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Endophytic Bacteria; Diversity, Characterization and Role in Agriculture

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Abstract:

Entophytic bacteria have an important role in the growth process and health of the plant host. Nevertheless, also some endophytic bacteria are existing in seeds and have not been studied yet. In addition, some Entophytic bacteria are important in plant tolerance to environmental stresses. They can colonize the internal tissues of the host and are able to use a variety of different relations including symbiotic, mutualism, communalistic, and trophobiotic. They have the ability for plant hormone production like auxin, indole acetic acid, and gibberellin; also some endophytic bacteria have the ability for siderophore creation, phosphate solubilization, nitrogen fixation, protease, and hydrogen cyanide formation.. Moreover, they produce compounds that could have possible usage in drug, agriculture or engineering. They have the ability to removesoil toxins thus, improving phytoremediation and soil fertility. Further, most of endophytic bacteria are diazotrophs and associated with the *Proteobacteria*, and a varied range has been detected agreeing to the *nifH* gene which codes for nitrogenase enzyme, structures recovered from plant materials, however a limited part of these genes looks to be stated. The endophytes discussed in this review are isolated from surface-disinfested plant tissue, and that do not damage the plant. Moreover, endophytes appear to be in-between saprophytic bacteria and plant pathogens, they are either saprophytes growing to be pathogens, or extremely grown plant pathogens with protective accommodation and nutrient provisions, but not killing their host. Generally, endophytic bacteria are partial under biotic and abiotic influences, with the plant itself being one of the main prompting influences.

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INTRODUCTION

The word Endophyton consists of two parts (endon =inside, phyton = plant), these microorganisms spend most of their lifecycle inside the plant tissue without affecting harm or illness, [1]. They have been supposed to be weakly strong plant pathogens. Recent research proved that they have several beneficial effects on the plant host [2]. These bacteria have the ability to colonize plant tissues without negatively affecting the host. Endophytic genera for example *Azoarcus, Burkholderia, Gluconobacter, Klebsiella, Pantoea, Herbaspirillum, Rahnella,* and *Pseudomonas* are capable to increase the biomass in inoculated plants [3]. Positive entophytic bacteria have been concerned in the recent years for their progressive effect on yield manufacture [4]. Moreover, numerous studies have revealed that entophytic bacteria can be used as bio-control agents for numerous plant pathogens [5]. Some authors divided endophytic into two groups, facultative and obligate bacteria. Further studies [6] describe endophytes as microbes containing bacteria, fungi, and protists that inhabit the plant's inner structures. In some situations, they also accelerate the seedling process, and help plants establish under contrary conditions [7]. Many researchers have justified the role of endophytic bacteria for the defense against biotic or abiotic pressure in addition to the developmental advancement of the plant [8]. These endophytic bacteria encourage plant development increasing the germination rate; leaf part; chlorophyll, nitrogen and protein contents;, hydraulic activity; growth parameters; and yield, and help plant tolerate abiotic exhausting factors like drought, salinity, etc. These bacteria promote plant growth either directly through hormones production, biological nitrogen fixation, phosphate solubilization, potassium solubilization, or indirectly through stimulating opposition to pathogens [9]. Also, endophytic bacteria can colonize as biological agents against the effect of phytopathogens, making them function asbio control agents [9]. Several studies have exposed that endophytic microorganisms have the capability to mechanise plant pathogens [10, 11]. Different factors can impact the endophytic community structure of the plant, like growth time, genotype, biological status, tissue, and environmental conditions, plant skin and genotype production, and play their role in the selection of different bacterial groups that assist with plants [12-15]. Endophytic bacteria in agriculture have a massive importance in diminishing the environmental influences affected by chemical fertilizers [16].

