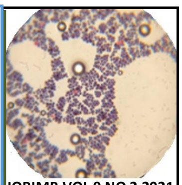
JOBIMB, 2021, Vol 9, No 2, 43-47
DOI: https://doi.org/10.54987/jobimb.v9i2.617

HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index



JOBIMB VOL 9 NO 2 2021
S. aureus in sachet water

Nitrogen Fixing Bacteria and Their Application for Heavy Metal Removal: A Mini Review

Lawal, I.[1]*, Yusuf, I.[2], Fardami, A.Y[3]., Bashir, A.[1] and Musa, A.[1]

[1]Department of Biological Sciences, College of Natural and Applied Sciences, Al-Qalam University Katsina, Katsina state-Nigeria.
[2]Department of Microbiology, Faculty of Life Sciences, College of Natural and Pharmaceutical Sciences, Bayero University Kano, P.M.B 3011, Kano State- Nigeria.
[3]Department of Microbiology, Faculty Science, Usmanu Danfodio University Sokoto, Sokoto State- Nigeria.
.
*Corresponding author:
I. Lawal,
Department of Biological Sciences,
College of Natural and Applied Sciences,
Al-Qalam University Katsina,
Katsina state,
Nigeria.
Email: lawalibrahim646@gmail.com

HISTORY

Received: 25th Nov 2021
Received in revised form: 13th Dec 2021
Accepted: 24th Dec 2021

KEYWORDS

Nitrogen fixing bacteria
Plant growth promotion
Biosorption
Phytohormones
Heavy metals

ABSTRACT

Nitrogen is a critical component of biological systems and typically serves as a constraint on production in both aquatic and terrestrial environments, although its shortage has been compensated for through the process of biological nitrogen fixation. Nitrogen fixation is a critical microbial activity that utilises nitrogenase enzymes to convert dinitrogen (N2) gas to ammonia (NH_3). It is carried out by a diverse spectrum of bacteria known as nitrogen fixing bacteria. These include free-living bacteria such as *Azotobacter, Bacillus, Beijerickia,* and *Clostridium*, associative bacteria such as *Azospirillum, Enterobacter*, and *Pseudomonas,* and bacteria that form symbiotic associations with legumes such as *Rhizobium* and actinorrhizal plants such as *Frankia.* These bacteria contribute significantly to plant growth by producing phytohormones (such as auxins, cytokinins, gibberelins, and indole acetic acid), reducing the incidence of plant diseases through the production of siderophores and cell wall degrading enzymes, and increasing phosphorus nutrition via phosphate solubilization. Additionally, they remove heavy metal ions from solutions through a process called biosorption, which is a feasible, natural, environmentally benign, and economically viable technique of remediating heavy metal-contaminated environments.

INTRODUCTION

Nitrogen is the most important nutrient for plant development and production in agribusiness[1]. To sustain life, it is one of the most crucial elements; a primary source for protein, nucleic acid, and other organic nitrogeneous substances synthesis. Chemically, nitrogen fixation occurs when airborne molecular nitrogen (N_2) is transformed into ammonia (NH_3) or other nitrogen compounds, most often in soil or aquatic systems [2], although it may also be used for industrial purposes. Most microbes can't utilise atmospheric nitrogen since it is molecular dinitrogen, an inert and nonreactive chemical. Nitrate is converted to ammonia (NH_3) by the nitrogenase protein complex in a microbiological process known as diazotrophy or nitrogen fixation (Nif)[3]. In contrast, the process of nitrogen mineralization in the soil is relatively sluggish, accounting for just one to three percent of the total soil nitrogen[4].

There appears to be a large number of heterotrophic bacteria living in soil, and these bacteria appear to be responsible for major nitrogen reductions [5]. One of the most significant microorganisms in agricultural soil is the nitrogen-fixing bacteria (also known as nitrobacteria) [6]. Certain species of *Enterobacter, Azospirillum,* and *Pseudomonas* have been shown to be mutualistic or symbiotic with diverse types of plants, including *Frankia, Rhizobium,* and certain *Azospirillum* and *Enterobacter* strains [7]. Soil microorganisms known as diazotrophs, including bacteria such as *Azotobacter* and archaea, fix nitrogen organically. Plant groupings, particularly legumes, have symbiotic partnerships with nitrogen-fixing bacteria. When nitrogen is fixed by rice roots, the link between diazotrophs and plants is sometimes referred to as associated relationship pr symbiotic. Some termites and fungi are able to fix nitrogen via a process known as nitrogen fixation. [8] Lightning-induced NOx emissions cause it to spontaneously arise in the atmosphere. Because of their capacity to convert nitrogen from the

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

atmosphere into a form that plants can utilise, these bacteria are among the most well-known [9]. As a result, it is imperative that the world's resources are tapped as quickly as possible, and the process of biological nitrogen fixation is a key component. A method for converting atmospheric nitrogen into fixed nitrogen is biological nitrogen fixation [10].

