

Enhancement of plant growth by using salt tolerated endophytic bacteria – A Review

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Abstract

Endophytic bacteria have the ability to promote growth and inhibit plant disease, and as they are in intimate contact with the plant, they are an attractive choice as plant growth promoters and biological control agents. Selection for competitive ability is an area in which there has been little research to date, and the challenge is to encourage the establishment of beneficial bacterial communities within the host plant, early in the crop's development or by artificially introducing specific genetic components that confer some long-lasting benefit. The study of plant-associated microorganisms is of great importance for biotechnological applications, for example, biological control of plant pathogens, plant growth promotion or isolation of active compounds. Understanding the diversity of plant bacterial associations and their role in plant development is necessary if these associations are to be manipulated to increase crop production, conserve biodiversity and sustain agroecosystems in relation to as well as under dry farming conditions that may help in overcoming abiotic stress. Worldwide salinity is one of the most severe abiotic stresses limiting plant growth and productivity. In view of ever increasing population, it has become necessary to cultivate not only saline soil but also coastal saline to step up crop productivity. An alternate strategy to improve crop plants for salt tolerance is to introduce salt-tolerant plant growth promoting bacteria (PGPB) that enhance plant growth in saline soil. It is suggested that root-colonizing bacteria that produce phytohormones may stimulate plant growth and help in nutrient recycling in the rhizosphere and thus the microbes can alleviate the effects of salinity in the environment. In addition, endophytic PGPB might also increase nutrient uptake by plants from soils and thereby reduce the need for fertilizers. The present review focuses on the evaluation of saline - tolerant endophytic bacterial strains to stimulate saline tolerance and promote growth of plants leading to better productivity in saline soil.

1. Introduction

India is having a large and diverse agricultural sector, accounting, on average, for about 16% of GDP and 10% of export earnings. India's arable land area of 159.7 million hectares (394.6 million acres) is the second largest in the world. However, the progress of organic agriculture in India is very slow. Only 41,000 ha. of areas has been converted to organic that is a mere 0.03% of the cultivated area. Even though, India has shown remarkable progress in recent years and has attained self-sufficiency in food staples, the productivity of Indian farms for the same crop is very low. To reach the global standards qualitatively and quantitatively sustainable agriculture that adopts organic and biodynamic cropping system is essential. Greater productivity and competitiveness are anticipated to come from increased efficiency through the acquisition and management of new biotechnologies and crop production strategies.

A renewed interest in the internal colonization of healthy plants by (non-rhizobial) endophytic bacteria has arisen as their potential for exploitation in agriculture becomes apparent. Exploitation of endophyte-plant interactions can result in the promotion of plant health and can play a significant role in low-input sustainable agriculture applications for both food and non-food crops. Considering the enormous potential of the endophytic microorganisms, a research program has been framed to study the role of these microorganisms in the plant growth promotion of crop plants with the following objectives.

Endophytic bacteria have been isolated from every plant studied so far (G. Strobel *et al.*, 2008) including both monocotyledonous and dicotyledonous plants, and ranging from woody tree species to herbaceous crop plants (Lodewyckx *et al.*, 2010). Also, endophytic bacteria have been isolated from different plant structures such as seeds, tubers, roots, stems, leaves, and fruits. The almost 3,00,000 plant species that exist on our planet, only a few of these plants have been completely tested for the presence of endophytic bacteria within their tissues. Consequently, the possibility exists to find new and beneficial endophytic bacteria. Additional potential exists for the subsequent discovery of novel metabolites with many potential biotechnological applications in agriculture, medicine, pharmaceutical, and in the field of environmental protection (Ryan *et al.*, 2008).

2. History and Definition of Endophytes

The term endophyte (Gr. endon, within; phyton, plant) was first coined by De Bary and an endophyte is a bacterial or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- and/or intra-cellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease (Wilson *et al.*, 1995). Bacterial endophytes have been known for >100 years. The presence of bacteria resident within the tissues of healthy plants was first reported as early as 1926 (Hallmann *et al.*, 1997). In 1926, Perotti recognized endophytic growth as a particular stage in the life of bacteria, described as an advanced stage of infection and as having a close relationship with mutualistic symbiosis. Perotti was the first to describe the occurrence of non-pathogenic flora in root tissues. Since then, endophytes have been defined as microorganisms that could be isolated from surface-sterilized plant organs (Perotti, 1926). Since 1940's, there have been numerous

reports on endophytic bacteria in various plant tissues (Hallmann *et al.*, 1997). In the 1980s, endophytic bacteria having nitrogen fixing ability were found in graminaceous plants (Barbara Reinhold-Hurek & Hurek, 1998).

