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ORIGINAL ARTICLE **OPEN ACCESS**

Isolation and Identification of Nitrogen Fixing Bacteria from Thoseghar Soil Samples

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ABSTRACT
Nitrogen is one of the most important nutrients. Biochemical nitrogen fixation for plant growth and development boosts agricultural production and food security.Microorganisms in the soil have a diversity of relationships some species able to fix nitrogen such as Azospirillum spp., Bacillus spp., Klebsiella spp., and Azotobacter spp. etc. Rhizobium fixes ammonia, which host legumes use as a nitrogen source, and gives Rhizobium a carbon supply. The symbiosis between the roots of higher plants and their root nodules is advantageous to both parties and crucial to the nitrogen cycle.Fertilizers can be used to maintain the fertility of such soil. Poor soil demonstrated low fertility, which has an impact on plant growth. particularly nitrogen. Through serial dilution and four-quadrant streaking on YEMA media, we were able to isolate Nitrogen fixing spp. from a soil sample from Thoseghar. Morphological characteristics and biochemical characteristics were done for microbial identification. Further, 16s rRNA was done for the confirmation of nitrogen-fixing bacteria. In this article, we concluded that the use of Nitrogen Fixing bacteria boosts plant fertility and is generally accepted as the best alternative to chemical fertilizers.
Keywords: *Nitrogen fixation, Klebsiella, Bacillus,Rhizobium, Fertilizer, YEMA.*

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INTRODUCTION:

In the metabolic system of plants, nitrogen is prominently present. Protein, of which nitrogen is a crucial component, is connected to all key functions in plants. In order to increase crop output, nitrogen application is therefore necessary and obligatory [7].Plant growth depends on nitrogen because ammonia and nitrites cannot be found as nitrogen in the environment. They are typically provided in the form of nitrogen or a mixture these genuine nitrogen cropping techniques provide nitro fertilizers, which are used in industrial products. The use of these fertilizers has had an adverse impact on both human health and the environment globally [1]. By associating symbiotically with leguminous plants and utilizing the nitrogenase enzyme some bacteria are able to fix atmospheric nitrogen into the soil and promote the growth of plants. The capacity of bacteria and cyanobacteria to fix atmospheric nitrogen for utilization by plants is constrained. Plants fix nitrogen as a result of the collaboration of these small organisms [1]. There is a well-known relationship between Rhizobium bacteria and legumes called symbiosis. Legumes that fix nitrogen help plants flourish when the soil is deficient in that mineral, and by leaving behind plant residues, they also improve the soil's nitrogen status for nearby crops [2]. For adequate nitrogen fixation, selecting an effective Nitrogen Fixing strain and improving its quality is crucial. This study's primary goal was to identify more productive strains. During the course of our research, we were able to isolate two bacterial strains that were both capable of producing a variety of growth factors and made a significant contribution to biological nitrogen fixation[3].

MATERIAL AND METHODS

Sample collection:

In the SATARA district of Thoseghar, soil samples were collected. With the aid of a spatula, two distinct soil samples were gathered from various locations in Thoseghar and stored in polythene bags. And moved to the Applied Microbiology Laboratory at Yashavantrao Chavan Institute of Science SATARA (Autonomous).

Preparation of sample and Isolation of nitrogen-fixing bacteria:

Before being examined, the sample is held at room temperature. For the purpose of isolating Nitrogen Fixing bacteria, 1 gm of soil sample (including RICE and JAMUN) was added to 100 ml distilled water. 1 ml from it added to 9 ml sterile distilled water tubes. Serial dilutions were then performed up to a factor of

ten, and 0.1 ml of each soil sample was spread on a YEMA (yeast extract mannitol agar) plate. The plate was then incubated for 24 hours. Growth was seen on distributed plates after 24 hours. Following that, a suspension was produced and streaked using the four-quadrant streaking method on YEMA (yeast extract mannitol agar) plates. Plates were incubated for 24 hours at 37 °C**.**

Morphological and Biological Characterization:

An isolated organism from various soils has a colony character that colony is pinkish-white in color with a smooth consistency, a round form, and impenetrable opacity.

Gram staining:

The microscopic field shows a Gram-negative, pink colored from the Jamun soil sample and a Gram-positive, violet color, rod shape bacteria from the rice soil sample.

Biochemical tests:

Biochemical tests were performed such as indole test, methyl red test, Voges-Proskauertest, citric acid test, urease test, catalase test, H_2S test, and various sugars such as glucose, lactose, D-mannose, xylose,sucrose, etc.are used to identify nitrogen-fixing bacteria from soil samples.

Identification of bacterial isolates by 16S rRNA:

Then, we proceed to a confirmatory test using 16S rRNA to identify isolated bacteria from soil whether isolated bacteria are able to fix nitrogen or not to identify such bacteria by using 16S rRNA sequencing.

