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Research Article

Association of Rhizospheric/Endophytic Bacteria with Plants: A Potential Gateway to Sustainable Agriculture

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ABSTRACT

Application of associative bacteria for sustainable agriculture holds immense potential. These bacteria are known to enhance growth and yield of plants by fixing atmospheric nitrogen, solubilization of phosphate, production of phytohormones and siderophores, possession of antagonistic activity as well as reducing the level of stress ethylene in host plants. Colonization of these bacteria can be tracked by tagging them with certain molecular markers such as β-glucuronidase (*gus*) or green fluorescent protein (*gfp*) followed by electron microscopy or laser scanning confocal microscopy. Associative bacteria and endophytes may express genes differentially to colonize and establish the plant interior. They may also use 'quorum sensing' molecules for colonization process. Present review aims to highlight various plant growth promoting properties, ecology and updates of molecular mechanisms involved in interaction between associative bacteria and plants as well as immune responses triggered by these bacteria in plants.

INTRODUCTION

The over increasing population of the world has already touched the number of 6.8 billion. To feed this burgeoning population, farmers heavily rely on the use of chemical fertilizers especially inorganic nitrogen. Application of inorganic fertilizer has many repercussions, as it leads to ground and surface water contamination due to leaching and denitrification, which is detrimental for human and animal health. Secondly, manufacturing of industrial nitrogen fertilizer uses non-renewable resources like natural gas and coal and causes production of green house gases viz., CO_2 and NO_2 contributing to global warming (Bhattacharjee et al., 2008). Therefore, it's high time to opt for alternative fertilizers which can be used in sustainable agricultural practices without affecting the environment. Application of plant growth promoting associative bacteria can be a potential option for enhancing growth and yield of plant in sustainable manner.

On the basis of area of colonization, Plant Associated Bacteria (PAB) can be grouped into associative bacteria that include rhizospheric (in vicinity of root) and rhizoplanic (on surface of root) bacteria and, endophytic bacteria. Term 'endophytic bacteria' is referred to those bacteria, which colonizes in the interior of the plant parts, viz, root, stem or seeds without causing any harmful effect on host plant (Hallmann et al., 1997). These bacteria may promote plant growth in terms of increased germination rates, biomass, leaf area, chlorophyll content, nitrogen content, protein content, hydraulic activity, roots and shoot length, yield and tolerance to abiotic stresses like draught, flood, salinity etc. PAB can promote plant growth directly through Biological Nitrogen Fixation (BNF), phytohormone production, phosphate solubilization, inhibition of ethylene biosynthesis in response to biotic or abiotic stress (induced systemic tolerance) etc., or indirectly through inducing resistance to pathogen (Bhattacharya and Jha, 2012). Present review aims to focus on plant growth promoting abilities of rhizospheric and endophytic bacteria and their molecular aspects. PAB has been classified as the plant growth promoting bacteria on the basis of basic mechanisms through which it stimulates plant growth as PGPB, which induces plant growth directly and; bio-controller, which protects plants by inhibiting growth of pathogen and/or insect (Fig. 1) (Backman and Sikora, 2008). In the present review, discussion regarding PGPB has excluded rhizobia associated with leguminous plants.

