#### **Abstract**

Endophytes are bacterial and fungal **Sreelakshmi Rajesh** members which can be detected inside the tissues of a healthy plant without producing any types of symptoms. Bacterial endophytes include a group of microbes which can be found inside a plant that help improve plant growth and derive better nutrients. This Plant Growth Promoting Bacterial group is less explored than the rhizospheric bacteria which are found in the soil near the plant root. A mechanism underlying direct and indirect plant growth promotion contributes to the great potential of E mail: drmaumar@gmail.comendophytic bacteria in a wide range of realworld applications. A better understanding of morphological and genomic level details is needed to reveal unexplored qualities which would definitely add to the future prospects.

**Keywords:** Endophytes, Plant Growth Promoting Bacteria, Siderophore, ACC deaminase, Ethylene, Rhizosphere

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#### **I. INTRODUCTION**

Endophytes are endosymbiotic microorganisms colonizing the internal tissues of healthy host plants [1] and possess the ability to improve the quality and growth rate of their respective hosts [2]. Their colonization does not produce any disease symptoms or morphological changes like gall formation of plant tissues [3]. Most of the plants on earth are host to one or more types of endophytes [4]. These endophytes can be either bacteria or fungi [5,6]. Their population density in a host plant can vary from hundreds to more than 9 x 10<sup>9</sup> bacteria per gram of plant tissue [7,8, 9]. They can be either obligate or a facultative and the obligate types cannot be cultured due to their specificity of growth conditions. On the other hand, facultative endophytes can be cultured outside the plant tissue using artificial nutrient media [10, 11]. Endophytes form an important part of the micro-ecosystem inside plant tissues [12]. The most explored endophytes are non-pathogenic fungi that provide a number of useful characteristics to their host plant. However, bacterial endophytes remain an unexplored group [13]. Any bacteria which could be isolated from a surface-sterilized plant or extracted from its tissues can be called an endophyte if it does not affect the plant negatively. Bacteria can positively promote plant growth whereas studies show that plants are able to select these beneficial bacterial members in their microbiome including those inside the plant tissues [14, 15, 16]. There is no shred of evidence suggesting that these bacteria take advantage in this relationship [17], but certainly, they get protection from pathogens in adverse times. They could also communicate much better than the rhizospheric bacteria at times of stress [18,19].

Plant growth promoting bacteria include diverse group of genera like *Acetobacter, Achromobacter, Anabaena, Arthobacter, Azoarcos, Azospirillum, Azotobacter, Bacillus, Bukholderia, Clostridium, Enterohacter, Flavobacterium, Frankia, Hydrogenophaga, Kluyvera, Microcoleus, Phyllobacterium, Pseudomonas, Serratia, Staphylococcus, Streptomyces, Vibrio, Rhizobium* etc [20]. The presence of different endophytic bacterial species depends upon the host plant, bacteria, and biotic and abiotic factors [21, 22]. *Bacillus* and *Pseudomonas* are two bacterial genera frequently reported from agricultural crops [23]. Endophytes generally belong to  $\alpha$ -,  $\beta$ -, and γ- proterobacteria out of which γ- proterobacteria is the most prevailing and common subgroup [21]. Endophytic bacteria may be culturedependent and or independent. Normally culture-dependent endophytic bacteria belong to Proterobacteria [5]. These bacteria can colonize almost every part of a plant including underground and aboveground parts [24] and are isolated from leaves, stems, flowers, fruits, seeds, roots and tubers [22]. Most of them have a phase in their life cycle that alternates between plant and soil. In order to get a clear picture of the endophytic diversity of a plant, metagenomics approaches are the most accepted and novel method. This can unravel the actual amount of culturable and non- culturable endophytic composition without compromise. Amplification of bacteria specific genomic regions and application of bioinformatic tools are combined to enumerate the bacterial composition inside plant organs [25, 26].

#### **II. PLANT GROWTH PROMOTING BACTERIA**

Plant growth-promoting bacteria (PGPB) are not always seen as associated with plants but can be seen in bulk soil. They wait until appropriate colonization mechanisms evolve in the host plant. Gram-negative PGPB (non-spore-forming bacteria) in the absence of their host form cysts and flocs that are large and visible aggregates that help them to withstand desiccation and reduce cell metabolism majority of PGPB store large amounts of polyhydroxybutyrate which is then used up in terms of nutrient scarcity [20]. They produce a large amount of secondary metabolites and hydrolytic enzymes [27]. An increase in the production of plant growth hormones and increasing availability of plant nutrients like nitrogen and phosphorus are some of the mechanisms that underlie plant growth-promoting activity of endophytes [28].

