Biological Control of Bacterial Wilt Disease-Causing Pathogens: A Sustainable Approach for Increasing Crop Production

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Abstract

India is the second largest global producer of vegetables and accounts for about 15 % of the world's vegetables production. The production of vegetables is affected by infection of crops with several diseases throughout their life cycle. Among the diseases, bacterial wilt caused by *Ralstonia solanacearum* in crops such as tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), chili (*Capsicum annum*), tobacco (*Nicotiana tabacum*), eggplant (*Solanum melongena*), and pepper (*Capsicum annum*) is a major disease contributing to production loss of 10.80–92.62 % per unit area in India. The incidence of this disease is much severe during summer due to high temperature (28–36 °C) and high moisture (50–100 %), which favor the activity of the pathogen (*R. solanacearum*). Currently, adopted disease management practices like chemical application, use of resistance varieties, and manual removal of infected plants are of limited success to control the disease. The use of naturally occurring microorganisms in the rhizosphere of crop plants as a biocontrol agent offers an alternate source, and is gaining greater importance nowadays. Many effective plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas* spp., *Bacillus* spp., *Burkholderia* spp., *Serratia* spp., and *Streptomyces* spp. are abundant in rhizospheric soil. Moreover, rhizospheric soils are regarded as a source of

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R. N. Kharwar et al. (eds.), *Microbial Diversity and Biotechnology in Food Security,*
DOI 10.1007/978-81-322-1801-2_34,

natural, effective, and valuable antagonists for the purpose of biological control. The use of PGPR to suppress pathogen (*R. solanacearum*)-causing bacterial wilt in crops has lately become successful, and thus is gaining greater importance. The PGPR control disease by producing siderophore, hydrogen cyanide (HCN), secondary metabolites/antibiotics such as pyoluteorin, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol (2,4-DAPG), 1,2-benzene dicarboxylic 46 acid, bis(2-ethylexyl) ester, 2,6-di-T-butyl-4-methelyne-2,5-cyclohexadiene-1, and antifungal enzymes such as cellulase, chitinase, and protease. Production of antibiotics is one of the primary mechanisms involved in disease suppression. Among many antibiotics, 2,4-DAPG, a polyketide produced by bacteria showing broad-spectrum antiviral, antifungal, antibacterial, antitumor activities, and phototoxic properties, has received considerable attention. Thus, the 2,4DAPG-producing genotypes can be exploited to suppress bacterial wilt disease in crop plants.

Keywords

Bacterial wilt diseaseBiocontrol · Plant growth-promoting rhizobacteria (PGPR) · Crop protection agents

34.1 Introduction

The global food demand is increasing with progressive increase of population; however, the production of food is greatly affected due to damage caused by plant diseases. The use of chemicals to control the pathogens causing plant diseases is resulting in resistance development in pathogens as well as various adversatives to the environment. In order to overcome these problems, use of naturally occurring beneficial microorganisms present in rhizospheric soil as biological control agents is a more reliable and effective technique. Plant growth-promoting rhizobacteria (PGPR), which are eco-friendly in nature and effectively suppress the disease-causing plant pathogens, have proven to be beneficial. Biological control is thus considered as an alternative approach to control plant diseases and increase crop production in sustainable agricultural management system.

34.2 Bacterial Wilt

Bacterial wilt caused by *Pseudomonas solanacearum* was first reported by E. F Smith (1897; Rolfs 1898). The disease incidence was observed in many solanaceous species and several other plant families (Kelman 1954; Kucharek 1998). The occurrence of this bacterial pathogen has been reported across the world. Because of its extensive host range, it is known as a dangerous pathogen among the bacterial diseases (Buddenhagen et al. 1962; Hayward 1993). Among the vegetable crops, bacterial wilt caused by *Ralstonia solanacearum* in tomato is a serious disease and a major constraint in the production of tomatoes in tropical, subtropical, and warm temperate regions of the world (Buddenhagen et al. 1962; Hayward 1993). In India, bacterial wilt disease contributes to production loss of 10.80–92.62 % (Mishra et al. 1995). The problem is severe, especially in summer season due to the presence of high temperature (28–36 °C) and high moisture (50–100 %). Crop plants infected with wilt pathogens show stunted growth, poor fruit set, and complete wilting symptoms (Kucharek 1998). Bacterial wilt is initially characterized with wilting of upper leaves, followed by complete wilting of the plants. Brown discoloration in the vascular tissues of the lower stem of the wilted plants can also be observed. If the stem of a wilted plant is cut and immersed in clear water, it shows visible white or yellowish bacterial ooze.