ISOLATION OF ENTOPHYTIC BACTERIA

Entophytic bacteria commonly inhabit the intercellular spaces; athough, they have also been isolated from the seeds of the plants [17, 18]. To isolate and describe the endophytic bacteria from diverse plant types surface disinfection methods are the common procedures using serial washing with 70% ethanol [19] for sorghum (cultivars RS626 and Dekalb 61), while Soybean, wheat, and prairie plants were treated for 10 s with 2% sodium hypochlorite having 0.1% Tween 20 to remove the disinfectant [20]. Another method for isolating entophytic bacteria is vacuum removal [21]. A vacuum method was used to extract the xylem sap from the roots of grapevine plants [22] and cotton [23]. Also, a centrifugation technique was applied to gather the intercellular liquid of plant soft tissue [24], by dipping the stem section (3-4 cm lenth) in ethanol alcohol then flaming it to prevent surface contamination, and centrifuging up to 3000 x g in suitable tubes. This technique is still favorable for soft plant tissues., the process of sterilizing the surface of the plant is necessary before the centrifugation process because the plant tissue would come in contact with the removed liquid through the centrifugation process. On the other hand, entophytic bacteria isolated from young seeds stay able to provide an aneglect of the total count of bacteria because their seed layer could be not enough distinguished to keep the entophytes from the apparent-disinfecting solution [25]. For the perfect tissues of plant *Arabidopsis thaliana* Cd, and pressure plucking out was used to isolate endophytic bacteria from seeds. This technique was applied to separate the xylem fluid from the roots of undying plants similar to grapevine [26, 27], numerous studies have established that the quantity and diversity of cultivable seed entophytes varies in different seeds [28, 29].

DISTRIBUTION OF ENTOPHYTES BACTERIA

Endophytic bacteria can enter in the plants by initial lateral roots breaking through the epidermis and cortex, thus obviously creating a ‘throughway’ for bacteria to go in at these places. Also, they may enter the cortex and xylem vessels that pass photosynthates (phloem) nutrients and water (xylem) [42]. They inhabit in the root and can move to young branches and leaves by leakage in the plant, this can result in injuries (e.g., leaf scabs, root breaks) for example a product of grassy or other physical damage [42]. For example, *Burkholderia sp*. strains PsJN colonize the root rhizodermis cells, inner soft tissue, specific inner parts, and grapevine plant leaves [43, 44]. Table **1** shows the distribution of

Table 1: Entophytic Bacterial Types Usually Isolated from Different Parts of the Diverse Plants' Yields

Part of the Plant	Endophyte Species	Plant types	Ref.
Seed	*Bacillus*, *Flavobacterium*, *Pseudmonas*	Cereal, vegetables, and woody plants,	[30]
Root	*Pseudomanas*, *Erwinia*	Alfalfa (*Medicago sativa L.*) Corn (*Zea mays L.*)	[31]
Root, radicle, stem, unpenedflowers, boll	*Erwinia, Bacillus, Clavibacter*	Cotton (*Gossypium hirsutum L.*)	[32]
Root	*Agrobacterium*, *Burkholderia*, *Serratia*	Cotton (*Gossypium hirsutum L.*)	[33]
Root	*Burkholderia*, *Enterobacter*	Corn (*Gossypium hirsutum L.*)	[34]
Root	*Pseudomanas*, *Bacillus*, *Enterobacter*, *Agrobacterium*, *Chryseobacterium*, *Burkholderia*	Cucmber (*Cucmissativis L.*)	[35]
Root	*Pseudomanas*, *Enterobacter*, *Bacillus*, *Serratia*	Rough lemon (Citrus jambhiri Lush.)	[36]
Root	*Bacillus*, *Erwinia*, *Pseudomonas*, *Corynebacterium*, *Lactobacillus*	Segar beet (*Beta vulgaris L.*)	[37]
Tuber	*Bacillus*, *Micrococcus*, *Pseudomanas*, *Bacillus*, *Flavobacterium*, *Xanthomonas*, *Agrobacterium*	Potato	[38]
Stem	*Enterobacter*, *Klebsiella*, *Pseudomanas*	Corn (*Zea mays L.)*	[39]
Stem	*Bacillus*	Corn (*Zea mays L.*)	[34]
Stem	*Bacillus*	Cotton (*Gossypium hirsutum L.*)	[34]
Stem	*Enterobacter*, *Pseudomonas*, *Rhodococcus*	Grapevine	[22]
Fruit	*Pseudomonadaceae*, *Enterobacteriaceae, Achromobacteriaceae*, *Micrococcaceae*	Tomato	[40, 41]
Fruit	Achromobacteriaceae, Micrococcaceae	Cucumber	[40, 41]

