



Bacterial communities associated with the surface of sweet pepper and their selection for biocontrol.

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Thesis accepted for the degree **Doctor of Philosophy in Science
with Biology** at the North-West University

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Graduation: May 2021

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DECLARATION

I, the undersigned, declare that this thesis submitted to the North-West University for the degree of Doctor of Philosophy in Science with Biology in the Faculty of Science, Agriculture and Technology, School of Environmental and Health Sciences, and the entirety of the work contained herein is my original work with the exception to the citations and that this work has not been submitted at any other University for the award of any degree.

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DEDICATION

This work is dedicated to my devoted mother, **Mrs. Avhatakali Salminah Netshilema**. You have been the pillar of my strength, helping me through many difficult moments. Your constant encouragement and sustenance has enabled me to reach this far. I love you always.

ACKNOWLEDGEMENTS

I would like to give God Almighty all the glory, honour and adoration for giving me the strength and wisdom through the Holy Spirit to complete this work.

I also hereby give my credit and sincere appreciation to the following people and organizations, without whom, this project would not have been conceivable:

Professor Olubukola Oluranti Babalola: I am so much thankful for giving me the opportunity to pursue my PhD under your supervision. You really inspired me through your work ethics and instilling a culture of excellence in your research team.

Dr Olayinka Ayobami Aiyegoro: Thank you for giving me a chance to work in your laboratory and for allowing me to learn to the level I am today. You made it endurable throughout.

Mr Phathutshedzo Ramudingana: I do acknowledge your assistance in collection of samples to the laboratory.

Dr Martin Maboko: Thank you for the assistance throughout the cultivation process.

Dr Casper Nyaradzai Kamutando: I do appreciate the time you spared to give me one on one lessons in data analysis using R. Thank you for making me understand the R language.

Dr Oliver Mogase Bezuidt: I do appreciate your support and assistance in Bioinformatics.

Mrs Christa Coetzee: Thank you so much for assisting in procuring all the material for my research project.

Dr Teresa Goszczynska: I appreciate all the information you provided on *Ralstonia solanacearum* BD 261 pathogenic strain.

Agriculture Research Council and National Research Foundation, South Africa: Thank you for the research funds awarded to my research project.

Agriculture Research Council GI Microbiology and Biotechnology Unit team: I appreciate the friendly environment you always created, I always felt at home.

Dr Takalani Mulaudzi: Thank you for being such a wonderful sister, I do acknowledge your unending love and support.

Ms Baatseba Mafoko: Thank you so much for calm advice and always believing in me.

My family, the Netshilema and Rasivhaga families: Thank you for being a wonderful family to me. I do appreciate you for positioning me on the path of excellence and to challenge life when necessary.

Mrs Maemu Shiela Rasivhaga: Goodbyes are not forever, goodbyes are not the end. They simply mean I will miss you. May your soul continue to rest in peace.

Mrs Avhatakali Netshilema: You are such a wonderful mother, I appreciate your support in all the decisions I have made in my life so far.

Mrs Muthevhuli Stella and Miss Ndou Tshimangadzo Sylvia: Thank you for supporting my vision from the days of its inception, you both know where it all started.

Nedididi Ndivhuwo and Nedididi Ndweleni: Friends like you are what make life worthwhile.

Hangwani Mamphogoro: Thank you for being the reason that I worked this hard.

Ebenezer: "Thus far the Lord has helped me despite my weakness and mistakes". 1 Samuel 7:

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LIST OF ARTICLES PUBLISHED AND MANUSCRIPT SUBMITTED FOR PUBLICATION

Chapter 2: Exploitation of epiphytic bacterial antagonist for the management of postharvest diseases of sweet pepper and other fresh produce. *This chapter has been published in this format in the Journal of Biocontrol Science and Technology.*

Authors: Tshifhiwa Paris Mamphogoro, Olubukola Oluranti Babalola and Olayinka Ayobami Aiyegoro

Candidate's Contributions: designed the study, managed the literature searches, and wrote the first draft of the manuscript.

Chapter 3: Sustainable management options for bacterial wilt of sweet peppers (*Capsicum annuum*) and other Solanaceous crops. *This chapter has been published in this format in the Journal of Applied Microbiology.*

Authors: Tshifhiwa P. Mamphogoro, Olubukola O. Babalola and Olayinka A. Aiyegoro

Candidate's Contributions: designed the study, managed the literature searches, and wrote the first draft of the manuscript.

Chapter 4: Bacterial communities associated with the surface of fresh sweet pepper (*Capsicum annuum*) and their potential as biocontrol. *This chapter has been published in this format in Scientific Reports.*

Authors: Tshifhiwa Paris Mamphogoro, Martin Makgose Maboko, Olubukola Oluranti Babalola and Olayinka Ayobami Aiyegoro

Candidate's Contributions: designed the study, managed the literature searches, wrote the protocol, carry out the laboratory work, performed all the analyses, interpreted of results and wrote the first draft of the manuscript.

Chapter 5: Epiphytic bacteria from the sweet pepper antagonistic *in vitro* to *Ralstonia solanacearum*. This chapter has been submitted in this format for publication in *Scientific Reports*.

Authors: Tshifhiwa Paris Mamphogoro, Casper Nyaradzai Kamutando, Martin Makgose Maboko, Olayinka Ayobami Aiyegoro and Olubukola Oluranti Babalola

Candidate's Contributions: designed the study, managed the literature searches, wrote the protocol, carry out the laboratory work, performed all the analyses, interpretation of results and wrote the first draft of the manuscript.

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ABSTRACT

Biocontrol agents (especially, microbial antagonists) can sustainably and effectively protect yield losses in crops. In sweet peppers (*Capsicum annum*), one of the most nutritionally rich fruit crop, widely grown worldwide, productivity is threatened by microbial pathogens, particularly those that cause post-harvest spoilage. The identities as well as the roles that could be played by microbial antagonistic microorganisms in protecting yield losses for this important crop are poorly established. The aim of this project was: i) to investigate how the effect of growing conditions (hydroponic system versus direct sowing), inorganic pesticides treatment (i.e., application of a fungicide) and maturity status (green versus red), could influence the structure and composition of bacterial communities on the surfaces of fresh pepper fruits; ii) to predict the phenotypic changes in the microbiota of pepper samples; iii) to identify bacterial taxa with potential to minimize postharvest losses of peppers; and also, iii) to identify bacterial antagonists of *R. solanacearum*, residing on the surfaces of red and green sweet pepper fruits. To achieve this, amplicon sequencing, targeting the 16S rRNA marker gene, and microbial functions assays to depict the identities and the potential antagonistic functions of bacteria were employed. Amplicon sequencing showed bacteria belonging to the phylum *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*, to be enriched in the fungicide-treated compared to fungicide-untreated samples, in open field compared to the hydroponic system samples, and in the green compared to the red samples. Phenotypic predictions (at phylum level) detected high abundance of potentially pathogenic, biofilm forming and stress tolerant bacteria on samples grown on open soils than those from hydroponic systems. Furthermore, bacterial species of genera mostly classified as fungal antagonists including; *Acinetobacter*, *Agrobacterium* and *Burkholderia* were the most

abundant on the surfaces. Microbial isolations and functional analysis successfully identified four potential antagonists from the surface of the sweet pepper fruit surfaces viz. *Bacillus cereus* strain HRT7.7, *Paenibacillus polymyxa* strain SGT5.3, *Serratia marcescens* strain SRT9.1 and *Enterobacter hormaechei* strain SRU4.4, and these indicated antagonism against *R. solanacearum* (the most devastating pathogen of peppers). Optimisation studies under different carbon and nitrogen sources revealed that these potentially antagonistic isolates can effectively suppress *R. solanacearum* at 3% (w/v) starch and 2,5% (w/v) tryptone at pH of 7 and temperature of 30°C. The mode of action exhibited by the strains against the pathogen was secretion of lytic enzymes (i.e., cellulase and protease). Furthermore, the antagonists also displayed plant growth-promoting (PGP) capabilities through phosphate solubilisation and siderophores production. Overall, results demonstrated the ability of sweet peppers to accommodate different microbial taxa on its fruit surfaces, and that some of the microbial constituents can potentially protect the plant under disease pressures from potential pathogenic microbial strain. Results also unequivocally indicated the potential of agronomic choices (especially, site selection/medium of growth) in reducing risk of disease pressures on plants. These results presents a starting point in development of effective biological control as well as integrated pest management (IPM) measures in peppers, for optimization of yield and its protection, globally.

Keywords: Bacterial wilt, bacterial antagonist, biocontrol, Epiphytes, Sweet peppers, 16S rRNA genes, postharvest loss, *Ralstonia solanacearum*, Solanaceous crops

CHAPTER 1

1.1 General Introduction

Peppers (*Capsicum*), are an economically essential crops (native to Mexico), that belong to the nightshade *Solanaceae* family (Samuels 2015). The *Capsicum* genera consists of 20 to 27 species, five of which are domesticated (i.e., *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*). Amongst the domesticated species, *C. annuum* is the most widely cultivated, worldwide (Aguilar-Meléndez *et al.* 2009). Its fruits are berry shaped and may be green, yellow, or red when ripe. Moreover, the fruits can be distinguished by its pungency, which varies from cultivar to cultivar typically higher in smaller types than larger fruit thick-fleshed types (Oboh and Rocha 2007). Pepper fruits are denoted by several names, which include sweet pepper, green pepper, yellow peppers and red pepper (Frank *et al.*, 2001).

Peppers are among the most widely grown vegetable crops, ranked second to tomatoes, on a global scale. Pepper is a vital commercial crop (Nadeem *et al.* 2014), and is considered a significant vegetable, not only because of its high nutritious status (Schreinemachers *et al.* 2018), but also because of its medicinal value (Sultana *et al.* 2013), and as well as its industrial uses (Wubalem, 2019).

The crop is classified as an annual because of its sensitivity to frost, and requires similar conditions as cultivation of tomatoes and eggplants (DeWitt and Bosland 1993; Bosland and Votava 2000). A single pepper plant can yield between ten and twenty pods (DeWitt and Bosland, 1993). Pepper fruits can be harvested before or after reaching maturity, and due to this, its growing period normally ranges from 80 to 100 days. Peppers are an excellent constituent of many flavouring agents and also are important source of vitamin C,

beta-carotene and other natural antioxidants, which neutralise free radicals that causes cell damage (Pietta 2000; Knet *et al.* 2002). Sweet pepper can be used medically for the treatment of colds and fevers (Dagnoko *et al.*, 2013). It also contains lycopene, a carotenoid that has been inversely associated with cancer (Story *et al.* 2010).

The crop is cultivated in many countries, not only for local consumption, but also for export for foreign currency generation. It covers about 1.93 million hectares of land dedicated for crop production, worldwide (Penella and Calatayud 2018). In 2017, global pepper production reached over 35 million tons (Penella *et al.* 2017). Regardless of its popularity, productivity of peppers is threatened by microbial pathogens, especially those that cause postharvest losses (Singh and Sharma 2018). Therefore, control of these pathogenic microorganisms is vital to protect yield losses and also to preserve quality of the pepper fruits (DeWitt and Bosland, 1993; Bosland and Votava, 2000).

Over the years, synthetic pesticides were commonly used to protect fresh produce from damage by postharvest pathogens (Damalas and Eleftherohorinos 2011). Nevertheless, there are concerns and reported proofs of hazardous impacts on the environment and consumers' health, traceable to the use of chemicals (Dasgupta *et al.* 2007). Furthermore, numerous chemicals, for example; Parathion, Lindane and Dichlorodiphenyltrichloroethane (DDT) (Acero *et al.* 2008; Roberts *et al.* 2012), have been removed from the market because of possible toxicological risks (Cao *et al.* 2012; Wilson and Wisniewski 1989; Zong *et al.* 2010). Therefore, healthier and more environmentally friendly alternatives to synthetic chemicals in the management of postharvest decays of fresh produce should be advocated (Ragsdale and Sisler 1994).

Of recent, a consensus seems to have built on the utilization of biological control agents (BCAs), as one of the most effective and sustainable method to control pathogenic

microorganisms (Jiang *et al.* 2009). Considerable success has been made employing rhizobacteria microorganisms as biocontrol for several postharvest diseases on various fruits and vegetables (Mari and Guizzardi 1998; Janisiewicz and Korsten 2002, Singh *et al.* 2003). However, biocontrol microorganisms associated with the surfaces fruits crops (i.e., epiphytes) have not been explored, yet this is important to understand the roles they may play in disease control and promotion of crop productivity. Therefore, this study aims to fingerprint bacterial taxa residing on the surfaces of sweet pepper fruits, at different stages of maturity, grown under disease-prone and disease-free growing media, under pesticide and non-pesticide control environments. The study also seeks to identify, isolate and characterize potential antagonistic bacterial species from the fruit surfaces. We hypothesize that sweet pepper plants recruits diverse microbial populations on its surfaces, some of which can be pathogenic whilst others are antagonistic, but diversity of these microbes is affected by agronomic management practices. We also hypothesise that potential bacterial antagonists can be isolated from surfaces of sweet pepper fruits, particularly those grown on high risk soil media such as the open soil environment.

CHAPTER 2

Exploitation of epiphytic bacterial antagonists for the management of post-harvest diseases of sweet pepper and other fresh produce – a viable option

Abstract

Postharvest loss of sweet pepper and other fresh produce is a major challenge throughout the world. The control of the loss of these valuable farm produce is primarily based on the use of synthetic fungicides. Nevertheless, there are concerns based on the impact of these chemicals on the environment and human health. Hence, the need for a much safer and environmentally friendly alternative. Among the various biological alternatives, the use of bacterial antagonistic strain is becoming popular throughout the globe. Bacterial antagonists are now controlling a number of postharvest pathogens. Several modes of action have been suggested by which microbial antagonists inhibit the growth of postharvest pathogens. However, very little is known about the overall diversity of microbial communities on harvested produce; and how these communities can serve as a foundation for research on postharvest biocontrol. Competition for nutrients and space is the most widely accepted mechanism of action for bacterial antagonists. In addition, antibiosis through the production of antibiotics, mycoparasitism through the production of cell wall lytic enzymes, production of volatile organic compounds and induced resistance are other modes of bacterial antagonist actions by which they suppress the activity of postharvest pathogens.

Keywords: bacterial antagonist; biocontrol; epiphytes; pathogen; Postharvest loss; synthetic fungicides.

1. Introduction

Sweet pepper (*Capsicum annuum*), one of the most widely used food in the world, is a fruit-bearing vegetable that belongs to the nightshade *Solanaceae* family. It is the world's second most important nutritious and universally consumed vegetable grown after tomato (Khan et al., 2005; Nkansah et al., 2017). The dietary benefits of pepper in human nutrition provide a source of essential vitamins and minerals that complement starchy staple foods (Phillips et al., 2006; Wahyuni et al., 2013). Consumption of vitamin C from pepper is associated with a significantly reduced risk of cancer (Gallicchio et al., 2008). Although widely grown and consumed, the yield of pepper and other fresh produce in some parts of the world remains low in comparison to other parts of the world. This is attributed to postharvest loss, which is a major challenge throughout the world, resulting in a huge loss of food production (FAOSTAT, 2018). Studies have shown that ~20–33% of the total fruit and vegetables produced globally are being lost to postharvest pest invasion (FAO, 2011; Okawa, 2015). Infections of pepper and other vegetables, such as tomatoes and grapes in the field as well as after harvest results in postharvest decay, and these losses are often more severe due to inadequate cold storage and transportation facilities (Dukare et al., 2018). Furthermore, even in the developing world, the pathogenic decay of crop vegetables is estimated at 20–25% (Sharma et al., 2009). The high level of losses is related to the high moisture content (~70–95% water) and low pH in vegetable crops (Droby et al., 1992).

In addition to economic losses and quality, descent fresh produce infected with microbial pathogens, especially by genera of *Alternaria*, *Fusarium*, *Penicillium*, *Erwinia*, *Xanthomonas*, *Pseudomonas* and *Clostridium* produce toxins, that poses an imminent health risk. For example, *Penicillium expansum* in a variety of harvested fruits produces copious

potential carcinogenic metabolites such as chaetoglobosins, citrinin and patulin, while *Pseudomonas syringe* produces toxins such as coronatine, syringomycin, tabtoxin, which contribute significantly to bacterial virulence in plants. Other toxins such as ochratoxins, fumonisin, aflatoxins, albicidins, borrelidin and phaseolotoxins are also produced in fruits and vegetables contaminated with *Alternaria*, *Aspergillus*, *Fusarium* and *Xanthomonas* (Andersen et al., 2004; Bender et al., 1999; Bignell et al., 2014; Sanzani et al., 2016).

Conventionally, synthetic fungicides which are applied either in the field or after harvesting, are generally used to control postharvest microbial spoilage of fruits and vegetables (Vitoratos et al., 2013). However, the excessive use of various synthetic fungicides in postharvest disease control has been reduced in the last decade due to the following reasons: (i) toxicological problems related to human health; (ii) development of new pathogen biotypes; (iii) increasing levels of fungicides residues in agricultural produce; (iv) emergence of pathogen resistance to many basic fungicides; and (v) negative environmental impacts (Droby, 2006). Consequently, the global trend is shifting towards the research for safer and eco-friendly alternative approaches to control postharvest loss of fruits and vegetables.

Biocontrol through antagonistic microbes, which is the reduction in disease-causing activity of a pathogen in its dormant state by one or more organisms that occur naturally or by the introduction of antagonists in nature, is an emerging alternative and attractive method for postharvest diseases management (Dukare et al., 2011; Janisiewicz & Korsten, 2002; Liu et al., 2013). The application of antagonist microbes in postharvest disease control offers certain advantages in comparison to synthetic fungicides. These include environmental friendliness, safer application method, no toxic residues, low cost of production and ease of delivery. As a result, it is hypothesised that the competition of biocontrol products with the conventional agrochemicals will be on the increase in the nearest future. Despite the large

number of research being conducted in the field of microbial antagonists, the number of efficient bacteria used as microbicides against postharvest diseases of fruits and vegetables remains limited (Nicot et al., 2011).

The current review presents a widespread knowledge available on the use of bacterial antagonist involved in controlling postharvest diseases of fresh produce, including mechanisms of their actions and highlights how fruit microbiome can serve as a foundation for research on postharvest biocontrol.

2. Significance of postharvest pathogens and postharvest disease development

Postharvest diseases growth could result from the microbial contagion, especially fungal and bacterial infection, accounting for significance loss of fresh produce. Fungal disease activity severely hits several quality traits of fresh produce during the postharvest phase. The microbial genera of *Penicillium*, *Alternaria*, *Aspergillus*, *Colletotrichum*, *Botrytis*, *Dothiorella*, *Lastodiplodia*, *Rhizopus*, *Phytophthora*, *Erwinia*, *Xanthomonas*, *Ralstonia* and *Pseudomonas* are major postharvest pathogens species responsible for the postharvest loss of fresh produce (Liu et al., 2013; Ongena & Jacques, 2008; Prasannath, 2013; Pusey, 1994; Rahman et al., 2012). The disease symptoms gradually accumulate in the infected fruits or crop on the growing field as well as during the transportation and storage.

Postharvest infection triggered by *Botrytis cinerea*, *Penicillium digitatum* and *Penicillium italicum* is responsible for the grey mould disease in apples (El-Ghaouth & Wilson, 2003); however, in peach blue mould, infection is a result by pathogenic activity of *Penicillium expansum* (Chen et al., 2008). *Rhizopus stolonifer*, which causes Rhizopus rot infection, is

responsible for a major postharvest loss in peaches and plums at maturity (Wang et al., 2013). The crown rot disease is a multifaceted infection of *Colletotrichum musae* in banana (Lassois et al., 2010). Change in postharvest quality of red apples is due to the development of core rot disease by *Alternaria alternate* (Shtienberg, 2012). *Colletotrichum gloeosporioides* is responsible for the fruit borne disease, which account for economic losses through the postharvest invasion of mango during storage (Jacobi & Giles, 1997). Soft rot disease, which gives habitually foul-smelling during storage, caused by *E. carotovora*, is a major postharvest problem in potatoes (Cladera-Olivera et al., 2006). *Pseudomonas syringae* is the causal agent of bacterial speck disease in tomatoes produced under field growing conditions and greenhouse growing conditions (Bashan & Bashan, 2002). Bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* is responsible for significant loss of tomato and pepper (Jones et al., 2004). *Ralstonia solanacearum* is the causal agent of bacterial wilt disease, is the most devastating pathogens causing postharvest loss of peppers (Lebeau et al., 2010). In the same vain *Erwinia carotovora*, sub sp. *carotovora* is responsible for causing soft rot diseases in several economically important vegetables and horticultural plants (Akbar et al., 2014).

Several biotic and abiotic stresses including ripening, harvesting and mechanical injuries often stimulate the postharvest disease progress. The process initiates when microbial pathogens germinate and penetrate the host tissue through wounds (Alkan & Fortes, 2015). Likewise, pathogens enter through the lenticels; pedicel–fruit interphase and reside endophytically in the stem ends. The pathogens also penetrate directly in the host cuticle throughout the fruit-growing period. Some microbes reside tranquilly at the initial introduction site of unripe fruits and they remain inactive and unidentified by visual examination until the fruits ripen (Prusky et al., 2009). When the fruits ripe, pathogens grow

aggressively and in the process, growing pathogenic microbes damage the host tissues and absorb nutrients from the host, leading to decomposition of the tissues. The intrinsic disease resistance mechanism protecting the fruits becomes weak during the ripening and then fruits become susceptible to pathogen attacks (Prusky et al., 2009). Therefore, postharvest disease management becomes important to prevent quantitative and overall quality loss of the harvested crop.

3. The role of microbiome in fruit disease resistance: a frontline for post harvest biocontrol in fresh produce

Microorganisms are an important part of the composition of fruits and vegetables and they are found as epiphytes on the surface of crops. The majority of these microorganisms are not pathogenic; however, their role and functions in disease resistance after harvest is largely unknown. Information about their ecology, colonisation and growth in harvested commodities is also lacking. The realisation that fruit surfaces harbour beneficial microorganisms fostered the field of biological control using epiphytic microorganisms (Droby et al., 2016).

Microbial communities have an essential role in ecosystem processes, including disease resistance (Delgado-Baquerizo et al., 2015). Unfortunately, comprehensive description of microbial diversity present in an ecosystem cannot be obtained by using the standard method alone. Concerning biocontrol of postharvest diseases, this limitation has led to an incomplete understanding of the effect that whole microbial communities play in the physiology of a plant and its interactions with the environment and other organisms (Abdelfattah et al., 2017; Berg et al., 2016). The use of amplicon sequencing and metagenomics have provided a fundamental breakthrough in comparing and discovering new

microbial communities (Ursell et al., 2012). A study by Abdelfattah et al. (2016) demonstrated that the alpha and beta diversity of the fungal microflora of harvested apples differed significantly between fruits parts; this strongly suggests that the microflora associated with different portions of the apple fruit need to be considered when designing biocontrol systems for the management of postharvest diseases.

The effect of the epiphytic microbiome of harvested fruits on fruit physiology and its susceptibility to pathogen attack remains to be explored. Plant-associated microorganisms have been reported to produce various phytohormones such as auxins, indole-3-acetic acid (IAA), cytokinins, ethylene and gibberellins (Ghosh et al., 2011; Gutierrez-Manero et al., 2001; Spaepen, 2015). These hormones have the ability to suppress fungal pathogens, especially those caused by *Fusarium oxysporum* f. sp. *cucumerinum* (Garzón et al., 2017). Microorganisms also have the potential to produce secondary metabolites capable of directly or indirectly inhibit 2-methyl-1-propanol against fungus *Pseudogymnoascus destructans* (Micalizzi et al., 2017). Microbiome studies and meta-omics offer the opportunity to explore a new frontier that can have a major impact on the development of biocontrol agents, and in the understanding of microbiome networks which may serve as a system framework for identifying microbial assemblages for disease management (Poudel et al., 2016).

4. Essential of bacterial antagonists

Antagonism refers to a phenomenon where by the action of any organisms suppresses the normal growth and activity of a pathogen in its vicinity. These organisms can control the pathogens of crops and are referred to as 'Biological Control Agents' (Heydari & Pessarakli, 2010). A number of antagonistic bacteria possessing activity against preharvest and postharvest loss microorganism have been reported. These organisms prevent, inhibit or kill

the propagules of pathogen growing on fruit surface by producing pathogen-specific antibacterial/and antifungal compounds which can control further possibility of fruit spoilage during storage. Numerous antagonistic bacterial species have been identified and artificially deployed on several horticultural commodities commissioning both direct and indirect inhibitory mechanisms in the suppression of pathogen growth (Wisniewski et al., 2016).

4.1 Sources of bacterial antagonists

Most of the bacterial antagonists are naturally present on the surface of fruits and vegetables. Many of them have been isolated and identified as suitable biocontrol agents for the management of postharvest pathogens (Vero et al., 2011). Apart from the fruit surface, microbes can be attained from other closely related surroundings, such as roots and soil (Long et al., 2005; Zhao et al., 2012). A list of bacterial antagonists from the different source used as biocontrol agents for the management of postharvest invasion of fruits is reported in Table 1.

Table 1. Proposed modes of action of some bacterial antagonists for effective control of postharvest diseases of fresh produce^a.

Antagonist	Host	Target pathogen	Mode of action	References
<i>Bacillus subtilis</i> M4	Apple	<i>Botrytis cinerea</i>	Antibiotic production	Ongena et al. (2005)
<i>Bacillus subtilis</i> Jaas ed1	Egg plant	<i>Verticillium Wilt</i>		Lin et al. (2009)
<i>Streptomyces albidoflavus</i> S1	Strawberry	<i>Verticillium wilt</i>		Berg et al. (2000)
<i>Pseudomonas corrugate</i> P94	Tomato	<i>Botrytis cinerea</i>		Guo et al. (2007)
<i>Paenibacillus polymyxa</i>	Strawberry	<i>Botrytis cinerea</i>		Haggag et al. (2013)
<i>Bacillus amyloliquefaciens</i>	Citrus	<i>Penicillium italicum</i>	Lytic enzyme production	Hao et al. (2011)
<i>Paenibacillus polymyxa</i>	Apple	<i>collectotrichum</i> <i>goleosporioides</i>		Kim et al. (2016)
<i>Serratia plymuthica</i> IC14	Cucumber	<i>Botrytis cinerea</i>		Kamensky et al. (2003)

<i>Bacillus circulans</i> GI 070	Grapes	<i>Botrytis cinerea</i>		Paul et al. (1997)
<i>Bacillus thuringiensis</i>	Citrus	<i>Guignardia citricarpa</i>	Induction of host defence	Lucon et al. (2010)
<i>Serratia marcescens</i> 90–166	Cucumber	<i>Botrytis cinerea</i>		Meziane et al. (2005)
<i>Streptomyces globisporus</i>	Citrus	<i>Penicillium italicum</i>	Production of volatile compounds	Li et al. (2010)
<i>Pseudomonas putida</i> Cha94	Pepper	<i>Botrytis cinerea</i>	Competition for space and nutrients	Park et al. (1999)
<i>Rhanelia aquatilis</i>	Apple fruit	<i>Botrytis cinerea</i>		Calvo et al. (2007)
<i>Pantoea agglomerans</i>	Apple	<i>Penicillium expansum</i>		Morales et al. (2008)
<i>Paenibacillus polymyxa</i>	Strawberry	<i>Botrytis cinerea</i>		Helbig et al. (2001)
<i>Bacillus pumilus</i>	Tomato	<i>Botrytis cinerea</i>		Elad et al. (1994)
<i>Bacillus cereus</i> CH2	Egg plant	<i>Verticillium Wilt</i>	Mycoparasitism	Li et al. (2008)
<i>Pseudomonas putida</i> B E2	Strawberry	<i>Verticillium Wilt</i>		Berg et al. (2001)
<i>Serratia plymuthica</i> HROC48	Strawberry	<i>Verticillium Wilt</i>		Kalbe et al. (1996)

^aThere are several published reports on these alternative strategies for control of postharvest of fresh produce. And effort was made to list representatives references, and we apologise to investigators whose specific results could not be cited due to space limitations.

There are two available approaches for using bacteria as antagonists for the control and management of postharvest losses of fresh produce. These include (i) the use of beneficial bacteria that already exist on the surfaces of fruits or vegetables, e.g. natural bacterial antagonists present naturally on the surface of vegetables which control postharvest decay against disease-causing *Botrytis cinerea* (Sharma et al., 2009); and (ii) the artificial introduction of bacterial antagonists against postharvest pathogens. For example, *Bacillus* sp. are effective in controlling Botrytis rot diseases caused by *Botrytis cinerea* (Pusey & Wilson, 1984). Of the two approaches, researches have revealed that the latter is a more effective technology. The reasons are that (i) the environment for the storage of harvested commodity is often controlled and maintained; (ii) the ability to deliver biological control agents to the intending site of action is enhanced in the postharvest application; and (iii) certain

commodities harvested for fresh-market consumption require a short-term period of protection against postharvest infections (Wisniewski & Wilson, 1992).

It is obvious that great advances have been made in recent years in testing alternative control measures, especially the use of microorganisms for control of postharvest pathogens (Castoria et al., 2003; Wisniewski & Wilson, 1992). However, as various biological control agents do not survive in the new habitats they are introduced, therefore, search for new biocontrol agents is of paramount importance.

4.2. Conditions for the selection of ideal bacterial antagonist

An effective potential antagonist should possess desirable traits for use as a postharvest loss biocontrol agent. The antagonists for postharvest disease control should be able to control the disease at low concentration, genetically stable and compatible with other physical and chemical treatment (Sharma et al., 2009). The antagonists should be effective against a broad spectrum of pathogens in a variety of fruits and vegetables and survive under adverse environmental conditions, unable to grow at human body temperature (37°C) and does not cause any infections in humans (Liu et al., 2013). Additionally, antagonist must use low-cost nutrition for growth, longer shelf-life, easy to dispense, resistant to pesticides and not produce metabolites that are deleterious to human health and host fruits (Nunes, 2012). In the same vein, the antagonists should be able to grow, survive and multiply in the environment favourable for the pathogen (Janisiewicz & Korsten, 2002). Furthermore, antagonists isolated from the same locale with the pathogen are appropriate for disease management (Manso & Nunes, 2011). Antagonists with better adaptive features compared to that of pathogens under the same environmental condition offer better pathogenic control. High viable cell count of an antagonist is another measure for selection as biocontrol

agents (Janisiewicz, 1997). Based on these traits, bacteria appear to be an exceptional candidate for biocontrol agents (Kobayashi & Palumbo, 2000). Hence, researches have focused on isolation, identification and characterisation for their potential use in the management of postharvest loss of fresh produce. Additionally, bacteria are the most suitable biological control agents because of their inherent characteristics such as quick growth survival and proliferation in postharvest fruit surface (Dukare, 2017).

5. Mode of actions of bacterial antagonists

Several studies have demonstrated the antimicrobial potential of many microbial antagonists against postharvest pathogens (Gbadeyan et al., 2016; Nunes, 2012; Wisniewski et al., 2016). The understanding on the mechanisms of action of microbial antagonists is limited to understanding the interactions between the antagonists, host tissue and the pathogens, 'tritrophic interactions' taking place on the infected site of the fruit produce (El Ghaouth et al., 2004). There are various mechanisms, operating in a tritrophic interaction system, to overpower pathogen infection, as shown in Figure 1 and Table 1. The principal biocontrol mechanisms displayed by antagonists include competition for nutrients and space, antibiosis through antibiotic production, mycoparasitism, production of cell wall lytic enzymes and induction of host resistance (Di Francesco et al., 2016; El Ghaouth et al., 2004; Sharma et al., 2009). Recent studies have elucidated the roles of volatile compound production in suppressing the activity of postharvest microbial pathogens on fruits (Liu et al., 2013). More than one mechanism is often employed in order to have effective postharvest biological control. The mechanisms of action of bacterial antagonists are discussed below.

5.1. Competition for nutrients and space

Competition for nutrients such as carbohydrates, amino acids, vitamins and minerals as well for space at the wound site between the microbial antagonists and the pathogen considered a vital mode by which microbial antagonists suppresses postharvest pathogens causing decay in fruits and other fresh produce (Jamalizadeh et al., 2011; Sharma et al., 2009; Spadaro & Droby, 2016; Spadaro & Gullino, 2004).

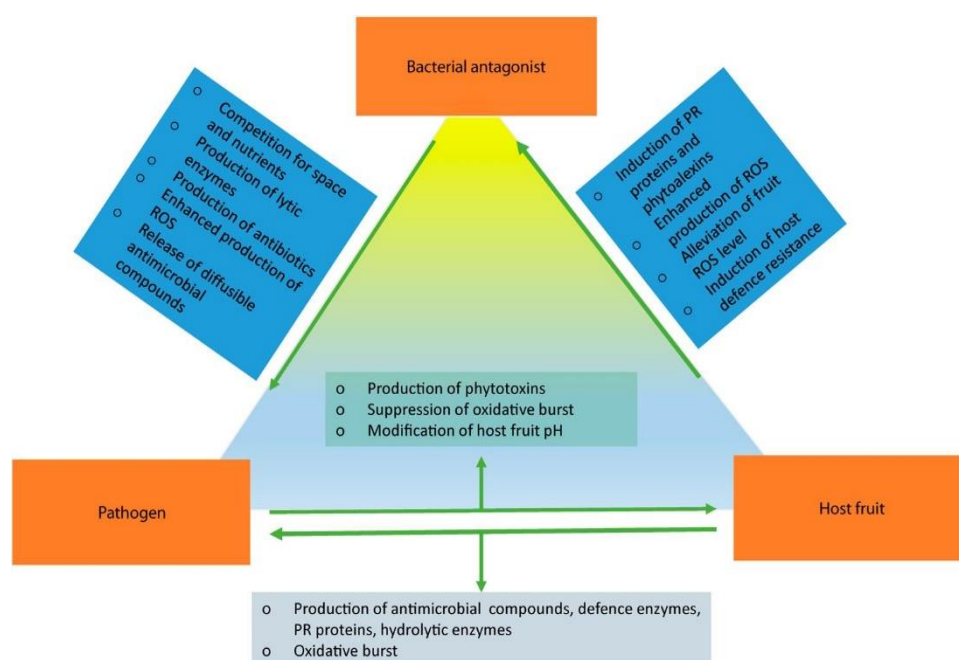


Figure 1. Schematic representation of the possible mechanisms of biocontrol actions involved in tritrophic system, describing the interaction between microbial antagonists, pathogen and host fruits.

This method has been described in several biocontrol studies for antagonists such as *Serratia plymuthica* and *P. agglomerans* (Meziane et al., 2006; Poppe et al., 2003). From the microbial perspective, plant surfaces are frequently nutrient-limited environment. Therefore for more effectiveness, antagonist should have the ability to rapidly colonise the fruit wounds prior to colonisation by pathogen (Droby et al., 2009; Sharma et al., 2009). Under nutrient starvation,

both the antagonist and the pathogens compete with one another for the nutrient and space, the antagonists diminish the available nutrients in the wound site and make nutrients inaccessible for the pathogens to germinate, grow and infect the plant surface. This process of competition is considered to be an indirect interaction between the pathogen and the biocontrol agent whereby the pathogens are excluded by the depletion of food base and by the physical occupation of the site (Lorito et al., 1994).

Although in some cases rapid colonisation of wound site depends on the antagonist concentration and the host fruit spaces, certain antagonists prefer certain nutrient types, non-pathogenic natural microbiota residing on fruit surface can release toxic metabolite suppressing pathogens (Di Francesco et al., 2016; Galvez et al., 2010). This mechanism has been mostly observed in bacterial antagonists such as *Xanthomonas maltophilia*, *Bacillus pumilus*, *Lactobacillus* spp. and *Pseudomonas* spp. in the control of *B. cinerea* in bean and tomato plants (Elad et al., 1994). Given the limited nutritional resources at the leaf and fruit surfaces, the efficiency of phyllosphere colonisation with nutrient uptake by bacteria is a key feature for successful antagonism by exhausting the available substrates and thus, reducing pathogen (Andrews, 1992; Huang & Erickson, 2005).

5.2. Antibiosis by antibiotic production

Antibiosis is the phenomenon whereby antagonists secrete chemical compounds that inhibits potential pathogens in close contiguity. Antibiosis involves the production of an antibiotic by microorganisms that have a direct effect on the growth of plant pathogen and it is assumed as the second most important mechanism by which microbial antagonists suppress diseases on leaf surfaces and in fruit wounds after competition for nutrients and space (El Ghaouth et al., 2004; Raaijmakers et al., 2002; Sharma et al., 2009). Several bacterial genera suppressing

postharvest microbial pathogen growth by producing antibiotics have been reported; these includes *Bacillus*, *Burkholderia*, *Pseudomonas*, *Enterobacter*, *Lysobacter* and *Streptomyces* (Raaijmakers et al., 2002; Raaijmakers & Mazzola, 2012). However, little information is available about bacterial production of antimicrobial compounds under field conditions.