The control of bacterial wilt pathogens is a much difficult task using chemical methods (Kucharek 1998). Management of bacterial wilt pathogen through integrated management approach like changing cultural practices, crop rotation, and use of resistant cultivars has provided some limited success, but failed to increase tomato production at commercial level (Kucharek 1998). The efficacy of current disease management methods employed for suppression of bacterial wilt pathogen is limited. No conventional practices are known to provide effective control of this soil-borne pathogen. Suppression of soil-borne wilt-plant pathogens using PGPR with increased crop production has been variously documented (Ciampi-Panno et al. 1989; Toyota and Kimura 2000). These can therefore be utilized as biocontrol agents. The PGPR have no side effects; hence, they are eco-friendly in nature (Bowen and Rovira 1999; Whipps 1997).

34.3 *Ralstonia solanacearum*

In the nineteenth century, for the first time, *R. solanacearum* was reported to cause bacterial wilt in potato, tobacco, tomato, and groundnut in Asia, southern USA, and South America. The pathogen was then described as *Bacillus solanacearum* by E. F. Smith (Rolfs 1898). Yabuuchi et al. (1992) described the pathogen as *Burkholderia solanacearum*. *R. solanacearum* is a Gram-negative rod with a polar tuft of flagella, and often produces nonfluorescent but diffusible brown pigments. Based on rRNA homology, *R. solanacearum* belongs to pseudomonad's group II and the *b*-subclass of proteobacteria. Different phenotypic and genotypic diversity in the same pathogen has been identified. The species are mainly divided into five races and five biovars depending on their host range and ability for oxidation/utilization of certain carbon sources (Hayward 1964). *R. solanacearum* mostly persists through soil, surface water, and infected crop residues (Granada and Sequeira 1983). The pathogen is also carried in the seeds of crop plants such as tomato and eggplant (Shakya 1993).

The most devastating and widespread bacterial wilt disease in many crop plants of tropical environment is due to the infection of *R. solanacearum* (He et al. 1983). This pathogen has worldwide distribution with host range of more than 50 different plant families (Hayward 1993). *R. solanacearum* gained its nuisance importance in the world due to its destructive nature, wide host range, and geographical distribution. It affects a wide range of economically important crops such as tomato, potato, eggplant, chili, and non-solanaceous crops such as banana and groundnut in India. The disease limits both the commercial and domestic level production.

34.4 Diversity of *R. solanacearum*

Buddenhagen et al. (1962) divided this pathogen into three races. Race 1 infects many solanaceous plants such as tomato, tobacco, pepper, and other plants including some weeds. It has a high temperature optimum (35 °C). Race 2 occurs mainly in tropical areas of South America and attacks bananas and *Heliconia* spp. (causing the so-called Moko disease), and in the Philippines (causing the so-called bugtok disease on plantains). In high tropics and subtropical and temperate areas, Race 3 is present and infects potato, tomato, occasionally capsicum and some solanaceous weeds like *Solanum nigrum* and *Solanum dulcamara* (Pradhanang et al. 2000; Strider et al. 1981; Janse et al. 2004). This race has a low temperature optimum (27 °C) and worldwide distribution, and appears to be mostly biovar 2A of RFLP group 26 (Cook and Sequeira 1994) and 27 (found in Chile and Colombia), or biovar 2T (sometimes also called 2N, found in tropical areas of South America). Race 4 infection is severe in *Zingiber* and *Morus*.

Another type of classification based on the pathogen's capacity to utilize or oxidize hexoses mannitol, dulcitol, and sorbitol classify *R. solanacearum* into four groups, while three groups are created on its utilization ability of disaccharides lactose, maltose, and cellobiose. Biovar I oxidizes hexose alcohols but not disaccharides, whereas biovar II oxidizes only disaccharides. Biovar III oxidizes both disaccharides and hexose alcohols, while biovar IV oxidizes only alcohols (Hayward 1964). Shrestha (1977) and Adhikari (1993) reported race 3 and the biovar II in

potato from mid-to-high hill regions, and race 1 (biovar III) in eggplants, peppers, tomatoes, and marigolds from lowland areas of Nepal. Titatarn (1986) classified the bacterial wilt pathogen of potato as biovar III and IV from mid hills, and biovar II from high hills of Thailand.

