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# Isolation and characterization of cultivable endophytic micro biome associated with wild fern *Azolla*

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**Abstract**

Endophyte constitute the important part of plant microbiome have been shown to benefit plant health in a variety of ways. The present investigation was aimed to isolate and characterize endophytic bacteria from wild fern Azolla. Within this study total of 15 endophytic bacteria were isolated from *Azolla* by standard microbiological culture methods. Based on 16S rDNA sequences, the isolated species comprising bacteria commonly associated with soil and plants. The genera *Bacillus polymyxa, Bacillus thuringiensis, Proteus mirabilis and Azotobacter* species were isolated from fern. All isolates were screened for the production of enzymes. The result of the study revealed that maximum isolates have positive protease, urease and amylase activity and this was followed cellulase activities. The result of the present study indicated that among the above four novel strains of endophytic bacterial isolates *Bacillus thuringiensis* and *Bacillus polymyxa* has wide range of agriculture application and potential biological control agents, Future research will analyse that these isolates can be used for biological control, growth promotion, medical application or the synthesis of enzymes for biotechnology.

**Key words :** Endophyte, *Bacillus* sp, Phylogenetic, Biochemical, plant growth promoting potential and *Azolla.*

Plants are one of the most important hosts for diverse groups of bacteria that colonise different plant tissues and categorized (endosphere–endophytes), outer plant surfaces (phyllosphere–epiphytes) and root surfaces (rhizosphere) forming the plant microbiota[51].

The interaction of microbes with plants may bebeneficial, neutral (commensalism) or detrimental (parasitism or pathogenicity)[6]. Previous studies have indicated that endophytic bacteria have been reported to promote plant growth by a number of different mechanisms producing substances such as phytohormones and antibiotics which support plant growth and provide protection against pathogens (Plant Growth Promoting Bacteria, PGPB)[18,24,44]. Including phosphate solubilization activity[54,55], production of phytohormones[28], nitrogen fixation[13,42], siderophore biosynthesis[30] and supplying essential nutrients to the host plant[40,42,52]. In addition, several endophytic bacteria have been shown to facilitate various phytoremediation strategies[7,40]. This property can help improve crop production–one of today's most important issues due to rising human population and the shortage in resources, therefore, it is worthwhile to learn about plant-associated micro biomes ferns from the genus *Azolla,* which are valuable plants in terms of potential microbiome hosts and are crucial to several industries[5]. The presence of bacteria in the leaves of *Azolla* was found for the first time by Bottomley in 1920 [45]. Carrapiço[10] and Zheng *et al*.,[58] found that the bacteria are prevalent in the leaf cavities of *Azolla* throughout the course of its life cycle and are strongly linked to the trichomes, studies proposed that bacteria could be a third partner in the *Azolla-Anabaena* association[10,27,37]. There is some information about bacteria as a third partner in symbiosis, The presence of bacteria in Azolla sp. leaves was first reported by [10,11,36]. Later on isolation and cultivation of endophytes of *Azolla filiculoides* was carried out by Banach *et al*.,[4]. But still there is lack of detailed information on endophyte harbour in different species and a huge gap in knowledge microbiome inhabiting *Azolla* sp. Thus, the novelty and main goal of the study was to isolate, identify, and describe unrecognized bacteria constituting the core microbiome of *Azolla,* and Genotyping of endobacterial diversity provides the molecular mechanisms of *Azolla-Anabaena* symbiosis and its coevolution. Since the fern is used in agriculture and water treatment, it would be useful to discover its microbiome, which may help to elucidate its role in the symbiotic system *Azolla*-microorganisms and indicate its possible applications in industrial application.

