

Review Article

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## Inside the Plants: Endophytic Bacteria and their Functional Attributes for Plant Growth Promotion

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### ABSTRACT

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Endophytic bacteria are the association of bacterial microbe resides inside the plant tissues. They are reported to alleviate several biotic and abiotic stresses of plant. They are also found to be promote growth of plant through their several functional attributes viz., nitrogen fixation, phosphate solubilization, siderophore production and by producing plant growth regulators. They are easily available for plant for their maximum utilization since, they are nurtured inside the plant host which is free from the external adverse and changeable environment. In this review effort will take to summarize the role of endophytic bacteria and their utilization as an alternative for agricultural based practices in sustainable manner.

### Introduction

Almost all plants of the world are a potential inhabitants of indigenous microbes principally recognized as endophytic microbes which can reside inside their tissue (Figure 1) without

giving any visible external symptoms which responsible for nutrient assimilation and their processing, induction of defense system, and synthesis of secondary metabolites. They may

be actinomycetes, bacteria or fungi. They colonize internal tissues either as obligate or in facultative manner with host plants without causing any immediate negative or external symptom to host and reported to show the beneficial effects, put forward opportunities for discovering products and processes with potential applications in agriculture, medicine and biotechnology (Pandey *et al.*, 2012; Pandey *et al.*, 2014; Pandey *et al.*, 2015; Pandey *et al.*, 2016; Pandey *et al.*, 2016a). Bacterial endophytes stimulate plant growth, directly or indirectly thereby increasing their yield and several parameters utilized by living things for their life prospects (Gray and Smith, 2005; Pandey *et al.*, 2012; Pandey *et al.*, 2015; Pandey *et al.*, 2016). Researches have been conducted on the plant growth-promoting abilities and the forest regeneration with the help of various micro-organisms. Plant Growth Promoting Endophytic Bacteria (PGEb) is a group of bacteria that enhances the plant growth via Phosphate solubilization (Senthilkumar *et al.*, 2009), Nitrogen fixation (Rediers *et al.*, 2003), Indole acetic acid (IAA) production (Armando *et al.*, 2009), Siderophore production (Logeshwaran *et al.*, 2009) and by producing plant growth regulators (Armando *et al.*, 2009) (Figure 2).

Moreover, a number of other useful effects which have been attributed to the plants by bacterial endophytes include enhanced uptake of minerals (Ratul *et al.*, 2013), alteration of nitrogen accumulation and their metabolism (Kelemu *et al.*, 2011). It has been suggested that they may play a vital role in improving the host plant growth (Bent and Chanway, 1998; Izumi *et al.*, 2007). Findings of the bacterial endophytes from the meristematic tissues of different varieties of the strawberry were reported and characterized for indole acetic acid (IAA) production, phosphate solubilization (solubilizing inorganic phosphate) and their potential for plant growth promotion under greenhouse

conditions which revealed the ability of the bacterial endophytes to enhance the biomass, root number, leaf number, petiole length and dry weight of the aerial portion (Armando *et al.*, 2009). The role of the wheat endophytic bacterium *Pseudomonas aeruginosa* PW09 was tested for their plant growth-promoting traits, antagonism against soil-borne pathogens and the ability to withstand a relatively high salt concentration (NaCl) for imparting possible benefits to the cucumber seedlings (Pandey *et al.*, 2012). Bacterial endophytes provide a wide range of benefits to the plant host against biotic and abiotic stresses (Hurek and Hurek, 2011). In return, the bacterial endophytes may be benefited by the various secondary metabolites and the growth regulators produced by the host plants (Schulz and Boyle, 2006). Szilagyi-Zecchin *et al.*, (2014) were isolated six endophytic bacterial isolates from corn (*Zea mays* L.) roots identified as *Bacillus* sp. and as *Enterobacter* sp. by 16S rRNA gene sequencing. Out of six strains, four strains CNPSo 2476, CNPSo 2477, CNPSo 2478 and CNPSo 2480 were found to be positive towards the nitrogen fixation ability and two *Bacillus* strains (CNPSo 2477 and CNPSo 2478) were able to produce IAA, siderophores and lytic enzymes. *Enterobacter* sp. CNPSo 2480 and *Bacillus* sp. CNPSo 2481 were reported to increase the root volume by 44% and 39%, respectively against pathogenic fungi *Fusarium verticillioides*, *Colletotrichum graminicola*, *Bipolaris maydis* and *Cercosporazea-maydis* and hence, identified as a good candidate for future biological inoculants in corn. Ozaktan *et al.*, (2015) were investigated the endophytic bacterial isolates in cucumber plants to control Fusarial wilt of cucumber caused by *Fusarium oxysporum* f. sp. *Cucumerinum* (FOC) and plant growth promotion under *in vitro* conditions. They were successfully isolated a total number of 112 endophytic bacterial strains belong to Gram-negative and Gram-

