

Review

# Beneficial properties, colonization, establishment and molecular diversity of endophytic bacteria in legumes and non legumes

Surjit Singh Dudeja\* and Rupa Giri

Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, 125004, India.

Received 30 November, 2013; Accepted 26 February, 2014

Different genera of bacteria are present as endophytic in roots and nodules of legumes and roots of non legumes grown in different parts of the world. A number of these endophytic inhabitants vary from few to large numbers. Predominantly, endophytes belongs to three major phyla, Actinobacteria, Proteobacteria and Firmicutes and includes members of *Streptomyces*, *Azocareus*, *Gluconobacter*, *Pseudomonas*, *Serratia*, *Stenophomonas*, *Bacillus*, *Paenibacillus* and *Enterobacter*. Majority of the endophytes show plant growth promotion and have other beneficial traits like enhancement of biological nitrogen fixation, phytohormone production, phosphate solubilization, inhibition of ethylene biosynthesis in response to biotic or abiotic stress and above all have biocontrol activity. These endophytes colonize inside the root or nodules. It seems no host specificity exist between different endophytic bacteria and plant host. These opportunist bacteria can enter the plant tissue whenever they have the opportunity either after dissolving the cell wall or through crack entry. Beneficial effects of bacterization of these endophytes have been shown in different plant host under green house and even under field conditions. Therefore endophytic bacteria are better alternate to sustain crop productivity.

**Key words:** Nodules, endophytic bacteria, legumes, roots, genera.

## INTRODUCTION

The plant root defines the interface between a multi cellular eukaryote and soil, one of the richest microbial ecosystems on Earth. Notably, soil bacteria are able to multiply outside and inside roots and modulate plant growth and development (Bulgarelli et al., 2012). Plants are constantly involved in interactions with a wide range of bacteria. These plant associated bacteria colonize the

rhizosphere (rhizobacteria), the rhizoplane (epiphytes) and the inside of plant tissues (endophytes). Endophytes are sheltered from environmental stresses and microbial competition by the host plant and they seem to be ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo, 2000). The term

\*Corresponding author. E-mail: [ssdudeja@gmail.com](mailto:ssdudeja@gmail.com). Tel: 91 1662-24319091 or 94160 43190. Fax: 91 1662-284310.

'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 and Berg., 2006; Ryan et al., 2008).

Symbiotic interactions are the driving force in ecosystems; symbiosis ranges from parasitism to mutualism and includes everything in between. The fitness outcomes for plants differ accordingly: if a plant is highly susceptible to pathogens, its fitness is likely to be low in pathogen rich environments; if a plant cooperates with mutualists, it is likely to thrive even in adverse environments. Bacteria, which colonize the interface between living plant roots and soil, namely the rhizosphere, are abundant symbiotic partners of plants. These so called rhizobacteria are said to be plant growth promoting (PGP). Although all the approximately 300,000 plant species have been estimated to harbor one or more endophytes, few relationships between plants and these endophytes have been studied in detail; the legume-rhizobia symbiosis is an exception (Strobel et al., 2004). The mutualistic interaction of legumes with rhizobia involves finely tuned recognition steps which ultimately lead to the production of root nodules in which the plants accommodate the bacteria (Oldroyd et al., 2005). For other endophytic rhizobacteria, the processes of host-microbe signaling and colonization, and the mechanisms leading to mutual benefit are less well characterized.

In recent years, interest in endophytic micro organisms has increased, as they play a key role in agricultural environment and are promising because of their potential use in sustainable agriculture. Endophytes can also be beneficial to their host by producing a range of natural products that could be used in medicine, agriculture and industry (Ruby and Raghunath, 2011). There is increasing interest in developing the biofertilizers for enhancing crop productivity (Saini et al., 2013a). A challenge is posed for systematic optimization for the application of suitable endophytic isolates and the amount of fertilizer to be added to obtain maximum output. One of the major challenges includes selection of plant genotype and age and compatible associative bacteria. Understanding 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, multiple 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. Extensive and intensive research on the understanding of associative and endophytic ecology will be major determinant to

maximize benefit from these bacteria. Keeping these points in mind, the present status of these aspects is being reviewed.

## ENDOPHYTIC MICROBES IN DIFFERENT HOSTS

Endophytic bacteria have been found in almost every plant studied (Ryan et al., 2008). Since the first reliable reports on the isolation of endophytic bacteria from surface sterilized plants (Samish and Etinger-Tulczynska, 1963; Mundt and Hinkle, 1976) more than 200 bacterial genera from 16 phyla have been reported as endophytes. These include both culturable and unculturable bacteria belonging to Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Cholorobi, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospira, Planctomycetes, Proteobacteria, Spirochaetes and Verrucomicrobiae (Sun et al., 2008; Mengoni et al., 2009; Manter et al., 2010; Sessitsch et al., 2012). However, the most predominant and studied endophytes belong to three major phyla (Actinobacteria, Proteobacteria and Firmicutes) and include members of *Streptomyces* (Suzuki et al., 2005), *Azoarcus* (Krause et al., 2006), *Gluconobacter* (Bertalan et al., 2009), *Pseudomonas*, *Serratia* (Taghavi et al., 2009), *Stenotrophomonas* (Ryan et al., 2008), *Bacillus* (Deng et al., 2011; Kumar et al., 2013; Saini et al., 2013b), *Enterobacter* (Taghavi et al., 2010) and *Burkholderia* (Weilharter et al., 2011). Saini et al. (2013a) isolated a total of 166 endophytic bacteria from root of legumes, chickpea (*Cicer arietinum*), pea (*Pisum sativum*), and lucerne (*Medicago sativa*), non-legumes wheat (*Triticum aestivum*), oat (*Avena sativa*) and also from nodules of chickpea.

## Occurance of endophytes in legumes

The population density of endophytes is highly variable, depending mainly on the bacterial species and host genotypes and also on the host developmental stage, inoculum density, and environmental conditions (Pillay and Nowak, 1997; Tan et al., 2006). There are many reports on occurrence of endophytic bacteria from roots and nodules of legume plants such as alfalfa, bean, chickpea, clover, cowpea, pea, peanut, soyabean, *Acacia*, *Argyrolobium*, *Conzattia*, fenugreek, *Hedysarum*, *Kennedia*, *Leucaena*, *Lotus*, mungbean, *Mimosa*, *Medicago*, *Melilotus*, *Ornithopus*, *Onobrychis*, *Oxytropis*, *Psoralea*, *Scorpiurus*, *Sesbania*, *Tetragonolobus* and *Vicia* (Muresu et al., 2008; Dudeja et al., 2012, Dudeja and Nidhi 2014). Bacteria isolated from legume tissues include *Bacillus*, *Paracoccus*, *Sphingomonas*, *Inquilinus*, *Pseudomonas*, *Serratia*, *Mycobacterium*, *Nocardia*, *Brevibacillus*, *Staphylococcus*, *Lysinibacillus*, *Bosea*,

*Rhodopseudomonas*, *Phyllobacterium*, *Ochrobactrum*, *Starkeya*, *Agromyces*, *Ornithinococcus*, *Actinobacterium*, *Paenibacillus*, *Methylobacterium*, *Pedobacter*, *Aerococcus*, *Stenotrophomonas*, *Streptomyces*, *Dyella* and others. Endophytic bacteria in a single plant host are not restricted to a single species but comprise several genera and species. It seems that the bacteria that best adapt to living inside plants are naturally selected. Endophytes are recruited out of a large pool of soil or rhizospheric species and clones.

