

Endophytic nitrogen-fixing bacteria: Untapped treasurer for agricultural sustainability

Kusam Lata Rana¹, Divjot Kour², Tanvir Kaur¹, Rajeshwari Negi¹, Rubee Devi¹, Neelam Yadav³, Pankaj Kumar Rai⁴, Sangram Singh⁵, Ashutosh Kumar Rai⁶, Ashok Yadav⁷, R. Z. Sayyed⁸, Ajar Nath Yadav^{1*}

¹Microbial Biotechnology Laboratory, Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India.

²Department of Microbiology, Akal College of Basic Sciences, Eternal University, Baru Sahib, Himachal Pradesh, India.

³Department of Food Technology, Gopi Nath P.G. College, Veer Bahadur Singh Purvanchal University, Ghazipur, Uttar Pradesh, India.

⁴Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India.

⁵Department of Biochemistry, Dr. Ram Manohar Lohia Avadh University, Faizabad, Uttar Pradesh, India.

⁶Department of Biochemistry, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Kingdom of Saudi Arabia.

⁷Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India.

⁸Department of Microbiology, PSGVP Mandal's Arts, Science and Commerce College, Shahada, Maharashtra, India.

ARTICLE INFO

Article history:

Received on: June 17, 2022

Accepted on: September 12, 2022

Available online: January 22, 2023

Key words:

Biological nitrogen fixation,
Endophytic bacteria,
plant growth promotion,
Sustainable agriculture.

ABSTRACT

Nitrogen (N) is one of the vital elements required for proper growth and development of plants. In the earth's atmosphere, N is available in the form of nitrogen gas (N₂) and mostly plants utilize N in the form nitrate (NO₃⁻) and ammonium ion (NH₄⁺) which are fixed through the biological process known as N₂ fixation. As N is one of the elements most likely to be limiting to plant growth, this phenomenon provides an alternative to the implementations of chemical fertilizers as source of nutrients which have resulted in the ammonia volatilization, leading to significant impact on global warming in the atmosphere which, further, diverts the focus of scientist to find out eco-friendly technology. Globally, the demand for introducing eco-friendly practices for improving sustainable agriculture productivity has been increased. Since long time, microbes play an important role in providing pollution-free environment. Endophytic microbes being present inside the specific tissues of plants mostly empower in the growth of plants. The endophytic nitrogen-fixing microbe has been well characterized from leguminous as well non-legume crops. Endophytic bacteria belong to different phyla such as Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. The predominant N₂-fixing endophytic *Burkholderia*, *Rhizobium*, *Pseudomonas*, *Bradyrhizobium*, *Bacillus*, *Frankia*, *Enterobacter*, and *Azospirillum* have been reported from different host plant. Nitrogen-fixing endophytic bacteria has a wide variety of application for maintaining growth of plant, crop yield, and health of soil for sustainable agriculture. The present review focuses on major developments on biodiversity of N-fixing endophytic microbiomes and their role for plant growth promotion and soil health for agroenvironmental sustainability.

1. INTRODUCTION

Nitrogen (N) is one of the essential macronutrients that help in the plant growth and development. It is also an important component of proteins, nucleic acids, protoplasm, chlorophyll, and other nitrogenous compounds [1]. Nitrogen is abundantly available in earth's atmosphere but cannot be utilized by the plants so it must be converted into ammonia (NH₃) or related nitrogenous compounds. This conversion of atmospheric N (N₂) is referred as N₂-fixation. The N₂ fixation process

is considered as the most important process influencing primary productivity, after photosynthesis. This process is carried out by physicochemical and biological process (through microbes) that fixes 10% and 90% natural nitrogen, respectively [2,3]. Biological fixation of nitrogen occurs in certain restricted plant species due to microbe's specific niche. Over 102 years, the process of biological N₂ fixation (BNF) is studied in legumes and is restricted to prokaryotes, as N₂ fixation is an anaerobic process [4].

To make nitrogen available to non-leguminous plants, it is supplied as external source and is prepared by the Haber-Bosch process. This process of manufacturing ammonia has made the most valuable invention and saved millions people worldwide [5]. About 450 million tonnes of nitrogen fertilizer per year, in the form of anhydrous ammonia, ammonium nitrate, and urea are produced by Haber process. Haber-Bosch is perhaps the most significant invention of the

*Corresponding Author:

Dr. Ajar Nath Yadav,

Microbial Biotechnology Laboratory, Department of
Biotechnology, Dr. Khem Singh Gill Akal College of

Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India.

E-mail: ajarbiotech@gmail.com

20th century; yet, it has numerous harmful effects, including reduced biodiversity, formation of aquatic algal blooms, which also contributes to air pollution as well as climate change ultimately contributing to global warming [6]. Therefore, the utilization of alternative resources as microbes in forms of bioinoculant, which are cost effective and eco-friendly, is essential. In recent time, much interest is focused on endophytic microbes as potential biofertilizers. The significance of biofertilizers is to enhance the plant growth, through combination of BNF, along with solubilization of various macronutrients [phosphorus (P) and potassium (K)] and micronutrients such as zinc (Zn) from soil, synthesis of hormones, and inhibiting the growth of various phytopathogens [7].

Microbes residing inside the tissue of plant host for at least part of its life cycle without causing apparent disease are referred to as endophytes [8]. Endophytic microbes are better in comparison to rhizospheric microbes as they provide N directly to the host plant [7]. Endophytic microbes are guarded from abiotic stress condition such as change in pH, temperature, water availability, and nutrient plus biotic stress such as competition [9,10]. Endophytic microbes colonize various parts of plant (roots, stem, leaves, flowers, fruits, and seeds) and transferred from one generation to next one either by vertical or horizontal transmission [11]. Cavalcante and Dobereiner [12] first laid the foundation of isolation of a N₂-fixing bacteria *Gluconacetobacter diazotrophicus* from internal tissues of sugarcane, latter on agriculturally important crops such as rice, wheat, maize, and kallar grass received much attention of scientists for isolation of N₂-fixing endophytic microbes. Endophytic microbes associated with leguminous and non-leguminous plants facilitate the growth, crop yield, and soil health. Endophytic microbes have been reported such as *Acetobacter diazotrophicus* (sugarcane) [13], *Acinetobacter lwoffii* (wheat) [14], *Azoarcus* (rice) [15], *Azospirillum brasilense* (maize) [16], *Acinetobacter calcoaceticus* (soybean) [17], and *Bradyrhizobium* (peanut) [18]. Present review presents an overview and recent advances in the understanding mechanism of N₂-fixing endophytic bacteria and their biotechnological applications for plant growth and soil health for agricultural sustainability.

2. BIODIVERSITY AND DISTRIBUTIONS

The microbial biodiversity is higher as compared to vertebrates, insects, and plants. Microbes have adapted to diverse habitat and reported from natural as well as from extreme environment. The diverse microbial groups were reported for their association with several host plants as plant microbiome (epiphytic, endophytic, and rhizospheric) [19]. The predominant and abundant group of microorganisms on earth reported as endophytic bacteria belongs to the phylum Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria [Figure 1a and b]. The phylogenetic tree was made using the MEGA 4 software to know the taxonomical affiliations for endophytic microbes isolated from diverse sources. The phylum Proteobacteria is further grouped into different classes such as α -, β -, and γ - proteobacteria. The member of phylum Proteobacteria with nitrogen-fixing capability has been reported as most dominant from diverse host plants. Least number of N₂-fixing endophytic bacteria has been reported from the phylum Bacteroidetes, Actinobacteria, and from Firmicutes [Figure 2]. On review of different host plants, the most dominant genera includes *Burkholderia*, *Rhizobium*, *Pseudomonas*, *Bradyrhizobium*, *Bacillus*, *Frankia*, *Enterobacter*, *Azospirillum*, *Klebsiella*, *Herbaspirillum*, and *Acinetobacter* which have been regarded as nitrogen-fixing endophytic bacteria. The relative distributions of N-fixing endophytic genera have been analyzed using studies from different host plants [Figure 3].

Among different genera, *Burkholderia* belongs to the phylum Proteobacteria and class β -proteobacteria has been reported to colonize maize (*Zea mays*) [20], soybean (*Glycine max*) [21], sugarcane (*Saccharum officinarum*) [22], and rice (*Oryza sativa*) [23]. *Burkholderia* sp., in a study, was reported to fix atmospheric N along with solubilization of P and also synthesizes indole-3-acetic acid (IAA) [21]. Further, novel species of genera *Burkholderia* as *Burkholderia unamae* MTI-641^T and *Burkholderia silvatlantica* SRMrh-20_T were isolated as endophytes from sugarcane [22]. The second most predominant genera *Rhizobium* also belongs to the phylum Proteobacteria and class α - proteobacteria has been reported to colonize rice [24], soybean [25], faba bean (*Vicia faba*) [26], clover (*Trifolium*) [27], beans (*Phaseolus vulgaris*) [28], and wild rice [29]. The various studies reported the plant growth promoting (PGP) potential of *Rhizobium* sp. as it plays key role in fixation of atmospheric nitrogen [30]. *Rhizobium vallis* CCBAU 65647^T and *Rhizobium hidalguense* FH14^T were reported as novel N₂-fixing endophytic species from *P. vulgaris* [31]. Another predominant genera *Pseudomonas* belong to the phylum Proteobacteria and class γ - proteobacteria has been reported to colonize different host such as sugarcane [32], maize [33], rice [34], and soybean [17]. One of the species of *Pseudomonas* as *Pseudomonas putida* showed various multifarious PGP attributes N₂ fixation, production of phytohormones and siderophores has been reported by Zhao, Xu [17].

2.1. Rhizobia

During the 17th century, on roots of legume, people observed “bumps” which laid the foundation for discovery of microorganisms inside nodules responsible for fixing atmospheric N. The organism inside nodule has been identified as vibrio-like or bacteria-like named as rhizobia [35]. *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium*, are collectively referred to as rhizobia. Symbiotic N₂-fixing rhizobia belong to the family Rhizobiaceae. In 1889, the first species of genus rhizobia was identified as *Rhizobium leguminosarum*. Rhizobia are diverse group of soil bacteria established *Rhizobium*-legume symbiosis. The symbiotic association of rhizobia with leguminous plants is a complex process which results in the development of nodule like structure. The nodules are the house for N₂-fixing endophytic microorganisms. It has been already documented in leguminous plants that *Rhizobia* species (*Azorhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*) act as natural endophytes and perform BNF to great extent [36]. The species of rhizobia is not only limited as an endophyte to leguminous plants, the member of rhizobia has been reported to colonize the roots region of non-legumes wheat, maize, and barley. Roots of rice were found to be attached by rhizobia. The bean plant inoculated with transformed *Rhizobium* resulted in an increased N content of seeds, nitrogenase activity, and leaf content [28]. *Azorhizobium caulinodans* has been reported in roots of rice [36]. *Azorhizobium* is symbiotic bacteria form nodule on the stem, fixes N₂, and produces large amount of IAA [37]. *Bradyrhizobium* reported to enhance the P, K, N, and organic content in soil and fixes N₂ efficiently in *Mucuna* seeds [38]. *R. pisi* (*Trifolium*) [27] and *R. smilacinae* (*Smilacina japonica*) [39] have been isolated from different hosts.

2.2. Frankia

The member of *Frankia* belongs to family *Frankiaceae* in the order Actinomycetales, which has been reported as symbiotic association with higher plants. The genus *Frankia* consisting of high G + C% are Gram-positive grows at a temperature of 28–30°C and are reported as slow growing N₂-fixing endophytes [40]. *Frankia* inhabits vital

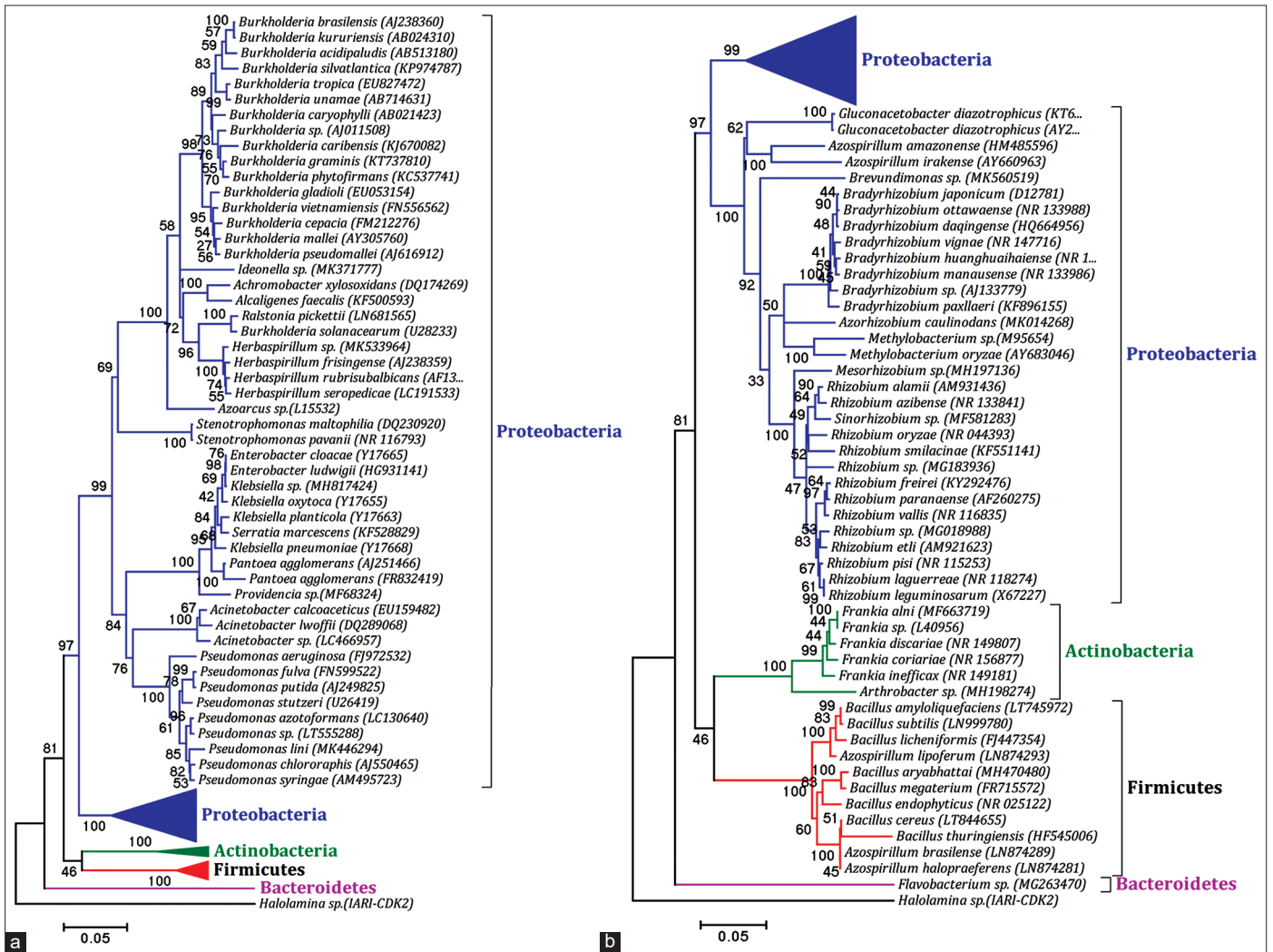


Figure 1: (a) Phylogenetic tree showing the relationship among different groups of nitrogen-fixing endophytic microbiomes isolated from different host plants, (b) Phylogenetic tree showing the relationship among different groups of nitrogen-fixing endophytic microbiomes isolated from different host plants.