positive equally from the healthy internal tissues of cucumber grown in greenhouse and field conditions in turkey and evaluated their plant growth promoting traits *in vitro* such as production of IAA, HCN, siderophore, phosphate solubilization. 30% of endophytic bacterial isolate able to produce IAA ranges between 20-125  $\mu\text{g ml}^{-1}$ , 46 % of endophytic bacterial isolate able to produce siderophore ranging from 3 to 19 mm zones, 16% able to produce HCN, 29% endophytic bacterial isolate able to solubilize phosphate and more than 53% of endophytic bacterial isolate able to inhibit the mycelial growth of *Fusarium oxysporum* f. sp. *Cucumerinum*.

### Functional attributes

#### Nitrogen fixation

A Brazilian scientist in 1986 discovered nitrogen fixing endophytic bacteria *Glucanacetobacter diazotrophicus* in the sugarcane stem (Cavalcante and Dobereiner, 1988). Further, in several investigations few more endophytic bacteria were added in the list such as *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Azospirillum lipoferum*, *Klebsiella pneumoniae* and *Azorhizobium caulinadans* (Schloter *et al.*, 1994; Boddey *et al.*, 1995). *Glucanoacetobacter diazotrophicus* were discovered in sugarcane, in sweet potato and in pineapple (Silva-Froufe, 2009). Endophytic bacteria were also reported from the legume nodule such as *Rhizobium (Agrobacterium) rhizogenes* and *R. leguminosarum* bv. *trifolii* in red clover nodules (Sturz *et al.*, 1997). Although much nitrogen fixing bacterial endophytes was isolated from the sugarcane but it is still controversial which are responsible to fix nitrogen in the plant, the level of nitrogen fixed by endophytes and what proportion of fixed nitrogen contributed to plant (Giller and Merckx, 2003). Identification of nitrogen fixation *in vitro* by the endophytic bacteria

helps in plant growth promotion when applied to the plant in the field conditions (Dobereiner and Day, 1976; James, 2000). Nitrogen (N), the most common nutrients required for the optimal growth of the plant remains unavailable to the plants yet its presence is 78% in the atmosphere. To make it available to the plants it is essential to convert it into the ammonia by biological Nitrogen Fixation by the microorganisms using a complex enzyme system of nitrogenase. The Biological Nitrogen Fixation accounts about the 60% of the earth's total nitrogen fixation and provide an economically and the environmentally friendly substitute to the synthetic fertilizers (Ladha, 1997). Endophytic diazotrophic bacteria GR-3 from a semi-aquatic grass (*Typha australis*) was isolated and characterized. They grow abundantly without supply of any nitrogen source. Their *nifH* gene amplification by PCR and dinitrogenase reductase detected by western blot confirmed the diazotrophic nature (Jha and Kumar, 2007).

The nitrogenase reductase gene sequences (*nifH*) from the bacterial strain isolated from the *Brachiaria* forage grasses were identified and the DNA sequence analysis demonstrated that the *nifH* gene sequences were highly similar to the  $\text{N}_2$ -fixing organisms (Kelemu *et al.*, 2011). Carrell and Frank, (2014) studied the nitrogen fixation role of endophytic bacteria associated with *Pinus flexilis* and *Picea engelmannii* and suggest that above ground endophytic bacteria fix nitrogen and affect conifer shoot tissue growth. *Pinus flexilis* and *Picea engelmannii* was a suitable candidate to study the role of nitrogen fixation because they have generally grown in a subalpine, nutrient-limited environment. They found that both conifers are dominated by the same phylotype related with *Glucanacetobacter diazotrophicus* and other  $\text{N}_2$  fixing acetic acid bacterial endophytes.