Bacteria that fix nitrogen facilitate this transition [11]. Many nitrogen-fixing bacteria have been isolated from various environments, including bacteria from the genera *Azospirillum, Bacillus, Azotobacter, Clostridium*, and Onyeze et al. [12], *Klebsiella* [13], and *Rhizobium* [14], all of which were isolated from soil [12,13,15] and from compost [16]. Additional to this, these bacteria are capable of promoting plant growth by synthesising plant growth promoting hormones such as cytokinins, auxins, and geberellins, inhibiting phytopathogenic organisms through production of fungal cell wall degrading enzyme, decreasing disease incidence through the secretion of antibiotic-like substances, and increasing phosphorus nutrition by phosphate solubilization [4]. This study provides an overview of the major group of bacteria known as nitrogen fixing bacteria in light of their contribution.

Biological Nitrogen Fixation

The principal means by which vegetation in native habitat get nitrogen necessary for development is through a process known as biological nitrogen fixation (BNF) [17]. Nearly one-third of the biologically fixed nitrogen (BNF) in terrestrial and aquatic ecosystems is produced by a very diversified group of Prokaryotes (Bacteria and Archaea) known as diazotrophs.[18, 11]. In addition to free-living bacteria from the *Azotobactera, Burkholderia, Bacillus, Azospiruliuma* and *Clostridium* genera, there are symbiotic bacteria linked with *Rhizobium*, actinorhizal plants like *Frankia*, and cyanobacteria connected with the cycad genus *Cyanobacteria* [19]. It is only methanogens that can fix nitrogen in Archaea[20]. nifH, a gene encoding a subunit of the iron protein of nitrogenase, is highly conserved throughout nitrogen-fixing groups and is an appropriate molecular identifier for these bacteria. Nitrogen-fixing bacteria express the nitrogenase enzyme complex[21].

Researchers researching the phylogeny, variety, and abundance of nitrogen-fixing bacteria use it as a marker gene of choice [22]. The only known natural mechanism for reducing N_2 to NH_3 is the enzyme complex found in bacteria, called bacterial nitrogenase [23]. The fixation of one molecule of nitrogen into two molecules of ammonia by BNF is one of the most expensive metabolic activities, requiring 16 molecules of ATP [23]. BNF is a cost-effective and environmentally friendly method for achieving long-term agricultural output sustainability [24].

Biological Associations of Nitrogen Fixing Bacteria

Symbiotic Nitrogen Fixing Bacteria

To fix biological nitrogen, heterocystic cyanobacteria and cycads [19], *Frankia* and the rhizome nodules of woody nonlegumes [25], as well as rhizobial bacteria (such as *Rhizobium*) and legumes (alfalfa, soybean) form symbiotic associations. This is how biological nitrogen fixation works [16]. When natural nitrogen is scarce, the symbiotic relationship between rhizobial bacteria and legumes has been shown to satisfy nitrogen demands for proliferation [26]. It is among the microorganism relationships that has been investigated the most thoroughly and evaluated the most exhaustively [27]. Legumes and Rhizobia work together to create Nod factors, which are then signalled by flavonoids released by the roots of legumes. These Nod factors aid plants in identifying microorganisms. Nodules are formed when legumes engage with rhizobia, resulting in bacterial infection. Rhizobia can fix nitrogen in the nodule because it has a low oxygen content[28, 29]. The legumes' carbon is exchanged for nitrogen fixed by the rhizobia inside the nodules [30].

Non-Symbiotic Nitrogen Fixing Bacteria

Bacteria that fix nitrogen without forming a symbiotic relationship with plants are known as non-symbiotic nitrogen fixers and can be found in soil or water, either in connection with plants or as free-living organisms.

