acagaagaaaagattcaaaaggcgatggatgctgttattgctggtagaactagtttcgtg
attgcgcaccgtttaaagacaattttgaattcagataagatagttgtcttaaaagacggg
aaggtaattgaagaaggtagccatgaaaagctattaaaagataaaggattttactacaaa
ctttacactgatcagatggctttcgaataa

>fig|6666666.426754.peg.1768 **Heterodimeric efflux ABC transporter, multidrug resistance => LmrD subunit of LmrCD** [Lactobacillus acidophilus PNW3]
MGDTQKAIKYFIKYLKPYWKGIIIVVVLSLVSSGAQVVAPAFLGQAVTDLTTYLSDLRKG
TASLSTFYSALWSMLFFYVLSQVAIFIAWMVMSKFNADSTNDMRENLFSKFQRMLVRYFD
THQDGKLLSLFTSDLDNIFNAMNNAIFEIISQGIMFIGTIIIMFTVNAQLAWTTIATTPL
ILIVSVFIMRKARIYLDKQQDEIGDLNGYITEQINGEKVIITNNLRKESVDGFKIYNNRV
RNAMFKGQFYSGILFPLMNGLNLLNLAIVIAMGAWMIISGQVTQAVGLGLIVTFVEYSQT
YFQPLTQITSIYSMIQLALTGARRLATVEEQKDEGTVPNGKILDGLKKSVKLEDVHFGYD
KNKEILHGVSISVDKGKSVAVVGPTGSGKTTIMNLLNRFYDVNSGKVTFDGTDVRDIKLE
SLRNNVGIVLQDSILFSGTIADNIRYGKPDASMDEVVSAAKQANIHDFIESLPDGYETQV
SDSSSVFSTGQKQLVSIARTILINPDFLILDEATSNVDTVTEEKIQKAMDAVIAGRTSFV
IAHRLKTILNSDKIVVLKDGKVIEEGSHEKLLKDKGFYYKLYTDQMAFE

Appendix N

The sequences of nucleotide and amino acid putative genes associated with toxic biochemicals

Lactobacillus reuteri PNW1

>fig|1598.593.peg.2069 **Arginine deiminase (EC 3.5.3.6)** [Lactobacillus reuteri PNW1]
atgcaaagtccaattcatgttacatcagaaattgggaagcttaagacagttatgttgcat
cgaccaggtaaggaaatcgaaaatgtttatcctgaaattcttcaccggatgcttgtagat
gatattccatacttaccgatcgctcaagaagaacatgacctttttgcgcaaacattacgt
gataacggcgcagaagtcttgtatcttgaagatttattaacagacgcactggcagatgat
aatatcaaagatgaattccttgaaaagatcatcgctgaatctggttatgctgctggagca
atccatgacggcttaaaggaattcttgcttagtttcagtactaaggatatggttaacaag
attattgctggtgtacgtaaagatgaaattaaaacaaagtatgcttcgcttgccgaatta
gcagaagataaggattacccattctatatggatccaatgccaaatgcatactttacgcgt
gaccaacaggcatgtatcggggatggaattaccattaatcacatgacctttaaggcacgt
caacgcgaatctctctttactgagtacatcattaagcacaataagcgttttgctgacaag
ggtgttgaagtatggcgaaaccgttatcctgaaggccggattgaaggtggagatgaatta
gttctcagcgatcatgtattagcaattggtatttctcaacgaacatctgcaaaggctatt
acagaattagctgaaagtctctttgaaaagtctgattacgatacagtaattgctatccat
attcctcacaatcacgcaatgatgcaccttgatactgtgtttacaatgattaactatgat
caatttacagttcatccagcaatcttacgtgatggtggacatgttgatgcatacattatg
cacccaggtaataatggtgaaatttcaattactcatgaaacaaatcttaaagaaatttg
aagaaggcacttgataagccggaaattgatttaattcctactggtggcggtgatccaatt
attgccccacgtgaacagtggaatgacggttctaacacgctagcaattgcccctggtgta
gttgtaacttatgatcggaactatgtctcaaacgatttgctgcgtaagcatggcattctc
gttcacgaggttcgttcaagcgaattatcacggggtcgtggtggcccacggtgcatgtca
tgtccaattgttcgtgaagatctcaagaaataa

