INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND PUBLIC HEALTH RESEARCH (ISSN – 2767-3774)
VOLUME 05 ISSUE 12 Pages: 91-106
OCLC –1242424495

International Journal of Medical Science and Public Health Research

Journal Website: https://ijmsphr.com/index.php/ijmsphr

Research Article

THE LAST 15-YEAR PROSPECTS OF THE CREATION OF BIOSTIMULATORS BASED ON NITROGEN FIXING MICROORGANISMS

Submission Date: December 13, 2024, **Accepted Date:** December 18, 2024,
Published Date: December 23, 2024
Crossref Doi: https://doi.org/10.37547/ijmsphr/Volume05Issue12-08

Rakhmatova Madina
PhD student, Gulistan State University, Department of Biology, Gulistan city, Uzbekistan

Qushiyev Khabibjon
Professor, Gulistan State University, Department of Biology, Gulistan city, Uzbekistan

Khusanov Tokhir
PhD, Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan

ABSTRACT

In order to create ecologically safe food products by improving the bioecological system based on microorganisms, it is important to breed nitrogen-fixing and phosphate-mobilizing bacteria on the basis of biotechnological methods and create biopreparations from strains for use in agriculture. As a result of the influence of various abiotic, biotic and anthropogenic factors of the environment on plants, the cultivation of useful plants in agriculture causes a number of problems every day. As a result of intensive demographic growth, the population's demand for food is increasing. The decrease in productivity due to the low production of plant products may lead to the problem of food shortage in the future. As a result of the use of large amounts of synthetic fertilizers to increase productivity, soil fertility has decreased and salinity has increased. Therefore, many studies are currently focused on improving plant-microorganism-soil system mediation relationships. Representatives of other classes of plants need nitrogen fixers. Treating them with biostimulators prepared on the basis of associative and free-living AFB living in the rhizosphere allows not to use synthetic fertilizers beyond the permissible limit in the future, thus improving soil fertility and growing environmentally friendly products.

KEYWORDS

Nitrogen fixation, phosphate mobilization, bacteria, microbiome, symbiont, rhizosphere, ex-situ, biostimulator.

INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND PUBLIC HEALTH RESEARCH (ISSN – 2767-3774)
VOLUME 05 ISSUE 12 Pages: 91-106
OCLC –1242424495

INTRODUCTION

There is a lot of evidence to show that the role of microorganisms in plant growth development is very important [41]. The application of beneficial microorganisms could be a valuable alternative to achieve this objective. Several soil microorganisms that possess plant growth promotion have been reported. These microorganisms could improve crop productivity by enhancing nutrient uptake, nutrient cycling, nitrogen fixation, phytohormone production, and resistance against abiotic and biotic stresses [1]. Transferring one or more microbial species to plants that lack them might be the way to reduce plant disease susceptibility, increase nutrient availability, improve abiotic stress tolerance, and ultimately increase crop yields [10].

This is because microbial species may help fix atmospheric nitrogen, solubilize phosphate, produce phytohormones or enzymes that can modulate plant growth and development, and induce resistance systems against pathogens [11].

The authors found that the enriched indigenous Streptomyces sp. UPMRS4 can prime the rice crops against Pyricularia oryzae, reducing blast disease severity by 67.9%. Similarly, Wu et al. [12].

Demonstrated that the enriched indigenous Pseudomonas mosseli BS011 could effectively prevent rice blast disease caused by Magnaporthe oryzae [3]. Yoolong et al. demonstrated that Oryza sativa cv. KDML105 roots inoculated with the ACC deaminase-producing Streptomyces venezuelae ATCC 10712 prior to salt stress showed increased salt tolerance than the uninoculated rice [15].

The microbial osmolytes work synergistically with those produced by plants to maintain plant health. Inoculation of osmotic-stressed crops with PGPR strains has been shown to increase glycine betaine content than those without inoculation. For instance, Gou et al. found that maize inoculated with Klebsiella variicola F2, Raoultella planticola YL2, and Pseudomonas fluorescen YX2 produced a higher accumulation of glycine betaine and choline than control, resulting in improved water relations and plant growth under drought conditions [16,48].

Many soil microorganisms are capable of synthesizing volatile organic compounds (VOCs) [5]. VOCs produced by microorganisms can regulate the plant physiological and metabolic processes to promote plant growth and improve stress tolerance [6]. The authors showed that VOCs emitted by Bacillus subtilis GB03 increased the total leaf area in Arabidopsis thaliana and confers salt tolerance by downregulating HKT1 expression in roots, thus decreasing Na+ accumulation. tetrahydrofuran-3-ol, 2-heptanone and 2-ethyl-1-hexanol emitted by Bacillus sp. stimulate growth on A. thaliana and tomato [7].

Dimethyl disulphide emitted by Pseudomonas stutzeri increases growth on tomato [8]. Many plants growth-promoting rhizobacteria (PGPR) produce indole-3-acetic acid (IAA) that could influence root growth and architecture [9].

Manipulation of the bacterial microbiome and the production of bioinoculants enables scientists to affect plant beneficial activities such as limits to growth, the action of phytopathogens, and promoting plant growth and health, thereby potentially reducing the use of chemical fertilizers [36]. Rhizospheric or endophytic bacteria that promote plant growth are known as plant growth-promoting bacteria (PGPB) [37].

INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND PUBLIC HEALTH RESEARCH (ISSN – 2767-3774)
VOLUME 05 ISSUE 12 Pages: 91-106
OCLC –1242424495

Yang et al. [43] found that soil application of microbial biofertilizer significantly increased wheat production while reducing the amount of chemical fertilizer required and enhancing the soil phosphorus and potassium availability. Erdemci demonstrated that wheat seeds pre-coated with microorganisms showed improved growth and yield [44]. Nonetheless, although the exogenous microbial inoculations had increased soil nutrients up to 90 days, the beneficial impact of microbial biofertilizers may decrease over time [45].