### Phosphate solubilization

Phosphorus (P) is a major nutrient required for the optimal plant growth has their role in the metabolic processes, signal transduction, macro-molecular biosynthesis, photosynthesis and respiration (Khan *et al.*, 2009). According to a report globally 5.7 billion hectares land deficient in phosphorous (Khan *et al.*, 2009). It is noted that the concentration of soluble phosphorous in the soil is very low approximately 400-1200 mg kg<sup>-1</sup> of the soil (Rodriguez and Fraga, 1999). Phosphorus is present in the soils as organic and inorganic forms. Their organic forms are found in the humus and the other decayed plant, animal and the microbial part (Richardson, 1994). In labile organic compounds phosphorous can be gradually mineralized as available inorganic phosphorous (McKenzie and Roberts, 1990). The majority of available phosphorous rapidly forms complex with the other elements in the field and it is unavailable to the plants due to their immobilization (Vassileva *et al.*, 1998). Immobilization could be slowed down phosphorous availability to the plants and hence the addition of phosphate fertilizers is required to overcome the phosphorous demands to the developing plants (Omar, 1998). The addition of phosphatic fertilizers is very costly and ecologically unfair to the environment. This has led to explore economic and environmental friendly possible substitute for improving plant growth via PGPEB, which provide the available phosphorous to the plants by various mechanisms (Yadav and Dadarwal, 1997; Senthilkumar *et al.*, 2009). These bacteria secrete different types of organic acids which lowers the pH in the soil surrounded by the plant's root and consequently release the bound forms of phosphate in the soils and hence can take by the plant for increasing efficiency of biological nitrogen fixation and provide availability of other element via producing plant growth promoting substances (Yadav and Dadarwal, 1997). Long *et al.*,

(2008) isolated seventy seven endophytic bacterial isolates from the stem, leaves and roots of *Solanum nigrum* uprooted from two native habitats in Jena, Germany. Out of seventy seven isolates, six isolates were solubilize inorganic phosphate efficiently. Phosphate solubilizing bacterial endophytes from the meristematic tissues of strawberry attribute in plant growth, which is signified by the biomass accumulation and improvement in the development of other plant parts (Armando *et al.*, 2009). ThamizhVendan *et al.*, (2010) isolated 18 endophytic isolates from ginseng plants in which 9 isolates reported to have phosphate solubilizing ability, detected by extracellular solubilization of precipitated tricalcium phosphate with glucose as a sole source of carbon. 8 isolate out of 18 isolates, isolated from tomato showed phosphate solubilization activity (Patel *et al.*, 2012).

Vitorino *et al.*, (2012) evaluate the capacity of endophytic bacteria from the root of *Hyptis maritima* Epling, a *Lamiaceae* to solubilize calcium phosphate in GELP medium and iron phosphate in the modified Reyes basal medium. Of the 42 endophytic bacterial isolates 20% able to solubilize the phosphate up to a certain extent. It is observed that endophytic bacteria of root zone available the soil phosphorous to the plant and thereby increasing vegetation and improves the plant growth. Endophytic bacteria from the three rice cultivar RD6, Chainat 1 and Black glutinous rice of organic paddy field in Han Tao Village, Muang District, Udon Thani were isolated for screening their phosphate solubilization activity. Their potentiality was screened in Pikovskaya's Agar medium and colony diameter and clear zone diameter were measured to calculate Phosphate solubilisation index and solubilization efficiency after incubation period of at 30 °C for 7 days. The isolates of CHR2I02, CHR3I01, CHR4I07, BRR1I04 and BRR3I01 can reported to solubilize tricalcium

phosphate in Pikovskaya's Agar medium. Estimation of quantity of phosphate solubilization was determined by Pikovskaya's broth culture method and absorbance was taken in spectrophotometer for calculation of solubilize phosphate with respect the standard curve (Duangpaenga *et al.*, 2013).