The most common effective antimicrobial compounds produced by bacteria are lipopeptides of iturin produced by *Bacillus subtilis* and *Pseudomonas cepacia* (now known as *Burkholderia cepacia*) (Abano & Sam-Amoah, 2012). Pyrrolnitrin produced by *Pseudomonas cepacia* have also been deployed for suppressing *B. cinerea* and *P. expansum* in apples (Di Francesco et al., 2016), and syringomycin produced by *P. syringae* have been used successfully for the suppression of green mould in citrus (Sharma et al., 2009). Many compounds produced by *Burkholderia* sp. exhibit antifungal activity, including cepaciamides A and B, altericidin and glidobactins which play an important role in inhibiting pathogens in tomato leaves (Schmidt et al., 2009; Tenorio-Salgado et al., 2013). In addition, other *Bacillus* species synthesises antibacterial and antifungal metabolites such as bacillomycin, gramicidin, fengycin and surfactin (Cho et al., 2003; Arrebola et al., 2010). Antibiotic compounds inhibit the growth and development of plant pathogens through various mechanisms, including destruction and alteration of cell membrane structures, prevention of the formation of initiation complexes on the subunits of the ribosomal protein synthesis and inhibition cell wall synthesis (De Souza et al., 2003).

Though antibiotic-producing microbial antagonists are used in postharvest disease control, the role of antibiotic-mediated antibiosis in some biocontrol systems has not been completely decoded (Nunes, 2012). Therefore, more emphasis is placed on the use of non-antibiotic-producing microbial antagonists to control postharvest pathogens. This approach

has wider reception and avoid the fast emergence of pathogen resistance to the antimicrobial compounds (Di Francesco et al., 2016; Sharma et al., 2009).

5.3. Mycoparasitism through production of cell wall lytic enzymes

Mycoparasitism involve the ability of antagonistic bacteria to attach with the hyphae of microbial pathogens to produce extracellular cell wall lytic enzymes. Mycoparasitism of antagonist is contingent to the sequential occurrence of the following measures: lytic enzymes secretion, mutual recognition by antagonist and pathogen, active growth of antagonists into the host and close contact of antagonist and the pathogen (Talibi et al., 2014). The production of extracellular cell wall-degrading enzymes such as chitinases, glucanases, cellulases, proteases and lipases is associated with the biocontrol abilities of bacteria. Secretion of these enzymes results in the suppression or detoxifying virulence factors of plant pathogens (Bouizgarne, 2013; Maksimov et al., 2011; Neeraja et al., 2010). Chitinase contributes significantly to biocontrol activities of *Sclerotium rolfsii* by *Serratia marcescens* (Ordentlich et al., 1988). Lipases contribute to direct suppression of plant pathogens by degrading cell wall components of pathogenic fungi. Likewise, the production of extracellular antifungal hydrolytic enzymes such as β -1,3-glucanase, cellulase and protease by halophilic bacteria such as *B. subtilis*, *B. pumilus*, *B. licheniformis* and *Staphylococcus equorum*, moderately suppressed the growth of *B. cinerea* grey mould pathogen of strawberry (Essghaier et al., 2009). In the same vein, the control of *B. cinerea* by *Bacillus cereus* strain IO8 has been mediated by chitinase production (Hammami et al., 2013). However, for another for *Bacillus cereus* strain B02 effects on DNA synthesis, mitochondrial membrane potential and the reactive oxygen quantity in the pathogen hyphae caused its antifungal activity (Li et al., 2012). Correspondingly, the role of chitinases was also revealed in *Bacillus thuringiensis*

UM96, to account for the defence of *Medicago truncatula* from *B. cinerea* infection (Martínez-Absalón et al., 2014). Joo (2005) demonstrated antifungal activity of purified chitinase from *Streptomyces halstedii* AJ-7 against various red pepper fungal pathogens. Equally, *Serratia plymuthica* and *S. marcescens* produces chitinases and proteases, which lead to the inhibition of pathogens such as *Fusarium oxysporum*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani* (Frankowski et al., 2001; Kamensky et al., 2003).

Although lytic enzymes might be effective against a wide spectrum of phytopathogens, their non-specificity may result in the suppression of beneficial microorganisms existing in particular environments (Pretorius et al., 2015). Pathogen growth inhibition can also be achieved indirectly by changing the growth conditions on plant surfaces, to make them unsuitable for successful infection. For example, increasing the growth pH of *B. pumilus* NCIMB 13374 and *P. fluorescens* NCIMB 13373 from a pH of 6 to ~8 inhibited the growth of *B. cinerea* in strawberries (Swadling & Jeffries, 1998).

5.4. Production of volatile organic compounds

Bacterial antagonists produce several antimicrobial metabolites including low molecular weight lipophilic compounds called volatile organic compounds (VOCs). These compounds play an important role in suppressing pathogen growth (Mari et al., 2016; Zheng et al., 2013). In recent years, the effects of VOCs on plants pathogens have been progressively studied and found to be one of the key mechanisms for used as biological control of plant pathogens; they include alkanes alkenes, alcohols, esters, ketones and sulphur compounds, (Effmert et al., 2012; Ryu et al., 2004; Schöller et al., 2002). Many of bacterial VOCs inhibit fungal growth, impair fungal spores and hyphae, and promote plant growth (Kai et al., 2007, 2009; Weisskopf, 2014). VOCs from one bacterial strain do not cause the same inhibitory effect on

different fungal pathogens. The responses may depend on the specific fungus–bacterial combination (Kai et al., 2009). VOCs produced by *B. pumilus* and *B. thuringiensis* have been reported to reduce ~88.5% anthracnose contagions in mangoes (Zheng et al., 2013).

Production of VOCs from *Streptomyces* species can prevent the growth of *B. cinerea*. For example, VOCs from *Streptomyces platensis* F-1 reduce Botrytis fruit rot in strawberry, they correspondingly decreased the level of leaf blight in rice and oilseed rape (Wan et al., 2008). In tomato fruits, VOCs produced by *Streptomyces globisporus* JK-1 grown on autoclaved wheat seeds showed inhibitory effects on growth of *B. cinerea* (Li et al., 2010).

Likewise, within the genera *Bacillus* and *Paenibacillus*, the potential role of different VOCs as one of the mode of action to inhibit *B. cinerea* infection has been established (Berrada et al., 2012; Zhang et al., 2013). Different degrees of inhibitory effects of VOCs from *Paenibacillus polymyxa* and *Bacillus* sp. (*B. subtilis* BLO2, *B. pumilus* BSH-4 and ZB13) were observed in vitro on *S. sclerotiorum*, and *Cercospora kikuchii* (Liu et al., 2008). Similarly, a study by Chen et al. (2008) demonstrated the antagonistic effects of these compounds generated by *B. subtilis* on mycelial growth and the conidial germination of *B. cinerea* and other fungal pathogens. The volatiles benzothiazol and citronellol produced by *P. polymyxa* strain BMP-11 inhibited mycelial in vitro growth of fungal pathogens (Zhao et al., 2011).

In addition to their strong antimicrobial inhibitory possibility, bacteria also emit VOCs which can promote plant growth and improve plant tolerance to abiotic stress (Bhattacharyya et al., 2015; Kanchiswamy et al., 2015). VOC-producing bacteria are well suited to control fungal decay under postharvest storage conditions in controlled environment, as biofumigants, although safety issues associated with these biochemicals need to be evaluated. Extensive researches concerning VOCs effects on grey mould have been performed under controlled conditions and thus their advantages and drawbacks for field applications

must be considered. The effects of environmental parameters, particularly air movements may be of major importance. Strong air currents could significantly decrease the concentration of produced VOCs and limit their efficacy. Moreover, adding nutrients into the soil such as carbon sources may promote bacterial production of VOCs (Fiddaman & Rossall, 1994). A possible drawback of this mode of action is the inhibitory effects of certain VOCs at high concentrations on plant growth (Bailly & Weisskopf, 2012).

5.6. Induced system resistance

Induced systemic resistance (ISR) refers to the ability of the plant to induce host defence responses because of some biotic or abiotic inducing agent from pathogens. When plants and pathogens interact, it could result in a response that is compatible with both of them and lead to infection. On the other hand, it could also result into a response that is not compatible with both organisms and lead to resistance from the plant. Induction of host defences can be localised or systemic in nature. This corresponds to a state of defence in the whole plant, preparing it to respond more quickly and intensely to a pathogen attack (Bloemberg & Lugtenberg, 2001). ISR is mediated by jasmonic acid and ethylene signalling pathways, which are produced following applications of some non-pathogenic rhizobacteria. Some of the most striking examples of bacterial determinants and types of disease resistance induced by biological control agents include a *Bacillus mycoides* strain capable of producing peroxidase, chitinase and β -1,3-glucanase in sugar beet. *Bacillus subtilis* GB03 and IN937 producing 2,3-butanediol in *Arabidopsis*; *Pseudomonas putida* strains producing a lipopolysaccharide in *Arabidopsis*; and *Serratia marcescens* 90-166 producing siderophore in cucumber (Bargabus et al., 2003; Meziane et al., 2005; Press et al., 2001; Ryu et al., 2004). Several compounds produced by bacteria including volatiles, siderophores, flagellin and lipopeptides are known

to elicit ISR against *B. cinerea* in many plant species (Ongena et al., 2005; Ongena & Jacques, 2008). In tomatoes and beans, *Pseudomonas aeruginosa* 7NSK2 produces a siderophore, pyochelin and the antibiotic pyocyanin, which trigger the ISR against fungal pathogen *B. cinerea* (Audenaert et al., 2002). The ability of several bacteria, including *Micromonospora*, *Saccharothrix algeriensis* and *P. fluorescens* have recently been shown to induce plant systemic resistance and then reduce *B. cinerea* infections (Gruau et al., 2015; Martínez-Hidalgo et al., 2015). *Stilbenic phytoalexins* are induced mostly in the early growth stages in grape berries, which progressively lose their potential for stilbene synthesis towards fruit maturity. Furthermore, this mode of action is also associated with metabolic costs and energetic trade-offs within host plants. These costs may include allocation from plant growth and development towards defence, as well as ecological costs such as negative effects on symbiotic interactions (Walters et al., 2013; Walters & Heil, 2007).

6. Conclusion and future works

The use of synthetic fungicides has been the traditional strategy for the management of postharvest diseases in the horticultural commodity. Owing to the serious growing concern for environmental pollution and health hazards that widespread of chemical pesticides has created in the world, pursuit for alternative safe methods is obvious. Biological control of plant diseases has been the subject of numerous research projects in recent years (Bargabus et al., 2004; Chisholm et al., 2006). Among the various microorganisms deployed for biological control, bacterial antagonists have the ability to grow quickly, survive and proliferate in postharvest fruit surface can be utilised as best candidates for the biocontrol agents. Controlling of postharvest diseases by employing antagonistic bacterial biocontrol agents has

been demonstrated to be the most suitable strategy to replace the synthetic fungicides, which are recommended for limited use in postharvest crop pathogens control.

Microbial pathogens are among the most important factors that cause serious damages and losses of fruits and vegetables. Biological control using bacterial antagonists to manage plant diseases seems to be a promising alternative strategy and have successfully been applied to control some diseases on different plants and crops (Heydari & Pessarakli, 2010). However, complete elimination of chemical pesticides for controlling plant diseases in modern agriculture may be impossible, but a logical reduction in their application is feasible. To have a sustainable agricultural system with minimum contamination and risks to the environment, a combination of all available methods should be applied to manage pest problems and integrated pest management (IPM) (Barzman et al., 2015; Ehi-Eromosele et al., 2013) can achieve this. The implementation of IPM strategies may be the safest solution for management of pest problems including fungal diseases in every cropping system and with no doubt, biological control is one of the most important components of IPM, which can lead us towards a sustainable agricultural system in the future. This review reported the success of some bacterial biocontrol agents and the mechanism associated with the control of postharvest pathogens in fresh produce. Advanced molecular techniques are now being used to characterise the diversity, abundance and activities of microbes that live in and around the plants, including those that significantly affect plant health (Joshi & Gardener, 2006). However, much remains to be learned about the microbial ecology of both plant pathogens and their microbial antagonists in different agricultural systems. Many of the bacterial genera in this review have been observed to be antagonists against fungal plant pathogens. However, a further investigation of these beneficial bacteria will help in characterising the effects of their antagonists against bacterial pathogens of pepper and other fresh produce.

Acknowledgements

Acknowledgements to the Agricultural Research Council for providing the Agroprocessing Competitive Funding [Cost Centre PO2000032] and for the PhD bursary to T.P.M. The authors would like to thank Prof Cuthbert Banga for the article proofread.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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CHAPTER 3

Sustainable management strategies for bacterial wilt of sweet peppers (*Capsicum annuum*) and other Solanaceous crops

Summary

Pepper bacterial wilt is caused by the bacterial pathogen, *Ralstonia solanacearum*. It is the most destructive disease of many Solanaceous crops such as potatoes, tobacco, pepper, tomatoes and eggplant and is a significant source of crop loss worldwide. Physical, cultural and chemical controls have been employed to combat this destructive disease. However, none of these strategies has been able to control the disease completely due to the broad host range and genetic diversity of the pathogen, its prolonged survival in the soil and survival on vegetation as a latent infection. Owing to co-management strategies, biological control is the best approach for human health and environmental friendly motivations. It makes use of various antagonistic rhizobacteria and epiphytic species such as *Bacillus cereus*, *Pseudomonas putida*, *Bacillus subtilis*, *Paenibacillus macerans*, *Serratia marcescens*, *Bacillus pumilus* and *Pseudomonas fluorescens*, which compete with and ultimately inhibit the growth of the pathogen. The possible mechanisms of biocontrol by these species involve multifaceted interactions between the host, pathogen and the antagonists. These can involve competition for nutrients and space, plant-mediated systemic resistance, siderophore production and production of extracellular cell wall degrading enzymes to inhibit or suppress the growth of the bacterial wilt agent.

Keywords: bacterial wilt, biological control, management, pathogen, *Ralstonia solanacearum*, Solanaceous crops, sweet pepper.

Introduction

Bacterial wilt is a widespread destructive disease caused by *Ralstonia solanacearum* that affects many economically important crops, including sweet pepper (Knapp *et al.* 2004). It is one of the most challenging diseases, causing severe damage to pepper plants throughout the world, especially in the tropical and subtropical regions, and parts of the warm temperate regions (Du *et al.* 2017). The disease is known as ‘Green wilt’ disease since the leaves of the infested plant stay green when the plant begins to show wilt symptoms (Jiang *et al.* 2017). It also results in substantial losses in other crops like tomato, eggplant, potato, tobacco and banana (Elphinstone 2005). The pathogen has been reported to invade more than 450 plant species from 54 botanical families, the most susceptible hosts being solanaceous crops (Lebeau *et al.* 2011; Kurabachew and Ayana 2016). Cultivation of solanaceous crops, especially pepper and tomato, plays a significant role in many developing countries in Africa as a source of income and enhanced social and nutritional status. Besides, they provide various employment opportunities because their management is labour intensive.

Currently, the majority of the Solanaceous crops in the world are produced on smallholder farms (Haverkort *et al.* 2012). The productivity of these crops may be limited by biotic constraints to include bacterial wilt. In addition to different abiotic constraints such as, low levels of irrigation, soil erosion and degradation, low levels of agrochemical input (fertilizer, pesticide and improved seeds), inadequate agricultural research and extension and constraints in market development (Yabuuchi *et al.* 1995).

Ralstonia solanacearum is highly widespread in most African countries, causing substantial crop yield losses (OEPP/PPO 2004). *Ralstonia solanacearum* is an extraordinarily diverse and complex species. The pathogen is subdivided into five races based on its ability to

infect different plant species and six biovars based on its ability to oxidize hexoses, alcohols and sorbitol as well as utilization of disaccharides (Xue *et al.* 2011; Chandrashekara *et al.* 2012). Pepper bacterial wilt is mostly caused by biovar 2 (a biological variation of group of bacterial strains distinct from other strains of the same species based on physiologic characteristics) of *R. solanacearum* which belong to race 3. The biovar has a broad host range, which guarantees the long-term existence of the pathogen in the soil, even in the absence of susceptible crops including on weeds and on other non-solanaceous plants (Momol *et al.* 2002; Yao and Allen 2006). Recently, a hierarchical classification system was proposed to separate the complex *R. solanacearum* species into four phylotypes (designated I–IV) based on ancestral relationship and geographical distribution of the pathogen (Safni *et al.* 2014; Prior *et al.* 2016). *Ralstonia solanacearum* currently is the most intensively studied phytopathogenic bacterium due to its devastating lethality in pepper and other agriculturally important crops.

Regardless of the development of various strategies to control bacterial wilt, effective environmentally friendly and human health control measures are still lacking for most of the crops. Herein we review the history and status of bacterial wilt, the approaches used to control bacterial wilt disease and the use of biological control particularly bacterial antagonists as an environmentally friendly method to suppress the disease in pepper and other Solanaceous crops

Dispersal of the pathogen

Dissemination of *R. solanacearum* occurs through several means; however, environmental factors are the main cause of development, spread and distribution of the disease. Weather conditions such as humidity and temperature have a substantial effect on disease

development and have been intensively studied as predictors of disease outbreaks caused by bacteria and fungi (Aslam and Mukhtar 2018; Lopes and Rossato 2018).

Ralstonia solanacearum can spread over long distances on vegetative propagating materials, surviving for about 2–3 years with vegetative organs undoubtedly being a vital source of inoculum (Coutinho 2005). Infested wet soil and weeds, contaminated irrigation water, contaminated farm equipment, waste from the crop processing industry as well as latently infected crops such as potato tubers and tomato seeds all have a high potential risk to house *R. solanacearum* (van Elsas et al. 2001). Crop residues in fields that were infected by *R. solanacearum* also serve as a source of disease inoculum in the surrounding area (Wang and Lin 2005). Furthermore, insects have been considered as vectors that naturally spread *R. solanacearum* race 3 (Tomlinson et al. 2009; Tahat and Sijam 2010). Hence, extensive distribution and long saprophytic survival in the environment makes the control of the disease caused by *R. solanacearum* more difficult.

Epidemiology and survival of the pathogen

Ralstonia solanacearum is the most destructive soil-borne pathogen and commonly enters plant roots from the soil, through open or natural wounds where secondary roots emerge. Once inside the host, the bacterium colonizes the root cortex and vascular parenchyma, then it multiplies swiftly, ultimately entering the xylem vessel and spreading into the stem, leaves and fruits (Gupta and Thind 2006; Yuliar et al. 2015). The pathogen moves up through the vascular system and the infected xylem, eventually blocking water transportation, causing wilting (Wang and Lin 2005). Degradation of the xylem vessels and adjacent tissues directly lead to the death of the plant (Hayward 2000). Particularly, the infected plants die rapidly within 3–4 days (Yuliar et al. 2015).

After the death of the plant, *R. solanacearum* cells are discharged into the soil from the infected roots and spread to neighbouring plants. When the environmental conditions are more favourable, the bacterium cells multiply rapidly and with exopolysaccharide slime they cause reduced sap flow leading to wilting of the entire plant (Denny 2006; Mukhtar *et al.* 2008). When the conditions are slightly favourable (i.e. under low temperatures and extremely high alkaline environments), the disease develops slowly, characterized by stunting the development of adventitious roots. Plants later fall and die due to extra degradation of vessels and contiguous tissues. The bacterium returns to the soil after plant death, living as a saprophytic organism until it infects a new host plant. Development of bacterial wilt in tomatoes and peppers is mainly promoted by high soil moisture and temperature (Wang and Lin 2005).

Symptoms and signs of bacterial wilt

Plants infected with *R. solanacearum* can show symptoms a few days after infection and are characterized by sudden wilting and yellowing of the leaves, followed by undersized growth and eventually death of the plants. In most cases, the stem near the root produces many adventitious root buds, which is also an indication of infection at the vascular bundle. Substantial attack of the cortex may result in the advent of water-soaked lesions on the exterior surface of the stem. If a diseased stem is cut crosswise, tiny drops of yellowish viscous or dirty white or milky bacterial ooze emanate, indicating infection by bacterial cells at the vascular bundles (Champoiseau *et al.* 2009; Karim *et al.* 2018). Even though diseased plants can be found dispersed in the field, there are various symptoms of bacterial wilt, and under normal circumstances, the preliminary symptom in mature pepper plants is similar to that observed in tomato and potato. It means that appearance of flaccidness on the fresh leaves

normally occurs 2–5 days after infection with *R. solanacearum* and wilting of upper leaves during the scorching days followed by recovery during the evening and early hours of the morning (Momol *et al.* 2001).

The wilted leaves maintain their green colour and they do not fall as the disease spreads. Under hot, humid situations, complete wilting occurs and the plant will die (Mihovilovich *et al.* 2017). A plant infected with *R. solanacearum* may undergo latency, which may lead the plant into expressing all these symptoms or none of them, even under conditions that are favourable for the pathogen (Monther and Kamaruzaman 2010). Further symptoms of bacterial wilt are characterized by discolouration of the vascular system from streaky light yellow to dark brown (Harveson *et al.* 2015).

Economic impact of bacterial wilt

The considerable economic damage caused by the pathogen can be ascribed to its wide host range and its broad topographical dispersal in some warm temperate regions of the world (Elphinstone 2005). The pathogen causes considerable yield loss subject to the cultivar, soil type, climate, cropping practices and pathogen strain (Elphinstone 2005).

The world's human population is projected to reach 10·5 billion by 2050. This will translate into more mouths to feed, with the highest demand in the poor communities of the world. It has been calculated that food supplies would need to increase by 60% to meet the expected food demand (Tilman *et al.* 2011; Ray *et al.* 2013). Therefore, increasing agricultural productivity while minimizing food loss is critical in ensuring global food security. About 1·3 billion tons of food are globally wasted or lost per year. Reduction in these losses would increase the amount of food available for human consumption, enhancing global food security. Microbial (bacteria) spoilage is the main cause of postharvest losses of many crops

including pepper, accounting for a 14% decrease in crop production worldwide. Thus, a reduction of plant diseases will contribute to increased crop production. Among the plant diseases, soil-borne diseases are estimated to account for 10–20% of yield losses annually (Savary *et al.* 2012; Yuliar *et al.* 2015).

Ralstonia solanacearum is ranked as the second most destructive among the 10 most deadly bacterial species affecting economically important crops (Mansfield *et al.* 2012). The pathogen has been reported to cause severe yield losses in many solanaceous crops, with 88% loss of tomatoes reported in Uganda, and 70% loss of potato in India and other countries in varying degrees (Katafiire *et al.* 2005). Bacterial wilt was reported to affect 50–100% of potatoes in Kenya (Muthoni *et al.* 2012). In Ethiopia, the percentage of bacterial wilt incidence is almost 100% on pepper, 63% on potato and 55% on tomato (Assefa *et al.* 2015). In the case of potato, since most wilted potato plants do not produce marketable tuber, crop yield losses from the diseases could be very high (Kurabachew and Ayana 2016).

Although there is no overall information on economic impacts of the pathogen on solanaceous crops worldwide, substantial losses of approximately 75% in potato and destruction of tomato harvest due to its susceptibility to bacterial wilt have been reported (Elphinstone 2005; Hayward 2005). Damages are intensifying because agriculture is now extending to countries where susceptible crops have not been cultivated previously. The presence of *R. solanacearum* in fields discourages the planting of many vegetables on home and family gardens, leading to a significant reduction in food sources (Hayward 2000). In many parts of the world, especially Africa, smallholder farmers do not grow genetically modified crops; hence, the crops they cultivate are more susceptible to bacterial wilt. The pathogen has been known to have high survival and damaging risk to other vegetation around the globe. Cost-effective postharvest treatments include, among others, controlled-atmosphere

storage, various pesticides and waxes which were employed to control the disease (Kader 2003; Wu 2010). However, most of these treatments are relatively expensive and/or pose some risks for humans and/or the environment (Cao *et al.* 2012). Hence, there is an urgent need for proper and more effective management against this pathogen globally.

Isolation of *Ralstonia solanacearum* from diseased plants

The bacterium is generally isolated from the stem of diseased plant necrotic vascular tissue, which is soaked in 2 ml of sterile water for 5 min. The tissues are seeded on yeast peptone glucose agar or tetrazolium chloride agar (Schaad *et al.* 2001; EPPO 2004) and left for incubation at 28°C for 48 h until growth of colonies becomes observable (Champoiseau *et al.* 2009). Morphological identification of *R. solanacearum* isolated is based on white with pink centres or red centre and whitish periphery (Pradhanang *et al.* 2000). Identification of the latent infection can be done using an immuno fluorescence antibody staining or selective plating on South African selective medium (SMSA) combined with elective PCR assays, and ELISA tests. The pathogen can also be isolated from difficult substrates like soil, waste or surface using several available selective media (OEPP/EPPO 2004).

Methods used for bacterial wilt control

According to Kurabachew and Ayana (2016), bacterial wilt is a difficult disease to control, especially once it is established in the soil. This is because of its broad host range, ability to survive for long period in soil; this is due to the broad host range and genetic diversity of the pathogen, its prolonged survival in the soil and survival on vegetation as a latent infection (Saddler 2005; Lemessa and Zeller 2007). Bacterial wilt control has been possible through

various methods as shown in Table 1, which include physical, cultural, chemical and biological control practices (Mbega et al. 2013; Kurabachew and Ayana 2016).

Table 1 Proposed mechanisms and approaches for management of bacterial wilt diseases

Methods	Examples			Mechanisms employed	References
physical	Solarization, treatment and disinfection	hot water	biological soil	Killing <i>R. solanacearum</i> with high or low temperatures	Fock et al. (2000), Boshou (2005), Posas et al. (2007) and Dahal et al. (2010).
Cultural	Resistant cultivar, amendment, crop rotation, and grafting		soil	Limited pathogen movement from the primary xylem to other xylem tissues, induced uptake and distribution of nutrients, reduced disease inoculum, and induced resistant plant	Islam and Toyota (2004), Elphinstone (2005), Hwang et al. (2005), Shimpi et al. (2005), Oliver et al. (2006), Ordonez et al. (2006), Texeira et al. (2006), Janvier et al. (2007), Igawa et al. (2008), Posas and Toyota (2010), Amorim et al. (2011), Pontes et al. (2011), Paret et al. (2012), Yuan et al. (2012), Terbalnche and De Villiers (2013).
Chemicals	Algicide (3-3-Indolyl acid), fumigants, acibenzolar-S-methyl, chitosan and sodium chloride	botanic		Induce systemic resistant, increase the amount of soil microorganisms or increase tolerance to <i>R. solanacearum</i> , antibacterial and bacteriostatic	Dannon and Wydra (2004), Khanum et al. (2005), Pradhanang et al. (2005), Haciasalihoglu et al. (2007), Ndakidemi (2007), Boonhan et al. (2008), Vincelli and Tisserat (2008), Nakaune et al. (2012), Mbega et al. (2013), Kurabachew and Wydra (2014), and Yuliar et al. (2015).

Biological	<i>Bacillus amyloliquefaciens</i> , <i>Bacillus cereus</i> , <i>Burkholderia</i> <i>nodosa</i> , <i>B. pyrrocinia</i> , <i>B.</i> <i>sacchari</i> , <i>B. tericola</i> , <i>Chryseobacterium</i> <i>daechongense</i> , <i>C. indologenes</i> , <i>Chryseomonas luteola</i> , <i>Clostridium sp.</i> , <i>Delftia</i> <i>acidovorans</i> , <i>Flavobacterium</i> <i>johnsoniae</i> , <i>Paenibacillus</i> <i>marcerans</i> , <i>Pseudomonas</i> <i>brassicacearum</i>	competition for nutrient and space, antibiosis, plant mediated systemic resistance, parasitism siderophore production, production of extracellular enzymes, and decrease root colonization	Guo et al. (2004), Ji et al. (2004), Kawabata et al. (2005), Ling et al. (2006), Wydra and Dannon (2006), Alvarez et al., (2007), Messiha et al. (2007), Hu et al. (2010), Li et al. (2011), Ding et al. (2013), Huang et al. (2013), Hyakumachi et al. (2013), Chen et al. (2014) and Li et al. (2014).
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Physical control

Numerous methods of physical control have been developed and proved useful for controlling *R. solanacearum*. These methods include soil solarization, hot water and bio-fumigation, known as biological soil disinfection (Yuliar *et al.* 2015).

Solarization of soil

Soil solarization is done by spreading a transparent plastic mulch sheet over the soil during long periods of high ambient temperature. This helps to trap the radiant energy of the sun, thereby warming the topsoil layer, which in turn eradicates insects, pathogens, weed seeds and seedlings and nematodes (Ploeg and Stapleton 2001). Vinh *et al.* (2005) discovered that solarization of the soil using plastic mulches for 60 days before planting tomatoes reduced the incidence of bacterial wilt. Solarization of the soil improves soil structure and increases the availability of nitrogen and other essential plant nutrients (Ploeg and Stapleton 2001). The main drawback of solarization is its negative potential impact on valuable soil microbes since they will encounter the same fate as their harmful counterparts (Wang *et al.* 2006).

Disinfection of soil through heating

This is mostly done as a preplanting treatment, and post-planting procedure. Hot water between a temperature of 70 and 90°C can be poured into the soil before planting to increase soil temperature to levels lethal for weed seeds, pests and pathogens. It is an environmentally friendly procedure, as it does not disturb soil microflora completely, for example, heat-resistant, spore forming bacteria can survive and regenerate the soil after cooling, in return strengthening resistances against plant disease (Katan 2000; Runia and Molendijk 2010).

Biological soil disinfection

Biological soil disinfestation is the process of farm working to eradicate soil-borne plant pathogens before planting crops. The process requires neither higher temperature nor long temperature incubation to stimulate activities of indigenous microbes in the soil through addition of organic materials (Blok *et al.* 2000; Goud *et al.* 2004). The treatment comprises of four steps including: (i) flooding soil by irrigation, (ii) covering the soil with plastic film to induce reduced soil conditions, (iii) introduction of easily decomposable organic materials (e.g. rice straw, wheat bean and rice bran) to soil and (iv) using volatile chemicals released from plant residues. Biofumigation using wheat bran or molasses proved to be effective against a broad range of soil-borne plant pathogens including *R. solanacearum*, *Phomopsis sclerotioides*, *F. redolens* and *Verticillium dahliae* as well as the nematodes such as *Meloidogyne incognita* (Blok *et al.* 2000; Shinmura 2004; Takeuchi 2004).

Cultural control

Cultural control encompasses farming techniques that will help to raise the quality and quantity of the crop yield and decreases the influence of diseases (Ajilogba and Babalola 2013).

Cultivar resistance

Growing cultivars that are highly resistant to bacterial wilt is the most effective, economical and environmentally friendly approach to disease control (Yuliar *et al.* 2015). Breeding of cultivars that are resistant to bacterial diseases has been practised mostly for crops such as potato, eggplant, tomato, peanut and pepper. For example, potato genotype BP9, introduced to *Solanum tuberosum* and *Solanum phureja* have reduced incidence of bacterial wilt by about 90–100% (Fock *et al.* 2000).

Arabidopsis NPR1 gene introduced into a tomato cultivar successfully reduced bacterial wilt by 70% twenty-eight days after inoculation (Lin *et al.* 2004). *NPR1* gene plays an essential role in the plant's response to pathogen challenge by establishing systemic acquired resistance and induced systemic resistance (Pieterse *et al.* 1998); it also functions as the master key in relation to plant defence-signalling network, facilitating a cross-talk between the salicylic acid (SA) and jasmonic acid/ethylene (JA/ET) responses. In *Arabidopsis thaliana*, expression of *NPR1* guarantees a swift response to salicylic acid (SA) (Cao *et al.* 1998). Resistant plants invaded by *R. solanacearum* displayed tolerance of the vascular tissues to bacterial wilt disease (Kurabachew and Ayana 2016). As much as the cultivar resistance has shown great attributes in reducing the bacterial wilt of solanaceous crops, public acceptance is needed before the commercial use of such genetically modified crops. Furthermore, reduction of bacterial wilt in many plants has generally been inversely

proportional to the yield and quality of the crops (Yuliar *et al.* 2015). Moreover, the complexity of *Ralstonia* strains has led to the development of resistant defences, which are effective in some growing regions and are ineffective in other regions (Narusaka *et al.* 2013).

Crop rotation

This is an affordable method to manage plant diseases and it involves cultivating different crops on the same farm, in alternate seasons (Ajilogba and Babalola 2013). Continuous cultivation of similar crops may lead to the establishment of certain populations of plant pathogens; for example, tomatoes planted in the same farm year after year will encourage disease-causing organisms to proliferate in the soil. Crop rotation breaks this detrimental effect and results in the reduction of diseases instigated by soil-borne pathogens (Janvier *et al.* 2007; Larkin 2008; Neshev 2008). For example, potato cultivation in rotation with carrots, millet, sweet potatoes or sorghum has been shown to decrease the incidence of bacterial wilt while increasing potato yield compared to that of mono-cultured tubers (Katafiire *et al.* 2005). For crop rotation to effectively manage soil-borne pathogens, the pathogen must be wholly eradicated from the farmland by replacing the contaminated soil with garden-fresh soil from another part of the farm (Neshev 2008).

Soil amendment

The use of organic matter as an alternative to suppress bacterial wilt has beneficially influenced crop productivity via improving the chemical, biological and physical properties of soil, which influences plant health positively (Bailey and Lazarovits 2003). Degradation of organic matter may affect the survival of disease-causing agents directly by releasing inhibitory substances in the soil, limiting the available nutrients. It may also increase microbial activities; thus enhancing the possibility of competition effects (Bailey and Lazarovits 2003;

Raaijmakers and Mazzola 2012). These activities can lead to stimulation of micro-organisms with antagonistic activities against pathogens (Akhtar and Malik 2000).

In addition, soil amendments often contain biologically active molecules such as growth regulators, vitamins and toxins, which can directly or indirectly affect micro-organisms. Lemaga *et al.* (2001) reported that organic amendment of soil with *Leucaena diversifolia* and *Sesbania sesbana*, combined with inorganic fertilizer, reduced the incidence of bacterial wilt while increasing potato tuber yield.

The application of silicon fertilizer and sugarcane bagasse (an alternative silicon source) has also been reported to reduce bacterial wilt populations and bacterial wilt incidence, while increasing tomato fruit yield (Getachew *et al.* 2011). Soil amendments with farmyard manure (FMY) compost or coco peat have been found to enhance tomato yield compared to un-amended soil, while significantly reducing bacterial wilt incidence by 81% in tomato (Yadessa *et al.* 2010). This might be due to an improvement in soil microbial activities and physicochemical characteristics of the organic amended soil, to the advantage of crop growth. Thus, soil amendment could be useful in managing *R. solanacearum* in the main Solanaceous crop growing regions of the world. Yamazaki *et al.* (2000) reported that increased calcium concentration in tomato plants reduced the population of *R. solanacearum* in the stems of the tomato.

Grafting

Grafting is an asexual plant propagation technique that joins parts from two different plants in such a way that they will unite and successively grow as one plant. Therefore, a grafted plant is a composite of elements derived from two or more plants (Hartmann 2002). It involves joining the upper part of a plant (scion) of a desirable cultivar onto a resistant

rootstock of another compatible species (McAvoy *et al.* 2012). The main purpose of grafting vegetables globally has been to produce crops that are resistant to soil-borne pathogens including *Ralstonia*, *Phytophthora*, *Fusarium*, *Monosporascus*, *Pyrenochaeta* and *Verticillium* (King *et al.* 2008; Louws *et al.* 2010).

Chemical control

This involves the use of chemicals to control soil-borne pathogens, pests and weeds. Some of these chemicals include benomyl, carbendazim, propiconazole and flubendazole. Various chemical methods have been used to control bacterial wilt over the years. However, due to the complex nature of the pathogen, no method is useful when applied alone, and economic considerations often influence the chemicals selected (Yuliar *et al.* 2015). Pesticides such as fumigants (metam sodium, 1,3-dichloropropene and chloropicrin), algicide (3-[3-indolyl] butanoic acid) and plant activators (validoxylamine and validamycin A) have been applied to manage bacterial wilt incidence. The use of methyl bromide coupled with 1,3-dichloropropene has reduced bacterial wilt incidence by 72%–100% while significantly increasing tomato yield in the field by 1.7- to 2.5-fold (Santos *et al.* 2006).

Pesticides have been reported to offer a more significant net benefit than other approaches of combating bacterial wilt; *albeit* not always (Edwards-Jones 2008). Ignorance and improper application of pesticides in the environment may result in some of the pesticides remaining in the environment for several years, becoming a contaminant in the soil and groundwater, and causing toxicity to the farmers and consumers (Dasgupta *et al.* 2007; Acero *et al.* 2008). Therefore, the use of chemicals like antibiotics to control plant pathogens has been seriously questioned because of the impact on human health and the environment, and the development of resistant organisms (OEPP/EPPO 2004).