Thus, there exist different biovars of *R. solanacearum,* which can adapt to a wide range of environments and may cause severe damage to crop production. There is an urgent need to understand the nature of interactions of pathogens to develop effective biocontrol agents to suppress its activity. The use of PGPR for suppressing bacterial wilt-causing pathogen is an important strategy of disease management and has a lot of scope to isolate, identify, and improve plant growth-promoting rhizobacterial strains specific to biovars of *R. solanacearum*.

34.5 Rhizospheric Soil

The entire soil mass along with roots is called rhizosphere (Hiltner 1904), and the bacteria present in the rhizosphere are called rhizobacteria, whose composition is influenced by root exudates (Kennedy 1999). Root exudates that contain different chemical compounds such as carbohydrates (sugars and oligosaccharides), vitamins, organic acids, flavonoids, enzymes, hormones, nucleotides, and volatile compounds (Prescott et al. 1999) influence the rhizobacterial community. The effect of rhizocomposition of the soil microbial population can be determined comparing the population density (colonies forming units) of the rhizospheric soil (*R*) and the bulk soil (*S*), for which the "*R*/*S* ratio" is employed (Atlas and Bartha 1997). The rhizospheric effect is more for bacteria, than that for fungi. Plant type and root exudates also affect the *R*/*S* ratio. The release of root exudates can be affected by various plant, soil, and environmental factors (Bowen and Rovira 1999; Whipps and Lynch 1986; Mc Cully 1999).

The colonization of individual microorganisms in the rhizosphere and its surviving capacity depends on the competence of the individual bacterial strains (Weller et al. 1988). Cellular activities like motility, chemotaxis, prototrophy, and the ability to sequester iron and oxygen (Lugtenberg et al. 2001) also contribute to the establishment of microorganisms in the rhizosphere. Other factors contributing colonization of bacteria are their ability to withstand physical stresses including heat, desiccation, and the presence of reactive oxygen molecules (Miller and Wood 1996; Sarniguet et al.1997; Schnider-Keel et al. 2001). The genes, which play a role in colonization, are *sss* (phenotypic variation; Sanchez-Contreras et al. 2002), *gacS, gacA, rpoS, algU* (global regulators; Sanchez-Contreras et al. 2002; Sarniguet et al. 1997; Schnider-Keel et al. 2001), *dsbA* (periplasmic disulfide-bond-forming protein), and *ptsP* (organic nitrogen utilization; Mavrodi et al. 2006). Moreover, plant genotype greatly influences the microbial community structure of the rhizosphere (Grayston et al. 1998; Smith and Goodman 1999; Smith et al. 1999; Weiland et al. 2001; Marschner et al. 2004). The presence of specific bacteria in the rhizosphere is decided by the type and nature of crop plants (Larkin et al. 1993; Smith and Goodman 1999, Raaijmakers and Weller 2001; Weiland et al. 2001; Marschner et al. 2004; Bergsma-Vlami et al. 2005). This proves the importance of the trait relationship between plant species and genotypes in the selection of specific groups of microbes in rhizospheric soil (Bergsma-Vlami et al. 2005; Landa et al. 2003; Mazzola et al. 2004; Okubara et al. 2004). Among the four *phlD*-positive bacterial genotypes (B, D, E, and L) present in the fields of wheat and barley affected by take-all decline (TAD) disease, genotype D was the most abundant in the soil in Washington State, USA (Raaijmakers and Weller 2001). However, depending upon the geographic location and the host plant, multiple genotypes of bacterial population have been reported in other crops like pea, flax, corn, and soybean (de Souza et al. 2003; Raaijmakers and Weller 2001; Landa et al. 2003). Plants also show a differential response to introduced and indigenous biocontrol agents (Landa et al. 2002; Maurhofer et al. 1995; Mazzola et al. 2004; Okubara et al. 2004). Hence, it may be inferred that microenvironment of the rhizospheric soil decides the diversity of microorganisms.

Microorganisms present in rhizospheric soil play an important role in promoting plant growth, directly as well as indirectly, by protecting them

from disease-causing pathogens. There is a wide diversity of these beneficial bacteria associated with the rhizospheric soil. Proper agriculture practices should be followed to maintain a favorable microenvironment to build up beneficial plant growth-promoting microorganisms in the soil and sustain these communities for a long period. The sustainability of these colonized bacteria in the rhizosphere for a long duration is thus a key factor for a biocontrol agent to successfully protect the plant against soil-borne plant pathogens.