Associative Nitrogen Fixing Bacteria

Bacteria belonging to the genera *Azospirillum, Paenibacillus* and *Herbaspirillum* [31], Klebsiella[13], Pseudomonas [32], and Enterobacter execute associative nitrogen fixation [33]. Free-living and symbiotic nitrogen fixers are separated by the associative nitrogen fixers in terms of the amount of nitrogen fixed [34]. On the roots of corn, wheat, and sugarcane, they have been most commonly found, according to the literature[35]. As their exudates include carbon, they aid the associated bacteria in obtaining a supply of carbon for nitrogen fixation [36].

Free-living Nitrogen Fixing Bacteria

Symbiotic bacteria play an important role in nitrogen fixation; nevertheless, free-living nitrogen fixers provide a significant quantity of nitrogen to ecosystems [37]. Proteobacteria, Firmicutes, Archaea and Cyanobacteria are only few of the heterotrophs that may convert nitrogen to nitrous oxide in the absence of a food source [38]. The oxidation of organic molecules provides the energy for this process. In compared to symbiotic nitrogen fixation, free-living diazotrophs are thought to provide a very small quantity of total fixed nitrogen. The free-living nitrogen fixers fix around one-tenth of the total atmospheric nitrogen fixed by the symbiotic relationship[24, 37].

Symbiotic nitrogen fixers fix more nitrogen than free-living and associated nitrogen fixers, on the other hand [29]. Symbiotic nitrogen fixation utilises the host plant as a carbon source of power and a shield against oxidation process damages of the nitrogenase enzyme [29]. In the absence of oxygen, anaerobes and facultative anaerobes convert nitrogen from the atmosphere into ammonia. It is possible for *Azospirillum* bacteria to fix nitrogen in environments with low oxygen levels. Nitrogen fixing in aerobic bacteria like *Azotobacter* happens by reducing the intracellular oxygen content. The heterocyst of cyanobacteria is where nitrogen fixing takes place [39]. With a rise in oxygen in the atmosphere, nitrogen fixation reduces [37].

Plant Growth Promotion by Nitrogen Fixing Bacteria

Plant growth stimulants have been linked to nitrogen-fixing microorganisms. Using both direct and indirect mechanisms, they encourage plant development [40]. Nitrogen fixing bacteria boost plant development by releasing phytohormones such as indole acetic acid (IAA), cytokinin's [41], gibberellins [42], and auxins [42] in addition to the nitrogen fixation described above [43, 44]. Direct mechanisms include release of a molecule lessening phytopathogenic organism effects and producing siderophore (low molecular weight iron chelating compounds), cell wall disintegrating enzymes such as chitinase, and antibiotic-like substances such as sulfonamide [45].

Solubilization of insoluble phosphorus by *Rhizobium* bacteria and species of *Azotobacter* have been shown to boost plant development in both legumes and non-legumes, respectively [46]. These bacteria are more beneficial than free-living or associative nitrogen fixing bacteria because they are well protected within the nodule tissue and encounter little or no competition from indigenous soil microorganisms. Production of

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

DOI: https://doi.org/10.54987/jobimb.v9i2.617

indoleacetic acid by *Rhizobium*, *Azotobacter* and Bacillus, Paenibacillus and Pseudomonas has been reported [47, 48] and plays a central role in plant growth and development and acts as a signalling molecule which is involved in plant signal processing and motility or signalling [49] Alcaligenes sp. [49] has also been reported to produce indoleacetic acid [50]. Pseudomonas [51,49] and *Azotobacter* [52,53] have all been shown to produce siderophore (a low molecular weight iron chelating compound). Azospirillum [54] and *Rhizobium* [15, 55]. It has been shown that phytopathogens are suffocated by siderophores because they are unable to acquire iron from the soil, and this has been linked to siderophores [40].