### **Siderophores production**

Term siderophores belongs to a molecule which chelates the Iron. Generally less than about 1000 molecular weight and are produced by many microorganisms (Neilands, 1995). Their biosynthesis is influenced by the presence of iron; for example, their synthesis is repressed if the presence of iron is abundant in the environment (Neilands, 1982). Several Gram-positive and Gram-negative bacteria were identified which produces a variety of siderophores having a peptide backbone with several non- protein amino acid (Ahemad and Khan, 2010a, b). Production of siderophore is of very important because of the leading role of iron in the nitrogen fixation and assimilation processes (Logeshwaran *et al.*, 2009). The iron enzyme nitrogenase, leghemoglobin, ferredoxin and the hydrogenase constitute major protein in the bacterial and infected plant cells (Verma and Long, 1983). The ferric complexes are too huge for passive diffusion. Hence, several proteins such as TonB, ExbB and ExbD form energy rich, complex with the ferric – siderophore complex and creates an electrochemical gradient across the cytoplasmic membrane which help in translocation of iron complex from the bacterial outer membrane into the periplasmic space where they binds to the associated protein and actively transported across the cytoplasmic membrane by an ATP dependent transporter system (Stintzi *et al.*, 2000). According to the Stintzi *et al.*, 2000 translocation of the ferric complex involved two mechanisms. The first one is the common

iron- siderophore method and the second one involved of shuttle system. In the first one method after binding of the ferric-siderophore to the protein receptor, a conformational change occurs in the binding protein, which after delivery of the ferric-siderophore complex into the periplasmic space returns to its normal conformation whilst second one involves formation of the shuttle for ferric – siderophore delivery. *Pseudomonad's* produces high-affinity  $Fe^{3+}$ -binding siderophores under the iron-limiting conditions (Sharma *et al.*, 2003).

Many bacterial endophytes were reported to secrete some extracellular Fe-chelating low molecular weight metabolites called the siderophores under iron limiting conditions (Senthilkumar *et al.*, 2009; Logeshwaran *et al.*, 2009). Siderophore-secreting PGPEB inside the plant tissues help in the transport of  $Fe^{3+}$  inside the plant cell and contribute to the plant growth and productivity via synthesis of ATP, DNA precursor and the heme (Schwyn and Neilands, 1987; Neilands, 1995; Logeshwaran *et al.*, 2009). Its deficiency can inhibit the DNA synthesis, alter the growth of cell and morphology and hamper with the metabolic processes such as photosynthesis and the mitochondrial reactions (Chincholkar *et al.*, 2000). Ferrichrome and Aerobactin is the hydroxamate-type siderophore produced by the many bacteria, including endophytic bacteria (Hofte, 1993; Buyer *et al.*, 1991; Logeshwaran *et al.*, 2009). Siderophore production was analyzed by the Chrome Azural S (CAS) plate assay, FeCl test, Carboxylate test, and the Hydroxamate test (Logeshwaran *et al.*, 2009). The role of siderophores in the inhibition of both fungal and bacterial pathogens was identified (Compant *et al.*, 2005). Competition for nutrients, antibiosis (the production and release of molecules that either kill target pathogens or inhibit their growth) is a well-known mechanism by which microbes can control the plant diseases (Compant *et al.*,

2005). Siderophore production by endophytic bacteria *Methylobacterium* spp. isolated from the citrus plant were evaluated by chromeazurool agar assay test (CAS), Csáky test (hydroxamate-type) and Arnow test (catechol-type) and found that all 37 strains of *Methylobacterium* spp. were positive for chromeazurool agar assay test. Interestingly *Methylobacterium* spp. found to be positive towards the hydroxamate-type of siderophore production (Csáky assay), but negative towards the catechol-type siderophores production (Arnow assay) (Lacava *et al.*, 2008).