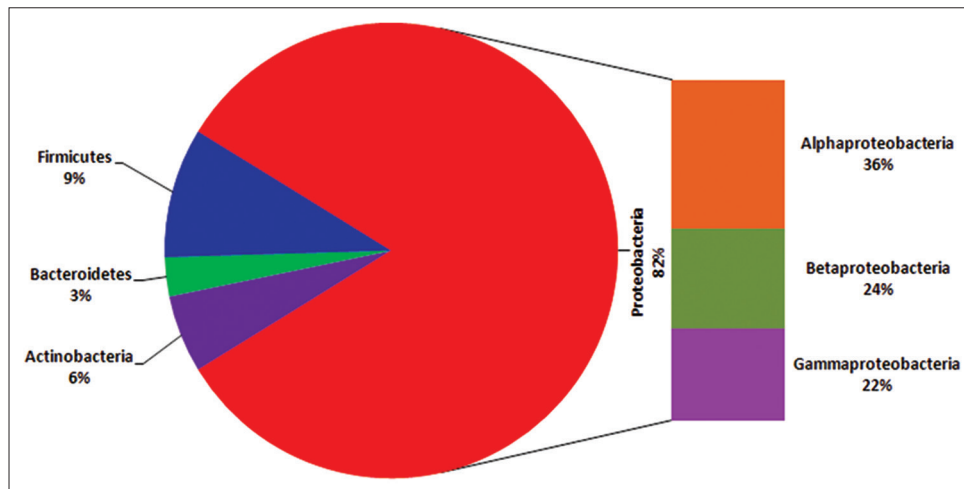


Figure 2: Abundance and relative distribution of nitrogen-fixing endophytic microbes belonging to diverse phylum.

ecological niche in the living plant tissue and also express nitrogenase enzyme, thereby fix the atmospheric N_2 and transferred fixed N_2 to their host [41]. In the recent time, various studies revealed nodule

of actinorhizal plants harbor a broad range of bacteria [42]. In non-leguminous plants, *Frankia* was known for development of N_2 -fixing nodules [43]. *Frankia* mostly established symbiotic association with

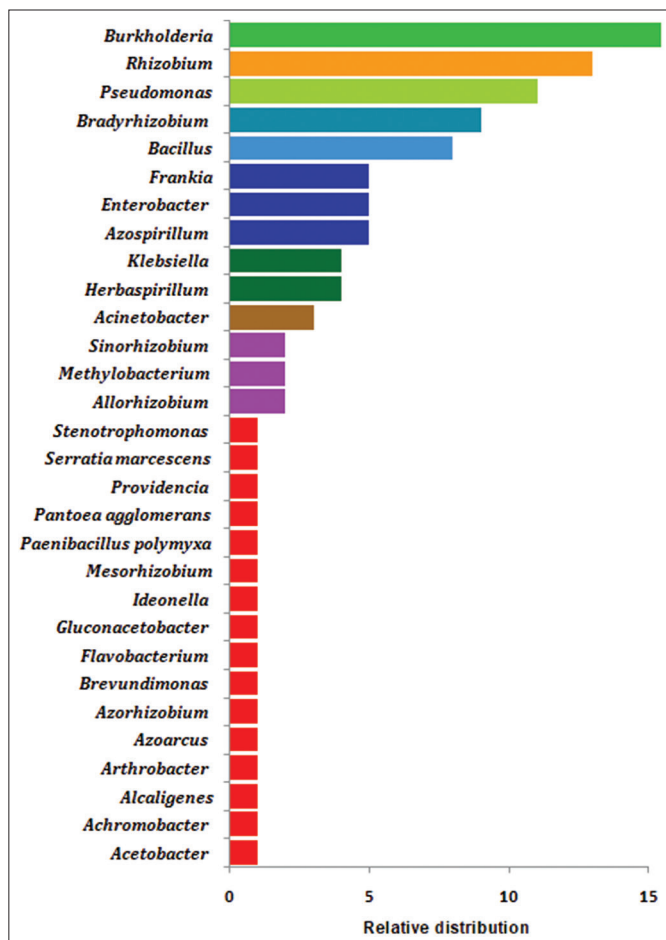


Figure 3: Relative distribution of predominant genera of nitrogen-fixing endophytic microbes reported from different host plants.

higher plants such as *Alnus*, *Elaeagnus*, *Coriaria*, *Casuarina*, *Datisca glomerata*, *Elaeagnaceae*, and *Myrica* species allows the availability of element such as N and P [44]. Different species of *Frankia* such as *F. elaeagni*, *F. alni*, *F. inefficax*, *F. discariae*, *F. coriariae*, and *F. discariae* has been reported from more than 24 host plants [45]. In a study, *Frankia* has been reported as N_2 -fixing bacteria isolated using culture-independent methods from *O. sativa* [46], *G. max* [47]. In a study, inoculation of *Casuarina equisetifolia*, *Acacia nilotica*, and *Eucalyptus tereticornis* plant with *Frankia*, arbuscular mycorrhizal fungi, *Rhizobium*, and *Azospirillum* has been done as a single inoculation or as consortium which finally revealed increased output with consortium than single inoculation. The plants absorb more P from soil, which developed more nodules with high nitrogenase activity as compared to control [48].

2.3. *Azospirillum*

Azospirillum is associative N_2 -fixing rhizobacteria also known to synthesize various antifungal, antibacterial, and siderophore compounds that are a Gram negative, aerobic, N_2 -fixing, and non-nodule forming bacteria belonging to family Spirilaceae [49]. *Azospirillum* is a potential N_2 -fixing microsymbiont as well as associative diazotrophs of grasses which also penetrate the root of gramineous plant species [50]. The endophytic nature of *Azospirillum* in rice plant has been demonstrated by Rodrigues, Rodrigues [51]. Under the genus *Azospirillum*, many species were discovered such as *A. lipoferum*, *A. amazonense*, *A.*

brasiliense, and *A. halopraeferens* [52]. The inoculation of plant with *Azospirillum* increases the water absorption and nutrient uptake which happen due to more development of root system. The inoculation of plants with *Azospirillum* results in the better root system development with highest efficiency in water and nutrients absorption from soil [53]. The *Azospirillum* genera demonstrated its potential application in agricultural systems, up to 30% in productivity [54]. The endophyte *A. lipoferum* reported to produce phytohormones such as abscisic acid and gibberellins which result in the alleviation of water scarcity [55]. *O. sativa* inoculated with bacterial endophyte, *Azospirillum* sp. B510, resulted in improved resistance against phytopathogens *Magnaporthe oryzae* and *Xanthomonas oryzae* [56]. The application of rice plants with *Azospirillum* resulted with an improved aerial biomass along with an increased content of N by 16 and 50 kg ha⁻¹ [57]. The *Azospirillum* promoted the plant growth by secretion of various growth promoters (IAA, gibberellins, and cytokinin), uptake of various nutrients and enhances the development of root of plant [58].

The inoculation of non-leguminous plant with growth-promoting N_2 -fixing endophytic bacteria is achieving its value globally. The *Z. mays* treated with *A. brasiliense* strain Ab-V5 designated that the treatment was proficient in stimulating growth of plant and also increases crop productivity [59]. In an experiment, the rice plant co-inoculated with *Rhizobia* and *A. brasiliense* efficiently promoted seed germination, increased wetland rice production which may, further, decrease production costs and negative impacts on environment caused by usage of N fertilizers [60]. The inoculation of legumes with *Rhizobia* and azospirilla could over-improve the activity of plants, in comparison with a single inoculation. Over the year, significant attainments were gained from the research that is the employment of azospirilla as a commercial inoculants for cereals or legumes as well as non-legume crops [61]. *Azospirillum* sp. B510 originally isolated from rice demonstrated its skill to activate the innate immune system in tomato plant against *Pseudomonas syringae* and *Botrytis cinerea* the causal agent of bacterial leaf spot and gray mold, respectively [62]. *Azospirillum* sp. B510 induces systemic disease resistance in the host and further study signified that involvement of ethylene (ET) signaling is necessary for endophyte-mediated ISR in rice [63]. For increasing the production of different agricultural product, *Azospirillum* could be used as a potential biocontrol agents and biofertilizers [64].

2.4. *Herbaspirillum*

Herbaspirillum is an endophytic N_2 -fixing organism known to colonize the intercellular spaces of roots of maize, rice, sorghum, and sugarcane. *Herbaspirillum seropedicae* has been first reported as N_2 -fixing bacterium associated with the roots of *O. sativa*, *Sorghum bicolor*, and *Z. mays* [65]. The endophytic nature of *Herbaspirillum* was confirmed by fluorescence and electron microscopy as the DNA sequence of bacteria was tagged with green fluorescent protein (GFP) [66]. Eighty different strains of *H. seropedicae* fix 31–45% of N in rice from atmosphere. Under aseptic condition, N_2 fixation by *Herbaspirillum* was 33–58 mg tube⁻¹ [67]. In wheat plant, *H. seropedicae* acts as an endophytic diazotrophs and also utilizes diverse range carbon sources [68]. *H. seropedicae* is well known diazotrophic endophytic bacteria that interact with various species of plant belonging to family Graminae. In addition to diazotrophy, *H. seropedicae* possesses complete machinery for siderophores, auxin, and ACC deaminase synthesis which makes it suitable for enhancement of plant growth [69]. *H. seropedicae* is an endophytic diazotrophs reported for colonization with various plants and also increase their productivity [70]. *H. seropedicae* colonizes several non-leguminous plants, uses various mechanisms for BNF,

solubilization of minerals, synthesis of phytohormones, and by acting as a biocontrol agent. The treatment of *Z. mays* L., with combination of humic substances and *H. seropedicae* resulted with variation of sugar and N metabolism, and better net photosynthesis along with better production of maize about 65% under field conditions [71].

Miscanthus sinensis inoculated with *Herbaspirillum frisingense* GSF30^T significantly promoted root and shoot growth affected the architecture of root by modulating phytohormone signaling pathways which ultimately affected the architecture of root [72]. The wetland rice inoculated with biological fertilizer (mycorrhizal fungi and *H. seropedicae*) extensively improved the productivity and also enhanced fortification and root growth. Integrated nutrient management with biological and chemical fertilizers advances the yield of *O. sativa* L., also health and fertility of soil [73]. In a study, *H. seropedicae* has been demonstrated for its colonization with the roots of *Triticum aestivum*. The transcriptomic analysis of *H. seropedicae* attached to roots of wheat did not show iron metabolism genes up-regulated; further, suggesting the effect of bacteria on plant iron metabolism is more complex [74].

2.5. *Gluconacetobacter*

Firstly, the diazotrophs responsible for N₂ fixation in root and stem of sugarcane was named as *Saccharobacter nitrocaptans* [12] and then transformed to *A. diazotrophicus* [75]. However, later on, *A. diazotrophicus* was reclassified into a new genus *Gluconacetobacter* [76]. The bacterium is a small, Gram-negative, aerobic, and rod shaped, which showed pellicle formation in N-free semi-solid medium. *G. diazotrophicus* reported as diazotrophic endophyte provides significant amount of N₂ to plants and also shows some other physiological properties of diazotrophs such as tolerance to high sucrose and salt and low pH conditions [77]. *G. diazotrophicus* is acid-tolerant endophyte need high concentration of sugar in the medium to grow [78] and that requirement was fulfilled in the sugarcane [79]. The endophytic nature of *G. diazotrophicus* as it was labeled with immunogold defined its occurrence on exterior and interior of *S. officinarum* plant tissues was confirmed by James, Reis [80]. The intercellular spaces of sugarcane stems were filled with the N₂-fixing endophytic bacterium *G. diazotrophicus* [81]. The close relationship between addition of N fertilizer to sugarcane and isolation frequencies of *G. diazotrophicus* was suggested by Fuentes-Ramirez, Jimenez-Salgado [82]. When sugarcane plant was fertilized with low amount of 120 kg of N/ha, densities of isolation elevated up to 70%. [67,83].

Further, Munoz-Rojas, Fuentes-Ramirez [84] analyzed that the antagonistic activity between 55 *G. diazotrophicus* strain bacteriocin-like molecules (approximately 3400 Da) with low molecular weight is responsible for such antagonism. Bacteriocin suppresses the growth of *Xanthomonas albilineans* [85]. The antagonistic property of some strains of *G. diazotrophicus* is responsible for colonization of sugarcane plant. Within the plant, there may be antagonistic interaction between endophytic bacteria [84]. *G. diazotrophicus* produces a Bacteriocin (lysozyme) that controlled the growth of the *Xanthomonas albilineans* and showed antifungal activity against *Helminthosporium carbonum* and *Fusarium* sp. [86].

Inoculation of micropropagated sugarcane plant with *G. diazotrophicus* and *Burkholderia vietnamiensis* resulted in improved yield of sugarcane [87]. In an investigation Sevilla, Burris [13] investigated the ability of *G. diazotrophicus* to promote the growth of rice, wheat, and maize. They noticed that the *G. diazotrophicus* colonization is restricted to only root tissues in rice, wheat, and maize. The major supplier of

fixed N₂ to sugarcane is *A. diazotrophicus*. *A. diazotrophicus* fixes N₂ when sucrose concentration high and it helps in enhancement of hormone production such as auxin [88]. *A. diazotrophicus* also found associated with sweet potato and Cameroon grass [89]. Inoculation with *A. diazotrophicus* resulted in a significant improvement in height of N-limited sugarcane plants in comparison with un-inoculated plants [13]. The unique characteristic of this bacterium is its capability to fixed up 48% of its N and make it available to the yeast. This reveals that the association *A. diazotrophicus* may directly benefit its host [90].

The constitutive exoenzyme levansucrase (*LsdA*) was identified in *G. diazotrophicus* strains. The enzyme hydrolyzed sucrose to produce oligofructans and levan. Further, *G. diazotrophicus* grows on sucrose, signifying that the *G. diazotrophicus* as an endophytic species utilizes plant sucrose through levansucrase and for the catabolism of glucose in *G. diazotrophicus* extracellular glucose oxidation is considered as the foremost route [91]. About 150 Kg N ha⁻¹ yr⁻¹ fixed by *G. diazotrophicus* in sugarcane has been reported by Muthukumarasamy, Cleenwerck [92]. The colonization of *G. diazotrophicus* strain PAL5 in the interior of root tissue of rice was confirmed by light microscopy as the PAL 5 marked with the GFP plasmid pHRGFPTC. The plasmids pHRGFPTC are valid tools for supervising the colonization of sugarcane and hydroponic rice with strain PAL5 Rouws, Meneses [93]. The colonization of rice roots endophytically by strain PAL5 was specified by Alquéres, Meneses [94]. In response to phytopathogens attack, plants activate their defense system by secretion of reactive oxygen species (ROS). At an early stage of colonization, of rice plants with the strain PAL5, the bacterium upregulated the ROS-detoxifying genes transcript levels. This ROS scavenging enzymes of PAL5 also participate in the colonization of rice plants. *G. diazotrophicus* endophytically colonizes the roots of *Arabidopsis thaliana*. Inoculation by this strain extensively promoted plant biomass leaves number, total leaf area, and improved water use efficiency in *A. thaliana* [95].

2.6. *Burkholderia*

The genus *Burkholderia* belongs to the β-proteobacteria class of phylum Proteobacteria. The genus *Burkholderia* divided into two main clusters on the basis of phylogenetically: the first one comprises chiefly of human, animal, and plant pathogens e.g. *Burkholderia cepacia* complex (Bcc) and the second one comprises plant-beneficial-environmental (PBE) [96]. In the early 1980s, the strains of *B. cepacia* were recovered from cystic fibrosis patients [97]. *B. cepacia* species includes seven closely related genomic species, now termed as “*cepacia* complex” [98]. Species of environment origin was, then, further joined to this genus including *B. graminis*, *B. caribensis*, and *Burkholderia kururiensis*. In recent times, the PBE cluster has been the center of study, due to their versatile ability of N₂ fixation, nodulation in legume, and degradation of recalcitrant compounds [99]. The three species of *Burkholderia* as *B. caryophylli*, *B. gladioli*, and *B. solanacearum* are identified as phytopathogens, whereas; *B. pseudomallei*, *B. mallei*, and *B. pickettii* are primary pathogens of animals and humans [75].

B. acidophilus, *B. kururiensis*, *B. phytofirmans*, and *B. unamae* have been isolated from surface sterilized plants [100-103]. During 1991, *B. phytofirmans* PsJN isolated from roots of onion infected with fungus *Glomus vesiculiferum* by Dr. Jerzy Nowak. Initially, the strain was classified as *Pseudomonas* sp. [104]. Later on, PsJN strain was reclassified as *B. phytofirmans*, recent research reported plant inoculated with strain PsJN that produces enhanced root system, large amount of chlorophyll, and phenolics compound in the plant [102]. *B. phytofirmans* found to be antagonistic to potato pathogen *Rhizoctonia solani*. Colonization of *Vitis vinifera* L., *Solanum tuberosum*, and

Solanum lycopersicum L. by PsJN reported to enhanced the root system, leaf content, and water use efficiency [105-107]. *B. phytofirmans* PsJN reported to stimulate resistance in grapevine against the pathogen *Botrytis cinerea* causing gray mold diseases [108]. *B. phytofirmans* PsJN has been recovered from roots of *Saccharum* sp., and agricultural soil in Netherlands [109]. The gfp-marked strain PsJN visualized as an endophyte inside young berries of *V. vinifera* L. [110]. *B. phytofirmans* PsJN has the largest genome comprising two chromosomes and one plasmid. The strain PsJN comprises quorum-sensing system that manages the switch of bacterium from free-living to the symbiotic system [111]. In another interesting study, *B. phytofirmans* strain PsJN transformed with *nif* operon containing genomic DNA. The gene was horizontally transferred from one of the known N₂-fixing bacterium, *B. phymatum* STM 815 [112].

A massive number of endophytic bacteria have been isolated from the roots of rice. *Burkholderia* sp. strains revealed that bacteria colonizes to the root surface and then invade into the intercellular spaces [113]. The isolation of *Burkholderia* from the roots of rice was demonstrated by Singh, Mishra [114]. Further, the *B. cepacia* from the stem of *Eucalyptus* was proficient in inhibiting *Magnaporthe grisea*, *Fusarium moniliforme*, and *Rhizoctonia solani* [115]. *B. vietnamiensis* (MGK3) had the maximum nitrogenase activity determined by its ability to reduce acetylene [23]. As reported by Govindarajan, Balandreau [87] sugarcane plant inoculated with *Burkholderia* MG43 resulted in increase in the yield of sugarcane saving the cost of ~140 kg ha⁻¹ N fertilizer, later in a study by Govindarajan, Balandreau [23] *B. vietnamiensis* (MGK3) inoculated to rice plants resulted with enhanced grain yield of rice by 5.6-12.6%. Grain yield of rice plant in a field trial increased up to 8 t ha⁻¹ when inoculated with *B. vietnamiensis* [116].