RESULT

Nitrogen-fixing bacteria were isolated (fig.1 and 2) from soil samples (Rice and Jamun) on YEMA (Yeast extract mannitol agar). Gram nature of the isolated colonies was performed (fig. 3 and4). Biochemical tests were also performed for both colonies isolated from Rice and Jamun soil samples (table 1 and 2). With the help of 16S rRNA sequencing, isolated strain was identified as *Klebsiella variicola* and *Bacillus simplex* which are able to fix Nitrogen and increased crop productivity.

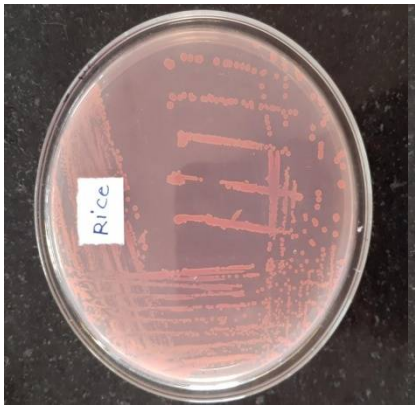
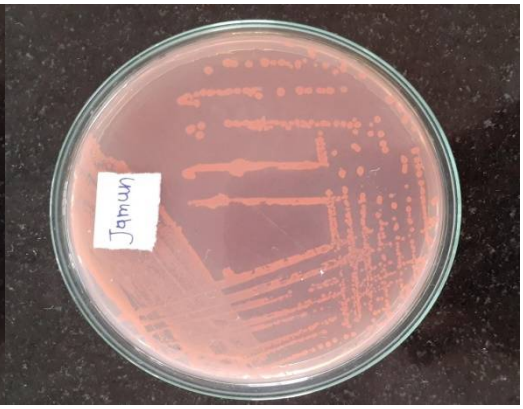


Figure 1:Isolation of bacteria from Jamun soil



Figur 2:Isolation of bacteria from Jamun soil

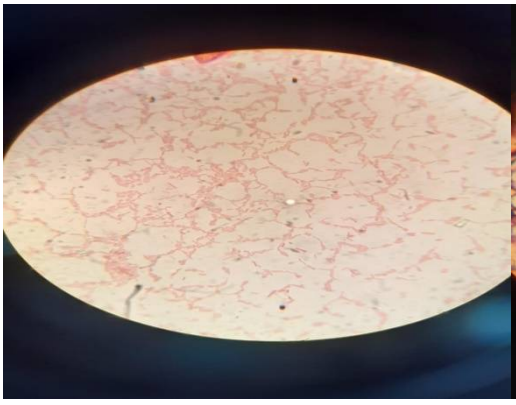


Figure 3:Gram staining of bacteria isolated from Jamun soil

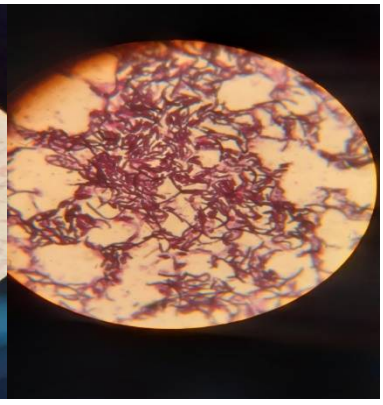


Figure 4: Gram staining of bacteria isolated from Rice soil

Sr.No	Test	Results
1	Indole	_
2	Methyl Red	+
3	Voges-Praskauer	+
4	Simmon Citrate	+
5	Oxidase	_
6	Catalase	+
7	Nitrate Reduction	+
8	H2S	_
9	Urease	+
10	Glucose	+
11	Lactose	_
12	Sucrose	+
13	Maltose	+
14	Mannitol	+
15	D-Sorbital	+
16	Fructose	+
17	Rhamnose	+

Table 1: Biochemical tests for bacterial colony isolated from Rice soil

Table 2: Biochemical tests for bacterial colony isolated from Jamun soil

Sr.No	Test	Result
1	Indole	_
2	Methyl Red	+
3	Voges-Praskauer	+
4	Simmon Citrate	_
5	Oxidase	_
6	Catalase	+
7	Nitrate Reduction	+
8	H2S	_
9	Urease	+
10	Glucose	+
11	Lactose	_
12	Sucrose	+
13	Maltose	+
14	Mannitol	+
15	D-Sorbital	_
16	Fructose	+
17	Rhamnose	_

