Growth Improvement and Management of Vegetable Diseases by Plant Growth-Promoting Rhizobacteria

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Abstract

Vegetables are an important part of human dietary systems. They contain several important nutrients including vitamins, antioxidants, etc. and affect immensely the human health. Vegetables are cultivated and consumed globally on a large scale and serve as the food of choice for millions of people across the globe. During cultivation, most of the vegetable crops are, however, often attacked by various insect pests and pathogenic microorganisms, thereby causing severe diseases, leading to huge yield losses. The agricultural practitioners depend heavily on chemical fertilizers to supply nutrients to vegetables while they apply pesticides to manage insect pests and to concurrently enhance vegetable production. The injudicious application of agrochemicals including pesticides into vegetable production practices adversely affects the soil fertility and consequently the plant health, thus making it unfit for human consumption. In order to protect the crops and to minimize yield losses due to phytopathogens, an alternate and inexpensive approach involving the use of plant growth-promoting rhizobacteria (PGPR) has been introduced into the vegetable production system. The application of PGPR formulations into the vegetable production strategies has been found to protect them from various diseases leading to improved yield and quality of the vegetables. The present chapter focuses on the disease incidence among some of the popularly grown vegetables and the role of PGPR in suppression of common vegetable diseases.

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5.1 Introduction

The population of the world is expanding consistently. It has been projected to increase up to nearly 8.2 billion by the year 2025 and is expected to reach around 9.3 billion in 2050 (DESA 2000). With limited resources available, it has become extremely difficult to feed such a hugely expanding human population. Among various food items, supplying vegetables to human population is also a major challenge. So, in order to overcome the vegetable demands, efforts are directed toward enhancing the production of vegetables worldwide. Vegetables being rich in various nutrients are consumed by millions of people globally. The field-grown vegetable crops are, however, highly prone to attack by several fungal and bacterial phytopathogens leading to huge economic losses to the growers. To overcome the nuisance caused by the phytopathogens, the vegetable growers adopt many strategies such as the use of disease-resistant varieties (Witek et al. 2016), crop rotation (Ikeda et al. 2015) and other disease control measures, but all these methods have not been successful and effective. Apart from such methods, vegetable growers also apply various agrochemicals to avoid yield losses due to phytopathogens (Srivastava and Sharma 2014). Such chemicals, however, cause serious environmental pollution and consequently result in a deleterious impact onto the vegetables (Gafar et al. 2013). Therefore, to minimize/reduce the use of chemicals in vegetable production practices and to improve the yield and quality of vegetables, growers are advised to use plant growth-promoting rhizobacteria: an inexpensive and sustainable approach for vegetable production (Zaidi et al. 2015). Although literature on disease management of vegetables using PGPR is very limited, some bioformulations comprising various PGPR, having biocontrol potential, have been tried against some vegetable diseases in order to minimize the severity of the diseases (Loganathan et al. 2014) while simultaneously maximizing the yield of vegetable crops. In this chapter, an attempt has been made to highlight the diseases affecting the commonly grown vegetables and their management by plant growth-promoting rhizobacteria.

5.2 Rationale for Using PGPR in the Management of Vegetables Diseases

Bacterial and fungal pathogens, in general, are a major threat to the sustainability, quality and yield of vegetables. Therefore, to minimize the yield losses caused by phytopathogens and hence to optimize vegetable production, the vegetable growers adopt various practices such as proper sanitation of the planting fields, crop rotation, use of disease-resistant cultivars and indiscriminate spraying of pesticides, etc. without considering their toxic impact on plants and via food chain on human health. Despite adopting so many methods including the excessive use of chemicals in vegetable production, considerable success has not been achieved in combating plant diseases. Therefore, to enhance the production of healthy vegetables and to reduce the yield losses due to pathogen attack, focus in recent times has been shifted toward the use of inexpensive, eco-friendly and viable alternative like PGPR in the management of vegetable diseases. By following this, the growth, yield and quality of many vegetables due to PGPR application have substantially been increased (Table 5.1). In the following section an attempt is made to highlight some of the serious diseases of most commonly grown and consumed vegetables and their management through the use of PGPR inoculations.

Table 5.1 Diseases of some common vegetables and their management by PGPR

Disease	Affected host plant	Causative agent	Principle antagonist	Active biomolecules	Reference
Fusarium wilt	Tomato, brinjal	*Fusarium oxysporum* f. sp. *lycopersici*; *F. oxysporum* f. sp. *melongenae*	*Bacillus subtilis*; *Trichoderma* sp.	Enzymes; secretion of extracellular cell wall-degrading enzymes	Loganathan et al. (2014), Abdel-Monaim et al. (2014)
Bacterial wilt	Tomato, brinjal	*Ralstonia solanacearum*	*Bacillus amyloliquefaciens*; *Pseudomonas fluorescens*	Antibiotics and secondary metabolites; rhizosphere colonization	Singh et al. (2016), Chakravarty and Kalita (2012)
Root rot	Okra	*Rhizoctonia solani*	*Pseudomonas fluorescens*	Siderophores, HCN and indole acetic acid	Adhikari et al. (2013)
Damping-off	Cucumber	*Pythium ultimum*	*Pseudomonas fluorescens*; *Pseudomonas* sp.; *Bacillus subtilis*	Antibiotics and metabolites	Khabbaz and Abbasi (2014)
Bacterial spot	Pepper	*Xanthomonas campestris* pv. *vesicatoria*	Lactic acid bacteria	Siderophores	Shrestha et al. (2014)
Black rot	Crucifers	*Xanthomonas campestris* pv. *campestris*	*Bacillus* sp.	Antibiosis	Luna et al. (2002)
Downy mildew	Cucumber	*Pseudoperenospora cubensis*	Consortium of *Achromobacter* sp.; *Streptomyces* sp. and *Bacillus licheniformis*	Induced systemic resistance	Sen et al. (2014)
Late blight	Potato; pepper	*Phytophthora infestans*; *Phytophthora capsici*	*Chaetomium globosum*; *Burkholderia cepacia*	Endo and exoglucanases; antimicrobial activity of organic acids	Shanthiyaa et al. (2013), Sopheareth et al. (2013)
Early blight	Potato	*Alternaria solani*	*Trichoderma harzianum* + *Pseudomonas fluorescens*	ND	Mane et al. (2014)