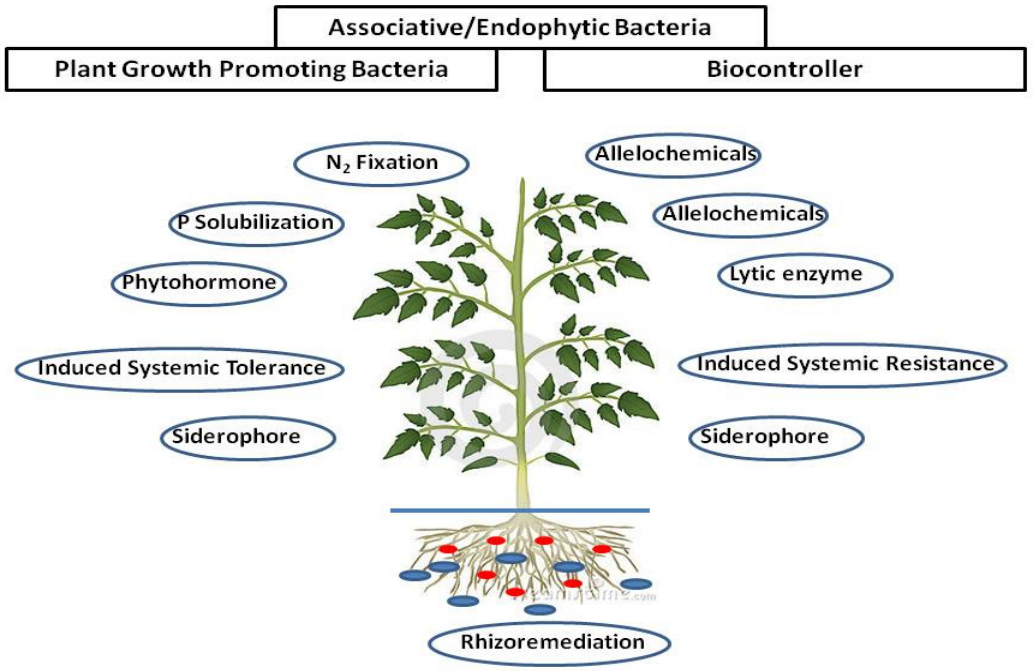

Figure 1: Properties of associative/endophytic bacteria for plant growth improvement. Based on the properties, associative/endophytic bacteria have been classified as Plant Growth Promoting Bacteria (PGPB) and biocontrol bacteria. PGPB may benefit associated plants through providing nutrition (nitrogen, phosphorous and iron), production of plant hormone and may enable plant tolerate abiotic stressors. Biocontrol bacteria (right panel in figure) protect plants from invasion of pathogenic microorganisms through antagonism and/or induced systemic resistance.

Plant Growth Promoting Bacteria

Associative bacteria as well as endophytic bacteria use same mechanisms to influence plant growth. However, they differ in efficiency through which they exert their beneficial effect. Based on various properties, plant growth promoting bacteria can be classified as biofertilizers, rhizoremediators, phytostimulators and stress controllers. Bacterial fertilizer is referred to the bacteria that supply nutrition to the associated plant. They may benefit plants by providing utilizable nitrogen through fixation of atmospheric nitrogen or they make free phosphate available from insoluble source of phosphate. Plant growth promotion due to solubilization of zinc compound driven by *Gluconoacetobacter* has also been reported Beneficial properties of these bacteria are described below in brief (Lugtenberg and Kamilova, 2009).

Biological Nitrogen Fixation: Many associative and endophytic bacteria are now known to fix atmospheric nitrogen and supply it to the associated host plants. A variety of nitrogen fixing bacteria like *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Derxia*, *Enterobacter*, *Gluconoacetobacter,* *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Serratia* and *Zoogloea* have been isolated from the rhizosphere of various crops, which contribute fixed nitrogen to the associated plants. For instance, contribution of 20 Kg N ha^{-1} by *Azotobacter paspali* was demonstrated using ^{15}N dilution technique (Baldani and Baldani, 2005; Reinhold-Hurek and Hurek, 2011). In recent years, application of endophytic bacterial inoculants supplying N requirement efficiently to the various host plants including cereal crops have drawn attention for increasing plant yield in sustainable manner. Additionally, some of the rhizobial isolates have also been found to colonize non-legume plant as an endophyte and benefit the associating host (Rothballer et al., 2008). In terms of benefiting through nitrogen fixation, endophytic bacteria are considered to be better than that of rhizospheric one as they provide fixed nitrogen directly to their host plant and fix nitrogen more efficiently due to lower oxygen pressure in the interior of plants than that of soil.

When diazotrophic bacteria establishes endophytic association with plants, total content of plant nitrogen rises which may be due to the biological nitrogen fixation or increased ability of nitrogen uptake from soil. In a well- organized study in Brazil suggested that 60-80% of the accumulated nitrogen in different varieties of sugarcane namely, CB45-3, SP70-1143 and Krakatau, was contributed by BNF (Boddey, 1995). Combination of nitrogen-fixing bacteria (viz.,*Rhizobium. trifolii* and *Burkholderia* MG43) and reduced amount of chemical fertilizer can achieve overall yield equivalent to the yield that was obtained from recommended full dose of chemical fertilizer **(**Bhattacharjee et al., 2008). *Gluconoacetobacter diazotrophicus* is the main contributor of endophytic BNF in sugarcane, which according to nitrogen balance studies fix as high as 150 Kg N $ha^{-1}yr^{-1}$ (Muthukumarasamy et al., 2005). However, contribution of BNF to host may vary with the genotype of host. Proteomic analyses of sugarcane variety SP70-1143 grown with *G. diazotrophicus* revealed up-regulated expression of ammonia lyase which indicates increased metabolism resulted from increased uptake of nitrogen contributed by bacteria (Lery et al., 2011). Up-regulation of genes for nitrogen metabolism during plant-bacteria interaction was also evident in differential gene expression studies carried out earlier (Nogueira et al., 2001). Another nitrogen-fixing endophyte of considerable interest is *Azoarcus*. This diazotroph inhabits the roots of kallar grass (*Leptochloa fusca*), which yields 20-40 t of hay ha^{-1} yr^{-1} without the addition of any N fertilizer in saline sodic, alkaline soils having low fertility (Ladha and Reddy, 2000). Percent contribution of plant nitrogen as a result of BNF by few associating endophytic bacteria has been given in table 1.