2.7. Bacillus and Bacillus-Derived Genera

Bacillus and *Bacillus*-derived genera (BBDG) are ubiquitous in nature and have been reported worldwide as plant microbiome, soil microbiome, and extremophiles. *Bacillus* is a genus of Gram-positive, endospore-forming, and rod shaped bacterium. Under unfavorable environmental conditions, it is identified that *Bacillus* species can form dormant spores (endospore). The endospores remain viable for longer period of time and are resistant to heat, sunlight, and chemicals. *Bacillus* species might be obligate aerobes or facultative anaerobes and is well known for its agricultural application (as an effective biocontrol agent) such as *Bacillus thuringiensis* prove as a biocontrol agent globally and industrial applications. Co-inoculation of pea and lentil plant with *B. thuringiensis* KR1 with *R. leguminosarum* PR1 resulted in enhanced growth of plant, increases the number of nodules [117]. *Bacillus amyloliquefaciens* as an endophyte in the internal tissues of *T. aestivum* was described firstly by Verma, Yadav [14].

One of the significant applications of BBDG in agricultural field is used as a biocontrol agent. Cabbage seedlings inoculated with *B. thuringiensis* resulted as a biocontrol agent against *Xanthomonas campestris* pv. *campestris* causing black root of cabbage [118]. One of the most destructive diseases in the production of coconut is basal stem rot caused by *Ganoderma lucidum*. The *Bacillus subtilis* EPC8 secluded from roots tissues of coconut (*Cocos nucifera*) showed strong inhibition of pathogen [119]. The ability of *Bacillus subtilis* EDR4 also reported to suppress the hyphal growth of *Sclerotinia sclerotiorum* [120]. In another study, *B. licheniformis* CHM1 has been reported to protect *Z. mays* and *V. faba* against *Bipolaris maydis* and *Rhizoctonia solani*. Under *in vitro* conditions, the culture filtrate of *B. licheniformis* CHM1 also inhibited the growth of *B. cinereapers*,

Colletotrichum gossypii, *Dothiorella gregaria*, *Fusarium oxysporum*, *Gibberella zeae*, and *Rhizoctonia solani* [121]. *Bacillus megaterium* was reported as first quinclorac degrading endophytic bacterium and can be used as good bioremediation bacterium. The degradation of quinclorac naturally in soil is very slow process [122]. *B. megaterium* BMN1 isolated from root nodules of *Medicago sativa* exhibited promotion of plant growth by synthesis of IAA and by solubilization of inorganic phosphate [123,124].

2.8. Pseudomonas

Pseudomonas is ubiquitous in nature and is a Gram-negative, rod-shaped, aerobic, non-spore forming, and polar-flagellated bacterium. Several studies described the association of *Pseudomonas* endophytically with variety of plants host for example *Arachis*, *Artemisia*, *Populus*, *Brassica*, *Triticum*, *Pennisetum*, and *Trifolium* [125-131]. *Pseudomonas* reported to enhance the growth of plant through a variety of mechanism such as phosphorous solubilization, production of phytohormones, BNF, and stimulation of induced systemic resistance [132]. *Pseudomonas* has been reported for exhibiting biocontrol activity as by synthesizing different compounds for example HCN, pyoluteorin, pyrrolnitrin, and phenazines chiefly phenazine-1-carboxamide and phenazine-1-carboxylic acid [133].

A diazotrophic endophyte, *P. chlororaphis*, isolated from the *Sophora alopecuroides* root nodules which have been confirmed through microscopic analysis expression of the *gfp* gene. The endophyte *P. chlororaphis* reported as potential growth promoting agent. The combined inoculation of *P. chlororaphis* with *Mesorhizobium* sp. showed significant effect on growth of plant such as production of siderophore, IAA, and solubilization of phosphorous [134]. Looking at the plant growth enhancing abilities of genus *Pseudomonas*, Tariq, Shafiqe [135] reported that endophytic *P. aeruginosa* showed significant inhibition of the root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium solani*, both under *in vivo* and *in vitro* conditions in wheat. In a study conducted on *P. stutzeri* strain, A15 under greenhouse experiment reported to induce five-fold enhancement in the biomass of *O. sativa* seedlings compared to uninoculated seedlings [136].

Other endophytes associated to genus *Pseudomonas* were isolated from root and seeds of maize plants. The strain *P. putida* FMZR9, *P. lini* MRR2, *P. aeruginosa* NFTR, *P. fulva* MRC41, *P. aeruginosa* FTR, and *P. montelli* FMZR2 showed tolerance against drought, salinity, and temperature [137]. *P. azotoformans* one of the drought resistant strains reported to nurture phytoremediation effectiveness of *Trifolium arvense* grown on contaminated soil containing multi-metal Cu, Zn, and Ni under stress of drought [138]. Rice seeds reported to harbor bacterial endophytes identified as *P. putida* (VWB3) by 16S rDNA sequencing. The bacterial endophyte prominently influenced the growth of seedlings, length of root and shoot, formation of secondary root hair, and also acted as biocontrol agent [139].

2.9. Other N₂-Fixing Endophytic Bacteria

The other endophytic N₂-fixing bacteria belong to genera *Acinetobacter*, *Achromobacter*, *Azoarcus*, and *Stenotrophomonas*. *Acinetobacter* belongs to a genus of Gram-negative bacteria that are an important soil microorganism and *A. lwoffii* was reported as an endophyte from wheat [14]. *A. calcoaceticus* DD161 isolated from *G. max* L. reported for its strongest inhibitory activity against *Phytophthora sojae* 01 which, further, causes the morphological abnormal changes of fungal mycelia [17]. *Achromobacter xylooxidans* isolated from wheat variety

Malviya-234, which demonstrated considerable nitrogenase activity, production of IAA, and solubilization of P [140].

In a study by Ladha and Reddy [141], association of *Azoarcus* with Kallar grass (*Leptochloa fusca*) roots reported for enhancing the yield up to 20–40 t ha⁻¹yr and *Azoarcus* also expresses *nif* genes and nitrogenase protein was demonstrated by [142]. Malik and co-workers reported by utilization of ¹⁵N isotope dilution and ¹⁵N natural abundance, kallar grass fix up to 26% of content of N [143]. *Stenotrophomonas maltophilia* described as endophytic association exerting advantageous result on the growth of plant (N₂ fixation and phytohormone induction) and anti-fungal activity [144]. Further, the species of the genus *Stenotrophomonas* that fixes N₂ in Sugarcane is *Stenotrophomonas pavanii* that is Gram-negative and non-motile and do not form spores [145].

3. N₂-FIXING NOVEL ENDOPHYTIC BACTERIA

BNF is one of the possible biological alternatives to N-fertilizers (urea) and could lead to more productive and agricultural sustainability without harming the environment. Several endophytic bacteria are now known to increase the availability of N for their host plants. Different novel N₂-fixing endophytic bacteria have been isolated from several crops, which contribute fixed N₂ to the associated plants. *Acetobacter* belonging to phylum proteobacteria reported as novel species *A. diazotrophicus* LMG 7603 and *Acetobacter nitrogenifigens* RG1^T which have been isolated from *S. officinarum* and Kombucha tea [146]. Novel species of N-fixing endophytic bacteria belonging to different genera as *A. diazotrophicus* LMG 7603 [147], *B. unamae* MTI-641^T [148], *B. silvatlantica* SRMrh-20^T [22], *S. pavanii* ICB 89^T [145],

Table 1: Nitrogen-fixing novel species of microbes reported from diverse crop species.

Novel microbes	Host/association	References
<i>A. nitrogenifigens</i> RG1 ^T	Kombucha tea	Dutta and Gachhui [146]
<i>A. melinis</i> TMCY 0552 ^T	<i>M. minutiflora</i>	Peng, Wang [164]
<i>B. mimosarum</i> PAS44 ^T	<i>M. pigra</i>	Chen, James [165]
<i>B. phytofirmans</i> PsJN ^T	<i>A. cepa</i>	Sessitsch, Coenye [102]
<i>B. silvatlantica</i> SRMrh-20 ^T	<i>S. officinarum</i>	Perin, Martínez-Aguilar [22]
<i>E. oryzae</i> Ola 51 ^T	<i>O. latifolia</i>	Peng, Zhang [166]
<i>E. sacchari</i> SP1 ^T	<i>S. officinarum</i>	Zhu, Zhou [149]
<i>F. endophyticum</i> 522 ^T	<i>Z. mays</i>	Gao, Lv [167]
<i>F. coriariae</i> BMG5.1 ^T	<i>C. japonica</i>	Nouioui, Ghodhbane-Gtari [45]
<i>G. kombuchae</i> RG3 ^T	Kombucha tea	Dutta and Gachhui [168]
<i>Gluconacetobacter</i> sp. PA12	<i>O. sativa</i>	Loganathan and Nair [151]
<i>H. lusitanum</i> P6-12 ^T	<i>P. vulgaris</i>	Valverde, Velazquez [169]
<i>K. phytohabitans</i> KLBMP 1111 ^T	<i>J. curcas</i> L.	Xing, Bian [170]
<i>K. arsenatis</i> CM1E1 ^T	<i>P. laevigata</i>	Román-Ponce, Wang [171]
<i>M. phragmitis</i> MP23 ^T	<i>P. karka</i>	Behera, Ramana [172]
<i>M. nidulans</i> LMG 21967 ^T	<i>C. glaucoides</i>	Rozahon, Ismayil [173]
<i>O. oryzae</i> MTCC 4195 ^T	<i>O. sativa</i>	Tripathi, Verma [152]
<i>P. herberti</i> R33 ^T	<i>H. sendtneri</i>	Guo, Zhou [174]
<i>P. wexxiniae</i> 373 ^T	<i>Z. mays</i>	Gao, Lv [175]
<i>P. diazotrophicus</i> LS 8 ^T	<i>O. sativa</i>	Zhang, Peng [153]
<i>P. diazotrophica</i> R5-392 ^T	<i>J. curcas</i>	Madhaiyan, Jin [176]
<i>R. hidalgonense</i> FH14 ^T	<i>P. vulgaris</i>	Yan, Yan [31]
<i>R. oryzae</i> Alt 505 ^T	<i>O. alta</i>	Peng, Yuan [24]
<i>R. pongamiae</i> VKLR-01 ^T	<i>P. pinnata</i>	Kesari, Ramesh [177]
<i>R. populi</i> K-38 ^T	<i>P. euphratica</i>	Rozahon, Ismayil [173]
<i>R. smilacinae</i> PTYR-5 ^T	<i>S. japonica</i>	Zhang, Shi [39]
<i>R. vallis</i> CCBAU 65647 ^T	<i>P. vulgaris</i>	Wang, Wang [160]
<i>S. kummerowiae</i> CCBAU 25048 ^T	<i>K. stipulacea</i>	Lin, Wang [178]
<i>S. pavanii</i> ICB 89 ^T	<i>S. officinarum</i>	Ramos, Van Trappen [145]

B. silvatlantica: *Burkholderia silvatlantica*, *A. nitrogenifigens*: *Acetobacter nitrogenifigens*, *A. melinis*: *Azospirillum melinis*, *B. mimosarum*: *Burkholderia mimosarum*, *B. phytofirmans*: *Burkholderia phytofirmans*, *E. oryzae*: *Enterobacter oryzae*, *E. sacchari*: *Enterobacter sacchari*, *F. endophyticum*: *Flavobacterium endophyticum*, *F. coriariae*: *Frankia coriariae*, *G. kombuchae*: *Gluconacetobacter kombuchae*, *H. lusitanum*: *Herbaspirillum lusitanum*, *K. phytohabitans*: *Kibdelosporangium phytohabitans*, *K. arsenatis*: *Kocuria arsenatis*, *M. phragmitis*: *Mangrovibacter phragmitis*, *M. nidulans*: *Methylobacterium nidulans*, *O. oryzae*: *Ochrobactrum oryzae*, *P. herberti*: *Paenibacillus herberti*, *P. wexxiniae*: *Paenibacillus wexxiniae*, *P. diazotrophicus*: *Phytobacter diazotrophicus*, *P. diazotrophica*: *Pleomorphomonas diazotrophica*, *R. hidalgonense*: *Rhizobium hidalgonense*, *R. oryzae*: *Rhizobium oryzae*, *R. pongamiae*: *Rhizobium pongamiae*, *R. populi*: *Rhizobium populi*, *R. smilacinae*: *Rhizobium smilacinae*, *R. vallis*: *Rhizobium vallis*, *S. kummerowiae*: *Shinella kummerowiae*, *S. pavanii*: *Stenotrophomonas pavanii*, *M. minutiflora*: *Melinis minutiflora*, *M. pigra*: *Mimosa pigra*, *A. cepa*: *Allium cepa*, *S. officinarum*: *Saccharum officinarum*, *O. latifolia*: *Oryza latifolia*, *Z. mays*: *Zea mays*, *C. japonica*: *Coriaria japonica*, *O. sativa*: *Oryza sativa*, *P. vulgaris*: *Phaseolus vulgaris*, *J. curcas*: *Jatropha curcas*, *P. laevigata*: *Prosopis laevigata*, *P. karka*: *Phragmites karka*, *C. glaucoides*: *Crotalaria glaucoides*, *H. sendtneri*: *Herbertus sendtneri*, *O. alta*: *Oryza alta*, *P. pinnata*: *Pongamia pinnata*, *P. euphratica*: *Populus euphratica*, *S. japonica*: *Smilacina japonica*, *K. stipulacea*: *Kummerowia stipulacea*.

Enterobacter sacchari SPI^T [149], and *Klebsiella variicola* strain DX120E [150] has been reported from sugarcane.

Novel bacterial species such as *Gluconacetobacter* sp. PA12 [151], *Ochrobactrum oryzae* MTCC 4195^T [152], *Phytobacter diazotrophicus* LS 8^T [153], *Acinetobacter oryzae* B23^T [154], *Enterobacter oryzendophyticus*, and *E. oryziphilus* [155] has been reported as N₂-fixing endophytic bacteria from rice. Rhizobia are N₂-fixing soil bacteria that fully or partially satisfy the N demand of the host plant belonging to leguminous family plant. The diversity of rhizobia from *Sesbania rostrata* [156], alfalfa [157], *P. vulgaris* L. and *Leucaena* sp. [158], and *Pisum sativum* [159] has been studied and characterized for N-fixing attributes. Further, novel species of genera *Rhizobium* as *R. vallis* CCBAU 65647^T [160] and *R. hidalgonense* FH14^T [31] has been reported for forming effective nodules in *P. vulgaris* [Table 1].

4. GENOMICS AND MECHANISMS OF N₂ FIXATION

Symbiotic microbes from leguminous plants provide substantial benefit in the growth through N metabolism. “Symbiome” is the protein family involved in N₂ fixation, nodulation, production of exopolysaccharides, and transport of oxygen. Sevilla, Meletus [161] identified various genes involved in the N₂-fixing process such as *nif*HDK, *nif*A, *nif*B, *nif*E, *nif*V, and *ntr*BC. *Rhizobium* carries plasmid genes for nodulation (*nod*, *nol*, and *noe*) in close proximity with *nif* and *fix* genes [162]. *Rhizobium* secretes the lipochitin oligosaccharides as the nodulation factor responsible for maintaining symbiotic association of rhizobia with legume [163].

Many endophytes reported to fix atmospheric N₂ as demonstrated by the transcriptional activator *nif*A, involved in activation of N₂-fixing (*nif*) operon, and regulator *mod*E, involved sensing and uptake of molybdate at nanomolar concentrations [179]. About 26% of investigated community of endophytic microbes were detected with harboring the *nif*A activator, which concluded that endophytes have the higher capacity to promote growth of plant by N₂ fixation process. Non-symbiotic microbes mostly include endophytes and free-living microbes such as *Azotobacter* and *Azospirillum* [180]. Different approaches have been used to study the interaction of endophytes with host plant in fixing atmospheric N₂ and providing fixed N₂ to plant such as by acetylene reduction assay, ¹⁵N isotope dilution experiments, and ¹⁵N₂ reduction assays. BNF takes place under normal temperature and pressure and is catalyzed by the enzyme nitrogenase. A new N₂-fixing endophyte, that is, *Pantoea* isolated from sugarcane, able to generate H₂, develop in aerobic and anaerobic conditions, show, adjustability to higher pH, higher temperature, higher osmotic pressure, reduced acetylene, and also revealed the development of pellicle in semisolid LGI-P medium [181]. Verma, Yadav [14] reported diverse N₂-fixing bacteria belonging to genera *Acinetobacter*, *Bacillus*, *Pseudomonas*, and *Stenotrophomonas*. The gene association with N₂ fixation such as *nif* HDK, *nif*V, *nif*E, and *nif*A has already been sequenced and detected in *A. diazotrophicus* [161]. The amplification of *nif*H gene fragment by polymerase chain reaction (PCR) the N₂-fixing endophytes was identified [140, 182].