Gibberellin is a key phytohormone in the growth and functioning of plants. *Bacillus* species such as *Bacillus cereus* MJ-1, *Bacillus macroides* CJ-29, and *Bacillus pumilus* have been shown to produce gibberellin [42]. Cytokinins [41], a phytohormone that regulates cytokinesis, growth, and development in plants, have been shown to be produced by nitrogen-fixing bacteria [56]. Nitrogen-fixing Bacillus megaterium [41] produced cytokinin (UMCV1). Bacillus sp. has also been shown to produce antibiotics and to degrade cell walls [57]. Antibiotics are made up of a variety of low-molecular-weight secondary metabolites that are harmful to other microorganisms, such as plant pathogens, as well as to themselves [58]. Nitrogen-fixing Bacillus has produced several antibiotics, including amphisin, 2,4-diacetylphloroglucinol (DAPG), hydrogen cyanide, phenazine, and pyrolnitrin [57, 58]. Growth-promoting properties of the cell wall-degrading enzymes generated by several nitrogen-fixing bacteria, most often Bacillus. It is possible to destroy the cell wall of many phytopathogenic mushrooms with enzymes such as chitinase, cellulase, beta 1,3 glucanase, protease and lipases, reducing the frequency of plant illnesses [59].

Biosorption of Heavy Metals by Nitrogen Fixing Bacteria

The importance of heavy metal pollution remediation technology cannot be overstated in a community that is able to meet both its economic and environmental needs. It's costly, requires a lot of chemicals, and results in harmful chemical sludge when using conventional techniques to remove metals from aqueous solutions [60]. Natural and ecologically friendly adsorbents, such as biomass from microbes and plants, are essential for metal adsorption, a process known as "biosorption." Toxic metal levels can be reduced to ecologically reasonable standards through biological therapy based on microbial and plant biomass [61].
Toxic metal ions have been removed from various matrices using microbial biomass [61-63].

Microbial biomass materials have been demonstrated to successfully adsorb heavy metals from even extremely dilute aqueous solutions, and this has implications for the biosorption of heavy metals from wastewater by biological materials [64]. Complexation, coordination, physical adsorption, chelation, ion exchange, inorganic precipitation, or a mix of these activities are some of the ways metal biosorption mechanisms operate [65]. For microorganisms to remove metal through biosorption mechanisms, several variables must come into play, including metal ion composition and ionic strength as well as cell wall composition, physiology, and physicochemical conditions like pH and temperature [62,63].

Various heavy metals can be removed or absorbed from solutions by different kinds of nitrogen-fixing bacteria and their products. Despite their importance in plant host specificity [66, 67] and growth promotion, rhizobial exopolysaccharides have only lately been studied for their metal sorption capability. At concentrations of 15.5, 20 and 25mg, *Azotobacter*'s extracellular polymer was able to bioadsorb Cu, Zn, and Fe. After the Chernobyl nuclear disaster, researchers Douka and Xenoulis [68] found that nodulated pasture legumes significantly reduced radioactive metal concentrations. *Rhizobium trifoli*i was reported to lower UO_2 2 contents by 60% in a 0.4 mM solution with a maximal sorption capacity of 0.25 mmol UO_2 2+/g, according to Cotoras et al.[68]. Using *Azotobacter chrococcum* XU1 exopolysaccharide, Rasulov et al. [70] explored the biosorption of metal ions such as lead (Pb) and mercury (Hg) from solution and observed substantial removal of these ions from solution. Chromium (Cr) sorption by *Azotobacter* s8, *Bacillus subtilis*, and *Pseudomonas aeruginosa l*ive cells was studied by Kurniawan et al. [71]. Adsorption capabilities of 135.3 and 167.5 mg/g for cadmium and cobalt, respectively, were achieved by *Rhizobium leguminosorium* in research by Abd-Alla et al.[72]. N_2 fixation bacteria were found to create polysaccharides and other biopolymers that have metal-binding capabilities [73]. Polysaccharides such as peptidoglycans, water-soluble and amphipathic exopolysaccharides (EPS), teichoic and teichuronic acids as well as capsular polysaccharides and polyglutamic acid are among the most common (LPS). If the cations are bound to the bacteria via electrostatic interactions, they are usually interacting with negatively charged functional groups such as uronic acids (EPS from *Bradyrhizobium japonicum* or alginate or teichuronic acid or emulsan or LPS from various sources), membrane phosphoryl groups or carboxylic amino acid groups. Cation binding by positively charged polymers [74] or coordination with hydroxyl groups may also play a role in electrostatic interactions[75, 76]. Eukaryotic polymers, such as chitin and chitosan, have been shown to bind cations, perhaps through chelation and coordination with hydroxyl groups [74]. LPS attaching metal ions to cell walls may not be directly linked to O-antigen side chains, but rather, the B-band LPS may alter cell surface characteristics, allowing metals to precipitate in certain areas of cell surface, as hypothesised by Langley and Beveridge[77].