some endophyte bacteria in different parts of the plants azeotropic *Paenibacillus polymyxa* P2b-2R, widely inhabits the external surface and inside of roots, branches and spines of lodge pole pine (*Pinus contorta Dougl*. var. *latifolia Engelm*) [45]. There are signals that bacteria are present in soil with soil microorganisms such as rhizobium [46]. Also, endophytic bacteria can be distributed in phyllosphere epiphytes through normal openings (e.g., stomata, hydathodes), insect, wounds, and pathogens [47], and colonize the shoot layer cells, palisade mesophyll cells, xylem vessels in addition to spaces among soft mesophyll coating cells [48]. Entophytic bacteria are divided into obligate endophytes, which highly depend on the plant host for their growth, and facultative endophytes, with irregular biphasic lives among plants and soil [49]. Endophytic bacteria are noticed in different organs, like fruits, seeds, and flowers but in a few numbers [50-52]. [53] found *Pseudomonas* spp. and *Bacillus* spp. in grapevine colonizing the layer of epidermis and xylem of the fruit, intercellular spaces of tissue cells, and alongside cell walls into the seeds [53] found *Streptomyces mutabilis* strain IA1colonize the area inside the caryopsis in the seed of wheat plants.

COLONIZATION

Endophyte bacteria can establish and live inside the plant without causing any destruction of the plant [1]. It could connect to the root's superficial layer, which is possibly essential in reaching to suitable places at lateral root appearance areas or other buds produced by wounds or manual injuries. Endophytic bacteria might adjust gene expression while inhabiting in the plants [54, 55]. *Burkholderia kururiensis* M130 can infect and colonize the rice plant by genes coding proteins associated with bacterial motility, chemotaxis, and bond [54]. Entophytic bacteria are also producing exopolysaccharides (EPS), which may enable the addition of bacterial cells on the root superficial layer and might be significant in the primary stages of endophytic establishment. *Gluconacetobacter diazotrophicus* Pal5 produces EPS as a necessary influence for rice root superficial supplement and colonization [56]. Bacterial endophytes most frequently inhabit intercellular spaces in the plant, as these zones have the productivity of carbohydrates, amino acids, and inorganic nutrients [6, 3]. There are several types of plant surroundings which include tropic, marine, xerophytic, temperate, antarctic, mangrove, geothermal soils, deserts, littoral forests for natural colonization [57, 58]. The endophytic bacteria enters in plants through the epidermis, cortex, and endodermis, also it can enter the phloem and xylem vessels that transport photosynthetic materials [42]. ROS- detoxification occurs early during the interaction process once the endophyte gains access to the plant. Through primary phases of rice root colonization, endophyte diazotrophic *Gluconacetobacter diazotrophicus* is expressed. ROS- produces superoxide dismutase (SOD) and glutathione reductase (GR) in higher amounts. Furthermore, SOD and GR mutants of *G. diazotrophicus* might not establish in rice roots associate with the supposition that ROS-restricting genes are essential in the first stages of colonization [59]. Suggested that wheat grass were colonized by *Actinobacteria*, *Firmicutes,* and *Gammaproteobacteria* moving from the seeds and by exogenous bacteria capable to colonize the plant components. The interaction between Endophytes and plants is determined by the bacterial genes and the ecological situations [60, 42] and seems to be familial and fairly preserved [21]. Examined the microbial endo-phytic communal of seeds from the wild forebear to domestic maize harvested in diverse areas. Colonization patterns in plant entophytic have been studied by many methods and have been described as ‘obligate,’ ‘facultative’ or ‘passive’ depending on whether they involve plant tissue to alive and duplicate [61]. Obligate entophytic bacteria be present producing beginning from the seeds and are not live in soils, Facultative entophytic usually occur in soil, these bacteria stay inside the cortex nevertheless some transfer in the central cortex and xylem [62], while the passive endophytic, it is capable to inhabit and intervention to the part of the plant via wounds and cleft on the plant [63].

DETECTION OF ENTOPHYTIC BACTERIA

a. Plating Method

A simple method is used to enumerate entophytic bacteria by using simulated (selective or non-selective) media. The population can be determined by surface sterilization above the ground and crushing the tissues and serial dilution is done for counting the colonies grown in the media [64, 65] and the total count of bacteria (CFU)/g) in the root and shoot. This method cannot measure the population size of introduced bacteria. Using an artificial medium would bring only a fraction of practical microbes capable to absorb the feeding of the medium [66].

b. Sustainable Staining

This method is suitable to detect the entophytic bacteria in the roots of plants inoculated with bacteria. By using 2 ml from 2, 3, 5- T.T.C for 3-4 hours, bacteria convert the dye to red-color as a result of reduction of T.T.C to from azans [67]. This method has been successfully practical to discover bacteria in surface-cleansing corn roots [66] and to notice *Azospirillum brasilense* and *Bacillus polymxa* in surface sterilized maize plants in a previous study by the author, (Figure **1**) [68]. Colonization of bacteria was detected like a muddy zone nearby the roots [69]. The connection of diastrophic *Herbaspirillum seropedicae*, to the root faces of maize is influenced by LPS (liposaccharide) [70]. [45] found that diazotrophic bacterial strain *Paenibacillus polymyxa* P2b-2R extensively colonized the surface and interior of roots, and stems.