3. Colonization of Plants by Endophytic Bacteria

There is a number of ways by which endophytic bacteria can get access to a plant's interior.

Rhizoplane colonization

Colonization of the plant's interior by bacteria generally starts with their establishment in the rhizosphere. The early events of this process such as recognition and chemotaxis have been extensively reviewed by (Lugtenberg & Kamilova, 2009). Following rhizosphere colonization, bacteria attach to the rhizoplane, i.e. the root surface. A number of mutational studies showed that the attachment of bacterial cells to the root is a crucial step for subsequent endophytic establishment. Several bacterial surface components can be involved in this process. For *Azoarcus* sp. BH72, an endophytic diazotroph of rice, type IV pili encoded by *pilAB* are required for attachment to the root surfaces (Dorr *et al.*, 1998). A mutant impaired in the expression of *pilAB* fails to colonize successfully roots and shoots of rice plants (Barbara Reinhold-Hurek *et al.*, 2006).

Bacterial entry

The preferable sites of bacterial attachment and subsequent entry are the apical root zone with the thin-walled surface root layer such as the cell elongation and the root hair zone (zone of active penetration), and the basal root zone with small cracks caused by the emergence of lateral roots (zone of passive penetration) (Fig.) At these sites, bacteria are often arranged in microcolonies comprising several hundreds of cells (Zachow *et al.*, 2010). For active penetration, endophytic bacteria have to be well-equipped with cellulolytic enzymes that hydrolyze the plant's exodermal cell.

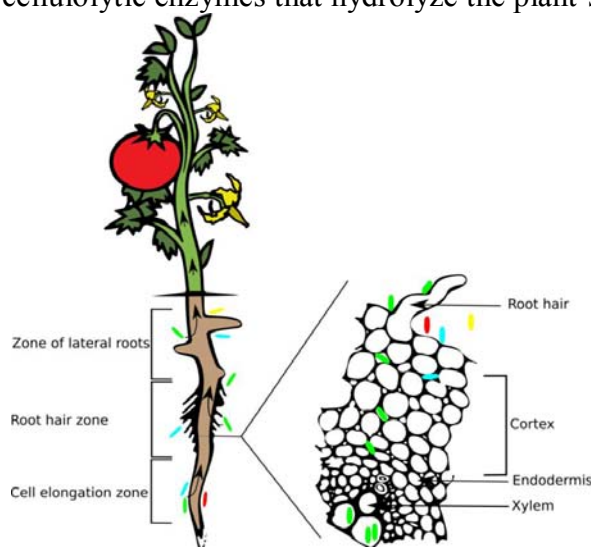


Figure 1 : Plant colonization by endophytic bacteria

Bacteria can enter a plant at several root zones as indicated above. Endophytes can either remain at the site of entry (indicated in blue) or move deeper inside or occupy the intercellular space of the cortex and xylem vessels (indicated in green). Red and yellow represent rhizospheric bacteria that are unable to colonize inner plant tissues walls.

Bacterial cell-wall degrading enzymes are also known to be involved in the elicitation of defense pathways in plants as many proteins that are involved in defense and repair are associated with plant cell walls (Norman-Setterblad *et al.*, 2000). Induction of such a response usually results in decreasing the spread of pathogens inside a plant (Iniguez *et al.*, 2005). Since this is not the case for endophytes, endophytic bacteria must be able to escape the plant immune responses even reduce it to some extent. Genomic analysis of sequenced endophytes confirmed this concept. The exact mechanism of this process remains to be elucidated.

Colonization of the plant cortex

Once bacterial cells have crossed the exodermal barrier, they can remain at the site of entry as it has been shown for *Paenibacillus polymyxa* in *Arabidopsis* (Timmusk *et al.*, 2005) or move deeper inside and occupy the intercellular space of the cortex (Compant *et al.*, 2005; Gasser I *et al.*, 2011, E.K. James *et al.*, 1994, Roncato-Maccari *et al.*, 2003)(Fig.2). It is uncommon for endophytic bacteria to penetrate plant cells and cause the formation of specific morphological structures like root-nodule bacteria do. However, recently Haung *et al.* showed that *Bacillus subtilis* GXJM08 colonizes the root of the leguminous plant *Robinia pseudoacacia* L. in a mode similar to that used by rhizobia. The most dramatic changes include deformation of the root hair (swelling, dichotomous branching), development of infection threads with bacteria between the cell walls of root cortical cells, and formation of bacteroids inside plant cortical cells. It is unknown whether these strain could fix N like the root-nodule bacteria do. It would also be of interest to determine whether other non-symbiotic bacteria can induce similar morphological changes in this plant (B. Huang *et al.*, 2011).