>fig|1598.593.peg.2069 **Arginine deiminase (EC 3.5.3.6)** [Lactobacillus reuteri PNW1]
MQSPIHVTSEIGKLKTVMLHRPGKEIENVYPEILHRMLVDDIPYLPIAQEEHDLFAQTLR
DNGAEVLYLEDLLTDALADDNIKDEFLEKIIAESGYAAGAIHDGLKEFLLSFSTKDMVNK
IIAGVRKDEIKTKYASLAELAEDKDYPFYMDPMPNAYFTRDQQACIGDGITINHMTFKAR
QRESLFTEYIIKHNKRFADKGVEVWRNRYPEGRIEGGDELVLSDHVLAIGISQRTSAKAI
TELAESLFEKSDYDTVIAIHIPHNHAMMHLDTVFTMINYDQFTVHPAILRDGGHVDAYIM
HPGNNGEISITHETNLKEILKKALDKPEIDLIPTGGGDPIIAPREQWNDGSNTLAIAPGV
VVTYDRNYVSNDLLRKHGILVHEVRSSELSRGRGGPRCMSCPIVREDLKK

>fig|1598.593.peg.1197 **Ornithine carbamoyltransferase (EC 2.1.3.3)** [Lactobacillus reuteri PNW1]
atggcttttaatttacgtaatcgtagtttcttaactcttgcagattttaacactcgggaa
atggaatacatgcttgatcttgctgaagatttaaagaaggcaaaatatgctggttatgaa
ggcaagaatcttaaaggtaagaacattgctttaatttttgaaaagagttctactcgtact
cgttgttctttttgaagttggtgctaaggatgaaggtgctcatgtaacttaccttggccca
tcaggttcacacatcggtcacaaggaatctgttaaggatactgcacgagttcttggtgga
atgtttgatggtatcgaataccgtggattctcacaacgcaatgttgaaattttagctaag
tactctggtgtaccagtttggaatggtttaactgacgaagaccacccaacacaagtactt
gctgacttcttaaccgctcacgaagtattgaagaagccttacaaggacatcaagtttgcc
tttgtaggtgacggtcaagacaatgtttcaaatgcattgatgcttggtgccgctgtaatg
gggatggaataccacgttgttactcctaaggaattggaaccaactaaggaaactctagat
aaggctaatgaaattgctgccaagactggtgctaagattgttgttactaatgacatcaaa
gaaggtgtcaaaggtatggacgtaatctatgctgatgtttgggtatcaatgggtgaatct
gatgatatgtgggaaaagcggatcaaccttcttaagccttaccaagtaacaatggatgtt
atgaaggctactgaaaaccctaatgttctctttgaacactgtcttcctgcattccacaac

ctcgacactgaagttggtaaggaaattgaaaagaagtttggcttaaaggaaatggaagtt
actgacgaagtattcgaaagtgaacattcagttgtcttccgtgaagccgaaaaccggatg
cacactatcaaggctgtaatggttgcaactttgggtgaacaaaactaa

>fig|1598.593.peg.1197 **Ornithine carbamoyltransferase (EC 2.1.3.3)**
[Lactobacillus reuteri PNW1]
MAFNLRNRSFLTLADFNTREMEYMLDLAEDLKKAKYAGYEGKNLKGKNIALIFEKSSTRT
RCSFEVGAKDEGAHVTYLGPSGSHIGHKESVKDTARVLGGMFDGIEYRGFSQRNVEILAK
YSGVPVWNGLTDEDHPTQVLADFLTAHEVLKKPYKDIKFAFVGDGQDNVSNALMLGAAVM
GMEYHVVTPKELEPTKETLDKANEIAAKTGAKIVVTNDIKEGVKGMDVIYADVWVSMGES
DDMWEKRINLLKPYQVTMDVMKATENPNVLFEHCLPAFHNLDTEVGKEIEKKFGLKEMEV
TDEVFESEHSVVFREAENRMHTIKAVMVATLGEQN

