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CONTRIBUTION OF ENDOPHYTIC AND RHIZOSPHERE BACTERIA TO WATER SHORTAGE AND SALT STRESS TOLERANCE OF AVOCADO SEEDLINGS (*Persea americana* MILL.).

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“CONTRIBUTION OF ENDOPHYTIC AND RHIZOSPHERE BACTERIA TO WATER SHORTAGE AND SALT STRESS TOLERANCE OF AVOCADO SEEDLINGS (*Persea americana* MILL.)”

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A mi abuela, a mis padres, hermanos, sobrinos, amigos y para ti mi amor, Mabel

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Summary and thesis outline

Plant inoculation with indole acetic acid (IAA)- and 1-aminocyclopropane-1-carboxylate deaminase (ACCD)- producing bacteria often has a positive effect on stress alleviation in plants. We isolated, characterized and formulated halotolerant endophytic and rhizosphere bacterial consortia from avocado trees with the aim of developing biofertilizers to improve avocado production under salt stress and water shortage. First, greenhouse experiments were conducted to investigate the effects of selected consortia on growth, biomass and superoxide dismutase (SOD) activity, using wheat seedlings under salt stress (0.25 and 0.45 M NaCl) as test plant. Later, inoculation trials were conducted in commercial nursery to investigate the effects of selected bacterial consortia on growth, biomass, lipid peroxidation (TBARS) and SOD activity of avocado seedlings exposed to salt (2% NaCl) and water shortage (50% less irrigation). Among 309 isolates, 17.4% were characterized as halotolerant IAA- and ACCD-producing bacteria. Based on differences in their IAA production and ACCD activities, four consortia were formulated with members of five genera: *Enterobacter*, *Serratia*, *Microbacterium*, *Pseudomonas* and *Achromobacter*. Inoculation with halotolerant bacterial consortia significantly ($P \geq 0.05$) increased the emergence, growth, biomass and SOD activity of wheat seedlings exposed to salt stress. Similarly, bacterial consortia mitigated effects of water shortage and salt stresses on avocado seedlings, increasing their growth, biomass, trunk diameter, chlorophyll content and SOD activity and decreasing TBARS. Avocado is naturally associated with halotolerant IAA- and ACCD-producing bacteria able to mitigate stress effects on plants. Our results showed the beneficial effect of bacterial inoculation on avocado plants under stress, which potentially could be used as biofertilizer in the field. However, further field studies are required to evaluate their effects on avocado yields under increasingly stressful conditions expected from global warming.

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CHAPTER I

General Introduction

1. General Introduction.

1.1. Introduction.

Global climatic change is increasing warm events in some world areas, resulting in reduced rainfalls, with subsequent desertification, land degradation and drought (DLDD) (WWAP, 2012). Drought due to shortage of water is critical for crop production in large agronomic areas worldwide and it is usually coped with extensive irrigation (Golldack et al., 2011). Poor quality water is often used for irrigation, so that eventually salt builds up in the soil, which consequently triggers soil salinization (Bui, 2013). Nowadays, over 800 million ha land throughout the world are salt affected, corresponding to 6-7% of the world's total land area and around 20% of the world's irrigated lands (Ahmad, 2014; Munns and Tester, 2008; Panta et al., 2014). Although irrigated land corresponds only to 15% total cultivated land, its importance lies in this land producing one third of the world's food (Munns and Tester, 2008). The DLDD are the main problems in modern agriculture that adversely affect plant development and have a crucial impact on agricultural productivity and yields (Athar and Ashraf, 2009).

Plants vary widely in their tolerance/sensitivity to abiotic stress, with avocado plants (*Persea americana* Mill.) showing a great drought-sensitivity along with the highest salt-sensitivity among cultivated fruit tree species (Bernstein and Meiri, 2004; Oster et al., 2007). Chile is one of main producers of avocado worldwide with sales of over US$ 170 million in 2015, and avocado production is thus of great economic importance for Chilean agriculture. In this context, the global demand for avocados has significantly grown up during the last decades, resulting in an increase of avocado orchards in central Chile from 23,800 ha in 2003 to 36,355 h in 2013 (Muñoz 2015). In contrast, during recent years, Chilean avocado production has been decreased from 263,476 t in 2009 to 164,720 t in 2013 (Muñoz 2015) mainly due to adverse environmental factors, particularly an extended drought that has been affecting the central Chile for nearly 5 years. Based on global warming estimations, the occurrence of drought in central

Chile could become increasingly severe with long term climate projections predicting a decrease of 20-25% in rainfall by 2040 (Neuenschwander, 2010). To solve the water limitation, orchards in Chile increasingly rely on irrigation, which is triggering and increasing soil salinization. Consequently, it is expected that in some Chilean areas the avocado production will no longer be viable unless that water shortage and salt stress tolerance can be increased. Accordingly, it is essential to find and develop strategies to ameliorate the detrimental effects of water shortage and salt stress on growth and development of avocado trees in order to maintain or/and enhance fruit production under new and changing climate scenarios.

From a physiological point of view, both salt stresses and water shortage are involved in a reduction of the osmotic potential of soil, with consequent impact on water and nutrient uptake, which decreases cellular elongation with subsequent plant growth inhibition (Khan et al., 2014; Munns and Tester, 2008). As a direct consequence of the stress, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase genes are induced in plant roots; therefore, ACC is transported by the xylem to shoots where it is oxidized into ethylene (Jackson, 1997). The increased ethylene levels trigger root growth inhibition and initiation of senescence, with consequent reduction of chlorophyll content, and finally plant death (Barnawal et al., 2014; Gepstein and Thimann, 1981; Glick et al., 1998).

An attractive and environmentally friendly strategy to mitigate stress effects on crops is the use of plant growth-promoting bacteria (PGPB) soil inoculants. The PGPB may be associated with host plant i) living freely in the plant rhizosphere (called plant growth-promoting rhizobacteria: PGPR) or ii) colonizing and residing inside of plant tissues without being pathogenic plants (called endophytic bacteria) (Gray and Smith, 2005; Hallmann et al., 1997). Most PGPB that have been tested produce the phytohormone indole acetic acid (IAA), which can directly increase root growth of host plant (Patten and Glick, 2002). Whereas, some PGPB strains are also able to produce the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD),

which catalyzes the hydrolysis of immediate precursor of ethylene, ACC to ammonia and α-ketobutyrate (αKB). Therefore, ACCD-producing bacteria prevent the increase of ethylene triggered for stressful conditions, and this avoids the so-called 'stress ethylene', characterized for inhibitions of root growth (Penrose and Glick, 2003). Earlier studies have showed the positive effects of IAA- and ACCD-producing PGPB on alleviation of both water shortage and salt stress in some crops plants, such as: cereals (maize, rice and wheat), pasture (ryegrass), medicinal plants (*Limonium sinense*), legumes (pea), among others (Arshad et al., 2008; Bal et al., 2012; Chakraborty et al., 2013; Cohen et al., 2009; Egamberdieva, 2009; Ji and Huang, 2008; Qin et al., 2013; D. P. Singh et al., 2011).

Accordingly, we hypothesized that inoculation of avocado plants with IAA- and ACCD-producing PGPB both PGPR as endophytic bacteria could stimulate tolerance of avocado plants growing under stress conditions. The findings of this study will provide a greater understanding of the behavior of bacterial communities associated with avocado trees. Secondly, the discovery of bacteria with the ability to alleviate water shortage and salt stress will allow the development of a phytostimulator inoculum that could be used in avocado orchards to improve stress tolerance.

1.2. Hypotheses and objectives.

1.2.1. Hypotheses.

- Endophytic and rhizosphere bacteria producers of IAA and ACCD improve water shortage and salt stress tolerance of avocado seedlings (*Persea americana* Mill.)

1.2.2. General objective.

- To evaluate the contribution of endophytic and rhizosphere bacteria to water shortage and salt stress tolerance of avocado seedlings (*Persea americana* Mill.).

1.2.3. Specific objectives.

- To isolate IAA- and ACCD-producing bacteria from endosphere and rhizosphere of avocado trees.
- To characterize, select and identify IAA- and ACCD-producing endophytic and rhizosphere bacteria.
- To determine the contribution of selected IAA- and ACCD-producing bacteria on salt stress tolerance of wheat seedlings, used as test plant.
- To determine the contribution of selected IAA- and ACCD-producing bacteria on water shortage and salt stress tolerance of avocado seedlings.

CHAPTER II

Review: *Endophytic bacteria in phytostimulation*

Paper in preparation

Endophytic bacteria in phytostimulation.

Abstract

Endophytic bacteria are microorganisms living within the tissues of plants without causing substantive damage to the host. Endophytic bacteria ubiquitously inhabit all plant species and they have been isolated from virtually all plant tissues studied. Little is known about the ecology of endophytic bacteria and their interaction mechanisms with host plant. In recent years, numerous studies have shown that endophytic bacteria can help to remove contaminants, suppress plant pathogens and mainly promote plant growth. Different mechanisms of plant growth promoting (PGP) such as biofertilization and phytostimulation have been proposed. Phytostimulation is a PGP mechanism that occurs when endophytic bacteria synthesizes or metabolizes some compounds, such as phytohormones and/or enzymes, which affect plant metabolism and influence its development. It has been shown that different bacteria strains are able to produce some phytohormones such as abscisic acid, auxins, gibberelins, cytokinins and jasmonates and some enzymes such as 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD), which regulates plant ethylene levels. Plant inoculation with phytostimulator-producing bacteria have shown promising, but sometimes inconsistent, results in PGP and plant stress tolerance. This work provides an overview on endophytic bacteria ecology, while discussing critically the phytostimulation mechanism of endophytic bacteria.

Keywords. Endophytic bacteria; phytostimulation; phytohormone; ACCD.

2.1. Introduction.

Naturally a wide number and diversity of bacteria interact detrimental neutral or beneficially with plants, being named as plant growth-promoting bacteria (PGPB) those strains able to provide some benefits to plants. The PGPB are diverse in the habitats occupying, with a large number colonizing and living freely in the rhizoplane and rhizosphere of plants, termed plant growth-promoting rhizobacteria (PGPR). Other group are symbiotically related with host plant living intracellularly into specialized root structures or nodules, such as rhizobia associated with legume and *Frankia* sp. with woody plant. A third group corresponds to PGPB able to colonize and reside the inner of plants tissues without being symbiotic or plant pathogen. These group of bacteria, in which is focused this review, are commonly referred as endophytic bacteria (Cheng et al., 2010; Gray and Smith, 2005; Hallmann et al., 1997).

Endophytic bacteria are able to enhance the plant growth by both indirect and direct mechanism. The indirect mechanisms consist in endophytic biocontrol, preventing and decreasing the deleterious effects of phytopathogenic microorganisms. Thus, endophytic bacteria produce antimicrobial agents, or exclude competitively pathogen organisms, or/and establish systemic resistance in plants (Bashan and De-Bashan, 2005; Dodd et al., 2010; Dudeja et al., 2011). In contrast, the direct mechanisms is the ability of bacteria to provide substances, which plants would usually obtain in lower concentrations, this is carried out by two bacterial process i) biofertilization and ii) phytostimulation. Biofertilization is the bacterial ability to increase supply or availability of important nutrients for host plant by nitrogen fixing, phosphate solubilizing or siderophores producing. Whereas, phytostimulation is the bacterial synthesis of compounds such as phyotohormones or enzymes affecting overall metabolism and consequently the host plant development (Arora, 2013; Glick et al., 2007b; Lugtenberg and Kamilova, 2009; Ping and Boland, 2004). The bacterial ability to produce phyotohormones,

such as abscisic acid (ABA), auxins, cytokinins (CKs), gibberellins (GAs) and jasmonic acid (JA) and enzymes such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (ACCD), have been widely described (Dodd et al., 2010; Glick, 2005; Lugtenberg and Kamilova, 2009; Martínez et al., 2010; J. S. Singh et al., 2011). However, the most of the studies and reviews published about bacterial phytostimulatory effects on plants have been mainly focused in PGPR, considering endophytic bacteria only briefly. Thereby, the main objective of the present review is provide an overview on ecology of endophytic bacteria, while discussing critically the phytostimulation mechanisms of endophytic bacteria.

2.2. Endophytic bacteria.

Although, etymologically, the word “endophyte” means “in the plant” (endon Greek, within; phyton, plant) (Senthilkumar et al., 2011a; Sturz et al., 2000), the term "endophyte” has not been easily defined being widely discussed by some author (Bacon and White, 2000; Hallmann et al., 1997; Kado, 1992; Quispel, 1992; Wilson, 1995). A widely accepted definition corresponds to the used firstly by Bacon and White, (2000) who defined endophyte as microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects. However, multiple publications are based on the criterion of Hallmann et al., (1997) who, in practical terms, have considered as endophytic bacteria those that could be isolated from disinfected-surface or extracted from inside of apparently healthy plants. Otherwise, Reinhold-Hurek and Hurek, (1998) asserted that to confirm a ‘true endophytic bacterium’, together with the isolation from surface-disinfected tissues, also is necessary microscopic evidence to visualize tagged-bacteria inside plant tissues. The term ‘putative endophyte’ it is recommended to describe bacteria that have not been validated microscopically.

The ability of some bacterial strains to colonize the internal environment of the plant would confer an ecological advantage over bacteria that colonize plants only epiphytically. Internal

tissues of plants would provide a more protective environment than plant surfaces, where bacteria are exposed to extreme environmental conditions, such as temperature, osmotic potentials, and ultraviolet radiation (Hallmann et al., 1997). Endophytic bacteria have been isolated from a wide variety of plant tissues such as fruits (de Melo Pereira et al., 2012), seeds (Mundt and Hinkle, 1976; Vega et al., 2005), nuts (Wilhelm et al., 1998) flowers (Misaghi and Donndelinger, 1990), as well as from stems (Rai et al., 2006), leaves (Ibañez et al., 2012; Kishore et al., 2005) and specially from roots (Compant et al., 2005b; Ibañez et al., 2012; Kirchhof et al., 1997; McInroy and Kloepper, 1995). Endophytic bacteria would inhabit ubiquitously all plant species, with a particular plant being considered as a complex micro-ecosystem, thereby the wide variety and quantity of species represents a vast and relatively untapped ecological niche (Lodewyckx et al., 2002; Senthilkumar et al., 2011a).

Ecologically, endophytic bacteria can be classified either as 'obligate' or 'facultative' (Bacon and Hinton, 2006; Hardoim et al., 2008; Senthilkumar et al., 2011). Obligate endophytic bacteria are those that cannot survive well in soil but can successfully colonize the internal roots and aerial parts of plants and generally their transmission to other plants occurs vertically from seeds (McInroy and Kloepper, 1995) and vegetative planting material (Dong et al., 1994; Sturz, 1995). Whereas, facultative are able to colonize both the surface and inner of plant, surviving well in the environment surrounding the plant (phylloplane, rhizoplane and/or rhizosphere). The classification as 'obligate' is still discussed, because many 'obligate' strains can be cultured *in vitro* without to need their host (B Reinhold-Hurek and Hurek, 1998). Hardoim et al., (2008) has proposed to classify endophytic bacteria as: i) competent, those having the genetic machinery required for colonizing and persisting in the endosphere; ii) opportunistic, are competent rhizosphere colonizers but only entering root tissue coincidentally; and iii) passenger, those that enter to the plant by accident in the absence of selective forces maintaining them in the internal tissue of the plant.

The main infection site correspond to emergence points of lateral roots and the differentiation and elongation zone, next to root tip (Reinhold-Hurek and Hurek, 2011; Senthilkumar et al., 2011), although it has been shown that endophytic bacteria can colonize the plant from different sites such as stomata, hydathodes, nectarthode, lenticels, germinating radicles, broken trichomes or wounds (Beattie, 2006; Hallmann et al., 1997; Sturz et al., 2000). In general, a successful endophere colonization should start with the successful rhizosphere colonization, which is controlled by numerous chemical signals (Bais et al., 2004). In this way, root cap and apical epidermal cells of root hairs secrete sugars, amino acids, amides, aliphatic and aromatic acids, phenolics compounds, fatty acids, vitamins, sterols, enzymes and proteins, hormones as well as acyl homoserine lactone, saponin, scopoletin, nucleotides, etc., several of these compounds are chemoattractants and others nutrients for the microorganisms living in or nearby the rhizosphere (Faure et al., 2008; Lugtenberg and Dekkers, 1999). Many biotic and abiotic factors affect root exudation (Berg and Smalla, 2009), even this exudation can vary in different root zones (Kuzyakov, 2002), allowing the selection of specific and different bacterial communities in different root zones, and in some way limiting the potential colonizing species (For more details to see Bais et al., 2004; Faure et al., 2008).

The ecological role of endophytic bacteria remains largely unexplored, because analysis of their functions is hindered by difficulties in cultivating most bacteria (Nikolic et al., 2011; Sessitsch et al., 2012). Endophytic bacteria diversity have traditionally been studied by cultivation-dependent methods from internal tissues, but their performance is relatively poor. The development of novel cultivation-independent techniques have allowed important progress of knowledge on diversity, ecology, dynamics and structure of the endophytic communities, although this knowledge remains limited (Hardoim et al., 2008; Reinhold-Hurek and Hurek, 2011; Sessitsch et al., 2002). Nevertheless, the combination of both techniques is necessary. Currently, it is known that endophytic community composition is dynamic and varies depending on factors such as temperature, agricultural practices, host genotype, and plant

growth development (McInroy and Kloepper, 1995; Pillay and Nowak, 1997; Rai et al., 2006; Seghers et al., 2004). Consequently, it is important to address and understand the ecological relevance of endophytic bacteria with the objective to develop successful inoculation strategies. However, further studies are needed to ascertain with certainty the biodiversity and dynamics of bacterial communities as well as their interactions and functions in host plants. It is important to address the ecological relevance of endophytic bacteria thereby developing successful inoculation techniques.

Although tools in microbial molecular ecology have advanced significant during the last years, there are only several studies related with mechanisms involved in endophytic-host and endophytic-endophytic interactions and just a little genes related with endophytic bacteria colonization and establishment into plant host have been described (Hardoim et al., 2008; Reinhold-Hurek and Hurek, 2011). Thus, Sessitsch et al., (2012) in a metagenomic study about endophytic bacteria of rice roots revealed that bacterial communities seem to be highly adapted to proliferate and spread within plants, suggesting that the endorhiza is an exclusive microhabitat requiring particular adaptations. This study found interested features related with plant host-endophytic bacteria interactions; including flagella, plant-polymer-degrading enzymes, protein secretion systems, iron acquisition and storage, quorum sensing, and detoxification of reactive oxygen species. In addition, Sessitsch et al., (2012) also showed that endophytic bacteria might be involved in the entire N cycle, with protein domains involved in N_2-fixation, denitrification, and nitrification were detected. Although this study provides an interesting approach about the mechanisms involved in endophytic bacteria-host interaction, these have not yet been fully elucidated. Genomic, proteomic and metagenomic approaches and other cultivation-independent techniques in addition to mutational analyses of endophytic bacteria and plant host might reveal more information about interaction mechanism.

2.3. Plant growth promoting endophytic bacteria.

An increased interest on biotechnological applications of endophytic bacteria has emerged in recent year particularly as a potential source of novel natural products (Qin et al., 2011; Ryan et al., 2008; Strobel et al., 2004), in phytoremediation (Rajkumar et al., 2009; Ma et al., 2011; Zhang et al., 2011), and as biocontrol agents (Compant et al., 2005). Nevertheless, most studies are focused on their PGPB mechanisms. The most studied and characterized PGP mechanism corresponds to N_2 -fixation by diazotrophic bacteria (James et al., 1997; Mattos et al., 2008; Vessey, 2003). Since nitrogen fixation was originally proposed as higher mechanism by which endophytic bacteria affected plant growth, considerable information has been reported about this mechanism (Lodewyckx et al., 2002). Among non-leguminous plants, several diazotrophic endophytes have been isolated and characterized including *Acetobacter* sp. (Sevilla et al., 2001), *Azoarcus* spp. (Hurek et al., 1994), *Serratia* spp. (Gyaneshwar et al., 2001), *Burkholderia* spp. (Divan Baldani et al., 2000), *Herbaspirillum* spp. (Elbeltagy et al., 2001; Gyaneshwar et al., 2002), *Pantoea* sp. (Loiret et al., 2004), *Klebsiella* sp. (Iniguez et al., 2004) and *Azospirillum* spp (Zhang et al., 1997). Significant progress has been carried out in biological nitrogen fixation with non-leguminous plants over the last years, but many bacteria fix N_2 but only a fraction is transferred to host plant (Bashan and de-Bashan, 2010) In this way. Gyaneshwar et al., (2001) inoculated a diazotrophic *S. marcescens* IRBG500 in rice, resulting in a significant increment in root length and root dry weight but not in total N of rice plants. Whereas, Elbeltagy et al., (2001) determined that *Oryza officinalis* inoculated with diazotrophic endophytic *Herbaspirillum* sp. B501 incorporated $^{15}N_2$-fixed in lower concentration compared with ^{15}N gas used. On the other hand, the N_2-fixing bacterium *Azotobacter paspali*, isolated from a subtropical grass species, improves growth of a variety of plants. Experiments with added inorganic N suggested that plant growth promotion is caused by the production of plant growth factors such as IAA, gibberellins, and cytokinins, rather than N_2 fixation (Lugtenberg and Kamilova, 2009). Endophytic bacteria have a wide spectrum of effects on hosts, which is

due at least in part to the production of secondary metabolites, such as phytohormones (Figure 2.1.) which alter the host's growth and phenotype. Many plant-associated bacteria are capable by themselves of synthesizing phytohormones (Figure 2.2.; Table 2.1.), which would be necessary as mediators in communications between plant host and its microflora (Hardoim et al., 2008; Tsavkelova et al., 2006). Further ecological and molecular studies are needed to elucidate this hypothesis.

2.4. Endophytic bacteria phytostimulation.

2.4.1. Phytohormones.

Phytohormones (Figure 2.1.) are crucial signaling molecules of low molecular weight that act as chemical messengers to coordinate, at least partly, all aspects of plant growth, development and defense (Piotrowska and Bajguz, 2011; Shan et al., 2012). A particular phytohormone acts displaying principally its action at distance, triggering specific biochemical, physiological, and morphological responses (Baca and Elmerich, 2007; Piotrowska and Bajguz, 2011). The phytohormone response will depend on its concentration within the tissue and on the sensitivity of the tissue to the hormone. The phytohormones correspond to diverse compounds that include those known traditionally as 'classical phytohormones': auxin, abscisic acid (ABA), cytokinin (CKs), gibberellin (GAs) and ethylene, and several compounds as brassinosteroids, jasmonic acid (JAs) and salicylic acid (SAs), that have been recognized as phytohormones in the last years (Liu et al., 2009; Santner and Estelle, 2009). Growth and development of plants involve the integration of both environmental and endogenous signals. Thus, there are two main sources of phytohormones available for the plants: i) endogenous, those produced by the plant tissues and ii) exogenous, those produced by plant associated microorganisms, like endophytic bacteria (Baca and Elmerich, 2007). Multiple endophytic bacteria strains able to produce phytohormones have been described. In the present review, each main phytohormone group

will be discussed in detail, but its biosynthesis will be considered only superficially because there are comprehensive reviews and books focused on plant hormone biosynthesis (Kende, 1993; Kudo et al., 2010; Taiz and Zeiger, 2010; Woodward and Bartel, 2005).

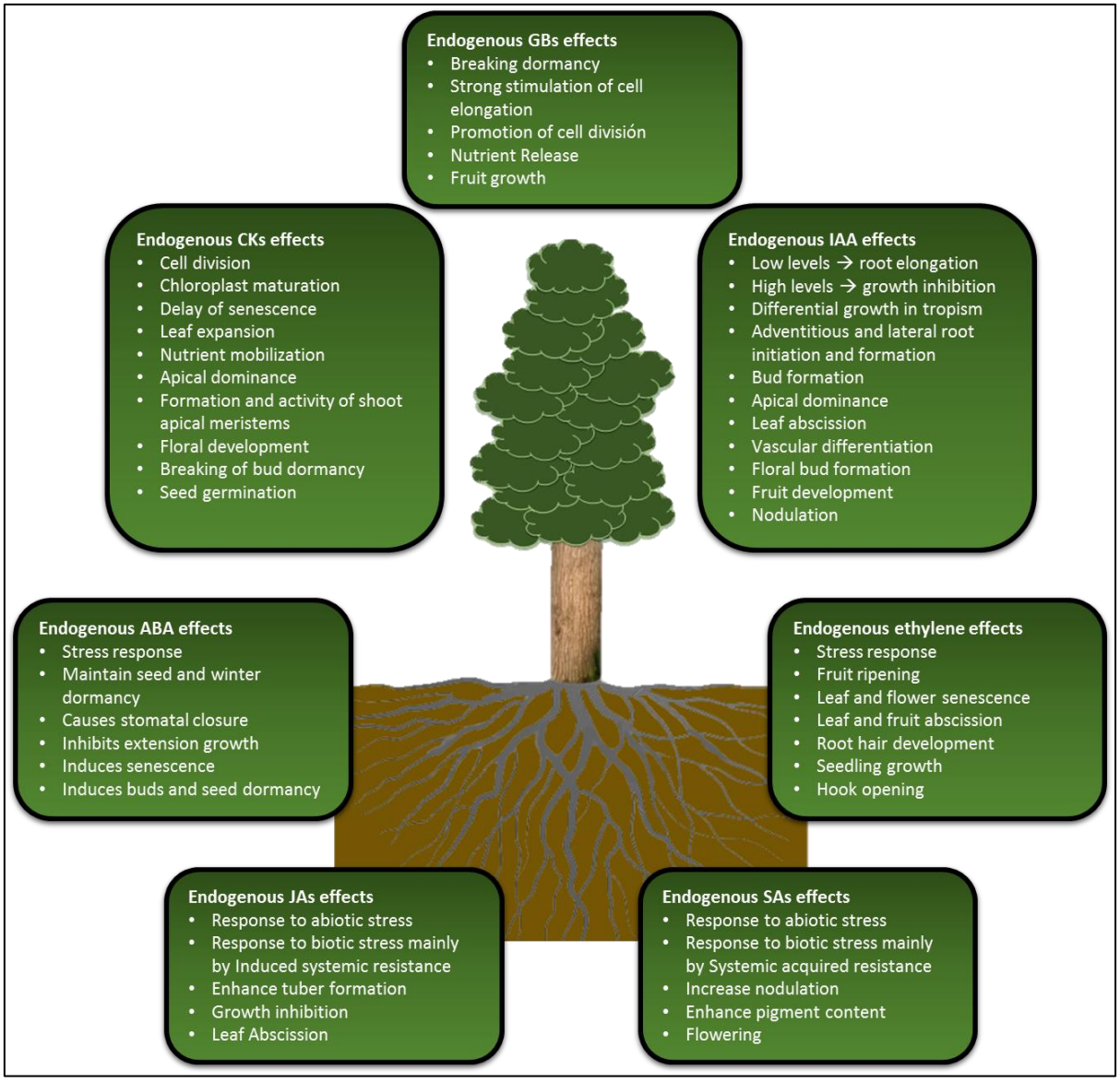

Figure 2.1. Effects of main phyotohormones on plant physiology and development.

2.4.1.1. Abscisic Acid (ABA).

Abscisic Acid is a 15-carbon sesquiterpenoid that plays important roles in many cellular processes including seed development, dormancy, germination, vegetative growth, and response environment stresses (Groppa et al., 2011; Piotrowska and Bajguz, 2011; Taiz and Zeiger, 2010). Increases in ABA levels have been reported in response to stresses, such as salt,

freezing, heat, drought and wounding, which trigger specific biochemical responses (Cohen et al., 2008; Piotrowska and Bajguz, 2011). The ABA has been detected in almost all classes of organisms tested from a range of cyanobacteria, algae, bryophytes, fungi, lichens, and higher plant (Hartung, 2010). However, the ABA production has not been extensively investigated in bacteria, even until recently it was well accepted that bacteria don not synthesize ABA. In recent years, some researchers have confirmed that some endophytic bacteria strains have the ability to produce ABA (Cohen et al., 2009, 2008; Forchetti et al., 2007; Piccoli et al., 2010; Sgroy et al., 2009). In this way, Forchetti et al., (2007) detected *in vitro* ABA production by endophytic bacteria identified as *Bacillus pumilus* and *Achromobacter xiloxidans* (or *Alcaligenes* sp.), which were isolated from *Helianthus annuus* roots. These selected endophytic strains were also able to increase the ABA production when were exposed *in vitro* to drought with water potentials −1.60 and −2.03 MPa. Likewise, Cohen et al., (2008) determined that *Azospirillum brasiliense* Sp 245 was able to produce ABA in a the chemically-defined medium NFb. Under normal conditions, the bacterium produced 73 ng ml^{-1} of media, but with decreased water potential (Ψa –0.7 MPa) the ABA production was increased by 245 ng ml^{-1} media. Similarly, Feng et al., (2006) determined that endophyte bacteria *Pantoea agglomerans* YS19 isolated from rice produced different phytohormones in LB medium, among which ABA with 675 ng ml^{-1} was the most abundant. Whereas, Sgroy et al., (2009) isolated seven endophytic bacteria strains *(Lysinibacillus fusiformis*, *Bacillus subtilis*, *B. pumilus*; *Brevibacterium halotolerans*, *B. licheniformis*, *Achromobacter xylosoxidans Pseudomonas putida*) associated with *Prosopis strombulifera* (halophyte plant), all these strains were able to produce ABA in a chemically defined medium, being *P. putida* which produced a higher amount of ABA (4.27 μg ml^{-1}). Cohen et al., (2008) also showed that inoculation of *Arabidopsis thaliana* with *A. brasilense* Sp 245 enhanced two-fold the plant's ABA content. Cohen et al., (2009) determined that *Azospirillum lipoferum* producer of ABA reversed effects of the ABA inhibitor fluridone (F) on inoculated *Zea mays*, both well-watered and under drought stress plants. Therefore, when F

treated *Zea mays* were inoculated with *A. lipoferum s*howed similar length to the control in well-watered plants, or even promoted shoot length above control in plants under drought. On the other hand, nothing is known about the biochemical mechanism of bacterial ABA production (biosynthesis and metabolism) or about a possible function of ABA for bacteria. Considerable research is required in this field (Dodd et al., 2010; Hartung, 2010; Rosenblueth and Martínez-Romero, 2006).

2.4.1.2. Auxins.

Auxins belong to diverse chemical compounds, most of which have an aromatic system such as indole, phenyl or naphthalene ring with a side chain containing an attached carboxyl group (Bajguz and Piotrowska, 2009). Indole-3-acetic acid (IAA), is by far the most abundant and physiologically relevant, and therefore studied auxin, which is a heterocyclic compound containing a carboxymethyl group, an acetic acid (Taiz and Zeiger, 2010). The endogenous IAA regulates several developmental plant processes such as initiation, growth and distribution of roots, stem elongation, apical dominance, fruit development, tropistic responses, flowering, fruit ripening and senescence, pigment formation, biosynthesis of various metabolites, and resistance to biotic stress, among others (Baca and Elmerich, 2007; Bajguz and Piotrowska, 2009; Bashan and de-Bashan, 2010; Dias et al., 2008; Dodd et al., 2010; Taiz and Zeiger, 2010).

Table 2.1. Main phyotohormones produced by endophytic bacteria species and host plant from where they were isolated.

Phyto-hormone	Bacterial species	Plants Host	References
ABA	*Achromobacter xiloxidan, A. xylosoxidans, Acinetobacter johnsonii, Arthrobacter koreensis, Azospirillum brasilense, A. lipoferum, Bacillus aquimaris, B. licheniformis, B.pumilus, B. subtilis, Bradyrhizobium japonicum, Brevibacterium halotolerans, Chryseobacterium* sp., *Lysinibacillus fusiformis, Pantoea agglomerans, Pseudomonas putida*	*A. thaliana*, maize, *Prosopis strombulifera*, rice, sunflower, sugar beat, wheat	Boiero et al., 2007; Cohen et al., 2009, 2008; Egorshina et al., 2011; Feng et al., 2006; Forchetti et al., 2010, 2007; Perrig et al., 2007; Piccoli et al., 2010; Sgroy et al., 2009; Shi et al., 2009
Auxins (IAA)	*Acetobacter diazotrophicus, Achromobacter sp. A. xylosoxidans, Acinetobacter sp. A. calcoaceticus, A. johnsonii, A. junii, A. radioresistens, Aeromonas veronii, Agrobacterium* sp., *Arthrobacter koreensis,Arthrobacter sp., Azorhizobium* sp., *Azospirillum brasilense, Bacillus* sp., *Bacillus amyloliquefaciens, B. cereus , B. firmus, B. flexus, B. ginsengihumi, B. horneckiae, B. idriensis, B. licheniformis, B. megaterium, B. muralis, B. mycoides, B.oleronius, B. psychrosaccharolyticus, B. pumilus, B. simplex, B. subtilis, B. thuringiensis, Brachybacterium sp., Bradyrhizobium elkanii, B. japonicum, Brevibacillus parabrevis, Brevibacterium casei, Brevundimonas sp., Burkholderia* sp., *B. caledonica, B. cenocepacia, B. cepacia, B. glathei, B. kururiensis, B. phenazinium, B. phytofirmans, B. sediminicola, B. vietnamiensis, Cellulomonas* sp., *Chryseobacterium sp., C. indologene, Cronobacter sakazakii, Curtobacterium* sp., *C. citreum, C. plantarum, Devosia sp., Dyella koreensis, D. marensis, Ensifer meliloti, Enterobacter* sp., *E. aerogenes, E. agglomerans, E. cloacae, E.ludwigii, Escherichia* sp., *Flavobacterium gleum, Gluconacetobacter sp., G. diazotrophicus, Haererehalobacter sp. , Halomonas sp., Herbaspirillum* sp., *H.frisingense, H. hiltneri, H. seropedicae, Klebsiella* sp. *K. oxytoca, K. pneumoniae, Kocuria* sp., *Lysinibacillus, fusiformis, L. sphaericus, Mesorhizobium* sp. *M. fujisawaense, Methylobacterium populi, Methylobacterium* sp., *Microbacterium* sp., *Microbacterium arborescens M. ginsengisoli, M. kitamiense, M. phyllosphaerae, M. oleivorans, M. takaoensis, M. testaceum, Micrococcus luteus, Micromonospora* sp. *Nocardioides* sp., *Ochrobactrum anthropic*	*Aster tripolium, Beta vulgaris, Bidens pilosa, Brassica napus, Capsicum annuum, Calystegia soldanella, Catharanthus roseus,* Chinese cabbage, *Citrus sinensis,* clover, coffee, *Commelina communis, Conyza canadensis,* cotton, *Cymbidium eburneum, Daucus carota,* deepwater rice, Echinacea plants, *Elsholtzia splendens, Elymus mollis, Glehnia littoralis, Heracleum sosnowskyi, Lespedeza* sp. *Lycopersicon esculentum, Mosla chinensis,* Onion, Palm tree, *Oryza alta, Oryza sativa, Panax ginseng, Panicum miliaceum Persea americana, Piper nigrum,* plant grown in a copper mine, poplar tree, *Populus trichocarpa, Prosopis strombulifera, Salicornia brachiata, Solanum lycopersicum, S. nigrum, S.tuberosum, Sorghum sudanense,* soybean, sugarcane, strawberry, sunflower, *Vicia faba, Vitis vinifera,* wheat, winter rye, *yellow lupine, Zea mays*	Ait Barka et al., 2006; Amaresan et al., 2011; Andreolli et al., 2016; Barra et al., 2016; Bastian et al., 1998; Beneduzi et al., 2013; Bhore et al., 2010; Blaha et al., 2006; Boiero et al., 2007; Caballero-Mellado et al., 2004; Chen et al., 2010; Cohen et al., 2009, 2008; Compant et al., 2005b; de Melo Pereira et al., 2012; Dias et al., 2008; Egorshina et al., 2011; Elbeltagy et al., 2000; Estrada-De Los Santos et al., 2001; Etesami and Alikhani, 2016; Etesami et al., 2014; Faria et al., 2013; Feng et al., 2006; Forchetti et al., 2010, 2007; Fuentes-Ramirez et al., 1993; Gasser et al., 2011; Gillis et al., 1989; Govindarajan et al., 2008; Ibañez et al., 2012; Jasim et al., 2013; Jha et al., 2012; Jha and Kumar, 2009; Johnston-Monje and Raizada, 2011; Karthikeyan et al., 2012; Lata et al., 2006; Lee et al., 2004; Li et al., 2008, 2016; Liu et al., 2011; Long et al., 2008; Malfanova et al., 2011; Mattos et al., 2008; Mendes et al., 2007; Merzaeva and Shirokikh, 2010; Mirza et al., 2001; Montañez et al., 2012; Onofre-Lemus et al., 2009; Palaniappan et al., 2010; L Perin et al., 2006; L. Perin et al., 2006; Perrig et al., 2007; Piccoli et al., 2010; Rasche et al., 2006a, 2006b; Rashid et al., 2012; Reis et al., 2004; Saïdi et al., 2013; Sessitsch et al., 2005; Sgroy et al., 2009

Table 2.1. Main phyotohormones produced by endophytic bacteria species and host plant from where they were isolated (continued).

