

The Role of Microbial Inoculants in Integrated Crop Management Systems

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Received: 2 December 2014 / Accepted: 24 December 2014
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Abstract One of the major issues facing humankind is global food security. A changing climate, coupled with a heightened consumer awareness of how food is produced and legislative changes governing the usage of agrichemicals for improving plant health and yield, means that alternative, more integrated and sustainable approaches are needed for crop management practices. To this end, there is increasing recognition of the value of the role of microbial inoculants in agriculture. The focus of this review is to understand how plant-growth-promoting bacteria and arbuscular mycorrhizal fungi can play a part in improving crop yield by promoting the health status of the plant through the sequestration of various nutrients and in the control of plant diseases.

Keywords Arbuscular mycorrhizal fungi · Biofertilisers · Biological control · Plant-growth-promoting bacteria · Sustainable agriculture · Volatile organic compounds

Introduction

The major twenty-first-century challenge is how to sustainably feed a burgeoning population. Successful crop management is essential for food security, and plant diseases are a serious threat to agricultural crops. For example, plant pathogens such as *Rhizoctonia solani*, *Phytophthora infestans* and *Pectobacterium carotovorum* pose a

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threat to the health and yield of potato crops (Powelson and Rowe 2008; Walter et al. 2008; Tsrör 2010; Baz et al. 2012). The implementation of integrated crop management practices for the control of plant diseases requires effective crop management strategies. Common farming practices include using certified seed and crop rotation to reduce the risk of prolonged contact with potato-associated pathogens, soil solarisation (i.e., using the Sun's energy (heat) to increase the soil temperature to kill or weaken pathogens) and biofumigation, in which naturally occurring plant volatiles such as glucosinolates released into the soil from decomposing *Brassica* material are used to suppress pathogens (Ghyselinck 2013). Plant pathogens are also frequently managed and controlled with agrichemicals, although they often provide only short-term solutions due to the emergence of new or virulent pathogens. This drives the search for new agrichemicals for target pathogens, but this approach is unlikely to provide a long-term and sustainable solution. These current control measures do not provide a completely effective solution for the prevention and treatment of plant diseases, which remain a continuous threat. In addition, the large amounts of fertilisers used to improve yields are not always completely absorbed by the plants and can result in environmental harm, with leaching and runoff from the soil surface into groundwater leading to the eutrophication of aquatic ecosystems and possible effects on humans (Adesemoye and Kloepper 2009).

Legislative changes in relation to the control and use of agrichemicals (especially in Europe) coupled with demand for more sustainably-produced food products, are dictating a more integrated approach for crop management practices throughout the world. Scientists are left with the challenge of developing new technologies to overcome these problems, such as genetic modification (GM) or conventional plant breeding techniques for the development of disease-resistant crop varieties. However, these approaches are also not without their problems. The strict regulatory procedures regarding registration, coupled with the lack of consumer acceptance of GM crops particularly in Europe and the whole debate on GM crop production, keep these products out of the market. With respect to conventional breeding, it can take a long time to introduce a desirable trait by breeding alone. Pathogens to new varieties may emerge which drive the search for alternative crop management practices (Tester and Langridge 2010). Holistic integrated plant health management is defined as “*the application of a totally integrated set of production practices that results in a quality crop produced in a manner that optimises economic return and minimises environmental impacts and undesirable societal effects*” (Rowe and Powelson 2008). A holistic approach must satisfy production efficiency, economic viability, environmental compatibility and social responsibility. To this end, the use of plant-growth-promoting bacteria (PGPB)/bacterium-based biological control agents (BCAs) and arbuscular mycorrhizal fungi (AMF) are among the methods to improve yields and control disease as part of an integrated crop management strategy (Borriss 2011; Jung et al. 2012; Ghyselinck et al. 2013; Mitter et al. 2013; Senés-Guerrero et al. 2014; Velivelli et al. 2014b).

Plant-Growth-Promoting Bacteria

Soil is a dynamic living source of microorganisms (e.g., bacteria and fungi) that compete for limited space, water and nutrients. Approximately 20 to 40% of

photosynthetic carbon fixed by a plant is released as root exudate into the surrounding soil, attracting microorganisms and creating a dynamic ecological niche known as the rhizosphere (Jones et al. 2009; Raaijmakers et al. 2009), a term coined by the German agronomist Lorenz Hiltner in 1904 (“rhizo”=root and “sphere”=area of influence) (Hartmann et al. 2008). The rhizosphere represents a microbial “hot spot”, in which positive, neutral and negative interactions take place with the host plant which can affect plant health and growth. There are three distinct, but interlinking components recognised in the rhizosphere: the rhizospheric soil or ectorrhizosphere (soil adhering to the root surface or the soil nearby the root), the rhizoplane (surface of the root) and endorhizosphere (plant internal tissues) (Morgan et al. 2005). Kloepper and co-workers subsequently termed the bacterial community that colonise and interact with the rhizosphere/roots as “rhizobacteria” (Kloepper et al. 1980). In the case of endophytic bacteria, one additional step involved is the endophytic colonisation of plant internal tissues, such as roots (Compant et al. 2010). In general, the bacterial concentration in the rhizosphere is more than in bulk soil but lower than in laboratory medium (Lugtenberg and Kamilova 2009). Some bacteria promote plant growth directly, others exert their effect indirectly via biological control of plant diseases, and some use both mechanisms; these are called plant-growth-promoting bacteria (PGPB). Although many of the exact mechanisms by which bacteria promote growth or control plant diseases remain to be determined, several known mechanisms have been established. Direct plant growth promoters can be classified into biofertilisers, rhizoremediators, phyto-stimulators, or stress controllers. Indirect plant growth promoters that reduce the number of pathogens do so via antibiosis, lytic enzyme production, competition for nutrients and induced systemic resistance (ISR) in the host plant (Lugtenberg and Kamilova 2009; Pliego et al. 2011; Glick 2012; Velivelli et al. 2014a). In recent years, another indirect mechanism of biological control which is becoming more studied is the effect of bacterial volatile organic compounds (VOCs) on plants and pathogens (Ryu et al. 2003; Ryu et al. 2004; Effmert et al. 2012).