Senthilkumar *et al.*, (2009) isolated a total of 137 endophytic bacteria from the surface sterilized root, stem and of soybean and screened their siderophore production ability. The isolate HKA-72 and HKA-113 were reported to synthesize Fe (III) chelating siderophores which is indicated by the formation of a yellow-gold halo around the endophytic bacterial colonies grown on CAS-agar plates. Siderophore production was very important for Fe supply and endophytic bacterial growth as endophytic bacteria have to compete with the plant cell for growth. It is one of the mechanisms used to compete with the plant pathogens. Loaces *et al.*, (2011) studied the communities of siderophore producing endophytic bacteria from the grains, roots, and leaves of *Oryza sativa* cultivated on Uruguayan soils. They reported that approximately 10% of the heterotrophic bacteria were able to produce siderophores in roots and leaves of young plants, but most of the heterotrophic bacteria were actively produced siderophore in mature plants. Siderophore production provides competitive advantages to endophytic bacteria for colonization of plant tissues and hence, excludes other microorganism from the colonization for the same ecological niche

(Loaces *et al.*, 2011). Siderophore production in nodule forming bacteria was investigated by Verma *et al.*, (2012), they inoculate the nodule forming bacteria in the YEM media and conducted Chrome AzuroolSulfonate (CAS) assay for formation of orange halos in blue agar plate.

### **Production of plant growth regulators (PGR)**

As we know hormones are organic compounds effective in very minute concentration, which after synthesis transported to another location where they interact with specific target tissue and regulate physiological functions of plant host and hence referred as plant growth regulators or Phyto-hormones (auxins, gibberellins, ethylene, cytokinins, and abscisic acid (ABA)). Different bacterial groups were reported to produce the IAA (indole-3-acetic acid), the most important auxin which regulates plant development such as cell expansion, division, differentiation, gene regulation and other tropic response (Ratul *et al.*, 2013).

Hung *et al.*, (2007) isolated endophytic bacteria from the surface sterilized root, nodules and stems of wild and cultivated soybean variety. Endophytic bacteria were reported to produce IAA significantly, in which 15 isolates able to produce more than 25  $\mu\text{g ml}^{-1}$  in the presence of tryptophan. Bacterial endophytes from different varieties of strawberry were identified for indole acetic acid (IAA) production and their potential for plant growth promotion such as enhance the biomass, root number, leaf number, petiole length and dry weight of the aerial portion (Armando *et al.*, 2009).

Fig.1 Plant part showing localization of endophytic and epiphytic microbes

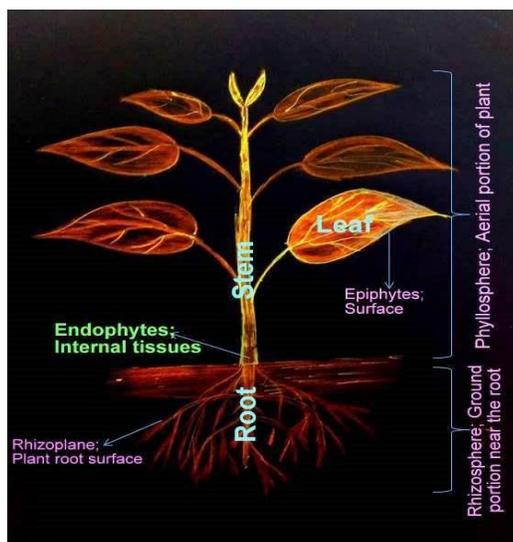
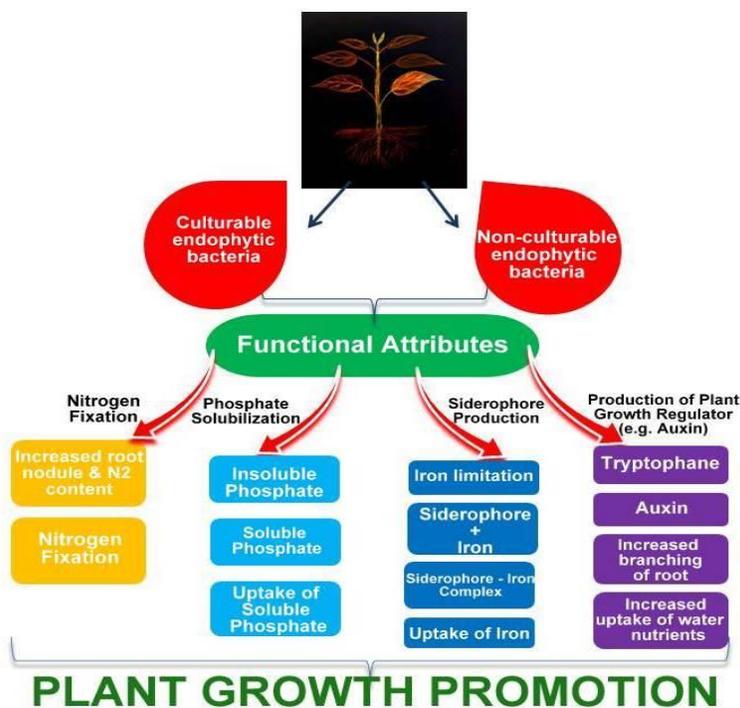