Phyto-hormone	Bacterial species	Plants Host	References
Auxins (IAA)	*Paenibacillus glucanolyticus, P. lentimorbus, P. macerans, P. validus, P. xylanexedens, Pantoea* sp., *P. agglomerans, P. ananatis, P. ananas, P. brenneri, P. punctata, P. stewartii, Pseudomonas,* sp., *P. aeuroginosa, P. boreopolis, P. brassicacearum, P. fluorescens, P. fulva, P. huttiensis, P. lutea, P. marginalis, P. savsananoi, P. pseudoalcaligenes, P. putida, P. stutzeri, P. thivervalensis, P.toloasi, Pseudoxantomonas* sp., *Rhanella sp., R.aquatilis , Ralstonia* sp., *Rhizobium* sp., *Rhizobium albertimagni, Rhizobium grahamii, Rhizobium huautlense, Rhizobium lusitanum, Rhizobium nepotum, Rhizobium pusense, Rhizobium rediobacter, Rhizobium tropici, Rhodanobacter* sp., *Rhodococcus equi, Serratia* sp., *S. nematodiphila, S. marcescens, S. plymuthica, S. proteamaculans, Shinella kummerowiae, Sphingomonas* sp., *Sphingopyxis* sp., *Sporosarcina aquimarina, Staphylococcus epidermidis, S. pasteuri , Stenotrophomonas* sp., *S. chelatiphaga, S. maltophilia, Streptomyces* sp., *S. griseoplanus, S. umbrinus, Thalassospira permensis, Variovorax paradoxus, Vibrio alginolyticus, Virgibacillus* sp., *Zhihengliuella* sp.		Shi et al., 2011, 2010, 2009; Shin et al., 2007; M. K. Singh et al., 2011; Subramanian et al., 2015; Sun et al., 2015, 2010; Szymańska et al., 2016; Taghavi et al., 2010, 2009; Trivedi et al., 2011; Vandamme et al., 2002; Vendan et al., 2010; Verma et al., 2001; Wang et al., 2011; Weyens et al., 2011; Xin et al., 2009; Yaish et al., 2015; Yim et al., 2009; Yue et al., 2007; Y. Zhang et al., 2011
CKs	*Acinetobacter johnsonii, Azospirillum brasilense , Bacillus pumilus, B. subtilis, Bradyrhizobium japonicum, Brevibacterium halotolerans, Chryseobacterium* sp., *Paenibacillus polimaxa, Pantoea agglomerans, Pseudomonas putida, P. resinovoran*	*Gynura procumbens,* hogweed, poplar, *Prosopis strombulifera,* soybean, sugar beet, wheat	Bhore et al., 2010; Boiero et al., 2007; Egorshina et al., 2011; Malfanova et al., 2011; Perrig et al., 2007; Piccoli et al., 1997; Sgroy et al., 2009; Shi et al., 2009; Weyens et al., 2011
GAs	*Acetobacter diazotrophicus, Achromobacter xylosoxidans, Acinetobacter johnsonii, Arthrobacter koreensis, A. brasilense, Azospirillum lipoferum, B. licheniformis, B. pumilus, B. subtilis, Bradyrhizobium japonicum, Brevibacterium halotolerans, Chryseobacterium* sp., *Herbaspirillum seropedicae, Lysinibacillus fusiformis, Pantoea agglomerans*	Hogweed, maize, *Prosopis strombulifera,* soybean, sugar beet, sugarcane, wheat	Bastian et al., 1998; Boiero et al., 2007; F. Cassán et al., 2001; Cohen et al., 2009; Egorshina et al., 2011; Malfanova et al., 2011; Perrig et al., 2007; Piccoli et al., 2010; Sgroy et al., 2009; Shi et al., 2009

Table 2.1. Main phyotohormones produced by endophytic bacteria species and host plant from where they were isolated (continued).

Phyto-hormone	Bacterial species	Plants Host	References
JAs	*Achromobacter xiloxidans, Arthrobacter koreensis, Bacillus pumilus*	*Prosopis strombulifera,* Sunflower	Forchetti et al., 2010, 2007b; Piccoli et al., 2010
SAs	*Achromobacter xylosoxidans, Bacillus cereus, B.ginsengihumi, B. licheniformis, B. pumilus, B.subtilis, Burkholderia cepacia, Burkholderia* sp., *Burkholderia vietnamiensis, Enterobacter cloacae,, Herbaspirillum* sp., *Lysinibacillus fusiformis, Methylobacterium* sp., *M. fujisawaense, M. populi, Microbacterium oleivorans, Paenibacillus glucanolyticus, Pseudomonas* sp., *P. validus, P. fluorescens, Pantoea* sp., *P.agglomerans, P. fulva, P. savsananoi, P. putida, P. toloasi, Serratia* sp., *S. plymuthica, S. proteamaculans, Stenotrophomonas* sp., *S. chelatiphaga, Streptomyces* sp., *S. griseoplanus, Variovorax paradoxus*	*Citrus sinensis, Helianthus annuus,* olive	Forchetti et al., 2010; Mercado-Blanco et al., 2004; Trivedi et al., 2011

Table 2.2. ACCD-producing endophytic bacteria

Effects on Plant	Bacterial species	Plants Host	References
Enzyme ACCD promotes plant growth by sequestering and cleaving plant-produced ACC thereby lowering the level of ethylene in the plant. Decreased ethylene levels allow the plant to be more resistant to a wide variety of environmental stresses.	*Achromobacter* sp., *A. xylosoxidans*, *Acinetobacter* sp., *A. calcoaceticus A. radioresistens*, *Aeromicrobium sp.*, *Aeromonas veronii*, *Arthrobacter* sp., *A. nitroguaiacolicus*, *Bacillus* sp., *B. anthracis*, *B. endophyticus*, *B. ginsengihumi*, *B. horneckiae*, *B. idriensis*, *B. licheniformis*, *B. megaterium*, *B. oleronius*, *B. psychrosaccharolyticus*, *B. pumilus*, *B. simplex*, *B. subtilis*, *Brachybacterium* sp., *Bradyrhizobium elkanii*, *Brevibacterium casei*, *B. halotolerans*, *Brevundimonas vesicularis*, *Burkholderia* sp., *B. caledonica*, *B. cepacia*, *B. glathei*, *B. kururiensis*, *B. phenazinium*, *B. phymatum*, *B. phytofirmans*, *B. sediminicola*, *B. silvatlantica*, *B. sordidicola*, *B. terricola*, *B. tunerum*, *B. tropica*, *B. unamae*, *B. vietnamiensis*, *Caulobacter vibrioides*, *Cellulomonas* sp., *Cronobacter sakazakii*, *Curtobacterium* sp., *Devosia* sp., *Dyella koreensis*, *D. marensis*, *Enterobacter* sp., *E. aerogenes*, *E. agglomerans*, *E. asburiae*, *E. cloacae*, *E. cancerogenus*, *E. ludwigii*, *Erwinia persicina*, *Escherichia* sp., *Haererehalobacter* sp., *Halomonas* sp., *Herbaspirillum* sp., *H. seropedicae*, *Klebsiella* sp., *K. oxytoca*, *K. pneumonia*, *Mesorhizobium* sp., *Methylobacterium* sp., *M. fujisawaense*, *M. populi*, *Microbacterium* sp., *M. arborescens*, *M. ginsengisoli*, *M. oleivorans*, *M. takaoensis*, *M. testaceum*, *Micrococcus luteus*, *Micromonospora* sp., *Nocardioides* sp., *Ochrobactrum anthropic*, *Paenibacillus* sp., *P. glucanolyticus*, *P. lentimorbus*, *P. macerans*, *P. pabuli*, *P. polimaxa*, *P. validus*, *P. xylanexedens*, *Pantoea* sp. *P. agglomerans*, *P. ananatis*, *P. ananas*, *P. stewartii*, *Pseudomonas* sp., *P. aeuroginosa*, *P. brassicacearum*, *P. congelans*, *P. fluorescens*, *P. fulva*, *P. huttiensis*, *P. lutea*, *P. oleovorans*, *P. marginalis*, *P. savsananoi*, *P. pseudoalcaligenes*, *P. putida*, *P. stutzeri*, *P. thivervalensis*, *P. toloasi*, *Ralstonia* sp., *Rhizobium lusitanum*, *Rhizobium* sp., *R. rediobacter*, *R. tropici*, *Rhodococcus* sp.*R. equi*, *Serratia* sp., *S. nematodiphila*, *S. marcescens*, *S. plymuthica*, *S. proteamaculans*,, *Sphingobium yanoikuyae*, *Sphingomonas* sp., *Staphylococcus epidermidis*, *S. warneri*, *Stenotrophomonas* sp., *S. chelatiphaga*, *S. maltophilia*, *Variovorax paradoxus*, *Vibrio alginolyticus*, *Zhihengliuella* sp.	*Aspalathus carnosa*, *Aster tripolium*, *Beta vulgaris*, *Bidens pilosa*, *Brassica napus*, *Calystegia soldanella*, *Capsicum annuum*, *Catharanthus roseus*, chinese cabbage, clover, coffee, *Commelina communis*, cotton, *Citrus sinensis*, *Conyza*, *Canadensis*, *Cymbidium eburneum*, *Daucus carota*, deepwater rice, Echinacea plants, *Elsholtzia splendens*, *Elymus mollis*, *Glehnia littoralis*, *Gynura procumbens*, *Heracleum sosnowskyi*, *Lespedeza* sp., *Lycopersicon esculentum*, *Machaerium lunatum*, *Mosla chinensis*, Onion, *Oryza alta*, *O. sativa*, Palm tree, *Panax ginseng*, *Panicum miliaceum*, plant grown in a copper mine, *Persea Americana*, *Piper nigrum*, poplar trees, *Populus trichocarpa*, *Prosopis strombulifera*, rice, *Salicornia brachiate*, *Solanum lycopersicum*, *S. nigrum*, *S. tuberosum*, *Sorghum sudanense*, Soybean, strawberry, sugarcane, sunflower, *Vicia faba*, yellow lupine, *Vitis vinifera*, wheat, winter rye, *Zea mays*,	Ait Barka et al., 2006; Amaresan et al., 2011; Andreolli et al., 2016; Barra et al., 2016; Bastian et al., 1998; Beneduzi et al., 2013; Bhore et al., 2010; Blaha et al., 2006; Caballero-Mellado et al., 2004; Chen et al., 2010; Compant et al., 2005b; de Melo Pereira et al., 2012; Dias et al., 2008; Egorshina et al., 2011; Elbeltagy et al., 2000; Estrada-De Los Santos et al., 2001; Etesami and Alikhani, 2016; Etesami et al., 2014; Faria et al., 2013; Feng et al., 2006; Forchetti et al., 2010, 2007; Gasser et al., 2011; Govindarajan et al., 2008; Ibañez et al., 2012; Jasim et al., 2013; Jha et al., 2012; Jha and Kumar, 2009; Johnston-Monje and Raizada, 2011; Karthikeyan et al., 2012; Lata et al., 2006; Li et al., 2008, 2016; Liu et al., 2011; Long et al., 2008; Malfanova et al., 2011; Mattos et al., 2008; Mendes et al., 2007; Merzaeva and Shirokikh, 2010; Mirza et al., 2001; Montañez et al., 2012; Onofre-Lemus et al., 2009; Palaniappan et al., 2010; L Perin et al., 2006; L. Perin et al., 2006; Rasche et al., 2006a, 2006b; Rashid et al., 2012; Reis et al., 2004; Saïdi et al., 2013; Sessitsch et al., 2005; Sgroy et al., 2009; Shi et al., 2009; Shin et al., 2007; M. K. Singh et al., 2011; Sun et al., 2015, 2010; Szymańska et al., 2016; Taghavi et al., 2010, 2009; Trivedi et al., 2011; Vandamme et al., 2002; Vendan et al., 2010; Verma et al., 2001; Wang et al., 2011; Weyens et al., 2011; Xin et al., 2009; Yaish et al., 2015; Yim et al., 2009; Yue et al., 2007; Y. Zhang et al., 2011

The IAA effects on plant seedling are dosage-dependent and root tissues development can be affected both negatively or positively depending on the exogenous IAA concentration applied (Cassán et al., 2011; Gravel et al., 2007; Spaepen et al., 2007). Thus, evident increases in the IAA content it have been correlated with some plants pathologies, such as tumor- and gall-inducing mediated by *Agrobacterium tumefaciens, A. rhizogenes* and *Pseudomonas savastanoi*, (Cassán et al., 2011; Nussaume and Robaglia, 2003; Spaepen et al., 2007). Whereas, enhanced root plant proliferation it has been attributed to lower endophytic bacteria production of IAA (Goudjal et al., 2013). Long et al., (2008) determined that exogenously applying IAA to *Solanum nigrum* seeds in the range of 100 µg ml^{-1} to 10 mg ml^{-1} inhibited seedling root growth. In contrast, applying 1 µg ml^{-1} of IAA to seeds significantly increased the root growth of seedlings compared with the control. Similar results where showed inoculating seeds with different IAA-producing bacteria strains. Of these, two strains increased root length in the range between 1.1 and 11 µg ml^{-1} of IAA and three strains with IAA levels ranged from 93 to 154 µg ml^{-1}, inhibited root growth. The plant inoculation with IAA-producing endophytic bacteria also triggers qualitative root architecture changes according to the IAA level, similar to previously described rhizobacteria effects (Ali et al., 2009). Thus, a high level of IAA stimulates lateral and adventitious root formation. However, too high IAA levels could cause inhibition of root length and finally inhibition of plant development. Therefore, optimum level of IAA is to be adjusted (Singh et al., 2013). This root system proliferation triggers increased ability of nutritional uptake allowing mine more nutrients from the soil. In this way, Singh et al., (2013) determined significant increase in nitrogen, phosphorous, and potassium uptake by *Oryza sativa* inoculated with the IAA-overproducing mutants endophytic *Burkholderia cepacia* Strain RRE25 compared with the wild type. In another hand, Merzaeva and Shirokikh, (2010) isolated some IAA-producing coryneform species (*Curtobacterium plantarun*, *C. plantarum*, and *Cellulomonas* sp.) from root of winter rye. The inoculation of these strains on winter rye seeds

increased the germination ability and allow intensive seedling growth *in vitro*. In addition, they showed that the IAA synthesis depends on the growth phase of bacteria, composition and acidity of nutrient medium, tryptophan concentration, and aeration conditions. Moreover, Shi et al., (2009) determined that IAA-producing endophytic bacteria *Bacillus pumilus*, *Chryseobacterium indologene* and *Acinetobacter johnsonii*, significantly increased plant height fresh and dry weights and number of leaves per plant, as well as levels of phytormones of sugar cane plants, compared with control plants. Lee et al., (2004) determined that *Gluconacetobacter diazotrophicus* strain mutant, with reduced ability to produce IAA, did not promoting plant growth compared with the wild type. Marulanda et al., (2009) showed that endophytic bacteria IAA-producing *Bacillus megaterium* improved water content of maize plants, which would help to plant growth under drought stress conditions. On the other hand, endophytic bacteria are able to increase the nodulation of leguminous plants. In this context, the IAA-producing endophytic bacteria *Bacillus megaterium* LNL6 isolated from root nodules of *Lesperdeza* sp. showed significant increase in nodule activity of (nodule leghemoglobin content, nodulated root ARA and total plant nitrogen content) *Bradyrhizobium japonicum* MN110 compared to solitary inoculation of *B. japonicum* (Subramanian et al., 2015)

The IAA is largely the most documented phytohormone production by endophytic bacteria. In this context, endophytic strains belonging to class α- (Andreolli et al., 2016; Montañez et al., 2012) β- (Trivedi et al., 2011; Zhang et al., 2011) and γ- Proteobacteria (Barra et al., 2016; Montañez et al., 2012) as well as Actinobacteria (Andreolli et al., 2016; Szymańska et al., 2016); Flavobacteriia (Elbeltagy et al., 2000; Shi et al., 2009) and Bacilli (Faria et al., 2013; Yaish et al., 2015) have shown the ability to produce IAA. In the same way, Table 2.1. shows the wide variety of IAA-producing bacterial species with *Bacillus, Burkholderia, Enterobacter, Pantoea* y *Pseudomonas* among more reported genera.

The evidence shows that similar to rhizobacteria, most of endophytic bacteria would produce IAA. In this way, 100% of isolates from *Persea Americana* (Barra et al., 2016), 96% of isolated from sugarcane Ibañez de Santi Ferrara *et al*., (2012), 93% of isolates from organic rice Phetcharat and Duangpaeng, (2012) and 86% of isolated from *Glycine max* (Hung et al., 2007) correspond to IAA-producing endophytic bacteria. The wide range of IAA production is a well-documented phenomenon. Barra et al., 2016; Jha et al., 2012; Thokchom et al., 2014 described IAA production ranging between 1.7–33.7 mg ml^{-1} 30–100 mg ml^{-1} and 0.5–12.0 mg ml^{-1} by endophytic bacteria isolated from *P. Americana, Salicornia brachiate* and *Citrus reticulate* and, respectively.

Accordingly, the main plant growth promoting effect triggered by IAA-producing endophytic bacteria is a change in root arquitecture which leads to enhanced water and mineral uptake by the host plant, allowing the plant to improve stress tolerance. In addition, IAA-producing endophytic bacteria are able to improve seed germination, whereby bacterial phytostimulation would be crucial in early developmental stages of plants (Bashan and de-Bashan, 2010; Bastian et al., 1998; Verma et al., 2001). Most studies on production and function of this phyotohormone have been performed in rhizobacteria; therefore further investigations in IAA-producing endophytic bacteria are required.

2.4.1.3. Cytokinin (CKs).

Cytokinins are a class of phytohormone that regulate principally cell division and differentiation in meristematic tissues of higher plants. Thus, CKs are defined as molecules that induce cytokinesis in the presence of auxins, and are classified according their biological activity(Kudo et al., 2010; Taiz and Zeiger, 2010). Naturally occurring CKs are N^6-substituted adenine derivatives that contain an isoprenoid or an aromatic derivative side chain (Bajguz and Piotrowska, 2009). The most prevalent CKs are those with an unsaturated isoprenoid side chain,

particularly those with a *trans*-hydroxylated N^6-side chain, *trans*-zeatin and its derivatives (Mok and Mok, 2001). Other CKs described with high biological activity are isopentenyl adenine (iP), kinetin (K) and benzylaminopurine (BAP) (Cassán et al., 2011). Depending on the chemical structure of their molecules, CKs exhibit diverse physiological activities (Tsavkelova et al., 2006). In addition to their action as inducers of cytokinesis, it has been shown that CKs has effects on many other physiological and developmental processes, including leaf senescence, nutrient mobilization, apical dominance, formation and activity of shoot apical meristems, floral development, root proliferation, reproductive competence, the breaking of bud dormancy, and seed germination (Kudo et al., 2010; Taiz and Zeiger, 2010). The CKs are produced in plant is meristematic regions including the roots, local and long-distance transport systems are involved in regulating CKs action (Dodd et al., 2010; Kudo et al., 2010).

Although the microbial production of CKs began with models of phytopathogenic microorganisms, nowadays, some researches are being carried out on PGPB (Cassán et al., 2011). The bacteria ability to synthesize CKs has been shown in some rizhobacteria species in culture media, such as *Azotobacter chroococcum, A. beijerinckii, A. vinelandii, Pseudomonas fluorescens* and *P. putida* (Nieto and Frankenberger, 1989); *Paenibacillus polymaxa* (Bent et al., 2001; Timmusk et al., 1999); *P. fluorescens, P. putida P. chlororaphis* and *Burkholderia cepacia* (García de Salamone et al., 2001), *Bacillus subtilis* (Arkhipova et al., 2007, 2005); *B. licheniformis, B. subtilis* and *Pseudomonas aeruginosa* (Hussain and Hasnain, 2009). However, little information is available on the CKs production by endophytic bacteria and their effects on plants (Table 2.1.). Thus, Sgroy et al., (2009) reported Zeatin production for four endophytic bacteria strains isolated from roots of the halophyte *Prosopis strombulifera* growing under extreme salt condition. These strains corresponded to *Bacillus subtilis B. pumilus, Brevibacterium halotolerans* and Pse*udomonas putida* producing 25.1, 1.36, 0.89 and 22.31 μg ml^{-1} Zeatin in chemically defined medium, respectively. On the other hand, Bhore et al., (2010)

by of cucumber cotyledon greening bioassay determined that *Pseudomonas resinovorans* and *Paenibacillus polymaxa* isolated from leaves of *Gynura procumbens* (Lour.) Merr. produced compounds that act CKs -like.

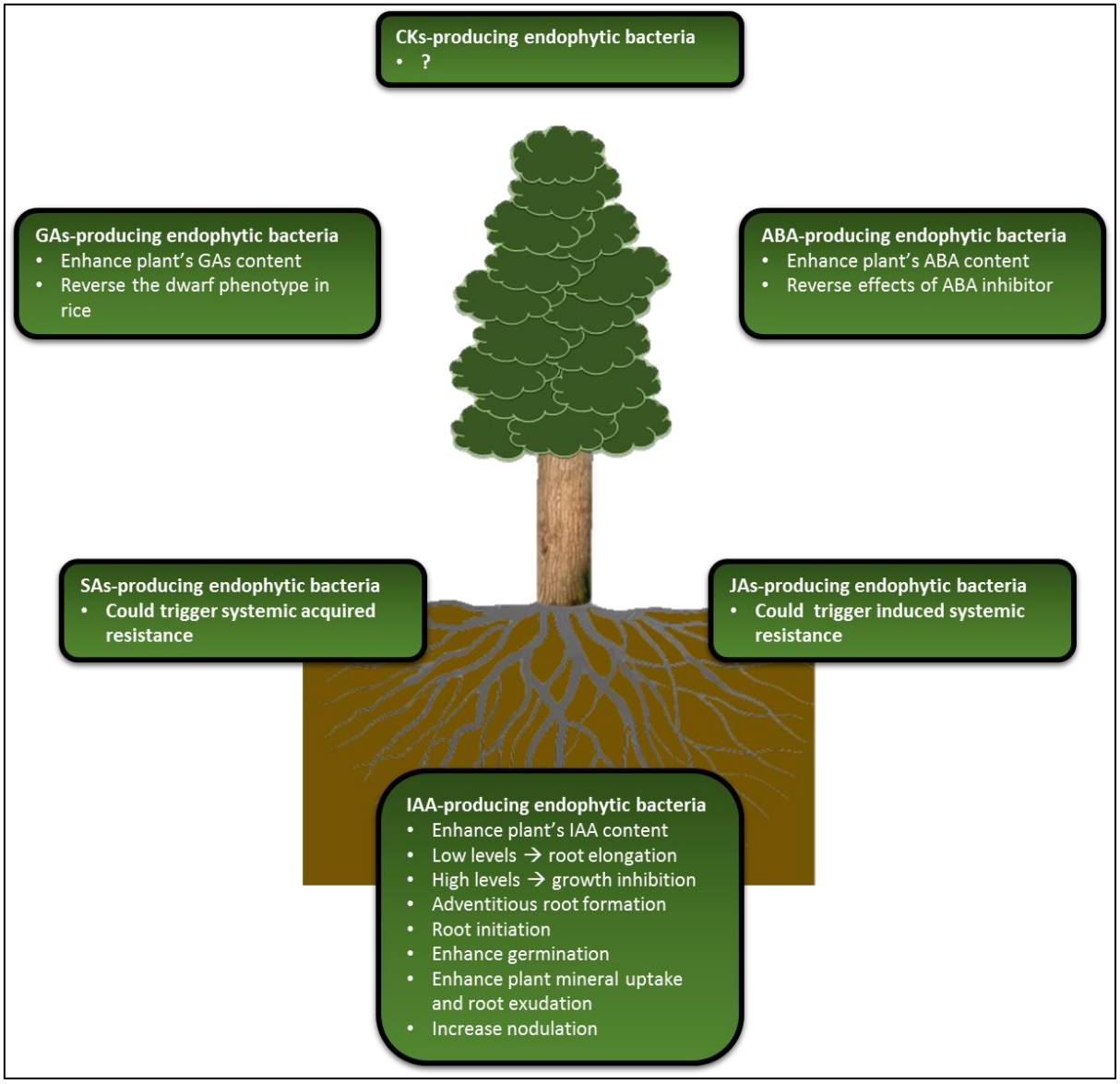

Figure 2.2. Effects of inoculation with phyotohormone-producing endophytic bacteria on plant physiology and development.

2.4.1.4. Gibberellins (GAs).

Gibberellins are a large group of related compounds, which chemically correspond to tetracyclic diterpenoid acids with structures based on the *ent*-gibberellane skeletal (shaped of 20 carbon units), but they are synthesized via *ent*-kaurene (Bömke and Tudzynski, 2009;

Piotrowska and Bajguz, 2011; Taiz and Zeiger, 2010). The gibberellins have the full complement of 20 carbons (C_{20}-GAs) or are composing for 19 carbons (C_{19}-GAs) (Taiz and Zeiger, 2010). All bioactive GAs are C_{19}-GAs, although not all C_{19}-GAs are bioactive (Bömke and Tudzynski, 2009; Huang et al., 1998). Gibberellins are involved in a wide number of developmental and physiological processes in plants, including seed germination, stem elongation, stem and leaf growth, pollen development, induction of flowering, flower and fruit growth and senescence (Bömke and Tudzynski, 2009; Bottini et al., 2004; Sponsel and Hedden, 2004; Yamaguchi, 2008).

The GAs were detected first in culture filtrates of *Fusarium moniliforme* (teleomorph *Gibberella fujikuroi*), pathogen fungus of rice plants, by Kurosawa (1926) (cited in Bottini et al., 2004). Whereas, the first GA (GA_1) of plants was discovered by MacMillan and Suter, (1958) in *Phaseolus coccineus* seeds. A growing number of fungal and plants GAs have been posteriorly identified, and numbered as gibberellin A_X (or GA_X), where X corresponded to a number, according to the order they were discovered (MacMillan, 2001; Taiz and Zeiger, 2010). Nowadays, over 130 GAs have been identified, with GA_1, GA_3, GA_4 and GA_7 as the most bioactive in higher plants (Piotrowska and Bajguz, 2011; Sponsel and Hedden, 2004).

Early studies demonstrated production of Gibberellins-like substances by some bacterial species such as *Bacillus japonicum* (Katznelson and Cole, 1965), *Azotobacter chroococcurn* (Brown and Burlingham, 1968), *Azotobacter paspali* (Barea and Brown, 1974) and *Azospirillum brasilense* (Tien et al., 1979), but the techniques used for their identification and quantification were of poor reliability. These "Gibberellins-like substances" is referring to compounds or extracts with GAs biological activity, but whose chemical structure has not been completely defined (Bottini et al., 2004; Taiz and Zeiger, 2010). The actual confirmation of GAs (GA_1 and GA_4) production was carried out in GC-MS culture media analyzes of *Rhizobium phaseoli* by Atzorn et al., (1988). Posteriorly, rhizobacterial GAs production has been identified

in *Azospirillum lipoferum* (GA_1 and GA_3) (Bottini et al., 1989), *Acetobacter diazotrophicus,* (GA_1 and GA_3) and *Herbaspirillum seropedicae* (GA_3) (Bastian et al., 1998) *A. brasilense* (GA_1 and GA_3) (Janzen et al., 1992) *B. licheniformis* (GA_1, GA_3, GA_4 and GA_{20}) and *Bacillus pumilus* (GA_1, GA_3, GA_4 and GA_{20}) (Gutierrez-Manero et al., 2001).

Exogenous GAs application on plants induce significant increases in plant height. In this way, GA_3 causes such extreme stem elongation in dwarf plants resembling the tallest varieties of the same species (Taiz and Zeiger, 2010). Precisely, GA_3 was the principal GA produced by *Azospirillum* spp (Bottini et al., 1989). Therefore, it has been expected that plants inoculation with GAs-producing bacteria could enhance their growth. Fulchieri et al., (1993) confirmed this hypothesis determining that *A. lipoferum*, a known endophytic bacteria, significantly increased roots gibberellin content along with improving root hair growth and density of maize seedling. Studies performed in genetic and chemically induced GAs deficient seedlings of maize and rice showed reversing dwarfism after being inoculated with endophytic bacteria *Azospirillum* spp (Lucangeli and Bottini 1996, 1997; Cassán et al., 2001). In addition, Cassán et al., (2001a) determined that *A. lipoferum* and *A. brasilense* are able to produce [17,17-$^2H_2$] GA_1, from [17,17-$^2H_2$] GA_{20}, in seedlings of rice dy mutant, showing *in vivo* the capacity to perform 3β-hydroxylation. It has have been demonstrated that *Azospirillum* spp. are also able to realize gibberellin-glucoside/glucosyl ester deconjugation *in vivo* (Piccoli et al., 1997; Cassán et al., 2001b). These results support the idea that plant growth response to *Azospirillum* spp. infection may occur by a combination of both GAs production and GAs deconjugation. (Piccoli et al., 1997). However, there is evidence that ABA and GAs have some antagonistic roles in the plant (Nemhauser et al., 2006), Cohen et al., (2009) showed in a study on maize plants treated with fluridone (F) and prohexadione-Ca (P) (inhibitors of ABA and GAs respectively), that ABA levels were enhanced and drought effect was neutralized when the plant was inoculated with *A. lipoferum*, suggesting that bacterial GAs are also important in stress alleviation.

On the other hand, Shi et al., (2009) isolated GAs-producing endophytic bacteria identified as *Bacillus pumilus*, *Chryseobacterium indologene* and *Acinetobacter johnsoni* from sugar beet roots, these strains produced up to 701.8 (after 24 h of incubation), 321.5 (after 144 h of incubation) and 1497.0 (after 144 h of incubation) μg mL^{-1}, respectively of chemically-defined medium supplemented with tryptophan. These strains significantly increased height, dry weights and leaf number of inoculated sugar beet compared with control plants. The GAs content of sugar beet also was significantly increased, although these decreased at the same control level after 20 days. Moreover, Chi et al., (2005) showed that endophytic rhizobia strains (*Sinorhizobium meliloti* 1021 and *Azorhizobium caulinodans* ORS571) tagged with green fluorescent protein *(gfp)* increased significantly root and shoot biomass, photosynthetic rate, stomatal conductance, transpiration velocity, water utilization efficiency, and leaf area, of inoculated rice, although bacteria ability to produce GAs was not tested the GA_3 and IAA levels of rice were significantly increased. Sgroy et al., (2009) isolated and characterized some GA_3-producing endophytic bacteria strains from halophyte *Prosopis strombulifera*, which corresponded to *Lysinibacillus fusiformis* (36.5 μgml^{-1}), *Bacillus subtilis* (21.3 μgml^{-1}), *B. pumilus* (3.85 μgml^{-1}), *B. licheniformis* (75.5 μgml^{-1}), *Achromobacter xylosoxidans* (50 μgml^{-1}), *Brevibacterium halotolerans* (90.0 μgml^{-1}). On the other hand, Piccoli et al., (2010) detected GA_1 and GA_3 by diazotrophic endophyte *Arthrobacter koreensis* isolated from roots of halophyte *Prosopis strombulifera*.

To conclude endophytic bacteria effects on growth and yield of many crop plants could be explained, at least in part, by: (1) GAs production by endophytic bacteria, (2) deconjugation of GAs- glucosyl conjugates exuded by the plant and (3) 3β-hydroxylation by bacterial enzymes of inactive 3-deoxy gibberellins present in plant to activate forms such as GA_1, GA_3 and GA_4. (Bottini et al., 2004). Although it has been shown that some endophytic strains have the ability to produce GAs *in vivo* and *in vitro* and these bacteria are able of promoting plant growth, there is insufficient evidence for involvement of bacterial GAs in promoting growth.

2.4.1.5. Salicylic acid (SAs).

Salicylic acid (SA) or ortho-hydroxybenzoic acid belongs to a varied group of phenolic compounds widely distributed among plant species. The SAs are presents in plants as free phenolic acids and as conjugate forms, which may be generated by glucosylation, methylation or hydroxylation of the aromatic ring (Bandurska, 2013). It has been found that SA have important roles during the plant response to abiotic stresses such as drought, chilling, heavy metal toxicity, heat, and osmotic stress (Rivas-San Vicente and Plasencia, 2011). The SAs are also involved in other plant physiological process such an increase nodulation, enhance pigment content, flowering, among others (Hayat and Ahmad, 2013). However, it is well known that SA is a natural endogenous signal mediating involved in plant defense response against pathogen infection (Kawano et al., 2013; Rivas-San Vicente and Plasencia, 2011). In this way, the most important documented function of endogenous SA is mediation in systemic acquired resistance (SAR) of plants (Hardoim et al., 2012; Ton et al., 2002). Thus, SAR is nonspecific defense mechanisms to protect plants against bacterial, viral and fungal pathogenic, which is induced by (local) exposure to pathogens. Once induced, SAR is active against a broad range of pathogens (Hardoim et al., 2012). Exogenous application of SA to plants lead to protection against a range of plant pathogens by SAR. Thus, it is possible to assume that the SA-producing PGPB could also elicit SAR through the production of SA. However, the evidence with rhizobacteria is not enough to definitively determine this assertion (Bakker et al., 2014).

Only a few studies have shown the ability of endophytic strains to produce SAs. In this way, the endophytic bacteria *Achromobacter* sp.SF2, *Bacillus* sp SF3 and *Bacillus* sp. SF4 isolated from *Helianthus annuus* produced 16, 238 and 270 pmol ml^{-1} of SAs *in vitro* (Forchetti et al., 2010). These strains inhibited growth of pathogenic fungi *Sclerotinia* sp. *Alternaria* sp. and *Verticillum* sp. Whereas, Trivedi et al., (2011) isolated thirty five strains from root of Valencia orange (*Citrus sinensis*) tree that produced between 2.12 and 8.33 µg ml^{-1} of SA. Nevertheless,

none of these studies showed effects of SA-producing bacteria in plants. The effects of SA-producing endophytic bacteria on plant response remains largely unexplored.

2.4.1.6. Jasmonic acid (JA).

Jasmonic acid and derivatives, collectively called jasmonates (JAs) are cyclopentanone derivatives biosynthesized from linolenic acid by the octadecanoid pathway (Delker et al., 2006; Pozo et al., 2005). The JAs are inducers of a variety of physiological processes such as seed germination, pollen development, ethylene synthesis, senescence and tuber formation (Piotrowska and Bajguz, 2011). Jasmonic acid is regarded as a phytohormone responsible for the activation of a signal transduction pathway in response to different kinds of biotic and abiotic stress (Piotrowska and Bajguz, 2011; Pozo et al., 2005). Thereby, JA increase (as well as ethylene) production is an early symptom of active defense in plants (Pieterse et al., 2000). As mentioned above, beneficial bacteria can induce an enhanced defensive ability in plant providing protection against a broad spectrum of pathogen microorganisms and even herbivore insects. Therefore, JAs together with ethylene are important regulators of the so-called induced systemic resistance (ISR) (Pozo et al., 2005; Van der Ent et al., 2009). Thus, ISR is an enhanced defensive capacity developed by a plant, phenotypically similar to SAR, which correspond to activation of latent resistance mechanisms that are expressed upon subsequent, so-called "challenge" inoculation with a pathogen (Maksimov et al., 2015; van Loon et al., 1998). Miché et al., (2006) showed that addition of JAs in rice decreased the physiologically successful colonization by the diazotrophic *Azoarcus* sp., suggesting that plant defense responses might also regulate endophytic entrance of the plant. In this way, *Arabidopsis thaliana* plants deficient in JA-mediated defenses experienced greater epiphytic bacterial diversity. Furthermore, there was a positive relationship between total community size and diversity, indicating that relatively susceptible plants should, in general, harbor higher bacterial diversity (Kniskern et

al., 2007). In addition, Gond et al., (2015) determined that pre-treatment of Indian corn plants with endophytic *Bacillus amyloliquefaciens* subsp. Subtilis trigger up-regulation of defense JA-induced gene of plants against *Fusarium moniliformeanalysis.*

The JA-producing bacteria ability has not been extensively investigated. In this way, Forchetti et al., (2007) identified and described for the first time bacterial production of JA in endophytic strains isolated from *Helianthus annuus* L. These strains were identified as *Achromobacter* sp. and two *Bacillus* spp., which produced approximately 3-5 pmol ml^{-1} in culture media at very low water potential. Whereas, Piccoli et al., (2010) reported JA production by endophytic *Arthrobacter koreensis* isolated from *Prosopis strombulifera*. The available literature about the JAs production of endophytic bacteria, as well as, their role in plant defense response against pathogens and endophytes microorganism is virtually zero. Significant studies are needed in this area in order to determine the actual exogenous JAs effects.

2.4.2. Modulation of plant ethylene levels.

Ethylene is a gaseous phytohormone involved in multiple plant physiological roles, which could promote or inhibit growth depending on the cell type and plant species (Dodd et al., 2010). The ethylene involves a variety of processes including seed germination, tissue differentiation, primordial shoot and root formation, root elongation, rooting of cuttings, lateral bud development, flowering initiation, anthocyanin synthesis, flower opening and senescence, pollination, fruit ripening and degreening, the production of volatile organic compounds in fruits, storage product hydrolysis, leaf and fruit abscission, microbe–plant interactions and stress response (Gray and Smith, 2005; Hardoim et al., 2008; Lin et al., 2009; Piotrowska and Bajguz, 2011).

Plant ethylene biosynthesis occurs through a relatively simple and well documented metabolic pathway (Argueso et al., 2007; Kende, 1993; Yang and Hoffman, 1984). Ethylene is derived from the amino acid methionine, which is metabolized to S-adenosylmethionine (AdoMet) by S-adenosylmethionine synthetase. Posteriorly, AdoMet is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) and 5'-deoxy-5'methylthioadenosine (MTA) by the enzyme 1-aminocyclopropane-1-carboxylase synthase (ACCS). Finally, the enzyme ACC oxidase (ACO) catabolizes the conversion of ACC to ethylene, CO_2, and cyanide (Argueso et al., 2007; Piotrowska and Bajguz, 2011).