Implementing a sustainable strategy to enhance crop tolerance against abiotic stresses is of great importance to increase global food production. Several studies have shown that microbiome engineering could reduce plant oxidative stress by producing microbial 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase to modulate plant ethylene stress hormone. Plants synthesize ethylene via the actions of ACC synthase to produce ACC, an ethylene precursor. However, a high level of ethylene may inhibit their root elongation. Soil microbiota that produces ACC deaminase can degrade plant-produced ACC, resulting in reduced ethylene levels in the plants [46,47].

Table 1. Mechanisms of plant resistance to stress based on the relationship between plants and microorganisms [42].

Mechanisms of resistance of plants to drought stress by inoculation of some nitrogen-fixing (AFB) and phosphate-mobilizing (FMB) microorganisms			
Plants	**Microbes**	**Effect/Mechanism**	**References**
Maize (Ze a mays)	Azospirillum lipoferum	Increase accumulation of soluble sugar, free amino acids and proline. Affect the growth of root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry	Bano et al. (2013)
	Bacillus Spp.	Increased accumulation of proline, sugars, free amino acids and decrease electrolyte leakage. It also reduces the activity of antioxidants enzyme (catalase, glutathione peroxidase)	Vardharajula et al. (2011)
Wheat (Triticum aestivum)	Azospirillum brasilense NO40	Bacterial mediated plant attenuated transcript level and improves homeostasis.	Kasim et al. (2013)
	Rhizobium leguminosarum (LR-30),	Catalase, exopolysaccharides and IAA produced by the Rhizobia improved the growth, biomass and drought tolerance index	Hussain et al. (2014)
	Mesorhizobium ciceri (CR-30 and CR-39), and		

	Rhizobium phaseoli (MR-2		
Lettuce	Azospirillum sp.	Promote aerial biomass, chlorophyll and ascorbic acid content, better overall visual quality, hue, Chroma and antioxidant capacity, and a lower browning intensity	Fasciglione et al. (2015)
Arabidopsis	Azospirilum Brasilense Sp 245	Improved plants seed yield, plants survival, proline levels and relative leaf water content; it also decreased stomatal conductance, malondialdehyde and relative soil water content	Cohen et al. (2015)
Brassica oxyrrhina	Pseudomonas libanensis TR1 and Pseudomonas reactans Ph3R3	Increased plant growth, leaf relative water and pigment content and decreased concentrations of proline and malondialdehyde in leaves	Ma et al., 2016a, Ma et al., 2016b
Medicago truncatula	Sinorhizobium medicae	Root nodulation and nutrient acquisition of nutrient during drought stress	Staudinger et al. (2016)
Mechanisms of resistance of plants to saline stress by inoculation of some nitrogen-fixing (AFB) and phosphate-mobilizing (FMB) microorganisms			
Plants	**Microbes**	**Effect/Mechanism**	**References**
Mung bea (Vigna radiate)	*Rhizobium*	ACC-deaminase for improving growth, nodulation and yield of mung bean under natural salt-affected conditions	Ahmad et al. (2011)
Wheat	*Azospirillum* Sp.	Increased shoot dry weight and grain yield. Plants accumulate some organic solutes (e.g. proline and soluble sugars) and inorganic ions to maintain osmotic adjustment	
Rice GJ-17	*Pseudomonas pseudoalcaligenes and Bacillus pumilus*	Reduced the toxicity of reactive oxygen species(ROS) and reduce lipid peroxidation and superoxide dismutase activity. Reduce lipid peroxidation and superoxide dismutase activity	Jha and Subramanian, (2014)
Rice	*Bacillus amyloliquefaciens* NBRISN13 (SN13)	Modulating differential transcription in a set of at least 14 genes	Nautiyal et al. (2013)

INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND PUBLIC HEALTH RESEARCH (ISSN – 2767-3774)
VOLUME 05 ISSUE 12 Pages: 91-106
OCLC –1242424495

Barley (*Hordeum vulgare* L.)	*Hartmannibacter diazotrophicus* E1 9	Increased root and shoot dry weight. ACC-deaminase activity of and lower ethylene content	Suarez et al. (2015)
lettuce seeds	*Azospirillum*	Promoted higher biomass, ascorbic acid content antiox- idant capacity, and a lower browning intensity	Fasciglione et al. (2015)

Mechanisms of heavy metal stress tolerance of plants by inoculation of some nitrogen-fixing (AFB) and phosphate-mobilizing (FMB) microorganisms

Heavy Metals	Plants	Microbes	Effect/Mechanism	References
Cd, Zn and Cu	*Sedum*	*Bacillus pumilus E2S 2,*	Production of IAA, siderophores, ACC deaminase and solubilization of Phosphorus. Increased water extractable Cd and Zn contents in soil, Improved plant growth and metal uptake	Ma et al. (2015)
Cd and Pb	*Lettuce*	*Bradyrhizobium japonicum*	IAA production enhance the growth and increased the shoot root lengths and dry biomas	Seneviratne et al. (2016)
Zn	*Brassica juncea*	*Rhizobium leguminosarum*	Induced metal chelation, toxicity attenuation and microbial-assisted phytoremediation.	Adediran et al. (2015)

Types of nitrogen fixers

Nitrogen-fixing endophytic microorganisms mainly form symbiotic relationships in the roots of representatives of the leguminous family (Fabaceae, Leguminosae). The most common among legumes are: dry bean, chickpea (Cicer arietinum L.) and cowpea (Vigna unguiculata L.), lentil (Lens esculenta L.), pigeon pea (Cajanus cajan L.), and peanut (Arachis hypogea L.). All these legumes are capable of nitrogen-fixation and are often grown in intercropping or for crop rotation. Nitrogen fixed by symbiotic association of soybean root system with soil bacteria (Rhizobia) has a significant contribution to the growth, development, and maturity stages.[35]

Species belonging to Azotobacter chroococcum and Azotobacter vinelandii are more abundant in tropical soils, while Azotobacter beijerinckii species were often reported in acidic soils. Nitrogen-fixing bacteria are divided into 5 groups according to genome analysis.