Mechanisms of Plant-Growth-Promoting Bacteria

Direct Plant Growth Promotion

Biofertilisers

PGPB enhance nutrient availability to the plant through either nitrogen fixation, phosphorus and potassium solubilisation, sequestering iron and sulphur oxidation, collectively termed as biofertilisers.

Nitrogen Fixation Nitrogen (N), one of the essential nutrients for plants and a limiting factor for agricultural ecosystems, constitutes 78% of the Earth’s atmosphere. Plants cannot use atmospheric nitrogen directly; hence, the form of nitrogen in the soil needs to be converted to another form to be available for plants, such as nitrate (NO_3^-) or ammonium (NH_4^+). Farmers heavily rely on nitrogen fertilisers to meet the demands of their crops. The production of nitrogen fertilisers places a substantial strain on the planet’s non-renewable resource reserves such as coal and petroleum. The production

of industrial nitrogen fertilisers not only depletes fossil fuels; any excess nitrogen fertiliser in the soil such as nitrate can be lost as a result of denitrification, but can also leach into the groundwater leading to eutrophication (Diaz and Rosenberg 2008). Biological nitrogen fixation (BNF), reducing the atmospheric nitrogen into ammonia through the nitrogenase enzyme complex, offers an alternative way to replace nitrogenous fertilisers. The organisms responsible for BNF are broadly classified into symbiotic and non-symbiotic nitrogen-fixing bacteria (Franche et al. 2009). The symbiotic nitrogen-fixing bacteria, such as *Rhizobium* spp., are being used on legume crops, to fix atmospheric nitrogen. In the legumes, root exudates such as flavonoid compounds attract and act as signals for *Rhizobium* spp., to secrete *Nod* factors, which are then perceived by legume plant hairs to induce root nodules for the *Rhizobium* spp., to fix atmospheric nitrogen (Brencic and Winans 2005; Peix et al. 2010). In addition to symbionts, non-symbiotic free-living bacteria such as *Azospirillum* spp. were reported to fix nitrogen in non-leguminous plants. However, the significance of global non-symbiotic nitrogen fixation to plant growth enhancement appears to be minor. In addition, various mechanisms create the growth-promoting effects (e.g., auxin) along with supplying nitrogen to the plant (Lugtenberg and Kamilova 2009).

Phosphate Solubilisation One of the macronutrients most vital for plant development is phosphorus (P). Unlike nitrogen, which can be taken up by the plants from the large atmospheric source, P is a non-renewable resource. The use of phosphate fertilisers has received a great deal of attention due to the rising costs of these chemicals, which preclude farmers in developing nations from using them. These higher prices are partially due to intense competition for high-quality rock phosphate from other industries such as food preservative productions, and supplies of this resource are declining at a rapid pace (Bashan et al. 2013a).

The majority of the phosphorus is insoluble and precipitated in soil; therefore, it is available to plants in a low proportion. There are two types of insoluble P present in soils, namely, inorganic mineral and organic forms. The former includes tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate. The latter includes inositol phosphate (soil phytate), which accounts up to 50% of the total organic P in soil, and other organic P compounds in the form of phosphomonoesters, phosphodiester and phosphotriesters (Rodríguez and Fraga 1999). Phosphorus is taken up by the plants, mostly in the form of ortho-phosphates, such as the monobasic (H_2PO_4^-) under alkaline conditions and the dibasic (HPO_4^{2-}) under acidic conditions. Therefore, to attain the highest possible level of plant productivity, large quantities of soluble P fertiliser are used in agriculture (Vessey 2003). Soil pH can significantly impact phosphorus availability. Under acidic and alkaline soil conditions, phosphorus is rapidly fixed into insoluble forms such as aluminium phosphate (Al-P), iron phosphate (Fe-P) and calcium phosphate (Ca-P), respectively, thereby being unavailable to plants. The excessive application of P fertilisers can have a negative impact on the environment. The more severe environmental consequences include eutrophication and hypoxia of lakes and estuaries (Park et al. 2009).