Root associated 72 endophytic bacteria from peanut plants grown in the main producing areas of six provinces in China were isolated (Wang et al., 2013). The 16S rRNA gene sequences and phylogenetic analysis revealed that 49 isolates belonged to Bacilli and 23 isolates to Gammaproteobacteria. Similarly, 39 endophytic bacterial strains were isolated from the nodule of *Lespedeza* sp. grown in two different locations of South Korea (Palaniappan et al., 2010). The strains were identified by using 16S rRNA gene sequence as *Arthrobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Dyella*, *Methylobacterium*, *Microbacterium*, *Rhizobium* and *Staphylococcus*.

Various rhizobial and non-rhizobial strains were isolated from root nodules of two widespread south eastern Australian tree legumes, *Acacia salicina*, *Acacia stenophylla*. This legume was nodulated primarily by *Bradyrhizobium*, while the results indicate significant associations with other root nodule forming bacterial genera, including *Rhizobium*, *Ensifer*, *Mesorhizobium*, *Burkholderia*, *Phyllobacterium* and *Devosia* (Hoque et al., 2011).

However in the majority, *Streptomyces* was present in *Stemona* earthnet samples (Wei and Wu, 2012). Endophytic bacteria from roots and nodules of fieldpea and chickpea being grown in Northern India were isolated. A total of 75 endophytic bacteria roots and nodules of fieldpea (Narula et al., 2013a) and 88 from roots and nodules of chickpea showed that 50% in roots and 93.4% in nodules were Gram positive and most of the isolates were spore formers (Saini et al., 2013b). More number of bacteria were present in nodules as compared to the roots of fieldpea as well as chickpea. The endophytic strain Zong1 isolated from root nodules of the legume *Sophora alopecuroides* was closely related to *Pseudomonas chlororaphis* (Zhao et al., 2013). Tagging with *gfp* gene indicated that strain Zong1 may colonize in root or root nodules.

Co-inoculation with Zong1 and SQ1 (*Mesorhizobium* sp.) showed significant effects as compared to single inoculation for siderophore production, phosphate solubilization, organic acid production, IAA production and antifungal activity *in vitro*. These results suggest that *P. chlororaphis* Zong1 and *Mesorhizobium* sp. SQ1 have better synergistic or additive effect and growth index in growth assays under greenhouse conditions is higher

than those of single inoculation.

### Occurrence of endophytes in non-legumes

There are many examples of reported microbial endophytes and plants harbouring them, including rice, banana, wheat, sugarcane, carrot, maize, potato, coffee, oats and citrus plants (Sturz et al., 2000; Rosenblueth and Martínez-Romero, 2006; Kumar et al., 2013). Population of endophytes from different tissues of a plant was different qualitatively and quantitatively (Qiao et al., 2006). Both Gram positive and negative bacteria were isolated, with a greater percentage (68%) being Gram negative.

The highest number of bacteria among the berry tissues sampled was isolated from the seed, and includes *Bacillus*, *Burkholderia*, *Clavibacter*, *Curtobacterium*, *Escherichia*, *Micrococcus*, *Pantoea*, *Pseudomonas*, *Serratia* and *Stenotrophomonas* (Vega et al., 2005). Inside the roots and stems of sugarcane plants, genera of *Burkholderia*, *Pantoea*, *Pseudomonas* and *Microbacterium* were present (Mendes et al., 2007). A total of 192 bacterial endophytes from roots of rice (*Oryza sativa*) formed 52 operational taxonomic units based on the similarity of the ARDRA banding profiles (Marquez-Santacruz et al., 2010); *Streptomyces* sp. was isolated from roots of a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105) (Rungin et al., 2012).

Rice endophytic bacteria were identified as two species of *Penibacillus*, three species of *Microbacterium*, three *Bacillus* species, and four species of *Klebsiella* (Jia et al., 2014). Rice seeds treated with the plant growth-promoting bacteria (PGPB) showed improved plant growth, increased height and dry weight and antagonistic effect against fungal pathogens. Berseem clover (*Trifolium alexandrinum*) endophytic bacteria for rice plant growth-promotion were selected on the basis of root colonization bioassay and a plant growth promoting trait (Etesami et al., 2013).

A significant relationship among indole-3-acetic acid producing isolates, the size of root colonization, and plant growth was observed. Endophytic bacteria from *Panax notoginseng* exhibited antagonistic properties against pathogens and all the 104 antagonistic bacteria belong to four clusters: Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes/Chlorobi.

Members of the Firmicutes, in particular the *Bacillus* sp., were predominant in all analyzed tissues (Ma et al., 2013). All these studies indicated that wide range of microbes exist as endophytic in roots and nodules of legumes and roots of non legumes. It seems that no host specificity of endophyte is existing as entry of a particular bacterial genera in roots or nodules, dependent upon availability of microflora in that particular soil which may be affected by soil and environmental conditions.

## BENEFICIAL CHARACTERISTICS OF BACTERIAL ENDOPHYTES

Endophytes play crucial role in plant growth promotion by having beneficial impact on host plant. These bacteria may promote plant growth in terms of increased germination rates, biomass, leaf area, chlorophyll content, nitrogen content, protein content, hydraulic activity, root and shoot length, yield and tolerance to abiotic stresses like drought, flood, salinity, etc. Plant associated bacteria can promote plant growth directly through biological nitrogen fixation, phytohormone production, phosphate solubilization, inhibition of ethylene biosynthesis in response to biotic or abiotic stress (induced systemic tolerance) etc., or indirectly by inducing resistance to pathogen (Bhattacharya and Jha, 2012). Bacterial endophytes have diverse positive effects on their hosts. Some examples include nitrogen fixation, antibacterial and antifungal activities, as well as plant growth promotion (Tan et al., 2006; Rijavec et al., 2007). Other roles such as synthesis of novel chemicals, resistance to heavy metals and xenobiotic degradation have been observed in endophytes (Siciliano et al., 2001). Jha et al. (2013) reviewed plant growth promoting abilities of rhizospheric and endophytic bacteria and their molecular aspects. Plant growth promoting bacteria has been classified on the basis of basic mechanisms through which they stimulates plant growth as PGPB, which induces plant growth directly and; biocontroller, which protects plants by inhibiting growth of pathogen and/or insect (Backman and Sikora, 2008). The different beneficial characteristics of different endophytes reported (Faria et al., 2013) are being discussed here.