TABLE 1: **Contribution of biological nitrogen fixation by associative/endophytic bacteria**

Endophytic bacteria	Associating plant	% Ndfa*	Reference
Rhizobium leguminosarum bv. *trifolii*	Rice	19 to 28	Yanni et al., 1997; Biswas et al., 2000
Burkholderia	Rice	31	Baldani and Baldani, 2005
Herbaspirillum	Rice	19-47	Ladha and Reddy, 2000
Azospirillum	Rice	19-47	Ladha and Reddy, 2000
G. diazotrophicus, H. seropedicae, H. rubrisubalbicans, A. amazonense and Burkholderia sp	Sugarcane	29	Oliveira et al., 2002
K. pneumoniae 324	Rice	42	Iniguez et al., 2004
Burkholderia vietnamiensis	Rice	40-42	Govindrajan et al., 2008

*Nitrogen derived from air

At the molecular level, role of endophytic bacteria supplying fixed nitrogen to host was ascertained using non-nitrogen fixing *Klebsiella pneumoniae* where the rice plants inoculated with non- nitrogen fixing *K. pneumoniae* in nitrogen-deficient media showed signs of nitrogen deficiency on the contrary to the wild type counterpart (Iniguez et al., 2004). Nitrogen-fixation ability of endophytic bacteria *ex-planta* or *in-planta* is measured or detected on the basis of *nif* genes, encoding nitrogenase enzyme or by immunological detection of nitrogenase using antibody raised against nitrogenase enzyme (Nogueira et al., 2001). Presence of structural genes namely *nifH* or *nifD* in associative as well as endophytic bacteria have been detected by polymerase chain reaction using pair of universal primers (Jha and Kumar, 2009; Reinhold-Hurek and Hurek, 2011). Expression of *nif* genes has also been demonstrated by reverse transcription PCR (RT-PCR) from plants inoculated with *Azoarcus* BH72 and plants growing in field and in other associative diazotrophic bacteria (Terakado-Tonooka et al., 2008; You et al., 2005).

Phosphate Solubilization: Phosphate is known to be the second most limiting compound for plant growth. Although most of the soil is rich in phosphate but they are in insoluble form and cannot be utilized by plants or other soil organisms. A vast number of PGPB with phosphate solubilizing property have been reported which include members belonging to *Burkholderia*, *Enterobacter*, *Pantoea*, *Pseudomonas*, *Citrobacter* and *Azotobacter* (Park et al., 2010). Some plant growth promoting bacteria solubilize phosphate from organic or inorganic bound phosphates and facilitate plant growth. Possible mechanisms for solubilization from organic bound phosphate involve either enzymes namely C-P lyase, non- specific phosphatases and phytases. However, most of the bacterial genera solubilize phosphate through the production of organic acids such as gluconate, ketogluconate, acetate, lactate, oxalate, tartarate, succinate, citrate and glycolate (Khan et al., 2009). Type of organic acid produced for P solubilization may depend upon the carbon source utilized as substrate. Highest P solubilization has been observed when glucose, sucrose or galactose has been used as sole carbon source in the medium (Khan et al., 2009; Park et al., 2010). Genetics and biochemical basis of acid secretion specifically gluconic acid in bacteria such as *Erwinia herbicola*, *Pseudomonas cepacia* and *Enterobacter asburiae* have been reviewed by Rodriguez *et al.* (Rodrıguez et al., 2006). Production of gluconic acid results from the conversion of glucose to gluconic acid by an enzyme glucose dehydrogenase (Gcd). Gcd is a cell-envelope bound enzyme which depends on cofactor pyrroloquinoline quinine (PQQ).

Production of Phytostimulating Compounds

PGPB exert its effects through the production of substances which stimulate plant growth. These substances include phytohormones namely auxins, cytokinins, gibberellins, certain volatiles and the cofactor pyrroquinoline quinine (PQQ). Several associative bacteria have been shown to produce auxins chiefly IAA, which enhances lateral root growth formation and thus increase nutrient uptake by plants and root exudation, which in turn stimulates bacterial colonization and thus amplify the inoculation effect. Plant growth promotion as a result of IAA has been documented in several plants in recent years (Spaepen et al., 2007). However, beneficial effects of bacterial IAA depend upon the optimum concentration, which may vary for different plants. The role of phytohormone produced by associative bacteria in the promotion of plant growth during stress conditions such as salinity or draught has also been demonstrated recently (Egamberdieva, 2009). Since, indigenously produced phytohormone in plants declines in salt stress condition, salt tolerant associative bacteria may enhance plant growth by supplying phytohormones synthesized by them. Similarly, IAA producing bacteria may enhance growth of plant in drought condition by stimulating formation of well- developed root system enough for providing sufficient water from soil. Moreover, the role of IAA in response to stress is evident from its increased production of IAA in *Azospirillum* sp. during carbon limitation and acidic pH (Spaepen et al., 2007).