Colonization of the xylem

Only a few bacteria can penetrate the endodermal barrier and invade the xylem vessels (Compant *et al.*, 2005, Gasser I. *et al.*, 2002, Roncato-Maccari *et al.*, 2003)(Fig. 2). This usually happens through unsubsided endodermal cells in the apical root zone and/or in the basal root zone, where the emerging lateral roots interrupt the continuity of the Casparian band in the wall of endodermal cells. The long-distance transport of water, ions and low-molecular-weight organic compounds, such as sugars, organic and amino acids, takes place in the xylem (Sattelmacher, 2001). Though the concentration of available nutrients is relatively low and represents 0.006 - 0.034 $\mu\text{mol/g}$ of fresh weight for some sugars (Madore & Webb, 1981), it has been calculated that they are sufficient to support the growth of endophytic bacteria (Sattelmacher, 2001). Direct evidence that bacterial endophytes feed on plant nutrients came from several radioactive labeling experiments. For example, after incubation of potato plants with $^{13}\text{CO}_2$, detected the isotope label first in the plant's

photosynthetic metabolites and subsequently in diverse bacterial endophytes (Rasche *et al.*, 2009).

Colonization of the reproductive organs

It is likely that the concentration of available nutrients in xylem is decreasing along the plant axis. This can explain the facts that the diversity and population density of endophytic bacteria decreases with the distance from the root and that only a small number of bacteria reaches the upper parts of shoots, the leaf apoplast and reproductive organs, such as flowers, fruits and seeds (Compant *et al.*, 2011, Frnkranz *et al.*, 2012). The presence of endophytic bacteria in reproductive organs of plants was confirmed by cultivation (Furnkranz *et al.*, 2012, Granr *et al.*, 2003, Mundt & Hinkle, 1976, Okunishi *et al.*, 2005, Samish *et al.*, 1963) and by microscopic visualization (Compant *et al.*, 2011, Coombs & Franco, 2003).

Other ways of plant colonization

Although the rhizosphere is assumed to be the main source of endophytic colonizers, other sites of entry cannot be ignored. Some bacteria can enter a plant through stomata as has been shown for *Gluconobacter diazotrophicus* on sugarcane (E K James *et al.*, 2001) and for *Streptomyces galbus* on rhododendron (Suzuki, Lopez, & Lnnerdal, 2005). In the latter case, production of non-specific wax-degrading enzymes might have facilitated the leaf surface colonization and the subsequent endophytic establishment of this microbe. Bacteria can also enter a plant through flowers, fruits, and seeds. However, this is mostly known for specialized phytopathogens and was not shown for (non-pathogenic) bacterial endophytes (Suzuki *et al.*, 2005).

4. Nitrogen Fixation By Endophytes

In 1986, Brazilian scientists (Cavalcante & Dobereiner, 1988) discovered N₂-fixing endophytic bacteria in sugarcane stem called *Gluconacetobacter diazotrophicus*. Their pioneering work was confirmed by other scientists in USA, UK, and Germany and led to the identification of two other N₂-fixing endophytes, *Herbaspirillum seropedicae* and *H. rubrisubalbicans* (Boddey *et al.*, 1995). Endophytic diazotrophs seem to constitute only a small proportion of total endophytic bacteria (Barraquio *et al.*, 1997; Martinez, L. *et al.*, 2003). Such microbes include *Azospirillum lipoferum*, *Klebsiella pneumoniae*, and *Azorhizobium caulinadans*. Endophytic diazotrophic bacteria that have been discovered in other plants include some specific diazotrophs, *Glucanoacetobacter diazotrophicus* in sugarcane, sweet potato, and pineapple (da Silva-Froufe *et al.*, 2009) *Herbaspirillum* sp. in sugarcane and rice, and *Azoarcus* sp. in rice and Kallar grass (E K James, 2000).