### Phytohormone production and root growth promotion by endophytes

Plant hormones are chemical messengers that affect a plant's ability to respond to its environment. There are five major groups of hormones: auxins, gibberellins, ethylene, cytokinins, and abscisic acid. Indole-3-acetic acid (IAA) is a member of the group of phytohormones and is generally considered the most important native auxin (Ashrafuzzaman et al., 2009). It functions as an important signal molecule in the regulation of plant development including organogenesis (root growth), tropic responses, cellular responses such as cell expansion, division, differentiation, and gene regulation (Ryu and Patten, 2008). The production of auxin like compounds increases seed production and germination along with increased shoot growth and tillering (Kevin, 2003). Hung and Annapurna (2004) isolated 65 bacterial endophytes from stem, root and nodule of two soyabean varieties, *Glycine max* and *Glycine soja* and 56 isolates were capable of producing IAA in different concentrations. Similarly, a total of 28 endophytic bacteria were

isolated from *Sophora alopecuroides* root nodules and one endophytic isolate along with *Mesorhizobium* produced good amount of IAA (Zhao et al., 2011).

Root growth promotion assay is a good parameter to find out whether the endophytes are producing phytohormones or not. A total of 166 endophytic bacteria from roots of legumes, chickpea (*Cicer arietinum*), pea (*Pisum sativum*), and lucerne (*Medicago sativa*) and non-legumes wheat (*Triticum aestivum*) and oat (*Avena sativa*) and nodules of chickpea were isolated. Majority of the endophytes were found to promote the growth of chickpea roots in root growth promotion assay in agar plates, however chickpea nodule endophytic bacteria were better root growth promoters as compared to others (Saini et al., 2013a). Similarly, field pea root growth promotion assay showed that 63.3% nodule endophytic bacteria out of 60 isolates were root growth promoters (Narula et al., 2013b).

### Phosphate solubilization by endophytes

Another important beneficial attribute of endophytic bacteria is P solubilization. The improvement of soil fertility is one of the most common strategies to increase agricultural production. The biological nitrogen fixation is very important in enhancing the soil fertility. In addition to biological nitrogen fixation, phosphate solubilization is equally important. Phosphorus (P) is one of the major essential macronutrient for biological growth and development. Microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble P to an accessible form, like orthophosphate, is an important trait in a plant growth promoting bacteria for increasing plant yields (Rodriguez et al., 2006).

The use of phosphate solubilizing bacteria as inoculants increases the P uptake by plants (Chen et al., 2006). Phosphate solubilizing bacteria secrete organic acids and phosphatases to solubilize insoluble phosphate to soluble form (Kim et al., 1998). The most efficient phosphate solubilizers belong to genera *Bacillus*, *Rhizobium* and *Pseudomonas* amongst bacteria, and *Aspergillus* and *Penicillium* amongst fungi. A total of 98 non-symbiotic endophytic bacterial strains were isolated from soybean root nodules grown in Heilong Jiang province of China and most of the strains could solubilize mineral phosphate (Li et al., 2008). Matsuoka et al. (2013) isolated endophytic bacteria (e.g. *Bacillus* sp., *Streptomyces luteogriseus* and *Pseudomonas fluorescens*) from *Carex kobomugi* roots, which exhibited both inorganic phosphate solubilization and siderophore production under Fe or P limiting conditions. Their results suggested that colonization of root tissue by these bacteria contribute to the Fe and P uptakes by C.

*kobomugi* by increasing availability in the soil.

Another study showed that a total of 38.3% out of 60 nodule endophytic bacteria from fieldpea were phosphate solubilizers and isolate PNE15 was the best phosphate solubilizer among all isolates (Narula et al., 2013a). In cases of chickpea, 12 endophytic bacteria from roots and 76 from the nodules were also screened for P solubilization. Results showed that 41.7% of isolates from roots and 73.6% from nodules were solubilizing phosphate and CRE3, and CNE215, were highest P solubilizers (Saini et al., 2013b). Further, 136 nodule and 90 root endophytic bacterial isolates were obtained from roots and nodules of legumes and non-legumes. In legume roots, 47.8% and in nodules 56% of bacterial endophytes were solubilizing P (Kumar et al., 2013).

### Metabolite production by endophytes

Various types of secondary metabolites like antibiotics, organic acids, ammonia, enzymes and growth hormones (type of organic acids) are produced by the bacterial endophytes. These metabolites beneficially affect the plant directly or indirectly. Ammonia fulfills the demand of nitrogen of plants and organic acids helps in solubilization of insoluble nutrients. A number of different scientists have reported metabolite production by endophytes. Hung et al. (2007) reported that out of 109 bacterial endophytes, 33% were producing pectinase enzyme and 51% of endophytes were producing cellulase enzyme from soyabean. Similarly, out of 91 bacterial isolates from roots of coastal sand dune plants, 23 produced protease, 37 produced pectinase, and 38 produced chitinase (Dong-Sung et al., 2007). Organic acid producing endophytic bacterial strains have been isolated by Forchetti et al. (2007) from roots of sunflower. Three strains that were grown in control medium produced jasmonic acid (JA), 12-oxo-phytodienoic acid (OPDA) and abscisic acid (ABA). These three strains did not differ in amount of JA or OPDA produced, however ABA content was higher than that of JA, and production of both ABA and JA increased under drought condition. Li et al. (2013) isolated eleven bioactive alkaloids produced from *Pseudomonas brassicacearum* subsp. *neaurantiaca*, an endophytic bacterium from *Salvia miltiorrhiza*. All these compounds were isolated from this bacterium for the first time. The antifungal and antibacterial activities of these compounds were evaluated. The results indicate that some cyclodipeptides may play an important role in plant-bacteria interactions.

Narula et al. (2013a), found that out of a total of 60 field pea nodule endophytic bacteria, 83.3% were ammonia producers and isolate PNE15 was the best ammonia producer and 32% were organic acid producers and isolate PNE17 was found to be the best organic acid producer. Saini et al. (2013b), isolated 88 endophytic

bacteria from roots and nodules of chickpea. Large number of bacterial endophytes from roots (75%) and nodules (80.3%) were ammonia producing with CRE 12 and CNE76 being highest ammonia producer.