34.6 Plant Growth-Promoting Rhizobacteria (PGPR)

Naturally occurring bacteria in the rhizospheric soil beneficial to crop growth and development are often referred to as PGPR (Kloepper et al. 1989; Glick 1995). The major groups of PGPR in rhizospheric soil are *Azotobacter, Azospirillum, Pseudomonads, Acetobacter, Burkholderia, Enterobacter,* and *Bacillus* spp. (Brown 1974; Elmerich 1984; Kloepper et al. 1988, 1989; Bashan and Levanony 1990).

34.7 PGPR's Role in Relation to Plants

PGPR play a vital role in the promotion of plant growth, directly by fixing nitrogen and increasing phosphorus availability through solubilization of organic and inorganic phosphorus (Kim et al. 1998; El-Tarabily et al. 2008; Sabannavar and Lakshman 2009; Hariprasad et al. 2009). The phytohormones such as auxins, cytokinins, and gibberellins produced by the PGPR directly influence root and shoot growth (Asghar et al. 2002; Tanushree et al. 2007), and also indirectly by suppressing plant disease-causing organisms. A great diversity of biocontrol PGPR agents are reported in rhizospheric soil (Maria et al. 2005; Keel et al. 1996; Landa et al. 2002; Raaijmakers and Weller 2001; Bergsma-Vlami et al. 2005). Among the PGPRs, *Pseudomonas fluorescens* is widely studied as a biocontrol agent against seed and soil-borne plant pathogens. The control of bacterial wilt and bacterial blight of potato with inoculation of *P. fluorescens* has been recorded in field and laboratory trials (Ciampi-Panno et al. 1989). The strains, *P. fluorescens* effectively controls *Fusarium* wilt of radish (Leeman et al. 1995), bacterial wilt of tobacco (Liu et al. 1999) and cucumber (Liu et al. 1999), *Sclerospora graminicola* in pearl millet (Umesha et al. 1998), *Xanthomonas oryzae* pv. *oryzae* in rice (Vidhyasekaran et al. 2001), eucalyptus wilt (Ran et al. 2005), and bacterial wilt in chili (Umesha et al. 2005).

34.8 Mechanisms of Biological Control

Different mechanisms involved in biological control of plant pathogens by PGPR have been documented. They include a variety of cell wall-degrading enzymes, competition, plant ethylene levels, systemic acquired resistance, hydrogen cyanide (HCN), siderophore, and antibiotic production.

34.8.1 Antifungal Enzymes

Some PGPR strains produce antifungal enzymes like chitinase, b-1,3-glucanase, protease, and lipase that can lyse cell wall of fungi and prevent disease infection in plants (Chet and Inbar 1994). *Pseudomonas stutzeri* strain, which produces extracellular chitinase and laminarinase effectively lyse the cell walls of *Fusarium solani* mycelia and control root rot (Lim et al. 1991). Similarly, *Pseudomonas cepacia* enzymes damage fungal mycelia of *Rhizoctonia solani, Sclerotium rolfsii,* and *Pythium ultimum* (Fridlender et al. 1993). Furthermore, Chernin et al. (1995) showed chitinolytic activity in the PGPR strain *Enterobacter agglomerans* antagonistic to fungal pathogens *R. solani, Trichoderma harzianum,* and *Rhizobium meliloti* transformed with chitinase gene from the bacterium *Serratia marcescens*. These transformants displayed increased antifungal activity (Chet and Inbar 1994). Similarly, *P. fluorescens* transformed with chitinase gene was effective

against the pathogen *R. solani* (Koby et al. 1994). *Cladosporium werneckii, P. cepacia,* and *P. solanacearum* hydrolyzes fusaric acid produced by *Fusarium,* which upon infection can damage the plants (Toyoda and Utsumi 1991).

34.8.2 Competition

The effective competitive nature of PGPR to utilize available nutrients efficiently and the ability to proliferate on the root surface plays an important role in disease suppression (Kloepper et al. 1988; O'Sullivan and O'Gara 1992). A limited surface area of leaf is invaded by phytopathogenic bacteria that cause disease in crop plants. This can be controlled by PGPR that can compete successfully with pathogens for these sites and thus often reduce disease incidence. The persistence and competition of a bacterium in the rhizosphere is influenced by a number of factors such as soil composition (Heijnen and van Elsas 1994; Bashan et al. 1995) and temperature (Sun et al. 1995; Chiarini et al. 1994). The rhizospheric soil contains a wide diversity of microorganisms, preferentiality those strains that are able to utilize an unusual carbon or nitrogen source such as an opine, a 1-aminocyclopropane carboxylate (ACC), or a xenobiotic compound (such as a herbicide or pesticide), and are able to proliferate and persist longer than other microorganisms in such rhizospheric soils (Jacobson et al. 1994). Effective suppression of *P. ultimum* by *Pseudomonas* spp. was dependent on the latter's ability to utilize seed exudates for the production of inhibitory compounds (Stephens et al. 1993). The saprophytic *Pseudomonas syringae* protected pears against gray mold and blue mold caused by *Botrytis cinerea* and *Penicillium expansum,* respectively, due to its high competitive nature (Janisiewicz and Marchi 1992).