Endophytic bacteria are found in legume nodules as well. In red clover nodules, some species of rhizobia were found, including *Rhizobium (Agrobacterium) rhizogenes*, in addition to *R. leguminosarum* which is the normal clover symbiont (Sturz, Christie, Matheson, & Nowak, 1997). Some γ -Proteobacteria are co-occupants with the specific rhizobia in *Hedysarum* plant nodules (Yacine Benhizia *et al.*, 2004). In most cases, the endophytic bacteria are unable to form nodules. Kallar grass grows in N-

poor soils in Pakistan and a diversity of *Azoarcus* sp. have been recovered from it (B. Reinhold-Hurek *et al.*, 1993).

5. Phosphate Solubilization By Endophytes

Endophytic bacteria possess the capacity to solubilize phosphates, and it was suggested by the authors that the endophytic bacteria from soybean might also participate in phosphate assimilation (Kuklinsky-Sobral *et al.*, 2004). Seventy-seven endophytic bacterial isolates were isolated from roots, stems and leaves of black nightshade plants (*S. nigrum*) grown in two different native habitats in Jena, Germany by Long *et al.* and six isolates were able to solubilize inorganic phosphate (Long, Schmidt, & Baldwin, 2008).

Thamizh Vendan *et al.* reported that 9 out of 18 endophytic isolates from ginseng plants had phosphate solubilizing ability by detecting extracellular solubilization of precipitated tricalcium phosphate with glucose as sole source of carbon (Vendan, Yu, Lee, & Rhee, 2010). Out of 18 endophytic isolates obtained from tomato by Patel *et al.*, eight showed phosphate solubilization activity. Results revealed that majority of the PGPR strains have phosphate solubilizing activity (Patel *et al.*, 2012).

6. Production of Plant Growth-Regulators By Endophytes

Research has been conducted on the plant growth-promoting abilities of various endophytic bacteria. They increase plant growth through the improved cycling of nutrients and minerals such as nitrogen, phosphate and other nutrients. These include phosphate solubilization activity (S C Verma *et al.*, 2001, Wakelin *et al.*, 2004), indole acetic acid production (Lee *et al.*, 2004) and the production of a siderophore (Costa., Jose M., 1994). Endophytic organisms can also supply essential vitamins to plants (Pirttila *et al.*, 2004). Moreover, a number of other beneficial effects on plant growth have been attributed to endophytes and include osmotic adjustment, stomatal regulation, modification of root morphology, enhanced uptake of minerals and alteration of nitrogen accumulation and metabolism (Compant *et al.*, 2005). The recent areas where these plant growth-promoting bacterial endophytes are being used are in the developing areas of forest regeneration and phytoremediation of contaminated soils.

Endophytic bacteria were isolated from surface-sterilized stems, roots, and nodules of wild and cultivated soybean varieties by Hung *et al.*. Except nine, all from *G. max*, IAA production was observed in the rest 56 endophytes. Fifteen produced IAA of more than $25\mu\text{g ml}^{-1}$ in the presence of the precursor tryptophan (Hung *et al.*, 2007).

7. Interactions of Endophytes With Pathogens

Endophytic bacteria can lessen or prevent the deleterious effects of certain pathogenic organisms. Diseases of fungal, bacterial, viral origin and in some instances even

damage caused by insects and nematodes can be reduced following prior inoculation with endophytes (Ellen L. Berg *et al.*, 2006, A. V. Sturz *et al.*, 2000). The widely recognized mechanisms of biocontrol mediated by PGPB are competition for an ecological niche or a substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens (Bloemberg & Lugtenberg, 2001) and/or abiotic stresses.

Endophytic bacterial biocontrol agents can be divided into two groups: (i) strains that extensively colonize the internal plant tissues and suppress invading pathogens by niche occupation, antibiosis, or both, and (ii) strains that primarily colonize the root cortex where they stimulate general plant defense/resistance mechanisms. More extensive and continuous colonization of plants might be required for endophytes of the first type because coincidence with pathogen propagates would be necessary for antagonism.

It is believed that certain endophytic bacteria trigger a phenomenon known as induced systemic resistance (ISR), which is phenotypically similar to systemic-acquired resistance (SAR). SAR develops when plants successfully activate their defence mechanism in response to primary infection by a pathogen, notably when the latter induces a hypersensitive reaction through which it becomes limited in a local necrotic lesion of brown desiccated tissue (van Loon *et al.*, 1998). ISR is effective against different types of pathogens but differs from SAR in that the inducing bacterium does not cause visible symptoms on the host plant (van Loon *et al.*, 1998).

8. Synthesis of Allelochemicals By Endophytes

Offensive endophyte colonization and defensive retention of rhizosphere niches are enabled by the production of bacterial allelochemicals, including iron-chelating siderophores, antibiotics, biocidal volatiles, lytic enzymes, and detoxification enzymes (Glick, 1995).