### Siderophore production by endophytic bacteria

Bacterial siderophores are low molecular weight compounds with high iron (III) chelating affinity (Sharma and Johri, 2003) that are responsible for the solubilization and transport of iron (III) into bacterial cells. Iron is an essential mineral and its sequestration by specific bacterial siderophores may induce the development of plant disease (Nachin et al., 2003). Acquisition of iron from siderophores produced by other microbial species has been described for *Escherichia coli*, *Salmonella typhimurium* (Martinez et al., 1990) and *Actinobacillus pleuropneumoniae* (Diarra et al., 1996).

Paulo et al. (2006) evaluated the ability of *Methylobacterium* sp., isolated as citrus endophytic bacteria to produce siderophores. All strains of *Methylobacterium* sp. tested were CAS-positive for siderophores production and the siderophores production tested by the CAS-agar assay revealed that 66% of CVC-symptomatic, 55% of uninfected, 20% of asymptomatic and 10% of tangerine strains of *Methylobacterium* sp., showed very high production.

Catherine et al. (2012) isolated 43 bacterial endophytes and assessed siderophore production. Distinct orange halos were observed with all the 12 *Pseudomonas* isolates with *Flavimonas oryzihabitans* isolates having the largest orange halos. They suggested that *Pseudomonas* isolates could therefore be considered high siderophore producers.

### Biocontrol activities of endophytic bacteria

Endophytes play an important role in protection of host plants from infection by phytopathogens. Endophytes are not subjected to the competition from soil microbes and colonize in the plant tissue. They have the ability to penetrate plant cells, stimulate plant defense response and produce antifungal metabolites *in situ*. A large number of endophytic microbes have been studied for their potential role as biocontrol agents against *Fusarium* (Lixiang et al., 2004). *Fusarium* wilt is a fungal disease, which affects a broad range of plants. The biological approach to control *Fusarium oxysporum* is becoming popular in many crop plants. Edkona et al. (2013) isolated five endophytic bacteria, exhibiting potential to control *F. oxysporum* from black pepper roots.

Ma et al. (2013) isolated endophytic bacteria from five different parts (root, stem, petiole, leaf and seed) of *Panax notoginseng* and evaluated antagonistic activity

against *F. oxysporum*, *Ralstonia* sp. and *Meloidogyne hapla*, three major pathogens associated with root-rot disease complex of *P. notoginseng*. Out of the 104 antagonists, 51 strains showed antagonistic activities to one pathogen only, while 43 and 10 displayed activities towards two and all three pathogens, respectively. The most dominant species in all tissues were *Bacillus amyloliquefaciens* subsp. *plantarum* and *Bacillus methylotrophicus*, which were represented by eight strains with broad antagonistic spectrum to all three test pathogens of root-rot disease complex of *P. notoginseng*. Similarly, 11 endophytic bacterial isolates from roots and nodules, and roots of non legumes also produced siderophores but showed low biocontrol activity against plant pathogens (Giri and Dudeja, 2014).

## COLONIZATION AND HOST SPECIFICITY OF ENDOPHYTES

Recent studies show that endophytes are not host specific (Cohen, 2006). Single endophytes can invade a wide host range. Carroll and Petrini (1983) suggested that some strains of the same fungus isolated from different parts of the same host differ in their ability to utilize different substances. So endophytes can be isolated from different plants belonging to the different families and classes and grow under different ecological and geographical conditions. Host endophyte relationship may be variable from host to host and endophyte. Some research showed that host plant and endophyte relationship are able to balance pathogen-host antagonism and are not truly symbiotic one (Schulzt et al., 1999).

Criteria to recognize true endophytic bacteria require not only the isolation from surface disinfected tissues but also microscopic evidence to visualize bacteria inside plant tissues (Sagarika et al., 2010). Naturally occurring endophytes can be visualized by fluorescence *in situ* hybridization (FISH) combined with confocal laser scanning microscopy using specific probes, as well as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and phase contrast microscopy (Amann et al., 1990; Loy et al., 2007). SEM studies confirmed abundant bacterial colonization of the proximal parts of wheat root surface. Nautiyal (2000) has reviewed other developments including use of different markers in the study of root colonization. Microscopic studies of *gfp* tagged endophytic inoculants revealed highly heterogeneous colonization patterns.

Colonization of bacteria in rhizosphere or on plant surface is a complex process which involves interplay between several bacterial traits and genes. The colonization is multistep 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 intra-cellularly. 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 (*gfp*) or  $\beta$ -glucosidase (*gus*) followed by 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).

## Recognition/chemotaxis

The sequence of events leading to colonization of a plant by a bacterium that is to become endophytic is presumably similar, at least in the early stages, to that observed for rhizoplane or rhizosphere bacteria. Bacteria belonging to the 'root colonizing rhizosphere competent bacteria' e.g. *P. fluorescens*, *Azospirillum brasilense* and *Bacillus subtilis*, all common rhizosphere inhabitants are often found as colonizers of the internal tissue of plants (Hallmann and Berg, 2006). Lugtenberg and Dekkers (1999) found that bacterial colonization of roots often starts with the recognition by bacteria of specific compounds that are secreted by the root tissue. De Weert et al. (2002) observed tomato roots secreting organic as well as amino acids in their exudates which provide chemo-attractants for *P. fluorescens* strain WCS365, but sugars had no effect on the chemotactic response.

Many biotic and abiotic factors affect root exudation. Spatial and temporal exudation patterns have been observed along the axes of the roots, creating differential niches for diverse soil bacteria (Kuzyakov, 2002). Hence, one might hypothesize that different root zones (the cork zone, root hair, elongation zone, differentiation zone and root cap) create a range of spatial niches that select specific bacterial communities, allowing establishment of interactions with the plant. For instance, colonization of wheat roots by *A. brasilense* strain 245 occurs preferentially at the root hair zone and at the sites of lateral root emergence (Broek et al., 1999) while colonization of rice roots by *Azoarcus* sp. strain BH72 occurs preferentially in the zones of division and elongation just behind the root cap (Hurek et al., 1994) or for rhizobial species at those of lateral root emergence (Chi et al., 2005).

In a study, 11 most efficient isolates were selected out

of more than 200 endophytic bacteria isolated previously from roots of chickpea (*Cicer arietinum*), field pea (*Pisum sativum*), lucerne (*Medicago sativa*), wheat (*Triticum aestivum*) and oat (*Avena sativa*) and nodules of chickpea and field pea (Giri and Dudeja, 2013a). To know their extent of establishment in different host and non-host tissues root exudates of four hosts' chickpea, field pea, wheat and oat were collected at 7 and 14 days growth. Root exudates of chickpea, field pea, wheat and oat attracted different endophytic bacteria to different extent. Isolate ORE27 exhibited maximum chemotactic ratio towards root exudates of all crops followed by isolate WRE4 towards oat, wheat and chickpea root exudates. Presence of root exudates, promoted the growth of different endophytes in MS medium tubes. In field pea roots, maximum endophytic colonization after 21 days was observed followed by chickpea, oat and wheat. All the 11 endophytic bacteria entered pea roots, 10 in chickpea, eight in oat and wheat roots in MS medium tubes. No host specificity among 11 endophytic bacteria and four hosts could be observed at any of the stage of root colonization.

