Int.J.Curr.Microbiol.App.Sci (2018) *7*(2): 1899-1911

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 02 (2018)**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2018.702.229

Endophytes: An Environmental Friendly Bacteria for Plant Growth Promotion

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Keywords

Biofertilizers, Endophytes, Ecofriendly, Fertilizers, Rhizobial, Nonrhizobial

Article Info

Accepted:
17 January 2018
Available Online:
10 February 2018

A B S T R A C T

Environment pollution and the food shortage are the biggest problem faced by human beings. As the world population increase food requirement also increases. To fulfil the requirement of food the farmers are dependent on the chemical fertilizers which are harmful for environment. Thus the alternate to these problems are the use of biofertilizers. Both rhizobial and nonrhizobial endophytes are used as the biofertilizers as they have potential to increase the crop yield by producing plant growth promoting activity like Indole acetic acid (IAA), enzyme 1- aminocyclopropane-1-carboxylate (ACC) deaminase, potassium solubilization, phosphate solubilisation, siderophore production and most importantly the nitrogen fixation. As these endophytes have potential to meet the food requirements and as well as do not pollute the environment thus can be called as ecofriendly bacteria.

Introduction

Traditionally, plants have been considered as single organisms but in nature, plants associate with diverse organisms. The network of interactions between different symbiotic microorganisms and the host plant likely is likely to have a huge importance for the plant growth and development. Plant growth involves the synergistic activity of many different life forms in a highly complex environment. Thus the field of growing plant is a complex of microbial activity, comprising soil microbes, atmospheric microbes, plant surface microbes, an internal plant-colonizing microbes or endophytes. As the plant constitute vast and diverse niches for endophytic organisms, these endophytes living inside plants might intimately interact with cells of the host and release plant-growth-promoting compounds. This synergistic interaction between plant and endophytes has been called as double-fitness trait which is active in the plant endophyte partnership. This interaction is beneficial for both plant and endophytes, as the plants provide protection and photo assimilates on which endophytes are heavily dependent and in return endophytes promote growth of plants by their different mechanisms. Plant-endophyte interactions that promote plant development and plant health have been the subject of considerable interest. Among the endophytic microorganisms, endophytic bacteria occupy internal tissue of plants without causing damage to their hosts. Endophytic bacteria includes both rhizobial and nonrhizobial endophytes.

Why endophytes are called as ecofriendly?

As the world population increases, it is insufficient to fulfill the food demand of populations. So to feed the burgeoning population of the world, farmer heavily rely on the use of chemical fertilizers. These chemical fertilizers increase yield in agriculture but are expensive and harm the environment. They deplete nonrenewable energy resources, *i.e.* manufacturing of industrial nitrogen fertilizers uses non-renewable resources like coal and natural gas and causes production of greenhouse gases contributing to global warming (Bhattacharjee *et al.,* 2008). These fertilizers also have side effects, such as leaching out and polluting water basins, destroying micro-organisms and friendly insects, making the crop more susceptible to attack of diseases, reducing soil fertility, thereby causing irreparable damage to overall system. Therefore, it's high time to opt for alternative fertilizers which can be used in sustainable agricultural practices without affecting the environment. Application of plant growth promoting endophytic bacteria (PGPBEs) can be a potential option for enhancing growth and yield of leguminous plant in sustainable manner. The potential of PGPBEs to improve plant health has led to a great number of studies examining their applied use as inoculants, primarily in agricultural crops (Kloepper and Schroth, 1978; Hallmann *et al.,* 1997; Kuklinsky-Sobral *et al.,* 2004). As they are safe to environment so they are called ecofriendly (Fig. 1).

History of endophytic microorganisms

Heinrich Friedrich link (1809) was the German botanist who first describes the endophytes. At that time he gave term "Endophytae" and described as a distinct group of parasitic fungi living in plants. But De Bary (1866) was first to coined the term endophyte and Vogl (1898) was first who reported the mycelium lives in the grass seed *Lolium temulentum*. In 1904, in Germany Freeman identified an endophytic fungus, in *Persian darnel* (annual grass). Perotti (1926) was the first to describe the occurrence of non-pathogenic flora in root tissues and Henning and Villforth (1940) reported the presence of bacteria in the leaves, stems and roots of apparently healthy plants. Since 1940's there have been several reports on endophytic bacteria in various plant tissues (Hallmann *et al.,* 1997). In the 1980's endophytic bacteria having nitrogen fixing ability were found in graminaceous plants (Reinhold-Hurek and Hurek, 1998). Evidence of plant associated microbes has been discovered in the fossilized tissues of stems and leaves (Taylor and Taylor, 2000).