Fig.2 Plant growth promoting functional attributes of endophytic bacteria



Endophytic bacterial isolate from sugar beet roots reported to produce indole-3-acetic acid (IAA) in vitro and reported to positively affect the plant height, their fresh and dry weights, leaves per plant, along with levels of phyto-hormones compared with control plants (Shi *et al.*, 2009). Thamizhvendan *et al.*, (2010) isolated 12 endophytic bacteria which

were reported to synthesize significant amount of IAA in nutrient broth provided with tryptophan as precursor.

Miliūtė and Buzaitė (2011) were isolated endophytic bacteria from the apple tree buds and investigated their role in production of plant hormone IAA. They reported that nine

isolates were able to produce IAA with concentration varied between 0.12- 0.24 micrograms per milligram of protein in culture. They compared the IAA production ability of apple tree buds endophytic bacterial isolates with several other endophytic bacteria associated various sources in the presence of tryptophan as a precursor, in which *Pseudomonas stutzeri* isolated from *Echinacea* able to produce 18.8 µg/ml of IAA (Lata *et al.*, 2006), actinomycetes were able to produce 77–83 µg/ml and coryneforms (winter rye) able to produce 94–95 µg/ml of IAA (Merzaeva and Shirokikh, 2008), *Methylobacterium* (red and white clover) were reported to synthesize 6–13.3 µg/ml (Omer *et al.*, 2004), different endophytes from *Solanum nigrum* were able to synthesize in the range of 1.1–154 µg/ml of IAA (Long *et al.*, 2008) and *Bacillus thuringiensis* were able to synthesize 1.53–9.71 of the IAA (Raddadi *et al.*, 2008). Vitorino *et al.*, (2012) investigated the endophytic bacteria from the root of *Hyptis marruboides* Epling, a Lamiaceae to synthesize IAA in DYGS medium supplemented with tryptophan, among the endophytic bacterial isolates 52% were able synthesize IAA. Isolates RF18 (95.13 µg/ml), RG9 (39.28 µg/ml), RF13 (16.21 µg/ml) and RG24 (11.96 µg/ml) were identified as a prominent strain to significantly produce phytohormone IAA under test conditions. A total of seventy one endophytic bacterial isolates were isolated from the duckweed A1 and A2 characterized as *Landoltia punctata* on the basis of morphological characters and the phylogenetic analysis of the *atpF-atpH* intergenic region sequences. Endophytic bacteria were studied for their plant growth promotion ability by the production of phytohormone indole-3-acetic acid (IAA). Out of seventy one isolates 27 were identified as positive for IAA production in which *Deinococcus* sp. L2-88 was reported to produce the highest quantity of IAA *i.e.*

713.2+11.6 µg/ml. All the isolates were characterized molecularly on the basis of 16S rRNA gene sequence and found to be belongs to the phyla *Actinobacteria* (10 isolates), *Deinococcus-Thermus* (1 isolate), *Firmicutes* (55 isolates) and *Proteobacteria* (5 isolates) (Kittiwongwattana, 2015).

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