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Research Article

Genetic Identification of Salt-Tolerant Rhizobium Strain Isolated from *Faba bean*

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Abstract:

Soil salinity is an environmental concern for global agriculture, it is a limited factor for symbiosis and legume growth and yield. Inoculation with salt-tolerant Rhizobium mitigate significantly the effect of salt on symbiosis and plant growth. Rhizobium is part of complex microbiomes that exist endophytically in root nodules of leguminous plants with high host specification. In this study, the aim is to isolate salt-tolerant Rhizobium strain and molecular identification. We isolated Rhizobium strains from *Vicia faba* that were treated with salt soil extract and irrigated with NaCl (100 mM). The isolate was tested on different concentrations of NaCl (100 and 150 mM NaCl). Rhizobium isolate that is tolerant to salinity identified with molecular technique. The 16S ribosomal RNA gene sequence alignment showed that the strain isolated bacteria is *Rhizobium rosettiformans* with a similar rate of 97.27%. Such Rhizobium strains could be useful in stimulating good bean genotype cultivation in moderately saline soils.

1. Introduction

The use of beneficial rhizobacteria (PGPR) in alleviating salinity stress and enhancing plant tolerance and protection has been extensively investigated (Qin et al., 2016). This kind of biological management is inexpensive, environmentally friendly, and fast-acting on plant growth. According to (Nabti et al., 2015), this method offers a great deal of potential for growing plants in saline-alkaline soil.

In stressful conditions, salt-tolerant bacteria (such as *Rhizobium, Azospirillum, Pseudomonas, Flavobacterium, Arthrobacter*, and *Bacillus*) increase agricultural productivity (Almaghrabi et al., 2014). In addition to their characteristics that encourage plant growth, bacteria isolated from inside plants that are stressed by salt show distinct coping mechanisms. They may therefore help to reduce stress (Shrivastava and Kumar, 2015). Osmoprotectant buildup in the cytoplasm is one of these tactics (Gontia-Mishra and Sharma, 2012). The phenomena mentioned above showcase the ability of endophytic bacteria to mitigate salt stress by generating exo-polysaccharides, which impede plant sodium adsorption (Milošević et al., 2012) as well as through auxin synthesis (Yaish et al. 2015).

Albert Bernhard Frank, a German botanist, initially described the Rhizobium bacterium in 1889 (Hassen et al., 2020). According to (Hansen et al, 2020), these bacteria are a component of the intricate microbiomes that endophytically reside in the root nodules of leguminous plants. Through processes aided by the nitrogenase enzyme, symbiotic *Rhizobium* species found in leguminous plant root nodule symbioses endophytically fix molecule-level nitrogen (N2) (Flores-Tinoco et al., 2020).

Rhizobium rosettiformans bacterial aerobic, motile, rod-shaped, gram-negative bacteria (0.6–0.761.3–1.5 mm) and rosette-forming. The genomic size DNA G + C content *Rhizobium rosettiformans* 62.3 mol% (Kaur et al., 2011). Colonies are cream-colored, round with full margins, and, when grown on, 0.5–1.2 mm in diameter YEM agar at 28 uC for 48 h. The existence of just one flagellum is revealed by electron transmission microscopy. robust expansion on TSA but sluggish growth on NA grows at 25–40 °C, pH 5–9 and 0–3 % (w/v) NaCl. Optimum conditions are 28 °C, pH 7.0, and 1.0 % NaCl (Kaur et al., 2011).

It was discovered that the Rhizobia strain *of Peteryoungia rosettifformans* was a natural production of an exopolysaccharide (RhrBR46) connected to glucuronan (Christophe et al., 2023). The medium-weight poly-glucuronic acid (1.85 × 105 Da) produced by a *Peteryoungia rosettiformans* strain has been identified. After being obtained at a hexachlorocyclohexane dump site in Lucknow, India, in the past (Kaur et al., 2011), it was recently reclassified under the unique genus *Peteryoungia* based on Rhizobiaceae phylogenomics investigations (Rahi et al., 2021).

The study's objective is to obtain salt-tolerant Rhizobium strains from Faba beans by isolating microorganisms from saline soils. These strains may help promote the development of high-quality genotypes of legumes in moderately salinized soils.