Definition of endophytes

Researchers defined endophytes in various ways and these definitions have been modified as the research field advanced. Tervet and Hollis (1948) defined endophytes as microorganisms which have ability to live inside the plants without showing any disease symptoms. Petrini (1991) defined endophytes as "all organisms inhabiting plant organs that at some time in their life cycle can colonize internal plant tissues without causing apparent harm to their host". Kado (1992) defined 'Endophytic reside within the living organism without doing causing harm or gaining benefit other than securing residency. Microorganisms living within plant tissues for all or part of their life cycle without causing any visible symptoms of their presence are defined as endophytes (Wilson, 1993). Bacon and White (2000) describes “endophytes are microorganisms which have ability to colonize living, internal tissue of plant without causing any immediate negative effects”. Schulz and Boyle (2006) defined endophytic bacteria are those bacteria that colonize the internal tissues of plants without causing any external sign of infection or showing negative effect on their host. The definition of endophytes given by Hallmann *et al.,* (1997) seems to be most appropriate. They defined endophytic bacteria resides inside the surface sterilized plant tissues or can be isolated from inside the plant tissue and having no harmful effect on the host plants. But this definition cannot be applied to nonculturable endophytes therefore definitions of endophytes by Bulgarelli *et al.,* 2013 seems to be more appropriate definitions which defines endophytes as a set of microbial genomes located inside plants organs.

Isolation of bacterial endophytes

In general endophytic bacterial population are lower than rhizospheric bacterial pathogens. (Hallmann *et al.,* 1997; Rosenblueth and Martínez-Romero, 2004). The endophytic environment gives the protection from outer environment to those bacteria which colonize the internal tissue of plants. These bacteria generally colonize the internal tissue of plants, and they have been isolated from all the parts of plants including seeds (Posada and Vega, 2005). Endophytic bacteria have been isolated from both leguminous and nonleguminous plants. Endophytic bacteria can be isolated and characterized from internal tissues of plant by sterilizing the surface with sodium hypochlorite or similar agents (Lodewyckx *et al.,* 2002; Miche and Balandreau, 2001). Kobayashi and Palumbo (2000) reported 10^2 to 10^4 viable endophytic bacteria in of sample. 65 bacterial endophytes were isolated from stem roots and nodules by Hung and Annapurna (2004). Similarly Saini *et al.,* (2015) isolated endophytic bacteria from roots (12 isolates) and nodules (76 isolates) of chickpea legume grown under CCS Haryana Agricultural University farm. Ghodhbane-Gtari *et al.,* (2014) isolated *Actinobacterium* strain BMG51109a from surface sterilized root nodules of *Casuarina glauca* collected from Tunisia.

Plant growth promotion by ecofriendly endophytes

Endophytic bacteria differs from rhizospheric bacteria in terms of living inside the plant tissues but employ similar plant growth promotion mechanisms as that of rhizospheric organisms. As endophytes lives inside the plant tissue therefore, it is likely that endophytic plant growth promoting bacteria will be superior to similar non-endophytic bacterial in terms of protection and promotes plant growth under a wide range of environmental conditions. Plant-growth-promoting bacterial endophytes (PGPBEs) facilitate plant growth *via* three interrelated mechanisms: phytostimulation, biofertilization and biocontrol (Bloemberg and Lugtenberg, 2001).

Phytostimulation includes ACC (1-aminocyclopropane-1-carboxylate) utilization and indole acetic acid production, biofertilization includes nitrogen fixation, phosphate solubilisation and potassium solubilisation and bioconrol include siderophore production (Fig. 2).

Phytostimulation

Phytostimulation is the direct promotion of plant growth through the production of phytohormones (Bloemberg and Lugtenberg, 2001). The most highly studied example of phytostimulation involves lowering plant hormone ethylene levels by the enzyme 1-

aminocyclopropane-1-carboxylate (ACC) deaminase and indole acetic acid (IAA) production.