Many of the problems described above can be avoided by applying phosphate-solubilising bacteria (e.g., *Bacillus* and *Pseudomonas* spp.) as biofertilisers, to solubilise bound phosphorus from organic and inorganic mineral forms. Phosphate-solubilising bacteria solubilise inorganic P through the synthesis of organic acids, such as gluconic

and citric acid, whose hydroxyl and carboxyl groups are able to either chelate phosphate-bound cations (Ca^{2+} , Fe^{3+} and Al^{3+}) or indirectly solubilise P by decreasing soil pH through the release of protons (H^+), thus converting insoluble phosphorus into soluble forms that plants can absorb (Bashan et al. 2013b). Plants can also assimilate phosphorus through the mineralisation of organic P. Many enzymes are directly involved in the release of soluble P from organic P, including phytases, phosphatases, phosphonatasases and C-P lyases. Phytases are responsible for the hydrolysis of phosphomonoester bonds in phytic acid, releasing inorganic orthophosphate. Phosphatases release phosphorus through the dephosphorylation of phosphoester or phosphoanhydride bonds in organic matter. Phosphonatasases and C-P lyase cleave C-P bonds from organophosphates, thereby releasing phosphorus (Rodríguez et al. 2006).

Iron Sequestration All organisms require iron, the fourth most common element on Earth. Nonetheless, the reduced form Fe^{2+} , unstable under aerobic conditions at physiological pH (i.e., 7.4), is easily oxidised to the ferric form Fe^{3+} , which is not directly absorbable by plants and bacteria due to limited solubility (Gamalero and Glick 2011; Beneduzi et al. 2012). Consequently, bacteria and plants have developed sophisticated strategies to acquire iron. Bacteria produce various iron-chelating compounds referred to as siderophores which exhibit a high affinity for ferric iron. The iron-limitation-dependent membrane receptors of bacteria recognise and bind the Fe^{3+} -siderophore complex formed, which is subsequently released and remains biologically active in the cytoplasm of bacteria, in the form of Fe^{2+} (Lugtenberg and Kamilova 2009). Siderophores secreted by the bacteria are divided into four categories namely, carboxylates, hydroxamates, phenol catecholates and pyoverdines (Crowley 2006). The beneficial effects of bacterial siderophores on plant growth have been well documented (Sharma et al. 2003). A significant increase in the uptake of iron was observed when rice was inoculated with a siderophore-producing strain, *Pseudomonas putida* (Sharma et al. 2013). Reduction and chelation are further strategies that plants use for the uptake of iron. Associated primarily with dicots and non-graminaceous monocots, the reduction strategy entails the secretion of H^+ and organic acids to acidify the rhizosphere which reduces Fe^{3+} to Fe^{2+} , thus enhancing iron availability. The chelation strategy, primarily utilised by grasses and graminaceous plants including wheat, barley, rice and maize, is based on the release of low-molecular-weight iron-chelating molecules known as phytosiderophores (e.g., mugineic acid) that can bind Fe^{3+} which is subsequently assimilated by the root cells (Gamalero and Glick 2011; Bulgarelli et al. 2013).

Potassium Solubilisation In addition to P solubilisation, another important plant macronutrient is potassium (K). K-solubilising bacteria, such as *Bacillus* spp., solubilise rock K minerals (e.g., micas, illite and orthoclase) through the synthesis of organic acids or directly solubilise K by chelating silicon ions (Sheng and He 2006; Parmar and Sindhu 2013). A significant increase in the growth and the uptake of K was observed when aubergine was co-inoculated with *Bacillus mucilaginosus* and *Bacillus megaterium* (Han and Lee 2005), indicating their potential role as K solubilisers.

Sulphur Oxidation Similarly, in recent years, there has been increased interest in the role of sulphur-oxidising bacteria. For instance, plants utilise sulphur as sulphate, but sulphur binds to organic molecules in the soil and becomes unavailable to plants.

Sulfur-oxidising bacteria such as *Thiobacillus* spp. increase the sulphur oxidation rate and produce sulphates that can then be taken up by plants (Anandham et al. 2011; Smyth et al. 2011; Mohamed et al. 2014).

Rhizoremediation

In rhizoremediation, bacteria degrade soil pollutants, and their survival is promoted by utilising root exudates as a nutrient source. For example, *Pseudomonas putida* PCL1444 degrades pollutants and protects grass seeds against naphthalene phytotoxicity, permitting the plant to grow normally in polluted soil; mutants incapable of degrading naphthalene did not protect plants (Kuiper et al. 2001; Lugtenberg and Kamilova 2009).

Phytohormones and Stress Controllers

It is well known that plant growth responses, including the establishment of root architecture, are modulated by a group of chemicals known collectively as phytohormones. Production of phytohormones is one of the most important features of plant growth and defence. The major phytohormones are auxins, cytokinins, gibberellins (GAs), abscisic acid (ABA), ethylene (ET), brassinosteroids (BRs), jasmonic acid (JA), salicylic acid (SA) and a more recently characterised group of plant hormone, strigolactones (SLs) (Cheng et al. 2013). Because of their potential role in the regulation of growth, bacteria that produce plant growth regulators are also important when they interact with plants. Phytostimulation results from microbial modulation of the plant hormonal balance. Auxins, cytokinins and GAs will be discussed under “phytohormones” whereas ABA and ethylene will be discussed under “stress controllers”.