2. Materials and Methods

2.1. Experimental design and extract

Vicia faba seeds were planted in pit moss and treated with salt soil extract (The salt clay was brought from Hamaul village, Kafr El-Sheikh, Egypt. One hundred grams of salt soil were added to one liter of distilled water and placed on a stirrer for 15 minutes. 50 ml of soil suspension was added to each pot). After seedlings appeared, they were treated with 100 mM NaCl. After six weeks from planting, nodules from the root were collected.

2.2. Isolation of Rhizobium from *Vicia faba* plants with saline soil

Vicia Faba plants were uprooted carefully to get intact. These were brought into the laboratory without any delay. Healthy nodules with pink colure were collected. Isolation of rhizobium was achieved on yeast extract mannitol agar media (YEMA) as described by (Rajendran et al., 2008). Rhizobial isolates were collected from the root nodules of faba bean plants. In this, healthy, unbroken, firm, and pink nodules were selected for the isolation. Nodules were surface sterile in 3% NaOCl for 4 min, rinsed five times in sterile distilled water, and cursed in a drop of sterile water on a sterilized petri dish. A loopful of the cursed nodule was streaked on yeast extract mannitol agar YEMA medium contained 10 gL-1mannitol, 1 gL-1 yeast extract, 0.5 gL-1 K_2HPO_4, 0.2 gL^{-1} magnesium sulfate ($MgSO_4$), 0.1 g L_{-1} sodium chloride (NaCl), 18 g L^{-1} agar, and 0.025 gL^{-1}congo red, 100 and 150 mM NaCl, with a pH of 7 (Vincent, 1970). Congo red pigment was added to the medium to ensure the purity of growth; then, the plates were incubated at 28 °C for three days.

2.3. Influence different concentrations of salt on Rhizobium isolate

The growth isolate was tested on different concentrations of salt. They inoculated on yeast extract mannitol agar medium containing no salt as control, 100 mM NaCl, and 150 mM NaCl. After 24 hours, the growth was recorded on the different plates.

2.4. Extraction of DNA, PCR amplification, and 16S rRNA gene sequencing:

DNA of the bacterial isolates was extracted by the sarkosyl method (Maniatis et al., 1982). On a 0.8% agarose gel, the quantity and quality of the extracted DNA were evaluated. The PCR reaction mixture following instructions supporting MyTaq Red Mix, 2x.BIOLINE. The PCR amplification of the 16S rRNA gene was accomplished using a thermal cycler (Applied Biosystems 2720 ABI, Foster City, USA). For 16S rRNA gene amplification, forward primer (27F:5′- AGAGTTTGATCMTGGCTCAG-3′) and reverse primer 1492R:5′- TACGGYTACCTTGTTACGACTT -3 were used (Frank et al., 2008). The PCR amplification condition was initial denaturation for 3 min at 95°C, then 35 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 53°C, extension for 90 s at 72°C, followed by a final extension step for 7 min at 72 °C. The agarose gel (1 %) stained with RedSafe DNA stain was used to resolve PCR products. QIAquick PCR purification kit (QIAgen Inc., USA) was used to purify PCR products following the manufacturer's protocol. Purified PCR products were sequenced with forward 16S rRNA primer using Macrogene, Inc. (Seoul, South Korea). The partial 16S rDNA gene sequences of 16S rRNA were BLAST searched with the NCBI database (Altschul et al., 1990).

3. Results

Screening the isolates of bacteria on the different concentrations of salt

Figure (1) shows the effect of salinity on the percentage of bacterial growth. Salinity reduced the growth of bacteria by increasing the salt from 100 to 150 mM. Table (1) shows the percentage and time for the growth of the rhizobia isolates. The isolate showed normal growth with a percentage of 100% after 24 hours of culture. NaCl (100 mM) showed growth with a percentage of 75% after 72 hours. NaCl with 150 mM showed growth with a percentage of 50% after 72 hours.

Molecular identification of Rhizobium isolate based on 16sRNA

Table (2) shows the sequence of rhizobium isolate. Alignment of these sequences with the sequence for the other identified species was done as shown in Figure (2). The definition and similarity of rhizobium are shown in Table (3).

With the use of the 16 sRNA primers, the bacterial 16 s region was amplified from the genomic DNA. PCR yielded a product with ~971 bp. A sequence search was applied utilizing the BLAST standard nucleotide-nucleotide basic local alignment search tool to identify the bacterial strain from 16s sequencing. To validate the initial identification of the tested sequence, the homology should be more than 95% with the referenced culture.

The outcomes from alignment exhibited similarity recorded 97.27% with the reference strain *Rhizobium rosettiformans* (Table 3, Fig 2).