Ethylene plays a key role in environment responses having a direct bearing on a plant's fitness for adaptation and reproduction. Ethylene production is regulated by a wide range of environmental factors and also by other phytohormones. (Lin et al., 2009). Ethylene induces response to different stresses, which help enhancing plant survival under adverse conditions (Glick, 2005; Stearns, 2003). The increased ethylene level produced as response to trauma inflicted by stress (as chemicals, temperature, drought, flooding, ultraviolet light, insect damage, salt, disease and mechanical wounding) triggers some of classical stress symptoms of plants and, in many instances, producing deleterious effects. The term ''stress ethylene'' was coined to describe the acceleration of ethylene biosynthesis associated with stresses (Glick, 2005, 2004; Glick et al., 2007a, 1998; Penrose and Glick, 2003; Stearns, 2003). Distinct plants respond differently to stress, thereby having a range of ethylene sensitivity (Glick et al., 2007a). In this way, Stearns, (2003) proposed that ethylene is produced in two peaks, in response to stresses. Thus, the first peak, smaller and closer in time to onset of stress, would trigger the plant protective response. Whereas, the second ethylene peak is often concomitant with the appearance of visible plant damage, such as senescence, chlorosis and leaf abscission (Glick et al., 2007a; Stearns, 2003). The second peak of ethylene occurs as a consequence of increased transcription of ACC synthase genes triggered by environmental and developmental signals

(Glick et al., 2007a). Some bacteria and fungi also produce ethylene, although its role in these organisms is less well understood (Lin et al., 2009).

Many PGPB contain the 1-aminocyclopropane-1-carboxylate deaminase (ACCD) enzyme, which is encoding for *acdS* gene. This enzyme cleaves the ethylene precursor ACC to α-ketobutyrate and ammonium (Blaha et al., 2006; Glick et al., 1998; Hontzeas et al., 2006). The ACCD is a sulfhydryl multimeric enzyme with a monomeric subunit molecular mass of 35–42 kDa that use pyridoxal 5-phosphate as an essential cofactor (Glick, 2005). The ACCD-producing bacteria act as sink to ACC, using the ACC released by plants as C and N source, consequently, avoiding ethylene levels rise above the point affecting plant growth (J. S. Singh et al., 2011). According to an model suggested by Glick et al., (1998), the bacterial ACCD production is strongly related with bacterial IAA production. In this way, the PGPB is firstly binding to plant surface (usually seeds or roots) in response to plants tryptophan exudates, which is used to synthesis and secretion of bacterial IAA. This IAA in conjunction with the endogenous plant IAA can either stimulate plant cell proliferation and/or elongation (see 2.4.1.2. Auxins). Alternatively, IAA can stimulate the enzyme ACCS producing more ACC (Glick et al., 2007a). A significant portion of the ACC may be exuded from plant roots or seeds, taken up by the bacterium and subsequently cleaved by the bacterial enzyme ACCD (Glick et al., 1998). The ACCD is not known currently to be excreted from the bacterial cytoplasm (Hardoim et al., 2008). Therefore, endophytic bacteria with locally high ACCD activities could be excellent plant-growth promoters under stress events, because they might act *in situ* inside the plant efficiently blocking ethylene production. This mechanism has not been completely elucidated in endophytic bacteria (Hardoim et al., 2008).,

Some studies have shown that ACCD-producing endophytic bacteria can improve growth of a wide range of plants under biotic and abiotic stresses, such as heavy metals (Zhang et al., 2011), pathogens (Sturz et al., 1999), salt (Barra et al., 2016; Karthikeyan et al., 2012) and drought

(Naveed et al., 2013). In this way, Sgroy et al., (2009) determined the presence of some endophytic bacteria strains with ACCD activity associated with *Prosopis strombulifera* (halophyte plant). These strains correspond to: *Bacillus subtilis, B. halotolerans, B. licheniformis*, *B. pumilus*, *Achromobacter xylosoxidans,* and *Pseudomonas putida*. Onofre-Lemus et al.,(2009) determined that endophytic bacteria *Burkholderia unamae, B. silvatlantica* and *B. kururiensis* produced ACCD. These researches have shown that shoot and root dry weights of tomato plants inoculated with *Burkholderia unamae* strain were significantly higher than those plants inoculated with the MTl-641^{T} ACCD-negative mutant strains and non-inoculated plants. In addition, the chlorophyll contents of plants inoculated with both strains were statistically increased compared with those of non-inoculated plants. Moreover, no statistical differences were found among control plants without stress and plants grown either in the presence of NaCl or with water saturation treatments (Onofre-Lemus et al., 2009). Similar results were showed by Sun et al., (2009) who contrasted and tested a mutant of *Burkholderia phytofirmans* PsJN deficient in ACC deaminase activity. This mutant had no detectable ACCD activity, lost its ability to promote canola root elongation, synthesized a decreased level of siderophores and produced an increased amount of IAA. On the other hand, Wang et al., (2000) isolated two ACCD-producing bacteria identified as *Pseudomonas fluorescens* biovar. IV and *Erwinia herbicola*, both strains showed positive plant growth-promoting activity when inoculated into cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum)* and rapeseed (*Brassica napus*). Similarly, Taghavi et al., (2009) isolated two strains of ACCD-producing endophytic bacteria from poplar trees (*Populus* spp.) identified as *Burkholderia cepacia* and *B. vietnamiensis*, showing *B. cepacia* promissory plant growth-promoting effect on poplar trees. On the other hand, Karthikeyan et al., (2012) showed that ACCD-producing *Achromobacter xylosoxidans* AUM54 inoculated on *Catharanthus roseus* growing at 150 mM NaCl, reduced the plant´s ethylene level. This bacterium also increased germination percentage, vigor index, plant height and root dry weight of *Catharanthus roseus*

growing at different NaCl levels (0, 50, 100 and 150 mM NaCl). Inoculation of *Vitis vinifera* with *Burkholderia phytofirmans* Strain PsJN increased gravepine growth and physiological activity at a low temperature (Ait Barka et al., 2006). Although plant inoculation with ACCD-producing endophytic bacteria has shown promising results in PGP and stress alleviation significant field studies are needed to evaluate the potential use of these bacteria in sustainable agriculture.

2.5. Conclusions and perspectives.

Endophytic bacteria have shown interesting biotechnological applications and nowadays are studied as a potential source of novel natural products, in phytoremediation and as biocontrol agents. Nevertheless, most studies are focused on their PGPB mechanisms. Multiples endophytic strains have shown PGP activity, which has increased interest in them due to their potential uses in sustainable agriculture. Nowadays, analyzing the available literature on phytostimulation appears as the main effect triggered by endophytic bacteria. Bacterial production of phytohormones and enzyme ACCD are main properties in phytostimulation. Many plant-associated bacteria are able by themselves of synthesizing phytohormones (Table 2.1.), which would be necessary as mediators in communications between plant host and its microflora (Hardoim et al., 2008; Tsavkelova et al., 2006). With endophytic *Bacillus pumilus* and *Pantoea agglomerans* showing the ability to produce the main phytohormones described in this review (ABA, IAA, CKs, GAs) together with ACCD activity (see Table 2.1. and Appendix 2.1.). The IAA is the most studied and described phytohormone produced by bacteria. Although effects on plant of IAA-producing bacteria have shown, their real impacts have been difficult to assess and the results are sometimes contradictory. Creating bacterial mutants with altered IAA production and using auxin-resistant plant mutants have confirmed the importance of IAA in selected plant–bacteria interactions, however, more research is needed in this

direction (Dobbelaere et al., 1999; Dodd et al., 2010; Lee et al., 2004). Other phytohormones, such as ABA, CKs, GAs, JAs and SA are also produced by endophytic bacteria (Figure 2.2., Table 2.1.), but their production has been mostly shown *in vitro* (in culture media) and very few studies have shown synthesis in their natural habitats and real contribution on plant growth (Baca and Elmerich, 2007; Bashan and de-Bashan, 2010; Bastian et al., 1998; Cohen et al., 2009; Karadeniz et al., 2006; Piccoli et al., 2010). Although considerable progress has been carried out in this area, there is still insufficient evidence for involving other bacterial phytohormones in promoting growth. Researches on plant and bacterial mutants are needed, but it is necessary to know the metabolic routes of some bacterial phytohormones before. Most studies have been focused on a particular bacterial phytohormone, but considering that many bacteria have one or more phytostimulator mechanism it is difficult to attribute PGP effects to a specific bacterial phytohormone (Hussain and Hasnain, 2011; Long et al., 2008; Ping and Boland, 2004). The "Multiple Mechanism Theory" has emerged based on the assumption that there is not a single mechanism involved in PGP (Bashan and de-Bashan, 2010). To get a clearer role of bacterial phytohormones in PGP, it is necessary to demonstrate simultaneous impact of two or more phytohormones in such phenomena.

Plant inoculations with ACCD-producing bacteria have showed promising results in plant growth and specifically in stress alleviation of such plants (such as drought, salinity and heavy metals) (Belimov et al., 2009; Burd et al., 1998; Ma et al., 2009; Mayak et al., 2004a, 2004b; Zahir et al., 2008). However, most researches have been carried out in rhizobacteria and only a few studies have been performed in endophytic bacteria (Onofre-Lemus et al., 2009; Sgroy et al., 2009; Taghavi et al., 2009; Weyens et al., 2011). Nowadays, it have been postulated that ACC-producing bacteria would be selected naturally by the plant, which would facilitate their plant inoculation (Hardoim et al., 2008; Reinhold-Hurek and Hurek, 2011). Due to the promising results of ACCD-producing bacteria, significant efforts have been made to introduce ACCD genes into plants (which has been quite successful) to regulate their ethylene level

particularly under stressed conditions (Saleem et al., 2007). However, genetic modification of all plant species is not possible due to many handicaps, which ACCD-producing endophytic bacteria could prove to be a cost effective and environment friendly strategy to ensure sustainable agriculture. In this way, further field studies are needed to determine endophytic bacteria effect on crop productivity, therefore apply these bacteria on a commercial scale.

Accordingly, endophytic bacteria contribute to plant growth and plant stress resistance, which would allow using these bacteria as crop inoculum in order to increase their productivity. Endophytic bacteria would have a comparative advantage over PGPR because of their ability to colonize plant tissues internally where they would have less competition and a more favorable environment. Knowledge about endophytic bacteria ecology is very limited and significant studies are needed in this area. The successful use of endophytic bacteria in crop production will depend on our ability to maintain, manipulate, and modify beneficial populations under field conditions. Identification and evaluation of all growth-promoting components in endophytic bacteria strains is important to improve their efficiency as crop inoculum. Therefore, endophytic bacteria will become an interesting tool in sustainable agriculture but substantial efforts are still needed.

CHAPTER III

"Formulation of bacterial consortia from avocado (*Persea americana* Mill.) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress"

Appllied Soil Ecology 102, 80–91 (2016)

Formulation of bacterial consortia from avocado (*Persea americana* Mill.) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress.

Abstract

Inoculation of plants with bacteria that produce indole acetic acid (IAA) and 1-aminocyclopropane-1-carboxylate deaminase (ACCD) often has a positive effect on alleviation of salt stress in plants. Here, we isolated, characterized and formulated halotolerant bacterial consortia from avocado trees with the aim of developing biofertilizers to improve avocado production on saline soils. Using wheat as a test plant, experiments were conducted to investigate the effects of selected bacterial consortia on growth, biomass and superoxide dismutase (SOD) activity of wheat seedlings exposed to salt stress (0.25 M and 0.45 M NaCl) under greenhouse conditions. Among 309 isolates, 17.4% were characterized as halotolerant IAA- and ACCD-producing bacteria. Based on differences in their IAA production and ACCD activities, four consortia were formulated using members of five genera: *Enterobacter*, *Serratia*, *Microbacterium*, *Pseudomonas* and *Achromobacter*. Inoculation with selected halotolerant bacterial consortia significantly ($P \geq 0.05$) increased the emergence, growth, biomass and SOD activity of wheat seedlings exposed to salt stress. Avocado trees and their rhizosphere soils harbor halotolerant IAA- and ACCD-producing bacteria with the potential to mitigate the salt stress effects on plants. While wheat was useful for screening, further studies are necessary to validate the effects of selected bacterial consortia on avocado growth and yields under saline conditions.

Keywords. Avocado; bacterial consortia; endophytic bacteria; rhizosphere bacteria; salt stress.

3.1. Introduction.

Soil salinity is a serious problem that affects plant growth, crop yield and productivity on over 800 million hectares of land around the world (6% of total world land area) (Munns and Tester, 2008). The main causes of soil salinization are improper irrigation/drainage practices when using saline water supplies. Physiologically, salt stress impairs seed germination and plant development by osmotic stress and ion toxicity (Munns and Tester, 2008). Plants vary widely in their tolerance/sensitivity to salt stress, with avocado (*Persea americana* Mill.) being the most salt-sensitive of cultivated fruit tree species (Oster et al., 2007). Prior research has shown that rhizosphere soils of avocado trees harbor a variety of plant growth promoting bacteria that may increase plant tolerance to salt stress (Nadeem et al., 2012), but their efficacy for improving plant salt tolerance has not yet been examined.

Chile is one of main producers of avocado worldwide with sales of over US$ 160 million in 2014, and avocado production is thus of great economic importance for Chilean agriculture. In this context, the global demand for avocados has significantly increased during the last decade, resulting in an increase of avocado orchards in central Chile, from 23,800 h in 2003 to 36,355 h in 2013. However, during recent years, Chilean avocado production has decreased from 263,476 t in 2009 to 164,720 t in 2013 (Muñoz, 2015) mainly due to adverse environmental factors, particularly frost and drought events that have affected central Chile. Based on global warming estimations, the occurrence of drought in central Chile could become increasingly severe with long term climate projections predicting a decrease of 20-25% in rainfall by 2040 (Neuenschwander, 2010). To solve the water limitation, orchards in Chile increasingly rely on irrigation, which is increasing soil salinization. It is expected that in some areas avocado production will no longer be viable unless stress tolerance can be increased. Therefore, it is important to find strategies to ameliorate the salt stress effects on avocado trees in order to maintain or increase fruit production under new climate scenarios.

A substantial number of the bacteria are mutualistic with their plant hosts and may exert beneficial effects on plant growth, stress tolerance, and disease suppression (Drogue et al., 2012; Pii et al., 2015). Therefore, an attractive and environmentally friendly strategy to mitigate stress effects on crops is the use of plant growth-promoting bacteria (PGPB) soil inoculants. Most PGPB (both endophytic and rhizosphere bacteria) that have been tested produce the phytohormone indole acetic acid (IAA), which can increase seed germination rates and root growth (Patten and Glick, 2002). Some PGPB strains are also able to produce the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD), which catalyzes the hydrolysis of the immediate precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC) to ammonia and α-ketobutyrate (αKB). Therefore, ACCD-producing bacteria can prevent the increase of stress-ethylene that normally would inhibit root growth under stressful conditions (Penrose and Glick, 2003). Multiple studies have showed the positive effects of IAA- and ACCD-producing PGPB on alleviation of salt stress in some crop plants, such as cereals (wheat and rice), pasture (ryegrass), and medicinal plants (*Limonium sinense*), among others (Bal et al., 2012; Chakraborty et al., 2013).

One of the difficulties in isolating individual strains or consortia that may serve as biofertilizers for avocado is the long growth period that is required to evaluate tree responses to inoculation in field studies. Here, we hypothesized that halotolerant bacterial consortia isolated from avocado trees are also able to ameliorate salinity stress in other plant species such as wheat, and thereby provide a useful method to prescreen for efficient PGPB formulations that can then be tested in field trials with mature trees. To test our hypothesis, we isolated, characterized and formulated consortia of halotolerant IAA- and ACCD- producing bacteria from the endosphere (leaf and root tissues) and rhizosphere (rhizoplane and soil adhering to roots) from avocado trees, and investigated their potential to be used as inoculants to mitigate salt stress on plants through inoculation assays with wheat seedlings (*Triticum aestivum* L.) as test plants.

3.2. Materials and Methods.

3.2.1. Sample Collection.

Samples of leaves, roots and rhizosphere soils were collected from avocado trees located at a commercial orchard 'Jorge Schmidt & Co. Ltd.' in Valparaíso Region, Chile (32°47'S and 70°47'W). Three healthy avocados trees were selected in three different locations of the orchard (U1S3, U3S3 and U14S4). Each location contained 5-6-year-old Hass avocado trees that had been grafted on Mexícola rootstocks. The trees were planted in rows at 3 m intervals between trees and 3 m spacing between rows. Trees were irrigated to field capacity when soil moisture fell below -50 cbars as measured using tensiometers. Irrigation used well water having an electric conductivity (EC) of 0.654 dS m^{-1}, and was applied using 12 gal h^{-1} mini-sprinkler emitters. Weed management was limited to occasional mowing between rows. Within rows, avocado self mulches and the dense tree canopy precluded weed growth in the areas that were sampled. Soil on this site was characterized as a sandy loam. The chemical fertilizers applied in avocado orchards were 680 kg N ha^{-1} (applied in three split doses as urea [46%] or ammonium nitrate [22%]), 300~380 kg Zn ha^{-1} (applied as zinc sulfate) and 30 kg Bo ha^{-1} (applied as boric acid). When weed control is required the post-emergent herbicide terbuthylazine (50%) was applied at manufacturer recommended dose (1.75~2.25 kg ha^{-1}). The samples were collected in May, when the avocado trees were in middle of the spring leaf flush period. For isolation of endophytic bacteria, branches and roots samples were collected from plants at each site and the samples were placed into sterile bags. For isolation of rhizobacteria, soil aggregates adhering to the roots were collected. Root and soil samples were collected using a clean spade to excavate intact roots from soil to a depth of 0-20 cm, after which sub samples were placed into sterile plastic tubes. All samples were collected in triplicate and stored at 4°C and immediately transported to the laboratory for soil and microbiological analyses.

Table 3.1. Selected chemical properties of rhizosphere soil samples.

	Sampling Area		
Properties	**U1S3**	**U3S3**	**U14S4**
N (mg kg^{-1})	13	7	14
P (mg kg^{-1})	27	35	54
K (mg kg^{-1})	246	235	438
pH (H_2O)	6.55	6.92	5.95
Organic matter	2.18	1.38	2.64
K ($cmol^+ kg^{-1}$)	0.63	0.6	1.12
Na ($cmol^+ kg^{-1}$)	0.19	0.14	0.25
Ca ($cmol^+ kg^{-1}$)	6.45	5.08	9.28
Mg ($cmol^+ kg^{-1}$)	1.08	0.94	1.44
Al ($cmol^+ kg^{-1}$)	0.02	0.02	0.02
CEC*($cmol^+ kg^{-1}$)	8.37	6.78	12.11
Al saturation (%)	0.24	0.29	0.17
Electrical conductivity (dS m^{-1})	0.69	0.81	0.99

*Calculated as (Al×100)/CEC, where CEC=cation exchange capacity = Σ (K, Ca, Mg, Na and Al).

3.2.2. Isolation of endophytic and rhizosphere culturable bacteria.

Leaf and root samples were repeatedly washed with tap water and immediately surface disinfected with 70% ethanol for 3 min followed by 2.5% sodium hypochlorite (NaClO) solution for 5 min. After disinfection, the tissues were thoroughly washed with sterile distilled water (SDW), after which portions of the wash samples were spread on to Luria Bertani (LB) agar plates (10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, 5 g l^{-1} NaCl and 15 g l^{-1} agar) to check for bacterial contamination by non-endophytic bacteria. Then, surface disinfected samples were ground with a mortar and pestle and 1.5 g of leaf and root samples were transferred to sterile 15 ml Falcon tubes containing 5 and 3 ml of sterile saline solution (SSS; 0.85% NaCl), respectively. This suspension was designated as ‘tissue suspension’ and was used for quantification and isolation of culturable endophytic bacteria.

To isolate rhizobacteria for further screening, 2 g of each rhizosphere sample was suspended in 50 ml of SSS and subjected to sonication at 150 watts for 30 s to detach bacterial cells from the soil particles. This suspension was named ‘rhizosphere suspension’ and was used for counting

and isolation of culturable rhizobacteria. The chemical properties of the rhizosphere soil samples are shown in Table 3.1. General properties of the soils included a slightly acidic to near-neutral pH (pH 5.95-6.92) with low organic matter content (1.38-2.64%) and low Al saturation (0.17-0.19%). The N, P and K contents ranged 7-14 mg kg^{-1}, 27-54 mg kg^{-1} and 235-438 mg kg^{-1}, respectively. With respect to the electrical conductivity (E.C.), the E.C. of rhizosphere soil samples were relatively high (0.69-0.99 dS m^{-1}; respectively), which is above the threshold reported to affect avocado yields (<0.57 dS m^{-1}) (Oster et al., 2007).

Serial dilutions (up to 10^{-8}) in SSS from both tissue and rhizosphere suspensions were plated onto LB (10 g l^{-1} D-glucose, 5 g l^{-1} yeast extract, 10 g l^{-1} tryptone, 15 g l^{-1} agar) and NM-1 agar (0.5 g l^{-1} D-glucose, 0.5 g l^{-1} polypeptone, 0.5 g l^{-1} Na-glutamate, 0.5 g l^{-1} yeast extract, 0.44 g l^{-1} KH_2PO_4, 0.1 g l^{-1} $(NH_4)_2SO_4$, 0.1 g l^{-1} $MgSO_4 \cdot 7H_2O$, 15 g l^{-1} agar; (Nakamura et al., 1995). Both media were supplemented with 100 µg l^{-1} of cycloheximide to prevent fungal growth (Calbiochem, San Diego, USA). The plates were incubated at 30°C for one week, after which the colonies were counted. Single colonies showing different phenotypes (color, brightness, form, elevation and margin) were randomly selected and transferred to fresh media, purified by streaking on agar and stored at -20°C (30% glycerol).

3.2.3. Putative ACCD-producing bacteria.

Culturable endophytic and rhizosphere bacteria were tested for their ability to grow in culture medium with ACC (Calbiochem®) as a sole N source according to the procedures described by Penrose and Glick (2003). Briefly, fresh cultures of each bacterial strain were added to 5 mL DF medium (4 g l^{-1} KH_2PO_4, 6 g l^{-1} Na_2HPO_4, 0.2 g l^{-1} $MgSO_4 \cdot 7H_2O$, 2 g l^{-1} gluconic acid, 2 g l^{-1} citric acid, and 1 ml trace elements containing 0.001 g l^{-1} $FeSO_4 \cdot 7H_2O$, 0.01 g l^{-1} H_3BO_3, 0.011 g l^{-1} $MnSO_4 \cdot H_2O$, 0.125 g l^{-1} $ZnSO_4 \cdot 7H_2O$, 0.078 g l^{-1} $CuSO_4 \cdot 5H_2O$, 0.01 g l^{-1} MoO_3; with $(NH_4)_2SO_4$ (2 g l^{-1}) as sole N source) and incubated at 30 °C for 2 days with shaking (180

rpm). After 2 days, 0.1 ml aliquots from each culture were removed, washed and transferred to test tubes with 5 ml DF medium containing ACC (3 mM) as a sole N source and incubated at 30 °C for 10 days with shaking. Bacterial growth was monitored daily and those that grew in ACC-supplemented DF medium were considered as putative ACCD-producing bacteria and used for further analysis.

3.2.4. IAA-producing bacteria.

Putative ACCD-producing bacteria were screened for IAA production according to procedure described by Patten and Glick, (2002) with minor modifications. Briefly, 50 µL of bacterial inoculum (adjusted to an optical density of 0.8 at 600 nm) were dispensed to 5 ml of fresh LB broth supplemented with 5 mM L-tryptophan and incubated at 30 °C for 48 h on an orbital shaker. Then, 50 µl aliquot of the supernatant were mixed with 200 µl of Salkowski's reagent (150 ml of 95-98% H_2SO_4, 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$ and 250 ml of SDW) and incubated at room temperature for 30 min. Color development was monitored at 535 nm using Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific Inc.). The IAA concentration in supernatant was determined by comparison with a standard curve prepared with known concentrations of pure IAA (Sigma-Aldrich, Co.).

3.2.5. Halotolerant bacteria.

The salt tolerances of selected putative ACCD-producing bacteria were tested. Serial dilutions (up to 10^{-10}) were prepared from fresh bacterial cultures in LB broth and plated onto LB agar plates at NaCl concentrations of 0.5 (0.86 M), 2.5% (0.43 M), 5.0% (0.86 M), 7.5% (1.29 M) and 10.0% (1.72 M) as suggested by Nadeem et al. (2012). The agar plates were incubated at 30°C for 4 days and single colonies grown on agar plates were re-inoculated fresh agar plates

with the same NaCl concentrations. Those isolates that were able to grow in LB agar supplemented with ≥ 5.0% NaCl were considered to be halotolerant, putative ACCD-producing bacteria.

3.2.6. ACCD activity.

The ACCD activities in selected bacteria showing positive results for three traits assayed were confirmed following the procedures described by Penrose and Glick (2003), which measures the amount of alpha-ketobutyrate (αKB) generated by the hydrolysis of ACC. The amount (µmole) of αKB produced was determined by comparison with a standard curve prepared with known concentrations of pure αKB (Sigma-Aldrich, Co.). Those isolates that showing ACCD activity were used for formulation of bacterial consortia.

3.2.7. Formulation and preparation of bacterial consortia.

Twelve halotolerant IAA- and ACCD-producing bacteria were selected to formulate four consortia based on three criteria: isolation source (endosphere or rhizosphere), production of IAA and activity of ACCD. The four formulated consortia (three strain each) were grouped, as follows: i) C1: endophytic bacteria with higher IAA production and ACCD activity, ii) C2: endophytic bacteria with lower IAA production and ACCD activity, iii) C3: rhizosphere bacteria with higher IAA production and ACCD activity, and iv) C4: rhizosphere bacteria with lower IAA production and ACCD activity (see Table 3.2.).

In parallel, members of each consortium were identified based on partial sequencing of 16S rRNA genes. Chromosomal DNA was extracted from overnight cultures using a Gentra Puregene Yeast/Bact. Kit (Qiagen, Inc.) according to the manufacturer instructions. The 16S rRNA gene fragments were amplified by PCR using the universal primers set 27f (AGA GTT

TGA TCC TGG CTC AG) and 1492r (TAC GGY TAC CTT GTT ACG ACT T). The PCR amplification was conducted as follows: a hotstart at 94°C for 5 min, then 35 cycles at 94 °C for 1 min, at 52 °C for 1 min, and final extension 72 °C for 2 min. The PCR products were sequenced in both directions by Macrogen, Inc. (Seoul, Korea). The consensus nucleotide sequences were compared with the GenBank database from the National Center for Biotechnology Information (NCBI) using BLAST tools (http://www.ncbi.nlm.nih.gov/BLAST). The nucleotide sequences of the 16S rRNA gene segments were deposited in GenBank database under accession numbers KR066642 - KR066653.

Soil inoculants employing bacterial consortia were prepared from overnight cultures in LB broth. The exponential phase cells were centrifuged at 6,000 × *g* for 15 min (4°C), washed repeatedly with SSS and suspended in 10% skim milk. Samples were frozen overnight at -80°C and later dried for 24 h in FreeZone Freeze Dry Systems (Labconco) according to Schwab et al. (2007). We used skim milk because their cryoprotective effects are widely described. Lactose/sugars in milk act as dehydrating agent reducing the amount of intracellular water. The colloidal structure also protects microorganisms. Milk also exerts its protective effect by raising the glass transition temperature of the samples (Jagannath et al., 2010). To estimate the bacterial survival after the freeze-drying process, the lyophilized samples were suspended in SDW, and serial aliquots (up to 10^{-20}) were plated onto LB agar. Plates were incubated at 30°C for 4 days and the colonies were counted and compared with counts of equivalent volume of cells suspension before freeze-drying process. The lyophilized bacterial cells were stored at room temperature until their use in inoculation assays.

3.2.8. Inoculation assay.

Wheat (*Triticum aestivum* L.) was chosen as the test plant for evaluation of formulated bacterial consortia because it is fast growing, can grow in a variety of conditions, and seeds are easily obtained and extensively used in pot experiments such as these that are conducted to demonstrate PGPB effects on plant growth. Wheat seeds were sorted to eliminate broken, small, and infected seeds. The seeds were disinfected with 70% ethanol for 5 min, washed several times with SDW, treated for 20 min with NaClO, and rinsed with several changes of SDW. Ten seeds were sown per plastic pot, each containing 1 kg of sterile soil (Andisol, Freire series). To decrease soil bacterial load, the soil (70% water holding capacity) was put in plastic bags and was subjected to heat treatments using a microwave (10 min at 2,450 MHz) for three consecutive days according to that described by Borie and Rubio, (1999) with minor modifications. Later, lyophilized bacterial consortia were dissolved in SDW at a final concentration of 10^8 CFU ml^{-1} and 30 ml was directly inoculated in soils. This inoculation method was named as 'lyophilized cell inoculation (LCI)'. In parallel, disinfected seeds were coated with a mixture of adhesive solution (arabic gum) and dolomite as coating material as described by Cartes et al. (2011), plus a suspension of lyophilized bacterial consortia at final concentration of 10^8 CFU g^{-1} of seed. Ten coated seeds were sown per pot as described above. This inoculation method was named as 'coated seed inoculation (CSI)'.

The inoculation assay was a complete randomized factorial design with three factors (5×3×2), and four replications pert treatment. The experimental factors were: i) 4 bacterial consortia (C1, C2, C3 and C4) plus uninoculated control, ii) two salt (NaCl) levels (0.25 and 0.45 M) plus control without NaCl (0 M), and iii) two inoculation methods (LCI and CSI). The pots were placed in a growth chamber at 25°C for 7 days with 80% relative humidity and a 16:8 h day:night cycle. After germination (7 d), the pots were transferred to the greenhouse and the

plants were grown at 20°C for 5 wks. During the experiment, the seedlings were watered every 3 days with SDW or sterile NaCl solution according to each treatment.

3.2.9. Emergence, growth, biomass and superoxide dismutase activity of seedlings.

A growth chamber experiment was conducted to count the numbers of seeds that germinated in each pot and calculate the percentage of seedling emergence. After the seedlings were transferred to the greenhouse, the seedlings were harvested 35 days after germination and plant growth (shoot and root length) was measured. Roots and shoots were separated and dried at 65ºC for 48 h for determination of plant dry weight biomass (DW). In parallel, subsamples of both root and shoot (0.1 g) of fresh seedlings were stored (-80 ºC) to determine the enzyme superoxide dismutase (SOD; EC 1.15.1.1) activity. To measure SOD activity, the stored subsamples were frozen in liquid N and ground with a mortar and pestle. The proteins were extracted with 50 mM potassium phosphate buffer (pH 7.0), centrifuged at 11,000 × *g* for 15 min (4°C), and then the supernatant was used as a 'enzyme crude extract'. The SOD activity was determined according to procedure described by Donahue et al. (1997) that measures inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of reduction of NBT at 560nm. SOD activity was calculated on a protein basis with the total amount of protein determined in the enzyme crude extract according to the Bradford' colorimetric assay.

3.2.10. Statistical analysis.

The data for bacterial counts, IAA-production and ACCD-activity were analyzed by Student's-T test. Plant growth data following inoculation with PGPB were analyzed using three-way

ANOVA and the means were compared by Duncan's multiple comparison test for mean separation. In all analysis, differences at $P \leq 0.05$ were considered as significant differences between treatments.

3.3. Results.

3.3.1. Culturable bacterial counts and isolation of putative ACCD-producing bacteria.

The results revealed significant differences ($P \leq 0.05$) in the bacterial cell counts between culturable endophytic leaf and rhizosphere bacteria from avocado tree samples (Figure 3.1.a). Significant ($P \leq 0.05$) higher abundance of culturable bacteria was observed in rhizosphere soil samples ($0.26\text{-}3.80 \times 10^5$ CFU g^{-1}) compared with endosphere samples ($1.53\text{-}2.30 \times 10^3$ CFU g^{-1}) with both culture media (LB and NM-1 agar).

A total of 309 isolates were obtained (19 from leaves, 103 from roots and 187 from rhizosphere soils) based on their phenotype. Ninety-five isolates (30.7%) showed the ability to grow in DF culture media with ACC as sole N source, and therefore were considered as putative ACCD-producing bacteria, corresponding to the 19.3%, 26.3%, and 37.4% of isolates from leaves, roots and rhizosphere soils, respectively. Noteworthy, 75 putative ACCD-producing bacteria (24.3%) grew in less than 5 days and these strains were selected for further analysis.

Table 3.2. Characteristics of selected halotolerant IAA- and ACCD-producing strains.

Isolate	Isolation source	IAA (μg ml^{-1}) [a]	Salt tolerance[b]	ACCD activity[c]	Closest relatives or cloned sequences[d] (accession no.)	Similarity (%)	Accesion no.
Consortium 1							
Enterobacter sp. 12	Endosphere	8.4 ± 1.6	5.0	2.7 ± 0.18	Endophytic *Enterobacter* sp. from root nodules of soybean (DQ988939)	97	KR066645
Enterobacter sp. 126	Endosphere	13.5 ± 0.9	10.0	2.60 ± 0.42	*Enterobacter ludwigii*.from soil (KF836496)	99	KR066643
Serratia sp. 73	Endosphere	14.6 ± 2.1	5.0	2.06 ± 0.18	*Serratia* sp. from soil (EU414474)	99	KR066653
Consortium 2							
Microbacterium sp. 35	Endosphere	3.8 ± 0.3	10.0	0.91 ± 0.08	*Microbacterium hydrocarbonoxydans* from citrus roots (HQ219958)	99	KR066649
Pseudomonas sp. 33	Endosphere	4.7 ± 1.1	7.5	0.75 ± 0.04	*Pseudomonas fluorescens* from saline rhizosphere soil (HF678366)	99	KR066650
Serratia sp. 16	Endosphere	6.1 ± 1.4	5.0	0.65 ± 0.05	Endophytic *Serratia grimesii* from garlic (HM217122)	99	KR066652
Consortium 3							
Enterobacter sp. 172	Rhizosphere	63.2 ± 0.9	7.5	3.63 ± 0.11	*Enterobacter* ludwigii from rhizosphere of rice plants (LC015547)	99	KR066644
Enterobacter sp. 206	Rhizosphere	17.3 ± 2.3	7.5	3.54 ± 0.75	*Enterobacter* sp. from soil (KJ482903)	99	KR066647
Enterobacter sp. 198	Rhizosphere	21.8 ± 2.7	7.5	3.44 ± 0.37	Endophytic *Enterobacter* sp. from tobacco (JF783987)	99	KR066646
Consortium 4							
Enterobacter sp. 357	Rhizosphere	9.6 ± 0.4	5.0	0.78 ± 0.18	*Enterobacter cloacae* from soil (KF322131)	92	KR066648
Serratia sp. 343	Rhizosphere	10.8 ± 0.5	5.0	0.25 ± 0.03	*Serratia marcescens* from soil (KM252937)	99	KR066651
Achromobacter sp. 249	Rhizosphere	9.2 ± 0.5	5.0	0.21 ± 0.03	*Achromobacter xylosoxidans* from rhizosphere soil (KM488321)	100	KR066642

[a] values are means ± SE of three experiments

[b] expressed as percentage (%) of NaCl, which did not affect bacteria growth rate

[c] expressed as μmol α-ketobutyrate mg^{-1} protein h^{-1}. Values are means ± SE of three experiments

[d] based on partial sequencing of 16S rDNA gene and comparison with those present in GenBank by using Blastn (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

3.3.2. IAA-producing bacteria.

The IAA production by 75 selected putative ACCD-producing bacteria is shown in Figure 3.1b. Seventy-one isolates (94.7%) were able to produce the phytohormone IAA. The IAA production varied 1.7-63.2 µg ml^{-1}. Although there were no significant differences ($P \leq 0.05$) between rhizosphere and endophytic bacteria, the rhizobacteria generally produced higher amounts of IAA (average of 16.1±1.5 µg ml^{-1}) than the endophytic bacteria (average of 10.7±1.7 µg ml^{-1}).

3.3.3. Halotolerant bacteria.

The salt tolerance assay revealed that 54 of 75 (72%) of selected putative IAA- and ACCD-producing bacteria were able to grow on LB agar plates supplemented with ≥ 5% NaCl. Most of the selected putative ACCD-producing bacteria (77.8%) isolated from the leaf endosphere were halotolerant, with 5 isolates able to grow at 10% NaCl (data not shown). Similarly, 70.2% of selected putative ACCD-producing rhizobacteria were halotolerant, with only 2 isolates able to grow at 10% NaCl.

It is noteworthy that the higher percentages of halotolerant putative ACCD-producing bacteria were found in U14S4 (82.6%) followed by U3S3 (80.8%) and U1S3 (61.5%) sampling sites. These results are in correspondence with relative differences in the EC values of these soils (Table 3.1.).

3.3.4. ACCD activity.

ACCD activity was tested for 54 halotolerant putative ACCD-producing bacteria. All of the tested isolates were shown to be ACCD-producing bacteria, having ACCD activities ranging

between 0.18-3.63 μmol αKB mg protein^{-1} h^{-1} (Figure 3.1c). No significant differences ($P \leq 0.05$) in ACCD activity were found between endophytic and rhizosphere bacteria. However, the results showed higher ACCD activity in rhizobacteria (average of 1.88±0.18 μmol αKB mg protein^{-1} h^{-1}) than endophytic bacteria (average of 1.43±0.22 μmol αKB mg protein^{-1} h^{-1}). Noteworthy, 9 of 10 isolates with the highest ACCD activities (>3 μmol αKB mg protein^{-1} h^{-1}) were isolated from rhizosphere soils.