### 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 inhibiting the other (Bais et al., 2006; Compant et al., 2011). Root hair regions and emergence points are preferred site for colonization (Lugtenberg and Kamilova, 2009). Colonization of roots by microorganisms may further induce release of exudates, and create 'biased' rhizosphere by exuding specific metabolic products. In some rhizospheric bacteria, root exudates induce flagellar motility that leads their colonization on plant surfaces. Lugtenberg and Kamilova (2009) demonstrated the role of bacterial major outer membrane protein (MOMP) in early host recognition 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. Preston et al. (2001), identified SSIII secretion system in *P. fluorescens* SBW25 by *in vitro* expression technology (IVET), a promoter trapping technique.

Using 11 most efficient isolates selected out of more than 200 endophytic bacteria isolated previously from roots of chickpea (*Cicer arietinum*), field pea (*Pisum sativum*), lucerne (*Medicago sativa*), wheat (*Triticum*

*aestivum*) and oat (*Avena sativa*) and nodules of chickpea and field pea, root colonization studies were done (Giri and Dudeja, 2013b). Extent of establishment in different host and non-host tissues four host chickpea, field pea, wheat and oat with these bacteria in sterilized sand in disposable coffee cups was studied. To induce nodulation in chickpea and field pea were also inoculated with respective rhizobia, apart from different endophytes. In chickpea at 15 days, isolate ORE 27, at 30 days, isolates CNE 215 and ORE 35 and at 50 days in total, 6 bacteria were able to enter the roots and exit as endophytic. In the case of field pea all the 11 bacteria entered the roots after 30 days of growth, whereas in wheat and oat bacteria entrance was detected at 50 days and not at 15 or 30 days. In wheat 4 and in oat 6 bacterial isolates were detected as endophytic. Even at 50 days, neither in chickpea nor in field pea nodules, no bacterial isolate was able to enter. In chickpea roots maximum endophytic colonization was observed by isolate PNE 92, in field pea by isolates CNE1 and PNE 17; in wheat and oat, isolate ORE 27 showed highest root endophytic colonization. No host specificity among endophytic bacteria and different hosts could be observed.

### Entry in plant tissue as endophyte

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 (Hardoim 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.

Preito et al. (2011) found that *Azospirillum* may also colonize endophytically through wounds and cracks of the plant root. 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 endo rhizosphere, which was evident from the observation that *Azoarcus* strain lacking endoglucanase was not effective in colonizing the rice plants. The endoglucanase loosen larger cellulose fibers, may help in entry 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 endogluca-

canase, exoglucanases may also help in colonization process. Reinhold-Hurek and Hurek (2011) identified an exoglucanase having cellobiohydrolase and  $\beta$ -glucosidase activity, playing a key role in colonization process of *Azoarcus* sp. BH72. 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). Understanding of molecular mechanism and conditions limiting the colonization process need to be elucidated for exploiting the beneficial endophytic or associative interaction with plants.

### BENEFICIAL EFFECTS OF ENDOPHYTIC BACTERIA AS INOCULANTS

Plant growth promotion has been shown for many endophytic bacteria (Zachow et al., 2010; Malfanova et al., 2013). Direct plant growth promotion mediated by endophytes is mostly based on providing essential nutrients to plants and production and/or regulation of phytohormones. Large number of endophytes has been reported in various plant tissues and these endophytes possess different beneficial properties. Bacterization of these endophytes promotes plant growth and in case of legumes helps in fixation of more nitrogen and is important aspect for the success of these endophytes in agriculture ecosystems. However, only a few studies using endophytes as inoculants under pot culture conditions are available. No report under field conditions could be observed. Such studies therefore need more attention.

Bacterization of bacterial endophytes promoted growth of red clover more often when applied in combination with *R. leguminosarum* biovar *trifolii* than when applied singly (Sturz et al., 1997). However, *Bacillus megaterium*, *Bordetella avium* and *Curtobacterium luteum* consistently promoted growth either individually or in combination with *R. leguminosarum* biovar *trifolii*. Nodulation was promoted when *R. leguminosarum* biovar *trifolii* was coinoculated with *Bacillus insolitus*, *B. brevis* or *Agrobacterium rhizogenes*.

A total of 166 endophytic bacteria isolated from root of chickpea (*Cicer arietinum*), pea (*Pisum sativum*) and lucerne (*Medicago sativa*), and non-legumes wheat (*Triticum aestivum*) and oat (*Avena sativa*) and nodules of chickpea were found to promote the growth of chickpea roots in chickpea root growth promotion assay in agar plates (Saini et al., 2013b), however chickpea nodule endophytic bacteria were better root growth promoters as compared to others. Selected 79 endophytic bacterial isolates were inoculated together with *Mesorhizobium* in chickpea under pot culture conditions and showed enhanced plant growth, nodula-

tion and nitrogen fixing parameters in chickpea, particularly, endophytic bacterial isolates in combination with *Mesorhizobium* than *Mesorhizobium* alone. Plant growth promoting endophytic isolates isolated from nodules of chickpea showed the highest growth promotion and enhanced nitrogen fixation in terms of shoot dry weight and shoot N contents. The most efficient isolates CNE1036 was identified as *Bacillus subtilis* and isolate LRE 3 was identified as *Bacillus amyloliquefaciens* by sequencing of amplified 16S rDNA. Similarly, bacterial endophytes isolated from field pea were inoculated along with standard *R. leguminosarum* biovar *trifolii* strain PS-43 and some of the co-inoculations showed enhanced nodulation, root growth, plant growth and nitrogen content in shoot of fieldpea (Narula et al., 2013a).

Two strains of *Paenibacillus macerans* promoted plant growth of *Cymbidium eburneum* orchid under greenhouse conditions (Faria et al., 2013). None of the treatments had a deleterious effect on growth of inoculated plants. Their results suggested that these bacterial effects could be potentially useful to promote plant growth during seedling acclimatization in orchid species other than the species of origin. Due to the presence of multiple traits in endophytic bacteria, enhancement in crop productivity in all the tested crops was observed.

### CONCLUSION

The root system, which was traditionally thought to provide anchorage and uptake of nutrients and water, is a chemical factory that mediates numerous underground interactions. Plants release enormous amount of chemicals through their roots, at a significant carbon cost, to combat pathogenic microorganisms and attract beneficial ones. Roots of legume and non-legume plants normally associate with diverse microorganisms. Some of bacteria and fungi are capable of entering the plant roots as endophytes and establishes a mutualistic association. Many reports found in literature strongly suggest that endophytes have an excellent potential to be used as plant growth promoters with legumes and non-legumes. Plants constitute vast and diverse niches for endophytic organisms. The processes of host-microbe signaling and colonization, and the mechanisms leading to mutual benefits are less well characterized. It seems no host specificity between host and endophytic bacterial population exist. However interaction studies at molecular level are required to exactly pin point the existence of host specificity and quantum of benefits derived by the host.