34.8.3 Plant Ethylene Levels

In response to fungal infection, plants synthesize excess amount of ethylene, which leads to senescence, abscission of leaf or fruit, disease development, inhibition of growth and synthesis of antifungal enzyme (Abeles et al. 1992). Reports of this kind are available for various plants such as wheat plant infected by *Septoria nodorum* (Abeles et al. 1992), *Verticillium* wilt of tomato (Cronshaw and Pegg 1976), *B. cinerea* infection in roses, carnations, tomatoes, peppers, French beans, and cucumbers (Elad 1988). The PGPR *Pseudomonas putida* GR12–2producing enzyme ACC deaminase modulates the level of ethylene (Glick 1995; Jacobson et al. 1994) and promotes plant growth.

34.8.4 Systemic-Acquired Resistance

The infection of plant disease is also controlled by inoculating plants with PGPR strains, which induce plant defense system (systemic acquired resistance; van Peer et al. 1991; Tuzun and Kloepper 1994). *P. putida* and *S. marcescens*' inoculation protects cucumber plants from bacterial angular leaf spot disease caused by *P. syringae* pv. *Lachrymans* (Liu et al. 1995). The diacetyl phloroglucinol (DAPG)-induced systemic resistance (ISR) in *Arabidopsis thaliana* with root inoculation of *P. fluorescens* strain CHA0 protects the leaves from *Peronospora parasitica* infection (Iavicoli et al. 2003). DAPG significantly influences the net efflux (i.e., exudation) of amino acids from roots of plant species like alfalfa, maize, wheat, and medicago (Philips et al. 2004).

34.8.5 HCN

Some of the PGPR produce low-molecular-weight antifungal metabolites like HCN (Dowling and O' Gara 1994). *Pseudomonas* spp. produce antifungal metabolite substance HCN, which inhibits *Thielabiopsis basicola,* the causative agent of black root rot of tobacco (Voisard et al. 1989). An extensive role of HCN produced by PGPR in suppressing disease causing pathogen has been reported by various workers (Vansuyt et al. 2007; Chincholkar et al. 2007; Ramette et al. 2003).

34.8.6 Siderophore

Iron present in bound form in the soil is unavailable to the plants for direct assimilation. Some of the PGPR strains are able to produce a natural iron chelating agent siderophore, which has high affinity towards iron (Castignetti and Smarrelli 1986) making it readily available to the plants (Neilands and Leong 1986; Briat 1992). Thus, it affects the proliferation of phytopathogens and controls disease spread in plant (O' Sullivan and O' Gara 1992). The effect of siderophore on disease suppression depends upon the type of PGPR, the siderophore affinity to iron, specific crop plant, type of pathogen and soil composition. Bacteria that strongly control pathogen in vitro may not perform well under field conditions. Siderophore produced by *Pseudomonas putida* helps in suppression of *Fusarium oxysporum* and *Pythium* spp. infections in tomato and *Gaeumannomyces graminis* var. *tritici* infection in wheat plants (Vandenburgh and Gonzalez 1984; Buysens et al. 1994; Elsherif and Grossmann 1994).

34.8.7 Antibiotics

The primary mechanism involved in biocontrol of disease causing pathogens by PGPR is through the production of antibiotics. Bacteria synthesize variety of antibiotics, agrocin, 2,4-diacetylphloroglucinol (2,4-DAPG), herbicolin, oomycin, phenazines, pyoluteorin and pyrrolnitrin. They are highly specific in their action; a particular antibiotic acts on specific phytopathogens. The evidence of direct involvement of antibiotic production in PGPR-mediated disease-suppression has been reported by various workers (Carmi et al. 1994; Thomashow and Weller 1988; Haas et al. 1991; Keel et al. 1992; Pierson et al. 1994). The biocontrol agent *P. fluorescens* (BL915) produces the antibiotic pyrrolnitrin, which acts on *R. solani* and prevents damping-off of cotton plants. An increased production of pyoluteorin and 2,4-DAPG antibiotics by *P. fluorescens* protects cucumber plants against the disease caused by *P. ultimum* (Maurhofer et al. 1992; Schnider et al. 1994). The enhanced antibiotic production by PGPR strains through modification of global regulation of genes in *P. fluorescens* (CHAO) encoding the housekeeping sigma factor has improved the protection against *P. ultimum*-induced damping-off of cucumbers (Maurhofer et al. 1995; Schnider et al. 1995).

