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# Use of Plant Growth-Promoting Rhizobacteria (PGPR) to Improve Mycorrhization, Nutrient acquisition and Growth of Vegetable Plants Affected by Soilborne Pathogens



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## 1. General Introduction.

Soilborne pathogens are leading to enormous yield decline and are, thus, one the big challenges for agriculture worldwide. Compant et al. (2005), stated that soilborne pathogenic microorganisms affecting plant health are the main and constant menace to food production worldwide. Over the past decades, agricultural production has increased and farmers rely on chemical fertilizers and pesticides as a relatively dependable method of protecting plants against soilborne pathogens. By contrast, excessive and imbalanced use of agrochemicals has adverse effects to the soil in terms of decreasing organic carbon and reducing microbial population (Naseby et al., 2000). Moreover, use of chemical pesticides has increased the resistance of the target organisms (Goldman et al., 1994). In spite of the great importance of soilborne pathogens, the control of many of these diseases by chemical pesticides is still limited. The control of soilborne pathogens is importance not only for intensive conventional farming (shortening of crop rotations and monoculture) but also for organic farming. Hence, there is a considerable need for alternative methods to control soiborne pathogens both in conventional and organic farming.

Monoculture of crops is not considered as a sustainable practice because of the abundant of soilborne pathogens population which can lead to poor root growth and decreased productivity (Posma et al., 2008). Enrichment of soilborne pathogens population in response to the steady input of qualitatively unique organic material (roots of the same crop species) is one explanation that can account for the common yield decline with crop monoculture system. There is a variation in susceptibility of different crop species to the different species of root and stem infecting soilborne pathogens uniquely adapted to the roots of their hosts (Chng et al., 2005; Cook and Weller, 2004; Malvick and Grunden, 2008).

Beneficial and pathogenic microorganisms are in competition with each other in the soil environment (Sikora and Reimann, 2004). Every soil has an antagonistic potential against specific pathogen, parasite or deleterious agent by activities of antagonistic microbes (Sikora, 1992), where the antagonistic potential can be range from extremely low to total suppressiveness (Baker and Cook, 1982). "Therefore, a certain level of biocontrol is the rule rather than the exception in agriculture soils" (Reimann, 2005).

There is increasing interest in an application of beneficial bacterial and fungal as biocontrol agents for managing soilborne pathogens, partly due to public concerns about negative effects of chemical pesticides and fumigants, but also because of a lack of effective controls for soilborne pathogens (Cook, 1993). In response to environmental and health concerns about extended use of chemical pesticides, there is a considerable interest in finding alternative control approaches as a part of an in integrated pest management system for crop diseases (Ellis et al., 1999; Raupach and Kloepper, 1998). However, many biocontrol agents are inconsistent in their effect from site to site and from time to time, and this is still the primary problem to commercial development. The efficacy of biological control is occasionally inadequate. Also the variability in control efficacy might be high due to an insufficient understanding of the principal mechanisms for a successful biological control. Among others, this will include, competition for nutrients and colonization sites, secretion of inhibitory compounds (antibiosis) and hyperparasitism as well as induced resistance in plants (Chet et al., 1990; Elad, 2000; Lugtenberg and Kamilova, 2009; Martinez et al., 2009; Vallad and Goodman, 2004). It is assumed that highly effective biocontrol agents should combine two or more of these mechanisms (Jung et al., 2003; Fridlender et al., 1993).

#### 1.1. Plant growth-promoting rhizobacteria (PGPR)

The rhizosphere was defined one century ago by Hiltner (1904) as the narrow zone of soil subjected to the influence of living roots. It is characterized by intense bacterial activity as a result of release of root exudates. The bacterial community is commonly referred to as rhizobacteria. Numerous species of soil bacteria grow in, on, or around roots and stimulate plant growth by a plethora of mechanisms. These bacteria are collectively defined as "plant growth promoting

rhizobacteria" (PGPR). Furthermore, PGPR were defined by Kloepper and Schroth (1978) as soil bacteria colonizing the roots with a subsequent enhanced plant growth after inoculation of seeds. Implicit for the colonization process are the abilities to survive the inoculation of seeds, to multiply in the spermosphere (region surrounding the seed) in response to seed exudates, to attach to the root surface, and to colonize the developing root system (Kloepper, 1993).

As reviewed by Kloepper et al. (1999) and, more recently, by Gray and Smith (2005), some of these PGPRs can also enter into the root tissue and enhance endophytic populations. Many of them are able to cross the endodermis, from the root cortex to the vascular system, and subsequently thrive as endophytes in stems, leaves, tubers, and other organs (Bell et al., 1995, Compant et al., 2005, Gray and Smith., 2005, Hallman et al., 1997, Ruppel et al., 1992, Ruppel et al., 2006). The extent of an endophytic colonization of host plant organs and tissues reflects the ability of bacteria to selectively adapt to these specific ecological niches (Gray and Smith., 2005, Hallman et al., 1997).