Figure 1: Colonization of maize roots by *Azosprillum Brasiliense.*

C-Electron Microscopy

Transmission electron microscopy (TEM) and Scan Electron microscopy (SEM) have been commonly used to observe and localize the entophytic bacteria inside plant tissue. Both electron microscopy fluorochromes and fluorescent stain were applied to test the particular bacterial endophytic establishments in the plant soft tissue. FISH, GFP group, GUS stain, and fluorogenic stain are communal structures related to microscopy to examine the establishment of endophytic bacterial in plants [64-65]. There is diverse microscopy proving that this procedure can be used to investigate the communication among the endophytic bacteria and the plant. Auto-fluorescence formed cell walls mainly in the leaf matters can end the usage of these systems however giving small focuses of lightening might increase the appearance of an image. These are used to detect entophytic bacteria in several plant species, like pea plants [71], rice (*Oryza sativa L.*) [72], for observing *Burkholderia kururiensis* in banana [73], for detection of *Brachybacterium, Micrococcus, Kocuria and Staphylococcus spp* in Sugar beet [68, 74]. Isolated the *Paenibacillus polymyxa* from maize using this technique (Figure **2**).

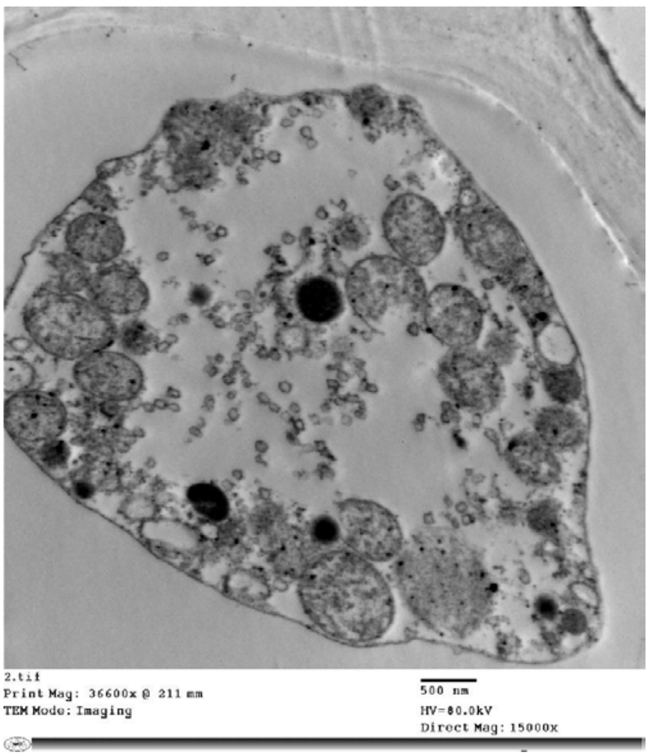

Figure 2: Transmission electron microscopy (TEM) of root hair for maize root colonization by *Bacillus polymxa.*

D-Immunological Staining

Immunological detection procedures have developed in microbial biology essentially for the pursuit of endophytic bacteria. For a dependable use of these methods, the Antibodies can be raised against bacteria by injecting them into rabbits or mice. Immunological methods are applied for the concurrence, quantification, and enhancement of specific antibodies. Immunological procedures could be associated with PCR methods using image treating of epifluorescence micrographs or laser scanning microscopy. It is applied to study complex environmental samples [75].

E-Nucleic Acid Hybridization

The inside of a plant tissue can be recognized through in situ hybridization by using polymerase chain reaction (PCR) methods to approve the presence of bacteria in the shoot and root tissue. Nucleic acid analyses is characterized through the connection of a haptin (i.e., biotin or digoxygenin), with an antibody leading to an apparent indicator, i.e., colloidal golden [76]. These methods are critical to discover endophytic bacteria in plant fluid [77] used the polymerase chain reaction (PCR) in rice inoculated with *Azoarcus sp* to approve these bacteria occurrences in the shoot and root tissues. Using rRNA for the examination of particular endophytic bacteria is another dominant device as nucleic acids have extremely well-maintained and limited areas [78].