Plant growth-promoting bacteria (PGPB) comprise two types of soil bacteriarhizospheric and endophytic bacteria. Rhizospheric bacteria are the ones found around the root of plants and endophytic species are found colonized inside a host plant [29]. Both these types have similar mechanisms of plant growth promotion. However, the significant difference is that the endophytic bacteria once stabilized inside a host plant are resistant towards variations in soil pH, water content or temperature. These are the major limiting factors in the case of rhizospheric bacteria [28] isolation and characterization.

## **III. ENDOPHYTIC BACTERIAL COLONIZATION INSIDE A PLANT**

After bacterial cell inoculation, they colonize the rhizosphere of the host plant [30] and slowly attach to the root surface by forming a string of cells in a pattern [31]. They gradually colonize the entire root surface and some rhizodermal cells through the creation of microcolonies and biofilms of bacteria [32]. In order to attain successful endophytic colonization, the endophytic bacterial species must competently colonize the rhizosphere and rhizoplane of the plant [33] along with other rhizospheric members [34]. Adaptation of bacteria with the nutrients available in root exudates of target plants is inevitable [35].

Bacterial adhesion to cell surface structures is controlled by polysaccharides, pili and bacterial adhesins [36]. Every endophytic bacterium has its own colonization pattern, site preference and specialized mechanism for penetration [37]. Bacterial attachment to plants can happen by chemotaxis where bacteria migrate towards the root within hours of attachment. This occurs by hydrophobic interaction and lectin recognition with the bacteria and plant cells [20]. Bacterial penetration can occur through active and passive modes [16]. In the passive mode, bacteria enter into plants either through the emerging points of lateral roots or wounds [38]. remain "invisible" to the plant's immune system since they enter through the plant's natural cracks. The lateral root emergence point includes the epidermis, cortex, endodermis, casparian strip and pericycle which serve as the highway for endophytic microbe entry [39]. Active penetration by a proficient endophyte is through dedicated machinery of attachment and proliferation involving lipopolysaccharides, flagella, pili, twitching motility and quorum sensing [38, 39, 40, 41].

Bacteria move from cell to cell through the release of cell wall degrading enzymes, pectinases and cellulases [42] and then spread to above-ground tissues [39]. This movement inside the host is with the help of bacterial flagella and plant transpiration stream [42,43]. The final endophytic bacterial sink from the plant roots is the leaf tissues. They can also gain entry into this destination from the phyllosphere through leaf stomata [44]. The number and diversity of endophytic bacteria in the root will be higher and only a few reach shoots and reproductive organs [45, 39]. It might be the vascular tissues which pave the way for endophytic bacteria to the reproductive structures [46].



**Table 1:** Plant Growth-Promoting Endophytic Bacteria Isolated From Various Plants



### **IV. MECHANISM OF GROWTH PROMOTION**

Mechanisms hired by plant growth promoting bacterial endophytes are analogous to that of rhizospheric bacteria [33].This plant growth strategy can happen in different ways, either through direct or indirect mechanisms. Indirect mechanism involves providing increased disease resistance by inhibiting phytopathogens [71, 72, 73, 74]. Nitrogen fixation, siderophore production, and phytohormone synthesis are some of the direct mechanisms of growth promotion [75]. They increase the plant's stress tolerance level against high salinity, pesticide load, droughts and metal toxicity [20]. Endophytic bacteria are also reported to promote plant growth by changing stomatal responses, modification of nitrogen accumulation and metabolism and osmotic pressure regulation thereby altering plant physiology [76,77]. Some actinobacterial species improve soil fertility by producing siderophores, solubilizing phosphate, or by the producing amylase, chitinase, cellulase, invertase, lipase, keratinase, peroxidase, pectinase, protease, phytase and xylanase that improve the availability of natural fertilizers [78]. *Rhizobacteria* found as endophytes in plant roots continue to induce a stimulating activity in the colonized areas [79, 80]. This stimulation of plant growth occurs by increased plant health or by influencing its physiology. This can be attributed to the extra protection rendered by endophytes from pathogens directly or indirectly or by making them less vulnerable to phytophagous insects [81]. For example, *Streptomycetes*, an agriculturally important endophytic soil bacterium produces a metabolite which upsurges host plant defence and reduces disease symptoms in adverse conditions [82]. They have got a number of antibacterial and antifungal metabolites and plant growth promoting (PGP) traits [83]. Results have shown that more than 60% of the antimicrobial and plant growth- promoting compounds originate from this genus [84]. The antibiotic production is often species specific and helps plant protection against pathogens whereas; *Streptomyces* receive plant exudates that promote its growth and development [85]. Bacterium *Pseudomonas putida* GR 12-2 is a documented and explored plant growth promoting rhizobacteria [86]. Later it was found that it contains an enzyme 1- aminocyclopropane- 1- carboxylate (ACC) deaminase that stimulate plant growth, specifically root elongation by sequestering and hydrolyzing ACC from germinating seeds and thereby ethylene [87,74].

