Investigating the role of mycorrhizal fungi and associated bacteria in promoting growth of citrus seedlings

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ABSTRACT

South Africa is the world's second largest exporter of fresh citrus and is ranked 14th in citrus production. Fungal pathogens such as *Phytophthora* and *Pythium* cause economic losses as a result of root rot and brown rot. Mycorrhizal fungi are specialized members of the fungal community forming a mutualistic relationship with plant roots. Mycorrhizal fungal structures are known to associate with other soil microorganisms and these may contribute to improved plant growth. A diverse group of bacteria that interact with the mycorrhizal fungi are known as Mycorrhizal Helper Bacteria (MHB). The aim of this study was to investigate the role of arbuscular mycorrhiza and associated bacteria isolated from spores and determine whether they had any plant growth promoting potential.

A total of 19 bacteria were isolated from arbuscular mycorrhizal spores and were molecularly identified as belonging to several *Bacillus, Micrococcus, Onchrobactrum* and S*taphylococcus* sp. All bacterial isolates were tested for plant growth promotion abilities. One *Bacillu*s isolate was able to solubilise phosphate. Four isolates *Micrococcus* sp, *Micrococcus leteus*, *Ochrobacterum* sp and *Ochrobacterum antropi* were able to produce Indole Aectic Acid and three isolates showed potential to reduce growth of *Phytophthora nicotianae*, *P. citrocola* and *P. citrophthora* in *in vitro* plate cultures. Further tests using culture supernatants of the *Bacillus* sp, *Micrococcus* sp and *Bacillus cereus* confirmed their ability to inhibit or reduce growth of the three *Phytophthora* species in a 96 well bioassay. *Bacillus* sp and *Bacillus cereus* were able to inhibit *Phytophthora* spp by 95 to 100 % and *Micrococcus* spp was able decrease pathogen growth by 60 to 94 %. These bacterial isolates were further evaluated for plant growth promoting abilities on citrus rough lemon seedlings alone or in combination with arbuscular mycorrhizal inoculum. Bacterial and mycorrhizal inoculants influence the increase in shoot and root biomass. *Bacillus cereus* in combination with mycorrhizal inoculum significantly increased seedling shoot to root ratio while root biomass was significantly increased with mycorrhizal inoculation. Due to the short duration of the trial mycorrhizal colonisation could not be assessed. It is evident that selected combinations of bacteria and mycorrhizal fungi could promote citrus seedling growth and potentially improve seedling health. Further studies under nursery conditions are recommended.

This thesis is dedicated to my parents who have been there for me throughout my studies and never giving up on me

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ABBREVATIONS

ANOVA analysis of variance

BLAST basic local alignment search tool

Bp base pairs

cm centimetre

dH2O distilled and deionized water

DNA deoxyribose nucleic acid

dNTP deoxyribose nucleotide triphosphate

e.g. for example

EDTA ethylene diamine tetra acetic acid

g gram

hr(s) hour(s)

i.e. that is

IAA indole acetic acid

ITS internal transcribed spacer

l/L litre

mg milligram

mg/ml milligram/millilitre

MHB mycorrhizal helper bacteria

Min(s) minute(s)

N nitrogen

NCBI National Centre for Biotechnology Information

P phosphorus

PCR polymerase chain reaction

PGPR plant growth promoting bacteria

PSB phosphate solubilising bacteria

rDNA ribosomal deoxynucleic acid

rpm rotation per min

Sec seconds

sp. species (singular)

spp. species (plural)

TAE tris-acetic acid-EDTA

TE tris-HCl EDTA

V volts

vol/vol volume / volume

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Mycorrhizal fungi

The word mycorrhiza originated from the Greek *mycos*, meaning 'fungus' and *rhiza*, meaning 'root' and are the specialized members of the fungal population that colonise the rhizosphere (Smith and Read, 2008). Many soil microorganisms form relationships with plants that range from being parasitic, commensal or mutualistic. Among these, the most widespread mutualistic relationship is between mycorrhizal fungal hyphae and plant roots (Smith and Read, 2008). These fungi are known to carry out many critical ecosystem functions such as improvement of plant establishment, enhancement of plant nutrient uptake, plant protection against cultural and environmental stress and improvement of soil structure (Jeffries *et al*., 2003; Read and Perez- Moreno, 2003; Sikes *et al*., 2009). In return the fungus receives carbohydrates in the form of sugars and growth factors from the plant (Smith and Read, 2008). As a result of these nutrient uptake-enhancing properties, mycorrhizal fungi have been used to stimulate plant growth in agriculture, horticulture and forestry. Under plant nursery conditions, the addition of mycorrhizal fungi at an early stage of plant growth has been shown to yield larger plants in comparison with uninoculated plants (Camprubi *et al*., 1993; Wu and Zou, 2009).

The fungi require carbon in the form of sugars to grow and complete their life cycles hence the symbiotic relationship with roots. The carbon is made available to the fungus by way of photosynthates (Bago *et al*., 2003). Mycorrhizal fungi are different from other fungi as they have the ability to create an interface for nutrients exchange which occurs within living cells of the plant (Brundrett, 2004) and this is a characteristic of the symbiosis. The function of mycorrhizal fungi goes beyond nutrient acquirement, they also provide several benefits to the host plant which include access to less labile nutrients from organic sources, enhanced water uptake, resistance to pathogenic fungi and harmful bacteria as well as protection against heavy metal in the soil (Al-Karaki *et al*., 2004; Filion *et al*., 1999; Hildebrandt *et al*., 1999; Marschner and Dell, 1994; Newsham *et al*., 1995b).

Mycorrhizal fungi also interact with microorganisms colonising roots including, both symbiotic and free-living nitrogen fixing bacteria which develop activities involved in plant growth promotion and plant protection (Barea *et al*., 2002; Dashti *et al.* 1998; Tilak *et al*., 2006). The dependence on mycorrhizal fungi varies with plant species, particularly the root morphology, and conditions of soil and climate so plants with thick poorly branched roots and with few root hairs, are usually more dependent on mycorrhizal fungi for normal growth and development (Smith and Read, 2008). Approximately 90% of all plant species are colonised by mycorrhizal fungi. The fungal network which exploits the soil environment and influences microbial populations is called the mycorrhizophere and extends beyond the rhizosphere (Frey-Klett *et al.*, 2007; Schüβler *et al.*, 2001; Wang *et al.*, 2008)

1.2 Mycorrhizosphere

The mycorrhizosphere is defined as the zone which is influenced by mycorrhizal fungi and plant roots in the soil. Different microbial communities are found in the mycorrhizosphere consisting of bacteria, yeast and other fungi as compare to the bulk soil (Fitter and Garbaye, 1994). The rhizosphere is the thin layer of soil which surrounds the root and directly influences the root, root hairs and microorganisms on the surface of the root. The hyphosphere is influenced by mycorrhizal fungal hyphae (Azcon – Aguilar and Barea, 1996; Linderman, 1988). Different microbial interactions are found in the hyphosphere and the rhizosphere and these differences are due to the quantitative and qualitative differences in the nutrients derived from plant root and fungi (Andrade *et al.*, 1997; Andrade *et al.*, 1998).

Soil microorganisms found in the mycorrhizosphere can produce compounds such as plant hormones that increase root cell permeability increasing root exudation (Bowen and Rovira, 1991). This would stimulate mycorrhizal fungal mycelia in the rhizosphere or facilitate root penetration of the fungi (Azcon –Aguilar and Barea, 1996). The colonisation of plants by mycorrhizal fungi can influence bacterial communities (Azaizeh *et al.*, 1995) associated with the root both directly and indirectly. Direct interactions include provision of energy rich carbon compounds derived from host assimilates, which are transported to the mycorrhizosphere and released into the soil. These compounds include nutrients as well as other inhibitory or stimulatory compounds (Johansson *et al.*, 2004). Indirect interactions can also take place in the form of mycorrhizal mediated effects on the host plant growth, root exudation and soil structure which subsequently influence microbial communities. The mycorrhizophere and the associated microorganisms in combination may lead to the improvement of plant nutrition, growth and disease resistance (Frey-Klett *et al.*, 2007; Hobbie, 1992; Söderström, 1992).

Andrade *et al.*, (1997) reported differences in the composition of the microflora in the rhizophere and hyphosphere soils of different mycorrhizal fungi, suggesting preferential associations between some bacteria and the conditions provided by their individual fungal partner. Mycorrhizal symbiotic status affects the chemical composition of root exudates while the development of a mycorrhizal hyphal network in the soil also introduces physical modification in the environment surrounding the root. These changes affect the rhizopheric microbial communities in the mycorrhizosphere (Linderman, 1988).

1.3 Types of mycorrhizal fungi

Mycorrhizal fungi are classified according to structural characteristics and their fungal associates. Different plant species associate with certain fungi in a unique manner which results in specific mycorrhizal associations (Johansson *et al*, 2004). There are seven different types of mycorrhizas broadly divided into endomycorrhizas and ectomycorrhizas based on whether the fungal hypha penetrates the root cortical cells (Smith and Read, 2008).

Ectomycorrhizal fungi have a significant proportion of their fungal partner's biomass external to the root. This comprises two parts, the sheath or mantle of fungal hyphae that wrap around the outside of the root and the extraradical hyphae and hyphal structures that extend into the surrounding soil. The association is characterized by a hyphal sheath covering the root tip and a 'Hartig' net of hyphae surrounding the plant cells within the root cortex. In some cases the hyphae may also penetrate the plant cell (Dighton, 2009). This type of mycorrhizal association is usually the dominant species in natural forest and woodlands and some produce mushrooms (e.g., *Russula*, *Hebeloma*, *Cortinarius,*) and truffles (e.g., *Tuber*). These fungi associate with a limited number of tree species in all biomes (Horton *et al*. 1999). Tree genera include most coniferous trees, larch, birch, beech, oak, and eucalypts (Wang and Qiu, 2006).

In endomycorrhizal associations the fungal component is usually found inside the roots. Several types of endomycorrhizal are found and this includes specialised ericoid and orchid mycorrhizal. In one such endomycorrhizal type the fungal hyphae penetrate the host cortical cell walls pushing the plasma membrane aside and branches into arbuscules. These arbuscules are finely branched tree like structures that maximize the area of contact between the fungus and the host cell content. Some species also produce vesicles which are sac-like structures emerging from hyphae which serve as storage of lipids (Smith and Read, 2008; Harrison, 2005). The increased contact area between fungus and plant cell enhances the rate of nutrient and carbon exchange between the two partners (Smith and Read, 2008). This endomycorrhizal type called the arbuscular mycorrhizas is relevant in this study.

1.3.1 Arbuscular mycorrhiza

Arbuscular mycorrhizas are the most common mycorrhizal type and are associated with a wide variety of host plants. These fungi are obligately biotrophic meaning that they obtain their carbon solely from the host plant (Bago *et al*., 2003; Douds *et al*., 2000; Graham, 2001). They were reclassified on the basis of DNA sequences into a separate fungal phylum, the Glomeromycota and have subsequently undergone taxonomic revision (Krüger *et al.*, 2009; Morton and Msiska, 2010; Smith and Read, 2008).

The arbuscular mycorrhizal (AM) fungi exist in the soil as spores. Germination can be stimulated by favourable soil conditions such as moisture, this is termed the asymbiotic phase as it is independent of the host plant (Buee *et al*., 2000) In the presence of root exudates from the host plant presymbiotic mycelia growth is accelerated and branching increases in order to ensure contact with the root (Paskowski, 2006). After germination and once contact between the symbionts is established the fungi forms an appressorium as a hyphal swelling on the root surface where pressure builds up. Penetration of the root epidermal cells follows due to the production of enzymes such as endoglucanases (Garcia-Romera *et al.*, 1991a; Garcia-Romera *et al*., 1991b). Hyphal filaments run between cortical root cells and subsequently penetrate through cortical cell walls. The host plasma membrane is pushed aside and covers the fungal hyphae which branches into characteristic arbuscules, tree-like structures. This maximizes the area of contact between the fungus and host cell contents (Fig 1.1). During this symbiotic phase the large surface area facilitates nutrient and carbon exchange between the fungal and plant components of the symbiosis. Extraradical hyphae will extend out of the root and exploit the soil for nutrients and new spores will be formed to continue the life cycle. In the absence of a host the growth of these fungi is relatively short, lasting between 20 to 30 days. In the presences of roots mycelium develops and colonise 60 to 90% of the length of the root system under favourable conditions. About 80% of all terrestrial plant species form this type of symbiosis (Newman and Reddell, 1987, Smith and Read, 2008).

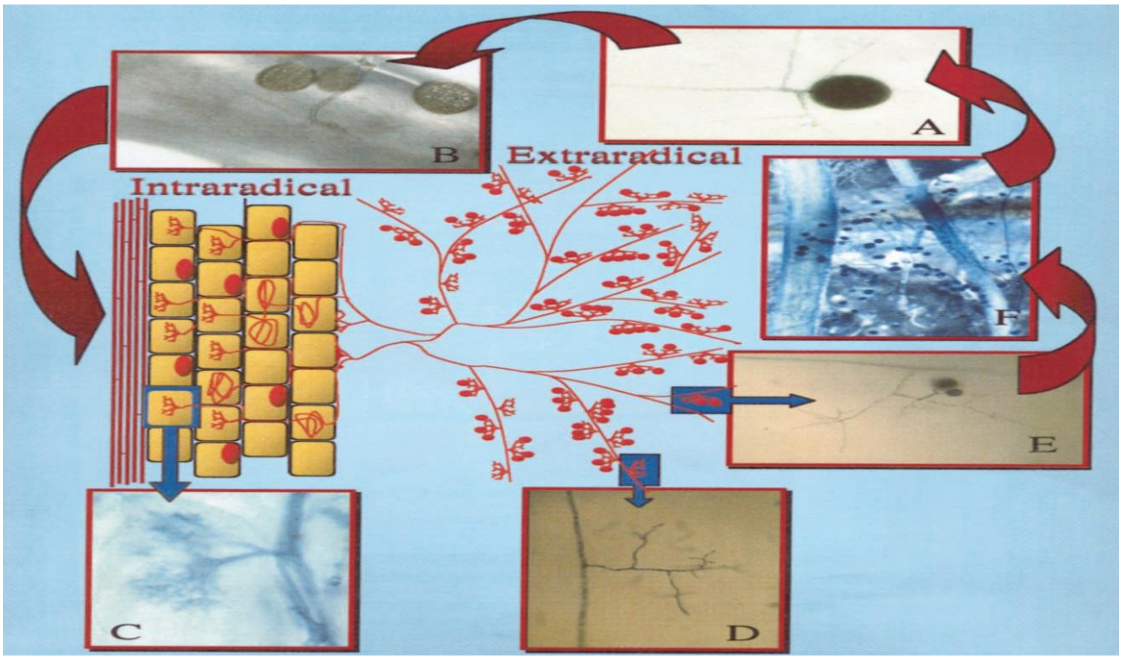

Figure 1.1: Life cycle of arbuscular mycorrhizal fungi, A- spore germination in the presence of root host plant. B - Penetration of mycorrhizal fungus into root tissue. C- Formation of arbuscules within cortical cell. D – Extraradical hyphal network for absorption of nutrient. E – Formation of resting spores (Bago *et al.,* 2003).

Characteristic fungal structures develop within the roots resulting in changes in the rate of root growth and branching. These differences are discernible only by detailed comparison with non-mycorrhizal plants. The colonisation structures facilitate nutrients and carbon exchange between the plant and the fungus (Harrison, 2005).

Two types of colonisation structures have been recorded and are referred to as the *Arum* or *Paris* type. During the *Arum* type of colonisation the fungus spreads in the root cortex by intercellular hyphae which extend along the well-developed intercellular spaces. Short side branches penetrate the cortical cell wall and branch dichotomously in the cell lumen and produce a multi- branched arbuscules. The *Paris* type colonisation is characterised by the extensive development of intracellular coiled hyphae which spreads directly from cell to cell (Brundett and Kendrick 1990; Smith and Read, 2008) (Fig 1.2a and b).

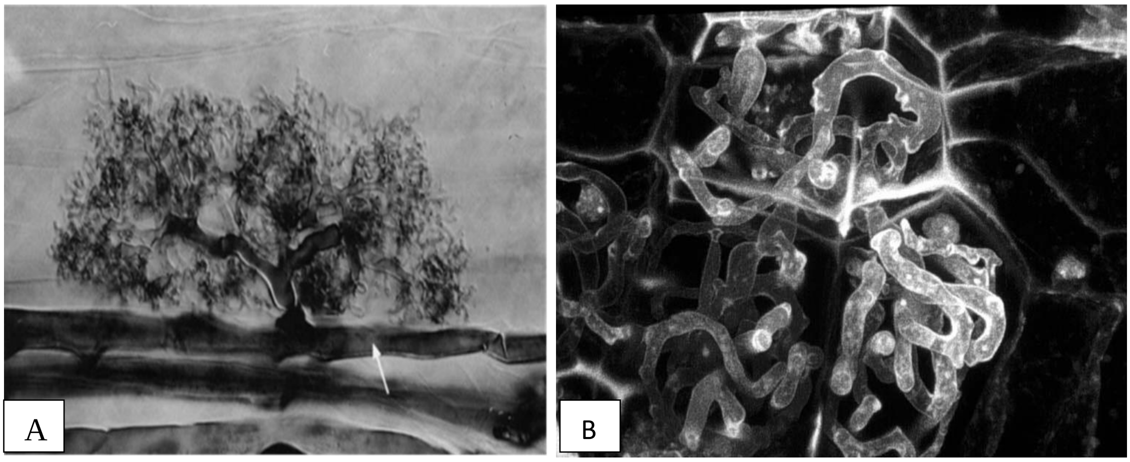

Figure 1.2: A) Light microscope of a *Arum* colonisation strategy arbuscule of *Glomus mosseae* within cortical cell of *Allium porrium* B) Scanning electron microscope of *Paris* type intracellular coils of *Glomus intraradices* in cortical cells of *Panax quinquifolius* (Smith and Read, 2008).

Colonisation of roots by AM fungi can arise from three main sources of inoculum in soil, spores, colonised root fragments and hyphae. These are collectively termed propagules (Smith and Read, 2008). The pattern of growth in the root varies depending on the partners involved. Regardless of the colonisation strategy the external mycorrhizal mycelium in association with other soil microorganism's forms water stable aggregates necessary for good soil quality and an increased capacity for nutrient acquisition thus resulting in stronger plants able to resist stress (Azcon-Aguilar and Barea, 1996).

1.4 Beneficial effects by arbuscular mycorrhizal fungi

1.4.1 Nutrient acquisition

Mycorrhizal fungi play a significant role in mobilizing nutrients from organic substrates. The main effect of significance to host plants is the mobilization of nutrients such as N and P from structural and other polymers which are otherwise unavailable to plant roots (Marschner & Dell, 1994; Tian et al., 2009). Several studies have shown that plants grown in the presence of mycorrhizal fungi grow larger than those grown in their absence. Mostly this has been attributed to increased phosphorus uptake (Barea *et al*., 2002; Dashti *et al.*, 1998).

Nutrients that are available in soil can be acquired by plants through two pathways, which are directly from the soil through a plant-specific pathway or through uptake and transport using the AM fungal symbiotic pathway (Smith and Read, 2008). Nutrients are required by plants in varying amounts (Nagy *et al.*, 2009).

The interaction between the symbionts creates a novel interface at the cellular level through which carbon and phosphorus as well as other nutrients are exchanged. In AM fungi phosphate acquisition is usually achieved through membrane integral proteins including phosphate transporters and the P-type H^+- ATPase. Following uptake, transport within fungal tissues is in the form of polyphosphate granules (Dighton, 2009). Within the arbuscular mycorrhizas, specific M+PT4 protein production is closely associated with arbuscule formation and phosphate uptake.

1.4.1.1 Phosphorus

Phosphorus (P) is one of the important nutrients for plant development and growth. It is involved in processes such as energy generation and photosynthesis, and is an important component of cell membranes and nuclei acids (Vance *et al*; 2003). Phosphorous in the environment exists as inorganic orthophosphate ions (H_2PO^{4-}) primarily involved in inert complexes with cations such as iron ($FePO_4$) and aluminium ($AlPO_4$), calcium ($CaPO_4$) phosphates and organic molecules such as lecithin which cannot be absorbed directly from the soil by the plant roots (Smith and Read, 2008; Ticconi and Abel 2004). Phosphorous is required by both symbionts in relatively large amounts, but it is poorly mobile in soil and it is only available in very low concentrations (Quilambo, 2003).

Plants have developed a number of different strategies to enhance phosphorus acquisition. One of these is increasing the root-soil interface through the development of root hairs which increases the general surface area and their growth is influenced by Pi availability (Ma *et al.*, 2001). The second strategy is usually achieved by roots secreting organic acids such as malate and citrate to solubilise phosphate trapped in complexes which compete with Pi for cation-binding partners (Johnson and Loeppert, 2006). The last strategy is the formation of a symbiotic association with mycorrhizal fungi. The hyphal network exploits the soil environmentally absorbing phosphate and translocating it to the roots (Javot *et al.*, 2007; Smith *et al.*, 2003). AM fungi can also facilitate the uptake of phosphorous by secreting enzymes such as phosphatases to hydrolyse and release phosphorous from organic complexes (Schachtman *et al*., 1998). After the phosphate has been acquired from the soil the fungi distributes it to the tissues throughout the plant using phosphate transporters (Javot *et al*., 2007; Smith *et al.*, 2003).

Studies have shown that the extraradical mycelium is responsible for acquisition of phosphate by using active Pi transporters. The fungal hyphae phosphate is stored in the vacuoles of the extraradical hyphae in the form of polyphosphate granules (Ezawa *et al*., 2002).The polyphosphate granules are then transferred to intraradical compartments by means of tubular vacuolar network (Bago *et al*., 2003), which is later translocated to the intraradical hyphae and is released to the plant (Jakobsen *et al*., 1992).

Phosphate transporters are usually specific genes that are found in the AM fungi such as GvPT isolated from *Glomus versiforme*, GiPT isolated from *Glomus intraradices* and GmosPT isolated from *Glomus mosseae* and all are expressed in extraradical hyphae. GmosPT has also been shown to be expressed in intraradical hyphae (Harrison and Buuren, 1995; Maldonado-Mendoza *et al*., 2001). Benedetto *et al.,* 2005 identified and characterised the phosphate transporter gene GmosPT from *Glomus mosseae.* Using Polymerase Chain Reaction (PCR) with PT1 and PT2 primers they obtained a DNA sequence that showed high significant phylogenetic similarity with GiPT and GvPT. Reverse transcriptase PCR experiment were carried out to study GmosPT expression profiles in different AM fungal life stages when exposed to high and low Pi concentrations. GmosPT showed an expression pattern similar to GiPT where high expression was observed in the extraradical mycelium, concluding that GmosPT plays a role in Pi transfer during interactions with the host plant.

The effects of phosphate uptake by AM fungi are usually observed in an increase in root length and shoot dry matter and improvement of plant nutrition (Smith and Read, 2008). Stewart *et al*., (2005) inoculated strawberry plants with different arbuscular mycorrhiza species using *Glomus intraradices*, *Glomus mosseae* and *Glomus etunicatum* under typical strawberry nursery conditions and in soil high in phosphate. They found that AM fungi were able to colonise the strawberry root and reported increased plant productivity compared to controls which were not inoculated with AM fungi concluding that the AM hyphal network, increased the surface area in the strawberry roots making it easier to absorb Pi whereas non-mycorrhizal roots systems may be unable to absorb P effectively resulting in poor growth (Javot *et al*., 2007). Chen *et al* (2004) investigated whether AM fungal colonisation can increase plant P-uptake of a plant community. Their results showed that the total P uptake of plants increased in the plot occupied by the plant mixtures. Hu *et al*., (2010) investigated AM inoculation impacts on plant growth, crop yield, and P acquisition of wheat (*Triticum aestivum* L.) as well as mycorrhizal colonisation. A 45% increase in P acquisition was reported in wheat inoculated with AM fungi when compared to the uninoculated control plants. Guissou, (2009) investigated the effects of an AM fungus on the growth of tamarind and jujube seedlings in a P-deficient soil under nursery conditions by inoculating the tamarind and jujube seedlings with *Glomus aggregatum.* Their findings showed that tamarind and jujube seedlings grew very poorly in the absence of *G. aggregatum* and the inoculated seedlings had greater shoot height and total biomass production. The total dry weight of the fruit tree species was increased 3 and 4 fold by *G. aggregatum*, in both plant species compared to the controls. Jujube inoculated seedlings had the highest AM root colonisation and higher P concentrations (8.33 fold) in shoots and inoculated tamarind seedlings recorded a1.62 fold increase in shoot P concentration, indicating greater mycorrhizal dependency in jujube seedlings. Mohammad *et al.,* (2003) evaluated the effect of AM fungi (*G. intraradices*) on plant growth of Barley (*Hordeum vulgar* L) and nutrient uptake in saline soils with different salt and phosphorus concentrations over a period of five weeks. An increase in the dry weight and the height of the plants was observed. Phosphorus concentration in the plants was higher in the mycorrhizal barley (8.84 mg plant $^{-1}$) compared to the non-inoculated plants which was about 4.96 mg plant $^{-1.}$

1.4.1.2 Nitrogen

Nitrogen (N) is one of the most important macronutrients which significantly increase plant growth and yield production. It is essential for protein synthesis. AM fungal associations have been shown to be able to alleviate the effects of different stresses on plant growth and yield production by significantly increasing the uptake of water and nutrients including N by the host plant (Daei *et al*. 2009; Feddermann *et al*., 2010; Jaskson *et al*., 2008; Miransari, 2011). Nitrogen is absorbed by plant roots as both nitrate (NO^{3-}) and ammonium (NH^{+}) compounds from the soil. The hyphal network is also involved in uptake and transfer of organic N although it is still not clear whether this always occur in amounts that are significant for whole plant nutrition (Jackson *et al*., 1997; Smith *et al.*, 2003). Using labelled N, it has been shown that the hyphae of AM fungi are able to utilize inorganic N efficiently

(Ames *et al*., 1983; Frey and Schüepp 1992; Johansen *et al*. 1993) and transfer it to the host plant. Nitrate is highly mobile and readily available in the soil solution whereas ammonium is comparatively immobile. The AM symbiosis is thought to influence the uptake and translocation of NH^{4+} rather than NO^{3-} (Chambers *et al*., 1980). Since NO^{3-} ions are the main form of N in many agricultural soils they reduce the importance of mycorrhizal fungi in such soils. However NO^{3-} mobility is severely limited by drought due to low concentrations and reduced diffusion rate (Azcon *et al*., 1996). Under such conditions the role of AM fungi in NO^{3-} transport to the root surface may be significant. Arbuscular mycorrhizal fungi also produce enzymes such as glutamine synthase that are required for the assimilation of NO^{3-} into amino acids (Marschner 1995; Tian *et al*., 2009).

Direct transport of N via the extraradical hyphae of AM fungi has been shown in systems where the mass flow and diffusion of N from a compartment containing root-distant hyphae to another compartment containing roots and root-near hyphae was reduced or prevented (Hawkins *et al*., 2000; Johansen *et al*., 1994). Johansen *et al.* (1994), in their experiment showed the external hyphae of *Glomus intraradices* were able to absorb NO^{3-} and NH^{4} and provide them to cucumber plants. The mobility of inorganic P is much lower than inorganic N including $NO3^{-,}$ indicating that the role of AM fungi in absorbing N may be of less significance than P.

Haystead *et al*., (1987) investigated whether AM fungal (*Glomus mosseae*) colonisation of perennial ryegrass (*Lolium prenne* L) and clover (*Trifolium repens*) roots can enhance the exchange of nitrogen between the root systems. Their experiment was to determine whether N applied to the clover plants was translocated to the grass plants growing in the same pot and whether this transfer was enhanced by mycorrhizal colonisation. Their findings showed that mycorrhizal colonisation had a significant effect as a source of nitrogen assimilated by the grass plant and the grass shoot nitrogen was derived from the labelled clover source. Feng *et al*., (2002) investigated plants colonised by *Glomus mosseae* and their utilisation of certain pools of soil N, which are not available to non-mycorrhizal plants. The mycorrhizal inoculum consisted of spore, mycelium and root segments of arbuscular mycorrhizal fungi propagated in *Trifolium repens* pot culture. Nitrogen concentration in shoots was not significantly affected by arbuscular mycorrhizal fungi but the overall nitrogen content was significantly higher in mycorrhizal plants than in non-mycorrhizal plants. The investigators concluded that arbuscular mycorrhizal fungi may facilitate plant acquisition of nitrogen from sources which were not or less available to non-mycorrhizal plants. Tawaraya *et al*., (2007) determine the effects of AM colonisation of roots of *Aloe vera* on nitrogen uptake and growth in peat soil. The *Aloe vera* plants were inoculated with one of two AM fungi, *Glomus clarum* or *Gigaspora decipiens*. Roots in the treatments of both AM fungi were colonised up to 95%, 12 months after transplantation. The nitrogen content was higher in the plants inoculated with *G. clarum* (3.05 mg/g) and *G. decipiens* (3.35 mg/g) compared to the controls which had a concentration of 1.24 mg/g.

1.4.1.3 Water acquisition

Drought is one of the major factors affecting plant growth and yield in dry land areas and AM fungi have been suggested as a factor in increasing tolerance to drastic environmental condition such as drought (Smith and Read 2008; Sylvia *et al*., 1993)resulting in improved water relations of the host plants. It has been suggested that this phenomenon could be due to improvement of hydraulic conductivity, increase transpiration rate, lowering stomatal resistance, reducing leaf elasticity and increasing effective rooting length and depth (Auge *et al*., 1987; Bethlenfalvay *et al*., 1988; Linderman, 1988).

Subramanian *et al.,* (1995) investigated maize (*Zea mays*) growth under drought conditions in a greenhouse experiment where they inoculated maize with the AM fungus *Glomus intraradices*. During the period of drought stress the investigators measured stomatal resistance, transpiration rate and leaf potential of the plant. The mycorrhizal plants had increased leaf water potential, reduced transpiration rate and increased green leaf area (27.5% higher) than the non mycorrhizal plants. Al-Karaki *et al*, (2004) investigated the effects of AM inoculated wheat (*Triticum aestivum L.*) cultivars grown in the field under well watered and water-stressed conditions. Wheat seeds were planted with or without the AM fungi *Glomus mosseae* or *G. etunicatum*. Mycorrhizal colonisation was higher in well-watered plants than water-stressed plants and plants inoculated with *G. etunicatum* generally had higher colonisation than plants colonised with *G. mosseae* under both soil moisture conditions. Porcel and Ruiz Lozano (2004) investigated the increased tolerance of mycorrhizal soya beans to drought resulting from increased root length and depth. Ruiz-Lozano *et al* (1995) evaluated the tolerance of lettuce plants (*Lactuca sativa* L) under drought stress conditions where they inoculated plants with different AM fungal isolates. Seven fungal species belonging to the genus *Glomus* behaved differently but *G. deserticola* had the most effect in improving water deficit tolerance and was associated with the lowest level of growth reduction (9%) under stress conditions. The growth of plants colonised by *G. occultum* was reduced by 70% after a progressive drought stress period. Concluding that *G. deserticola* is more adapted to drought stress conditions and is a better source of inoculum under these conditions.

1.4.3.4 Plant defence

Pathogenic fungi affect plant health which can lead to economic loss by reducing the yield in the agricultural industry. These pathogens can be root-infecting fungi (Azcon-Aguilar and Barea, 1996). The role of AM fungi in the reduction of plant pathogens is relatively well known. The AM association can protect the host plant in a number of ways. The fungi may be provided a direct physical barrier to protect the root. Biochemical defence mechanisms in which secondary metabolites of fungi defend the mycorrhizal root against pathogen have also been suggested. AM fungal hyphae contain similar surface molecules as pathogenic fungi, including chitin, glucans and oligomers which act as general elicitors of plant defence responses (Azcon-Aguilar and Barea, 1996; Harrison, 2005; Whipps, 2004). AM fungi can reduce the effect of pathogenic fungi in roots by competing with the pathogen fungi in a host plant roots. Pathogenic fungi and AM fungi exploit common resources within the root, including infection sites, space and photosynthates within the root (Larsen and Bodker, 2001; Whipps 2004) and interference competition may also arise due to carbon availability within intercellular spaces and the rhizosphere (Graham 2001).

Another mechanism by which AM fungal colonisation can assist the plant against pathogens is that colonisation can influence the root structure of the host plant which would lead to morphological changes such as more branched root system, allowing for the rapid replacement of damaged or diseased roots (Alah *et al*., 2005; Paszkowski *et al*., 2002; Wehner *et al*., 2009). Interactions between changes in the root system and protection of plant roots from pathogen attack have been demonstrated in several species of AM fungi such as *G. mosseae* and *G. etunicatum* (Matsubara *et al*., 1995; Trotta *et al*., 1996; Vigo *et al*., 2000). Another protective mechanism may be due to the influence of AM fungi on mycorrhizospheric microbial communities, which in turn lead directly to the reduction of fungal pathogen populations in the soil (Larsen *et al*., 2003).

Larsen *et al.,* (2011) evaluated the interaction of AM fungi and tomato *(Lycopersicon esculentu*) challenged with *Pythium aphanidermatum.* They used *G. intraradices G. mosseae* and *G. clariodeum. Pythium* recovery from roots colonised by *G. intraradices* and *G. claroideum* was 71% and 76% lower than from roots of non- inoculated plants, whereas the *Pythium* recovery from roots colonised by *G. mosseae* did not differ from that of the non-inoculated plants.

Watenarojanaporn *et al.,* (2011) investigated AM colonisation of citrus plants by AM fungi to determine whether it can reduce infection of *Phytophthora nicotianae* root rot in citrus. They isolated different AM fungi from the rhizosphere of a citrus orchard and used them to inoculate three commercial citrus cultivars, C-35 citrange (rootstock), Shogun and Tangerine to screen for effective AM fungi. AM colonisation in citrus roots differed for each isolate. *Acualospora tuberculata* were the most effective AM species colonising all citrus cultivars, followed by *G. etunicatum,* A. *scrobiculata, G. caledonium, G. aggregatum* and *G. clarum,* while the lowest colonisation rates were found in roots colonised with *Sclerocystis sp.* and *G. calospora*. Shogun roots inoculated with *G. etunicatum* or *A. tuberculata* or *A. scrobiculata* showed the best colonisation rates of 60.13%, 62.36%, and 60.10%, respectively whereas *Sclerocystis* sp. revealed the least colonisation rate of 17.68%. Tangerine roots inoculated with *G. etunicatum* or *A. tuberculata* had significantly higher percentage colonisation (50.73% and 50.17%, respectively). C-35 citrange inoculated roots, showed the greatest colonisation rate produced by *A. tuberculata* at 70.89%, followed by *G. etunicatum*, *A. scrobiculata*, and *G. caledonium*. The rootstocks infected by both *P. nicotianae* and AM fungi showed reduced root injury and those inoculated with AM strain showed healthy roots which were more straight and longer than those of *P. nicotianae* treatment (Watenarojanaporn *et al.,*2011).

1.7 Mycorrhiza helper bacteria

Mycorrhizal interactions are not limited to the plant and fungus. It also involves interactions between other soil microorganisms. The interaction between mycorrhizal fungi and other microorganisms have been shown to contribute towards the symbiotic relationship and are able to promote mycorrhizal development (Garbaye, 1994). A diverse group of bacteria that interact with the mycorrhizal fungi are known as Mycorrhizal Helper Bacteria (MHB) (Frey-Klett *et al*., 2007).

MHB can be found from taxonomically diverse bacterial groups and these bacteria sometimes are closely associated with the mycorrhizal fungus because they are usually fungal-specific but not plant specific (Garbaye, 1994). Duponnois and Garbaye (1991) and Garbaye, (1994) proposed several possible mechanisms in which the MHB could be beneficial to mycorrhizal fungi, these included: MHB could soften the cell wall and the middle lamella between the cell root cortex by producing specific enzymes such as endoglucanases and hydrolases thereby making fungal penetration easier; the MHB interferes with the plant-fungus recognition mechanism where the attachment of bacteria to either the root or fungi or both can improve the cell wall properties or facilitate the establishment if the symbiosis; the MHB assist the fungus during asymbiotic stages in the soil and lastly the metabolic activity of the MHB in the soil can modify the physio- chemical properties in a way that could assist in mycorrhizal colonisation.