ND Not determined

5.3 How Plant Growth-Promoting Rhizobacteria Combat Phytopathogen Attack: A General Perspective

Plant growth-promoting rhizobacteria (Kloepper and Schroth 1978) are certain beneficial bacteria that colonize plant roots and improve the performance of crop plants through enhanced nutrient uptake from soil and several other mechanisms. They are known to antagonize several plant pathogenic microorganisms by releasing antimicrobial metabolites (George et al. 2015) and also by chelating the iron present in the soil, thus creating a competition for iron requirement by plant pathogens (Haas and Défago 2005; Haas and Keel 2003; Raaijmakers et al. 2002). Plant growth-promoting rhizobacteria are effective antagonists toward various bacterial (Liu et al. 2016), fungal (Kumari and Khanna 2014) and viral diseases (Li et al. 2016) attacking the crops. Some PGPR secretes antibiotics, for example, pyrrolnitrin, pyoluteorin, 2,4-DAPG, etc. and inhibit the growth of plant pathogens (Beneduzi et al. 2012). The biocontrol activity of many disease-suppressive microorganisms is also attributed to stimulation of defence-related mechanisms within the host plants, what is better known as induced systemic resistance (ISR). Some PGPR combine different mechanisms of antagonism and plant growth promotion and are therefore able to suppress a wide range of plant diseases while simultaneously enhancing plant growth and development (Vassilev et al. 2006). For instance, *Pseudomonas fluorescens* CHA0 has been reported to synthesize antifungal compounds like 2,4-diacetylphloroglucinol (DAPG) (Keel et al. 1992; Keel et al. 1990) and pyoluteorin (PLT) (Maurhofer et al. 1994; Maurhofer et al. 1992). These compounds in turn have been found to suppress various soilborne plant diseases (Haas and Keel 2003).

Although several strains of PGPR have been reported as suitable candidates for plant disease suppression, PGPR belonging to the genus *Pseudomonas* have received considerable attention as potential biocontrol agent (Cabanás et al. 2014). The process of plant growth promotion and disease control by *Pseudomonas* sp. are interlinked involving various direct and indirect mechanisms that include synthesis of some metabolites like auxins, cytokinins, gibberellins, etc., ACC deaminase activity, production of iron-chelating compounds (siderophores), antibiotics and numerous cyanogenic and volatile compounds. Other mechanisms may include mineral phosphate solubilization, competition for nutrients and induced systemic resistance (Lucy et al. 2004; Adesemoye et al. 2008). These beneficial bacteria are able to improve the yield of vegetable crops, thereby reducing economic losses with minimum cost inputs involved (Dias et al. 2013). In addition to *Pseudomonas* sp. acting as effective biocontrol agent in the agricultural system, some strains of *Bacillus subtilis* are also known to inhibit the growth of phytopathogenic fungi by producing certain wide-spectrum antibiotics and thermostable metabolites as a disease control measure (Mercado-Flores et al. 2014). To understand the importance of PGPR in vegetable disease suppression and eventually plant growth promotion, the present section highlights some of the active biomolecules secreted by PGPR which are involved in combating the attack of phytopathogens.

5.3.1 Release of Siderophores

Siderophores are low molecular weight (200–2000 Daltons) compounds released by PGPR (Gupta et al. 2015) which chelate iron present within the soil system and transport it through the bacterial cells. Siderophores are secreted by many bacterial genera, for example, *Bacillus* (Bharucha et al. 2013), *Pseudomonas* (Luján et al. 2015), etc. to solubilize iron from the surrounding environment, thus forming a ferric-siderophore complex that can diffuse through the cell and be returned to the cell surface (Andrews et al. 2003). Thus, siderophores play an important role in the control of some soilborne plant pathogens through competition for iron nutrition (Loper and Buyer 1991). Since siderophores are known to sequester iron (III) present within the surroundings, they limit its availability to the pathogens and ultimately suppress their growth and disease-causing ability (Schroth et al. 1984). Among most of the siderophores released by the bacteria, those produced by pseudomonads, for example, pyoverdin (Peek et al. 2012), can inhibit the growth of plant pathogenic bacteria and fungi (Ruiz et al. 2015). Moreover, a pseudobactin siderophore produced by *P. putida* strain B10 has been found to suppress *Fusarium oxysporum* in the soil and the diseases caused by this pathogenic fungus by limiting the supply of iron. Also, recent studies have demonstrated the suppression of soilborne fungal pathogens with the help of iron-chelating siderophores by fluorescent pseudomonads, thus making it unavailable to other pathogenic microorganisms (Vanitha and Ramjegathesh 2014; Dwivedi and Johri 2003). Production of siderophores is therefore considered as one of the most potent mechanisms of disease suppression and an indirect means of growth promotion employed by numerous PGPR. Besides, iron-chelating siderophores (Beneduzi et al. 2012), various antibiotics (Sivasakthi et al. 2014) and cyanogenic compounds (Sureshbabu et al. 2016) are also produced by PGPR strains that aid in combating the phytopathogens attack and promoting plant growth and development by alleviating the disease severity. Some of the other biomolecules involved in disease suppression are discussed in the following sections.