In addition to IAA, some of the associative bacteria have ability to produce other phytohormones such as cytokinin and gibberellin. Cytokinin produced by *Bacillus megatarium* UMCV1, a rhizospheric bacterium, was found to promote biomass production in *Arabidopsis thaliana* through the inhibition of primary root growth followed by increased lateral root formation and root hair length of host plant (López-Bucio et al., 2007). Interestingly, few isolates are capable of producing more than one phytohormone. Moreover, few bacteria namely *B. subtilis*, *B. amyloliqufaciens* and *E. cloacae* promote plant growth through the production of volatile organic compounds (VOCs) such as acetoin and 2,3-butanediol. VOCs of PGPR were found to enhance plant growth by regulating auxin homeostasis in plants which was evident from induction of genes encoding enzymes of metabolism of IAA (Zhang et al., 2008).

Induced Systemic Tolerance

A few PGPB enable the associating plants to tolerate abiotic stresses such as drought, salt, nutrient deficiency or excess, extremes of temperature and, presence of toxic metals. Thus, physical and chemical changes in plants resulted from PGPB-induced tolerance to abiotic stresses has been termed recently as 'Induced Systemic Tolerance' (IST). IST is elicited through the production of bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase, antioxidants, cytokinin or VOCs (Fig. 2).

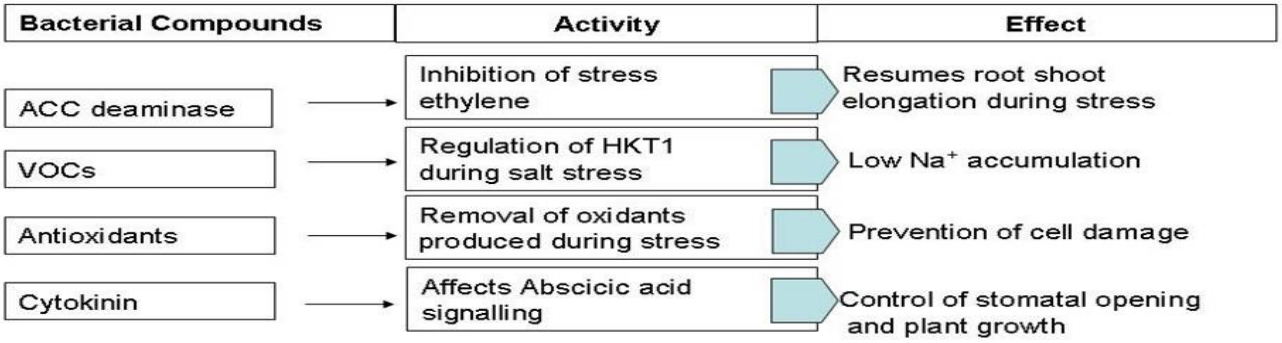

Figure 2: Various components of induced systemic tolerance. PGPB may help associated plant reduce effect of stressors through reducing level of stress ethylene due to presence of ACC deaminase activity, release of antioxidants, volatile organic compounds and plant hormone cytokinin.

PGPB equipped with ability to synthesize ACC deaminase (ACCD) reduce level of stress ethylene produced in plants in response to various biotic and abiotic stressors. ACCD degrades ACC, an immediate precursor of ethylene, to α-ketobutyrate and ammonia (Yang et al., 2009). In addition to ACC deaminase mediated IST, other mechanisms also exist to confer IST in response to stresses. In salt stress, level of Na^+ elevates, which decreases plant growth and productivity. The ion transporter high-affinity K^+ transporter 1 (HKT1) regulates Na^+ import in roots. VOC of *Bacillus subtilis* GB03 confer salt tolerance by down- and up-regulating HKT1 in roots and shoots respectively, and result in low Na^+ accumulation throughout the plant in comparison to control. Other PGPB mediated IST include production of cytokinin which affects abscicic acid (ABA) signaling of plants during stress and augmented production of antioxidant catalase (Yang et al., 2009).

Rhizoremediation

Bacteria with the ability to degrade organic pollutant can be used for remediation of soil. Although pollutant degrading bacteria characterized in laboratory environment may not thrive well in pollutant rich natural environment due to requirement of energy for primary metabolism. Aforementioned problem can be overcome with the use of associative and endophytic bacteria possessing ability to degrade soil pollutant. Since, PGPR colonizes in rhizosphere or rhizoplane; they obtain their source of energy from root exudates for primary metabolism and degrade efficiently organic xenobiotics present in the vicinity. For instance, *P. putida* PCL1444 effectively utilizes root exudates, degrades naphthalene around the root, protects seeds from being killed by naphthalene, and allows the plant to grow normally. Similarly, *in-situ* inoculation of *P. putida* W619-TCE reduced evapotranspiration of trichloroethylene by 90% under field condition (de Bashan et al., 2012). In a recent report, endophytic bacteria isolated from seeds of *Nicotiana tabacum* has been found to be potential candidate for reducing cadmium phytotoxicity (Mastretta et al., 2009). Application of endophytic bacteria for degrading the pollutants like petroleum, toluene and other organic solvent as well as protecting the plants from metals is of significant importance. In addition, endophytic bacteria engineered with genes encoding enzymes for degradation of pollutants can be better exploited for remediation of soil (de Bashan et al., 2012).