ACC utilization by endophytes

Under normal conditions, ethylene helps in germination of seeds root hair growth and fuit ripening (Abeles *et al.,* 1992; Siddikee *et al.,* 2010). But, during the stress response, plant produce high level of ethylene that acts opposite to normal function and is harmful to the plant growth. The bacterial endophytes have capability to produce ACC deaminase enzyme which promotes the plant growth by converting ACC to ammonia and alpha ketobutyrate. Rhizobial strains that produce active ACC deaminase, contained a relatively low level of ethylene activity (approximately 2-8%) compared with the amount of enzyme activity generally found in free-living soil bacteria (Zahir *et al.,* 2003). Various *Bacillus* strain (*Bacillus circulans, Bacillus firmus*, *Bacillus globisporus*) have potential to produce ACC deaminase, reported to stimulate the root elongation in *Brassica camprestris* (Ghosh *et al.,* 2003). Apart from the bacillus strain *Pseudomonas spp*. also have ability to produce ACC deaminase and there by increased the dry matter contents of root when inoculated in seeds (Reed and Glick, 2005). Some of the studies reported that some ACC deaminase producing bacteria have potential to promote plant growth in various stressful conditions like drought, saline conditions and flooding.

High salt conditions suppresses the growth of plants because during the stress conditions ACC production by plants increases. Bacterium *Achromobacter piechaudii* isolated from soil of *Lycium shawii* have capacity to produce ACC deaminase. Under salt stress conditions, this bacterium is inoculated with the seed of tomato, were significantly reduced ACC production and increased the dry and fresh tomato seedlings weight (Mayak *et al.,* 2004). Etesami *et al.,* (2104) showed in their studies that under the flooded conditions ACC deaminase producing *Pseudomonas fluorescence* increased the rice seedlings significantly reported that ACC deaminase containing *P. fluorescens* REN1 increased *in vitro* root elongation and endophytically colonized the root of rice seedlings significantly, under constant flooded conditions.

Production of phytohormones

Among the five major classes of phytohormone auxin, gibberellins, ethylene, abscisic acid and cytokinins, indole acetic acid (IAA) is the most abundant phytohormone which belongs to the auxin family, improves plant growth (Tsakelova *et al.,* 2006). IAA helps the plants by production of longer roots and also increase density of roots which helps is more nutrient uptake (Datta and Basu, 2000). Various studies revealed that endophytic bacteria promote plant growth significantly by production of phytohormones.

These phytohormones are important for plant growth as they regulate the various biological processes, fruit development, cell division and senescence. Indole acetic acid induce cell elongation by increasing the permeability of water in to the cells, increasing ostomic contents of the cells and by decreasing wall pressure. It also induces the flowering and fruiting and delay the abscission of leaves (Zhao, 2010).

It is reported that about 80% of the naturally occurring microorganisms in soil are capable for indole acetic acid production (Patten and Glick, 1996; Khalid *et al.,* 2004; Loper and Schroth, 1986). Some rhizobial species are known to produce indole acetic acid in the rhizospheric soil (Bhattacharyya and Jha, 2012; Spaepen *et al.,* 2009).

Biofertilization

Increasing the supply of utilizable form of nutrients to plants for growth promotion is defined as biofertilization (Bashan, 1998). Nitrogen fixation and phosphorus solubilisation is the best studied example of biofertilization (Bloemberg and Lugtenberg, 2001).