34.8.7.1 Diacetylphloroglucinol (DAPG)

Antibiotic DAPG is a polyketide compound produced by bacteria. Among the antibiotics, it has received particular attention because of its broad-spectrum antiviral, antifungal, antibacterial, and antitumor activity and phytotoxic properties (Keel et al. 1992; Shanahan et al. 1992; Thomashow and Weller 1995; Bangera and Thomashow 1999; Isnansetyo et al. 2003; de Souza et al. 2003; Raaijmaker et al. 2002; Haas and Keel 2003). Production of 2,4-DAPG by *Pseudomonas* spp. is extensively studied (Shanahan et al. 1993, Bangera and Thomashow 1999; Schnider-Keel et al. 2000; Raaijmakers et al. 2002). This particular genotype of bacteria (*Pseudomonas* spp.) is associated with specific crop rhizosphere (Raaijmakers and Weller 2001; Landa et al. 2002; Okubara et al. 2004; Bergsma-Vlami et al. 2005). The DAPG-producing bacteria are highly rhizosphere competent (Mc Spadden et al. 2000; Raaijmakers and Weller 2001). This characteristic is an essential prerequisite of DAPG-producing organisms for successful biocontrol of plant diseases (Raaijmakers et al. 1995; Johnson 1994; Raaijmakers and Weller 1998; Lugtenberg et al. 2001). The competitive nature of DAPG-producing bacteria increases their ability to establish and maintain population densities sufficient to suppress disease in rhizosphere of pea and wheat (Landa et al. 2003; Raaijmakers and Weller 2001). DAPG-producing PGPR are found to be more competitive in nature compared to that of other bacteria (Mavrodi et al. 2006). Competence is important since direct correlation exists between the population size of the biocontrol strain and the level of biocontrol (Johnson 1994; Raaijmakers et al. 1995, 1998).

The role of DAPG produced by *Pseudomonas* strains has been reported in biological control of *Fusarium* crown and root rot, *Pythium* root rot (Rezzonico et al. 2007; Sharifi-Tehrani

et al. 1998), black root rot of tobacco caused by *T. basicola* (Stutz et al. 1986; Keel et al. 1996; Ramette et al. 2003), and pea wilt caused by *F. oxysporum* (Landa et al. 2003). The dominating DAPG-producing *Pseudomonas* spp. in the rhizospheric soil of wheat effectively suppresses TAD disease of wheat caused by *G. graminis* var. *tritici* (Raaijmakers et al. 1997; Mc Spadden et al. 2000; Weller et al. 2002; de Souza et al. 2003).

34.8.7.2 Diversity of 2,4-DAPG-Producing PGPR in Rhizospheric Soil

To study the diversity among the DAPG-producing rhizobacteria, various methods such as amplified ribosomal DNA restriction analysis (ARDRA; Keel et al. 1996; Mc Spadden et al. 2000; Picard et al. 2000), random amplified polymorphic DNA (RAPD; Raaijmakers and Weller 2001; Mavrodi et al. 2001), colony hybridization (Raaijmakers et al. 1997), direct characterization with whole-cell repetitive sequence-based PCR (rep-PCR; Mc Spadden et al. 2000; Landa et al. 2002), restriction fragment length polymorphism (RFLP; Mavrodi et al. 2001; Mc Spadden et al. 2001; Ramette et al. 2001; Wang et al. 2001), and BOX-PCR (McSpadden et al. 2001) analyses have been employed. Recently, the denaturing gradient gel electrophoresis (DGGE; Bergsma-Vlami et al. 2005) and allele-specific primer-based techniques (de la Fuente et al. 2006) have been developed to detect highly competitive and indigenous DAPG producers in natural environments. The diversity study of 200 *phlD*-positive strains collected across the world by BOX-PCR could distinguish 18 genotypes (A–Q and T; McSpadden et al. 2000; Landa et al. 2002) and were close to groups distinguished by RFLP (Mavrodi et al. 2001; McSpadden et al. 2001). Additional groups of DAPG-producing organisms (genotypes PspC, PspD, PspF, and PspZ) and (genotypes R and S) are given by Bergsma-Vlami et al. (2005). These techniques reveal the existence of diverse genotypic and phenotypic populations of DAPG-producing *P. fluorescens* in the environment (Thomashow and Weller 1995; Keel et al. 1996; McSpadden et al. 2000; Lee and Kim 2001; Weller et al. 2002; Isnansetyo et al. 2003). Identification of potential biocontrol agents capable of controlling disease-causing pathogens can be the core area of interest in maintaining sustainable agriculture production.