### Conflict of Interests

The author(s) have not declared any conflict of interests.



## REFERENCES

- Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA (1990). Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl. Environ. Microbiol.* 56:1919-1925.
- Ashrafuzzaman M, Hossen FA, Ismail MR, Hoque MA, Islam MZ, Shahidullah SM, Meon S (2009). Efficiency of plant growth promoting Rhizobacteria (PGPR) for the enhancement of rice growth. *Afr. J. Biotechnol.* 8(7):1247-1252.
- Backman PA, Sikora RA (2008). Endophytes: An emerging tool for biological control. *Biol. Control* 46: 1-3.
- Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57:233-66.
- Bertalan M, Albano R, Pádua V, Rouws L, Rojas C, Hemery A, Teixeira K (2009). Complete genome sequence of the sugarcane nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus* Pal5. *BMC Genomics* 10:450-461.
- Bhattacharya PN, Jha DK (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J. Microbiol. Biotechnol.* 28:1327-1350.
- Broek A, Lambrecht M, Eggermont K, Vanderleyden J (1999). Auxins upregulate expression of the indole-3-pyruvate decarboxylase gene in *Azospirillum brasilense*. *J. Bacteriol.* 181:1338-1342.
- Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E, Peplies J, Gloeckner FO, Amann R, Eickhorst T, Schulze-Lefert P (2012). Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 488 (7409):91-95.
- Carroll GC, Petrini O (1983). Patterns of substrate utilization by some fungal endophytes from coniferous foliage. *Mycologia* 75:53-63.
- Catherine N N, Vivienne NM, Akio T, Catherine WM (2012). Isolation and identification of endophytic bacteria of bananas (*Musa* sp.) in Kenya and their potential as biofertilizers for sustainable banana production. *Afr. J. Microbiol. Res.* 6(34):6414-6422.
- Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.* 34 (1):33-41.
- Chi F, Shen SH, Cheng HP, Jing YX, Yanni YG, Dazzo FB (2005). Ascending migration of endophytic Rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. *Appl. Environ. Microb.* 71:7271-7278.
- Cohen SD (2006). Host selectivity and genetic variation of *Discula umbrinella* isolates from two oak species: analyses of intergenic spacer region sequences of ribosomal DNA. *Microbiol. Ecol.* 52: 463-469.
- Compant S, Mitter B, Colli-Mull JG, Gangl H, Sessitsch A (2011). Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microb. Ecol.* 62:188-197.
- De Weert S, Vermeiren H, Mulders IHM, Kuiper I, Hendrickx N, Bloembergen GV, Vanderleyden J, De Mot R, Lugtenberg BJJ (2002). Flagella-driven chemotaxis towards exudate components is an important trait for tomato root colonization by *Pseudomonas fluorescens*. *Mol. Plant-Microbe Interact.* 15:1173-1180.
- Deng Y, Zhu Y, Wang P, Zhu L, Zheng J, Li R, Ruan L, Peng D, Sun M (2011). Complete genome sequence of *Bacillus subtilis* BSn5, an endophytic bacterium of *Amorphophallus konjac* with antimicrobial activity for the plant pathogen *Erwinia carotovora* subsp. *carotovora*. *J. Bacteriol.* 193:2070-2071.
- Diarra MS, Mckee JA, Dolence EK, Darwish I, Miller MJ, Malouin F, Jacques M (1996). Growth of *Actinobacillus pleuropneumoniae* is promoted by exogenous hydroxamate and catechol siderophores. *Appl. Environ. Microbiol.* 62:853-859.
- Dong-Sung S, Park MS, Jung S, Lee MS, Lee KH, Bae KS, Kim SB (2007). Plant growth-promoting potential of endophytic bacteria isolated from roots of coastal sand dune plants. *J. Microbiol. Biotechnol.* 17(8):1361-1368.
- Dudeja SS, Giri R, Saini R, Suneja-Madan P, Kothe E (2012). Interaction of endophytic microbes with legumes. *J. Basic Microbiol.* 52:248-60
- Dudeja SS, Nidhi (2014). Molecular Diversity of Rhizobial and Non rhizobial Bacteria from Nodules of Cool season Legumes In: *Biotechnology: Prospects and Applications* [Editors: R.K. Salar, S.K. Gahlawat, P. Siwach and J.S. Duhan] published by Springer-Verlag, Germany. DOI 10.1007/978-81-322-1683-4\_10, © Springer India 2014
- Edkona EJ, WS King, SLC Teck M, Jiwan ZFA, Aziz FR, Kundat OH, NMA Majid (2013). Antagonistic activities of endophytic bacteria against Fusarium wilt of black pepper (*Piper nigrum*). *Int. J. Agric. Biol.* 15:291-296
- Etesami H, Mirsyedhosseini H, Alikhani HA (2013). Rapid screening of berseem clover (*Trifolium alexandrinum*) endophytic bacteria for rice Plant seedlings growth-promoting agents. *ISRN Soil Science Article ID 371879*, 9 pages <http://dx.doi.org/10.1155/2013/371879>
- Faria DC, Dias AC, Melo IS, de Carvalho, Costa FE (2013). Endophytic bacteria isolated from orchid and their potential to promote plant growth. *World J. Microbiol. Biotechnol.* 29(2):217-21.
- Forchetti G, Masciarelli O, Alemanno S, Alvarez D, Abdala G (2007). Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Appl. Microbiol. Biotechnol.* 76:1145-1152.
- Giri R, Dudeja SS (2013a). Host specificity of plant endophytic bacterial interactions: Root colonization in liquid medium. *J. Microbiol. Res.* 1(6):75-82
- Giri R, Dudeja SS (2013b). Host specificity of plant endophytic bacterial interactions: Root and nodule colonization under sterilized sand conditions in disposable coffee cups. *Cent. Eur. J. Exp. Biol.* 2 (4):22-26.
- Giri R, Dudeja SS (2014). Beneficial properties, establishment, identification and beneficial effects of root and nodule endophytic bacteria in chickpea and wheat crops. *Microbiol. Res.* (Submitted)
- Hallmann J, Berg G (2006). Spectrum and population dynamics of bacterial root endophytes. In: *Microbial Root Endophytes*. Schulz. B. J. E., Boyle, C. J. C. and Sieber T. N. (eds.), Berlin, Heidelberg: Springer. 9: 15-31.
- Hardoim PR, van Overbeek LS, Elsas JD (2008). Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol.* 16:463-471.
- Hoque MS, Broadhurst LM, Thrall PH (2011). Genetic characterisation of root nodule bacteria associated with *Acacia salicina* and *Acacia stenophylla* (*Mimosaceae*) across Southeastern Australia. *Int. J. Syst. Evol. Microbiol.* 61(2):299-309.
- Hung PQ, Annapurna K (2004). Isolation and characterization of endophytic bacteria in soybean (*Glycine* sp.). *Omonrice* 12:92-101.
- Hung PQ, Kumar SM, Govindsamy V, Annapurna K (2007). Isolation and characterization of endophytic bacteria from wild and cultivated soybean varieties. *Biol. Fertil. Soils* 44:155-162.
- Hurek T, Reinhold-Hurek, van Montagu M, Kellenberger E (1994). Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. *J. Bacteriol.* 176:1913-1923.
- Jha PN, Gupta G, Jha P, Mehrotra R (2013). Association of Rhizospheric/Endophytic Bacteria with Plants: A Potential Gateway to Sustainable Agriculture. *Greener J. Agric. Sci.* 3 (2):73-84.
- Jia SH, Gururanib MA, Chuna SC (2014). Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiol. Res.* 169: 83- 98.
- Kevin VJ (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255: 571-586.
- Kim KY, Jordan D, McDonald GA (1998). Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. *Biol. Fertil. Soils* 26:79-87.
- Kobayashi D, Palumbo JD (2000). Bacterial endophytes and their effects on plants and uses in agriculture. In: *Microbial Endophytes*. Bacon, C.W., White, J.F. (eds.), Marcel, Dekker, New York. pp. 199-233.
- Kumar V, Pathak DV, Dudeja SS, Saini R, Giri R, Narula S, Anand RC