5.3.2 Production of Cyanogenic Compounds

Production of cyanogenic compounds like hydrogen cyanide (HCN) by PGPR (Lukkani and Reddy 2014) is yet another active biomolecules that aid in successfully controlling various plant diseases by inhibiting the growth and proliferation of plant pathogenic bacteria and fungi, thereby assisting in plant growth promotion. Interestingly, the phenomenon of cyanogenesis by PGPR was predominantly thought to be associated with pseudomonads, and it enhanced in the presence of glycine added as an additional supplement to the culture media (Lakshmi et al. 2015). Cyanide, a highly toxic secondary metabolite is produced by most microorganisms including PGPR (Fouzia et al. 2015) and fungi (Ng et al. 2015) as a means of defence mechanism to safeguard the crops from the pathogens and, therefore, indirectly promotes the growth of plants. Mechanistically, hydrogen cyanide synthesized mostly by *Pseudomonas* (Reetha et al. 2014) and *Bacillus* species inhibits the electron transport chain and the energy supply to the bacterial cell and eventually thus cause the death of the pathogenic microbes. For instance, certain rhizobacterial strains have been reported to have the ability to synthesize HCN by which they restrict the growth of phytopathogens and, hence, exert positive effects on seedling root growth of various plants (Kremer and Souissi 2001).

5.3.3 Production of Antibiotics

Antibiotic production is an important mechanism of antagonism associated with PGPR to fight the target phytopathogens (Glick et al. 2007). Plant growth-promoting rhizobacteria are known to synthesize a vast array of antibiotics, as yet another major defence tool that provides protection to plants from nuisance of phytopathogens (Ulloa-Ogaz et al. 2015). And hence one or more antibiotics produced by the PGPR (Wang et al. 2015) play a prime role in disease suppression. The mechanism of antibiosis is to produce low molecular weight compounds that may pose deleterious impacts on the metabolism of pathogenic microorganisms and thus retards their growth. Several studies have shown that the production of certain antibiotics like pyrrolnitrin, phycocyanin, 2,4-diacetylphloroglucinol (DAPG) (Meyer et al. 2016), etc. by various microbial genera belonging to PGPR can cause suppression of phytopathogens (Subba Rao 1993; Glick 1995). Since then a variety of antibiotics have been isolated from various bacterial strains that could eventually inhibit the synthesis of cell walls of the pathogenic microflora (Dilantha et al. 2005). Also, the antibiotics damage the membrane integrity of the cells and the formation of initiation complexes on the small subunit of the ribosome (Maksimov et al. 2011). For example, 2,4-diacetylphloroglucinol (DAPG) is an effective and extensively studied antibiotic produced by pseudomonads that has been reported to damage the membrane of *Pythium* sp. and causes inhibition of zoospore formation (De Souza et al. 2003). Pseudomonads also produce some other antibiotics like phenazine that possesses redox activity and is capable of suppressing *F. oxysporum* and *Gaeumannomyces graminis* (Chin-A-Woeng et al. 2003). Besides *Pseudomonas* sp., several strains of *Bacillus* also produce antibiotics like polymyxin, circulin and colistin that are active against numerous Gram-positive and Gram-negative bacteria, as well as many plant pathogenic fungi (Maksimov et al. 2011).

5.3.4 Secretion of Lytic Enzymes

Several lytic enzymes are released by PGPR (Gupta et al. 2015) that are able to destruct/lyse the cell walls of fungal pathogens. Secretion of lytic enzymes, e.g. chitinase (Shrivastava et al. 2016), glucanase (Figueroa-Lopez et al. 2016), β-1,3-glucanase (El-Gamal et al. 2016), cellulases (Ashwini and Srividya 2014), proteases (Illakiam et al. 2013), lipases (Tiru et al. 2013), etc. is yet another mode of defence adopted by PGPR to protect plants from damage caused by phytopathogens. These lytic enzymes can degrade the cell wall of the pathogenic fungi and ultimately cause their death. Since the fungal cell walls are mainly composed of chitin and beta-glucans, the beneficial antagonistic PGPR could inhibit the growth of pathogenic fungi by degrading their cell walls through these lytic enzymes. Symbiotic nitrogen-fixing plant growth-promoting rhizobacteria, *Sinorhizobium fredii* strain KCC5, and free-living PGPR, *P. fluorescens* strain LPK2, have been reported to produce lytic enzymes such as chitinase and beta-glucanases, which have been found to inhibit the growth of *Fusarium udum* leading consequently to manage the fusarium wilt disease caused by the fungus (Kumar et al. 2010).

5.3.5 Induced Systemic Resistance (ISR)

Some PGPR do not directly inhibit the pathogens, instead they activate the host plants to develop resistance against specific attacking pathogen, through a mechanism commonly known as induced systemic resistance (ISR). Principally, ISR is defined as the mechanism of enhanced resistance at specific sites of plant tissue at which disease induction has occurred. Only when a potent pathogen attacks the host plant, the defence mechanism of ISR is activated in its response. In other words, ISR is a condition of enhanced defence developed by a plant when appropriately stimulated by an attacking pathogen (Van Loon et al. 1998). There are numerous biotic and abiotic agents that can protect crops from pathogenic microorganisms by eliciting ISR (Da Rocha and Hammerschmidt 2005; Reglinski and Walters 2009; De Vleesschauwer and Höfte 2009). Of these, the biotic agents include a varied range of plant growth promoters including *Bacillus* sp. (Jourdan et al. 2009; Kloepper et al. 2004), *Pseudomonas* sp. (Bakker et al. 2007), *Serratia* sp. (Press et al. 1997; Schuhegger et al. 2006), *Trichoderma* sp. (Koike et al. 2001; Segarra et al. 2009), *Piriformospora indica* (Shoresh et al. 2010), *Penicillium simplicissimum* (Elsharkawy et al. 2012), *Phoma* sp. (Sultana et al. 2009), non-pathogenic *F. oxysporum* (Fravel et al. 2003) and arbuscular mycorrhizal fungi (Pozo et al. 2009). However, the ISR is not specific against particular pathogen but may play a major role in controlling plant diseases. The major role in providing systemic resistance by plants to various plant pathogens is primarily due to plant hormones jasmonic acid and ethylene. The crosstalk between these two molecules leads to enhanced resistance to pathogens.