1.A proteobacteria

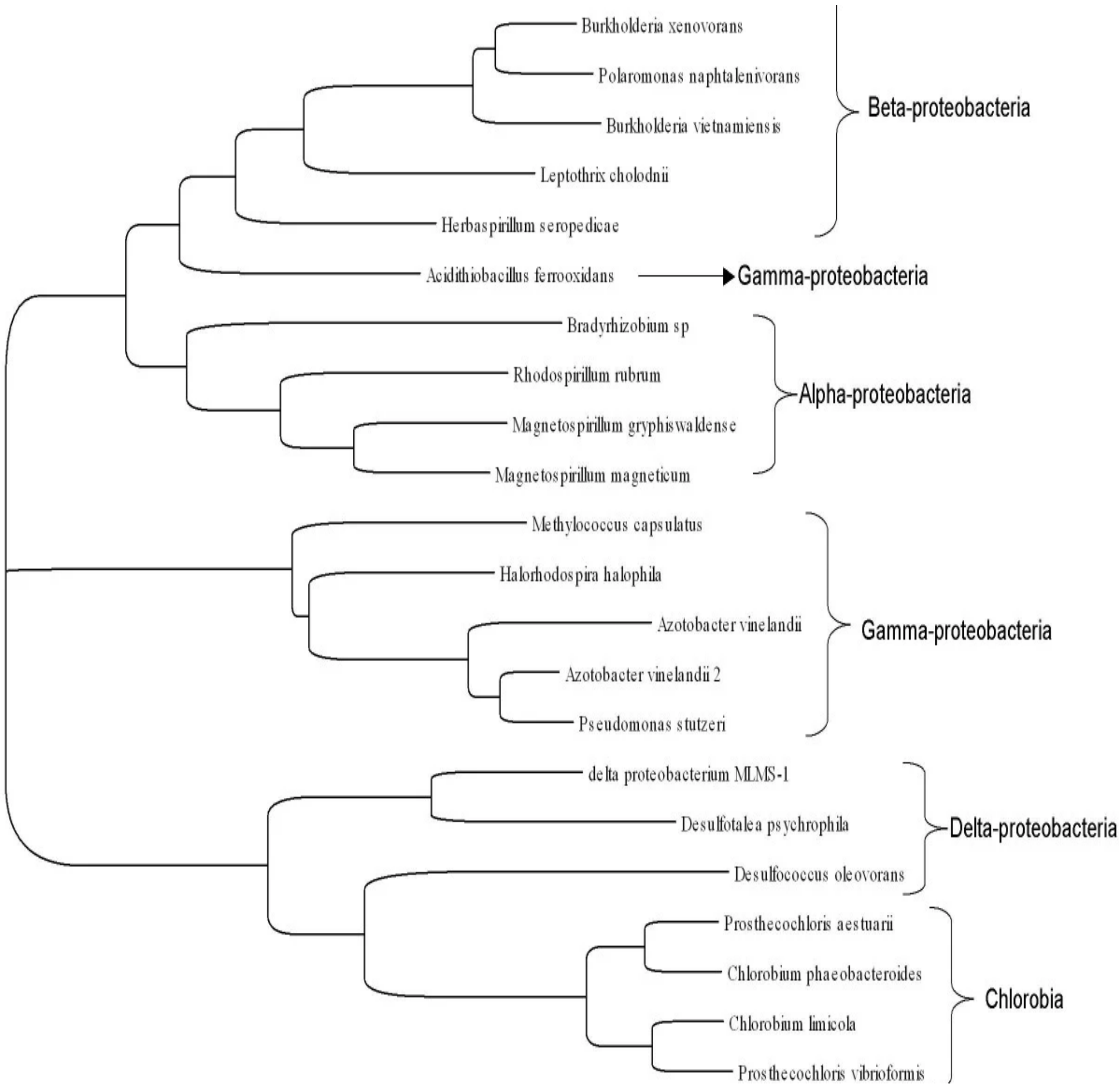
2.B proteobacteria

3.C proteobacteria

4.D proteobacteria

INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND PUBLIC HEALTH RESEARCH (ISSN – 2767-3774)
VOLUME 05 ISSUE 12 Pages: 91-106
OCLC –1242424495

5. E Chlorobia



1- fig. Group 5 of nitrogen-fixing bacteria based on genome analysis [120].

Plant root microbiome

Several bacterial species constitute the microbiome in the root tissues of legumes and in the root rhizosphere of non-legumes. The root microbiome (microbial communities present in plant roots and the rhizosphere) is the primary determinant for these beneficial effects [3].

Microbiomes are critical in the food production system due to their potential benefits in improving environmental health and protecting crops from pathogen attacks [2].

There are two approaches to engineer the plant microbiome: direct inoculation of exogenous beneficial microorganisms and re-inoculation of ex-situ enriched indigenous beneficial microorganisms. Direct inoculation of exogenous microorganisms with antagonistic activities against phytopathogens is the most common strategy to enhance the crop microbiome [10].

The ability of diverse bacterial endophytes to promote plant-growth occurs as a consequence of either direct or indirect mechanisms. Direct promotion of plant growth occurs when a bacterium either facilitates the acquisition of essential nutrients or modulates of level of hormones within a plant. Nutrient acquisition facilitated by PGPB typically includes nitrogen, phosphorus and iron. Modulation of hormone levels may entail PGPB (Plant Growth-Promoting Bacteria) synthesizing one or more of the phytohormones auxin, cytokinin and gibberellin [37].

Control of plant-bacteria relationship by genes

There is evidence that the association of endogenous microorganisms with plants is controlled by genes. Ali et al., by comparing the complete genomes of nine Proteobacterial endophytes. At this point, only some of those genes have been experimentally shown to be involved in endophytic colonization.