Biological control

Biological control involves the killing of one living organism by another (Sharma *et al.* 2009). It has emerged as a promising alternative to chemical use, particularly as an integrated part of pest management, to reduce the use of synthetic fungicide. For example, antagonistic rhizosphere inhabiting bacteria have been used to improve plant growth, as well control of plant diseases (Zhang *et al.* 2007; Sharma *et al.* 2009).

Biological control agents exhibit a number of characteristics that have increased their use in preference to the use of chemicals. Such features include reduced input of nonrenewable resources, their potential to be self-sustaining and spread after initial establishment and the ability to provide long-term disease suppression (Whipps 2001; Whipps and Gerhardson 2007). Various studies reported that biocontrol of bacterial wilt disease may be accomplished by making use of antagonistic rhizobacteria and epiphytic bacteria such as *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Paenibacillus macerans*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Serratia marcescens* (Kurabachew *et al.* 2007; Alyie *et al.* 2008).

Table 2. Some of the biocontrol agents verified to control bacterial disease in the field environment

Microorganisms	Inoculation method	Mechanisms	BE (%)	References
<i>Acinetobacter</i> sp. Xa6	Soaking seedling roots in the bacterial suspension	Rhizocompetence and root colonization	57-67% in tomato	Xue et a.(2013)
<i>Bacillus</i> sp. (RCh6)	Deeping seedlings in antagonist suspension	Production of inhibitory compounds	81% in the egg plant	Ramesh and Phadke (2012)
<i>Bacillus amyloliquefaciens</i> SQR-7 and SQR-101	Pouring and stem injection	Production of indole acetic acid and siderophores	18-60% in tobacco	Yuan et al. (2014)
<i>Bacillus amyloliquefaciens</i> Bg-C31	Poured bacterial suspension on plant	Production of antibacterial proteins	60-80% in Capsicum	Hu et al. (2010)
<i>Enterobacter</i> sp. Xy3	Soaking seedling roots in the	Rhizocompetence and root colonization	57-67% in tomato	Xue et al. (2013)

Conclusion

Like many other Solanaceous crops, peppers are susceptible to bacterial wilt disease. Bacterial wilt is a severe disease to control due to high variability of the pathogen, high capacity of the pathogen to survive in complex environments, survival in vegetation as latent infection and long survival on soil (Denny 2006). Chemical pesticides have conventionally been used to control bacterial diseases. However, pesticides lose effectiveness with time and most of these treatments are relatively expensive and pose some risks to humans and the environment. Notwithstanding the limited effectiveness of some of the management strategies, such as growing resistant crop varieties, grafting and bio-fumigation in controlling the disease, none of these approaches have been able to entirely suppress the disease (Momma 2008; Ajilogba and Babalola 2013). Moreover, the potential loss of methyl bromide and other chemicals as a soil fumigant, combined with pathogen resistance to commonly used pesticides, makes the disease difficult to control (Momma 2008). Therefore, there is a need for new and more effective means of controlling bacterial wilt disease. It is evident that significant advances have been made in recent years in testing alternative control measures, especially the use of micro-organisms (biocontrol) for the control of bacterial wilt. However, as micro-organisms are adapted to the environment where they were isolated, many biological control agents do not thrive in the new habitats they are introduced into; hence new biocontrol agents are needed to overcome this problem.

Acknowledgements

The South African Agricultural Research Council- Agro processing Competitive Funding (Cost centre PO2000032) to O.A.A. supported this work. The authors are grateful to the Agricultural

Research Council for the PhD bursary to T.P.M. and the North West University, for the research collaboration platform.

Declaration of Interest

The authors declare no conflict of interest.

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CHAPTER 4

Bacterial communities associated with the surface of fresh sweet pepper (*Capsicum annuum*) and their potential as biocontrol

Abstract

Fresh produce vegetables are colonized by different bacterial species, some of which are antagonistic to microbes that cause postharvest losses. However, no comprehensive assessment of the diversity and composition of bacteria inhabiting surfaces of fresh pepper plants grown under different conditions has been conducted. In this study, 16S rRNA amplicon sequencing was used to reveal bacterial communities inhabiting the surfaces of red and green pepper (fungicides-treated and non-fungicides-treated) grown under hydroponic and open field conditions. Results revealed that pepper fruit surfaces were dominated by bacterial phylum *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and, *Bacteroidetes*. The majority of the bacterial operation taxonomic units (97% similarity cut-off) were shared between the two habitats, two treatments, and the two pepper types. Phenotypic predictions (at phylum level) detected a high abundance of potentially pathogenic, biofilm-forming, and stress-tolerant bacteria on samples grown on open soils than those from hydroponic systems. Furthermore, bacterial species of genera mostly classified as fungal antagonists including; *Acinetobacter*, *Agrobacterium*, and *Burkholderia* were the most abundant on the surfaces. These results suggest that peppers accommodate substantially different bacterial communities with antagonistic activities on their surfaces, independent of employed agronomic strategies and that the beneficial bacterial strains maybe more important for peppers established on open fields, which seems to be more vulnerable to abiotic and biotic stresses.

Introduction

Fresh products such as apples, grapes, peaches, and tomatoes are known to harbour diverse bacterial populations^{1–3}. Plant species, geographic location, climatic conditions, ripening stage and application of agrochemicals, are some of the factors that determine distribution of microorganisms on the surface of these products⁴. Bacterial species that colonise fruit surfaces (epiphytes) are introduced from the soil to the host plants by insects, air currents and other animal species^{5–7}. Among these microorganisms, some are beneficial to plants, for example, several *Sphingomonas* strains induce resistance to Fusarium head blight caused by *Fusarium culmorum* in the host plant⁸; while others are phytopathogens (e.g., *Phoma* and *Pantoea*) known to cause economic losses^{1,9}. Therefore, understanding the diversity and ecology of epiphytic bacteria may be important to develop new biocontrol agents¹⁰.

Previously, the identities of the members of microbial communities were established using culture-dependent methods¹¹. However, these methods are known to underestimate microbial diversity, as only 0.1–8.4% of environmental bacteria are considered cultivable^{12,13}. Data gathered using these methods only provide limited information on the vast majority of microbes present in a given sample. Nowadays, the diversity of bacterial communities is usually assessed by culture-independent techniques that include the analysis of the 16S rRNA gene fragments¹⁴. Such methods have allowed for instance the investigation of the microbial diversity of tomato, grape, peach and apple fruits^{15,16}. However, information on bacterial communities associated with the surface of fresh sweet pepper fruits is still limited, despite this being vital in identifying microbes that can antagonize the effects of pathogenic strains which may contribute to postharvest losses.

The primary goal of this study was to investigate, using 16S rRNA gene Illumina amplicon sequencing, how the effect of growing conditions (hydroponic system versus direct sowing), inorganic pesticides treatment (i.e., application of a fungicide) and maturity status (green versus red), could influence the structure and composition of bacterial communities on the surfaces of fresh pepper fruits. Additionally, we aimed to predict the phenotypic changes in the microbiota of pepper samples and also, to identify bacterial taxa with potential to minimize postharvest losses of peppers.

We hypothesized that, regardless of agronomic management approaches, pepper fruits can accommodate antagonistic bacteria on its surfaces that can potentially minimize damage that maybe induced by its potential pathogens, and that some of these antagonists, may contribute in reduction of postharvest loses.

Results and discussion

Analysing the bacterial communities associated with the surface of *Capsicum annuum* fruits, we obtained 1,586,400 bacterial high-quality reads, which resulted in 1,137 OTUs (97% cut-off). The majority of bacterial OTUs were shared between the habitats, treatments and pepper sample types (56.4%, 58.9% and 59.4%, respectively) (Supplementary Fig. S1). Microbial diversity (Supplementary Fig. S2) tended to be higher in the fungicide-treated compared to fungicide-untreated samples, in open field compared to the hydroponic system samples, and in the green compared to the red samples, although they did not differ significantly ($P > 0.05$). This implies that microbial diversity on the surfaces of peppers is not affected by growth stage, growing system and treatment with fungicides. For habitats, diversity results were as expected, as it is well known that the organic matter in the soil is an important source of nutrients for microorganisms and contains higher levels of fungal and

bacterial propagules than hydroponic systems¹⁷. The diversity in treatments is in agreement with a study by Schaeffer, *et al.*¹⁸, which showed fungicides application on nectar have no observable effect on bacterial OTU richness or community compositions. Furthermore, higher bacterial populations observed on the immature (green) fruit surfaces compared to the mature samples corroborated with findings by Palumbo, *et al.*¹⁹, who found greatest bacterial diversity on early summer mature almond fruits than on the late summer mature almonds. The possible explanation for this observation is that the intact hulls during the immature growing stage of fruits will still be metabolically active and therefore, could be ideal sources of carbon and water for microbial survival.

A total of 17 distinct bacterial phyla were detected across all 80 samples. The most abundant sequences in all the 80 samples were affiliated with the phylum *Proteobacteria* (71%), followed by *Firmicutes* (13%), *Actinobacteria* (7%) and *Bacteroidetes* (5%) (Fig. 1). Other phyla were also represented, although in lower proportions. There were significant differences in *Proteobacteria* abundance between the two habitats, with the phylum being more abundant in open soil as compared to the hydroponic habitat (Kruskal-Wallis: $P < 0.001$), but non-significant differences were observed between the two treatment groups (Kruskal-Wallis: $P = 0.55$) and the two pepper sample types (Kruskal-Wallis: $P = 0.53$). For *Firmicutes*, significant differences in abundance were shown between the habitats (Kruskal-Wallis: $P < 0.001$) and treatments (Kruskal-Wallis: $P = 0.03$), but non-significant differences in abundance were noted between the pepper sample types (Kruskal-Wallis: $P = 0.71$). Moreover, abundance of *Actinobacteria* did not differ between habitats (Kruskal-Wallis: $P = 0.34$), treatments (Kruskal-Wallis: $P = 0.68$) and pepper sample types (Kruskal-Wallis: $P = 0.34$). Additionally, significant differences in abundance were shown between habitats (Kruskal-Wallis: $P < 0.001$) and pepper sample types (Kruskal-Wallis: $P = 0.03$) for the *Bacteroidetes*,

while abundances between treatment groups were not significant (Kruskal-Wallis: $P = 0.06$). The trend in abundance of *Proteobacteria* in habitats could be explained by the fact that this phylum is commonly identified as being copiotrophic (i.e., they thrive in conditions of elevated carbon availability and exhibit relatively rapid growth rates and compete successfully when organic resources are abundant), possibly because they associate with nematodes soil layers where organic matter, plant roots, and other resources are more abundant^{20,21}. *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* have been shown to be widely represented on the surfaces of fruits of other plants such as grape²². They represent various taxonomic groups and different ecological statuses, such as antagonist, symbionts (especially, endophytes) and saprophytes²³. Their dominance on fruit surfaces could be attributed to the fruit's ability to use a wide variety of carbon sources such as carbohydrates, amino acids, and lipids, which could help resist different environmental changes that occur during fruit development^{24,25}.

Potential prediction of phenotypic functions of bacterial communities (at phylum level) on the surfaces of the different pepper samples detected nine potential microbial phenotypes including; aerobic, anaerobic, facultative anaerobic, mobile elements carriers, biofilm forming, Gram-negative, Gram-positive and pathogens (Fig. 2; Supplementary Table S1). In general, aerobic bacteria were more abundant on fungicide-treated compared to untreated samples and this was opposite for anaerobic bacterial populations. This could suggest that the rise in abundance of aerobic bacteria is associated with the capability of degrading fungicides by these bacteria as described by Megadi *et al.*²⁶. On another note, potentially pathogenic bacteria showed to be more overrepresented on surfaces of both, immature (green) and mature (red) peppers grown on open field (both fungicide-treated and untreated). This was not the case with peppers grown under the hydroponic system, and this

clearly demonstrates that, growing peppers using the hydroponic system maybe an effective agronomic management strategy in comparting yield constraining effects of microbial pathogens of peppers. Therefore, using the hydroponics technology, crops can be grown with minimal negative effects on ecosystems and biodiversity, which are usually profound in cropping systems dependent on synthetic pesticides for control of pests and diseases^{27–29}. Although the initial investment of hydroponic systems is huge³⁰, it may tend to be a cheaper method of growing high-value, horticultural crops such as peppers, since production costs will be minimized by reduction in pesticide requirements, which are generally very expensive^{31,32}. Hydroponic systems have been adopted in production of some high value crops such as tomato and lettuce³³ and in seedling production in nurseries^{33,34}.

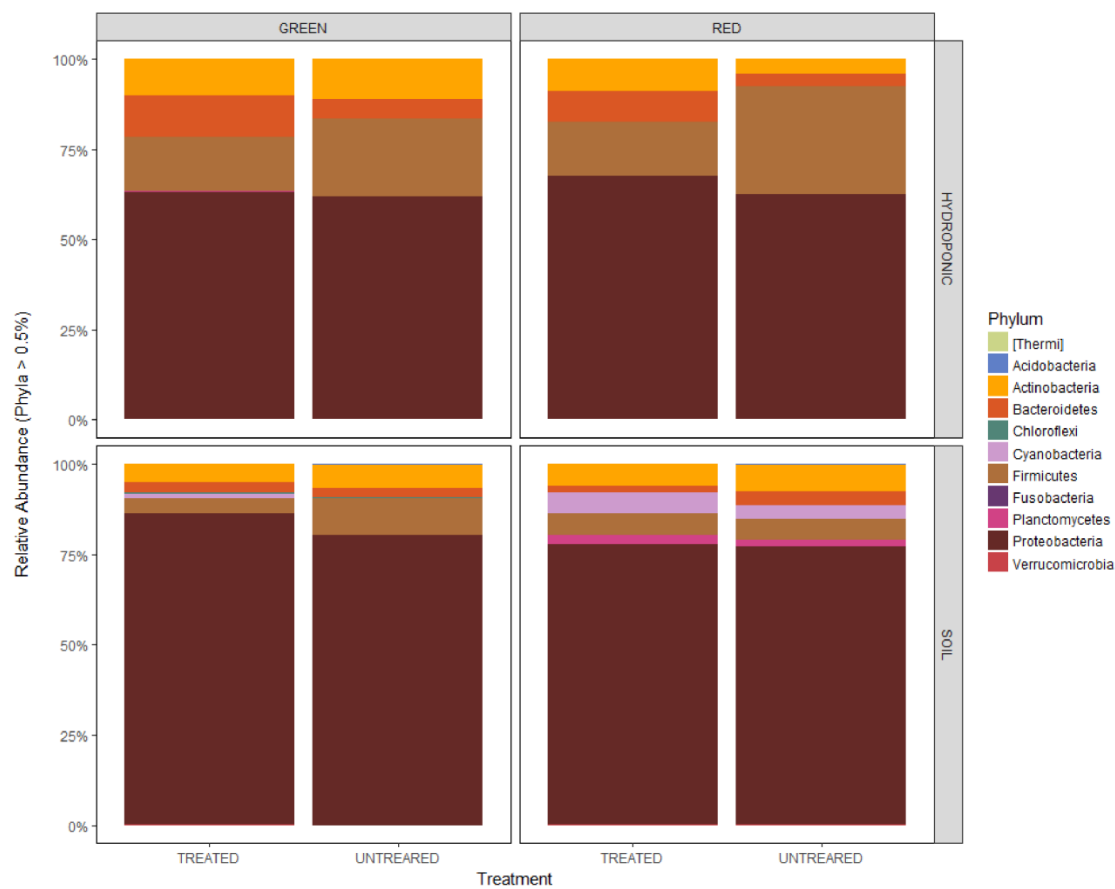


Figure 1. Mean relative abundances of taxa (phylum); (a) between hydroponic and soil habitats samples, (b) green and red samples, (c) treated and untreated samples. The abundance of each taxon calculated as the percentage of sequences per location for a given microbial group.

Additionally, it is not surprising that stress tolerance functions were predicted to be more abundant on surfaces of peppers grown on open field than those grown on the hydroponic system. It is widely known that hydroponic systems raise plants free from most abiotic stresses (e.g., drought and nutrient stress) as well as biotic stresses (diseases and weeds). In addition, it is highly likely that the biofilm forming function, predicted to be present on surfaces of peppers grown on open field than hydroponic-produced peppers, is necessary to compartment the various crop growth constraining factors known to be more common on the open fields. It is also highly probable that bacterial antagonists to deter potential pathogens could be exhibited by biofilm function. Likewise, pathogenicity can also be promoted by biofilm formation. Biofilms are defined as a collective of one or more types of microorganisms that can grow on many different surfaces³⁵.

Differences in abundance of all the predicted nine phenotypic functions were significant (Supplementary Table S1), implying that the bacterial communities with these functions were affected by how pepper plants were managed, (e.g., fungicide treatment versus non-fungicide treatment or hydroponic versus open soil planting). It is also worth mentioning that most of these predicted functions are present in the bacterial phylum, *Proteobacteria* and *Firmicutes* (Fig. 1, Supplementary Fig. 2). According to our knowledge, no phenotypic functions on surface bacterial communities for fresh produce grown in hydroponic and open field soil or in other farming practices such as in organic and conventional practices have been reported before.

At the genus level, significant differences in abundance of *Microbispora*, *Sphingobium*, *Paenibacillus* and *Lactococcus* were noted between the treated and untreated red and green peppers produced in hydroponics. A similar observation was recorded for peppers grown in

soil (Table 1). *Microbispora* species have the ability to produce phenazine-1-carboxylic acid, which is capable of controlling southern blight disease caused by the phytopathogenic fungus, *Sclerotium rolfsii*, which causes large economic losses in many crops such as *Zea mays*³⁶. *Sphingobium* species were reported to produce a volatile inhibitory compound 2-methyl-1-propanol against fungus *Pseudogymnoascus destructans*³⁷, while *Paenibacillus* is known to be capable of producing various plant hormones, antibiotics and hydrolytic enzymes with ability to suppress *Fusarium* wilt of cucumber (*Cucumis sativus*), which is caused by *Fusarium oxysporum* f. sp. *cucumerinum* in non-sterile, soil-less potting medium³⁸. The bacterial genus *Lactococcus*, a bacteriocin producing Lactic acid bacteria (LAB), isolated from fresh fruits *Chryso-phyllum cainito* (star apple) and *Solanum stramonifolium* (pea eggplant), was reported to show inhibitory activities against both Gram-positive pathogens such as *Bacillus cereus* and *Staphylococcus aureus* and the Gram-negative pathogens (e.g., *Salmonella typhimurium*)^{39,40}.

Other well-known bacterial genera such as: *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Curtobacterium*, *Enterococcus*, *Flavobacterium*, *Lactobacillus*, *Methylobacterium*, *Microbacterium*, *Novosphingobium*, *Pseudomonas*, *Sphingomonas* and *Weissella*, were represented by the majority of sequences, but no significant differences in abundance for these genera were observed between the hydroponic-green-treated (HGT) and the hydroponic-green-untreated (HGU) samples, the soil-green-treated (SGT) and the soil-green-untreated (SGU) pepper samples, the hydroponic-red-treated (HRT) and the hydroponic-red-untreated (HRU) samples as well as between the soil-red-treated (SRT) and the soil-red-untreated (SRU) pepper samples (Fig. 3, Supplementary Table S2a,b).

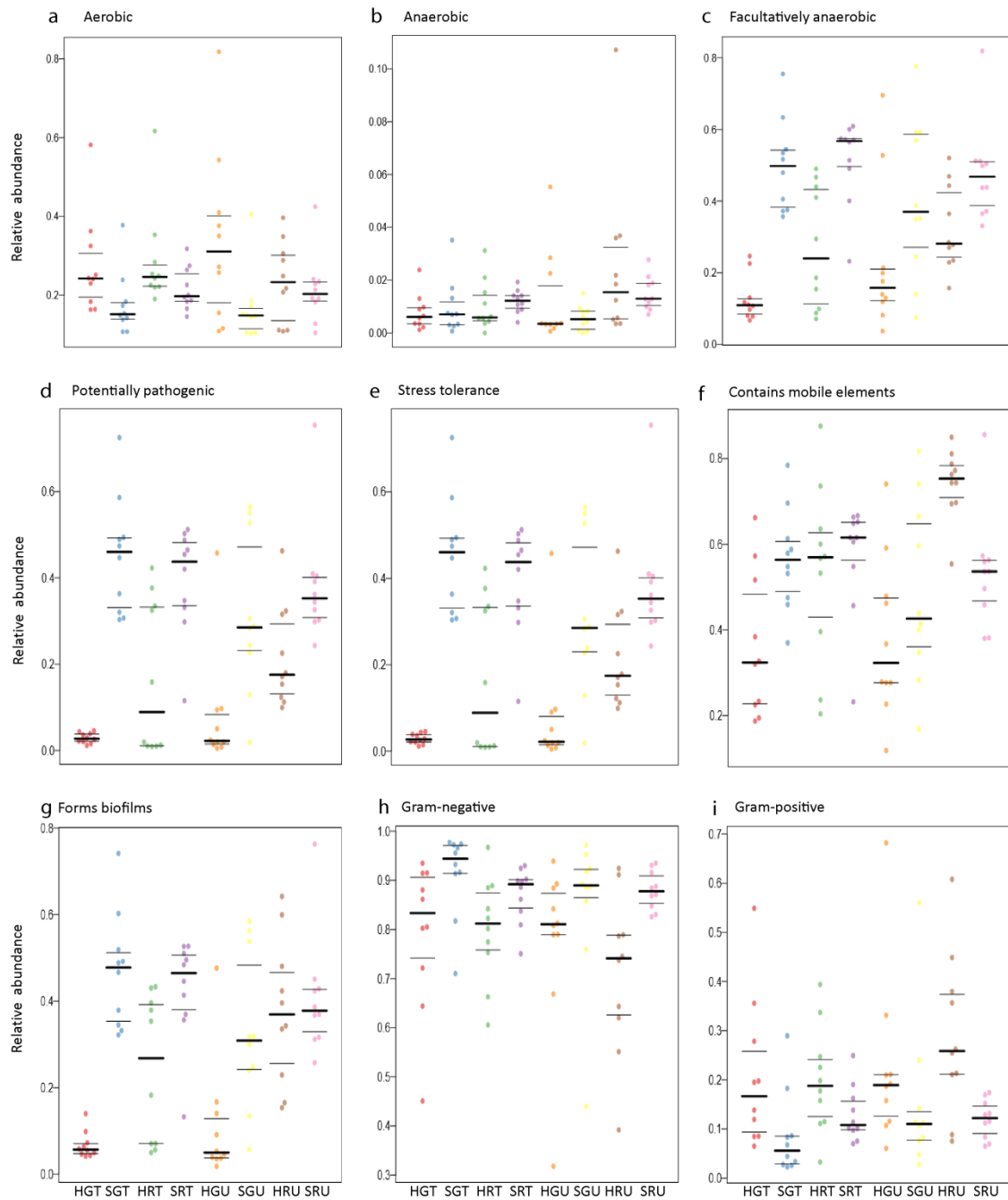


Figure 2. Phenotypic prediction based on BugBase analysis. Prediction of phenotypic differences from 16S rRNA sequence data associated with aerobic, potentially pathogenic, stress tolerance, mobile element, biofilms formation, Gram-negative bacteria and Gram-positive bacteria from sample between hydroponic and soil treated and untreated pepper samples.

These genera are known to have an antagonistic action against fungal pathogens, reducing cucumber Fusarium wilt, *Fusarium oxysporum*, and other fungal pathogens while stimulating growth of other vegetable and fruit crops such as cucumbers and chickpeas^{41–46}.

Genus	HGT		HGU		P-value
	Mean	St. error	Mean	St. error	
<i>Brevundimonas</i>	0.310	0.039	0.000	0.000	<0.001
<i>Chitinophaga</i>	0.317	0.009	0.174	0.020	<0.001
<i>Chryseobacterium</i>	0.377	1.123	0.170	0.015	<0.001
<i>Clostridium</i>	0.414	0.025	0.214	0.038	<0.001
<i>Microbispora</i>	6.929	0.042	1.577	0.014	<0.001
<i>Myroides</i>	0.660	0.083	0.000	0.000	<0.001
<i>Ochrobactrum</i>	0.088	0.001	0.000	0.000	<0.001
<i>Paenibacillus</i>	1.178	0.008	0.359	0.105	<0.001
<i>Pedobacter</i>	0.383	0.041	0.000	0.000	<0.001
<i>Phenylobacterium</i>	0.417	0.028	0.217	0.051	0.002
<i>Sphingobacterium</i>	0.721	0.010	0.213	0.017	0.026
	SGT		SGU		
<i>Sphingobium</i>	0.363	0.004	0.118	0.001	0.001
	HRT		HRU		
<i>Agromyces</i>	0.373	0.001	0.014	0.025	<0.001
<i>Azospirillum</i>	0.340	0.010	0.141	0.017	<0.001
<i>Bacteroides</i>	0.523	0.000	0.000	0.091	<0.001
<i>Cellvibrio</i>	0.384	0.000	0.000	0.045	<0.001
<i>Chitinophaga</i>	0.474	0.015	0.000	0.000	<0.001
<i>Clostridium</i>	0.355	0.000	0.000	0.016	<0.001
<i>Corynebacterium</i>	0.477	0.006	0.126	0.037	<0.001
<i>Exiguobacterium</i>	0.400	0.004	0.104	0.044	<0.001
<i>Geobacillus</i>	1.158	0.052	0.230	0.120	<0.001
<i>Paenibacillus</i>	1.645	0.008	0.642	0.097	<0.001
<i>Phenylobacterium</i>	0.370	0.012	0.149	0.039	<0.001
<i>Serratia</i>	0.358	0.000	0.000	0.603	<0.001
	SRT		SRU		
<i>Agromyces</i>	0.688	0.000	0.000	0.008	<0.001
<i>Clostridium</i>	0.488	0.004	0.015	0.056	<0.001
<i>Comamonas</i>	0.701	0.004	0.115	0.037	<0.001
<i>Corynebacterium</i>	0.950	0.008	0.133	0.242	0.002
<i>Geobacillus</i>	0.406	0.014	0.200	0.027	<0.001
<i>Klebsiella</i>	0.364	0.001	0.098	0.054	<0.001
<i>Lactococcus</i>	2.312	0.116	0.657	0.007	0.001

Table 1. Comparison of bacterial genera showing significance differences; between hydroponic treated and untreated green samples, soil treated and untreated green pepper samples, hydroponic treated and hydroponic untreated red pepper samples, and between soil treated and untreated red pepper samples. Average relative abundance of sequences assigned to genus (Mean) constituting 0.3% or more sequences in either of the sample, standard error of the corresponding average (St. error) and p-value ($p < 0.05$ significant) describing the significance of the differential abundance observed between the two sample sources.

These findings suggest that the abundance of these bacterial genera on surfaces of peppers are not affected by changes in growing conditions, maturity stage and pesticide treatment. Hence, these antagonists may be recommended for use in integrated pest management (IPM) programs, where both biological and chemical methods of pest control are recommended⁴⁷. Similar results were obtained when the fruit surface bacterial communities living on apple fruits under conventional and organic management were compared, where only low abundance groups differed between the two environments⁴⁸. A study by Telias, *et al.*¹⁵ also showed that these bacterial genera were highly abundant and variable on the surfaces of tomato fruits, but with no significant differences detected between the tomato fruit samples sprayed with surface water and groundwater. *Acinetobacter*, *Pseudomonas* and *Sphingomonas* were also identified in high abundance in the phyllosphere of some Atlantic rainforest tree species and cottonwood^{15,49}, as well as on the leaves of field-grown tomatoes⁵⁰.

In general, the abundance of all genera was consistently higher in pesticides-treated compared to pesticides-untreated pepper samples grown under both hydroponic and open field conditions. The same scenario was observed in the case of fruit maturity, where the relative abundances of all genera were higher in green compared to red sample types. For treatments, similar trends were observed in studies conducted by Johnsen, *et al.*⁵¹, which showed that some microbial groups are capable of using the applied pesticides as a source of energy and nutrients to multiply. For instance, benomyl insecticides have been found to stimulate *Pseudomonas* sp, which use the insecticide as a carbon source for growth⁵². Some pesticides inhibit certain groups of microorganisms and outnumber other groups by releasing them from competition. For example, a study by Hussain, *et al.*⁵³.

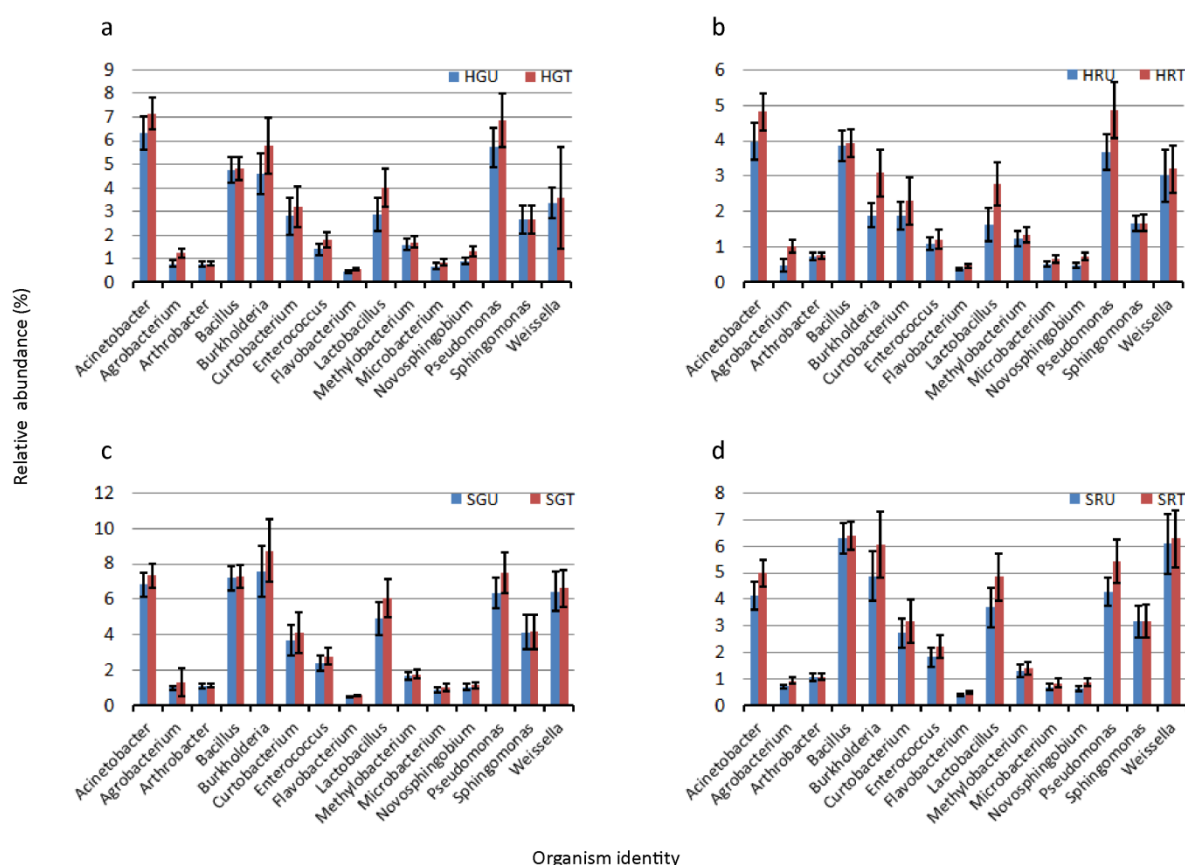


Figure 3. Relative proportion of bacterial antagonists (mean ≥ 0.3); (a) between hydroponic green untreated and hydroponic treated green samples, (b) hydroponic red untreated and hydroponic red treated samples, (c) soil green untreated and soil green treated samples, (d) soil red untreated and soil red treated samples. Error bars indicate mean $\pm$ SE.

demonstrated that fungicide applications inhibited fungal activity of *Fusarium* and *Colletotrichum*, which led to a rapid flush in bacterial activity of *Bacillus*, *Acinetobacter* and *Rhodobacter*. Trends observed for fruit maturity could be explained by the fact that, (i) a cyclic changes are observed in temperature and water availability during fruit development in early summer and (ii) progressive desiccation of fruits during maturation in late summer causing pepper to become less susceptible to many bacterial species. These conditions are selective for few species including *Bacillus* as described by Nicholson, *et al.*⁵⁴.

Ordinating bacterial communities data using NMDS plots grouped the bacterial communities separately according to their habitats, treatments and sample type, observing distinct microbial assemblages (Fig. 4). Permutation tests revealed significant effects of habitats, pepper types and treatments on bacterial community structure and composition (i.e., PERMANOVA_{Habitat}, $F_1 = 23.99$, $P < 0.001$; PERMANOVA_{Treatment}, $F_1 = 2.89$, $P < 0.001$; and, PERMANOVA_{Type}, $F_1 = 8.80$, $P < 0.001$). Although differences in pepper surface bacterial community structure have been reported between organic and conventional farming practices¹⁵, no differences have been reported for hydroponic and open field soil. Results from the present study indicate that the bacterial community in hydroponic surface pepper is distinct from those in open field soil surface pepper, regardless of pesticides application and pepper types.

In conclusion, we have demonstrated that pepper (*Capsicum annum*) harbours diverse bacterial communities on its surfaces, independent of growing conditions, sample treatment, and sample type, which influenced their composition and abundances. Some of these bacteria are potential antagonists, which may interact with and inhibit postharvest pathogens. The likely biocontrol mechanisms by these genera involve multifaceted interactions between the host, pathogen and the antagonists which include production of extracellular cell wall degrading enzymes, competition for space and nutrients, production of various plant hormones, mycoparasitism, and production of volatile organic compounds^{36–38,41–44,46,55}. A large group of taxa were common across habitats, treatments and sample type. These taxa represented more than 50% bacterial phylotypes. Phenotypic predictions (at phylum level) seemed to suggest that the agronomic decision of whether to grow peppers on hydroponics or on open fields can be key as a disease control measure, as potentially pathogenic bacteria were predicted to be more abundant on samples grown on open fields than those from

hydroponic systems. This finding demonstrated that hydroponic systems can be key in reducing production costs, in the long-run, as well as in preserving the integrity of ecosystems, which have for long, been under threat from high-input crop production systems that rely much of heavy inorganic pesticide and fertilizer applications. Additionally, many of the bacterial genera observed in high abundance in samples collected on plants grown under hydroponic and open field conditions are known to contain bacterial strains with plant growth promoting abilities for example *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Curtobacterium* and *Microbacterium*^{56–59} and those that act as antagonists against fungal plant pathogens^{42–46}.

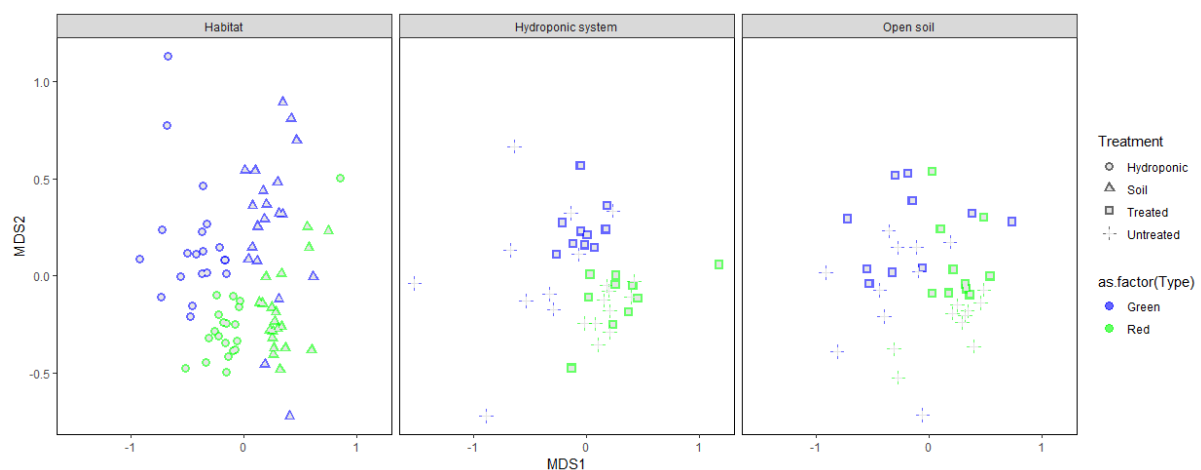


Figure 4. An NMDS plot showing differences in bacterial structure; (a) between hydroponic and soil habitat, (b) green and red samples under hydroponic habitat, (c) green and red samples under soil habitat.