Nitrogen (N_2) fixation

Nitrogen plays a important role in plant growth and is usually absorbed by plants as nitrate or ammonium ions. It is the essential elements of metabolism of plants and very important for making proteins, enzymes and chlorophyll structure of plants. Soils which are deficient in nitrogen, usually result in low crop yield. Farmers supply nitrogen to agricultural land in the form chemical fertilizers such as urea and ammonium nitrate. Low amount of these chemical fertilizers is used to stimulate the growth of plants and also required to induce the activity of nitrogen fixing bacteria (Jefing *et al.,* 1992). But these chemical fertilizers harm the environment in various ways, therefore, researchers are focused to develop the ecofriendly agriculture practices, for example by applying the biofertilizers like endophytic bacteria. *Rhizobium is* the best example to fix nitrogen in a sustainable manner. These microorganisms were traditionally considered to be responsible for legume infection process, however rhizobia can behave as endophytes in nodules and the isolated rhizobial strain from nodule have ability to again infect the similar legumes from which they were recovered and also promote their growth. Lei *et al.,* (2008) isolated the endophytic rhizobia from the vicia nodules. Two of the endophytes *R. leguminosarum* and *Mesorhizobium loti* were isolated from the clover nodules and when these were coinoculated, showed increase in plant growth (Strutz *et al.,* 1997).

As the legumes possess nodules on their roots where this bacterium *Rhizobium* locates with a defined function of converting the atmospheric N_2 into plant available form, called biological nitrogen fixation. Increases in amount of biological nitrogen fixation can result from the use of different commercial or wild strains. Significant increase in biological nitrogen fixation suggests that more nitrogen can be fixed if they are inoculated more often or with more effective rhizobia. In this manner, an appreciable quantity of biological nitrogen is accumulated in the soil which can be used by similar crop and the subsequent one. Two main reason for the low crop yield is unavailability of good quality seeds and effective rhizobial inoculation. Seed treated with native rhizobia in soil lacking effective rhizobia is useful for improving nodulation and crop yield (Rupela and Dart, 1979; Patil and Sbinde, 1980; Shamim and Ali, 1987; Shah *et al.,* 1994). Chickpea respond positive to rhizobial inoculation when grown in soils that contain native rhizobia (Sharma *et al.,* 1983). However with the introduction of improved varieties or sowing of this crop in new areas, inoculations with effective rhizobial strain is essential for getting higher yields. Apart from the rhizobial endophytes there are some promising nonrhizobial endophytic biofertilizers that include the members of *Azoarcus, Achromobacter, Burkholderia, Gluconoacetobacter, Herbaspirillum, Klebsiella* and *Serratia* (Rothballer *et al.,* 2008; Frache *et al.,* 2009). The sufficient nitrogen supply by endophytic diazotropic bacteria in kallar grass and sugarcane suggest that endophytic bacteria can have potential to fix biological nitrogen fixation in sufficient amount. *Gluconoacetobacter diazotrophicus* (*Acetobacter diazotrophicus*) is an endophytes, has potential to fix nitrogen in sugarcane and can fix nitrogen approximately 150 Kg N ha^{-1} $year^{-1}$ (Dobereiner *et al.,* 1993; Muthukumarassamy *et al.,* 2005).