F-Autoradiography

Autoradiography methods are also used to study the dynamic endophytic bacteria in the plant. The incorporation of radioactive material happens exclusively in molecules that are dynamically produced when. Endophytic bacteria are cultured in normal growth media containing (I5NH4) SO4 [79]. These bacteria can be tracked inside the plant by labeling on them the active labeled bacteria can be checked by mass spectrometry or autoradiography.

The Beneficial Effect of Endophytic Bacteria on Crops

1. Biological Nitrogen Fixation (BNF)

Nitrogen is necessary for plant growth. About 30–50% of the N in plants comes from the biological fixation of N_2 by soil microbes [80]. A large number of bacterial genes intricated in N cycling of rice roots, shows that the - nitrification and ammonia oxidation procedures in rice roots can be exposed to the impact of the endophytic root micro biome [81]. Endophytic bacteria arevery important in N cycling and there is a signal that N2 fixation by foliar endophytic bacteria has happened in numerous subalpine conifer species [82]. Some bacteria have been isolated and described as nitrogen-fixing endophytes from nonleguminous plants in previous times. Rice, sugarcane, and maize must be the most generally exposed grasses to find out if endophytic bacteria can be a part of the nitrogen essential for these crops. Rice has a wide range of endophytic diazotrophs [83]. The involvement of diazotrophic endophytic communal of rice has been identified by [84]. The genera *Herbaspirillum* [85] and Burkholderia [86] were primarily designated as the highest diazotrophic endophytes isolated from rice. *Azoarcus*as though was eventually isolated from Kallar grass [87], which could inhabit the rice in laboratory trials [77]. There is a diversity of diazotrophic bacteria such as Pseudomonas, Stenotrophomonas, Xanthomonas, Acinetobacter, Rhanella, Enterobacter, Shinella, Agro- bacterium, and Achromobacter [88], which were isolated from the sugarcane plant. The capability of *G. diazotrophicus* for growing and fixing nitrogen in sugarcane internal tissues has been proved [89]. Its positive effect on the plant construction relies on the bacterial mass, and then bacterial transduction may be essential for the PGP possessions of *G. diazotrophicus*. In recent times, the incidence of Gluconacetobacter-similardiazotrophs was found in different plants like sweet potato, tea, carrot, radish, and rice, with extensive physical spreading [90]. Among the species that have been isolated Stenotrophomonas and Burkholderia belong to nitrogen fixers bacteria [91] nitrogen fixing bacterial endophytes have a wide host variety. Nitrogen fixing bacteria are frequently present in Gramineae plants but are not special. *Herbaspirillum seropedicae* was established in a diversity of crops, including maize, sorghum, sugarcane, and other Gramineae plants [85, 92]. [93] isolated *Herbaspirillum* strain from rice it may establish in the sugarcane plant and the Burkholderia sp. isolated from onion can also grow in grapes [43]. Inoculation of sugarcane with a mixture of bacterial *Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae, H. rubrisubalbicans, Azospirillum amazonense* and *Burkholderia* species amplified 30% of its nitrogen content [94].

2. Plant Growth-Promoting Endophytic Bacteria

Endophytes bacteria also have paramount importance in the plant growth through direct and indirect, mechanisms. Directly, they backup the plant growth by phosphate solubilization activity [95, 96], siderophore production [97] and indole acetic acid (IAA) production [98]. Also, these bacteria can create plant hormones and improve other useful microorganisms [99]. Indirectly, they improve plant growth by prompting plant protection responses. PGPR endophytic bacteria-induced stomata regulation, osmotic instruction, and variation in the morphology of root, improve the absorption of minerals [100, 101]. They are being used for phytoremediation of contaminated soils. Phytohormones are recognized as auxins, gibberellins, and cytokinins which stimulate morphological modifications of roots, enhancing the absorption of minerals and water [102, 103]. Microorganisms such as *Acinetobacter* sp., *Azospirillum* sp., *Azotobacter* sp., *Pseudomonas* sp., and *Bacillus* sp. create phytohormones like indoleacetic acid, cytokinins, and mixtures that simulate the behavior of jasmonates in existence of the plant [104].