34.8.7.3 Factors Influencing Production of DAPG

DAPG production can be modulated by a diverse array of abiotic and biotic factors, including carbon and nitrogen sources, metal ions and metabolites released by bacteria, fungi, and plants (Duffy and Défago 1999; Maurhofer et al. 2004; Notz et al. 2001). The impact of environmental factors on the production of DAPG has been studied both in vitro and in situ for a number of *Pseudomonas* strains (Duffy and Defago 1999; Notz et al. 2001; Shanahan et al. 1992). The mineral elements glucose, sucrose, fructose, and mannitol promote the production of DAPG in *P. fluorescens* (Shanahan et al. 1992). Zinc sulfate and ammonium molybdate supports 2,4-DAPG production; in contrast, organic phosphate and sorbose inhibit its synthesis (Shanahan et al. 1992). Fusaric acids produced by *F. oxysporum* inhibit the production of DAPG by *P. fluorescens* (Duffy and Defago 1999). The bacterial and plant metabolite salicylate inhibits DAPG production (Schnider-Keel et al. 2000). DAPG production is controlled by four global regulators in *Pseudomonas* spp. The two-component regulatory system composed of the sensor kinase GacS (formerly designated LemA) and the cognate response regulator GacA is required for the synthesis of 2,4-DAPG (Blumer et al. 1999; Corbel and Loper 1995; Laville et al. 1992; Whistler et al. 1998). Furthermore, its synthesis is influenced by the relative level of the housekeeping sigma factor RpoD and the stationary-phase and stress sigma factor RpoS. Mutational studies on *rpoS* gene of *P. fluorescens* indicated that overproduction of antibiotics 2,4-DAPG and pyoluteorin effectively suppressed certain root diseases (Maurhofer et al. 1992; Sarniguet et al. 1995; Whistler et al. 1998). The strain *P. fluorescens* Q2–87 contains gene *phlACBD* cluster involved in biosynthesis of 2, 4-DAPG and can gainfully be manipulated in PGPR to increase the production of 2,4-DAPG and its biocontrol efficiency.