For years, scientists are looking at the possibility of using microorganisms like bacteria that can resist, detoxify, and absorb metals to clean up metal-polluted settings [78]. Biosorbents and biosorption systems that are efficient, natural, eco-friendly, and financially feasible may be one answer to bioremediation of settings contaminated by heavy metals [79].

CONCLUSION

Nitrogen-fixing bacteria convert atmospheric nitrogen into plant-available form and are essential to soil health and plant growth. Plant growth promotion is promoted by the synthesis of various hormones and enzymes as well as the inhibition of phytopathogens by these microbes. A process known as biosorption provides an efficient, natural, eco-friendly, and economically viable method of removing heavy metal ions from solutions that are polluted with them. Reviewers found that these particular microbes have a variety of responsibilities in maintaining a healthy ecosystem.

REFERENCES

1. Anas M, Liao F, Li, YR. Fate of nitrogen in agriculture and environment: agronomic, ecophysiological and molecular approaches to improve nitrogen use efficiency. Biol Res, 2020; 53(47): 75-89.
2. Zahran HH. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev. 1999; 64(4): 968-989.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

3. Egamberdieva D, Kucharova Z. Cropping effects on microbial population and nitrogenase activity in saline arid soil. Turk J Biol. 2008; 32: 85-90.
4. Escobar C, Horna Y, Carreño C, Mendoza G. Caracterización de cepas nativas de *Azotobacter spp.* y su efecto en el desarrollo de *lycopersicum esculentum* mill. "tomate" en lambayeque. Sci Agropecuaria. 2011; 2: 36-49.
5. Wagner SC. Biological nitrogen fixation. Nat Edu Know, 2012; 3(10):15.
6. Vats S, Srivastava P, Saxena S. Beneficial effect of nitrogen-fixing bacteria for agriculture of the future. In book: Soil Nitrogen Ecology. 2021; 305-325.
7. Rifat H, Safdar A, Ummay A, Rabia K, Iftikhar A. Soil beneficial bacteria and their role in plant growth promotion: A Rev Ann Microbiol. 2010; 60(4): 579-598.
8. Panos S, Jane V, Rousk K, Cillier M. Potential for nitrogen fixation in the fungus-growing termite symbiosis. Frontiers Microbiol. 2016; 7: 1-5.
9. Philippot L, Germon JC. Contribution of bacteria to initial input and cycling of nitrogen in soils. 2010; 159-176. *In* Buscot F, Varma A. (Ed.) Microorganisms in soils: Roles in genesis and functions. *S*pringe*r*, New York.
10. Dahal B, Nanda-Kafle G, Perkins L, Brozel VZ. Diversity of free-living nitrogen fixing *streptomyces* in soils of the badlands of south dakota. Microbiol Res. 2017;195: 31-39.
11. Tai XS, Mao WL, Liu GX, Chen T, Zhang W, Wu XK, Long HZ, Zhang BG, Zhang Y. High diversity of nitrogen-fixing bacteria in the upper reaches of the Heihe River, northwestern China. BiogeoSci. 2013; 10: 5589–5600.
12. Onyeze R, Onah G, Cecilia I. Isolation and characterization of nitrogen fixing bacteria in the soil. Int J Life Sci. 20132(3): 438-445.
13. Emmyrafedziawati AKR, Stella M. Identification of free living nitrogen fixing bacteria isolated from efb compost, molecular detection of nifh gene and measurement of nitrogenase activity. J Trop Agri Food Sci. 2018; 46(1): 39-46.