>fig|1598.593.peg.408 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
atgagtgagaaaaaaggtattaatacgacggaactaacccttttaattgtgggatcaaca
attggttcgggagtatttggaattaccagtgatttagctaccgcggcggctcccggacca
gcaatcattgcctggataatagtaggggtcggcgttttgactttagtgctctccctaact
aatctttcgcaaaagcggccagaccttgattcagggattttcagctatgcggcagctgct
tttggtccgctgggcgaatttatttctggttgggcatactggctttcagcgtggctcggc
aatattgcttttgccacgattatgatgagcgcccttggaacgtttttccccagcctattt
gccaatggtcaaaacttattctcaattattgttgcgattattttaacctgggttttgaca
tttttggtaaatcgagggatcgaatcagcggccttcatcaattcaattgggacaatctgc
aagattattccattagtcgtctttgttatctgtgtgattcttggttttaaggctaagatc
tttactgctgatttctggggcaatgtaacacagaatctcagttcaaaaatcgaaactggt
tcaatctataaccaagtaaaggcctcaattatggtaatgatgtggctatttgtcggcgtt
gaaggtgcctcagttctttctagtcgtgccaaaacacggactgatgctgaacgggcttcc
atcttcggtcttgtaagcctactcgttatctacattgtggtttctattctaccatacggg
gtaatgacccgcgcccagttagcagctggtggtcaaccggcgcttggtgctatcatgcaa
catctggttggaacgtggggtgccgccctgatcagtatcggattgatcatctcagtatta
gtttcgtggctttcgtggacaatgctcccagctgaatcattaatgctaatggctgatgat
gaaacccttccaacatcatggggtaagctaaatgacaaaaaagcgccaacacgggcatta
ataatcaccggggttcttcaatcaatcttcctcttctctttacttttcactacttatgct
tataactttgcatatacgctttgtacggctgcgattttgatctgttacctccttgttggc
atttaccagatgatgtacagttggcaacaccaagaatggggacaatttgtcattggacta
attgccaccctctttgaactgatggcgatggttctctctggctggaaggaaataatggtt
gtttcagtcggttttattcccggattcttcttctatgtccaagcatgcaaggaattccat
cacccaatcacagtaaaggaaaaatgggcaatggggattatcgctgtctttgcggttatt
tccctagtattagtaatcaacggaacaatttcaattaattaa

>fig|1598.593.peg.408 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
MSEKKGINTTELTLLIVGSTIGSGVFGITSDLATAAAPGPAIIAWIIVGVGVLTLVLSLT
NLSQKRPDLDSGIFSYAAAAFGPLGEFISGWAYWLSAWLGNIAFATIMMSALGTFFPSLF
ANGQNLFSIIVAIILTWVLTFLVNRGIESAAFINSIGTICKIIPLVVFVICVILGFKAKI
FTADFWGNVTQNLSSKIETGSIYNQVKASIMVMMWLFVGVEGASVLSSRAKTRTDAERAS
IFGLVSLLVIYIVVSILPYGVMTRAQLAAGGQPALGAIMQHLVGTWGAALISIGLIISVL
VSWLSWTMLPAESLMLMADDETLPTSWGKLNDKKAPTRALIITGVLQSIFLFSLLFTTYA
YNFAYTLCTAAILICYLLVGIYQMMYSWQHQEWGQFVIGLIATLFELMAMVLSGWKEIMV
VSVGFIPGFFFYVQACKEFHHPITVKEKWAMGIIAVFAVISLVLVINGTISIN