Biocontroller

World agriculture faces a great loss every year incurred from infection by pathogenic organisms. Application of microorganism for the control of diseases seems to be one of the most promising ways. Biocontrol systems are eco-friendly, cost-efficient and involved in improving the soil consistency and maintenance of natural soil flora. To act efficiently, the biocontrol agent should remain active under large range of conditions viz., varying pH,

temperature and concentrations of different ions. Biocontrol agents limit growth of pathogen as well as few nematodes and insects. Biocontrol bacteria can limit pathogens directly by producing antagonistic substances, competition for iron, detoxification or degradation of virulence factors; or indirectly by inducing Systemic Resistance (ISR) in plants against certain diseases, signal interference, competition for nutrients and niches and interference with activity, survival, germination and sporulation of the pathogen (Lugtenberg and Kamilova, 2009).

Antagonism: Associative/endophytic bacterial biocontrol agents may inhibit growth of fungal pathogens by one or more of the several mechanisms, which include production of antibiotics, siderophore and lytic enzymes.

A vast array of antagonistic chemical compounds has been identified in bacterial biocontrol agents. Gram negative biocontrol agents such as *Pseudomonas* produce HCN, pyoleutorin (PLT), pyrrolnitrin (PRN), 2,4-diacetylphloroglucinol (2-DAPG) and phenazines (PHZ) chiefly phenazine-1-carboxylic acid and phenazine-1-carboxamide Lugtenberg and Kamilova, 2009). The Role of each antibiotic produced by bacterial biocontrol agent in conferring control of fungal pathogen may vary in different species. Control of *Sclerotinia sclerotiorum* by *P. chlororaphis* PA23 is primarily executed by PRN while PHZ (phenazine-1-carboxylic acid, 2-hydroxyphenazine) helps in the development of biofilm formation (Selin et al., 2010). On the contrary, PHZ (phenazine-1-carboxamide) produced by *P. chlororaphis* strain 1391 was identified to be responsible for controlling tomato fruit and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Few other biochemicals having pathogen inhibiting activity include gluconic acid, 2-hexyl-5-propyl resorcinol, munumbicin, and few VOCs (2,3-butanediol) produced by biocontrol agent (Backman and Sikora, 2008). The level of antibiotic synthesis depends upon nutritional factors viz., type of carbon source utilized, trace elements and availability of other nutrients as well as non-nutritional factors like environmental influences. Regulation of antibiotic production in biocontrol bacterial agents involves GacA/GacR or GrrA/GrrS, RpoD, and RpoS, N-acyl homoserine lactone (AHL) derivatives, and positive auto regulation (Compant et al., 2005).

Under iron-limiting condition, some of the biocontrollers secrete siderophore, which chelates available iron of the soil and sometime from cohabiting microorganism, and deprive pathogenic fungi from this element (Compant et al., 2005). In addition to the role of siderophore in biocontrol, bacterial siderophore has been implicated in iron nutrition of crop plants and heavy metal phytoextraction. Production of siderophore by diazotrophic bacteria seems physiologically more important since the role of catecholate type of siderophore has been implicated in transport of Mo under iron starved condition in *Azospirillum lipoferum*. Because nitrogen-fixing bacteria require both iron and Mo for the activity of nitrogenase, the role of siderophore seems pivotal for any diazotrophic bacteria especially under iron deficiency (Rajkumar et al., 2010).

Bacteria may limit growth of other microorganisms also through the production of hydrolytic enzymes such as chitinase, β-1, 3-glucanase, protease and, laminarinase etc. For instance, *Serratia marcescens* and *Paenibacillus* sp. secrete chitinase to exert antifungal activity against *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *cucumerinum* respectively. *Bacillus cepacia* destroys *Rhizoctonia solani*, *R. rolfsii*, and *Pythium ultimum* through the production of β-1, 3-glucanase. Secretion of protease and chitinase was found to be the possible mechanism for antagonistic activity of endophytic bacteria *Enterobacter* and *Pantoea* against fungal pathogen *Fusarium oxysporum* f.sp. *vasinfectum* (Backman and Sikora, 2008; Compant et al., 2005).

Induced Systemic Resistance: Certain bacterial interactions with root enables the associated plant to develop resistance against potent pathogens. This phenomenon is termed as Induced Systemic Resistance (ISR) and has been noted to be exhibited by both associative and endophytic bacteria (Table 2) (van Loon, 2007). It was first noticed in carnation and cucumber where inoculation with selected PGPB (rhizobacteria) reduced susceptibility to wilt and foliar disease respectively. In contrast to many biocontrol mechanisms, extensive colonization of the root system is not required for ISR to be exerted (Lugtenberg and Kamilova, 2009).