The process of recognition between the host plant and mycorrhizal fungus includes the reception of the plant signals by fungal mycelium, chemotrophic hyphal extension growth to the prospective colonisation sites and characteristic changes in mycelia and hyphal morphology which results in establishment in the root. MHB may stimulate fungal growth during the asymbiotic and presymbiotic phases such as spore germination and mycelia growth through soil and on the root surface. Root susceptibility to colonisation may also be enhanced by MHB resulting in increased colonisation (Bowen, 1993). Examples of MHB include strains of Gram-negative Proteobacteria (*Agrobacterium* and *Pseudomonas*), Gram-positive Firmicutes (*Bacillus, Brevibacillus, Paenibacillus*) and Gram-positive Actinomycetes (*Rhodococcus, Streptomyces, Arthrobacter*) (Frey-Klett *et al*., 2007).

The presence of MHB may enhance spore germination by affecting the spore wall through production of stimulants such as CO_2 or by affecting the AM fungal capacity to absorb P and thus support fungal growth. *Pseudomonas* isolated from fungal spores has been shown to stimulate germination of *Glomus versiforme* (Mayo *et al*., 1986). The presences of MHB can assist in stress alleviation, controlling of pathogens and increase nutrient availability (Barea *et al*., 2002; Frey-Klett *at al*., 2007). The presences of MHB can also stimulate the formation of mycorrhizal fungi and hence the term MHB. Budi *et al*., (1999) showed *Paenibacillus* spp isolated from *Glomus masseae* spores were able to stimulate mycorrhizal formation in sorghum. Xavier and Germid, (2003) showed that *Bacillus* spp isolated from *G. clarum* spores increased colonisation by 28 % in pea roots. Pivato *et al*., (2009) tested if bacterial isolates isolated from mycorrhizal spores and showed they were able to promote mycorrhizal development in *Medicago truncatula*. *P.fluorescens* C7R12 promoted growth and root colonisation of *G mosseae* BEG12, indicating that *P. fluorescens sp* acted as MHB.

There is an increasing interest in agriculture for safe and environmentally friendly alternatives to control plant diseases to replace conventional pesticides. MHB could potentially be used in nurseries to promote mycorrhizal development in seedlings and prevent attack by phytopathogens (Frey-Klett and Garbaye 2005). Li *et al*., (2007) found that *Paenibacillus* spp isolated from AM spores had biological control abilities against *Phythium* which caused damping off in cucumbers reducing disease by 78 %.

Bharadwaj *et al.,* (2008) in investigating the role of MHB on how they affect potato growth and colonisation of potato roots by *G mosseae* in a greenhouse and field experiment found that AM colonisation of potato roots was higher in the presences of *Pseudomonas* sp by over 50 % in the greenhouse and *Stenotrophomonas* and *Arthrobacter* isolates were highly effective in the field experiment by increasing the biomass by 15 %. *Pseudomonas* sp also showed an inhibitory effect against both bacterial and fungal pathogens such as *Erwina carotova*, *Phythophtora* and *Verticilluim* sp. *Pseudomonas* sp are known to produce protease, siderophores and Indole Acetic Acid which are possible mechanisms that contributed to their positive response.

Alam *et al*., (2011) reported synergetic effects of the plant growth promoting rhizobacteria (PGPR) *Bacillus subtilis* and AM fungi which increased the total yield of *Pelargonium graveolens* (rose geranium). *Glomus aggregatum, G. intraradices* and *G. mosseae* were used alone and in combination with *Bacillus subtilis* to evaluate their capacity in increasing productivity of *Pelargonium graveolens* in this study. Inoculation with *G. mosseae* increased in total biomass and essential oil content. Freita and Vildoso (2004) performed three greenhouse trials with MHB to see whether they can promote citrus plant growth using fluorescent *Pseudomonas* and *Bacillus* spp, and seven other rhizospheric bacteria. There were tested in three rootstocks seedlings: Cleopatra mandarin (*Citrus reshni*), Rangpure lime (*Citrus limonia*) and Volkamerian lemon (*Citrus volkameriana*). Depending on the rootstock, seven *Pseudomonas,* one isolate of *Bacillus* and one of other rhizospheric bacteria increased the root or shoot dry weight, indicating a high proportion of growth promoters among the fluorescent pseudomonads.

Although MHB have shown positive results in promoting plant growth and colonisation by AM fungi, the evidence is not always as clear cut. Pirlak and Kose, (2008) investigated the growth promoting effects of foliar and root application of *Bacillus* and *Psuedomonas* strains on strawberry yield. Results indicated significant increase in plant growth compared to the controls although the bacterial effect did not influence the average fruit weight and yield.

1.8 Plant growth promoting rhizobacteria

Rhizobacteria are found at the root soil interface. Interactions between plant roots and rhizobacteria that increase plant growth have been termed plant growth promoting rhizobacteria (PGPR) through any beneficial activity concerning plant growth improvement (Kennedy *et al*., 1997; Kloepper *et al*., 1991; Pattern and Glick, 2002).

A number of bacterial species have been shown to have plant growth activity and belong to the genera Pseudomonas*, Bacillus, Rhizobium, Burkholderia, Enterobacter, Arthrobacter, Flavobacterium* and *Azospirillium* spp *(*Kim *et al*., 2010; Kohler *et al*., 2009; Okon and Kapulnik 1986). PGPR have a positive effect on plant growth and their influence can be either direct or indirect (Rodriguez and Faga, 1999). Indirect growth promotion is due to the decrease or prevention of deleterious effects of pathogenic microorganisms due to the synthesis of antibiotics or siderophores by the rhizobacteria. Direct promotion mechanisms may be due to the production of phytohormones like Indole Acetic Acid (IAA) (Pattern and Glick, 2002), nitrogen fixation, synthesis of enzymes that modulate the level of plant hormones, production of amino- cyclopropanel carboxylate (ACC) deaminase to reduce the level of ethylene in the roots of the developing plants thereby increasing the root length and growth, phosphate solubilisation and nutrient uptake and growth under water stress (Malekzadeh *et al*. 2011; Marschner and Dell 1994; Sarig *et al*., 1988).

PGPR are able to assist plants in acquiring nutrients from soil and such as *Rhizobium* and *Azospirillium* sp are able to increase the AM fungal development by affecting root colonisation as well as by enhancing plant N and P uptake (Artursson *et al*, 2005). The production of extracellular polysaccharides by *Azospirillium* and *Rhizobium* significantly enhance attachment of the bacterial strains to mycorrhizal roots and AM fungal structures (Miransari, 2011). The growth promotion activities of these rhizobacteria have been related to root formation, nutrient cycling, seedling emergence and nodulation (Kloepper *et al*., 1991). Figueiredo *et al*., (2008) evaluated the effects of PGPR on nodulation and nitrogen fixation on growth of *Phaseolus vulgaris* (bean) in a greenhouse experiment for 40 days. Seeds were inoculated and/or co-inoculated with *Rhizobium tropici*, *Bacillus endophyticus, B. pumilus, B. subtilis, Paenibacillus lautus, P. macerans and P. polymyxa.* Five bean seeds were sown in each pot and inoculated with 5 ml pot^{-1} of liquid culture containing *Rhizobium sp*. and *Bacillus sp*. and/or *Paenibacillus spp.* Beans co-inoculated with *Rhizobium tropici* and *Paenibacillus polymyxa* had higher leghaemoglobin concentrations, nitrogenase activity and N_2 fixation efficiency indicating that the dual inoculation stimulated nodulation as well as nitrogen fixation. Esitken *et al*., (2006) investigated the growth promoting effects of *Pseudomonas* BA-8 and *Bacillus* OSU-142. Experimental plants were grown over a period of 2 years and after harvesting a significant increase in yield was obtained in the plants that were treated with *Pseudomonas* compared to control plants. Orhan *et al*., (2006) evaluated plant growth effects of *Bacillus* OSU-142 and *Bacillus* M3 on raspberry fruit yield, and inoculated the plant roots with the bacterial suspensions using root dip. Results showed the yield was significantly increased by *Bacillus* M3 compared to the control but there was no increase in plants that were treated with *Bacillus* OSU-142. Alagawadi and Gaur (1988) inoculated chickpea with *Rhizobium sp* and *Pseudomonas sp* in trying to increase yield and the PGPR were able to increase the nodulation, dry matter content yield and nitrogen and phosphorus uptake significantly over un-inoculated plants.

PGPR have been shown to have the ability to solubilise phosphate contributing to nutrient acquisition (Richardson *et al*., 2009). Kim *et al*., (1997) evaluated the synergetic effects of AM fungi and phosphate solubilising bacteria (PSB) on P uptake and tomato growth. Tomato seedlings were inoculated with the *Glomus etunicatum* alone and in combination with *Enterobacter agglomerans.* Inoculation with *E. agglomerans* and *G. etunicatum* alone and *E. agglomerans + G. etunicatum* in combination increased plant growth by 40 % compared with the control. Significantly higher soluble phosphorus concentrations were observed in treatments of *E. agglomerans* and *E. agglomerans + G. etunicatum* compared to uninoculated seedling. There was no significant difference in soluble P concentration in the rhizosphere between treatments with time. De Ferreira *et al*., (2008) investigated the role of phosphate solubilising bacteria (PSB) in promoting rice growth in pot trails. Shoot height and root length increased but there was no significant increase in the shoot dry matter.

PGPR have been shown to alter several hormonal pathways which may lead to different morphological changes in plant roots, such as lateral root initiation, elongation and root hair development (Beneduzi *et al*., 2008). Indole Acetic Acid (IAA) production is considered as the most important tool in screening for beneficial plant growth promoting bacteria as there have been several studies that showed IAA producing bacteria to have a positive effect on plant growth (Shahab *et al.,* 2009). IAA is produced from tryphophan via the intermediate indole acetic amide. PGPR synthesise IAA predominantly by alternate tryptophan dependent pathway through indole pyruvic acid (Pattern and Glick, 2002).

Ahmad *et al*., (2003) evaluated *Pseudomonas* and *Azotobacter* isolates for IAA production and their effects on root elongation was evaluated in different concentrations of tryptophan, of 0, 1, 2, and 5 mg/ml. Without tryptophan, the root elongation of germinating seeds of *Sesbania aculeata* and *Vigna radiata* was highest with *Azotobacte*r isolate Azs9, followed by Azs1 and Azs6, compared to the control, whereas the root length decreased in *Pseudomonas* isolates at 1 and 2 mg/ml tryptophan concentrations in *S. aculeate* and in *V. radiata*. In the case of *V. radiata* at 2 mg/ml of tryptophan only Azs9 showed significant root elongation. At a 5mg/ml tryptophan concentration in both *S. aculeata* and *V. radiata* the root elongation decreased in the presence of all isolates, which lead to the conclusion that tryptophan at a 5 mg/ml concentration was toxic in the presence of test bacteria.

Pattern and Glick, 2002 investigated if IAA was involved in the stimulation of root growth by *Pseudomonas putida* GR12-2. The *ipdc* gene encoding indolepyruvate decarboxylase, which catalyzes a key step in the indole pyruvic acid pathway for IAA synthesis, was isolated. An IAA-deficient mutant was constructed by insertional mutagenesis of *ipdc*. Mung beans were used as the test plant. Their results concluded that IAA produced by *P. putida* GR12-2 had a significant impact on the ability of this bacterium to stimulate the growth of the primary roots of mung beans. The roots from seeds treated with wild-type *P. putida* GR12-2 were on average 35% longer than the roots from uninoculated control seeds, the lengths of roots from seeds treated with the IAA deficient mutant were not different from the lengths of roots from uninoculated control seeds. IAA produced from the wild-type strain had no effect on shoot length. PGPR inoculation was also shown to promote cell division of wheat roots (Levanony and Bashman 1986) and increased the diameter and length of lateral roots in maize and enhanced root hair production (Kapulnik and Okon 1983).

Some reports have shown that the PGPR have biological control activity (Field *et al*., 2008). Some PGPR, especially if inoculated on the seed before planting, are able to establish themselves on the crop roots and use scarce resources which then lead to limiting or preventing the of pathogenic microorganisms (Khalid *et al.*, 2004, Kloepper 1991). Siderophore production is usually an important mechanism for biological activity of PGPR. Siderophores are synthesised by PGPR to compete with fungal pathogens for iron uptake as they are iron chelators that are usually produced by bacteria under conditions of iron deficiency (Saha *et al.*, 2012). Siderophores display a selectivity for iron that is reflected in the corresponding complex stability constant that is higher with Fe^{3+} than with Al^{3+} or with bivalent cations like $Ca^{2+,}$ Cu^{2+} or Zn^{2+} (Wandersman and Delepelaire, 2004). PGPR excrete desferrisiderophores in order to scavenge iron from the environment (Robinson *et al*., 2003).

Wahyudi *et al* (2011), using *Bacillus* sp as a plant growth promoter in a soybean greenhouse experiment, found that 12 *Bacillus* isolates had the ability to promote growth in plants due to the production of siderophores. In a study by Datta *et al.*, (2011) PGPR isolated from the rhizosphere of chilli that were evaluated for plant promoting characteristics in pot trails. Although the strains were not identified, C2 and C32 were able to increase growth and productivity in the experiment concluding that it could be due to the fact that C2 and C32 were able to produce siderophores. Strain C2 and C32 also showed some biological control activity against *Fusarium sp.*

1.9 Pathogens of Citrus

Citrus fruit are amongst the most important crops in terms of total yield worldwide and rank first in international fruit trade in terms of value. More than seven million hectares of citrus are planted throughout the world (Cacciola and Magnano di San Lio, 2008). South Africa is the world’s second largest exporter of fresh citrus and is ranked the 14th in citrus production (Sibiya, 2010). According to the Citrus Growers Association of Southern Africa, South Africa produced 1.64 % of the world fresh citrus in 2010. The genera *Phytophthora* and *Pythium* cause the most serious soil borne diseases of plants. Both genera belong to the class oomycetes and the most prevalent *Phytophthora* sp that affect citrus plants are *Phytophthora* *citrophthora*, *Phytophthora nicotianae* and *Phytophthora palmivora* and for *Pythuim* sp, *Phythuim ultimum* the one that is most harmful in citrus. These species can cause brown rot and gummosis on the trunk of the trees (Graham *et al*., 1998). Root rot is most harmful when plants are grafted onto a susceptible rootstock resulting in a slow decline of the tree; the leaves turn light green and may drop depending on the level of infection. Root rot can be difficult to detect by visual inspection because it has symptoms similar to those caused by other factors such as reduced soil aeration and excessive salt content in irrigation water. The disease destroys the feeder roots of the susceptible rootstock and the pathogen infects the root cortex which turns soft and separates from the stele limiting nutrient uptake (Coccialia and Magnano Di San Lio, 2008). These species if not controlled can cause high yield losses, when the pathogens are present in high populations (Bowman *et al*., 2007). The presence of *Phytophthora* sp is usually associated with periods of prolonged wetness exceeding 7 days and temperatures ranging from 23^{o}C to 32^{o}C (Graham and Timmer, 1992).

Outbreaks of *Phytophthora* have been reported in Florida. In trying to find clear explanation of the *Phytophthora* spp that was causing an outbreak of citrus brown rot in Florida during the period of 1994 to 1995 Graham *et al*., (1998) characterised the isolates using morphological methods, pathogenicity and the symptoms on citrus fruit, mating behaviour and cellulose acetate electrophoresis for enzyme analysis. The cause of that outbreak was not caused by *P. nicotianae* as suspected but by the *P. palmivora*. Methods used to detect and identify *Phytophthora* species involved baiting from the soil or isolation by means of selective media from infected plant tissue or soil then followed by pure-culturing the organism and identifying based on their morphological characteristics (Timmer *et al*., 1998) but these procedures are time and labour consuming, and have subsequently been replaced by molecular methods.

Molecular detection methods developed include PCR with species specific primers, nested PCR as well as real time PCR (Ippolito *et al*., 2002; Ippolito *et al.*, 2004). These methods are more sensitive, highly specific and rapid. Ippolito *et al.,* (2004) described the development of PCR primers from the internal transcribed space ITS (internal transcribed spacer) regions and their use in a nested PCR assay to detect *Phytophthora* species in citrus roots and soil. Bowman *et al*, (2007) investigated whether molecular methods based on the ITS–fingerprinting can be effectively used for the detection and identification of *Phytophthora* spp of citrus under controlled conditions of greenhouse. They found PCR amplification of ribosomal ITS regions is a valuable tool for detecting and identifying the *Phytophthora* spp.

1.10 Management for citrus pathogens

The common and wide spread occurrence of *Phytophthora* spp that are pathogenic to citrus continue to have a great influence on production and control can be achieved by adapting safer cultural practices and using preventative measures (Cacciola and Magnona di San Lio, 2008). *Phytophthora* diseases are mostly managed by fungicides, use of tolerant rootstocks and biological control agents which reduce infections and increase yield (Colburn and Graham, 2007). Control of root rot of citrus begins with the production of disease free seedlings since most *Phytophthora* associated with citrus root rot in the field probably originates from infested nursery stock (Ippolito *et al.*, 2002; Menge and Nemec, 1997).

The most commonly used agrochemicals are metalaxyl and Al-ethyl-phosphyte (fosetyl –Al). These fungicides are effective against both *P. citrophthora* and *P. nicotianae* but there are slight differences in the range of action, fosetyl-Al is more effective against *P. citrophthora* whereas metalaxyl is more effective against *P. nicotianae* (Cacciola and Magnona di San Lio, 2008). Afek and Sztejnberg (1989) in their research of determining the mode of action of fosetyl-Al in citrus found that low concentrations of fosetyl-Al act against *P. citrophthora* by increasing the plant defence mechanisms against the pathogens and that higher concentration are fungistatic. There is an increasing concern about the use of chemicals in the environment and pathogens have acquired a degree of resistance to chemical fungicides (Levy *et al.*, 1983).

Resistance of metalaxyl has been detected in *Phytophthora* species like *P. nicotianae* as well as in *Pythium* species so the restriction on the use of soil fumigants and the emergence of metalaxyl- resistance populations have shifted the focus of *Phytophthora* disease control to alternative strategies including the use of biological control agents. Studies regarding the use of alternative methods have investigated the efficacy of various fungal and bacterial biological control agents against species of *Phytophthora* and *Pythium* causing root rot in citrus. *Chaetomium*, *Penicillium* and *Trichoderma* species are biological control agents that have shown promising results. Field trials have shown that *Chaetomium* formulated bioproducts have promising results as a broad spectrum mycofungicide to control many diseases and *Penicillium funiculasum* could inhibit the growth of *P. parasitica* and *P. citrophthora* (Soytong *et al.*, 2001; Fang and Tsao, 1995). Kean *et al.*, (2010) compared chemical fungicides and biofungicide products against citrus root rot caused by *Pythium ultimum*, they tested biological fungicides from *Chaetomium* spp and *Trichoderma* spp and used metalaxyl as a chemical fungicide. Their results showed that fungicides from biological products can work as effectively as the chemical fungicides (Kean *et al*., 2010).

MOTIVATION

Citrus is an economically important agricultural product in South Africa as it is the world's second largest exporter of fresh citrus and is ranked the 14th in citrus production (Sibiya, 2010). Production is heavily reliant on chemical inputs and as orchards are in production for several years, sustainability of soil and is environmentally of importance. This study investigates the role of arbuscular mycorrhizal fungi and their associated bacteria as an alternative form of growth promotion in seedlings and associated bacteria as a form of biological control against citrus pathogens. AM fungi are the most common mycorrhizal type and are associated with a wide variety of host plants. AM fungi are different from other fungi as they have the ability to create an interface for nutrients exchange which occurs within living cells of the plant (Brundrett, 2004), but the function of mycorrhizal fungi goes further than nutrient acquirement, they also provide several other benefits to the host plant which include access to less labile nutrients from organic and complexed inorganic sources, enhanced water uptake, resistance to pathogenic fungi and harmful bacteria as well as protection against heavy metal in the soil (Martino *et al.*, 2009). The use of AM fungi in agriculture is also of importance as the established mutualistic symbiosis acts as a living bridge between soil and plants. It has the potential to sustainable increase citrus production by providing alternative pathways of nutrient plant uptake due to the increased access to the soil environment. The AM fungi affect the physicochemical characteristics of the substrate and contribute to the formation and maintenance of soil structure, enmeshing soil particles by means of extraradical hyphae and their exudates and residues (Tisdall and Oades, 1982) and produce glomalin, a protein extracted from the soil as glomalin-related soil protein, which plays a key role in soil stability (Bedini *et al.*, 2009; Rillig *et al.*, 2002). Although there are many mycorrhizal benefits the promotion of plant growth in association with interacting bacteria was of interest in this study.

HYPOTHESIS

The combined use of arbuscular mycorrhizal fungi and associated bacteria can improve citrus growth and health.

OBJECTIVES

1. Isolation of bacteria from AM fungal spores
2. Assessment of antagonist activity of bacterial isolates against *Phytophthora.*
3. Evaluation of plant growth promoting properties of bacterial isolates
4. Identification of selected bacterial isolates
5. Investigating whether bacterial isolates associated with AM fungi are important for mycorrhizal establishment and plant growth.

CHAPTER 2
MATERIALS AND METHODS

2.1 Isolation of arbuscular mycorrhizal spores and associated bacteria

Composite soil samples were collected from organically managed soils under citrus trees and spores were extracted using the wet sieving and decanting method (Smith and Dickson, 1997). Three hundred grams of soil was weighed and placed in a beaker. A soil solution was made by adding approximately 500 ml of water to the soil. The solution was stirred to free spores from particles and allowed to settle for 45 sec. The supernatant was decanted through a nest of sieves having mesh sizes of 425 µm, 250 µm, 125 µm and 45 µm. The sieves were then washed and the debris from the 425 µm discarded. The debris from the remainder of the sieves were washed into 50 ml centrifuge tubes and filled with water. The spores were then centrifuged using Megafuge 1.0R centrifuge for 5 min at 1900 rpm. The supernatant was discarded. The pellet was resuspended in 60% sucrose solution and centrifuged for a further 5 min. The supernatant was filtered through a Buchner funnel onto a grided filter paper disc and rinsed with distilled water. The filter paper was then transferred to the lid of a clean petri dish. The spores were examined under a Leica S 4E dissecting microscope and collected for bacterial isolations. Six extractions were done in oder to obtain surficent spores

2.1.1 Bacteria associated with AM spores

2.1.1.1 External bacterial isolation

Ten spores of the AM fungi were placed in a 1.5 ml microcentrifuge tube with 0.2% sterile saline/Tween solution. The tube was vortexed and the spores were allowed to soak. A sample from the solution was then diluted to 10^{-3} with sterile distilled water and 0.1 ml aliquots were spread plated on Nutrient Agar (NA) Biolab (Catalogue No HG000C1.500) plates and incubated at $37^{o}C$ for 72 hrs. Single distinct colonies were selected and discontinuously streaked onto NA plates to establish pure cultures. Colony morphology was recorded.

2.1.1.2 Internal bacterial isolation

Ten AM fungal spores isolated from the soil were selected and surfaced sterilised by soaking them in a solution of ampicillin (50µg/ml), chloramphenicol (50µg/ml) and rifampicin

(100µg/ml) for 30 min in a 1.5 ml microcentrifuge tube. The tubes were centrifuged in a Hangzhou Allsheng Instrument Super Mini Centrifuge at 10 000 rpm for 15 min to pellet the spores and the antibiotic solution was removed. Thereafter chloramine T was added to the spores and left for soak for 30 min. Spores were subsequently rinsed 5 times after centrifugation in sterile 0.2% saline. After the last rinse spores were crushed using a sterile micropestle to release the internal bacteria into the sterile saline. The saline containing the crushed spores was serial diluted to 10^{-3} and 0.1 ml aliquots were spread plated onto NA plates. The plates were incubated at 37^{o}C for 72 hours. Single distinct colonies were selected and discontinuously streaked onto NA plates to establish pure cultures. Colony morphology was recorded.

Pure cultures of the externally and internally isolated bacteria were inoculated in 0.5 ml of Nutrient Broth (NB) Biolab (Catalogue No HG000C24.500) and incubated overnight at 37^{o}C. An equal quantity of sterile 50% glycerol solution was added to the culture and vortexed. Replicates of all the cultures were stored in a -20^{o}C and -80^{o}C freezer.

2.2 Morphological identification

2.2.1 Gram staining

Gram staining is a procedure used to distinguish between Gram positive and Gram negative bacteria resulting from their differences in the chemical and physical properties of the cell wall. Gram stains were performed on all isolates to ensure that the isolates were purified as well as to determine the shape and Gram reaction of the cells. For each isolate, a smear was prepared by transferring a drop of water on the slide and picking a colony from the pure isolate. The smear was heat fixed. The smear was flooded with crystal violet stain for 60 sec turning the bacteria a dark purple colour. The smear was gently rinsed to remove excess stain with a stream of water. The smear was flooded with iodine solution and left to stand for 30 sec. The iodine which fixed the crystal violet solution was poured off and rinsed with water. The excess water from the surface was gently shaken off. The smear was decolorized with 94 % ethanol for 5 sec. The ethanol dissolves the lipids in the cell wall. The smear was then counterstained with a safranin solution for 20 sec. After the smear was rinsed, it was allowed to dry. Bacterial isolates were examined under oil immersion on a Nikon YS100 compound microscope. Gram positive bacteria have a thicker peptidoglycan (dissacharrides and amino acids) layer than the Gram negative bacteria (Black, 2008). During the crystal violet staining Gram positive cells retain the stain due to their peptidoglycan layer but in Gram negative the ethanol wash disrupt the outer lipopolysaccharide layer and the crystal violet- iodine complex is washed away through the thin layer of peptidoglycan. The Gram negative cells become colourless until counterstained with safranin in which they retain the pink colour (Black, 2008). Gram reaction as well as cell shape was recorded. Gram stain reaction was used to determine the morphology for identification purposes

2.3 Evaluation of bacteria properties

2.3.1 Phosphate solubilisation

The bacterial isolates were grown overnight in NB at 37^{o}C in a 1.5 ml microcentrifuge tube for 24 hrs. Their concentrations were measured at a wavelength of 660 nm in UV mini-120 UV-VIS spectrophotometer. The concentrations were adjusted to an OD reading of 0.5 by diluting with sterile 0.2% saline. Phosphate-solubilising potential of the bacterial isolates was tested using modified phosphate medium-NBRIP (National Botanical Research Institute's Phosphate growth medium, (Mehta and Nautiyal, 2001). The media contained 10 g glucose, 5 g $CaHPO_4.2H_2O$, 5 g $MgCl_2.6H_2O$, 0.25 g $MgSO_4.7H_2O$, 0.2 g KCL, 0.1 g $(NH_4)_2$ SO_4 and 15 g of agar in 1 L. The media was autoclaved and poured into Petri dishes. On each plate four wells were punched using a sterile glass puncher and 50 µl of the bacterial suspension was added to each well (Fig 2.1). The plates were incubated for 24 hrs at 37^{o}C. Phosphate solubilisers produce clearing zones around the colonies in media containing insoluble $CaHPO_4.2H_2O$ (Rodriguez and Fraga, 1999). Five replicates of each bacterial isolate were used and controls were inoculated with sterile saline.

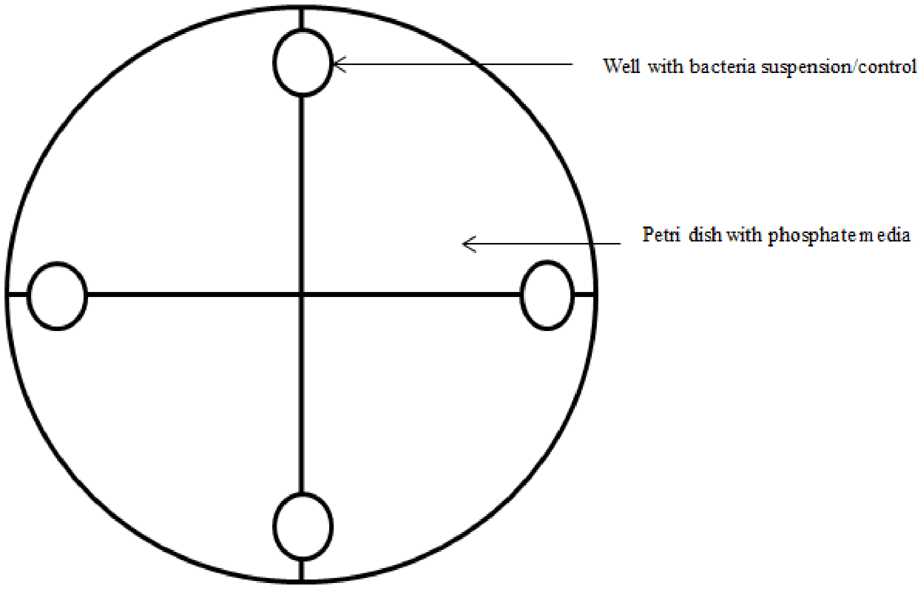

Figure 2.1: Experimental plate design used for testing phosphate solubilisation method

2.3.2 Indole acetic acid production

The bacterial isolates were grown in 1.5 ml DEV Tryptophan Broth (Merck Catalogue No: 1106940500) for 3 days at 37^{o}C. This media contains L-tryptophan which is a precursor for the formation of Indole Acetic Acid (IAA). Cells were centrifuged at (4500 rpm for 20 min) and 500 ml of the supernatant was added to 600 µl, Kovacs Reagent (Merck Catalogue No: 1092930100). A colour change to red was indicative of IAA production (Cappuccino and Sherman, 2005). Five replicates for each bacterial isolate were performed. Un-inoculated broth was used as a control.

2.3.3 Fungal pathogen inhibition

Fungal pathogen inhibition experiments were conducted to determine if any of the bacterial isolates had an inhibitory effect against the citrus pathogens, *Phytophthora citrophthora* (PPRI 10961), *P. citrocola* (PPRI 10963), and *P. nicotianae* (PPRI 10962*)* obtained from National Collection of Fungi. *Phytophthora* isolates were grown on Potato Dextrose Agar (PDA) (Bidab Catalogue No HG00C100.500) for 7 days at 25^{o}C. Bacteria were grown in NB at 37^{o}C for 24 hrs in 1.5 ml microcentrifuge tube. The optical densities of the bacterial culture were measured at 660 nm in a spectrophotometer, and were adjusted to an OD reading with the lowest concentration value by diluting with sterile saline. The lowest OD for the isolates was 0.5. A volume of 50 µl of the bacterial isolates was inoculated into wells on PDA plates at four points along a perpendicular axis. A plug of the fungal pathogen *(Phytophthora*) was placed in the middle of the PDA plate (Fig 2.2). The fungal growth was measured (mm) along the two perpendicular axes for approximately 14 days. Five replicates of each bacterial isolate were used as well as five control plates which contain sterile saline instead of bacterial isolates. Growth curves were plotted to determine the effect of bacterial isolates.

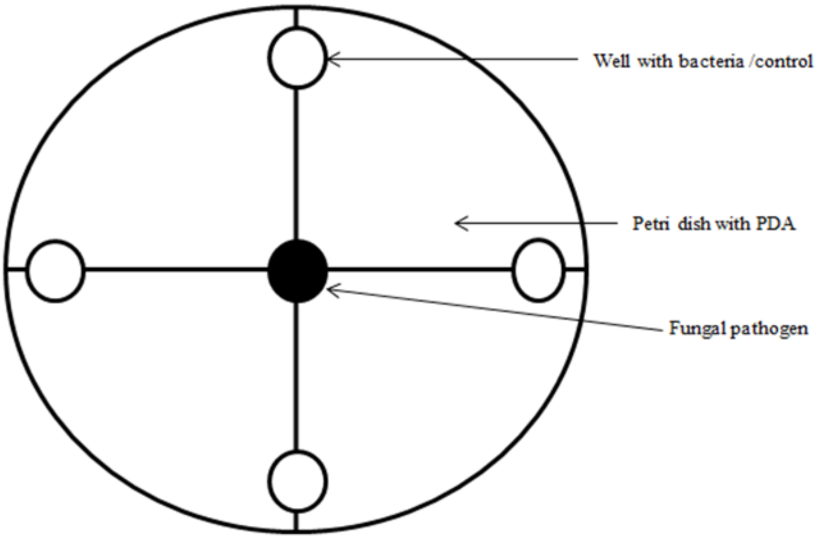

Figure 2.2 Experimental plate design used for fungal growth inhibition studies

2.3.4 Metabolite inhibition assay

From the initial fungal inhibition results three bacterial isolates were tested against *Phytophthora citrophthora, P.citrocola,* and *P.nicotianae.* These three bacterial isolates were tested for the presence of secreted compounds that could have any inhibitory effect on these pathogens. The bacterial isolates were grown overnight in NB at 37^{o}C in 1.5 ml microcentrifuge tube. The cells were centrifuged at 10.000 rpm for 5 min to pellet the cells and the supernatant was retained. The fungal pathogens were grown in 2.5% Malt Extract Broth (MEB) Bidab (Catalogue No HG00C10.500) and incubated at 28^{o}C on a rotary shaker for 7 days. A blender was used to homogenise each isolate and their OD was measured at 600 nm and were adjusted to 0.5 OD by diluting with 2.5% MEB.

The fungal inhibition experiment was set up on a 96 well micro-titre plate. In column 1A to 1E the wells were filled with 100 µl of NB as a control, column 2A to 2E were filled with 100µl of MEB as a control, column 3A to 3E wells were filled with 100 µl of bacterial suspension 1, 4A to 4E wells were filled with 100 µl of bacterial suspension 2, 5A to 5E wells were filled with 100 µl of bacterial suspension 3, 6A to 6E wells were filled with 100 µl *Phytophthora citrophthora,* 7A to 7E wells were filled with 50 µl bacterial suspension 1 plus 50 µl *P. citrophthora,* 8A to 8E wells were filled with 50 µl bacteria 2 and 50 µl *P. citrophthora*, 9A to 9E wells were filled with 50 µl bacterial suspension 3 and 50 µl *P. citrophthora*, 10A to 10E wells were filled with sterile distilled water as an additional control and to maintain humidity. This experiment was repeated for, *P. citrocola,* and *P. nicotianae.* Five replicates (A - E) were made for each fungal isolates Table 2.1. The plates were incubated at 28^{o}C and the optical density was measured for 5 days using a Bio-Tek Instruments Power Wave X at an absorbance of 600 nm.

Table 2.1: Metabolites inhibition assay. Experimental design of microtitre plates.

	1	2	3	4	5	6	7	8	9	10
	NB	**MEB**	**BS1**	**BS2**	**BS3**	**F**	**BS1+F**	**BS2+F**	**BS3+F**	**s.dH$_2$O**
A-E	**NB Control**	**MEB Control**	**Supernatant**			**Fungal control**		**Treatments**		**Additional control**

NB - Nutrient Broth, MEB - Malt Extract Broth, BS1- Bacterial suspension of isolate E5, BS2- Bacterial suspension of isolate E9, BS3- Bacterial suspension of isolate I19, F - Fungal pathogen, s.dH2O - Sterile distilled water

Percentage inhibition of pathogen growth was calculated.

% I = 100 (C-T)/C

Where I= inhibition of pathogen growth,

C= growth of pathogen and

T= treatment growth (Prasanna *et al*., 2010)

2.4 Molecular identification of selected bacterial isolates`

2.4.1.DNA Extraction

The bacteria isolates were grown overnight in NB at 37^{o}C in 1.5 ml microcentrifuge tubes. DNA was extracted using the ZR Fungal/Bacterial DNA Mini-Prep kit (Catalogue No D6005) according to the manufacturer's instructions. A 200 µl bacterial suspension was placed in a Bashing Bead lysis tube before the additton of 750 µl of lysis solution to the tube. The tube was centrifuged for 5 min at 10, 000 rpm in-order to break the cell wall and remove the lipid membrane. The ZR BashingBead^{TM} lysis tube was then centrifuged in a microcentrifuge at 10,000 rpm for 1 min.