Fig.1 Benefit of using endophytes over chemical fertilizers

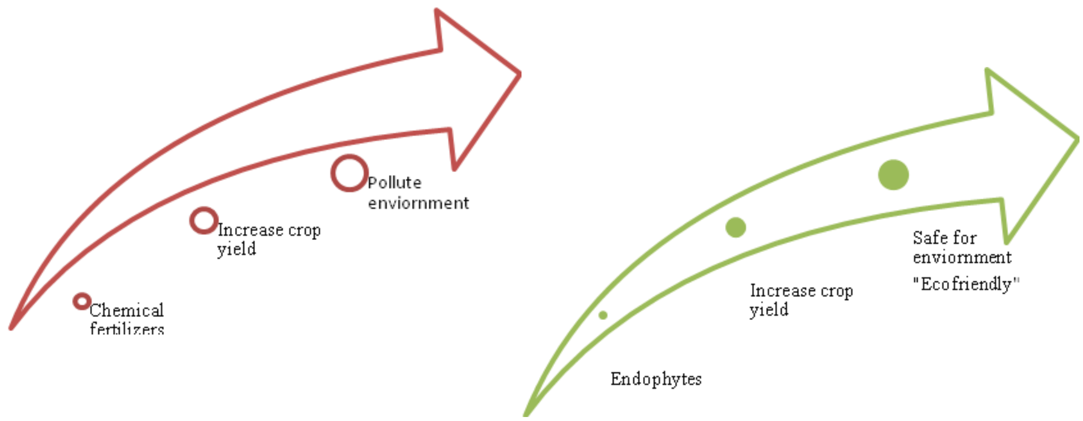


Fig.2 Plant growth promoting activity of bacterial endophytes

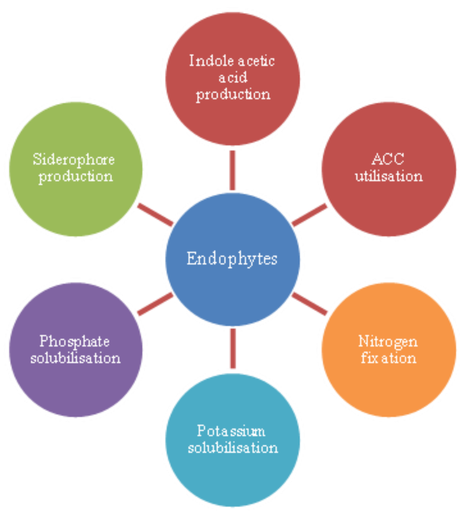
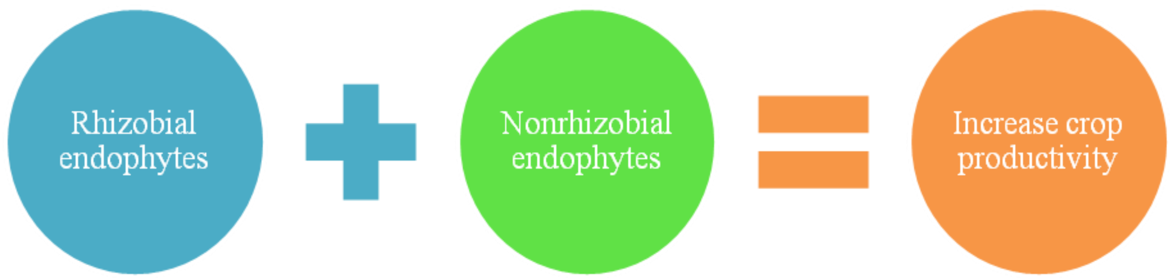


Fig.3 Rhizobial and nonrhizobial culture as bioinoculant



These investigations suggest that both rhizobial as well as nonrhizobial endophytic bacteria have potential to increase the productivity of plants. Thus these can used as bioinoculant and can improves more productivity when coinoculated (Fig. 3).

Phosphate solubilisation

Phosphorous is one of the most important nutrient for plants and also second most limiting factor. Phosphorous plays a central role in genetic structures, photosynthesis and energy transfer (Kim *et al.,* 1998). It is a limiting factor because it is found in soil in bound form that cannot be utilized by plants quickly. Thus in agriculture practices farmer utilizes the fertilizers which heavily contaminate the environment. Thus alternate to this is use of micro-oraganisms such as ecofriendly bacteria. Various research publications also showed that alternate to this problem is to use phosphate solubilizing microorganisms as a biofertilizers (Richardson, 2001).

Bacteria like *Bacillus, Pseudomonas* and *Rhizobium* have capability to solubilise the phosphorus (Sridevi *et al.,* 2007; Rodriguez and Fraga, 1999). A strain of *Bacillus megaterium* when applied to seed of chickpea plant also significantly increased the plant height, nodule dry weight, root, shoot, nitrogen percentage, chlorophyll content pod number seed protein and total biomass yield as compared to control and mineral fertilizers applications in the field as well as controlled environment conditions (Elkoca *et al.,* 2007). A study by Oteino *et al.,* (2015) described that endophytic *Pseudomonas* isolates showed that many endophytic strains differ in their potential to solubilize phosphorus when inoculated to pea plant grown in soil and also showed beneficial plant growth promoting effects under the limiting soluble phosphate. Bacterium *Mesorhizobium (Rhizobium)* when applied to soil containing insoluble phosphorous have capability to enhance the chickpea plant growth by solubilising the phosphorus (Peix *et al.,* 2001). The studied reveals that his strain has efficiency to mobilize phosphorous when TCP (tricalcium phosphate) was added in the experimental soil. Also this study by Peix *et al.,* (2001) showed that when the bacterial culture was inoculated in the soil, the soluble phosphorus content was increased as compared to the uninoculated soil and also magnesium content, calcium content, plant dry matter was significantly increased in comparison studies.