All organisms need Fe^{3+} for growth. Under conditions of Fe^{3+} limitation, many bacteria secrete Fe^{3+} chelating compounds, called siderophores. The siderophore Fe^{3+} complex is subsequently bound to Fe^{3+} limitation-inducible outer membrane protein receptors and the Fe^{3+} ion is transported into the bacterial cell, in which it becomes biologically active as Fe^{2+} . An example of a siderophore is pyoverdine or pseudobactin, the pigment responsible for the fluorescence of fluorescent pseudomonads. Fe^{3+} is poorly soluble under aerobic conditions at neutral and alkaline pH. Some bacteria produce siderophores which are sufficiently strong to bind Fe^{3+} to the extent that fungi in their neighbourhood cannot grow anymore under iron limitation and siderophore producing bacteria can then act as biocontrol agents (Leong, 1986), as exemplified by the control of *Erwinia carotovora* by *P. fluorescens* strains (Kloepper *et al.*, 1980).

The cyanide ion is exhaled as HCN and metabolize to lesser degree into other compounds. HCN first inhibits the electron transport, and the energy supply to the cell is disrupted, leading to the death of organisms. It inhibits proper functioning of enzymes and natural receptors' reversible mechanism of inhabitation, and it is also known to inhibit the action of cytochrome oxidase. HCN is produced by many

rhizobacteria and is postulated to play a role in biological control of pathogens. Production of HCN by a certain strain of fluorescent pseudomonads has been involved in the suppression of soil – borne pathogens. Suppression of black root rots of tobacco and take all of the wheat by *P.fluorescens* strain CHAO was attributed to the production of HCN. *P.fluorescens* HCN inhibited the mycelial growth of *Pythium* in vitro. The cyanide producing strain CHAO stimulated root hair formation indicating that the strain induced an altered plant physiological activity. Four of the six PGPR strains that induced systemic resistance in cucumber against *Colletotrichum orbiculare* produced HCN. Fluorescent *Pseudomonas* strain RRSI isolated from rajanigandha (tuberose) produced HCN, and the strain improved seed germination and root length. Pessi and Haas reported that low oxygen concentrations are a prerequisite for the activity of the transcription factor ANR which positively regulates HCN biosynthesis (Pessi & Haas, 2000).

Biocontrol activity of microorganisms involving synthesis of allelochemicals has been studied extensively for endophytic bacteria (Lodewyckx *et al.*, 2002), since they can synthesize metabolites with antagonistic activity toward plant pathogens (Chen, C. *et al.*, 1995). A variety of endophytes also exhibit hyperparasitic activity, attacking pathogens by excreting cell wall hydrolases (Chernin *et al.*, 2002). The ability to produce extracellular chitinases is considered crucial for the endophyte, *Serratia marcescens* to act as antagonist against *Sclerotium rolfii* (Ordentlich, A. and Y. Elad, 1988), and for *Paenibacillus* sp. strain 300 and *Streptomyces* sp. strain 385 to suppress *Fusarium oxysporum* f. sp. *cucumerinum*. It has been also demonstrated that extracellular chitinase and laminarinase synthesized by the endophytic *Pseudomonas stutzeri* digest and lyse mycelia of *F. solani* (Lim *et al.*, 1991). Chitinase produced by *S. plymuthica* C48 inhibited spore germination and germ-tube elongation in *Botrytis cinerea* (Frankowskiet *al.*, 2001).

9. Outlooks of Entire Review

Salinity is a serious environmental issue, as it limits crop growth and drastically reduces productivity. In view of ever increasing global population, we are more constrained than ever before to augment crop productivity. Therefore, in addition to arable land, saline land needs to be cultivated for increased yield output. This review accentuates the perception of the endophytic bacteria in saline environment.

The natural condition of plants seems to be in a close interaction with endophytes. Endophytic bacteria evolved biochemical pathways, resulting in the production of each of the five classes plant growth hormones (auxins, abscisins, ethylene, gibberellins and kinetins). In the endophyte - host interactions the minimum contribution of the plant to the endophyte is one of providing nutrition.

Endophytes seem promising to increase crop yields, remove contaminants, inhibit pathogens and produce fixed nitrogen or novel substances. The challenge and goal are to be able to manage microbial communities to favor plant colonization by beneficial bacteria. This would be amenable when a better knowledge on endophyte ecology and their molecular interactions is attained. The contributions of this research field may have economic and environmental impacts.

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