14. Abar TH, Alemayehu LA. Isolation and characterization of rhizobia from rhizosphere and root nodules of cowpea, elephant and lab lab plants. Int J Novel Res Inter Stud. 2017; 4(4): 1-7.
15. 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.
16. Zgadzaj R, Ruben G, Jensen DB, Koprwova A, Paul S, Radutou S. Root Nodule Symbiosis in lotus japonicu drives the establishment of distinctive rhizosphere, root and nodule bacterial communities. Proc Nat Acad Sci USA. 2016; 113(49) :763-775.
17. Zehr JP, Jenkins BD, Short SM, Steward GF. Nitrogenase : A cross-system comparison. Environ Microbiol 2003; 5, 539-554.
18. Debrosses GJ, Stougaard J. Root nodulation: a paradigm for how plant –microbe symbioses influence host development pathways. Cell Host Microb. 2011; 10 : 348 -358.
19. Linblad P. Cyanobacteria in symbiosis with cycads. In Cyanobacteria in symbiosis. 2002; (pp. 195-205). Springer, Dordrecht.
20. Dixon RA, Khan D. Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol. 2004; 2(8). 621-31.
21. Deslippe J, Egger K. Molecular Diversity of nifH genes from bacteria associated with high arctic dwarf shrubs. Microbial Ecol. 2006; 51: 516–525.
22. Gaby JC, Buckley DH. A comprehensive evaluation of pcr primers to amplify the nifh gene of nitrogenase. PLOS ONE. 2012; 7(7) : 142-149.
23. Seefeldt LC, Hoffman BM, Dean DR. Mechanism of Mo-dependent nitrogenase. Annual Rev of Biochem 2009; 78, 701.
24. Dahal B. Diversity of free living nitrogen fixing bacteria in the badland of south Dakota. Open Public Res Access Institutional Repository and Information Exchange. 2016; 4-8.
25. Van Nguyen T, Pawlowski K. *Frankia* and Actinorrhizal plant. symbiotic nitrogen fixation . In Book. Rhizotrophs : Plant Growth Promotion to Bioremediation. 2017; 237- 261.
26. Ribeiro CW, Alloing G, Mandon K, Frendo P. Redox regulation of differentiation in symbiotic nitrogen fixation. Biochem Biophys Acta (BBA). 2015; 8: 1850.
27. Chang C, Damiani I, Puppo, A, Frendo P. Redox changes during the legume–*Rhizobium* symbiosis. Mol Plant. 2009; 2, 370-377.
28. Oldroyd GED. Speak, Friend, and Enter: signalling systems that promote nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Ann Rev Biochem. 2013; 78, 701.
29. Mus F, Mathew BC, Kevin G, Amaya GC, Varney AG, Evangelia DK, Ponaraje MR, Giles EDO, Philps SP, Michael KU, Christoper AV, Jean A and John WP. Symbiotic nitrogen fixation and its challenges of its extension to non legumes. Mini Rev. Appl Environ Microbiol. 2016; 115 :293-320.
30. Nelson SM, Sadowsky MJ. Secretion system and sgnal exchange between nitrogen fixing rhizobia and legumes. Front Plant Sci. 2015; 6: 491.
31. Baldani J, Baldani V. History on biological nitrogen fixation Res in graminaceous plants. special emphasis on brazilian experience. A Rev An Acad Bras Cienc . 2005; 77(3) : 549- 79.
32. Li H, Rajesh KS, Sing P, Song Q, Xing Y, Yang L, Li R. Genetic diversity of nitrogen fixing and plant growth promoting pseudomonas species isolated from sugar cane rhizosphere. Front Microbiol Microbiotechnol. 2017; 45-52.