The supernatant of volume (400µl) was transferred into a Zymo-Spin^{TM} IV Spin Filter and centrifuged at 7,000 rpm for 1 min, after which 1200 µl of Binding Buffer was added to the filtrate in the collection tube to bind to the DNA while other molecues remain in the filtrate and 800 µl of the fitrate was transferred to a Zymo–Spin^{TM} IIC Column and was centrifuged at 10,000 rpm for 1 min. The procedure was repeated with the remaining mixture before adding 200 µl DNA Pre-Wash Buffer to the Zymo- Spin IIC Column to wash away the impurities, prior to centrifuging at 10,000 rpm for 1 minute in a new collection tube. Thereafter 500 µl DNA Wash Buffer were added to the Zymo-Spin IIC Column which was centrifuged at 10,000 rpm for a min. The empty tube was centrifuged again to remove excess buffer. The Zymo- Spin Column was transferred into a clean 1.5 ml microcentrifuge tube prior to adding 100 µl of DNA Elution Buffer directly to the column matrix and centrifuging at 10,000 rpm for 30 sec for final DNA elution. extracted DNA (10 µl) was evaluated using electrophoresis in a 0.5 µg/ml ethidium bromide stained 1% (w/v) agarose gel and a 100 bp DNA molecular marker (Promega Catalogue no G210A) was used to estimate the size of the DNA. The gel was run for 45 min at 100V. Visualization was carried out on a UV Fluorescence Uvitec Gel Doc System.

2.4.2 Polymerase Chain Reaction amplification

Polymerase Chain Reaction (PCR) is a technique used for amplification of a double stranded DNA template by using short single stranded DNA fragments which are called primers in a presence of an enzyme *Taq* polymerase (Black, 2008). Amplification of the 16S rDNA bacterial gene was carried out with a reaction volume of 50 µl with 5 µl template DNA, 2 µl of Fd1 and 2 µl of rP2 primers (Weisburg *et al*., 1991), 25 µl of KAPA Biosystems (Catalogue KK1024) ready mix containing Kapa *Taq* DNA polymerase (0.05 U/ul 1.25 µl per 25 µl), reaction buffer, $MgCl_{2,}$ 0.4 mM for each dNTP, loading dye and 16 µl of sterile distilled water.

Table 2.2: Oligonucleotides for 16S rDNA gene sequence analysis used in this study

Primer	Sequence	Reference
Fd1	5'AGAGTTTGATCCTGGCTCAG 3'	Weisburg *et al*., 1991
rP2	5'ACGGCTACCTTGTTACGACTT 3'	Weisburg *et al*., 1991

Amplification was carried out using a 2720 Thermal Cycler (Applied Biosystems) with the following thermal profile: Initial denaturation step at 95^{o}C for 90 s, in this step the double stranded DNA separates into two strands followed by a 30 cycle denaturation step at 95^{o}C for 45 s. Annealing at 50^{o}C for 45 s with 30 cycles and in this step the primers bind to the desired known site on the single DNA strands. Extension at 72^{o}C for 60 s with 30 cycles, this step is where the *Taq* polymerase synthesizing the DNA elongating the strand (Black 2008). The PCR conditions are presented in Table 2.3

Table 2.3: PCR cycling conditions used for amplification of bacterial 16s DNA

Conditions	Temperature (^{o}C)	Time (seconds)	Cycles
Initial Denaturation	95	90	1
Denaturation	95	45	30
Annealing	50	45	30
Extension	72	60	30
Final Extension	72	120	1

2.4.3 Agarose gel electrophoresis

The evaluation of 16s DNA amplification after PCR was achived by electrophoresis through 1% (w/v) agarose gel containing 1X Tris-Acetate-EDTA (TAE) buffer, 2 µl (concentration 0.5 µg/ml) ethidium bromide for 45 min at a current of 42 Amps and 100V and 5µl of DNA tamplate was used. The size of the amplified DNA was determined using Promega Lambda/*EcoR1 + HindII* (Catalogue No G1731) marker. The gel was visualized under UV fluorescence on a Uvitec gel doc (Moore *et al.*, 1987). Expected number of base pairs was about 1500 bp

2.4.4 DNA purification

During PCR amplification residual reaction components such as dNTPs, primers and enzymes can interfere with the DNA sequencing process.The PCR products were purified using a Promega Wizard® SV PCR clean up kit (Catalogue A9281/2/5) following the manufacturer's instruction. The PCR product was transferred into a 1.5 ml microcentrifuge tube and 2 times the volume of the membrane binding buffer was added to the PCR product. The mixture was then transferred to a SV micro-column with a collection tube and incubated at room temperature for 1 min. The samples were then centrifuged at 16,000 rpm for 1 min. The flow through was discarded and 700 µl of membrane wash solution was added to the column and centrifuged at 16,000 rpm for 1 min. Another 500 µl of the membrane wash solution was added and centrifuged as previously described. The empty column was centrifuged again for 1 min to remove the residual. The column was transferred to a new 1.5 ml microcentrifuge and 50 µl of nuclease free water was added, prior to incubation on the bench at room temperature for 1 min. The tubes were then centrifuged at 16,000 rpm for 1 min in order to elute the purified DNA. The purified DNA was then stored at -20^{o}C. To evaluate the purity of the PCR product 5µl of the PCR product was electrophoresed through 1% (w/v) agarose gel containing 1X Tris-Acetate-EDTA (TAE) buffer, 2 µl (concentration 0.5 µg/ml) ethidium bromide for 45 min at a current of 42 Amps and 100V and 5µl of DNA tamplate was used. The gel was visualized under UV fluorescence on a Uvitec gel doc (Moore *et al.*, 1987)

2.4.5 Sequencing

PCR products were sent for sequencing to Inqaba Biotechnology, Pretoria, South Africa sequencing facility. Big Dye V3.1 was used to sequence the sample using Sanger sequencing. The sequences were read using Finch TV software. Nucleotide sequences were identified using Basic Local Alignment Search (BLAST). BLAST is a searching program on the National Centre for Biotechnology Information (NCBI) site http://www.ncbi.nlm.nih.gov

2.5: Growth evaluation of citrus plants

2.5.1 Inoculation of the citrus seedlings with AM fungi and bacterial isolates

Three bacterial isolates showing potential plant growth promotion properties were selected for use in greenhouse trials. Bacterial isolates were grown overnight in Nutrient Broth at 37^{o}C in 500 ml flasks and their concentrations were measured at 660 nm spectrophotometrically. Concentrations were adjusted to an OD reading of 0.5 using sterile saline

Citrus seedlings of rough lemon were obtained from the Citrus Foundation Block in Uitenhage, South Africa were inoculated in pots of pasteurized (1:1) sand: compost mixture. The bottoms of the pots were covered with stones rinsed in NaHOCl to aid drainage. Seedlings were inoculated with the selected bacterial isolate (15 ml per pot) after transplant. Mycorrhizal inoculum (10 ml) was obtained from Mycoroot (Pty) Ltd, South Africa (MPN 100 propagule in 1g) and applied at transplant; control pots were inoculated with water (Table 2.3.) Each treatment had five replicate pots. Experiments took place in a mycorrhizal tunnel at Rhodes University with temperature min/max temperature of 20 - 30^{o}C. Pots were watered daily with UV sterilised water and placed under natural light.

Table 2.3: Growth evaluation experimental design

Treatment 1			Treatment 2		
Replicates	**Isolate**	**Mycoroot**	**Replicates**	**Isolate**	**Mycoroot**
5	**I5**	**+**	**5**	**I5**	**-**
5	**E9**	**+**	**5**	**E9**	**-**
5	**I19**	**+**	**5**	**I19**	**-**
5	**I5 + E9 + I19**	**+**	**5**	**I5+ E9+I19**	**-**
5	**Control (s.dH_2O)**	**+**	**5**	**Control (s.dH2O)**	**-**

2.5.2 Shoot height- growth parameters

Initial shoot heights of the seedlings were measured (cm) after planting. Measurements were taken on a weekly basis for five weeks. At harvest, the plants were removed and the roots separated from the shoots. Shoots and roots were weighed on an Adam® Highland HCB 1002 balance for wet weight. The samples were oven dried at 60°C for 96 hrs in a Memmet oven and dry weights were recorded. A subsample of 1g of the fresh root material was removed for AM fungal colonisation assessment. The root dry weight was corrected for the subsample removed after moisture determinations by adding the removed weight back to the initial weight of the roots. Total biomass (shoots and roots) as well as shoot to root ratios was calculated.

2.5.3 AM fungal colonisation

The colonisation of the plant roots was evaluated by using a method described by Koske and Gemma, 1989 and Smith & Dickson, 1997. This method involves using 1-3 cm long sections of the root which are cleared with 5% KOH solution that remove the cytoplasm and all coloured material from the plant cells. The roots in the KOH were then heated in a water bath at just below boiling for 30 min. The KOH solution was discarded and the roots were rinsed with distilled water. The roots were then bleached in an alkaline H_2O_2 solution for 15 min. When the roots were white, the bleach was poured off and the roots rinsed with water. The roots were acidified to ensure the binding of Sigma Trypan Blue (Catalogue 23850) to the mycorrhizal structures by using 0.1M HCl solution. The HCl was removed and the roots were covered with lactoglycerol solution containing 0.05% trypan blue and stained overnight at room temperature. The stain was poured off and distained with lactoglycerol without the Trypan Blue for 24 hrs. The roots were then viewed under the compound microscope at 100 x magnification. Colonisation was characterised by the formation of intercellular hyphae and intracellular arbuscules (McGonigle *et al.*, 1990). Percentage colonisation was calculated using the following equation.

$$\textbf{Percentage colonisation} = \frac{\textbf{colonised cells}}{\textbf{50}} \times \textbf{100}$$

(Smith and Dickson, 1997)

2.6. Data Analyses

All experiment data was collected from the replicates, with N being equal to 3-5 depending on the experiment. Means and Standard deviations were calculated using Microsoft excel 2010. Data were recorded and statistically analyzed using one-way analysis of variance (ANOVA) by using SPSS software (Windows version 18). Duncan's multiple range tests were performed at $P < 0.05$ on each of the significant variables measured (Duncan 1955).

CHAPTER 3
RESULTS

3.1 Isolation of arbuscular mycorrhizal spores and associated bacteria

Arbuscular mycorrhizal spores were extracted from the soil using the wet sieving and decanting method (Smith and Dickson, 1997). Yellow brown spores where extracted from the soil samples obtained from the organically managed soils. From the isolated spores (Fig 3.1), the spores were mostly oval shaped, yellow in colour and had subtending hyphae (Fig 3.1A and B). Spore diameter ranged from 88.7µm to 123.5µm. These were tentatively placed in the *Glomus* genus.

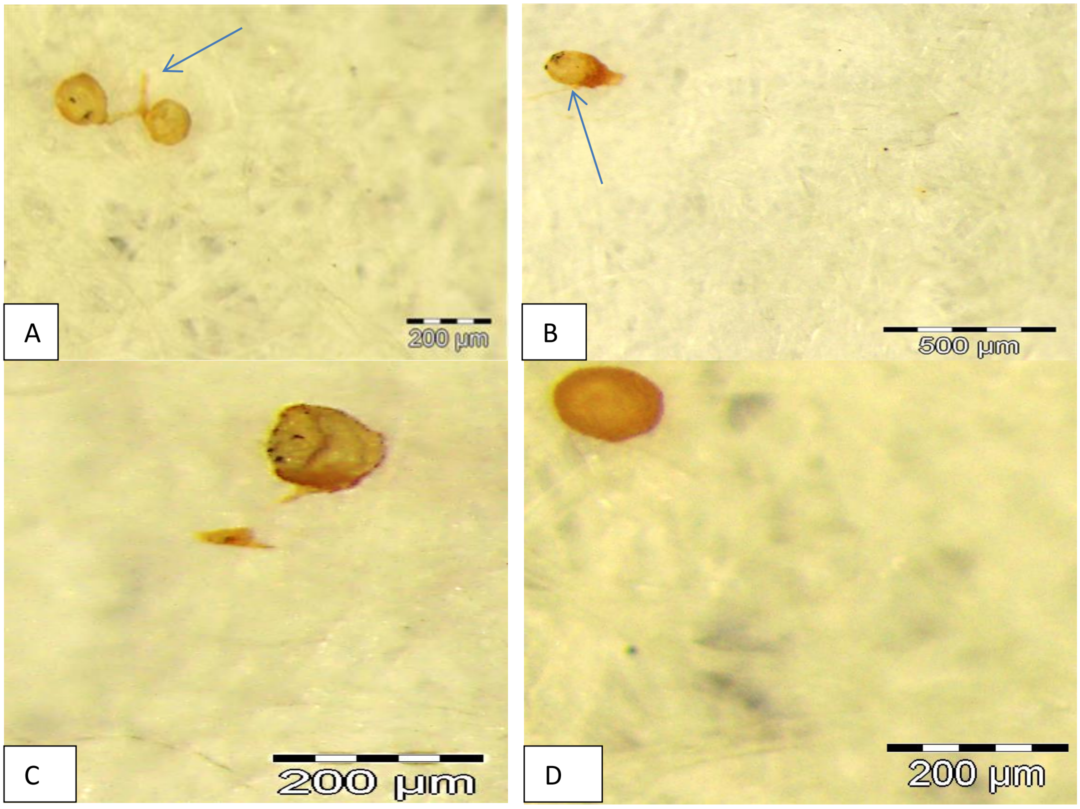

Figure 3.1: (A-D) Arbuscular mycorrhizal spores isolated from soil samples, arrows show subtended hyphae indicating characteristic features of the genus *Glomus* developing from the spores.

From the AM fungal spores 19 bacteria were isolated and purified, 12 isolates from the external spore surface and 7 from internally (Fig 3.2).

3.2 Identification of bacterial isolates.

3.2.1 Morphological

The bacterial isolates were separated and coded on base on whether isolated externally (E) or internally (I). The colony morphology was recorded as indicated in Fig 3.2 and Table 3.1. Bacterial identification is based on many factors such as colony shape, colour on nutrient agar and chemical composition of cell walls (Gram Staining). The Gram reaction distinguishes between Gram positive and negative bacteria. Gram positive bacteria have a thick peptidoglycan layer, which retains the crystal violet stain and remain dark purple colour. Gram negative bacteria have thin peptidoglycan layer and retain the pink colour as a result of safrinin (Black, 2008). Gram reaction and cell phenology are shown in Table 3.1.

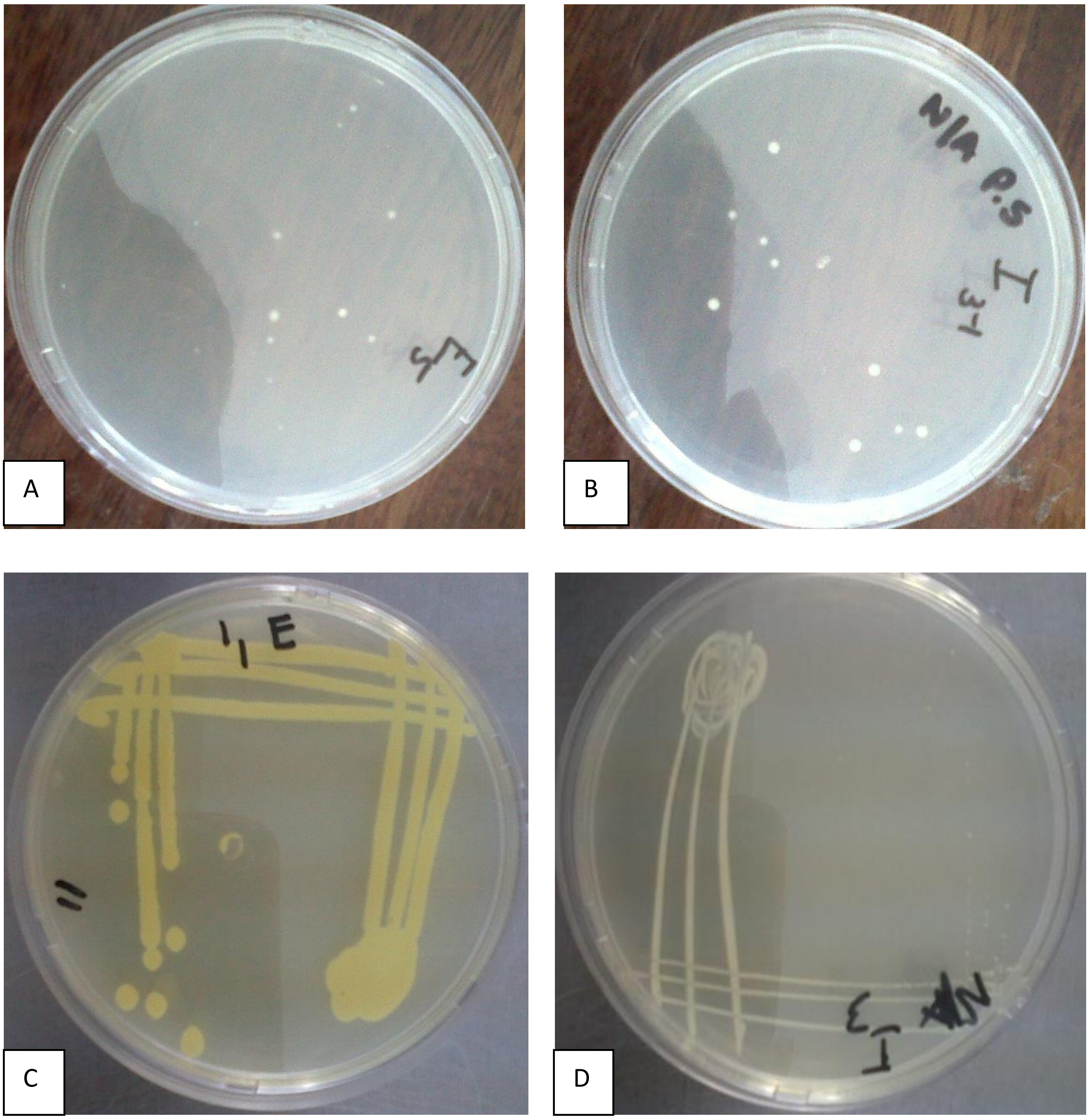

Figure 3.2: (A-D) A selection of bacterial isolates on a Nutrient Agar plates. The colour on the purified colony ranged from yellow to cream. The isolates were coded A isolate E1, B isolate E7, C isolate E9, D isolate I17

Table 3.1: Morphological identification of bacterial isolates by Gram reaction, cell phenology and colony appearance on Nutrient Agar plates

Isolate	**Appearance on nutrient agar plate**		**Gram Reaction**	
	Shape	**Color**	**+/-**	**Cell phenology**
E1	Irregular	Cream	+	Rod
E2	Round	Yellowish	+	Cocci
E3	Irregular	Cream	+	Rod
E4	Irregular	Cream	+	Rod
I5	Irregular	Cream	+	Rod
E6	Irregular	Cream	+	Rod
I7	Irregular	Cream	+	Rod
E8	Irregular	Cream	+	Rod
E9	Round	Yellow	+	Rod
E10	Round	Whitish	-	Rod
E11	Round	Yellow	+	Cocci
I12	Irregular	Cream	+	Rod
I13	Irregular	Cream	+	Rod
E14	Round	Whitish	_	Rod
E15	Round	Yellowish	+	Cocci
E16	Irregular	Cream	+	Rod
I17	Round	Yellowish	+	Cocci
I18	Irregular	Cream	+	Rod
I19	Irregular	Cream	+	Rod

Of the externally associated bacteria 7 were Gram positive rods, 2 were Gram negative cocci, 1 was a Gram negative cocci, 2 were Gram positive cocci. In the internally associated bacteria 6 were Gram positive rods and 1 was Gram positive cocci, no Gram negative bacteria were isolated.

3.2.2 Molecular identification

For accurate results the isolates were further identified using molecular techniques. Extracted DNA was amplified by PCR using molecular probes (primers) that bind only the targeted DNA. The targeted gene was 16S rDNA. The sequencing of the 16S rDNA was done to identify bacterial isolate to genus and species. Fig 3.3a and 3.3b are agarose gels of amplified PCR after clean-up. The gel represents the selection of isolates isolated from the spores and all are approximately 1500 bp in size.

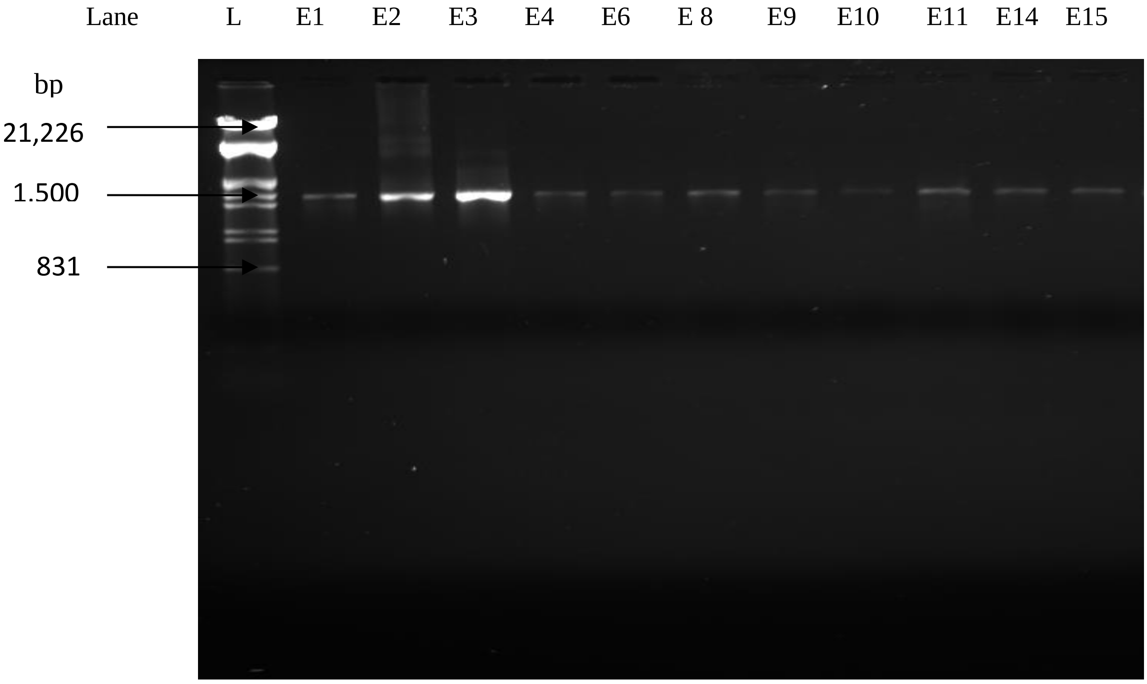

Figure 3.3a: PCR product of bacterial isolates as visualized in 1% agarose gel stained with (50mg/m) ethidium bromide. Lane 1 (L) is the marker and subsequent are isolates lanes, E1, E2, E3, E4, E6, E 8, E9, E10, E11 E14 and E15, indicating successful PCR. The estimate sizes for the bacterial isolates are approximately 1500 bp.

PCR amplification was also conducted using no DNA template (not shown in clean up gel) no band was observed indicating the success of the amplification and lack of contamination.

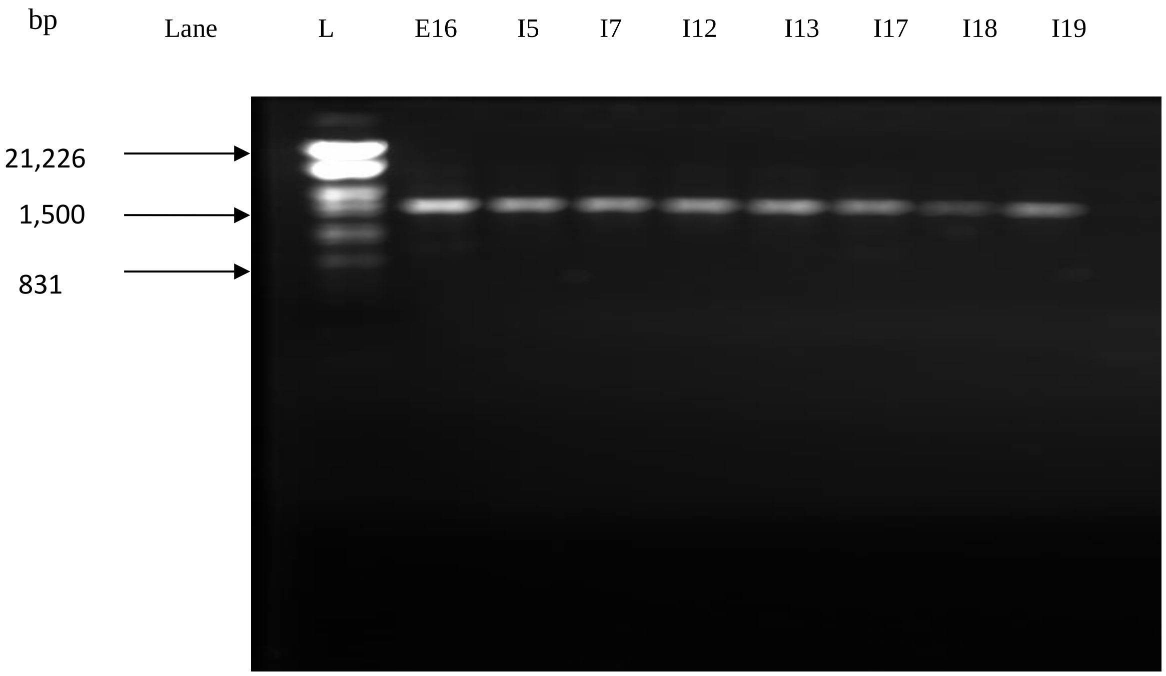

Figure 3.3b: PCR product of bacterial isolates as visualized in 1% agarose gel stained with (50mg/m) ethidium bromide. Lane 1 (L) is the marker and subsequent are isolates lanes, E16, I5, I7, I12, I13, I17, I18, and I19 indicating successful PCR. The estimate sizes for the bacterial isolates are approximately 1500 bp

PCR products were returned after sequencing and the sequences were read using Finch Tv software and submitted to GenBank. All 19 isolates were identified to at least genus level. 12 of the bacterial isolates were identified to be from the genus *Bacillus* (various species), 3 as *Staphylococcus* and 2 each as *Micrococcus* and *Ochrobactrum* (Table 3.2).

Table3.2: Molecular identification of bacterial isolates. Sequences were identified using BLAST from the NCBI.

Isolate	Accession No	Description	% Identity	E-Value	% Coverage
E1	HM566459.1	*Bacillus sp*	98	0.0	99
E2	JQ522980.1	*Staphylococcus sp*	98	0.0	99
E3	JN 700135.1	*Bacillus thuringiensis*	98	0.0	98
E4	EU621383.1	*Bacillus cereus*	96	0.0	98
I5	HE660034.1	*Bacillus spp*	98	0.0	97
E6	JN700135.1	*Bacillus thuringiensis*	98	0.0	97
I7	JX010994.1	*Bacillus sp*	99	0.0	97
E8	JX010994.1	*Bacillus sp*	99	0.0	99
E9	JQ433937.1	*Micrococcus sp*	99	0.0	99
E10	EF587906.1	*Ochrobactrum sp*	95	0.0	96
E11	HQ220042.1	*Micrococcus leteus*	95	0.0	97
I12	HM566469.1	*Bacillus sp*	97	0.0	99
I13	HM567066	*Bacillus sp*	98	0.0	99
E14	FJ374126.1	*Ochrobactrum onthropi*	99	0.0	97
E15	EU071603.1	*Staphylococcus sp*	98	0.0	99
E16	GU826149.1	*Bacillus cereus*	98	0.0	97
I17	EU939709.1	*Staphylococcus pasteuri*	99	0.0	95
I18	HQ857776.1	*Bacillus antracis*	99	0.0	98
I19	HQ317144.1	*Bacillus cereus*	97	0.0	98

3.3 Evaluation of plant growth promotion properties of bacterial isolates

3.3.1 Phosphate solubilisation

Of all the isolates tested only one isolate, E5 (Fig 3.4 B) produced a clear zone around the well in which the bacteria was inoculated, indicating the solubilisation of the insoluble phosphate source (Table 3.3).

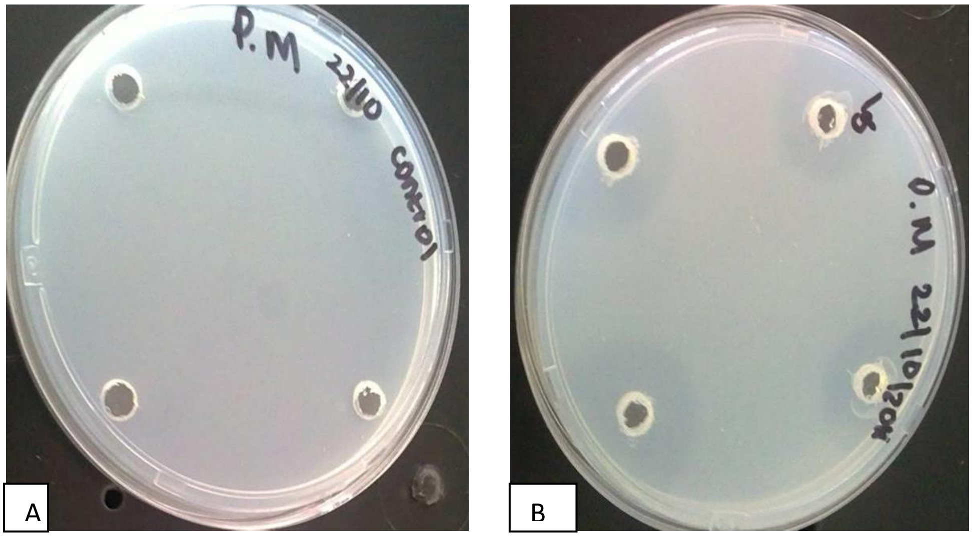

Figure 3.4: A- Control, B- Phosphate solubilisation as indicated by development of a clear zone around the wells. Isolate I5 after incubation for 24 hours at 37°C formed a clear zone around the colony in the media.

3.3.2 Indole acetic acid (IAA) production

All nineteen bacterial isolates were tested for IAA production by growing on media containing L-tryptophan which is a precursor for the formation of IAA. A colour change to red was indicative of IAA production. After introduction to Kovac reagent four isolates E9, E11, E14, E10 turned red as shown in (Fig 3.5 and table 3.3).

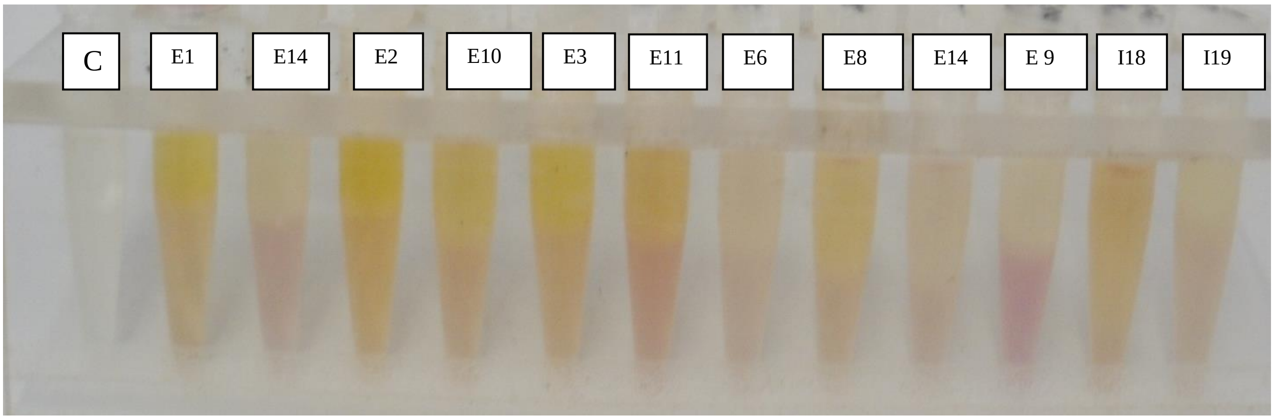

Figure 3.5: Test for the production of Indole Acetic Acid isolates E9, E11, E14, E10 were able to produce IAA as a colour change was observed after addition of Kovacs Reagent.

Table 3.3 Bacterial isolates shown to be phosphate solubilisers and Indole Acetic Acid producers

Isolate	**Phosphate test**	**IAA test**
E9	–	+
E11	–	+
E14	–	+
E10	–	+
I5	+	–

(- negative reaction, + positive reaction)

3.4 Fungal Pathogen Inhibition

The 19 bacterial isolates were tested for potential inhibitory effects against citrus pathogen, *Phytophthora citrophthora, P. citrocola* and *P. nicotianae.* There were significant differences observed in *P. citrophthora* (Fig 3.6) as P value was 0.026 but with *P. nicotianae* (Fig 3.9) and *P. citrocola* (Fig 3.12) there were no significant differences as the P value was greater than 0.05. Growth curves were done for three isolate, I5, E9 and I19 as they showed the potential to inhibiting all the fungal isolates. There were significant differences observed in *P. citrophthora* (Fig 3.8) and *P. nicotianae* (Fig 3.11) as P value was less than 0.005 but with *P. citrocola* (Fig 3.14) there were no significant differences as the P value was greater than 0.05.This experiment was done over a period of 10 days.