#### **V. DIRECT PLANT GROWTH PROMOTION**

#### **1. Production of Phytostimualtors**

Bacterial endophytes promote plant growth by increasing the production of phytohormones like ethylene, abscisic acid, cytokinins, gibberellins and auxins [88, 89, 90, 91, 92].

Several bacterial endophytes nearly 80% have been reported to produce auxin [93, 89] and most of them use tryptophan as a precursor [91]. Auxins are important plant growth hormones helping in lateral root formation and plant growth promotion at times of stress. Many rhizospheric bacteria are reported to produce and secrete gibberellins in the rhizosphere. Gibberellins are important in cell elongation, cell division and seed germination [89].

Bacterial endophytes produce an enzyme 1- aminocyclopropane- 1- carboxylate (ACC) deaminase which helps to reduce plant hormone ethylene in plants by breaking down ACC to  $\alpha$ - ketobutyrate and ammonia [94, 95, 12, 96, 74, 97]. Ethlyene is a major plant hormone which plays a significant role in times of seed germination, root initiation, fruit ripening, flower wilting, leaf abscission and in times of stress [98]. This hormone is normally synthesized in small amounts in plants except at the time of fruit ripening. During stress conditions like salinity, wounding, extremes of temperature, pathogen attack, flooding, drought, nutritional stress, heavy metal pollution, organic pollutants etc [99, 100, 101] plants undergo tremendous ethylene biosynthesis and is termed as "stress ethylene"[98,99]. Significant amount of damage that happens to the plant is due to the concentration of stress ethylene and not from the direct consequence of stress [99, 102] that can help to reduce levels of ethylene and promote plant growth can be used in times of stress to the plants. However, treatment with certain chemicals can cause negative effects to the plant and environment [98, 18]. Bacterial endophytes having capability to produce ACC deaminase enzyme can be successfully used to reduce ethylene content and increase plant growth activity in times of stress [ 30]. Bacterial endophyte, *Achromobacter xylosoxidans* AUM54, is reported to produce ACC deaminase thereby reducing ethylene levels in *Catharanthus roseus* grown in saline soil [103].

There are endophytes like *Azocarus sp.* that are known for fixing nitrogen [93, 104, 5, 62]. They are able to bind with atmospheric nitrogen and convert it into ammonia that can be used up by the plant.

Phosphorus is an important mineral needed for plant growth which plants cannot directly absorb. Rhizospheric bacteria are known to produce enzymes that act on phosphorus from organic and inorganic molecules to make them available to plants [90, 105, 106]. Most of the endophytes too have the property of performing this function. Phosphorus mobilization can be actively done by endophytes when they are still in their rhizoplane or rhizosphere soil i.e., when they have not entered the root interior. Nine out of eighteen endophytes isolated from ginseng stem could solubilize mineral phosphate [93].

Iron is a mineral which is largely inaccessible as it is poorly soluble in water [107]. But, these mineral ions are needed by all organisms. Bacteria secrete some low- molecular weight molecules called siderophores that have a greater affinity towards ferric ions [108]. Siderophores have been important since they are found to have a significant role in plant disease suppression [109,110,111]. Endophytic bacteria are reported to produce siderophores in vitro [112] and may produce these metabolites inside root to cope with the highly iron depleted micro environments [5].

## **VI. INDIRECT PLANT GROWTH PROMOTION**

### **1. Disease Resistance**

Endophytic bacteria have evolved various mechanisms to suppress disease occurrence in host plants like rhizosphere bacteria [107, 113, 114,115, 116, 117, 118, 119, 120, 121, 122]. Many of such mechanisms were found in in vitro studies. For example, many of the isolated endophytic bacteria were able to produce antibiotics against some fungal pathogens in vitro. *Pseudomonas, Streptomyces* and *Bacillus* were found to be the endophytic bacterial antagonists in potato [123].