However, a further investigation of these beneficial bacteria using culture-based approaches will help in isolating and characterizing the effects of the antagonists against bacterial pathogens of pepper. Overall, peppers can accommodate different bacterial taxa on its surfaces, some of which with beneficial functional attributes such as pathogenic microbe

antagonism, but these beneficial functions will be more important for plants grown under open soils, since they will be more exposed to both, biotic and abiotic stress factors.

Materials and methods

Study sites and crop management.

Sweet peppers were grown in the summer and autumn seasons from October 2014 to March 2015, at the Agricultural Research Council-Vegetable and Ornamental Plants (ARCVOP), Roodeplaat, Pretoria, South Africa (25°59'S; 28°35'E and at an altitude of 1200 m above sea level). Plants were grown under both hydroponic (40% black and white shade net structure) and field conditions. The mean temperature for hydroponic growing conditions were 33 °C day/15 °C night. In the open field, temperatures of 34.5 °C day/15 °C night were recorded. The experimental design was a 2 (treatments) × 2 (growing conditions) × 2 (maturity stages) factorial, with ten replicates (n = 80). The treatments were fungicide-treated (T) and fungicide-untreated (U); the growing conditions hydroponic (H) and field (S); and the two maturity stages red (R) and green (G).

For the field experiment, seven-week-old sweet-pepper seedlings of cultivar 'King Arthur' of indeterminate growth habit were transplanted onto 20 cm-high ridges, with an intra-row spacing of 0.3 m and an inter-row spacing of 1.5 m. Plants were pruned to three stems and supported by horizontal twines to box the plants between horizontal twine until the height of 1.5 m. The soil was composed of a mixture of sandy, clay and loam (68%, 8% and 24%, respectively). The chemical composition of the soil (pH 7.3) was as follows: 73.1 mg.kg⁻¹ phosphorus (P), 182 mg.kg⁻¹ potassium (K), 978 mg.kg⁻¹ calcium (Ca), 189 mg.kg⁻¹ magnesium (Mg), and 51.1 mg.kg⁻¹ sodium (Na). Nitrogen was applied at the rate of 180 kg.ha⁻¹ and was incorporated into the soil by banding with three split applications. The first application of

nitrogen was at transplanting (50%), the second four weeks after transplanting (WAT) at WAT (25%) and the last at eight WAT (25%). Superphosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$) and potassium sulphate (K_2SO_4) were applied at planting at the rate of $20 \text{ kg} \cdot \text{ha}^{-1}$ (10.5% P) and $40 \text{ kg} \cdot \text{ha}^{-1}$ (42% K), respectively. Drip irrigation supplied 550 mm water. The total rainfall received during the growing season was 40 mm.

For the hydroponic experiment, sweet-pepper seedlings as above were transplanted into 10 L plastic bags filled with sawdust as a growing medium. The drip irrigation system, with one dripper per plant, delivering 2.1 litres of nutrient solution per hour was used to fertilize the plants as described by Maboko and Du Plooy⁶⁰. The plants were pruned to three stems at four WAT. Each stem was trellised by twisting twine around the main stem and fixing it to a stay wire 2 m above the ground surface to support the plant. Side branches were removed weekly to maintain the three-stem system.

For both hydroponic and field conditions, after two WAT plants were sprayed with the following fungicides to control powdery mildew, blight and leaf spot: COPPER-COUNT N (5 mL/L), SPOREKILL (1 mL/L), BINOMYL (50 g mL/L), BRAVO (210 mL/L) and RIDMOL (360 mL/L). Insecticides ACTARA (50 mL/L), HUNTER (40 mL/L), DIOZINON (160 mL/L), BIOMECTINE (60 mL/L), and SAVAGE (40 mL/L)) were also applied to control white flies, red spider mites and aphids.

Sample collection and processing.

Fresh, intact and healthy green and red (10 and 14 weeks after planting, respectively) sweet pepper fruit samples were aseptically collected, stored in sterile Ziploc bags and kept at 4 °C in the lab. A total of 80 samples were harvested: 10 Hydroponic-Green-Treated (HGT), 10 Hydroponic-Red-Treated (HRT), 10 Hydroponic-Green-Untreated (HGU), 10 Hydroponic-Red-

Untreated (HRU), 10 Soil-Green-Treated (SGT), 10 Soil-Red-Treated (SRT), 10 Soil-Green-Untreated (SGU) and 10 Soil-Red-Untreated (SRU). Microbial biofilms on the surfaces of the pepper fruits were retrieved using sterile cotton swabs soaked in a solution containing 0.15 M NaCl and 0.1% Tween 20, as described by Paulino, *et al.*⁶¹. The swabs were then transferred to micro centrifuge tubes and stored at -80 °C until DNA extraction was performed.

DNA extraction and fragment amplification and high- throughput sequencing.

Genomic DNA was isolated from the 80 samples using the ZR Fungal/Bacterial DNA extraction kit (ZYMO Research, Irvine, CA, USA) according to the manufacturer's instructions. Bacterial 16S rRNA gene amplicons were amplified using primers, 515F (5'-GTGYCAGCMGCCGCGGRA-3') and 909 R (5'-CCCCGYCAATTCMTTTRAG-3'), targeting the V4 hypervariable region⁶². PCR was conducted in a single step using a barcoded forward primer and HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA). The thermocycling conditions were initial denaturation at 94 °C for 3 minutes, followed by 28 cycles of 94 °C for 30 seconds, 53 °C for 40 seconds and 72 °C for 1 minute, then a final elongation step at 72 °C for 5 minutes. PCR products were separated by electrophoresis on 2% agarose gel to observe the expected band sizes. All samples were pooled in equal proportions and purified using calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA). Sequencing was performed on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the Molecular Research LP next generation sequencing service (<http://www.mrdnalab.com>, Shallowater, TX, USA) according to the manufacture's guidelines.

Bioinformatics analysis.

The generated 16S rRNA gene sequence data was analyzed using QIIME v1.9.1⁶³. Joined sequences <200 bp long, with more than two ambiguous bases, had a quality score of <25 or more than one mismatch to the sample-specific barcode or to the primer sequences, were discarded. Chimeric sequences were discarded using USEARCH V6.1⁶⁴. Good quality reads were clustered into operational taxonomic units (OTUs) at 97% similarity level based on the *Greengenes* reference sequence database (version 13.8) and the *de novo* OTU picking algorithm. The taxonomic affiliations of the OTUs were determined using the naive Bayesian rRNA classifier⁶⁵ at the 80% confidence level. Singletons, chloroplast and archaea species were filtered out from the OTU table and each sample was randomly subsampled (rarefied) to 28,646 reads, which was the lowest number of sequences obtained in a given sample.

Statistical analyses.

Alpha diversity was assessed by computing richness and Shannon index using the '*diversity*' function in the Vegan⁶⁶ R package. Statistical differences were evaluated using Kruskal-Wallis tests⁶⁷. The number of shared OTUs between communities/samples was visualized using the '*venn*' function in gplots (cran.r-project.org/package = gplots). The OTU table was Hellinger-Transformed and the Bray-Curtis distances was used to generate a dissimilarity matrix. The structure of the microbial communities was visualized using non-metric multidimensional scaling (nMDS) plots. Permutational analysis of variance (PERMANOVA)⁶⁸ using the '*Adonis*' function in the Vegan R package was used to test for differences in bacterial composition and structure. BugBase (<http://github.com/danknights/bugbase>) was used to calculate differences between both groups in terms of microbial phenotypes.

Data availability

The raw Illumina sequencing reads for this project have been submitted to the National Centre for Biotechnology Information Short Read Archive (SRA) database with accession no. PRJNA529905. This Targeted Locus Study (TLS) project have been deposited at DDBJ/EMBL/GenBank under the accession KDDL000000000. The version described in this paper is the first version, KDDL010000000.

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Acknowledgements

The authors would like to thank Mr Phathutshedzo Ramudingana and Mr Silence Chiloane for assistance in sampling. We thank Prof Angel Valverde and Dr Nyaradzai Kamutando for their

constructive criticism on the manuscript. This work was supported by funding from the Agriculture Research Council and National Research Foundation, South Africa. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

Conceived and designed the experiment: O.A.A., M.M.M. and O.O.B. Performed the experiments and analysed the data: T.P.M. Supervised the Research and contributed research material: O.A.A., M.M.M. and O.O.B. Wrote the paper: T.P.M., O.A.A., M.M.M. and O.O.B.

Competing interests

The authors declare no competing interests.

Supplementary information

Figure S1

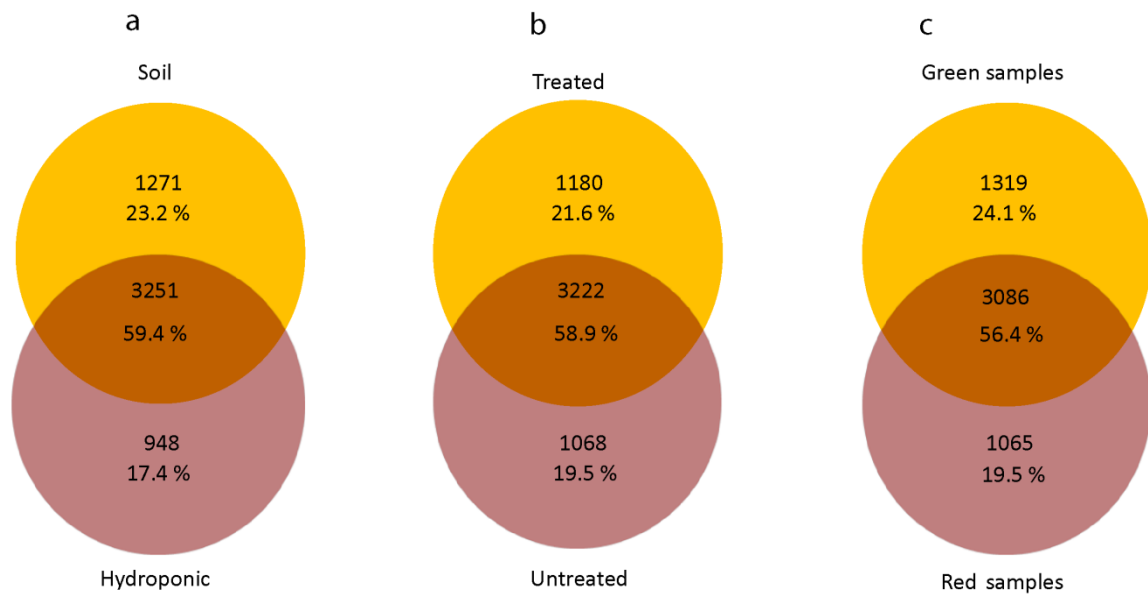


Figure S1. Venn diagram showing the number of shared phylotypes A) between hydroponic and soil habitats, B) treated and untreated samples, and C) green and red samples communities.

Figure S2

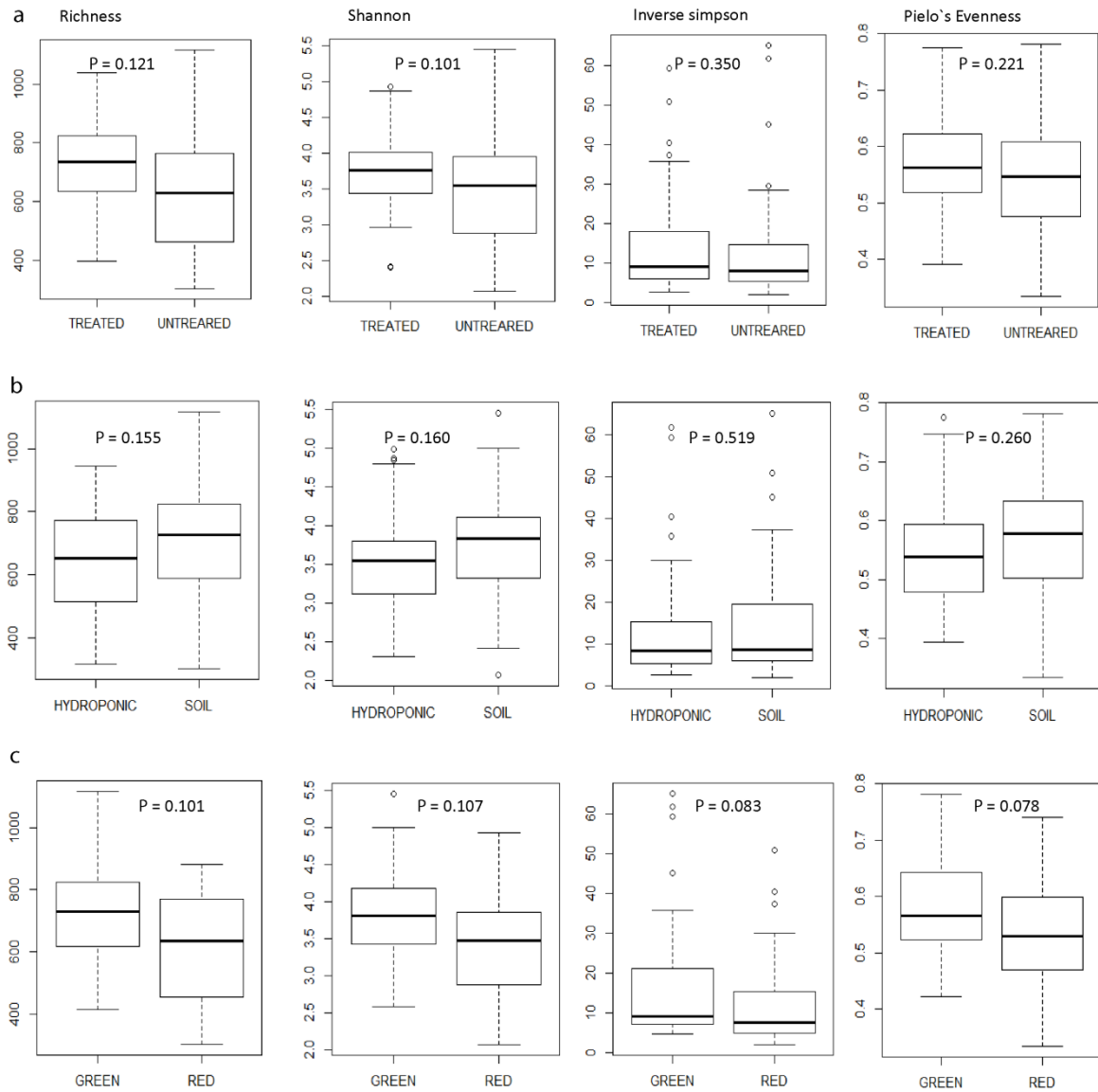


Figure S2. Diversity measures (richness, Shannon, inverse Simpson and Pielou's evenness) of bacterial OTUs (both 97% cut-off) a) between treated and untreated samples b) hydroponic and soil habitats and c) green and red samples.

Figure S3.

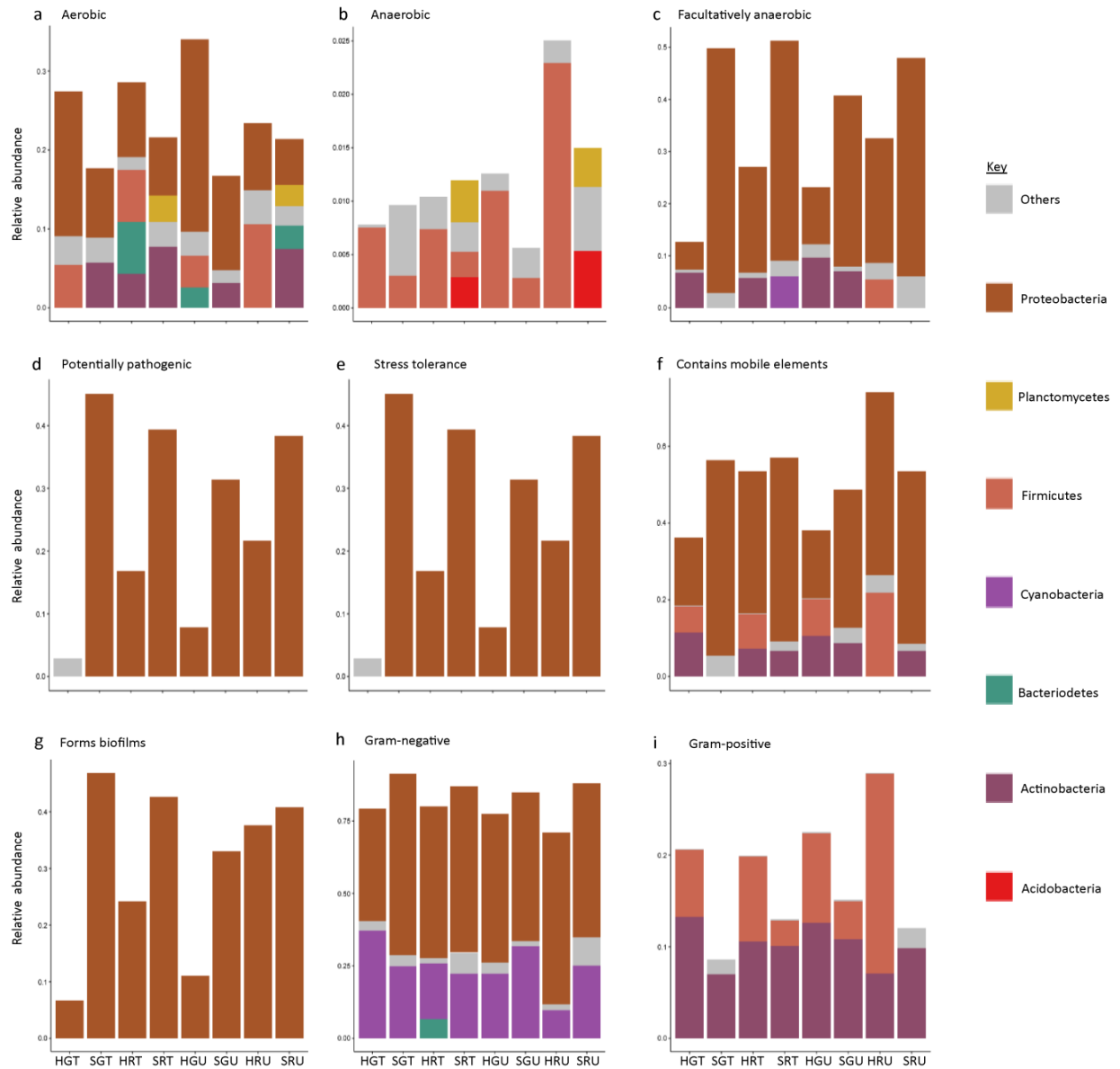


Figure S3. BugBase OTU contribution phyla plots for phenotypic functions predictions; relative abundance plots of phyla predicting phenotypic functions between hydroponic and soil treated and untreated pepper samples.

Table S1

Phenotypes	HGT/PROP	SGT/PRO P	HRT/PROP	SRT/PROP	HGU/PRO P	SGU/PRO P	HRU/PRO P	SRU/PRO P	P-value
Aerobic	0.275	0.177	0.286	0.216	0.341	0.117	0.234	0.214	0.007
Anaerobic	0.008	0.009	0.010	0.012	0.013	0.025	0.015	0.006	0.035
Contains mobile elements	0.362	0.564	0.536	0.570	0.382	0.487	0.742	0.534	<0.001
Facultative anaerobe	0.126	0.497	0.269	0.513	0.232	0.408	0.325	0.479	<0.001
Forms biofilms	0.067	0.469	0.242	0.426	0.111	0.330	0.377	0.408	<0.001
Gram-negative	0.793	0.914	0.800	0.869	0.775	0.849	0.710	0.879	0.003
Gram-positive	0.207	0.086	0.199	0.130	0.225	0.151	0.289	0.120	0.003
Potential pathogenic	0.029	0.451	0.168	0.394	0.079	0.314	0.217	0.384	<0.001
Stress tolerance	0.029	0.451	0.168	0.394	0.079	0.314	0.217	0.384	<0.001

Table S1. Relative abundance of nine potential phenotypes predicted by BugBase in fungicide treated and untreated samples (HGT, hydroponic green treated; SGT, soil green treated; HRT, hydroponic red treated; SRT, soil red treated; HGU, hydroponic green untreated; SGU, soil green untreated; HRU, hydroponic red untreated; SRU, soil red untreated; PROP, Proportion).

Table S2 (a)

Genus	HGU mean	HGU SE	HGT mean	HGU SE	p-value	HRU mean	HRU SE	HRT mean	HRT SE	P-value
<i>Acinetobacter</i>	6.321	0.689	7.16	0.684	0.389	3.981	0.519	4.819	0.515	0.254
<i>Agrobacterium</i>	0.791	0.123	1.231	0.194	0.057	0.479	0.196	1.023	0.185	0.045
<i>Arthrobacter</i>	0.778	0.096	0.795	0.091	0.898	0.74	0.103	0.756	0.098	0.911
<i>Bacillus</i>	4.745	0.535	4.826	0.487	0.421	3.845	0.434	3.927	0.387	0.888
<i>Burkholderia</i>	4.595	0.879	5.788	1.189	0.421	1.896	0.356	3.093	0.665	0.115
<i>Curtobacterium</i>	2.804	0.771	3.205	0.853	0.728	1.866	0.393	2.302	0.656	0.569
<i>Enterococcus</i>	1.399	0.253	1.785	0.325	0.350	1.089	0.196	1.212	0.268	0.712
<i>Flavobacterium</i>	0.438	0.04	0.536	0.051	0.133	0.368	0.041	0.465	0.043	0.105
<i>Lactobacillus</i>	2.86	0.698	4.002	0.828	0.293	1.641	0.464	2.783	0.611	0.139
<i>Methylobacterium</i>	1.602	0.229	1.704	0.229	0.753	1.226	0.218	1.329	0.219	0.739
<i>Microbacterium</i>	0.689	0.116	0.838	0.144	0.422	0.517	0.081	0.666	0.109	0.274
<i>Novosphingobium</i>	0.893	0.125	1.313	0.200	0.077	0.469	0.077	0.725	0.107	0.054
<i>Pseudomonas</i>	5.721	0.842	6.862	1.120	0.417	3.665	0.507	4.862	0.785	0.202
<i>Sphingomonas</i>	2.665	0.586	2.675	0.587	0.990	1.669	0.228	1.679	0.227	0.975
<i>Weissella</i>	3.365	0.636	3.553	2.157	0.934	3.016	0.731	3.204	0.66	0.849

Table S2 (a). Bacterial genera (antagonists) in pepper fruit surface samples; between hydroponic untreated and treated green samples, and between hydroponic untreated and treated red samples (Average relative abundance of sequences assigned to that genus (mean) constituting 0.4% or more sequences in in each the samples, standard error of the corresponding average (SE) and p-value describing the significance of the differential abundance observed between the two sample sources. Hydroponic-green-treated (HGT); hydroponic-green-untreated (HGU); hydroponic-red-treated (HRT); hydroponic-red-untreated (HRU)).

Table S2 (b)

Genus	SGU mean	SGU SE	SGT mean	SGT SE	p-value	SRU mean	SRU SE	SRT mean	SRT SE	P-value
<i>Acinetobacter</i>	6.817	0.681	7.322	0.656	0.594	4.143	0.511	4.981	0.504	0.245
<i>Agrobacterium</i>	0.999	0.131	1.312	0.775	0.691	0.709	0.058	0.942	0.121	0.084
<i>Arthrobacter</i>	1.111	0.127	1.128	0.123	0.924	1.074	0.134	1.090	0.129	0.932
<i>Bacillus</i>	7.199	0.674	7.280	0.626	0.929	6.299	0.571	6.381	0.524	0.916
<i>Burkholderia</i>	7.559	1.459	8.753	1.768	0.603	4.865	0.935	6.058	1.244	0.445
<i>Curtobacterium</i>	3.668	0.869	4.103	1.132	0.761	2.730	0.55	3.165	0.813	0.658
<i>Enterococcus</i>	2.392	0.411	2.779	0.483	0.543	1.819	0.354	2.206	0.426	0.486
<i>Flavobacterium</i>	0.472	0.052	0.569	0.062	0.232	0.401	0.044	0.499	0.054	0.161
<i>Lactobacillus</i>	4.91	0.961	6.052	1.092	0.434	3.692	0.745	4.834	0.875	0.322
<i>Methylobacterium</i>	1.677	0.246	1.779	0.247	0.770	1.302	0.235	1.404	0.236	0.759
<i>Microbacterium</i>	0.871	0.162	1.019	0.191	0.555	0.699	0.128	0.847	0.157	0.466
<i>Novosphingobium</i>	1.057	0.159	1.149	0.166	0.689	0.633	0.099	0.889	0.141	0.216
<i>Pseudomonas</i>	6.323	0.872	7.484	1.151	0.423	4.267	0.537	5.428	0.815	0.236
<i>Sphingomonas</i>	4.154	0.969	4.164	0.969	0.994	3.158	0.611	3.169	0.610	0.989
<i>Weissella</i>	6.433	1.116	6.621	1.045	0.902	6.084	1.141	6.272	1.069	0.904

Table S2 (b). Bacterial genera (antagonists) in pepper fruit surface samples; between soil untreated and treated green samples, and between soil untreated and treated red samples (Average relative abundance of sequences assigned to that genus (mean) constituting 0.4% or more sequences in each of the samples, standard error of the corresponding average (SE) and p-value describing the significance of the differential abundance observed between the two sample sources. Soil-green-treated (SGT); soil-green-untreated (SGU); soil-red-treated (SRT); soil-red-untreated (SRU)).

CHAPTER 5

Epiphytic bacteria from sweet pepper antagonistic *in vitro* to *Ralstonia solanacearum*

Abstract

Biological control of plant pathogens, particularly using microbial antagonists, is posited as the most effective, environmentally-safe, and sustainable strategy to manage plant diseases. But, the roles of antagonists in controlling bacterial wilt, a disease caused by the most devastating and widely distributed pathogen of sweet peppers (i.e., *R. solanacearum*) are poorly understood. Here, amplicon sequencing and several microbial function assays were used to depict the identities and the potential antagonistic functions of bacteria isolated from 80 red and green sweet pepper fruit samples, grown under hydroponic and open soil conditions, with some plants, fungicide-treated while others were untreated. Amplicon sequencing revealed the bacterial strains; *Bacillus cereus* strain HRT7.7, *Enterobacter hormaechei* strain SRU4.4, *Paenibacillus polymyxa* strain SRT9.1 and *Serratia marcescens* strain SGT5.3, as potential antagonists of *R. solanacearum* strain BD 261. Optimization studies under different carbon and nitrogen sources revealed that maximum inhibition of the pathogen was produced at 3% (w/v) starch and 2,5% (w/v) tryptone at pH of 7 and temperature of 30°C. Mode of action exhibited by the antagonistic isolates includes the production of lytic enzymes (i.e., cellulase and protease enzymes) and siderophores, as well as, solubilization of phosphate. Overall, results demonstrated that maximum antimicrobial activity of bacterial antagonists could only be achieved under specific environmental conditions (e.g., available carbon and nitrogen sources, pH, and temperature levels), and that,

bacterial antagonists can also potentially indirectly promote crop growth and development through nutrient cycling and siderophore production.

Keywords: antagonists, biological control, epiphytes, sweet pepper, 16S rRNA genes, *Ralstonia solanacearum*

Introduction

Sweet pepper (*Capsicum annum*), a heat-loving vegetable species, is grown worldwide, with an estimated fruit yield of 26 million tonnes, annually¹. In a world where some communities or households, can be food secure but nutritionally insecure^{2,3} peppers can bridge this gap, as they harbour important nutritional attributes. For example risks of human diseases such as cancer, heart diseases and diabetes, were reported to be minimized by polyphenols and flavonoids^{4,5}, biochemicals highly concentrated in peppers^{6,7,8}. Pepper fruits are usually used for spicing because of their ideal flavour⁹. Due to these reasons, demand for sweet pepper fruits may increase, and this calls for intervention measures that can promote the productivity of this important crop on a global scale.

Yield and fruit quality of sweet peppers were previously reported to be influenced by the genotype¹⁰ as well as the farming system (i.e., agronomic management)¹¹. Both, the crop genotype and crop management practices are traditionally known to affect yield development and crop quality, but this happens in an environment endowed with abiotic and biotic stress factors. For example, under disease stress, some plants may tolerate or resist infections genetically, either through physiological or biochemical mechanisms^{12,13}. In order to withstand disease pressures, the plant genotype was also reported as instrumental in shaping the surrounding microbial communities, to harbour mostly those microbial taxa with plant growth-promoting potentials, including antagonists of the pathogenic taxa^{14,15}.

The top ten, most problematic bacterial pathogens of crops were previously listed, with *Pseudomonas syringe* pathovars and *Ralstonia solanacearum*, topping the list¹⁶. In sweet peppers, *R. solanacearum* is regarded as the most damaging and yield constraining pathogen^{17,18}. *R. solanacearum* causes a disease known as bacterial wilt. Apart from peppers,

this pathogen also attacks, over 200 other plant species, and it is distributed worldwide, where it was observed to induce a destructive economic impact¹⁹. Affected plants usually shows rapid and fatal wilting symptoms²⁰. Over the years, farmers have struggled to control this pathogen because of its abilities to; grow endophytically, survive in deeper soil horizons, travel along with water, as well as its ability to relate with weeds²¹. Therefore, more pragmatic approaches to control this pathogen, especially those measures that are sustainable and environmentally friendly, need to be developed.

Biological control agents (especially, antagonists), are widely accepted as sustainable and ideal for protecting the integrity of ecosystem functions and biodiversity^{22,23,24}. For instance, field evaluations of the bacterial antagonists, *Bacillus amyloliquefaciens* SQR-7 and SQR-101 and *B. methylotrophicus* SQR-29, against *R. solanacearum*, showed biocontrol efficacy (BE) of 18-60% in tobacco²⁵. Elsewhere, the antagonist, *Ralstonia pickettii* QL-A6 indicated BE of 73% against *R. solanacearum* in tomato plants²⁶. In our recent study, 16S rRNA amplicon sequencing data of samples collected from surfaces of red and green sweet pepper fruits, grown under hydroponic (fungicide treated + untreated) and open soil (fungicide treated + untreated) systems, revealed several bacterial taxa with potential to antagonize pathogenic microorganisms²⁷. However, details on the antagonists that can suppress the most important pathogens of sweet peppers such as *R. solanacearum*, are not yet available.

Therefore, this study aims to isolate, characterize and evaluate potential bacterial antagonists, residing on the surfaces of red and green sweet pepper fruits, sampled from plants grown under different management conditions (i.e., hydroponic and open soil conditions, but either fungicide-treated or untreated) for their ability to suppress *R. solanacearum*. We hypothesize that sweet pepper fruits harbour some specific bacterial strains on their surfaces that inhibit the proliferation of pathogenic strains such as *R.*

solanacearum, and that, these antagonists are more important on plants grown under open soil conditions, where disease pressures are commonly high.

Results

Isolation and identification of potent bacterial strains

Bacterial isolations yielded a total of 800 colonies (i.e., isolates) that showed unique morphologies, in terms of colour, shape and texture. Specially, the 800 isolates consisted of 10 colonies selected from each of the 80 sweet pepper fruit samples [i.e., 10 HGT (hydroponic green treated) + 10 HRT (hydroponic red treated) + 10 HGU (hydroponic green untreated) + 10 HRU (hydroponic red untreated) + 10 SGT (soil green treated) + 10 SRT (soil red treated) + 10 SGU (soil green untreated) + 10 SRU (soil red untreated)] (Table S1). Among the 800 isolated strains, only four exhibited inhibitory effects against the *R. solanacearum* BD 261 plant pathogenic strain. These antagonistic strains were identified as HRT7.7, SGT5.3, SRT9.1 and STU4.4 (Table S1; Fig S1).

Taxonomic identities of these four potential antagonistic strains were depicted using 16S rRNA gene sequencing, and homology searches of these sequences on the NCBI platform showed interesting results. Briefly, the isolate identified as HRT7.7 showed highest sequence similarity with *Bacillus cereus* MN589698 (99%), SRU4.4 shared the highest sequence similarity with *Enterobacter hormaechei* MN428803 (98%), SGT5.3 showed close identity with *Serratia marcescens* MN155793.1 (99%), while SRT9.1 showed to be similar to *Paenibacillus polymyxa* MK791706 (99%). Based on these similarities, we, therefore, refer the isolates: HRT7.7 as *Bacillus cereus* strain HRT7.7; SRT9.1 as *Paenibacillus polymyxa* strain SRT9.1; SGT5.3 as *Serratia marcescens* strain SGT5.3; and lastly, SRU4.4 as *Enterobacter hormaechei* strain SRU4.4 (Table 1). More interestingly, phylogenetic analysis of the 16S rRNA gene

sequences confirmed that the isolated antagonistic strains clustered with other genera of *Bacillus*, *Enterobacter*, *Paenibacillus* and *Serratia* (Figure 1).

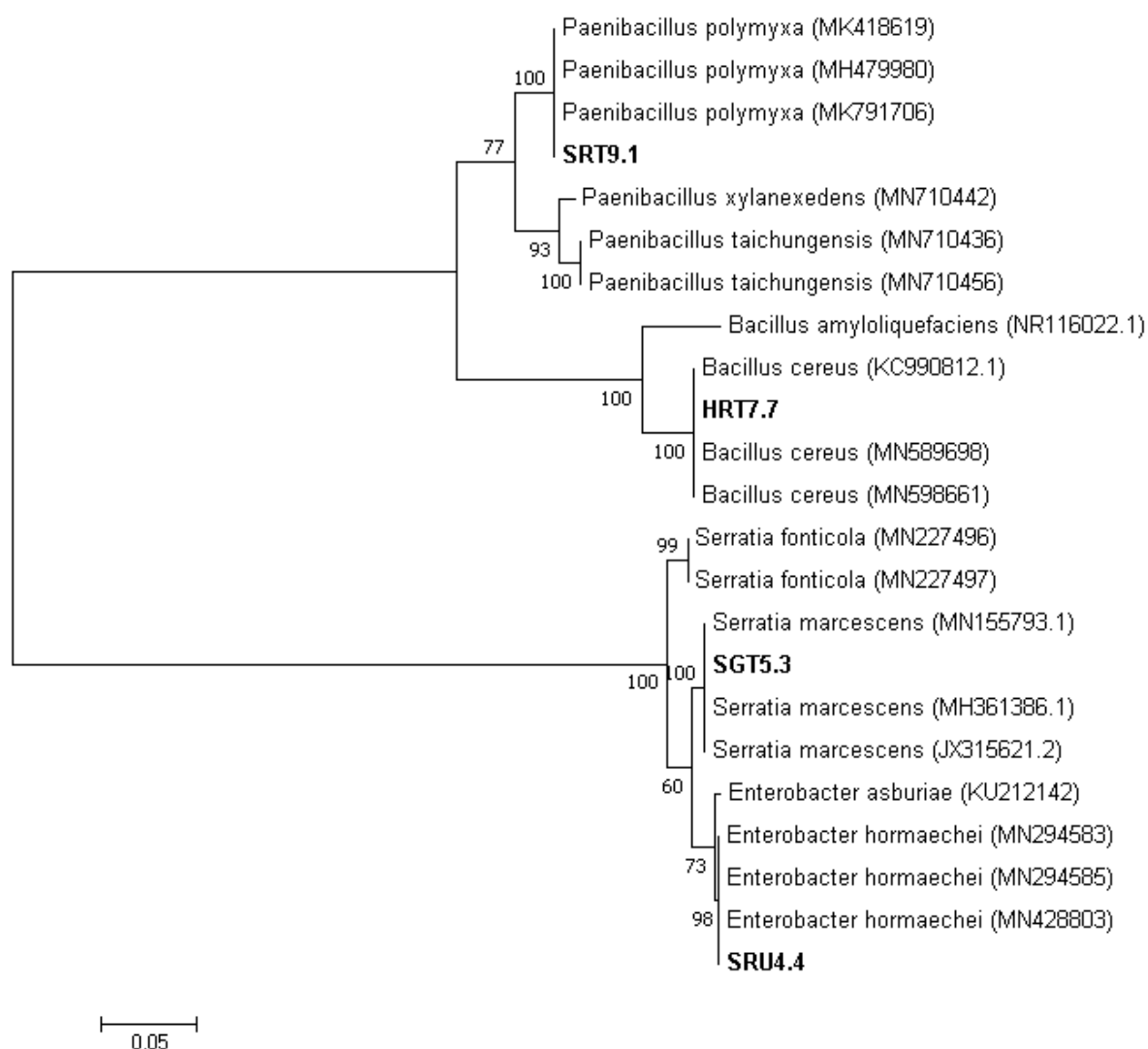


Figure 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of potential antagonistic strains showing the relationship of closest type strain sequences. The phylogenetic tree was constructed using the neighbour-joining algorithm.