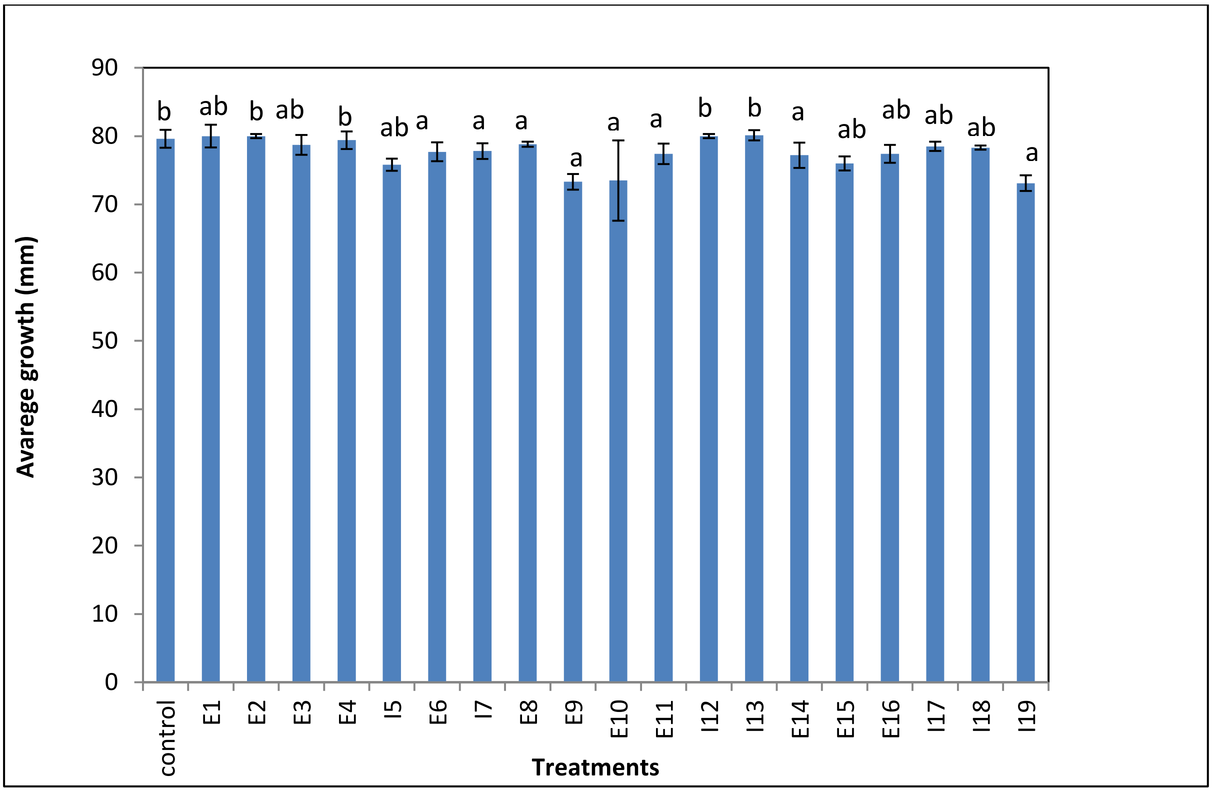

Figure 3.6: Effects of *Phytophthora citrophthora*, growth in dual culture of bacterial isolates after 10 days. The bacterial effect on pathogen $F_{(28,161)}$=1.889; P = 0.026. Bar represents treatment means ± standard error. The letters represents the significant differences ($p<0.05$) by Duncan's Multiple Range Test; n=5

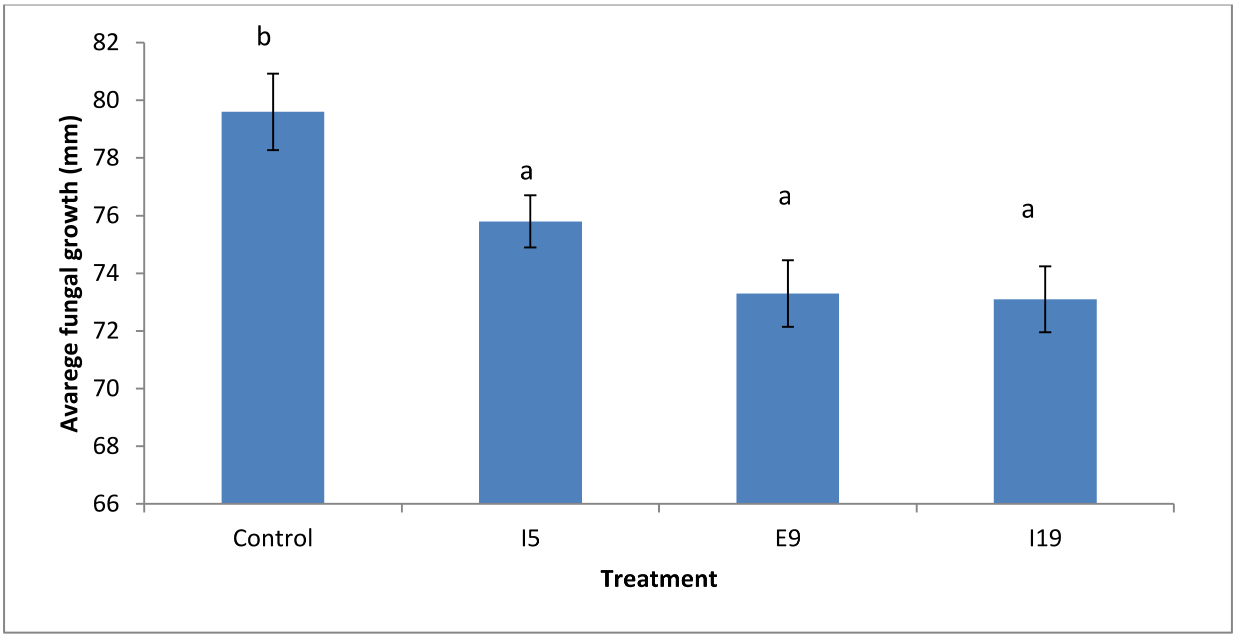

Figure 3.7: Bacteria I5, E9 and I19 shown to have some inhibitory effect on *in vitro* growth of *Phytophthora citrophthora* although it was not significant different, bars represent treatment means ± standard error

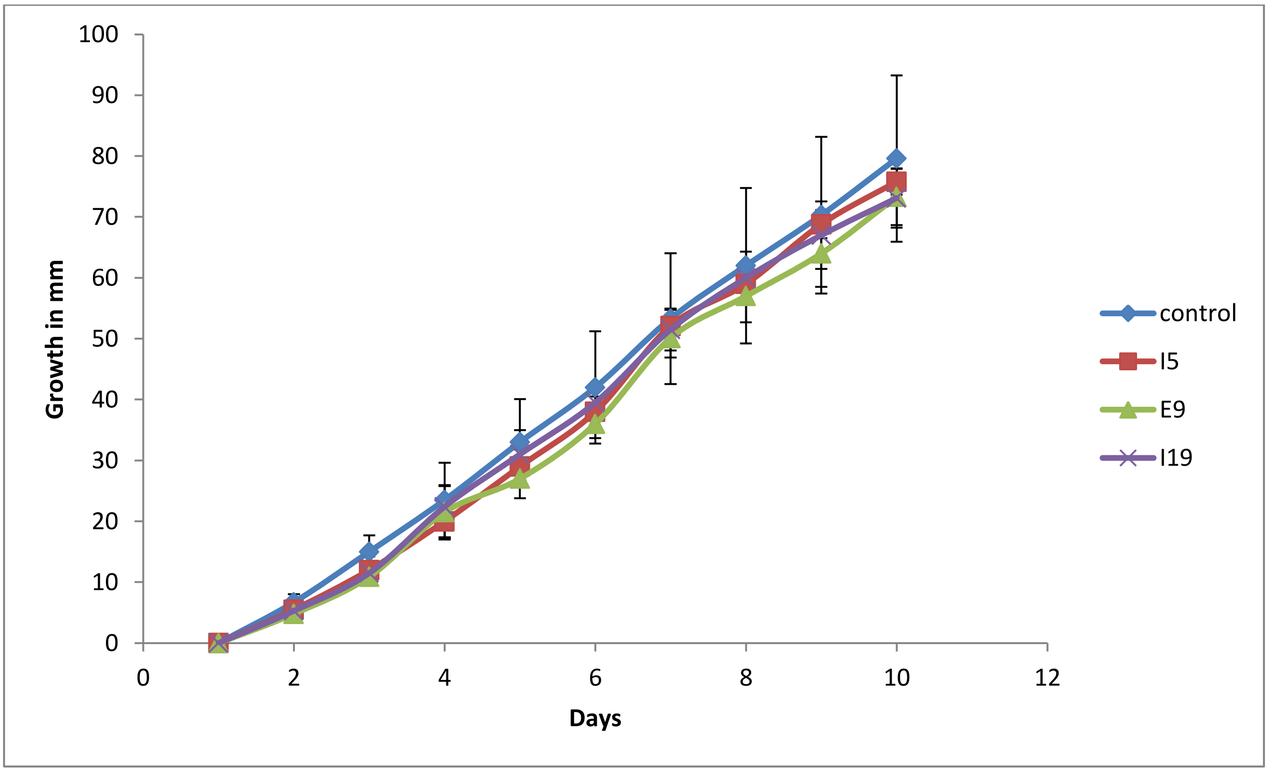

Figure 3.8 .Growth curves of *Phytophthora citrophthora* and bacterial isolates I5, E9, I19 effects on the citrus pathogen. The bacterial effect on fungi growth F $_{(45.817)}$ = 7.015, P =0.003

Bar represent treatment errors ± standard deviation. Significantly differences ($p<0.05$) by Duncan's Multiple Range Test; n=5

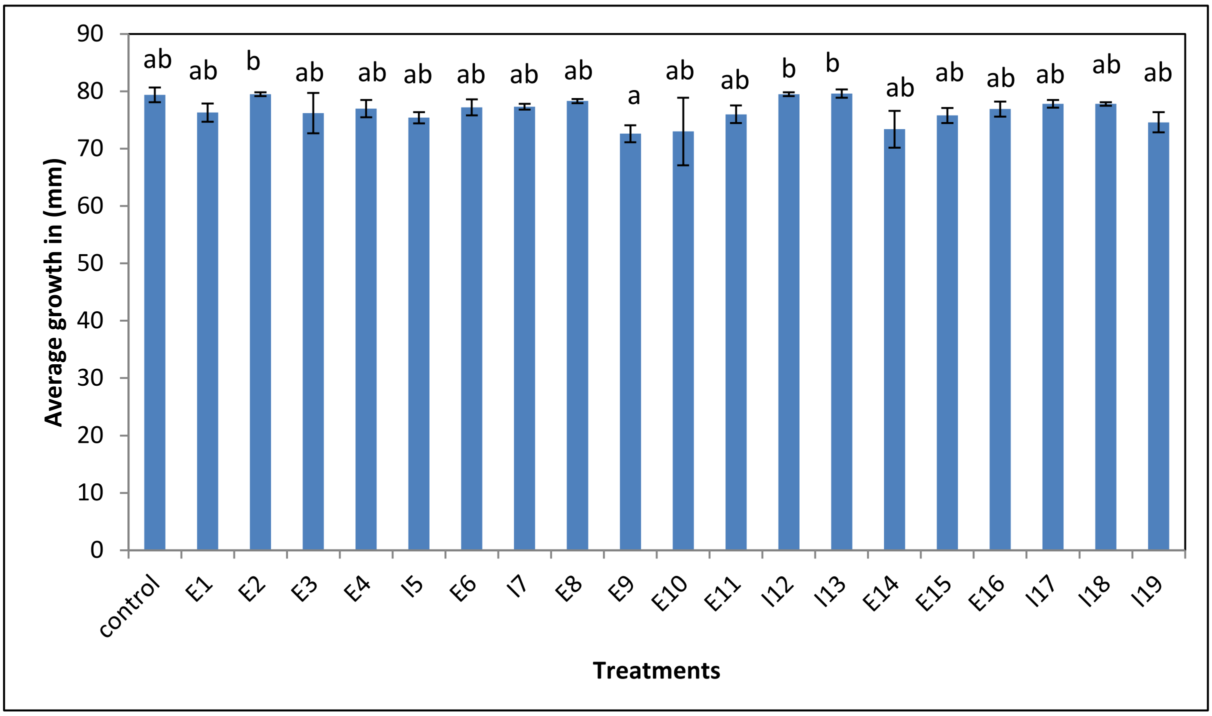

Figure 3.9: Effects of *Phytophthora nicotianae*, growth in dual culture of bacterial isolates after 10 days. The bacterial effect on pathogen $F_{(22,708)}$= 1.143; P = 0.327. Bar represent treatment means ± standard error. The letters represents the significant differences ($p<0.05$) by Duncan's Multiple Range Test; n= 5

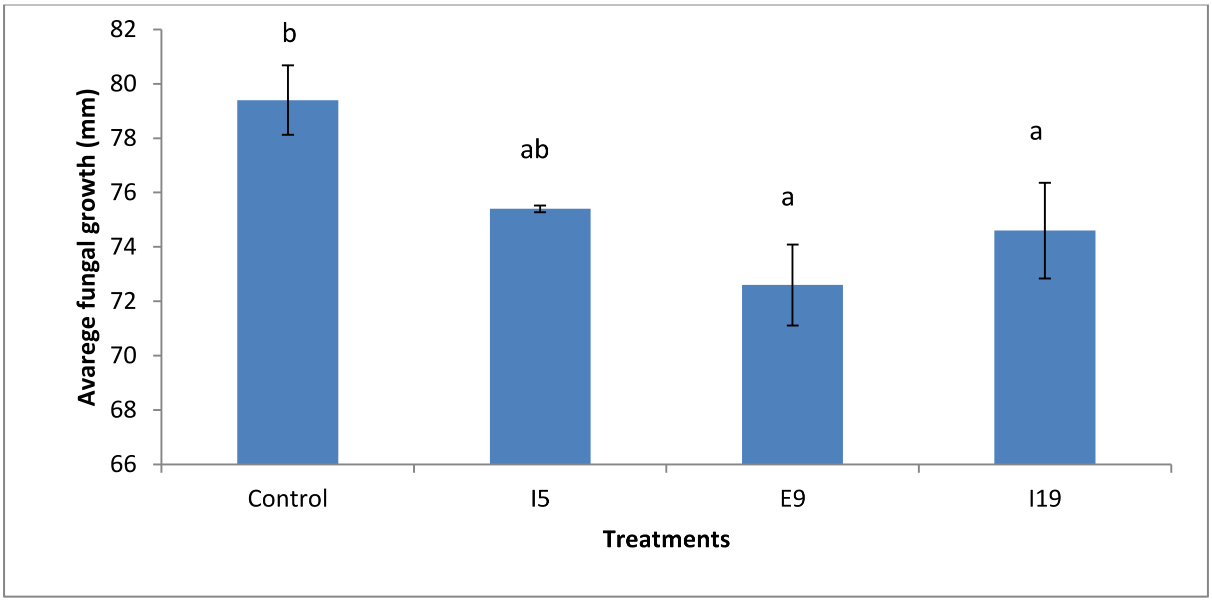

Figure 3.10: Bacteria I5, E9 and I19 showed to have some inhibitory effect on *in vitro* growth of *Phytophthora nicotianae* bars represent treatment means ± standard error

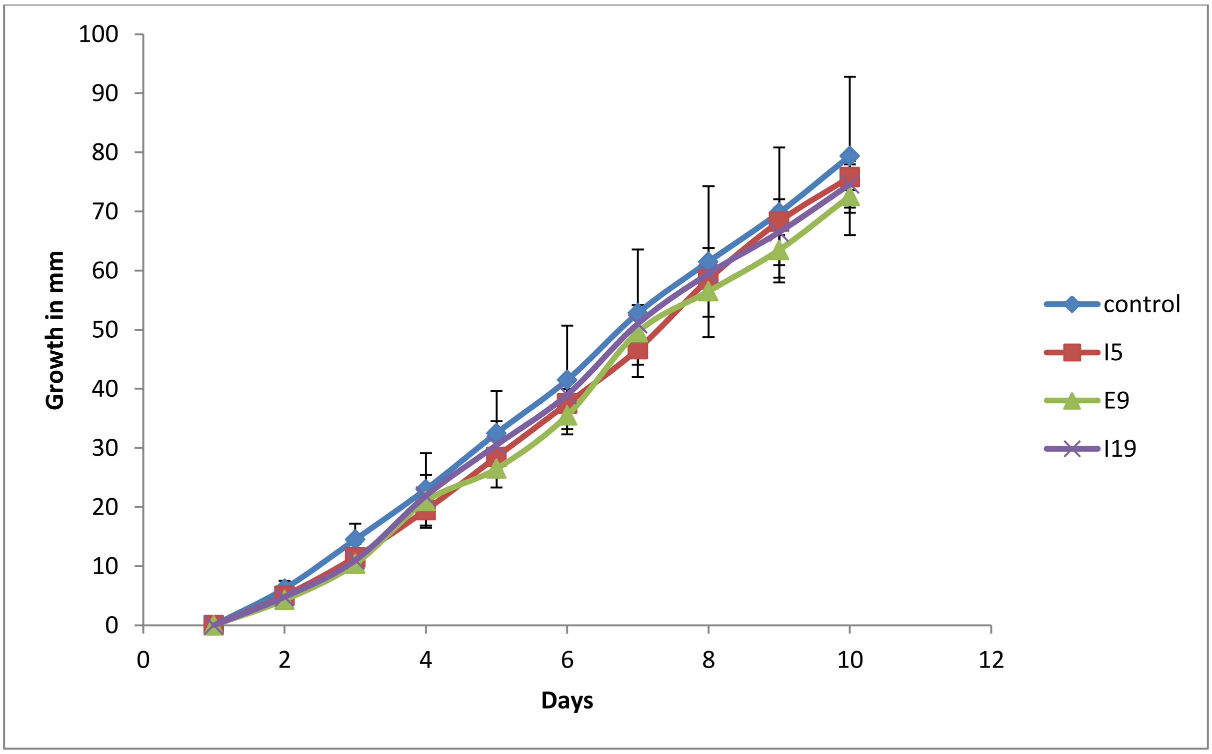

Figure 3.11 Growth curves of *Phytophthora nicotianae* and bacterial I5, E9, I19 effects on the citrus pathogen. The bacterial effect on fungi growth F $_{(40,733)} =$ 4.130 P = 0.024. Bars represent treatment means ± standard deviation. Significantly differences ($p<0.05$) by Duncan's Multiple Range Test; n=5

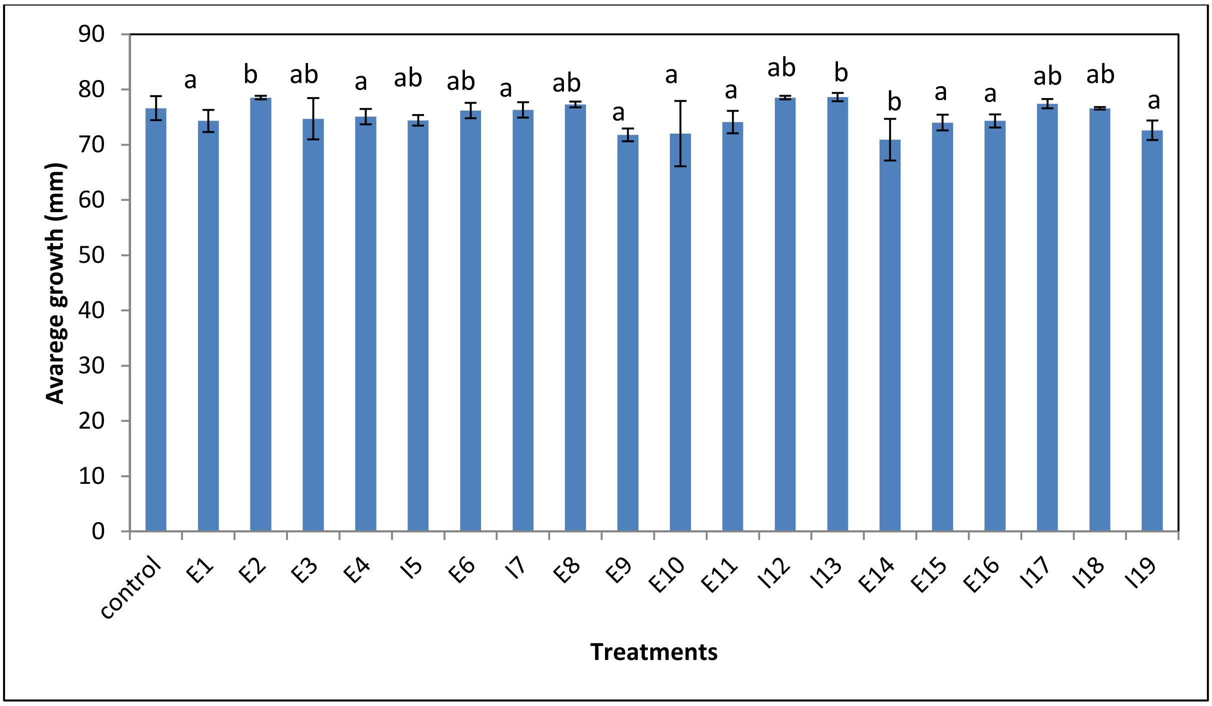

Figure 3.12: Effects of *Phytophthora citrocola*, growth in dual culture of bacterial isolates after 10 days bacterial effect on pathogen $F_{(26,563)}$=1.153; P = 0.319. Bar represents treatment means ± standard error. The letters represents the significant differences ($p<0.05$) by Duncan's Multiple Range Test; n= 5

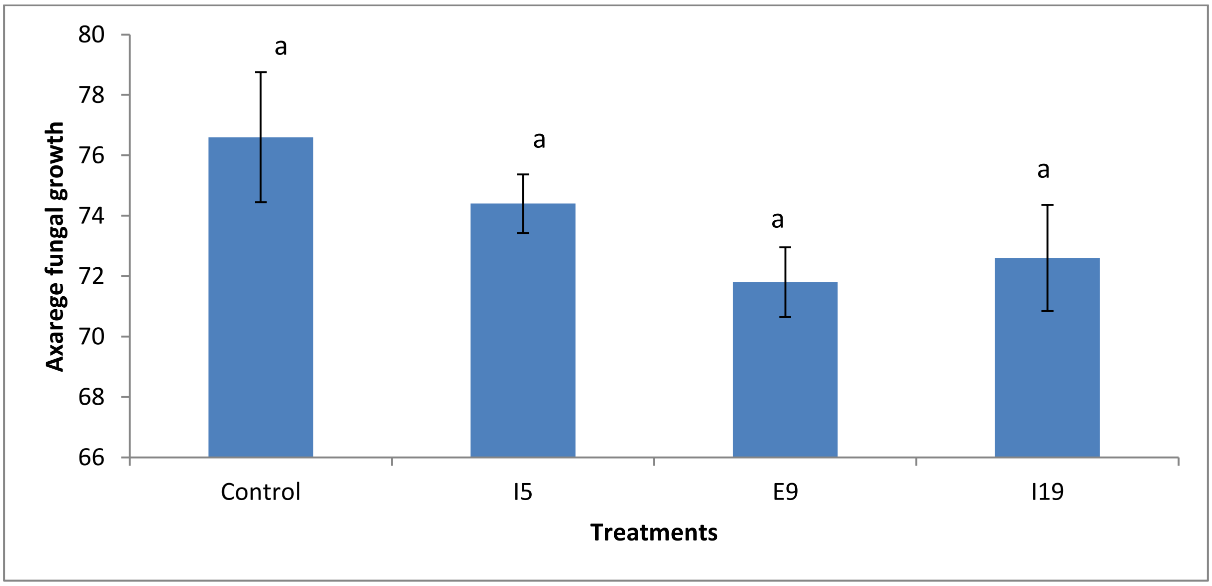

Figure 3.13: Bacteria I5, E9 and I19 showed no significant effect on *in vitro* growth of the *Phytophthora citrocola* bars represent treatment means ± standard error.

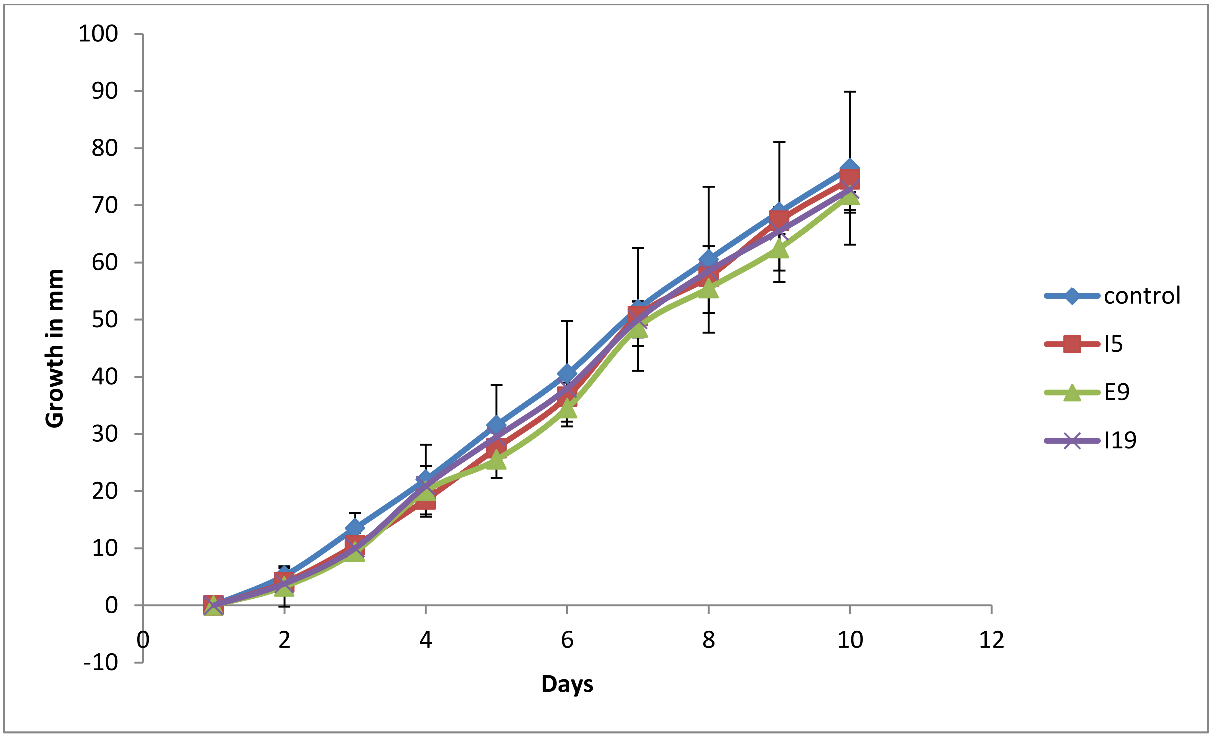

Figure 3.14 Growth curves of *Phytophthora citrocola* and bacterial I5, E9, I19 effects on the citrus pathogen. The bacterial effect on fungi growth F $_{(22,717)}$ = 1.814, P = 0.185. Bar represent treatment means ± standard deviation. Significantly differences ($p<0.05$) by Duncan's Multiple Range Test; n=5

3.5: Metabolite inhibitory assay

From the initial fungal inhibition results three bacterial isolates I5, E9, I19 were shown to have some potential in inhibiting the fungal pathogens and were further tested against *Phytophthora citrophthora*, *P. citrocola*, and *P. nicotianae*. These three bacterial isolates were tested for the presence of secreted compounds that could have any inhibitory effect on these fungal pathogens. Supernatant from the two bacterial isolates, I5 and I19, significantly inhibited the growth of all three fungal pathogens (Fig 3.15, 3.16 and 3.17). The supernatant from the bacterial isolate E9 also reduced fungal growth but not completely inhibit growth.

3.5.1 Phytophthora citrophthora

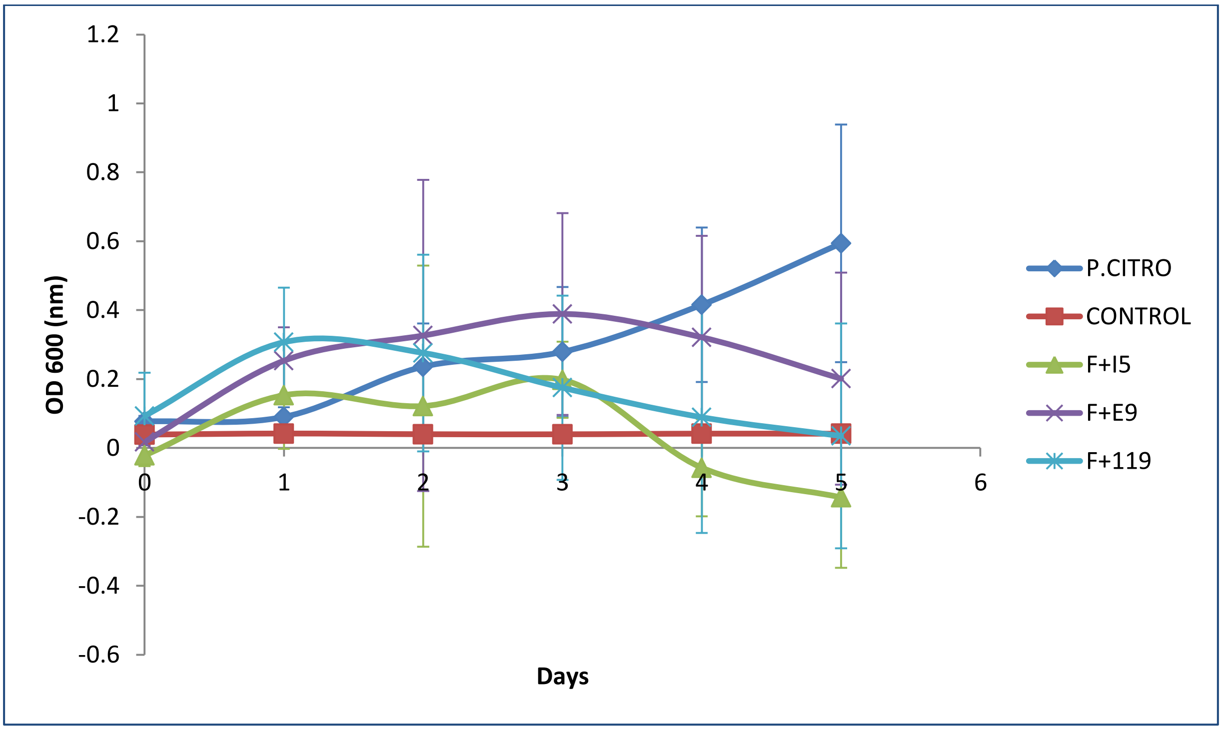

Figure 3.15 Growth curve of *Phytophthora citrophthora* interaction with Bacterial supernatant from isolates I5, E9 and I19. F $_{(4,25)}$ = 21.838; P 0.001, n=5. Daily means ± standard deviations are represented.

Isolate I5 significantly inhibited the growth of *Phytophthora citrophthora* by 67% in day 3 but by day 5 the growth was inhibited by 100%, I19 inhibited the growth by 94.5% in day 5 and E9 reduced growth 94% by day 5.

3.5.2: Phytophthora nicotianae

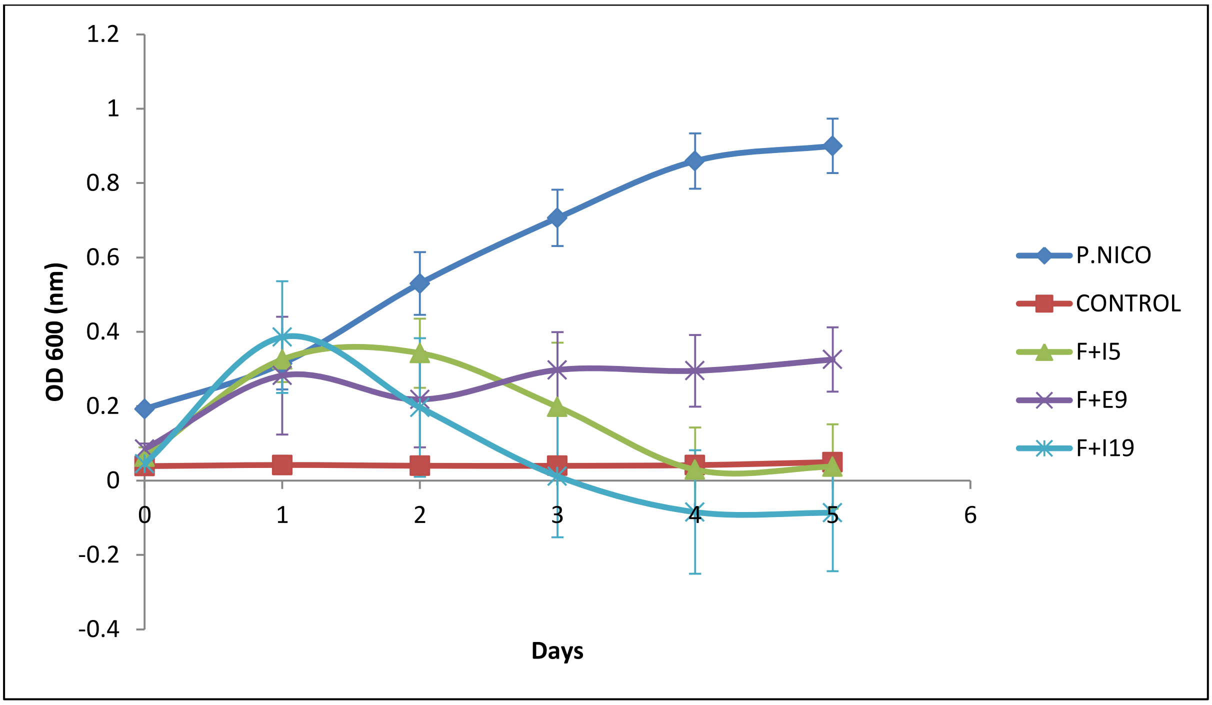

Figure 3.16: Growth curve of *Phytophthora nicotianae* interaction with Bacterial supernatant from isolates I5, E9, I19, $F_{(9.40)} = 4.866$: $P = 0.004$, $n = 5$. Daily means ± standard deviations are represented.

Isolate I5 significantly inhibited the growth of *Phytophthora nicotianae* by 96.6% in day 3, I19 inhibited the growth by 98.6% in day 3 and E9 reduced growth by 65.7 % and the effect was mainly in day 4 and 5

3.5.3 Phytophthora citrocola

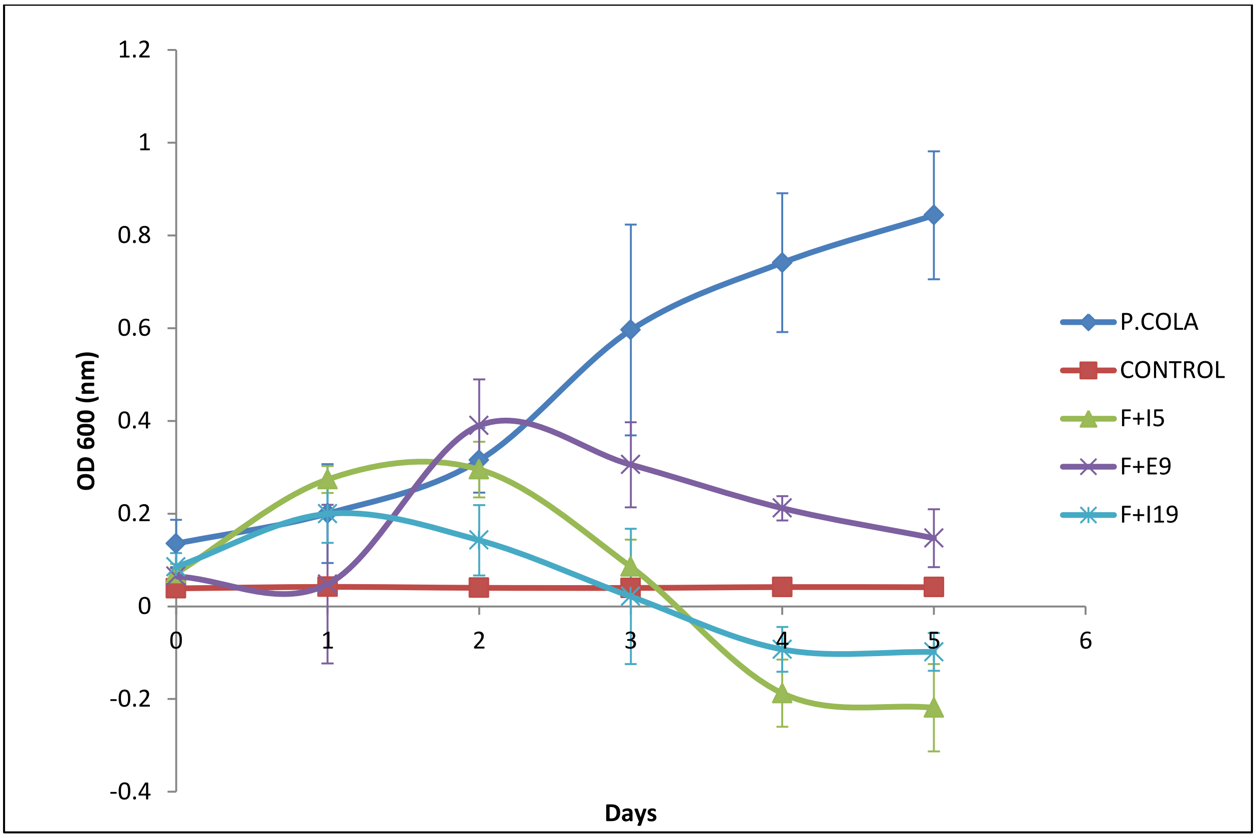

Figure 3.17: Growth curve of *Phytophthora citrocola* interaction with bacterial supernatant from isolate I5, E9 and I19. F $_{(4.25)}$ = 374.31 P =0.001, n=5. Daily means ± standard deviations are represented.

Isolate I5 significantly inhibited the growth of *Phytophthora citrocola* by 96 % within 3 days, I19 also inhibited the growth by 98.6% within 3 days and E9 reduced growth by 65.7% in 5 days.

3.6 Growth evaluation of citrus growth

Bacterial isolates I5, E9, I19 were used for citrus growth evaluation trails as they showed some plant growth promoting properties. The effect of the treatment on plant growth was assessed by recording shoot heights measured (cm) weekly for five weeks. As seedlings were of variable sizes all initial height were subtracted from the weekly measure. The data represents increase in shoot growth. Treatment BM4 and B4 consist of all three isolates I5, E9, I19.