>fig|1598.593.peg.866 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
gtgcaggaaacacaaacgaagaaaaaacatcattttcatatgccctctgcgtttacaatt
ttatttttaattattatttttaattgcaattttaacgtggattataccagcagggacatat
gagacggataaagctggaaatattatttctggcacatataaggcggtgacaaataaaccc
cagggaatttgggatatctttatggcacccgttatcgggatggtcggtgataaaaagaca
gatggagctatctcagtatcactatttattcttgttatcggtggattcctaggagtggtt
aataagacaaatgcattaaacgaagggatcggttcaatcgttcgccggtataagggacga
gaaaaacaattgattccaattttaatgcttctgtttgctcttggtggatcaacatatgga
atgggggaagaaacgattgcattctatcctctcttgatccccgtgatgatgggtgttggc

tttgattcaattgtagcggttgcgattgctttagtgggaagtcaagttggttgtttagca
tcaactgttaacccgtttgccacaggagtagcttctcaaacgttaaatatttctcctggt
gatggtttagtttcccgggtaatccttttaattattacgataacgattagtattatttat
gtttaccactatgcatcggtaattgaaaaagatccaactaaatcattagtttacaatcaa
cgagcagaagatgaaaagcacttttagtcaaggctgatcctaatgacgaccataagatg
accggtcgtcaaaagacagttatttggctattcggaattacttttgttgtgatgattcta
ggactaattccatggtcaaaccttaatagccattggacatttttgataaatttacaaag
tggttggttaatattccattcctaggcgatttattagggcatgatatggcacccttgga
acatggtacttcaatgaaattaccatgctcttcctctttatgtcggtcttgattatggct
gtttaccatatgaaggaaagtgaatttattgacgctttcatgtcaggaatgggagatttc
ttaagcgttgcaattattgtggcagttgcacggggaattcaagtaataatgaataatggg
atgatcaccggaacagttcttcactggggagaattgggccttcatggattatcacaaacg
atctttattattttgacttacattttctatattccaatgtcattcctaattccatcaacg
tctgggttagctgcggcaacgatgggaattatcgggccaatggggcattttgcacatgtt
tcagggagccttgttattaccgcttatcaagcagcttcaggatgggttaaccttattacc
ccaacttcaggagttgttatgggagcattagcaattgcgcatattaatgtcggtatttgg
tggaagtggatgctgaaattaatgatctatctctttattgctacttgcttgttcttagga
attgctgccttgctctag

>fig|1598.593.peg.866 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
MQETQTKKKHHFHMPSAFTILFLIIILIAILTWIIPAGTYETDKAGNIISGTYKAVTNKP
QGIWDIFMAPVIGMVGDKKTDGAISVSLFILVIGGFLGVVNKTNALNEGIGSIVRRYKGR
EKQLIPILMLLFALGGSTYGMGEETIAFYPLLIPVMMGVGFDSIVAVAIALVGSQVGCLA
STVNPFATGVASQTLNISPGDGLVSRVILLIITITISIIYVYHYASVIEKDPTKSLVYNQ
RAEDEKHFLVKADPNDDHKMTGRQKTVIWLFGITFVVMILGLIPWSNLNSHWTFFDKFTK
WLVNIPFLGDLLGHDMAPFGTWYFNEITMLFLFMSVLIMAVYHMKESEFIDAFMSGMGDF
LSVAIIVAVARGIQVIMNNGMITGTVLHWGELGLHGLSQTIFIILTYIFYIPMSFLIPST
SGLAAATMGIIGPMGHFAHVSGSLVITAYQAASGWVNLITPTSGVVMGALAIAHINVGIW
WKWMLKLMIYLFIATCLFLGIAALL