5.3.6 Competition

The ability to compete for limited space and scarcely available nutrients within the rhizosphere is another defence mechanism that has evolved within PGPR strains. The plant growth-promoting rhizobacteria sometimes compete with the plant pathogenic microbes for various nutrients present in trace amounts which can limit the growth of the disease-causing pathogens. The beneficial microflora of the rhizosphere, especially the pseudomonads, are efficient colonizers (Zhao et al. 2013) which very efficiently colonize the surface of plant roots and in turn limit the growth of pathogenic microbes. Moreover, the growth-promoting rhizobacteria, when inoculated onto seeds or soils, compete for the available nutrients. Through active uptake of essential nutrients, the PGPR inhibits the growth of pathogenic fungi and bacteria by limiting the availability of nutrients to competing microbiota. Summarily, various beneficial soilborne PGPR such as *Pseudomonas* sp. and *Bacillus* sp. endowed with massive potential of protecting plants against pathogenic microorganisms involving a wide range of mechanisms, such as competition for space and nutrients, production of secondary metabolites, release of antibiotics and bacteriocins, production of iron-chelating siderophores, secretion of lytic enzymes and elicitation of induced systemic resistance (ISR) (Pieterse et al. 2014), could be used to protect crops including vegetables from negative impact of phytopathogens.

5.4 Some Examples of Growth Promotion and Vegetable Disease Management by PGPR Wilt Disease: A General Perspective

Bacterial wilt is a common disease among vegetables and affects mainly tomato, eggplant, potato, tobacco and pepper. The causal organism of bacterial wilt is *Ralstonia solanacearum* which is highly devastating for the crops (Hayward 1991). Moreover, nearly 450 different species of other crops serve as suitable hosts for this bacterial pathogen (Swanson et al. 2005). *Ralstonia solanacearum* thrives mainly in the tropical and subtropical regions of the world (Kelman 1998) and is known to cause enormous yield losses of the vegetable crops. Attempts have been made to control the menace caused by bacterial wilt using PGPR formulations having antagonistic abilities against *R. solanacearum* (Nguyen and Ranamukhaarachchi 2010).

5.4.1 Diseases of Tomato and their Management

5.4.1.1 Bacterial Wilt of Tomato

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop grown and consumed worldwide. It is a rich source of vitamin A and C and is most popular among vegetables because of high nutritive value. Among various diseases, bacterial wilt is the most common and destructive disease of tomato caused by *R. solanacearum* (Tahat and Kamaruzaman 2010). The yield loss of the crops due to this pathogen ranges from 2 to 90% in various agro-climatic conditions (Mishra et al. 1995). To overcome the losses caused due to bacterial wilt, various strategies including the use of agrochemicals have been adopted to control the disease (Singh et al. 2012). However, application of these chemicals has not been found effective enough to control the disease; rather such chemicals following deposition in soils have resulted in deleterious impact on soil fertility and plant health. Thus, growers, in order to avoid chemicals threat, rely on biological control measures for the management of bacterial wilt disease (Singh et al. 2013). In this regard, several antagonistic bacteria, such as *P. fluorescens*, *P. putida*, *Bacillus* sp., etc., have been used to control wilt disease in tomato (Singh et al. 2016; Toua et al. 2013). Among various bacterial antagonists, *Bacillus* spp. including *B. amyloliquefaciens*, *B. coagulans*, *B. cereus*, *B. licheniformis*, *B. pumilus*, *B. subtilis* and *B. vallismortis* have been used extensively for controlling the disease effectively (Tan et al. 2013). In a study various strains of *Bacillus* including *B. amyloliquefaciens* DSBA-11 and DSBA-12, *B. cereus* JHTBS-7, *B. pumilus* MTCC-7092 and *B. subtilis* DTBS-5 were selected to test their comparative antagonistic ability to control wilt disease as well as growth promotion of tomato. The results revealed minimum disease intensity (17.95%) and maximum biocontrol efficacy (68.19%) in tomato plants inoculated with *B. amyloliquefaciens* DSBA-11. The intensity of the disease was, however, a little higher in case of other treatments, for example, *B. amyloliquefaciens* strain DSBA-12 which showed the disease intensity up to 20.81% while *B. subtilis* strain DTBS-5 could reduce the intensity of the disease up to 21.63% after 30 days of initiation of infection by *R. solanacearum*. Furthermore, the population of *R. solanacearum* decreased in *Bacillus*-treated plants. Also, *Bacillus* strains improved other growth parameters of tomato plants. For instance, maximum shoot length (39.50 cm) was recorded in *B. subtilis* DTBS-5-inoculated plants which was followed by *B. amyloliquefaciens* DSBA-11 (38.50 cm) and *B. amyloliquefaciens* DSBA-12 (38.40 cm). Likewise, root length was maximum in plants inoculated with *B. amyloliquefaciens* strain DSBA-11, followed by *B. amyloliquefaciens* DSBA-12 after 30 days of inoculation. Similarly, the dry matter accumulation in root and shoots also enhanced correspondingly (Singh et al. 2016).