*Sample collection, isolation and cultivation of entophytes :*

Fresh samples of *Azolla* Willd species were collected from Badgonda forest Mhow Region M.P and maintained in biotechnology lab SAGE university Indore, Plant leaves were surface–sterilized for removal of epiphytes by stepwise immersion in 70% ethanol for 1 min, then in 2.5% sodium hypochlorite for 2 min and finally in 70% ethanol for 1 min, followed by five rinses in sterile distilled water. Few randomly chosen plants were cut into small pieces with a sterilised razor blade 1-3 mm long and put on TSA plates and Ashby agar media plate, plate was incubated at 28°C – 30°C for 24-48 hrs to until growth observed and visible colony is counted[14]. After 24-48 hrs bacterial cultures with different phenotypic character, such as colony morphology, colony, colour, cell shape and were repeatedly streaked to achieve bacterial isolates.[3]

*Preservation and maintenance of culture :*

Based on colony morphology, a typical single colony of endophytic bacteria was chosen from the plates and transferred to nutrient agar slants to establish and maintain a pure culture as per standard protocol[8] and were maintained at 4°C till further used[15]. Preliminary identification of endophytic bacteria was carried by morphological, biochemical and enzymatic characteristics of bacteria like cell shape and size, gram staining, spore formation, motility, colony colouring, Citrate, Catalase, Indole, MR, VP, Casein, Amylase, Urease, Cellulase According to Bergey's Manual of Determinative Bacteriology (1994). For further identification, two isolates from each target bacterial group were chosen for gene sequencing.

*Phenotypic and genotypic characterization of selected isolates :*

The genomic DNA of endophytic bacteria was extracted[1] and 16S rDNA was amplified in polymerase chain reaction (PCR) using the genomic DNA as template and primers 16s Forward Primer (395F) AAGGTCTGGAGCACGCTTAT, 16s Reverse Primer (396R) CAACCGTGCCGTGGA-ATTAT. The PCR reactions were performed in 20 µl volumes containing 2 µl of the genomic DNA sample, 1× PCR buffer containing; 0.16 mM dNTP, 20 pmol of forward and reverse primers and 0.75 U Taq DNA polymerase (MBI, Fermentas, Lithuania). with the PCR conditions as follows: an initial denaturation at 95°C for 6 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 1 min, and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min. PCR products were analysed using 2% agarose gel electrophoresis, purified and was further subjected to sequencing. Phylogenetic tree reconstruction was conducted in MEGA version 6. The neighbor-joining method[33] based phylogenetic tree was inferred from evolutionary distances calculated using the Maximum Composite Likelihood method[50] sequence data was checked by BLAST analysis[57].

*Endophytic bacterial isolation :*

The surface-disinfected leaf of the *Azollae* was effectively used to isolate and purify a number of endophytic bacteria were investigated (Figure: 1). Total of 15 endophytic bacteria that were successfully isolated and purified from the surface-disinfected leaf of *Azolla*. Four isolates (A, B, C, D) were further investigated. Gram staining indicated A was a Gram negative-bacterium and the isolate was beige-pigmented on TSA agar, The isolate B was whitish on TSA agar plate, C was a Gram-Positive bacterium and was yellow-pigmented on TSA agar, D, was a Gram negative-bacterium and the isolate was creamy white colony on Ashby's agar (Figure: 2).

*Morphological and biochemical characterizations of endophytic isolates :*

The isolated bacteria displayed a unique colony with a range of colours and shapes, including orange, yellowish orange, white, and cream, *etc.* On the basis of results of the gram's staining, growth on selective and differential media and biochemical analysis the isolated bacterial stain is identified using the software PIBWIN-2007. We observed variations in the colony shape, which is

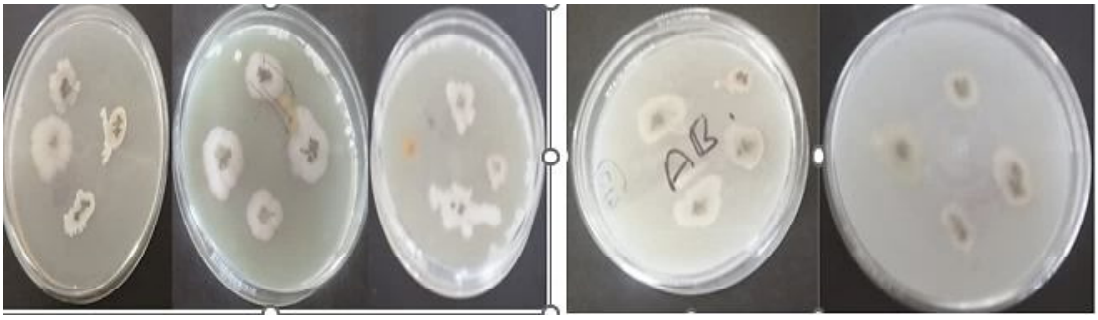

Figure 1 colonies of endophyte bacteria observed on TSA medium and on Ashbay medium plates