Biological fixation of atmospheric N₂ into ammonia was achieved by an enzyme complex called as nitrogenase present in the diazotrophic bacteria. Nitrogenase is a complex enzyme structure consisting of two subunits dinitrogenase and dinitrogenase reductase. Dinitrogenase/Component I (Larger component protein of the Mo-dependent nitrogenase is called as MoFe protein) containing molybdenum, iron and inorganic sulfur, and dinitrogenase reductase/Component II (The smaller component protein, called the Fe protein) containing

iron and inorganic sulfur. The Fe protein transfers electrons from a reducing agent, such as ferredoxin to the MoFe protein [183]. The *nif* gene encodes the nitrogenase complex. The nitrogenase enzyme is synthesized by prokaryotes. It is found in both main domains of prokaryotes, in the archaea and in the bacteria. Pan and Vessey [184] described in term of changes of atmospheric pO₂ *G. diazotrophicus* that has a switch-off/switch-on mechanism for the protection of nitrogenase enzyme system.

About 20 genes were found to be responsible in the complex N₂ fixation mechanism for both genetic regulation of the process and nitrogenase protein synthesis [185,186]. To control the expression of genes including the *nif* system in the diazotrophs, a general N regulation (*ntr*) system is important. Key proteins which are involved in N control system are as follows: A uridylyltransferase (UTase) encoded by *glnD*, a small tetrameric effector protein (PII) encoded by *glnB*, NtrB encoded by *ntrB*, and a transcriptional activator NtrC encoded by *ntrC* [187]. Ureta and Nordlund [188] presented that the evidence for conformational protection of nitrogenase against O₂ in *G. diazotrophicus*, in which protein is involved, is putative FeSII Shethna. To confirm the microbe in sample immunolabeling, *in situ* hybridization, and reporter genes have been used to identify the expression of nitrogenase proteins and/or nitrogenase encoding (*nif*) genes. The endophytic expression of *nif*H by *Alcaligenes faecalis* A15 and *Azospirillum irakense* within epidermal cells of roots has been shown by Vermeiren, Willems [189] and similar results have been obtained with *Azoarcus* [190].

Reis and Döbereiner [191], in his study, observed that nitrogenase enzyme was protected against inhibition by oxygen, ammonium, and some amino acids by 10% sucrose. Further, Madhaiyan, Poonguzhali [192] described the reduction in nitrogenase activity of pure cultures of *G. diazotrophicus* by the addition of pesticides to the growth medium. N-fixing system is sensitive to molecular oxygen. This sensitivity results from the instability of nitrogenase when exposed to oxygen. Diazotrophs use protective mechanism to protect nitrogenase [193]. Leghemoglobin is the protective protein associated nitrogenase. Leghemoglobin bind with the oxygen and keep nitrogenase enzyme active from oxygen. During the process of N₂ fixation, sufficient amount of oxygen is required by rhizobia for respiration and prevention of oxygen supply to the nitrogenase complex take place at same time as leghemoglobin has high affinity for binding to oxygen [194].

During symbiotic association of rhizobia with host plant, flavonoids and phytohormones act as signaling molecule is produced by legume plant and activates the nodulation factor (*nod*) in rhizobia. N-fixing bacteria initiate the division of cortical cell of root and lead to nodule organogenesis [37]. Through the process of transformation the gene of Hb transferred from *Vitreoscilla* sp. to *Rhizobium etli* resulted in higher respiration rate of rhizobia [195]. Without the defense of leghemoglobin, the *Rhizobium* bacterioids will only fix N₂ when external oxygen concentration is decreased to about 0.01 atm [196]. The structural genes for nitrogenase *nif* HDK in *G. diazotrophicus* are organized in a cluster (GDI0425–GDI0454), along with other N₂ fixation-related genes, for instance *fix* ABCX, *mod* ABC and *nif* AB. In the regulation and metabolism of oxygen *fix*, gene family is involved. Other related genes, *ntrX*, *ntrY*, and *ntrC* (GDI2263, GDI2264, and GDI2265) are confined somewhere else in the chromosome in a 5.2 kb cluster. Thaweenut, Hachisuka [197] described the expression of *nif*H genes in the stems and roots of sugarcane plants by reverse transcription (RT)–PCR. The inoculation of *Oryza officinalis* with endophytic

Herbaspirillum sp. B501 and in planta N₂ fixation was studied using acetylene reduction assay and ¹⁵N₂ gas incorporation approaches [66]. The colonization of rice plant with endophytic *Burkholderia* resulted in 372 µg N per plant an increase in N content in grains. The N₂ was derived by biological N₂ fixation [113]. Roncato-Maccari, Ramos [198] reported in the intercellular region of vascular tissue and root cortex of *T. aestivum*, *S. bicolor*, *O. sativa*, and *Z. mays* roots observed with the expression of *nifH* gene encoding for iron protein of nitrogenase.

In a study, 343 endophytes were isolated from stem, root, and leaves of soybean cultivar. The two methodologies were used for screening N₂-fixing endophytes. About 60% of endophytes grow on NFb medium and 21% of endophytes revealed containing *nifH* gene by PCR analysis. The endophytic isolates identified as *A. calcoaceticus*, *Burkholderia* sp., *Pseudomonas* sp., and *Ralstonia* sp. [21]. Further, Jha and Kumar [140] selected only those wheat plants showing acetylene reduction assay positive for endophytic bacteria isolation. One *T. aestivum* variety Malviya-234 revealed the occurrence of endophytic diazotrophic bacteria the highest nitrogenase activity was found in *A. xylosoxidans* (WM234C-3) which was 1.70 µmol C₂H₄ mg⁻¹ protein h⁻¹. *Enterobacter ludwigii* and *Enterobacter cloacae* reported to fix N₂ by acetylene reduction assay and also have the potential of solubilization of phosphorous [199]. Hongrittipun, Youpensuk [200] reported *B. cepacia* (CS5) and *Citrobacter* sp. (CR9) isolated from rice as an endophyte and their inoculation in rice seedlings increases the concentration of N₂ in roots of rice. Endophytic species *Azoarcus* sp. (Kallar grass), *Burkholderia* sp. (Rice), *G. diazotrophicus* (Sugarcane), and *Herbaspirillum* sp. (Rice) were reported to benefit their host plant by BNF. The commercially available N₂-fixing biofertilizers are *Rhizobium*, *Bradyrhizobium*, *Azospirillum*, and *Azotobacter* [201].

5. PGP ATTRIBUTES OF ENDOPHYTIC N₂-FIXING MICROBES

Endophytic microbes, along with N₂-fixing attributes, also help in the uptake of various nutrients from soil and transfer to the plants and also synthesize various growth promoting compounds. The endophytic microbes directly or indirectly affect the growth of plants. The endophytic microbes in a direct way effectively enhance the growth of plants by solubilizing nutrients such as P, K, and Zn from soil as well as stimulate the better development of plant root which absorbs nutrients from soil, synthesize phytohormones which enhance the plant growth at different stages [Table 2]. Indirectly endophytic microbes enhances the plant growth by functioning as biocontrol agent, that is, suppresses various phytopathogens, release of siderophores, production of hydrogen cyanide, antibiotics and enzymes such as chitinases, and β-1,3-glucanase [202-204].

5.1. Solubilization of Macronutrients and Micronutrients

The most essential macronutrients for growth of plant are P and K. In soil, the phosphorous exists in two different forms organic and inorganic. The most important attributes performed by PGP endophytic microbes are the conversion of insoluble phosphates into soluble form [205]. In the soil, the concentration of P and K is very low, mineral compounds of phosphorus usually contain aluminium, iron, manganese, and calcium. The K in the soil exists in the form of feldspar (90–98%) and mica [206]. The phenomenon of solubilization of phosphate from soil by endophytic microbes mostly dependent on the pH and type of soil [207]. Endophytic N₂-fixing bacteria belonging to genera *Achromobacter* [140], *Acinetobacter* [21], *Bacillus* [14], *Burkholderia* [21], *Methylobacterium* [208], and *Pantoea* [209]

have been reported for phosphate-solubilization. The phosphorous solubilizing microbe dissolves the phosphorous through synthesis of different types of acids such as (acetate, lactate, oxalate, tartarate, succinate, citrate, gluconate, keto-gluconate, and glycolate) [210]. In case of Gram-negative bacteria, the enzyme glucose dehydrogenase contributes significantly to mineral phosphate solubilization [211]. The soil contains P in the form of organic substrates, which can be hydrolyzed to inorganic P by the enzyme alkaline phosphatases [212], phytase [213], phosphonoacetate hydrolase [214], D-α-glycerophosphatase [215], and C-P lyase [216] and subsequently available for plant nutrition. Endophytic bacteria *A. calcoaceticus* and *Burkholderia* sp. isolated from soybean cultivars showed significant growth promotion of plant by solubilization of mineral phosphate [21].

In a study Jha and Kumar [140] reported, *A. xylosoxidans* one of the diazotrophic endophytic bacteria isolated from surface-sterilized roots and culms of wheat variety Malviya-234 showed appreciable level of P solubilization ability. Verma, Yadav [14], in their findings, isolated 247 bacteria from wheat from five different sites of northern hills zone of India. The isolated strains were screened for P-, K-, and Zn-solubilization. The P, K, and Zn- solubilizing isolate was identified as *B. amyloliquefaciens*. Singh, Rajawat [231], in a study, evaluated the role of endophytes in the Zn fortification in wheat genotypes. Two endophytic bacteria *Bacillus subtilis* DS-178 and *Arthrobacter* sp. DS-179 inoculated in wheat resulted in two folds higher yield as compared to un-inoculated control. These Zn solubilizing endophytic bacteria also enhance the translocation and improvement of Zn to grains in wheat. In the modern strategies for biofortification, the endophytic microbes resulted as a better option.

5.2. Phytohormones Production

The hormones are the chemical messengers or an organic substance being produced in any one part and are transferred to another part that there influences a specific physiological process which included auxins, gibberellins, cytokinins, abscisic acid, and ethylene [232]. The various reports have demonstrated the ability of endophytic bacteria such as *A. xylosoxidans* [140], *A. calcoaceticus*, *B. amyloliquefaciens* [17], *Burkholderia* sp. [21], *E. cloacae* [17], *Klebsiella oxytoca* [224], *Methylobacterium oryzae* [30], *Pantoea agglomerans* [233], and *Pseudomonas fluorescence* [34] isolated from different host plant to synthesize different phytohormones. Ambawade and Pathade [234] reported *Bacillus siamensis* as a newly isolated endophytic bacteria from the banana have the ability to produce a significant amount of gibberellic acid with and without tryptophan-supplemented in media. One of the beneficial endophytic bacteria reported for its potential in N₂ fixation along with production of phytohormones especially IAA with important crops such as sugarcane [80], rice [93], *A. thaliana* [95], and sorghum [235], and various other important crops are *G. diazotrophicus*. In a study by Rodrigues, Soares [236] identified the genes and pathways involved in biosynthesis of IAA by *G. diazotrophicus* and finally concluded that *G. diazotrophicus* PAL5^T synthesizes IAA through the IPyA pathway in cultures supplemented with tryptophan and provides data for the contribution of an L-amino acid oxidase gene cluster in the IAA biosynthesis.

In a study by Defez, Andreozzi [182] reported endophytic bacteria *E. cloacae* RCA25 and *K. variicola* RCA26 which were isolated from roots and leaves of rice plants. *K. variicola* RCA26 had the highest nitrogenase activity. The two isolates, *E. cloacae* RCA25 and *K. variicola* RCA26, and two model bacteria (*H. seropedicae* z67 and *Sinorhizobium fredii* NGR234) were transformed to enhance the IAA biosynthesis. In addition to IAA, the endophytic bacteria

Table 2: Endophytic microbes from cereal crops with multifarious plant growth promoting properties.

Endophytic microbes	Host	NF	P	K	Zn	IAA	Sid	References
<i>A. diazotrophicus</i>	Sugarcane							Sevilla, Burris [13]
<i>A. xylooxidans</i>	Wheat							Jha and Kumar [140]
<i>A. calcoaceticus</i>	Soybean							Kuklinsky-Sobral, Araújo [21]
<i>A. lwoffii</i>	Wheat							Verma, Yadav [14]
<i>A. faecalis</i>	Rice							You and Zhou [217]
<i>A. amazonense</i>	Sugarcane							Oliveira, Urquiaga [218]
<i>A. brasilense</i>	Maize							Swędrzyńska and Sawicka [16]
<i>A. brasilense</i>	Sugarcane							de Bellone and Bellone [52]
<i>A. brasilense</i>	Wheat							Saubidet, Fatta [219]
<i>B. amyloliquefaciens</i>	Rice							Verma, Yadav [14]
<i>B. amyloliquefaciens</i>	Soybean							Zhao, Xu [17]
<i>B. amyloliquefaciens</i>	Wheat							Verma, Yadav [14]
<i>B. aryabhatai</i>	Rice							Ji, Gururani [220]
<i>B. cereus</i>	Soybean							Zhao, Xu [17]
<i>B. megaterium</i>	Soybean							Subramanian, Kim [30]
<i>B. subtilis</i>	Soybean							Bai, Zhou [221]
<i>B. thuringiensis</i>	Soybean							Bai, Zhou [221]
<i>B. japonicum</i>	Soybean							Subramanian, Kim [30]
<i>Brevundimonas</i>	Maize							Montañez, Abreu [33]
<i>B. silvatlantica</i>	Sugarcane							Perin, Martínez-Aguilar [22]
<i>B. vietnamiensis</i>	Rice							Govindarajan, Balandreau [23]
<i>E. agglomerans</i>	Rice							James, Gyaneshwar [222]
<i>E. cloacae</i>	Rice							Ladha, Barraquio [223]
<i>E. cloacae</i>	Soybean							Zhao, Xu [17]
<i>E. cloacae</i>	Sugarcane							Mirza, Ahmad [224]
<i>Gluconacetobacter</i>	Maize							Eskin [225]
<i>Gluconacetobacter</i>	Sugarcane							Suman, Shrivastava [226]
<i>Herbaspirillum</i>	Maize							de Bellone and Bellone [52]
<i>H. seropedicae</i>	Maize							Oliveira, Urquiaga [218]
<i>H. seropedicae</i>	Rice							Baldani, Baldani [113]
<i>H. seropedicae</i>	Sugarcane							Ladha, Barraquio [227]
<i>K. oxytoca</i>	Soybean							Mirza, Ahmad [224]
<i>K. oxytoca</i>	Sugarcane							Roesch, Camargo [228]
<i>K. planticola</i>	Rice							Ladha, Barraquio [223]
<i>K. pneumonia</i>	Maize							Govindarajan, Kwon [32]
<i>K. pneumonia</i>	Sugarcane							Gyaneshwar, James [229]
<i>M. oryzae</i>	Rice							Subramanian, Kim [30]
<i>M. oryzae</i>	Soybean							Kumar, Tomar [208]
<i>P. polymyxa</i>	Maize							Subramanian, Kim [30]
<i>P. agglomerans</i>	Rice							Puri, Padda [230]
<i>P. agglomerans</i>	Soybean							Verma, Ladha [209]
<i>P. fluorescens</i>	Rice							Montañez, Abreu [33]
<i>P. putida</i>	Soybean							Mbai, Magiri [34]
<i>R. leguminosarum</i>	Rice							Govindarajan, Kwon [32]
<i>S. marcescens</i>	Rice							Dakora, Matiru [25]

NF: Nitrogen fixation, P: Phosphorus solubilization, K: Potassium solubilization, Zn: Zinc solubilization, IAA: Indole-3-acetic acid, Sid: Siderophores, *A. diazotrophicus*: *Acetobacter diazotrophicus*, *A. xylooxidans*: *Achromobacter xylooxidans*, *A. calcoaceticus*: *Acinetobacter calcoaceticus*, *A. lwoffii*: *Acinetobacter lwoffii*, *A. faecalis*: *Alcaligenes faecalis*, *A. amazonense*: *Azospirillum amazonense*, *A. brasilense*: *Azospirillum brasilense*, *B. amyloliquefaciens*: *Bacillus amyloliquefaciens*, *B. aryabhatai*: *Bacillus aryabhatai*, *B. cereus*: *Bacillus cereus*, *B. megaterium*: *Bacillus megaterium*, *B. subtilis*: *Bacillus subtilis*, *B. thuringiensis*: *Bacillus thuringiensis*, *B. japonicum*: *Bradyrhizobium japonicum*, *B. silvatlantica*: *Burkholderia silvatlantica*, *B. vietnamiensis*: *Burkholderia vietnamiensis*, *E. agglomerans*: *Enterobacter agglomerans*, *E. cloacae*: *Enterobacter cloacae*, *H. seropedicae*: *Herbaspirillum seropedicae*, *K. oxytoca*: *Klebsiella oxytoca*, *K. planticola*: *Klebsiella planticola*, *K. pneumonia*: *Klebsiella pneumonia*, *M. oryzae*: *Methylobacterium oryzae*, *P. polymyxa*: *Paenibacillus polymyxa*, *P. agglomerans*: *Pantoea agglomerans*, *P. fluorescens*: *Pseudomonas fluorescens*, *P. putida*: *Pseudomonas putida*, *R. leguminosarum*: *Rhizobium leguminosarum*, *S. marcescens*: *Serratia marcescens*.

Bacillus aryabhatai (MF693121.1), *B. megaterium*, and *Bacillus cereus* were isolated from nodules of *Vigna radiata*. Further, the study confirmed the presence of trp-dependent pathway for IAA production in the isolates and the *in vitro* application of bacterial isolates on plant roots resulted in increase in root length as well as number of lateral roots [237]. One of the most widely studied and best-characterized phytohormone is IAA. *Bradyrhizobium* sp. confirmed as an endophyte in rice plant and demonstrated its potential in enhancing plant growth by N₂ fixation, production of ACC deaminase, and IAA.