5.4.1.2 Fusarium Wilt of Tomato

Fusarium wilt caused by *Fusarium* sp. causes severe tomato yield losses. Yellowing and wilting of the lower leaves are the initial symptoms of the disease that could be visible on the plant (Khan and Khan 2002). The fungus invades the host tissue and the microconidia and grows intercellularly within the xylem of the stem and root of the host plant. The xylem tissue is then infected by the fungus resulting in severe damage to the xylem. The damage caused to xylem leads to disruption of water transportation within the plant, which results in death of the infected tomato plant (Burgess et al. 2008). On the other hand, the conidia forms chlamydospores that fall back into the soils (Jones 2000) which germinates under amenable environmental conditions, and thus the reproductive cycle of the fungus continues. The management of *Fusarium* wilt is however a big challenge for tomato growers (Srinon et al. 2006). The use of fungicides and other chemicals has not been a practical method for controlling the disease. Rather, disease management through biocontrol mechanisms involving PGPR is considered an effective and suitable approach. For controlling the disease, several microorganisms like species of *Pseudomonas* and *Bacillus* have been used as successful antagonists against this disease. Of all the antagonists, *Bacillus* sp. has been found very effective in plant disease management (Jacobsen et al. 2004). To substantiate this further, a study conducted by Ajilogba et al. (2013) revealed a significant growth inhibition of *Fusarium solani* by four *Bacillus* strains, namely, *B. amyloliquefaciens*, *B. cereus*, *B. pumilus* and *B. subtilis*. A 95.2% reduction in the growth of *F. solani* was observed when tomato plants were inoculated with *B. Amyloliquefaciens*. Despite the variation in effectiveness of each bacteria strain, all four strains of *Bacillus* served as potential antagonists and successfully protected tomato plants from fusarium wilt disease. Mechanistically, the antagonistic potential of *B. amyloliquefaciens* strain was attributed to the release of various metabolites and antifungal compounds by the test bacterial strains used in this study (Dihazi et al. 2012). Several other studies have also revealed the production of a variety of antibiotics like as zwittermicin, bacillomycin, fengycin, bacilysin and difficidin by *B. amyloliquefaciens* strains which explains the possible mechanism of resistance to fusarium wilt of tomato, thereby leading to improved growth and yield (Athukorala et al. 2009; Chen et al. 2009).

5.4.1.3 Bacterial Wilt of Brinjal

Bacterial wilt of brinjal (*Solanum melongena*) is yet another important disease caused by plant pathogenic bacterium *R. solanacearum* and is a major challenge to brinjal production causing severe losses in crop yield. Several strategies like crop rotation and introduction of resistant cultivars, etc. have been employed for the management of wilt disease, but complete control of the disease has not been achieved so far, since the survivability of the pathogen in soil is longer, and therefore, the same pathogen can reinfect the healthy plants under favourable environmental conditions. Moreover, the strain exists in diverse forms due to which the development of resistant cultivars has become difficult and ineffective (Wang et al. 1998). To minimize the yield losses caused by *R. solanacearum*, application of hazardous chemicals to soil, modification of soil pH, soil solarization, and the use of plant essential oils (e.g. thymol) or phosphoric acid (Norman et al. 2006) have been practised over the years. However, these methods have not been found successful due to one or other reasons (Champoiseau Patrice et al. 2009). Thus, there is an urgent need to overcome this disease so as to safeguard the vegetables and minimize the adverse impact on the environment. In this regard, biological strategies to control plant diseases have been suggested (Lwin and Ranamukhaarachchi 2006). Among various PGPR, strains of *P. fluorescens* are well-known for suppressing soilborne diseases caused by phytopathogens (O'Sullivan and O'Gara 1992). To assess the potential of *P. fluorescens* as a biocontrol agent against bacterial wilt, a study was conducted and the efficacy of *P. fluorescens*-based bioformulations in disease suppression was determined under pot and field trials. During the experiment, the population density of *P. fluorescens* at 30 days after transplanting increased significantly up to 60 days. Besides reducing the disease severity, *P. fluorescens*-based bioformulation also improved the growth and yield attributes of brinjal. Various biological parameters like leaf area, average fruit weight, yield/plant, no. of fruits/plant, no. of branches/plant and plant height were enhanced in the presence of *P. fluorescens* (Chakravarty and Kalita 2011). The formulations when applied to seed, root and soil were more effective in reducing the incidence and severity of bacterial wilt disease in brinjal which could possibly be due to the correct placement of the antagonist *P. fluorescens* on the seed, from where it migrated to the elongating roots (Burr et al. 1978), on the roots which is the best location for colonization by microbes (Anuratha and Gnanamanickam 1990) and on the soil, the collection of both beneficial and pathogenic microorganisms (Dupler and Baker 1984). Thus, the strategy adopted by *P. fluorescens* for disease management including both its colonization on the root surface of brinjal plants and its ability to survive and establish within the soil provides a competitive advantage to the antagonists over the native soil/rhizosphere microflora (Loper et al. 1985).

5.4.1.4 Fusarium Wilt of Brinjal

Fusarium wilt of eggplant is one of the most destructive diseases caused by *F. oxysporum* f. sp. *melongenae*. The pathogenic fungus is soilborne and causes disease in healthy eggplants by invading the vascular bundles. The invasion of vascular bundles ultimately results in severe wilting and finally the death of the plants which occur due to blocking of the xylem tissue and collapsing of the water transport system within the plant (Altinok 2005). Since the spores of *Fusarium* are resistant to environmental stress and can survive in the soil for many years, it becomes difficult to control the fungal growth and spread of the disease through conventional disease management strategies. Thus, the application of beneficial PGPR as biocontrol agents has become important, since they are endowed with multiple disease resistance mechanisms. Realizing the importance of PGPR, a study was conducted to assess the biocontrol potential of certain PGPR isolates against Fusarium wilt disease in brinjal. Among the PGPR isolates, *Pseudomonas aeruginosa* (P07-1), *P. putida* (P11-4), *P. aeruginosa* (85A-2), *Bacillus amyloliquefaciens* (76A-1) and *B. cereus* (B10a) could significantly reduce the incidence of the disease by up to 85%. Interestingly, the PGPR strains exhibited some traits of disease suppression that ultimately led to the inhibition of the mycelial growth of the pathogenic fungus. The percentage of inhibition varied from 38 to 72% depending upon the potentiality of each PGPR strain. Moreover, of all the PGPR strains, *P. aeruginosa* (P07-1) and *P. putida* (P11-4) successfully colonized within the seedlings of eggplant and eliminated the chances of entry of the fungal mycelium within the host tissue and thus prevented the disease incidence. The experiment further revealed that the PGPR isolates could suppress the disease more efficiently when applied singly, rather than when used in combination. Also, the eggplants exhibited the property of induced systemic resistance which was triggered by the PGPR strains in response to *F. oxysporum* f. sp. *melongenae*. The brinjal plants could synthesize several enzymes like peroxidase (POX, EC 1.11.1.7), polyphenol oxidase (PPO, EC 1.14.18.1) catalase (CAT, 1.11.1.6) along with several lytic enzymes capable of degrading the fungal cell wall. The production of enzymes could be a possible mechanism of resistance against Fusarium wilt in brinjal. The study, thus, demonstrated the use of beneficial PGPR that could serve as antagonists and enhance disease resistance for sustainable production of brinjal (Altinok et al. 2013).