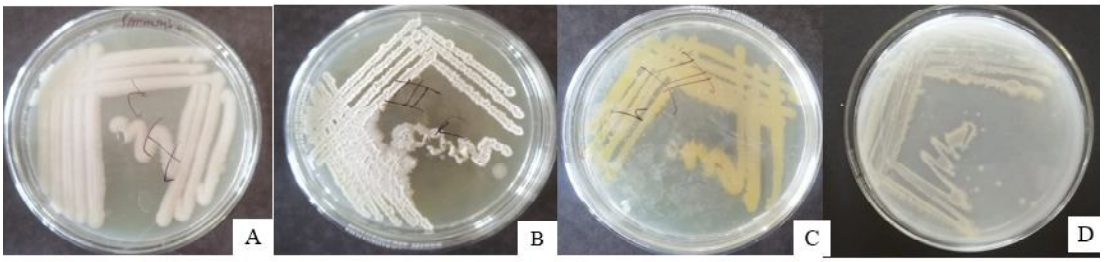


Figure 2 Purified cultures of bacterial endophytes (A-C) from TSA medium and (D) from Ashby's medium

suggestive of the isolates' inclusion in several taxonomic groupings. Out of the 4 isolates, 2 Gram-negative and 2 Gram-positive rods in terms of cell morphology and gram staining the isolates had colonies that ranged in size from around 1 mm to about 0.5 mm. 50% had small size, and 50% are large in size, Circular shape was dominant in the studied pool of microorganisms. Endophytes did not form irregular and filamentous colonies, we observed three types of colony texture: mucoid (MUC), viscid and butyrous (BUT). In terms of colony transparency, we divided microorganisms into opaque (OPQ) and translucent (TRANS), Isolated microorganisms displayed two types of pigmentation – white-cream-beige, whitish and yellow, Next studied characteristic was colony elevation: flat (F) and raised (R), Margin was the last morphological traitwe observed colonies with entire and undulate (Figure 1).

Table-1. Morphological characteristics of the isolated strains of endophytic bacteria

| S. no | Strain | Colour | Size | Form | Margin | Texture | Elevation | Opacity | Size | Gram staining |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | Creamy | Large | Circular | Ent | Mucoid | Flat | TRANS | Bacilli | G- |
| 2 | B | Whitish | Large | Circular | Und | Viscid | Raised | OPQ | Bacilli | G+ |
| 3 | C | Yellow | Large | Circular | Ent | Mucoid | Raised | OPQ | Bacilli | G+ |
| 4 | D | Creamy | Small | Circular | Ent | But | Flat | TRANS | Bacilli | G- |

G+: Gram positive, G-: Gram Negative

Table-2. Biochemical characteristics of the isolated strains of endophytic bacteria

| S. no | Strain | Citrate | Catalase | Indole | Methyl red | Voges pursar | Casein | Amyl-ase | Ure-ase | Pecti-nase | Cellu-lase | Gelatin Hydrol-ysis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | + | - | + | - | - | - | + | + | + | + | + |
| 2 | B | + | + | + | + | - | - | + | - | + | + | + |
| 3 | C | + | + | + | + | - | - | + | + | + | + | + |
| 4 | D | - | + | + | - | + | - | + | + | + | + | - |

The biochemical analysis of isolates showed that all isolates can produce the enzyme amylase, pectinase and cellulase, however, three isolates showed positive results for the gelatin and urease hydrolysis test. The isolates cultural characteristic is shown in (Table-2).