5.3. Fe-Chelating Compounds Production

Siderophores (hydroxamate, catecholate and carboxylates) are high-affinity, iron-chelating organic compounds with low molecular masses, synthesized by microbes, and plants under low iron conditions. In the recent years, siderophores producing microbes have gained much attention due to their potential applications in environmental research. Microbes synthesizing the siderophores provide the bioavailability of Fe to plants for enhancing their growth [238]. The production of siderophores is generally specific at the genus level, for example, *Pseudomonas* sp. produces only pyoverdins, ornibactin by *Burkholderia* sp., and mycobactin by *Mycobacterium* sp. [239]. The production of siderophores by microbes confers the benefits to inhabit plant and eliminate other microbes from the similar ecological niche. Siderophores-producing bacteria under *in vitro* inhibition assays showed that *Burkholderia* was good antagonistic as they suppress fungal pathogens [240]. Various researches have shown the practicality of using eco-friendly approaches as microbes for improving the health and productivity of crop. From the roots of tomato plant, about 12 endophytic bacteria have been isolated and screened for PGP attributes and most of the root endophytes *Pseudomonas*, *Rhizobium*, *Rhodococcus*, and *Agrobacterium* have been detected for siderophores production. As, production of siderophores is considered as one of the biocontrol trait as lower availability of iron limits the growth of phytopathogens [241].

One of the endophytic bacterium, *Pseudomonas brassicacearum* strain Zy-2-1 was reported for producing siderophores and its inoculation with *Sinorhizobium meliloti* on *Medicago lupulina* resulted in enhancement of fresh weight of roots, total dry weight, nodule number and fresh weight and N content. The results demonstrated the usage of *P. brassicacearum* for remediation of heavy metal-contaminated soils [242]. A total of 276 endophytic bacteria were isolated from the root nodules of *G. max* L. Out of 276 endophytic bacteria, *A. calcoaceticus* resulted in the production of 54.33 ± 0.093 µg mL⁻¹ siderophores and also causes the morphological abnormal changes of *P. sojae* 01 by acting as strongest inhibitor [17]. The contamination of arsenic in rice plants causes many negative impacts. In a study by Dolphen and Thiravetyan [243] reported, *Bacillus pumilus* had a capability to reduce accumulation of arsenic in rice grains to levels below those achieved by *Pseudomonas* sp. and *B. thuringiensis*.

5.4. ACC Deaminase

In agriculture practices, the plant hormone ethylene performs a significant function. Ethylene is one of the organic molecule and at a very low concentration (0.05 µL/L), ethylene functions as a proficient plant growth regulator [244]. The decreased concentration of ethylene produces significant increase in promoting the growth of roots [245]. In *V. vinifera* and *S. tuberosum*, the endophyte *Burkholderia* sp. synthesizes high levels of ACC deaminase [107]. *Burkholderia* strain PsJN^T shows high ACC deaminase activity [246]. The novel species *B. phytofirmans* PsJN^T showed highest activity

of 1-aminocyclopropane-1-carboxylate deaminase ACC and lower the level of ethylene [102]. Plant seeds and roots treated with ACC deaminase containing bacteria demonstrated with 2–4 folds decrease in ACC and ethylene synthesis [247]. *Pseudomonas* sp. producing ACC deaminase resulted in enhanced height and weight of root and total biomass of maize plant [248]. The use of ACC deaminase producing bacteria reduces the level of ethylene along with mitigates the salt stress reported in maize plant [249]. *H. frisingense* strain GSF30^T was isolated from *M. sinensis* C4 as an endophytic diazotrophs [250]. *A. xylooxidans* AUM54 isolated from *Catharanthus roseus* was identified as highly salt tolerant endophytic bacteria [251]. The inoculation of two rice cultivar IR42 and IAC4440 with isolates of *A. brasilense* sp245 and *B. kururiensis* M130 resulted in a significant increase in the number of roots after 10 days. The cultivar IR42 was colonized more with bacteria and shows more BNF. The different ethylene receptors (ER) were observed in two cultivars of rice. Data suggested different bacteria could trigger expression of different ER in rice genotypes [252].

One of the useful tools used by microbes is the production of ACC deaminase enzyme to lessen the adverse effect of environmental condition on plants. Infection of legume plant with *Rhizobia* resulted in increased production of ethylene; increased ethylene level inhibited the nodule formation on root. Some *Rhizobial* strain produces “rhizobitoxine” a phytotoxin that inhibits the production of enzyme ACC synthase. ACC is precursor for ethylene production [253]. *R. leguminosarum* RP2 strain isolated from nodules of pea synthesized detectable amount of ACC deaminase [254]. Psychrotolerant endophytic bacteria *Arthrobacter*, *Bacillus*, *Methylobacterium*, *Providencia*, *Pseudomonas*, and *Flavobacterium* isolated from *T. aestivum* show ACC deaminase activity [14]. During the process of nodulation in the roots of plant, the hormone ethylene and its precursor 1-aminocyclopropane-1-carboxylate (ACC) were observed to get accumulated in the root [30]. Heavy metal toxicity in soil decreased the fertility of soil and is detrimental for plants growth. The heavy metals are rapidly absorb by the roots of plants and translocate to shoot and leave leading to reduced growth of plant and finally the death of plant [255].

The ACC deaminase enzyme secreted by microbes lowers the ethylene level. The ethylene accumulation significantly increases during the communication of pathogen with plant. PGP bacteria synthesizes the enzyme ACC deaminase, hence reduces the level of ethylene [256]. The decreased concentration of ethylene in combination with the hormone auxin has beneficial effect on the host plant. *R. leguminosarum* (RPN5) produces intracellular ACC deaminase and utilizes ACC as a sole source of N [257]. In a study, two ACC deaminase expressing endophytic bacteria *Pseudomonas* sp. strains, OFT2 and OFT5, were studied as they uphold the growth, photosynthesis, water content in the leaf, and ionic balance of tomato plants. The inoculated OFT5 decreased salt-induced ethylene production by tomato seedlings and also enhanced shoot uptake of P, K, and Mg as well as Mn, Fe, Cu, and Zn [258].

6. CONCLUSION

Over the worldwide, improvements are required regarding the inputs of organics and BNF. Endophytic bacteria have been isolated from a large number of both leguminous and non-leguminous plants and play an integral role in the growth and development of plants and also help in restoration of available nutrient. For the enhancement of delivering fixed N₂ to plants, BNF has been proven to be an effective mechanism. The symbiotic association of rhizobia is practically limited to legumes and more interest is focused on N₂ fixation in non-legume crops. Over the

past few decades, mostly, the research is focused on studying the traits and characteristics of bacterium, and recently, advancement in molecular biology and biochemical analysis opened the new gateway for scientist to study bacterium at its gene level and further provide more details about the process of bacterial endophyte colonization. In the future, development of genetically modified microbes will be a better strategy over transgenic plants for enhancing plant performance and genetic modification of bacterium is better option as modification of higher organism is a complex phenomenon. It is a vital need to develop more effective endophytic strains with longer shelf lives for achieving better sustainable agriculture. More focus should be laid on designing the tools through which identification of genes responsible for endophytic association in non-legumes can be made possible. The creation of artificial symbiosis and endophytic association among N₂-fixing microbes and crops of agricultural importance is an important criterion for reducing the usage of chemical fertilizers. Recent advancement in the fields of biotechnology, microbiology, molecular biology, and bioinformatics has unlocked the way to discover novel genes involved in N₂ fixation.

7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. FUNDING

There is no funding to report.

9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

12. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

- do Vale Barreto Figueiredo M, do Espírito Santo Mergulhão AC, Sobral JK, de Andrade Lira M, de Araújo AS. Biological nitrogen fixation: Importance, associated diversity, and estimates. In: Arora NK, editor. *Plant Microbe Symbiosis: Fundamentals and Advances*. New Delhi: Springer; 2013. p. 267-89.
- Rondon MA, Lehmann J, Ramirez J, Hurtado M. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol Fert Soils* 2007;43:699-708.
- Kaur T, Devi R, Kour D, Yadav A, Yadav AN, Dikilitas M, *et al.* Plant growth promoting soil microbiomes and their potential implications for agricultural and environmental sustainability. *Biologia* 2021;76:2687-709.
- Kahindi JH, Karanja NK. Essentials of nitrogen fixation biotechnology. *Biotechnology* 2009;8:54.
- Rockström J, Steffen W, Noone K, Persson Å, Chapin F 3rd, Lambin E, *et al.* A safe operating space for humanity. *Nature* 2009;461:472-75.
- Bhattacharjee RB, Singh A, Mukhopadhyay S. Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: Prospects and challenges. *Appl Microbiol Biotechnol* 2008;80:199-209.
- Cocking EC. Endophytic colonization of plant roots by nitrogen-fixing bacteria. *Plant Soil* 2003;252:169-75.
- Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, *et al.* The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev* 2015;79:293-320.
- Rosenblueth M, Martínez-Romero E. Bacterial endophytes and their interactions with hosts. *Mol Plant Microb Interact* 2006;19:827-37.
- Yadav AN, Kour D, Kaur T, Devi R, Yadav A. Endophytic fungal communities and their biotechnological implications for agro-environmental sustainability. *Folia Microbiol* 2022;67:203-32.
- Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN, Kumar V, *et al.* Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh B, editor. *Advances in Endophytic Fungal Research*. Cham: Springer; 2019. p. 105-44.
- Cavalcante VA, Dobereiner J. A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. *Plant Soil* 1988;108:23-31.
- Sevilla M, Burris RH, Gunapala N, Kennedy C. Comparison of benefit to sugarcane plant growth and 15N₂ incorporation following inoculation of sterile plants with *Acetobacter diazotrophicus* wild-type and nif mutant strains. *Mol Plant Microb Interact* 2001;14:358-66.
- Verma P, Yadav AN, Khannam KS, Panjiar N, Kumar S, Saxena AK, *et al.* Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum*) from the Northern hills zone of India. *Ann Microbiol* 2015;65:1885-99.
- Hurek T, Reinhold-Hurek B, Van Montagu M, Kellenberger E. Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. *J Bacteriol* 1994;176:1913-23.
- Swędrzyńska D, Sawicka A. Effect of inoculation with *Azospirillum brasilense* on development and yielding of maize (*Zea mays* ssp. *saccharata* L.) under different cultivation conditions. *Pol J Environ Stud* 2000;9:505-9.
- Zhao L, Xu Y, Lai X. Antagonistic endophytic bacteria associated with nodules of soybean (*Glycine max* L.) and plant growth-promoting properties. *Braz J Microbiol* 2018;49:269-78.
- Castro S, Permigliani M, Vinocur M, Fabra A. Nodulation in peanut (*Arachis hypogaea* L.) roots in the presence of native and inoculated rhizobia strains. *Appl Soil Ecol* 1999;13:39-44.
- Yadav AN, Kumar V, Dhaliwal HS, Prasad R, Saxena AK. Microbiome in crops: Diversity, distribution, and potential role in crop improvement. In: Prasad R, Gill SS, Tuteja N, editors. *Crop Improvement Through Microbial Biotechnology*. San Diego: Elsevier; 2018. p. 305-32.
- Estrada P, Mavingui P, Courmoyer B, Fontaine F, Balandreau J, Caballero-Mellado J. A N₂-fixing endophytic *Burkholderia* sp. associated with maize plants cultivated in Mexico. *Can J Microbiol* 2002;48:285-94.
- Kuklinsky-Sobral J, Araújo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environ Microbiol* 2004;6:1244-51.
- Perin L, Martínez-Aguilar L, Paredes-Valdez G, Baldani J, Estrada-

- De Los Santos P, Reis V, et al. *Burkholderia silvatlantica* sp. nov., a diazotrophic bacterium associated with sugar cane and maize. *Int J Syst Evol Microbiol* 2006;56:1931-7.
23. Govindarajan M, Balandreau J, Kwon SW, Weon HY, Lakshminarasimhan C. Effects of the inoculation of *Burkholderia vietnamensis* and related endophytic diazotrophic bacteria on grain yield of rice. *Microb Ecol* 2008;55:21-37.
 24. Peng G, Yuan Q, Li H, Zhang W, Tan Z. *Rhizobium oryzae* sp. nov., isolated from the wild rice *Oryza alta*. *Int J Syst Evol Microbiol* 2008;58:2158-63.
 25. Dakora F, Matiru V, King M, Phillips D. Plant growth promotion in legumes and cereals by lumichrome, a rhizobial signal metabolite. In: Finan TM, O'Brian MR, Layzell DB, Vessey K, Newton WE, editors. *Nitrogen Fixation: Global Perspectives*. Wallingford, UK: CABI Publishing; 2002. p. 321-2.
 26. Tian CF, Wang ET, Wu LJ, Han TX, Chen WF, Gu CT, et al. *Rhizobium fabae* sp. nov., a bacterium that nodulates *Vicia faba*. *Int J Syst Evol Microbiol* 2008;58:2871-5.
 27. Ramírez-Bahena MH, García-Fraile P, Peix A, Valverde A, Rivas RL, Igual JM, et al. Revision of the taxonomic status of the species *R. leguminosarum* (Frank 1879) Frank 1889AL, *Rhizobium phaseoli* Dangeard 1926AL and *Rhizobium trifolii* Dangeard 1926AL. *R. trifolii* is a later synonym of *R. leguminosarum*. Reclassification of the strain *R. leguminosarum* DSM 30132 (= NCIMB 11478) as *Rhizobium pisi* sp. nov. *Int J Syst Evol Microbiol* 2008;58:2484-90.
 28. Glick BR. Resource acquisition. In: Glick BR, editor. *Beneficial Plant-bacterial Interactions*. Cham: Springer International Publishing; 2015. p. 29-63.
 29. Zhang X, Sun L, Ma X, Sui XH, Jiang R. *Rhizobium pseudoryzae* sp. nov., isolated from the rhizosphere of rice. *Int J Syst Evol Microbiol* 2011;61:2425-9.
 30. Subramanian P, Kim K, Krishnamoorthy R, Sundaram S, Sa T. Endophytic bacteria improve nodule function and plant nitrogen in soybean on co-inoculation with *Bradyrhizobium japonicum* MN110. *Plant Growth Regul* 2015;76:327-32.
 31. Yan J, Yan H, Liu LX, Chen WF, Zhang XX, Verástegui-Valdés MM, et al. *Rhizobium hidalgonense* sp. nov., a nodule endophytic bacterium of *Phaseolus vulgaris* in acid soil. *Arch Microbiol* 2017;199:97-104.
 32. Govindarajan M, Kwon SW, Weon HY. Isolation, molecular characterization and growth-promoting activities of endophytic sugarcane diazotroph *Klebsiella* sp. GR9. *World J Microbiol Biotechnol* 2007;23:997-1006.
 33. Montañez A, Abreu C, Gill PR, Hardarson G, Sicardi M. Biological nitrogen fixation in maize (*Zea mays* L.) by ¹⁵N isotope-dilution and identification of associated culturable diazotrophs. *Biol Fert Soils* 2009;45:253-63.
 34. Mbai F, Magiri E, Matiru V, Nganga J, Nyambati V. Isolation and characterization of bacterial root endophytes with potential to enhance plant growth from Kenyan Basmati rice. *Am Int J Contemp Res* 2013;3:25-40.
 35. Hirsch AM. Brief History of the Discovery of Nitrogen Fixing Organisms; 2009. Available from: http://www.mcd.edu/research/hirsch/imagesb/historydiscovery/n2fixing_organisms.pdf [Last accessed on 2022 Sep 29].
 36. Gopalakrishnan S, Sathya A, Vijayabharathi R, Varshney RK, Gowda CL, Krishnamurthy L. Plant growth promoting rhizobia: Challenges and opportunities. *3 Biotech* 2015;5:355-77.
 37. Gourion B, Berrabah F, Ratet P, Stacey G. *Rhizobium*-legume symbioses: The crucial role of plant immunity. *Trends Plant Sci* 2015;20:186-94.
 38. Youssef M, Eissa M. Biofertilizers and their role in management of plant parasitic nematodes. A review. *E3 J Biotechnol Pharm Res* 2014;5:1-6.
 39. Zhang L, Shi X, Si M, Li C, Zhu L, Zhao L, et al. *Rhizobium smilacinae* sp. nov., an endophytic bacterium isolated from the leaf of *Smilacina japonica*. *Antonie van Leeuwenhoek* 2014;106:715-23.
 40. Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F. *The Prokaryotes: Actinobacteria*. Berlin, Germany: Springer; 2014.
 41. Soe KM, Bhromsiri A, Karladee D, Yamakawa T. Effects of endophytic actinomycetes and *Bradyrhizobium japonicum* strains on growth, nodulation, nitrogen fixation and seed weight of different soybean varieties. *Soil Sci Plant Nutr* 2012;58:319-25.
 42. Lechevalier MP. Taxonomy of the genus *Frankia* (Actinomycetales). *Int J Syst Evol Microbiol* 1994;44:1-8.
 43. Coombs JT, Franco CM. Visualization of an endophytic *Streptomyces* species in wheat seed. *Appl Environ Microbiol* 2003;69:4260-2.
 44. Benson DR, Silvester W. Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. *Microbiol Rev* 1993;57:293-319.
 45. Nouioui I, Ghodhbane-Gtari F, Rohde M, Klenk HP, Gtari M. *Frankia coriariae* sp. nov., an infective and effective microsymbiont isolated from *Coriaria japonica*. *Int J Syst Evol Microbiol* 2017;67:1266-70.
 46. Tian X, Cao L, Tan H, Han W, Chen M, Liu Y, et al. Diversity of cultivated and uncultivated actinobacterial endophytes in the stems and roots of rice. *Microb Ecol* 2007;53:700-7.
 47. Ikeda S, Okubo T, Kaneko T, Inaba S, Maekawa T, Eda S, et al. Community shifts of soybean stem-associated bacteria responding to different nodulation phenotypes and N levels. *ISME J* 2010;4:315.
 48. Shah S, Shah R, Xu H, Aryal U. Biofertilizers: An alternative source of nutrients for sustainable production of tree crops. *J Sustain Agric* 2007;29:85-95.
 49. Bohlool B, Ladha J, Garrity D, George T. Biological nitrogen fixation for sustainable agriculture: A perspective. *Plant Soil* 1992;141:1-11.
 50. Ladha J, Barraquio W, Watanabe I. Immunological techniques to identify *Azospirillum* associated with wetland rice. *Can J Microbiol* 1982;28:478-85.
 51. Rodrigues EP, Rodrigues LS, de Oliveira AL, Baldani VL, dos Santos Teixeira KR, Urquiaga S, et al. *Azospirillum amazonense* inoculation: Effects on growth, yield and N₂ fixation of rice (*Oryza sativa* L.). *Plant Soil* 2008;302:249-61.
 52. de Bellone SC, Bellone C. Presence of endophytic diazotrophs in sugarcane juice. *World J Microbiol Biotechnol* 2006;22:1065-8.
 53. Reis VM, Baldani JI, Baldani VL, Dobereiner J. Biological dinitrogen fixation in gramineae and palm trees. *Crit Rev Plant Sci* 2000;19:227-47.
 54. Dalla Santa OR, Hernández RF, Alvarez GL, Ronzelli P Jr., Soccol CR. *Azospirillum* sp. inoculation in wheat, barley and oats seeds greenhouse experiments. *Braz Arch Biol Technol* 2004;47:843-50.
 55. Cohen AC, Travaglia CN, Bottini R, Piccoli PN. Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botany* 2009;87:455-62.
 56. Yasuda M, Isawa T, Shinozaki S, Minamisawa K, Nakashita H. Effects of colonization of a bacterial endophyte, *Azospirillum* sp. B510, on disease resistance in rice. *Biosci Biotechnol Biochem* 2009;73:2595-9.
 57. De Salamone IE, Di Salvo LP, Ortega JS, Sorte PM, Urquiaga S, Teixeira KR. Field response of rice paddy crop to *Azospirillum* inoculation: Physiology of rhizosphere bacterial communities and the genetic diversity of endophytic bacteria in different parts of the plants. *Plant Soil* 2010;336:351-62.
 58. Trabelsi D, Mhamdi R. Microbial inoculants and their impact on soil microbial communities: A review. *Biomed Res Int* 2013;2013:863240.
 59. Matsumura EE, Secco VA, Moreira RS, dos Santos OJ, Hungria M, de Oliveira AL. Composition and activity of endophytic bacterial communities in field-grown maize plants inoculated with *Azospirillum brasilense*. *Ann Microbiol* 2015;65:2187-200.
 60. Hahn L, Sá EL, Filho BD, Machado RG, Damasceno RG, Giongo A. Rhizobial inoculation, alone or coinoculated with *Azospirillum*