>fig|1598.593.peg.1924 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
atgatgggagaagcaacagcagggaaaaagcatcggtttaagttaaaaatgccaggtgct
tttacgattttattctttctaacggtgattgcagtgatgttgacttggattgttccagcg
ggaagctattctaagttagcatatgtcaatgatcatctccaagtaacacaaccaactggg
gaaaaaattagttatccggcaacacaggcaacattagataaattgcacattcagattgat
gtcgaccagtttaaatctggtgcaatcaataagccgatttcagtaccgggaacttataag
cgacttgcacagcatcctgcttcggtcagcgatatttttttctagtatggtcgacggtacg
attgaatcagttgatattatgatttttatcctagtcttaggtggtttgattggtgttgtt
aaagcaagtggagcctttgaatctgggctggccgcactatctaccaaaactaaggggaaa
gaatttattttagtgttcctcgtgacagttttactagcacttggcggaaccctatgtggg
attgaagaagaagcggttgccttttatccaatcttagccccagtttttattgcgatgggt
tatgattcaattgtttgtgttggggctattttcttagcaagttcgcttggaacaacattt
tcagtaatcaatccgttttcagttgtcattgcgtcaaatgctgctgggacaacttttacg
caaggaatggcaggacgaatttttggtttggtaattgcagttacttgtttattattttac
ctgcattggtatgcacgaaaagtacaacagaacccccagttttcttattcctatgatgac
cgagaaaaatttgatcaaatgtggggaatgactactagcgaagaaaaagatcagcggttt
actttaaaaaagaaaattattttaattttatttgcttgtgctttcccaattatgatttgg
ggagtaatggccaaaggatgggcctttcctaacatggcatcagctttcttaacaattgct
attattattatgtttttaacttgtttcgggcgccaagacggtttaggcgaatataagacc
acaacagctttttctgatggagcagctagtttagtcggtgtgtcattaattattggtttg
gcacggggaattaataaagtgcttaatgacggctatatttctgatacaattctctatgct
tcttcaaaaatggttgcgcatatgaacggatcatttttcattattgtgatgatgttggta
ttctttgtcttaggctttattgttccatcatcatctggtttagcagtgctagcaatgcca
attttagcgccgctagctgatacggttcacattccacggtatacagttgttactgcatat
caatttggtcaatatgcaatgttattcttagcaccgactggattagtaatggcaactttg
caaatacttgatatgcgatattcccattggttgcggttcgtgtggccggtagttgtcttt
gttttaatctttggtggtggattgttggtaacagaagtattaattgcaggataa

>fig|1598.593.peg.1924 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
MMGEATAGKKHRFKLKMPGAFTILFFLTVIAVMLTWIVPAGSYSKLAYVNDHLQVTQPTG
EKISYPATQATLDKLHIQIDVDQFKSGAINKPISVPGTYKRLAQHPASVSDIFSSMVDGT
IESVDIMIFILVLGGLIGVVKASGAFESGLAALSTKTKGKEFILVFLVTVLLALGGTLCG
IEEEAVAFYPILAPVFIAMGYDSIVCVGAIFLASSLGTTFSVINPFSVVIASNAAGTTFT
QGMAGRIFGLVIAVTCLLFYLHWYARKVQQNPQFSYSYDDREKFDQMWGMTTSEEKDQRF
TLKKKIILILFACAFPIMIWGVMAKGWAFPNMASAFLTIAIIIMFLTCFGRQDGLGEYKT
TTAFSDGAASLVGVSLIIGLARGINKVLNDGYISDTILYASSKMVAHMNGSFFIIVMMLV
FFVLGFIVPSSSGLAVLAMPILAPLADTVHIPRYTVVTAYQFGQYAMLFLAPTGLVMATL
QILDMRYSHWLRFVWPVVVFVLIFGGGLLVTEVLIAG