Table 2. Genes/mutants and products involved in legume regulation of nodule number [112]

Gene/mutant	Gene product	Site of production	Site of action	Comments	References
GmNARK; *GsNARK*; *LjHAR1*; *MtSUNN*; *PsSYM29*	LRR-RK	Shoot/root	Shoot/root	Acts in shoot (AON) and root (NO_3− inhibition) (LRR-RK)	Sagan and Duc (1996); Krusell *et al.* (2002); Men *et al.* (2002); Nishimura *et al.* (2002*a*); Searle *et al.* (2003); Schnabel *et al.* (2005)
GmNIC1	CLE	Root	Root	NO_3− induced (CLE pre-propeptide)	Reid *et al.* (2011)
GmRIC1/2; *LjCLE-RS1/2*; *MtCLE12/13*	CLE	Root	Probably the shoot	Rhizobia-induced (CLE prepropeptide)	Okamoto *et al.* (2009); Mortier *et al.* (2010); Reid *et al.* (2011); Lim *et al.* (2011)
LjASTRAY	bZIP TF		Root	Also acts in photomorphogenesis (transcription factor)	Nishimura *et al.* (2002*b*)
LjCLV2; *PsSYM28*	CLV2	Shoot/root	Shoot/root ?	May interact with other AON LRR RKs	Sagan and Duc (1996); Krusell *et al.* (2011)

Gene/mutant	Gene product	Site of production	Site of action	Comments	References
				(truncated LRR-receptor protein)	
LjETR1	ETR1	Shoot/root	Shoot/root	Ethylene receptor (two-component receptor)	Gresshoff *et al.* (2009); Lohar *et al.* (2009)
LjKLV	LRR-RK	Shoot/root ?	Shoot/root ?	May interact with other AON LRR RKs	Oka-Kira *et al.* (2005)
LjPLENTY	Unknown	Root	Root	Hypernodulation phenotype	Yoshida *et al.* (2010)
LjRDH1	Unknown	Root	Root		Ishikawa *et al.* (2008)
LjTML	Unknown	Root	Root		Magori *et al.* (2009)
MtEFD	AP2-EREBP TF	Root	Root	Positively regulates CK levels (transcription factor)	Vernié *et al.* (2008)
MtLSS	Unknown	Shoot/root ?	Shoot/root ?	Possible epigenetic factor of *MtSUNN*	Schnabel *et al.* (2010)
MtSKL	EIN2	Root	Root	\ethylene response factor	Penmetsa and Cook (1997); Penmetsa *et al.* (2008)
PsNOD1 and *2*	Unknown				Gelin and Blixt (1964)
PsNOD3; *Mt RDN1*	RDN1	Root	Root	Affects CLE synthesis and/or transport	Jacobsen and Feenstra (1984); Engvild (1987); Novák *et al.* (1997); Li *et al.* (2009); Schnabel *et al.* (2011)
PsNOD4 and *5*	Unknown		Shoot		Sidorova and Shumnyi (1998, 2003)
PsNOD6	Unknown		Shoot		Sidorova and Shumnyi (1998)

There are other areas for bacteria of entry too, such as the lenticels, stomas, wounds, ruptures, and nodules. Endophytes can also be inherited by vertical transmission, through seeds [37]. Some researchers have proposed that the internal microbiome of plants has an advantage over external bacteria (phyllosphere or rhizosphere) in that it not affected by soil conditions including the presence of bacterial predators. Moreover, it has been proposed that (chemical) communication is more effective inside of the plant's

INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND PUBLIC HEALTH RESEARCH (ISSN – 2767-3774)
VOLUME 05 ISSUE 12 Pages: 91-106
OCLC –1242424495

tissues [38,39], because lower concentrations of metabolites secreted by the bacterial endophytes may exert a greater effect on the plant.

What is the phyllosphere?

Virtually every plant part is colonized by microorganisms, including bacteria, archaea, fungi, collectively designated as the plant–microbiome or phytomicrobiome. Depending on the plant part it colonizes, the phytomicrobiome is often referred to as endophytic (inside plant parts), epiphytic (on aboveground plant parts), or rhizospheric (in the soil closely associated to the roots) [41]. A common type of epiphytic phytomicrobiome is the phyllosphere.

There is also a phyllosphere structure in the conditions of symbiosis between plants and bacteria. The phyllosphere comprises the surface and the apoplast of leaf tissue The great importance of the phyllosphere microbiome on biocontrol, and the promotion of plant growth, has been suggested for years [40]. Dominant nitrogen fixing microbes reported are Beijerinkia, Azotobacter, and Derxia etc. in the phyllosphere of wheat, cotton and maize [51].

Examples include plantgrowth-promoting and disease-suppressive phyllobacteria, probiotics and fermented foods that support human health, as well as microbials that remedy foliar contamination with airborne pollutants, residual pesticides, or plastics. Phyllosphere microbes promote plant biomass conversion into compost, renewable energy, animal feed, or fiber [56].

There is increasing evidence that micro-organisms on seeds or roots can become endophytic in the roots, enter the vascular system and be transferred internally to the aerial parts of plants where they establish as phyllosphere endophytes. In general, greater numbers of bacteria are found on lower than upper leaf surfaces [51].

The best studied of these is nitrogen fixation. Measured rates of bacterial nitrogen fixation in the phyllosphere vary widely, but in the phyllosphere of trees in some tropical habitats rates of over 60 kg N have been reported, although amounts fixed in the phyllosphere of temperate trees is generally considerably lower [53]. Furthermore, nitrogen fixation or the presence of nitrogen-fixing bacteria has been reported in the phyllosphere of many crop plants [54,55].

N_2 fixation was found to occur mostly on the leaf surfaces (not in the leaf interior) and cyanobacteria associated with epiphytes are likely to represent the key N_2-fixing bacteria in this environment. In addition, bacteria such as diazotrophic γ-proteobacteria may be involved in N_2 fixation processes [57].