- brasilense*, promotes growth of wetland rice. Rev Bras Ciênc Solo 2016;40:e0160006.
61. Cassán F, Díaz-Zorita M. The Contribution of the use of *Azospirillum* sp. in sustainable agriculture: Learnings from the laboratory to the field. In: Castro-Sowinski S, editor. Microbial Models: From Environmental to Industrial Sustainability. Singapore: Springer Singapore; 2016. p. 293-321.
 62. Fujita M, Kusajima M, Okumura Y, Nakajima M, Minamisawa K, Nakashita H. Effects of colonization of a bacterial endophyte, *Azospirillum* sp. B510, on disease resistance in tomato. Biosci Biotechnol Biochem 2017;81:1657-62.
 63. Kusajima M, Shima S, Fujita M, Minamisawa K, Che FS, Yamakawa H, *et al.* Involvement of ethylene signaling in *Azospirillum* sp. B510-induced disease resistance in rice. Biosci Biotechnol Biochem 2018;82:1522-6.
 64. Mohapatra D, Singh N, Rath S. Prospects and application of *Azospirillum* spp. as a natural agricultural biofertilizer. In: Kumar P, Patra JK, Chandra P, editors. Advances in Microbial Biotechnology. New Jersey: Apple Academic Press; 2018. p. 169-90.
 65. Saikia SP, Jain V. Biological nitrogen fixation with non-legumes: An achievable target or a dogma? Curr Sci 2007;92:317-22.
 66. Elbeltagy A, Nishioka K, Sato T, Suzuki H, Ye B, Hamada T, *et al.* Endophytic colonization and in planta nitrogen fixation by a *Herbaspirillum* sp. isolated from wild rice species. Appl Environ Microbiol 2001;67:5285-93.
 67. Dos Reis FB Jr., Reis VM, Urquiaga S, Döbereiner J. Influence of nitrogen fertilisation on the population of diazotrophic bacteria *Herbaspirillum* spp. and *Acetobacter diazotrophicus* in sugar cane (*Saccharum* spp.). Plant Soil 2000;219:153-9.
 68. Catalán AI, Ferreira F, Gill PR, Batista S. Production of polyhydroxyalkanoates by *Herbaspirillum seropedicae* grown with different sole carbon sources and on lactose when engineered to express the lacZlacY genes. Enzyme Microb Technol 2007;40:1352-7.
 69. Pedrosa FO, Monteiro RA, Wasseem R, Cruz LM, Ayub RA, Colauto NB, *et al.* Genome of *Herbaspirillum seropedicae* strain SmR1, a specialized diazotrophic endophyte of tropical grasses. PLoS Genet 2011;7:e1002064.
 70. Chubatsu LS, Monteiro RA, de Souza EM, de Oliveira MA, Yates MG, Wasseem R, *et al.* Nitrogen fixation control in *Herbaspirillum seropedicae*. Plant Soil 2012;356:197-207.
 71. Canellas LP, Balmori DM, Médiçi LO, Aguiar NO, Campostrini E, Rosa RC, *et al.* A combination of humic substances and *Herbaspirillum seropedicae* inoculation enhances the growth of maize (*Zea mays* L.). Plant Soil 2013;366:119-32.
 72. Straub D, Yang H, Liu Y, Tsap T, Ludewig U. Root ethylene signalling is involved in *Miscanthus sinensis* growth promotion by the bacterial endophyte *Herbaspirillum frisingense* GSF30T. J Exp Bot 2013;64:4603-15.
 73. Hoseinzade H, Ardakani M, Shahdi A, Rahmani HA, Noormohammadi G, Miransari M. Rice (*Oryza sativa* L.) nutrient management using mycorrhizal fungi and endophytic *Herbaspirillum seropedicae*. J Integr Agric 2016;15:1385-94.
 74. Pankievicz V, Camilios-Neto D, Bonato P, Balsanelli E, Tadra-Sfeir M, Faoro H, *et al.* RNA-seq transcriptional profiling of *Herbaspirillum seropedicae* colonizing wheat (*Triticum aestivum*) roots. Plant Mol Biol 2016;90:589-603.
 75. Maheshwari DK, Annapurna K. Endophytes: Crop Productivity and Protection. Berlin, Germany: Springer; 2017.
 76. Yamada Y, Hoshino KI, Ishikawa T. The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA: The elevation of the subgenus *Gluconoacetobacter* to the generic level. Biosci Biotechnol Biochem 1997;61:1244-51.
 77. Stephan M, Oliveira M, Teixeira K, Martinez-Drets G, Döbereiner J. Physiology and dinitrogen fixation of *Acetobacter diazotrophicus*. FEMS Microbiol Lett 1991;77:67-72.
 78. Döbereiner J. Isolation and identification of root associated diazotrophs. In: Skinner FA, Boddey RM, Fendrik I, editors. Nitrogen Fixation with Non-legumes. Dordrecht: Springer; 1989. p. 103-8.
 79. Muthukumarasamy R, Revathi G, Lakshminarasimhan C. Influence of N fertilisation on the isolation of *Acetobacter diazotrophicus* and *Herbaspirillum* spp. from Indian sugarcane varieties. Biol Fert Soils 1999;29:157-64.
 80. James E, Reis V, Olivares F, Baldani J, Döbereiner J. Infection of sugar cane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. J Exp Bot 1994;45:757-66.
 81. Dong Z, Heydrich M, Bernard K, McCully M. Further evidence that the N (inf2)-fixing endophytic bacterium from the intercellular spaces of sugarcane stems is *Acetobacter diazotrophicus*. Appl Environ Microbiol 1995;61:1843-6.
 82. Fuentes-Ramirez LE, Jimenez-Salgado T, Abarca-Ocampo I, Caballero-Mellado J. *Acetobacter diazotrophicus*, an indoleacetic acid producing bacterium isolated from sugarcane cultivars of Mexico. Plant Soil 1993;154:145-50.
 83. Muthukumarasamy R, Revathi G, Seshadri S, Lakshminarasimhan C. *Gluconacetobacter diazotrophicus* (syn. *Acetobacter diazotrophicus*), a promising diazotrophic endophyte in tropics. Curr Sci 2002;83:137-45.
 84. Munoz-Rojas J, Fuentes-Ramirez LE, Caballero-Mellado J. Antagonism among *Gluconacetobacter diazotrophicus* strains in culture media and in endophytic association. FEMS Microbiol Ecol 2005;54:57-66.
 85. Piñón D, Casas M, Blanch Ma, Fontaniella B, Blanco Y, Vicente C, *et al.* *Gluconacetobacter diazotrophicus*, a sugar cane endosymbiont, produces a bacteriocin against *Xanthomonas albilineans*, a sugar cane pathogen. Res Microbiol 2002;153:345-51.
 86. Blanco Y, Arroyo M, Legaz M, Vicente C. Isolation from *Gluconacetobacter diazotrophicus* cell walls of specific receptors for sugarcane glycoproteins, which act as recognition factors. J Chromatogr A 2005;1093:204-11.
 87. Govindarajan M, Balandreau J, Muthukumarasamy R, Revathi G, Lakshminarasimhan C. Improved yield of micropropagated sugarcane following inoculation by endophytic *Burkholderia vietnamiensis*. Plant Soil 2006;280:239-52.
 88. Boddey R, Urquiaga S, Reis V, Döbereiner J. Biological nitrogen fixation associated with sugar cane. Plant Soil 1991;137:111-7.
 89. Baldani J, Caruso L, Baldani VL, Goi SR, Döbereiner J. Recent advances in BNF with non-legume plants. Soil Biol Biochem 1997;29:911-22.
 90. Cojho EH, Reis VM, Schenberg AC, Döbereiner J. Interactions of *Acetobacter diazotrophicus* with an amylolytic yeast in nitrogen-free batch culture. FEMS Microbiol Lett 1993;106:341-6.
 91. Alvarez B, Martínez-Drets G. Metabolic characterization of *Acetobacter diazotrophicus*. Can J Microbiol 1995;41:918-24.
 92. Muthukumarasamy R, Cleenwerck I, Revathi G, Vadvivelu M, Janssens D, Hoste B, *et al.* Natural association of *Gluconacetobacter diazotrophicus* and diazotrophic *Acetobacter peroxydans* with wetland rice. Syst Appl Microbiol 2005;28:277-86.
 93. Rouws L, Meneses C, Guedes H, Vidal M, Baldani J, Schwab S. Monitoring the colonization of sugarcane and rice plants by the endophytic diazotrophic bacterium *Gluconacetobacter diazotrophicus* marked with *gfp* and *gusA* reporter genes. Lett Appl Microbiol 2010;51:325-30.
 94. Alquéres S, Meneses C, Rouws L, Rothballer M, Baldani I, Schmid M, *et al.* The bacterial superoxide dismutase and glutathione reductase are crucial for endophytic colonization of rice roots by *Gluconacetobacter diazotrophicus* PAL5. Mol Plant Microbe Interact 2013;26:937-45.
 95. de Souza AR, De Souza S, De Oliveira M, Ferraz T, Figueiredo F,

- Da Silva N, et al. Endophytic colonization of *A. thaliana* by *Gluconacetobacter diazotrophicus* and its effect on plant growth promotion, plant physiology, and activation of plant defense. *Plant Soil* 2016;399:257-70.
96. Stopnisek N, Bodenhausen N, Frey B, Fierer N, Eberl L, Weisskopf L. Genus-wide acid tolerance accounts for the biogeographical distribution of soil *Burkholderia* populations. *Environ Microbiol* 2014;16:1503-12.
 97. Isles A, Maclusky I, Corey M, Gold R, Prober C, Fleming P, et al. *Pseudomonas cepacia* infection in cystic fibrosis: An emerging problem. *J Pediatr* 1984;104:206-10.
 98. Vandamme P, Holmes B, Vancanneyt M, Coenye T, Hoste B, Coopman R, et al. Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. *Int J Syst Evol Microbiol* 1997;47:1188-200.
 99. Pérez-Pantoja D, Donoso R, Agulló L, Córdova M, Seeger M, Pieper DH, et al. Genomic analysis of the potential for aromatic compounds biodegradation in Burkholderiales. *Environ Microbiol* 2012;14:1091-117.
 100. Aizawa T, Ve NB, Nakajima M, Sunairi M. *Burkholderia heleia* sp. nov., a nitrogen-fixing bacterium isolated from an aquatic plant, *Eleocharis dulcis*, that grows in highly acidic swamps in actual acid sulfate soil areas of Vietnam. *Int J Syst Evol Microbiol* 2010;60:1152-7.
 101. Baldani V, Oliveira E, Balota E, Baldani J, Kirchoff G, Döbereiner J, et al. *Burkholderia brasiliensis* sp. nov., uma nova espécie de bactéria diazotrófica endofítica. *An Acad Bras Cienc* 1997;69:116.
 102. Sessitsch A, Coenye T, Sturz A, Vandamme P, Barka EA, Salles J, et al. *Burkholderia phytofirmans* sp. nov., a novel plant-associated bacterium with plant-beneficial properties. *Int J Syst Evol Microbiol* 2005;55:1187-92.
 103. Coenye T, Laevens S, Willems A, Ohlén M, Hannant W, Govan J, et al. *Burkholderia fungorum* sp. nov. and *Burkholderia caledonica* sp. nov., two new species isolated from the environment, animals and human clinical samples. *Int J Syst Evol Microbiol* 2001;51:1099-107.
 104. Frommel MI, Nowak J, Lazarovits G. Growth enhancement and developmental modifications of *in vitro* grown potato (*Solanum tuberosum* spp. *tuberosum*) as affected by a nonfluorescent *Pseudomonas* sp. *Plant Physiol* 1991;96:928-36.
 105. Barka EA, Belarbi A, Hachet C, Nowak J, Audran JC. Enhancement of *in vitro* growth and resistance to gray mould of *Vitis vinifera* co-cultured with plant growth-promoting rhizobacteria. *FEMS Microbiol Lett* 2000;186:91-5.
 106. Nowak J, Asiedu S, Lazarovits G, Pillay V, Stewart A, Smith C, et al. Enhancement of *in vitro* growth and transplant stress tolerance of potato and vegetable plantlets co-cultured with a plant growth promoting pseudomonad bacterium. In: Chagvardieff CP, editor. *Ecophysiology and Photosynthetic In Vitro Cultures*. Aix en Provence: CEA; 1995. p. 173-80.
 107. Pillay V, Nowak J. Inoculum density, temperature, and genotype effects on *in vitro* growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. *Can J Microbiol* 1997;43:354-61.
 108. Barka EA, Gognies S, Nowak J, Audran JC, Belarbi A. Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. *Biol Control* 2002;24:135-42.
 109. Luvizotto DM, Marcon J, Andreote FD, Dini-Andreote F, Neves AA, Araújo WL, et al. Genetic diversity and plant-growth related features of *Burkholderia* spp. from sugarcane roots. *World J Microbiol Biotechnol* 2010;26:1829-36.
 110. Compant S, Kaplan H, Sessitsch A, Nowak J, Ait Barka E, Clément C. Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: from the rhizosphere to inflorescence tissues. *FEMS Microbiol Ecol* 2007;63:84-93.
 111. Mitter B, Petric A, Shin MW, Chain PS, Hauberg-Lotte L, Reinhold-Hurek B, et al. Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. *Front Plant Sci* 2013;4:120.
 112. Lowman S, Kim-Dura S, Mei C, Nowak J. Strategies for enhancement of switchgrass (*Panicum virgatum* L.) performance under limited nitrogen supply based on utilization of N-fixing bacterial endophytes. *Plant Soil* 2016;405:47-63.
 113. Baldani VD, Baldani JI, Döbereiner J. Inoculation of rice plants with the endophytic diazotrophs *Herbaspirillum seropedicae* and *Burkholderia* spp. *Biol Fert Soils* 2000;30:485-91.
 114. Singh RK, Mishra RP, Jaiswal HK, Kumar V, Pandey SP, Rao SB, et al. Isolation and identification of natural endophytic rhizobia from rice (*Oryza sativa* L.) through rDNA PCR-RFLP and sequence analysis. *Curr Microbiol* 2006;52:345-9.
 115. Procópio R, Araújo W, Maccheroni W Jr., Azevedo J. Characterization of an endophytic bacterial community associated with *Eucalyptus* spp. *Genet Mol Res* 2009;8:1408-22.
 116. Van VT, Berge O, Ke SN, Balandreau J, Heulin T. Repeated beneficial effects of rice inoculation with a strain of *Burkholderia vietnamiensis* early and late yield components in low fertility sulphate acid soils of Vietnam. *Plant Soil* 2000;218:273-84.
 117. Mishra PK, Mishra S, Selvakumar G, Bisht J, Kundu S, Gupta HS. Coinoculation of *Bacillus thuringiensis*-KR1 with *Rhizobium leguminosarum* enhances plant growth and nodulation of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.). *World J Microbiol Biotechnol* 2009;25:753-61.
 118. Wulf E, Mguni C, Mansfeld-Giese K, Fels J, Lübeck M, Hockenhull J. Biochemical and molecular characterization of *B. amyloliquefaciens*, *B. subtilis* and *B. pumilus* isolates with distinct antagonistic potential against *Xanthomonas campestris* pv. *campestris*. *Plant Pathol* 2002;51:574-84.
 119. Rajendran L, Karthikeyan G, Raguchander T, Samiyappan R. Cloning and sequencing of novel endophytic *Bacillus subtilis* from coconut for the management of basal stem rot disease. *Asian J Plant Pathol* 2008;2:1-14.
 120. Chen Y, Gao X, Chen Y, Qin H, Huang L, Han Q. Inhibitory efficacy of endophytic *Bacillus subtilis* EDR4 against *Sclerotinia sclerotiorum* on rapeseed. *Biol Control* 2014;78:67-76.
 121. Wang H, Wen K, Zhao X, Wang X, Li A, Hong H. The inhibitory activity of endophytic *Bacillus* sp. strain CHM1 against plant pathogenic fungi and its plant growth-promoting effect. *Crop Prot* 2009;28:634-9.
 122. Liu M, Luo K, Wang Y, Zeng A, Zhou X, Luo F, et al. Isolation, identification and characteristics of an endophytic quinclorac degrading bacterium *Bacillus megaterium* Q3. *PLoS One* 2014;9:e108012.
 123. Khalifa AY, Almalki MA. Isolation and characterization of an endophytic bacterium, *Bacillus megaterium* BMN1, associated with root-nodules of *Medicago sativa* L. growing in Al-Ahsaa region, Saudi Arabia. *Ann Microbiol* 2015;65:1017-26.
 124. Munjal V, Nadakkakath AV, Sheoran N, Kundu A, Venugopal V, Subaharan K, et al. Genotyping and identification of broad spectrum antimicrobial volatiles in black pepper root endophytic biocontrol agent, *Bacillus megaterium* BP17. *Biol Control* 2016;92:66-76.
 125. Gupta C, Kumar B, Dubey R, Maheshwari D. Chitinase-mediated destructive antagonistic potential of *Pseudomonas aeruginosa* GRC 1 against *Sclerotinia sclerotiorum* causing stem rot of peanut. *Biocontrol* 2006;51:821-35.
 126. Chung BS, Aslam Z, Kim SW, Kim GG, Kang HS, Ahn JW, et al. A bacterial endophyte, *Pseudomonas brassicacearum* YC5480,