PGPR are a group of bacteria that actively colonize the roots and increase plant growth and yield (Wu et al., 2005). PGPR enhance plant growth by direct and indirect mechanisms, but the specific mechanisms involved have not all been well characterized. Direct mechanisms of PGPR to enhance plant growth have been reported by a variety of mechanisms: production of siderophores that chelate iron and other micronutrients making them available to the plant root, solubilization of mineral nutrients such as phosphorus and micronutrients, synthesis of phytohormones and fixation atmospheric nitrogen including its transfer to the higher plant (Egamberdiyeva., 2007., Shaharoona et al., 2006., Glick, 1995, Kloepper., 1993, Vessey, 2003, Ryu et al., 2005). This also include mechanisms against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds (Ahmad et al., 2006., Bharanthi et al., 2004., Jeun et al., 2004).

at the root surface in the presence of PGPR has also been reported by Bashan et al., (1991) and Bertrand et al., (2000). PGPR strains may use one or more of these above mentioned mechanisms in the rhizosphere.

The improvement of mycorrhization in the roots of the plant by PGPR inoculation might be one of the indirect mechanism underlying the disease suppression of soilborne diseases by PGPR. There was an inverse relationship between increasing of mycorrhizal infection in the roots and disease index in the plants. PGPR can promote mycorrhizal functioning. Recently for example, Villegas and Fortin (2001) showed an interesting specific synergistic interaction between the P solubilizing bacterium *P. aeruginosa* and the AMF *Glomus intraradices*. Furthermore, PGPR also can improves the population of beneficial rhizobacteria such as fluorescent *Pseudomonas* spp. and Mn-reducers bacteria but decreased the population of Mn-oxidizers bacteria. Induction of the systemic resistance against many soilborne pathogens, insect and nematodes, is also another recent indirect mechanism of action of PGPR (Ramamoorty et al., 2001; Zehnder et al., 2001).

In figure 1.1, Haas and Défago., (2005) illustrate how PGPRs, plants and soil can interact synergistically to stimulate healthy plant growth through a range of the above explained mechanisms. These interactions might be of crucial importance for sustainable, low-input agricultural cropping systems relying on biological processes rather than agrochemicals to maintain soil fertility and plant health.



Figure 1.1. Interactions between biocontrol plant growth-promoting rhizobacteria (PGPR), plants, pathogens and soil. These elements interact with one another through biotic and abiotic signals. (Source : Haas and Défago., 2005). ISR = Induced systemic resistance

# 1.2. Arbuscular mycorrhizal fungi (AMF)

The word mycorrhiza was coined by Frank (1885) and means "fungus-root" being derived from the Greek word "*mykes*", meaning mushroom and "*rhiza*" meaning root. Frank (1885) already recognized the enormous potential and importance of his findings, calling the fungus the "wet nurse" of the tree. Nowadays we know that mycorrhizas are the most widespread associations between fungi and higher plants, occurring on roots of more than 80% of all terrestrial plants (Sieverding, 1991). Fossils of the first land plants have revealed that filamentous fungi, resembling the *glomalean* mycorrhizal fungi, were present in their root tissues (Brundrett., 2002, Redecker et al.,2000).

The mycorrhizal association between fungi and roots of plants has remained very successful through the evolution. The great majority of existing land plant species (80% *Angiospermae*, 100% *Gymnosperms* and 70% *Pteridophytes*) in nature are associated with one or several mycorrhizal fungi. Mycorrhizal formation is not only restricted to the *Glomales* but can also be found within *Basidiomycota*. In most cases, the basis of the mutualism is that the plant provides the major source of fixed carbon, whereas the fungus provides the host with mineral nutrients, water and suppression root pathogens (Smith and Read, 1997).

Seven types of mycorrhizas are known, but several are very similar (Brundrett, 2002). The two most frequency types of mycorrhizal fungi are: (1) the endomycorrhizal fungi with the often used name "Arbuscular Mycorrhizal Fungi" (AMF) and (2) ectomycorrhizal fungi (EMF). AMF are classified as *Zygomycetes*, order *Glomales*, family *Endogonaceae* with the genera *Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora*, *Paraglomus* and *Archaeospora* (Morton and Redecker, 2001), and are the most widespread species in natural ecosystems.

AMF are obligate symbionts that colonize the roots of most cultivated plant species. Mycorrhizal symbiosis are found in nearly all types of ecological situations and most plant species are able to naturally form this symbiosis (Smith and Read, 1997). This association, which normally occurs naturally when plantlets are transplanted into the field, favours plant establisment, enhancement of nutrient uptake and protection against cultural and environmental stresses (Barea et al., 1997).