>fig|1598.593.peg.2066 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
gtgaaggaaaagaagttaaatctcttcctcttaatcaccttaattgtaggaacgattatt
ggtggtgggatttttaatagtccgaccgatttaattttgaaagcaaatccaatggctgca
ttagttgcctggttaattggtggcttcgggattttgatgctagtactagtttttacaag
ctttctgttattaagccggagatgaacggtggaatttatacttatgcaaaagaaggcttt
ggtaactatattggttttaactccttttggggatattggatgggagcagtttttggtaac
attgcctttatctcgctctttttttaagaccttaaatagtatgctcgggacgcatcaactt
tcgccattaatgtgttttatcgggggctcaattattctgtggggatacactgcgattaca
tggtttggtgtccgcgaggcaagtatccttaatgccgtcattacgattattaagatctta
ccgcttttattagttgttattgttggtgtctttgcattccagccgcatatttttaatgtt
cctgattggacaaacattcttgccgttaatcaaacgcaagtcccatttaaagaccagatt
agtggtgcgatgagtactattgtgtggtgttttgttgggattgaagcagctgtttcaatg
tcacgtcgggcaaagcagccacgtgatgtggggttagcaacgattattagttttgtaatt
gtattggtcctctatatttcaatctctattattccaatggggattctacctgctaaagaa
cttagccagacttcagttccactggcagcagtgcttagtcatacagcattaggaatagta
gggagtcttattattaaaattggcttactgatttctctattaggggcattattaagttgg
tttatgattggaccagaaattgcatacgtaaccagttatgatcgggatatgccgcgagca
tttaaacttgttaatcgccatggtgttcctggctttgcattaattgtatatacaattatt
atgcaagtatgtttactagttttactgcttccacagcttcaaatggcctatacaattgca
tatactttagctgcgacgatgaccttagttgcttacctgctctctgcgttatatgggttg
aagctagcaattagcgaaaaaatgggccttggctttaagattattgctacgttagcttct
ctttattccgtctatatgttagttgcttcgggacttgaatatgtatttgcaagtgcgatt
atttttgcgctaggaataccattatttgccggggctcctaataaaatgagtaagaaagaa
aggtggttagcaacaattattgccttagcagctgtgattgcgctgatgttgattattact
ggaaagattaatttttaa

>fig|1598.593.peg.2066 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
MKEKKLNLFLLITLIVGTIIGGGIFNSPTDLILKANPMAALVAWLIGGFGILMLVLVFYK
LSVIKPEMNGGIYTYAKEGFGNYIGFNSFWGYWMGAVFGNIAFISLFFKTLNSMLGTHQL
SPLMCFIGGSIILWGYTAITWFGVREASILNAVITIIKILPLLLVVIVGVFAFQPHIFNV
PDWTNILAVNQTQVPFKDQISGAMSTIVWCFVGIEAAVSMSRRAKQPRDVGLATIISFVI
VLVLYISISIIPMGILPAKELSQTSVPLAAVLSHTALGIVGSLIIKIGLLISLLGALLSW
FMIGPEIAYVTSYDRDMPRAFKLVNRHGVPGFALIVYTIIMQVCLLVLLLPQLQMAYTIA
YTLAATMTLVAYLLSALYGLKLAISEKMGLGFKIIATLASLYSVYMLVASGLEYVFASAI
IFALGIPLFAGAPNKMSKKERWLATIIALAAVIALMLIITGKINF

>fig|1598.593.peg.2067 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
atggatgaacaaaagaaaggtattggtagaggcgaattgatcgccttaattgtaagttca
tgtatcggtaccggaatatttggtattactagtgatgtggcagcggcggcggcaccaggt
cctgccctgttagcttggatttttgttggaattggtttttgatgttagttctctcgcta
aataatttatctgaaaagcgaccagatcttacttcagggattttctcatatgctggtgcc
ggttttgggccattaggtgaatttatttctggatggtcttactggttatccgcatggctg
ggaaatattgcttttgccaccatgttgatgagttcaattggaacgttcttcccaaccttt
aaaggtggtcaaaatttaccatcgattattattgccatcatcttctgttggctattaaca
atcttagttaacaatggtgttgaaagtgcatcttttgttaacatgattggaacaatctgc
aaggttttaccattagttatctttattattatgatggttgtttgcttcaaggctgggatg