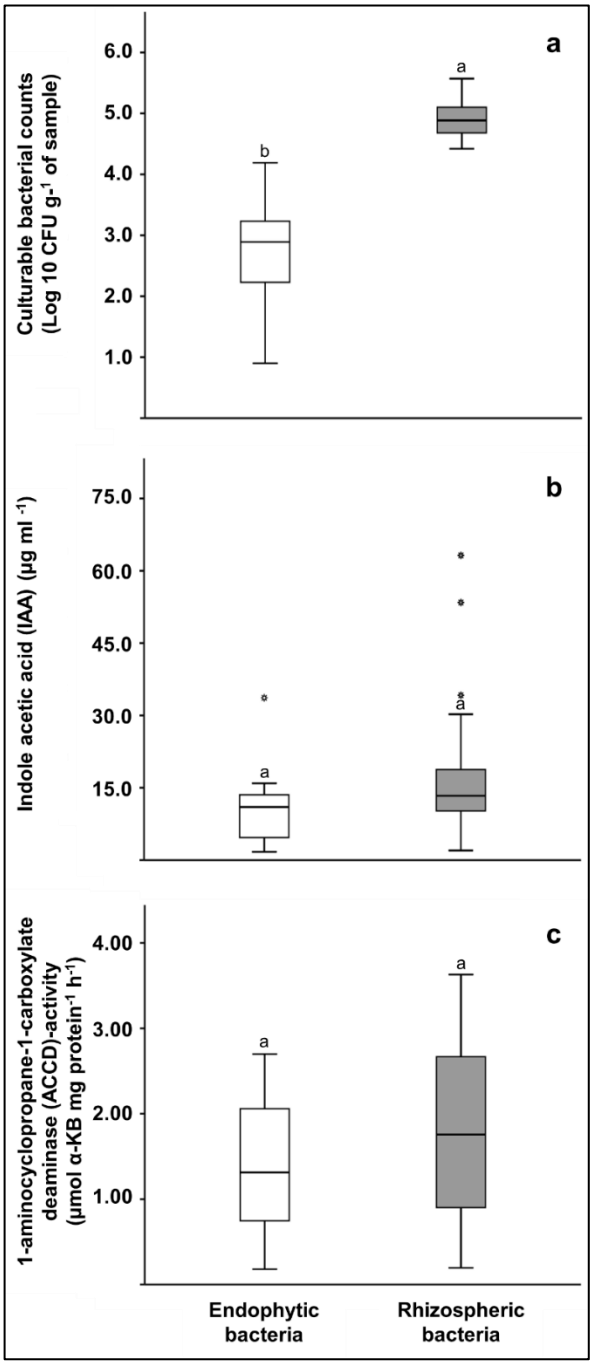

Figure 3.1. (**a**) Culturable bacterial counts in endosphere and rhizosphere of avocado tree samples. (**b**) Indole acetic acid (IAA) released by endophytic and rhizosphere bacteria isolated from avocado trees. (**c**) 1-aminocyclopropane-1-carboxylate deaminase (ACCD)-activity of endophytic and rhizosphere bacteria isolated from avocado trees. The center line of each box represents the median, the top and bottom of boxes represent the 25th and 75th percentile of data, respectively, and the top and bottom of the error bars represent the 5th and 95th percentile of data, respectively. α-KB: α-ketobutyrate. Asterisks represent outliers.

3.3.5. Formulation and preparation of bacterial consortia.

Detailed descriptions of the bacterial consortia are provided in Table 3.2. Consortium C1 was formulated with endophytic bacteria with higher ACCD activity (2.0-2.7 μmol αKB mg^{-1} protein h^{-1}) and higher IAA production (8.5-14.7 mg^{-1} IAA ml^{-1}). Consortium C2 was formulated with endophytic bacteria with lower ACCD activity (0.65-0.91 μmol αKB mg^{-1} protein h^{-1}) and lower IAA production (3.8-6.1 mg^{-1} IAA ml^{-1}). Consortium C3 was formulated with rhizosphere bacteria with higher ACCD activity (3.4-3.6 μmol αKB mg^{-1} protein h^{-1}) and higher IAA production (17.3-63.2 mg^{-1} IAA ml^{-1}). Consortium C4 was formulated with rhizosphere bacteria with lower ACCD activity (0.21-0.78 μmol αKB mg^{-1} protein h^{-1}) and lower IAA production (9.1-10.8 mg^{-1} IAA ml^{-1}).

With respect to the identification based on 16S rRNA gene sequencing (Table 3.2.), the results showed that C1 was formulated with *Enterobacter* sp. 12, *Serratia* sp. 73, and *Enterobacter* sp. 126; C2 was formulated with *Serratia* sp. 16, *Pseudomonas* sp. 33 sp. and *Microbacterium* 35; C3 formulated with *Enterobacter* sp. 172, *Enterobacter* sp. 198 and *Enterobacter* sp. 206; C4 formulated with *Achromobacter* sp. 249, *Serratia* sp. 343 and *Enterobacter* sp. 357. Taxonomic assignments were performed at genus level (≥95% similarity) with those present in Genbank database. It is noteworthy that among 6 isolates with high IAA production and high ACCD activity, 5 isolates corresponded to members of the genus *Enterobacter*.

Bacterial survival rates (BSR) measured as colony forming units on agar plates streaked with the consortia before and after lyophilization process are shown in Table 3.3. The results showed a decrease in cell numbers from 10^9-10^{10} CFU ml^{-1} to 10^6-10^7 CFU ml^{-1} produced by lyophilization process, equivalent to BSR ≥65%. The higher BSR were observed with *Pseudomonas* sp. 33 (78.2 %) and *Enterobacter* sp. 172 (77.4%). In contrast, the lower BSR were observed with *Serratia* sp. 73 (65.9%) and *Achromobacter* sp. 249 (66.5%).

Table 3.3. Bacterial counts of selected halotolerant IAA- and ACCD-producing strains before and after lyophilization process.

Isolates	Before (log CFU ml^{-1})	After (log CFU ml^{-1})	BSR* (%)
Consortium 1			
Enterobacter sp. 12	9.47 ± 0.25[a]	7.24 ± 0.37	76.5
Enterobacter sp. 126	10.22 ± 0.52	7.17 ± 0.27	70.2
Serratia sp. 73	9.43 ± 0.22	6.21 ± 0.32	65.9
Consortium 2			
Microbacterium sp. 35	9.88 ± 0.42	7.34 ± 0.94	74.3
Pseudomonas sp. 33	8.97 ± 1.21	7.02 ± 0.29	78.3
Serratia sp. 16	8.84 ± 0.25	6.16 ± 1.02	69.7
Consortium 3			
Enterobacter sp. 172	9.54 ± 0.78	7.38 ± 0.93	77.4
Enterobacter sp. 206	9.72 ± 0.54	7.07 ± 0.35	72.7
Enterobacter sp.198	9.86 ± 1.14	7.37 ± 0.45	74.8
Consortium 4			
Enterobacter sp. 357	10.43 ± 0.48	7.97 ± 1.18	76.4
Serratia sp. 343	9.12 ± 0.34	6.71 ± 0.88	73.6
Achromobacter sp. 249	9.81 ± 0.31	6.52 ± 0.74	66.5

*bacterial survival ratio = (after / before) × 100

[a] values are means ± SE of three experiments

3.3.6. Emergence, growth, biomass and superoxide dismutase activity of seedlings.

The percentages of seedling emergence are illustrated in Figure 3.2. In general and independent of inoculation methods, the percentages of seedling emergence were decreased with increasing soil salinity. However, the percentage of seedling emergence was only significantly decreased ($P \leq 0.05$) for uninoculated seeds irrigated with 0.45 M NaCl using the CSI method. With 0.45 M NaCl treatment, inoculation of seeds with both C2 and C4 significantly increased ($P \leq 0.05$) seedling emergence by up to 70% as compared to 43% emergence for uninoculated seeds treated with the CSI method. Similarly, with 0.45 M NaCl treatment, the C1 consortium significantly increased ($P \leq 0.05$) the percentage of seedling emergence (92.5%) as compared to to uninoculated seeds (67.5%) using the CSI method. In general, significantly ($P \leq 0.05$) higher percentages of seedling emergence (Table 3.4.) were obtained with LCI (67.5-92.5%) than CSI (42.5-90%).

Figure 3.3. and Figure 3.4. show growth and biomass of inoculated wheat seedlings. Independent of inoculation methods, the results showed that growth and biomass of shoots were significantly ($P \leq 0.05$) lower in uninoculated seedlings irrigated with 0.25 and 0.45 M NaCl compared with uninoculated seedlings in the no-salt control treatment. Similar effects were observed with respect to growth and biomass of roots, except for growth of roots from plants inoculated with the LCI method. With respect to the effects of the consortia, Table 3.4. shows that bacterial inoculation produced significant ($P \leq 0.05$) changes on all parameters analyzed in the present study. Therefore, inoculation with either C1 or C2 resulted in significantly ($P \leq 0.05$) greater shoot and root growth for plants exposed to the 0.45 M NaCl treatment, except for root growth of seedlings inoculated with C3 using LCI (Figure 3.3.). In respect to biomass, with 0.45 M NaCl treatment, inoculation of plants with C1, C2 and C3 by SCI method resulted in significant ($P \leq 0.05$) greater dry weight of shoots and roots as compared with uninoculated seedlings. The C1 consortium inoculated by LCI also significantly increased ($P \leq 0.05$) the shoot length of seedlings irrigated with 0.25 NaCl M. Similarly, with the 0.45 M NaCl treatment, the inoculation with C2 and C3 increased significantly ($P \leq 0.05$) the shoot and root biomass, respectively, of seedlings inoculated by LCI method compared with uninoculated control. With 0.25 M NaCl treatment, the C1 and C3 increased significantly ($P \leq 0.05$) the shoot biomass in both inoculation methods and the root biomass of seedlings inoculated by CSI method (Figure 3.4.). In is noteworthy that C4 significantly increased ($P \leq 0.05$) the shoot biomass of no-salt stressed wheat seedlings (control). In general, our results show significant difference ($P \leq 0.05$) between both inoculation methods (Table 3.4.). Therefore, shoot length as well as shoot and root dry weight of seedlings inoculated by CSI method were significantly higher ($P \leq 0.05$) than those inoculated by LCI method. These same parameters were significantly affected ($P \leq 0.05$) by the interaction between SL x BC. In the same way, shoot length was also significantly influenced ($P \leq 0.05$) by the interaction between IM x SL x BC as well as by IM x SL (Table 3.4.).

Similar to percentages of emergence and growth of seedlings, the SOD activity was strongly affected by 0.45 M NaCl treatment (Figure 3.5.). In this way, SOD activity was significantly increased ($P \leq 0.05$) in shoots (27.1-31.0 U mg^{-1} protein) and roots (320.5-350.1 U mg^{-1} protein) of uninoculated seedlings in relation to shoot (23.1-26.9 U mg^{-1} protein) and root (280.6-297.1 U mg^{-1} protein) of uninoculated seedlings without NaCl treatment. Independent of inoculation method, C1 and C3 significantly ($P \leq 0.05$) increased the SOD activity in roots and shoots at 0.45 M NaCl, with the exception of C1 in CSI and C3 in LCI. Similarly, at 0.25 M NaCl in SCI method, the SOD activity of shoots was significantly increased ($P \leq 0.05$) by C3 and C1. Whereas, at 0.25 M NaCl, root SOD activity was significantly ($P \leq 0.05$) increased by C2 and C3 in LCI and SCI methods, respectively, compared with uninoculated controls. It should be noted that C1 was the only consortium able to increase the SOD activity of seedling controls (irrigated with distilled water) in LCI method. Similar to growth and biomass, the results showed a significant difference ($P \leq 0.05$) between inoculation methods. However, the shoot and root SOD activity were significantly ($P \leq 0.05$) higher in seedlings inoculated by LCI than those inoculated by CSI method. The results of three-way ANOVA, illustrated in Table 3.4., show that SOD activity of shoot and root were significantly affected ($P \leq 0.05$) by interactions between IM x BC as well as SL x BC. Whereas, the interactions among IM x SL x BC only significantly influenced ($P \leq 0.05$) the SOD activity of roots.

3.4. Discussion.

In the present study, culture based methods using colony forming unit plate counts revealed bacterial loads of 1.53-2.30×10^3 CFU g^{-1} of tissue and 0.26-8.30×10^5 g^{-1} of soil in the endosphere and rhizosphere of avocado trees, respectively (Figure 3.1a). The endophytic bacterial population densities were within the range previously described for banana tissues (10^2-10^5 CFU g^{-1} tissue) (Kuklinsky-Sobral et al., 2005), but lower than those shown for

grapevine tissues (10^3-10^7 CFU g^{-1} tissue) (Compant et al., 2011). To our knowledge, there are no published studies describing the indigenous endophytic bacteria present inside the root tissues of avocado plants. With respect to rhizobacteria cell densities, a recent study of mature avocado trees in California (USA) described bacterial loads ranging from 10^4-10^6 CFU g^{-1}, which is similar to our study (Nadeem et al., 2012).

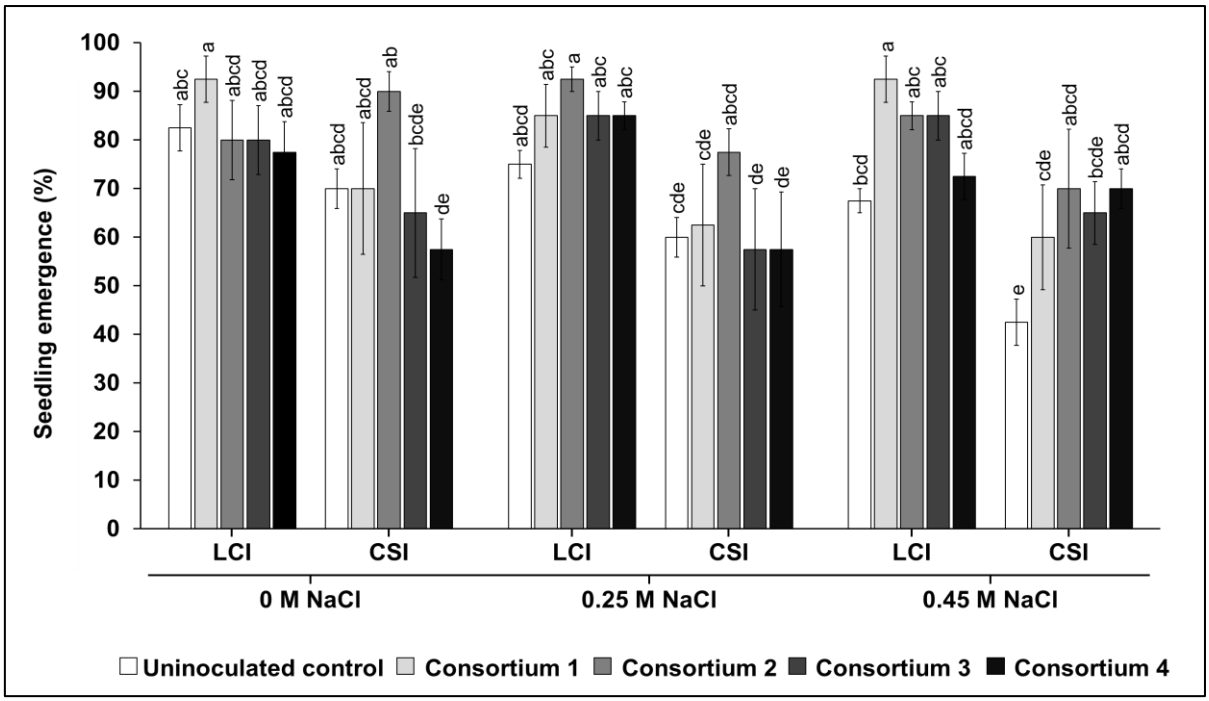

Figure 3.2. Effect of NaCl treatments on the percentage of seedling emergence of plants inoculated with bacterial consortia by using lyophilized cell inoculation (LCI) and coated seed inoculation (CSI) methods. Vertical bars represent average (n=4) ± standard error. Different letters denote significant difference ($P \leq 0.05$; Duncan's test) between consortia treatments.

Endophytic and rhizosphere bacteria with ACCD activities have been shown to be excellent plant-growth promoters, because they ameliorate plant stress by efficiently blocking ethylene production (Hardoim et al., 2008). There is wide variability in ACCD degrading activity depending on the bacterial strain. Penrose and Glick (2003) described levels of ACCD activity at ≥20 nmol αKB mg $protein^{-1}$ h^{-1} as sufficient to promote host plant growth. Among all of the isolates examined here, 30.7% were considered as putative ACCD-producing bacteria, which is similar to the results published by Barnawal et al. (2014) who founded 26.7% putative

ACCD-producing rhizobacteria associated with plants naturally growing on saline soils. However, our findings are higher than other previously reported values, such as Trivedi et al. (2011) and Qin et al. (2013) who determined that 19% and 10.3% of endophytic bacteria isolated from *Citrus sinensis* and *Limonium sinense* were able to grow in DF culture media, respectively.

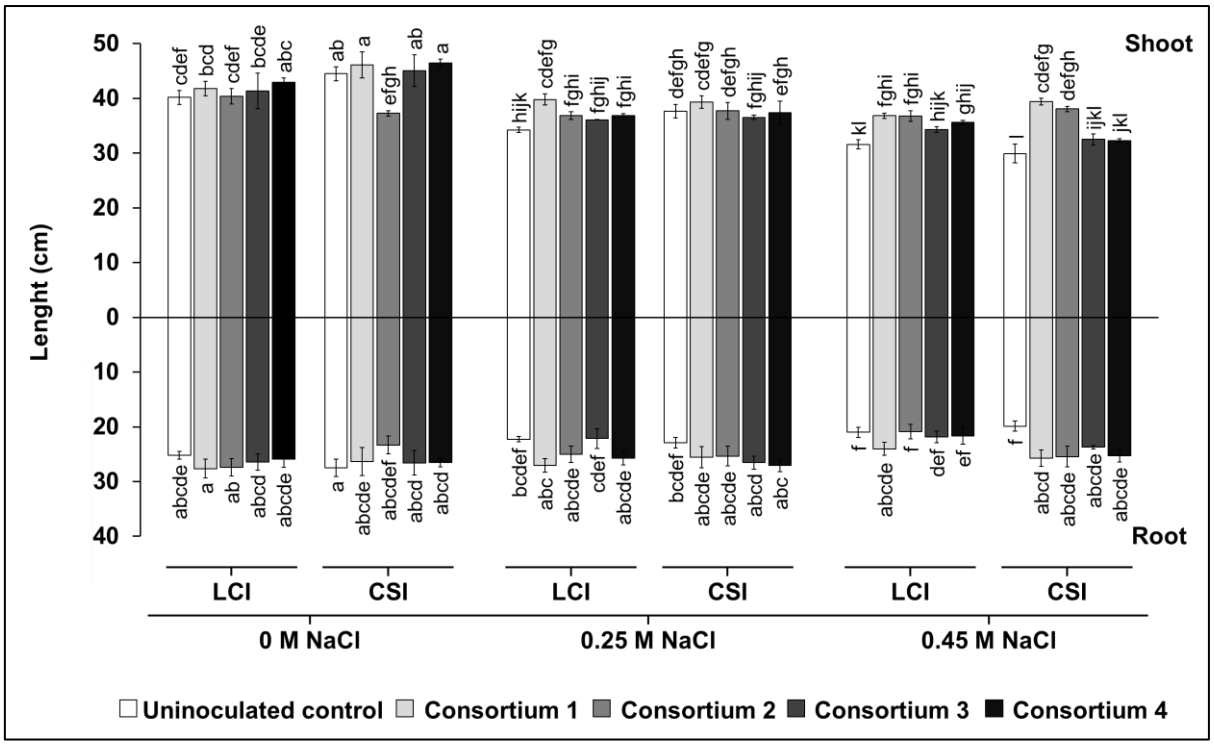

Figure 3.3. Effects of NaCl treatments on shoot and root growth (length) of wheat seedlings inoculated with bacterial consortia by using lyophilized cell inoculation (LCI) and coated seed inoculation (CSI) methods. Vertical bars represent average (n=4) ± standard error. Different letters denote significant difference ($P \leq 0.05$; Duncan's test) between consortia treatments.

In relation to IAA production, 100% and 95.7% of the endophytic bacteria and rhizosphere bacteria, respectively, produced IAA at concentrations ranging between 1.7-33.7 µg ml^{-1} and 1.7-63.2 µg ml^{-1}, respectively (Figure 3.1b). Similar results were reported by Ibañez et al. (2012) who determined that 96% of endophytic bacteria and 100% of rhizosphere bacteria associated with sugarcane produced IAA. Similarly, a previous study performed on avocado rhizosphere soil showed that 100% of the culture-isolated strains were able to produce IAA

(Nadeem et al. 2012). Moreover, we observed that the levels of IAA production are highly variable among different strains. The wide range of IAA production is a well-documented phenomenon. Thokchom et al. (2014) and Jha et al. (2012) described IAA production ranging between 0.5-12.0 μg ml^{-1} and 30-100 μg ml^{-1} by endophytic bacteria isolated from *Citrus reticulate* and *Salicornia brachiate*, respectively. Whereas, Rashid et al. (2012) and Ibañez et al. (2012) described IAA production ranging between 3.75-143.3 μg ml^{-1} and 0.03-17.73 μg ml^{-1} by rhizobacteria.

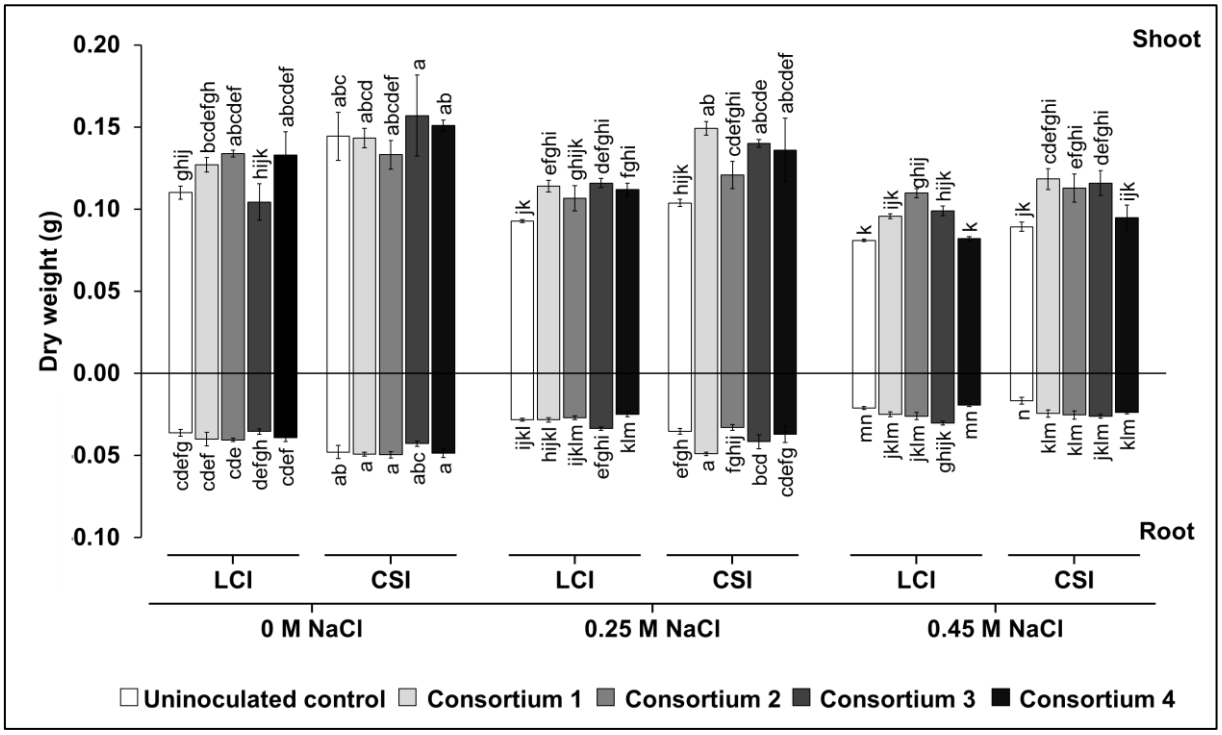

Figure 3.4. Effects of NaCl treatments on shoot and root biomass (dry weight) of wheat seedlings inoculated with bacterial consortia by using lyophilized cell inoculation (LCI) and coated seed inoculation (CSI) methods. Vertical bars represent average (n=4) ± standard error. Different letters denote significant difference ($P \leq 0.05$; Duncan's test) between consortia treatments.

In relation to halotolerance of isolates, 72% of IAA- and ACCD-producing bacteria showed salt tolerance at ≥ 5% on agar plate. Coincidently, the isolates with higher percentages of salt tolerance were isolated from soils with higher E.C. Another recent study also has reported the presence of some halotolerant strains in rhizosphere of avocado trees (Nadeem et al. 2012).

These authors isolated rhizobacteria strains that grew in a concentration of 5% NaCl (5 isolates) and 10% NaCl (4 isolates), which also showed ACCD activity. It should be noted that Siddikee et al. (2010) showed that 69% of halotolerant bacteria (1.75 M NaCl) isolated from soil and halophytic plants were also ACCD-producing bacteria. This result is consistent with Duan et al. (2009) who reported that ACCD-producing bacteria are commonly founded in stressful habitats, suggesting that ACCD-producing bacteria are naturally selected by plants under stress conditions.

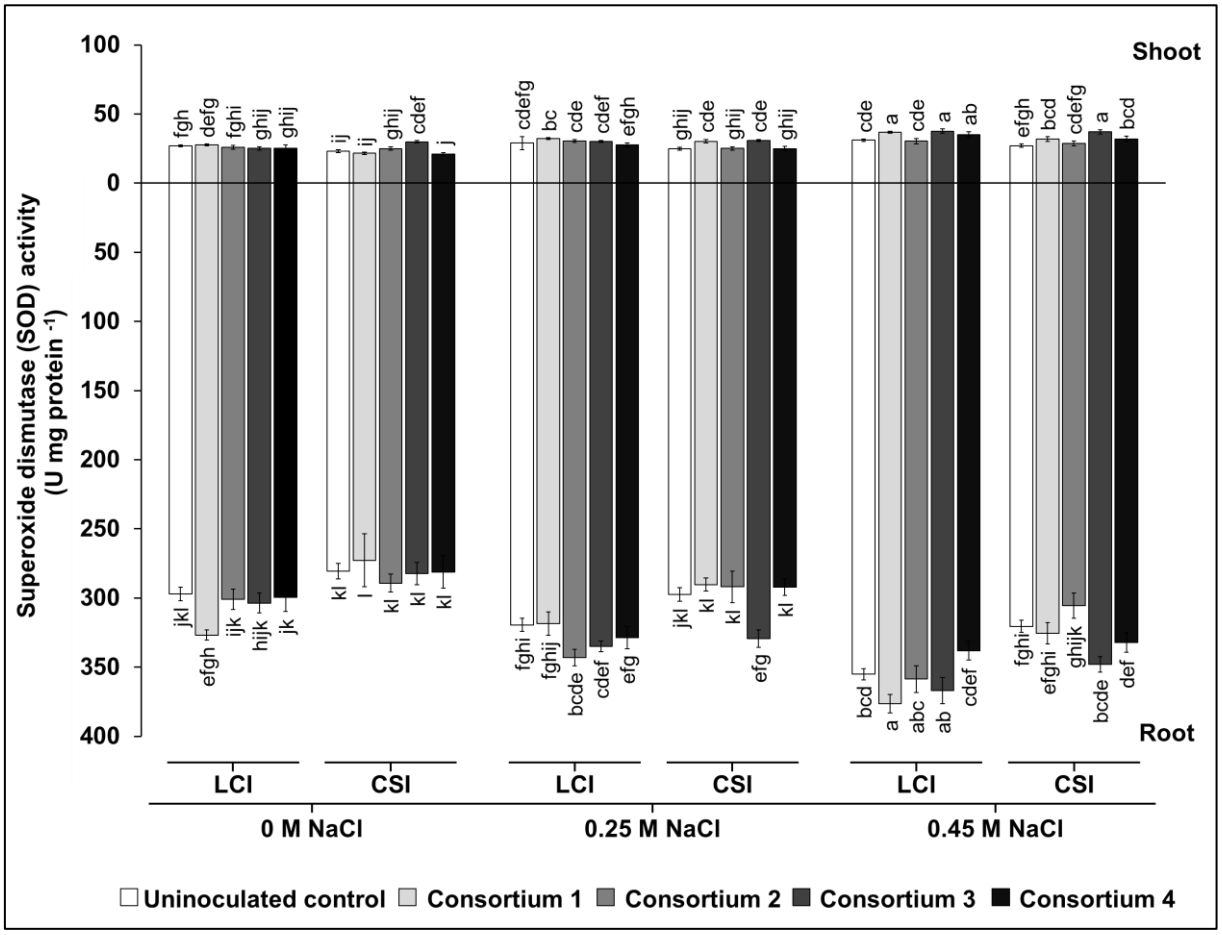

Figure 3.5. Effect of NaCl treatments on shoot and root superoxide dismutase activity of wheat seedlings inoculated with bacterial consortia by using lyophilized cell inoculation (LCI) and coated seed inoculation (CSI) methods. Vertical bars represent average (n=4) ± standard error. Different letters denote significant difference ($P \leq 0.05$; Duncan's test) between consortia treatments.

Table 3.4. *F*- and *P*-values of three-way ANOVA of seedling emergence and length, dry weight and SOD activity of shoot and root of wheat seedlings inoculated with four bacterial consortia by two different methods under different salt levels. *P*-values are significant at <0.05 (*n*=4).

	Main-factor effects						**Significant interactions**							
	IM		**SL**		**BC**		**IM x SL**		**IM x BC**		**SL x BC**		**IM x SL x BC**	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Seedling emergence (%)	41.814	<0.05	1.377	0.258	4.333	<0.05	1.104	0.336	1.356	0.256	1.073	0.389	0.838	0.572
Shoot length	4.176	<0.05	93.639	<0.05	8.282	<0.05	3.527	<0.05	1.005	0.409	5.361	<0.05	2.136	<0.05
Root length	2.809	0.097	12.799	<0.05	3.253	<0.05	1.898	0.156	0.789	0.535	0.820	0.587	1.319	0.244
Shoot dry weight	41.343	<0.05	42.826	<0.05	6.189	<0.05	1.329	0.271	1.966	0.108	2.228	<0.05	1.000	0.443
Root dry weight	60.380	<0.05	182.756	<0.05	5.083	<0.05	21.005	<0.05	2.086	0.091	4.267	<0.05	1.498	0.172
SOD activity of Shoot	26.808	<0.05	85.472	<0.05	14.594	<0.05	0.267	0.766	5.160	<0.05	4.114	<0.05	1.247	0.281
SOD activity of Root	103.301	<0.05	103.017	<0.05	4.702	<0.05	0.666	0.516	4.020	<0.05	2.280	<0.05	2.125	<0.05

IM: inoculation methods; SL: salt levels; BC: bacterial consortia inoculation.

ACCD activities of the halotolerant endophytic IAA-producing bacteria (Figure 3.1c) described here showed a higher range of ACCD activity (0.18-2.70 μmol αKB mg protein^{-1} h^{-1}) than those described by Jha et al. (2012), who isolated halotolerant endophytic strains with ACCD activity (0.12-0.98 μmol αKB mg protein^{-1} h^{-1}) from plant halophyte *Salicornia brachiate*. Whereas, the ACCD activity our rhizosphere isolates (0.20-3.63 μmol αKB mg protein^{-1} h^{-1}) was similar to those obtained by Siddikee et al. (2010), who isolated from rhizosphere of six halophytic plants 36 halotolerant rhizobacteria with ACCD activity ranging from 0.69 to 4.90 μmol αKB mg protein^{-1} h^{-1}. However, our rhizosphere isolates showed lower ACCD activity than those strains with ACCD activity (4.91-185.51 μmol αKB mg protein^{-1} h^{-1}) isolated from rhizosphere of avocado tree described by Nadeem et al. (2012).

Twelve bacterial strains were selected and four bacterial consortia were formulated, the members of each consortia were identified based on partial sequencing of 16S rRNA genes (Table 3.2.). The selected isolates belonged to five different bacterial genera: *Enterobacter*, *Serratia*, *Pseudomonas*, *Microbacterium* and *Achromobacter*. The most efficient IAA- and ACCD-producing strains were identified as *Enterobacter* genus, which came from two different sampling sites and were isolated from roots, leaves and rhizosphere. These interesting results demonstrated the wide distribution of IAA- and ACCD-producing *Enterobacter* spp. and their close association with avocado trees. These findings allow us to assume that IAA- and ACCD-producing *Enterobacter* spp. could efficiently colonize avocado plants. Previous studies have reported the occurrence of IAA- and ACCD-producing *Enterobacter* spp. associated with diverse plant species, such as *Citrus reticulate* (Thokchom et al., 2014), *Populus* spp. (Taghavi et al., 2009), *Piper nigrum* (Jasim et al., 2013), and commonly show plant growth-promoting abilities. Thus, Thokchom et al. (2014) determined significantly increased shoot length, shoot and root dry biomass of *Citrus reticulate* seedlings inoculated with *Enterobacter* spp. over the uninoculated controls. Here, the selected halotolerant endophytic strains with lower IAA production and ACCD activity were identified as *Microbacterium* sp., *Pseudomonas* sp. and

Serratia sp. These genera have already been previously described as IAA- and ACCD-producing endophytic bacteria (Qin et al., 2013; Trivedi et al., 2011). At the same time, the selected halotolerant rhizosphere bacteria with lower IAA production and ACCD activity were identified as *Achromobacter* sp., *Serratia* sp. and *Enterobacter* sp. Some previous studies have shown the occurrence of IAA- and ACCD-producing *Achromobacter* spp. associated with *Catharanthus roseus* (Karthikeyan et al., 2012) and *Citrus reticulata* (Thokchom et al., 2014). In this way, IAA- and ACCD-producing *Achromobacter xylosoxidans* was reported to increase biomass and growth of *Ocimum sanctum* plants during waterlogging stress (Barnawal et al., 2012). Independently of ACCD activity and IAA production, the taxonomic affiliations of our selected isolates are commonly associated with PGPB. With respect to freeze-drying process, the bacteria viabilities were lower than 78% these are acceptable for our purpose, the transport and inoculation of bacterial consortia. Freeze-drying survival data bacteria show great variation, which reflects the numerous factors influencing this process: species, cell concentration, freeze-drying medium, physiological state, freeze-drying parameters, and rehydration (Palmfeldt et al., 2003). Accordingly, further studies are needed to determine the optimal conditions for freeze-drying process of bacteria.

Results of the inoculation experiments showed that all of the constructed consortia increased the percentages of wheat seedling emergence under salt stress conditions, particularly consortia C1, C2 and C4 (Figure 3.2.). Seed germination is mediated by a complex hormonal network, where the salt repressive effect on germination could be related to a decline in endogenous levels of growth phytohormones, such as IAA (Egamberdieva, 2009; Finkelstein, 2010). Accordingly, the increase in the percentage of seedling emergence promoted by some consortia could be attributed to ability of bacteria to produce IAA. In this way, Egamberdieva (2009) determined that wheat seeds inoculated with three different IAA-producing *Pseudomonas* spp. strains increased germination rates (27-33%) when watered with 0.1 M NaCl. Similarly, Kaya

et al. (2009) showed that inoculation with the IAA-producing *Curtobacterium plantarum*, increased the percentage of seedling emergence of winter rye seeds by 17%.

Inoculation assay results also showed that growth and biomass of roots and shoots were gradually reduced with increasing salt stress (Figure 3.3. and Figure 3.4.). The reduction in shoot growth is probably due to hormonal signals generated by the roots (Munns and Tester, 2008). As a consequence of salt stress, ACC synthase genes are induced in the roots of wheat plants and ACC is therefore transported by the xylem to shoots where it is oxidized to ethylene (Jackson, 1997). At the same time, the ACC is accumulated and secreted by roots, which would stimulate the proliferation of both native or inoculated ACCD-producing bacteria (Grichko and Glick, 2001). This higher production of ethylene under salt stress conditions involves a decline of growth and biomass in plants. In the present study, inoculation with C1 and C2 increased the growth and biomass of shoots and roots in seedlings exposed to 0.45 M NaCl. Similarly, inoculation with C3 and C4 increased the biomass and growth, respectively, of shoots and roots in seedlings exposed to salt stress. In the same way, the interaction between salt levels and bacterial consortia (Table 3.4.) affected significantly the shoot length as well as shoot and root weight. The above suggested that as the salt levels increased, the protective effect of bacterial consortia concomitantly increased. These results might be due to the hydrolysis of ACC by the bacterial enzyme ACCD, which decrease the detrimental ethylene levels (Barnawal et al., 2014; Grichko and Glick, 2001).

The consortia formulated with bacteria that produced the highest amounts of IAA and ACCD (C1 and C3) were significantly more effective for mitigating salt stress effects and improving the wheat growth than those formulated with bacteria having lower IAA production and ACCD activity, particularly the C4. Previous studies have associated IAA synthesis by bacteria with plant growth stimulation under salt stress conditions (Egamberdieva, 2009; Ramadoss et al., 2013; Tank and Saraf, 2010). Moreover, the amino acid tryptophan, which is identified as the

main precursor for IAA, also stimulates IAA releasing by bacteria (Martens and Frankenberger, 1994; Sarwar and Kremer, 1995). In this way, Martens and Frankenberger, (1994) described the presence of tryptophan (20-29 nM g^{-1}) in root exudates of three wheat varieties; thereby, it is tempting to assume that bacterial IAA is in part dependent upon wheat-exuded tryptophan. However, the IAA effects on plant root system are dose-dependent, having inhibitory effects at high concentrations and stimulatory effects when endogenous plant IAA levels are low (Dobbelaere et al., 1999). Therefore, the concentration of IAA-producing bacterial inoculum is critical to determine the effect of this phytohormone. The fact that root elongation was observed in our experiments indicated that the applied concentrations of IAA-producing bacteria were within the range that promote the growth of wheat. However, further study using exogenous IAA alone would be necessary to determine the actual IAA-level where bacteria have the optimal results. With respect to ACC-deaminase, our results are in agreement with a previous study conducted by Shaharoona et al. (2006) who demonstrated a significant positive correlation between the level of ACCD activity of a group of rhizobacteria strains and root elongation of *Zea mays* L.