Assessing the antagonistic potential of the isolates, before and after enrichment, also revealed some interesting trends. Firstly, the four isolates and the control strain significantly differed ($p < 0.05$) in their ability to suppress the *R. solanacearum* BD 261 strain, both before and after enrichment (Table S2). Generally, before enrichment, all the isolates (including the

control) showed low potential in inhibiting the pathogenic strain. However, the strains, SRT9.1 (*Paenibacillus polymyxa* strain SRT9.1) and SRU4.4 (*Enterobacter hormaechei* strain SRU4.4), with inhibition zones of 8.133 mm and 9.1 mm, respectively, exhibited huge potential in suppressing the pathogenic strain before enrichment. After enrichment, a jump in antagonistic potential was shown for all the isolates, together with the control (Figure 2; Table S3). Interestingly, the inhibitory potential of the control was significantly ($p < 0.05$) lower than all of the newly identified antagonistic strains (Table S4).

Sample no	Strain Code ^a	Base pair length ^b	Species name	Accession no ^c	Similarity (%)
1	HRT7.7	1264 bp	<i>Bacillus cereus</i> strain HRT7.7	MN911398.1	99
2	SGT5.3	1254 bp	<i>Serratia marcescens</i> strain SGT5.3	MN911401.1	99
3	SRT9.1	1265 bp	<i>Paenibacillus polymyxa</i> strain SRT9.1	MN911399.1	99
4	SRU4.4	1255 bp	<i>Enterobacter hormaechei</i> strain SRU4.4	MN911400.1	98

Table 1. Molecular identification of 16S rRNA gene of epiphytic bacterial strains with *in vitro* antagonistic traits. (^aCode for the selected strains with antagonistic, ^bFragment length of selected strain and ^cGeneBank sequence accession numbers of selected strains).

Optimization for enhanced antagonistic activity

Determining the effects of the different treatment levels of pH, carbon and nitrogen sources, temperature, concentration of carbon and nitrogen sources (starch and tryptone) on antagonistic potential of the bacterial isolates from the sweet pepper fruit samples showed encouraging results. First, at these different treatment levels, the isolates indicated to differ significantly (i.e., $p < 0.05$) in how they can deter the functioning of the pathogenic strain, *R. solanacearum* strain BD 261, except for pH = 6 and the yeast extract treatments (Table 2). The highest antagonistic activity was observed at a neutral pH (pH = 7), but pH levels above 6, all seemed to enhance inhibitory activities of the antagonistic strains, with inhibitory zones

above 10 mm, in most cases (Figure 3A; Table S5). Furthermore, the effects of the isolate at the different pH levels significantly differed with that of the control (Table S6).

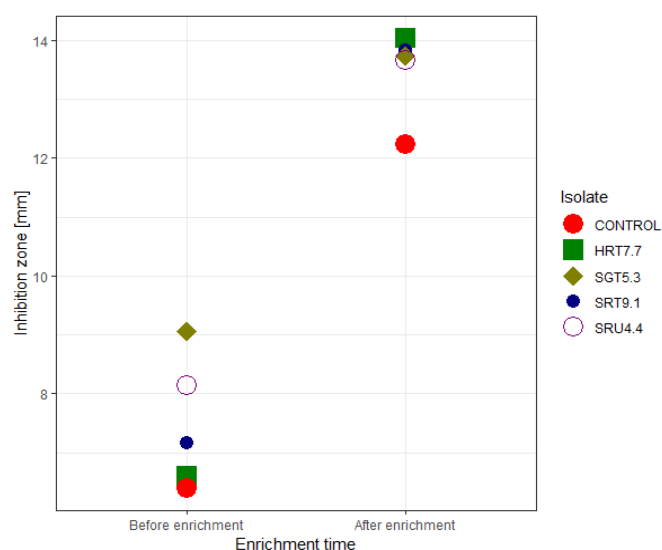


Figure 2. A scatter plot showing inhibition zones of the sweet pepper fruits isolates against the *R. solanacearum* BD 261, before and after enrichment

Carbon sources, including lactose, fructose and starch, indicated the highest potential in promoting the antagonistic potential of the isolates. But, starch proved to be the ideal carbon source, with inhibition zones above 13.5 mm for all the isolates (Figure 3B; Table S5). Of note, all the isolates significantly differed with the control in their ability to inhibit the pathogenic strain when supplied with starch (Table S6). Additionally, the antagonists seemed to favour starch at higher concentrations for optimal activity (Figure 3E).

Source of Variation	Degrees of Freedom	pH				
		5	6	7	8	9
Replication	2	0.002	0.42467	0.32067	0.9613	0.4687
Treatment	4	4.071***	2.65	2.053*	27.907** *	7.036** *
Residual error	8	0.032	0.733	0.35317	1.4853	0.2845

Table 2 (a). Analysis of variance (ANOVA) for antagonistic activity of the sweet pepper fruit isolates against the *R. solanacearum* BD 261 strain, at different treatment levels of pH.

Source of variation	Degree of Freedom	Carbon sources				
		Glucose	Starch	Lactose	Maltose	Fructose
Replication	2	0.26467	0.162	0.05067	0.2167	0.42467
Treatment	4	1.278**	1.562**	2.489***	5.304***	1.674**
Residual error	8	0.14967	0.14367	0.114	0.1525	0.2005

Table 2 (b). Analysis of variance (ANOVA) for antagonistic activity of the sweet pepper fruit isolates against the *R. solanacearum* BD 261 strain, at different treatment levels of carbon sources.

Source of variation	Degrees of freedom	Nitrogen source				
		Glycine	Yeast extract	Tryptone	(NH ₄) ₂ SO ₄	NH ₄ CL
Replication	2	0.1147	0.14467	0.042	0.15267	0.66467
Treatment	4	6.631***	0.34933	0.413*	0.676*	1.893*
Residual error	8	0.1672	0.39883	0.08867	0.161	0.38217

Table 2 (c). Analysis of variance (ANOVA) for antagonistic activity of the sweet pepper fruit isolates against the *R. solanacearum* BD 261 strain, at different treatment levels of nitrogen sources

Source of Variation	Degrees of Freedom	Temperature (°C)				
		25	28	30	35	37
Replication	2	0.1647	0.3247	0.1167	0.126	0.2847
Treatment	4	20.464***	14.142***	8.259***	14.451***	3.424**
Residual error	8	0.2738	0.3163	0.0775	0.0677	0.3388

Table 2 (d). Analysis of variance (ANOVA) for antagonistic activity of the sweet pepper fruit isolates against the *R. solanacearum* BD 261 strain, at different treatment levels of temperature.

Source of variation	Degree of freedom	Starch concentration (%)					
		0.5	1	1.5	2	2.5	3
Replication	2	0.0347	0.206	0.006	0.08867	0.0107	0.1607
Treatment	4	7.367***	5.353***	2.297***	1.922***	3.383***	7.034**
Residual error	8	0.0563	0.0693	0.05933	0.04783	0.0557	0.544

Table 2 (e). Analysis of variance (ANOVA) for antagonistic activity of the sweet pepper fruit isolates against the *R. solanacearum* BD 261 strain, at different treatment levels of starch concentrations.

Source of Variation	Degree of Freedom	Tryptone concentration (%)					
		0.5	1	1.5	2	2.5	3
Replication	2	0.0127	0.026	0.0507	0.1047	0.3227	0.1847
Treatment	4	9.837***	12.944***	12.612***	28.236***	10.561***	6.884***
Residual error	8	0.1552	0.0443	0.1498	0.1755	0.2652	0.1563

Table 2 (f). Analysis of variance (ANOVA) for antagonistic activity of the sweet pepper fruit isolates against the *R. solanacearum* BD 261 strain, at different treatment levels of tryptone concentration.

Although the nitrogen sources, (NH₄)₂SO₄, yeast extract and tryptone revealed immense potential in the aiding activity of the antagonists against the pathogenic strain, *R.*

solanacearum BD 261, tryptone was observed as the ideal nitrogen source (Figure 3C). Inhibitory zones of the isolates, together with the control, were all above 12.5 mm for the tryptone treatment, zones were above those observed for the other nitrogen sources (Table S5). For this treatment, no meaningful differences in activity were detected between the isolates and the control (Table S6). But, as observed for tryptone, it is important to note that, the activity of the isolates, under an environment enriched with tryptone, tends to be much higher at higher concentration levels (Figure 3E). Lastly, temperatures ranging from 27-35°C were observed as ideal for promoting the activity of the antagonists against the pathogenic strain. However, maximum activity was observed at a temperature of 30°C (Figure 3F).

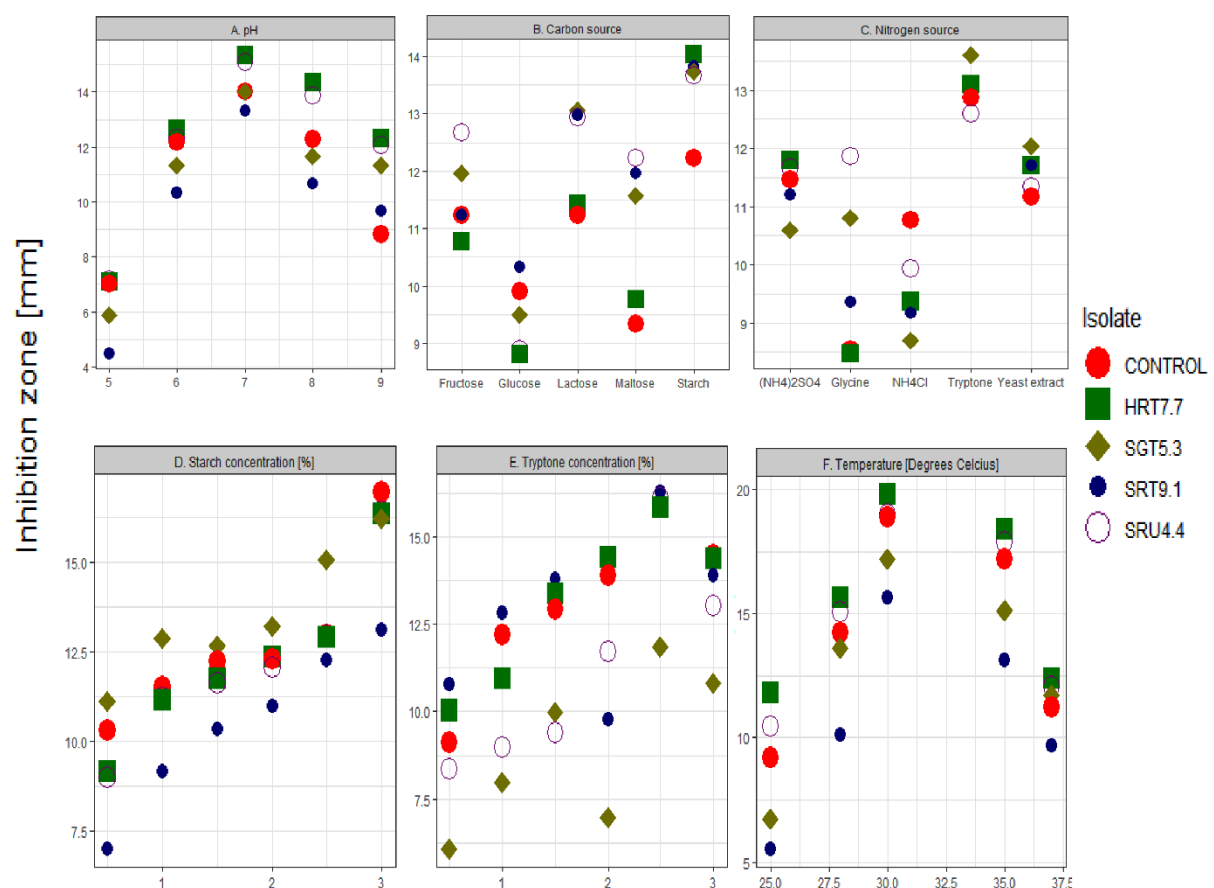


Figure 3. A scatter plot showing inhibition zones of the sweet pepper fruits isolates against *R. solanacearum* strain BD 261, at different treatment levels of pH, carbon sources and nitrogen sources, starch, tryptone concentrations and temperature.

Determination of antimicrobial traits of the antagonists

Bacillus cereus (HRT7.7), *Paenibacillus polymyxa* (SRT9.1), *Serratia marcescens* (SGT5.3) and *Enterobacter hormaechei* (SRU4.4) were evaluated for secondary metabolites production associated with antimicrobial activity, including cellulase and protease, on LB plates containing, CMC and skim milk. Clear zones around the isolates exhibit their high cellulase and proteolytic activity (Figure 4a). Additionally, solubilization of insoluble phosphate and siderophore production were also depicted by the clear zone halos around wells containing colonies and the yellow-orange halos formation around the CAS agar plates (Figure 4b). The assays clearly showed that the isolates potentially antagonize *R. solanacearum* BD 261 using lytic enzymes and siderophore production, as well as by solubilizing phosphate, as their mode of action (Table 3).

Discussion

Biological control (particularly, using antagonists) is poised as the most sustainable and environmentally safe, disease control strategy in crop production^{22,23,24}. However, the roles of antagonists in controlling bacterial wilt, a disease caused by the most devastating and widely distributed pathogen of sweet peppers (i.e., *R. solanacearum*) are poorly understood. Here, potential bacterial antagonists were isolated from 80 red and green sweet pepper fruit samples, grown under hydroponic and open soil conditions, with some plants, fungicide-treated while others were untreated. Amplicon sequencing of the identified potential antagonists, together with microbial activity assays, depicted the identities of the isolates against *R. solanacearum* and revealed the optimal conditions of activity, as well as the mode of action of the isolates against the pathogenic strains.

Firstly, identification of the isolates; *Bacillus cereus* strain HRT7.7, *Paenibacillus polymyxa* strain SRT9.1, *Serratia marcescens* strain SGT5.3 and *Enterobacter hormaechei* strain SRU4.4, as antagonists of *R. solanacearum*, was not surprising since several strains in the genera; *Bacillus*, *Enterobacter*, *Serratia* and *Paenibacillus*, were previously reported to suppress *R. solanacearum* in-vitro²⁸. The ability of these strains to inhibit the growth of phytopathogenic bacteria, as observed in this study, places them as suitable biocontrol agents in crop production.

Different studies have demonstrated that temperature is one of the significant factor that influences microbial antagonists growth and activity^{29,30}. Our results also demonstrated temperature as an essential parameter in determining the antagonistic activity of bacterial antagonists against *R. solanacearum* BD 261. Although temperatures that ranges between 27-35°C showed to be ideal for antagonistic activity, 30°C was observed to be the most optimal temperature. This finding has several implications of decision-making in crop production. For instance, since the antagonists prefer averagely high temperatures of maximum activity, this suggests that application of the bacteria as a biological control measure on the crop should be made in the afternoon when temperatures are high. But for horticultural crops like sweet peppers, which are predominantly grown under controlled environments (e.g., greenhouses), after applying these antagonists, it would be good to maintain temperatures at 30°C (i.e., the optimal temperature), in order to encourage maximum suppression of the pathogen. These high temperatures will not affect the sweet pepper plants physiologically since the plants are thermophilic in nature¹.

In agreement with Passari *et al.*³¹, present results exhibited antagonistic activity against *R. solanacearum* strain at a wide pH range (Figure 3A), with maximum antimicrobial activity at pH 7. At an optimal pH level, cell growth and enzyme production (e.g., lytic

enzymes) are produced³¹. Several previous studies reported that near-neutral pH is appropriate for most bacteria to synthesize antagonistic substances³².

Apart from supporting microbial growth, the amendment of medium with carbon and nitrogen sources is known to strongly influence antimicrobial activity and synthesis of antimicrobial metabolites by microbial strains³³. The present study depicted that carbon and nitrogen sources (particularly, high concentration of starch and tryptone) in growth medium play an important role in encouraging antagonistic activity against *R. solanacearum* strain (Figure 3). Interestingly, these results strongly agreed with previous studies, in-which antimicrobial activity of *B. cereus*³⁴, *E. hormaechei*³⁵, *P. polymyxa*³⁶ and *S. marcescens*³⁷, were shown to be strongly influenced by the medium with carbon and nitrogen sources. These findings could as well help agro-chemical companies that will be interested in packaging these potential antagonists as bio-control pesticides. For instance, in formulations, antagonistic bacteria can be mixed with the most important carbon and nitrogen sources identified in this study (i.e., starch and tryptone), as this will improve on the efficacy of these bio-pesticides against the *R. solanacearum* pathogen.

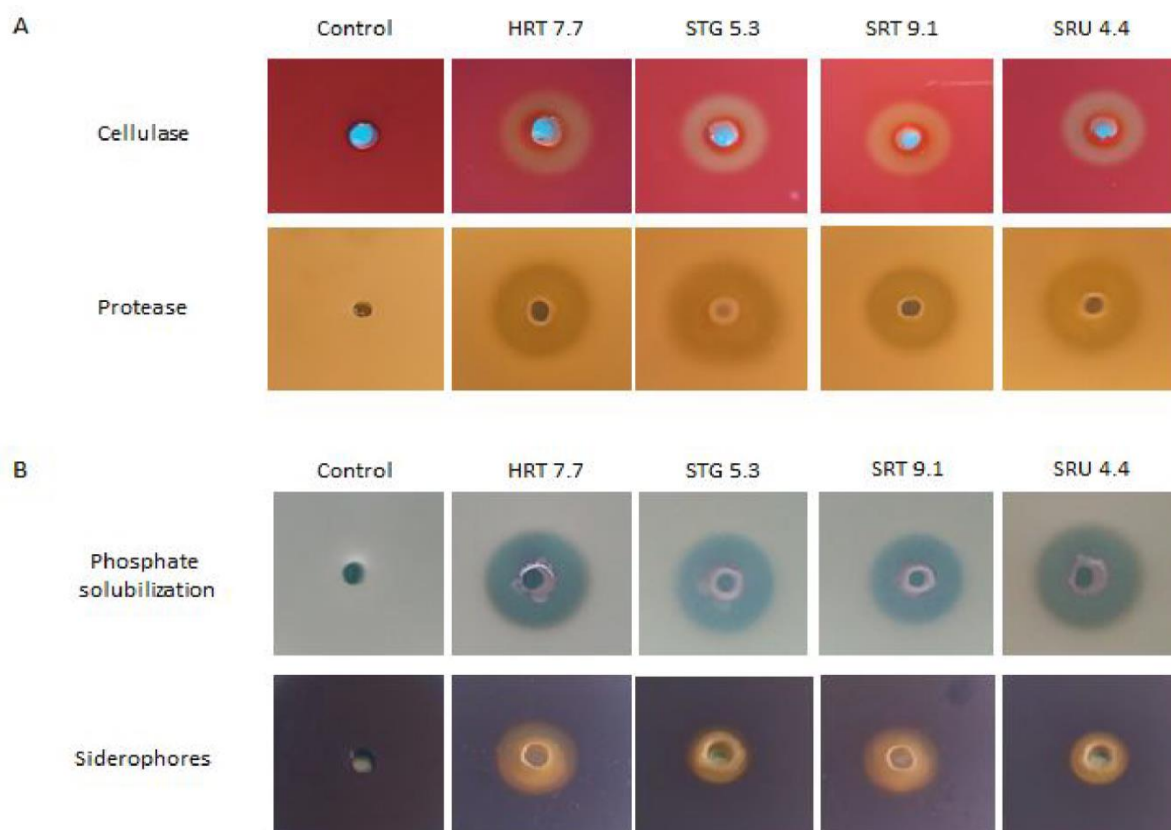


Figure 4. Production of antimicrobial traits by *Bacillus cereus* HRT7.7, *Paenibacillus polymyxa* SRT9.1, *Serratia marcescens* SGT5.3 and *Enterobacter hormaechei* SRU4.4. (A) Production of cellulase and protease, (B) phosphate solubilization and siderophore production.

Previous studies by El-Sayed *et al.*³³ and Dhar Purkayastha *et al.*³⁸ reported that several antagonistic bacteria (e.g., *Bacillus* spp, *Paenibacillus* spp, *Serratia* spp and other *Enterobacter* spp.) secrete lytic enzymes including; amylase, cellulases and chitinases, which are capable of degrading chitin. The secretion of these enzymes is considered as the major and the most effective antagonistic mode of action deployed by various bacteria against plant phytopathogens³⁹. Apart from suppressing pathogenic microbes, antagonists also indirectly promote plant growth and development through organic matter decomposition, phosphate solubilization and siderophore production⁴⁰. The present results corroborated with these previous accessions, as siderophores and phosphate solubilization potential was also shown

(Figure 4). In addition, cellulase and protease activity depicted by the isolates against *R. solanacearum* strain was also reported in previous studies^{41,42}. Based on information available to us, this is the first report of isolation of a comprehensive range of epiphytic bacteria with antagonistic potential, from the surface of a fruit crop.

Isolates	Lytic enzyme production ^a		Siderophore production ^b	Phosphate solubilization ^c
	Cellulase	protease		
<i>Bacillus cereus</i> strain HRT7.7	++++	+++++	++++	+++++
<i>Paenibacillus polymyxa</i> strain SGT5.3	++++	+++++	+++	+++++
<i>Serratia marcescens</i> strain SRT9.1	++++	++++	++++	++++
<i>Enterobacter hormaechei</i> strain SRU4.4	++++	+++++	+++	+++++

Table 3. Specific modes of action by antagonistic bacteria against *R. solanacearum* strain BD 261 (^aDiameter of clear zone due to the production of lytic enzymes ++++ + ≥ 14 mm, +++ ≥ 6 mm, ++ ≥ 5 mm; ^bDiameter of yellow halo on CAS agar plates ++++ ≥ 6 mm, +++ ≥ 5 mm; ^cDiameter of clear zones as a results of phosphate solubilisation ++++ + ≥ 12 mm, +++ ≥ 8 mm).

In conclusion, we have successfully isolated effective antagonistic strains from the surfaces of fresh red and green sweet pepper fruits viz. *Bacillus cereus* strain HRT7.7, *Paenibacillus polymyxa* strain SRT9.1, *Serratia marcescens* strain SGT5.3 and *Enterobacter hormaechei* strain SRU4.4. These strains exhibited a strong antagonistic activity for suppressing *R. solanacearum* strain BD 261 *in vitro*, by secreting lytic enzymes such as cellulase and protease. The strains further exhibited capability of solubilizing phosphate and siderophores production, making them good candidates as biocontrol and noble plant growth-promoting (PGP) agents. As *in vitro* studies should be considered before the commencement of any green house and field studies, the present study delivers a piece of convincing evidence that surface fresh pepper fruits (especially, from plants grown under open soil environments) harbour bacteria with ability to offer plant protection against phytopathogens. Future investigation of these beneficial strains will involve analysis of the

expression of defense-related genes such as phenylalanine ammonia lyase in pepper plants and evaluation of their ability to control *R. solanacearum* strain BD 261 and other pathogens *in vivo* under different environmental conditions and cultural practices. In order to understand the pathways and mechanisms of suppressing the pathogen, further studies will encompass analysing antagonist strains whole-genome sequencing. Also in future, the establishment of the relationship between metabolite or antioxidant production by the sweet pepper fruits treated with these antagonistic strains and the level (i.e., growth and antibacterial activity), is of paramount importance since all plants deploy inherent mechanisms to resist or tolerate, both the abiotic and biotic stresses.

Materials and methods

Study sites and crop management

Sweet peppers were planted in October 2014 and were maintained until March 2015, at the Agricultural Research Council- Vegetable and Ornamental Plants Institute (ARC-VOP), in Roodeplaat, Pretoria, South Africa (Latitude = 1,200, Longitude = 25° 59' S; 28° 35' E). Plants were grown in hydroponic system as well as under open field conditions. The mean temperature under hydroponic growing conditions were 33°C day/15°C night. In the open field, average temperatures of 34.5°C day/15°C night were recorded. The experimental design was a 2 [treatments, i.e., fungicide-treated (T) and untreated (U)] x 2 [growing conditions, i.e., hydroponic (H) and open field (S)] x 2 [maturity stages, i.e., green (G) and red (R) colour] factorial, with ten replicates, thereby making-up a total of 80 planting stations.

Cultivation practices of sweet pepper for the field and hydroponic growing conditions were previously reported in detail by Mamphogoro *et al.*²⁷. Two weeks after transplanting (WAT), plants were treated with COPPER-COUNT N (5 ml/L), SPOREKILL (1 ml/L), BINOMYL

(50g ml/L), BRAVO (210 ml/L) and RIDMOL (360ml/L), to control against powdery mildew, blight and leaf spot. Insecticides such as ACTRA (50 ml/L), HUNTER (40 ml/L), DIOZINON (160 ml/L), BIOMECTINE (60 ml/l) and SAVAGE (40 ml/L)), were also applied to control white flies, red spider mites and aphids.

Sample collection, processing and isolation of potential antagonists

A total of 80 (i.e., 10 HGT + 10 HRT + 10 HGU + 10 HRU + 10 SGT + 10 SRT + 10 SGU + 10 SRU), fresh, intact and health green and red sweet pepper fruits were aseptically collected in sterile Ziploc bags and kept at 4°C in the lab. Bacterial biofilms on the surfaces of the pepper fruits were recovered using sterile cotton swabs soaked in a solution containing 0.15M NaCl and 0.1% Tween 20, as described by Paulino *et al.*⁴³. The swabs were vortexed in sterile Eppendorf tubes containing saline solution (0.85% Na₂Cl). The supernatant was serially diluted and one hundred µL aliquots from the 10⁻¹ and 10⁻² dilutions were plated on Trypticase soy agar (TSA). The plates were incubated for 48 h at 30°C under aerobic conditions, and ten colonies per plate with unique morphologies were selected based on differences in colour, shape and texture, for further purification (i.e., n=800, 80 swabs x 10 colonies) (see Table S1). Purified colonies were streaked on TSA and incubated at 37°C for 24 h, and stored on a Trypticase soy broth (TSB) medium containing 50% glycerol at - 80°C for further use.

Plant bacterial pathogen

The plant pathogenic bacteria *R. solanacearum* strain BD 261⁴⁴, isolated from wilted tomato plants, was acquired from the culture bank of the ARC's Plant Protection Biosystems Laboratories, in Pretoria, South Africa (www.arc.agric.za/arc-ppri). The pathogen was maintained on 2-3-5 triphenyl tetrazolium chloride (TZC), in McCartney bottles at 4°C until use. Stock cultures of the test pathogen were prepared for use throughout the study and

maintained in the culture collection of the ARC's Gastro Intestinal Microbiology and Biotechnology Laboratories, which are under the Animal Production Institute, Irene (www.arc.agric.za/arc-api).

Multiplication of potential antagonists and the pathogen

Multiplication of potential antagonists and pathogen followed procedures in Facelli *et al.*⁴⁵, but with minor modifications. Isolates grown on TSA medium and pathogen from TZC medium were re-cultured on sucrose peptone broth (SPB) medium containing (g l⁻¹); sucrose (20), peptone (5), K₂HPO₄ (0.5), MgSO₄·7H₂O (0.25), and pH 7.2-7.4. The growth of potential antagonists and pathogen isolates was observed at 30°C on shaking for 48 h⁴⁶.

***In vitro* Screening of isolates for antagonism**

Antibacterial activity screening of potential antagonists against *R. solanacearum* strain BD 261 was conducted before and after enrichment using an optimized spot-on-lawn assay⁴⁷. Briefly, 200 µL of *R. solanacearum* strain BD 261 cell culture (OD₆₀₀ ~ 0.4) grown in SPB medium was grown on cooled King's B agar medium plates which were containing (g l⁻¹); protease peptone (20), MgSO₄·7H₂O (1.5), K₂HPO₄ (1.5), glycerol (10 ml) and agar (15), at a pH of 7.2. Plates were dried for 40-50 minutes, and five wells (5mm in diameter) were made per plate using a cork borer, with 50 µL of each potential bacterial antagonist grown in SPB (i.e., OD₆₀₀ ~ 0.4) was added into each well. Fifty (50) µL cell culture of *Bacillus stratosphericus* (LT743897) (OD₆₀₀ ~ 0.4) grown in SPB was used as a positive control. The inhibition zone of the bacterial isolates on *R. solanacearum* strain BD 261 was measured after 48 h of incubation at 30°C. The experiments were performed at least three times.

Data (i.e., inhibition zones) collected before and after enrichment, was subjected firstly, to analysis of variance (ANOVA) using the 'aov' function in the agricolae v1.3-1 R

package⁴⁸. Statistical differences between the isolates and the positive control in suppressing the pathogen were predicted using the Tukey's HSD test, using the '*TukeyHSD*' function in the agricolae R package. In order to have a clear picture of how the potential antagonists could suppress *R. solanacearum* BD 261, before and after enrichment, and also, to visualize how they differ in performance in comparison with the control, a scatter plot was used. Scatter plots were graphed using the '*ggplot*' function in the ggplot2 v3.0.0 R package⁴⁹.

Molecular identification of potential antagonistic strains

PCR amplification of 16S rRNA genes

Total genomic DNA of potential antagonistic strains was extracted from pure cultures using the Quick-DNA Fungal/ Bacterial Miniprep Kit Zymo Research D6005 according to the manufacturer's instructions (www.zymoresearch.com), before polymerase chain reaction (PCR). In general, one μL of genomic DNA was used as a template to amplify the full-length of the 16S rRNA gene⁵⁰. An approximately 1.5-kb fragment part of the 16S rRNA gene was amplified using the universal primer pair (27F:5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R:5'-GGTTACCTTGTTACGACTT-3')⁵¹. Amplifications were performed in 20 μL reaction volumes containing, 10 μL One Taq 2X Master Mix with Standard Buffer (NEB, catalogue No. M048S, Invitrogen, USA; 1X), 1 μL of both primers: 27F and 1492R with a concentration of 10 μM , 7 μL Nuclease free water (Catalogue No. E476) and 1 μL DNA template (10-30ng/ μL).

PCR was performed using Thermal Cycler (MJ Mini Personal Thermal Cycler, Bio-Rad; www.bio-rad.com). The PCR conditions were initial denaturation at 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds, 68°C for 1:30 minutes, and then a final elongation step at 68°C for 5 minutes. The amplified genes were ran on 1% agarose gel electrophoresis CSL-AG500 (Cleaver Scientific Ltd; www.cleverscientific.com),

stained with EZ-vision Bluelight DNA Dye with the size markers (10kb Fast DNA ladder NEB N3238, Invitrogen, USA; www.amresco-inc.com) and then cleaned with ExoSAP, a mixture of Exonuclease I NEB M0293L and Shrimp Alkaline Phosphatase NEB M0371 (Invitrogen, USA).

Sequencing and bioinformatics analysis of the 16S rRNA amplicons

The cleaned amplicons were sequenced at Inqaba Biotechnical Industries (Pty) Limited (www.inqababiotec.co.za) in the forward and reverse direction, using the Nimagen, BrilliantDye Terminator Cycle Sequencing Kit V3.1, BRD3 100/1000, following the manufacturer's instructions. Amplicons were then purified with the Zymo Research, ZR-96 DNA Sequencing Clean-up Kit D4053. Purified fragments were analyzed on the ABI 3500XL Genetic Analyzer with a 50cm array, using POP7 (Applied Biosystems, ThermoFisher Scientific; www.thermofisher.com) for each reaction for every sample. The sequence chromatogram generated by the ABI 3500XL Genetic Analyzer were analysed using the FinchT v1.4 software, and the obtained results were compared with the related 16S-rDNA sequences identified by Basic Local Alignment Search (BLAST) search program on the National Center for Biotechnology Information (NCBI), National Library of Medicine, USA (<https://blast.ncbi.nlm.nih.gov/>)⁵².

Sequence alignments were performed using the CLUSTLW algorithm in MEGA v6.06⁵³ with default settings, and phylogenetic trees were constructed using the neighbor-joining method⁵⁴. Reliability of the phylogenetic tree was evaluated through bootstrap analysis with 1000 re-samplings using a p-distance model, with numbers on branches indicating percentage level of bootstrap support (i.e., only values greater than 20% are shown) as described by Saitou and Nei⁵⁴. The 16S rRNA gene sequences obtained in this study has been deposited in the GenBank under accession numbers MN911398.1– MN911401.1.

Optimization for improved activity of potential antagonistic strains

The screened bacterial antagonistic strains were inoculated into SPB enriched with different compositions of carbon sources (including; fructose, glucose, lactose, maltose and starch) and nitrogen sources (i.e., ammonium sulfate, ammonium chloride glycine, yeast and tryptone) at different pH levels (ranging from 5-9, adjusted with 1N HCL and 1N NaOH), and incubated at 30°C on shaker for 48 h as in Costa *et al.*⁵⁵ and Durairaj *et al.*⁵⁶. After incubation, supernatants of the potential antagonistic isolates (OD₆₀₀ ~ 0.4), were poured onto the 5 mm wells of King's B agar plates containing a suspension of the *R. solanacearum* strain BD 261 cell culture (OD₆₀₀ ~ 0.4), and observed for pathogen inhibition. The antagonists were then cultured at concentrations [0.5, 1, 1.5, 2, 2.5, 3 % (w/v)] with optimized carbon and nitrogen sources and pH. Potent strains displaying highest potential for *R. solanacearum* strain BD 261 suppression at the highest concentration of optimized carbon, nitrogen sources, and pH were allowed to grow at 25, 28, 30, 35 and 37°C for 24-48 h together with *R. solanacearum* strain BD 261 using the perforated agar plate technique using a protocol described by Nguyen and Ranamukhaarachchi⁴⁶. The antagonists were further cultured at concentrations [0.5, 1, 1.5, 2, 2.5, 3 % (w/v)] with optimized carbon and nitrogen sources, pH and temperature that exhibited maximum pathogen inhibition with slight modification⁴⁶. The plates were for 24-48 h, and the inhibition zones were measured. The experiments were performed at least three times.

Gathered data (i.e., inhibition zones) for each of the different treatment levels (i.e., pH, carbon and nitrogen sources, temperature, etc.), was subjected to analysis of variance (ANOVA) using the 'aov' function in the agricolae v1.3-1 R package⁴⁸. Differences in mean performance (i.e., inhibition of *R. solanacearum* strain BD 261) between the isolates and the control were detected using the Tukey's HSD test, using the 'TukeyHSD' function in the

agricolae R package⁴⁸. In order to visualize how the isolates differ in performance (i.e., pathogen suppression), in comparison with the control isolate, at the different levels of enrichment, a scatter plot was used. Scatter plots were graphed using the '*ggplot*' function in the ggplot2 v3.0.0 R package⁴⁹.

Determination of potential antimicrobial traits

Cellulase activity

Cellulase activity was determined according to a method by Teather and Wood⁵⁷ with minor modifications. Briefly, the antagonistic strains supernatants (50 µL) were inoculated into the wells of carboxymethyl cellulose (CMC) agar medium containing (g l⁻¹); KH₂PO₄ (1.0), MgSO₄·7H₂O (0.5), NaCl (0.5), FeSO₄·7H₂O (0.01), MnSO₄·H₂O (0.01), NH₄NO₃(0.3), CMC (10) and agar (15). After incubation at 25°C for 72 h, plates were flooded with 0.1% Congo red for 20 min and then with 1M NaCl for 20 minutes. Double – distilled water (ddH₂O) was used as the negative control. Production of cellulase was recognized by a zone formation around the colonies.

Protease activity

Protease activity was determined by inoculating antagonistic strain's supernatants (50 µL) were added into wells of LB agar medium containing 3% skim milk powder and incubated at 28°C for 72 h. Double – distilled water (ddH₂O) was used as the negative control. A clear zone around the test strains after incubation was used as an indicator for protease production⁵⁸.

Detection of phosphate solubilization

Phosphate solubilisation was carried out in a minimal medium, according to Nautiyal⁵⁹ with slight modifications. This medium containing (g l⁻¹); glucose (10), Ca₃(PO₄)₂ (5), (NH₄)₂SO₄ (0.5),

NaCl (0.2), MgSO₄·7H₂O (0.1), KCl (0.1), yeast extract (0.2), MnSO₄·H₂O (0.001), FeSO₄·7H₂O (0.001) and agar (20) at pH 6.8. After cooling the media to 50°C, supernatants of the antagonistic strains (50 µL) were added on the wells of the medium plates and incubated for 72 h at 25°C. Double – distilled water (ddH₂O) was used as the negative control. Phosphate solubilisation was determined by observing a clear zone around the colonies.

Siderophore production

Production of siderophores was assessed by the universal modified chemical assay using the Chrome azurol S (CAS) agar medium prepared according to Schwyn and Neilands⁶⁰. The CAS agar plates were used to detect for presence of siderophores in culture supernatants of the potential antagonistic strains. The CAS agar plates consist of two main components (i.e., the *CAS indicator solution* and the Basal agar medium). CAS indicator solution was prepared by dissolving 60.5 mg CAS in 50 ml distilled water, mixed with 10 ml of Fe⁺³ (27 mg FeCl₃·6H₂O, and 83 ml conc. HCl in 100 ml ddH₂O). Additionally, 72.9 mg hexadecyltrimethylammonium bromide (HDTMA) dissolved in 40 ml distilled water was also slowly added while stirring to give a dark blue 100 ml total volume. The solution was autoclaved before use.