Indole-3-Acetic Acid (IAA) It is the auxin commonly produced by plants. It is a well-known phytohormone and up to 80% of bacteria are able to produce it (Patten and Glick 1996; Khalid et al. 2004). Plants have been shown to gain increased access to soil nutrients and water as a result of increases in root branching due to IAA releases by bacteria (Vessey 2003). The reasons why bacteria synthesise IAA are not clearly understood, although it has been suggested that bacteria obtain nutrients, such as the amino acid tryptophan, from root exudates, but when tryptophan levels are higher, bacterial growth can be negatively affected. Tryptophan is a precursor of IAA, and bacteria can diminish this negative effect by detoxifying excess tryptophan by converting it to IAA. This bacterial IAA can supplement the endogenous pool of the plant to modify plant growth and behaviour. The result can be increased production of nutrients from the roots, which supports bacterial growth (Bar and Okon 1992; Teixeira et al. 2007). Multiple biosynthetic pathways have been proposed in plant-associated bacteria including tryptophan-dependent or tryptophan-independent pathways. The tryptophan-dependent pathways include the (1) indole-3-acetamide (IAM), (2) tryptamine (TAM), (3) indole-3-pyruvate (IPyA), (4) tryptophan side-chain oxidase (TSO) and (5) indole-3-acetonitrile (IAN). Although tryptophan-independent biosynthesis is not well known in bacteria, this pathway has been shown to exist in *Azospirillum*

brasilense and, moreover, it accounts for 90% of the IAA produced in circumstances where no exogenous tryptophan is supplied. However, little genetic or biochemical evidence is available to support this proposed pathway of IAA biosynthesis in bacteria (Spaepen et al. 2007). Bacteria are known to use different pathways to synthesise IAA, and plant-bacterial interactions can raise plant IAA levels to either optimal or supra-optimal levels, leading to promotion or inhibition of normal plant growth, respectively. From the point of view of soil bacteria, one of the benefits of IAA in the plant is an enhancement of lateral and adventitious root growth, which results in increased uptake of mineral nutrients and enhanced production of root exudates, which can stimulate bacterial growth and proliferation around the actively growing root. Root growth in radish was shown to be enhanced by the production of IAA by *Pseudomonas fluorescens* WCS365 when radish plants exuded higher amounts of tryptophan than tomato, sweet pepper or cucumber (Kamilova et al. 2006). In the presence of L-tryptophan, the increase in plant growth and colonisation of maize was observed when inoculated with IAA-producing strain *Burkholderia phytofirmans* PsJN (Naveed et al. 2014). In another experiment, the growth of *Lemna* (duckweed) was enhanced by the production of IAA by *Bacillus amyloliquefaciens* FZB42—a fivefold increase in IAA levels in the presence of 5 mM tryptophan. Mutants of FZB42 deficient in the *trp* genes involved in the synthesis of IAA produced reduced amounts of IAA and were less efficient at promoting growth, suggesting that synthesis of IAA is tryptophan dependent and functionally related to growth (Idris et al. 2007).

Cytokinin It is an important phytohormone involved in plant growth. Cytokinins regulate diverse processes such as cell division, chlorophyll synthesis, seed germination, apical dominance, leaf expansion and delayed senescence (Muller and Sheen 2007). The balance between auxins and cytokinins results in an interaction between these two classes of phytohormones with outcomes that affect shoot and root architecture. For example, when a callus is exposed to these hormones, a high auxin/cytokinin ratio leads to root development, while a low auxin/cytokinin ratio leads to shoot development (Nordström et al. 2004). The production of cytokinin has been observed in various bacteria such as *Bacillus* spp., *Pseudomonas* spp., *Paenibacillus* spp., *Azospirillum* spp. and *Bradyrhizobium* spp. (Arkhipova et al. 2007; Perrig et al. 2007; Cassán et al. 2009). Although the mechanism of plant growth by cytokinin-producing bacteria is not clearly understood, it has been speculated that bacterial cytokinins can combine with the endogenous cytokinin pool of the plant to modify plant growth and development. The beneficial effects of bacterial cytokinins on plant growth have been well documented. Lettuce plants inoculated with cytokinin-producing *Bacillus* spp. showed increased shoot and root growth (Arkhipova et al. 2005). The vital role played by cytokinin-producing bacteria, *Pseudomonas fluorescens* G20-18, in modulating plant growth has been validated by mutants with reduced production of cytokinin and normal levels of auxin, where the mutants did not stimulate growth of wheat and radish plants (Garcia de Salamone et al. 2001; Garcia de Salamone et al. 2006). A significant increase in fresh weight, chlorophyll content, cotyledon size and cell division was observed when cucumber plants were inoculated with cytokinin-producing bacteria, *Bacillus licheniformis* (Hussain and Hasnain 2009). In recent years, there has also been increased interest in the role of cytokinin-producing bacteria in alleviating drought stress (Arkhipova et al. 2007; Liu et al. 2013). Although there are

studies describing cytokinin-producing bacteria and their role in plant growth, much remains to be done in this area of research.