Here, the beneficial effect of C2, which was formulated with strains showing lower IAA production and ACCD activity, could partially be attributed to its endophytic nature, because as endophytic bacteria establish a more intimate relationship with the plant host than rhizosphere bacteria. Endophytic bacteria encounter a protective environment in which the supply of nutrients is possibly constant, providing a suitable niche where they could have a better survival and therefore more prolonged activity (Hardoim et al., 2008).

On the other hand, an increase of SOD activity in plants under environmental stress is correlated with an increase in the need for protection against damage associated with cellular oxidative stress (Qiu et al. 2014). In the present study, some of the bacterial consortia were able to further increase the SOD activity of shoot and root of wheat seedlings (Figure 3.5., Table 3.4.). Our

results clearly demonstrated that both endophytic and rhizosphere bacterial consortia with higher IAA production and ACCD activity (C1 and C3) were also able to significantly increase SOD activity, which could result in lower oxidative stress in plants. Similar to growth parameters, the interaction between salt levels and bacterial consortia (Table 3.4.) significantly affected the SOD activity, demonstrating that SOD activity is mostly increased by bacterial consortia when higher is salt level. Based on our results, we cannot conclude whether consortia inoculation improved the salt plant tolerance with concomitant increase of SOD activity by plant, or vice versa, whether consortia inoculation induced an increase of SOD activity by plant with the concomitant salt plant tolerance. There is little and contradictory information regarding to the mechanism by which PGPB are able to increase the activity of antioxidant enzymes. Studies have demonstrated that the inoculation with PGPB increases the SOD activity of plants under salt stress conditions, such as in *C. roseus* inoculated with the ACCD-producing bacteria *A. xylosoxidans* (Karthikeyan et al., 2012) and in the *Solanum melongena* inoculated with the bacterial strain *Pseudomonas* sp. DW1 (Fu et al., 2010). In this context, Gururani et al. (2012) determined by real-time PCR analyses that inoculation of *Solanum tuberosum* with two *Bacillus* spp. improved the expression (RNAm) of genes encoding antioxidant enzymes (including SOD) of plants under salt and heavy-metal stress. In contrast, a proteomic approach of *Cucumis sativus* under anoxic stress revealed that inoculation with ACCD-producing bacteria *Pseudomonas putida* UW4 triggered a down-regulation of the enzyme SOD (Li et al., 2013).

Finally, despite that our results suggest the potential of selected bacterial consortia to be used as inoculants to mitigate salt stress in plants, experiments with avocado seedlings are required under greenhouse and field conditions, because it have been reported that colonization and activity of PGPB can be specifically regulated by type of root exudates, competition with autochthonous bacterial populations, and age, variety and type of plant, among others (Drogue et al., 2012; Pii et al., 2015).

3.5. Conclusions.

Our results demonstrated the association of avocado trees and their rhizosphere soils with halotolerant endophytic and rhizosphere bacteria with variable activity of the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD) and production of the phytohormone indole acetic acid (IAA). Among 309 isolates, 54 isolates (17.4%) were characterized as halotolerant IAA- and ACCD-producing bacteria with range of 1.7-63.2 μg ml^{-1} and 0.18-3.63 μmol αKB mg $protein^{-1}$ h^{-1}, respectively. Based on isolation source, IAA production and ACCD activity of isolates, 4 consortia were formulated containing members of five genera: *Enterobacter*, *Serratia*, *Microbacterium*, *Pseudomonas* and *Achromobacter*. In general, plant inoculation with the formulated bacterial consortia ameliorated the effect of salt (NaCl) stress on the emergence, growth and biomass of wheat seedlings under growth chamber and greenhouse conditions. At higher salt stress, bacterial consortia from endosphere were more efficient than those from rhizosphere to promote the growth and biomass of seedlings. The inoculation methods also affected seedling emergence, growth and biomass of seedlings under salt stress. Further work is required to validate the utility of promising bacterial consortia for improving tolerance and yields of avocado trees under field conditions.

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CHAPTER IV

"Bacterial consortia inoculation mitigates the water shortage and salt stress in an avocado (*Persea americana* ***Mill.) nursery"***

Submitted to Applied soil ecology

Bacterial consortia inoculation mitigates the water shortage and salt stress in an avocado (*Persea americana* Mill.) nursery.

Abstract

Chile is one of main producers of avocado (*Persea americana* Mill.) worldwide; however, during recent years, its production has decreased mainly attributed to abiotic stresses such as drought and soil salinity. Here, we evaluated the contribution of halotolerant bacterial consortia to water shortage and salt stress tolerance of avocado seedlings under field conditions. Inoculation trials were conducted in a commercial nursery to investigate the effects of two endophytic (C1 and C2) and two rhizosphere (C3 and C4) halotolerant bacterial consortia on growth, biomass, superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS) of avocado seedlings exposed to salt (2% NaCl) and water shortage (50% less irrigation). Ours results revealed that inoculation with C4 significantly ($P \leq 0.05$) increased aerial and root length; aerial and root fresh weight and chlorophyll content of salt stressed seedling; and the aerial length and root fresh weight of seedlings under water shortage. Similarly, the C4 significantly ($P \leq 0.05$) increased SOD activity in leaves of both the control and seedlings grown under salt stress and water shortage and also decreased TBARS content in leaves of control plants and of seedlings grown under salt stress. Whereas, C3 increased significantly ($P \leq 0.05$) aerial and root length and root fresh weight of salt stressed seedlings; and also increased the trunk diameter and chlorophyll content of seedlings under water shortage. Similarly, C3 significantly ($P \leq 0.05$) stimulated SOD activity of leaves as compared to the control seedlings and also reduced the TBARS content of leaves and roots of avocado seedlings under salt stress. In contrast, the endophytic consortia were less efficient than rhizosphere consortia. Thus, C1 only increased the trunk diameter and chlorophyll content of salt stressed seedlings and C2 increased the chlorophyll content of avocado seedlings under water shortage. Our study showed

the beneficial effect of bacterial inoculation on avocado plants in nursery conditions under water shortage and salt stress, and identified consortia that potentially could be used as avocado biofertilizers.

Keywords: Bacterial consortia; avocado; water shortage; salinity; stress

4.1. Introduction.

Water shortages and decreases in water quality are increasingly affecting crop production in world areas where crops are grown in arid and semi-arid regions in the globe. Over 800 million hectares of land are now salt affected, corresponding to 20% of the world's irrigated lands producing an estimated 40% of the world's food supply (Munns & Tester, 2008; Zahir et al., 2009). In this context, avocado (*Persea americana* Mill.) is considered to be the most salt-sensitive of cultivated fruit trees, and has a high water requirement of some 4 acre feet of water per year for normal production (Bernstein & Meiri, 2004; Chartzoulakis et al., 2002). Chile is one of the largest avocado producing and exporting countries worldwide. As a result of rapid market growth, the area planted with this crop has increased from 23,800 ha in 2003 to 36,355 h in 2013. However, despite this increased planting, Chilean avocado fruit yields have decreased during recent years, from 263,476 t in 2009 to 164,720 t in 2013 (Muñoz, 2015) due to drought conditions that have affected central Chile, and increased reliance on saline groundwater supplies. Water shortages in central Chile are predicted to become increasingly severe, with long term climate projections predicting a decrease of 20-25% in rainfall by 2040 (Neuenschwander, 2010). Therefore, it is important to find strategies to ameliorate stress effects on avocado plants to increase yields under changing climate conditions expected to be occur with global warming.

Physiologically, both water shortage and salinity cause decreases in the soil water potential, leading to diminished root growth, water and nutrient uptake, and subsequent lower plant growth and crop yields (Khan et al., 2014; Munns & Tester, 2008). As a direct consequence of these and other environmental stresses, plants produce increased amounts of ethylene, that generates so called 'stress ethylene'. The increased ethylene levels cause root growth inhibition and initiation of senescence in a feedback loop that finally leads to plant death. Simultaneously, abiotic stress leads to oxidative stress due to the increasing levels of reactive oxygen species (ROS) as a direct result of the imbalance in electron transport rates and metabolic consumption activity of reducing equivalents in the plant cells (Kasim et al., 2012). These ROS react with several macromolecules including chlorophyll, proteins, DNA and lipids, leading to peroxidation of membrane lipids, which can be used as an general indicator of stress-induced damage at the cellular levels (Kasim et al., 2012).

An environmentally friendly strategy to mitigate stress effects on crops is the use of plant growth-promoting bacteria (PGPB) inoculants. Most PGPB produce the phytohormone indole acetic acid (IAA) that can directly increase root growth, allowing enhanced access to water and nutrients required by the host plant (Patten & Glick, 2002). Many PGPB strains also produce the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD), which catalyzes the hydrolysis of the immediate precursor of ethylene, 1- aminocyclopropane-1-carboxylic acid (ACC) to ammonia and a-ketobutyrate (aKB). Therefore, ACCD-producing PGPB can prevent the increase of stress-ethylene that otherwise would inhibit root growth under stressful conditions (Penrose & Glick, 2003). However, the beneficial effects of PGPB have generally been validated only in laboratory and/or greenhouse trials. In the field, results from studies examining the use of PGPB have been less consistent. This inconsistency in field results is likely associated with many factors including poor inoculum survival due to starvation, predation, and competition with indigenous bacteria, difficulty in obtaining good distribution of the inoculant in the soil profile, and variation in fitness in relation to variations in soil

properties, among others. Altogether, this has limited the widespread commercial adoption of PGPB biotechnologies. Inoculation trials with PGPB under field conditions are thus essential to assess inoculant performance and their potential ability to decrease the effects of environmental stresses on crop plants.

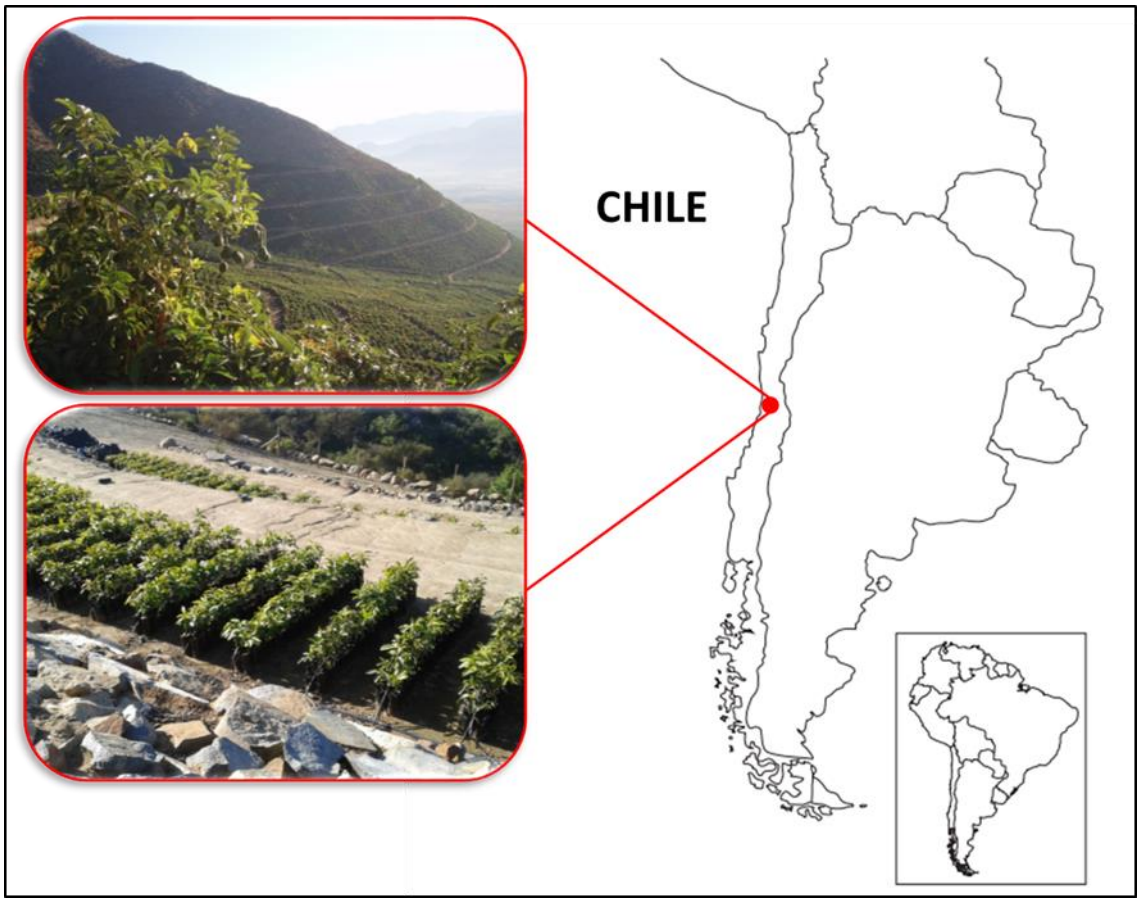

Figure 4.1. Field nursery assay carried out on commercial orchard of avocado 'Jorge Schmidt & Company Limited' located in the vicinity of the Llay Llay town, Valparaíso Region, Chile (32°51'19.5"S and 71°00'22.0"W).

Previously, we isolated some endophytic and rhizosphere bacterial strains from avocado trees, and formulated four halotolerant consortia with IAA- and ACCD- producing bacteria (Table 1). Inoculation trials under greenhouse conditions with these bacterial consortia showed soil drenches with inoculants increased the emergence, growth, biomass and superoxide dismutase (SOD) activity of wheat seedlings exposed to salt stress (Barra et al., 2016). Therefore, the objective of the present study was to validate the contribution of these bacterial

consortia on growth and antioxidant activity of avocado trees grown under salt stress and water shortage conditions in a commercial nursery from central Chile.

4.2. Materials and Methods.

4.2.1. Halotolerant bacterial consortia and inocula preparation.

Six endophytic bacterial strains and six rhizosphere bacterial strains were previously isolated from sterilized tissues and rhizosphere soil of avocado plants grown in a commercial orchard 'Jorge Schmidt & Co. Ltd.' in Valparaíso Region, Chile (32°47'S and 70°47'W) according to the process described by Barra et al. (2016). These isolates were identified based on partial sequencing of 16S rRNA genes. The gene segments were deposited in GenBank database under accession numbers KR066642 - KR066653. In parallel, four bacterial consortia (Table 1) were formulated with three IAA- and ACCD- producing bacteria each, as follows: Consortium C1 was isolated from avocado endosphere tissues and featured high IAA production and ACCD activity. Consortium C2 was formulated with *Serratia* sp. st. 16, *Pseudomonas* sp. st. 33 and *Microbacterium* sp. st 35, all also isolated from avocado endosphere tissues. The C2 was characterized by lower IAA production and low ACCD activity as compared to C1. Consortium C3 was formulated with strains from avocado rhizosphere soil and consisted of *Enterobacter* sp. st. 172, *Enterobacter* sp. st. 198 and *Enterobacter* sp. st. 206. Among all four consortia examined, C3 had the highest measured IAA production and ACCD activity. Consortium C4 was formulated with rhizosphere isolates *Achromobacter* sp. st. 249, *Serratia* sp. st. 343 and *Enterobacter* sp. st. 357 and had with lower IAA production and ACCD activity as compared to C3, the other rhizosphere consortium (Barra et al., 2016).

Inocula were prepared by growing the twelve bacterial strains separately in 2 L flasks containing 800 ml LB broth at 30°C for 16 h under shaking (120 rpm). The cells were collected by centrifugation (6,000 × g for 15 min at 4°C), washed twice with sterile saline solution

(0.85%) and then suspended in 10% skim milk. Bacterial cells were freeze dried separately with a Freeze Dry Systems (FreeZone, Labconco) according to procedure described by Jagannath et al., (2010) and the lyophilized bacterial strains were stored at room temperature for further inoculation assays.

4.2.2. Avocado tree nursery trial.

A field assay was conducted in an outdoor nursery of a commercial orchard (Jorge Schmidt & Company Ltd.) located near Llay Llay town, Valparaíso Region, Chile (32°51'19.5"S and 71°00'22.0"W) (Figure 1). The company is currently the largest Chilean producer of avocados cv 'Hass', exporting about 85% of their production to the European and USA market and 15% remaining in the local market.

One hundred and fifty vigorous avocado seedlings cv 'Hass' recently grafted on 'Mexícola' rootstocks of ~30 cm were selected from the commercial nursery. The seedlings were planted in plastic 5-L bags filled with sandy loam soil according to company protocol. The seedlings were irrigated to field capacity when soil moisture fell below -50 cbars as measured using tensiometers. Salinity of the water used for irrigation measured as electric conductivity (EC) was 0.654 dS m^{-1}. The chemical fertilizers applied in avocado orchards were 680 kg N ha^{-1} (applied in three split doses as urea [46%] or ammonium nitrate [22%]), 340 kg Zn ha^{-1} (applied as zinc sulfate) and 30 kg B ha^{-1} (applied as boric acid). The seedlings were randomly pooled in three groups of fifty seedlings each group. All seedlings were well irrigated and maintained under the same regimen for one month before inoculation with the consortia. Plant growth was restricted to the period between March of 2014 and March of 2015 with annual average temperature and total rainfall of 14.4 ºC and 236.8 mm in the region.

For the inoculation process, the twelve lyophilized strains were dissolved in sterile distilled water at final concentration of 10^8 CFU ml^{-1} according to agar plate-counting methods and the strains belonging to each of the four consortia (C1, C2, C3 and C4) were proportionally mixed. Roots of ten avocado seedlings of each group (treatment) were inoculated with freshly prepared inoculum once a month by drench application of 100 mL of each bacterial consortia. Responses to the inoculation treatments were compared to a control group consisting of ten uninoculated plants. Each group was subjected to different treatments for one year as follows: i) Control; seedlings irrigated according to company protocol (irrigated to field capacity twice a week); ii) Salt stress treatment; seedlings irrigated once a month with 340 mM (2%) NaCl solution for one year (Castro et al., 2009) and iii) Water shortage treatment; the seedlings were irrigated at half normal irrigation rates.

The trees were arranged in the nursery using a completely randomized factorial design. The experimental treatments were: i) 4 bacterial consortia (C1, C2, C3 and C4) plus uninoculated control (with 10 replicates each); ii) one salt stress treatment, one water shortage treatment plus well-irrigated control without NaCl (0 M).

4.2.3. Growth parameters determination.

After one year, leaf chlorophyll contents were measured and data were collected on the trunk diameter, shoot and root length, and shoot and root biomass. Immediately prior to harvest, leaf chlorophyll contents were quantified for the first fully expanded leaves by nondestructive sampling technique using a hand-held chlorophyll meter SPAD-502 (Minolta, K. Arano & Co. Ltd, Tokyo, Japan). This instrument provides a relative measurement of leaf chlorophyll through the evaluation of the changes of the transmittance in the 600–700 nm region of the visible spectra and in the near infrared region (San-Francisco et al., 2005). Mean leaf

chlorophyll content for each treatment was derived from three readings taken at the base, middle and tip of leaf 1, 2 and 3.

Trunk diameters were measured 2 cm above the graft union of each tree using dial calipers according Mickelbart & Arpaia (2002). For biomass determinations, plants were carefully removed from the pots and the root surfaces were carefully cleaned several times first with tap water and then with distilled water. Each plant was divided into root and shoot portions, and growth parameters including root and shoot length and biomass were measured for the harvested avocado plants. Fine roots (<1 mm) and fully expanded leaves were carefully separated, frozen in liquid N and kept at 80ºC to determine superoxide dismutase (SOD) activity and lipid peroxidation.

4.2.4. Superoxide dismutase activity.

Fresh subsamples of both roots and leaves of seedlings were stored (-80 ºC) and then processed to determine activities of the SOD dismutase (EC 1.15.1.1). To measure SOD activity, the stored subsamples were frozen in liquid N and ground with a mortar and pestle. The proteins were extracted with 50 mM potassium phosphate buffer (pH 7.0), centrifuged at 11,000 × *g* for 15 min (4°C), and the supernatant was collected as a crude extract. The SOD activity was determined according to the procedure described by Donahue et al. (1997) that measures inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of reduction of NBT at 560nm. SOD activity was calculated on a protein basis with the total amount of protein determined in the enzyme crude extract according to the Bradford' colorimetric assay.

4.2.5. Lipid peroxidation measurements.

Lipid peroxidation was determined by monitoring the thiobarbituric acid reactive substances (TBARS) in shoot tissues following the modified protocol of Du and Bramlage, (1992). For this purpose, 30 mg root and leaf tissues were homogenized and macerated with 500 ml 0.2% trichloroacetic acid (TCA) and then centrifuged at 10,000 × g for 5 min. Later, 200 ul of supernatant was mixed with 800 µl 0.5% thiobarbituric acid (TBA) in 20% TCA, the mixture was incubated at 95 ºC for 30 min, and then, it was rapidly cooled. The absorbance was measured at 440, 532 and 600 nm, to correct the interference generated by TBARS-sugar complexes. Finally, the malondialdehyde concentration was estimated by using an extinction coefficient of 84.152 $M^{-1}cm^{-1}$.

4.2.6. Statistical analysis.

The analysis of variance was performed with two factors (3×5): stress treatment (salt, water shortage and control) and consortia inoculation (C1, C2, C3, C4 and control), using ten replications per treatment. Data obtained from each treatments were analyzed statistically using the one-way ANOVA and the means were compared by the Duncan's test for multiple comparisons. Difference at $P \leq 0.05$ was considered as significant between treatments. The analyses were conducted using the IBM SPSS 21 software.

4.3. Results.

4.3.1. Vegetative growth.

The effects of the consortia inoculation on growth and biomass accumulation for roots and shoots of avocado seedlings that were subjected to salt stress for one year are illustrated in

Figure 2. In general, aerial and root lengths of seedlings under salt stress were significantly ($P \leq 0.05$) decreased by 23.0% and 27.9%, respectively, compared with the well-irrigated seedlings in the control treatment. Avocado seedlings grown under water shortage had significantly ($P \leq 0.05$) 28.2% and 32.0% shorter aerial part and root lengths, respectively, than control seedlings grown in nonsalinized soil (Figure 2a). With respect to biomass accumulation, only the fresh weights of aerial part were significantly ($P \leq 0.05$) lower by 25.5% in salt stress treatment as compared with the control. Whereas, avocado seedlings grown under water shortage, showed significantly ($P \leq 0.05$) lower fresh weights of aerial part and roots by 36.4% and 34.0%, respectively, compared with the well-irrigated seedlings in the control treatment (Figure 2b).

With respect to the inoculation of the trees with bacterial consortia, the results were variable depending on the compositions of the individual consortia for all of the growth parameters that were measured. Of the four consortia, the C3 and C4 were the more efficient increasing the growth and biomass accumulation of avocado plants under both treatment (Figure 2a and 2b). In general, inoculated seedlings showed greater root lengths than uninoculated avocado seedlings. Therefore, C3 and C4 increased significantly ($P \leq 0.05$) by 34.4% and 64.4% the root lengths and by 19.8% and 20.6% the aerial parts of avocado seedlings under salt stress in comparison to the uninoculated control trees. In water shortage treatment, only C4 increased significantly ($P \leq 0.05$) by 26.2% the aerial lengths of avocado seedlings in comparison to the uninoculated control trees. In relation to biomass, the inoculated seedlings subjected to salt stress and water shortage conditions also had greater aerial and root fresh weights than uninoculated seedlings. However, significant differences were only found for some bacterial consortia, especially C4. Thus, compared to the inoculated control, the consortium C4 significantly increased ($P \leq 0.05$) the aerial and root fresh weights of seedlings under salt stress by 43.1% and 59.4%, respectively, and the root fresh weights of seedlings under water shortage

by 68.4%. Similarly, the C3 significantly increased ($P \leq 0.05$) the root fresh weights of seedlings under salt stress by 54.6%.

On the other hand, the results illustrated in Figure 2c shows that trunk diameters of well-irrigated control and seedlings growing under salt stress and water shortage were not significantly different ($P \leq 0.05$). Although in general trees inoculated with the bacterial consortia had greater trunk diameters than the uninoculated seedlings, only C1 and C3 were able to significantly increase ($P \leq 0.05$) the trunk diameters of seedlings growing under salt stress and water shortage by 15.9% and 16.1%, respectively, in comparison to the control treatment.

4.3.2. Chlorophyll content of avocado seedlings.

The chlorophyll content of inoculated avocado seedlings grown under salt stress and water shortage are illustrated in Figure 3a. Thus, the results show that chlorophyll contents were significantly ($P \leq 0.05$) decreased in avocado seedlings grown under salt and water shortage by 22.6% and 21.1%, respectively, as compared with uninoculated seedlings. All inoculated seedlings with the bacterial consortia had greater chlorophyll contents than the uninoculated seedlings under both salt stress and water shortage. However, in salt stress, only C1 and C4 were able to increase significantly ($P \leq 0.05$) the chlorophyll content by 23.4% and 25.4%, respectively. In contrast, in water shortage, only the C2 and C3 increased chlorophyll content significantly ($P \leq 0.05$) by 22.1% and 19.6%, respectively, when compared to the uninoculated control treatment.

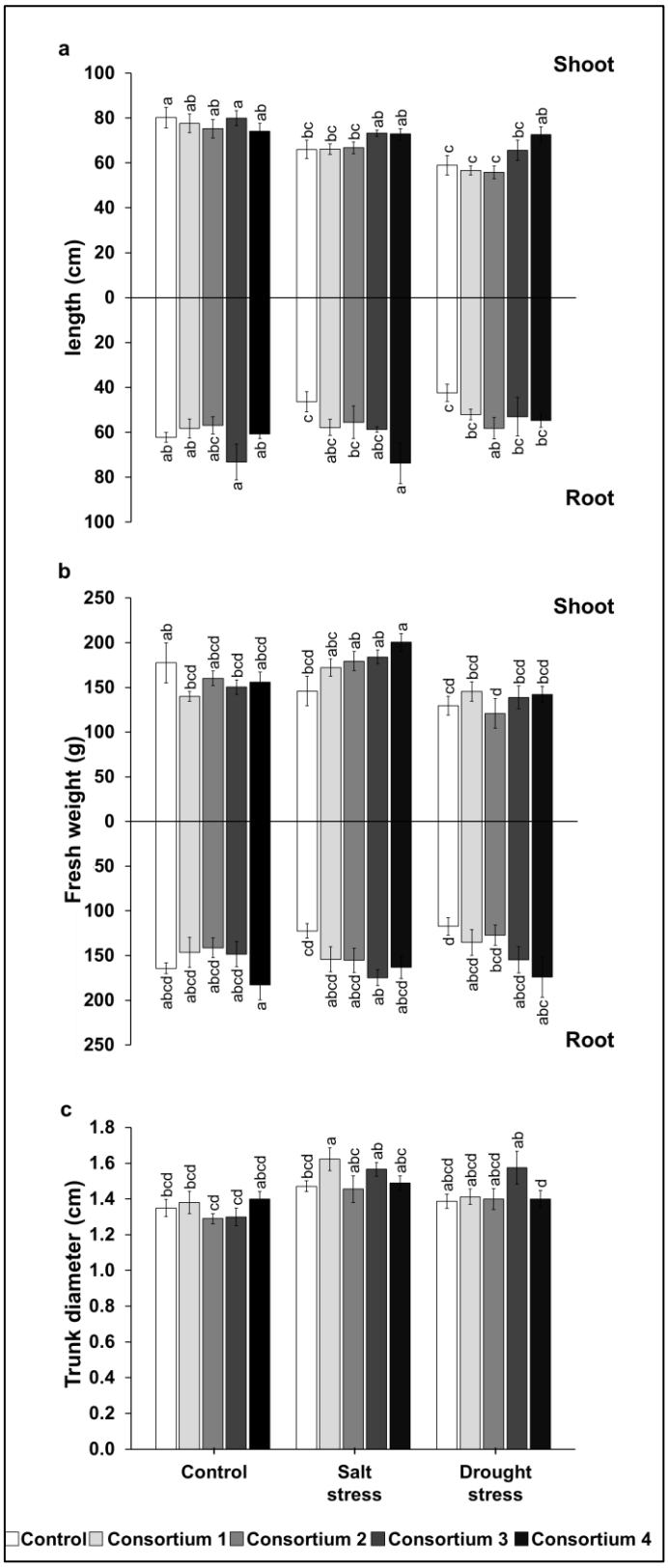

Figure 4. 2. Effects of water shortage and salt stress treatments on (**a**) growth (length); (**b**) biomass accumulation (fresh weight) of both root and shoot and (**c**) trunk diameter of avocado seedlings inoculated with four selected bacterial consortia. Vertical bars represent average (n=10) ± standard error. Different letters denote significant difference ($P \leq$ 0.05; Duncan's test) between consortia treatments.

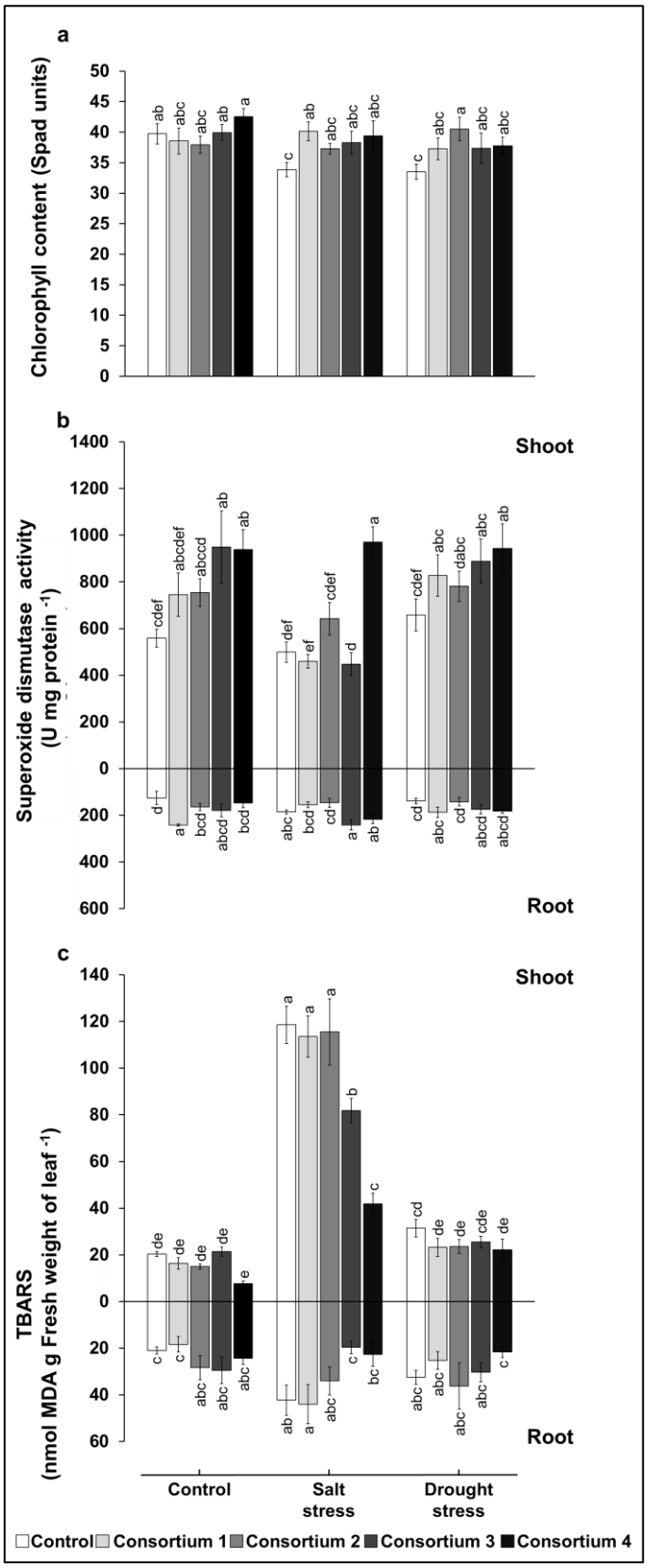

Figure 4.3. Effects of water shortage and salt stress treatments on (**a**) leaf chlorophyll content (**b**) root and shoot superoxide dismutase (SOD) activity and (**c**) root and shoot TBARS content of avocado seedlings inoculated with four selected bacterial consortia. Vertical bars represent average (n=10) ± standard error. Different letters denote significant difference ($P \leq 0.05$; Duncan's test) between consortia treatments.

4.3.3. Superoxide dismutase (SOD) activity.

The effects of salt stress and water shortage on SOD activity of root and leaves of inoculated avocado seedlings are illustrated in Figure 3b. The SOD activities of avocado leaves were not significantly different ($P \leq 0.05$) between the well-irrigated control treatment and avocado seedlings that were subjected to salt and water shortage. In contrast to results with leaf tissues, root SOD activities of uninoculated seedlings under salt stress were significantly greater than the control by 48%. Inoculation of avocado plants with the selected bacterial consortia showed variable results. Inoculation with C4 significantly ($P \leq 0.05$) increased SOD activity in leaves by 68%, 95% and 43% of both the control and seedlings grown under salt stress and water shortage, respectively. Similarly, C3 significantly stimulated SOD activity ($P \leq 0.05$) as compared to the control seedlings by 70%. The other tested consortia also appeared to increase SOD activities of the leaves, as compared to control seedlings and seedlings grown under water shortage conditions, although no significant differences were found with respect to the uninoculated control. Curiously, the root SOD activities of avocado seedlings under stress were not significantly affected ($P \leq 0.05$) by bacterial inoculation. Only C1 increased the SOD activity of avocado seedling in the control treatment by 93%.

4.3.4. Lipid peroxidation.

The effects of salt stress and water shortage treatments on lipid peroxidation of leaves and roots of avocado seedlings were determined by measuring changes in the content of thiobarbituric acid reactive substances (TBARS). The results illustrated in Figure 3c show that TBARS in both leaves and roots of avocado seedlings subjected to salt stress were significantly ($P \leq 0.05$) increased by 482% and 101% as compared with the well-irrigated control. Although TBARS contents in leaves and roots of avocado seedlings under water shortage stress were increased by 54%, there were no significant differences ($P \leq 0.05$) with seedlings in the well-irrigated

control treatment. With respect to the effects of bacteria consortia inoculation, C4 was again the most efficient bacterial consortia for decreasing the effects of stress as determined by TBARS content (Figure 3c). In this context, C4 decreased significantly ($P \leq 0.05$) TBARS content in leaves of control plants and of seedlings grown under salt stress by 62% and 65%, respectively. Similarly, C3 significantly reduced ($P \leq 0.05$) the TBARS content of leaves and roots of avocado seedlings under salt stress by 31% and 53%.

4.4. Discussion.

Plants vary widely in their sensitivity to salt and drought stresses, with avocado plants being the most salt-sensitive of cultivated fruit tree species (Oster et al., 2007). Results of the present study confirmed that both water shortage and salt stress significantly affected root and aerial length, aerial fresh weight and chlorophyll content of avocado seedlings. In contrast, under the conditions of this experiment, only water shortage reduced the root fresh weights. In agreement with these findings, Bernstein and Meiri (2004) determined that root and shoot growth of avocado plants decreased by 43% and 10%, respectively, when irrigated with 15 mM NaCl. With respect to water shortage, there are conflicting results in the literature. Thus, a field experiment in avocado plants carried out by Oster et al. (2007) determined that water shortage had no significant effects on the growth and biomass accumulation of mature avocado trees: while, yield was significantly ($P \leq 0.05$) decreased. Whereas, Chartzoulakis et al. (2002) determined in a greenhouse assay that moderate water stress reduced significantly ($P \leq 0.05$) the total plant leaf area by 69% and the total plant dry weight by 80% of avocado plants cv. ‘Hass’. On the other hand, it is well documented that plants exposed to stressful environments often have decreased chlorophyll contents, such that this parameter is widely used as an index to indicate the abiotic sensitivity level in plants (Nadeem et al. 2006; Zahiret al. 2009; Qiu et al. 2014). Similar to our findings, decreases in the chlorophyll contents of avocado plants under

salt stress has previously been reported by Mickelbart and Arpaia (2002). With respect to trunk diameter, our results showed that trunk diameters were not significantly ($P \leq 0.05$) affected for trees subjected to salinity and water shortage. Similar results were reported by Silber et al. (2013), who determined in a field study that the trunk diameters of avocado plants were not affected by irrigation water shortage.

Our results also showed that leaf and root TBARS were increased under salt stress by five and two fold, respectively, as compared with the well-irrigated control, demonstrating that the integrity of the cell membranes was seriously damaged. It should be noted although the root and leaf TBARS contents of seedlings under water shortage were over 54% greater than the control, this difference was not significant. Increased SOD activity is correlated with increased protection from damage associated with oxidative stress (Qiu et al., 2014). Contrary to expectations, in the present study no significant differences were found in the SOD activity between control and stressed seedlings. Only the root SOD activities of salt stressed plants were significantly increased. These results could be due to direct damage of proteins integrity, and therefore SOD integrity, produced by ions. However, variation in antioxidant concentrations are dependent on the severity and duration of the stress and the species and age of the plant. Differences in protective enzyme activities are known for a number of species (Hernandez & Almansa, 2002; Abogadallah, 2010). For example, overexpression of SOD occurs in stressed wheat seedlings (Sairam & Srivastava, 2002; Ruan, 2002; Sairam et al., 2005; Barra et al., 2016). Whereas, others researchers have determined a decrease in SOD activity in wheat seedlings under salt and drought stresses (Qiu et al., 2014; Gallé et al., 2013). In contrast, Yu and Rengel (1999) described that SOD activity in lupin was not affected by salt stress, whereas it was increased by 17% in plants subjected to drought stress, indicating that different mechanisms may be involved in oxidative stress injury caused by drought and salt.