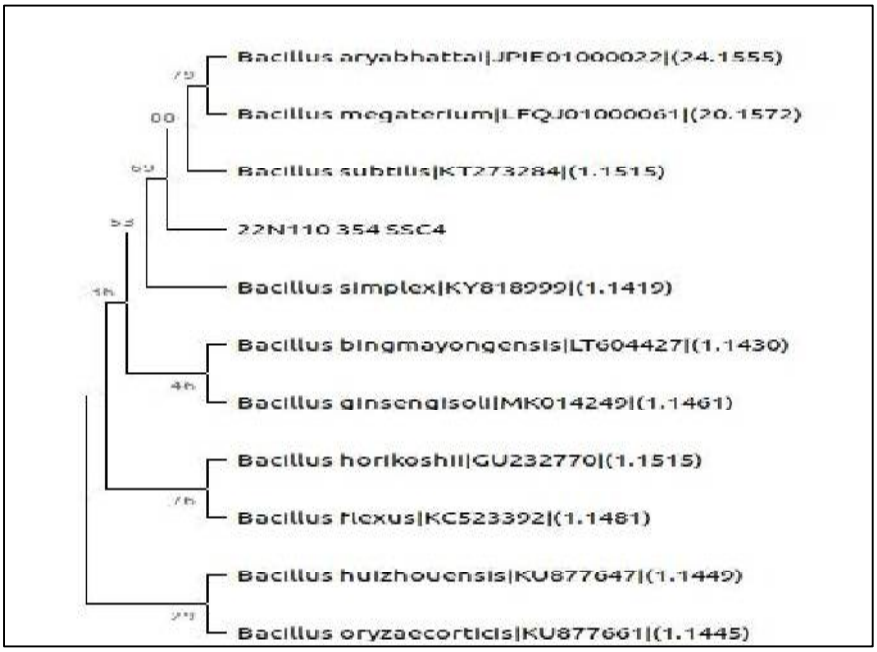


Figure 5: Phylogenetic Tree of identified bacteria isolated from rice soil (*Klebsiella variicola*)

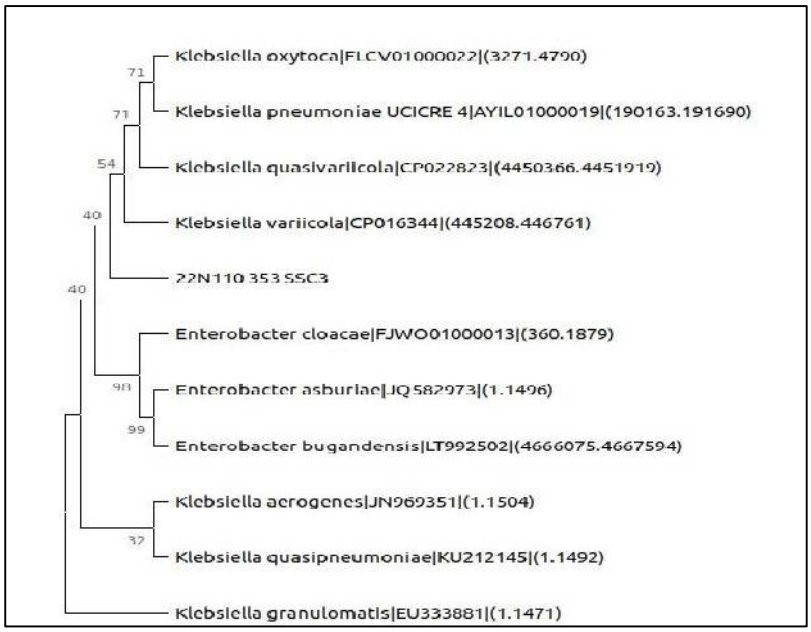


Figure 6:Phylogenetic Tree of identified bacteria isolated from rice soil (*Bacillus simplex*)

DISCUSSION

As environmental changes influenced by the people, where it has been found sustainable environment friendly alternatives. As we developed a new way for leading towards agricultural aspects. We were

enriched nitrogen fixing bacteria from the land of Thoseghar, it is land of varieties of soil sample respectively. In some studies nitrogen fixing bacteria in kind of reactors includes low nitrogen waste water. However, we isolated two strains which are capable for nitrogen fixation technology at the industrial revolution [8].In contrast to clinical isolates, the *K.variicola* environmental isolates consistently exhibit low expected pathogenicity [10]. The genome analysis supported and confirmed the screening in-vitro results, showing that *K.variicola* UC4115 functioned as PGPR and that the strain actually contained many of the signature genes that are functionally related to the traits that promote plant growth[12].The nifH gene's role in these heterotrophic Bacillus strains' molecular basis for nitrogen fixation has been investigated. and found that the heterotrophic Bacillus sp. may be capable of fixing nitrogen [11]. When the right energy source is available along with the ideal temperature, pH, and controlled O_2 concentration, nitrogen fixation is a biological process that is well defined and understood, at least to some extent, in pure culture and in vitro. Nevertheless, it is difficult to conclusively demonstrate that a particular strain is to blame for some of the nitrogen ingested by plants, especially in field settings [9].Above isolates *Bacillus simplex* and *Klebsiella variicola* are able to fix atmospheric nitrogen which boost plant growth and development for better yield.