TABLE 2: **Biocontrol activity of associative/endophytic bacteria**

Endophytic Isolates	Plants	Pathogenic Fungi/Bacteria
P. fluorescens EP1	sugarcane	*Colletotrichum falcatum*
Burkholderia phytofirmans PsJN	Grapevine	*Botrytis cinerea*
Burkholderia phytofirmans PsJN	Tomato	*Verticllium dahlia*
P. Denitrificans 1-15	Oak	*Ceratocystis fagacearum*
P. putida 5-48	Oak	*Ceratocystis fagacearum*
P. fluorescens 63-28	tomato	*F. oxysporum* f. sp. *radicis-lycopersici*
P. fluorescens 63-28	pea	*Pythium ultimum* and *F. oxysporum* f. sp. *pisi*
Bacillus pumilus SE34	Pea	*F. oxysporum* f. sp. *Pisi*
Bacillus pumilus SE34	cotton	*F. oxysporum* f. sp. *Vasinfectum*
Bradyrhizobium Sp. Strain ORS278	*Arabidopsis thaliana*	transcriptome analysis based study
Paenibacillus alvei K165	*A. thaliana*	*Verticillium dahlia*
Actinobacteria	*A. thaliana*	Quantitative PCR analysis based study
Bacillus cereus AR156	*A. thaliana*	*Pseudomonas syringae*

The bacterial products that elicit induction of ISR are diverse and can induce in plants which possibly possess receptor for respective ligands. These elicitors may be lipopolysaccharides, flagella, siderophores, antibiotics, VOCs or quorum-sensing signals. Majority of ISR activated by PGPB is mediated by jasmonate or ethylene (van Loon, 2007). Mechanisms of ISR in Pseudomonas has been reviewed recently (Jankiewicz and Kołtonowicz, 2012). In a recent study, plant growth promoting *Bacillus cereus* AR156 was found to trigger ISR in *A. thaliana* through SA- and JA/ET-signaling pathways in an (Non-expressor of PR1) NPR1-dependent manner (Niu et al., 2011). Development of ISR may induce various genes to strengthen the host plant mechanically or metabolically. It involves fortification of plant cell wall strength, alteration of host physiology or metabolic responses and, enhanced synthesis of plant defense chemicals such as phenolic compounds, pathogenicity related protein (PR-1, PR-2, PR-5), chitinases, peroxidases, phenyl alanine ammonia lyase, phytoalexins, oxidase and/or chalcone synthase. These metabolic products protect the host plant from future infections from pathogens. Local immune response induced by PGPR has also been demonstrated in few studies. However, pattern of local immune response depends on genotype of plants and respective bacterial species associated with them (Compant et al., 2005).

Biocontrol against Nematode: Few rhizobacteria acting as a biological control agent against plant-parasitic nematodes have also been reported (Tian et al., 2007). Antagonistic activity by aerobic endospore-forming bacteria (AEFB) (mainly *Bacillus* spp.) and *Pseudomonas* spp against nematodes is well known. It is mainly exerted by the means of metabolic by-products, enzymes and toxins including 2, 4-DAPG (*P. fluorescens*), hydrogen sulphide, chitinase, and hydrogen cyanide.

Colonization

Colonization of bacteria in rhizosphere or on plant surface is a complex process, which involve interplay between several bacterial traits and genes. The colonization is multi-step process and includes (a) migration towards root surface, (b) attachment, (c) distribution along root and (d) growth and survival of the population. For endophytic bacteria one additional step is required that is entry into root and formation of microcolonies inter-or intracellularly. Each trait may vary for different associative/endophytic bacteria. Colonization of bacteria is traced by tagging the putative colonizing bacteria with a molecular marker such as auto fluorescent marker (e.g., green fluorescent protein (*gfp*)) or β-glucosidase (*gus*) followed by microscopy (electron or confocal laser scanning microscopy) (Reinhold-Hurek and Hurek, 2011). Fluorescent in-situ hybridization with real time PCR analysis can also be used for tracking bacterial colonization and its quantification (Lacava et al., 2006). Understanding of molecular mechanism involved in associative or endophytic colonization process is not well understood. Recent reports based on the genomic data and other similar reports have suggested resemblance of colonization methods between pathogenic bacteria and PGPB (Hardoim et al., 2008).

Root Colonization

Root colonization is the first and the critical step in establishment of plant-microbe association. Microorganisms move towards rhizosphere in response to root exudates, which are rich in amino acids, organic acids, sugars, vitamins, purines/ pyrimidines and other metabolic products. In addition to providing nutritional substances, plants start cross-talk to microorganisms by secreting some signals which cause colonization by

some bacteria while inhibits the other (Bais et al., 2006; Compant et al., 2011). The patterns of chemoattractant especially organic acids may vary in different isolates/strains. Malate, succinate and fructose are considered to be the strongest chemoattractants.