Inoculation of avocado seedlings with the bacterial consortia had variable but several significant effects on growth and biochemical markers of stress in the plant tissues. Some of the selected consortia were able to enhance growth, biomass accumulation and chlorophyll content of stressed seedlings. Thus, the consortia formulated with rhizobacteria, C4 and C3, were the more efficient alleviating the stress effects. Thereby, C4 significantly increased aerial and root length; aerial and root fresh weight and chlorophyll content of salt stressed seedlings. The C4 also increased aerial length and root fresh weight of seedlings under water shortage. Whereas, C3 increased aerial and root length and root fresh weight of salt stressed seedlings; and also increased the trunk diameter and chlorophyll content of seedlings under water shortage. In contrast, the endophytic were less efficient than rhizosphere consortia. Thus, C1 only increased the trunk diameter and chlorophyll content of salt stressed seedlings and C2 increased the chlorophyll content of avocado seedlings under water shortage.

The observed growth promotion of avocados seedlings by these bacteria in the present study can be attributed to two main reasons. First, the IAA released by the isolates may directly stimulate root cell elongation and lateral root growth, increasing the root surface area, and consequently, the ability to acquire water and nutrients (Vessey, 2003; Marques et al., 2010). In addition, previous studies have also shown that application of exogenous IAA to plants directly stimulates chlorophyll production (Sharma & Sardana, 2012; Hayat et al., 2001), probably by improved iron acquisition. Siderophore production may also contribute to iron mobilization in the rhizosphere. Secondly, in accord with general models, the avocado plants subjected to stress conditions will accumulate and cycle ACC in the rhizosphere, which is released in the root exudates and reabsorbed as with organic acids. This temporary extracellular ACC provides a nitrogen source for proliferation of both native or inoculated ACCD-producing bacteria (Grichko & Glick, 2001). The ACCD-producing bacteria act as a sink for ACC, the immediate biosynthetic precursor of ethylene, thereby decreasing plant ethylene levels and its detrimental effects on plant development (Glick et al., 2007).

Our findings also suggest that bioaugmentation with certain PGPB introduced through the irrigation water may enhance the oxidative stress tolerance of avocado seedlings. Here, inoculation with C4 significantly increased SOD activity and decreased TBARS content in leaves of salt stressed seedlings; increased the SOD activity of plants under water shortage; and decreased the TBARS content of leaves from nonstressed seedlings. These results clearly demonstrated ability of C4 to ameliorate stress by inducing physiological protection of plants against oxidative damage, being able to decrease by over 60% the lipid peroxidation both in control and salt stressed plants. This effect is attributed to SOD activity, which was widely increased in both treatments. In addition, lower cellular damage would have induced the higher growth and biomass accumulation observed in this study.

Despite these results, we cannot conclude the degree to which the increase in SOD activity is a consequence of the improvement in stress tolerance (led by stress ethylene reduction), or vice versa, whether the improvement in salt stress tolerance is a consequence of the increase in SOD activity. A third option would be that the bacterial consortia independently stimulate both mechanisms. There is little and somewhat contradictory information regarding the mechanism by which PGPB are able to increase the activity of antioxidant enzymes, such as SOD. In this context, Wang et al. (2012) determined that inoculation with consortia bacteria (formulated with *Bacillus cereus, B. subtilis* and *Serratia* sp.) conferred induced systemic tolerance to drought stress in cucumber plants, by protecting plant cells, maintaining photosynthetic efficiency and root vigor and increasing some of antioxidant activities, without involving the action of ACCD to lower plant ethylene levels. Whereas, Gururani et al. (2012) determined by quantitative PCR approach that inoculation of *Solanum tuberosum* with two *Bacillus* spp. improved the gene expression of different ROS-scavenging enzymes of plants under salt and heavy-metal stress. Whereas, a proteomic approach of *Cucumis sativus* under anoxic stress revealed that inoculation with ACCD-producing bacteria *P. putida* UW4 triggered a downregulation of enzyme (Li et al., 2013). Despite this, our results are consistent with previous

research showing that inoculation with PGPB increase the SOD activity and decrease TBARS of cotton plants under salt stress conditions inoculated with *Klebsiella oxytoca* (Wu et al., 2014) and soybean growing under drought stress inoculated with *Azospirillum brasilense* and *Azotobacter chrococcum*.

It is noteworthy that in a prior research, the bacterial inoculants used here significantly ($P \geq 0.05$) increase the emergence, growth, biomass and SOD activity of wheat seedlings exposed to salt stress (Barra et al., 2016). In contrast to present study, the previous results obtained in wheat plants showed that endophytic consortia and C3 were more efficient decreasing the salt stress effects than C4 (Barra et al., 2016). The lower effects of endophytic consortia observed in avocado plants would be because were inoculated in the rhizosphere soil where endophytic bacteria were less competitive than rhizobacteria. Further studies using different inoculation methods are necessary to confirm this hypothesis.

Consortium C4, the most effective bacteria consortium, was formulated with halotolerant rhizobacterial strains with lower IAA production and ACCD activity, which were identified as *Achromobacter* sp., *Serratia* sp. and *Enterobacter* sp. Some previous studies have shown the occurrence of IAA- and ACCD-producing *Achromobacter* spp. that were associated with *Catharanthus roseus* (Karthikeyan et al., 2012) and *Citrus reticulata* (Thokchom et al., 2014). IAA- and ACCD-producing *Achromobacter xylosoxidans* also has been reported to increase the biomass and growth of *Ocimum sanctum* plants subjected to waterlogging stress (Barnawal et al., 2012). Similarly, previous studies have reported the occurrence of IAA- and ACCD-producing *Enterobacter* spp. associated with diverse plant species, such as *Citrus reticulate* (Thokchom et al., 2014), *Populus* spp. (Taghavi et al., 2009), *Piper nigrum* (Jasim et al., 2013), showing also plant growth-promoting abilities. Thus, Thokchom et al. (2014), in a greenhouse experiment determined significantly increased shoot length, shoot and root dry biomass of *Citrus reticulate* seedlings inoculated with *Enterobacter* spp. over the uninoculated control. In

addition, some studies have described both endophytic and rhizosphere *Serratia* spp., with PGP capacity, associated with some plants such as rice (Gyaneshwar et al., 2001) bean (Saïdi et al., 2013) poplar trees (Taghavi et al., 2009) and tomato and peppers (Amaresan et al., 2011). Furthermore, Zahir et al., (2009) determined that ACCD-producing halotolerant *Serratia proteamaculans* significantly improved the growth and yield of wheat under salt-stress condition. Independently of ACCD activity and IAA production, the taxonomic affiliations of our selected isolates are commonly associated with PGPB.

In general plant inoculation studies with bacterial consortia have been carried out under greenhouse conditions. In this way, it is important to note that isolates used in this study have been tested in avocado seedlings grown in a nursery located outdoors and within the commercial avocado orchard, and therefore, with the same environmental conditions of avocado crops. For these reasons, the results of our study are a close approximation to actual field conditions. The present study is the first showing the positives effects of bacterial inoculation on growth of any tree fruit growing in a nursery under stress conditions. However, with the final objective of formulating a biofertilizers product, the next step would be to produce inoculants testing different vehicles for soil inoculation. Finally, the selected consortia still need to be tested in actual field trials with bearing trees to determine the survival of the isolates in the rhizosphere, and their economic effects on avocado growth under stress conditions.

4.5. Conclusions.

Our results show that avocado trees harbor halotolerant IAA- and ACCD-producing bacteria that are able to mitigate the effects of water shortage and salt stress on avocado tree seedlings grown in a commercial nursery. In this way, the selected IAA- and ACCD-producing consortia increased SOD activity, which resulted in lower oxidative damage and consequently, higher growth, biomass accumulation and chlorophyll contents. Therefore, our isolates could be used

as a suitable bioinoculants for avocado plants subjected to water scarcity or grown under salt affected area. To our knowledge, this is the first study showing the beneficial effect of bacterial inoculation on growth of avocado plants under both water shortage and salt stress conditions. Our findings in nursery conditions endorse the need for further field studies on avocado yields of with mature orchards, and hold promise for enhancing avocado trees tolerance under increasingly stressful conditions expected from global warming.

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Chapter V

General discussion and conclusions

5.1. General discussion.

Drought due to shortage of water is critical for crop production in large agronomic areas worldwide and it is usually coped with extensive irrigations (Golldack et al. 2011). Poor quality water is often used for irrigation, so that eventually salt builds up in the soil, which consequently triggers soil salinization (Bui 2013). Nowadays, over 6% of the world's total land area and around 20% of the world's irrigated lands are salt affected (Munns and Tester 2008; Ahmad 2014; Panta et al. 2014). Although irrigated land corresponds only to 15% total cultivated land, its importance lies in this land producing one third of the world's food (Munns and Tester 2008). Drought and salinity are the two main environmental factors that adversely affect plant growth and development and have a crucial impact on agricultural productivity and yields (Athar and Ashraf 2009).

Among Chilean crops, avocado is known to be the most salt-sensitive cultivated fruit tree, being also sensitive to water shortage (Chartzoulakis et al. 2002; Bernstein and Meiri 2004). Avocado production is of great economic importance for Chile, However, the avocado production has decreased considerably, mainly due to rainfall decline. This problem could be increased because it has been projected that rainfall will continue declining as concequence of global climatic change; consequently, greater irrigation rates will be required with subsequent soil salinization (Neuenschwander 2010). Therefore, development of sustainable strategies to improve avocado crop yields under stress conditions is crucial for adjusting agricultural production to climate change. An attractive and environmental friendly strategy to mitigate stress effects on crops is the use of PGPB soil inoculants. In this way, we hypothesized endophytic and rizospheric PGPB producers of IAA and ACCD improve water shortage and salt stress tolerance of avocado seedlings. To evaluate this hypothesis we isolated, characterized, identified and selected a group of halotolerant IAA- and ACCD- producing endophytic and rhizosphere bacteria associated with avocado trees growing in a commercial orchard. To our knowledge, there are no previous

published studies describing the indigenous endophytic bacteria present inside tissues of avocado plants.

Twelve bacterial strains were selected and four bacterial consortia were formulated with three strain each (Table 3.2.). The selected isolates belonged to five different bacterial genera: *Enterobacter*, *Serratia*, *Pseudomonas, Microbacterium* and *Achromobacter*. The most efficient IAA- and ACCD-producing strains were identified as *Enterobacter* genus, which came from two different sampling sites and were isolated from roots, leaves and rhizosphere soil. These interesting results demonstrated the wide distribution of IAA- and ACCD-producing *Enterobacter* spp. and their close association with avocado trees. These findings allowed us to assume that IAA- and ACCD-producing *Enterobacter* spp. could efficiently colonize avocado plants. Independently of ACCD activity and IAA production, the taxonomic affiliations of our selected isolates have been commonly associated with PGPB of *Citrus reticulate, Populus* spp. *Piper nigrum Catharanthus roseus* (Jasim et al., 2013; Karthikeyan et al., 2012; Qin et al., 2013; Taghavi et al., 2009; Thokchom et al., 2014; Trivedi et al., 2011). With respect to freeze-drying process, the bacteria viabilities were lower than 78% these are acceptable for our purpose, the transport and inoculation of bacterial consortia. However, further studies are needed to determine the optimal conditions for freeze-drying process of bacteria.

Wheat plants were chosen as the test plant for evaluation and validation *in vitro* of bacterial consortia effects on mitigation salt stress. Results of the inoculation experiments showed that all of the formulated consortia increased the percentages of wheat seedling emergence under salt stress conditions, particularly consortia C1, C2 and C4 (Figure 3.2.). Growth and biomass of roots and shoots were gradually reduced with increasing salt stress, which generally is due to increased ethylene levels. In general, our formulated consortia increased the growth and biomass of shoots and roots in seedlings exposed to salt stress. The consortia formulated with bacteria that produced the highest amounts of IAA and ACCD were significantly more effective

for mitigating wheat salt stress effects and improving their growth than those formulated with bacteria having lower IAA production and ACCD activity. In addition, our results clearly demonstrated that both endophytic and rhizosphere bacterial consortia with higher IAA production and ACCD activity were also able to significantly increase wheat SOD activity, which could result in lower oxidative stress in plants.

Posterior to validation in greenhouse with wheat as test plant, the bacterial consortia were tested in a nursery trial of avocado plant growing under salt and water shortage. Results of the present study confirmed that water shortage and salt stress significantly affected root and shoot length and chlorophyll content of avocado plants. Some of the selected consortia, specially the rhizosphere consortia, were able to enhance significativelly growth and biomass accumulation of both control and stressed avocado seedlings and chlorophyll content of stressed seedlings, with C4 being the most efficient consortium for lowering biochemical measures of stress effects. Thereby, the C4 significantly increased aerial and root length; aerial and root fresh weight and chlorophyll content of salt stressed seedling; and the aerial length and root fresh weight of seedlings under water shortage. Whereas, C3 increased significantly ($P \leq 0.05$) aerial and root length and root fresh weight of salt stressed seedlings; and also increased the trunk diameter and chlorophyll content of seedlings under water shortage. In contrast, the endophytic consortia were less efficient than rhizosphere consortia. Thus, C1 only increased the trunk diameter and chlorophyll content of salt stressed seedlings and C2 increased the chlorophyll content of avocado seedlings under water shortage.

The observed growth promotion of wheat in greenhouse assay as well as avocado seedlings in nursery trial by selected consortia can be attributed to two main reasons. First, the IAA released by the isolates may directly stimulate root cell elongation and lateral root growth, increasing the root surface area, and consequently, the ability to acquire water and nutrients (Marques et al., 2010; Vessey, 2003). Secondly, in accord with general models, the avocado plants that were

subjected to stress conditions will accumulate and cycle ACC in the rhizosphere which is released in the root exudates and reabsorbed as with organic acids. This temporary extracellular ACC provides a nitrogen source for proliferation of both native or inoculated ACCD-producing bacteria (Grichko and Glick, 2001). The ACCD-producing bacteria act as a sink for ACC, the immediate biosynthetic precursor of ethylene, thereby decreasing plant ethylene levels and its detrimental effects on plant development (Glick et al., 2007a).

In this study also was showed that TBARS of avocado leaves and roots were increased under salt stress by five and two fold, respectively, as compared with the well-irrigated control, demonstrating that integrity of cell membranes were seriously damaged. Increased SOD activity is correlated with increased protection from damage associated with oxidative stress (Qiu et al., 2014). Contrary to expectations, only the root SOD activities of salt stressed plants were significantly increased. These results could be due to the direct damage of proteins integrity (and therefore SOD integrity) produced by ions. However, variation of antioxidant concentrations will be dependent on the severity and duration of the stress and the species and age of the plant. Differences in protective enzyme activities are known for a number of species (Abogadallah, 2010; Hernández and Almansa, 2002). The effects of inoculation with selected bacterial consortia showed interesting results that suggest bioaugmentation with certain PGPR that are introduced through the irrigation water may enhance the oxidative stress tolerance of avocado seedlings. Here, inoculation with C4 significantly increased SOD activity and decreased TBARS content in leaves of salt stressed seedlings; increased the SOD activity of drought stressed plants; and decreased the TBARS content of leaves from nonstressed seedlings. These results clearly demonstrated ability of C4 to ameliorate stress by inducing physiological protection of plants against oxidative damage, being able to decrease by over 60% the lipid peroxidation both in control and salt stressed plants. This effect is attributed to SOD activity, which was widely increased in both treatments. Inoculation with C3 also showed interested results, decreasing TBARS content in leaves and roots of salt stressed seedlings and

increased SOD activity of control seedling leaves. However, there was no correlation with SOD activity in the salt stress treatment, where the SOD activity was not significantly increased. Further studies are necessary to clarify this contradiction. Despite these results, we cannot conclude the degree to which the increase in SOD activity is a consequence of the improvement in stress tolerance (led by stress ethylene reduction), or vice versa, whether the improvement in salt stress tolerance is a consequence of the increase in SOD activity. A third option would be that the bacterial consortia stimulate independently both mechanisms. There is little, and somewhat contradictory, information regarding the mechanism by which PGPB are able to increase the activity of antioxidant enzymes, such as SOD.

It is noteworthy that in contrast to results in avocado, the results obtained in wheat plants showed that endophytic consortia and C3 were more efficient decreasing the salt stress effects than C4 (Barra et al., 2016). The lower effects of endophytic consortia observed in avocado plants would be because they were inoculated in the rhizosphere soil where endophytic bacteria were less competitive than rhizobacteria. In the same way, we attribute this effect to specifity relationship between strains of C4 with avocado roots. Further studies using different inoculation methods and marked strains to determine the specificity of the relationship are necessary to confirm these hypotheses.

Finally, it is important to note that this is the first study showing the positives effects of bacterial inoculation on growth of any tree fruit growing under stress in nursery conditions. The isolates used in this study have been tested in avocado seedlings growing in a nursery located outdoors and within the commercial avocado orchard, and therefore, with the same environmental conditions of avocado crops. For these reasons, the results of our study are a close approximation to actual field conditions. However, with the final objective of formulating a biofertilizers product, the next step would be to produce inoculants testing different vehicles for soil inoculation. Finally, the selected consortia still need to be tested in field trials with

bearing trees to determine the survival of the isolates in the rhizosphere, and their economic effects on avocado growth under stress conditions.

5.2. General conclusions and future directions.

This is the first report describing the presence of endophytic bacteria containing phytostimulator mechanisms in avocado trees. Our results have demonstrated the close association of avocado trees and their rhizosphere soils with halotolerant bacteria, with 92.0% endophytic and 67.1% rhizosphere bacteria able to grow at concentrations equal to or above than 5% NaCl. These strains showed variable activity of the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD) and production of the phytohormone indole acetic acid (IAA). Among 309 isolates, 17.4% were characterized as halotolerant IAA- and ACCD-producing bacteria with range of 1.7-63.2 μg ml^{-1} and 0.18-3.63 μmol αKB mg $protein^{-1}$ h^{-1}, respectively. Based on isolation source (endosphere or rhizosphere), IAA production (higher or lower) and ACCD activity (higher or lower) of isolates, four bacterial consortia with three strain each were formulated containing members of five genera: *Enterobacter, Serratia*, *Microbacterium*, *Pseudomonas* and *Achromobacter*. The twelve selected strains showed 65.9-78.3% viability, immediately after of freeze drying process. In general, wheat plant inoculation by different methods with the four formulated bacterial consortia ameliorated the effect of salt (NaCl) stress determined by the emergence, growth and biomass of wheat seedlings under growth chamber and greenhouse conditions. At higher salt stress, bacterial consortia from endosphere were more efficient than those from rhizosphere to promote the growth and biomass of seedlings. The inoculation methods also affected seedling emergence, growth and biomass of seedlings under salt stress. Similarly, the inoculation of bacteria consortia increase the superoxide dismutase (SOD) activity of wheat seedlings under salt stress.

Our study show that halotolerant IAA- and ACCD-producing bacteria, isolated from adult avocado trees, are able to mitigate the effects of water shortage and salt stress on avocado tree seedlings grown in a commercial nursery under field conditions. In this way, the selected IAA- and ACCD-producing consortia increased SOD activity, which resulted in lower oxidative damage and consequently, higher growth, biomass accumulation and chlorophyll contents.

Thus, we demonstrated that use of beneficial bacteria is a promising approach to control salt stress in wheat and water shortage and salt stress in avocado seedings. We also suggest that bacterial inoculation has a strong impact on several plant stress tolerance mechanisms that altogether result in improved homeostatic mechanisms upon stress challenge. This may be due to a combination of morphological, physiological, and metabolic effects on the host plant brought by the beneficial bacteria. Therefore, our isolates could be used as a suitable bioinoculants for avocado plants subjected to water scarcity or grown under salt affected area, although, it is still necessary to determine optimal inoculations conditions in the field. To our knowledge, this is the first study showing the beneficial effect of bacterial inoculation on growth of avocado plants under both water shortage and salt stress conditions. Finally, our findings in nursery conditions endorse the need for further field studies on avocado yields with mature orchards, and hold promise for enhancing avocado trees tolerance under increasingly stressful conditions expected from global warming.

References

References.

Abogadallah, G.M., 2010. Antioxidative defense under salt stress. Plant Signal. Behav. 5, 369–374.

Ahmad, P., 2014. Physiological mechanisms and adaptation strategies in plants under changing environment. Springer, New York. 376 pp.

Ait Barka, E., Nowak, J., Clément, C., 2006. Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia* phytofirmans strain PsJN. Appl. Environ. Microbiol. 72, 7246–7252.

Ali, B., Sabri, A., Ljung, K., Hasnain, S., 2009. Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum* L. Lett. Appl. Microbiol. 48, 542–547.

Amaresan, N., Jayakumar, V., Kumar, K., Thajuddin, N., 2011. Isolation and characterization of plant growth promoting endophytic bacteria and their effect on tomato (*Lycopersicon esculentum*) and chilli (*Capsicum annuum*) seedling growth. Ann. Microbiol. 62, 805–810.

Andreolli, M., Lampis, S., Zapparoli, G., Angelini, E., Vallini, G., 2016. Diversity of bacterial endophytes in 3 and 15 year-old grapevines of *Vitis vinifera* cv. *Corvina* and their potential for plant growth promotion and phytopathogen control. Microbiol. Res. 183, 42–52.

Argueso, C., Hansen, M., Kieber, J., 2007. Regulation of ethylene biosynthesis. J. Plant Growth Regul. 26, 92–105.

Arkhipova, T., Prinsen, E., Veselov, S., Martinenko, E., Melentiev, A., Kudoyarova, G., 2007. Cytokinin producing bacteria enhance plant growth in drying soil. Plant Soil 292, 305–315.

Arkhipova, T., Veselov, S., Melentiev, A., Martynenko, E., Kudoyarova, G., 2005. Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. Plant Soil 272, 201–209.

Arora, N.K., 2013. Plant Microbe Symbiosis: Fundamentals and Advances. Springer India, New Delhi. 459 pp.

Arshad, M., Shaharoona, B., Mahmood, T., 2008. Inoculation with *Pseudomonas* spp. containing ACC-Deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of Pea (*Pisum sativum* L.). Pedosphere 18, 611–620.

Athar, H., Ashraf, M., 2009. Strategies for crop improvement against salinity and drought stress: An Overview, in: Ashraf, M., Ozturk, M., Athar, H. (Eds.), Salinity and water stress: Improving Crop Efficiency. Springer, Netherlands, pp. 1–16.

Atzorn, R., Crozier, A., Wheeler, C., Sandberg, G. 1988. Production of gibberellins and Indole 3-acetic acid by *Rhizobium phaseoli* in relation to nodulation of *Phaseolus vulgaris* roots. Planta 175, 532–538

Baca, B., Elmerich, C., 2007. Microbial production of plant hormones, in: Elmerich, C., Newton, W. (Eds.), associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations. Springer, Netherlands, pp. 113–144.

Bacon, C., Hinton, D., 2006. Bacterial endophytes: the endophytic niche, its occupants, and its utility, in: *Gnanamanickam*, S.S. (Ed.), Plant-Associated Bacteria. Springer, Netherlands, pp. 155–194.

Bacon, C., White. J. 2000. Microbial endophytes. Marcel Dekker Inc., New York, N.Y. 500 pp.

Bais, H., Park, S.-W., Weir, T., Callaway, R., Vivanco, J., 2004. How plants communicate using the underground information superhighway. Trends Plant Sci. 9, 26–32.

Bajguz, A., Piotrowska, A., 2009. Conjugates of auxin and cytokinin. Phytochemistry 70, 957–969.

Bakker, P.A.H.M., Ran, L.X., Mercado-Blanco, J., 2014. Rhizobacterial salicylate production provokes headaches. Plant Soil 382, 1–16.

Bal, H.B., Nayak, L., Das, S., Adhya, T.K., 2012. Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil 366, 93–105.

Bandurska, H., 2013. Salicylic Acid: An update on biosynthesis and action in plant response to water deficit and performance under drought, in: Hayat, S., Ahmad, A., Alyemeni, M.N. (Eds.), Salicylic Acid. Springer Netherlands, Dordrecht, pp. 1–14.

Barea, J.M., Brown, M.E., 1974. Effects on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. J. Appl. Microbiol. 37, 583–593.

Barnawal, D., Bharti, N., Maji, D., Chanotiya, C.S., Kalra, A., 2014. ACC deaminase-containing *Arthrobacter protophormiae* induces NaCl stress tolerance through reduced ACC oxidase activity and ethylene production resulting in improved nodulation and mycorrhization in *Pisum sativum*. J. Plant Physiol. 171, 884–894.

Barnawal, D., Bharti, N., Maji, D., Chanotiya, C.S., Kalra, A., 2012. 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase-containing rhizobacteria protect *Ocimum sanctum* plants during waterlogging stress via reduced ethylene generation. Plant Physiol. Biochem. 58, 227–235.

Barra, P.J., Inostroza, N.G., Acuña, J.J., Mora, M.L., Crowley, D.E., Jorquera, M.A., 2016. Formulation of bacterial consortia from avocado (*Persea americana* Mill.) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress. Appl. Soil Ecol. 102, 80–91.

Bashan, Y., de-Bashan, L.E., 2010. How the plant growth-promoting bacterium *Azospirillum* Promotes Plant Growth — A Critical Assessment. Adv. Agron. 108, 77–136.

Bashan, Y., De-Bashan, L.E., 2005. Bacteria/plant growth-promotion, in: Hillel, D. (Ed.), Encyclopedia of soils in the environment. Elsevier Ltd, Oxford U.K, pp. 103–115.

Bastian, F., Cohen, A., Piccoli, P., Luna, V., Baraldi, R., Bottini, R., 1998. Production of indole-3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media. Plant Growth Regul. 24, 7–11.

Beattie, G., 2006. Plant-associated bacteria: survey, molecular phylogeny, genomics and recent advances, in: Gnanamanickam, S.S. (Ed.), Plant-Associated Bacteria. Springer Netherlands, Netherlands, pp. 1–56.

Belimov, A., Dodd, I., Hontzeas, N., Theobald, J., Safronova, V., William, D., 2009. Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. New Phytol. 181, 413–423.

Beneduzi, A., Moreira, F., Costa, P.B., Vargas, L.K., Lisboa, B.B., Favreto, R., Baldani, J.I., Passaglia, L.M.P., 2013. Diversity and plant growth promoting evaluation abilities of bacteria isolated from sugarcane cultivated in the South of Brazil. Appl. Soil Ecol. 63, 94–104.

Bent, E., Tuzun, S., Chanway, C.P., Enebak, S., 2001. Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. Can. J. Microbiol. 47, 793–800.

Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68, 1–13.

Bernstein, N., Meiri, A., 2004. root growth of avocado is more sensitive to salinity than shoot growth. J. Am. Soc. Hortic. Sci. 129, 188–192.

Bhore, S., Ravichantar, N., Loh, C.Y., 2010. Screening of endophytic bacteria isolated from leaves of Sambung Nyawa [*Gynura procumbens* (Lour.) Merr.] for cytokinin-like compounds. Bioinformation 5, 191–197.

Blaha, D., Prigent-Combaret, C., Mirza, M., Moënne-Loccoz, Y., 2006. Phylogeny of the 1-aminocyclopropane-1-carboxylic acid deaminase-encoding gene acdS in phytobeneficial and pathogenic Proteobacteria and relation with strain biogeography. FEMS Microbiol. Ecol. 56, 455–470.

Boiero, L., Perrig, D., Masciarelli, O., Penna, C., Cassán, F., Luna, V., 2007. Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Appl. Microbiol. Biotechnol. 74, 874–880.

Bömke, C., Tudzynski, B., 2009. Diversity, regulation, and evolution of the gibberellin biosynthetic pathway in fungi compared to plants and bacteria. Phytochemistry 70, 1876–1893.

Borie, F., Rubio, R., 1999. Effects of arbuscular mycorrhizae and liming on growth and mineral acquisition of aluminum-tolerant and aluminum-sensitive barley cultivars. J. Plant Nutr. 22, 121–137.

Bottini, R., Cassán, F., Piccoli, P., 2004. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Appl. Microbiol. Biotechnol. 65, 497–503.

Bottini, R., Fulchieri, M., Pearce, D., 1989. Identification of gibberellins A1, A3, and iso-A3 in cultures of *Azospirillum lipoferum*. Plant Physiol. 90, 45–47.

Brown, M., Burlingham, S., 1968. production of plant growth substances by *Azotobacter chroococcum*. J. Gen. Microbiol. 135–144.

Bui, E.N., 2013. Soil salinity: A neglected factor in plant ecology and biogeography. J. Arid Environ. 92, 14–25.

Burd, G., Dixon, D., Glick, B., 1998. A plant growth-promoting bacterium that decreases Nickel toxicity in seedlings. Appl. Environ. Microbiol. 64, 3663–3668.

Caballero-Mellado, J., Martinez-Aguilar, L., Paredes-Valdez, G., Estrada-de los Santos, P., 2004. *Burkholderia unamae* sp. nov., an N2-fixing rhizosphere and endophytic species. Int. J. Syst. Evol. Microbiol. 54, 1165–1172.

Cartes, P., Gianfreda, L., Paredes, C., Mora, M.., 2011. Selenium uptake and its antioxidant role in ryegrass cultivars as affected by selenite seed pelletization. J. soil Sci. plant Nutr. 11, 1–14.

Cassán, F., Bottini, R., Schneider, G., Piccoli, P., 2001. *Azospirillum brasilense* and *Azospirillum lipoferum* hydrolyze conjugates of GA20 and metabolize the resultant aglycones to GA1 in seedlings of rice dwarf mutants. Plant Physiol. 125, 2053–2058.

Cassán, F., Perrig, D., Sgroy, V., Luna, V., 2011. Basic and technological aspects of phytohormone production by microorganisms: *Azospirillum* sp. as a model of Plant Growth Promoting Rhizobacteria, in: Maheshwari, D.K. (Ed.), Bacteria in Agrobiology: Plant Nutrient Management. Springer Berlin Heidelberg, pp. 140–182.

Cassán, F.D., Lucangeli, C.D., Bottini, R., Piccoli, P.N., 2001. *Azospirillum* spp. metabolize [17,17-2H2] gibberellin A20 to [17,17-2H2] gibberellin A1 in vivo in dy rice mutant seedlings. Plant Cell Physiol. 42, 763–767.

Castro, M., Iturrieta, R., Fassio, C., 2009. Rootstock effect on the tolerance of avocado plants cv. Hass to NaCl stress. Chil. J. Agric. Res. 69, 316–324.

Chakraborty, U., Chakraborty, B., Chakraborty, A., Dey, P., 2013. Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerant bacteria. World J. Microbiol. Biotechnol. 29, 789–803.

Chartzoulakis, K., Patakas, a., Kofidis, G., Bosabalidis, a., Nastou, a., 2002. Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. Sci. Hortic. (Amsterdam). 95, 39–50.

Chen, L., Luo, S., Xiao, X., Guo, H., Chen, J., Wan, Y., Li, B., Xu, T., Xi, Q., Rao, C., Liu, C., Zeng, G., 2010. Application of plant growth-promoting endophytes (PGPE) isolated from *Solanum nigrum* L. for phytoextraction of Cd-polluted soils. Appl. Soil Ecol. 46, 383–389.

Cheng, Z., McConkey, B.J., Glick, B.R., 2010. Proteomic studies of plant–bacterial interactions. Soil Biol. Biochem. 42, 1673–1684.

Chi, F., Shen, S., Cheng, H., Jing, Y., Yanni, Y., Dazzo, F., 2005. Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl. Environ. Microbiol. 71, 7271–7278.

Cohen, A.C., Bottini, R., Piccoli, P.N., 2008. *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. Plant Growth Regul. 54, 97–103.

Cohen, A.C., Travaglia, C.N., Bottini, R., Piccoli, P.N., 2009. Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. Botany 87, 455–462.

Compant, S., Duffy, B., Nowak, J., Clenment, C., Ait Barka, E., 2005a. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71, 4951–4959.

Compant, S., Mitter, B., Colli-Mull, J.G., Gangl, H., Sessitsch, A., 2011. Endophytes of grapevine flowers, berries, and seeds: Identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. Microb. Ecol. 62, 188–197.

Compant, S., Reiter, B., Nowak, J., Sessitsch, A., Clément, C., Barka, E.A., 2005b. Endophytic colonization of *Vitis vinifera* L. by plant growth- promoting bacterium *Burkholderia* sp. Strain PsJN. Appl. Environ. Microbiol. 71, 1685–1693.

de Melo Pereira, G.V., Magalhães, K.T., Lorenzetii, E.R., Souza, T.P., Schwan, R.F., 2012. A multiphasic approach for the identification of endophytic bacterial in strawberry fruit and their potential for plant growth promotion. Microb. Ecol. 63, 405–417.

Delker, C., Stenzel, I., Hause, B., Miersch, O., Feussner, I., Wasternack, C., 2006. Jasmonate biosynthesis in *Arabidopsis thaliana*--enzymes, products, regulation. Plant Biol. (Stuttg). 8, 297–306.

Dias, A., Costa, F., Andreote, F., Teixeira, M., Assumpção, L., Araújo, W., Azevedo, J.L., Melo, I.S., 2008. Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. World J. Microbiol. Biotechnol. 25, 189–195.

Divan Baldani, V.L., Baldani, J.I., Döbereiner, J., 2000. Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. Biol. Fertil. Soils 30, 485–491.

Dobbelaere, S., Croonenborghs, A., Thys, A., Broek, A. Vande, Vanderleyden, J., 1999. Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production on wheat. Plant Soil 6, 155–164.

Dodd, I., Zinovkina, N., Safronova, V., Belimov, A., 2010. Rhizobacterial mediation of plant hormone status. Ann. Appl. Biol. 157, 361–379.

Donahue, J.L., Okpodu, C.M., Cramer, C., Grabau, E.A., Alscher, R.G., 1997. Responses of Antioxidants to Paraquat in Pea Leaves. Plant Physiol. 113, 249–257.

Dong, Z., Canny, M.J., McCully, M.E., Roboredo, M.R., Cabadilla, C.F., Ortega, E., Rodes, R., 1994. A Nitrogen-fixing endophyte of sugarcane stems (A new role for the apoplast). Plant Physiol. 105, 1139–1147.

Du, Z., Bramlage, W.J., 1992. Modified thiobarbituric acid assay for measuring lipid oxidation in sugar-rich plant tissue extracts. J. Agric. Food Chem. 40, 1566–1570.

Dudeja, S., Giri, R., Saini, R., Suneja-Madan, P., Kothe, E., 2011. Interaction of endophytic microbes with legumes. J. Basic Microbiol. 51, 1–13.

Egamberdieva, D., 2009. Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol. Plant. 31, 861–864.

Egorshina, A.A., Khairullin, R.M., Sakhabutdinova, A.R., Luk'yantsev, M.A., 2011. Involvement of phytohormones in the development of interaction between wheat seedlings and endophytic *Bacillus subtilis* strain 11BM. Russ. J. Plant Physiol. 59, 134–140.

Elbeltagy, A., Nishioka, K., Sato, T., Suzuki, H., Ye, B., Hamada, T., Isawa, T., Mitsui, H., Minamisawa, K., 2001. Endophytic colonization and in planta. Nitrogen fixation by a *Herbaspirillum* sp. isolated from wild rice species. Appl. Environ. Microbiol. 67, 5285–5293.

Elbeltagy, A., Nishioka, K., Suzuki, H., Sato, T., Sato, Y.I., Morisaki, H., Mitsui, H., Minamisawa, K., 2000. Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. Soil Sci. Plant Nutr. 46, 617–629.

Estrada-De Los Santos, P., Bustillos-Cristales, R., Caballero-Mellado, J., 2001. *Burkholderia*, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. Appl. Environ. Microbiol. 67, 2790–2798.

Etesami, H., Alikhani, H.A., 2016. Rhizosphere and endorhiza of oilseed rape (*Brassica napus* L.) plant harbor bacteria with multifaceted beneficial effects. Biol. Control 94, 11–24.

Etesami, H., Hosseini, H.M., Alikhani, H.A., Mohammadi, L., 2014. Bacterial biosynthesis of 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase and Indole-3-Acetic Acid (IAA) as endophytic preferential selection traits by rice plant seedlings. J. Plant Growth Regul. 33, 654–670.

Faria, D.C., Dias, A.C.F., Melo, I.S., de Carvalho Costa, F.E., 2013. Endophytic bacteria isolated from orchid and their potential to promote plant growth. World J. Microbiol. Biotechnol. 29, 217–221.

Faure, D., Vereecke, D., Leveau, J.H.J., 2008. Molecular communication in the rhizosphere. Plant Soil 321, 279–303.

Feng, Y., Shen, D., Song, W., 2006. Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. J. Appl. Microbiol. 100, 938–945.

Finkelstein, R.R., 2010. The Role of Hormones during Seed Development and Germination, in: Davies, P. (Ed.), Plant Hormones: Biosynthesis, Signal Transduction, Action Springer Netherlands, Dordrecht, pp. 549–573.

Forchetti, G., Masciarelli, O., Alemano, S., Alvarez, D., Abdala, G., 2007. Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. Appl. Microbiol. Biotechnol. 76, 1145–1152.

Forchetti, G., Masciarelli, O., Izaguirre, M.J., Alemano, S., Alvarez, D., Abdala, G., 2010. Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi. Curr. Microbiol. 61, 485–493.

Fu, Q., Liu, C., Ding, N., Lin, Y., Guo, B., 2010. Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium *Pseudomonas* sp. DW1 on growth of eggplant (*Solanum melongena* L.) seedlings under salt stress. Agric. Water Manag. 97, 1994–2000.