ttcacagcagatttctggggccgagttgccaataatgcttctcacggcactacaactggg
tctgtttgggaacaaatgaaaggtaccctaatgaccttaatttgggtatttatcggtgtt
gaaggtgcttctgttatggcaagtcgggcaaagtcccttacagccgctcgtgaagcttct
ctcatcagttttggtcttttagtagttatctatgtattaatttcaattttaccttatggt
gcattaactcgggcagaattagccggcatgggacaaccagctattggtcatgttcttcaa
gcaaccgttggtagttggggctcaattttaatcaatgttggtttaatcatctcaacaatt
gtttcctggctttcttggacaatgctcccagccgaaacaacaatgttagttgccgatgat
aaggcaatgccaaaaatctggggaaaggttaatgccaaaaaagcaccaactgcttccttg
atgattacagcggtattgcaaacaatcttcttgttctcattgcttttcactgataaagcc
tatgaattcgcatattccttatgtagtgcagcaattttattctcatacttatttgttgga
ctttaccaaatgaaatacagttctgcacaccaagaatggggacaatttacaattggtttg
ctctctgctgcattcatgttcgcatgtatgttccttgctggatggcaagaagtcttatta
gtttcaattagttttattcctggattctacatttactacttagcatgtaaagaaaataat
cgtaaagttacaactgctgaaaagtggacaatggcgctaattttaatcctaagtgttatt
gcaatttggcttgttgctaatggaactattgccatcggctag

>fig|1598.593.peg.2067 **Arginine/ornithine antiporter ArcD** [Lactobacillus reuteri PNW1]
MDEQKKGIGRGELIALIVSSCIGTGIFGITSDVAAAAAPGPALLAWIFVGIGFLMLVLSL
NNLSEKRPDLTSGIFSYAGAGFGPLGEFISGWSYWLSAWLGNIAFATMLMSSIGTFFPTF
KGGQNLPSIIIAIIFCWLLTILVNNGVESASFVNMIGTICKVLPLVIFIIMMVVCFKAGM
FTADFWGRVANNASHGTTTGSVWEQMKGTLMTLIWVFIGVEGASVMASRAKSLTAAREAS
LISFGLLVVIYVLISILPYGALTRAELAGMGQPAIGHVLQATVGSWGSILINVGLIISTI
VSWLSWTMLPAETTMLVADDKAMPKIWGKVNAKKAPTASLMITAVLQTIFLFSLLFTDKA
YEFAYSLCSAAILFSYLFVGLYQMKYSSAHQEWGQFTIGLLSAAFMFACMFLAGWQEVLL
VSISFIPGFYIYYLACKENNRKVTTAEKWTMALILILSVIAIWLVANGTIAIG