33. Lin L, Li Z, Hu C, Zhang X, Chang S, Yang L, Li Y, Qianli A. Plant growth promoting nitrogen fixing enterobacteria in association with sugarcane plants growing in guangxi, China. Microb Environ. 2012; 27(4) : 391-8.
34. Smercina DN, Evans SE, Friesen ML, Tiemann LK. To fix or Not to fix: Controls on free living nitrogen fixation in the rhizosphere. Appl Environ Microbiol. 2019; 85: 02546-18.
35. Ohyama T, Momose A, Ohtake N, Sueyoshi K, Sato T, Yasuhiro N, Constancio A, Asis Jr, Ruam S, Ando S. nitrogen fixation in sugar cane. Adv Biol Ecol Nitro Fix. 2013; 36(1): 49-61.
36. Diogo PC, Armendo CFD, Cotta SR. Changes of bacterial communities in the rhizospher of sugarcane under elevated concentration of atmosphere C02. GCB Bioenergy .2017; 10(2).
37. Unkovich M, Baldock J. Measurement of asymbiotic N2 fixation in Australian agriculture. Soil Biol Biochem. 2008; 40, 2915-2921.
38. Zhan J, Sun Q. Diversity of free-living nitrogen-fixing microorganisms in wastelands of copper mine tailings during the process of natural ecological restoration. J Environ Sci. 2011; 23, 476-487.
39. Pankaj S. Understanding the philosophy of heterocyst and nitrogen fixation in cyanobacteria or blue green algae. Nat Sci .2008; 6(1) : 28-35.
40. Zaidi A, Khan MS, Saima S, Rizvi A. Role of nitrogen-fixing plant growth promoting rhizobacteria in sustainable production of vegetables: current perspective. In book: Microbial Strategies for Vegetable Production. 2017; 49-79.
41. Oritz-Castro R, Valencia-Cantero E, Lopez-Bucio IJ. Plant growth promotion by *Bacillus megaterium* involves in cytokinins signaling. Plant Signal Behav. 2008; 3: 263-265.
42. Joo GJ, Kim YM, Kim JT, Rhee IK, Kim JH, Lee IJ. Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red peppers. J Microbiol. 2005; 43: 510-515.
43. Khalid A, Arshad M, Zahir ZA. () Growth and Yield Response of Wheat to Inoculation With Auxin Producing Plant Growth Promoting Rhizobacteria. Pak J Bot, 2003;35:483–498.
44. Khalid, A., Arshad, M and Zahir, ZA. Screening Plant growth promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol. 2004; 96:473–480.
45. Glick BR, Cheng Z, Duan J. Promotion of Plant Growth by ACC Deaminase-producing Soil Bacteria. Eur J Plant Pathol, 2007;119: 329-339.
46. Nosrati, R., Owlia, P and Saderi, H. Phosphate solubilization characteristics of efficient nitrogen -fixing soil *Azotobacter* strains. Iran J Microbiol. 2007; 6:285–295.
47. Çakmakçi R, Erat M, Erdoğan ÜG, Dönmez MF. The influence of PGPR on growth parameters, antioxidant and pentose phosphate oxidative cycle enzymes in wheat and spinach plants. J Plant Nutr Soil Sci. 2007; 170:288–295.
48. Beneduzi A, Peres D, Vargas LK, Bodanese-Zanettini MH, Passaglia LMP. Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing Bacilli Isolated from rice fields in South Brazil. Appl Soil Ecol. 2008; 39:311–320.
49. Dell'Amico E, Cavalca L, Andreoni V. Improvement of Brassica napus growth under cadmium stress by cadmium resistant rhizobacteria. Soil Biol Biochem. 2008; 40:74–84.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