Potassium solubilisation

Potassium is one of the most essential micronutrient and has important role in development of plants. If the potassium is not available to plants, the plants will show characters weak root development, stunted growth, small seeds and low yield. The soil contains the potassium in large amount but problem is that it is found in unavailable form which cannot be utilized by plants directly. Thus the farmers rely on agrochemical which pollute the environment as well as increase the cost of agriculture. Microorganisms are used now days to solve the above problem to make potassium availability to plants (Rogers *et al.,* 1998; Groudev, 1987). A number of bacteria namely *Pseudomonas, Rhizobium, Bacillus* and *Paenibacilus* have been reported in studies to make unavailable potassium to available form for plants (Badr *et al.,* 2006). Inoculation of plants with this potassium solubilising bacteria resulted in higher mobilization of potassium from insoluble form thus helps in wheat and maize plant growth promotion (Singh *et al.,* 2010). Thus the application of potassium solubilising bacteria can reduce the environment pollution by agrochemicals and can support the crop production in ecofriendly way (Sheng *et al.,* 2013).

Biocontrol

Microorganisms protect plants from phytopathogen by various mechanisms termed as biocontrol. Endophytes produce siderophore is one of the mechanisms of plant protection as wells as plant growth promotion.

Siderophore production

Iron (Fe) is the essential micronutrient for plants as it is required for many processes such as photosysntheis, DNA synthesis, respiration and nitrogen fixation. Iron is the present in large amount in soil as it is the fourth most abundant element of earth crust. But this iron is not available to plants because most of it is found in insoluble form. If the iron is not available to plants, it results in chlorosis. So this problem can be solved by the endophytic bacteria as they have ability to dissolve this insoluble iron by producing siderophore and hence making the availability to the plants. The microorganisms produce siderophore in iron limiting conditions, making insoluble form of iron to soluble forms it is defined as low molecular weight chelating compounds which chelate iron from the environment. There are various type of siderophore produce by endophytes such as rhizobactin, enterobactin and ferrioxamine. As the siderophore produced by endophytes help the plants by making iron available to plant, second it can act as a biocontrol agent by removing the iron from phytopathogen in the iron limiting conditions. *Fusarium Oxysprium* is a phytopathogen and its growth is inhibited by the indigenous strain Bacillus *Subtilis* by producing siderophore thus can act as a biocontrol agent and indirectly promote plant growth (Patil *et al.,* 2014).

Field application of endophytes

Some research has shown that endophytes have a great potential to increase the yield of different plants when they are inoculated. Coinoculation of rhizobial endophytes and nonrhizobacterial endophytes had reported to increase the yield of plant in a significant manner (Harish *et al.*, 2009). To use the rhizobial and nonrhizobial coinoculation in the field, one thing must considered before applying in field, that these endophytes are compatible with each other and do not inhibit the growth of each other. Santiago *et al.*, (2017) used compatible bioinoculants in their experiment to increase the potato crop yield. Various studies reported that single inoculations can increase the yield of crops but when both rhizobial and nonrhizobial culture applied to a crop, the percentage of crop yield increase from single inoculations Stajković *et al.*, (2009). The most important benefit of using these endophytic biofertilizers to field is that it can be useful to subsequent crops also as these bacterial endophytes have ability to multiply. Thus the farmer has benefits, to use these endophytes as biofertilizers rather than using the fertilizers as these were ecofriendly, cheap and can be used for subsequent crops in a sustainable manner.

Agriculture has a great importance in the livelihood of the human being. But now days, to increase the agriculture productivity chemical fertilizers are used in high quantity which are polluting the environment. As Environment pollution is the one of the biggest problem in the world so alternate of this is to use the ecofriendly endophytes which have potential to increase the agriculture productivity as well as safe for environment.

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How to cite this article:

Priyanka Batra, Monika Barkodia, Umang Ahlawat, Rekha Sansanwal, Tanisha Sharma and Leela Wati. 2018. Endophytes: An Environmental Friendly Bacteria for Plant Growth Promotion. *Int.J.Curr.Microbiol.App.Sci.* 7(02): 1899-1911.
doi: https://doi.org/10.20546/ijcmas.2018.702.229