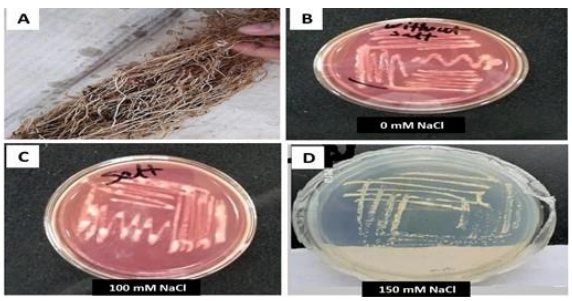

Figure (1) shows the effect of NaCl on the growth of isolates, the nodules of the root, (B) the growth of isolate on the plate without salt, (C) the growth of isolate on the plate with 100 mM NaCl, (D) the growth of isolate on the plate with 150 mM NaCl.

Table1. The effect of different concentrations of salt on the growth of isolates on YEM medium.

Salt Concentrations	Growth time			Growth Concentration%
	24	48	72	
No Salt	✓	✓	✓	100
100mMNaCl	×	✓	✓	75
150mMNaCl	×	×	✓	50

Table 2. Definition and Sequence of Rhizobium isolate.

>H221228-001_A24_R1_F.ab1 971

TCTGTGAATGGG-
GAGCTTACACATGCAAGTCGAGCGCCCCG-
CAAGGGGAGCGGCAGAC-
GGGTGAGTAACGCGTGGGAATCTACCGTG
CCCTACGGAATAGCTCCGGGAAACTG-
GAATTAATACCGTATACGCCCTACGGGG-
GAAAGAT-
TTATCGGGGTATGATGAGCCCGCGTTGGA
TTAGCTAGTTGGTGGGGTAAAGGCCTAC-
CAAGGCGACGATCCATATCTGGTCTGA-
GAG-
GATGATCAGCCACATTGGGACTGAGACAC
GGCCCAAACTCCTACGGGAGGCAGCAG-
TGGGGAATATTGGACAATGGGCGCAA-
GCCTGATCCAGCCATGCCGCGTGAGTGAT
GAAGGTCTTAG-
GATTGTTTAGCTCTTTCACCGGTGAA-
TATAAGGTCGTGTAACCTGATAA-
GAACCCTTGTTGTCATCGCTGCTCACTTGC
TCGTTGAGTT-
GTGTGGGGCTCCCTTTTTCTGGTGTCC-
TATCTGTACGCGTTACGTGGTT-
GATGTTTTTTGTCCGGGTGGTTATCTGGCT
TTACATTCTGTGTGGTCACTCTTTT-
GTCCTTTGTTGTTGTGCTGTT-
GGCGGTTACTTGTT-
GTTGTGTTCTGAGTGGTTGGTATATGTAGT
TTGTGGGTGTCTGTTTTTGCTTTT-
GTGCTGTGTGGCTTGTCTCCTGCTTTTGTT-
GTTGTT-
GGATCTTATTGGTTTATTTTGGTGGGTATT
TTTGGTTGGTTTTATTTGTACTGTGTTTTTT-
GCTCCTTTATGGTTTTTGTGTTTGTT-
GGGTT-
GGGTTTGTGTCTTTCTTTTTTATGGTTTTTT
GTTCTGGTTTTATGGCAGTATTGTGTTT-
GTTGTGTATGCCCTGTGTTTGTTTTGTT-
GATTT-
GATCTTATTTGTCTTGTGCGTTGTGTTGCT
GTATTTGTTCTTTGGTATATTTCTTGTGTT-
GTTTTTGTTTATCTCTGGCTTTGTTT-
GTGTCGTATTATTGTGTTGTCGGTCT