Root exudation

Plants secrete various molecules from roots into the rhizosphere to support microbial activity or magnetize the diversity of soil microbiota. This secretion process is known as root exudation [4].

Plants have been found to release 5–20% of net photosynthetically fixed C into the rhizosphere. These rhizodeposits include inorganic (CO_2 from cell respiration and H^+ efflux) and a variety of complex organic compounds like sloughed-off cells and tissue, intact root border cells, mucilage (polysaccharides) and proteins, all of them classified as high molecular weight compounds. Also, part of the rhizodeposits is the insoluble and soluble low molecular weight (LMW) organic compounds, collectively known as root exudates, which are actively or passively released by growing roots. Root exudates can be classified in different classes such as sugars, amino acids, and amides, organic acids, as well as aromatic and phenolic acids. From the plant point of view, the goal of shaping the rhizosphere microbiome is to attract preferred partners like plant growth promoting microorganisms through the exudation of specific carbon compounds that can be used as feed and to deter pathogens or unwanted competitors for nutrients through the exudation of antimicrobial compounds such as volatiles or proteins [41].

The symbiotic relationship between soil bacteria, collectively known as rhizobia (which includes the genera Rhizobium, Bradyrhizobium, Mesorhizobium, and Sinorhizobium), and legume roots generates nodules (a new differentiated special organ) that fix atmospheric nitrogen through the action of the nitrogenase enzyme [22].

BNF by plant–rhizobia symbiotic systems is mediated by endosymbiotic interaction when plants develop root nodules; in legumes and rhizobia, gram-negative alpha proteobacteria are the most common microbial species that associate (endo-symbiotic interaction) with legumes of the Fabaceae (Papilionaceae) family [26,27,28].

The structure of legume nodules

The rhizobium bacteria residing in nodules fix atmospheric nitrogen gas to NH_3, which plants can assimilate via glutamine synthase to form glutamine. In response, the bacteria derive plant carbohydrates, mainly as malate for food and an energy source for nitrogen fixation. Nodules are very complex structures, containing several processes which operate and interact at distinct levels. The process of nodule formation requires a coordinated exchange of signals between the two symbiotic partners [31]. Depending on whether or not the meristem remains active for the life of the nodule, two main types of nodules are formed on the various legume species,

1. Indeterminate

2. Determinate.

In the case of determinate nodules, nodular meristematic activity is terminated early and is usually initiated sub-epidermally in the outer cortex, thus giving rise to spherical nodules [32]. In indeterminate nodules, the inner cortex undergoes cell division (anticlinally) followed by periclinal divisions in the pericycle. Here, cylindrical nodules are formed due to more persistent meristems [33,34].

There are two nitrogen fixers based on their association developed with plants: the symbionts and the free-living nitrogen fixers. Several symbiotic nitrogen fixers have been identified, including Allorhizobium, Azoarcus, Azorhizobium, Bradyrhizobium, Burkholderia, Frankia, Mesorhizobium, Rhizobium, and Sinorhizobium [13]. For free-living nitrogen fixers, the more notable ones are Azoarcus, Azospirillum, Azotobacter, Gluconacetobacter, and Herbaspirillum [14]. Free-living diazotrophs correspond to a small fraction of the plant rhizospheres ecosystem, and they belong to alphaproteobacterial (Rhizobia, Bradyrhizobia, Rhodobacteria), betaproteobacteria (Burkholderia, Nitrosospira), gammaproteobacterial (Pseudomonas, Xanthomonus), firmicutes, and cyanobacteria [24]. For this extreme sensitivity to oxygen, obligate anaerobes such as Clostridium pasteurianu are ideal candidates for nitrogen fixation; however, facultative anaerobes such as Klebsiella oxytoca are also capable of fixing nitrogen but only when the oxygen is absent in the system [29]. Obligate aerobes, such as Azotobacter vinelandii can also shield nitrogenase from oxygen and

INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND PUBLIC HEALTH RESEARCH (ISSN – 2767-3774)
VOLUME 05 ISSUE 12 Pages: 91-106
OCLC –1242424495

perform nitrogen fixation by consuming oxygen via cytochrome oxidases [29,30].

Biostimulants

Biostimulant: A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganism [19].

Microorganisms which are used as biofertilizers have ability to convert atmospheric nitrogen in to ammonia and phosphate solubilization in plant rhizosphere.

Biofertilizers are substances containing living organism, which when inoculated with seed or plant enhance plant growth and development. Stress tolerant PGPM actively participate in mainly in nutrient mobilization and fix atmospheric nitrogen. It is a potential substitute for inorganic fertilizers and pesticides. Most common bacteria such as Azospirillum, Acetobacter, Azotobacter, and Pseudomonas are some active microbes. In addition, Pseudomonas and Bacillus spp. act as potent biocontrol and plant growth promoting strains under stress condition. They provide protection and disease resistance to plants from pathogens. These microbes improve the nutrient availability, competition for nutrient and induced systemic resistance [42].

The primary function of PBs is not to provide nutrients or target pests and pathogens, but to enhance crop quality, ameliorating plants nutrient use efficiency and increasing their resistance to stress [17].

They do not provide nutrients to plants and do not target pests and pathogens, but they encourage nutrient uptakes or help foster plant growth and development. PBs have been classified based on their mode of action [18], contents [19], or a combination of both [20]. However, under the FPR, PBs are classified into two categories: microbial PBs and non-microbial PBs.

Many bacteria and fungi have been explored for their potential to be applied as biostimulants. Of these, Rhizobium and rhizospheric PGPR are the major reported group. To date, more than 20 commercially available microbial PBs are derived from PGPR. [21]

Mobilizing phosphate rock into the environment at rates vastly faster than the natural cycle has not only polluted many of the world's freshwater bodies and oceans, but has also created a human dependence on a single nonrenewable resource [49].