Exudate composition is in turn influenced by physiological status of plant, the presence of microbes and products from rhizobacteria such as phenazines, 2,4-DAPG, zearalenone and exopolysaccharide. Sloughed up root cap cells also have large impact on plant-microbe interaction. In addition to chemotaxis, electrotaxis (electrogenic ion transport at the root surface) is also considered as a possible mechanism for initiating rhizobacterial colonization. Root hair regions and emergence points are preferred site for colonization (Lugtenberg and Kamilova, 2009).

Colonization of root by microorganism may further induce release of exudates, and create 'biased' rhizosphere by exudating specific metabolic products. In some rhizospheric bacteria, root exudates induce flagellar motility that leads their colonization on plant surfaces. During root colonization process, movement of associative bacteria is followed by their adhesion on plant root which may be mediated by glycosylated polar flagellum, Role of bacterial major outer membrane protein (MOMP) in early host recognition has been recognized in earlier report, where MOMPs from *Azospirillum brasilense* showed stronger adhesion to extracts of cereals than extracts of legumes and tomatoes. It suggests involvement of MOMPs in adhesion, root adsorption and cell aggregation of the bacterium (Lugtenber and Kamilova, 2009). On the other hand, involvement of type IV pili and twitching motility has been identified in tomato root colonization by *Pseudomonas* using *pilA* and *pilT* mutant, *pilA* is the gene encoding prepilin, structural component of type IV pili and *pilT* encodes for protein required for pilus contraction that is responsible for twitching motility (Lugtenberg and Kamilova, 2009; Reinhold-Hurek and Hurek, 2011). Preston *et al.* (2001) identified SSIII secretion system III (SSIII) (hrp) in *P. fluorescens* SBW25 that is by *in-vitro* expression technology (IVET), a promoter trapping technique. Moreover, role of two component regulatory system ColR/ColS in competitive root colonization in *P. fluorescence* has been demonstrated. ColR/ColS system regulates methyltransferase/*WapQ* operon, and thus maintains the integrity of outer membrane for efficient colonization (de Weert et al., 2009).

Endophytic Colonization

Primary mechanism for colonization of endophytic bacteria is similar to that of associative one. Twitching motility and type IV pile were found to be essential for successful colonization of *Azoarcus*, obligate endophytic bacteria (Böhm et al., 2007; Reinhold-Hurek and Hurek, 2011). In addition, Bilal *et al.* (1993) suggested that cell-surface protein and Ca^{2+} dependent twitching motility may be implicated in specific interaction with plants. Chemical composition of lipopolysaccharides (LPS) present on the surface of bacteria might be determinative for successful colonization in host plants (Serrato et al., 2010). Requirement for plant signal such as flavonoid present in root exudates of host plant was also observed for stimulation of endophytic colonization of wheat and *Brassica napus* plants by *Azospirillum brsilense* and *A. caulinodans* respectively (Lugtenberg and Kamilova, 2009).

Majority of natural isolates associated with plants form biofilm in the rhizosphere, on the surface of plant as well as in the endorhizosphere. LapA (large adhesion protein A), a cell surface protein, or its homologue is supposed to be putative adhesion needed for the adhesion of *Pseudomonads* on plant roots (Lugtenberg and Kamilova, 2009). Entry of endophytic bacteria in plant roots is known to occur (a) through wounds particularly where lateral or adventitious roots occur; (b) through root hairs and (c) between undamaged epidermal cells (Harodoim et al., 2008). Chi *et al.* (2005) demonstrated that the colonization of *gfp*-tagged rhizobia in crop plants begin with surface colonization of the rhizoplane at lateral root emergence, followed by endophytic colonization within roots, and then ascending endophytic migration into the stem base, leaf sheath, and leaves where they develop high populations. *Azospirillum* may also colonize endophytically through wounds and cracks of the plant root (Preito et al., 2011; Reinhold-Hurek and Hurek, 2011).

Endophytic bacteria may colonize root tissues and spread actively in aerial parts of plants through expressing moderate amount of degradative enzymes such as pectinases and cellulases. Utilization of aforesaid enzymatic activities for colonization by *Azospirillum irakense*, *Azoarcus* sp. and others has been demonstrated as one of the efficient methods to get entry into the host plant. Endoglucanase is one of the major determinants for the colonization of endorhizosphere, which was evident from the observation that *Azoarcus* strain lacking endoglucanse was not effective in colonizing the rice plants. The endoglucanase loosen larger cellulose fibers, which may help entering to the plant. A homologue of endoglucanase gene has also been identified in *P. stutzeri* A1501, which occasionally colonizes cortex of crop plants. In addition to endoglucanse, exoglucanases may also help in colonization process. An exoglucanase having cellobiohydrolase and β- glucosidase activity was identified to be key player in colonization process of *Azoarcus* sp. BH72 (Reinhold-Hurek and Hurek, 2011). In *Elaegnus* and *Mimosa*, the endophyte penetrates the radial walls presumably by digesting the middle lamella and then proceeds between cells and through intercellular spaces. In contrast to above examples, genes encoding plant cell wall degrading enzymes has not been found in endophytic bacteria *Herbaspirillum seropedicae* strain SmR1 (Pedrosa et al., 2011).