Fuentes-Ramirez, L.E., Jimenez-Salgado, T., Abarca-Ocampo, I.R., Caballero-Mellado, J., 1993. *Acetobacter diazotrophicus*, an indoleacetic acid producing bacterium isolated from sugarcane cultivars of México. Plant Soil 154, 145–150.

Fulchieri, M., Lucangeli, C., Bottini, R., 1993. Inoculation with *Azospirillum lipoferum* affects growth and gibberellin status of corn seedling roots. Plant Cell Physiol. 34, 1305–1309.

García de Salamone, I.E., Hynes, R.K., Nelson, L.M., 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can. J. Microbiol. 47, 404–411.

Gasser, I., Cardinale, M., Müller, H., Heller, S., Eberl, L., Lindenkamp, N., Kaddor, C., Steinbüchel, A., Berg, G., 2011. Analysis of the endophytic lifestyle and plant growth promotion of *Burkholderia terricola* ZR2-12. Plant Soil 347, 125–136.

Gepstein, S., Thimann, K., 1981. The role of ethylene in the senescence of oat leaves. Plant Physiol. 68, 349–354.

Gillis, M., Kersters, K., Hoste, B., Janssens, D., Kroppenstedt, R.M., Stephan, M.P., Teixeira, K.R.S., Dobereiner, J., De Ley, J., 1989. *Acetobacter diazotrophicus* sp. nov., a nitrogen-fixing acetic acid bacterium associated with sugarcane. Int. J. Syst. Bacteriol. 39, 361–364.

Glick, B.R., 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol. Lett. 251, 1–7.

Glick, B.R., 2004. Bacterial ACC deaminase and the alleviation of plant stress. Adv. Appl. Microbiol. 56, 291–312.

Glick, B.R., Cheng, Z., Czarny, J., Duan, J., 2007a. Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur. J. Plant Pathol. 119, 329–339.

Glick, B.R., Penrose, D.M., Li, J., 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theor. Biol. 190, 63–68.

Glick, B.R., Todorovic, B., Czarny, J., Cheng, Z., Duan, J., McConkey, B., 2007b. Promotion of plant growth by bacterial ACC deaminase. CRC. Crit. Rev. Plant Sci. 26, 227–242.

Golldack, D., Lüking, I., Yang, O., 2011. Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellular transcriptional network. Plant Cell Rep. 30, 1383–1391.

Gond, S.K., Bergen, M.S., Torres, M.S., White, J.F., Kharwar, R.N., 2015. Effect of bacterial endophyte on expression of defense genes in Indian popcorn against *Fusarium moniliforme*. Symbiosis 66, 133–140.

Goudjal, Y., Toumatia, O., Sabaou, N., Barakate, M., Mathieu, F., Zitouni, A., 2013. Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity. World J. Microbiol. Biotechnol. 29, 1821–1829.

Govindarajan, M., Balandreau, J., Kwon, S.-W., Weon, H.-Y., Lakshminarasimhan, C., 2008. Effects of the inoculation of *Burkholderia vietnamensis* and related endophytic diazotrophic bacteria on grain yield of rice. Microb. Ecol. 55, 21–37.

Gravel, V., Antoun, H., Tweddell, R.J., 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: Possible role of indole acetic acid (IAA). Soil Biol. Biochem. 39, 1968–1977.

Gray, E., Smith, D., 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol. Biochem. 37, 395–412.

Grichko, V.P., Glick, B.R., 2001. Amelioration of flooding stress by ACC deaminase-containingplant growth-promoting bacteria. Plant Physiol. Biochem. 39, 11–17.

Groppa, M.D., Benavides, M.P., Zawoznik, M.S., 2012. Root hydraulic conductance, aquaporins and plant growth promoting microorganisms: A revision. Appl. Soil Ecol. 61, 247–254.

Gururani, M.A., Upadhyaya, C.P., Baskar, V., Venkatesh, J., Nookaraju, A., Park, S.W., 2012. Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum*

through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. J. Plant Growth Regul. 32, 245–258.

Gutierrez-Manero, F.J., Ramos-Solano, B., Probanza, A., Mehouachi, J., Tadeo, F., Talon, M., 2001. The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiol. Plant. 111, 206–211.

Gyaneshwar, P., James, E.K., Mathan, N., Reddy, P.M., Reinhold-hurek, B., Jagdish, K., 2001. Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. J. Bacteriol. 183, 2634–2645.

Gyaneshwar, P., James, E.K., Reddy, P.M., Ladha, J.K., 2002. *Herbaspirillum* colonization increases growth and nitrogen accumulation in aluminium-tolerant rice varieties. New Phytol. 154, 131–145.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W., Kloepper, J., 1997. Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43, 895–914.

Hardoim, P., Nissinen, R., Elsas, J.D. Van, 2012. Ecology of bacterial endophytes in sustainable agriculture, in: Maheshwari, D.K. (Ed.), Bacteria in Agrobiology: Plant Probiotics. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 97–126.

Hardoim, P.R., van Overbeek, L.S., Elsas, J.D. Van, 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol. 16, 463–471.

Hartung, W., 2010. The evolution of abscisic acid (ABA) and ABA function in lower plants, fungi and lichen. Funct. Plant Biol. 37, 806–812.

Hayat, S., Ahmad, A., 2013. Salicylic acid a plant hormone. Springer Netherlands, Dordrecht. 401 pp

Hayat, S., Ahmad, A., Mobin, M., Fariduddin, Q., Azam, Z., 2001. Carbonic anhydrase, photosynthesis, and seed yield in mustard plants treated with phytohormones. Photosynthetica 39, 111–114.

Hernández, J.A., Almansa, M.S., 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol. Plant. 115, 251–257.

Hontzeas, N., Hontzeas, C.E., Glick, B.R., 2006. Reaction mechanisms of the bacterial enzyme 1-aminocyclopropane-1-carboxylate deaminase. Biotechnol. Adv. 24, 420–426.

Huang, S., Raman, A.S., Ream, J.E., Fujiwara, H., Cerny, R.E., Brown, S.M., 1998. Overexpression of 20-oxidase confers a gibberellin-overproduction phenotype in *Arabidopsis*. Plant Physiol. 118, 773–781.

Hung, P.Q., Kumar, S.M., Govindsamy, V., Annapurna, K., 2007. Isolation and characterization of endophytic bacteria from wild and cultivated soybean varieties. Biol. Fertil. Soils 44, 155–162.

Hurek, T., Reinhold-Hurek, B., Van Montagu, M., Kellenberger, E., 1994. Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. J. Bacteriol. 176, 1913–1923.

Hussain, A., Hasnain, S., 2011. Interactions of bacterial cytokinins and IAA in the rhizosphere may alter phytostimulatory efficiency of rhizobacteria. World J. Microbiol. Biotechnol. 27, 2645–2654.

Hussain, A., Hasnain, S., 2009. Cytokinin production by some bacteria: Its impact on cell division in cucumber cotyledons. African J. Microbiol. Res. 3, 704–712.

Ibañez, F., Machado Oliveira, Z., Soto Gonzales, H.H., Segal Floh, E.I., Ramos Barbosa, H., 2012. Endophytic and rhizosphere enterobacteria isolated from sugar cane have different potentials for producing plant growth-promoting substances. Plant Soil 353, 409–417.

Iniguez, A.L., Dong, Y., Triplett, E.W., 2004. Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. Mol. Plant-Microbe Interact. MPMI 17, 1078–1085.

Jackson, M., 1997. Hormones from roots as signals for the shoots of stressed plants. Trends Plant Sci. 2, 22–28.

Jagannath, A., Raju, P.S., Bawa, A.S., 2010. Comparative evaluation of bacterial cellulose (nata) as a cryoprotectant and carrier support during the freeze drying process of probiotic lactic acid bacteria. LWT - Food Sci. Technol. 43, 1197–1203.

James, E., Olivares, F., Baldani, J., Dobereiner, J., 1997. *Herbaspirillum*, an endophytic diazotroph colonizing vascular tissue in leaves of Sorghum bicolor L. Moench. J. Exp. Bot. 48, 785–797.

Janzen, R.A., Rood, S.B., Dormaar, J.F., McGill, W.B., 1992. *Azospirillum brasilense* produces gibberellin in pure culture on chemically-defined medium and in co-culture on straw. Soil Biol. Biochem. 24, 1061–1064.

Jasim, B., John Jimtha, C., Jyothis, M., Radhakrishnan, E.K., 2013. Plant growth promoting potential of endophytic bacteria isolated from *Piper nigrum*. Plant Growth Regul. 71, 1–11.

Jha, B., Gontia, I., Hartmann, A., 2012. The roots of the halophyte *Salicornia brachiata* are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. Plant Soil 356, 265–277.

Jha, P., Kumar, A., 2009. Characterization of novel plant growth promoting endophytic bacterium *Achromobacter xylosoxidans* from wheat plant. Microb. Ecol. 58, 179–188.

Ji, Y.-X., Huang, X.-D., 2008. Amelioration of salt stress on annual ryegrass by ACC deaminase-containing plant growth-promoting rhizobacteria. 2008 2nd Int. Conf. Bioinforma. Biomed. Eng. 4104–4107.

Johnston-Monje, D., Raizada, M.N., 2011. Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology. PLoS One 6, 1–22.

Kado, C., 1992. Plant pathogenic bacteria. Ballows A., Truper G., Dworkin M., Harder W., Schleifer K., Ballows A., Truper G., Dworkin M., Harder W., Schleifer K. (eds) The prokaryotes. Springer, New York, pp 660–662.

Karadeniz, A., Topcuoğlu, Ş., İnan, S., 2006. Auxin, gibberellin, cytokinin and abscisic acid production in some bacteria. World J. Microbiol. Biotechnol. 22, 1061–1064.

Karthikeyan, B., Joe, M.M., Islam, M.R., Sa, T., 2012. ACC deaminase containing diazotrophic endophytic bacteria ameliorate salt stress in *Catharanthus roseus* through reduced ethylene levels and induction of antioxidative defense systems. Symbiosis 56, 77–86.

Kasim, W.A., Osman, M.E., Omar, M.N., Abd El-Daim, I.A., Bejai, S., Meijer, J., 2012. Control of drought stress in wheat using plant-growth-promoting bacteria. J. Plant Growth Regul. 32, 122–130.

Katznelson, H., Cole, S.E., 1965. Production of gibberellin-like substances by bacteria and actinomycetes. Can. J. Microbiol. 11, 733–741.

Kawano, T., Hiramatsu, T., Bouteau, F., 2013. Signaling role of salicylic acid in abiotic stress responses in plants, in: Hayat, S., Ahmad, A., Alyemeni, M.N. (Eds.), Salicylic Acid. Springer Netherlands, Dordrecht, pp. 249–275.

Kaya, C., Tuna, A.L., Yokas, I., 2009. The role of plant hormones in plants under salinity stress, in: Ashraf, M., Ozturk, M., Athar, H.R. (Eds.), Salinity and water stress. Improving crop efficiency, Tasks for vegetation science. Springer Netherlands, Dordrecht, pp. 45–50.

Kende, H., 1993. Ethylene biosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44, 283–307.

Khan, P.S.S.V., Nagamallaiah, G. V, Rao, M.D., Sergeant, K., Hausman, J.F., 2014. Abiotic stress tolerance in plants, in: Emerging technologies and management of crop stress tolerance. Elsevier Inc., pp. 23–68.

Kirchhof, G., Reis, V., Baldani, J., Eckert, B., 1997. Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. Plant Soil 194, 45–55.

Kishore, G.K., Pande, S., Podile, a R., 2005. Phylloplane bacteria increase seedling emergence, growth and yield of field-grown groundnut (*Arachis hypogaea* L.). Lett. Appl. Microbiol. 40, 260–268.

Kniskern, J.M., Traw, M.B., Bergelson, J., 2007. Salicylic acid and jasmonic acid signaling defense pathways reduce natural bacterial diversity on *Arabidopsis thaliana*. Mol. plant-microbe Interact. MPMI 20, 1512–1522.

Kudo, T., Kiba, T., Sakakibara, H., 2010. Metabolism and long-distance translocation of cytokinins. J. Integr. Plant Biol. 52, 53–60.

Kuklinsky-Sobral, J., Araújo, W.L., Mendes, R., Pizzirani-Kleiner, A.A., Azevedo, J.L., 2005. Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. Plant Soil 273, 91–99.

Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sci. 165, 382–395.

Lata, H., Li, X.C., Silva, B., Moraes, R.M., Halda-Alija, L., 2006. Identification of IAA-producing endophytic bacteria from micropropagated *Echinacea* plants using 16S rRNA sequencing. Plant Cell. Tissue Organ Cult. 85, 353–359.

Lee, S., Flores-Encarnacion, M., Contreras-Zentella, L., Gracia-Flores, L., Escaramilla, J., Kennedy, C., 2004. Indole-3-acetic acid biosynthesis is deficient *Gluconacetobacter diazotrophicus* strains with mutation in cytochcrome c biogenesis genes. J. Bacteriol. 186, 5284–5391.

Li, J., McConkey, B.J., Cheng, Z., Guo, S., Glick, B.R., 2013. Identification of plant growth-promoting bacteria-responsive proteins in cucumber roots under hypoxic stress using a proteomic approach. J. Proteomics 84, 119–131.

Li, J.H., Wang, E.T., Chen, W.F., Chen, W.X., 2008. Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biol. Biochem. 40, 238–246.

Li, Y., Wang, Q., Wang, L., He, L.-Y., Sheng, X.-F., 2016. Increased growth and root Cu accumulation of *Sorghum sudanense* by endophytic *Enterobacter* sp. K3-2: Implications for *Sorghum sudanense* biomass production and phytostabilization. Ecotoxicol. Environ. Saf. 124, 163–168.

Lin, Z., Zhong, S., Grierson, D., 2009. Recent advances in ethylene research. J. Exp. Bot. 60, 3311–3336.

Liu, Q., Zhang, Y.-C., Wang, C.-Y., Luo, Y.-C., Huang, Q.-J., Chen, S.-Y., Zhou, H., Qu, L.-H., Chen, Y.-Q., 2009. Expression analysis of phytohormone-regulated microRNAs in rice, implying their regulation roles in plant hormone signaling. FEBS Lett. 583, 723–728.

Liu, X., Jia, J., Popat, R., Ortori, C.A., Li, J., Diggle, S.P., Gao, K., Cámara, M., 2011. Characterisation of two quorum sensing systems in the endophytic *Serratia plymuthica* strain G3: differential control of motility and biofilm formation according to life-style. BMC Microbiol. 11, 26.

Lodewyckx, C., Vangronsveld, J., Porteous, F., Edward, R.B., Taghavi, S., Mezgeay, M., Lelie, D. Van Der, 2002. Endophytic bacteria and their potential applications. CRC. Crit. Rev. Plant Sci. 21, 583–606.

Loiret, F.G., Ortega, E., Kleiner, D., Ortega-Rodes, P., Rodes, R., Dong, Z., 2004. A putative new endophytic nitrogen-fixing bacterium *Pantoea* sp. from sugarcane. J. Appl. Microbiol. 97, 504–511.

Long, H.H., Schmidt, D.D., Baldwin, I.T., 2008. Native bacterial endophytes promote host growth in a species-specific manner; Phytohormone manipulations do not result in common growth responses. PLoS One 3, 1–10.

Lugtenberg, B., Dekkers, L., 1999. What makes *Pseudomonas* bacteria rhizosphere competent? Environ. Microbiol. 1, 9–13.

Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. Annu. Rev. Microbiol. 63, 541–556.

Ma, Y., Prasad, M.N. V, Rajkumar, M., Freitas, H., 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol. Adv. 29, 248–258.

Ma, Y., Rajkumar, M., Freitas, H., 2009. Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. J. Environ. Manage. 90, 831–837.

MacMillan, J., 2001. Occurrence of Gibberellins in Vascular Plants, Fungi, and Bacteria. J. Plant Growth Regul. 20, 387–442.

MacMillan, J., Suter, P.J., 1958. The occurrence of gibberellin A1 in higher plants: Isolation from the seed of runner bean (*Phaseolus multiflorus*). Naturwissenschaften 45, 46.

Maksimov, I. V., Veselova, S. V., Nuzhnaya, T. V., Sarvarova, E.R., Khairullin, R.M., 2015. Plant growth-promoting bacteria in regulation of plant resistance to stress factors. Russ. J. Plant Physiol. 62, 715–726.

Malfanova, N., Kamilova, F., Validov, S., Shcherbakov, A., Chebotar, V., Tikhonovich, I., Lugtenberg, B., 2011. Characterization of *Bacillus subtilis* HC8, a novel plant-beneficial endophytic strain from giant hogweed. Microb. Biotechnol. 4, 523–532.

Marques, A.P.G.C., Pires, C., Moreira, H., Rangel, A.O.S.S., Castro, P.M.L., 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. Soil Biol. Biochem. 42, 1229–1235.

Martens, D., Frankenberger, W.T., 1994. Assimilation of exogenous 2'-14C-indole-3-acetic acid and 3'-14C-tryptophan exposed to the roots of three wheat varieties. Plant Soil 166, 281–290.

Martínez, O., Jorquera, M., Crowley, D., Gajardo, G., Mora, M. de la L., 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J. Soil Sci. Plant Nutr. 10, 293–319.

Marulanda, A., Barea, J.-M., Azcón, R., 2009. Stimulation of plant growth and drought tolerance by native microorganisms (AM Fungi and Bacteria) from dry environments: mechanisms related to bacterial effectiveness. J. Plant Growth Regul. 28, 115–124.

Mattos, K. a, Pádua, V.L.M., Romeiro, A., Hallack, L.F., Neves, B.C., Ulisses, T.M.U., Barros, C.F., Todeschini, A.R., Previato, J.O., Mendonça-Previato, L., 2008. Endophytic colonization of rice (*Oryza sativa* L.) by the diazotrophic bacterium *Burkholderia kururiensis* and its ability to enhance plant growth. An. Acad. Bras. Cienc. 80, 477–493.

Mayak, S., Tirosh, T., Glick, B., 2004a. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci. 166, 525–530.

Mayak, S., Tirosh, T., Glick, B., 2004b. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol. Biochem. 42, 565–572.

McInroy, J., Kloepper, J., 1995. Population dynamics of endophytic bacteria in field-grown sweet corn and cotton. Can. J. Microbiol. 41, 895–901.

Mendes, R., Pizzirani-Kleiner, A.A., Araujo, W.L., Raaijmakers, J.M., 2007. Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. Appl. Environ. Microbiol. 73, 7259–7267.

Mercado-Blanco, J., Rodríguez-Jurado, D., Hervás, A., Jiménez-Diaz, R.M., 2004. Suppression of *Verticillium wilt* in olive planting stocks by root-associated fluorescent *Pseudomonas* spp. Biol. Control 30, 474–486.

Merzaeva, O., Shirokikh, I., 2010. The production of auxins by the endophytic bacteria of winter rye. Appl. Biochem. Microbiol. 46, 44–50.

Miché, L., Battistoni, F., Gemmer, S., Belghazi, M., Reinhold-Hurek, B., 2006. Upregulation of jasmonate-inducible defense proteins and differential colonization of roots of *Oryza sativa* cultivars with the endophyte *Azoarcus* sp. Mol. plant-microbe Interact. MPMI 19, 502–511.

Mickelbart, M. V, Arpaia, M.L., 2002. Rootstock influences changes in ion concentrations, growth, and photosynthesis of "Hass" avocado trees in response to salinity. J. Am. Soc. Hortic. Sci. 127, 649–655.

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

Misaghi, I., Donndelinger, C., 1990. Endophytic bacteria in symptom-free cotton plants. Phytopathology 80, 808–811.

Mok, D.W.S., Mok, M.C., 2001. Cytokinin metabolism and action. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 89–118.

Montañez, A., Blanco, A.R., Barlocco, C., Beracochea, M., Sicardi, M., 2012. Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects in vitro. Appl. Soil Ecol. 58, 21–28.

Mundt, J., Hinkle, N., 1976. Bacteria within ovules and seeds. Appl. Environ. Microbiol. 32, 694–698.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651–681.

Muñoz, M., 2015. Boletín frutícola, Abril 2015. Santiago, Chile. 15 pp.

Nadeem, S.M., Shaharoona, B., Arshad, M., Crowley, D.E., 2012. Population density and functional diversity of plant growth promoting rhizobacteria associated with avocado trees in saline soils. Appl. Soil Ecol. 62, 147–154.

Nakamura, K., Ishikawa, S., Kawaharasaki, M., 1995. Phosphate uptake and release activity in immobilized polyphosphate-accumulating bacterium *Microlunatus phosphovorus* strain NM-1. J. Ferment. Bioeng. 80, 377–382.

Naveed, M., Hussain, M.B., Zahir, Z. A., Mitter, B., Sessitsch, A., 2013. Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. Plant Growth Regul. 73, 121–131.

Nemhauser, J.L., Hong, F., Chory, J., 2006. Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell 126, 467–475.

Neuenschwander, A., 2010. El Cambio Climático en el sector silvoagropecuario de Chile. Fundación para la Innovación Agraria, Santiago, Chile. 123 pp.

Nieto, K.F., Frankenberger, W.T., 1989. Biosynthesis of cytokinins by *Azotobacter chroococcum*. Soil Biol. Biochem. 21, 967–972.

Nikolic, B., Schwab, H., Sessitsch, A., 2011. Metagenomic analysis of the 1-aminocyclopropane-1-carboxylate deaminase gene (*acd*S) operon of an uncultured bacterial endophyte colonizing *Solanum tuberosum* L. Arch. Microbiol. 193, 665–676.

Nussaume, L., Robaglia, C., 2003. Tales from the underground: molecular plant – rhizobacteria interactions. Plant, cell Environ. 199, 189–199.

Onofre-Lemus, J., Hernández-Lucas, I., Girard, L., Caballero-Mellado, J., 2009. ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, a widespread trait in *Burkholderia* species, and its growth-promoting effect on tomato plants. Appl. Environ. Microbiol. 75, 6581–6590.

Oster, J.D., Stottlmyer, D.E., Arpaia, M.L., 2007. Salinity and water effects on “Hass” avocado yields. J. Am. Soc. Hortic. Sci. 132, 253–261.

Palaniappan, P., Chauhan, P., Saravanan, V., Anandham, R., Sa, T., 2010. Isolation and characterization of plant growth promoting endophytic bacterial isolates from root nodule of *Lespedeza* sp. Biol. Fertil. Soils 46, 807–816.

Palmfeldt, J., Rådström, P., Hahn-Hägerdal, B., 2003. Optimisation of initial cell concentration enhances freeze-drying tolerance of *Pseudomonas chlororaphis*. Cryobiology 47, 21–29.

Panta, S., Flowers, T., Lane, P., Doyle, R., Haros, G., Shabala, S., 2014. Halophyte agriculture: Success stories. Environ. Exp. Bot. 107, 71–83.

Patten, C., Glick, B., 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. 68, 3795–3801.

Penrose, D.M., Glick, B.R., 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol. Plant. 118, 10–15.

Perin, L., Martínez-Aguilar, L., Castro-González, R., Estrada-De Los Santos, P., Cabellos-Avelar, T., Guedes, H. V, Reis, V.M., Caballero-Mellado, J., 2006. Diazotrophic *Burkholderia* species associated with field-grown maize and sugarcane. Appl. Environ. Microbiol. 72, 3103–3110.

Perin, L., Martínez-Aguilar, L., Paredes-Valdez, J., Baldani, P., Estrada-de los Santos, V., Reis, M., Caballero-Mellado, J., 2006. *Burkholderia silvatlantica* sp. nov., a diazotrophic bacterium associated with sugar cane and maize. Int. J. Syst. Evol. Microbiol. 56, 1931–1937.

Perrig, D., Boiero, M.L., Masciarelli, O. a, Penna, C., Ruiz, O. a, Cassán, F.D., Luna, M. V, 2007. Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. Appl. Microbiol. Biotechnol. 75, 1143–1150.

Phetcharat, P., Duangpaeng, A., 2012. Screening of endophytic bacteria from organic rice tissue for indole acetic acid production. Procedia Eng. 32, 177–183.

Piccoli, P., Lucangeli, C., Schneider, G., Bottini, R., 1997. Hydrolysis of [17,17-$2H_2$] gibberellin A20-glucoside and [17,17-$2H_2$] gibberellin A20-glucosyl ester by *Azospirillum lipoferum* cultured in a nitrogen-free biotin-Based chemically-defined medium. Plant Growth Regul. 23, 179–182.

Piccoli, P., Travaglia, C., Cohen, A., Sosa, L., Cornejo, P., Masuelli, R., Bottini, R., 2010. An endophytic bacterium isolated from roots of the halophyte *Prosopis strombulifera* produces ABA, IAA, gibberellins A1 and A3 and jasmonic acid in chemically-defined culture medium. Plant Growth Regul. 64, 207–210.

Pieterse, C.M.J., Van Pelt, J. A., Ton, J., Parchmann, S., Mueller, M.J., Buchala, A.J., Métraux, J.-P., Van Loon, L.C., 2000. Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. Physiol. Mol. Plant Pathol. 57, 123–134.

Pillay, V.K., Nowak, J., 1997. Inoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. Can. J. Microbiol. 43, 354–361.

Ping, L., Boland, W., 2004. Signals from the underground: bacterial volatiles promote growth in *Arabidopsis*. Trends Plant Sci. 9, 263–266.

Piotrowska, A., Bajguz, A., 2011. Conjugates of abscisic acid, brassinosteroids, ethylene, gibberellins, and jasmonates. Phytochemistry 72, 2097–2112.

Pozo, M.J., Loon, L.C., Pieterse, C.M.J., 2005. Jasmonates - Signals in Plant-Microbe Interactions. J. Plant Growth Regul. 23, 211–222.

Qin, S., Xing, K., Jiang, J.-H., Xu, L.-H., Li, W.-J., 2011. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl. Microbiol. Biotechnol. 89, 457–473.

Qin, S., Zhang, Y.-J., Yuan, B., Xu, P.-Y., Xing, K., Wang, J., Jiang, J.-H., 2013. Isolation of ACC deaminase-producing habitat-adapted symbiotic bacteria associated with halophyte Limonium sinense (Girard) Kuntze and evaluating their plant growth-promoting activity under salt stress. Plant Soil 374, 753–766.

Qiu, Z., Guo, J., Zhu, A., Zhang, L., Zhang, M., 2014. Exogenous jasmonic acid can enhance tolerance of wheat seedlings to salt stress. Ecotoxicol. Environ. Saf. 104, 202–208.

Quispel, A., 1992. A search for signals in endophytic microorganisms. Verma D. (ed) Molecular signals in plant-microbe communications. CRC, Boca Raton, FL, pp 471–490.

Rai, R., Dash, P.K., Prasanna, B.M., Singh, A., 2006. Endophytic bacterial flora in the stem tissue of a tropical maize (*Zea mays* L.) genotype: isolation, identification and enumeration. World J. Microbiol. Biotechnol. 23, 853–858.

Rajkumar, M., Ae, N., Freitas, H., 2009. Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77, 153–160.

Ramadoss, D., Lakkineni, V.K., Bose, P., Ali, S., Annapurna, K., 2013. Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. Springer plus 2, 6.

Rasche, F., Marco-Noales, E., Velvis, H., Overbeek, L.S., López, M.M., Elsas, J.D., Sessitsch, A., 2006a. Structural characteristics and plant-beneficial effects of bacteria colonizing the shoots of field grown conventional and genetically modified T4-lysozyme producing potatoes. Plant Soil 289, 123–140.

Rasche, F., Velvis, H., Zachow, C., Berg, G., Van Elsas, J.D., Sessitsch, A., 2006b. Impact of transgenic potatoes expressing anti-bacterial agents on bacterial endophytes is comparable with the effects of plant genotype, soil type and pathogen infection. J. Appl. Ecol. 43, 555–566.

Rashid, S., Charles, T.C., Glick, B.R., 2012. Isolation and characterization of new plant growth-promoting bacterial endophytes. Appl. Soil Ecol. 61, 217–224.

Reinhold-Hurek, B., Hurek, T., 2011. Living inside plants: bacterial endophytes. Curr. Opin. Plant Biol. 14, 435–443.

Reinhold-Hurek, B., Hurek, T., 1998. Interactions of gramineous plants with *Azoarcus* spp. and other diazotrophs: Identification, localization, and perspectives to study their function. CRC. Crit. Rev. Plant Sci. 17, 29–54.

Reinhold-Hurek, B., Hurek, T., 1998. Life in grasses: diazotrophic endophytes. Trends Microbiol. 6, 139–144.

Reis, V.M., Santos, P.E. los, Tenorio-Salgado, S., Vogel, J., Stoffels, M., Guyon, S., Mavingui, P., Baldani, V.L.D., Schmid, M., Baldani, J.I., Balandreau, J., Hartmann, A., Caballero-Mellado, J., 2004. *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant-associated bacterium. Int. J. Syst. Evol. Microbiol. 54, 2155–2162.

Rivas-San Vicente, M., Plasencia, J., 2011. Salicylic acid beyond defence: Its role in plant growth and development. J. Exp. Bot. 62, 3321–3338.

Rosenblueth, M., Martínez-Romero, E., 2006. Bacterial endophytes and their interactions with hosts. Mol. plant-microbe Interact. MPMI 19, 827–837.

Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J., Dowling, D.N., 2008. Bacterial endophytes: recent developments and applications. FEMS Microbiol. Lett. 278, 1–9.

Saïdi, S., Chebil, S., Gtari, M., Mhamdi, R., 2013. Characterization of root-nodule bacteria isolated from *Vicia faba* and selection of plant growth promoting isolates. World J. Microbiol. Biotechnol. 29, 1099–1106.

Saleem, M., Arshad, M., Hussain, S., Bhatti, A.S., 2007. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J. Ind. Microbiol. Biotechnol. 34, 635–648.

Santner, A., Estelle, M., 2009. Recent advances and emerging trends in plant hormone signalling. Nature 459, 1071–1078.

Sarwar, M., Kremer, R., 1995. Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. Plant Soil 172, 261–269.

Schwab, C., Vogel, R., Gänzle, M.G., 2007. Influence of oligosaccharides on the viability and membrane properties of *Lactobacillus reuteri* TMW1.106 during freeze-drying. Cryobiology 55, 108–114.

Seghers, D., Wittebolle, L., Top, E.M., Verstraete, W., Siciliano, S.D., 2004. Impact of agricultural practices on the Zea impact of agricultural practices on the *Zea mays* L. endophytic community. Appl. Environ. Microbiol. 70, 1475–1482.

Senthilkumar, M., Anandham, R., Madhaiyan, M., Venkateswaran, V., 2011a. Bacteria in Agrobiology: Crop Ecosystems, Bacteria in Agrobiology: Crop Ecosystems. Springer, Berlin, Heidelberg.

Senthilkumar, M., Anandham, R., Madhaiyan, M., Venkateswaran, V., Sa, T., Maheshwari, D.K., 2011b. Bacteria in agrobiology: Crop Ecosystems, Bacteria in Agrobiology: Crop Ecosystems. Springer, Berlin, Heidelberg.

Sessitsch, A., Coenye, T., Sturz, A. V., Vandamme, P., Barka, E.A., Salles, J.F., Elsas, J.D. Van, Faure, D., Reiter, B., Glick, B.R., Wang-Pruski, G., Nowak, and J., 2005. *Burkholderia phytofirmans* sp. nov., a novel plant-associated bacterium with plant-beneficial properties. Int. J. Syst. Evol. Microbiol. 55, 1187–1192.

Sessitsch, A., Hardoim, P., Döring, J., Weilharter, A., Krause, A., Woyke, T., Mitter, B., Hauberg-Lotte, L., Friedrich, F., Rahalkar, M., Hurek, T., Sarkar, A., Bodrossy, L., van Overbeek, L., Brar, D., van Elsas, J.D., Reinhold-Hurek, B., 2012. Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Mol. plant-microbe Interact. MPMI 25, 28–36.

Sessitsch, A., Reiter, B., Pfeifer, U., Wilhelm, E., 2002. Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and Actinomycetes-specific PCR of 16S rRNA genes. FEMS Microbiol. Ecol. 39, 23–32.

Sevilla, M., Burris, R.H., Gunapala, N., Kennedy, C., 2001. Comparison of benefit to sugarcane plant growth and $^{15}N_2$ incorporation following inoculation of sterile plants with *Acetobacter diazotrophicus* wild-type and nif − mutant strains. Mol. Plant-Microbe Interact. 14, 358–366.

Sgroy, V., Cassán, F., Masciarelli, O., Del Papa, M.F., Lagares, A., Luna, V., 2009. Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-

regulating (PSHB) bacteria associated to the halophyte *Prosopis strombulifera*. Appl. Microbiol. Biotechnol. 85, 371–381.

Shaharoona, B., Arshad, M., Zahir, Z.A., 2006. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). Lett. Appl. Microbiol. 42, 155–159.

Shan, X., Yan, J., Xie, D., 2012. Comparison of phytohormone signaling mechanisms. Curr. Opin. Plant Biol. 15, 84–91.

Shi, Y., Lou, K., Li, C., 2011. Growth promotion effects of the endophyte *Acinetobacter johnsonii strain* 3-1 on sugar beet. Symbiosis 54, 159–166.

Shi, Y., Lou, K., Li, C., 2010. Growth and photosynthetic efficiency promotion of sugar beet (*Beta vulgaris* L.) by endophytic bacteria. Photosynth. Res. 105, 5–13.

Shi, Y., Lou, K., Li, C., 2009. Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. Biol. Fertil. Soils 45, 645–653.

Shin, D., Myung, S., Jung, S., 2007. Plant growth-promoting potential of endophytic bacteria isolated from roots of coastal sand dune plants. J. Microbiol. 17, 1361–1368.

Siddikee, M., Chauchan, P., Anandham, R., Gan, G., T, S., 2010. Isolation, Characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. J. Microbiol. Biotechnol. 20, 1577–1584.

Silber, A., Naor, A., Israeli, Y., Assouline, S., 2013. Combined effect of irrigation regime and fruit load on the patterns of trunk-diameter variation of “Hass” avocado at different phenological periods. Agric. Water Manag. 129, 87–94.

Singh, D.P., Prabha, R., Yandigeri, M.S., Arora, D.K., 2011. Cyanobacteria-mediated phenylpropanoids and phytohormones in rice (*Oryza sativa*) enhance plant growth and stress tolerance. Antonie Van Leeuwenhoek 100, 557–568.

Singh, J.S., Pandey, V.C., Singh, D.P., 2011. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. Agric. Ecosyst. Environ. 140, 339–353.

Singh, M.K., Singh, D.P., Mesapogu, S., Babu, B.K., Bontemps, C., 2011. Concomitant colonization of *nif*H positive endophytic *Burkholderia* sp. in rice (*Oryza sativa* L.) promotes plant growth. World J. Microbiol. Biotechnol. 27, 2023–2031.

Singh, R.K., Malik, N., Singh, S., 2013. Improved nutrient use efficiency increases plant growth of rice with the use of IAA-overproducing strains of endophytic *Burkholderia cepacia* strain RRE25. Microb. Ecol. 66, 375–384.

Spaepen, S., Vanderleyden, J., Remans, R., 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol. Rev. 31, 425–448.

Sponsel, V., Hedden, P., 2004. Gibberellin biosynthesis and inactivation, in: Davis, P. (Ed.), Plant Hormones. Biosynthesis, Signal Transduction, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 63–94.

Stearns, J., 2003. Transgenic plants with altered ethylene biosynthesis or perception. Biotechnol. Adv. 21, 193–210.

Strobel, G., Daisy, B., Castillo, U., Harper, J., 2004. Natural products from endophytic microorganisms. J. Nat. Prod. 67, 257–268.

Sturz, A. V, Christie, B.R., Nowak, J., 2000. Bacterial endophytes: potential role in developing sustainable systems of crop production. CRC. Crit. Rev. Plant Sci. 19, 1–30.

Sturz, A. V., 1995. The role of endophytic bacteria during seed piece decay and potato tuberization. Plant Soil 175, 257–263.

Sturz, A. V., Christie, B.R., Matheson, B.G., Arsenault, W.J., Buchanan, N.A., 1999. Endophytic bacterial communities in the periderm of potato tubers and their potential to improve resistance to soil-borne plant pathogens. Plant Pathol. 48, 360–369.

Subramanian, P., Kim, K., Krishnamoorthy, R., Sundaram, S., Sa, T., 2015. Endophytic bacteria improve nodule function and plant nitrogen in soybean on co-inoculation with *Bradyrhizobium japonicum* MN110. Plant Growth Regul. 76, 327–332.

Sun, L., Wang, X., Li, Y., 2016. Increased plant growth and copper uptake of host and non-host plants by metal-resistant and plant growth-promoting endophytic bacteria. Int. J. Phytoremediation. 3, 494–501.

Sun, L.-N., Zhang, Y.-F., He, L.-Y., Chen, Z.-J., Wang, Q.-Y., Qian, M., Sheng, X.-F., 2010. Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper-tolerant plant species on copper mine wasteland. Bioresour. Technol. 101, 501–509.

Sun, Y., Cheng, Z., Glick, B.R., 2009. The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN. FEMS Microbiol. Lett. 296, 131–136.

Szymańska, S., Płociniczak, T., Piotrowska-Seget, Z., Złoch, M., Ruppel, S., Hrynkiewicz, K., 2016. Metabolic potential and community structure of endophytic and rhizosphere bacteria associated with the roots of the halophyte *Aster tripolium* L. Microbiol. Res. 182, 68–79.

Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N., Barac, T., Vangronsveld, J., van der Lelie, D., 2009. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl. Environ. Microbiol. 75, 748–757.