- (2013). Legume nodule endophytes more diverse than endophytes from roots of legumes or non legumes in soils of Haryana, India. *J. Microbiol. Biotechnol. Res.* 3 (3):83-92.
- Kuzyakov Y (2002). Review: factors affecting rhizosphere priming effects. *J. Plant Nutr. Soil Sci.* 165:382-396. <http://dx.doi.org/10.1103/PhysRevB.66.235114>
- Lacava PT, Li WB, Ara WL, Azevedo JL, Hartung JS (2006). Rapid, specific and quantitative assays for the detection of the endophytic bacterium *Methylobacterium mesophilicum* in plants. *J. Microbiol. Methods* 65:535-541.
- Li JH, Wang ET, Chen WF, Chen WX (2008). Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. *Soil Biol. Biochem.* 40:238-246.
- Li XJ, Tang HY, Duan JL, Gao JM, Xue QH (2013). Bioactive alkaloids produced by *Pseudomonas brassicacearum* subsp. *Neourantiaca*, an endophytic bacterium from *Salvia miltiorrhiza*. *Nat. Prod. Res.* 27(4-5):496-9.
- Lixiang C, Zhiqi Q, Xin D, Hongming T, Yongcheng L, Shining Z (2004). Isolation of endophytic actinomycetes from roots and leaves of banana (*Musa acuminata*) plants and their activities against *Fusarium oxysporum* sp. *cubense*. *World J. Microbiol. Biotechnol.* 20:501-504.
- Loy A, Maixner F, Wagner M, Horn M (2007). probeBase-an online resource for rRNA-targeted oligonucleotide probes: new features. *Nucleic Acids Res.* 35:800-804.
- Lugtenberg B, Kamilova F (2009). Plant-growth-promoting Rhizobacteria. *Annu. Rev. Microbiol.* 63:541-56.
- Lugtenberg BJJ, Dekkers LC (1999). What makes *Pseudomonas* bacteria rhizosphere competent? *Environ. Microbiol.* 1:9-13.
- Ma L, Cao YH, Cheng MH, Huang Y, Mo MH, Wang Y, Yang JZ, Yang FX (2013). Phylogenetic diversity of bacterial endophytes of *Panax notoginseng* with antagonistic characteristics towards pathogens of root-rot disease complex. *Antonie Van Leeuwenhoek* 103(2): 299-312.
- Malfanova N, Kamilova F, Validov S, Chebotar V, Lugtenberg B (2013). Is L-arabinose important for the endophytic lifestyle of *Pseudomonas*. *Arch. Microbiol.* 195:9-17.
- Manter DK, Delgado J, Holm DG, Stong R (2010). Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. *Microb. Ecol.* 60:157-166.
- Martinez JL, Delgado-Iribarren A, Baquero F (1990). Mechanisms of iron acquisition and bacterial virulence. *FEMS Microbiol.* 75: 45-56.
- Matsuoka H, Akiyama M, Kobayashi K, Yamaji K (2013). Fe and P solubilization under limiting conditions by bacteria isolated from *Carex kobomugi* roots at the Hasaki coast. *Curr. Microbiol.* 66(3):314-21
- Mendes R, Pizzirani-Kleiner AA, Araujo WL, Raaijmakers JM (2007). Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. *Appl. Environ. Microbiol.* 73(22): 7259-7267.
- Mengoni A, Pini F, Huang LN, Shu WS, Bazzicalupo M (2009). Plant-by-plant variations of bacterial communities associated with leaves of the nickel hyperaccumulator *Alyssum bertolonii* Desv. *Microb. Ecol.* 58: 660-667.
- Mundt JO, Hinkle NF (1976). Bacteria within ovules and seeds. *Appl. Environ. Microbiol.* 32:694-698
- Muresu R, Polone E, Sulas L, Baldan B, Tondello A, Delogu G, Cappuccinelli P, Alberghini S, Benhizia Y, Benhizia H, Benguedouar A, Mori B, Calamassi R, Dazzo FB, Squartini A (2008). Coexistence of predominantly nonculturable rhizobia with diverse, endophytic bacterial taxa within nodules of wild legumes. *FEMS Microbiol. Ecol.* 63:383-400.
- Nachin L, Loiseau L, Expert D, Barras F (2003). SufC: an unorthodox cytoplasmic A BC/ATPase required for [Fe-S] biogenesis under oxidative stress. *EMBO J.* 22:427-437.
- Narula S, Anand RC, Dudeja SS (2013a). Beneficial traits of endophytic bacteria from field pea nodules and plant growth promotion of field pea. *J. Food Legume* 26(3-4):73-79.
- Narula S, Anand RC, Dudeja SS, Kumar V, Pathak DV (2013b). Molecular diversity of root and nodule endophytic bacteria from field pea (*Pisum sativum* L.) *Legume Res.* 36(4):344-350.
- Oldroyd GED, Harrison MJ, Udvardi M (2005). Peace talks and trade deals: Keys to long-term harmony in legume-microbe symbioses. *Plant Physiol.* 137:1205-1210.
- Palaniappan P, Chauhan PS, Saravanan VS, Anandham R, Sa T (2010). Isolation and characterization of plant growth promoting endophytic bacterial isolates from root nodule of *Lespedeza* sp. *Biol. Fertil. Soils* 46:807-816.
- Pillay VK, Nowak J (1997). Inoculum density, temperature and genotype effects on epiphytic and endophytic colonization and *in vitro* growth promotion of tomato (*Lycopersicon esculentum* L.) by a pseudomonad bacterium. *Can. J. Microbiol.* 43:354-361.
- Preito P, Schilirò E, Maldonado-González MM, Valderrama R, Barroso-Albarracín JB, Mercado-Blanco J (2011). Root hairs play a key role in the endophytic colonization of olive roots by *Pseudomonas* sp. with biocontrol activity. *Microb. Ecol.* 62:435-445.
- Qiao H, Huang L, Kang Z (2006). Endophytic bacteria isolated from wheat and their antifungal activities to soil-borne disease pathogens. *Ying Yong Sheng Tai Xue Bao* 17(4): 690-694.
- Reinhold-Hurek B, Hurek T (2011). Living inside plants: bacterial endophytes. *Curr. Opin. Plant Biol.* 14:435-443.
- Rijavec T, Lapanje A, Dermastia M, Rupnik M (2007). Isolation of bacterial endophytes from germinated maize kernels. *Can. J. Microbiol.* 53: 802-808.
- Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2006). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant Soil* 287(1-2):15-21.
- Rosenblueth M, Martínez-Romero E (2006). Bacterial endophytes and their interactions with hosts. *Mol. Plant Microbe Interact.* 19:827-837.
- Ruby J, Raghunath M (2011). A Review: Bacterial Endophytes and their Bioprospecting. *J. Pharm. Res.* 4(3):795-799.
- Rungin S, Indananda C, Suttiviriya P, Kruasuwan W, Jaemsang R, Thamchaipenet A (2012). Plant growth enhancing effects by a siderophore-producing endophytic streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105). *Antonie Van Leeuwenhoek* 102(3):463-472.
- Ryan RP, Germaine K, Franks A, Ryan DJ (2008). Bacterial endophytes: recent developments and applications. *FEMS Microbiol. Lett.* 278:1-9.
- Sagarika M, Sharma GD, Deb B (2010). Diversity of endophytic diazotrophs in non-leguminous crops - A Review. *Assam Univ. J. Sci. Technol.* 6:109-122.
- Saini R, Dudeja SS, Giri R, Kumar V (2013b). Isolation, characterization and evaluation of bacterial root and nodule endophytes from chickpea cultivated in Northern India. *J. Basic Microbiol.* <http://dx.doi.org/10.1002/jobm.201300173>.
- Saini R, Kumar V, Dudeja SS, Pathak DV (2013a). Beneficial effects of inoculation of endophytic bacterial isolates from roots and nodules in chickpea. *Acta Agron Hungarica* (accepted).
- Samish Z, Etinger-Tulczynska R (1963). Distribution of bacteria within the tissue of healthy tomatoes. *Appl. Environ. Microbiol.* 11: 7-10.
- Schulz B, Rommert AK, Dammann U, Aust HJ, Strack D (1999). The endophyte-host interaction: a balanced antagonism? *Mycol. Res.* 105:1275-1283.
- Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Mitter B (2012). Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. *Mol. Plant-Microbe Interact.* 25:28-36.
- Sharma A, Johri BN (2003). Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. *Microbiol. Res.* 158:243-248.
- Siciliano SD, Fortin N, Mihoc A, Wisse G (2001). Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. *Appl. Environ. Microbiol.* 67:2469-2475.
- Strobel G, Daisy B, Castillo U, Harper J (2004). Natural products from endophytic microorganisms. *J. Nat. Prod.* 67:257-268. <http://dx.doi.org/10.1021/np030397v>; PMID:14987067.
- Sturz AV, Christie BR, Matheson BG, Nowak J (1997). Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biol. Fertil. Soils* 25:

- 13-19.
- Sturz AV, Christie BR, Nowak J (2000). Bacterial endophytes: potential role in developing sustainable systems of crop production. *Crit. Rev. Plant Sci.* 19:1-30.
- Sun L, Qiu F, Zhang X, Dai X (2008). Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. *Microb. Ecol.* 55:415-424.
- Suzuki T, Shimizu M, Meguro A, Hasegawa S, Nishimura T, Kunoh H (2005). Visualization of infection of an endophytic Actinomycete *Streptomyces galbus* in leaves of tissue-cultured *Rhododendron*. *Actinomycetologica* 19:7-12.
- Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, van der Lelie D (2009). Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Appl. Environ. Microbiol.* 75:748-757.
- Taghavi S, van der Lelie D, Hoffman A, Zhang YB, Walla MD, Vangronsveld J, Newman L, Monchy S (2010). Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp. 638. *PLoS Genet.* 6: e1000943.
- Tan Z, Hurek T, Reinhold-Hurek B (2006). Effect of N-fertilization, plant genotype and environmental conditions on *nifH* gene pools in roots of rice. *Environ. Microbiol.* 5:1009-1015.
- Vega FE, Pava-Ripoll M, Posada F, Buyer JS (2005). Endophytic bacteria in *Coffea arabica* L. *J. Basic Microbiol.* 45(5):371-380.
- Wang K, Yan PS, Ding QL, Wu QX, Wang ZB, Peng J (2013). Diversity of culturable root associated/endophytic bacteria and their chitinolytic and aflatoxin inhibition activity of peanut plant in China. *World J. Microbiol. Biotechnol.* 29(1):1-10.
- Wei Sheng, Wu XB (2012). Isolation, classification and biosynthetic potential of endophytic actinomycetes from *Stemona*. *Antonie Van Leeuwenhoek* 52(3):389-95.
- Weilharter A, Mitter B, Shin MV, Chain PSG, Nowak J, Sessitsch A (2011). Complete genome sequence of the plant growth-promoting endophyte *Burkholderia phytofirmans* strain PsJN. *J. Bacteriol.* 193:3383-3384.
- Zachow C, Fatehi J, Cardinale M, Tilcher R, Berg G (2010). Strain-specific colonization pattern of *Rhizoctonia antagonists* in the root system of sugar beet. *FEMS Microbiol. Ecol.* 74:124-35.
- Zhao L, Xu Y, Sun R, Deng Z, Yang W, Wei G (2011). Identification and characterization of the endophytic plant growth promoter *Bacillus cereus* strain MQ23 isolated from *Sophora alopecuroides* root nodules. *Braz. J. Microbiol.* 42:567-575.
- Zhao LF, Xu YJ, Ma ZQ, Deng ZS (2013) Colonization and plant growth promoting characterization of endophytic *Pseudomonas chlororaphis* strain Zong1 isolated from *Sophora alopecuroides* root nodules. *Braz. J. Microbiol.* 44:629-637.