Azoarcus sp., an obligate endophyte of Kallar grass, has been critically studied by using transposon

mutant expressing β-glucuronidase (GUS) constitutively as a reporter gene (in *Azoarcus* sp. BH72). *Azoarcus* sp. BH72 colonize apical region of roots behind the meristem intensively and penetrate the rhizoplane preferentially in the zone of elongation and differentiation. They colonize in the cortex region both inter- and intracellularly. In older parts of the roots, they also occur in air spaces. *Azoarcus* sp. is capable of invading even the stele of rice and xylem vessels suggesting systemic spreading into shoots through the transport in vessels (Hurek and Reinhold-Hurek, 2003). On the contrary, shoot colonization of Gramineae appears to be more pronounced by *G. diazotrophicus* and *H. seropedicae* (Jha et al., 2004). Furthermore, Compant and associates reported colonization of endophytic bacteria *Burkholderia phytofirmans* in epidermis and xylem of even reproductive organ of grapevine. In another study Preito and associates suggested that endophytic bacteria are confined within an organelle most likely vacuole which arises by narrowing of an internal membranous structure in roots (Preito et al., 2011).

Endophytic colonization is not as specific as of *Rhizobia* but successful endophytic colonization does involve a compatible host plant (Ryan et al., 2008). However, endophytic colonization indeed depends upon the physiological changes in plants and is restricted or slowed down by defense mechanism (Rosenblueth and Martínez-Romero, 2006). Colonization of *G. diazotrophicus* was found to be diminished in plants grown under high nitrogen fertilizer regime. This reduction in colonization was explained as a result of altered plant physiology in the presence of nitrogen fertilizer, which reduces sucrose concentration to be utilized by endophytic bacteria. Influence of organic amendment on endophytic population has also been demonstrated (Hallman et al., 1997). Plant defense response plays critical role in regulating colonization of endophytic bacteria. In dicotyledonous plants, salicylic acid (SA) and ethylene restricts endophytic colonization. Ethylene, a signal molecule of ISR in plants decreases endophytic colonization as observed in *Arabidopsis thaliana* inoculated with *K. pneumoniae* 342 (Iniguez et al., 2005). However, proteomic approach used to study colonization by bacteria indicated that jasmonic acid, not ethylene and SA, contribute in restricting endophytic colonization in grasses (Miché et al., 2006). Expression of jasmoic acid (JA) induced PR proteins (defense proteins) depends upon the compatibility of plant variety and endophytic bacteria. Antimicrobial peptides synthesized by some plants like rice and maize may reduce endophytic colonization (Hurek and Reinhold-Hurek, 2003). Understanding of molecular mechanism and conditions limiting the colonization process need to be elucidated for exploiting the beneficial endophytic or associative interaction with plants.

Future Prospects and Challenges

A thorough exploration of associative/endophytic bacteria and their obvious abilities to enhance plant growth and productivity indeed indicate the existence of natural associations of these bacteria and their beneficial impact which can be exploited to feed burgeoning population of the world. Despite the fact that a large number of associative and endophytic bacteria have shown plant growth promoting properties at laboratory and green house level, these bacteria fail to exhibit consistent performance under natural conditions. The factors that affect colonization and thus PGPB derived benefit to plant may be soil type, nutritional status of soil, host plant genotype and age as well as climatic conditions (Bhattacharya and Jha, 2012). High amount of available utilizable nitrogen reduces colonization of PGPB in natural condition and it may also reduce the process of nitrogen fixation due to regulatory mechanism acting in the diazotrophic isolates. Therefore, a challenge is posed for systematic optimization for the application of suitable PGPB isolates and the amount of fertilizer to be added to obtain maximum output. Use of compost may be useful at some extent which provides utilizable nitrogen to support growth of microorganism and make the plant evade from negative effects of PGPB colonization on it.

One of the major challenges includes selection of plant genotype and age, and compatible associative bacteria. Understanding of this compatibility would help to enhance productivity by using specific strain for inoculation. Since, the colonization of associative bacteria also depends upon seasonal changes and soil hydric stress, multiples field trials are required to optimize parameters for obtaining the maximum output. Another factor which is to be studied in details is the plant defense response which may limit or reduce the colonization of associative bacteria. In addition, colonization mechanism is still not well understood. Intelligent analysis of genomic and functional genomics studies can help manipulate the conditions to enhance colonization process and increased plant growth properties.

Lastly and most importantly, extensive and intensive research on the understanding of associative and endophytic ecology will be major determinant to maximize benefit from these bacteria.

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