A particularly large number of organic phosphates accumulates when phosphorus fertilizers are added to the soil or when the soil is sufficiently supplied with assimilable phosphorus. Then the so-called biological immobilization of phosphorus occurs: assimilable phosphorus is used by microorganisms and is included in the organic compounds of the microbial cell. Direct microbial mobilization of phosphates can serve as a basis for use in agricultural technologies in the form of biophosphorus fertilizers [50].

Mechanism of nitrogen fixation

Biological nitrogen fixation (BNF) refers to a microbial mediated process based upon an enzymatic “Nitrogenase” conversion of atmospheric nitrogen (N_2) into ammonium readily absorbable by roots. N_2-fixing microorganisms collectively termed as “diazotrophs” are able to fix biologically N_2 in association with plant roots. Specifically, the symbiotic rhizobacteria induce structural and physiological modifications of bacterial cells and plant roots into specialized structures called nodules. Other N2-fixing bacteria are free-living fixers that are highly diverse and globally widespread in cropland. They represent key natural source of nitrogen (N) in natural and agricultural ecosystems lacking symbiotic N fixation (SNF) [58].

Nitrogen fixation is a dynamic and high energy demanding process [23]. The pathway for the biological reduction of inert N2 into the reactive compound NH3 (ammonia) under micro-aerobic conditions is as follows:

$$N_2 + 8H^+ + 8e^- + 16Mg\text{-}ATP \rightarrow 2NH_3 + H_2 + 16Mg\text{-}ADP + 16\ P \quad (1)$$

Formula 1. Absorption of free nitrogen

CONCLUSION

As a result of the influence of various abiotic, biotic and anthropogenic factors of the environment on plants, the cultivation of useful plants in agriculture causes a number of problems every day. As a result of intensive demographic growth, the population's demand for food is increasing. The decrease in productivity due to the low production of plant products may lead to the problem of food shortage in the future. As a result of the use of large amounts of synthetic fertilizers to increase productivity, soil fertility has decreased and salinity has increased. Therefore, many studies are currently focused on improving plant-microorganism-soil system mediation relationships. Among these microorganisms, nitrogen-fixing bacteria dominate in terms of usefulness. Because these microorganisms not only fix nitrogen as nutrients from plants, but also plant phytohormones, iron chelating siderophos, antioxidants, exopolysaccharides, jasmonic acid, ACC deaminase, antibiotic and lytic enzymes, free protons, phosphotase, phytase and organic, inorganic has the property of synthesizing acids, H2S substances. therefore, creating biostimulators from these microorganisms and using them in agriculture is a guarantee of a high yield. Because azotobacteria are sufficient only in leguminous plants, they live in symbiosis with bacteria belonging to the genus Rhizobium. Representatives of other classes of plants need nitrogen fixers. Treating them with biostimulators prepared on the basis of associative and free-living AFB living in the rhizosphere allows not to use synthetic fertilizers beyond the permissible limit in the future, thus improving soil fertility and growing environmentally friendly products.

REFERENCES

1. Aamir M, Samal S, Rai A, Kashyap SP, Singh SK, Ahmed M, et al. Chapter 25 - Plant microbiome: diversity, distribution, and functional relevance in crop improvement and sustainable agriculture. In: White J, Kumar A, Droby S, editors., et al., Microbiome Stimulants for Crops. Woodhead Publishing; 2021. p. 417–36.

2. Orozco-Mosqueda MD, Rocha-Granados MD, Glick BR, Santoyo G. Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. Microbiol Res. 2018;208:25–31. https://doi.org/10.1016/j.micres.2018.01.005.

3. Lau, SE., Teo, W.F.A., Teoh, E.Y. et al. Microbiome engineering and plant biostimulants for sustainable crop improvement and mitigation of biotic and abiotic stresses. Discov Food 2, 9 (2022). https://doi.org/10.1007/s44187-022-00009-5

4. Baetz U, Martinoia E. Root exudates: The hidden part of plant defense. Trends Plant Sci. 2014;19(2):90–8. https://doi.org/10.1016/j.tplants.2013.11.006.

5. Fincheira P, Quiroz A, Tortella G, Diez MC, Rubilar O. Current advances in plant-microbe communication via volatile organic compounds as an innovative strategy to improve plant growth. Microbiol Res. 2021;247:126726. https://doi.org/10.1016/j.micres.2021.126726.

6. Ryu C-M, Farag MA, Hu C-H, Reddy MS, Wei H-X, Paré PW, et al. Bacterial volatiles promote growth in Arabidopsis. Proc Natl Acad Sci USA. 2003;100(8):4927–32. https://doi.org/10.1073/pnas.0730845100.

7. Jiang CH, Xie YS, Zhu K, Wang N, Li ZJ, Yu GJ, et al. Volatile organic compounds emitted by Bacillus sp JC03 promote plant growth through the action of auxin and strigolactone. Plant Growth Regul. 2019;87(2):317–28. https://doi.org/10.1007/s10725-018-00473-z.

8. Rojas-Solís D, Zetter-Salmón E, Contreras-Pérez M, Rocha-Granados MC, Macías-Rodríguez L, Santoyo G. Pseudomonas stutzeri E25 and Stenotrophomonas maltophilia CR71 endophytes produce antifungal volatile organic compounds and exhibit additive plant growth-promoting effects. Biocatal Agric Biotechnol. 2018;13:46–52. https://doi.org/10.1016/j.bcab.2017.11.007.

9. Vacheron J, Desbrosses G, Bouffaud M-L, Touraine B, Moënne-Loccoz Y, Muller D, et al. Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci. 2013;4:356. https://doi.org/10.3389/fpls.2013.00356.

10. Quiza L, St-Arnaud M, Yergeau E. Harnessing phytomicrobiome signaling for rhizosphere microbiome engineering. Front Plant Sci. 2015;6:11. https://doi.org/10.3389/fpls.2015.00507.

11. Kumar A, Verma JP. Does plant—microbe interaction confer stress tolerance in plants: A review? Microbiol Res. 2018;207:41–52. https://doi.org/10.1016/j.micres.2017.11.004.