Gibberellins (GAs) These are another class of phytohormones involved in cell division and stem elongation, seed germination, flowering, fruit growth and delay of senescence (MacMillan 2001). The production of GAs was observed in various bacteria such as *Bacillus* spp., *Azotobacter* spp., *Azospirillum* spp., and *Rhizobium* spp. (Gutiérrez-Mañero et al. 2001; Bottini et al. 2004; Perrig et al. 2007; Cassán et al. 2009). Although, the exact mechanism by which bacteria GA interacts with plant GA is not well understood, it has been hypothesised that enhanced GA levels in the plant result from either bacteria producing GA or deconjugating GA from root exudates, or activation of inactive GAs by bacteria. It was also speculated that GAs enhance plant growth by increasing root hair density, thereby being actively involved in the assimilation of water and nutrients from the soil (Fulchieri et al. 1993; Bottini et al. 2004). Under controlled greenhouse conditions, plant growth of Chinese cabbage, cucumber and crown daisy was significantly enhanced following inoculation with GA-producing *Acinetobacter calcoaceticus* SE370 (Kang et al. 2009). Although gibberellin production by plant-associated bacteria seems less widespread, more research is necessary to determine how these bacterial gibberellins interact with plant gibberellins, and their involvement in plant growth.

Abscisic Acid (ABA) It is another important phytohormone involved in the plant growth response to biotic and abiotic stresses (Cohen et al. 2008). Regulation of water loss is important for plants experiencing drought conditions. In response to drought stress, ABA acts as a signal mainly by inducing stomatal closure (Bauer et al. 2013). The production of ABA was observed in various bacteria such as *Azospirillum* spp. and *Bradyrhizobium* spp. (Boiero et al. 2007; Perrig et al. 2007; Cohen et al. 2008). The exact mechanism underpinning the interaction between ABA-producing bacteria and the plant and its effect on plant growth remains unclear. A significant increase in the levels of ABA was observed when *Arabidopsis thaliana* was inoculated with the ABA-producing bacteria, *Azospirillum brasilense* Sp 245 (Cohen et al. 2008), and the application of the ABA-producing bacteria *Azospirillum lipoferum* USA59b to maize plants resulted in reduced drought stress and promoted plant growth (Cohen et al. 2009). The studies conducted so far may open up many new avenues of research and have the potential to create opportunities for using bacterial strains that have the ability to synthesise phytohormones to improve plant performances under in vitro and field conditions.

Ethylene (ET) It is a gaseous phytohormone that is essential for the modulation of plant growth, including fruit ripening, flower senescence and seed germination. ET is also produced in response to biotic (e.g., pathogen attack) and abiotic stress (e.g., drought); optimal levels can have positive effects on the plant while increased levels inhibit root growth. ET is therefore referred to as “stress ethylene/hormone” (Glick et al. 2007b). To overcome these negative effects, some bacteria release 1-aminocyclopropane-1-carboxylate (ACC) deaminase (encoded by the *acdS* gene), which cleaves the ethylene precursor ACC to α -ketobutyrate and ammonia, thereby lowering ethylene levels (Glick 2012). When tomato plants were treated with bacteria expressing ACC

deaminase, reduced ethylene levels were observed in response to biotic stress, thereby stimulating plant growth (Glick et al. 2007b; Toklikishvili et al. 2010). Plant growth is not regulated by a single mechanism but by interrelated networks of mechanisms. Ethylene biosynthesis is regulated by auxin biosynthesis. Bacterial indole acetic acid (IAA) combined with endogenous plant IAA can either increase plant growth or induce ACC synthase to biosynthesise ACC, followed by ET biosynthesis. High levels inhibit auxin response factors (ARFs), thereby indirectly controlling auxin-stimulated plant growth and ACC synthase activity. However, in the presence of ACC-deaminase-producing bacteria, ET levels are decreased, which relieves the suppression of ARFs to promote auxin-stimulated growth (Glick et al. 2007a). Aside from its role in plant growth, ET is also a signalling molecule in induced systemic resistance (ISR) mediated by beneficial bacteria against pathogens (Van Loon 2007).

Indirect Plant Growth Promotion

Several theories exist to explain how PGPB control plant diseases at a mechanistic level, so-called biological control, and the PGPB showing this activity are called biological control agents (BCAs).