*Molecular identification :*

Phylogenetic tree is important and crucial tool for taxonomic classification and also suitable way for illustrating the evolutionary link between organisms, suggested by Gollery[13]. The DNA sequence of 16S rRNA

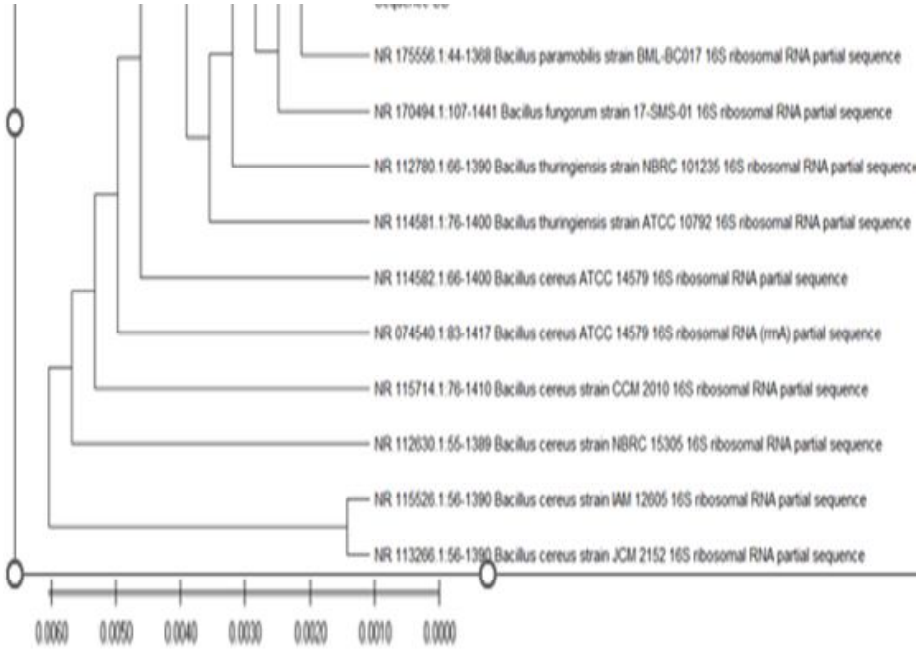


Figure 3 : Molecular phylogenetic showing the genetic relationship of the BB isolate to other isolates by using maximum likelihood method on basis of bacterial 16S ribosomal RNA sequencing. The numbers at the nodes are the bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree.

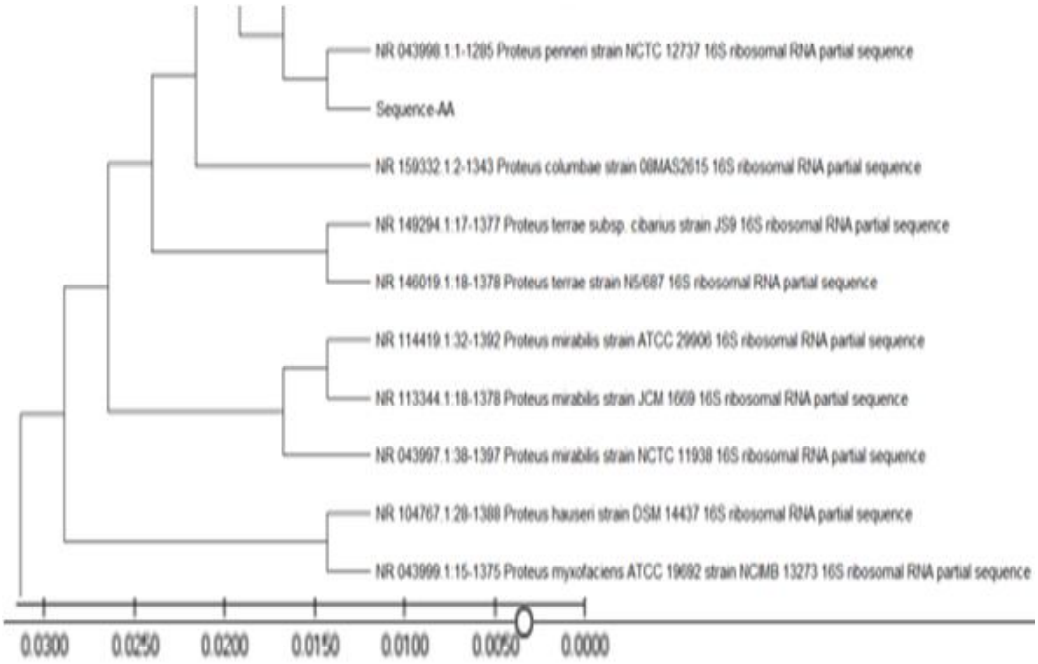


Figure 4: Molecular phylogenetic showing the genetic relationship of the BB isolate to other isolates by using maximum likelihood method on basis of bacterial 16S ribosomal RNA sequencing. The numbers at the nodes are the bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree.