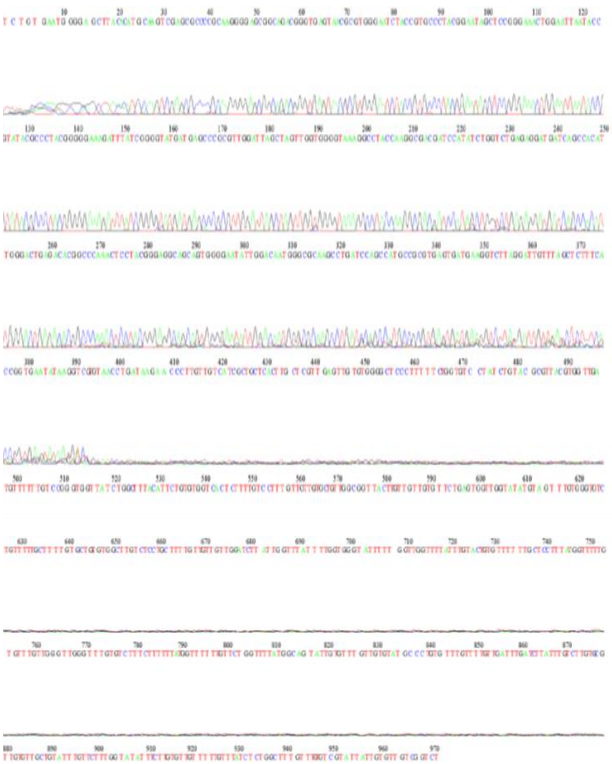

Figure 2: The degree of similarity of the isolated stain with other strains.

Table 3: Definition and similarity of bacterial strain

Sample name	Nearest match	Similarity	Accession No.
R1-F	*Rhizobium rosettiformans*	97.27%	MZ276328.1

4. Discussion

While the majority of rhizobia are host-specific, it is also true that multiple bacterial species can be isolated from a single legume species, and only a small number of hosts have been studied in terms of microsymbionts (Arora et al., 2001). The two families of these rhizobia are distinguished by their respective rates of growth. Rhizobia are classified into two groups: those that grow quickly and those that develop slowly (Lohis and Hansen, 1921).

In selective broth media, the mean generation time for slow-growing bacteria is larger than 6 hours, while that of fast-growing bacteria is less than 6 hours (Elkan, 1992). According to (Deshwal et al., 2003), Arachishypogaea L and slow- and fast-growing rhizobia are related. Both Rhizobium and Agrobacterium belong to the Rhizobiaceae family under the Eubacterial order. According to (Fred et al., 2002), Agrobacterium spp. colonies on the YEMA medium can be mistaken for fast-growing Rhizobium species. Agrobacterium absorbs YEMA medium containing congo red (1:400), according to (Allen and Allen, 1950). Conversely, when given any combination of arabinose, galactose, glucose, mannose, or xylose (pH 5-8),

Bradyrhizobium rapidly utilized hexose (galactose, gluconate, glucose, and mannose) and was able to reduce the pH (Padmanabhan et al., 1990). Biochemical tests provide the basis for characterizing Rhizobia. *Rhizobium joponicum* syn. and *Bradyrhizobium japonicum* were isolated from root nodules of soybean (*Glycine max* L.) on YEMA medium, and their morphological, cultural, and biochemical features were examined (Gachande and Khansole, 2011).

In addition to being essential for active nitrogen fixation, the symbiotic relationship between leguminous plants and rhizobia is also important for the production of crops. Improving nitrogen availability in sustainable agriculture production systems requires co-inoculation of rhizobia with plant growth-promoting bacteria (PGPB) to enhance legume nitrogen fixation. Numerous rhizobacteria have been shown to either directly or indirectly enhance plant growth by producing plant growth regulators and improving nutrient uptake (Kloepper, 1992; Glick, 1995) or by producing metabolites such as antibiotics, siderophores, and other compounds that inhibit the growth of phytopathogens (Glick, 1995). Additionally, PGPB promotes the growth of legumes, and certain strains improve nodulation and nitrogen fixation by altering the interactions between rhizobia and plants (Parmar and Dadarwal, 1999). The majority of these bacteria that help with nodules are free-living rhizobacteria, however some might be endophytic. Endophytic bacteria live inside their hosts' tissues, either intracellularly or intercellularly (Sturz et al., 2000). As a result, they may benefit from protection from external factors and competition from other microorganisms (Kobayashi and Palumbo, 2000).

In plants that do not develop root-nodule symbioses, such as sugarcane and spruce, *R. rosettiformans* is involved in root-associated nitrogen fixation (Burbano et al., 2011). According to reports, *Rhizobium rosettiformans* can help plants develop in both normal and drought-prone environments (Afzal et al., 2019). Results showed that Rhizobium strains have the potential to grow in a salt medium and these agree with another reference that showed that some *Rhizobium* strains can produce auxin and improve the growth of mung beans under drought-stressed conditions (Tanveer & Ali, 2022).