5.4.1.5 Diseases of Okra

Root Rot Disease

Okra (*Abelmoschus esculentus*) is one of the important summer vegetables of India with a high average productivity. Field-grown okra is attacked largely by a number of phytopathogens including bacteria, fungi, viruses, nematodes and various insect pests which adversely affect the production, and if the crop is not cured off the pathogens at the right time, it may lead to serious destruction resulting in heavy yield losses that may reach up to 80–90% (Hamer and Thompson 1957). Among various diseases of vegetables, root rot of okra incited by *Rhizoctonia solani* is one of the most serious and devastating diseases of okra and is a menace for its cultivation on a large scale. To highlight the potential of *Pseudomonas* strains as a biocontrol agent against root rot of okra, two isolates of *Pseudomonas flourescens* PF-7 and PF-8 were used in a study where they inhibited the mycelial growth of *R. solani* by 72.05 and 68.25%, respectively. On the other hand, the vigour index of okra was recorded maximum for isolate PF-8 (2415.7) followed by PF-7 (2063.25) (Adhikari et al. 2013). The strains of *P. fluorescens* produced secondary metabolites responsible for the inhibition of fungal growth and proliferation, as a major mechanism of biocontrol of *R. solani.* The other antagonistic attributes of *P. fluorescens* strains included production of pigments, iron-chelating siderophores, cyanogenic compounds like HCN, etc. Besides exhibiting biocontrol properties, *P. fluorescens* strains PF-7 and PF-8 also released certain plant growth-promoting substances like indole acetic acid and salicylic acid and could solubilize inorganic P. All these growth-promoting properties of *P. fluorescens* make this organism a suitable choice for the enhancement of okra production while limiting the root rot disease of okra.

5.4.1.6 Blight Diseases

Early Blight of Potato

Among the most important food crops of the world, potato (*Solanum tuberosum* L.) ranks third after rice and wheat (Anonymous 2012). Globally, India ranks fourth in terms of area under production and fifth overall in the world (Shailbala and Pathak 2008). Potato, popularly known as the king of vegetables, is cultivated mainly in the tropics and in subtropics during the cool and dry seasons. Cultivation of potato suffers heavily from attack of pathogenic microorganisms leading to enormous yield losses. Among various potato diseases, early blight is one of the most common foliar diseases of potato occurring worldwide (Christ 1990; Van der Walls et al. 2001) caused by *Alternaria solani*. In recent past, a constant increase in disease incidence on potato foliage caused by *A. solani* has been reported in various potato-growing areas (Vloutoglou and Kalogerakis 2000). Initial symptoms of the disease begin with premature defoliation of the potato plants, leading to reduction in the yield of potato tubers. The symptoms first occur on the lower senescing leaves, which later on become chlorotic and abscise prematurely. The disease appears as brown spots that enlarge slowly to completely destroy the leaves. The pathogenic fungus infects young seedlings to cause stem canker or collar rot. Sunken spots or cankers on older stems, dark leathery fruit spots, etc. are some of the other symptoms that appear on the potato plants simultaneously. Sometimes, lesions appear on upper stems and petioles, indicating the severity of the disease (Raziq and Ishtiaq 2010). The loss in yield of potato following infection by *A. solani* depends mainly on season of cropping, location of planting, type of cultivars and the stage of potato at which infection starts. Early blight may also result in other infections including dry rot of tubers, which reduces the quality and quantity of the tubers to be sold in the market (Nnodu et al. 1982). Rotem (2004) reported that high water content in the surrounding atmosphere is favourable for germination of conidia leading to augmentation of infection. Moreover, alternating low and high humidity in the environment also favours disease development (Van der Walls et al. 2001). The incidence of this disease is also enhanced through repeated and continuous production of potato (Olanya et al. 2009). Management of such lethal diseases is a challenge for potato growers. Even though fungicides can be used to circumvent such diseases, the adverse effects of fungicides and chemicals on plants have warranted to search for a safer and inexpensive method to control early blight disease while simultaneously enhancing the potato growth and productivity. Apart from the sole application of some fungi, for example, *Trichoderma* (Chet et al. 1981; Kumar and Mukerji 1996), a bioformulation comprising of *Trichoderma harzianum* and *Pseudomonas fluorescens* has been applied to potato plants along with the fungicide mancozeb to ward off the pathogenic fungus *A. solani*. The severity and incidence of the disease were greatly reduced in the presence of biocontrol agents. Also, the growth and yield of potato were enhanced significantly (Mane et al. 2014). Although the exact mechanism of control of early blight disease by composite culture of *T. harzianum* and *P. fluorescens* is not determined, these combinations were found effective against *A. solani* and, hence, could be developed as a substitute to chemical treatments.