were aligned by MEGA using either the Clustal W and results were compared with the existing sequence of 16S rRNA from other organisms in Gen Bank databases in the NCBI website. On the basis of 16S rRNA (hypervariable fragments V2-V4) analysis. We demonstrated 99% sequence similarity of sequences (Figs. 3&4). The identified endophytic isolates were *Proteus penneri* as shown in the tree having high similarity and homologous to *Proteus penneri* strain NCTC 12737 16S ribosomal RNA with accession number NR_043998.1. *Bacillus thuringiensis* as shown in the tree having high similarity and homologous to *Bacillus thuringiensis* strain IAM 12077 16S ribosomal RNA with accession number NR_043403.1. Based on genetic distance and phylogenetic trees, endophytic bacterial isolates obtained from *Azolla* plants are included in the genus of *Bacillus*.

Endophytic microorganisms have several functional properties that make them interesting tool for agricultural applications. Several studies are conducted to isolate the endophytic bacteria of *Azolla*'s in nature by different scientist since 1970. Our study has shown that *Azolla* is inhabited by bacteria present inside the plant (endophytes)[2,16,27,39]. During the present study, six endophytic bacteria were isolated from leaves using routine bacteriological procedures and these

were characterised for their physical, cultural and biochemical properties. Several studies are carried out to isolate endophytic bacteria from a wide range of plant species to observe under different ecological conditions[34,59]. Leaves being the major source of these bacteria as compared to other above ground parts since entry into the leaves (via the stomata) is easier than into the stems[13]. In this work, endophytic communities of leaves of wild *Azolla* species were studied. A total of 6cultivated endo-phytic bacteria were isolated from the plant material and out of four are screened further., approximately similar number of isolates were obtained in a work *with Azolla caroliniana* Willd (M. Grilli Caiola *et al*., 1988). Isolated four endophytic bacteria from *Azolla* cavity biochemical identified as *Bacillus Polymyxa, Bacillus thuringiensis, Proteus mirabilis and Azotobacter* species, The presence of certain genera in *Azolla* suggest that they are better adapted to live as endophytic bacteriain *Azolla* than in other genera, The genera isolated in this work that have not been previously reported as follows *P. pennerri, B. thuringiensis, B. polymyxa*.

In this study, bacteria found inside fern is *B. thuringiensis*, are to be known as potential biocontrol agents against insects ay be a useful way to explore new products agriculture biotechnology[48,49]. Similarly, *proteus penneri* that were inhibited in *Azoll*a are known to be pathogenic and having multidrug resistance and can be explore as new useful tool in field of medical microbiology in future.

*Paenibacillus polymyxa* isolated (formerly known as *Bacillus polymyxa*) which resides mainly in the soil, rhizosphere, and plant tissue[25], it is rod-shaped endospore-forming bacterium, facultatively anaerobic, neutrophilic, and sporulating and have several potential application in field of agriculture biocontrol agents against a variety of plant diseases, such as bacteria, fungi, oomycetes and antibiotic compound such as polymyxins and fusaricidins[12,19,23,26,46]. *P. polymyxa*, is regarded as a potential PGPR and have broad host rang, it has been isolated from several other plant species of wheat and barley[29] white clover, perennial ryegrass, crested wheatgrass,[20] green bean[38] andzea mays[9] *etc*. Due to these properties, the *P. polymyxa* strains have gained lots of research attention as important application in biofertilization, biocontrol, and biofuel applications[56].

Bacterial isolated from medicinal plant *Azolla* were able to produce important enzyme Amylase, urease, pectinase, cellulase, gelatin hydrolysis (Table-2.) Similar investigations indicated that endophytic bacteria exhibited plant beneficial traits and resource of different extracellular enzyme[22] enzyme have wide application in industries, in addition, endophytic microbes producing enzymes can help to counteract biotic stress; however, the role of such endophytes in abiotic stresses cannot be ruled out[22].