DOI: https://doi.org/10.54987/jobimb.v9i2.617

50. Spaepen S, Das F, Luyten E. Indole-3-acetic acid-regulated genes in *Rhizobium etli* CNPAF512: Res letter. FEMS Microbiol Lett. 2009; 291:195–200.
51. Dey R, Pal K K, Bhatt DM, Chauhan SM. Growth promotion and yield enhancement of peanut (Arachis hypogaea L) by application of plant growth promoting rhizobacteria. Microbiol Res. 2004; 159:371–394.
52. Muthuselvan I, Balagurunathan R. Siderophore production from *Azotobacter* sp. and its application as biocontrol agent. Int J Curr Res Rev. 2013; 5(11):23–35.
53. Prasad JS, Reddy RS, Reddy, PN. Isolation, screening and characterization of *Azotobacter* from rhizospheric soils for different plant growth promotion (pgp) and antagonistic activities and compatibility with agrochemicals: An *in vitro* study. Ecol Environ Conserv. 2014; 20:959–966.
54. Tortora ML, Díaz-Ricci JC, Pedraza RO. *Azospirillum brasilense* Siderophores with antifungal activity against *Colletotrichum acutatum*. Arch Microbiol. 2011; 193:275–286.
55. Datta B, Chakrabartty PK. Siderophore biosynthesis genes of *Rhizobium* sp. Isolated From *Cicer arietinum* L. 3 Biotech. 2014; 4: 391–401.
56. Aloni R, Aloni E, Ullrich CI. Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation , lateral root initiation, root apical dominance and root gravitropism. Annals of Botany. Oxford University Press. 2006; 46-54.
57. Benduzi A, Ambrosini A, Passaglia, LMP. Plant growth promoting rhizobacteria(pgp) : their potential as antagonist and biocontrol agent. Gen Mol Biol. 2016; 35(4): 1044-51.
58. Glick BR. Plant growth promoting bacteria: mechanisms and applications. Scientifica(Cairo). 2012; 963401.
59. Green-Ruiz C, Rodriguez-Tirado V, Gomez-Gil B. Cadmium and Zinc removal from aqueous solutions by *Bacillus jeotgali*: pH, salinity and temperature effects. Bioresour Technol. 2008; 99, 3864-3870.
60. Volesky B. Adv in biosorption of metals: selection of biomass types. FEMS Microbiol Rev. 1994; 14, 291-302.
61. Gabr RM, Hassan SHA, Shoreit AAM. Biosorption of lead and nickel by Living and Non-living cells of P*seudomonas aeruginosa* ASU 6a. Int Biodeterior Biodegrad. 2008; 62 : 195-203.
62. Hassan SHA, Kim SJ, Jung AY, Joo JH, Oh SE, Yang JE. Biosorptive capacity of Cd(II) and Cu(II) by lyophilized cells of *Pseudomonas stutzeri*. J Gen Appl Microbiol. 2009; 55: 27-34.
63. Volesky B. Biosorption process simulation tools. Hydrometallurgy. 2003; 71: 179-190.
64. Chen XC, Wang YP, Lin Q, Shi JY, Wu WX, Chen YX. Biosorption of copper(II) and zinc(II) from aqueous solution by *Pseudomonas putida* CZ1. colloids and surface. Biointerfaces. 2005; 46, 101-107.
65. Dudman WF. The polysaccharides and oligosaccharides of *Rhizobium* and the role in the infection process. In: Veeger C, Newton WE. (Eds.). Adv in Nitrogen Fixation Res. Nijhoff/Dr. W. Junk Publishers, The Hague, Netherlands. 1984; 397-404.
66. Wu G, Kang HB, Zhang XY, Shao HB, Chu LY, Ruan CJ. A critical Rev on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-Environ concerns and opportunities. J Hazard Material. 2010; 174:1-8.
67. Douka CE, Xenoulis AC. Beneficial effects of biological nitrogen fixation on the reduction of: I. metal toxicity of the biomass produced. II. radioisotope uptake following the chernobyl fallout. In: Bothe H, De Bruijn J, Newton WE. (Eds.). Nitrogen Fixation: Hundred Years After. Gustav Fisher, Stuttgart. 1988; 812.
68. Cotoras D, Millar M, Viedma P, Pimentel J, Mestre A. Biosorption of metal ions by *Azobacter vinelandii*. World J Microbiol Biotechnol. 1992; 8: 319-323.
69. Rasulov B, Yili A, Aisa H. Biosorption of metal ions by exopolysaccharide produced by *Azotobacter chrococcum XU1*. J Environ Protection. 2013; 20: 109-114.
70. Kurniawan BS, Muhammad FI, Furwanti IF. Biosorption of chromium by living cells of *Azotobacter* S8, *Bacillus subtilis* and *Pseudomonas aeruginosa* using batch system reactor. J Ecol Eng. 2019; 20(6); 184-189.
71. Abd-Alla M H, Fatthy MM, Abdel-wahab EE. Isolation and characterization of a heavy metal-resistant isolate of *Rhizobium leguminosarum* bv vicia potentially applicable for biosorption of Cd^{2+} and Co^{2+}. Int Biodeterior Biodegrad. 2012; 67, 48-55.
72. Gutnick DL, Bach H. Engineering bacterial biopolymer for biosorption of heavy metals: new product and new formulation. Appl Microbial Biotechnol. 2000; 54: 45-46.
73. Muzzarelli RA, Tubertini O. Chitin and chitosan as chromatographic supports and adsorbents for collection of metal ions from organic and aqueous solutions and sea-water. Talanta. 1969; 16, 1571-1577.
74. Beveridge TJ, Murray RG. Sites of metal deposition in the cell wall of *Bacillus subtilis*. J Bacteriol. 1980; 141, 876-887.
75. Zosim Z, Gutnick DL, Rosenberg E. Uranium binding by emulsan and emulsanosols. Biotechnol Bioeng. 1983; 25 : 1725-1735.
76. Langley S, Beveridge TJ. Effect of O-side-chain-lipopolysaccharide chemistry on metal binding. Appl Environ Microbiol. 1999; 65:489-498.
77. Lasat MM. Phytoextraction of toxic metals: A Rev of biological mechanisms, J Environ Qual. 2002; 31 : 109–120.
78. Garbisu G, Alkorta I. Phytoextraction: A Cost-effective Plant-based Technology for the Removal of Metals From the Environment. Bioresour Technol. 2002; 77: 229–236.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).