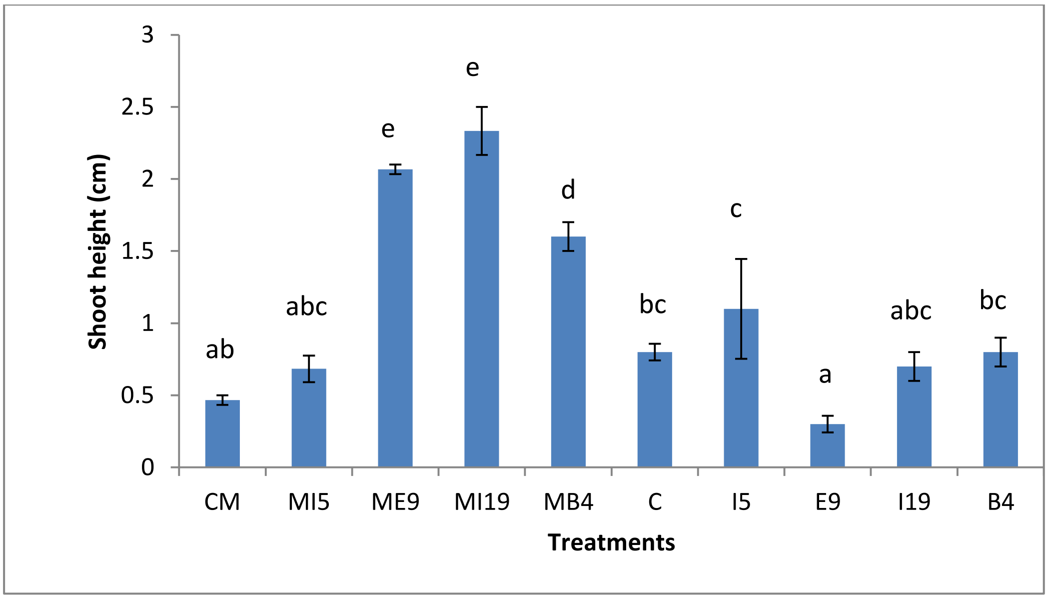

Figure 3.18: Citrus shoot height (cm) treated with bacterial isolates I5, E9 and I19 and in combination with arbuscular mycorrhizal fungi after five weeks. The treatment effect on rough lemon plant growth $F_{(24,228)} = 1.419$; P 0.001. Bar represents ± standard error. Different letters are for significantly differences ($p<0.05$) by Duncan's Multiple Range Test; n=3

In shoot height of the rough lemon plants significant differences were observed in isolates ME9, MI19 and MB4 in comparison with the control and I5. Although there was improvement in citrus plants isolate E9 was observed to be decreasing the growth of this plant. Significant differences in shoot height were recorded for the other treatments (Fig.3.18)

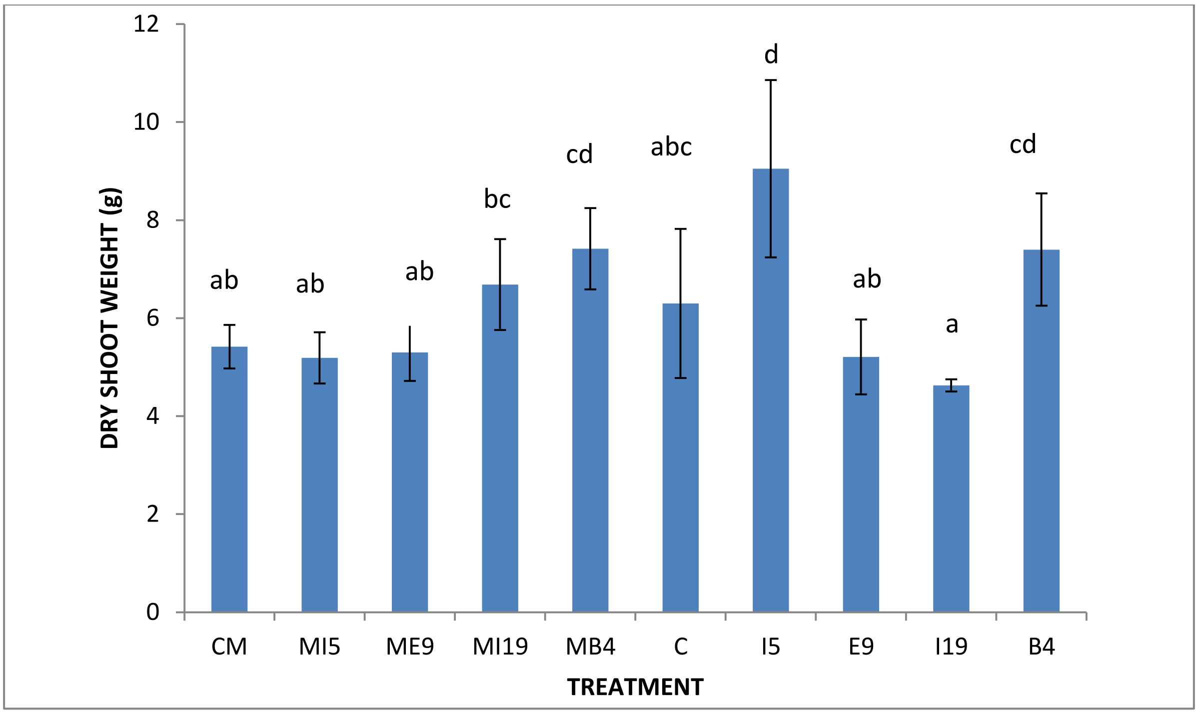

Figure 3.19: Citrus dry shoot weight (g) treated with bacterial isolates I5, E9 and I19 and in combination with arbuscular mycorrhizal fungi after five weeks. The treatment effect on rough lemon plant growth F $(_{9,\ 40})$ = 2.603, p=.0182 Bar represents ± standard errors. Different letters are significantly different (p<0.05) by Duncan's Multiple Range Test; n=3

In shoot dry weight of the rough lemon plants significant differences were observed in isolates MI19, MB4, I5 and B4 in comparison with the control. Significant differences in shoot dry weight were recorded for the other treatments (Fig.3.19)

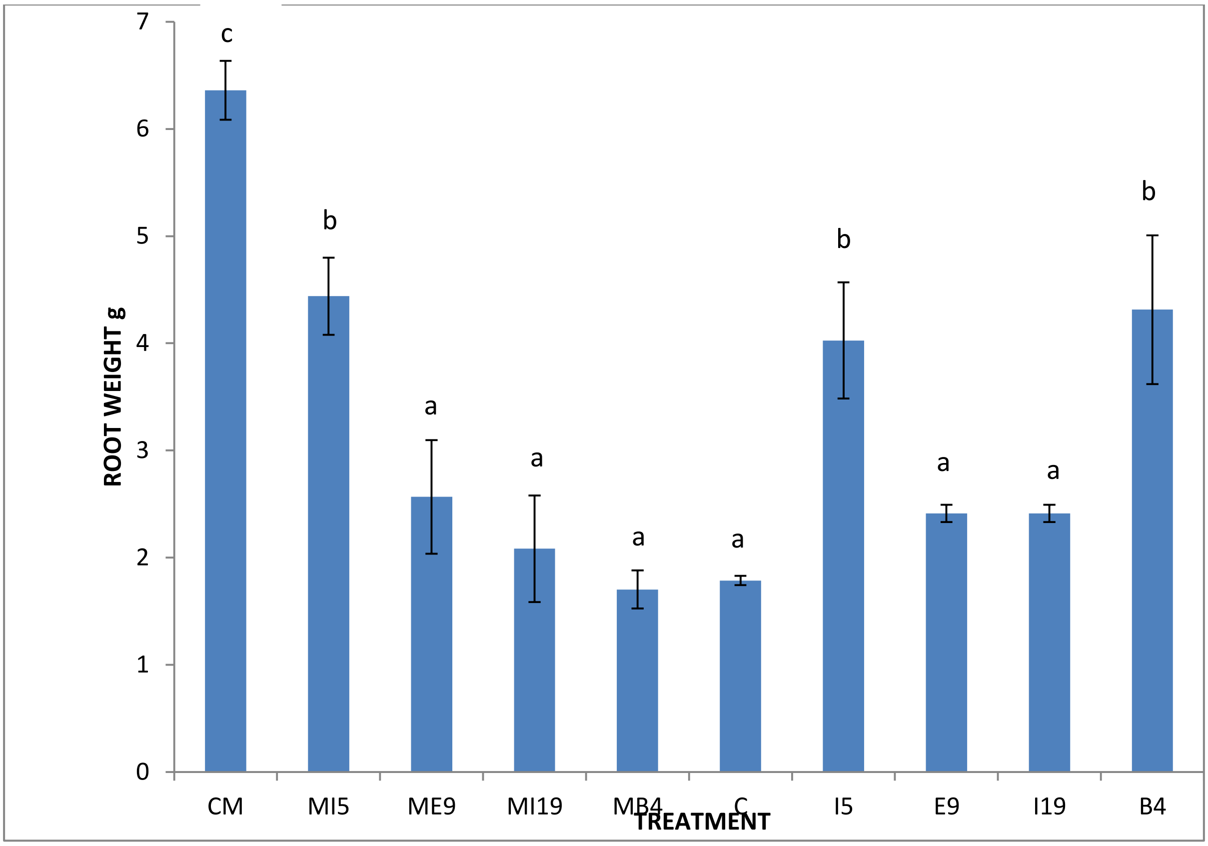

Figure 3.20: Citrus dry root weight treated with bacterial isolates I5, E9 and I19 and in combination with arbuscular mycorrhizal fungi after five weeks. The treatment effect on rough lemon plant growth F $_{(14,654)}$ =6.856, p=.0008. Bar represents ± standard errors. Different letters are significantly different ($p<0.05$) by Duncan's Multiple Range Test; n=3

Significant differences in root dry weight were observed in the rough lemon inoculated with MI15, I5 and B4 plants. The root dry weight of the citrus plants improved as a result of inoculated with bacterial isolates and there was no improvement observed in those inoculated with AM fungi. Significant differences in root dry weight were recorded for the other treatments (Fig.3.20).

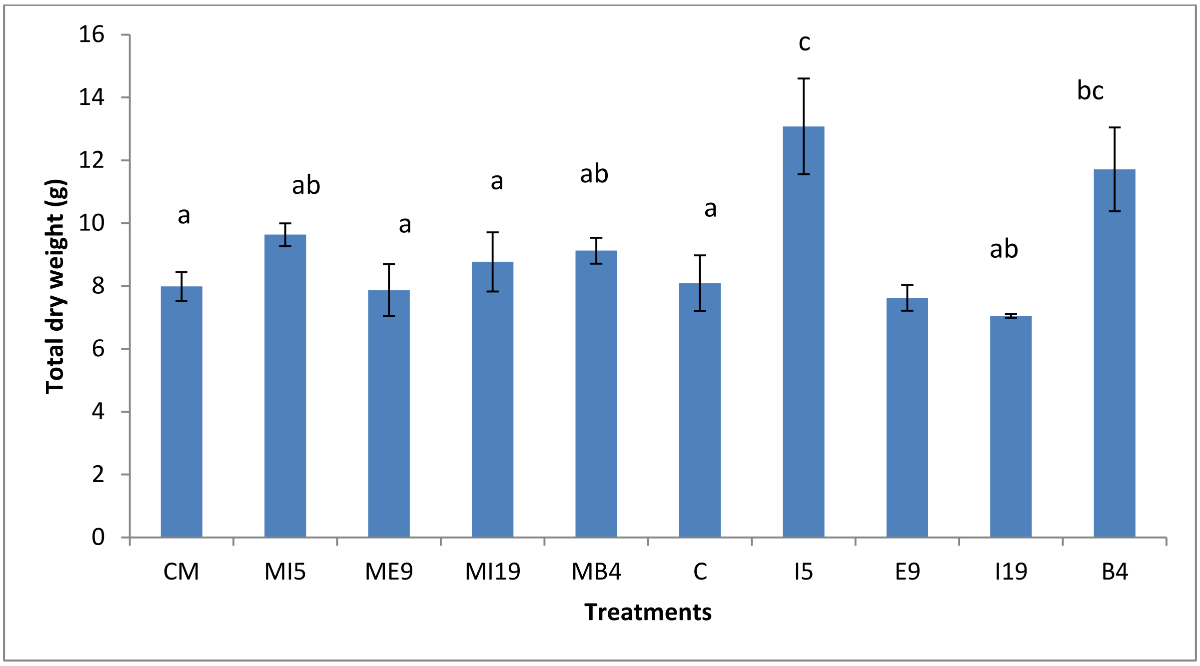

Figure 3.21: Citrus total dry weight treated with bacterial isolates I5, E9 and I19 and in combination with arbuscular mycorrhizal fungi after five weeks. The treatment effect on rough lemon plant growth F $_{(11,078)}$ =5.167, p=0.001. Bar represents ± standard errors. Different letters are significantly different ($p<0.05$) by Duncan's Multiple Range Test; n=3

3.7 Dry shoot and root analysis

Analysis of dry shoot and root weight were analysed using the ratio method where each plant dry shoots were divided by dry root weight (Figure 3.16). This was done to correct the plants initial weight as the plant varied in sizes.

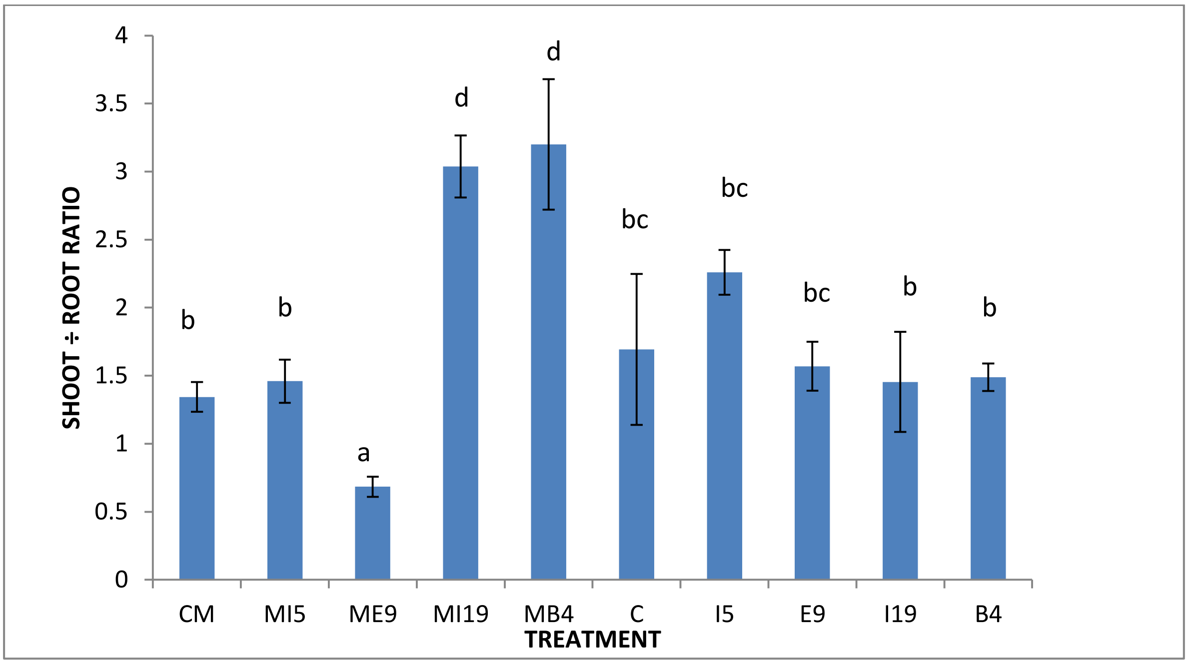

Figure 3.22: Citrus shoot height ratio (cm) treated with I5, E9 and I19 and arbuscular mycorrhizae after five weeks. The treatment effect on rough lemon plant growth (Mean, LS), current effect F $_{(11,440)}$ = 1.850; P 0.002. Bar represents ± standard errors. Different letters are significantly different ($p<0.05$) by Duncan's Multiple Range Test; n=3

CHAPTER 4
DISCUSSION

4.1 Isolation of arbuscular mycorrhizal spores and associated bacteria

This study shows the beneficial effect of mycorrhizal fungi and associate fungi in promoting growth of citrus, thus enhancing its productivity. Arbuscular mycorrhizal fungi exist in soil as spores (Harrison, 2005) and in this study these propagules were extracted in order to isolate and assess colonising bacteria. The spores isolated were tentatively identified as belonging to the genus *Glomus* (Fig 3.1). Mycorrhizal helper bacteria (MHB) in most cases have been found to colonise mainly the outer spore wall layer or extraradical hyphae and rarely penenetrate into inner layer (Bonfante-Fasolo and Schubert., 1987; Maia and Kimbrough. 1998) but some investigators have reported bacteria occurring in the cytoplasm of AM fungal spores (Bianciotto *et al*., 2000). Based on these reports bacteria were isolated from the external surface of the AM fungal spores and internally from the cytoplasm. For internal isolations spores were first surface sterilising with a series of antibiotics followed by crushing. During isolation of internal bacteria several attemps were made with little success perhaps indicating that sterilisation was too harsh or that spore walls were damaged allowing the antibiotics to enter the spore, several studies have found AM spores to have holes in their surface possibly due to predation Roessti *et al*., (2005). Fillippi *et al*., (1998) using a transimmission electron microscopy found that bacterial formed holes in the outer layer of *G.mosseae* spores and suggested that these were due to lytic activity.. After several attenpts a few isolates were successfully grown. However it must be mentioned that these isolates do not reflect the true population of internally residing bacteria as an unculturable component of the population, the endosymbiotic bacteria, have been previously mentioned (Bianciotto *et al*., 2000).

Athough identifying the AM fungi for this study was not an objective there has been some studies that suggested that the associated bacteria are AM spores specific. Toljander *et al.*, (2006) was able to isolate *Paenibacillus brasilensis* and *Psuedomonas fluorescents* bacteria associated with extraradical hyphae of *Glomus intraradices*. Mayo *et al*., (1986) found *Bacillus* and *Pseudomonas* spp to be associated with spores from *Glomus verisiforme.* Roesti *et al.*, (2005) assessed bacterial community associated with spores of *Glomus geosporum* and *Glomus constrictum.* The bacteria isolated included *Chondondromyces, Flexibacter, Lysobacteria* and *Pseudomonas.* However in order to determine the specific nature of these associations, studies comparing AM fungal species isolated from the same soils and under the same conditions would need to be compared. There is little evidence to suggest that the associated bacteria are not influence by the resident soil microbial populations. Root exudation is another factor that influences bacterial communities stiumlating their growth in the rhizosphere. AM fungal spore germination and presymbiotic growth towards the host root is also influenced by root exudations (Mayo *et al.,* 1986). The quality and composition of exudates differ from one plant to another and different bacterial communities could be stimulated depending on preference for distinct plant exadates. These differences could dictate the composition of bacterial communities on AM spore surface and require further investigation. The majority of plant–microbe interactions in the soil rely on the release of various compounds from roots that can affect the growth and activity of microbes (Hartmann *et al.*, 2009). Root exudates of mycorrhizal plants also contain hyphal exudates and deposition of AM fungal mycelial products that could serve as substrates for bacterial growth and impose a direct influence on bacterial communities in the mycorrhizosphere**.** These exudates could also influence the bacterial communities growing within the fungal network as well as on fungal surfaces. Studies comparing bacterial populations around mycorrhizal plant roots and un-inoculated plant roots have indicated changes in the bacterial populations (Harrison, 2005)

The purpose of the spore extraction was to isolate bacteria that were associated on or found in the spores (Garbaye, 1994). A total of 19 bacterial isolates were isolated from the external and internal spore surfaces. This is comparable to a study by Bharadwaj *et al.,* (2012) where they isolated 10 bacterial isolates from AM spores. Lecomte *et al.,* (2011) isolated 29 bacterial isolates from AM spores indicated that our numbers may have been low.

4.2 Identification of bacterial isolates

Identification and characterisation of bacteria is an important requirement in understanding the functional abilities of the bacteria and hence determine the possible mechanisms that could be contributing to the association with mycorrhizal fungi. The isolates were initially morphologically identified based on the colony appearance in nutrient agar plant, looking at colour, shape (Fig 3.2 and Table 3.1) and Gram reaction. This provides only a crude initial characterisation and generic identifications cannot be made with any certainty. Gram staining was performed to ensure that the isolates were purified and not mixed as well as to determine the shape and Gram reaction of the cells. The results (Table 3.1) confirmed that a selection of different bacteria were isolated being a combination of Gram positive rods, cocci and Gram negative rods. Xavier and Germinda, (2003) isolated both Gram positive and Gram negative rods which were naturally associated with *G. clarum* spores and Mayo *et al*., (1986) in their studies reported that Gram positive and Gram negative bacteria were found in colonising *G. verisiforme* spores.

Phenotypic identifications have some limitations as it is difficult to identify the isolate with any certainty. For accurate identification of the 19 bacterial isolates Polymerase Chain Reactions on extracted DNA were performed to amplify the double stranded DNA template (Black, 2008). In this study the 16S rDNA bacterial primers, Fd1 and rP2 were used. Fd1 and rP2 sequencing primers are useful for conserved regions within the 16S rDNA and are capable of amplifying a variety of bacterial taxa and a maximum number of nucleotides in the 16S rDNA (Weisburg *et al.,* 1991). Using the 16s rDNA sequencing can assist in the identification of isolates to generic and species levels. BLAST nucleotide searches of the 16s rDNA gene showed sequences homologies of 95 to 99 % maximum identification (Table 3.2). Sequence homologies of 95 to 96 % were indicative of genera matches while a 98 to 100 % sequence homologies is regarded suitable enough for the species determination (Lecomte *et al.,* 2011).

Isolates were shown after comparison to the GenBank database to belong to the genera *Bacillus, Micrococcus, Staphylococcus* and *Ochrobacterum* (Table 3.2). *Bacillus* and *Micrococcus* were dominant genera in this study. The Gram reactions of the isolates provided confirmation of the molecular identities. *Bacillus* has been reported to be associated with AM spores (Xavier and Germida, 2003; Cruz *et al*., 2008). *Bacillus, Micrococcus* and *Ochrobacterum* sp have been previously reported as plant growth promoting rhizobacteria (Chakraborty *et al*., 2009; Tsavkelova *et al.*, 2005) and little is documented about *Staphylococcus* being either associated with mycorrhizal spores or as a PGPR.

Zhao *et al*., (2012) isolated and characterized a plant rhizosphere bacterial strain, *Ochrobactrum* sp to evaluate its plant growth promotion capabilities by inoculating the isolate on cucumber in pot experiments. *Ochrobactrum* sp was able to increase the growth of cucumber in natural garden soil. Leaf and root lengths were significantly increased by 27% and 58%, respectively compared with the uninoculated control. Safa and Ali, (2011) inoculated mung bean with *Bacillus* sp in pot trails under natural environmental conditions and recorded maximum increase for shoot length (57%), shoot fresh weight (85%), shoot dry weight (96%), and seeds weight (19%). Dastager *et al*., (2010) isolated and identified *Micrococcus* sp from a forest soil and determined the plant growth promotion potential. It was observed that the cow pea seedlings which were inoculated with *Micrococcus* sp had a 100% and 39.2% increase in root and shoot lengths, respectively compared to uninoculated control. Increase in dry biomass, as well as number of roots were also observed. Kumar *et al*., (2012) isolated various plant growth promoting strains from the rhizosphere soils of French bean plants and identified that bacteria as *Bacillus* sp, *Micrococcus sp*, and *Pseudomonas* sp.

The isolates from this study were further tested for plant growth promoting abilities such as the phosphate solubilisation and Indole Acetic Acid production and tested for their ability to inhibit pathogenic fungal growth.

4.3 Phosphate solubilisation

Phosphate is a major nutrient for plants and microbes (Khan *et al*., 2010), but is mainly bound or complexed into inaccessible forms resulting in phosphate deficiencies. Soil bacteria are key solubilisers of phosphate in the soil. There are three factors that bacteria depend on in order to solubilise phosphate, these include nutritional, physiological and growth condition of the medium. In this study bacterial isolates were tested for phosphate solubilisation potential on a modified phosphate medium-NBRIP which is media supplemented with insoluble phosphate as the sole source of P (Mehta and Nautiyal, 2001). The solubilisation activity by the bacteria is related to its organic acid production and it has been reported to be affected by the carbon source (Fasim *et al.*, 2002). Reports show that P solubilising bacteria produce organic acids such as oxalic acid, gluconic acid, malic, lactic and citric acid which lead to acidification of the microbial cells and their surroundings, this releases P ions from the P mineral by H^+ substitution for Ca^{2+} (Singal *et al*., 1994; Vyas and Gulati, 2009; Bolan *et al*., 1996; Hue *et al*., 1986) and the zone of clearance or halo on the plate is due to the production of organic acids in the surrounding medium which lead to decrease of pH (Rodriguez *et al*., 2006; Puente *et al.*, 2004). Of the 19 isolates that were tested only one isolate was able to solubilise phosphate (Fig 3.4). During identification that isolate was identified as a *Bacillus* species. This phosphate solubilising Bacillus was isolated internally from crushed spores; this is of interest as one of the key and most studied benefits of mycorrhizal fungi is enhance P uptake and acquisition (Smith and Read, 2008). Bacteria within AM spores could be contributing significantly to this mycorrhizal benefit by assisting with the release of organic acids to solubilise external complexed sources of P. Several studies have reported *Bacillus* species as a phosphate solubiliser (Khan *et al*., 2010; Maheswar and Sathiyavani, 2012; Sultan *et al*., 2010; Veline and Brahmaaprakashi, 2011). Some B*acillus* strains have been found to produce mixtures of lactic, isovaleric and isobutyric acids (Rodriguez *et al*., 1999).

Not all *Bacillus* species are able to solubilise phosphate, Saidi *et al*., (2009), isolated and tested 20 species of *Bacillus* for phosphate solubilisation and only 5 was able to solubilise phosphate. Of the 11 *Bacillus* isolates identified in this study only one was positive for solubilisation. The ability of bacterial isolates to solubilise phosphate has been linked to enhanced plant growth. Gaind and Gaur (1991) experimenting on mung bean that were grown in a P-deficient field soil amended with insoluble rock phosphate and inoculated with *B. subtilis* showed significantly increased biomass, grain yield, and P uptake. Similarly, De Freitas, *et al.*, (1997) found a number of P solubilising *Bacillus* isolates and a *Xanthomonas maltophilia* isolate from *Brassica napus L.* rhizosphere which had positive effects on plant growth. A study by Datta *et al*., (1982) showed an increase in grain yield and P uptake in rice when inoculated with P-solubilizing strain of *B. firmus* in a P-deficient soil with rock phosphate.

Interestingly the isolate *Micrococcus luteus* did not solubilise phosphate contrary to several studies that have shown this species to be a phosphate solubiliser. Chibuogwa and Nmesoma (2011), isolated *Micrococcos luteus* from rhizosphere soil that was able to solubilise phosphate and their study were supported by the findings of Tsavkelova *et al*., (2004). Either the isolate from this study is a different strain or the methodology for screening is not adequate. The halo is difficult to distinguish and bromophenol blue can be added to the medium to indicate the change in pH (Mehta and Nautiyal, 2001) lengthening of the incubation time may also be required for some isolates. Isolates in the study were incubated at 30^{o}C for 72 hrs.

Acid phosphatase production by bacteria also plays a major role in mineralisation of organically bound phosphorus in soil (Rodriguez and Fraga, 1999). Enzyme production was not tested in this study. However these isolates may have contributed to P acquisition in other way not included in this study.

4.4 Indole Acetic Acid

The 19 bacterial isolates were tested for IAA production (Cappuccino and Sherman, 2005). Only 4 isolates (E9, E10, E11 and E14 Figure 3.5, Table 3.3) gave positive results. These bacterial species were identified to be *Micrococcus* sp, *Micrococcus luteus*, *Ochrobacterum* sp and *Ochrobacterum antropi.* IAA is considered to be an important phytohormone and functions in regulation of plant development (Sahab *et al*., 2009). It has been reported that IAA production by PGPR can vary among different species and strains, and is also influenced by culture conditions, growth stage and substrate availability (Mirza *et al*., 2001). Chakraborty *et al*., (2009) isolated *Ochrobactrum anthropi* TRS-2, from the rhizosphere of tea which tested positive for IAA production. Dastager *et al*., (2010) isolated and identified a *Micrococcus* sp from forest soils for evaluation of their effectiveness as a plant growth promoter. The isolate was able to produce IAA, it was further used in a pot trial of cow pea plant and IAA was positively shown to influence root growth and development, thereby enhancing nutrient uptake of the plant. The bacteria able to produce IAA were all isolated from the external AM spore surfaces, as IAA was detected in the supernatant it is probably that the release of IAA into the soil environment stimulates root development in order to increase the probability of contact between germinating AM spores and the host plant (Zafar *et al*., 2012). Whether IAA stimulates AM spore germination and hyphal proliferation it not known, but could be further investigated in future studies. Kumar *et al*., (2012) isolated a total of 30 bacteria we from French bean and *in vitro* screened for different plant growth promotion activities such as phosphate solubilisation, IAA production, ammonia production, ACC deaminase activity, HCN production and catalase. These isolates were identified as *Bacillus* sp, *Micrococcus* sp, and *Pseudomonas* sp which had plant promoting qualities as 5 could solubilise phosphate and 5 could produce Indole acetic acid.

4.5 Fungal inhibition

Phytophthora causes the most serious soil borne diseases in citrus plants (Martin and Lopez, 1999). Biological control offers an environmentally safe alternative to chemical use and is an attractive model for plant protection against plant diseases. Many biological agents are found by screening large number of microorganisms against plant pathogen *in vitro* (Anith *et al.*, 2003; Berg *et al.*, 2005). Antagonistic microorganisms have been selected through screening methods with *Bacillus* often showing antimicrobial activity (Kloepper, 1991; Xiao *et al.*, 2002). *Bacillus* spp. has enormous potential as biological control agents because they produce active antagonistic metabolites and also form endospores that survive under adverse environmental conditions (Handelsman *et al.,* 1990; Silo-Suh *et al*., 1994). *Bacillus* spp. have been shown to produce antibiotics such as Zwittermicin A or Kanosamine which are active against *Phytophthora* spp (Milner *et al*., 1995; Silo-suh *et al*., 1994;Yu *et al*., 2002) and these traits appear to contribute to establishment and persistence of the antagonist in the plant rhizosphere (Krebs *et al*., 1998).

In this study 3 *Phytophthora* spp were tested against 19 bacterial isolates to evaluate if they had potential in inhibiting fungal growth. The chosen citrus pathogen species were *P. citrophthora, P. citrocola,* and *P. nicotianae*. An *in vitro* dual culture experiment was done where each bacterial isolate was tested against each fungal pathogen and results obtained showed that there were significant differences observed in *P. citrophthora* (Fig 3.6) as P value was 0.026 but with *P. nicotianae* (Fig 3.9) and *P. citrocola* (Fig 3.12) there were no significant differences as the P value was greater than 0.05. Isolate I5, E9, I19 did show some inhibition potential when compared with the control and a growth curve was done for three isolate, I5, E9 *and there* were significant differences observed in *P. citrophthora* (Fig 3.8) and *P. nicotianae* (Fig 3.11) as P value was less than 0.005 but with *P. citrocola* (Fig 3.14) there were no significant differences as the P value was greater than 0.05. Isolate I5 identified as a *Bacillu*s E9 identified as *Micrococcus* spp. and I19 as *Bacillus cereus*, respectively, showed some inhibition. Some *Bacillus* spp. and *Micrococcus* spp. produce antifungal inhibitors but a differential effect was observed in this study.

Bharadwaj *et al*. (2008a, 2008b) isolated *Bacillus* from the surface of G. *mosseae* and *G. intraradices* and these were found to inhibit the growth of *Rhizoctonia solani* and *Verticillium dahlia* which are fungal pathogens of potato. Ahmed *et al.,* (2003) reported some inhibition of *Phytophthora* spp by *Bacillus* in a greenhouse experiment on pepper plants. This effect was only slightly evident in this study. Interesting isolates I5 and I19 were both identified as *Bacillus* spp. Although both isolates were from internal AM spores it is clear that they are different strains or possibly different species due to their differential effect on the fungal pathogens. Isolate I5 showed no inhibition to *P. nicotianae* while I19 had some inhibitory effect against both *P. citrophthora* and *P. nicotianae*. Isolate I5 was also a phosphate solubiliser while I19 was not (Figure 3.4). This antifungal plate experiment was conducted under conditions suitable for fungal growth (28^{o}C on PDA medium). These conditions may not have been favourable for the production of secondary antagonistic metabolites by the bacterial isolates. PDA has a high carbohydrate level compared to standard bacteriological medium such as Nutrient Agar. The bacteria were inoculated into wells a distance away from the centrally inoculated fungal plug, apart from conditions perhaps not being favourable for secondary metabolite, metabolites if produced, may not have been able to diffuse through the agar.

Three bacterial isolates I5 identified as *Bacillus* spp, E9 as *Micrococcus* spp and I19 as *Bacillus* sp were selected for their potential antifungal abilities by testing the effects of their metabolites released into the medium or supernatant. Many studies have implicated antifungal secondary metabolites produced by *Bacillus* in the control of plant diseases such as brown rot caused by pathogenic fungi like *Fusarium* sp (Kotan *et al.*, 2009, Lucas-García *et al.*, 2004; Sadfi *et al.*, 2002).

4.6 Metabolite inhibition assay

Isolates I5, E9, I19 were grown in nutrient broth and supernatant was collected and used in a 96 well plate assay. Metabolite secretions were tested against the three *Phytophthora* spp. The plate assay ensured that compounds in the supernatant were in constant contact with the fungal mycelium. These isolates were identified as *Bacillus* spp, *Micrococcus* and *Bacillus cereus* spp. and all supernatants showed some inhibitory effect against the fungal pathogens, *Phytophthora citrophthora, P.citrocola,* and *P.nicotianae* (Figs 3.15; 3.16; 3.17). Negative absorbance values recorded were a result of cell debris and precipitate accumulation in the wells.

Interaction between *P. citrophthora* and the supernatant of the three bacterial isolates is shown in figure 3.12. Supernatant from both *Bacillus* sp and *Bacillus cereus* inhibited fungal growth within 3 days. *Micrococcus* sp metabolites also reduced fungal growth although the pathogen was not inhibited completely. *Bacillus* (Isolate I5) metabolite, initially reduced growth during the first 2 days, but the effect was subsequently overcome. However after day 3, growth was again reduced and inhibited by day 4 and 5 (Fig 3.15). The growth curve observed was a form of diauxic growth curve which is when a species in this case the fungal pathogen has two growth peaks. *Bacillus* spp. are known to produce enzymes such as cellulose that have degrading properties against some pathogenic fungal cell wall components (Aktugano *et al.,* 2003; Jarnagin and Ferrari, 1992). Isolate I19 metabolites initially stimulated fungal growth, possibly a nutritional effect, but thereafter growth was reduced and later inhibited. Isolate E9 metabolites decreased fungal growth. The bioassay was run for 5 days; longer incubation time may have resulted in the eventual inhibition of growth by all isolates (Fig 3.15).

In the *P. nicotianae* inhibition assay interactions with the bacterial metabolites from isolates I5, E9 and I19 presented different results as seen in *P. citrophthora*. In combination with isolate I19 metabolite there was initial growth followed by reduced growth by day 3 with inhibition occurring by day 4. Isolate I5 metabolites stimulated initial growth followed by a gradual decrease in growth with inhibition occurring by day 4. Isolate E9 metabolites reduced fungal growth but did not show inhibition (Figure 3.16). Growth of *P. citrocola* was completed inhibited by isolate I5 metabolites, while isolate I19 metabolites showed initial growth followed by a reduction after day 2 and inhibition by day 4. Only slight reduction in fungal growth was recorded for metabolites from E9 no growth was observed between the initial day and day 1 and from day 1 to day 2 there was an increase in growth. Although fungal growth was reduced, it was not completely inhibited (Figure 3.17). From these results it is interesting to note that the two internally isolated bacterial supernatants from *Bacillus* sp and *Bacillus cereus* were more antagonistically activity against *Phytophthora*.