Antibiosis The production of antibiotics by plant-growth-promoting bacteria is a highly efficient method for inhibiting plant pathogens. The antibiotics produced by beneficial bacteria are low-molecular-weight compounds that suppress the growth of plant pathogens (Beneduzi et al. 2012), and two distinct categories of bacterial antibiotics have been recognised. One category includes the volatile antibiotics, such as hydrogen cyanide (HCN). HCN, a secondary metabolite produced by *Pseudomonas fluorescens* CHA0, was found to inhibit the development of *Thielaviopsis basicola*, which causes black root on tobacco plants (Siddiqui et al. 2006). In recent years, there has also been increased interest in the role of volatile organic compounds (VOCs) in biocontrol, such as dimethyl disulfide (DMDS) (Dandurishvili et al. 2011). The second category of bacterial antibiotic compounds includes the non-volatile diffusible antibiotics, such as 2,4-diacetylphloroglucinol (DAPG), pyoluteirin (PLT), phenazines (PHZ), pyrrolnitrin (PRN) (Fernando et al. 2006), biosurfactants, including cyclic lipopeptides (CLPs) such as viscosin, as well as surfactin, iturin and fengycin (Raaijmakers et al. 2006; Raaijmakers et al. 2010). 2,4-DAPG, produced by *Pseudomonas* spp., damages the membrane and is particularly inhibitory to zoospores of *Pythium* spp., a fungus that causes damping-off disease in the sugar beet (de Souza et al. 2003), and PLT, produced by *Pseudomonas fluorescens* Pf-5, suppressed damping-off caused by *Pythium ultimum* (Bender et al. 1999). PHZ have been found to inhibit plant pathogens by disrupting their cell membranes, which induces the generation of toxic superoxide radicals and hydrogen peroxide (Chin-A-Woeng et al. 2003).

Efficient root colonisation is one of the key mechanisms for all plant-microbe interactions, and a strong correlation exists between root colonisation and the biocontrol of plant pathogens. For example, a *Pseudomonas chlororaphis* PCL1391 mutant defective in root tip colonisation lost the ability to control tomato root rot, caused by *Fusarium oxysporum*, despite the fact that these mutants produced wild-type levels of PHZ (Chin et al. 2000). However, in some cases, root colonisation is not necessary to

control plant pathogens, as ISR can provide broad-spectrum resistance to pathogens without direct interaction (Kloepper et al. 2004; Bakker et al. 2007). PRN, produced by *Pseudomonas fluorescens* BL915, suppressed the damping-off effects of *Rhizoctonia solani* on cotton plants. However, mutants that do not produce PRN were ineffective against *Rhizoctonia solani* (Ligon et al. 2000). Biosurfactants target the cellular membrane of plant pathogens, including *Rhizoctonia solani*, and the CLPs are the best characterised biosurfactants in the context of biocontrol, motility and biofilm formation (Raaijmakers et al. 2006; Raaijmakers et al. 2010).

Lytic Enzyme Production In addition to antibiotics, beneficial bacteria can secrete extracellular enzymes, such as chitinases, β -1,3 glucanases, cellulases and proteases (Compant et al. 2005). As a linear polymer of β -(1,4)-N-acetyl glucosamine, chitin is a major cell wall constituent of most pathogenic fungi and is targeted by the lytic enzyme chitinase. The secretion of extracellular chitinases enables *Paenibacillus illinoisensis* KJA-424 to suppress the damping-off effect of *Rhizoctonia solani* on cucumber (Jung et al. 2003). Glucanases are another category of hydrolytic enzymes of degradation of β -1,3 glucans found in the cell walls of pathogenic fungi. *Pythium* spp., causing Bipolaris leaf spot, was suppressed by β -1,3 glucanase producing *Lysobacter enzymogenes* (Palumbo et al. 2005). The protease and cellulase produced by beneficial bacteria also play a key role in the suppression of plant pathogens. It has been demonstrated that the production of extracellular proteases by *Stenotrophomonas maltophilia* W81 suppressed the growth of *Pythium ultimum* (Dunne et al. 2000). Synergetic interactions between cell-wall-degrading enzymes and antibiotic-producing bacteria are known to exist. A cellulase-producing *Micromonospora carbonacea* in conjunction with antibiotic-producing *Streptomyces violascens* suppressed the root rot caused by *Phytophthora cinnamomi* (El-Tarabily et al. 1996; Fogliano et al. 2002).

Competition for Nutrients This represents one of the key biocontrol mechanism through which bacteria protect plants against pathogens in the rhizosphere. The competition for iron has been the focus of many studies establishing it as a crucial mechanism of biocontrol by bacteria. The Fe^{3+} in the rhizosphere is bound to siderophores secreted by beneficial bacteria, making iron unavailable to plant pathogens and, implicitly, inhibiting their proliferation (Gamalero and Glick 2011). It has been demonstrated that the production of siderophores by *Pseudomonas* spp. reduced *Fusarium* wilt, whereas mutant strains in the biosynthesis of siderophores were ineffective (Duijff et al. 1993). Competition for nutrients and niche (CNN) is an important plant disease biocontrol mechanism that occurs between bacteria and pathogens. Bacteria exerting biocontrol need to utilise the nutrients (e.g., root exudates) by competing and colonising the same niche on the root as indigenous pathogens (Lugtenberg and Kamilova 2009).