Lactobacillus acidophilus PNW3

>fig|6666666.426754.peg.700 **Ornithine decarboxylase (EC 4.1.1.17)** [Lactobacillus acidophilus PNW3]
atggatttttaaaaatagctattggaaataatgttagtatcgataaattaaaagattgg
acattagttccaattggtcaagctgaaaatagtgcggagcttgcagctattgtcattaag
caaaatgatatcttagctaaattaaaagctgatgaattaaaaaaacaatctgggtttccg
attcctgtgattgaagtagagcagcaagttgattcaaaagtaaaacaagatataattcaa
aaagcggagaactatcaacatgaaatggtgcccggtttttaactgatttgattaatttc
gctcaggctaaaccgattagttttactacgccgggtcaccataatggtcaataccttgat
ttgcatcccgctggtgtagtattcaataggttctttggcaagaatatgatgtttgcagat
acaagcgataccgttcctcaattgggtgatacgatgactcatacgggtacaccacttgac
gcagagaaaaaggcggcagaaacttatcatgctgataaagtgtatttctgtacgaatggt
acaactagtgctaattctatctgtgcaagtgcgcttttgtctgaaggtgatttagtatta
tttgaccgtaataatcacaagtccttatacaatagcgcattagttatgagcggtgctaag
cctgtatatattccaactgatcgcaatgctttgggattaattggcgaaatggaccctgac
tttttaacagaagataaaattagagctgaaattgccaaagttgatccaaaaaaagctaaa
ttaaagcgtccattcagattagccattgtgcaagctgaaacttatgatggtatattttat
gatgctaagtggattcttgatcgaatcggaaaactttgtgactacatcttattcgattgt
gcatggggcggatttgaagaatttgttccaattatgaaacacttatcaccattactcctt
aacctagggcctgatgatcctggtattttggtaactcaatcactgcacaagcaacaagtt
ggtatggctcaggcttctcaaattttgaagaaggactcccatattcaaggccaaaagcgc
tatgttgaccacaagcactttaaccatgaatacttgaagtttgttacttcttcatatgcc
tatccactttatgcatctttgactgttaactcttatgttactgctggtgaaggtaacaaa
aagtggtgggatgaaatcttgcgtatgggtattgaatggcgtaaggaacttctaaagaag
tctaaattatttaagccatttgttccagttaacttttagatattccgactaatgaattg
gcaactaatgctaagtattggaatatgagcaaagaagataattggcatgggtttactaag
atgggtaaaggcgaagcaatgatcgatccactcaagattacggtaaaaactcctggtatt
gatgtccagaatgctaaatatgaagaaacaggtattccaggtgcagttgtagcagaattc
ttgatggaaaatcatattattcgtgcaaagaacgacttgaattcgcttttgttcttgcta
acaccaggtgatactaaagaagaacttgataccttgcttgatgcatttttgaagtttgaa
aagtattataacgatgatggtttggtaaaggatgtccttccagttttgtacaaagaatat
cctgatcgctacaagggctacactgttaagcatctttgtcaagaaatgcacgaatactat

aaagaaaataatacttttgttttgcagcaagaactatttgaaaagccggggatgcaagat
tacaagatgactcctgcagaagctgatcaaatgtttaagcgtaatgaaaataaagtagtc
aaccttgaagatgtggtaggtgaaaccgcagctgaaggtgctttgccatatccaccggga
gtctttattgtagctcctggtgaaaagtggggcacaattgatcaaaagtacttcgaagtt
ttggcacatgcaattgaaaaatttccaggttttgtgccagaaattcaaggagtatatttg
gatcaaaatgctgatggtacgttgagagtacaagctgaagttattaagaaatag

>fig|6666666.426754.peg.700 **Ornithine decarboxylase (EC 4.1.1.17)** [Lactobacillus acidophilus PNW3]
MDFLKIAIGNNVSIDKLKDWTLVPIGQAENSAELAAIVIKQNDILAKLKADELKKQSGFP
IPVIEVEQQVDSKVKQDIIQKAENYQHEMVPGFLTDLINFAQAKPISFTTPGHHNGQYLD
LHPAGVVFNRFFGKNMMFADTSDTVPQLGDTMTHTGTPLDAEKKAAETYHADKVYFCTNG
TTSANSICASALLSEGDLVLFDRNNHKSLYNSALVMSGAKPVYIPTDRNALGLIGEMDPD
FLTEDKIRAEIAKVDPKKAKLKRPFRLAIVQAETYDGIFYDAKWILDRIGKLCDYILFDC
AWGGFEEFVPIMKHLSPLLLNLGPDDPGILVTQSLHKQQVGMAQASQILKKDSHIQGQKR
YVDHKHFNHEYLKFVTSSYAYPLYASLTVNSYVTAGEGNKKWWDEILRMGIEWRKELLKK
SKLFKPFVPVNFLDIPTNELATNAKYWNMSKEDNWHGFTKMGKGEAMIDPLKITVKTPGI
DVQNAKYEETGIPGAVVAEFLMENHIIRAKNDLNSLLFLLTPGDTKEELDTLLDAFLKFE
KYYNDDGLVKDVLPVLYKEYPDRYKGYTVKHLCQEMHEYYKENNTFVLQQELFEKPGMQD
YKMTPAEADQMFKRNENKVVNLEDVVGETAAEGALPYPPGVFIVAPGEKWGTIDQKYFEV
LAHAIEKFPGFVPEIQGVYLDQNADGTLRVQAEVIKK