The Basal agar medium consisted of a mixture of 10 ml MM9 salt stock solution which contained 30 g KH₂PO₄, 50 g NaCl and 100 g NH₄Cl in 1 L ddH₂O, 3.23 g PIPES and 12 g of NaOH, all dissolved in 75 ml using distilled water, with pH adjusted to 6.8. After adjusting the pH, 1.2 g agar was added while stirring. The resultant solution was then autoclaved. After cooling the media to 50°C, 10 ml blue dye solution, 3 ml of 10% Casamino acid solution, and 10 ml of 20% glucose as a carbon source, were slowly added along the glass wall with adequate agitation to blend thoroughly. The potential antagonistic strains supernatants (50 µL) were applied in a well on each CAS plate, and the plates were incubated at 25°C for 72 h.

Double – distilled water (ddH₂O) was used as the negative control. Observation of formation of yellow-orange halos around the bacterial colonies designated siderophore production.

Data availability

All data generated during this study are included in this article (and its Supplementary Information file).

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Acknowledgements

This work was supported by the South African Agricultural Research Council- Agroprocessing Competitive Funding [Cost centre PO2000032] and National Research Foundation, South Africa. The authors would like to thank Mr Phathutshedzo Ramudingana, Mr Silence Chiloane for assistance in sampling, and Dr Teresa Goszczynska for providing *R. solanacearum* BD 261 pathogenic strain. The authors are also grateful to the Agriculture Research Council for the PhD bursary to T.P.M and the North West University, for the research collaboration platform.

Author contributions

Conceived and designed the experiment: MMM OAA OOB. Performed the experiments TPM
Analysed the data: TPM CNK. Supervised the Research and contributed research material:
OAA MMM OOB. Wrote the paper: TPM CNK MMM OAA OOB.

Competing Interest

The authors declare no conflict of interest.

Supplementary information

Figure S1

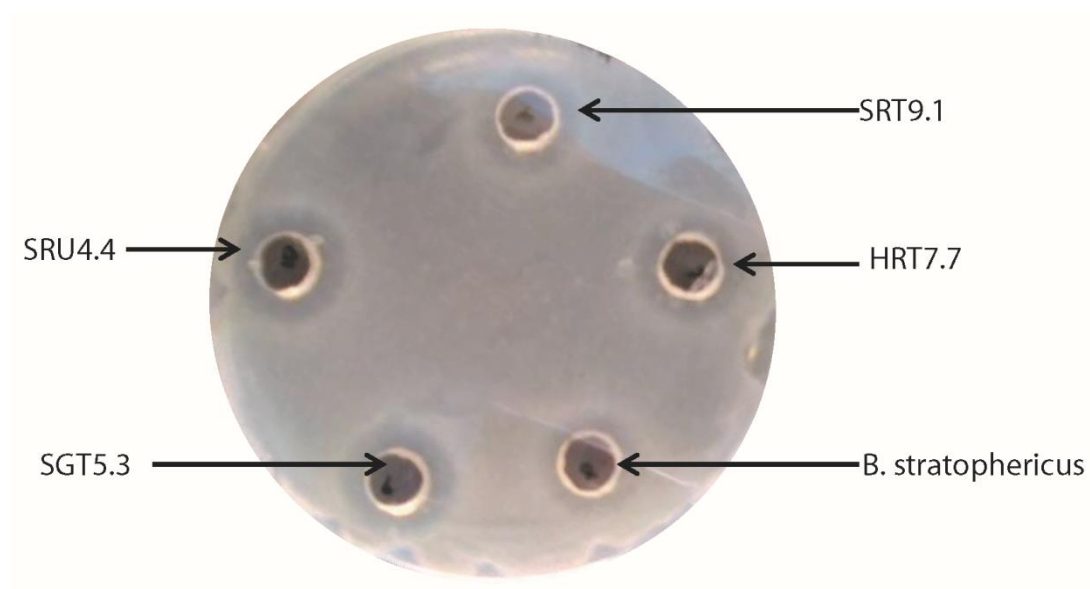


Figure S1. Antagonistic activity of HRT7.7, SGT5.3, SRT9.1, SRU4.4 and *Bacillus stratosphericus* (LT743897) positive control against *R. solanacearum* strain BD 261 pathogen.

Figure S2

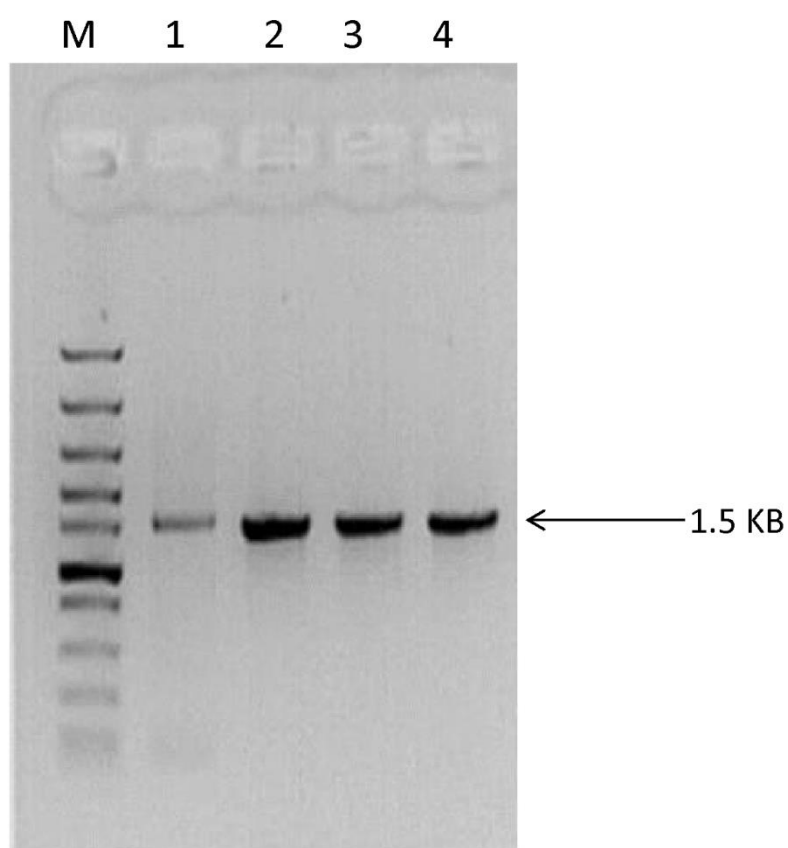


Figure S2. Agarose gel electrophoresis analysis of 16S rRNA genes amplified from four unknown bacterial isolates using primers 27F/1492R. PCR amplified products were run on 1% agarose gel. Lane M contains the DNA Ladder (NEB Fast DNA Ladder Mix 0.5 kb – 10 kb, catalogue number N3238S), lane 1: HRT7.7, lane 2: SGT5.3, lane 3: SRT9.1, lane 4: SRU4.4.

Table S1 (a)

Isolate number	Isolate code	Shape	Color	Texture	Inhibition against <i>R. solanacearum</i> strain BD 261
1	HGU1.1	Irregular	White	Smooth	Negative
2	HGU1.2	Irregular	White	Smooth	Negative
3	HGU1.3	Irregular	White	Smooth	Negative
4	HGU1.4	Irregular	White	Smooth	Negative
5	HGU1.5	Irregular	White	Smooth	Negative
6	HGU1.6	Irregular	White	Smooth	Negative
7	HGU1.7	Irregular	White	Smooth	Negative
8	HGU1.8	Irregular	White	Smooth	Negative
9	HGU1.9	Irregular	White	Smooth	Negative
10	HGU1.10	Irregular	White	Smooth	Negative
11	HGU2.1	Circular	Cream white	Smooth	Negative
12	HGU2.2	Circular	Cream white	Smooth	Negative
13	HGU2.3	Circular	Cream white	Smooth	Negative
14	HGU2.4	Circular	Cream white	Smooth	Negative
15	HGU2.5	Circular	Cream white	Smooth	Negative
16	HGU2.6	Circular	Cream white	Smooth	Negative
17	HGU2.7	Circular	Cream white	Smooth	Negative
18	HGU2.8	Circular	Cream white	Smooth	Negative
19	HGU2.9	Circular	Cream white	Smooth	Negative
20	HGU2.10	Circular	Cream white	Smooth	Negative
21	HGU3.1	Rod	Light yellow	Smooth	Negative
22	HGU3.2	Rod	Light yellow	Smooth	Negative
23	HGU3.3	Rod	Light yellow	Smooth	Negative
24	HGU3.4	Rod	Light yellow	Smooth	Negative
25	HGU3.5	Rod	Light yellow	Smooth	Negative
26	HGU3.6	Rod	Light yellow	Smooth	Negative
27	HGU3.7	Rod	Light yellow	Smooth	Negative
28	HGU3.8	Rod	Light yellow	Smooth	Negative
29	HGU3.9	Rod	Light yellow	Smooth	Negative
30	HGU3.10	Rod	Light yellow	Smooth	Negative
31	HGU4.1	Irregular	Cream	Smooth	Negative
32	HGU4.2	Irregular	Cream	Smooth	Negative
33	HGU4.3	Irregular	Cream	Smooth	Negative
34	HGU4.4	Irregular	Cream	Smooth	Negative
35	HGU4.5	Irregular	Cream	Smooth	Negative
36	HGU4.6	Irregular	Cream	Smooth	Negative
37	HGU4.7	Irregular	Cream	Smooth	Negative
38	HGU4.8	Irregular	Cream	Smooth	Negative
39	HGU4.9	Irregular	Cream	Smooth	Negative
40	HGU4.10	Irregular	Cream	Smooth	Negative
41	HGU5.1	Cocci	Cream white	Smooth	Negative
42	HGU5.2	Cocci	Cream white	Smooth	Negative
43	HGU5.3	Cocci	Cream white	Smooth	Negative
44	HGU5.4	Cocci	Cream white	Smooth	Negative
45	HGU5.5	Cocci	Cream white	Smooth	Negative
46	HGU5.6	Cocci	Cream white	Smooth	Negative
47	HGU5.7	Cocci	Cream white	Smooth	Negative
48	HGU5.8	Cocci	Cream white	Smooth	Negative
49	HGU5.9	Cocci	Cream white	Smooth	Negative

50	HGU5.10	Cocci	Cream white	Smooth	Negative
51	HGU6.1	Rod	Light yellow	Smooth	Negative
52	HGU6.2	Rod	Light yellow	Smooth	Negative
53	HGU6.3	Rod	Light yellow	Smooth	Negative
54	HGU6.4	Rod	Light yellow	Smooth	Negative
55	HGU6.5	Rod	Light yellow	Smooth	Negative
56	HGU6.6	Rod	Light yellow	Smooth	Negative
57	HGU6.7	Rod	Light yellow	Smooth	Negative
58	HGU6.8	Rod	Light yellow	Smooth	Negative
59	HGU6.9	Rod	Light yellow	Smooth	Negative
60	HGU6.10	Rod	Light yellow	Smooth	Negative
61	HGU7.1	Irregular	Yellow	Smooth	Negative
62	HGU7.2	Irregular	Yellow	Smooth	Negative
63	HGU7.3	Irregular	Yellow	Smooth	Negative
64	HGU7.4	Irregular	Yellow	Smooth	Negative
65	HGU7.5	Irregular	Yellow	Smooth	Negative
66	HGU7.6	Irregular	Yellow	Smooth	Negative
67	HGU7.7	Irregular	Yellow	Smooth	Negative
68	HGU7.8	Irregular	Yellow	Smooth	Negative
69	HGU7.9	Irregular	Yellow	Smooth	Negative
70	HGU7.10	Irregular	Yellow	Smooth	Negative
71	HGU8.1	Cocci	White	Smooth	Negative
72	HGU8.2	Cocci	White	Smooth	Negative
73	HGU8.3	Cocci	White	Smooth	Negative
74	HGU8.4	Cocci	White	Smooth	Negative
75	HGU8.5	Cocci	White	Smooth	Negative
76	HGU8.6	Cocci	White	Smooth	Negative
77	HGU8.7	Cocci	White	Smooth	Negative
78	HGU8.8	Cocci	White	Smooth	Negative
79	HGU8.9	Cocci	White	Smooth	Negative
80	HGU8.10	Cocci	White	Smooth	Negative
81	HGU9.1	Circular	Cream	Smooth	Negative
82	HGU9.2	Circular	Cream	Smooth	Negative
83	HGU9.3	Circular	Cream	Smooth	Negative
84	HGU9.4	Circular	Cream	Smooth	Negative
85	HGU9.5	Circular	Cream	Smooth	Negative
86	HGU9.6	Circular	Cream	Smooth	Negative
87	HGU9.7	Circular	Cream	Smooth	Negative
88	HGU9.8	Circular	Cream	Smooth	Negative
89	HGU9.9	Circular	Cream	Smooth	Negative
90	HGU9.10	Circular	Cream	Smooth	Negative
91	HGU10.1	Irregular	Yellow	Smooth	Negative
92	HGU10.2	Irregular	Yellow	Smooth	Negative
93	HGU10.3	Irregular	Yellow	Smooth	Negative
94	HGU10.4	Irregular	Yellow	Smooth	Negative
95	HGU10.5	Irregular	Yellow	Smooth	Negative
96	HGU10.6	Irregular	Yellow	Smooth	Negative
97	HGU10.7	Irregular	Yellow	Smooth	Negative
98	HGU10.8	Irregular	Yellow	Smooth	Negative
99	HGU10.9	Irregular	Yellow	Smooth	Negative
100	HGU10.10	Irregular	Yellow	Smooth	Negative
101	HGT1.1	Rod	Light yellow	Smooth	Negative
102	HGT1.2	Rod	Light yellow	Smooth	Negative
103	HGT1.3	Rod	Light yellow	Smooth	Negative

104	HGT1.4	Rod	Light yellow	Smooth	Negative
105	HGT1.5	Rod	Light yellow	Smooth	Negative
106	HGT1.6	Rod	Light yellow	Smooth	Negative
107	HGT1.7	Rod	Light yellow	Smooth	Negative
108	HGT1.8	Rod	Light yellow	Smooth	Negative
109	HGT1.9	Rod	Light yellow	Smooth	Negative
110	HGT1.10	Rod	Light yellow	Smooth	Negative
111	HGT2.1	Circular	Cream white	Smooth	Negative
112	HGT2.2	Circular	Cream white	Smooth	Negative
113	HGT2.3	Circular	Cream white	Smooth	Negative
114	HGT2.4	Circular	Cream white	Smooth	Negative
115	HGT2.5	Circular	Cream white	Smooth	Negative
116	HGT2.6	Circular	Cream white	Smooth	Negative
117	HGT2.7	Circular	Cream white	Smooth	Negative
118	HGT2.8	Circular	Cream white	Smooth	Negative
119	HGT2.9	Circular	Cream white	Smooth	Negative
120	HGT2.10	Circular	Cream white	Smooth	Negative
121	HG3.1	Irregular	Yellow	Smooth	Negative
122	HG3.2	Irregular	Yellow	Smooth	Negative
123	HG3.3	Irregular	Yellow	Smooth	Negative
124	HG3.4	Irregular	Yellow	Smooth	Negative
125	HG3.5	Irregular	Yellow	Smooth	Negative
126	HG3.6	Irregular	Yellow	Smooth	Negative
127	HG3.7	Irregular	Yellow	Smooth	Negative
128	HG3.8	Irregular	Yellow	Smooth	Negative
129	HG3.9	Irregular	Yellow	Smooth	Negative
130	HG3.10	Irregular	Yellow	Smooth	Negative
131	HGT4.1	Cocci	Cream	Smooth	Negative
132	HGT4.2	Cocci	Cream	Smooth	Negative
133	HGT4.3	Cocci	Cream	Smooth	Negative
134	HGT4.4	Cocci	Cream	Smooth	Negative
135	HGT4.5	Cocci	Cream	Smooth	Negative
136	HGT4.6	Cocci	Cream	Smooth	Negative
137	HGT4.7	Cocci	Cream	Smooth	Negative
138	HGT4.8	Cocci	Cream	Smooth	Negative
139	HGT4.9	Cocci	Cream	Smooth	Negative
140	HGT4.10	Cocci	Cream	Smooth	Negative
141	HGT5.1	Circular	White	Smooth	Negative
142	HGT5.2	Circular	White	Smooth	Negative
143	HGT5.3	Circular	White	Smooth	Negative
144	HGT5.4	Circular	White	Smooth	Negative
145	HGT5.5	Circular	White	Smooth	Negative
146	HGT5.6	Circular	White	Smooth	Negative
147	HGT5.7	Circular	White	Smooth	Negative
148	HGT5.8	Circular	White	Smooth	Negative
149	HGT5.9	Circular	White	Smooth	Negative
150	HGT5.10	Circular	White	Smooth	Negative
151	HGT6.1	Rod	Yellow	Smooth	Negative
152	HGT6.2	Rod	Yellow	Smooth	Negative
153	HGT6.3	Rod	Yellow	Smooth	Negative
154	HGT6.4	Rod	Yellow	Smooth	Negative
155	HGT6.5	Rod	Yellow	Smooth	Negative
156	HGT6.6	Rod	Yellow	Smooth	Negative
157	HGT6.7	Rod	Yellow	Smooth	Negative

158	HGT6.8	Rod	Yellow	Smooth	Negative
159	HGT6.9	Rod	Yellow	Smooth	Negative
160	HGT6.10	Rod	Yellow	Smooth	Negative
161	HGT7.1	Circular	Light yellow	Smooth	Negative
162	HGT7.2	Circular	Light yellow	Smooth	Negative
163	HGT7.3	Circular	Light yellow	Smooth	Negative
164	HGT7.4	Circular	Light yellow	Smooth	Negative
165	HGT7.5	Circular	Light yellow	Smooth	Negative
166	HGT7.6	Circular	Light yellow	Smooth	Negative
167	HGT7.7	Circular	Light yellow	Smooth	Negative
168	HGT7.8	Circular	Light yellow	Smooth	Negative
169	HGT7.9	Circular	Light yellow	Smooth	Negative
170	HGT7.10	Circular	Light yellow	Smooth	Negative
171	HGT8.1	Cocci	Cream	Smooth	Negative
172	HGT8.2	Cocci	Cream	Smooth	Negative
173	HGT8.3	Cocci	Cream	Smooth	Negative
174	HGT8.4	Cocci	Cream	Smooth	Negative
175	HGT8.5	Cocci	Cream	Smooth	Negative
176	HGT8.6	Cocci	Cream	Smooth	Negative
177	HGT8.7	Cocci	Cream	Smooth	Negative
178	HGT8.8	Cocci	Cream	Smooth	Negative
179	HGT8.9	Cocci	Cream	Smooth	Negative
180	HGT8.10	Cocci	Cream	Smooth	Negative
181	HGT9.1	Irregular	White	Smooth	Negative
182	HGT9.2	Irregular	White	Smooth	Negative
183	HGT9.3	Irregular	White	Smooth	Negative
184	HGT9.4	Irregular	White	Smooth	Negative
185	HGT9.5	Irregular	White	Smooth	Negative
186	HGT9.6	Irregular	White	Smooth	Negative
187	HGT9.7	Irregular	White	Smooth	Negative
188	HGT9.8	Irregular	White	Smooth	Negative
189	HGT9.9	Irregular	White	Smooth	Negative
190	HGT9.10	Rod	Light yellow	Smooth	Negative
191	HGT10.1	Rod	Light yellow	Smooth	Negative
192	HGT10.2	Rod	Light yellow	Smooth	Negative
193	HGT10.3	Rod	Light yellow	Smooth	Negative
194	HGT10.4	Rod	Light yellow	Smooth	Negative
195	HGT10.5	Rod	Light yellow	Smooth	Negative
196	HGT10.6	Rod	Light yellow	Smooth	Negative
197	HGT10.7	Rod	Light yellow	Smooth	Negative
198	HGT10.8	Rod	Light yellow	Smooth	Negative
199	HGT10.9	Rod	Light yellow	Smooth	Negative
200	HGT10.10	Rod	Light yellow	Smooth	Negative
201	HRU1.1	Circular	Yellow	Smooth	Negative
202	HRU1.2	Circular	Yellow	Smooth	Negative
203	HRU1.3	Circular	Yellow	Smooth	Negative
204	HRU1.4	Circular	Yellow	Smooth	Negative
205	HRU1.5	Circular	Yellow	Smooth	Negative
206	HRU1.6	Circular	Yellow	Smooth	Negative
207	HRU1.7	Circular	Yellow	Smooth	Negative
208	HRU1.8	Circular	Yellow	Smooth	Negative
209	HRU1.9	Circular	Yellow	Smooth	Negative
210	HRU1.10	Circular	Yellow	Smooth	Negative
211	HRU2.1	Irregular	Cream	Smooth	Negative

212	HRU2.2	Irregular	Cream	Smooth	Negative
213	HRU2.3	Irregular	Cream	Smooth	Negative
214	HRU2.4	Irregular	Cream	Smooth	Negative
215	HRU2.5	Irregular	Cream	Smooth	Negative
216	HRU2.6	Irregular	Cream	Smooth	Negative
217	HRU2.7	Irregular	Cream	Smooth	Negative
218	HRU2.8	Irregular	Cream	Smooth	Negative
219	HRU2.9	Irregular	Cream	Smooth	Negative
220	HRU2.10	Irregular	Cream	Smooth	Negative
221	HRU3.1	Rod	White	Smooth	Negative
222	HRU3.2	Rod	White	Smooth	Negative
223	HRU3.3	Rod	White	Smooth	Negative
224	HRU3.4	Rod	White	Smooth	Negative
225	HRU3.5	Rod	White	Smooth	Negative
226	HRU3.6	Rod	White	Smooth	Negative
227	HRU3.7	Rod	White	Smooth	Negative
228	HRU3.8	Rod	White	Smooth	Negative
229	HRU3.9	Rod	White	Smooth	Negative
230	HRU3.10	Rod	White	Smooth	Negative
231	HRU4.1	Cocci	Cream white	Smooth	Negative
232	HRU4.2	Cocci	Cream white	Smooth	Negative
233	HRU4.3	Cocci	Cream white	Smooth	Negative
234	HRU4.4	Cocci	Cream white	Smooth	Negative
235	HRU4.5	Cocci	Cream white	Smooth	Negative
236	HRU4.6	Cocci	Cream white	Smooth	Negative
237	HRU4.7	Cocci	Cream white	Smooth	Negative
238	HRU4.8	Cocci	Cream white	Smooth	Negative
239	HRU4.9	Cocci	Cream white	Smooth	Negative
240	HRU4.10	Cocci	Cream white	Smooth	Negative
241	HRU5.1	Circular	Yellow	Smooth	Negative
242	HRU5.2	Circular	Yellow	Smooth	Negative
243	HRU5.3	Circular	Yellow	Smooth	Negative
244	HRU5.4	Circular	Yellow	Smooth	Negative
245	HRU5.5	Circular	Yellow	Smooth	Negative
246	HRU5.6	Circular	Yellow	Smooth	Negative
247	HRU5.7	Circular	Yellow	Smooth	Negative
248	HRU5.8	Circular	Yellow	Smooth	Negative
249	HRU5.9	Circular	Yellow	Smooth	Negative
250	HRU5.10	Circular	Yellow	Smooth	Negative
251	HRU6.1	Cocci	White	Smooth	Negative
252	HRU6.2	Cocci	White	Smooth	Negative
253	HRU6.3	Cocci	White	Smooth	Negative
254	HRU6.4	Cocci	White	Smooth	Negative
255	HRU6.5	Cocci	White	Smooth	Negative
256	HRU6.6	Cocci	White	Smooth	Negative
257	HRU6.7	Cocci	White	Smooth	Negative
258	HRU6.8	Cocci	White	Smooth	Negative
259	HRU6.9	Cocci	White	Smooth	Negative
260	HRU6.10	Cocci	White	Smooth	Negative
261	HRU7.1	Rod	Light yellow	Smooth	Negative
262	HRU7.2	Rod	Light yellow	Smooth	Negative
263	HRU7.3	Rod	Light yellow	Smooth	Negative
264	HRU7.4	Rod	Light yellow	Smooth	Negative
265	HRU7.5	Rod	Light yellow	Smooth	Negative

266	HRU7.6	Rod	Light yellow	Smooth	Negative
267	HRU7.7	Rod	Light yellow	Smooth	Negative
268	HRU7.8	Rod	Light yellow	Smooth	Negative
269	HRU7.9	Rod	Light yellow	Smooth	Negative
270	HRU7.10	Rod	Light yellow	Smooth	Negative
271	HRU8.1	Circular	White	Smooth	Negative
272	HRU8.2	Circular	White	Smooth	Negative
273	HRU8.3	Circular	White	Smooth	Negative
274	HRU8.4	Circular	White	Smooth	Negative
275	HRU8.5	Circular	White	Smooth	Negative
276	HRU8.6	Circular	White	Smooth	Negative
277	HRU8.7	Circular	White	Smooth	Negative
278	HRU8.8	Circular	White	Smooth	Negative
279	HRU8.9	Circular	White	Smooth	Negative
280	HRU8.10	Circular	White	Smooth	Negative
281	HRU9.1	Cocci	Yellow	Smooth	Negative
282	HRU9.2	Cocci	Yellow	Smooth	Negative
283	HRU9.3	Cocci	Yellow	Smooth	Negative
284	HRU9.4	Cocci	Yellow	Smooth	Negative
285	HRU9.5	Cocci	Yellow	Smooth	Negative
286	HRU9.6	Cocci	Yellow	Smooth	Negative
287	HRU9.7	Cocci	Yellow	Smooth	Negative
288	HRU9.8	Cocci	Yellow	Smooth	Negative
289	HRU9.9	Cocci	Yellow	Smooth	Negative
290	HRU9.10	Cocci	Yellow	Smooth	Negative
291	HRU10.1	Irregular	Cream white	Smooth	Negative
292	HRU10.2	Irregular	Cream white	Smooth	Negative
293	HRU10.3	Irregular	Cream white	Smooth	Negative
294	HRU10.4	Irregular	Cream white	Smooth	Negative
295	HRU10.5	Irregular	Cream white	Smooth	Negative
296	HRU10.6	Irregular	Cream white	Smooth	Negative
297	HRU10.7	Irregular	Cream white	Smooth	Negative
298	HRU10.8	Irregular	Cream white	Smooth	Negative
299	HRU10.9	Irregular	Cream white	Smooth	Negative
300	HRU10.10	Irregular	Cream white	Smooth	Negative
301	HRT1.1	Cocci	White	Smooth	Negative
302	HRT1.2	Cocci	White	Smooth	Negative
303	HRT1.3	Cocci	White	Smooth	Negative
304	HRT1.4	Cocci	White	Smooth	Negative
305	HRT1.5	Cocci	White	Smooth	Negative
306	HRT1.6	Cocci	White	Smooth	Negative
307	HRT1.7	Cocci	White	Smooth	Negative
308	HRT1.8	Cocci	White	Smooth	Negative
309	HRT1.9	Cocci	White	Smooth	Negative
310	HRT1.10	Cocci	White	Smooth	Negative
311	HRT2.1	Rod	Light yellow	Smooth	Negative
312	HRT2.2	Rod	Light yellow	Smooth	Negative
313	HRT2.3	Rod	Light yellow	Smooth	Negative
314	HRT2.4	Rod	Light yellow	Smooth	Negative
315	HRT2.5	Rod	Light yellow	Smooth	Negative
316	HRT2.6	Rod	Light yellow	Smooth	Negative
317	HRT2.7	Rod	Light yellow	Smooth	Negative
318	HRT2.8	Rod	Light yellow	Smooth	Negative
319	HRT2.9	Rod	Light yellow	Smooth	Negative

320	HRT2.10	Rod	Light yellow	Smooth	Negative
321	HRT3.1	Circular	Yellow	Smooth	Negative
322	HRT3.2	Circular	Yellow	Smooth	Negative
323	HRT3.3	Circular	Yellow	Smooth	Negative
324	HRT3.4	Circular	Yellow	Smooth	Negative
325	HRT3.5	Circular	Yellow	Smooth	Negative
326	HRT3.6	Circular	Yellow	Smooth	Negative
327	HRT3.7	Circular	Yellow	Smooth	Negative
328	HRT3.8	Circular	Yellow	Smooth	Negative
329	HRT3.9	Circular	Yellow	Smooth	Negative
330	HRT3.10	Circular	Yellow	Smooth	Negative
331	HRT4.1	Irregular	Cream	Smooth	Negative
332	HRT4.2	Irregular	Cream	Smooth	Negative
333	HRT4.3	Irregular	Cream	Smooth	Negative
334	HRT4.4	Irregular	Cream	Smooth	Negative
335	HRT4.5	Irregular	Cream	Smooth	Negative
336	HRT4.6	Irregular	Cream	Smooth	Negative
337	HRT4.7	Irregular	Cream	Smooth	Negative
338	HRT4.8	Irregular	Cream	Smooth	Negative
339	HRT4.9	Irregular	Cream	Smooth	Negative
340	HRT4.10	Irregular	Cream	Smooth	Negative
341	HRT5.1	Circular	Orange	Smooth	Negative
342	HRT5.2	Circular	Orange	Smooth	Negative
343	HRT5.3	Circular	Orange	Smooth	Negative
344	HRT5.4	Circular	Orange	Smooth	Negative
345	HRT5.5	Circular	Orange	Smooth	Negative
346	HRT5.6	Circular	Orange	Smooth	Negative
347	HRT5.7	Circular	Orange	Smooth	Negative
348	HRT5.8	Circular	Orange	Smooth	Negative
349	HRT5.9	Circular	Orange	Smooth	Negative
350	HRT5.10	Circular	Orange	Smooth	Negative
351	HRT6.1	Irregular	White	Smooth	Negative
352	HRT6.2	Irregular	White	Smooth	Negative
353	HRT6.3	Irregular	White	Smooth	Negative
354	HRT6.4	Irregular	White	Smooth	Negative
355	HRT6.5	Irregular	White	Smooth	Negative
356	HRT6.6	Irregular	White	Smooth	Negative
357	HRT6.7	Irregular	White	Smooth	Negative
358	HRT6.8	Irregular	White	Smooth	Negative
359	HRT6.9	Irregular	White	Smooth	Negative
360	HRT6.10	Irregular	White	Smooth	Negative
361	HRT7.1	Irregular	Cream white	Smooth	Negative
362	HRT7.2	Rod	Cream white	Smooth	Negative
363	HRT7.3	Rod	Cream white	Smooth	Negative
364	HRT7.4	Rod	Cream white	Smooth	Negative
365	HRT7.5	Rod	Cream white	Smooth	Negative
366	HRT7.6	Rod	Cream white	Smooth	Negative
367	HRT7.7	Rod	Cream white	Smooth	Positive
368	HRT7.8	Rod	Cream white	Smooth	Negative
369	HRT7.9	Rod	Cream white	Smooth	Negative
370	HRT7.10	Cocci	Cream white	Smooth	Negative
371	HRT8.1	Cocci	Yellow	Smooth	Negative
372	HRT8.2	Cocci	Yellow	Smooth	Negative
373	HRT8.3	Cocci	Yellow	Smooth	Negative

374	HRT8.4	Cocci	Yellow	Smooth	Negative
375	HRT8.5	Cocci	Yellow	Smooth	Negative
376	HRT8.6	Cocci	Yellow	Smooth	Negative
377	HRT8.7	Cocci	Yellow	Smooth	Negative
378	HRT8.8	Cocci	Yellow	Smooth	Negative
379	HRT8.9	Cocci	Yellow	Smooth	Negative
380	HRT8.10	Cocci	Yellow	Smooth	Negative
381	HRT9.1	Spindle	White	Smooth	Negative
382	HRT9.2	Spindle	White	Smooth	Negative
383	HRT9.3	Spindle	White	Smooth	Negative
384	HRT9.4	Spindle	White	Smooth	Negative
385	HRT9.5	Spindle	White	Smooth	Negative
386	HRT9.6	Spindle	White	Smooth	Negative
387	HRT9.7	Spindle	White	Smooth	Negative
388	HRT9.8	Spindle	White	Smooth	Negative
389	HRT9.9	Spindle	White	Smooth	Negative
390	HRT9.10	Spindle	White	Smooth	Negative
391	HRT10.1	Irregular	Light yellow	Smooth	Negative
392	HRT10.2	Irregular	Light yellow	Smooth	Negative
393	HRT10.3	Irregular	Light yellow	Smooth	Negative
394	HRT10.4	Irregular	Light yellow	Smooth	Negative
395	HRT10.5	Irregular	Light yellow	Smooth	Negative
396	HRT10.6	Irregular	Light yellow	Smooth	Negative
397	HRT10.7	Irregular	Light yellow	Smooth	Negative
398	HRT10.8	Irregular	Light yellow	Smooth	Negative
399	HRT10.9	Irregular	Light yellow	Smooth	Negative
400	HRT10.10	Irregular	Light yellow	Smooth	Negative

Table S1 (a) The 400 morphologically distinct colonies isolated from the 80 green and red sweet pepper fruit samples grown under hydroponic conditions (fungicide-treated and untreated) at the ARC-Vegetables and Ornamental Center in South Africa, during the 2014-15 autumn and summer season, where negative means incapable of suppressing the pathogen and positive means capable of suppressing the pathogen.