Induced Systemic Resistance (ISR) There has been increased interest in the role of ISR as an indirect biocontrol mechanism. Colonisation of plant rhizosphere/roots by beneficial microorganisms (e.g., plant-growth-promoting bacteria—*Bacillus* and *Pseudomonas* spp.) confers broad-spectrum resistance to virulent pathogens and is known as induced systemic resistance (ISR) (Kloepper et al. 2004; Bakker et al. 2007; Van Loon

2007). In this process, plants are bioprimered for faster and stronger activation of cellular defence mechanisms when a pathogen attacks; these mechanisms include the oxidative burst, cell wall consolidation, aggregation of defence-associated enzymes and production of secondary metabolites (Conrath et al. 2006; Lakshmanan et al. 2012; Van de Mortel et al. 2012). For the successful and efficient induction of ISR, plants must recognise bacterial determinants. A number of microbe-associated molecular patterns (MAMPs) involved in ISR activation have been characterised, including lipopolysaccharide (LPS), antibiotics (such as 2,4-diacetylphloroglucinol), siderophores and volatile organic compounds (VOCs; such as 2,3-butanediol or tridecane) (Lee et al. 2012). Once ISR is activated in the plant, it persists for a significant period of time, even if the inciting bacterial population decreases (Van Loon 2007). A number of plant signalling hormones are involved in defence responses including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). In ISR, JA/ET-dependent signalling pathways are induced, which express defence-related proteins including plant defensins (Pieterse et al. 2009). Systemic acquired resistance (SAR), on the other hand, is triggered by pathogens, and the signal (SA) triggers systemic resistance to induce protection against successive infections by the same or different pathogen (Van Loon et al. 1998). SA-inducible genes ultimately lead to systemic accumulation of pathogenesis-related proteins such as *PR1*, *PR2* and *PR5* (Lakshmanan et al. 2012; Van de Mortel et al. 2012). The nature of the pathogen (e.g., biotrophic or necrotrophic) appears to dictate the type of phytohormone utilised in the signalling pathways for defence gene regulation; SA-dependent signalling pathways mediate normal biotrophic pathogenic resistance, while necrotrophic pathogens utilise JA/ET-dependent mechanisms (Glazebrook 2005). However, in some cases, ISR activation can also mobilise different signalling pathways, since certain ISR inducers have been reported to trigger an SA-dependent pathway (Tjamos et al. 2005; Van der Ent et al. 2009). Interactions between SA- and JA/ET-dependent signalling pathways are known to exist, which function as an efficient regulatory mechanism by which the plant adjusts its inducible defence reaction depending on pathogen type (Niu et al. 2011).

Volatile Organic Compounds (VOCs) The normal metabolic activity of any microorganism involves the production of a wide range of infochemicals, of which a large number are volatiles (Fig. 1). The main characteristics of volatiles are relatively low molecular mass (<300 Da), low boiling point and high vapour pressure (Vespermann et al. 2007). They often occur as blends as opposed to the emission of a single compound. These complex volatile blends can have either a positive or negative effect on the growth of plants, fungi and other organisms. The ability of bacteria to emit complex blends of volatiles has been highlighted by a number of reports over the past 10 years (Effmert et al. 2012). Direct contact between bacteria and the plant underlies most bacteria-plant interactions. However, it is now known that interactions between bacteria and plants can occur at significant distances and these interactions are facilitated by VOCs. This phenomenon was first observed by Ryu and co-workers and has led to many new directions in plant research. In cases where physical contact with the plant is impossible, certain bacteria (e.g., *Bacillus* spp.) rely on the production of VOCs (e.g., acetoin and 2,3-butanediol) to stimulate plant growth or trigger ISR against *Erwinia carotovora* (Ryu et al. 2003; Ryu et al. 2004).

The identification of various VOCs and their functions has been watershed moments in the study of plant-bacterial interactions, and determining the roles played by VOCs in the complex signalling system between plants/pathogens and bacteria has been the focus of a number of research groups. It was demonstrated that the VOCs (e.g., tridecane and hexadecane) produced by *Paenibacillus polymyxa* E681 stimulated plant growth in *Arabidopsis* and induced systemic resistance against *Pseudomonas syringae* and *Pectobacterium carotovorum* (Lee et al. 2012; Park et al. 2013). In addition to plant growth and ISR, it has been demonstrated that VOCs produced by bacteria inhibit the growth of various pathogens (Fernando et al. 2005; Yuan et al. 2012). The growth of *Rhizoctonia solani* was suppressed in a split plate assay by VOCs produced by *Bacillus* spp., and *Pseudomonas* spp. (Kai et al. 2007). The volatile metabolites emitted by *Bacillus amyloliquefaciens* NJN-6 inhibited the growth of *Fusarium oxysporum*. The volatiles produced by this bacterium were diverse, including benzothiazole, 2-nonanone and nonanal. Using two-compartment Petri dishes, it was shown that exposure to pure synthetic volatiles reduced the growth of phytopathogenic fungi (Yuan et al. 2012). A comprehensive understanding of the subject cannot be derived solely from in vitro experiments. It is necessary to address whether effects observed under controlled laboratory conditions are relevant in the field. Under greenhouse conditions, the soil drench application of VOCs, such as dimethyl disulfide (DMDS), released by *Bacillus cereus* C1L, protected tobacco and corn plants against *Botrytis cinerea* and *Cochliobolus heterostrophus*, respectively (Huang et al. 2012). Pre-treatment of cucumber seedlings with 3-pentanol and 2-butanone showed protective effects against *Pseudomonas syringae* and a sucking insect aphid (*Myzus persicae*) in open-field trials (Song and Ryu 2013). More recently, it has been demonstrated that