In the present work, a great number of endophytic bacilli bacteria isolated. The statement is in agreement with the present findings of [47]. *Bacillus* species have emerged as a complementary, efficient, and safe alternative to current crop management practices. One benefit of the genus for use in formulations is the capacity of *Bacillus* species

to generate spores, which are resistant to unfavourable environments. In addition to protecting plants from phytopathogenic microbes, insects, and nematodes, endophytic *Bacillus* species also induce resistance in plants and encourage plant growth without harming the environment[31]. Several studies in the literature confirm that certain *Bacillus* species are dominant inside plants, such as the species *B. toyonensis, B. megaterium, B. cereus, B. aryabhattai, B. stratosphericus,* and *B. cereus* isolated from tomato plants in Brazil[21,43]. The dominancy of *Bacillus* species of bacteria observed in our research leads a broad range of benefits for including prevention and control of diseases caused by pathogens, eliciting plant resistance, and promoting plant growth, Stress tolerant and resistant in a recent study,[47] From the available literature, it seems that the bacillus species could be more efficient biofertilizers and biocontrol agent and wide range of application in agriculture biotechnology.

Future works should therefore focus on finding the endophyte from wild species of Azolla. Four species are isolated in the study is *Bacillus polymyxa, Bacillus thuringiensis, Proteus mirabilis and Azotobacter* species. Endophytic bacterial isolates have different abilities related to plant growth promotion prevention and control of diseases caused by pathogens, eliciting plant resistance, and promoting plant growth, stress tolerant and resistant biofertilizers and biocontrol. Bacterial isolates have different abilities to produce different enzyme like, protease, lipase, amylase and cellulase and have wide industrial application. This study provides future encouragement for the plant growth promoting endophytic bacterial isolates (A, B, C, and D) for the improvement of eco-friendly biofertilizers and effective tool as biocontrol agent.

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**Conflict of Interest :**

There is no conflict of interest among the authors or institution.

References :

1. Aboul-Maaty, N. A.-F., andH. A.-S. Oraby, (2019). *Bulletin of the National Research Centre*, *43*(1): 25.
2. Adams, D.G., B. Bergman, S.A. Nierzwicki-Bauer, A. N. Rai, and A. Schüßler, (2006). Cyanobacterial-Plant Symbioses. In *The Prokaryotes* (pp. 331–363). Springer New York.
3. Anjum, N. and R. Chandra (2015). *Article in Asian Journal of Pharmaceutical and Clinical Research*, *8*.
4. Banach, A., A. Kuźniar, R. Mencfel, and A. Wolińska, (2019). *Applied Sciences*, *9*(10), 2143.
5. Banach, A. M., K. Banach, and Z. Stępniewska (2012). *In Acta Agrophysica 19*(2):
6. Banach, A. M., A. Kuźniar, J. Grządziel, and A. Wolińska (2020). *PLOS ONE*, *15*(5): e0232699.
7. Barac, T., S. Taghavi, B. Borremans, A. Provoost, L. Oeyen, J. V. Colpaert, J. Vangronsveld and D. van der Lelie (2004). *Nature Biotechnology*, *22*(5): 583–588
8. Basumatary, B., D. Das, B. N. Choudhury, P. Dutta, and A. Bhattacharyya, (2021).