Taghavi, S., van der Lelie, D., Hoffman, A., Zhang, Y.-B., Walla, M.D., Vangronsveld, J., Newman, L., Monchy, S., 2010. Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp. 638. PLoS Genet. 6, e1000943.

Taiz, L., Zeiger, E., 2010. Plant Physiology, Fifth Edit. ed. Sinauer Associates, Sunderland, MA. 622 pp.

Tank, N., Saraf, M., 2010. Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. J. Plant Interact. 5, 51–58.

Thokchom, E., Kalita, M.C., Talukdar, N.C., 2014. Isolation, screening, characterization, and selection of superior rhizobacterial strains as bioinoculants for seedling emergence and growth promotion of Mandarin orange (*Citrus reticulata* Blanco). Can. J. Microbiol. 60, 85–92.

Tien, T. M. Gaskins M. H., Hubbell., D. H. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* l.). Appl. Environ. Microbiol. 37, 1016–1024.

Timmusk, S., Nicander, B., Granhall, U., Tillberg, E., 1999. Cytokinin production by *Paenibacillus polymyxa*. Soil Biol. Biochem. 31, 1847–1852.

Ton, J., Van Pelt, J. a, Van Loon, L.C., Pieterse, C.M.J., 2002. Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in *Arabidopsis*. Mol. plant-microbe Interact. MPMI 15, 27–34.

Trivedi, P., Spann, T., Wang, N., 2011. Isolation and characterization of beneficial bacteria associated with citrus roots in Florida. Microb. Ecol. 62, 324–336.

Tsavkelova, E., Klimova, S., Cherdyntseva, T., Netrusov, A., 2006. Microbial producers of plant growth stimulators and their practical use: A review. Appl. Biochem. Microbiol. 42, 117–126.

Van der Ent, S., Van Wees, S.C.M., Pieterse, C.M.J., 2009. Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry 70, 1581–1588.

van Loon, L.C., Bakker, P. a, Pieterse, C.M., 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36, 453–483.

Vandamme, P., Goris, J., Chen, W.-M., de Vos, P., Willems, A., 2002. *Burkholderia tuberum* sp. nov. and *Burkholderia phymatum* sp. nov., nodulate the roots of tropical legumes. Syst. Appl. Microbiol. 25, 507–512.

Vega, F.E., Pava-Ripoll, M., Posada, F., Buyer, J.S., 2005. Endophytic bacteria in *Coffea arabica* L. J. Basic Microbiol. 45, 371–380.

Vendan, R.T., Yu, Y.J., Lee, S.H., Rhee, Y.H., 2010. Diversity of endophytic bacteria in ginseng and their potential for plant growth promotion. J. Microbiol. 48, 559–565.

Verma, S., Ladha, J., Tripathi, A., 2001. Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. J. Biotechnol. 91, 127–141.

Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255, 571–586.

Wang, C., Knill, E., Glick, B.R., Défago, G., 2000. Effect of transferring 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHA0 and its gacA derivative CHA96 on their growth-promoting and disease-suppressive capacities. Can. J. Microbiol. 46, 898–907.

Wang, C.-J., Yang, W., Wang, C., Gu, C., Niu, D.-D., Liu, H.-X., Wang, Y.-P., Guo, J.-H., 2012. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS One 7, e52565.

Wang, M.-X., Liu, J., Chen, S., Yan, S.-Z., 2011. Isolation, characterization and colonization of 1-aminocyclopropane-1-carboxylate deaminase-producing bacteria XG32 and DP24. World J. Microbiol. Biotechnol. 28, 1155–1162.

Weyens, N., Boulet, J., Adriaensen, D., Timmermans, J.-P., Prinsen, E., Oevelen, S., D'Haen, J., Smeets, K., Lelie, D., Taghavi, S., Vangronsveld, J., 2011. Contrasting colonization and plant growth promoting capacity between wild type and a gfp-derative of the endophyte *Pseudomonas putida* W619 in hybrid poplar. Plant Soil 356, 217–230.

Wilhelm, E., Arthofer, W., Schafleitner, R., Krebs, R., 1998. *Bacillus subtilis* an endophyte of chestnut (*Castanea sativa*) as antagonist against chestnut blight (*Cryphonectria parasitica*). Plant Cell. Tissue Organ Cult. 52, 105–108.

Wilson, D., 1995. Endophyte - The evolution of a term, and clarification of its use and definition. Oikos 73, 274–276.

Woodward, A.W., Bartel, B., 2005. Auxin: regulation, action, and interaction. Ann. Bot. 95, 707–735.

Wu, Z., Peng, Y., Guo, L., Li, C., 2014. Root colonization of encapsulated *Klebsiella oxytoca* Rs-5 on cotton plants and its promoting growth performance under salinity stress. Eur. J. Soil Biol. 60, 81–87.

WWAP (World Water Assessment Programme), 2012. The United Nations World Water Development Report 4: Managing Water under Uncertainty and Risk. Paris, France. 865 pp.

Xin, G., Zhang, G., Kang, J.W., Staley, J.T., Doty, S.L., 2009. A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. Biol. Fertil. Soils 45, 669–674.

Yaish, M.W., Antony, I., Glick, B.R., 2015. Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (*Phoenix dactylifera* L.) and their potential role in salinity tolerance. Antonie Van Leeuwenhoek 107, 1519–1532.

Yamaguchi, S., 2008. Gibberellin metabolism and its regulation. Annu. Rev. Plant Biol. 59, 225–251.

Appendix

Appendix 2.1. Publications (authors, affiliations)

1. **Barra, P.J.;** Crowley, D.E.; Inostroza, N.G.; Mora, M.L. & Jorquera, M.A. 2016. Endophytic bacteria in phytostimulation: A Review. In preparation.

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2. **Barra, P.J.;** Crowley, D.E.; Inostroza, N.G.; Mora, M.L. & Jorquera, M.A. 2016. Bacterial consortia inoculation alleviates the water shortage and salt stress in an avocado (*Persea americana* Mill.) nursery. Submitted to Appl. Soil Ecol.

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3. **Barra, P.J.;** Inostroza, N.G.; Acuña, J.J.; Mora, M.L.; Crowley, D.E. & Jorquera, M.A., 2016. Formulation of bacterial consortia from avocado (*Persea americana* Mill.) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress. Appl. Soil Ecol. 102, 80–91

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Appendix 2.2. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated.

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Acetobacter diazotrophicus		x	x					Sugarcane	Bastian et al., 1998; Fuentes-Ramirez et al., 1993	N fix
Achromobacter sp.		x					x	Palm tree, sugarcane	Beneduzi et al., 2013; Yaish et al., 2015	N fix, P sol, sid, Zn sol, K sol, *nif*H, chitinase,
Achromobacter xiloxidans	x				x			Sunflower	Forchetti et al., 2007	N fix, P sol, antifungal
Achromobacter xylosoxidans	*x*	x	x			x	x	Wheat, *Catharanthus roseus*, sunflower, *Prosopis strombulifera*	Forchetti et al., 2010; Jha and Kumar, 2009; Karthikeyan et al., 2012; Sgroy et al., 2009	N fix, P sol, Sid, ARA
Acinetobacter calcoaceticus		x					x	*Solanum nigrum, Elsholtzia splendens, Solanum tuberosum*	Long et al., 2008; Rasche et al., 2006a; Sun et al., 2010	antibacterial
Acinetobacter johnsonii	x	x	x	x				*Beta vulgaris*	Shi et al., 2011, 2009	P sol
Acinetobacter junii		x						*Elsholtzia splendens*	Sun et al., 2010	Sid, Arg descarbox
Acinetobacter radioresistens		x					x	*Solanum lycopersicum*	Rashid et al., 2012	NH_3
Acinetobacter sp.		x					x	Soybean , *Solanum nigrum, Aster tripolium*	Chen et al., 2010; Li et al., 2008; Szymańska et al., 2016	P sol, Sid.
Aeromicrobium sp.							x	*Solanum tuberosum*	Rasche et al., 2006b	
Aeromonas veronii		x					x	*Solanum nigrum*	Long et al., 2008	
Agrobacterium sp.		x						*Calystegia soldanella*	Shin et al., 2007	antifungal
Arthrobacter nitroguaiacolicus							x	*Lespedeza* sp.	Palaniappan et al., 2010	Sid
Arthrobacter koreensis (o sp)	x	x	x		x			*Prosopis strombulifera*	Piccoli et al., 2010	N fix
Arthrobacter sp.		x					x	*Commelina communis, Solanum tuberosum*	(Rasche et al., 2006b; Sun et al., 2010)	Sid, Arg descarbox
Azorhizobium sp.		x						Sugarcane	(Beneduzi et al., 2013)	N fix, Sid
Azospirillum brasilense	x	x	x	x					(Cohen et al., 2008; Perrig et al., 2007; Sgroy et al., 2009)	putrescine, spermine, spermidine, cadaverine
Azospirillum lipoferum	x		x						Cohen et al., 2009	

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Bacillus amyloliquefaciens		x						*Panax ginseng*	(Vendan et al., 2010)	P sol
Bacilus aquimaris	x							*Aster tripolium*	(Szymańska et al., 2016)	*nif*H
Bacillus anthracis							x	Palm tree	(Yaish et al., 2015)	Amonio;
Bacillus cereus		x				x		*Elsholtzia splendens*, *Panicum miliaceum*, *Citrus sinensis*, *Panax ginseng*, *Lycopersicon esculentum*	(Amaresan et al., 2011; Malfanova et al., 2011; Sun et al., 2010; Trivedi et al., 2011; Vendan et al., 2010)	P sol, Sid, antifungal, N fix, *Nif*H, chitinase
Bacillus endophyticus							x	Palm tree	(Yaish et al., 2015)	Amonio; sol K
Bacillus firmus		x						*Elsholtzia splendens*	(Sun et al., 2010)	Sid, arg descarbox
Bacillus flexus		x						*Panax ginseng*	(Vendan et al., 2010)	P Sol, Sid
Bacillus ginsengihumi		x				x	x	*Citrus sinensis*	(Trivedi et al., 2011)	Sid, P sol, N fix, chitinase, *Nif*H,
Bacillus horneckiae		x					x	*Solanum lycopersicum*	(Rashid et al., 2012)	Sid, NH_3
Bacillus idriensis		x					x	*Solanum lycopersicum*	(Rashid et al., 2012)	NH_3
Bacillus licheniformis	x	x	x			x	x	*Prosopis strombulifera*, *Piper nigrum*, *Citrus sinensis*, *Capsicum annuum*, *Aster tripolium*	(Sgroy et al., 2009)(Jasim et al., 2013)(Trivedi et al., 2011)(Amaresan et al., 2011)(Szymańska et al., 2016)	P sol, Sid, N fix, *Nif*H, chitinase,
Bacillus megaterium		x					x	Palm tree, strawberry, *Elsholtzia splendens*, *Capsicum annuum*, *Lespedeza* sp. , *Vicia faba*, *Panax ginseng*	(Amaresan et al., 2011; Dias et al., 2008; Palaniappan et al., 2010; Saïdi et al., 2013; Sun et al., 2015, 2010; Vendan et al., 2010; Yaish et al., 2015)	P sol, Sid, Arg descarbox
Bacillus muralis		x						*Vicia faba* -,	(Saïdi et al., 2013)	
Bacillus mycoides		x						*Aster tripolium*	(Szymańska et al., 2016)	
Bacillus oleronius		x					x	Palm tree	(Yaish et al., 2015)	Amonio;
Bacillus psychrosaccharolyticus		x					x	*Solanum lycopersicum*	(Rashid et al., 2012)	NH_3

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Bacillus pumilus	x	x	x	x	x	x	x	Sunflower, *Prosopis strombulifera, Beta vulgaris, Commelina communis, Panax ginseng, Lycopersicon esculentum, Solanum tuberosum*	(Amaresan et al., 2011; Forchetti et al., 2010, 2007; Rasche et al., 2006a; Sgroy et al., 2009; Shi et al., 2009; Sun et al., 2010; Vendan et al., 2010)	P sol, Sid N fix, antifungal, protease, antibacterial
Bacillus simplex		x					x	*Solanum lycopersicum*	(Rashid et al., 2012)	NH_3
Bacillus sp.		x					x	Strawberry, soybean, *Commelina communis, Solanum lycopersicum, Calystegia soldanella, Piper nigrum, Solanum tuberosum , Lycopersicon esculentum, Aster tripolium*	(Amaresan et al., 2011; de Melo Pereira et al., 2012; Dias et al., 2008; Jasim et al., 2013; Li et al., 2008; Rasche et al., 2006b; Rashid et al., 2012; Shin et al., 2007; Sun et al., 2010; Szymańska et al., 2016)	P sol, Sid, *nif*H, NH_3, Protease, Pectinase, Chitinase, celulase, antifungal
Bacillus subtilis	x	x	x	x			x	*Prosopis strombulifera,* Wheat, strawberry, *Heracleum sosnowskyi, Vicia faba, Panax ginseng, Brassica napus*	(de Melo Pereira et al., 2012; Dias et al., 2008; Egorshina et al., 2011; Etesami and Alikhani, 2016; Malfanova et al., 2011; Saïdi et al., 2013; Sgroy et al., 2009; Vendan et al., 2010)	N fix, P sol, Sid, Protease, antifungal
Bacillus thuringiensis		x						Palm tree	(Yaish et al., 2015)	NH_3.
Brachybacterium sp.		x					x	*Salicornia brachiata*	(Jha et al., 2012)	N fix, Sid, *nifH*
Bradyrhizobium elkanii		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	P sol, Sid
Bradyrhizobium japonicum	x	x	x	x					(Boiero et al., 2007[a])	
Brevibacillus parabrevis		x						*Citrus sinensis*	(Trivedi et al., 2011)	N fix, P sol, Sid chitinase
Brevibacterium casei		x					x	*Salicornia brachiata*	(Jha et al., 2012)	N fix, P sol, Sid, *nifH*
Brevibacterium halotolerans	x		x	x			x	*Prosopis strombulifera*	(Sgroy et al., 2009)	Antifungal, N fix, proteasa
Brevundimonas sp.		x						*Zea mays, Vitis vinifera*	(Andreolli et al., 2016; Montañez et al., 2012)	*nif*H

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Brevundimonas vesicularis							x	*Solanum tuberosum*	(Rasche et al., 2006b)	
Burkholderia caledonica		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	P sol, Sid
Burkholderia cenocepacia		x						Sugarcane	(Mendes et al., 2007)	Pyrrolnitrin, antifungal
Burkholderia cepacia		x				x	x	Sugarcane, yellow lupine, *Zea mays*, Rice, *Citrus sinensis*	(Mendes et al., 2007) (Taghavi et al., 2009)(Montañez et al., 2012)(M. K. Singh et al., 2011)(Trivedi et al., 2011)	N fix, P sol, Sid, Pyrrolnitrin, antifungal, *nif*H, ,Nitrogenase, *phl*D AHL, chitonase
Burkholderia glathei		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	Sid
Burkholderia kururiensis		x					x	Rice	(Estrada-De Los Santos et al., 2001; Mattos et al., 2008; Onofre-Lemus et al., 2009)	N fix
Burkholderia phenazinium		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	P sol, Sid
Burkholderia phymatum							x	*Machaerium lunatum*	(Onofre-Lemus et al., 2009) (Vandamme et al., 2002)	
Burkholderia phytofirmans		x					x	Onion, *Lespedeza* sp.	(Ait Barka et al., 2006; Compant et al., 2005b; Palaniappan et al., 2010; Sessitsch et al., 2005)	Sid
Burkholderia sediminicola		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	P sol, Sid
Burkholderia silvatlantica							x	Sugarcane	(Onofre-Lemus et al., 2009) (L. Perin et al., 2006)	N fix
Burkholderia sordidicola							x	*Lespedeza* sp.	(Palaniappan et al., 2010)	P sol, Sid
Burkholderia sp.		x				x	x	Soybean, *Elsholtzia splendens*, *Zea mays*, *Citrus sinensis*, sugarcane	(Beneduzi et al., 2013; Johnston-Monje and Raizada, 2011; Li et al., 2008; Sun et al., 2015, 2010; Trivedi et al., 2011)	N fix, P sol, Sid, arg descarbox, antifungal, *phl*D, AHL, cellulose, *nif*H, chitinaase
Burkholderia terricola							x	*Beta vulgaris* L	(Gasser et al., 2011)	Sid
Burkholderia tunerum							x	*Aspalathus carnosa*	(Onofre-Lemus et al., 2009) (Vandamme et al., 2002)	

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Burkholderia tropica							x	Sugarcane, *Zea mays*	(Reis et al., 2004) (Blaha et al., 2006) (L Perin et al., 2006)	N fix
Burkholderia unamae							x	*Zea mays*	(Onofre-Lemus et al., 2009)(Caballero-Mellado et al., 2004)	N-fix
Burkholderia vietnamiensis		x				x	x	*Populus trichocarpa*, *Zea mays*, coffee, rice, *Citrus sinensis*	(Estrada-De Los Santos et al., 2001; Govindarajan et al., 2008; Onofre-Lemus et al., 2009; M. K. Singh et al., 2011; Trivedi et al., 2011; Xin et al., 2009)	N fix, P sol, Sid, *nif*H, chitinase, nitrogenase, *phl*D, AHL
Caulobacter vibrioides							x	*Solanum tuberosum L.*,	(Rasche et al., 2006b)	
Cellulomonas sp.		x					x	Winter rye, *Zea mays*	(Merzaeva and Shirokikh, 2010)	P sol, acetoin, antifungal, cellulose, pectinase
Chryseobacterium sp.	x	x	x	x				*Beta vulgaris*	(Johnston-Monje and Raizada, 2011; Shi et al., 2011, 2009)	
Chryseobacterium indologene		x						*Beta vulgaris*	(Shi et al., 2010)	
Cronobacter sakazakii		x					x	*Salicornia brachiata*	(Jha et al., 2012)	N fix, P sol, Sid, *nifH*
Curtobacterium citreum		x						strawberry fruit	(de Melo Pereira et al., 2012)	
Curtobacterium plantarum		x						Winter rye	(Merzaeva and Shirokikh, 2010)	
Curtobacterium sp.		x					x	*Solanum tuberosum* , *Vitis vinifera*	(Andreolli et al., 2016; Rasche et al., 2006b)	Sid, NH_3
Devosia sp.		x					x	*Solanum lycopersicum*	(Rashid et al., 2012)	Sid, NH_3
Dyella koreensis		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	P sol
Dyella marensis		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	
Ensifer meliloti		x						*Vicia faba* -	(Saïdi et al., 2013)	
Enterobacter aerogenes		x					x	*Solanum. nigrum*	(Chen et al., 2010)	P sol, Sid

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Enterobacter agglomerans		x					x	*Solanum nigrum*, *Solanum nigrum*, *Daucus carota*, tap, *Panicum miliaceum*, *Lycopersicon esculentum*	(Long et al., 2008)(Malfanova et al., 2011)	
Enterobacter asburiae							x	*Solanum tuberosum*	(Rasche et al., 2006a)	antibacterial
Enterobacter cloacae		x				x	x	Palm tree , *Citrus sinensis*	(Yaish et al., 2015)(Trivedi et al., 2011)	NH_3 P sol, chitinase ,N fix, Sid
Enterobacter cancerogenus							x	*Solanum tuberosum*	(Rasche et al., 2006a)	antibacterial
Enterobacter ludwigii		x					x	strawberry fruit, plant grown in a copper mine	(de Melo Pereira et al., 2012; Y. Zhang et al., 2011)	P sol, Sid
Enterobacter sp.		x					x	Strawberry, sugarcane, *Zea mays*, *Piper nigrum*, poplar trees, poplar, *Solanum nigrum*, *Persea Americana*, *Sorghum sudanense*	(Barra et al., 2016; Chen et al., 2010; de Melo Pereira et al., 2012; Ibañez et al., 2012; Jasim et al., 2013; Johnston-Monje and Raizada, 2011; Li et al., 2016; Mirza et al., 2001; Montañez et al., 2012; Taghavi et al., 2009, 2010)	N fix, P sol, Sid pectinase, cellulase, Arg decarboxacetoin, *nif*H,
Erwinia persicina							x	*Solanum tuberosum*	(Rasche et al., 2006a)	Antibacterial
Escherichia sp.		x					x	Palm tree	(Yaish et al., 2015)	NH_3
Flavobacterium gleum		x						*Oryza alta*	(Elbeltagy et al., 2000)	
Gluconacetobacter diazotrophicus		x						sugarcane	(Beneduzi et al., 2013; Gillis et al., 1989; Lee et al., 2004)	N fix, P sol, Sid
Gluconacetobacter sp.		x						sugarcane	(Beneduzi et al., 2013)	N fix, P sol, Sid
Haererehalobacter sp.		x					x	*Salicornia brachiata*	(Jha et al., 2012)	N fix, P sol, Sid, *nif*H
Halomonas sp.		x					x	*Salicornia brachiata*	(Jha et al., 2012)	N fix, Sid, *nif*H
Herbaspirillum frisingense		x						*Zea mays*	(Montañez et al., 2012)	*nif*H
Herbaspirillum hiltneri		x						*Zea mays*	(Montañez et al., 2012)	P sol, *nif*H,
Herbaspirillum seropedicae		x	x			x	x	*Citrus sinensis*	(Bastian et al., 1998; Trivedi et al., 2011)	N fix, P sol, Sid, *nif*H

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Herbaspirillum sp.		x					x	*Commelina communis*	(Sun et al., 2010)	Sid
Klebsiella oxytoca		x					x	*Oryza sativa* , Palm tree , cotton, *Solanum tuberosum*	(Elbeltagy et al., 2000; Rasche et al., 2006a; Yaish et al., 2015; Yue et al., 2007)	P sol, NH_3, antibacterial
Klebsiella pneumoniae		x					x	*Piper nigrum, Solanum tuberosum*	(Jasim et al., 2013; Rasche et al., 2006a)	Sid, P sol, antibacterial
Klebsiella sp.		x					x	sugar cane, *Piper nigrum*	(Ibañez et al., 2012; Jasim et al., 2013)	sid
Kocuria sp.		x						*Vitis vinifera*	(Andreolli et al., 2016)	P sol
Lysinibacillus fusiformis	x	x	x			x		*Prosopis strombulifera, Citrus sinensis, Panax ginseng*	(Sgroy et al., 2009; Trivedi et al., 2011; Vendan et al., 2010)	N fix, P sol, Sid, chitimase
Lysinibacillus sphaericus		x						*Panax ginseng*	(Vendan et al., 2010)	Sid
Mesorhizobium sp.		x					x	*Salicornia brachiata*, *Vitis vinifera*	(Andreolli et al., 2016; Jha et al., 2012)	N fix, Sid, *nifH*
Methylobacterium fujisawaense		x				x	x	*Lespedeza* sp. *Citrus sinensis*	(Palaniappan et al., 2010; Trivedi et al., 2011)	N fix, P sol, Sid, AHL, *nifH*
Methylobacterium populi		x				x	x	Poplar tree, *Citrus sinensis*	(Taghavi et al., 2009; Trivedi et al., 2011)	Chitinase, Sid, P sol, N fix, AHL, *nifH*
Methylobacterium sp.		x				x	x	*Citrus sinensis, Vitis vinifera*	(Andreolli et al., 2016; Trivedi et al., 2011)	N fix, P sol, Sid, Chitinase, AHL, *nifH*
Microbacterium arborescens		x					x	*Solanum tuberosum, Citrus sinensis*	(Rasche et al., 2006b; Trivedi et al., 2011)	P sol, Sid, chitinase
Microbacterium ginsengisoli		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	P sol, Sid
Microbacterium kitamiense		x						*Commelina communis*	(Sun et al., 2010)	Sid
Microbacterium phyllosphaerae		x						*Panax ginseng*	(Vendan et al., 2010)	P sol, Sid
Microbacterium oleivorans		x				x	x	*Citrus sinensis, Solanum tuberosum*	(Rasche et al., 2006a; Trivedi et al., 2011)	N fix, P sol, Sid, Chitinase, antibacterial

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Microbacterium sp.		x					x	*Solanum lycopersicum, Solanum tuberosum, Persea americana, Aster tripolium*	(Barra et al., 2016; Rashid et al., 2012; Szymańska et al., 2016)	Sid, NH_3
Microbacterium takaoensis		x					x	*Solanum lycopersicum*	(Rashid et al., 2012)	Sid, NH_3
Microbacterium testaceum		x						Sugarcane, *Solanum tuberosum*	(Mendes et al., 2007; Rasche et al., 2006b)	Protease, α-glucanase
Micrococcus luteus		x						*Elsholtzia splendens, Panax ginseng*	(Sun et al., 2010; Vendan et al., 2010)	P sol, Sid
Micromonospora sp.		x						Winter rye	(Merzaeva and Shirokikh, 2010)	
Nocardioides sp.		x					x	*Solanum tuberosum*, *Vitis vinifera*	(Andreolli et al., 2016; Rasche et al., 2006b)	sid
Ochrobactrum anthropic		x						Deepwater rice	(Verma et al., 2001)	P sol, N fix
Paenibacillus glucanolyticus		x				x	x	Palm tree, *Citrus sinensis, Panax ginseng*	(Trivedi et al., 2011; Vendan et al., 2010; Yaish et al., 2015)	P sol, chitinase, *nif*H, N fix
Paenibacillus lentimorbus		x						*Cymbidium eburneum*	(Faria et al., 2013)	
Paenibacillus macerans		x						*Cymbidium eburneum*	(Faria et al., 2013)	
Paenibacillus pabuli							x	*Solanum tuberosum*	(Rasche et al., 2006a)	antibacterial
Paenibacillus polimaxa				x				*Gynura procumbens*	(Bhore et al., 2010)	
Paenibacillus sp.							x	*Solanum tuberosum*	(Rasche et al., 2006b)	
Paenibacillus validus		x				x		*Citrus sinensis*	(Trivedi et al., 2011)	P sol, chitinase, N fix, Sid
Paenibacillus xylanexedens		x					x	Palm tree	(Yaish et al., 2015)	

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Pantoea agglomerans	x	x	x	x		x	x	Rice, *Solanum nigrum*, deepwater rice, *Conyza, Canadensis*, *Piper nigrum*, *Solanum tuberosum* , *Zea mays*, *Vicia faba*, *Citrus sinensis*, *Solanum tuberosum*	(Feng et al., 2006; Jasim et al., 2013; Long et al., 2008; Montañez et al., 2012; Rasche et al., 2006a, 2006b; Saïdi et al., 2013; Trivedi et al., 2011; Verma et al., 2001; Y. Zhang et al., 2011)	N fix, P sol, Sid, *nif*H, chitinase, antibacterial
Pantoea ananatis		x					x	Sugarcane, plant grown in a copper mine	(Mendes et al., 2007)(Y. Zhang et al., 2011)	Antifungal, Sid, P sol
Pantoea ananas		x						*Oryza alta*	(Elbeltagy et al., 2000)	
Pantoea brenneri		x						*Vicia faba*	(Saïdi et al., 2013)	P sol.
Pantoea punctata		x						Strawberry	(de Melo Pereira et al., 2012)	Sid
Pantoea sp.		x				x	x	Soybean, *Zea mays*, *Citrus sinensis*, *Solanum tuberosum*, *Vitis vinifera*	(Andreolli et al., 2016; Johnston-Monje and Raizada, 2011; Li et al., 2008; Montañez et al., 2012; Rasche et al., 2006a; Trivedi et al., 2011)	N fix, P sol, Sid, acetoin, pectinase, antifungal, cellulose, *nif*H, chitinase, antibacterial
Pantoea stewartii		x					x	Sugarcane, plant grown in a copper mine	(Mendes et al., 2007; Y. Zhang et al., 2011)	P sol, Sid, Antifungal
Pseudomonas aeuroginosa		x					x	*Citrus sinensis*	(Trivedi et al., 2011)	N fix, P sol, Sid, *nif*H
Pseudomonas boreopolis		x						Deepwater rice	(Verma et al., 2001)	N fix
Pseudomonas brassicacearum		x					x	*Solanum nigrum*	(Long et al., 2008)	
Pseudomonas congelans							x	*Solanum tuberosum*	(Rasche et al., 2006a)	antibacterial
Pseudomonas fluorescens		x				x	x	*Solanum nigrum*, Sugarcane, *Solanum lycopersicum*, *Solanum tuberosum*, *Zea mays*, *Lycopersicon esculentum*, clover, olive	(Etesami et al., 2014; Long et al., 2008; Mendes et al., 2007; Montañez et al., 2012; Rasche et al., 2006b; Rashid et al., 2012; Wang et al., 2011)(Mercado-Blanco et al., 2004)	P sol, Sid, Pyrrolnitrin, protease, sid NH_3, *nif*H, antibacterial

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Pseudomonas fulva		x				x	x	Deepwater rice, *Citrus sinensis*, *Solanum tuberosum*	(Rasche et al., 2006a; Trivedi et al., 2011; Verma et al., 2001)	N fix, P sol, Sid, chitinase, *nif*H, *phi*D, antibacterial
Pseudomonas huttiensis		x					x	*Solanum tuberosum*	(Rasche et al., 2006b)	
Pseudomonas lutea		x					x	*Solanum nigrum*	(Long et al., 2008)	
Pseudomonas marginalis		x						*Vicia faba*	(Saïdi et al., 2013)	P sol, Sid
Pseudomonas oleovorans							x	*Solanum tuberosum*	(Rasche et al., 2006b)	
Pseudomonas pseudoalcaligenes		x					x	*Salicornia brachiata*	(Jha et al., 2012)	N fix, Sid, *nifH*
Pseudomonas putida	x	x		x		x	x	*Prosopis strombulifera*, poplar trees, *Salicornia brachiata*, *Citrus sinensis*, clover	(Etesami et al., 2014; Jha et al., 2012; Sgroy et al., 2009; Taghavi et al., 2009; Trivedi et al., 2011; Weyens et al., 2011)	N fix, P sol, Sid, AHL, *nifH*
Pseudomonas resinovoran				x				*Gynura procumbens*	(Bhore et al., 2010)	
Pseudomonas savsananoi		x				x	x	*Citrus sinensis*	(Trivedi et al., 2011)	N fix, P sol, Sid, *phl*D, AHL, chitinase
Pseudomonas sp.		x				x	x	Strawberry, Chinese cabbage, *Solanum nigrum* , plant grown in a copper mine, *Zea mays*, *Solanum lycopersicum*, *Elymus mollis*, *Glehnia littoralis*, *Piper nigrum*, *Salicornia brachiata*, *Solanum tuberosum*, *Citrus sinensis*, *Persea americana*	(Barra et al., 2016; de Melo Pereira et al., 2012; Jasim et al., 2013; Jha et al., 2012; Johnston-Monje and Raizada, 2011; Long et al., 2008; Rasche et al., 2006b; Rashid et al., 2012; Shin et al., 2007; Trivedi et al., 2011; Yim et al., 2009; Y. Zhang et al., 2011)	N fix, P sol, Sid, (ARA *nifH*), acetoin, pectinase, antifungal, cellulose, NH_3, Protease, Chitinase, AHL, *phl*D
Pseudomonas stutzeri		x					x	*Echinacea* plants, *Citrus sinensis*, *Aster tripolium*	(Lata et al., 2006; Szymańska et al., 2016; Trivedi et al., 2011)	N fix, P sol, Sid, *phl*D, Celulase
Pseudomonas thivervalensis		x					x	*Solanum nigrum*, *Mosla chinensis*	(Long et al., 2008; Y. Zhang et al., 2011)	P sol, Sid

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Pseudomonas toloasi		x				x	x	*Citrus sinensis*	(Trivedi et al., 2011)	P sol, sid, *phl*D, N fix
Pseudoxantomonas sp.		x						*Vitis vinifera*	(Andreolli et al., 2016)	NH_3, Sid
Rahnella aquatilis		x						*Heracleum* sp., *Vicia faba*	(Malfanova et al., 2011; Saïdi et al., 2013)	P sol, *nif*H
Rahnella sp.		x						*Zea mays*	(Montañez et al., 2012)	P sol, *nif*H
Ralstonia sp.		x					x	*Bidens pilosa*	(Y. Zhang et al., 2011)	P sol, Sid
Rhizobium albertimagni		x						*Vicia faba*	(Saïdi et al., 2013)	
Rhizobium grahamii		x						*Vicia faba*	(Saïdi et al., 2013)	
Rhizobium huautlense		x						*Vicia faba*	(Saïdi et al., 2013)	
Rhizobium lusitanum		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	
Rhizobium nepotum		x						*Vicia faba*	(Saïdi et al., 2013)	Sid
Rhizobium pusense		x						*Vicia faba*	(Saïdi et al., 2013)	
Rhizobium rediobacter		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	P sol, Sid
Rhizobium sp.		x					x	*Solanum tuberosum*, *Zea mays*, *Citrus sinensis*, *Vitis vinifera*	(Andreolli et al., 2016; Montañez et al., 2012; Rasche et al., 2006b; Trivedi et al., 2011)	P sol, Sid, chitinase, AHL, *nifH*,
Rhizobium tropici		x					x	*Lespedeza* sp.	(Palaniappan et al., 2010)	P sol, Sid,
Rhodanobacter sp.		x						*Calystegia soldanella*	(Shin et al., 2007)	antifungal
Rhodococcus equi		x					x	Palm tree, *Solanum lycopersicum*	(Rashid et al., 2012; Yaish et al., 2015)	NH_3
Rhodococcus sp.							x	*Solanum tuberosum*	(Rasche et al., 2006a)	antibacterial
Serratia nematodiphila		x					x	*Solanum nigrum*	(Chen et al., 2010)	
Serratia marcescens		x					x	*Elsholtzia splendens*, *Solanum tuberosum*, *Capsicum annuum*, *Aster tripolium*	(Amaresan et al., 2011; Rasche et al., 2006b; Sun et al., 2010; Szymańska et al., 2016)	P sol. Sid, chitinase, antifungal, *nif*H,
Serratia sp.		x				x	x	Soybean, *Citrus sinensis*, *Persea americana*	(Barra et al., 2016; Li et al., 2008; Trivedi et al., 2011)	N fix, P sol, Sid chitinase

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Serratia plymuthica		x				x	x	Wheat, *Citrus sinensis*, *Aster tripolium*	(Liu et al., 2011; Szymańska et al., 2016; Trivedi et al., 2011)	N fix, P sol, Sid chitinase
Serratia proteamaculans		x				x	x	Poplar tree, *Citrus sinensis*	(Taghavi et al., 2009; Trivedi et al., 2011)	N fix, P sol, Sid chitinase
Shinella kummerowiae		x						*Vicia faba*	(Saïdi et al., 2013)	
Sphingobium yanoikuyae							x	*Solanum tuberosum*	(Rasche et al., 2006b)	
Sphingomonas sp.		x					x	*Commelina communis*	(Sun et al., 2010)	Sid, Arg descarbox
Sphingopyxis sp.		x						Strawberry	(Dias et al., 2008)	P sol
Sporosarcina aquimarina		x						*Aster tripolium*	(Szymańska et al., 2016)	
Staphylococcus epidermidis		x					x	*Solanum tuberosum*, *Panax ginseng*	(Rasche et al., 2006a, 2006b; Vendan et al., 2010)	
Staphylococcus pasteuri		x						Palm tree, *Panax ginseng*	(Vendan et al., 2010; Yaish et al., 2015)	NH3
Staphylococcus warneri							x	*Lespedeza* sp.	(Palaniappan et al., 2010)	
Stenotrophomonas chelatiphaga		x				x	x	*Citrus sinensis*	(Trivedi et al., 2011)	N fix, P sol, Sid
Stenotrophomonas maltophilia		x				x	x	Poplar tres, *Solanum tuberosum*	(Rasche et al., 2006a, 2006b; Taghavi et al., 2009)	antibacterial
Stenotrophomonas sp.		x				x	x	*Zea* mays, *Citrus sinensis*	(Johnston-Monje and Raizada, 2011; Trivedi et al., 2011)	P sol, Sid acetoin, pectinase, antifungal, cellulose
Streptomyces griseoplanus		x						*Aster tripolium*	(Szymańska et al., 2016)	Sid, Celulase
Streptomyces sp.		x						Winter rye	(Merzaeva and Shirokikh, 2010)	
Streptomyces umbrinus		x						*Aster tripolium*	(Szymańska et al., 2016)	Sid, celulase
Thalassospira permensis		x						*Aster tripolium*	(Szymańska et al., 2016)	
Variovorax paradoxus		x				x	x	*Citrus sinensis*	(Trivedi et al., 2011)	N fix, P sol, Sid, *nif*H
Vibrio alginolyticus		x					x	*Salicornia brachiata*	(Jha et al., 2012)	N fix, P sol, Sid, *nif*H

Appendix 2.1. Phytohormone-producing endophytic bacteria described in the literature and host plant from where they were isolated (continued).

Bacterial species	ABA	IAA	GAs	CKs	JAs	SAs	ACCD	Plant host	Reference	Other PGP trait
Virgibacillus sp.		x						Strawberry	(Dias et al., 2008)	P sol
Zhihengliuella sp.		x					x	*Salicornia brachiata*	(Jha et al., 2012)	N fix, P sol, Sid, *nif*H

ABA: abscisic acid; **IAA**: Indole acetic acid; **GAs:** Gibberellins; **CKs:** cytokinins; **JAs**: JAsmonic acid; **SAs:** salicylic acid; **ACCD:** 1 aminocyclopropane 1 carboxylate deaminase; **N fix.** Nitrogen fixation; **P sol:** Phosphate solubilization; **Sid:** Siderophore production; ***nif*H:** detection of *nif*H gene, **NH_3:** production of **NH_3** *in vitro*; **antibacterial:** antibacterial activity *in vitro*; **antifungal:** antifungal activity *in vitro*; **Arg descarbox:** production of Arginine decarboxylase *in vitro.*