Biological control is an important alternative in managing soil borne plant disease as the use of naturally occurring organisms is safer for the environment than chemicals (Guo *et al*., 2004; Whipps and Van, 1999). Many potential biological control agents have been found by screening (Berg *et al*., 2005). Inhibition of growth in *Phytophthora* by *Bacillus* could be explained as due to the production of antibiotics or production of enzymes such as cellulases as previously mentioned. Phytophthora belongs to the fungal phylum Oomycota which is characterised by having cellulose as a major component of their cell walls, unlike of other fungi which have chitin (Bharadwaj *et al*. 2008a). *Bacillus* spp are used as biological control agents because they produce active antagonistic metabolites and are well known to be antibiotic producers with antagonistic activity against fungi pathogens (Kloepper *et al*., 2004; Krebs *et al*., 1998; Yu *et al.,* 2002).

Bacillus spp can also enhance their antagonistic effects against fungal pathogens by competition in a form of direct parasitism (Muninbazi and Bullerman, 1998; Walker *et al.*, 1998). This mechanism was not investigated in this study.

Some species of *Bacillus cereus* have been shown to produce a novel aminopolyol antibiotic Zwittermicin A that has a potential to supress *Phytophthora* spp (He *et al*., 1994). Handelsman *et al*., (1991) used *Bacillus* spp to inhibit the growth of *Phytophthora nicotianae* in tobacco seedlings and as seen in our study *Bacillus cereus* was also able to inhibit the growth of *Phytophthora nicotianae.* Budi *et al*., (2000) isolated *Paenibacillus* spp. strain B2 that produced hydrolytic enzymes with antagonistic effect on two pathogenic fungi, *P. parasitica* and *Fusarium oxysporum.* Their results showed that the presence of *Paenibacillus* sp. strain B2 resulted in disorganisation of cell walls of *P. parasitica* and *F. oxysporum* and concluded that the inhibitory effect of *Paenibacillus sp*. strain B2 on the growth of soil-borne fungal pathogens was probably derived from production of enzymes or the cellulolytic activity of *Paenibacillus* and possibly chitin degrading potential as *Fusarium* has chitin as a major component in their cell walls.

Although there are not many studies reporting on *Bacillus* and *Phytophthor*a interaction, antagonistic activity against pathogenic fungi by *Bacillus* have been reported in several studies, Saidi *et al*., (2009) reported some inhibition effect on *Fusarium* spp by *Bacillu*s in tomato plants.

4.7 Growth evaluation of citrus growth

Citrus roots have been known to form symbiotic associations with arbuscular mycorrhizal (AM) fungi (Rayner 1935). In this study citrus seedlings were inoculated with mycorrhizal inoculum (Mycoroot^{TM}) and bacterial isolates I5, E9 and I19 in order to evaluate their effect on plant growth promotion abilities as it is well documented that arbuscular mycorrhizal fungi colonisation of roots can enhance plant growth. Masri *et al.*, (1998) reported *Glomus mosseae* colonisation was able to enhanced growth, leaf transpiration and conductance of *Garcinia mangostana L.* and also caused a 67–88% improvement in P uptake efficiency compared to un-inoculated control plants. Yano-Melo *et al.* (1999) in their studies reported that *Glomus clarum* and *Glomus etunicatum* enhanced growth, photosynthesis and transpiration rates of *Citrus jambhiri* Lush. AM fungi increased root length of *Citrus jambhiri* and increased whole plant transpiration when soil was moist. Camprubi *et al.,* (1995) inoculated *Glomus intraradices* onto citrus rootstock, *Citrus reshni* and observed the growth was stimulated as the dry weight of the plant increased from 1.2 g to 1.4 g compared with control and the AM fungi was able to colonise the roots by 51 %.

The citrus seedlings in this study were removed after 5 weeks in order to reduce the spread of a pathogen that was picked up in the roots of plants. Although this pathogen was not identified it caused wilting, so it was possibly a *Fusarium* and was initially thought to have been introduced in the compost growth medium mixture even though the medium was pasteurised. However, surplus seedlings which were not planted out into pots and left in the seedling tray also shown similar symptoms without coming into contact with the compost mixture. This suggested that the infection came in with the seedlings and was already established, limiting the ability of the mycorrhizal fungi to colonise the plant roots.

Despite the reduced experimental time significant growth difference were recorded. Unfortunately mycorrhizal colonisation was difficult to assess microscopical, experiments usually run for a minimum of 8-12 weeks. Increased mycorrhizal percentage colonisation would have indicated whether any of the bacterial isolates had mycorrhizal helper abilities. This could not be claimed, however, plant growth promotion effects were recorded. *Bacillus* sp had the most significant growth effect compared to the control when assessing shoot height.

The bacterial treatments alone did not significantly increase shoot growth as measure by difference in shoot height from planting. The combination of mycorrhizal and *Micrococcus* (isolate E9) inoculation, and mycorrhizal and *Bacillus* (isolate I19) inoculation significantly increased shoot height by approximately 3 fold. Inoculation with mycorrhizal fungi and the three bacterial isolates combined (treatment MB4) significantly increased shoot height but growth was depressed when compared to the other two treatments. Indicating that the treatment combination with the other *Bacillus* sp (isolate I5) had a negative influence on shoot height, this is interesting as treatment with isolate I5 alone was significantly better than treatment with isolate E9. This suggests that competition between potential plant growth promoting isolates needs to be consider when selecting isolates for future commercial application.

The dry shoot weight of treatments with mycorrhiza and combined bacterial isolates (MB4), *Bacillus* sp (isolate I5) and combined bacterial isolates (B4) showed a significant increase

(Figure 3.19). This is contradictory to the shoot height data which indicates that although shoot growth may have been depressed the plants energy was channelled to increasing mass. At this early stage of growth it is difficult to determine whether this would have a positive or a negative influence as the sapling mature and eventually bear fruit. There are several studies that report *Bacillus* sp as a plant growth promoter and a biocontrol agent (Wahyadi *et al*., 2011). In this study, roots were observed to be infected with a pathogenic fungus, but plants were able to survive despite this challenge. It is tempting to assume that that the bacterial treatment could be aiding growth due to some biocontrol activity, however there is no evidence to support this.

Root dry weight was significantly increased by the mycorrhizal treatment (CM) showing approximately a 3 fold increase in weight compared to the uninoculated control (C) treatment. This suggests that initial mycorrhizal effect was on stimulating root growth rather than shoot growth. Treatments MI5 - mycorrhiza and *Bacillus* sp, *Bacillus* sp alone (isolate I5) and combined bacteria (B4) also significantly increased root weight but not as much as the mycorrhizal treatment alone (Figure 3.20). Stimulation of root growth is usually associated with the production of IAA, however only isolate E9 was shown to produce IAA, although this may have contributed increase in root weight of the combined bacterial treatment (B4) the other bacterial isolates were not shown to produce IAA (Figure 3.5). This suggests that other mechanisms or other hormones may be responsible.

Total biomass (shoot plus root dry weights) was significantly increased by inoculations with treatment I5 (*Bacillus* sp) and combined bacterial treatment (Fig 3.21), indicating overall the benefits of using multi-agent based inoculum. As shoot and root weight could not be corrected for initial weight of the seedling at planting the shoot to root ratio was also analysed, results indicated a significant increase with the mycorrhizal treatment in combination with *Bacillus* sp (isolate I19) and mycorrhizal treatment combined with all three bacterial isolates (MB4) (Figure 3.22), this indicates a strong contribution of shoot biomass. Mycorrhizal treatment with *Bacillus* sp (isolate E9) had a significantly lower shoot to root ration indicating the emphasis of the root biomass (Figure 3.22).

4.8 AM fungal colonisation

4.8.1 Period of inoculation

AM fungal colonisation was not confirmed, although some contribution to growth promotion was attributed to the presence of mycorrhizal inoculation. There are several factors such as the period of trial, size of the plant as the plants were mostly well established, and lastly the bacteria interaction with the AM fungi, which may have affected colonisation. Arbuscular mycorrhizal fungi colonising a host plant roots involves a series of complex morphogenic changes in the fungus such as spore germination, hyphal differentiation, root penetration, development within the plant and arbuscule formation and that usually take about 8 to 10 weeks depending of the roots of the plants (Harrison, 1995, Smith and Read, 2008). Other studies on AM inoculated plants have reported run pot trials for a period of at least 3 or more months depending on plant (Wu and Xia, 2006). Wu *et al.*, (2008) Inoculated citrus plant (*Poncirus trifoliata)* with *G.mosseae, G. veriformme* and *G. diaphanum* for 4 months. They observed citrus seedlings were infected by AM fungi and the average root colonisation varied from 13.64% to 67.21%. *G. mosseae*- inoculation showed the highest AM fungus colonisation and hyphal density and *G. diaphanum*-inoculation the lowest AM fungus colonisation. Enhanced growth due to mycorrhizal fungi is a result of the successful establishment of colonisation and arbuscules which are involved in bidirectional nutrient transfer as well as the development of extraradical hyphal which are involved in nutrient uptake (Smith and Read, 2008). The subsample of roots selected for staining was a small portion of the root system and the initial mycorrhizal fungal contact with roots and appressorium development may have gone unobserved. The remaining roots had already been oven dried for biomass determination eliminating any further microscopic observations. The influence of unidentified fungal pathogen could have also delayed mycorrhizal establishment. Competition for space within root cells has been recorded in interactions between AM fungi and *Phytophthora.* Early establishment of AM fungi and formation of arbuscules excluded the growth of pathogenic fungi in colonised cells and adjacent cells, this has been attributed to both competition for space as well as priming of the plants own defence mechanisms through the production of phenolic compounds and pathogenesis related proteins (Mandel *et al.*, 2010). It is not unlikely that should a fungal pathogen be the first to become established in root cells, they could exclude the formation of mycorrhizal colonisation. This is particularly relevant when considering timing of application of mycorrhizal inoculum, the earlier the mycorrhizal fungi become established the better. Under nursery conditions the inoculum should be applied at the time of seeding so that developing roots quickly become colonised and the growing seedling are able to fully benefit from the association. This is particularly important for fruit trees such as citrus which are grown for several years in orchards.

CHAPTER 5

CONCLUSION AND SUMMARY

5.1 Isolation of AM spores and associated bacteria

Different bacterial species were successfully isolated from AM fungal spores extracted from soils obtained from under citrus trees. This however does not represent the complete bacterial population that associates with AM spores as many bacterial species are unculturable or may not have been isolated under the growth conditions provided. AM fungal spore identification and density was not of importance in this study as the focus was on the bacteria that were associated with the AM fungal spores. Different bacterial species were obtained from the nutrient agar plate but the bacterial isolates lacked diversity. The same bacteria were isolated from nutrient agar plates even though spore extractions were done several times. However this lack of diversity could be attributed to the surrounding soil microbial populations and it would be of interest to compare bacterial populations from the rhizosphere and AM fungal surfaces. Only a small fraction (1 to 10%) of the total bacterial community associated with AM spores is culturable. Further investigations would need to consider other methods for microbial diversity studies these could include direct molecular approaches that avoid a cultivation step to give a broader picture of the bacterial communities. The use of PCR analysis for the 16S rDNA gene permits fingerprinting of the dominant bacteria of a given sample. Suggested molecular approaches could include 454 Pyrosequencing which is able to provide not only an indication of diversity but also identify dominant species (Tedersoo *et al*., 2010).

5.2 Morphological and Molecular identification

Although microscopic examination after Gram staining provided some morphological information of the isolated bacteria, molecular techniques using PCR and sequencing was more useful in identification. The main genera identified were *Bacillus*, *Micrococcus*, *Staphylococcus* and *Ochrobacterum*, with *Bacillus* species being the most dominant. The dominance of *Bacillus* was interesting as these bacteria produce endospores which ensure survival under adverse conditions (Mobberley *et al*., 2010).

5.3 Evaluation of bacteria growth properties

One *Bacillus* species isolated from crushed AM spores was a phosphate solubiliser and this interaction may of particular relevance to ability of mycorrhizal fungi to access complexed sources of P. The isolate has the potential to be a plant growth promoter due to the nutrient uptake-enhancing property given that phosphate is an essential nutrient required for plant growth. However it must not be forgotten that the bacteria themselves as well the mycorrhizal fungi also require phosphate for growth as it is an important component as membranes, nuclei acids and provides energy for metabolic processes (Rodriguez *et al*., 2006). *Micrococcus* and *Ochrobacterum* sp were found to be indole acetic acid producers. IAA is one of the most important phytohormone and function as important signal molecule in the regulation of plant development by playing a key role in both root and shoot development of a plant (Rodriguez and Fraga, 1999).

5.4 Fungal pathogen inhibition

Two methods of assessing fungal pathogen inhibition were used in this study. The plate method showed variable inhibition result but not complete inhibition, while the metabolite bioassay using the 96 well plates showed increased inhibition possibly due to the direct contact between the inhibitory compounds and the fungal mycelium. Inhibition was not attributed to nutrient competition or starvation as control treatments showed expected fungal growth curves. *Bacillus* species were more noticeable to have inhibition effect on fungal growth of *P. citrophthora* and *P. nicotianae*. The assays confirmed that *Bacillus* sp could be potential biological control agents against these citrus pathogens *Bacillus* sp have enormous potential as biological control agents because they produce active antagonistic metabolites and also form endospores that survive under adverse environmental conditions (Handelsman *et al.,* 1990; Silo-Suh *et al*., 1994). *Bacillus* sp have shown to produce antibiotics which have antagonistic activity against fungal growth (Yu *et al*., 2002) and these traits appear to contribute to establishment and persistence of the antagonist in the plant (Krebs *et al*., 1998). Development of commercial biological control products is also able to make use of the endospores in their formulation, resulting in products with a longer shelf life.

5.5 Growth evaluation of citrus plants

Although this trial was conducted for only a short period mycorrhizal inoculation did significantly affect root development and in combination with the bacterial isolates particularly the *Bacillus* sp showed plant growth promoting abilities. Selection of bacterial isolates and their combination with mycorrhizal fungi needs to be further investigated. Future pot trials should consider the timing of inoculation of both mycorrhizal fungi and bacteria as this could affect not only the establishment of the mycorrhizal association but also improve the health of seedlings. For longer term trials multiple applications of the bacterial isolates may also be considered in order to ensure population establishment.

5.6 Conclusion

All the objectives for this study have been met to some extent this included isolation of bacteria from AM fungal spores, assessment of antagonist activity of bacterial isolates against *Phytophthora,* evaluation of plant growth promoting properties of bacterial isolates, identification of selected bacterial isolates and investigating whether bacterial isolates associated with AM fungi are important for mycorrhizal establishment and plant growth. Although further investigations are required particularly in the nursery the combined use of arbuscular mycorrhizal fungi and associated bacteria have the potential to improve citrus growth and health.

REFERENCES

Afek, U., and Sztejnberg, A. (1989). Effects of fosetyl-Al and phosphorous acid on scoparone, a Phytoalexin Associated with Resistance of citrus to *Phytophthora citrophthora. Phytopathology*. **79**. 736-739

Ahmed, A., Ezziyyani, M., Egea-Gilabert, C and Candela, M.E. (2003). Selecting bacterial strains for use in the biocontrol of diseases caused by *Phytophthora capsici* and *Alternaria alternata* in sweet pepper plants. *Biologia Plantarum*. **47**. 569–574

Al-Karaki, G.N., McMicheal, B and Zak, J. (2004). Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza*. **14**. 263-269

Alagawadi, A. R and Gaur, A. C. (1988). Associative effect of *Rhizobium* and phosphate-solubilizing bacteria on the yield and nutrient uptake of chickpea. *Plant and Soil*. **105**. 241-246

Alam, M., Khaliq, A., Sattar, A., Shukla R S, Anwar, M and Dharni S. (2011). Synergistic effect of arbuscular mycorrhizal fungi and Bacillus subtilis on the biomass and essential oil yield of rose-scented geranium *(Pelargonium graveolens*). *Archives of Agronomy and Soil Science*. **57**. 889–898

Aktugano, G.E., Melentev, A.I., Kuzmina, L.Y., Galimzyanova, N.F and Shirokov, A.V. (2003). The chitinolytic activity of *Bacillus* bacteria antagonistic to phytopathogenic fungi. *Microbiology*. **72**. 356–360.

Ames, R. N., Reid, C. P. P., Porter, L. K and Cambardella, C. (1983). Hyphal uptake and transport of nitrogen from two 15N labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. *New Phytologist*. **95**. 381-396.

Andrade, G., Mihara., K.L, Linderman., R.G. and Bethlenfalvay, G.J. (1997) Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. *Plant and Soil*. **192**. 71–7

Andrade, G., Mihara, K. L., Linderman. R. G. and Bethlenfalvay, G. J. (1998). Soil aggregation status and rhizobacteria in the mycorrhizosphere. *Plant and Soil*. **202**. 89–96.9.

Anith, K.N., Radhakrishan, N.V and Manomohandas, T.P. (2003). Screening of antagonistic bacteria for biological control of nursery wilt of black pepper (*Piper nigrum*). *Microbiology Research*. **158.** 91–97.

Artursson, V., Finlay, R. D. and Jansson, J. K. (2005). Interactions between arbuscular mycorrhizal fungi and their potential for stimulating plant growth. *Environmental Microbiology*. **8**. 1-10

Auge, R. M., Schekel , K. A and Wample, R. L. (1987a). Rose leaf elasticity changes in response to mycorrhizal colonisation and drought acclimation. *Physiologia Plantarum.* **70**. 175-182.

Azaizeh, H. A., Marschner, A., Römheld, V. and Wittenmayer, L. (1995). Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil-grown maize plants. *Mycorrhiza.* **5**. 321–327.

Azcón-Aguilar, C and Barea, J. M. (1996). Arbuscular mycorrhizas and biological control of soil-borne plant pathogens. An overview of the mechanisms involved. *Mycorrhiza.* **6**. 457–464

Bago, B., Pfeffer, P. E., Abubaker, J., Jun, J., Allen, J.W., Brouillette, J., Douds, D. D., Lammers, P. J. and Shachar-Hill, Y. (2003). Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. *Plant Physiology*. **131**. 1496–1507

Barea, J.M., Azcón, R and Azcón-Aguilar, C. (2002). Mycorrhizosphere interactions to improve plant fitness and soil quality. *Antonie van Leeuwenhoek.* **81**. 343-351

Bedini, S., Pellegrino, E and Avio, L. (2009). Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. *Soil Biology and Biochemistry.* **41**.1491–1496

Benedetto, A., Magurno, F., Bonfante, P and Lanfranco, L. (2005). Expression profiles of a phosphate transporter gene (GmosPT) from the endomycorrhizal fungus *Glomus mosseae*. *Mycorrhiza*. **15**. 620–627.

Berg, G., Krechel, A., and Ditz, M. (2005). Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *Microbiology Ecology*, **51**. 215–229.

Bethlenfalvay, G. J., Brown, M.S., Ames, R.N and Thomas, R. E. (1988) Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphate uptake. *Plant Physiology*. **72**. 565–571

Bharadwaj, D.P., Lundquist, P-O., Persson, P and Alström, S. (2008a). Evidence for specificity of cultivable bacteria associated with arbuscular mycorrhizal fungal spores. *FEMS Microbiology Ecology*. **65**. 310–322

Bharadwaj, D. P., Lundquist, P. O and Alström, S. (2008b). Arbuscular mycorrhizal fungal spore-associated bacteria affect mycorrhizal colonization, plant growth and potato pathogens. *Soil Biology and Biochemistry*. **40**. 2494–2501

Bharadwaj, D.P., Alström, S and Lundquist, P-O. (2012). Interactions among Glomus irregulare, arbuscular mycorrhizal spore-associated bacteria, and plant pathogens under in vitro conditions. *Mycorrhiza.* **22**. 437–447

Bianciotto, B., Lumini, V Lanfranco, L., Minerdi, D., Bonfante, P and Perotto, S. (2000). Detection and identification of bacterial endosymbionts in arbuscular mycorrhizal fungi belonging to the family *Gigasporaceae*. *Applied Environmental Microbiology*. **66**. 4503–4509

Black, J. (2008). Microbiology Principles and Explorations 7th Edition. John Wiley and Sons Inc USA

Bolan, N.S., Currie, L.D and Baskaran S. (1996). Assessment of the influence of phosphate fertilizers on the microbial activity of pasture soils. *Biology Fertility Soils*. **21**.284‑292

Bonfante-Fasolo, P. and Schubert, A. (1987). Spore wall architecture of *Glomus* spp. *Canadian Journal of Botany*. **65**. 539–546.

Bowen, G. D and Rovira A. D. (1991). The rhizosphere. In Plant Roots, the Hidden Half. Eds. 641– 669. Marcel Dekker, New York

Bowman. K.D., Albrecht. U and Graham, J. H. (2007). Detection of *Phytophthora nicotianae* and *P.palmivora* in citrus roots using PCR-RFLP in comparison with other methods. *European Journal Plant Pathology*. **119**. 143-158

Brundett, M and Kendrick, B. (1990). The root and mycorrhazas of herbaceous woodland plnats. *New Phytologist*. **114**. 457- 468

Brundrett, M. (2004) Diversity and classification of mycorrhizal associations. *Biological Review*. **79**. 473-495

Buee, M., Rossignol, M., Jauneau, A., Ranjeva, R and Bécard, G. (2000). The présymbiotic growth of arbuscular mycorrhizal fungi is reduced by a branching factor partially purified from plant root exudates. *Phytopathology*. **6**. 693-698

Budi, S.W., Van Tuinen, D., Martinotti, G., Gianinazzi, S. (1999). Isolation from *Sorghum bicolor* mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soil-borne fungal pathogens. *Applied and Environmental Microbiology*. **65**. 5148–5150.

Cacciola S.O and Magnano Di San Lio, G. (2008). Management of citrus disease caused by *Phytophthora spp.* Integrated Management of disease caused by fungi. 61-84

Camprubi, A., Pinochet, J., Calvet, C and Estaun, V. (1993). Effects of the root lesion nematode *Pratylenchus vulnus* and the vesicular arbuscular mycorrhizal fungus *Glomus mosseae* on the growth of three plum rootstocks. *Plant and Soil.* **153**. 223–229

Camprubi, A., Calvet, C., and Estaun, V. (1995) Growth enhancement of *citrus reshni* after inoculation with *Glomus intraradices* and *Trichderma aureoviride* and associated effects on microbial populations and enzyme activity in plotting mixes. *Plant and Soil*. **173**. 233-238

Cappuccino, J.G. and Sherman, N., (2005). Microbiology A Laboratory Manual (9th Edition). Benjamin Cummings, San Francisco, USA.

Chakraborty, U., Chakraborty, B.N., Basnet, M and Chakraborty, A.P. (2009). Evaluation of *Ochrobactrum anthropi* TRS- 2 and its talc based formulation for enhancement of growth of tea plants and management of brown root rot disease. *Journal of Applied Microbiology*. **107**. 625–634.

Chambers, C. A., Smith, S. E and Smith F. A. (1980). Effects of ammonium and nitrate ions on mycorrhizal infection, nodulation and growth of *Trifolium subterrancum*. *New Phytologist* **85**. 47–62

Chen, X., Tang, J., Zhi, G. and Hu, S. (2004). Arbuscular mycorrhizal colonisation and phosphorous acquisition plants: effects of coexisting plant species. *Applied Soil Ecology*. **28**. 259-269

Citrus Growers Association Of Southern Africa- www.cga.co.za/pages/4643/05/05/2011

Colburn G.C and Graham J.H. (2007). Protection of Citrus rootstocks against *Phytophothora* spp with a Hypovirulent isolates of *Phytophythora nicotiane*. *Phytopathology*. **97**. 958-963

Cruz, A. F., Horii, S., Ochiai, S., Yasuda, Amand Ishii, T. (2008). Isolation and analysis of bacteria associated with spores of *Gigaspora margarita*. *Journal Applied Microbiology*. **104**. 1711-1717

Daei, G., Ardekani, M.R., Rejali, F., Teimuri, S and Miransari, M. (2009). Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. *Journal of Plant Physiologist*. **166**. 617–625

Dashti, N., Zhang, F., Hynes, R and Smith D. L (1998). Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean (*Glycine max* (L.) Merr.) under short season conditions. *Plant and Soil*. **200**. 205 –213.

Dastager, S., Deepa, C.K and Pandey, A. (2010). Isolation and characterisation of noval lantis growth promoting *Micrococcus* sp NII-0901 and its interaction with cowpea plant. *Plant Physiology and Biochemistry*. **48**. 987-992

Datta, M., Banik, S and Gupta, R.K. (1982). Studies on the efficacy of a phytohormone producing phosphate solubilizing *Bacillus firmus* in augmenting paddy yield in acid soils of Nagaland. *Plant and Soil*. **69**. 365–373

de Freitas, J. R., Banerjee., M. R and Germida, J. J. (1997). Phosphate solubilizing rhizobacteria enhance the growth and yeild but not phosphorus uptake of canola *(Brassica napus*). *Biology Fertility Soils*. **24**. 358-364.

de Souza, J. T., Boer, M., Waard, P., and Raaijmakers, J. M. (2003). Biochemical, genetic and zoosporicidal properties of cyclic lipopeptide surfactants produced by *Pseudomonas fluorescens*. *Applied and Environmental Microbiology*. **69**. 7161–7172

Dighton, J. (2009). Mycorrhizae. New Lisbon, USA. Marcel Dekker 153-162

Douds, D.D.J., Pfeffer, P.E and Shachar-Hill, Y. (2000). Application of *in vitro* methods to study carbon uptake and transport by AM fungi. *Plant and Soil*. **226**. 255–261.

Duncan DB (1955) Multiple range and multiple F-tests. *Biometrics* **11**. 1–42.

Duponnois, R and Garbaye, J. (1991). Mycorrhization helper bacteria associated with the Douglas fir - *Laccaria laccata* symbiosis: effects in aseptic and in glasshouse conditions. *Annals of Forest science*. **48**. 239-251

Esitken, A., Pirlak, L., Turan, M and Sahin, F. (2006). Effects of floral and foliar application of plant growth promoting rhizobacteria on yield, growth and nutrition of sweet cherry. *Scientia Horticulture.***110**. 324-327

Ezawa, T., Smith S. E and Smith F. A. (2002). P metabolism and transport in AM fungi. *Plant and Soil*. **244**. 221-230.

Fang, J.G. and Tsao, P.H. (1995). Efficacy of *Penicillium funiculosum* as a biological control agent against *Phytophthora* root rot of Azalea and Citrus. *Phytopathology*. **85**. 871-878.

Fasim, F., Ahmed, N., Parson, R and Gadd, G.M (2002). Solubilization of zinc salts by a bacterium isolated from air environment of a tannery. *FEMS Microbiology Letter*. **213**. 1–6.

Feddermann, N., Finlay, R., Boller, T and Elfstrand, M. (2010). Functional diversity in arbuscular mycorrhiza- the role of gene expression, phosphorous nutrition and symbiotic efficiency. *Fungal Ecology*. **3**.1–8.

Figueiredo, M.V.B., Burity, H.A., Martinez, C.R and Chanway, C.P. (2008). Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Applied Soil Ecology*. **40**.182–188

Filion, M., St-Arnaud, M. and Fortin, J.A. (1999) Direct interaction between the arbuscular mycorrhizal fungus Glomus intraradices and different rhizosphere microorganisms. *New Phytologist* **141**. 525–533.

Filippi, C., Bagnoli, G., Citernesi., AS and Giovannetti M. (1998) Ultrastructural spatial distribution of bacteria associated with sporocarps of *Glomus mosseae*. *Symbiosis.* **24**. 1–12.

Fitter, A.H and Garbaye, J. (1994). Interactions between mycorrhizal fungi and other soil organisms. *Plant and Soil* **159.** 123–132.

Freita, S. S and Vildoso, C. I. A. (2004). Rhizobacteria and growth promotion of citrus Plants. *Brazilian Journal Of Soil Science*. **28**. 987-994.

Frey, B and Schüepp, H. (1992). Transfer of symbiotically fixed nitrogen from berseem (*Trifolium alexandrium* L.) to maize via vesicular-arbuscular mycorrhizal hyphae. *New Phytologist*. **122**. 447–454

Frey-Klett P, Garbaye, J and Tarkka M. (2007). The mycorrhiza helper bacteria revisited. *New Phytologist*. **176**. 22–36

Gaind, S and Gaur, A.C. (1991) Thermo tolerant phosphate solubilizing microorganisms and their interaction with mung bean. *Plant and Soil*. **133**. 141–149

Garbaye, J. (1994). Helper bacteria: A new dimension to the mycorrhizal symbiosis. *New Phytologist.* **128**. 197-210

Garcia–Romera, I., Garcia-Garrido, J.M and Ocampo, J.A. (1991a). Pectrolytic activity in vesicular arbuscular mycorryzas during colonisation of lettuce. *Symbiosis*. **12**. 189- 198

Garcia–Romera, I., Garcia-Garrido, J.M and Ocampo, J.A. (1991b). Pectrolytic activity in vesicular arbuscular mycorryzas during colonisation of lettuce. *New Phytologist*. **114**. 47-468

Graham JH (2001) What do root pathogens see in mycorrhizas? *New Phytologist*. **148**. 357-359.

Graham, J. H., and Timmer, L. W. (1992). *Phytophthora* diseases of citrus, Plant Diseases of International Importance. *Diseases of Fruit Crops*. **3**. 250-269

Graham J.H, Timmer L.W, Drouillard D.L and Peever T.L. (1998) Characterization of Phytophora spp . Causing Outbreaks of Citrus Brown Rot in Florida. *Pytopathology*. **88**. 724-729

Guissou, T. (2009). Contribution of arbuscular mycorrhizal fungi to growth and nutrients uptake and tamarind seedlings in phosphate (P) deficient soil. *African Journal of Microbiology*. **3**. 643-650

Guo, J. H., Qi, H. Y., Guo, Y. H., Ge, H. L., Gong, L. Y., Zhang, L. X. (2004). Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. *Biological Control*. **29**. 66–72

Haystead, A., Malajczuk, N and Grove, T. S. (1988). Underground transfer of nitrogen between pasture plants infected with vesicular-arbuscular mycorrhizal fungi. *New Phytologis*t. **108**. 417-423.

Handelsman, J., Raffel, S., Mester, E. H., Wunderlich, L and Grau, C. R. (1990). Biological control of damping-off of alfalfa seedlings with *Bacillus cereus* UW85. *Applied Environmental Microbiology*. **56**. 713-718

Harrison, M.J. (2005). Signalling in arbuscular Mycorrhizal symbiosis. *Annual Review Microbiology*. **59**. 19-42

Harrison, M. J and van Buuren, M. L. (1995). A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature.* **378**. 626–629

Hartmann, A., Schmid, M., van Tuinen, D and Berg, G. (2009). Plant-driven selection of microbes. *Plant and Soil.* **321**. 235–257

Hawkins, H. J., Johansen, A and George, E. (2000). Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant and Soil.* **226**. 275–285

Hildebrandt, U., Kaldorf M and Bothe H. (1999). The zinc violet and its colonisation by arbuscular mycorrhizal fungi. *Journal Plant Physiology.* **154**. 171-179

Hobbie ,S .E. (1992). Effects of plant species on nutrient cycling. *Ecology*. **7**. 336–339

Horton, T. R., Bruns, T D and Parker T. (1999). Mycorrhizal fungi associated with Arctostaphylos facilitate the establishment of *Pseudotsuga menziesii* during succession. *Canadian Journal of Botany*. **77**. 93– 102.

Hu, J., Lin, X., Wang, J., Shen, W., Wu, S., Peng, S and Mao, T. (2010). Arbuscular mycorrhizal fungal inoculation enhances suppression of *Triticum aestivum* L wilt in greenhouse soils. *Pedosphere.* **20**. 586-593.

Ippolito, A., Schena, L and Nigro F. (2002). Detection of *Phytophthora nicotianae* and *P. Citrophthora* in citrus roots and soils nested PCR. *European Journal Of Plant Pathology.***108**. 855-868

Ippolito, A., Schena L., Nigro, F., Soleti Ligorio, V. and Yaseen, T. (2004). Real-time detection of *Phytophthora nicotianae* and *P. citrophthora* citrus roots and soil. *European Journal of Plant Pathology*. **110**. 833-843.

Jackobsen, I., Abbott, L. K and Robson, A. D. (1992). External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. I. Spread of hyphae and phosphorus inflow into roots. *New Phytologist*. **120**. 371–380

Jackson, R.B., Mooney, H.A and Schulze, E.D. (1997). A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of National Academy Science*. **94**. 7362–7366

Jarnagin, A.S and Ferrari, E. (1992). Extracellular enzymes, gene regulation and relationship studies. In: *Biology of bacilli, applications to industry* (eds. R.E. Doi and M. McGloughlin), pp. 189-224. Butterworth-Hoffman Press, Boston, U.S.A.

Javot, H., Pumplin, N and Harrison, M.J. (2007). Phosphate in the arbuscular mycorrhizal symbiosis:transport properties and regularly roles. *Plant, Cell and Environment*. **30**. 310-322

Jeffries P, Gianinazzi, S., Perotto, S., Turnau K and Barea J . M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology Fertility Soils*. **37**. 1–16

Johansen, A., Jakobsen, I and Jensen, E.S. (1993). Hyphal transport by a vesicular-arbuscular mycorrhizal fungus of N applied to the soil as ammonium or nitrate. *Biology and Fertility of Soils*. **16**. 66–70

Johnson, S.E and Loeppert, R.H. (2006). Role of organic acids in phosphate mobilization from iron oxide. *Soil Science Society of America Journal.* **70**. 222–234.

Johansen, J.F., Paul, L.R and Finlay, R.D. (2004). Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiology Ecology*. **48**. 1–13.

Kean, S., Soytong, K. and To-anum, C. (2010). Application of biological fungicides to control citrus root rot under field conditions in Combodia, *Journal of agricultural technology*. **6**. 219-230

Kennedy, I.R., Pereg-Gerk, L.L., Wood, C., Deaker, R., Gilchrist, R and Katupitiya, S. (1997). Biological nitrogen fixation in non-leguminous field crops: facilitating the evolution of an effective association between *Azospirillum* and wheat. *Plant and Soil*. **194**. 65-79.

Khalid, A., Arshad, M. and Zahir Z.A. (2004). Screening plants growth promoting rhizobacteria for improvement of growth and yield of wheat. *Journal of Applied Microbiology*. **96**: 473-480

Khan, M.S., Zaidi, A., Ahemad, M., Oves, M and Wani, P.A. (2010). Plant growth promotion by phosphate solubilizing fungi-current perspective. *Archives of Agronomy and Soil Science*. **56**. 73-98

Kim, K., Yim, W., Trivedi, P., Madhaiyan, M., Deka Boruah, H., Islam, M., Lee, G., Sa. T. (2010). Synergistic effects of inoculating arbuscular mycorrhizal fungi and *Methylobacterium oryzae* strains on growth and nutrient uptake of red pepper. *Plant and Soil*. **327**. 429–440

Kloepper, J.W., Zablotowicz, R.M., Tipping, E.M and Lifshitz, R. (1991). Plant growth promotion mediated by bacterial rhizosphere colonizers. In: Keister DL, Cregan PB (eds.) The Rhizosphere and Plant Growth. Kluwer Academic Publishing, Dordrecht, pp. 315-326.

Kohler., J, Caravaca., F and Roldan, A. (2009). Effect of drought on the stability of rhizosphere soil aggregates of *Lactuca sativa* grown in a degraded soil inoculated with PGPR and AM fungi. *Applied Soil Ecology*. **42**.160–165

Koske, R.E and Gemma, J.N. (1989). A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92**. 48-505

Kotan, R., Sahin, F., Demirci, E and Eken, C. (2009). Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. *Biological Control*. **50**.194–198

Krebs, B., Hoding, B., Kubart, S. M., Workie, A., Junge, H., Schmiedeknecht, G., Grosch, R., Bochow, H and Hevesi, M. (1998).Use of *Bacillus subtilis* as biocontrol agent. Activities and characterization of *Bacillus subtilis* strains. *Journal of Plant Disease and Protection*. **105**. 181-197

Krüger, M., Stockinger, H., Krüger, C and Schüßler, A. (2009). DNA-based species level detection of *Glomeromycota*: PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist*. **183**. 212-223

Kumer, P., Dubey, R.C and Maheshwari, D.K. (2012).Bacillus strain isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathology. *Microbiological Research.* **167**. 493-499

Larsen, J and Bødker, L. (2001). Interactions between pea root inhabiting fungi examined using signature fatty acids. *New Phytologist*. **149**. 487–493

Larsen, J., Ravnskov, S and Jakobsen, I.(2003).Combined effect of an arbuscular mycorrhizal fungus and a biocontrol bacterium against *Pythium ultimum* in soil. *Folia Geobotanica.* **38**. 145–154.