*Journal of Nematology*, *53*(1): 1–16.
9. Bolivar-Anillo, H.J., V.E. González-Rodríguez, J. M. Cantoral, D. García-Sánchez, I. G. Collado, and C. Garrido, (2021). *Biology*, *10*(6): 492.
10. Carrapiço, F. (1991). Are bacteria the third partner of the *Azolla-Anabaena* symbiosis? In *Nitrogen Fixation* (pp. 453–456). Springer Netherlands.
11. Carrapico, F. (2017). The Azolla–Anabaena–Bacteria Association: A Case of Symbiotic Abduction? In *Algal and Cyanobacteria Symbioses* (pp. 329–345). WORLD SCIENTIFIC (EUROPE).
12. Cheng, W., J. Yang, Q. Nie, D. Huang, C. Yu, L. Zheng, M. Cai, L.S. Thomashow, D. M. Weller, Z. Yu, and J. Zhang, (2017). *Scientific Reports*, *7*(1): 16213.
13. Compant, S., B. Duffy, J. Nowak, C. Cleìment and E.A. Barka (2005). *Applied and Environmental Microbiology*, *71*(9): 4951–4959.
14. El-Deeb, B., K. Fayez, and Y. Gherbawy, (2013). *Journal of Plant Interactions*, *8*(1): 56–64.
15. Garmana, A. N., E. Y. Sukandar, and I. Fidrianny, (2014). *Procedia Chemistry*, *13*: 164–169.
16. Gates, J.E., R.W. Fisher and R.A. Candler (1980). *Archives of Microbiology*, *127*(2): 163–165.
17. Gollery, M. (2005). Bioinformatics: Sequence and Genome Analysis, 2nd ed. David W. Mount. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2004, 692 pp., $75.00, paperback. ISBN 0-87969-712-1. *Clinical Chemistry*, *51*(11): 2219–2219.
18. Hardoim, P. R., L. S. Van Overbeek, G. Berg, A. M. Pirttilä, S. Compant, A. Campisano, M. Döring, and A. Sessitsch, (2015). *Microbiology and Molecular Biology Reviews*, *79*(3): 293–320.
19. He, Z., D. Kisla, L. Zhang, C. Yuan, K.B. Green-Church, and A. E. Yousef, (2007). *Applied and Environmental Microbiology*, *73*(1): 168–178.
20. Holl, F. (1988). *Soil Biology and Biochemistry*, *20*(1): 19–24.
21. Karthik, M., P. Pushpakanth, R. Krishnamoorthy, and M. Senthilkumar, (2017). *The Journal of Horticultural Science and Biotechnology*, *92*(6): 568–576.
22. Khan, A. L., R. Shahzad, A. Al-Harrasi, and I.-J. Lee, (2017). *Endophytic Microbes: A Resource for Producing Extracellular Enzymes* (pp. 95–110).
23. Khan, Z., S. G. Kim, Y. H. Jeon, H. U. Khan, S. H. Son, and Y. H. Kim, (2008). *Bioresource Technology*, *99*(8): 3016–3023.
24. Khanna, K., V. L. Jamwal, S. K. Kohli, S. G. Gandhi, P. Ohri, R. Bhardwaj, L. Wijaya, M. N. Alyemeni, and P. Ahmad, (2019). *Plant and Soil*, *436*(1–2): 325–345.
25. Lal, S., and S. Tabacchioni, (2009). *Indian Journal of Microbiology*, *49*(1): 2–10.
26. Langendries, S. and S. Goormachtig (2021). *Environmental Microbiology*, *23*(10): 5659–5669.
27. Lechno-Yossef, S., and Nierzwicki-Bauer, S.A. (n.d.-a). *Azolla-Anabaena* Symbiosis. In *Cyanobacteria in Symbiosis* (pp. 153–178). Kluwer Academic Publishers.
28. Lee, S., M. Flores-Encarnacion, M. Contreras-Zentella, L. Garcia-Flores, J.E. Escamilla and C. Kennedy (2004). *Journal of Bacteriology*, *186*(16): 5384–5391.
29. Lindberg, T., and U. Granhall, (1984). *Applied and Environmental Microbiology*,

*48*(4): 683–689.