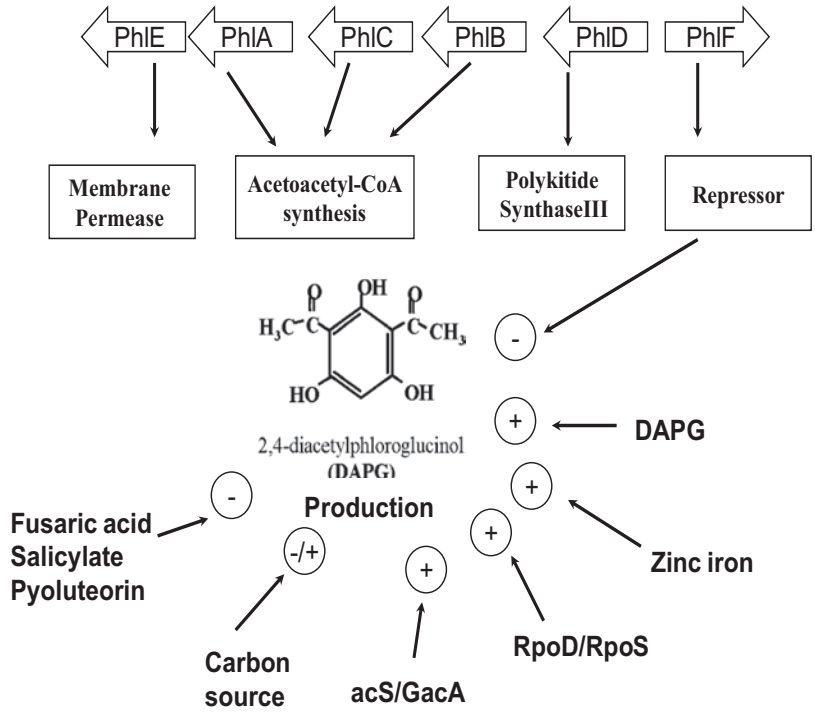

Fig. 34.1 Factors of 2, 4-influencing biosynthesis DAPG

34.8.7.4 Biosynthesis and Regulation of 2,4-DAPG

Six genes (*PhlE, PhlA, PhlC, PhlB, PhlD,* and *PhlF*) are involved in the biosynthesis and regulation of 2, 4-DAPG (Fig. 34.1). Among them, four genes *PhlA, PhlC, PhlB,* and *PhlD* (identified in *P. fluorescens* strains Q2–87, F113, CHA0, and Pf-5) are transcribed as a single operon (Bangera and Thomashow 1999; Fenton et al. 1992; Bangera and Thomashow 1999; Schnider-Keel et al. 2000; Mavrodi et al. 2001). *phlD* acts as an initiation gene for the production of 2,4-DAPG; it encodes a polyketide synthase (PKS), which is involved in the production of monoacetylphloroglucinol (MAPG; Bangera and Thomashow, 1999). MAPG is converted to 2,4-DAPG by the action of *PhlA, PhlC,* and *PhlB* genes (Shanahan et al. 1993). The *phlE* gene encodes a putative transmembrane permease (Bangera and Thomashow 1999), which is involved in the exportation of 2,4-DAPG from the cell. The *phlF* gene encodes a pathway-specific transcriptional repressor of the 2,4-DAPG biosynthetic operon (Bangera and Thomashow 1999; Schnider-Keel et al. 2000).

34.9 Possible Action of 2,4-DAPG-Producing PGPR in Suppression of Wilt Disease Incidence in Crop Plants

Biocontrol potential of 2,4-DAPG in bacterial wilt disease suppression is reported by many early workers (Jian-Hua Guo 2004; Ran et al. 2005; Lemessa and Zeller 2007; Naser et al. 2008; Qing-Yun et al. 2009, Rashmi 2010). The evidence in favor of suppression of bacterial wilt disease of crop plants (caused by *R. solanacearum*) through antagonistic effects of inoculated 2,4-DAPG-positive bacteria came from high population density of 2,4-DAPG-positive bacteria in the crop rhizosphere. The suppression of pathogen in the rhizospheric soil has re-

sulted in healthy and vigorous plant growth. An increased growth of the crop plants inoculated with PGPR strains, and the suppression of *R. solanacearum*-causing bacterial wilt has been well documented by Srinivasamurthy et al. (2012). Vincent and Mew (1998) found that an increase in soil pH and the presence of ammonium ion has a suppressing effect on the growth of *R. solanacearum*. The beneficial effects of high pH in reducing *Fusarium* wilt disease have been reported in a number of crops, including tomato (Woltz and Jones 1981). High pH reduces the availability of nutrients such as P, Mg, Mn, Cu, Zn, and Fe in organic growth media (Handreck and Black 1991), thus making pathogens more vulnerable (Woltz and Jones 1981).

Inoculation of 2,4-DAPG-positive bacteria decreases the population density of wilt disease-causing pathogen, resulting in almost absence of this disease in crop plants. The soil physical characteristics and rhizospheric composition of tomato crop plants support the activity of 2,4-DAPG-positive bacteria thereby increasing their population, which in turn suppresses *R. solanacearum.* Microbial competition of *P. fluorescens,* which plays a significant role in disease suppression, is decided by carbon source present in the media as well as on the role of root exudates (Lockwood 1988; Nelson 1990; Weller et al. 2002; Celia et al. 2004).

Rhizocompetence is a critical factor in the suppression of plant diseases (Dashti et al. 2000; Kamilov et al. 2005; Qing-Yun et al. 2009). Biocontrol efficiency of inoculated PGPR is influenced by the microenvironment of the rhizospheric soil (Weller et al. 2002; Celia et al. 2004). Rhizocompetence of *phlD*-positive bacteria and 2,4-DAPG are essential factors for the suppression of *R. solanacearum,* responsible for bacterial wilt disease. A direct relation exists between PGPR population density and prevention of wilt disease infection by *R. solanacearum* in tomatoes (Srinivasamurthy et al. 2012). Thus, the efficacy of biocontrol potential of *phlD*-positive bacteria can be exploited in future as a potential biocontrol measure in sustainable agriculture system to suppress bacterial wilt disease in a large number of crop plants.

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