12. Wu L, Xiao W, Chen G, Song D, Khaskheli MA, Li P, et al. Identification of Pseudomonas mosselii BS011 gene clusters required for suppression of rice blast fungus Magnaporthe oryzae. J Biotechnol. 2018;282:1–9. https://doi.org/10.1016/j.jbiotec.2018.04.016.

13. Turan M, Kıtır N, Alkaya Ü, Günes A, Tüfenkçi Ş, Yıldırım E, et al. Making Soil More Accessible to Plants: The Case of Plant Growth Promoting Rhizobacteria. In: Rigobelo EC, editor., et al., Plant Growth. New York: IntechOpen; 2016. p. 61–9.

14. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255(2):571–86. https://doi.org/10.1023/A:1026037216893.

15. Yoolong S, Kruasuwan W, Pham HTT, Jaemsaeng R, Jantasuriyarat C, Thamchaipenet A. Modulation of salt tolerance in Thai jasmine rice (Oryza sativa L cv KDML105) by Streptomyces venezuelae ATCC 10712 expressing ACC deaminase. Sci Rep. 2019;9(1):1275. https://doi.org/10.1038/s41598-018-37987-5.

16. Gou W, Tian L, Ruan Z, Zheng P, Chen F, Zhang L, et al. Accumulation of choline and glycinebetaine and drought stress tolerance induced in maize (Zea mays) by three plant growth promoting rhizobacteria (PGPR) strains. Pak J Bot. 2015;47(2):581–6.

INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND PUBLIC HEALTH RESEARCH (ISSN – 2767-3774)
VOLUME 05 ISSUE 12 Pages: 91-106
OCLC –1242424495

17. Del Buono D. Can biostimulants be used to mitigate the effect of anthropogenic climate change on agriculture? It is time to respond. Sci Total Environ. 2021;751:141763. https://doi.org/10.1016/j.scitotenv.2020.141763.

18. Bulgari R, Cocetta G, Trivellini A, Vernieri P, Ferrante A. Biostimulants and crop responses: A review. Biol Agric Hortic. 2015;31(1):1–17. https://doi.org/10.1080/01448765.2014.964649

19. du Jardin P. Plant biostimulants: Definition, concept, main categories and regulation. Sci Hortic. 2015;196:3–14. https://doi.org/10.1016/j.scienta.2015.09.021.

20. Basak A. Biostimulators–definitions, classification and legislation. In: Gawrońska H, editor. Monographs Series: Biostimulators in Modern Agriculture. General Aspects. Warsaw: Wieś Jutra; 2008. p. 7–17

21. Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH. Going back to the roots: The microbial ecology of the rhizosphere. Nat Rev Microbiol. 2013;11(11):789–99. https://doi.org/10.1038/nrmicro3109

22. Graham P.H., Vance C.P. Legumes: Importance and constraints to greater use. Plant Physiol. 2003;131:872–877. doi: 10.1104/pp.017004. [DOI] [PMC free article] [PubMed] [Google Scholar]

23. Rosenblueth M., Ormeño-Orrillo E., López-López A., Rogel M.A., Reyes-Hernández B.J., Martínez-Romero J.C., Reddy P.M., Martínez-Romero E. Nitrogen Fixation in Cereals. Front. Microbiol. 2018;9:9. doi: 10.3389/fmicb.2018.01794.

24. Morris J.J., Schniter E.J. Black Queen markets: Commensalism, dependency, and the evolution of cooperative specialization in human society. J. Bioecon. 2018;20:69–105. doi: 10.1007/s10818-017-9263-x.

25. S. Ali, J. Duan, T.C. Charles, B.R. Glick A bioinformatics approach to the determination of genes involved in endophytic behavior in Burkholderia spp J. Theor. Biol., 343 (2014), pp. 193-198

26. Schultze M., Kondorosi A.J. Regulation of symbiotic root nodule development. Annu. Rev. Genet. 1998;32:33–57. doi: 10.1146/annurev.genet.32.1.33.

27. Oldroyd G.E., Downie J.A. Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu. Rev. Plant Biol. 2008;59:519–546. doi: 10.1146/annurev.arplant.59.032607.092839.

28. Desbrosses G.J., Stougaard J. Root nodulation: A paradigm for how plant-microbe symbiosis influences host developmental pathways. Cell Host Microbe. 2011;10:348–358. doi: 10.1016/j.chom.2011.09.005.

29. Yates M., Jones C. Advances in Microbial Physiology. Volume 11. Elsevier BV; Amsterdam, The Netherlands: 1974. Respiration and Nitrogen Fixation in Azotobacter; pp. 97–135.

30. Poole R.K., Hill S. Respiratory protection of nitrogenase activity in Azotobacter vinelandii—Roles of the terminal oxidases. Biosci. Rep. 1997;17:303–317.

31. Biswas B., Gresshoff P.M. The role of symbiotic nitrogen fixation in sustainable production of biofuels. Int. J. Mol. Sci. 2014;15:7380–7397.

32. Wopereis J., Pajuelo E., Dazzo F.B., Jiang Q., Gresshoff P.M., De Bruijn F.J., Stougaard J., Szczyglowski K. Short root mutant of Lotus japonicus

with a dramatically altered symbiotic phenotype. Plant J. 2000;23:97–114.

33. Newcomb W., Sippell D., Peterson R.J. The early morphogenesis of Glycine max and Pisum sativum root nodules. Can. J. Bot. 1979;57:2603–2616.

34. Rolfe B.G., Gresshoff P.J., Biology P.M. Genetic analysis of legume nodule initiation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 1988;39:297–319.

35. Zapata F., Danso S.K.A., Hardarson G., Fried M. Time Course of Nitrogen Fixation in Field-Grown Soybean Using Nitrogen-15 Methodology1. Agron. J. 1987;79:172–176.