Table S1 (b)

Isolate number	Isolate	Shape	Color	Texture	Inhibition against <i>R. solanacearum</i> strain BD 261
401	SGU1.1	Circular	Cream	Smooth	Negative
402	SGU1.2	Circular	Cream	Smooth	Negative
403	SGU1.3	Circular	Cream	Smooth	Negative
404	SGU1.4	Circular	Cream	Smooth	Negative
405	SGU1.5	Circular	Cream	Smooth	Negative
406	SGU1.6	Circular	Cream	Smooth	Negative
407	SGU1.7	Circular	Cream	Smooth	Negative
408	SGU1.8	Circular	Cream	Smooth	Negative
409	SGU1.9	Circular	Cream	Smooth	Negative
410	SGU1.10	Circular	Cream	Smooth	Negative
411	SGU2.1	Rod	White	Smooth	Negative
412	SGU2.2	Rod	White	Smooth	Negative
413	SGU2.3	Rod	White	Smooth	Negative
414	SGU2.4	Rod	White	Smooth	Negative
415	SGU2.5	Rod	White	Smooth	Negative
416	SGU2.6	Rod	White	Smooth	Negative
417	SGU2.7	Rod	White	Smooth	Negative
418	SGU2.8	Rod	White	Smooth	Negative
419	SGU2.9	Rod	White	Smooth	Negative
420	SGU2.10	Rod	White	Smooth	Negative
421	SGU3.1	Irregular	Yellow	Smooth	Negative
422	SGU3.2	Irregular	Yellow	Smooth	Negative
423	SGU3.3	Irregular	Yellow	Smooth	Negative
424	SGU3.4	Irregular	Yellow	Smooth	Negative
425	SGU3.5	Irregular	Yellow	Smooth	Negative
426	SGU3.6	Irregular	Yellow	Smooth	Negative
427	SGU3.7	Irregular	Yellow	Smooth	Negative
428	SGU3.8	Irregular	Yellow	Smooth	Negative
429	SGU3.9	Irregular	Yellow	Smooth	Negative
430	SGU3.10	Irregular	Yellow	Smooth	Negative
431	SGU4.1	Cocci	Cream white	Smooth	Negative
432	SGU4.2	Cocci	Cream white	Smooth	Negative
433	SGU4.3	Cocci	Cream white	Smooth	Negative
434	SGU4.4	Cocci	Cream white	Smooth	Negative
435	SGU4.5	Cocci	Cream white	Smooth	Negative
436	SGU4.6	Cocci	Cream white	Smooth	Negative
437	SGU4.7	Cocci	Cream white	Smooth	Negative
438	SGU4.8	Cocci	Cream white	Smooth	Negative
439	SGU4.9	Cocci	Cream white	Smooth	Negative
440	SGU4.10	Cocci	Cream white	Smooth	Negative
441	SGU5.1	Rod	White	Smooth	Negative
442	SGU5.2	Rod	White	Smooth	Negative
443	SGU5.3	Rod	White	Smooth	Negative
444	SGU5.4	Rod	White	Smooth	Negative
445	SGU5.5	Rod	White	Smooth	Negative
446	SGU5.6	Rod	White	Smooth	Negative
447	SGU5.7	Rod	White	Smooth	Negative
448	SGU5.8	Rod	White	Smooth	Negative
449	SGU5.9	Rod	White	Smooth	Negative
450	SGU5.10	Rod	White	Smooth	Negative

451	SGU6.1	Circular	Light yellow	Smooth	Negative
452	SGU6.2	Circular	Light yellow	Smooth	Negative
453	SGU6.3	Circular	Light yellow	Smooth	Negative
454	SGU6.4	Circular	Light yellow	Smooth	Negative
455	SGU6.5	Circular	Light yellow	Smooth	Negative
456	SGU6.6	Circular	Light yellow	Smooth	Negative
457	SGU6.7	Circular	Light yellow	Smooth	Negative
458	SGU6.8	Circular	Light yellow	Smooth	Negative
459	SGU6.9	Circular	Light yellow	Smooth	Negative
460	SGU6.10	Circular	Light yellow	Smooth	Negative
461	SGU7.1	Irregular	White	Smooth	Negative
462	SGU7.2	Irregular	White	Smooth	Negative
463	SGU7.3	Irregular	White	Smooth	Negative
464	SGU7.4	Irregular	White	Smooth	Negative
465	SGU7.5	Irregular	White	Smooth	Negative
466	SGU7.6	Irregular	White	Smooth	Negative
467	SGU7.7	Irregular	White	Smooth	Negative
468	SGU7.8	Irregular	White	Smooth	Negative
469	SGU7.9	Irregular	White	Smooth	Negative
470	SGU7.10	Irregular	White	Smooth	Negative
471	SGU8.1	Rod	Cream	Smooth	Negative
472	SGU8.2	Rod	Cream	Smooth	Negative
473	SGU8.3	Rod	Cream	Smooth	Negative
474	SGU8.4	Rod	Cream	Smooth	Negative
475	SGU8.5	Rod	Cream	Smooth	Negative
476	SGU8.6	Rod	Cream	Smooth	Negative
477	SGU8.7	Rod	Cream	Smooth	Negative
478	SGU8.8	Rod	Cream	Smooth	Negative
479	SGU8.9	Rod	Cream	Smooth	Negative
480	SGU8.10	Rod	Cream	Smooth	Negative
481	SGU9.1	Irregular	Yellow	Smooth	Negative
482	SGU9.2	Irregular	Yellow	Smooth	Negative
483	SGU9.3	Irregular	Yellow	Smooth	Negative
484	SGU9.4	Irregular	Yellow	Smooth	Negative
485	SGU9.5	Irregular	Yellow	Smooth	Negative
486	SGU9.6	Irregular	Yellow	Smooth	Negative
487	SGU9.7	Irregular	Yellow	Smooth	Negative
488	SGU9.8	Irregular	Yellow	Smooth	Negative
489	SGU9.9	Irregular	Yellow	Smooth	Negative
490	SGU9.10	Irregular	Yellow	Smooth	Negative
491	SGU10.1	Circular	Light yellow	Smooth	Negative
492	SGU10.2	Circular	Light yellow	Smooth	Negative
493	SGU10.3	Circular	Light yellow	Smooth	Negative
494	SGU10.4	Circular	Light yellow	Smooth	Negative
495	SGU10.5	Circular	Light yellow	Smooth	Negative
496	SGU10.6	Circular	Light yellow	Smooth	Negative
497	SGU10.7	Circular	Light yellow	Smooth	Negative
498	SGU10.8	Circular	Light yellow	Smooth	Negative
499	SGU10.9	Circular	Light yellow	Smooth	Negative
500	SGU10.10	Circular	Light yellow	Smooth	Negative
501	SGT1.1	Rod	Yellow	Smooth	Negative
502	SGT1.2	Rod	Yellow	Smooth	Negative
503	SGT1.3	Rod	Yellow	Smooth	Negative
504	SGT1.4	Rod	Yellow	Smooth	Negative

505	SGT1.5	Rod	Yellow	Smooth	Negative
506	SGT1.6	Rod	Yellow	Smooth	Negative
507	SGT1.7	Rod	Yellow	Smooth	Negative
508	SGT1.8	Rod	Yellow	Smooth	Negative
509	SGT1.9	Rod	Yellow	Smooth	Negative
510	SGT1.10	Rod	Yellow	Smooth	Negative
511	SGT2.1	Irregular	White	Smooth	Negative
512	SGT2.2	Irregular	White	Smooth	Negative
513	SGT2.3	Irregular	White	Smooth	Negative
514	SGT2.4	Irregular	White	Smooth	Negative
515	SGT2.5	Irregular	White	Smooth	Negative
516	SGT2.6	Irregular	White	Smooth	Negative
517	SGT2.7	Irregular	White	Smooth	Negative
518	SGT2.8	Irregular	White	Smooth	Negative
519	SGT2.9	Irregular	White	Smooth	Negative
520	SGT2.10	Irregular	White	Smooth	Negative
521	SGT3.1	Circular	Cream	Smooth	Negative
522	SGT3.2	Circular	Cream	Smooth	Negative
523	SGT3.3	Circular	Cream	Smooth	Negative
524	SGT3.4	Circular	Cream	Smooth	Negative
525	SGT3.5	Circular	Cream	Smooth	Negative
526	SGT3.6	Circular	Cream	Smooth	Negative
527	SGT3.7	Circular	Cream	Smooth	Negative
528	SGT3.8	Circular	Cream	Smooth	Negative
529	SGT3.9	Circular	Cream	Smooth	Negative
530	SGT3.10	Circular	Cream	Smooth	Negative
531	SGT4.1	Rod	Cream white	Smooth	Negative
532	SGT4.2	Rod	Cream white	Smooth	Negative
533	SGT4.3	Rod	Cream white	Smooth	Negative
534	SGT4.4	Rod	Cream white	Smooth	Negative
535	SGT4.5	Rod	Cream white	Smooth	Negative
536	SGT4.6	Rod	Cream white	Smooth	Negative
537	SGT4.7	Rod	Cream white	Smooth	Negative
538	SGT4.8	Rod	Cream white	Smooth	Negative
539	SGT4.9	Rod	Cream white	Smooth	Negative
540	SGT4.10	Rod	Cream white	Smooth	Negative
541	SGT5.1	Circular	Light yellow	Smooth	Negative
542	SGT5.2	Circular	Light yellow	Smooth	Negative
543	SGT5.3	Circular	Light yellow	Smooth	Positive
544	SGT5.4	Circular	Light yellow	Smooth	Negative
545	SGT5.5	Circular	Light yellow	Smooth	Negative
546	SGT5.6	Circular	Light yellow	Smooth	Negative
547	SGT5.7	Circular	Light yellow	Smooth	Negative
548	SGT5.8	Circular	Light yellow	Smooth	Negative
549	SGT5.9	Circular	Light yellow	Smooth	Negative
550	SGT5.10	Circular	Light yellow	Smooth	Negative
551	SGT6.1	Irregular	Yellow	Smooth	Negative
552	SGT6.2	Irregular	Yellow	Smooth	Negative
553	SGT6.3	Irregular	Yellow	Smooth	Negative
554	SGT6.4	Irregular	Yellow	Smooth	Negative
555	SGT6.5	Irregular	Yellow	Smooth	Negative
556	SGT6.6	Irregular	Yellow	Smooth	Negative
557	SGT6.7	Irregular	Yellow	Smooth	Negative
558	SGT6.8	Irregular	Yellow	Smooth	Negative

559	SGT6.9	Irregular	Yellow	Smooth	Negative
560	SGT6.10	Irregular	Yellow	Smooth	Negative
561	SGT7.1	Rod	White	Smooth	Negative
562	SGT7.2	Rod	White	Smooth	Negative
563	SGT7.3	Rod	White	Smooth	Negative
564	SGT7.4	Rod	White	Smooth	Negative
565	SGT7.5	Rod	White	Smooth	Negative
566	SGT7.6	Rod	White	Smooth	Negative
567	SGT7.7	Rod	White	Smooth	Negative
568	SGT7.8	Rod	White	Smooth	Negative
569	SGT7.9	Rod	White	Smooth	Negative
570	SGT7.10	Rod	White	Smooth	Negative
571	SGT8.1	Circular	Cream white	Smooth	Negative
572	SGT8.2	Circular	Cream white	Smooth	Negative
573	SGT8.3	Circular	Cream white	Smooth	Negative
574	SGT8.4	Circular	Cream white	Smooth	Negative
575	SGT8.5	Circular	Cream white	Smooth	Negative
576	SGT8.6	Circular	Cream white	Smooth	Negative
577	SGT8.7	Circular	Cream white	Smooth	Negative
578	SGT8.8	Circular	Cream white	Smooth	Negative
579	SGT8.9	Circular	Cream white	Smooth	Negative
580	SGT8.10	Circular	Cream white	Smooth	Negative
581	SGT9.1	Cocci	Light yellow	Smooth	Negative
582	SGT9.2	Cocci	Light yellow	Smooth	Negative
583	SGT9.3	Cocci	Light yellow	Smooth	Negative
584	SGT9.4	Cocci	Light yellow	Smooth	Negative
585	SGT9.5	Cocci	Light yellow	Smooth	Negative
586	SGT9.6	Cocci	Light yellow	Smooth	Negative
587	SGT9.7	Cocci	Light yellow	Smooth	Negative
588	SGT9.8	Cocci	Light yellow	Smooth	Negative
589	SGT9.9	Cocci	Light yellow	Smooth	Negative
590	SGT9.10	Cocci	Yellow	Smooth	Negative
591	SGT10.1	Rod	Yellow	Smooth	Negative
592	SGT10.2	Rod	Yellow	Smooth	Negative
593	SGT10.3	Rod	Yellow	Smooth	Negative
594	SGT10.4	Rod	Yellow	Smooth	Negative
595	SGT10.5	Rod	Yellow	Smooth	Negative
596	SGT10.6	Rod	Yellow	Smooth	Negative
597	SGT10.7	Rod	Yellow	Smooth	Negative
598	SGT10.8	Rod	Yellow	Smooth	Negative
599	SGT10.9	Rod	Yellow	Smooth	Negative
600	SGT10.10	Rod	Yellow	Smooth	Negative
601	SRU1.1	Cocci	White	Smooth	Negative
602	SRU1.2	Cocci	White	Smooth	Negative
603	SRU1.3	Cocci	White	Smooth	Negative
604	SRU1.4	Cocci	White	Smooth	Negative
605	SRU1.5	Cocci	White	Smooth	Negative
606	SRU1.6	Cocci	White	Smooth	Negative
607	SRU1.7	Cocci	White	Smooth	Negative
608	SRU1.8	Cocci	White	Smooth	Negative
609	SRU1.9	Cocci	White	Smooth	Negative
610	SRU1.10	Cocci	White	Smooth	Negative
611	SRU2.1	Rod	Light yellow	Smooth	Negative
612	SRU2.2	Rod	Light yellow	Smooth	Negative

613	SRU2.3	Rod	Light yellow	Smooth	Negative
614	SRU2.4	Rod	Light yellow	Smooth	Negative
615	SRU2.5	Rod	Light yellow	Smooth	Negative
616	SRU2.6	Rod	Light yellow	Smooth	Negative
617	SRU2.7	Rod	Light yellow	Smooth	Negative
618	SRU2.8	Rod	Light yellow	Smooth	Negative
619	SRU2.9	Rod	Light yellow	Smooth	Negative
620	SRU2.10	Rod	Light yellow	Smooth	Negative
621	SRU3	Circular	Yellow	Smooth	Negative
622	SRU4	Circular	Yellow	Smooth	Negative
623	SRU5	Circular	Yellow	Smooth	Negative
624	SRU6	Circular	Yellow	Smooth	Negative
625	SRU7	Circular	Yellow	Smooth	Negative
626	SRU8	Circular	Yellow	Smooth	Negative
627	SRU9	Circular	Yellow	Smooth	Negative
628	SRU10	Circular	Yellow	Smooth	Negative
629	SRU11	Circular	Yellow	Smooth	Negative
630	SRU12	Circular	Yellow	Smooth	Negative
631	SRU4.1	Irregular	Cream	Smooth	Negative
632	SRU4.2	Irregular	Cream	Smooth	Negative
633	SRU4.3	Irregular	Cream	Smooth	Negative
634	SRU4.4	Irregular	Cream	Smooth	Positive
635	SRU4.5	Irregular	Cream	Smooth	Negative
636	SRU4.6	Irregular	Cream	Smooth	Negative
637	SRU4.7	Irregular	Cream	Smooth	Negative
638	SRU4.8	Irregular	Cream	Smooth	Negative
639	SRU4.9	Irregular	Cream	Smooth	Negative
640	SRU4.10	Irregular	Cream	Smooth	Negative
641	SRU5.1	Circular	Orange	Smooth	Negative
642	SRU5.2	Circular	Orange	Smooth	Negative
643	SRU5.3	Circular	Orange	Smooth	Negative
644	SRU5.4	Circular	Orange	Smooth	Negative
645	SRU5.5	Circular	Orange	Smooth	Negative
646	SRU5.6	Circular	Orange	Smooth	Negative
647	SRU5.7	Circular	Orange	Smooth	Negative
648	SRU5.8	Circular	Orange	Smooth	Negative
649	SRU5.9	Circular	Orange	Smooth	Negative
650	SRU5.10	Circular	Orange	Smooth	Negative
651	SRU6.1	Irregular	White	Smooth	Negative
652	SRU6.2	Irregular	White	Smooth	Negative
653	SRU6.3	Irregular	White	Smooth	Negative
654	SRU6.4	Irregular	White	Smooth	Negative
655	SRU6.5	Irregular	White	Smooth	Negative
656	SRU6.6	Irregular	White	Smooth	Negative
657	SRU6.7	Irregular	White	Smooth	Negative
658	SRU6.8	Irregular	White	Smooth	Negative
659	SRU6.9	Irregular	White	Smooth	Negative
660	SRU6.10	Irregular	White	Smooth	Negative
661	SRU7.1	Irregular	Cream white	Smooth	Negative
662	SRU7.2	Rod	Cream white	Smooth	Negative
663	SRU7.3	Rod	Cream white	Smooth	Negative
664	SRU7.4	Rod	Cream white	Smooth	Negative
665	SRU7.5	Rod	Cream white	Smooth	Negative
666	SRU7.6	Rod	Cream white	Smooth	Negative

667	SRU7.7	Rod	Cream white	Smooth	Negative
668	SRU7.8	Rod	Cream white	Smooth	Negative
669	SRU7.9	Rod	Cream white	Smooth	Negative
670	SRU7.10	Cocci	Cream white	Smooth	Negative
671	SRU8.1	Cocci	Yellow	Smooth	Negative
672	SRU8.2	Cocci	Yellow	Smooth	Negative
673	SRU8.3	Cocci	Yellow	Smooth	Negative
674	SRU8.4	Cocci	Yellow	Smooth	Negative
675	SRU8.5	Cocci	Yellow	Smooth	Negative
676	SRU8.6	Cocci	Yellow	Smooth	Negative
677	SRU8.7	Cocci	Yellow	Smooth	Negative
678	SRU8.8	Cocci	Yellow	Smooth	Negative
679	SRU8.9	Cocci	Yellow	Smooth	Negative
680	SRU8.10	Cocci	Yellow	Smooth	Negative
681	SRU9.1	Spindle	White	Smooth	Negative
682	SRU9.2	Spindle	White	Smooth	Negative
683	SRU9.3	Spindle	White	Smooth	Negative
684	SRU9.4	Spindle	White	Smooth	Negative
685	SRU9.5	Spindle	White	Smooth	Negative
686	SRU9.6	Spindle	White	Smooth	Negative
687	SRU9.7	Spindle	White	Smooth	Negative
688	SRU9.8	Spindle	White	Smooth	Negative
689	SRU9.9	Spindle	White	Smooth	Negative
690	SRU9.10	Spindle	White	Smooth	Negative
691	SRU10.1	Irregular	Light yellow	Smooth	Negative
692	SRU10.2	Irregular	Light yellow	Smooth	Negative
693	SRU10.3	Irregular	Light yellow	Smooth	Negative
694	SRU10.4	Irregular	Light yellow	Smooth	Negative
695	SRU10.5	Irregular	Light yellow	Smooth	Negative
696	SRU10.6	Irregular	Light yellow	Smooth	Negative
697	SRU10.7	Irregular	Light yellow	Smooth	Negative
698	SRU10.8	Irregular	Light yellow	Smooth	Negative
699	SRU10.9	Irregular	Light yellow	Smooth	Negative
700	SRU10.10	Irregular	Light yellow	Smooth	Negative
701	SRT1.1	Circular	Yellow	Smooth	Negative
702	SRT1.2	Circular	Yellow	Smooth	Negative
703	SRT1.3	Circular	Yellow	Smooth	Negative
704	SRT1.4	Circular	Yellow	Smooth	Negative
705	SRT1.5	Circular	Yellow	Smooth	Negative
706	SRT1.6	Circular	Yellow	Smooth	Negative
707	SRT1.7	Circular	Yellow	Smooth	Negative
708	SRT1.8	Circular	Yellow	Smooth	Negative
709	SRT1.9	Circular	Yellow	Smooth	Negative
710	SRT1.10	Circular	Yellow	Smooth	Negative
711	SRT2.1	Irregular	Cream	Smooth	Negative
712	SRT2.2	Irregular	Cream	Smooth	Negative
713	SRT2.3	Irregular	Cream	Smooth	Negative
714	SRT2.4	Irregular	Cream	Smooth	Negative
715	SRT2.5	Irregular	Cream	Smooth	Negative
716	SRT2.6	Irregular	Cream	Smooth	Negative
717	SRT2.7	Irregular	Cream	Smooth	Negative
718	SRT2.8	Irregular	Cream	Smooth	Negative
719	SRT2.9	Irregular	Cream	Smooth	Negative
720	SRT2.10	Irregular	Cream	Smooth	Negative

721	SRT3.1	Rod	White	Smooth	Negative
722	SRT3.2	Rod	White	Smooth	Negative
723	SRT3.3	Rod	White	Smooth	Negative
724	SRT3.4	Rod	White	Smooth	Negative
725	SRT3.5	Rod	White	Smooth	Negative
726	SRT3.6	Rod	White	Smooth	Negative
727	SRT3.7	Rod	White	Smooth	Negative
728	SRT3.8	Rod	White	Smooth	Negative
729	SRT3.9	Rod	White	Smooth	Negative
730	SRT3.10	Rod	White	Smooth	Negative
731	SRT4.1	Cocci	Cream white	Smooth	Negative
732	SRT4.2	Cocci	Cream white	Smooth	Negative
733	SRT4.3	Cocci	Cream white	Smooth	Negative
734	SRT4.4	Cocci	Cream white	Smooth	Negative
735	SRT4.5	Cocci	Cream white	Smooth	Negative
736	SRT4.6	Cocci	Cream white	Smooth	Negative
737	SRT4.7	Cocci	Cream white	Smooth	Negative
738	SRT4.8	Cocci	Cream white	Smooth	Negative
739	SRT4.9	Cocci	Cream white	Smooth	Negative
740	SRT4.10	Cocci	Cream white	Smooth	Negative
741	SRT5.1	Circular	Yellow	Smooth	Negative
742	SRT5.2	Circular	Yellow	Smooth	Negative
743	SRT5.3	Circular	Yellow	Smooth	Negative
744	SRT5.4	Circular	Yellow	Smooth	Negative
745	SRT5.5	Circular	Yellow	Smooth	Negative
746	SRT5.6	Circular	Yellow	Smooth	Negative
747	SRT5.7	Circular	Yellow	Smooth	Negative
748	SRT5.8	Circular	Yellow	Smooth	Negative
749	SRT5.9	Circular	Yellow	Smooth	Negative
750	SRT5.10	Circular	Yellow	Smooth	Negative
751	SRT6.1	Cocci	White	Smooth	Negative
752	SRT6.2	Cocci	White	Smooth	Negative
753	SRT6.3	Cocci	White	Smooth	Negative
754	SRT6.4	Cocci	White	Smooth	Negative
755	SRT6.5	Cocci	White	Smooth	Negative
756	SRT6.6	Cocci	White	Smooth	Negative
757	SRT6.7	Cocci	White	Smooth	Negative
758	SRT6.8	Cocci	White	Smooth	Negative
759	SRT6.9	Cocci	White	Smooth	Negative
760	SRT6.10	Cocci	White	Smooth	Negative
761	SRT7.1	Rod	Light yellow	Smooth	Negative
762	SRT7.2	Rod	Light yellow	Smooth	Negative
763	SRT7.3	Rod	Light yellow	Smooth	Negative
764	SRT7.4	Rod	Light yellow	Smooth	Negative
765	SRT7.5	Rod	Light yellow	Smooth	Negative
766	SRT7.6	Rod	Light yellow	Smooth	Negative
767	SRT7.7	Rod	Light yellow	Smooth	Positive
768	SRT7.8	Rod	Light yellow	Smooth	Negative
769	SRT7.9	Rod	Light yellow	Smooth	Negative
770	SRT7.10	Rod	Light yellow	Smooth	Negative
771	SRT8.1	Circular	White	Smooth	Negative
772	SRT8.2	Circular	White	Smooth	Negative
773	SRT8.3	Circular	White	Smooth	Negative
774	SRT8.4	Circular	White	Smooth	Negative

775	SRT8.5	Circular	White	Smooth	Negative
776	SRT8.6	Circular	White	Smooth	Negative
777	SRT8.7	Circular	White	Smooth	Negative
778	SRT8.8	Circular	White	Smooth	Negative
779	SRT8.9	Circular	White	Smooth	Negative
780	SRT8.10	Circular	White	Smooth	Negative
781	SRT9.1	Cocci	Yellow	Smooth	Positive
782	SRT9.2	Cocci	Yellow	Smooth	Negative
783	SRT9.3	Cocci	Yellow	Smooth	Negative
784	SRT9.4	Cocci	Yellow	Smooth	Negative
785	SRT9.5	Cocci	Yellow	Smooth	Negative
786	SRT9.6	Cocci	Yellow	Smooth	Negative
787	SRT9.7	Cocci	Yellow	Smooth	Negative
788	SRT9.8	Cocci	Yellow	Smooth	Negative
789	SRT9.9	Cocci	Yellow	Smooth	Positive
790	SRT9.10	Cocci	Yellow	Smooth	Negative
791	SRT10.1	Irregular	Cream white	Smooth	Negative
792	SRT10.2	Irregular	Cream white	Smooth	Negative
793	SRT10.3	Irregular	Cream white	Smooth	Negative
794	SRT10.4	Irregular	Cream white	Smooth	Negative
795	SRT10.5	Irregular	Cream white	Smooth	Negative
796	SRT10.6	Irregular	Cream white	Smooth	Negative
797	SRT10.7	Irregular	Cream white	Smooth	Negative
798	SRT10.8	Irregular	Cream white	Smooth	Negative
799	SRT10.9	Irregular	Cream white	Smooth	Negative
800	SRT10.10	Irregular	Cream white	Smooth	Negative

Table S1 (b) The 400 morphologically distinct colonies isolated from the 80 green and red sweet pepper fruit samples grown under open soil conditions (fungicide-treated and untreated) at the ARC-Vegetables and Ornamental Center in South Africa, during the 2014-15 autumn and summer season, where negative means incapable of suppressing the pathogen and positive means capable of suppressing the pathogen.

Table S2

Source of variation	Degrees of freedom	Before enrichment				After enrichment			
		Sum of Squares (SS)	Mean of Squares (MS)	F-value	P-value	SS	MS	F-value	P-value
Replication	2	0.105	0.053	0.471	0.641	0.324	0.162	1.1276	0.370322
Treatment	4	14.949	3.737	33.419	4.85E-05	6.2467	1.56167	10.8701	0.002553
Residuals	8	0.895	0.112			1.1493	0.14367		

Table S2 Analysis of variance (ANOVA) for bacterial colonies with potential antagonistic effects, isolated from sweet pepper fruit surfaces, against the *R. solanacearum* BD 261 pathogenic strain, before and after enrichment.

Table S3

Isolate	Treatment	Inhibition zone
SGT5.3	Before enrichment	9.066667
SRU4.4	Before enrichment	8.133333
SRT9.1	Before enrichment	7.166667
HRT7.7	Before enrichment	6.6
CONTROL	Before enrichment	6.4
HRT7.7	After enrichment	14.03333
SRT9.1	After enrichment	13.83333
SGT5.3	After enrichment	13.73333
SRU4.4	After enrichment	13.66667
CONTROL	After enrichment	12.23333

Table S3 Antagonistic potential of bacterial isolates from green and red sweet pepper fruit samples, grown under hydroponic and open soil conditions (but, either fungicide-treated or untreated) at the ARC-VOC, during the 2014-15 autumn and summer season in South Africa, against the *R. solanacearum* BD 261 strain, before and after enrichment.

Table S4

Treatment	Comparisons	Difference	Lower	Upper	P-value
Before enrichment	HRT7.7-CONTROL	0.2	-0.64975	1.049754	0.9323273
	SGT5.3-CONTROL	2.666667	1.816913	3.51642	0.0000091
	SRT9.1-CONTROL	0.766667	-0.08309	1.61642	0.0822164
	SRU4.4-CONTROL	1.733333	0.88358	2.583087	0.0003929
	SGT5.3-HRT7.7	2.466667	1.616913	3.31642	0.0000186
	SRT9.1-HRT7.7	0.566667	-0.28309	1.41642	0.2563173
	SRU4.4-HRT7.7	1.533333	0.68358	2.383087	0.0010483
	SRT9.1-SGT5.3	-1.9	-2.74975	-1.05025	0.0001829
	SRU4.4-SGT5.3	-0.93333	-1.78309	-0.08358	0.0302507
	SRU4.4-SRT9.1	0.966667	0.116913	1.81642	0.0247731
After enrichment	HRT7.7-CONTROL	1.8	0.768561	2.831439	0.0013578
	SGT5.3-CONTROL	1.5	0.468561	2.531439	0.0051652
	SRT9.1-CONTROL	1.6	0.568561	2.631439	0.0032684
	SRU4.4-CONTROL	1.433333	0.401894	2.464773	0.0070532
	SGT5.3-HRT7.7	-0.3	-1.33144	0.731439	0.8678983
	SRT9.1-HRT7.7	-0.2	-1.23144	0.831439	0.9650678
	SRU4.4-HRT7.7	-0.36667	-1.39811	0.664773	0.7675204
	SRT9.1-SGT5.3	0.1	-0.93144	1.131439	0.9973615
	SRU4.4-SGT5.3	-0.06667	-1.09811	0.964773	0.9994595
	SRU4.4-SRT9.1	-0.16667	-1.19811	0.864773	0.9818279

Table S4 Turkey's HSD mean comparisons of the bacterial isolates from green and red sweet pepper fruit samples, grown under hydroponic and open soil conditions (but, either fungicide-treated or untreated) at the ARC-VOC, during the 2014-15 autumn and summer season in South Africa, against the *R. solanacearum* BD 261 strain, before and after enrichment.

Table S5

Treatment	Treatment level	Isolate	Inhibition zone
pH	5	SRU4.4	7.166667
pH	5	HRT7.7	7.1
pH	5	CONTROL	7
pH	5	SGT5.3	5.866667
pH	5	SRT9.1	4.466667
pH	6	HRT7.7	12.66667
pH	6	SRU4.4	12.33333
pH	6	CONTROL	12.16667
pH	6	SGT5.3	11.33333
pH	6	SRT9.1	10.33333
pH	7	HRT7.7	15.33333
pH	7	SRU4.4	15.06667
pH	7	CONTROL	14.03333
pH	7	SGT5.3	14
pH	7	SRT9.1	13.33333
pH	8	HRT7.7	14.33333
pH	8	SRU4.4	13.86667
pH	8	CONTROL	12.3
pH	8	SGT5.3	11.66667
pH	8	SRT9.1	10.66667
pH	9	HRT7.7	12.33333
pH	9	SRU4.4	12.06667
pH	9	SGT5.3	11.33333
pH	9	SRT9.1	9.666667
pH	9	CONTROL	8.833333
Carbon source	Glucose	SRT9.1	10.33333
Carbon source	Glucose	CONTROL	9.9
Carbon source	Glucose	SGT5.3	9.5
Carbon source	Glucose	SRU4.4	8.9
Carbon source	Glucose	HRT7.7	8.8
Carbon source	Starch	HRT7.7	14.03333
Carbon source	Starch	SRT9.1	13.83333
Carbon source	Starch	SGT5.3	13.73333
Carbon source	Starch	SRU4.4	13.66667
Carbon source	Starch	CONTROL	12.23333
Carbon source	Lactose	SGT5.3	13.06667
Carbon source	Lactose	SRT9.1	12.96667
Carbon source	Lactose	SRU4.4	12.93333
Carbon source	Lactose	HRT7.7	11.43333
Carbon source	Lactose	CONTROL	11.23333
Carbon source	Maltose	SRU4.4	12.23333
Carbon source	Maltose	SRT9.1	11.96667
Carbon source	Maltose	SGT5.3	11.56667
Carbon source	Maltose	HRT7.7	9.766667
Carbon source	Maltose	CONTROL	9.333333
Carbon source	Fructose	SRU4.4	12.66667
Carbon source	Fructose	SGT5.3	11.96667
Carbon source	Fructose	CONTROL	11.23333
Carbon source	Fructose	SRT9.1	11.23333

Carbon source	Fructose	HRT7.7	10.76667
Nitrogen source	Glycine	SRU4.4	11.86667
Nitrogen source	Glycine	SGT5.3	10.8
Nitrogen source	Glycine	SRT9.1	9.366667
Nitrogen source	Glycine	CONTROL	8.533333
Nitrogen source	Glycine	HRT7.7	8.466667
Nitrogen source	Yeast extract	SGT5.3	12.03333
Nitrogen source	Yeast extract	HRT7.7	11.7
Nitrogen source	Yeast extract	SRT9.1	11.7
Nitrogen source	Yeast extract	SRU4.4	11.33333
Nitrogen source	Yeast extract	CONTROL	11.16667
Nitrogen source	Tryptone	SGT5.3	13.6
Nitrogen source	Tryptone	HRT7.7	13.1
Nitrogen source	Tryptone	SRT9.1	12.93333
Nitrogen source	Tryptone	CONTROL	12.86667
Nitrogen source	Tryptone	SRU4.4	12.6
Nitrogen source	(NH ₄) ₂ SO ₄	HRT7.7	11.8
Nitrogen source	(NH ₄) ₂ SO ₄	SRU4.4	11.66667
Nitrogen source	(NH ₄) ₂ SO ₄	CONTROL	11.46667
Nitrogen source	(NH ₄) ₂ SO ₄	SRT9.1	11.2
Nitrogen source	(NH ₄) ₂ SO ₄	SGT5.3	10.6
Nitrogen source	NH ₄ Cl	CONTROL	10.76667
Nitrogen source	NH ₄ Cl	SRU4.4	9.933333
Nitrogen source	NH ₄ Cl	HRT7.7	9.366667
Nitrogen source	NH ₄ Cl	SRT9.1	9.166667
Nitrogen source	NH ₄ Cl	SGT5.3	8.7
Temperature	25	HRT7.7	11.83333
Temperature	25	SRU4.4	10.46667
Temperature	25	CONTROL	9.2
Temperature	25	SGT5.3	6.733333
Temperature	25	SRT9.1	5.5
Temperature	28	HRT7.7	15.63333
Temperature	28	SRU4.4	15.06667
Temperature	28	CONTROL	14.23333
Temperature	28	SGT5.3	13.6
Temperature	28	SRT9.1	10.1
Temperature	30	HRT7.7	19.8
Temperature	30	SRU4.4	18.96667
Temperature	30	CONTROL	18.9
Temperature	30	SGT5.3	17.2
Temperature	30	SRT9.1	15.66667
Temperature	35	HRT7.7	18.4
Temperature	35	SRU4.4	17.86667
Temperature	35	CONTROL	17.2
Temperature	35	SGT5.3	15.13333
Temperature	35	SRT9.1	13.1
Temperature	37	HRT7.7	12.4
Temperature	37	SRU4.4	12.06667
Temperature	37	SGT5.3	11.7
Temperature	37	CONTROL	11.23333
Temperature	37	SRT9.1	9.666667
Starch	0,5	SGT5.3	11.13333
Starch	0,5	CONTROL	10.33333
Starch	0,5	HRT7.7	9.166667

Starch	0,5	SRU4.4	9
Starch	0,5	SRT9.1	7
Starch	1	SGT5.3	12.9
Starch	1	CONTROL	11.53333
Starch	1	SRU4.4	11.23333
Starch	1	HRT7.7	11.16667
Starch	1	SRT9.1	9.166667
Starch	1,5	SGT5.3	12.7
Starch	1,5	CONTROL	12.23333
Starch	1,5	HRT7.7	11.76667
Starch	1,5	SRU4.4	11.63333
Starch	1,5	SRT9.1	10.36667
Starch	2	SGT5.3	13.23333
Starch	2	HRT7.7	12.36667
Starch	2	CONTROL	12.3
Starch	2	SRU4.4	12.06667
Starch	2	SRT9.1	11
Starch	2,5	SGT5.3	15.06667
Starch	2,5	CONTROL	13
Starch	2,5	SRU4.4	12.96667
Starch	2,5	HRT7.7	12.93333
Starch	2,5	SRT9.1	12.26667
Starch	3	CONTROL	16.96667
Starch	3	SRU4.4	16.43333
Starch	3	HRT7.7	16.36667
Starch	3	SGT5.3	16.23333
Starch	3	SRT9.1	13.13333
Tryptone	0.5	SRT9.1	10.76667
Tryptone	0.5	HRT7.7	10.03333
Tryptone	0.5	CONTROL	9.1
Tryptone	0.5	SRU4.4	8.366667
Tryptone	0.5	SGT5.3	6.066667
Tryptone	1	SRT9.1	12.83333
Tryptone	1	CONTROL	12.2
Tryptone	1	HRT7.7	10.93333
Tryptone	1	SRU4.4	8.966667
Tryptone	1	SGT5.3	7.966667
Tryptone	1.5	SRT9.1	13.8
Tryptone	1.5	HRT7.7	13.36667
Tryptone	1.5	CONTROL	12.93333
Tryptone	1.5	SGT5.3	9.966667
Tryptone	1.5	SRU4.4	9.4
Tryptone	2	HRT7.7	14.4
Tryptone	2	CONTROL	13.9
Tryptone	2	SRU4.4	11.7
Tryptone	2	SRT9.1	9.766667
Tryptone	2	SGT5.3	6.966667
Tryptone	2.5	SRT9.1	16.26667
Tryptone	2.5	SRU4.4	16.1
Tryptone	2.5	CONTROL	15.83333
Tryptone	2.5	HRT7.7	15.83333
Tryptone	2.5	SGT5.3	11.83333
Tryptone	3	CONTROL	14.46667

Tryptone	3	HRT7.7	14.36667
Tryptone	3	SRT9.1	13.9
Tryptone	3	SRU4.4	13.03333
Tryptone	3	SGT5.3	10.8

Table S5 Antagonistic activity of sweet pepper fruit isolates, against the *R. solanacearum* BD 261 strain, at different treatment levels of pH, carbon sources and nitrogen sources, temperature, concentration of starch and tryptone