Lecomte. J., St-Arnaud, M and Hijri, M. (2011). Isolation and identification of soil bacteria growing at the expense of arbuscular mycorrhizal fungi. *Research letter*. **317**. 43–51

Levy., Y, Levi. R and Cohen Y. (1983) Buildup a pathogen subpopulation resistant to systemic fungicide under various control strategies: a flexible simulation model. *Phytopathology*. **73**. 1475-1480

Li, B., Ravnskov, S., Xie, G and Larsen, J. (2007). Biocontrol of *Pythium* damping-off in cucumber by arbuscular mycorrhiza-associated bacteria from the genus *Paenibacillus*. *Biological Control*. **52**. 863–875

Linderman, R.G. (1988). Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology*. **78**. 366-371

Lucas-Garcia, J.A., Probanza, A., Rumos, B., Barriuso, J and Gutierrez-Manero, F.J. (2004). Effect of inoculation with plant growth promoting rhizobacteria nodulation and growth Glycine max cv Osumi. *Plant and soil*. **267**. 143-153.

Ma, Z., Bielenberg D.G., Brown K.M and Lynch, J.P. (2001). Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. *Plant, Cell & Environment* **24**. 459–467.

Maia, L. C. and Kimbrough J. W. (1998). Ultrastructural studies of spores and hypha of a *Glomus* species. *International Journal of Plant Science*. **159**. 581–589

Maheswar and Sathiyavani, G. (2012). Solubilization of phosphate by bacillus sp from grounhut rhizosphere (*Arachishypogaea* L). *Journal of Chemical and Pharmaceutical Research*. **4**. 4007-4011

Maldonado-Mendoza, I.E., Dewbre, G. R and Harrison, M. J. (2001). A phosphate transporter gene from the extraradical mycelilum of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. *Molecular Plant-Microbe Interaction.* **14**. 1140–1148

Malekzadeh, E., Alikhani, H.A., Savaghebi-Firoozabadi, G.R and Zarei, M. (2011). Influences of arbuscular mycorrhizal fungi and an improvement growth bacterium on Cd uptake and maize growt in Cd polluted soils. *Spanish Journal of Agricultural Research*. **9**. 1213-1223

Marschner , H And Dell, B (1994) Nutrient uptake in mycorrhizai symbiosis. *Plant and Soil* **159**. 89-102.

Martin, F. N and Lopez, J.E. (1999). Soilborne plants disease caused by *Pythium* spp. Ecology, epidemiology and prospect for biological control. *Citrus Plant Review*. **18**. 111-181

Masri, M., Azizah, H., Mohd, R.I and Mamat, A.S. (1998). Arbuscular mycorrhiza enhances growth and reduces the nursery period of mangosteen (*Garcinia mangostana* L.) seedlings. J Trop Agric Food Sci 26:7–15

Matsubara,Y.,Tamura,H and Harada,T.(1995).Growth enhancement and *Verticillium wilt* control by vesicular-arbuscular mycorrhizal fungus inoculation in egg plant. *Journal of the Japanese Society for Horticultural Science.* **65**. 555–561

Mayo, K., Davis, R. E and Motta, J. (1986). Stimulation of germination of spores of *Glomus versiforme* by spore-associated bacteria. *Mycologia.* **78**. 426 – 431

Mehta, S and Nautiyal, S.C. (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Current Microbiology*. **43**.51-56

Menge, J. A and Nemec, S (1997) Citrus Soilborne disease of tropical Crops. 185-227 CAB International, Willingford ,UK

Milner, J.L., Raffel, S.J., Lethbridge, B.J and Handelsman, J., (1995). Culture conditions that influence accumulation of zwittermicin a by *Bacillus cereus* UW85. *Applied Microbiology and Biotechnology.* **43**. 685–691

Miransari, M. (2011). Interactions between arbuscular mycorrhizal fungi and soil bacteria. *Applied Microbiology Biotechnology.* **89**. 917-930.

Mirza, M.S., Ahmad, W., Latif, F., Haurat, J., Bally, R., Normand, P and Malik, K.A. (2001). Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane in vitro. *Plant and Soil.* **237**. 47–54.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L and Swan, J.A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*. **115**. 495-501

Mobberley, J., Authement, N. R., Segall, A. M., Edwards, R.A., Slepecky, R.A and Paul, J. H. (2010). Lysogeny and Sporulation in *Bacillus* Isolates from the Gulf of Mexico. *Applied and Environmental Microbiology*. **76**. 829–842

Mohammad, M. J., Malkawi, H.I and Shibli R. (2003).Effects of arbuscular mycorrhizal fungi and phosphorous fertilization on growth and nutrient uptake of barley grown on soils with different levels of salts. *Journal of Plant Nutrition*. **26**.125–137.

Morton, J.B and Msiska, Z. (2010). Phylogenies from genetic and morphological characters do not support a revision of Gigasporaceae (*Glomeromycota*) into four families and five genera. *Mycorrhiza.* **20**. 483–496

Muninbazi, C and Bullerman, L.B. (1998). Isolation and partial characterization of antifungal metabolites of *Bacillus pumilus*. *Journal of Applied Microbiology*. **84**. 959-968.

Nagy. R., Drissner, D., Amrhein, N., Jakobsen, I. and Bucher, M. (2009). Mycorrhizal phosphate uptake pathway in tomato is phosphorus-repressible and transcriptionally regulated. *New Phytologist* . **181**. 950–959

Newman, E.I and Reddell, P. (1987). The distribution of mycorrhizas among families of vascular plants. *New Phytologist*. **106**. 745-751

Okon, Y and Kapulnik, Y.(1986). Development and function of *Azospirillum* inoculated roots. *Plant and Soil*. **90**. 3-16

Orhan, E., Esitken, A., Ercisli, S., Turan, M and Sahin, F. (2006). Effects of plant growth promoting rhizobacteria on yield, growth and nutrient contents in organically growing raspberry. *Scientica horticulture*. **111**. 38-43

Patten, C.L and Glick, B.R. (2002). Role of *Pseudomonas putida* indole-acetic acid in development of the host plant root system. *Applied Environmental Microbiology*. **68**. 3795-3801.

Paszkowski, U. (2006). A journey through signalling in arbuscular mycorrhizal symbiosis. *New Phytologist*. **172**. 35- 46

Paszkowski, U., Kroken, S., Roux, C and Briggs, S.P. (2002). Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* **99**.13324–13329

Pirlak, L and Kose, M. (2009). Effect of plant growth promoting rhizobacteria on yield and some fruit properties of strawberry. *Journal of Nutrition*. **32**. 1173-1184

Pivato, B., Offre, P., Marchelli, S., Barbonaglia, B., Mougel, C., Lemanceau, P and Berta G. (2009). Bacterial effects on arbuscular mycorrhizal fungi and mycorrhiza development as influenced by the bacteria, fungi, and host plant. *Mycorrhiza.* **19**. 81–90

Porcel, R and ruiz-Lozano J.M. (2004). Arbuscular mycorrhizal influence on leaf water potential solute accumulation and oxidative stress in soyabean plants subjected to drought stress. *Journal of Experimental Botany*. **55**. 1743-1750

Puente, M. E., Bashan., Y., Li., C.Y and Lebsky, V.K. (2004). Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. *Plant Biology***. 6**.629–642

Quilambo, O.A. (2003). The vesicular arbuscular mycorrhizal symbiosis. *African Journal of Biotechnology*. **2**. 539-546

Rayner, M.C. (1935). Mycorrhizal habit in the genus *Citrus*. Nature. **136**. 516–517

Read, D. J and Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems: a journey towards relevance? *New Phytologist* **157**. 475–492.

Rillig, M.C., Wright, S.F and Eviner, V. (2002). The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant and soil*. **238**. 325-333.

Rodríguez, H and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biochemistry Advances*. **17**. 319-339.

Rodríguez, H., Fraga, R., Gonzalez, T and Bashan, Y. (2006). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil*. **287**. 15–21

Roesti, D., Ineichen, K., Braissant, O., Redecker, D., Wiemken, A. and Aragno M. (2005) Bacteria associated with spores of the arbuscular mycorrhizal fungi *Glomus geosporum* and *Glomus constrictum. Applied and Environmental Microbiology*. **71**. 6673–6679.

Ruiz-Lozano, JM., Azcon, R and Gomez, M. (1995) Effects of arbuscular mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses. *Applied Environmental Microbiology*. **61**. 456–60

Saidi, N., Kouki, S., M'hiri, F., Hajlaoui, M-R., Mahrouk, M., Ouzari, H., Jedidi, N and Hassen A. (2009). Characterization and selection of *Bacillus* sp. strains, effective biocontrol agents against *Fusarium oxysporum* f. sp. *radicis-lycopersici*, the causal agent of Fusarium crown and root rot in tomato. *Annals of Microbiology*. **59**. 191-198

Sadfi, N., Cherif, M., Hajlaoui, M.R and Boudabbous, A. (2002). Biological control of the potato tubers dry rot caused by *Fusarium roseum* var. sambucinum under greenhouse, field and storage conditions using *Bacillus* spp. isolates. *Journal of Phytopathology*. **150**. 640–648

Safa, A and Basharat, A. (2011). Evaluation of rhizobacteria as non-rhizobial inoculants. *Australian Journal of Crop Science*. **5**. 1723-1729

Sarig, S., Blum, A and Okon, Y. (1988). Improvement of water status and yield of field-grown grain sorghum (*Sorghum bicolor*) by inoculation with *Azospirillum brasilense*. *Journal Agricultural Science.* **110**. 271

Sikes, B.A., Cottenie, K and Klironomos, J.N. (2009). Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology*. **97**.1274–1280.

Silo-Suh, L. A., Lethbridge, B. J., Raffel, S. J., He, H., Clardy, J. and Handelsman, J. (1994). Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. *Applied Environmental Microbiology*. **60**. 2023-2030

Singal, R., Gupta, R and Saxena, R.K. (1994). Rock phosphate solubilization under alkaline conditions by *Aspergillus japonicus* and *A. foetidus*. *Folia Microbiologica*. **39**. 33–36

Shahab, S., Ahmed N and Khan N-S. (2009). Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. *African Journal of Agricultural Research*. **4**. 1312-1316.

Smith, S and Dickson, S. (1997). VA Mycorrhizas: Basic research techniques. Cooperative Research Centre for Soil and Land Management, Adelaide, Australia. 39

Smith, S.E., Smith, F.A and Jakobsen I. (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology.* **133**. 16–20.

Smith, S.E., Read, D.J., (2008). In: Mycorrhizal Symbioses. 3rd Edition Academic Press, New York, USA.

Schachtman, D.P., Reid, R.J and Ayling, S.M. (1998). Phosphorus uptake by plants: from soil to cell. *Plant Physiology.* **116**. 447–453

Schüßler, A., Schwarzott, D. and Walker, C. (2001b). A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research.* **105**. 1413–1421

Söderström B (1992) The ecological potential of the ectomycorrhizal mycelium. In Mycorrhizas in Ecosystems. Eds. D J Read, D H Lewis, A H Filter and I J Alexander. pp 77–83. CAB International, Wallingford, UK.

Soytong, K., Kanokmedhakul, S., Kukongviriyapan, V. and Isobe, M. (2001). Application of *Chaetomium* species as new broad spectrum biological fungicide for plant diseases control. *Fungal Diversity.* **7**. 1-15.

Subramanian, K.S., Charest, C., Dwyer, L.M and Hamilton, R. (1995). Arbuscular mycorrhizas and water relation in maize under drought stress at tasseling. *New Phytologist.* **129**. 643-650

Sultana, S. M., Maya, C., Peter, V and Omar, F. (2010). Biochemical characterisation of phospho bacterium isolated from rhizosphere of costus sp with special reference to *bacillus* spp. *International Journal of Pharmaceutical Science and Research.* **9**. 116-119

Sylvia, D. M., Hammond, L.C., Bennet, J. M., Hass, J.H and Linda, S.B. (1993). Field response of maize to a VAM fungus and water management. *Agronomy Journal*. **85**. 193–198

Tawaraya K., Turjaman M., EkamawantiH.(2007). Effect of arbuscular mycorrhizal colonisation of nitrogen and Phosphorus uptake and growth of *Aloe Vera* L. *New Horticulture Science.* **42**. 1737-1739

Tedersoo, L., Nilsson, R. H., Abarenkov, K., Jairus, T., Sadam, A., Saar, I., Bahram, M., Bechem, E., Chuyong G and Koljalg, U. (2010). 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytologist*. **188**. 291–301

Tian, H., Gai, J.P., Zhang, J.L, Christie, P and Li, X.L. (2009). Arbuscular mycorrhizal fungi in degraded typical steppe of inner Mongolia. *Land Degradation and Development*. **20**. 41-54.

Ticconi C.A. and Abel S. (2004) Short on phosphate: plant surveillance and countermeasures. *Trends in Plant Science.* **9**. 548–555.

Tilak, K. V., Ranganayaki, B. R and Manoharachari, C. (2006) Synergistic effects of plant-growth promoting rhizobacteria and Rhizobium on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*). *European Journal of Soil Science*. **57**. 67–71

Timmer, L. W., Graham, J. H., and Zitko, S. E. (1998). Metalaxyl-resistant isolates of *Phytophthora nicotianae*: Occurrence, sensitivity, and competitive parasitic ability on citrus. *Plant Disease Journal*. **82**. 254-261.

Tisdall, J.M and Oades, J.M. (1982). Organic matter and water stable aggregates in soil. *Journal of Soil Science*. **33**. 141-163

Toljander, J.F., Artursson, V., Paul, L.R., Jansson J.K and Finlay, R. D. (2006). Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species. *FEMS Microbiology Letter*. **254**. 34–40

Trotta, A., Varese, G.C., Gnavi, E., Fusconi, A., Sampo, S and Berta,G.(1996).Interactions between the soil borne root pathogen *Phytophthora nicotianae var. parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. *Plant and Soil.* **185**. 199–209.

Tsavkelova, E., Cherdyntseva, T and Netrusov, A. (2005). Auxin production by bacteria associated with orchid roots. *Microbiology*. **74**. 46-53

Vance, C. P., Uhde-Stone , C. and Allan D. L. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist.* **157**. 423–447.

Velineni, S and Brahamaprakash, G.P. (2011). Survival and phosphate solubilizing ability of *bacillus megaterium* in liquid inoculants under high temperature and desiccation stress. *Journal of Agricultural Science and Technology*. **13**. 795-802

Vigo, C., Norman, J.R and Hooker, J.E. (2000). Biocontrol of the pathogen *Phytophthora parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. *Plant Pathology*. **49**.509–514

Vyas, P and Gulati, A. (2009) Organic acid production in-vitro and plant growth promotion in maize under controlled environment by phosphate solubilizing fluorescent *Pseudomonas*. *BMC Microbiology*. **9**. 174

Walker, R., Powel, A.A and Seddon, B. (1998*). Bacillus* isolates from the spermosphere of peas and dwarf French beans with antifungal activity against *Botrytis cinerea* and *phythium* species. *Journal of Applied Microbiology*. **84**. 791-801.

Wandersman, C. and Delepelaire, P (2004). Bacterial iron sources: from siderophores to hemophores. *Annual. Review. Microbiol*ogy. **58**. 611–647.

Wang, Y.Y., Vestberg, M., Walker, C., Hurme, T., Zhang, X.P and Lindström, K. (2008). Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan Province of mainland China. *Mycorrhiza.* **18**. 59- 68

Wang, B and Qiu, Y.L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza.* **16**. 299-363

Weisburg, W.G., Barns, S.M., Pelletier, D.A and Lane, D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*. **173**. 697-707

Wehner, J., Antunes, P. M., Powell J. R., Mazukatow, J and Rilling, M.C (2009). Plant pathogen protection by arbuscular mycorrhizas: A role for fungal diversity. *Pedobiologia*. **53**. 197-201

Whipps, J.M. (2004). Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany*. **82**. 1198–1227.

Whipps, J. M and Van, J. D. (1997). Ecological considerations involved in commercial development of biological control agents for soil-borne diseases In: Modern soil microbiology. 525–545. New York: Marcel Dekker, Inc

Wu, Q.S and Xia, R.X. (2006). Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology*. **163**. 417–425

Wu, O. S., Xia, R. X. and Zou Y. N. (2008). Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *European Journal of Soil Biology* **44**.122–128

Wu, Q-S and Zou, Y-N. (2009). Arbuscular mycorrhizal symbiosis improves growth and root nutrient status of citrus subjected to salt stress. *Science Asia.* **35**. 388–391

Xavier, L.J.C and Germida, J.J. (2003). Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity. *Soil Biology and Biochemistry* **35**. 471–478.

Xiao, K., Kinkel, L.L and Samac, D.A. (2002). Biological control of Phytophthora root rots on alfalfa and soybean with Streptomyces. *Biological Control*. **23**. 285–295

Yano-Melo, A.M., Saggin, O.J., Lima-Filho, J.M., Melo, N.F and Maia, L. C. (1999). Effect of arbuscular mycorrhizal fungi on the acclimatization of micropropagated banana plantlets. *Mycorrhiza* **9**.119–123

Yu, G.Y., Sinclair, J.B., Hartman, G.L and Bertagnolli, B.L. (2002). Production of iturin a by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. *Soil Biology and Biochemistry*. **34**. 955-963

Zafar, M., Abbasi, M.K., Khan, M.A., Khauq, A., Sultan, T and Aslam, M. (2012). Effect of plant growth promoting rhizobacteria on growth nodulation and nutrient accumulation of lentil. *Pedosphere.* **22**. 848-859

APPENDICES

APPENDIX A

Modified phosphate medium-NBRIP (Mehta and Nautiyal, 2001).

Preparation in 1L

10 g glucose,

5 g $CaHPO_4.2H_2O$,

5 g $MgCl_2.6H_2O$,

0.25 g $MgSO_4.7H_2O$,

0.2 g KCL,

0.1 g $(NH_4)_2 SO_4$

15 g of agar

APPENDIX B

1% agarose gel

1g agarose powder

100 ml distilled water

TE (Tris/ EDTA) Buffer pH 8

Tris/ HCI pH 8	10mM
EDTA pH 8	10mM

Preparation in 1 liter

5X TBE (Tris-EDTA) Buffer, mix following:

- 5.3g of Tris base
- 27.5g of boric acid

- 20 ml 500 mM EDTA pH 8.0
- 1000 ml distilled water

To make make 1X TBE Buffer working solution

Add 200ml of 5X TBE in 800ml distilled water

Storage is at room temperature.

APPENDIX C

Roots staining solutions (Smith and Dickson, 1997)

5% KOH

100 g KOH

2 L distilled water

Alkaline Peroxide H_2O_2

3 ml NH_4OH (Ammonia)

30 ml 10% H_2O_2

576 ml distilled water

0.1 M HCI (MW36.46)

22.79 ml HCI

2 L distilled water

Lactoglycerol trypan blue stain

Lactic acid: Glycerol: Water (13:12:16)

520 ml lactic acid

480 ml Glycerol

640 ml distilled water

APPENDIX D

Alignment of isolated bacterial species with species from NCBI Genbank where the query is the isolated species and the subject is the species aligned with in the GenBank

E1 species alignment with Genbank *Bacillus sp*

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Query  4      CGCGNCTATACATGCAGTCGAGCGAATGGATTNAGAGCTTGCTCTYANGAAGTTAGCGGC  63
              |||| ||||||||||||||||||||||||||| |||||||||||| | ||||||||||||
Sbjct  2      CGCGTCTATACATGCAGTCGAGCGAATGGATTGAGAGCTTGCTCTCAAGAAGTTAGCGGC  61

Query  64     GGACNGNTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCG  123
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Sbjct  62     GGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCG  121

Query  124    GGGCTAATACNGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCT  183
              |||||||||| |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  122    GGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCT  181

Query  184    GTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGG  243
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Sbjct  182    GTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGG  241

Query  244    CAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA  303
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Sbjct  242    CAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA  301

Query  304    GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA  363
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Sbjct  302    GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA  361

Query  364    ACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAG  423
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  362    ACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAG  421

Query  424    TGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACG  483
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  422    TGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACG  481

Query  484    TGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAG  543
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Sbjct  482    TGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAG  541

Query  544    CGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCA  603
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             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  604   TTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAA  663
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  602   TTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAA  661

Query  664   ATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACA  723
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  662   ATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACA  721

Query  724   CTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA  783
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Sbjct  722   CTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA  781

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Sbjct  782   ACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAG  841

Query  844   CACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGC  903
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Query  904   ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGA  963
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Sbjct  902   ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGA  961

Query  964   CATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGC  1023
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  962   CATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGC  1021

Query  1024  ATGGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA-GTCCCGCAACGAGCGCACCC  1082
             |||| |||||||||||||||||||||||||||||||||| ||||||||||||||||| ||
Sbjct  1022  ATGG-TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC  1080

Query  1083  CTTGATCTTAGTTGCCATCATTAAGTTGGGCMCTCTACG-TGACTGCCGGTGAACAACCG  1141
             ||||||||||||||||||||||||||||||| ||||| | |||||||||||||  |||||
Sbjct  1081  CTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCG  1140

Query  1142  -AG-A-G-TGGG-ATGACGTCAA-TCATCCATGGCCCCTT  1175
              || | | |||| |||||||||| ||||| ||| ||||||
Sbjct  1141  GAGGAAGGTGGGGATGACGTCAAATCATC-ATG-CCCCTT  1178
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E2 species alignment with Genbank *Staphylococcus sp*

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Query  5    GGCGGGNCTAATACATGC-AGTCGAGCGAACAGATAAGGAGCTTGCTCCTTTGACGTTAG  63
            |||| | ||||||||||| |||||||||||||||||||||||||||||||||||||||||
Sbjct  10   GGCGTGCCTAATACATGCAAGTCGAGCGAACAGATAAGGAGCTTGCTCCTTTGACGTTAG  69

Query  64   CGGCGGACGGNTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAA  123
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Query  424  AAATGTGTAAGTAACTGTGCACATCTTGACGGTACCTGATCAGAAAGCCACGGCTAACTA  483
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Sbjct  550  AGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGT  609
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Query  604   CATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTG  663
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  610   CATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTG  669

Query  664   AAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGA  723
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  670   AAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGA  729

Query  724   CGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT  783
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  730   CGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT  789

Query  784   AAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTA  843
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  790   AAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTA  849

Query  844   AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCC  903
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  850   AGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCC  909

Query  904   GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTT  963
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  910   GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTT  969

Query  964   GACATCCTTTGACCGCTCTAGAGATAGAGTCTTCCCCTTCGGGGGACAAAGTGACAGGTG  1023
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  970   GACATCCTTTGACCGCTCTAGAGATAGAGTCTTCCCCTTCGGGGGACAAAGTGACAGGTG  1029

Query  1024  GTGCATGGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1083
             |||||||| |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1030  GTGCATGG-TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC  1088

Query  1084  A-CC-TTA-GCTTAGCTGCCATCATTA-GT-GGGCACTCTA-GCTGACTGCCCGATGACA  1137
             | || ||| |||||| ||||||||||| || |||||||||| | |||||||| | |||||
Sbjct  1089  AACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGG-TGACA  1147

Query  1138  GTC-GGAAGAATGGTGGG-ATGACGTTCAAATC  1168
               | ||| ||| |||||| ||||||| ||||||
Sbjct  1148  AACCGGAGGAA-GGTGGGGATGACGT-CAAATC  1178
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E3 species alignment with Genbank *Bacillus thuringiensis*

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Query  4      CGGCGT-CTATACATGCAAGTCGAGCGAATGGATTRAGAGCTTGCTCTYANGAAGTTAGC  62
              |||||| |||||||||||||||||||||||||||| |||||||||||| | |||||||||
Sbjct  9      CGGCGTGCTATACATGCAAGTCGAGCGAATGGATTGAGAGCTTGCTCTTATGAAGTTAGC  68

Query  63     GGCGGACGGNTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAA  122
              ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  69     GGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAA  128

Query  123    CCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCG  182
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  129    CCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCG  188

Query  183    GCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA  242
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  189    GCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA  248

Query  243    AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  302
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  249    AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  308

Query  303    CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA  362
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  309    CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA  368

Query  363    GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAAC  422
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  369    GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAAC  428

Query  423    AAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT  482
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  429    AAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT  488

Query  483    ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA  542
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  489    ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA  548

Query  543    AAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGG  602
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  549    AAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGG  608
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Query  603   TCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGT  662
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  609   TCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGT  668

Query  663   GAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTG  722
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  669   GAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTG  728

Query  723   ACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG  782
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  729   ACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG  788

Query  783   TAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATT  842
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  789   TAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATT  848

Query  843   AAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC  902
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  849   AAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC  908

Query  903   CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCT  962
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  909   CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCT  968

Query  963   TGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGG  1022
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  969   TGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGG  1028

Query  1023  TGCATGGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA  1082
             ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1029  TGCATGG-TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA  1087

Query  1083  ACCCTTGATCT-AGTTGCCATCATTA-GT-GG-CNCTCTAAGGTGACTGCCGGTGANAA-  1137
             ||||||||||| |||||||||||||| || || | ||||||||||||||||||||| || 
Sbjct  1088  ACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAA  1147

Query  1138  C-GGAGGAAGGTGGG-ATGACGTCA--TCATCATNGCCCTTATGACCTGGGCT  1186
             | ||||||||||||| |||||||||  |||||||  |||||||||||||||||
Sbjct  1148  CCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCT  1200
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E4 species alignment with Genbank *Bacillus cereus*

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Query  10   CTATACATGC-AGTCGAGCGAATGGATTNAGAGCTTGCTCTYANGAAGTTAGCGGCGGAC  68
            |||||||||| ||||||||||||||||| |||||||||||| | ||||||||||||||||
Sbjct  14   CTATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGAC  73

Query  69   GGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGC  128
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  74   GGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGC  133

Query  129  TAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCA  188
            ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  134  TAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCA  193

Query  189  CTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAAC  248
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  194  CTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAAC  253

Query  249  GATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACT  308
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  254  GATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACT  313

Query  309  CCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGC  368
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  314  CCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGC  373

Query  369  CGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCT  428
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  374  CGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCT  433

Query  429  AGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCC  488
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  434  AGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCC  493

Query  489  AGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCG  548
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  494  AGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCG  553

Query  549  CGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGG  608
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  554  CGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGG  613

Query  609  AAACTGGGAGACTTGAGTGCANAANAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGC  668
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                  ||||||||||||||||||||| || |||||||||||||||||||||||||||||||||||
Sbjct  614        AAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGC  673

Query  669        GTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTTCTGGTCTGTAACTGACACTG  728
                  ||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  674        GTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT-CTGGTCTGTAACTGACACTG  732

Query  729        AGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGYCCACGCCGTAAACG  788
                  ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  733        AGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG  792

Query  789        ATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCAC  848
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  793        ATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCAC  852

Query  849        TCCGCCTGGGGAGTACGGCCGCAAGGCTGAA-CTCAAAGGATTTGACGGGGGCCCGCACA  907
                  ||||||||||||||||||||||||||||||| ||||||||| ||||||||||||||||||
Sbjct  853        TCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACA  912

Query  908        AGCGGTG-AGCATGTG-TTTAATTCGAAGCA-CGCGAAGAACCTTACCAGGTCTTGACAT  964
                  ||||||| |||||||| |||||||||||||| ||||||||||||||||||||||||||||
Sbjct  913        AGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT  972

Query  965        CCTCTGA-AACC-TAGAGATAGG-C-TCTC-TTCGG-AGCAGAGTGACAGGTGGTGCATG  1018
                  ||||||| |||| |||||||||| | |||| ||||| |||||||||||||||||||||||
Sbjct  973        CCTCTGACAACCCTAGAGATAGGGCCTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATG  1032

Query  1019       GTTGTCGTCAGCTCGTGTCGTGAGATGTGGGTTA-GTCC-GCA-CGAGCGCACC-T-GAT  1073
                  |||||||||||||||||||||||||||||||||| |||| ||| |||||||||| | |||
Sbjct  1033       GTTGTCGTCAGCTCGTGTCGTGAGATGTGGGTTAAGTCCCGCAACGAGCGCACCCTTGAT  1092

Query  1074       CTAGTGCCATCATTAAGTGG-CACTCTACGTGACTGCCGNACAC-CGAGAGGTGGTATN-  1130
                  ||||||||||||| |  ||| ||||||| ||||||||   |||  |||||||||| ||
Sbjct  1093       CTAGTGCCATCAT-AGTTGGGCACTCTA-GTGACTGCGTGACAAACGAGAGGTGGGATGA  1150

Query  1131       CGTCATCAN-ATGCNCT-ATGACCTGGCTTACA  1161
                  ||||||||  |||| || |||||  ||||||||
Sbjct  1151       CGTCATCATCATGCCCTTATGACTGGGCTTACA  1183
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I5 species alignment with Genbank *Bacillus sp*

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Query  11    CTAATACATGCAAGTCGAGCGAATGGATTNAGAGCTTGCTCTNANGAAGTTAGCGGCGGA  70
             ||||||||||||||||||||||||||||| |||||||||||| | |||||||||||||||
Sbjct  38    CTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGA  97

Query  71    CGGNTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGG  130
             ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  98    CGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGG  157

Query  131   CTAATACYGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTC  190
             ||||||| |||||||||||||||| |||||||||||||||||||||||||||||||||||
Sbjct  158   CTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTC  217

Query  191   ACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAA  250
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  218   ACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAA  277

Query  251   CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGAC  310
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  278   CGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGAC  337

Query  311   TCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG  370
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  338   TCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACG  397

Query  371   CCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGC  430
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  398   CCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGC  457

Query  431   TAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGC  490
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  458   TAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGC  517

Query  491   CAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGC  550
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  518   CAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGC  577

Query  551   GCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTG  610
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  578   GCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTG  637

Query  611   GAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATG  670
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             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  638   GAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATG  697

Query  671   CGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTG  730
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  698   CGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTG  757

Query  731   AGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG  790
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  758   AGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG  817

Query  791   ATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCAC  850
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  818   ATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCAC  877

Query  851   TCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACA  910
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  878   TCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACA  937

Query  911   AGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT  970
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  938   AGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACAT  997

Query  971   CCTCTGAAA-CCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATG  1029
             ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  998   CCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATG  1057

Query  1030  GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA-CCCTTG  1088
             ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  1058  GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTG  1117

Query  1089  ATCTTAGTTGCCATCATTA-GTTGGGCACTCTAAGGTGACTGCCGGTGACAA-CCGGGAG  1146
             ||||||||||||||||||| |||||||||||||||||||||||||||||||| || ||||
Sbjct  1118  ATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCCGGAG  1177

Query  1147  -A-GGTGGGGGATGACGTTCAAATCATCATGCC--TNATGAACCTGGGCCTAC  1195
              | ||||||||||||||| ||||||||||||||  | |||| ||||||| |||
Sbjct  1178  GAAGGTGGGGGATGACGT-CAAATCATCATGCCCCTTATGA-CCTGGGC-TAC  1227
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E6 species alignment with Genbank *Bacillus thuringiensis*

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Query  4      CGGCGT-CTATACATGCAAGTCGAGCGAATGGATTRAGAGCTTGCTCTNANGAAGTTAGC  62
              |||||| |||||||||||||||||||||||||||| |||||||||||| | |||||||||
Sbjct  9      CGGCGTGCTATACATGCAAGTCGAGCGAATGGATTGAGAGCTTGCTCTTATGAAGTTAGC  68

Query  63     GGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAA  122
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  69     GGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAA  128

Query  123    CCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCG  182
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  129    CCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCG  188

Query  183    GCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA  242
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  189    GCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA  248

Query  243    AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  302
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  249    AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  308

Query  303    CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA  362
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  309    CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA  368

Query  363    GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAAC  422
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  369    GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAAC  428

Query  423    AAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT  482
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  429    AAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT  488

Query  483    ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA  542
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  489    ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA  548

Query  543    AAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGG  602
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  549    AAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGG  608

Query  603    TCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGT  662
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             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  609   TCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGT  668

Query  663   GAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTG  722
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  669   GAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTG  728

Query  723   ACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG  782
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  729   ACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG  788

Query  783   TAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATT  842
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  789   TAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATT  848

Query  843   AAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC  902
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  849   AAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC  908

Query  903   CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCT  962
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  909   CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCT  968

Query  963   TGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAG-TGG  1021
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  969   TGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGG  1028

Query  1022  TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA  1081
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1029  TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA  1088

Query  1082  CC-TTGATCTTAGTTGCCATCATTA-GT-GG-CNCTCTAAGK-GACTGCCGGTGACAA-C  1135
             || |||||||||||||||||||||| || || | |||||||  ||||||||||||||| |
Sbjct  1089  CCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAAC  1148

Query  1136  -G-AG-A-G-TGG--ATGACGTCA--TCATCANGCCCGT-ATGCACCTGG  1175
              | || | | |||  |||||||||  |||||| |||| | ||| ||||||
Sbjct  1149  CGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATG-ACCTGG  1197
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I7 species alignment with Genbank *Bacillus sp*

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Query  11    CCTATAGGCGGCTGGCTCCAAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTCTCGT  70
             ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1445  CCT-TAGGCGGCTGGCTCCAAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTCTCGT  1387

Query  71    GGTGTGACGGNNGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGC  130
             ||||||||||  ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1386  GGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGC  1327

Query  131   GATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAAC  190
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1326  GATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAAC  1267

Query  191   GGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAGC  250
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1266  GGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAGC  1207

Query  251   ACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCG  310
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1206  ACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCG  1147

Query  311   GTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAATGATGGCAACTAAGATCAAGGG  370
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1146  GTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAATGATGGCAACTAAGATCAAGGG  1087

Query  371   TTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCA  430
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1086  TTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCA  1027

Query  431   CCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCTCTAGGGTTTTCAGAGGATGTCA  490
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1026  CCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCTCTAGGGTTTTCAGAGGATGTCA  967

Query  491   AGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCG  550
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  966   AGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCG  907

Query  551   GGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTT  610
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  906   GGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTT  847
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Query  611   AATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTA  670
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  846   AATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTA  787

Query  671   CGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGT  730
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  786   CGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGT  727

Query  731   CAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTC  790
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  726   CAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTC  667

Query  791   ACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGA  850
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  666   ACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGA  607

Query  851   CCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTT  910
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  606   CCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTT  547

Query  911   TACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGT  970
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  546   TACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGT  487

Query  971   AGTTAGCCGTGGCCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACT  1030
             ||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  486   AGTTAGCCGTGGC-TTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACT  428

Query  1031  TGTTCTTCCCTAACAACAGAGTTT-ACGACCCGAAAGCCTTCATCCACTCACGCGACGAT  1089
             |||||||||||||||||||||||| |||||||||||||||||||| ||||||||| || |
Sbjct  427   TGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATC-ACTCACGCGGCGTT  369

Query  1090  GCCTCCGGTCAGACTT-CGTCAAT-GCGGAAGATTCC-TACTGACTGCCTCC  1138
             || |||| |||||||| |||| || |||||||||||| ||||| ||||||||
Sbjct  368   GC-TCCG-TCAGACTTTCGTCCATTGCGGAAGATTCCCTACTG-CTGCCTCC  320
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E8 species alignment with Genbank *Bacillus sp*

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Query  8     ACCNTTAGGCGGCTGGCTCCAAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTCTCG  67
             ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1446  ACC-TTAGGCGGCTGGCTCCAAAAGGTTACCCCACCGACTTCGGGTGTTACAAACTCTCG  1388

Query  68    TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCG  127
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1387  TGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCG  1328

Query  128   CGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAA  187
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1327  CGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAA  1268

Query  188   CGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAG  247
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1267  CGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAG  1208

Query  248   CACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCC  307
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1207  CACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCC  1148