36. A.O. Adesemoye, J.W. Kloepper Plant-microbes interactions in enhanced fertilizer-use efficiency Appl. Microbiol. Biotechnol., 85 (2009), pp. 1-12

37. G. Santoyo, G. Moreno-Hagelsieb, Ma del C. Orozco-Mosqueda, B.R. Glick Plant growth-promoting bacterial endophytes Microbiol. Res., 183 (2016), pp. 92-99

38. S. Ali, T.C. Charles, B.R. Glick Delay of flower senescence by bacterial endophytes expressing 1-aminocyclopropane-1-carboxylate deaminase J. Appl. Microbiol., 113 (2012), pp. 1139-1144

39. B.G. Coutinho, D. Licastro, L. Mendonça-Previato, M. Cámara, V. Venturi Plant-influenced gene expression in the rice endophyt: Burkholderia kururiensis M130 Mol. Plant Microbe Interact., 28 (2015), pp. 10-21

40. J.A. Vorholt Microbial life in the phyllosphere Nat. Rev. Microbiol., 10 (2012), p. 828

41. Quiza L, St-Arnaud M, Yergeau E. Harnessing phytomicrobiome signaling for rhizosphere microbiome engineering. Front Plant Sci. 2015;6:11. https://doi.org/10.3389/fpls.2015.00507.

42. Kumar A, Verma JP. Does plant—microbe interaction confer stress tolerance in plants: A review? Microbiol Res. 2018;207:41–52. https://doi.org/10.1016/j.micres.2017.11.004.

43. Yang WL, Gong T, Wang JW, Li GJ, Liu YY, Zhen J, et al. Effects of compound microbial fertilizer on soil characteristics and yield of wheat (Triticum aestivum L). Soil Sci Plant Nutr. 2020;20(4):2740–8. https://doi.org/10.1007/s42729-020-00340-9.

44. Erdemci I. Effects of seed microbial inoculant on growth, yield, and nutrition of durum wheat (Triticum durum L). Commun Soil Sci Plant Anal. 2021;52(7):792–801. https://doi.org/10.1080/00103624.2020.1869764.

45. Wang ZK, Chen ZY, Kowalchuk GA, Xu ZH, Fu XX, Kuramae EE. Succession of the resident soil microbial community in response to periodic inoculations. Appl Environ Microbiol. 2021;87(9):16. https://doi.org/10.1128/aem.00046-21.

46. Arif I, Batool M, Schenk PM. Plant microbiome engineering: expected benefits for improved crop growth and resilience. Trends Biotechnol. 2020;38(12):1385–96. https://doi.org/10.1016/j.tibtech.2020.04.015.

47. Rodriguez R, Duran P. Natural holobiome engineering by using native extreme microbiome to counteract the climate change effects. Front Bioeng Biotechnol. 2020;8:14. https://doi.org/10.3389/fbioe.2020.00568.

48. Gou W, Tian L, Ruan Z, Zheng P, Chen F, Zhang L, et al. Accumulation of choline and glycinebetaine and drought stress tolerance induced in maize (Zea

mays) by three plant growth promoting rhizobacteria (PGPR) strains. Pak J Bot. 2015;47(2):581–6.

49. Cordell, D.,White, S. (2014): Life's bottleneck: sustaining the world's phosphorus for a food secure future. Annu. Rev. Environ. Resour. 39, 161–188.

50. ФОСФАТ-СОЛЮБИЛИЗИРУЮЩАЯ АКТИВНОСТЬ РИЗОБАКТЕРИЙ ПШЕНИЦЫ (Triticum aestivum L.) // Universum: химия и биология : электрон. научн. журн. Кадырова Г.Х. [и др.]. 2022. 12(102). URL: https://7universum.com/ru/nature/archive/item/14609

51. Whipps, J.M., Hand, P., Pink, D. and Bending, G.D. (2008). Phyllosphere microbiology with special reference to diversity and plant genotype. J. Appl. Microbiol. 105: 1744 –1755.

52. Dugald E. Reid, Brett J. Ferguson, Satomi Hayashi, Yu-Hsiang Lin, Peter M. Gresshoff., Molecular mechanisms controlling legume autoregulation of nodulation Annals of Botany, Volume 108, Issue 5, October 2011, Pages 789–795, https://doi.org/10.1093/aob/mcr205

53. Freiberg, E. (1998) Microclimatic parameters influencing nitrogen fixation in the phyllosphere in a Costa Rican premontane rain forest. Oecologia 117, 9–18.

54. Murty, M.G. (1983) Nitrogen fixation (acetylene-reduction) in the phyllosphere of some economically important plants. Plant Soil 73, 151–153.

55. Miyamoto, T., Kawahara, M. and Minamisawa, K. (2004) Novel endophytic nitrogen-fixing clostridia from the grass Miscanthus sinensis as revealed by terminal restriction fragment length polymorphism analysis. Appl Environ Microbiol 70, 6580–6586.

56. Rangel, Lorena & Leveau, Johan. (2024). Applied microbiology of the phyllosphere. Applied Microbiology and Biotechnology. 108. 10.1007/s00253-024-13042-4.

57. Michael Fürnkranz, Wolfgang Wanek, Andreas Richter, Guy Abell, Frank Rasche & Angela Sessitsch., Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica The ISME Journal volume 2, pages561–570 (2008)

58. Aasfar A, Bargaz A, Yaakoubi K, Hilali A, Bennis I, Zeroual Y and Meftah Kadmiri I (2021) Nitrogen Fixing Azotobacter Species as Potential Soil Biological Enhancers for Crop Nutrition and Yield Stability. Front. Microbiol. 12:628379. doi: 10.3389/fmicb.2021.628379

59. Levicán, G., Ugalde, J.A., Ehrenfeld, N. et al. Comparative genomic analysis of carbon and nitrogen assimilation mechanisms in three indigenous bioleaching bacteria: predictions and validations. BMC Genomics 9, 581 (2008). https://doi.org/10.1186/1471-2164-9-581