Table S6

Treatment	Treatment level	Comparisons	Difference	Lower	Upper	P-value
pH	5	HRT7.7-CONTROL	0,1	-0,33329	0,533291	0,9365887
pH	5	SGT5.3-CONTROL	-1,13333	-1,56662	-0,70004	0,0000471
pH	5	SRT9.1-CONTROL	-2,53333	-2,96662	-2,10004	0
pH	5	SRU4.4-CONTROL	0,166667	-0,26662	0,599958	0,7161104
pH	5	SGT5.3-HRT7.7	-1,23333	-1,66662	-0,80004	0,0000222
pH	5	SRT9.1-HRT7.7	-2,63333	-3,06662	-2,20004	0
pH	5	SRU4.4-HRT7.7	0,066667	-0,36662	0,499958	0,984816
pH	5	SRT9.1-SGT5.3	-1,4	-1,83329	-0,96671	0,000007
pH	5	SRU4.4-SGT5.3	1,3	0,866709	1,733291	0,0000138
pH	5	SRU4.4-SRT9.1	2,7	2,266709	3,133291	0
pH	6	HRT7.7-CONTROL	0,5	-1,70172	2,701721	0,9399161
pH	6	SGT5.3-CONTROL	-0,83333	-3,03505	1,368387	0,7271857
pH	6	SRT9.1-CONTROL	-1,83333	-4,03505	0,368387	0,1164768
pH	6	SRU4.4-CONTROL	0,166667	-2,03505	2,368387	0,9989941
pH	6	SGT5.3-HRT7.7	-1,33333	-3,53505	0,868387	0,3345921
pH	6	SRT9.1-HRT7.7	-2,33333	-4,53505	-0,13161	0,0368369
pH	6	SRU4.4-HRT7.7	-0,33333	-2,53505	1,868387	0,9856936
pH	6	SRT9.1-SGT5.3	-1	-3,20172	1,201721	0,5875215
pH	6	SRU4.4-SGT5.3	1	-1,20172	3,201721	0,5875215
pH	6	SRU4.4-SRT9.1	2	-0,20172	4,201721	0,0796966
pH	7	HRT7.7-CONTROL	1,3	-0,28216	2,882155	0,1230041
pH	7	SGT5.3-CONTROL	-0,03333	-1,61549	1,548822	0,9999937
pH	7	SRT9.1-CONTROL	-0,7	-2,28216	0,882155	0,6093745
pH	7	SRU4.4-CONTROL	1,033333	-0,54882	2,615489	0,2725134
pH	7	SGT5.3-HRT7.7	-1,33333	-2,91549	0,248822	0,1108054
pH	7	SRT9.1-HRT7.7	-2	-3,58216	-0,41784	0,013092
pH	7	SRU4.4-HRT7.7	-0,26667	-1,84882	1,315489	0,9788156
pH	7	SRT9.1-SGT5.3	-0,66667	-2,24882	0,915489	0,6486194
pH	7	SRU4.4-SGT5.3	1,066667	-0,51549	2,648822	0,2480004
pH	7	SRU4.4-SRT9.1	1,733333	0,151178	3,315489	0,0306867
pH	8	HRT7.7-CONTROL	2,033333	0,704163	3,362503	0,003613
pH	8	SGT5.3-CONTROL	-0,63333	-1,9625	0,695837	0,546453
pH	8	SRT9.1-CONTROL	-1,63333	-2,9625	-0,30416	0,0156194
pH	8	SRU4.4-CONTROL	1,566667	0,237497	2,895837	0,0201124
pH	8	SGT5.3-HRT7.7	-2,66667	-3,99584	-1,3375	0,0004499
pH	8	SRT9.1-HRT7.7	-3,66667	-4,99584	-2,3375	0,0000294
pH	8	SRU4.4-HRT7.7	-0,46667	-1,79584	0,862503	0,7750091
pH	8	SRT9.1-SGT5.3	-1	-2,32917	0,32917	0,1723317
pH	8	SRU4.4-SGT5.3	2,2	0,87083	3,52917	0,0020284
pH	8	SRU4.4-SRT9.1	3,2	1,87083	4,52917	0,0000972
pH	9	HRT7.7-CONTROL	3,5	1,976751	5,023249	0,0001451
pH	9	SGT5.3-CONTROL	2,5	0,976751	4,023249	0,0021606
pH	9	SRT9.1-CONTROL	0,833333	-0,68992	2,356583	0,4236322
pH	9	SRU4.4-CONTROL	3,233333	1,710084	4,756583	0,0002828
pH	9	SGT5.3-HRT7.7	-1	-2,52325	0,523249	0,2684648
pH	9	SRT9.1-HRT7.7	-2,66667	-4,18992	-1,14342	0,0013253
pH	9	SRU4.4-HRT7.7	-0,26667	-1,78992	1,256583	0,9757069
pH	9	SRT9.1-SGT5.3	-1,66667	-3,18992	-0,14342	0,0309066

pH	9	SRU4.4-SGT5.3	0,733333	-0,78992	2,256583	0,537457
pH	9	SRU4.4-SRT9.1	2,4	0,876751	3,923249	0,0029191
Carbon source	Glucose	HRT7.7-CONTROL	-1,1	-2,2166	0,016599	0,0539435
Carbon source	Glucose	SGT5.3-CONTROL	-0,4	-1,5166	0,716599	0,7628118
Carbon source	Glucose	SRT9.1-CONTROL	0,433333	-0,68327	1,549933	0,7099035
Carbon source	Glucose	SRU4.4-CONTROL	-1	-2,1166	0,116599	0,0850214
Carbon source	Glucose	SGT5.3-HRT7.7	0,7	-0,4166	1,816599	0,3055849
Carbon source	Glucose	SRT9.1-HRT7.7	1,533333	0,416734	2,649933	0,0076404
Carbon source	Glucose	SRU4.4-HRT7.7	0,1	-1,0166	1,216599	0,9980604
Carbon source	Glucose	SRT9.1-SGT5.3	0,833333	-0,28327	1,949933	0,1773672
Carbon source	Glucose	SRU4.4-SGT5.3	-0,6	-1,7166	0,516599	0,4396842
Carbon source	Glucose	SRU4.4-SRT9.1	-1,43333	-2,54993	-0,31673	0,0118766
Carbon source	Starch	HRT7.7-CONTROL	1,8	0,768561	2,831439	0,0013578
Carbon source	Starch	SGT5.3-CONTROL	1,5	0,468561	2,531439	0,0051652
Carbon source	Starch	SRT9.1-CONTROL	1,6	0,568561	2,631439	0,0032684
Carbon source	Starch	SRU4.4-CONTROL	1,433333	0,401894	2,464773	0,0070532
Carbon source	Starch	SGT5.3-HRT7.7	-0,3	-1,33144	0,731439	0,8678983
Carbon source	Starch	SRT9.1-HRT7.7	-0,2	-1,23144	0,831439	0,9650678
Carbon source	Starch	SRU4.4-HRT7.7	-0,36667	-1,39811	0,664773	0,7675204
Carbon source	Starch	SRT9.1-SGT5.3	0,1	-0,93144	1,131439	0,9973615
Carbon source	Starch	SRU4.4-SGT5.3	-0,06667	-1,09811	0,964773	0,9994595
Carbon source	Starch	SRU4.4-SRT9.1	-0,16667	-1,19811	0,864773	0,9818279
Carbon source	Lactose	HRT7.7-CONTROL	0,2	-0,6554	1,0554	0,933794
Carbon source	Lactose	SGT5.3-CONTROL	1,833333	0,977933	2,688733	0,000261
Carbon source	Lactose	SRT9.1-CONTROL	1,733333	0,877933	2,588733	0,0004148
Carbon source	Lactose	SRU4.4-CONTROL	1,7	0,8446	2,5554	0,0004859
Carbon source	Lactose	SGT5.3-HRT7.7	1,633333	0,777933	2,488733	0,0006707
Carbon source	Lactose	SRT9.1-HRT7.7	1,533333	0,677933	2,388733	0,0011037
Carbon source	Lactose	SRU4.4-HRT7.7	1,5	0,6446	2,3554	0,0013084
Carbon source	Lactose	SRT9.1-SGT5.3	-0,1	-0,9554	0,7554	0,9945839
Carbon source	Lactose	SRU4.4-SGT5.3	-0,13333	-0,98873	0,722067	0,9840731
Carbon source	Lactose	SRU4.4-SRT9.1	-0,03333	-0,88873	0,822067	0,9999272
Carbon source	Maltose	HRT7.7-CONTROL	0,433333	-0,6593	1,525964	0,6943897
Carbon source	Maltose	SGT5.3-CONTROL	2,233333	1,140703	3,325964	0,0003864
Carbon source	Maltose	SRT9.1-CONTROL	2,633333	1,540703	3,725964	0,0000963
Carbon source	Maltose	SRU4.4-CONTROL	2,9	1,80737	3,99263	0,0000414
Carbon source	Maltose	SGT5.3-HRT7.7	1,8	0,70737	2,89263	0,0021009
Carbon source	Maltose	SRT9.1-HRT7.7	2,2	1,10737	3,29263	0,0004369
Carbon source	Maltose	SRU4.4-HRT7.7	2,466667	1,374036	3,559297	0,0001686
Carbon source	Maltose	SRT9.1-SGT5.3	0,4	-0,69263	1,49263	0,7491546
Carbon source	Maltose	SRU4.4-SGT5.3	0,666667	-0,42596	1,759297	0,3282263
Carbon source	Maltose	SRU4.4-SRT9.1	0,266667	-0,82596	1,359297	0,9237688
Carbon source	Fructose	HRT7.7-CONTROL	-0,46667	-1,79765	0,864313	0,775818
Carbon source	Fructose	SGT5.3-CONTROL	0,733333	-0,59765	2,064313	0,4172971
Carbon source	Fructose	SRT9.1-CONTROL	0	-1,33098	1,33098	1
Carbon source	Fructose	SRU4.4-CONTROL	1,433333	0,102354	2,764313	0,0337515
Carbon source	Fructose	SGT5.3-HRT7.7	1,2	-0,13098	2,53098	0,0824786
Carbon source	Fructose	SRT9.1-HRT7.7	0,466667	-0,86431	1,797646	0,775818
Carbon source	Fructose	SRU4.4-HRT7.7	1,9	0,569021	3,23098	0,0058727
Carbon source	Fructose	SRT9.1-SGT5.3	-0,73333	-2,06431	0,597646	0,4172971
Carbon source	Fructose	SRU4.4-SGT5.3	0,7	-0,63098	2,03098	0,4589236
Carbon source	Fructose	SRU4.4-SRT9.1	1,433333	0,102354	2,764313	0,0337515
Nitrogen source	Glycine	HRT7.7-CONTROL	-0,06667	-1,13027	0,996941	0,9995211
Nitrogen source	Glycine	SGT5.3-CONTROL	2,266667	1,203059	3,330274	0,0002736

Nitrogen source	Glycine	SRT9.1-CONTROL	0,833333	-0,23027	1,896941	0,1483032
Nitrogen source	Glycine	SRU4.4-CONTROL	3,333333	2,269726	4,396941	0,0000093
Nitrogen source	Glycine	SGT5.3-HRT7.7	2,333333	1,269726	3,396941	0,0002147
Nitrogen source	Glycine	SRT9.1-HRT7.7	0,9	-0,16361	1,963608	0,1089217
Nitrogen source	Glycine	SRU4.4-HRT7.7	3,4	2,336392	4,463608	0,0000077
Nitrogen source	Glycine	SRT9.1-SGT5.3	-1,43333	-2,49694	-0,36973	0,0086597
Nitrogen source	Glycine	SRU4.4-SGT5.3	1,066667	0,003059	2,130274	0,0492707
Nitrogen source	Glycine	SRU4.4-SRT9.1	2,5	1,436392	3,563608	0,0001195
Nitrogen source	Yeast extract	HRT7.7-CONTROL	0,533333	-1,05186	2,118528	0,7993757
Nitrogen source	Yeast extract	SGT5.3-CONTROL	0,866667	-0,71853	2,451862	0,4242048
Nitrogen source	Yeast extract	SRT9.1-CONTROL	0,533333	-1,05186	2,118528	0,7993757
Nitrogen source	Yeast extract	SRU4.4-CONTROL	0,166667	-1,41853	1,751862	0,9963923
Nitrogen source	Yeast extract	SGT5.3-HRT7.7	0,333333	-1,25186	1,918528	0,9537117
Nitrogen source	Yeast extract	SRT9.1-HRT7.7	1,78E-15	-1,5852	1,585195	1
Nitrogen source	Yeast extract	SRU4.4-HRT7.7	-0,36667	-1,95186	1,218528	0,9361165
Nitrogen source	Yeast extract	SRT9.1-SGT5.3	-0,33333	-1,91853	1,251862	0,9537117
Nitrogen source	Yeast extract	SRU4.4-SGT5.3	-0,7	-2,2852	0,885195	0,6109545
Nitrogen source	Yeast extract	SRU4.4-SRT9.1	-0,36667	-1,95186	1,218528	0,9361165
Nitrogen source	Tryptone	HRT7.7-CONTROL	0,233333	-0,52354	0,990203	0,8431593
Nitrogen source	Tryptone	SGT5.3-CONTROL	0,733333	-0,02354	1,490203	0,0586003
Nitrogen source	Tryptone	SRT9.1-CONTROL	0,066667	-0,6902	0,823536	0,9981818
Nitrogen source	Tryptone	SRU4.4-CONTROL	-0,26667	-1,02354	0,490203	0,772917
Nitrogen source	Tryptone	SGT5.3-HRT7.7	0,5	-0,25687	1,256869	0,2635794
Nitrogen source	Tryptone	SRT9.1-HRT7.7	-0,16667	-0,92354	0,590203	0,9458334
Nitrogen source	Tryptone	SRU4.4-HRT7.7	-0,5	-1,25687	0,256869	0,2635794
Nitrogen source	Tryptone	SRT9.1-SGT5.3	-0,66667	-1,42354	0,090203	0,0915807
Nitrogen source	Tryptone	SRU4.4-SGT5.3	-1	-1,75687	-0,24313	0,0098599
Nitrogen source	Tryptone	SRU4.4-SRT9.1	-0,33333	-1,0902	0,423536	0,6131437
Nitrogen source	(NH ₄) ₂ SO ₄	HRT7.7-CONTROL	0,333333	-0,73929	1,405955	0,8394874
Nitrogen source	(NH ₄) ₂ SO ₄	SGT5.3-CONTROL	-0,86667	-1,93929	0,205955	0,1315748
Nitrogen source	(NH ₄) ₂ SO ₄	SRT9.1-CONTROL	-0,26667	-1,33929	0,805955	0,9190543
Nitrogen source	(NH ₄) ₂ SO ₄	SRU4.4-CONTROL	0,2	-0,87262	1,272621	0,9695737
Nitrogen source	(NH ₄) ₂ SO ₄	SGT5.3-HRT7.7	-1,2	-2,27262	-0,12738	0,0272639
Nitrogen source	(NH ₄) ₂ SO ₄	SRT9.1-HRT7.7	-0,6	-1,67262	0,472621	0,4038196
Nitrogen source	(NH ₄) ₂ SO ₄	SRU4.4-HRT7.7	-0,13333	-1,20595	0,939288	0,9931605
Nitrogen source	(NH ₄) ₂ SO ₄	SRT9.1-SGT5.3	0,6	-0,47262	1,672621	0,4038196
Nitrogen source	(NH ₄) ₂ SO ₄	SRU4.4-SGT5.3	1,066667	-0,00595	2,139288	0,0514381
Nitrogen source	(NH ₄) ₂ SO ₄	SRU4.4-SRT9.1	0,466667	-0,60595	1,539288	0,6230927
Nitrogen source	NH ₄ Cl	HRT7.7-CONTROL	-1,4	-3,17976	0,379756	0,1460645
Nitrogen source	NH ₄ Cl	SGT5.3-CONTROL	-2,06667	-3,84642	-0,28691	0,0219732
Nitrogen source	NH ₄ Cl	SRT9.1-CONTROL	-1,6	-3,37976	0,179756	0,0835652
Nitrogen source	NH ₄ Cl	SRU4.4-CONTROL	-0,83333	-2,61309	0,946423	0,5615867
Nitrogen source	NH ₄ Cl	SGT5.3-HRT7.7	-0,66667	-2,44642	1,113089	0,734159
Nitrogen source	NH ₄ Cl	SRT9.1-HRT7.7	-0,2	-1,97976	1,579756	0,9953426
Nitrogen source	NH ₄ Cl	SRU4.4-HRT7.7	0,566667	-1,21309	2,346423	0,8279501
Nitrogen source	NH ₄ Cl	SRT9.1-SGT5.3	0,466667	-1,31309	2,246423	0,9040288
Nitrogen source	NH ₄ Cl	SRU4.4-SGT5.3	1,233333	-0,54642	3,013089	0,227658
Nitrogen source	NH ₄ Cl	SRU4.4-SRT9.1	0,766667	-1,01309	2,546423	0,6311073
Temperature	25°C	HRT7.7-CONTROL	2,633333	1,284391	3,982276	0,0005616
Temperature	25°C	SGT5.3-CONTROL	-2,46667	-3,81561	-1,11772	0,0009447
Temperature	25°C	SRT9.1-CONTROL	-3,7	-5,04894	-2,35106	0,0000309
Temperature	25°C	SRU4.4-CONTROL	1,266667	-0,08228	2,615609	0,0682335
Temperature	25°C	SGT5.3-HRT7.7	-5,1	-6,44894	-3,75106	0,0000016
Temperature	25°C	SRT9.1-HRT7.7	-6,33333	-7,68228	-4,98439	0,0000002

Temperature	25°C	SRU4.4-HRT7.7	-1,36667	-2,71561	-0,01772	0,0467533
Temperature	25°C	SRT9.1-SGT5.3	-1,23333	-2,58228	0,115609	0,0773461
Temperature	25°C	SRU4.4-SGT5.3	3,733333	2,384391	5,082276	0,0000285
Temperature	25°C	SRU4.4-SRT9.1	4,966667	3,617724	6,315609	0,0000021
Temperature	28°C	HRT7.7-CONTROL	1,4	-0,11533	2,915328	0,0736754
Temperature	28°C	SGT5.3-CONTROL	-0,63333	-2,14866	0,881995	0,6549673
Temperature	28°C	SRT9.1-CONTROL	-4,13333	-5,64866	-2,61801	0,0000325
Temperature	28°C	SRU4.4-CONTROL	0,833333	-0,68199	2,348661	0,4189773
Temperature	28°C	SGT5.3-HRT7.7	-2,03333	-3,54866	-0,51801	0,0089085
Temperature	28°C	SRT9.1-HRT7.7	-5,53333	-7,04866	-4,01801	0,0000022
Temperature	28°C	SRU4.4-HRT7.7	-0,56667	-2,08199	0,948661	0,7352732
Temperature	28°C	SRT9.1-SGT5.3	-3,5	-5,01533	-1,98467	0,0001388
Temperature	28°C	SRU4.4-SGT5.3	1,466667	-0,04866	2,981995	0,0589047
Temperature	28°C	SRU4.4-SRT9.1	4,966667	3,451339	6,481995	0,0000061
Temperature	30°C	HRT7.7-CONTROL	0,9	0,115031	1,684969	0,0236708
Temperature	30°C	SGT5.3-CONTROL	-1,7	-2,48497	-0,91503	0,0002392
Temperature	30°C	SRT9.1-CONTROL	-3,23333	-4,0183	-2,44836	0,0000007
Temperature	30°C	SRU4.4-CONTROL	0,066667	-0,7183	0,851636	0,9984225
Temperature	30°C	SGT5.3-HRT7.7	-2,6	-3,38497	-1,81503	0,0000056
Temperature	30°C	SRT9.1-HRT7.7	-4,13333	-4,9183	-3,34836	0,0000001
Temperature	30°C	SRU4.4-HRT7.7	-0,83333	-1,6183	-0,04836	0,036493
Temperature	30°C	SRT9.1-SGT5.3	-1,53333	-2,3183	-0,74836	0,0005587
Temperature	30°C	SRU4.4-SGT5.3	1,766667	0,981698	2,551636	0,000173
Temperature	30°C	SRU4.4-SRT9.1	3,3	2,515031	4,084969	0,0000006
Temperature	35°C	HRT7.7-CONTROL	1,2	0,443131	1,956869	0,0027886
Temperature	35°C	SGT5.3-CONTROL	-2,06667	-2,82354	-1,3098	0,0000322
Temperature	35°C	SRT9.1-CONTROL	-4,1	-4,85687	-3,34313	0,0000001
Temperature	35°C	SRU4.4-CONTROL	0,666667	-0,0902	1,423536	0,0915807
Temperature	35°C	SGT5.3-HRT7.7	-3,26667	-4,02354	-2,5098	0,0000005
Temperature	35°C	SRT9.1-HRT7.7	-5,3	-6,05687	-4,54313	0
Temperature	35°C	SRU4.4-HRT7.7	-0,53333	-1,2902	0,223536	0,2157238
Temperature	35°C	SRT9.1-SGT5.3	-2,03333	-2,7902	-1,27646	0,0000372
Temperature	35°C	SRU4.4-SGT5.3	2,733333	1,976464	3,490203	0,0000025
Temperature	35°C	SRU4.4-SRT9.1	4,766667	4,009797	5,523536	0
Temperature	37°C	HRT7.7-CONTROL	1,166667	-0,3723	2,705636	0,1676648
Temperature	37°C	SGT5.3-CONTROL	0,466667	-1,0723	2,005636	0,8505325
Temperature	37°C	SRT9.1-CONTROL	-1,56667	-3,10564	-0,0277	0,0456068
Temperature	37°C	SRU4.4-CONTROL	0,833333	-0,70564	2,372303	0,4328112
Temperature	37°C	SGT5.3-HRT7.7	-0,7	-2,23897	0,83897	0,5862983
Temperature	37°C	SRT9.1-HRT7.7	-2,73333	-4,2723	-1,19436	0,0011856
Temperature	37°C	SRU4.4-HRT7.7	-0,33333	-1,8723	1,205636	0,9487868
Temperature	37°C	SRT9.1-SGT5.3	-2,03333	-3,5723	-0,49436	0,00986
Temperature	37°C	SRU4.4-SGT5.3	0,366667	-1,1723	1,905636	0,9295463
Temperature	37°C	SRU4.4-SRT9.1	2,4	0,861031	3,93897	0,0031453
Starch	0,5	HRT7.7-CONTROL	-1,16667	-1,77943	-0,5539	0,0006863
Starch	0,5	SGT5.3-CONTROL	0,8	0,187234	1,412766	0,0106541
Starch	0,5	SRT9.1-CONTROL	-3,33333	-3,9461	-2,72057	0,0000001
Starch	0,5	SRU4.4-CONTROL	-1,33333	-1,9461	-0,72057	0,00023
Starch	0,5	SGT5.3-HRT7.7	1,966667	1,353901	2,579433	0,0000074
Starch	0,5	SRT9.1-HRT7.7	-2,16667	-2,77943	-1,5539	0,000003
Starch	0,5	SRU4.4-HRT7.7	-0,16667	-0,77943	0,4461	0,8923571
Starch	0,5	SRT9.1-SGT5.3	-4,13333	-4,7461	-3,52057	0
Starch	0,5	SRU4.4-SGT5.3	-2,13333	-2,7461	-1,52057	0,0000035
Starch	0,5	SRU4.4-SRT9.1	2	1,387234	2,612766	0,0000064

Starch	1	HRT7.7-CONTROL	-0,36667	-1,20214	0,468805	0,6160062
Starch	1	SGT5.3-CONTROL	1,366667	0,531196	2,202138	0,0022145
Starch	1	SRT9.1-CONTROL	-2,36667	-3,20214	-1,5312	0,0000232
Starch	1	SRU4.4-CONTROL	-0,3	-1,13547	0,535471	0,7613484
Starch	1	SGT5.3-HRT7.7	1,733333	0,897862	2,568805	0,0003418
Starch	1	SRT9.1-HRT7.7	-2	-2,83547	-1,16453	0,0001021
Starch	1	SRU4.4-HRT7.7	0,066667	-0,7688	0,902138	0,9987635
Starch	1	SRT9.1-SGT5.3	-3,73333	-4,5688	-2,89786	0,0000003
Starch	1	SRU4.4-SGT5.3	-1,66667	-2,50214	-0,8312	0,0004713
Starch	1	SRU4.4-SRT9.1	2,066667	1,231196	2,902138	0,0000768
Starch	1,5	HRT7.7-CONTROL	-0,46667	-1,05947	0,126134	0,1456397
Starch	1,5	SGT5.3-CONTROL	0,466667	-0,12613	1,059468	0,1456397
Starch	1,5	SRT9.1-CONTROL	-1,86667	-2,45947	-1,27387	0,0000089
Starch	1,5	SRU4.4-CONTROL	-0,6	-1,1928	-0,0072	0,0469917
Starch	1,5	SGT5.3-HRT7.7	0,933333	0,340532	1,526134	0,0029344
Starch	1,5	SRT9.1-HRT7.7	-1,4	-1,9928	-0,8072	0,0001147
Starch	1,5	SRU4.4-HRT7.7	-0,13333	-0,72613	0,459468	0,9418233
Starch	1,5	SRT9.1-SGT5.3	-2,33333	-2,92613	-1,74053	0,0000011
Starch	1,5	SRU4.4-SGT5.3	-1,06667	-1,65947	-0,47387	0,0010715
Starch	1,5	SRU4.4-SRT9.1	1,266667	0,673866	1,859468	0,0002677
Starch	2	HRT7.7-CONTROL	0,066667	-0,56923	0,702564	0,9964318
Starch	2	SGT5.3-CONTROL	0,933333	0,297436	1,569231	0,0048437
Starch	2	SRT9.1-CONTROL	-1,3	-1,9359	-0,6641	0,0003858
Starch	2	SRU4.4-CONTROL	-0,23333	-0,86923	0,402564	0,7476707
Starch	2	SGT5.3-HRT7.7	0,866667	0,230769	1,502564	0,008035
Starch	2	SRT9.1-HRT7.7	-1,36667	-2,00256	-0,73077	0,000255
Starch	2	SRU4.4-HRT7.7	-0,3	-0,9359	0,335898	0,5550775
Starch	2	SRT9.1-SGT5.3	-2,23333	-2,86923	-1,59744	0,0000032
Starch	2	SRU4.4-SGT5.3	-1,16667	-1,80256	-0,53077	0,0009204
Starch	2	SRU4.4-SRT9.1	1,066667	0,430769	1,702564	0,0018348
Starch	2,5	HRT7.7-CONTROL	-0,06667	-0,64716	0,513826	0,994939
Starch	2,5	SGT5.3-CONTROL	2,066667	1,486174	2,647159	0,0000028
Starch	2,5	SRT9.1-CONTROL	-0,73333	-1,31383	-0,15284	0,0131446
Starch	2,5	SRU4.4-CONTROL	-0,03333	-0,61383	0,547159	0,9996612
Starch	2,5	SGT5.3-HRT7.7	2,133333	1,552841	2,713826	0,0000021
Starch	2,5	SRT9.1-HRT7.7	-0,66667	-1,24716	-0,08617	0,0234426
Starch	2,5	SRU4.4-HRT7.7	0,033333	-0,54716	0,613826	0,9996612
Starch	2,5	SRT9.1-SGT5.3	-2,8	-3,38049	-2,21951	0,0000002
Starch	2,5	SRU4.4-SGT5.3	-2,1	-2,68049	-1,51951	0,0000024
Starch	2,5	SRU4.4-SRT9.1	0,7	0,119508	1,280492	0,0175321
Starch	3	HRT7.7-CONTROL	-0,6	-2,43699	1,236989	0,8151426
Starch	3	SGT5.3-CONTROL	-0,73333	-2,57032	1,103655	0,6896101
Starch	3	SRT9.1-CONTROL	-3,83333	-5,67032	-1,99635	0,0003258
Starch	3	SRU4.4-CONTROL	-0,53333	-2,37032	1,303655	0,8686132
Starch	3	SGT5.3-HRT7.7	-0,13333	-1,97032	1,703655	0,999147
Starch	3	SRT9.1-HRT7.7	-3,23333	-5,07032	-1,39635	0,0012713
Starch	3	SRU4.4-HRT7.7	0,066667	-1,77032	1,903655	0,9999452
Starch	3	SRT9.1-SGT5.3	-3,1	-4,93699	-1,26301	0,0017534
Starch	3	SRU4.4-SGT5.3	0,2	-1,63699	2,036989	0,9958745
Starch	3	SRU4.4-SRT9.1	3,3	1,463011	5,136989	0,0010853
Tryptone	0,5	HRT7.7-CONTROL	0,933333	-0,02303	1,8897	0,0565416
Tryptone	0,5	SGT5.3-CONTROL	-3,03333	-3,9897	-2,07697	0,0000083
Tryptone	0,5	SRT9.1-CONTROL	1,666667	0,7103	2,623033	0,0013724
Tryptone	0,5	SRU4.4-CONTROL	-0,73333	-1,6897	0,223033	0,1607907

Tryptone	0,5	SGT5.3-HRT7.7	-3,96667	-4,92303	-3,0103	0,0000007
Tryptone	0,5	SRT9.1-HRT7.7	0,733333	-0,22303	1,6897	0,1607907
Tryptone	0,5	SRU4.4-HRT7.7	-1,66667	-2,62303	-0,7103	0,0013724
Tryptone	0,5	SRT9.1-SGT5.3	4,7	3,743634	5,656366	0,0000001
Tryptone	0,5	SRU4.4-SGT5.3	2,3	1,343634	3,256366	0,0000981
Tryptone	0,5	SRU4.4-SRT9.1	-2,4	-3,35637	-1,44363	0,0000678
Tryptone	1	HRT7.7-CONTROL	-1,26667	-1,80856	-0,72478	0,0001253
Tryptone	1	SGT5.3-CONTROL	-4,23333	-4,77522	-3,69144	0
Tryptone	1	SRT9.1-CONTROL	0,633333	0,091442	1,175225	0,0211497
Tryptone	1	SRU4.4-CONTROL	-3,23333	-3,77522	-2,69144	0
Tryptone	1	SGT5.3-HRT7.7	-2,96667	-3,50856	-2,42478	0,0000001
Tryptone	1	SRT9.1-HRT7.7	1,9	1,358108	2,441892	0,0000033
Tryptone	1	SRU4.4-HRT7.7	-1,96667	-2,50856	-1,42478	0,0000024
Tryptone	1	SRT9.1-SGT5.3	4,866667	4,324775	5,408558	0
Tryptone	1	SRU4.4-SGT5.3	1	0,458108	1,541892	0,0008792
Tryptone	1	SRU4.4-SRT9.1	-3,86667	-4,40856	-3,32478	0
Tryptone	1,5	HRT7.7-CONTROL	0,433333	-0,53554	1,402202	0,6003992
Tryptone	1,5	SGT5.3-CONTROL	-2,96667	-3,93554	-1,9978	0,0000115
Tryptone	1,5	SRT9.1-CONTROL	0,866667	-0,1022	1,835535	0,0854781
Tryptone	1,5	SRU4.4-CONTROL	-3,53333	-4,5022	-2,56447	0,0000023
Tryptone	1,5	SGT5.3-HRT7.7	-3,4	-4,36887	-2,43113	0,0000033
Tryptone	1,5	SRT9.1-HRT7.7	0,433333	-0,53554	1,402202	0,6003992
Tryptone	1,5	SRU4.4-HRT7.7	-3,96667	-4,93554	-2,9978	0,0000008
Tryptone	1,5	SRT9.1-SGT5.3	3,833333	2,864465	4,802202	0,0000001
Tryptone	1,5	SRU4.4-SGT5.3	-0,56667	-1,53554	0,402202	0,3645711
Tryptone	1,5	SRU4.4-SRT9.1	-4,4	-5,36887	-3,43113	0,0000003
Tryptone	2	HRT7.7-CONTROL	0,5	-0,57933	1,579332	0,5707594
Tryptone	2	SGT5.3-CONTROL	-6,93333	-8,01267	-5,854	0
Tryptone	2	SRT9.1-CONTROL	-4,13333	-5,21267	-3,054	0,0000014
Tryptone	2	SRU4.4-CONTROL	-2,2	-3,27933	-1,12067	0,0003953
Tryptone	2	SGT5.3-HRT7.7	-7,43333	-8,51267	-6,354	0
Tryptone	2	SRT9.1-HRT7.7	-4,63333	-5,71267	-3,554	0,0000005
Tryptone	2	SRU4.4-HRT7.7	-2,7	-3,77933	-1,62067	0,0000697
Tryptone	2	SRT9.1-SGT5.3	2,8	1,720668	3,879332	0,0000507
Tryptone	2	SRU4.4-SGT5.3	4,733333	3,654001	5,812666	0,0000004
Tryptone	2	SRU4.4-SRT9.1	1,933333	0,854001	3,012666	0,00111
Tryptone	2,5	HRT7.7-CONTROL	-5,3E-15	-1,41342	1,413421	1
Tryptone	2,5	SGT5.3-CONTROL	-4	-5,41342	-2,58658	0,0000234
Tryptone	2,5	SRT9.1-CONTROL	0,433333	-0,98009	1,846754	0,8456609
Tryptone	2,5	SRU4.4-CONTROL	0,266667	-1,14675	1,680088	0,9682789
Tryptone	2,5	SGT5.3-HRT7.7	-4	-5,41342	-2,58658	0,0000234
Tryptone	2,5	SRT9.1-HRT7.7	0,433333	-0,98009	1,846754	0,8456609
Tryptone	2,5	SRU4.4-HRT7.7	0,266667	-1,14675	1,680088	0,9682789
Tryptone	2,5	SRT9.1-SGT5.3	4,433333	3,019912	5,846754	0,0000092
Tryptone	2,5	SRU4.4-SGT5.3	4,266667	2,853246	5,680088	0,000013
Tryptone	2,5	SRU4.4-SRT9.1	-0,16667	-1,58009	1,246754	0,9944029
Tryptone	3	HRT7.7-CONTROL	-0,1	-1,18156	0,98156	0,9978047
Tryptone	3	SGT5.3-CONTROL	-3,66667	-4,74823	-2,58511	0,0000045
Tryptone	3	SRT9.1-CONTROL	-0,56667	-1,64823	0,514893	0,4623262
Tryptone	3	SRU4.4-CONTROL	-1,43333	-2,51489	-0,35177	0,0096666
Tryptone	3	SGT5.3-HRT7.7	-3,56667	-4,64823	-2,48511	0,0000058
Tryptone	3	SRT9.1-HRT7.7	-0,46667	-1,54823	0,614893	0,6297938
Tryptone	3	SRU4.4-HRT7.7	-1,33333	-2,41489	-0,25177	0,0153128
Tryptone	3	SRT9.1-SGT5.3	3,1	2,01844	4,18156	0,0000208

Tryptone	3	SRU4.4-SGT5.3	2,233333	1,151773	3,314893	0,0003553
Tryptone	3	SRU4.4-SRT9.1	-0,86667	-1,94823	0,214893	0,1359563

Table S6 Turkey's HSD mean comparisons of antagonistic activity of the sweet pepper fruit isolates, against the *R. solanacearum* BD 261 strain, at different treatment levels of pH, carbon sources and nitrogen sources, temperature, concentration of starch and tryptone.

CHAPTR 6

Summarizing research answers and providing future prospects

6.1 Potential impact of the discoveries

Sweet pepper (*Capsicum annuum*), is one of the most extensively used fruit crop in the world. Regardless of its importance and popularity, fruit yield remains low in some parts of the world. Major yield losses are attributed to postharvest diseases, and these are commonly controlled using inorganic pesticides, known to harbour a several health and environmental risks (FAOSTAT 2018). Biocontrol agents, especially, microbial antagonist can potentially protect yield losses in pepper, in a sustainable manner, although this claim is poorly established. Establishing the identities of microorganisms residing on the surfaces of sweet pepper fruits is important in the documentation of potential biocontrol agents, which can potentially be exploited to control against pathogenic strains of peppers, thereby helping in protection of yield losses.

Our findings show that fresh sweet pepper fruits mostly associates with members of the genera including *Acinetobacter*, *Agrobacterium* and *Burkholderia*, which harbour some important strains with antagonistic potential against microbial plant pathogens of plants. Additionally, microbial functions assays and amplicon sequencing revealed the bacterial strains; *Bacillus cereus* strain HRT7.7, *Enterobacter hormaechei* strain SRU4.4, *Paenibacillus polymyxa* strain SRT9.1 and *Serratia marcescens* strain SGT5.3, as potential antagonists of *R. solanacearum* (the most damaging pathogen of peppers, globally). The potential antagonistic strains were identified, mostly on peppers sampled on plants grown under high risk production media (i.e., open soil environments), suggesting that crop management strategies

(e.g., site selection) can shape microbial communities that can be accommodated of plant surfaces. The findings will be key in the development of bacterial-based bio-pesticides for control of the most devastating pests of sweet peppers. Bio-pesticides are also an important ingredient in integrated pest management programs (IPMs), known to be effective in sustainable and effective disease control.

6.2 Future work

Potential bacterial biocontrol strains suppressing bacterial wilt causing pathogen (*Ralstonia solanacearum* BD 261) *in vitro* (i.e., *Bacillus cereus* strain HRT7.7, *Enterobacter hormaechei* strain SRU4.4, *Paenibacillus polymyxa* strain SRT9.1 and *Serratia marcescens* strain SGT5.3) have been wholly characterized. Future study of these valuable strains will involve expression of defense-related genes in pepper plants and evaluation of their ability to control *R. solanacearum* BD 261 and other pathogens *in vivo* under different environmental conditions and cultural practices. In addition, establishment of the relationship between metabolite or antioxidant production by the sweet pepper fruits treated with these antagonistic strains (alone or in combination) and the level of activity (i.e., growth and antibacterial activity), is of paramount importance since all plants deploy inherent mechanisms to resist or tolerate, both the abiotic and biotic stresses (He et al. 2018). In order to understand the mechanisms of pathogen suppression in sweet peppers, additional studies will encompass analysing antagonistic strains whole-genome sequencing.

Fingerprinting the bacterial diversity and determining functional potential of bacteria associated with sweet pepper treated with bacterial antagonists from this study in hydroponic and open soil may also be valuable, as then we would be able to identify whether there is a core microbiome associated with sweet pepper fruits. Identifying the core microbiome is essential

to decoding the ecology of microbial consortia (Shade and Handelsman 2012), because it has been recommended that these co-occurring organisms that appear in most assemblages associated with a specific habitat are important to the function of the community they are found.

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