Surprisingly, *R. rosettiformans*, which is involved in root-associated nitrogen fixation with sugarcane and spruce, was also found to have transcripts of this phylotype in Norway spruce roots that were sampled from a German forest habitat. The occurrence of a unique clade of *nif*H transcript sequences in spruce and sugarcane, which is widely dispersed, suggests a close relationship between the corresponding nitrogen-fixing bacteria and their host. Unexpectedly, *R. rosettiformans* has a majority of *nif*H expression in the roots of sugarcane and spruce, as *nif*H transcription in non-host conditions appears to be uncommon within the family Rhizobiales. Since *R. rosettiformans* did not exhibit any of the nodulation genes (*nod*A, *nod*C, and *nod*D) (Kaur et al., 2010), given that sugarcane and spruce lack symbiotic structures, likely, *R. rosettiformans'* association with these plants does not depend on nod genes. The phylotype that is most closely related to the incomplete nifH sequence from a grown bacterium is represented by the predominant rhizobial nifH fragment found in all samples. This isolate, which came from an Indian landfill, was recently named *Rhizobium rosettiformans*, a new species in the genus Rhizobium (Kaur et al., 2010).

5. Conclusions

The strategy of benefiting from isolated microorganisms from saline soils (Such as our isolated Rhizobium strain) and using them, could be useful in stimulating good legume genotype cultivation in moderately saline soils.

6. References

Afzal, I., Shinwari, Z. K., Sikandar, S., and Shahzad, S. (2019). Plant beneficial endophytic bacteria: Mechanisms, diversity, host range, and genetic determinants. Microbiological research, 221, 36-49.

Allen, E. K., and Allen, O. N. (1950). Biochemical and symbiotic properties of the rhizobia. Bacteriological reviews, 14(4), 273-330.

Almaghrabi, O. A., Abdelmoneim, T. S., Albishri, H. M., and Moussa, T. A. (2014). Enhancement of maize growth using some plant growth promoting rhizobacteria (PGPR) under laboratory conditions. Life Sci J, 11(11), 764-772.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. Journal of molecular biology, 215(3), 403-410.

Arora, N. K., Kang, S. C., and Maheshwari, D. K. (2001). Isolation of siderophore-producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. Current Science, 673-677.

Burbano, C. S., Liu, Y., Rösner, K. L., Reis, V. M., Caballero‐Mellado, J., Reinhold‐Hurek, B., and Hurek, T. (2011). Predominant nifH transcript phylotypes related to *Rhizobium rosettiformans* in field‐grown sugarcane plants and in Norway spruce. Environmental microbiology reports, 3(3), 383-389.

Christophe, G., Hou, X., Petit, E., Traikia, M., Le Cerf, D., Rihouey, C., ... and Dubessay, P. (2023). Description of the Wild Strain *Rhizobium rosettiformans* DSM26376, Reclassified under Peteryoungia rosettiformans comb. nov., for Producing Glucuronan. Polymers, 15(9), 2177.

Deshwal, V. K., Pandey, P., Kang, S. C., and Maheshwari, D. K. (2003). Rhizobia as a biological control agent against soil borne plant pathogenic fungi.

Elkan, G. H. (1992). Taxonomy of the rhizobia. Canadian Journal of Microbiology, 38(6), 446-450.

Flores‐Tinoco, C. E., Tschan, F., Fuhrer, T., Margot,

C., Sauer, U., Christen, M., and Christen, B. (2020). Co‐catabolism of arginine and succinate drives symbiotic nitrogen fixation. Molecular systems biology, 16(6), e9419.
Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A., and Olsen, G. J.(2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Applied and environmental microbiology, 74(8), 2461-2470.
Fred, E. B., Baldwin, I. L., and McCoy, E.(2002). Root nodule bacteria and leguminous plants (No. 5). UW-Madison Libraries Parallel Press.
Gachande, B. D., and Khansole, G. S. (2011). Morphological, cultural and biochemical characteristics of *Rhizobium japonicum* syn and *Bradyrhizobium japonicum* of soybean Bioscience Discovery Journal, 2(1), 1-4.

Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. Canadian journal of microbiology, 41(2), 109-117.
Gontia-Mishra, I., & Sharma, A. (2012). Exogenously supplied osmoprotectants confer enhanced salinity tolerance in rhizobacteria.Journal of Ecobiotechnology, 4(1), 11-13.