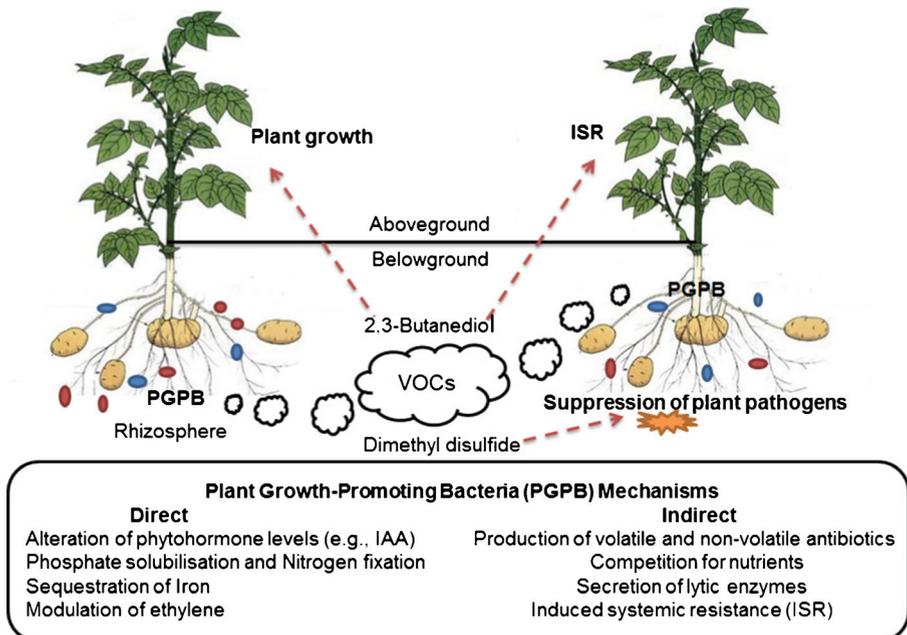


Fig. 1 Mechanisms of plant-growth-promoting bacteria (PGPB)

pre-treatment of pepper seedlings with 3-pentanol significantly reduced disease severity caused by *Xanthomonas axonopodis* and *Cucumber mosaic virus* under field conditions (Choi et al. 2014). These studies conducted to date should lead to new discoveries regarding the use of VOCs to control microbial pathogens under open-field conditions.

Arbuscular Mycorrhizal Fungi

In addition to PGPB, AMF are symbiotic biotrophs of the phylum *Glomeromycota* that contribute to plant defences. AMF form symbiotic associations with plants, including *Solanum tuberosum* (potato). AMF supply water and nutrients (e.g., phosphorus) through their extensive hyphal network in the soil from beyond where the plants roots extend and protect against biotic stress and abiotic stress. In return, the plant provides the carbohydrates necessary for completion of their life cycle (Schüßler et al. 2001; Senés-Guerrero et al. 2014). Phosphorus, zinc, copper, iron, nitrogen and potassium are transported to the host plant by AMF, and they also improve the soil structure and mitigate against drought and heavy metal stress (Goltapeh et al. 2008; Smith and Read 2008). The beneficial effects of AMF in the control of potato-associated diseases are well documented. Similar to ISR, AMF triggers mycorrhizal-induced resistance (MIR) to resist pathogens such as *Rhizoctonia solani* in potatoes (Pozo et al. 2009; Jung et al. 2012). Positive interactions between beneficial bacteria and AMF are known to exist, with beneficial bacteria enhancing the germination of AMF. The co-inoculation of papaya with *Pseudomonas* spp., and *Glomus* spp., showed enhanced protection against *Fusarium oxysporum* (Hernández-Montiel et al. 2013). This symbiotic relationship is considered a prospective solution to increase crop growth, yield and sustainability and avoids polluting the environment with agrichemicals.

VALORAM—a Case Study

The VALORAM project (<http://valoram.ucc.ie/>), an EU-FP7 project (Grant Agreement no. 227522), was a collaboration between five European and three Latin American partners. The project involved the bioprospecting of microbial diversity in the center of origin of potato, i.e., the Central Andean Highlands, by using a range of -omics technologies (metagenomics, transcriptomics, proteomics, metabolomics and volatilomics). On the one hand, cultivation-independent approaches were applied to investigate microbial diversity in this particular environment and to assess the drivers of microbial communities. In addition, yet untapped microbial resources were explored by metagenomic tools. To identify microorganisms, which can be further developed as microbial inoculants to support potato growth and health, a large collection of Andean bacteria and AMF was identified and is held in an International Culture collection, i.e., <http://bccm.belspo.be/>, in Belgium. The characteristics of the Andean potato-associated microflora and their influence on plant growth promotion and disease suppression in potato were determined in both in vitro and in vivo settings. A number of isolates have proved promising when tested under open-field conditions in the Central Andean Highlands. The VALORAM strain collection, held in Belgium, provides an ideal

opportunity for the preservation of potentially valuable Andean microbial isolates that may prove promising in the future in terms of the development of integrated crop management systems.

Acknowledgments The research project “VALORAM—Valorizing Andean microbial diversity through sustainable intensification of potato-based farming systems” was supported by European Commission’s Seventh Framework Program FP7/2007–2013 under grant agreement No 227522, 01/02/2009–31/01/2014.

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