CONCLUSION

In agriculture, nitrogen-fixing bacteria are crucial because they make nitrogen available to plants for improved growth and responsiveness. As far as we are aware, bacteria like *Klebsiella variicola* and *Bacillus simplex* have a role in promoting plant growth and making nutrients available from soil. Foods and fibres are in demand in agriculture now as a result of the green revolution. Its main focus is on nitrogen-fixing microorganisms in order to increase productivity

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Uddin, M. R., Tasmin, M. F., Hossin, M. S., Rashad, M. M., Hoque, M. F., & Islam, M. K. (2020). Isolation and identification of Rhizobium from non-saline coastal soils of Bangladesh and preparation of mother culture. Asian Journal of Medical and Biological Research, 6(2), 222-228. doi:10.3329/ajmbr.v6i2.48053.
2. Islam, Rabiul & Mosaib, Md. Golam & Alam, Md & Uddin, Md & Imran, Sayeed & Mahmud, Shahriar & Sohana, Shaharuq. (2020). Isolation, Identification and Characterization of Rhizobium species from soil of Cicer arietinum field of faridpur in Bangladesh.. International Journal of Current Research. 12. 10322-10325. 10.24941/ijcr.38456.04.2020.
3. Setu, L. J., Ahmmed, B., & Kibria, K. Q. (2019). Identification and characterization of Rhizobium bacteria. International Journal of Research and Analytical Reviews, 6(2). doi:10.1729/Journal.22212.
4. 4.Dhiman, M., Dhiman, V. K., Rana, N., & Dipta, B. (2019). Isolation and characterization of Rhizobium associated with root nodules of Dalbergia sissoo. International Journal of Current Microbiology and Applied Sciences, 8(03), 1910-1918. doi:10.20546/ijcmas.2019.803.227.
5. 5.Isolation and identification of Rhizobium species from root nodules of arachis hypogaea L. and Telfairia occidentalis in south-east, Nigeria. (2016). International Journal of Science and Research (IJSR), 5(6), 227-230. doi:10.21275/v5i6.nov163941.
6. 6.Beattie, G. A., & Handelsman, J. (1989). A rapid method for the isolation and identification of Rhizobium from root nodules. Journal of Microbiological Methods, 9(1), 29-33. doi:10.1016/0167-7012(89)90027-4.
7. Leghari, S.J. *et al.* (2016) “Role of nitrogen for plant Growth and Development: A Review ,” pp. 209–218.
8. Ospina-Betancourth C, Acharya K, Allen B, Entwistle J, Head IM, Sanabria J, Curtis TP. Enrichment of Nitrogen-Fixing Bacteria in a Nitrogen-Deficient Wastewater Treatment System. Environ Sci Technol. 2020 Mar 17;54(6):3539-3548. doi: 10.1021/acs.est.9b05322. Epub 2020 Mar 5. PMID: 32083474.
9. Oluwaseyi A. Ajala, Fidelis O. Ajibade, Oluwafisayo R. Oluwadipe, Nathaniel A. Nwogwu, Bashir Adelodun, Awoke Guadie, Temitope F. Ajibade, Kayode H. Lasisi, James R. Adewumi, Microbial impact on climate-smart agricultural practices, Microbiome Under Changing Climate, 10.1016/B978-0-323-90571-8.00009-2, (203-236), (2022).
10. Medina-Cordoba LK, Chande AT, Rishishwar L, Mayer LW, Valderrama-Aguirre LC, Valderrama-Aguirre A, Gaby JC, Kostka JE, Jordan IK. Genomic characterization and computational phenotyping of nitrogen-fixing bacteria isolated from Colombian sugarcane fields. Sci Rep. 2021 Apr 28;11(1):9187. doi: 10.1038/s41598-021-88380-8. PMID: 33911103; PMCID: PMC8080613.

11. 11.Yousuf J, Thajudeen J, Rahiman M, Krishnankutty S, P Alikunj A, A Abdulla MH. Nitrogen fixing potential of various heterotrophic Bacillus strains from a tropical estuary and adjacent coastal regions. J Basic Microbiol. 2017 Nov;57(11):922-932. doi: 10.1002/jobm.201700072. Epub 2017 Aug 8. PMID: 28787089.
12. 12.Lin, L., Wei, C., Chen, M. et al. Complete genome sequence of endophytic nitrogen-fixing Klebsiella variicola strain DX120E. Stand in Genomic Sci 10, 22 (2015). https://doi.org/10.1186/s40793-015-0004-2

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