- isolated from the root of *Artemisia* sp. producing antifungal and phytotoxic compounds. *Plant Pathol J* 2008;24:461-8.
127. Weyens N, Truyens S, Dupae J, Newman L, Taghavi S, Van Der Lelie D, et al. Potential of the TCE-degrading endophyte *Pseudomonas putida* W619-TCE to improve plant growth and reduce TCE phytotoxicity and evapotranspiration in poplar cuttings. *Environ Pollut* 2010;158:2915-9.
 128. Aeron A, Dubey R, Maheshwari D, Pandey P, Bajpai VK, Kang SC. Multifarious activity of bioformulated *Pseudomonas fluorescens* PS1 and biocontrol of *Sclerotinia sclerotiorum* in Indian rapeseed (*Brassica campestris* L.). *Euro J Plant Pathol* 2011;131:81-93.
 129. Pandey PK, Yadav SK, Singh A, Sarma BK, Mishra A, Singh HB. Cross-species alleviation of biotic and abiotic stresses by the endophyte *Pseudomonas aeruginosa* PW09. *J Phytopathol* 2012;160:532-9.
 130. Gupta G, Panwar J, Jha PN. Natural occurrence of *Pseudomonas aeruginosa*, a dominant cultivable diazotrophic endophytic bacterium colonizing *Pennisetum glaucum* (L.) R. Br. *Appl Soil Ecol* 2013;64:252-61.
 131. Sun K, Liu J, Gao Y, Jin L, Gu Y, Wang W. Isolation, plant colonization potential, and phenanthrene degradation performance of the endophytic bacterium *Pseudomonas* sp. Ph6-gfp. *Sci Rep* 2014;4:5462.
 132. Miller S, Mark G, Franks A, O'Gara F. *Pseudomonas*-plant interactions. In: *Pseudomonas: Model Organism, Pathogen, Cell Factory*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim; 2008. p. 353-76.
 133. Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. *Ann Rev Microbiol* 2009;63:541-56.
 134. Zhao LF, Xu YJ, Ma ZQ, Deng ZS, Shan CJ, Wei GH. Colonization and plant growth promoting characterization of endophytic *Pseudomonas chlororaphis* strain Zong1 isolated from *Sophora alopecuroides* root nodules. *Braz J Microbiol* 2013;44:629-37.
 135. Tariq T, Shafiqe HA, Sultana V, Ehteshamul-Haque S. Management of root rot disease of wheat with endophytic plant growth promoting *Pseudomonas* associated with healthy wheat roots. *Int J Biol Res* 2014;2:39-43.
 136. Pham VT, Rediers H, Ghequire MG, Nguyen HH, De Mot R, Vanderleyden J, et al. The plant growth-promoting effect of the nitrogen-fixing endophyte *Pseudomonas stutzeri* A15. *Arch Microbiol* 2017;199:513-7.
 137. Sandhya V, Shrivastava M, Ali SZ, Prasad VS. Endophytes from maize with plant growth promotion and biocontrol activity under drought stress. *Russ Agric Sci* 2017;43:22-34.
 138. Ma Y, Rajkumar M, Moreno A, Zhang C, Freitas H. Serpentine endophytic bacterium *Pseudomonas azotoformans* ASS1 accelerates phytoremediation of soil metals under drought stress. *Chemosphere* 2017;185:75-85.
 139. Verma SK, Kingsley K, Irizarry I, Bergen M, Kharwar RN, White JF. Seed vectored endophytic bacteria modulate development of rice seedlings. *J Appl Microbiol* 2017;122:1680-91.
 140. Jha P, Kumar A. Characterization of novel plant growth promoting endophytic bacterium *Achromobacter xylosoxidans* from wheat plant. *Microb Ecol* 2009;58:179-88.
 141. Ladha JK, Reddy P. *The Quest for Nitrogen Fixation in Rice*. Los Baños, Philippines: International Rice Research Institute; 2000.
 142. Egener T, Martin DE, Sarkar A, Reinhold-Hurek B. Role of a ferredoxin gene cotranscribed with the nifHDK operon in N₂ fixation and nitrogenase "switch-off" of *Azoarcus* sp. strain BH72. *J Bacteriol* 2001;183:3752-60.
 143. Malik K, Bilal R, Mehnaz S, Rasul G, Mirza M, Ali S. Association of nitrogen-fixing, plant-growth-promoting rhizobacteria (PGPR) with kallar grass and rice. In: Ladha JK, de Bruijn FJ, Malik KA, editors. *Opportunities for Biological Nitrogen Fixation in Rice and other Non-legumes*. Dordrecht: Springer; 1997. p. 37-44.
 144. Ahemad M, Khan MS. Effect of insecticide-tolerant and plant growth-promoting *Mesorhizobium* on the performance of chickpea grown in insecticide stressed alluvial soils. *J Crop Sci Biotechnol* 2009;12:217-26.
 145. Ramos PL, Van Trappen S, Thompson FL, Rocha RC, Barbosa HR, De Vos P, et al. Screening for endophytic nitrogen-fixing bacteria in Brazilian sugar cane varieties used in organic farming and description of *Stenotrophomonas pavanii* sp. nov. *Int J Syst Evol Microbiol* 2011;61:926-31.
 146. Dutta D, Gachhui R. Novel nitrogen-fixing *Acetobacter nitrogenifigens* sp. nov., isolated from Kombucha tea. *Int J Syst Evol Microbiol* 2006;56:1899-903.
 147. Gillis M, Kersters K, Hoste B, Janssens D, Kroppenstedt RM, Stephan MP, et al. *Acetobacter diazotrophicus* sp. nov., a nitrogen-fixing acetic acid bacterium associated with sugarcane. *Int J Syst Evol Microbiol* 1989;39:361-4.
 148. Caballero-Mellado J, Martínez-Aguilar L, Paredes-Valdez G, Santos PE-dl. *Burkholderia unamae* sp. nov., an N₂-fixing rhizospheric and endophytic species. *Int J Syst Evol Microbiol* 2004;54:1165-72.
 149. Zhu B, Zhou Q, Lin L, Hu C, Shen P, Yang L, et al. *Enterobacter sacchari* sp. nov., a nitrogen-fixing bacterium associated with sugar cane (*Saccharum officinarum* L.). *Int J Syst Evol Microbiol* 2013;63:2577-82.
 150. Lin L, Wei C, Chen M, Wang H, Li Y, Li Y, et al. Complete genome sequence of endophytic nitrogen-fixing *Klebsiella variicola* strain DX120E. *Stand Genom Sci* 2015;10:22.
 151. Loganathan P, Nair S. Crop-specific endophytic colonization by a novel, salt-tolerant, N₂-fixing and phosphate-solubilizing *Gluconacetobacter* sp. from wild rice. *Biotechnol Lett* 2003;25:497-501.
 152. Tripathi AK, Verma SC, Chowdhury SP, Lebuhn M, Gatteringer A, Schloter M. *Ochrobactrum oryzae* sp. nov., an endophytic bacterial species isolated from deep-water rice in India. *Int J Syst Evol Microbiol* 2006;56:1677-80.
 153. Zhang GX, Peng GX, Wang ET, Yan H, Yuan QH, Zhang W, et al. Diverse endophytic nitrogen-fixing bacteria isolated from wild rice *Oryza rufipogon* and description of *Phytobacter diazotrophicus* gen. nov. sp. nov. *Arch Microbiol* 2008;189:431-9.
 154. Chaudhary HJ, Peng G, Hu M, He Y, Yang L, Luo Y, et al. Genetic diversity of endophytic diazotrophs of the wild rice, *Oryza alta* and identification of the new diazotroph, *Acinetobacter oryzae* sp. nov. *Microb Ecol* 2012;63:813-21.
 155. Hardoim PR, Nazir R, Sessitsch A, Elhottová D, Korenblum E, van Overbeek LS, et al. The new species *Enterobacter oryziphilus* sp. nov. and *Enterobacter oryzendophyticus* sp. nov. are key inhabitants of the endosphere of rice. *BMC Microbiol* 2013;13:164.
 156. Dreyfus B, Garcia JL, Gillis M. Characterization of *Azorhizobium caulinodans* gen. nov., sp. nov., a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata*. *Int J Syst Evol Microbiol* 1988;38:89-98.
 157. Glazebrook J, Walker GC. A novel exopolysaccharide can function in place of the calcofluor-binding exopolysaccharide in nodulation of alfalfa by *Rhizobium meliloti*. *Cell* 1989;56:661-72.
 158. Martínez-Romero E, Segovia L, Mercante FM, Franco AA, Graham P, Pardo MA. *Rhizobium tropici*, a novel species nodulating *Phaseolus vulgaris* L. beans and *Leucaena* sp. trees. *Int J Syst Evol Microbiol* 1991;41:417-26.
 159. El-Hamdaoui A, Redondo-Nieto M, Rivilla R, Bonilla I, Bolanos L. Effects of boron and calcium nutrition on the establishment of the *Rhizobium leguminosarum*-pea (*Pisum sativum*) symbiosis and nodule development under salt stress. *Plant Cell Environ* 2003;26:1003-11.
 160. Wang F, Wang ET, Wu LJ, Sui XH, Li Jr Y, Chen WX. *Rhizobium vallis* sp. nov., isolated from nodules of three leguminous species. *Int*