3. As a Bio-Control Agent Against Plant Diseases

Endophytic bacteria have beans used as bio-control agent in agriculture [105]. These may be useful as bio-control agents by direct inhibition of pathogens and by indirect mechanisms. A direct mechanism is through the production of hydrogen cyanide (HCN), which plays a critical role in providing iron nutrition to the plants and antifungal metabolites [42]. [106] stated that endophytic bacteria produce hormones, which manage plant growth genes coding the proteins for bio-installation of indole acetic acid (IAA) [107], production of cytokinins (CKs) [108] as well as gibberellins (GAs) [109]. *Sphingomonas* sp. LK11 is an endophytic bacterium that improved the growth of tomato plants because it was intermediated by the creation of GAs and IAA [110]. This research sheds light on the indirect plant-bacterial interactions that encourage the growth of plants. The Siderophores are produced by endophytes when iron is deficient in the soil. Siderophore-producing plant growth-endophytes can be encouraging to chemical fertilizers [111]. Indirect resistance of endophytic bacteria through plant-induced resistance inhibits a large number of plant pathogens [112]. Endophytic bacteria antibiotics that control the cell metabolic rate and are an effective tool for controlling the plant pathogens. But, the efficacy of an antibiotic on one pathogen strain may differ in other strains of similar species because of the existence of genomic resistance tools, or the difference in environmental conditions [102]. Species such as *Pseudomonas* sp. and *Bacillu*s sp. create peptides bio surfactants that can be essential in competitive connections of diverse groups of microbes with nematodes and plants [106]. In many researches, biological control of maize, wheat, and legume plants has been studied against many diseases as well as the post-harvest control of plants like pepper and apple [113]. Endophytic bacteria could improve plant protection responses, and their systemic resistance (ISR) may be accepted as a device for disease managing in agriculture. Genera *Pseudomonas* and *Bacillus* might be the most common groups that produce ISR, however, it is not limited to these sp. only [114].

4. Increase Nutrient Uptake Productivity

Endophytes have the ability to solubilize some mineral deposits and fixed nitrogen.

A. Nitrogen

Endophytic bacteria characterize an exclusive class of bacteria that could inhabit the internal parts of a plant and provide benefits to the plant related to those provided by the rhizospheric microorganisms.

The legumes have the capability of using symbiotic associations with specific nitrogen-fixing bacteria therefore nitrogen fixation is called plant procedure. Though, the chief role is performed by nitrogenase enzyme synthesis in these associations, which is produced and confined in the endophyte named endosymbiont. Such bacteria are known as rhizobia and belong to diverse genres, such as Rhizobium, Sinorhizobium, Bradyrhizobium, Mesorhizobium, Azorhizobium, etc. Other endophytic species are from the genera Azospirillum, Herbaspirillum, Klebsiella, Acetobacter, etc. [115]. It is nteresting amazing to note that endophytes can be implicated in the complete nitrogencycle, like proteins implicated in N2-fixation, denitrification, and nitrification were noticed and then selectedfor genes expressed [81].

B. Phosphorus

The use of phosphate fertilizers, one of the macronutrients that regulate plant growth, in plant nutrition is important to increase plant growth [116]; Using normal sources of phosphate fertilizers is limited due to the low ion –exchange process of acidic tropical soils [117]. Additionally, use of soluble phosphate chemicals in fertilizing is expensive. Phosphate-dissolving bacteria play a major role in providing plants with phosphate in different environmental conditions [118, 119]. Endophytic PSB could be applied to efficiently utilize the nutrient as they dissolve the phosphate and make it accessible for absorption by plants [50]. These bacteria can colonize the root superficial layer and internal parts of the plants. Many studies have been conducted on common but different species of endophytic bacteria capable of solubilizing mineral phosphates [120]. The capability of phosphate-solubilizing bacteria shows that endophytic bacteria rhizobium function as growth stimuli [50].

C. Siderophore Production

Siderophores are small molecular with iron-chelating minor metabolites produced by different groups of endophytic bacteria for assistance in iron-restricted conditions. Siderophores created by endophytic bacteria help plants to grow by supplying iron to the plants. Endophytes produce two types of siderophores; Hydroxamate and catecholate [121]. Iron is an essential micronutrient for crops, complexes as a cofactor in numerous enzymatic reactions; it exists in the extremely indissoluble hydroxide form in the soil and is not available to plants. [122] found that siderophore making is a widespread attribute of endophytes [123].