Late Blight of Potato

Late blight disease of potato is another highly destructive disease and is one of the major constraints in potato cultivation (Chycoski and Punja 1996; Fry and Goodwin 1997; Song et al. 2003). In the mid 1800, the disease resulted in severe crop losses throughout Northern Europe including Ireland where it was responsible for the Irish famine (Elansky et al. 2001). Since then, it has spread very rapidly and, in the present time, attacks potatoes on a large scale wherever potatoes are cultivated. The annual losses of potato caused due to *Phytophthora infestans* have been estimated to € 12 billion worldwide, out of which a productivity and yield loss of approximately € 10 billion per annum has been estimated for the developing nations (Haverkort et al. 2009). The causal organism of this disease (*P. infestans*) produces lesions on potato plants which is small and chlorotic initially, but enlarge in size when the climatic conditions are humid, thereby destroying almost the entire plant. The most prominent disease symptom is the appearance of irregular pale green lesions around the tip and margins of the leaves that enlarges to form brown to purplish black necrotic spots. Also, a white mildew, consisting of sporangia and viable spores of the pathogen can be seen on the ventral side of the infected leaves. The stems of the potato plant also get affected by this disease and exhibit light to dark brown lesions. The entire affected crop appears blackened and may be destroyed within a week if the conditions are favourable for the growth and survival of the pathogen. The sporangia from the diseased foliage fall to the ground and reach the tubers to infect them. Irregular reddish brown to purple coloured spots appear as disease symptoms on the infected potato tubers. As a consequence, rotting of the potato tubers occurs when the favourable conditions arrive and results in heavy yield losses of potato (Flier et al. 2001), thereby leading to a reduction in global production of potato by approximately 15% (Anonymous 1997). The infected tubers may consequently be attacked by soft rot-causing bacteria upon storage. In conventional farming systems, late blight disease is controlled mainly through repeated and injudicious applications of various chemical protectants like fungicides that, after a long term usage, may pose serious threats to plant and soil health (Cooke et al. 2011; Axel et al. 2012). To overcome the losses caused by late blight disease, biocontrol measures have been introduced and employed nowadays as an effective alternate strategy for protection against such devastating diseases (Velivelli et al. 2014).

Considering these, a study was conducted where three *Pseudomonas* strains were tested for their protective ability against late blight disease of potato. The green house experiment revealed that *P. chlororaphis* strain R47 was the most active protectant PGPR. This strain possessed biocontrol potential against *P. infestans* when tested in vitro. However, the protective effect provided by *P. chlororaphis* strain R47 against *P. infestans*, its survival in the phyllosphere and its ability to colonize the potato rhizosphere in a very high number suggest that this strain could be used as a suitable antagonist to late blight of potato under field conditions. *P. chlororaphis* R47 responded to the pathogen most efficiently and showed the highest level of inhibition of *P. infestans* in vitro, followed by *P. fluorescens* R76 and *P. marginalis* S35. The prime mechanism of management of late blight of potato by *Pseudomonas* strains is through the secretion of some antifungal compounds that could probably inhibit the growth of *P. infestans*, thereby leading to a better potato production with highly minimized yield losses (Guyer et al. 2015). *Pseudomonas* strains, in general, have also been reported as the best producers of various antifungal metabolites (Hunziker et al. 2015). Together, these studies suggest that *Pseudomonas* isolates could be used as a potent biocontrol agent against *P. infestans* for potato cultivation on a large scale in different production systems.

Blight Disease of Pepper

Blight of pepper (*Capsicum annuum* L.) is caused by *Phytophthora capsici* and results in severe yield losses. The disease is soilborne in origin and affects the pepper plants cultivated worldwide across major pepper-growing countries like China (Ma et al. 2008), Mexico (Robles-Yerena et al. 2010), Turkey (Akgül and Mirik 2008), Spain (Silvar et al. 2006), The United States of America (Hausbeck and Lamour 2004) and Nigeria (Alegbejo et al. 2006). Although, the disease is difficult to control, yet there are numerous reports where disease has been controlled employing various chemical (Hausbeck and Lamour 2004) and microbial (Kim et al. 2010) fungicides. For example, some *Pseudomonas* isolates from various crops have been used to inhibit the growth of *P. capsici* in vitro and for the production of biosurfactant. Also, the efficacy of selected *Pseudomonas* strains against *P. capsici* was determined in two experiments where the antagonistic bacteria were applied to infected pepper plants along with fungicide acibenzolar-S-methyl (ASM) and mefenoxam, either singly or in combination. Bacterial strains were applied by soil drenching method whereas the fungicides were applied as foliar sprays. The application of four *Pseudomonas* strains resulted in significant reduction in the severity of pepper blight ranging from 48.4 to 61.3% in infected pepper. In another experiment, when *P. fluorescens* was applied along with olive oil, the biocontrol efficiency of the *Pseudomonas* isolates enhanced significantly, resulting in a significant decrease in the level of disease severity from 56.8 to 81.1%. The reduction in severity of disease and consequently the inhibition of germination of zoospores and hyphal growth of *P. capsici* was attributed to the synthesis of rhamnolipid-type biosurfactants by *Pseudomonas* sp. (D'aes et al. 2010). Besides this, other molecules that could be involved in disease management by *P. fluorescens* include the production of a vast array of antibiotics like phenazines, pyrrolnitrin, pyoluteorin and 2,4-diacetylphloroglucinol (Cui and Harling 2006), HCN, indolic compounds and siderophores, etc. Thus, it is established that the use of *P. fluorescens* strains possessing biosurfactant producing properties can be a successful and effective method of blight disease management and plant growth promotion in pepper plants while reducing the use of chemicals and fungicides in pepper production to a great extent (Özyilmaz and Benlioglu 2013).