30. Lodewyckx, C., J. Vangronsveld, F. Porteous, E. R. B. Moore, S. Taghavi, M. Mezgeay, and D. Van der Lelie, (2002). *Critical Reviews in Plant Sciences*, *21*(6): 583–606.
31. Lopes, R., S. Tsui, P. J. R. O. Gonçalves,, and M. V. de Queiroz, (2018). *World Journal of Microbiology and Biotechnology*, *34*(7): 94.
32. M. Grilli Caiola •, C. Forni*, & M. Castagnola••. (1988). *Symbiosis, Balaban Publishers, Rehovot/Philadelphia, 5*: 185–198.
33. N Saitou and M Nei. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*.
34. Nair, D. N., and S. Padmavathy, (2014). *The Scientific World Journal*, 1–11.
35. Nierzwicki-Bauer, S. A., and H. Aulfinger, (1990). *Current Microbiology*, *21*(2): 123–129.
36. Nierzwicki-Bauer, S. A., and H. Aulfinger, (1991). *Applied and Environmental Microbiology*, *57*(12): 3629–3636.
37. Peters, G. A., and J. C. Meeks, (1989). *Annual Review of Plant Physiology and Plant Molecular Biology*, *40*(1): 193–210.
38. Petersen, D. J., M. Srinivasan, and C. P. Chanway, (1996). *FEMS Microbiology Letters*, *142*(2–3): 271–276.
39. Plazinski, J. (1990). *FEMS Microbiology Letters*, *70*(1): 55–59.
40. Puente, M. E., C. Y. Li, and Y. Bashan, (2009). *Environmental and Experimental Botany*, *66*(3): 402–408.
41. Radhakrishnan, R., A. Hashem, and E. F. Abd_Allah (2017). *Frontiers in Physiology*, *8*.
42. Rashid, S., T. C. Charles, and B. R. Glick, (2012). *Applied Soil Ecology*, *61*: 217–224.
43. Rocha, F. Y. O., C. M. Oliveira, P. R. A. de, da Silva, L. H. V. Melo, de, Carmo, M. G. F. do, and J. I. Baldani, (2017). *Applied Soil Ecology*, *120*: 8–19.
44. Santoyo, G., G. Moreno-Hagelsieb, Ma. del Carmen Orozco-Mosqueda, and B. R. Glick, (2016). *Microbiological Research*, *183*: 92–99.
45. Si-Ping, Z., C. Bin, G. Xiong, and Z. Wei-Wen, (2008). *Chinese Journal of Agricultural Biotechnology*, *5*(3): 269–276.
46. Son, S. H., Z. Khan, S. G. Kim, and Y. H. Kim, (2009). *Journal of Applied Microbiology*, *107*(2): 524–532.
47. Sorokan, A., E. Cherepanova, G. Burkhanova, S. Veselova, S. Rumyantsev, V. Alekseev, I. Mardanshin, E. Sarvarova, R. Khairullin, G. Benkovskaya and I. Maksimov, (2020). *Frontiers in Microbiology*, *11*.
48. Strobel, G. A. (2002). *Critical Reviews in Biotechnology*, *22*(4): 315–333.
49. Strobel, G., B. Daisy, U. Castillo, and J. Harper, (2004). *Journal of Natural Products*, *67*(2): 257–268.
50. Tamura, K., M. Nei and S. Kumar (2004). *Proceedings of the National Academy of Sciences*, *101*(30): 11030–11035.
51. Turner, T. R., E. K. James and P. S. Poole, (2013). *Genome Biology*, *14*(6): 209.
52. Van Aken, B., C. M. Peres, S. L. Doty, J. M. Yoon, and J. L. Schnoor, (2004). *International Journal of Systematic and Evolutionary Microbiology*, *54*(4): 1191–1196.
53. Vasileva, E. N., G. A. Akhtemova, A. M. Afonin, Yu. Borisov, A., I. A. Tikhonovich, and V. A. Zhukov, (2020). *Ecological*

*Genetics*, *18*(2): 169–184.

54. Verma, S. (2001). *Journal of Biotechnology*, *91*(2–3): 127–141.
55. Wakelin, S. A., R. A. Warren, P.R. Harvey and M. H. Ryder, (2004). *Biology and Fertility of Soils*, *40*(1): 36–43.
56. Weselowski, B., N. Nathoo, A.W. Eastman, J. MacDonald, and Z.-C. Yuan, (2016). *BMC Microbiology*, *16*(1): 244.
57. Zhang, Z., S. Schwartz, L. Wagner, and W. Miller, (2000). *Journal of Computational Biology*, *7*(1–2): 203–214.
58. Zheng, W. W., M. Nilsson, B. Bergman, and U. Rasmussen, (1999). *Theoretical and Applied Genetics*, *99*(7–8): 1187–1193.
59. Zinniel, D. K., P. Lambrecht, N. B. Harris,, Z. Feng, D. Kuczmarski, P. Higley, C. A. Ishimaru, A. Arunakumari, R. G. Barletta, and A. K. Vidaver, (2002). *Applied and Environmental Microbiology*, *68*(5): 2198–2208.