Hansen, B. L., Pessotti, R. D. C., Fischer, M. S., Collins, A., El-Hifnawi, L., Liu, M. D., and Traxler, M. F. (2020). Cooperation, competition, and specialized metabolism in a simplified root nodule microbiome. MBio, 11(4), 10-1128.
Hassen, A. I., Lamprecht, S. C., and Bopape, F. L. (2020). Emergence of β-rhizobia as new root nodulating bacteria in legumes and current status of the legume–rhizobium host specificity dogma. World Journal of Microbiology and Biotechnology, 36, 1-13.
Kaur, J., Verma, M., and Lal, R. (2011). *Rhizobium rosettiformans* sp. nov., isolated from a hexachlorocyclohexane dump site, and reclassification of *Blastobacter aggregatus* Hirsch and Müller 1986 as *Rhizobium aggregatum* comb. nov. International journal of systematic and evolutionary microbiology, 61(5), 1218-1225.
Kaur, J., Verma, M., and Lal, R. (2010) *Rhizobium rosettiformans* sp. nov., isolated from hexachlorocyclohexane (HCH) dump site in India, and reclassification of *Blastobacter aggregatus* Hirsch and Muller (1985) as *Rhizobium aggregatum* comb. nov. Int J Syst Evol Microbiol.
Kloepper, J. W. (1992). Plant growth-promoting rhizobacteria as biological control agents. Soil microbial ecology: applications in agricultural and environmental management., 255-274.
Kobayashi, D. Y., and Palumbo, J. D. (2000). Bacterial Endophytes and Their. Microbial endophytes, 2000, 99-233.
Löhis, F., and Hansen, R. (1921). Nodulating bacteria of leguminous plant. J. Agric. Res, 20, 543-556.
Maniatis, T., Fritsch, E.F., & Sambrook, J. (1982). Molecular Cloning A Laboratory Manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N. Y.
Milošević, N. A., Marinković, J. B., and Tintor, B. B. (2012). Mitigating abiotic stress in crop plants by microorganisms. Zbornik Matice srpske za prirodne nauke, (123), 17-26.
Nabti, E., Schmid, M., and Hartmann, A. (2015). Application of halotolerant bacteria to restore plant growth under salt stress. In Halophiles: Biodiversity and sustainable exploitation (pp.235-259). Cham: Springer International Publishing.
Padmanabhan, S., Hirtz, R. D., and Broughton, W. J. (1990). Rhizobia in tropical legumes: cultural characteristics of Bradyrhizobium and Rhizobium sp. Soil Biology and Biochemistry, 22(1), 23-28.
Parmar, N., and Dadarwal, K. R. (1999). Stimulation of nitrogen fixation and induction of flavonoid‐like compounds by rhizobacteria. Journal of applied Microbiology, 86(1), 36- 44.
Qin, Y., Druzhinina, I. S., Pan, X., and Yuan, Z. (2016). Microbially mediated plant salt tolerance and microbiome-based solutions for saline agriculture. Biotechnology Advances, 34(7), 1245-1259.
Rahi, P., Khairnar, M., Hagir, A., Narayan, A., Jain, K. R., Madamwar, D., ... and Shouche, Y. (2021). *Peteryoungia gen. nov.* with four new species combinations and description of *Peteryoungia desertarenae* sp. nov., and taxonomic revision of the genus Ciceribacter based on phylogenomics of Rhizobiaceae. Archives of Microbiology, 203, 3591-3604.
Rajendran, G., Sing, F., Desai, A. J., and Archana, G. (2008). Enhanced growth and nodulation of pigeon pea by co-inoculation of Bacillus strains with Rhizobium spp. Bioresource technology, 99(11), 4544-4550.
Shrivastava, P., and Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi journal of biological sciences, 22(2), 123-131.
Sturz, A. V., Christie, B. R., and Nowak, J. (2000). Bacterial endophytes: potential role in developing sustainable systems of crop production. Critical reviews in plant sciences, 19(1), 1-30.
Tanveer, S., and Ali, B. (2022). Evaluation of Bacillus and rhizobium strains to enhance the growth of *Vigna radiata* (L.) under drought stress. Pak-Euro Journal of Medical and Life Sciences, 5(1), 101-112.
Vincent, J. M. (1970). The cultivation, isolation and maintenance of rhizobia. A manual for the practical study of the root-nodule bacteria, 1-13.
Yaish, M. W., Antony, I., and Glick, B. R. (2015). Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (*Phoenix dactylifera* L.) and their potential role in salinity tolerance. *Antonie VanLeeuwen*hoek, 107, 1519-1532.