Query  308   GGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAATGATGGCAACTAAGATCAAGG  367
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1147  GGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAATGATGGCAACTAAGATCAAGG  1088

Query  368   GTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGC  427
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1087  GTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGC  1028

Query  428   ACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCTCTAGGGTTTTCAGAGGATGTC  487
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1027  ACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCTCTAGGGTTTTCAGAGGATGTC  968

Query  488   AAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGC  547
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  967   AAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGC  908

Query  548   GGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCT  607
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  907   GGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCT  848
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Query  608   TAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTT  667
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  847   TAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTT  788

Query  668   ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTG  727
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  787   ACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTG  728

Query  728   TCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTT  787
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  727   TCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTT  668

Query  788   CACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATG  847
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  667   CACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATG  608

Query  848   ACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCT  907
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  607   ACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCT  548

Query  908   TTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACG  967
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  547   TTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACG  488

Query  968   TAGTTAGCCGTGGCTTTCTGGGTTAGGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCA  1027
             ||||||||||||||||||||| ||||| ||||||||||||||||||||||||||||||||
Sbjct  487   TAGTTAGCCGTGGCTTTCTGG-TTAGG-TACCGTCAAGGTGCCAGCTTATTCAACTAGCA  430

Query  1028  CTTGTTCTTCCCTAACA-CAGAGTTTTACGACYCGAAAGCCTTCATCACTCACGCGGCGG  1086
             ||||||||||||||||| |||||||||||||| ||||||||||||||||||||||||||
Sbjct  429   CTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGT  370

Query  1087  TGCTNCGTCAGACTT-CGTC-AT-GCCGA-GATGCC-TACTGCTGC-TCC-GTAGGGAGT  1139
             |||| |||||||||| |||| || || || ||| || ||||||||| ||| ||||| |||
Sbjct  369   TGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGG-AGT  311

Query  1140  CTGGG  1144
             |||||
Sbjct  310   CTGGG  306
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E9 species alignment with Genbank *Micrococcus sp*

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Query  2     CACCGGCTTCGGGTGTTACCGACTTTCGTGACTTGANGGGNGGTGTGTACAAGGCCCGGG  61
             |||||||||||||||||||||||||||||||||||| ||| |||||||||||||||||||
Sbjct  1401  CACCGGCTTCGGGTGTTACCGACTTTCGTGACTTGACGGGCGGTGTGTACAAGGCCCGGG  1342

Query  62    AACGTATTCACCGCAGCGTTGCTGATCTGCGATTACTAGCGACTCCGACTTCATGGGGTC  121
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1341  AACGTATTCACCGCAGCGTTGCTGATCTGCGATTACTAGCGACTCCGACTTCATGGGGTC  1282

Query  122   GAGTTGCAGACCCCAATCCGAACTGAGACCGGCTTTTTGGGATTAGCTCCACCTCACAGT  181
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1281  GAGTTGCAGACCCCAATCCGAACTGAGACCGGCTTTTTGGGATTAGCTCCACCTCACAGT  1222

Query  182   ATCGCAACCCATTGTACCGGCCATTGTAGCATGCGTGAAGCCCAAGACATAAGGGGCATG  241
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1221  ATCGCAACCCATTGTACCGGCCATTGTAGCATGCGTGAAGCCCAAGACATAAGGGGCATG  1162

Query  242   ATGATTTGACGTCGTCCTCACCTTCCTCCGAGTTGACCCCGGCAGTCTCCCATGAGTCCC  301
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1161  ATGATTTGACGTCGTCCTCACCTTCCTCCGAGTTGACCCCGGCAGTCTCCCATGAGTCCC  1102

Query  302   CACCACGACGTGCTGGCAACATGGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAAC  361
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1101  CACCACGACGTGCTGGCAACATGGAACGAGGGTTGCGCTCGTTGCGGGACTTAACCCAAC  1042

Query  362   ATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTGAACCCGCCCCAAAGGGGAA  421
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1041  ATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTGAACCCGCCCCAAAGGGGAA  982

Query  422   ACCGTATCTCTACGGCGATCGAGAACATGTCAAGCCTTGGTAAGGTTCTTCGCGTTGCAT  481
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  981   ACCGTATCTCTACGGCGATCGAGAACATGTCAAGCCTTGGTAAGGTTCTTCGCGTTGCAT  922

Query  482   CGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAG  541
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  921   CGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAG  862

Query  542   CCTTGCGGCCGTACTCCCCAGGCGGGGCACTTAATGCGTTAGCTGCGGCGCGGAAACCGT  601
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  861   CCTTGCGGCCGTACTCCCCAGGCGGGGCACTTAATGCGTTAGCTGCGGCGCGGAAACCGT  802
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Query  602   GGAATGGTCCCCACACCTAGTGCCCAACGTTTACGGCATGGACTACCAGGGTATCTAATC  661
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  801   GGAATGGTCCCCACACCTAGTGCCCAACGTTTACGGCATGGACTACCAGGGTATCTAATC  742

Query  662   CTGTTCGCTCCCCATGCTTTCGCTCCTCAGCGTCAGTTACAGCCCAGAGACCTGCCTTCG  721
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  741   CTGTTCGCTCCCCATGCTTTCGCTCCTCAGCGTCAGTTACAGCCCAGAGACCTGCCTTCG  682

Query  722   CCATCGGTGTTCCTCCTGATATCTGCGCATTCCACCGCTACACCAGGAATTCCAGTCTCC  781
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  681   CCATCGGTGTTCCTCCTGATATCTGCGCATTCCACCGCTACACCAGGAATTCCAGTCTCC  622

Query  782   CCTACTGCACTCTAGTCTGCCCGTACCCACCGCAGATCCGGGGTTAAGCCCCGGACTTTC  841
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  621   CCTACTGCACTCTAGTCTGCCCGTACCCACCGCAGATCCGGGGTTAAGCCCCGGACTTTC  562

Query  842   ACGACAGACGCGACAAACCGCCTACGAGCTCTTTACGCCCNATAANTTCCGGATAACGCT  901
             |||||||||||||||||||||||||||||||||||||||| |||| ||||||||||||||
Sbjct  561   ACGACAGACGCGACAAACCGCCTACGAGCTCTTTACGCCCAATAA-TTCCGGATAACGCT  503

Query  902   CGCACCCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCG  945
             ||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  502   CGCACCC-TACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCG  460
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E10 species alignment with Genbank *Ochrobactrum sp*

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Query  19    CCTGCCTCCTTGCGGTTAGCANAGCGCCTTCGGGTAAAACCAACTCCCATGGTGTGACGG  78
             ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  1397  CCTGCCTCCTTGCGGTTAGCACAGCGCCTTCGGGTAAAACCAACTCCCATGGTGTGACGG  1338

Query  79    GCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGC  138
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1337  GCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGC  1278

Query  139   GATTCCAACTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAG  198
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1277  GATTCCAACTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAG  1218

Query  199   ATTAGCTCACACTCGCGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAGC  258
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1217  ATTAGCTCACACTCGCGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAGC  1158
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Query  259   CCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTATCACC  318
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1157  CCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTATCACC  1098

Query  319   GGCAGTCCCCTTAGAGTGCCCAACTAAATGCTGGCAACTAAGGGCGAGGGTTGCGCTCGT  378
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1097  GGCAGTCCCCTTAGAGTGCCCAACTAAATGCTGGCAACTAAGGGCGAGGGTTGCGCTCGT  1038

Query  379   TGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTAT  438
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1037  TGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTAT  978

Query  439   CCGGTCCAGCCGAACTGAAAGACACATCTCTGTGTCCGCGACCGGTATGTCAAGGGCTGG  498
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  977   CCGGTCCAGCCGAACTGAAAGACACATCTCTGTGTCCGCGACCGGTATGTCAAGGGCTGG  918

Query  499   TAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCG  558
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  917   TAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCG  858

Query  559   TCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTT  618
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  857   TCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTT  798

Query  619   AGCTGCGCCACCGARGAGTAAACTCCCCAACGGCTAACATTCATCGTTTACGGCGTGGAC  678
             |||||||||||||| |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  797   AGCTGCGCCACCGAAGAGTAAACTCCCCAACGGCTAACATTCATCGTTTACGGCGTGGAC  738

Query  679   TACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATGGT  738
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  737   TACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATGGT  678

Query  739   CCAGTGAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACAC  798
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  677   CCAGTGAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACAC  618

Query  799   TCGGAATTCCACTCACCTCTACYATACTCRAGGACTTCCAGTATCAA-GGCAGTTCCGGG  857
             |||||||||||||||||||||| |||||| || |||||||||||||| ||||||||||||
Sbjct  617   TCGGAATTCCACTCACCTCTACCATACTCAAG-ACTTCCAGTATCAAAGGCAGTTCCGGG  559

Query  858   GTTGAGCCCCGGGATTTCACCMCTGACTTAAAGTYCGCCTACGTGCGCTTTACGCCCAGT  917
             ||||||||||||||||||||| |||||||||||| |||||||||||||||||||||||||
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Sbjct  558   GTTGAGCCCCGGGATTTCACCCCTGACTTAAAGTCCGCCTACGTGCGCTTTACGCCCAGT  499


Query  918   ARATCCGAACAACGCTAGCYCCCYTTCGTATTASCGCGGSTGCTGGCACGAAGTAATCGG  977
             | ||||||||||||||||| ||| ||||||||| ||||| |||||||||||||| | | |
Sbjct  498   AAATCCGAACAACGCTAGCCCCC-TTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCG  440


Query  978   GGGCTTCTWCTYCGGTTACGATCATTATCATCNNCGGNGAAAGAGCTT-ACATCCTNAGG  1036
             |||||||| || |||||||  |||||||| ||  ||| |||||||||| ||| ||  |||
Sbjct  439   GGGCTTCTTCTCCGGTTACCGTCATTATCTTCACCGGTGAAAGAGCTTTACAACCCTAGG  380


Query  1037  GTCTATCATNNCTYNCGCNGNATGGCNTGGATSNAGCNTGCGNCCAGTTGATCGATAT-C  1095
             | || ||||  ||  ||| | ||||| |||||   || |||| ||| |||  | |||| |
Sbjct  379   GCCT-TCATCACTCACGCGGCATGGC-TGGATCAGGCTTGCGCCCA-TTGTCCAATATTC  323


Query  1096  CCCACTGCTTGCCTC--GTAG-AGTCTGG-C-G-GTCTCA  1129
             ||||||||| |||||  |||| ||||||| | | ||||||
Sbjct  322   CCCACTGCT-GCCTCCCGTAGGAGTCTGGGCCGTGTCTCA  284
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E11 species alignment with Genbank *Micrococcus leteus*

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Query  25    CTCCSCCCAYAGGGNTTACGTSCTC-CCGGCTTCGGGTGTTACCGACTTTCGTGACTTGA  83
             |||| |||| | || ||| |  ||| |||||||||||||||||| |||||||||||||||
Sbjct  1418  CTCCCCCCACAAGGGTTA-GAGCTCACCGGCTTCGGGTGTTACCAACTTTCGTGACTTGA  1360


Query  84    CGGGSGGTGTGTACAAGGCCCGGGAACGTATTCACCGCASCGTTGCTGATCTGCGATTAC  143
             |||| ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  1359  CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGCTGATCTGCGATTAC  1300


Query  144   TAGCGACTCCGACTTCATGGGGTCGAGTTGCAGACCCCAATCCGAACTGAGACCGGCTTT  203
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1299  TAGCGACTCCGACTTCATGGGGTCGAGTTGCAGACCCCAATCCGAACTGAGACCGGCTTT  1240


Query  204   TTGGGATTAGCTCCACCTCACAGTATCGCAACCCATTGTACCGGCCATTGTAGCATGCGT  263
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1239  TTGGGATTAGCTCCACCTCACAGTATCGCAACCCATTGTACCGGCCATTGTAGCATGCGT  1180



Query  264   GAAGCCCAAGACATAAGGGGCATGATGATTTGACGTCGTCCTCACCTTCCTCCGAGTTGA  323
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1179  GAAGCCCAAGACATAAGGGGCATGATGATTTGACGTCGTCCTCACCTTCCTCCGAGTTGA  1120


Query  324   CCCCGGCAGTCTCCCATGAGTCCCCACCACGACGTGCTGGCAACATGGAACGAGGGTTGC  383
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                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1119   CCCCGGCAGTCTCCCATGAGTCCCCACCACGACGTGCTGGCAACATGGAACGAGGGTTGC  1060

Query  384    GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCAC  443
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1059   GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCAC  1000

Query  444    CTGTGAACCCGCCCCAAAGGGGAAACCGTATCTCTACGGCGATCGAGAACATGTCAAGCC  503
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  999    CTGTGAACCCGCCCCAAAGGGGAAACCGTATCTCTACGGCGATCGAGAACATGTCAAGCC  940

Query  504    TTGGTAAGGTTCTTCGCGTTGCATCGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCC  563
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  939    TTGGTAAGGTTCTTCGCGTTGCATCGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCC  880

Query  564    CCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGGCACTTAATG  623
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  879    CCCGTCAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGGCACTTAATG  820

Query  624    CGTTAGCTGCGGCGCGGAAACCGTGRAATGGTCCCCACACCTAGTGCCCAACGTTTACGG  683
              ||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  819    CGTTAGCTGCGGCGCGGAAACCGTGGAATGGTCCCCACACCTAGTGCCCAACGTTTACGG  760

Query  684    CATGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTCCTCAGCGTCAG  743
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  759    CATGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTCCTCAGCGTCAG  700

Query  744    TTACAGCCCAGAGACCTGCCTTCGCCATCGGTGTTCCTCCTGATATCTGCGCATTCCACC  803
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  699    TTACAGCCCAGAGACCTGCCTTCGCCATCGGTGTTCCTCCTGATATCTGCGCATTCCACC  640

Query  804    GCTACACCAGGAATTCCAGTCTCCCCTACTGCACTCTAGTCTGCCCGTACCCACCGCAGA  863
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  639    GCTACACCAGGAATTCCAGTCTCCCCTACTGCACTCTAGTCTGCCCGTACCCACCGCAGA  580

Query  864    ATCCGGGGTTAAGCCCCCGGACTTTCACGACAGACGCGACAACCSGCCTACGAGCTCTTT  923
               |||||||||||||||| |||||||||||||||||||||||| | |||||||||||||||
Sbjct  579    -TCCGGGGTTAAGCCCC-GGACTTTCACGACAGACGCGACAAACCGCCTACGAGCTCTTT  522

Query  924    ACRCCCAATAATTCCGGGATAACGCTCGCACCCTACGTATTACCGSSGCTGCTGGCMCGT  983
              || |||||||||||||| |||||||||||||||||||||||||||  ||||||||| |||
Sbjct  521    ACGCCCAATAATTCCGG-ATAACGCTCGCACCCTACGTATTACCGCGGCTGCTGGCACGT  463
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Query  984   AGTTAGCCGGTGCTTCTTCTGCAGGWACCGTCRCTTTYSGYTTCTTCCCTACTGAAAGAA  1043
             ||||||||||||||||||||||||| |||||| ||||  | ||||||||||||||||||
Sbjct  462   AGTTAGCCGGTGCTTCTTCTGCAGGTACCGTCACTTTC-GCTTCTTCCCTACTGAAAGA-  405

Query  1044  GGTTTACAACCCGAAGGCCGGCATYCCCT  1072
             |||||||||||||||||||| ||| ||||
Sbjct  404   GGTTTACAACCCGAAGGCCGTCAT-CCCT  377
```

I12 species alignment with Genbank *Bacillus sp*

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Query  4     CGGCANCTATACATGCAGTCGAGCGAATGGATTNAGAGCTTGCTCTNANGAAGTTAGCGG  63
             ||||| ||||||||||||||||||||||||||| |||||||||||| | |||||||||||
Sbjct  8     CGGCATCTATACATGCAGTCGAGCGAATGGATTGAGAGCTTGCTCTCAAGAAGTTAGCGG  67

Query  64    CGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACC  123
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  68    CGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACC  127

Query  124   GGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGC  183
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  128   GGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGC  187

Query  184   TGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAG  243
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  188   TGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAG  247

Query  244   GCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC  303
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  248   GCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC  307

Query  304   AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC  363
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  308   AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGC  367

Query  364   AACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAA  423
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  368   AACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAA  427

Query  424   GTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTAC  483
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  428   GTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTAC  487
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Query  484   GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAA  543
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  488   GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAA  547

Query  544   GCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC  603
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  548   GCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC  607

Query  604   ATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGA  663
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  608   ATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGA  667

Query  664   AATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGAC  723
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  668   AATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGAC  727

Query  724   ACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA  783
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  728   ACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA  787

Query  784   AACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAA  843
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  788   AACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAA  847

Query  844   GCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCG  903
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  848   GCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCG  907

Query  904   CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTG  963
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  908   CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTG  967

Query  964   ACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAG-TG-TG  1021
             |||||||||||||||||||||||||||||||||||||||||||||||||||||| || ||
Sbjct  968   ACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTG  1027

Query  1022  CATGGT-GTCGTCAGCTCGTGTCGTGAGATGT-GGGTTAAGTCCCGCAACGAGCGCAACC  1079
             |||||| ||||||||||||||||||||||||| |||||||||||||||||||||||||||
Sbjct  1028  CATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC  1087

Query  1080  CTTGATCT-AGT-GCCATCAT-A-GT-GGGCMCTCTAAG-TGACTGC-G-TGACAA-CCG  1130
             |||||||| ||| |||||||| | || |||| ||||||| ||||||| | |||||| |||
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Sbjct  1088  CTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCG  1147


Query  1131  GAG-A-GGTGGGAATGACGTCAA-TCATCATGCCC-TTATGACCTGG-CTAC-CACGTGC  1184
             ||| | |||||| |||||||||| ||||||||||| ||||||||||| |||| |||||||
Sbjct  1148  GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGC  1207


Query  1185  TACAATG-ACGCTAACAAAGANGCTNGCNANGAACCGCG  1222
             ||||||| ||| || |||||| ||| || | || |||||
Sbjct  1208  TACAATGGACGGTA-CAAAGA-GCT-GCAA-GA-CCGCG  1241
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I13 species alignment with Genbank *Bacillus sp*

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Query  3     GCGGCG-NCTATACATGCAGTCGAGCGAATGGATTNAGAGCTTGCTCTNANGAAGTTAGC  61
             ||||||  ||||||||||||||||||||||||||| |||||||||||| | |||||||||
Sbjct  7     GCGGCGTGCTATACATGCAGTCGAGCGAATGGATTGAGAGCTTGCTCTCAAGAAGTTAGC  66


Query  62    GGCGGACGGNTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAA  121
             ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67    GGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAA  126


Query  122   CCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCG  181
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  127   CCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCG  186


Query  182   GCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA  241
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  187   GCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA  246


Query  242   AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  301
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  247   AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGC  306


Query  302   CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA  361
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  307   CCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA  366


Query  362   GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAAC  421
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  367   GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAAC  426


Query  422   AAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT  481
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             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427   AAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT  486

Query  482   ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA  541
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  487   ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA  546

Query  542   AAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGG  601
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  547   AAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGG  606

Query  602   TCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGT  661
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  607   TCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGT  666

Query  662   GAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTG  721
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  667   GAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTG  726

Query  722   ACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG  781
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  727   ACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG  786

Query  782   TAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATT  841
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  787   TAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATT  846

Query  842   AAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC  901
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  847   AAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC  906

Query  902   CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCT  961
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  907   CGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCT  966

Query  962   TGACATCCTCTGAAAACYCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGG  1021
             ||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  967   TGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGG  1026

Query  1022  TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA-  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 
Sbjct  1027  TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA  1086
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Query  1081  CYCTTGATCT-AGT-GCCATCATTAAGT-GG-CACTCTA-G-TGACTGCCG-TGANAACC  1133
             | |||||||| ||| ||||||||||||| || ||||||| | ||||||||| ||| || |
Sbjct  1087  CCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAAC  1146

Query  1134  CGGAG-A-G-TGGG-ATGACGTCAA-TCATTCANGCC  1165
             ||||| | | |||| |||||||||| |||| || |||
Sbjct  1147  CGGAGGAAGGTGGGGATGACGTCAAATCAT-CATGCC  1182
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E14 species alignment with Genbank *Ochrobactrum onthropi*

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Query  12    TACACATGCAAGTCGAGCGCCCCGCAAGGGGAGCGGCAGACGGGTGAGTAACGCGTGGGA  71
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3     TACACATGCAAGTCGAGCGCCCCGCAAGGGGAGCGGCAGACGGGTGAGTAACGCGTGGGA  62

Query  72    ACGTACCTTTTGCTACGGAATAACTCAGGGAAACTTGTGCTAATACCGTATGTGCCCTTC  131
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  63    ACGTACCTTTTGCTACGGAATAACTCAGGGAAACTTGTGCTAATACCGTATGTGCCCTTC  122

Query  132   GGGGGAAAGATTTATCGGCAAAGGATCGGCCCGCGTTGGATTAGCTAGTTGGTGAGGTAA  191
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  123   GGGGGAAAGATTTATCGGCAAAGGATCGGCCCGCGTTGGATTAGCTAGTTGGTGAGGTAA  182

Query  192   AGGCTCACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACT  251
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  183   AGGCTCACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACT  242

Query  252   GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA  311
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  243   GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA  302

Query  312   GCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCACC  371
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  303   GCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTCACC  362

Query  372   GGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGC  431
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  363   GGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGC  422

Query  432   GGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGCGG  491
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  423   GGTAATACGAAGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGCGG  482
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Query  492   ACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGATACTGGAA  551
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  483   ACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGATACTGGAA  542

Query  552   GTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATT  611
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  543   GTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATT  602

Query  612   CGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAA  671
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  603   CGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAA  662

Query  672   GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTA  731
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  663   GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTA  722

Query  732   GCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGA  791
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  723   GCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGA  782

Query  792   GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA  851
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  783   GTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA  842

Query  852   TGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACCGGTCGCGGA  911
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  843   TGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATACCGGTCGCGGA  902

Query  912   CACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTGCATGGCTGTCGTCA  971
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  903   CACAGAGATGTGTCTTTCAGTTCGGCTGGACCGGATACAGGTGCTGCATGGCTGTCGTCA  962

Query  972   GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTTAGTTG  1031
             |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  963   GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTTAGTTG  1022

Query  1032  CCAGCATTTAGTTGGGCACTCTAAGGG-ACTGCCGGTGATA-GCCGAGAG-AAGGTGGGG  1088
             ||||||||||||||||||||||||||| ||||||||||||| |||||||| |||||||||
Sbjct  1023  CCAGCATTTAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGG  1082

Query  1089  ATGACGTCAAGTC-TCATGGTCCTTANGG-CTGG-CTACNCACGTGCTACAATG-TG-TG  1143
             ||||||||||||| |||||| ||||| || |||| |||| |||||||||||||| || ||
Sbjct  1083  ATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTG  1142
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Query  1144  ANCGGTGGGCAGCGAGCNCGCGAGT  1168
             | | ||||||||||||| |||||||
Sbjct  1143  A-CAGTGGGCAGCGAGCACGCGAGT  1166
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E15 species alignment with Genbank *Staphyloccocus sp*

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Query  13    GCTAGCTCCAAAGGTTACTCCACCGGCTTCGGGTGTTACAAACTCTCGTGGTGCGACTGG  72
             ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||| ||
Sbjct  1412  GCTAGCTCCAAAGGTTACTCCACCGGCTTCGGGTGTTACAAACTCTCGTGGTGTGACGGG  1353

Query  73    MGGTGTGTACAAGACCCGGGAACGTATTCACCGTAGCATGCTGATCTACGATTACTAGCG  132
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1352  CGGTGTGTACAAGACCCGGGAACGTATTCACCGTAGCATGCTGATCTACGATTACTAGCG  1293

Query  133   ATTCCAGCTTCATGTAGTCGAGTTGCAGACTACAATCCGAACTGAGAACAACTTTATGGG  192
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1292  ATTCCAGCTTCATGTAGTCGAGTTGCAGACTACAATCCGAACTGAGAACAACTTTATGGG  1233

Query  193   ATTTGCTTGACCTCGCGGTTTCGCTACCCTTTGTATTGTCCATTGTAGCACGTGTGTAGC  252
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1232  ATTTGCTTGACCTCGCGGTTTCGCTACCCTTTGTATTGTCCATTGTAGCACGTGTGTAGC  1173

Query  253   CCAAATCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCG  312
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1172  CCAAATCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCG  1113

Query  313   GCAGTCAACTTAGAGTGCCCAACTTAATGATGGCAACTAAGCTTAAGGGTTGCGCTCGTT  372
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1112  GCAGTCAACTTAGAGTGCCCAACTTAATGATGGCAACTAAGCTTAAGGGTTGCGCTCGTT  1053

Query  373   GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCAC  432
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1052  GCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCAC  993

Query  433   TCTGTCCCCCGAAGGGGAAAACTCTATCTCTAGAGGGGTCAGAGGATGTCAAGATTTGGT  492
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  992   TCTGTCCCCCGAAGGGGAAAACTCTATCTCTAGAGGGGTCAGAGGATGTCAAGATTTGGT  933

Query  493   AAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGTCCCCGT  552
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             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  932   AAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGTCCCCGT  873

Query  553   CAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTA  612
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  872   CAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTA  813

Query  613   GCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGA  672
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  812   GCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTACGGCGTGGA  753

Query  673   CTACCAGGGTATCTAATCCTGTTTGATCCCCCACCGCTTTTCGCACATCAGCGTCAGTTA  732
             ||||||||||||||||||||||||||||||| || ||||| |||||||||||||||||||
Sbjct  752   CTACCAGGGTATCTAATCCTGTTTGATCCCC-AC-GCTTT-CGCACATCAGCGTCAGTTA  696

Query  733   CAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTTCACCGCT  792
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  695   CAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTGCGCATTTCACCGCT  636

Query  793   ACACATGGAAATTCCACTTTCCTCTTTCTGCACTCRAGTTTTCCAGTTTCCCAATGAMCC  852
             |||||||||| ||||||||||||||| |||||||| ||||||||||||||| ||||| ||
Sbjct  635   ACACATGGAA-TTCCACTTTCCTCTT-CTGCACTCAAGTTTTCCAGTTTCC-AATGA-CC  580

Query  853   CTCCACGGTTGAGCCGTGGGCTTTCACATCANACTTnaaaaaaCCGCCTACGCNCGCTTT  912
             ||||||||||||||||||||||||||||||| |||| |||||||||||||||| ||||||
Sbjct  579   CTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT-AAAAAACCGCCTACGCGCGCTTT  521

Query  913   ACGCC-AATNATTTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACNN  971
             ||||| ||| ||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  520   ACGCCCAATAATT-CCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGT  462

Query  972   AGTTANCCCGTGGNCTTTCTGAATTNAGTACCGGTCAAGACGT  1014
             ||||| || |||| |||||||| ||  |||||| |||||||||
Sbjct  461   AGTTAGCC-GTGG-CTTTCTGA-TTAGGTACCG-TCAAGACGT  423
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E16 species alignment with Genbank *Bacillus cereus*

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Query  9     CCTATACATGCAGTCGAGCGAATGGATTNAGAGCTTGCTCTNANGAAGTTAGCGGCGGAC  68
             |||||||||||||||||||||||||||| |||||||||||| | ||||||||||||||||
Sbjct  21    CCTATACATGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGAC  80
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Query  69    NGNTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGC  128
              | |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  81    GGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGC  140

Query  129   TAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCA  188
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  141   TAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCA  200

Query  189   CTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAAC  248
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  201   CTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAAC  260

Query  249   GATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACT  308
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  261   GATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACT  320

Query  309   CCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGC  368
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  321   CCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGC  380

Query  369   CGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCT  428
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  381   CGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCT  440

Query  429   AGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCC  488
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  441   AGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCC  500

Query  489   AGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCG  548
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  501   AGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCG  560

Query  549   CGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGG  608
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  561   CGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGG  620

Query  609   AAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGC  668
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  621   AAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGC  680

Query  669   GTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGA  728
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct  681   GTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGA  740

Query  729   GGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGA  788
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  741   GGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGA  800

Query  789   TGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACT  848
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  801   TGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACT  860

Query  849   CCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA  908
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  861   CCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA  920

Query  909   GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC  968
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  921   GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC  980

Query  969   CTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAG-TG-TGCATGG  1026
             ||||||||||||||||||||||||||||||||||||||||||||||||| || |||||||
Sbjct  981   CTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGG  1040

Query  1027  GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA-CC-TTG  1084
              |||||||||||||||||||||||||||||||||||||||||||||||||||| || |||
Sbjct  1041  -TTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTG  1099

Query  1085  ATCTTAGT-GCCATCAT-A-GT-GG-CMCTCTAAGGTGACTGCCGGTGACAA-C-GGAG-  1136
             |||||||| |||||||| | || || | |||||||||||||||||||||||| | ||||
Sbjct  1100  ATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGG  1159

Query  1137  A-GGTGGG-ATGACGTCA--TCATCATGCCCCGTATGAC-TGGGC  1176
             | |||||| |||||||||  |||||||||||| |||||| |||||
Sbjct  1160  AAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGC  1204
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I17 species alignment with Genbank *Staphylococcus pasteuri*

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Query  17   TATAMTGCAAGTCGAGCGACAGATAAGGAGCTTGCTCCTTTGACGTTAGCGGCGGACGGG  76
            |||| |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  15   TATAGTGCAAGTCGAGCGACAGATAAGGAGCTTGCTCCTTTGACGTTAGCGGCGGACGGG  74
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Query  77   TGARNAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAA  136
            |||  |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  75   TGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAA  134

Query  137  TACCGGATAACATATTGAACCGCATGGTTCAATAGTGAAAGGCGGCTTTGYTGTCACTTA  196
            |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  135  TACCGGATAACATATTGAACCGCATGGTTCAATAGTGAAAGGCGGCTTTGCTGTCACTTA  194

Query  197  TAGATGGATCCGCGCCGTATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATA  256
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  195  TAGATGGATCCGCGCCGTATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATA  254

Query  257  CGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTA  316
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  255  CGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTA  314

Query  317  CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCG  376
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  315  CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCG  374

Query  377  TGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATCAGGGAAGAACAAATGTGTAAG  436
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  375  TGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATCAGGGAAGAACAAATGTGTAAG  434

Query  437  TAACTGTGCACATCTTGACGGTACCTGATCAGAAAGCCACGGCTAACTACGTGCCAGCAG  496
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435  TAACTGTGCACATCTTGACGGTACCTGATCAGAAAGCCACGGCTAACTACGTGCCAGCAG  494

Query  497  CCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAG  556
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  495  CCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAG  554

Query  557  GCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACT  616
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  555  GCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACT  614

Query  617  GGAAAACNTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCNGNGAAATGCGCR-  675
            ||||||| |||||||||||||||||||||||||||||||||||||| | |||||||||
Sbjct  615  GGAAAAC-TTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAG  673

Query  676  AGATATGGGAGGACCTCCNGTGYCGAA  702
            |||||||| |||| | || ||| ||||
Sbjct  674  AGATATGG-AGGAACACCAGTGGCGAA  699
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I18 species alignment with Genbank *Bacillus antracis*

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Query  5    GCANCTATACATGCAAGTCGAGCGAATGGATTNAGAGCTTGCTCTNANGAAGTTAGCGGC  64
            ||| |||||||||||||||||||||||||||| |||||||||||| | ||||||||||||
Sbjct  5    GCAGCTATACATGCAAGTCGAGCGAATGGATTGAGAGCTTGCTCTCAAGAAGTTAGCGGC  64

Query  65   GGACGGNTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCG  124
            |||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  65   GGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCG  124

Query  125  GGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCT  184
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  125  GGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCT  184

Query  185  GTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGG  244
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  185  GTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGG  244

Query  245  CAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA  304
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  245  CAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA  304

Query  305  GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA  364
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  305  GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA  364

Query  365  ACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAG  424
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  365  ACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAG  424

Query  425  TGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACG  484
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  425  TGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACG  484

Query  485  TGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAG  544
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  485  TGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAG  544

Query  545  CGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCA  604
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  545  CGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCA  604
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Query  605    TTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAA  664
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  605    TTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAA  664

Query  665    ATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACA  724
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  665    ATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACA  724

Query  725    CTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA  784
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  725    CTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA  784

Query  785    ACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAG  844
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  785    ACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAG  844

Query  845    CACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGC  904
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  845    CACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGC  904

Query  905    ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGA  964
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  905    ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGA  964

Query  965    CATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAG-TGGTGC  1023
              ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  965    CATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGC  1024

Query  1024   ATGGTTGTCGTCAGCTCGTGTCGTGAGATGT-GGGT-AAGTCCCGCAACGAGCGCA-CNC  1080
              ||||||||||||||||||||||||||||||| |||| ||||||||||||||||||| | |
Sbjct  1025   ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC  1084

Query  1081   TTGATCT-AGT-GCCATCATTAAGT-GGGCNCTCTA-G-TGACTGCCG-TGACAA-CCGG  1133
              ||||||| ||| ||||||||||||| |||| ||||| | ||||||||| |||||| ||||
Sbjct  1085   TTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGG  1144

Query  1134   AGGA-G-TGGG-ATGACGTCAA-TCATCATGCCC-T-ATGACTTGGCTTACACACCGTGC  1187
              |||| | |||| |||||||||| ||||||||||| | ||||| |||  ||||||| ||||
Sbjct  1145   AGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACAC-GTGC  1203

Query  1188   CTACAATGGACCGGT  1202
               |||||||||| |||
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Sbjct  1204  -TACAATGGAC-GGT  1216
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I19 species alignment with Genbank *Bacillus sp*

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Query  5     GGCGTCCNAA-NCATGCAAGTCGAGCGAATGGATTNAGAGCTTGCTCTNANGAAGTTAGC  63
             ||||||| ||  ||||||||||||||||||||||| |||||||||||| | |||||||||
Sbjct  7     GGCGTCCTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGC  66

Query  64    GGCGGACGGCGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAA  123
             ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  67    GGCGGACGG-GTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAA  125

Query  124   ACCGGGGCTAATACNGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTC  183
             |||||||||||||| |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  126   ACCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTC  185

Query  184   GGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACC  243
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186   GGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACC  245

Query  244   AAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGG  303
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246   AAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGG  305

Query  304   CCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGG  363
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  306   CCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGG  365

Query  364   AGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAA  423
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  366   AGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAA  425

Query  424   CAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC  483
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  426   CAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC  485

Query  484   TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGT  543
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  486   TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGT  545
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Query  544   AAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGG  603
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  546   AAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGG  605

Query  604   GTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGG  663
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  606   GTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGG  665

Query  664   TGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACT  723
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  666   TGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACT  725

Query  724   GACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC  783
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  726   GACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC  785

Query  784   GTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCAT  843
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  786   GTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCAT  845

Query  844   TAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAA-GGAATTGACGGGGGC  902
             |||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  846   TAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGC  905

Query  903   CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA-CGCGAAGAACCTTACCAGGTC  961
             |||||||||||||||||||||||||||||||||||||| |||||||||||||||||||||
Sbjct  906   CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTC  965

Query  962   TTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTG  1021
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  966   TTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTG  1025

Query  1022  GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGT-GGGTTAAGTCCCGCAACGAGCGCA  1080
             ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  1026  GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA  1085

Query  1081  ACCCTTGATCT-AGTTGC-ATCATTAAGT-GGGCACTCTACG-TGACTGCCG-TGACAAA  1135
             ||||||||||| |||||| |||||||||| |||||||||| | ||||||||| |||||||
Sbjct  1086  ACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAA  1145

Query  1136  CCG-AGGA-G-TGG--ATGACGTCAA-TCATCATGCCC-T-ATGACCTGGGCTTAC-CAC  1186
             ||| |||| | |||  |||||||||| ||||||||||| | ||||||||||||  || |||
Sbjct  1146  CCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCT-ACACAC  1204
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Query  1187  GGTGCTACA-TGGACCGTAACAGAGAG  1212
             | ||||||| ||||| ||| || ||||
Sbjct  1205  G-TGCTACAATGGACGGTA-CAAAGAG  1229
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