AMF has received attention as part of an increasing popular paradigm that considers an active and diverse soil biological community as essential for increasing the sustainability of agricultural systems. The ability of AMF to enhance host-plant acquisition of Phosporus (P), several micronutrients and water, has been recognized as the primary beneficial effects of mycorrhiza

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(Cardoso and Kuyper, 2006; Mukerji et al., 2006). Koide (1991) reported that the most prominent importance of AMF is for P nutrition of the host plant in soils with low phosphorus levels. However uptake of nitrogen, copper, zinc and other micronutrients are enhanced as well. The beneficial effect of AMF on plant nutrition is due to (i) increased root surface through extension of extraradical hyphae, (ii) decomposition of organic material and (iii) alteration of the beneficial microbial composition in the rhizosphere (Marschner, 1988, Hodge and Campbell, 2001). In general, the contribution of AMF to plant nutrient acquisition at low nutrient supply depends on the spatial distribution and the chemical status of nutrients in a soil. In undisturbed soils, the contribution of mycorrhizal hyphae to plant nutrient uptake will be high when (i) extraradical hyphae proliferate abundantly, (ii) hyphae have access to nutrients that are not chemically available to roots, or not homogenously distributed in the soil (iii) roots do not fully exploit the whole soil volume. This could be the case in plant species with coarse, less branched roots, in soils where root growth is restricted and for immobile nutrients in soils (George et al., 1994). In addition, mycorrhizas might improve soil aggregation (Andrade et al., 1998), increase drought resistance (Auge and Stodola, 1990) and heavy metal tolerance (Brundrett, 1991) and promote health plant growth by protect plants against pathogens (Linderman, 1992, Cardoso and Kuyper, 2006). Furthermore, rhizosphere interactions occur between AMF and other soil micro-organisms with effects on plant nutrient balances, such as nitrogen-fixing bacteria and plant growth-promoting rhizobacteria (Paula et al., 1993).

### 1.3. Interaction between PGPR and AMF .

The main reason for the lack of an effect of biocontrol in agriculture disease management are inconsistent result and insufficient antagonistic activities of single application of biocontrol agent (Reimann, 2005). For this reason, Stirling (1991), Sikora (1992) and Weller and Thomashow (1994) proposed one

possible strategy to increase the efficacy of biocontrol agents by application of multiple antagonists with different modes of action.

Plant growth-promoting rhizobacteria (PGPR) in the soil interact with the plants and a variety of soil microorganisms. Among the beneficial microorganisms, AMF are certainly the most widespread root-associated fungi in agricultural soils (Bethlenfalvay and Linderman, 1992), and increasing interest is focused on their synergistic interactions with PGPR and their antagonistic effect on pathogens (Barea et al., 1997). Some PGPR are known to induce a higher beneficial effect on the plant when co-inoculated with AMF. They include *Pseudomonas* spp. (Barea et al., 1998; Gamalero et al., 2004; Vázquez et al., 2000; Vosátka and Gryndler, 1999; Walley and Germida, 1997), *Bacillus* spp. (Medina et al., 2003; Neveen et al., 2008; Rodríguez-Romero et al., 2005; Tahmatsidou V et al., 2006), phosphate solubilizing bacteria (PSB) (Barea et al., 2002; Kim et al., 1988; Toro et al., 1996; Toro et al., 1997) and nodule forming N<sub>2</sub>-fixing Rhizobia or free-living Azospirillum spp. (Alarcón et al., 2002; Barea et al., 1996; Biro et al., 2000; Patreze and Cordeiro, 2004; Ratti et al., 2001; Wu et al., 2005). A synergistic effect of AMF and PGPR on plant growth could result either from a stimulation of bacteria by AMF or a stimulation of AMF growth by PGPR. These PGPR populations may be beneficial to AMF either by enhancing mycorrhizal colonization of roots or stimulating hyphal growth. These mycorrhiza helper bacteria (MHB) (Garbaye, 1994) can be beneficial to endomycorrhizae (Gryndler et al., 2000) or ectomycorrhizae (Frey-Klett et al., 1997). Their beneficial effects on AMF growth are exerted not only by improving the mycorrhizal colonization of roots or stimulating hyphal growth but also by favouring germination of AMF spores (Garbaye, 1994; Gryndler et al., 2000). Some bacteria can stimulate fungal growth. For example, AMF increased colonization of sugar cane by PGPR when present in mixed inocula (Boddey et al., 1991). It has been suggested that this synergism relies on physical contacts between PGPR and AMF, which has been demonstrated for several wild-type and genetically modified bacterial strains (Bianciotto et al., 1996).