D. Zinc Solubilization

Zinc is an essential micronutrient for micro flora in addition to plants. Zinc is present in the earth in proportion of 0.008%. It plays an important role in feeding both eukaryotic and prokaryotic bacteria as cofactor stimulating numerous enzyme reactions [124]. Endophytic bacteria have the ability to solve immobilize zinc such as zinc oxide, and zinc carbonate [125] found that, many genera of bacterial viz. *Thiobacillus thioxidans, Thiobacillus ferroxidans, Acinetobacter, Bacillus, Gluconacetobacter, Pseudomonas,* and facultative thermophilic iron oxidizers were zinc solubilizers. Lack of zinc in basic food crops, apart from the loss of yield, leads to a decrease in the zinc content in grains and thus may serious damage to human nutrition [126]. Use of zinc solubilizing bacteria (ZSB) could be an ecological method for the increase of Zn obtain ability in soil. Numerous Zinc solubilization bacteria have been described from warm and mild soils [127]. Usually, nitrogen-fixing bacteria such as *Rhizobium, Azospirillum,* and *Azotobacter* are not the zinc-solving bacteria while Acinetobacter, Bacillus, and Pseudomonas are zinc-solving bacteria [125]. They appear to be present in roots, stems, shoots, and grains of many plant types [1]. These zinc-solving endophytic bacteria nitrogen fixing bacteria may be used in association with nitrogen fixing bacteria in grain yields to meet double objectives of fixing atmospheric nitrogen besides zinc solubilization [128].

Adaptation of Endophytic Bacteria under Stress Condition

Endophytic bacteria have a major role in overcoming the negative effect of the presence of salts in the soil and promoting plant growth under stress conditions because they can adapt to harsh environmental conditions under drought or salt stresses or any other conditions [2]. [129] found that inoculations of grape plants by endophytic bacteria *Burkholderia phytofirmans*- PsJN improved the growth under cold stress conditions by increasing the photosynthetic activity and absorption of carbohydrates complex. The presence of endophytic bacteria in the plant made the plant adapt to high temperatures, which led to a decrease in cell damage, and increase in photosynthetic activity; and the accumulation of receptors associated with frost stress such as protein, phenolic compounds, and starch. They help in mechanical resistance by regulating plant hormones and detoxification in plants that improves growth under salt stress. Under osmotic stress there is a decrease in the rate of photosynthesis, and also the stomata are closed and the leaf area decreases, and the rate of carbon dioxide fixation inside the cell prevents light reactions in the end [130]. Reactive oxygen species (ROS) have very essential roles in plant cell cycle systems. ROS in plant cells mostly includes (superoxide anion radical), H2O2 (hydrogen oxide), OH (hydroxyl radical), and O2 (singlet oxygen), which are mostly produced from chloroplasts, mitochondria, and peroxisomes [131]. The bacterial cells produce the anti-oxidant compounds like (carotenoids, flavonoids, and otherphenolic) and anti-oxidative enzymes [superoxide dismutase (SOD), glutathionereeducates (GR), catalase (CAT) and peroxidases (POD)] to reduce the harmful effects of increased ROS concentrations [132].

1. ACC: Deaminase Activity

Ethylene (ET) is a major organizer of salinity stress; it is prepared from methionine by S-adenylyl-Methionine that is changed to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC oxidase [133]. Plants under stress conditions produce high concentrations of ET; this leads to a decrease in the plant growth and causes cell wall death [134]. High levels of ET in the plants under stress conditions reduce the cell division, DNA creation and decrease the growth of roots and aerial parts of the plants., Inoculation of plants with endophytic bacteria producing ACC deaminase (1-aminocyclopropane-1-carboxylic acid) enzyme, leads to a decrease in the amount of ethylene level in the plants, as well as ACC deaminase strengthens internal plant immunity and stress tolerance and encourages seed germination [102]. Endophytic bacteria use1-aminocyclopropane-1-carboxylate (ACC) as a carbon and nitrogen source under high pressure of ET by producing ACC [135, 136]. The ACC accumulates in the roots spreads directly to the buds [137, 138]. Endophytic diazotrophic like *Achromobacter xylosoxidans* AUM54 isolated from *Catharanthus roseus* can create ACC deaminase and decrease the ethylene levels under salinity stress [138]. Furthermore, *Achromobacter xylosoxidans* strain Ax 10 and *Pantoea agglomerans* Jp3-3 created ACC deaminase to reduce stress. *Brassica* sp, bacteria enhanced the growth of plants grown in copper- polluted soils and improved copper uptake by the plants [139]. Isolated entophytic bacteria isolated from *Commelina communis* plants grown-up on a soil affected with zinc, creating ACC deaminase increased the growth of rape plants in the lead-polluted soil.

