IJBPAS, July, 2023, 12(7): 2985-2992

ISSN: 2277–4998

International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# ISOLATION, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF PLANT GROWTH-PROMOTING BACTERIA FROM *SPINACIA OLERACEA* GROWTH SOIL SAMPLE

**RAHINI G[1] AND SUMATHI V[2*]**

**1:** Research Scholar, Annamalai University, Chidambaram, Cuddalore

**2:** Assistant Professor, Faculty of Science, Annamalai University, Chidambaram, Cuddalore

**[*]Corresponding Author: Dr. V. Sumathi: E Mail: Researchbio23@gmail.com**

**Received 10th July 2022; Revised 15th Sept 2022; Accepted 10th Oct. 2022; Available online 1st July 2023**

https://doi.org/10.31032/IJBPAS/2023/12.7.7208

## ABSTRACT

*Spinacia oleracea L.* (child spinach) is a fairly young Amaranthaceae family leaf vegetable. The crop is high in human supplements and includes considerable quantities of bioactive mixtures such as nutrients A, C, and minerals. PGPR bacteria from *Spinacia oleracea* grow soil are isolated and biochemical characteristics are identified in this study. The parameter of soil was analysed. Genomic DNA was extracted from cultured bacteria. The isolated genomic DNA was amplified using the 16s rRNA region. The new DNA sequences were identified at the genus and species levels with the BLAST software tool (NCBI, USA) and deposited in (The National Center for Biotechnology Information) NCBI, with accession numbers acquired from Genbank. MEGA 11.0 (Molecular Evolutionary Genetics Analysis) was used to perform phylogenetic analysis on the DNA sequences that were most similar to the query sequences. The phylogenetic tree was generated based on the Neighbour-joining method.

**Keywords: *Spinacia oleracea,* Amaranthaceae, soil, 16s rRNA region, NCBI, MEGA 11.0.**

## INTRODUCTION:

Spinach (*Spinacia oleracea L.*), a yearly plant, is a green, verdant vegetable that can be harvested in the spring **[1]**. The dietary benefit of new spinach, with 91 percent dampness content, shows between 0.4 and 0.6 percent lipid content, around 2.9 percent protein content, and high levels of fundamental amino acids, aside from sulfurous amino acids (methionine) and tryptophan **[2, 3]**. Despite the fact that the carbohydrate content is extremely low (2 percent -10 percent), the fiber content is high (2.2 percent). There are numerous medical advantages associated with the use of spinach, including its lipid-lowering properties and cardiovascular protection, antiobesity effects, hypoglycemic action, mitigating effects, anticancer properties, neuronal insurance, and antimacular degeneration, among others **[4]**. Because it contains a lot of phenolic or flavonoid compounds, chlorophylls, ferulic corrosive and caffeic corrosive, quercetin, patuletin, spinacetin, and jaceidin, which are primarily found in the leaves, with the highest levels in the middle of the year **[5]** new spinach is attributed cell reinforcement properties **[6]**.

The aim of the present investigation is to collect the PGPR bacteria from *Spinacia oleracea* soil samples, and PGPR microorganisms were isolated from *Spinacia oleracea* soil samples, then to describe their morphological, biochemical, and Molecular portrayal of PGPR microbes sequencing and phylogenetic analysis.

## MATERIALS AND METHODS

### *Collection of Soil*

Soil samples were taken from *Spinacia oleracea* soil. The top 7 cm of soil was discarded during collection, and the lower soil layer beneath this was collected. Aseptically collected in a neat and clean polythene packet and then brought to the laboratory and kept in an aseptic environment for use in the future

### *Isolation of Bacteria*

Bacterial strains were isolated from soil samples using a serial dilution technique using Nutrient Agar Media (Beef extract-3g; Peptone-5g; Agar-5g; Distilled water-1000ml., Sodium chloride-5g, pH-6.8). Plates were incubated at 280 degrees Celsius for 48-72 hours, and the number of bacterial colonies developed was recorded and inoculated separately in Nutrient Agar slants, which were kept at 40 degrees Celsius for further work.

### *Characterization of bacteria*

Characterization of isolated bacteria was carried out using standard methods such as morphological studies, cultural properties, staining nature, and biochemical peculiarities. Color, elevation, and colony edge are all morphological characteristics. The Gram nature of each isolate was initially determined by staining with crystal violet and safranin. Among the biochemical tests, amylase, catalase, gelatin hydrolysis, urease, and citrate utilisation were performed using standard techniques for bacterial characterization. (This is followed by Capuccino Sherman's Microbiology-A Laboratory Manual, 7th edition).