Bacterial endophytes are reported to use a mechanism known as Induced Systemic Resistance (ISR) [16, 89, 124, 125, 126, 127] through bacterial surface molecules, metabolites and volatiles [88, 89, 119] that is different from that of Systemic Acquired Resistance (SAR) [128]. Bacteria like *Bacillus amyloliquifasciens, Bacillus pumilus, Bacillus subtilis, Psuedomonas fluroscens, Psuedomonas syringae* and *Serratia marcescens* are some of the ISR inducing endophytes [129].

## **2. Adaptation Against Biotic and Abiotic Stress**

Role in phytohormone production and regulating plant metabolism makes bacterial endophytes a part of plant abiotic and biotic stress managing systems. They might be providing plants with some important crop adaptation strategy as endophytic bacteria have themselves got mechansims to overcome high salt, drought or water-logged conditions of soil [130]. Bacterial endophyte *Burkholderia phytofirmans* PsJN in Grapevine plants are reported to increase cold stress managing mechanism by varying use of carbohydrates and photosynthetic activity [131, 132]. In rice plants stress tolerance was increased due to endophytic bacteria *Pseudomonas pseudoalcaligenes* by secreting higher concentrations of glycine betain-like compounds [133].

Abscisic acid (ABA) is a plant derived hormone playing a great role in plant water balance and osmotic stress tolerance. Its values are found to be high when the plant is under stress condition. Endophytic bacterium *Azospirillum* sp. was reported to increase abscisic acid level in maize plants at times of water stress [134].

## **3. Biocontrol Activity of Bacterial Endophytes**

Advancement in strategies and incorporation of genomic level approaches in endophytic research has helped in better understanding the biocontrol potential of bacterial endophytes [135]. *Bacillus amyloliquefaciens* isolated from peanuts produce antimicrobial compounds that lead to decrease in the incidence of peanut bacterial wilt caused by *Ralstonia solanacearum* [136]. The same bacterial endophyte, but a different strain Bg- C31 isolated from mangrove produces antimicrobial proteins against *Ralstonia solancearum* causing capsicum bacterial wilt [137]. *Pseudomonas fluorescens* PICF7 from *Olea europea* act against *Verticillium* wilt caused by *Veticillium dahlia* by enhancing plant growth and induced systemic resistance [138,139]. *Serratia marcescens* UPM39B3 promoted growth in banana plants by deferring the onset of symptoms for 7- 10days against *Fusarium* wilt caused by *Fusarium oxysporum* [140].

### **4. Rhizoremediation**

The Removal of environmental pollutants using rhizospheric microbes is termed as rhizoremediation (Kuiper et al., 2004). It is referred to as phytoremediation [141] when degradation is carried out by plants and the role of microbes are unnoticed. Endophyte *Burkholderia cepacia* in *Lupinus luteus* L. (yellow pine) is genetically modified to improve organic pollutants remediation [142]

### **VII. PLANT GROWTH PROMOTION BY INOCULATION OF PGPB**

There are many bacteria that are used to exploit their plant growth promotion activity on a commercial scale. *Azosprillum* is one among the best-known symbiotic Plant growth promoting bacteria (PGPB). Experiments have shown that this bacterium can increase crop yield by 5- 30%, however inoculum establishment is very difficult. This may be due to some reasons:

- I. If the bacteria are not successfully attached to the root epidermal layer, growth substances produced by the bacteria will diffuse to the soil and will be used up by the microbes present in the soil.
- II. If the attachment is unsuccessful bacteria may get washed out from the rhizosphere soil of its host plant which reduces their chance of survival.
- III. Some other non-beneficial root microbes may have already colonized potential association sites of PGPB [143,144, 145].

## **VIII. CONCLUSION**

It is evident that bacteria play an important role in balancing the link between plant physiology and different ecosystems. Apart from rhizospheric bacteria, bacterial endophytes are better adapted with their hosts and can be used positively in plant growth promotion activities. However, endophytic bacterial species were less explored when compared to rhizospheric bacteria. Now, these plant growth promoters are of growing interest. Endophytes could be used as bio inoculants to promote plant growth and fitness in agricultural crops. They could also be used in industrial and medical applications like antibiotic production. However, like some rhizosphere bacteria, endophytes are found to be potential human-pathogenic bacteria which may cause some serious health issues. It is important to screen all the endophytes at initial stages of research itself.

#### **CONFLICT OF INTEREST**

The authors report no conflict of interest.

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