2. Drought Stress Tolerance

[140] Endophytic bacteria can help plants to tolerate drought. Plants need several mechanisms to adapt to adverse environmental conditions. Among these is the establishment of an interactive regulatory signaling for exchange in specific pathways with cofactors. Signaling during biotic or abiotic stresses involves an interactive regulatory network with frequent interchange between individual pathways and signal molecules/cofactors [141]. Phytohormones; ROS, Ca2+, NO2, phosphates, etc. serve as signaling molecules. Drought stress leads to osmotic pressure whereas in the case of salt, stress together with osmotic pressure either ionic or ion-toxicity influences also act on cells. The increase of the phytohormone abscise acid (ABA) is noted that encourage the adaptive reactions in plants during drought and salt stress [142]. Molecular units such as Ca^{2+} and NO- play significant roles in signaling during stress response pathways via hormones. Nitric oxide or Ca^{2+} signaling show a chief role in plant protection responses, ABA-independent stomata actions, and drought stress conditions [143]. Potato plants inoculated with endophytic *B. phytofirmans* PsJN showed a diverse effect of functionalities [144]. Reactive oxygen species (ROS) produced under drought stress are extremely reactive and harmful to the cell, and might lead potato plants to necrosis.I Inoculation of entophytic *B. phytofirmans* PsJN adjusted the environments and induced biological changes in plants via changes in gene expression. A positive influence was observed among bacterium on metabolic equilibrium which reduced the result of drought stress in wheat plants grownup under decreased irrigation conditions [145]. Endophytic *Pseudomonas pseudoalcaligenes* can induce resistance to rice plants under drought conditions by producing high concentrations of glycine [146, 147] as well as endophytic *Azospirillum* spp, when inoculated in maize plants led to improve the growth of maize by producing the abscise acid (ABA) IAA and gibberellins. ABA is phytohormone working on regulation of plant water balance and osmoticstress tolerance [148].

Commercial uses of Endophytic Bacterial Products

Endophytic bacteria are used as bio-fertilizer, plant strengthener, microbial bio-stimulators improving soil., the laws for use of these bacterial products vary from country to country, and the product must be presented as a plant protection product (PPP) [149]. In common, the bio-fertilizers are characterized by their practicality for example using them as liquid inoculants, added directly to the soil and seeds [150]. However, the inoculum must be preserved to maintain bacterial vitality for a long time [151]. Although used in previous years, the liquid inoculants are generally not applied today because it is expensive compared to new techniques. EU must focus to properly protect the product and maintain its capacity. The establishment of companies can contribute significantly to the success of the process of preserving the inoculant and keeping records of the inoculant process [152].

Encapsulation of endophytic bacteria with alginate

Alginate is the most common material used for the encapsulation of bacteria. Inoculates are used for many objectives such as application as biological control agents and mycoherbicides for increasing stability of plasmids in plant cells as well as in bacterial chemo taxis [153, 154]. The main purpose of the manufactured formulations is to save the cells and maintain activity for as long as possible. Encapsulated processes in agriculture industry require at least two different goals (i) maintaining encapsulated bacteria in the soil and bacterial competition and (ii) slowing the exit of bacteria to and colonize plant roots [155]. The characteristics of the alginate material are that it is nontoxic, natural, and biodegradable [156]. This carrier of alginate sodium was carefully chosen after optimization of the encapsulation process which includes evaluating the alive bacterial alive with regard to polymer characteristics, i.e., eco-friendly, bio-compatible, and inexpensive. Encapsulation technique must be carried out in several stages including capability to formula beads, small viscous solution) and fast drying. I In microencapsulation, the capsules have a size reaching between 1 to 1000 μm [157]. Figure **3** shows the *Azosprillum lipoferum* capsulated with sodium alginate [158-160].

Figure 3: Endophytic bacteria capsulated with sodium alginate.

CONCLUSIONS AND FUTURE PERSPECTIVES

Endophytic bacteria have proven to play a role in plant tolerance to difficult environmental conditions besides, it has great biological importance in improving soil fertility and quality. Entophytic bacteria have been applied as an alternative to mineral fertilization, as mineral fertilizer pollutes the atmosphere. A large group of these bacteria isolated from plants showed their great importance in maintaining plant growth and effectiveness in preserving agricultural ecosystems. The composition of the bacterial population depends on the genetic composition of the plant as well as on environmental biotic and abiotic stresses. The commercial use of endophytic bacteria to enhance the growth and production of crops is not as easy as plant growth-promoting bacteria (PGPR), so selected and approved studies must be conducted on how to use them in commercial formulations.

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