- J Syst Evol Microbiol 2011;61:2582-8.
161. Sevilla M, Meletzus D, Teixeira K, Lee S, Nutakki A, Baldani I, et al. Analysis of nif and regulatory genes in *Acetobacter diazotrophicus*. Soil Biol Biochem 1997;29:871-4.
 162. Black M, Moolhuijzen P, Chapman B, Barrero R, Howieson J, Hungria M, et al. The genetics of symbiotic nitrogen fixation: Comparative genomics of 14 rhizobia strains by resolution of protein clusters. Genes 2012;3:138-66.
 163. Maillat F, Poinsoit V, André O, Puech-Pagès V, Haouy A, Gueunier M, et al. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 2011;469:58-63.
 164. Peng G, Wang H, Zhang G, Hou W, Liu Y, Wang ET, et al. *Azospirillum melinis* sp. nov., a group of diazotrophs isolated from tropical molasses grass. Int J Syst Evol Microbiol 2006;56:1263-71.
 165. Chen WM, James EK, Coenye T, Chou JH, Barrios E, De Faria SM, et al. *Burkholderia mimosarum* sp. nov., isolated from root nodules of *Mimosa* spp. from Taiwan and South America. Int J Syst Evol Microbiol 2006;56:1847-51.
 166. Peng G, Zhang W, Luo H, Xie H, Lai W, Tan Z. *Enterobacter oryzae* sp. nov., a nitrogen-fixing bacterium isolated from the wild rice species *Oryza latifolia*. Int J Syst Evol Microbiol 2009;59:1650-5.
 167. Gao JL, Lv FY, Wang XM, Yuan M, Li JW, Wu QY, et al. *Flavobacterium endophyticum* sp. nov., a nif H gene-harboring endophytic bacterium isolated from maize root. Int J Syst Evol Microbiol 2015;65:3900-4.
 168. Dutta D, Gachhui R. Nitrogen-fixing and cellulose-producing *Gluconacetobacter kombuchae* sp. nov., isolated from Kombucha tea. Int J Syst Evol Microbiol 2007;57:353-7.
 169. Valverde A, Velazquez E, Gutierrez C, Cervantes E, Ventosa A, Igual JM. *Herbaspirillum lusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of *Phaseolus vulgaris*. Int J Syst Evol Microbiol 2003;53:1979-83.
 170. Xing K, Bian GK, Qin S, Klenk HP, Yuan B, Zhang YJ, et al. *Kibdelosporangium phytohabitans* sp. nov., a novel endophytic actinomycete isolated from oil-seed plant *Jatropha curcas* L. containing 1-aminocyclopropane-1-carboxylic acid deaminase. Antonie Leeuwenhoek 2012;101:433-41.
 171. Román-Ponce B, Wang D, Vásquez-Murrieta MS, Chen WF, Estrada-de los Santos P, Sui XH, et al. *Kocuria arsenatis* sp. nov., an arsenic-resistant endophytic actinobacterium associated with *Prosopis laegivata* grown on high-arsenic-polluted mine tailing. Int J Syst Evol Microbiol 2016;66:1027-33.
 172. Behera P, Ramana VV, Maharana B, Joseph N, Vaishampayan P, Singh NK, et al. *Mangrovibacter phragmitis* sp. nov., an endophyte isolated from the roots of *Phragmites karka*. Int J Syst Evol Microbiol 2017;67:1228-34.
 173. Rozahon M, Ismayil N, Hamood B, Erkin R, Abdurahman M, Mamtimin H, et al. *Rhizobium populi* sp. nov., an endophytic bacterium isolated from *Populus euphratica*. Int J Syst Evol Microbiol 2014;64:3215-21.
 174. Guo GN, Zhou X, Zhao R, Chen XY, Chen ZL, Li XD, et al. *Paenibacillus herberti* sp. nov., an endophyte isolated from *Herbertus sendtneri*. Antonie van Leeuwenhoek 2015;108:587-96.
 175. Gao JL, Lv FY, Wang XM, Qiu TL, Yuan M, Li JW, et al. *Paenibacillus wenxiniae* sp. nov., a nif H gene-harboring endophytic bacterium isolated from maize. Antonie Leeuwenhoek 2015;108:1015-22.
 176. Madhaiyan M, Jin TY, Roy JJ, Kim SJ, Weon HY, Kwon SW, et al. *Pleomorphomonas diazotrophica* sp. nov., an endophytic N-fixing bacterium isolated from root tissue of *Jatropha curcas* L. Int J Syst Evol Microbiol 2013;63:2477-83.
 177. Kesari V, Ramesh AM, Rangan L. *Rhizobium pongamiae* sp. nov. from root nodules of *Pongamia pinnata*. BioMed Res Int 2013;2013:165198.
 178. Lin DX, Wang ET, Tang H, Han TX, He YR, Guan SH, et al. *Shinella kummerowiae* sp. nov., a symbiotic bacterium isolated from root nodules of the herbal legume *Kummerowia stipulacea*. Int J Syst Evol Microbiol 2008;58:1409-13.
 179. Gisin J, Müller A, Pfänder Y, Leimkühler S, Narberhaus F, Masepohl B. A *Rhodobacter capsulatus* member of a universal permease family imports molybdate and other oxyanions. J Bacteriol 2010;192:5943-52.
 180. Bhattacharyya P, Jha D. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World J Microbiol Biotechnol 2012;28:1327-50.
 181. Loiret F, Ortega E, Kleiner D, Ortega-Rodés P, Rodes R, Dong Z. A putative new endophytic nitrogen-fixing bacterium *Pantoea* sp. from sugarcane. J Appl Microbiol 2004;97:504-11.
 182. Defez R, Andreozzi A, Bianco C. The overproduction of indole-3-acetic acid (IAA) in endophytes upregulates nitrogen fixation in both bacterial cultures and inoculated rice plants. Microb Ecol 2017;74:441-52.
 183. Santi C, Bogusz D, Franche C. Biological nitrogen fixation in non-legume plants. Ann Bot 2013;111:743-67.
 184. Pan B, Vessey JK. Response of the endophytic diazotroph *Gluconacetobacter diazotrophicus* on solid media to changes in atmospheric partial O₂ pressure. Appl Environ Microbiol 2001;67:4694-700.
 185. Jacobson MR, Cash VL, Weiss MC, Laird NF, Newton WE, Dean DR. Biochemical and genetic analysis of the nifUSVWZM cluster from *Azotobacter vinelandii*. Mol Gen Genet 1989;219:49-57.
 186. Arnold W, Rump A, Klipp W, Priefer UB, Pühler A. Nucleotide sequence of a 24,206-base-pair DNA fragment carrying the entire nitrogen fixation gene cluster of *Klebsiella pneumoniae*. J Mol Biol 1988;203:715-38.
 187. Merrick M, Edwards R. Nitrogen control in bacteria. Microbiol Mol Biol Rev 1995;59:604-22.
 188. Ureta A, Nordlund S. Evidence for conformational protection of nitrogenase against oxygen in *Gluconacetobacter diazotrophicus* by a putative FeSII protein. J Bacteriol 2002;184:5805-9.
 189. Vermeiren H, Willems A, Schoofs G, De Mot R, Keijers V, Hai W, et al. The rice inoculant strain *Alcaligenes faecalis* A15 is a nitrogen-fixing *Pseudomonas stutzeri*. Syst Appl Microbiol 1999;22:215-24.
 190. Egener T, Hurek T, Reinhold-Hurek B. Use of green fluorescent protein to detect expression of nif genes of *Azoarcus* sp. BH72, a grass-associated diazotroph, on rice roots. Mol Plant Microbe Interact 1998;11:71-5.
 191. Reis VM, Döbereiner J. Effect of high sugar concentration on nitrogenase activity of *Acetobacter diazotrophicus*. Arch Microbiol 1998;171:13-8.
 192. Madhaiyan M, Poonguzhali S, Hari K, Saravanan V, Sa T. Influence of pesticides on the growth rate and plant-growth promoting traits of *Gluconacetobacter diazotrophicus*. Pestic Biochem Physiol 2006;84:143-54.
 193. Kennedy I. Integration of nitrogenase in cellular metabolism. In: Hardy RW, Bottomley R, Burns RC, editors. A Treatise of Dinitrogen Fixation: Inorganic and Physical Chemistry and Biochemistry. New York: Wiley; 1979. p. 653-90.
 194. Coppola D, Giordano D, Tinajero-Trejo M, Di Prisco G, Ascenzi P, Poole RK, et al. Antarctic bacterial haemoglobin and its role in the protection against nitrogen reactive species. Biochim Biophys Acta Proteins Proteom 2013;1834:1923-31.
 195. Glick BR. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica 2012;2012:963401.
 196. Ronson CW, Primrose SB. Carbohydrate metabolism in *Rhizobium trifolii*: Identification and symbiotic properties of mutants. Microbiology 1979;112:77-88.
 197. Thaweenut N, Hachisuka Y, Ando S, Yanagisawa S, Yoneyama T. Two seasons' study on nifH gene expression and nitrogen fixation

- by diazotrophic endophytes in sugarcane (*Saccharum* spp. hybrids): Expression of nifH genes similar to those of rhizobia. *Plant Soil* 2011;338:435-49.
198. Roncato-Maccari LD, Ramos HJ, Pedrosa FO, Alquini Y, Chubatsu LS, Yates MG, *et al.* Endophytic *Herbaspirillum seropedicae* expresses nif genes in gramineous plants. *FEMS Microbiol Ecol* 2003;45:39-47.
 199. Shoebitz M, Ribaudó CM, Pardo MA, Cantore ML, Ciampi L, Cura JA. Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from *Lolium perenne* rhizosphere. *Soil Biol Biochem* 2009;41:1768-74.
 200. Hongrittipun P, Youpensuk S, Rerkasem B. Screening of Nitrogen Fixing Endophytic Bacteria in *Oryza sativa* L. *J Agric Sci* 2014;6:66.
 201. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 2003;255:571-86.
 202. Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN, *et al.* Endophytic fungi: Biodiversity, ecological significance and potential industrial applications. In: Yadav AN, Mishra S, Singh S, Gupta A, editors. *Recent Advancement in White Biotechnology through Fungi: Diversity and Enzymes Perspectives*. Vol. 1. Springer, Switzerland; 2019. p. 1-62.
 203. Rana KL, Kour D, Kaur T, Devi R, Negi C, Yadav AN, *et al.* Endophytic fungi from medicinal plants: Biodiversity and biotechnological applications. In: Kumar A, Radhakrishnan E, editors. *Microbial Endophytes*. Cambridge, MA: Woodhead Publishing; 2020. p. 273-305.
 204. Yadav AN, Singh J, Rastegari AA, Yadav N. *Plant Microbiomes for Sustainable Agriculture*. Cham: Springer; 2020.
 205. Kour D, Rana KL, Kaur T, Yadav N, Yadav AN, Kumar M, *et al.* Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and -mobilizing microbes: A review. *Pedosphere* 2021;31:43-75.
 206. McAfee J. Potassium, a Key Nutrient for Plant Growth. Department of Soil and Crop Sciences; 2008. Available from: <http://jimcmcafee.tamu.edu/files/potassium> [Last accessed on 2022 Sep 29].
 207. Rodríguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 1999;17:319-39.
 208. Kumar M, Tomar RS, Lade H, Paul D. Methylophilic bacteria in sustainable agriculture. *World J Microbiol Biotechnol* 2016;32:120.
 209. Verma SC, Ladha JK, Tripathi AK. Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. *J Biotechnol* 2001;91:127-41.
 210. Alori ET, Glick BR, Babalola OO. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Front Microbiol* 2017;8:971.
 211. Sashidhar B, Podile AR. Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase. *J Appl Microbiol* 2010;109:1-12.
 212. Kapri A, Tewari L. Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* spp. *Braz J Microbiol* 2010;41:787-95.
 213. Martínez-Viveros O, Jorquera M, Crowley D, Gajardo G, Mora M. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J Soil Sci Plant Nutr* 2010;10:293-319.
 214. McGrath JW, Wisdom GB, McMullan G, Larkin MJ, Quinn JP. The purification and properties of phosphonoacetate hydrolase, a novel carbon-phosphorus bond-cleavage enzyme from *Pseudomonas fluorescens* 23F. *Eur J Biochem* 1995;234(1):225-30.
 215. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann Microbiol* 2010;60:579-98.
 216. Otieno N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, *et al.* Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front Microbiol* 2015;6:745.
 217. You C, Zhou F. Non-nodular endorhizospheric nitrogen fixation in wetland rice. *Can J Microbiol* 1989;35:403-8.
 218. Oliveira Ad, Urquiaga S, Döbereiner J, Baldani J. The effect of inoculating endophytic N₂-fixing bacteria on micropropagated sugarcane plants. *Plant Soil* 2002;242:205-15.
 219. Saubidet MI, Fatta N, Barneix AJ. The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. *Plant Soil* 2002;245:215-22.
 220. Ji SH, Gururani MA, Chun SC. Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiol Res* 2014;169:83-98.
 221. Bai Y, Zhou X, Smith DL. Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. *Crop Sci* 2003;43(5):1774-81.
 222. James EK, Gyaneshwar P, Barraquío WL, Mathan N, Ladha JK. Endophytic diazotrophs associated with rice. In: Ladha JK, Reddy PM, editors. *The Quest for Nitrogen Fixation in Rice*. Makati City, Philippines: International Rice Research Institute; 2000. p. 119-40.
 223. Ladha J, Barraquío W, Watanabe I. Isolation and identification of nitrogen-fixing *Enterobacter cloacae* and *Klebsiella planticola* associated with rice plants. *Can J Microbiol* 1983;29:1301-8.
 224. Mirza MS, Ahmad W, Latif F, Haurat J, Bally R, Normand P, *et al.* Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane *in vitro*. *Plant Soil* 2001;237:47-54.
 225. Eskin N. Colonization of *Zea mays* by the Nitrogen Fixing Bacterium *Gluconacetobacter diazotrophicus*. Electronic Thesis and Dissertation Respository; 2012.
 226. Suman A, Shrivastava A, Gaur A, Singh P, Singh J, Yadav R. Nitrogen use efficiency of sugarcane in relation to its BNF potential and population of endophytic diazotrophs at different N levels. *Plant Growth Regul* 2008;54:1-11.
 227. Ladha J, Barraquío W, Revilla L. Isolation of endophytic diazotrophic bacteria from wetland rice. In: Ladha JK, de Bruijn FJ, Malik KA, editors. *Opportunities for Biological Nitrogen Fixation in Rice and other Non-legumes*. Dordrecht: Springer; 1997. p. 15-24.
 228. Roesch LF, Camargo FA, Bento FM, Triplett EW. Biodiversity of diazotrophic bacteria within the soil, root and stem of field-grown maize. *Plant Soil* 2008;302:91-104.
 229. Gyaneshwar P, James EK, Mathan N, Reddy PM, Reinhold-Hurek B, Ladha JK. Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. *J Bacteriol* 2001;183:2634-45.
 230. Puri A, Padda KP, Chanway CP. Can a diazotrophic endophyte originally isolated from lodgepole pine colonize an agricultural crop (corn) and promote its growth? *Soil Biol Biochem* 2015;89:210-6.
 231. Singh D, Rajawat MV, Kaushik R, Prasanna R, Saxena AK. Beneficial role of endophytes in biofortification of Zn in wheat genotypes varying in nutrient use efficiency grown in soils sufficient and deficient in Zn. *Plant Soil* 2017;416:107-16.
 232. Bayliss W, Starling EH. Croonian lecture: The chemical regulation of the secretory process. *Proc R Soc Lond* 1904;73:310-22.
 233. Li JH, Wang ET, Chen WF, Chen WX. Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang Province of China. *Soil Biol Biochem* 2008;40:238-46.
 234. Ambawade M, Pathade G. Production of gibberellic acid by *Bacillus siamensis* BE 76 isolated from banana plant (*Musa* spp.). *Int J Sci Res* 2015;4:394-8.
 235. Yoon V, Tian G, Vessey JK, Macfie SM, Dangi OP, Kumer AK, *et al.* Colonization efficiency of different sorghum genotypes by *Gluconacetobacter diazotrophicus*. *Plant Soil* 2016;398:243-56.
 236. Rodrigues EP, Soares CP, Galvão PG, Imada EL, Simões-Araújo JL,

- Rouws LF, *et al.* Identification of genes involved in indole-3-acetic acid Biosynthesis by *Gluconacetobacter diazotrophicus* PAL5 strain using transposon mutagenesis. *Front Microbiol* 2016;7:1572.
237. Bhutani N, Maheshwari R, Negi M, Suneja P. Optimization of IAA production by endophytic *Bacillus* spp. from *Vigna radiata* for their potential use as plant growth promoters. *Israel J Plant Sci* 2018;65:83-96.
238. Crowley DE. Microbial siderophores in the plant rhizosphere. In: Barton LL, Abadia J, editors. *Iron Nutrition in Plants and Rhizospheric Microorganisms*. Dordrecht: Springer Netherlands; 2006. p. 169-98.
239. Bultreys A. Siderotyping, a tool to characterize, classify and identify fluorescent pseudomonads. In: Varma A, Chincholkar SB, editors. *Microbial Siderophores*. Berlin, Heidelberg: Springer; 2007. p. 67-89.
240. Loaces I, Ferrando L, Scavino AF. Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. *Microb Ecol* 2011;61:606-18.
241. Abbamondi GR, Tommonaro G, Weyens N, Thijs S, Sillen W, Gkorezis P, *et al.* Plant growth-promoting effects of rhizospheric and endophytic bacteria associated with different tomato cultivars and new tomato hybrids. *Chem Biol Technol Agric* 2016;3:1-10.
242. Kong Z, Deng Z, Glick BR, Wei G, Chou M. A nodule endophytic plant growth-promoting *Pseudomonas* and its effects on growth, nodulation and metal uptake in *Medicago lupulina* under copper stress. *Ann Microbiol* 2017;67:49-58.
243. Dolphen R, Thiravetyan P. Reducing arsenic in rice grains by leonardite and arsenic-resistant endophytic bacteria. *Chemosphere* 2019;223:448-54.
244. Abeles FB, Morgan P, Saltveit Jr M. *Ethylene in Plant Biology*. San Diego: Academic Press; 1992.
245. Glick BR. The enhancement of plant growth by free-living bacteria. *Can J Microbiol* 1995;41:109-17.
246. Penrose DM, Glick BR. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol Plant* 2003;118:10-5.
247. Glick BR. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiol Lett* 2005;251:1-7.
248. Shaharoon B, Arshad M, Zahir ZA, Khalid A. Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. *Soil Biol Biochem* 2006;38:2971-5.
249. Nadeem SM, Zahir ZA, Naveed M, Arshad M. Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. *Can J Microbiol* 2007;53:1141-9.
250. Rothballer M, Eckert B, Schmid M, Fekete A, Schloter M, Lehner A, *et al.* Endophytic root colonization of gramineous plants by *Herbaspirillum frisingense*. *FEMS Microbiol Ecol* 2008;66:85-95.
251. Karthikeyan B, Joe MM, Islam MR, Sa T. ACC deaminase containing diazotrophic endophytic bacteria ameliorate salt stress in *Catharanthus roseus* through reduced ethylene levels and induction of antioxidative defense systems. *Symbiosis* 2012;56:77-86.
252. Vargas L, de Carvalho TL, Ferreira PC, Baldani VL, Baldani JI, Hemerly AS. Early responses of rice (*Oryza sativa* L.) seedlings to inoculation with beneficial diazotrophic bacteria are dependent on plant and bacterial genotypes. *Plant Soil* 2012;356:127-37.
253. Nascimento FX, Brígido C, Glick BR, Oliveira S. ACC deaminase genes are conserved among *Mesorhizobium* species able to nodulate the same host plant. *FEMS Microbiol Lett* 2012;336:26-37.
254. Ahmad E, Khan MS, Zaidi A. ACC deaminase producing *Pseudomonas putida* strain PSE3 and *Rhizobium leguminosarum* strain RP2 in synergism improves growth, nodulation and yield of pea grown in alluvial soils. *Symbiosis* 2013;61:93-104.
255. Mahanty T, Bhattacharjee S, Goswami M, Bhattacharyya P, Das B, Ghosh A, *et al.* Biofertilizers: A potential approach for sustainable agriculture development. *Environ Sci Pollut Res* 2017;24:3315-35.
256. Yadav AN, Rastegari AA, Yadav N. *Microbiomes of Extreme Environments, Volume 2: Biotechnological Applications in Agriculture, Environment and Industry*. Vol. 2. Boca Raton, USA: CRC Press, Taylor and Francis Group; 2020.
257. Kumar P, Dubey RC, Maheshwari DK, Bajpai VK. ACC deaminase producing *Rhizobium leguminosarum* RPN5 isolated from root nodules of *Phaseolus vulgaris* L. *Bangladesh J Bot* 2016;45:477-84.
258. Win KT, Tanaka F, Okazaki K, Ohwaki Y. The ACC deaminase expressing endophyte *Pseudomonas* spp. Enhances NaCl stress tolerance by reducing stress-related ethylene production, resulting in improved growth, photosynthetic performance, and ionic balance in tomato plants. *Plant Physiol Biochem* 2018;127:599-607.

How to cite this article:

Rana KL, Kour D, Kaur T, Negi R, Devi R, Yadav N, Rai PK, Singh S, Rai AK, Yadav A, Sayyed RZ, Yadav AN. Endophytic nitrogen-fixing bacteria: Untapped treasurer for agricultural sustainability. *J App Biol Biotech*. 2023;11(2):75-93. DOI: 10.7324/JABB.2023.110207