5.4.1.7 Diseases of Crucifers

Bacterial Soft Rot of Cabbage

Bacterial soft rot is another detrimental disease of vegetables occurring worldwide and affecting several economically important crop plants including crucifers (Pérombelon and Kelman 1980). The disease is caused by *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*), one of the most hazardous plant pathogenic bacterium (Kyeremeh et al. 2000) which hinders the production of Chinese cabbage (Kikumoto 2000). Several methods including biological approaches have been attempted to control/minimize the severity of soft rot diseases (Hayward 1991; Bernal et al. 2002). There are few reports available on the control measures of soft rot disease either by using microbial pesticide formulations (Takahara 1994), avirulent mutant strains of *Erwinia* (Takahara et al. 1993; Kyeremeh et al. 2000) or through fluorescent antagonistic bacterium (Togashi et al. 2000) as biocontrol agents. Moreover, disease-resistant transgenic cultivars of Chinese cabbage (Vanjildorj et al. 2009) showing resistance to soft rot have been developed by the growers in an attempt to eradicate this disease to avoid the yield losses. Among microbiological preparations for use against soft rot of cabbage, few bacterial formulations comprising of *Lactobacillus*, *Lactococcus* and *Paenibacillus* strains have been tried against the same disease. Biocontrol efficacies of these bacterial strains were tested against soft rot of cabbage and were found significantly effective as antagonists to the disease. The disease severity for the strains KLF01, KLC02 and

KPB3 was reported as 23, 20 and 20%, respectively, whereas the biocontrol efficacy of KLF01, KLC02 and KPB3 was 55, 60 and 62%, respectively, when tested in field trials. Among various strains used in the study, strain KPB3 proved to be the best biocontrol agent with the highest biocontrol efficacy (Shrestha et al. 2009). The factors affecting growth promotion and disease suppression by *Lactobacillus* and *Lactococcus* strains were suggested as the production of various antibacterial substances like acetic acid, lactic acid (Ariyapitipun et al. 1999), hydrogen peroxide (Chang et al. 1997) and bacteriocins (Klaenhammer 1982); furthermore, these bacterial strains could exhibit antagonistic effect (Visser et al. 1986) and antifungal activity (Laitila et al. 2002) against phytopathogens most probably due to the release of biomolecules mentioned earlier.

5.4.1.8 Diseases of Cucumber

Damping-Off and Root Rot of Cucumber

Damping-off and root rot diseases are mainly caused by an oomycete plant pathogen *Pythium* sp. and damage young seedlings of several horticultural and vegetable crops both under greenhouse and field conditions (Howard et al. 1994; Paulitz and Bélanger 2001). The causal organism of root rot of cucumber is *Pythium ultimum*. The oomycete pathogen generally attacks the juvenile tissues of bedding plants (Gravel et al. 2009), greenhouse transplants and floral crops (Moorman et al. 2002) and direct seeded field crops (Paulitz 2006; Leisso et al. 2009). The most favourable conditions for the growth of damping-off and root rot pathogen are cool and wet environment when it can cause infection of the seedlings in poorly drained soils and eventually kill the young seedlings either before or soon after emergence. Also, it has been reported that various young emerging plant organs like the radicle, hypocotyl, cotyledons, seed coat, endosperm and embryo are highly prone to attack by the pathogen-causing damping-off and root rot diseases (Paulitz et al. 1992). The severity of the disease caused by damping-off and root rot pathogens, however, can be reduced considerably provided some measures are taken to check or slow down the initial attacks by the phytopathogen. In this context, several fungicides such as captan, thiram, iprodione, fenaminosulf, fosetyl-Al and metalaxyl have been applied as seed treatments to control the disease (Leisso et al. 2009). But the biological control has been considered as a good and safe option for the management of damping-off and root rot diseases in both conventional and organic farming practices with least destruction to the environment (Jacobsen and Backman 1993; Georgakopoulos et al. 2002; Nagarajkumar et al. 2004). To further promote and popularize the use of biocontrol agents to eradicate/reduce this disease, several species of non-pathogenic bacteria belonging to the genera *Pseudomonas* and *Bacillus* have been used as potential antagonists to damping-off and root rot pathogen *P. ultimum*. In a study, the biocontrol potential of three most effective antagonistic bacteria was evaluated against seedling damping-off and root rot of cucumber caused by *P. ultimum*. Based on phenotypic characteristics, biochemical characterization and 16S rDNA gene sequence analysis, the three antagonistic bacteria were identified as *P. fluorescens* (9A-14), *Pseudomonas* sp. (8D-45) and *Bacillus* *subtilis* (8B-1). All of the three bacteria could promote plant growth and simultaneously suppress the effects of damping-off and root rot caused by *P. ultimum* on cucumber seedlings when tested in growth chamber trials. Interestingly, both pre- and post-planting application of bacterial treatment led to a decrease in damping-off and root rot severity in cucumber by 27–50%, thereby resulting in an improved growth (Khabbaz and Abbasi 2014). All the strains could successfully reduce the disease incidence when applied as seed treatment either singly or in combination. The production of antibiotics and some specific metabolites could probably be a possible reason of disease suppression by PGPR isolates. Additionally, the ISR may also be involved in providing protection to cucumber against damping-off and root rot disease (Van Loon et al. 1998; Powell et al. 2000; Van Loon 2007). This study thus suggests that various formulations of PGPR can be used to develop biofungicides to minimize the crop losses caused by seedling damping-off and root rot disease in cucumber and other vegetables of economic importance.

5.5 Conclusion and Future Prospects

Vegetables are grown on a large scale worldwide to fulfil human food demands. But unfortunately, most of the vegetable crops are lost due to bacterial and fungal phytopathogens that cause major diseases leading eventually to enormous yield losses. To minimize the yield loss in vegetables, several conventional approaches for plant disease management like developing resistant cultivars, crop rotation, field sanitization, spraying of fungicides, etc. have been practised over the years. But these methods have not been found fully effective in controlling plant diseases, and more so such strategies are expensive and labour intensive. Also, the use of fungicides and other chemicals adversely affects the quality and productivity of the vegetables. Thus, production of disease-free vegetables becomes a challenging task for the growers. In this context, biological control measures could be an effective alternate approach for containing vegetable diseases. Several plant growth-promoting rhizobacteria are known to suppress various diseases of vegetables by employing one or a combination of mechanisms leading eventually to enhancement in production. Application of such beneficial microbes is likely to reduce the use of chemicals in vegetable production practices in different production systems.

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