### Characterization of plant growth-promoting Rhizobacteria (PGPR) [7-11]

**Table 1**

| PGPR Bacteria Tests | |
|---|---|
| P Solubilization | Pikovskaya (1948) |
| IAA Production | Brik *et a*l (1991) |
| Ammonia Production | Dye method (1962) |
| ACC Deaminase activity | Dworkin and Foster, 1958; Penrose and Glick, 2003 |
| HCN Production | Bakker and Schipper (1987) |

**Molecular characterization of Bacteria isolates**

A modified organism by adding the unadulterated culture in the 250 ml funnel-shaped carafes containing 100 ml of the medium, the DNA extraction strategy was used to separate DNA from growths **[12]** the microscopic organisms developed in Nutrient stock. The carafes were hatched for 24 hours at 372 ° C with shaking. An adjusted CTAB strategy for microorganisms DNA extraction technique was utilized to seclude DNA from microscopic organisms **[13]**. The sequencing response was performed with ABI large color cycle sequencing Biosystems responses (Applied Biosystems) at Eurofins Genomics, Bangalore

**Data analysis**

The BLAST programming device was used to identify the new DNA groupings (NCBI, USA). The classifications were saved in the NCBI. MEGA 7.0 (Molecular Evolutionary Genetics Analysis) was used to perform phylogenetic analysis on the DNA groups that showed the most extreme similarity to question successions. The phylogenetic tree was constructed using the Neighbor-Joining method.

## RESULTS AND DISCUSSION:

In this study, PGPR bacteria isolated from *Spinacia oleracea* soil were enumerated. Morphological, biochemical, Molecular and PGPR characteristics were determined using various techniques. The bacterial isolates were tested for colony morphology and biochemical properties using the standard methods described in Cappuccino Sherman's "Microbiology- A Laboratory Manual" (7th edition). The PGPR tests were performed using standard methods, as shown in **Table 1**, **Table 2** shows the appearance of yellow coloration, bubble production, gelatin liquefaction, appearance of blue colour, and conversion of yellow to pink colour were all noted, **Table 3** shows the results of PGPR confirmation tests. The Molecular characterization are carried out with 16srRNa

### Biochemical characterization of Bacteria (Table 2)

### Molecular Identification:

### DNA Isolation, Amplification, and Sequencing

DNA isolation of cultured fungi was successful and purified using a sodium acetate-ethanol precipitant, and DNA isolation of bacteria was successful and purified using a modified CTAB - method. The isolated genomic DNA was amplified using PCR, with amplicons ranging in length from 800 -1200bp 16srRNA regions.

### Accession Number of PGPR bacteria

The isolated PGPR bacteria are successfully submitted and get accession numbers from Genbank. *Pseudomonas putida (*ON261599), *Bacillus subtilis (*ON261566*), Pseudomonas aeruginosa (*ON261400*), Bacillus paramycoides (*ON261399*)* and *Bacillus cereus (*ON261398*).*

### Phylogenetic Analysis

The Phylogenetic analysis shows *Pseudomonas putida* and *Pseudomonas aeruginosa* species in a single clade from the same branches, whereas *Bacillus cereus, Bacillus subtilis,* and *Bacillus paramycoides* are Present in three different clades.

### Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method **[14]**. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown below the branches **[15]**. The evolutionary distances were computed using the Maximum Composite Likelihood method **[16, 17]** and are in the units of the number of base substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1185 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 **[18, 19]**.

**Table 2: Isolation and characterization of bacteria**

| Biochemical Tests | Gram Staining | Indole | Methyl red | Citrate Utilization | Voges–Proskauer (VP) | Urease | Oxidase | Starch Hydrolysis | Catalase | Nitrate | Strain Name |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Principle | Cell wall composition | Deamination and hydrolysis by bacteria | Detects acid production | Detects capability of organisms to utilize citate as sole carbon | Detects acetylmethyl carbinol Production | Detects urease activity | Detects the presence of a cytochrome oxidase | Detects organism to hydrolyze starch | Detects presence of catalase | Detects a organism to reduce nitrate to | |
| ABA 1 | Positive | Negative | Positive | Positive | Positive | Positive | Positive | Negative | Positive | Positive | *Bacillus paramycoides* |
| ABA 2 | Positive | Negative | Positive | Positive | Positive | Positive | Positive | Negative | Positive | Positive | *Bacillus subtilis* |
| ABA 3 | Positive | Negative | Positive | Positive | Positive | Positive | Positive | Negative | Positive | Positive | *Bacillus cereus* |
| ABA 4 | Negative | Positive | Negative | Negative | Negative | Negative | Positive | Positive | Negative | Positive | *Pseudomonas aeruginosa* |
| ABA 5 | Negative | Positive | Negative | Negative | Negative | Negative | Negative | Positive | Negative | Positive | *Pseudomonas putida* |

**Table 3: PGPR tests of isolated bacterial strains**

| Sample Code | P Solubilization | IAA Production | Ammonia Production | ACC Deaminase activity | HCN Production | Catalase Activity |
|---|---|---|---|---|---|---|
| ABA 1 | Positive | Positive | Positive | Positive | Positive | Positive |
| ABA 2 | Positive | Positive | Positive | Positive | Positive | Positive |
| ABA 3 | Positive | Positive | Positive | Positive | Positive | Positive |
| ABA 4 | Positive | Positive | Positive | Positive | Positive | Positive |
| ABA 5 | Positive | Positive | Positive | Positive | Positive | Positive |

**Table 4: Bacterial isolates showing Quantitative P-solubilization & IAA Production**

| Sample Code | P Solubilization | IAA Production |
|---|---|---|
| ABA 1 | Positive | 421.51µg/ml |
| ABA 2 | Positive | 410.52µg/ml |
| ABA 3 | Positive | 412.63µg/ml |
| ABA 4 | Positive | 552.31µg/ml |
| ABA 5 | Positive | 514.32µg/ml |

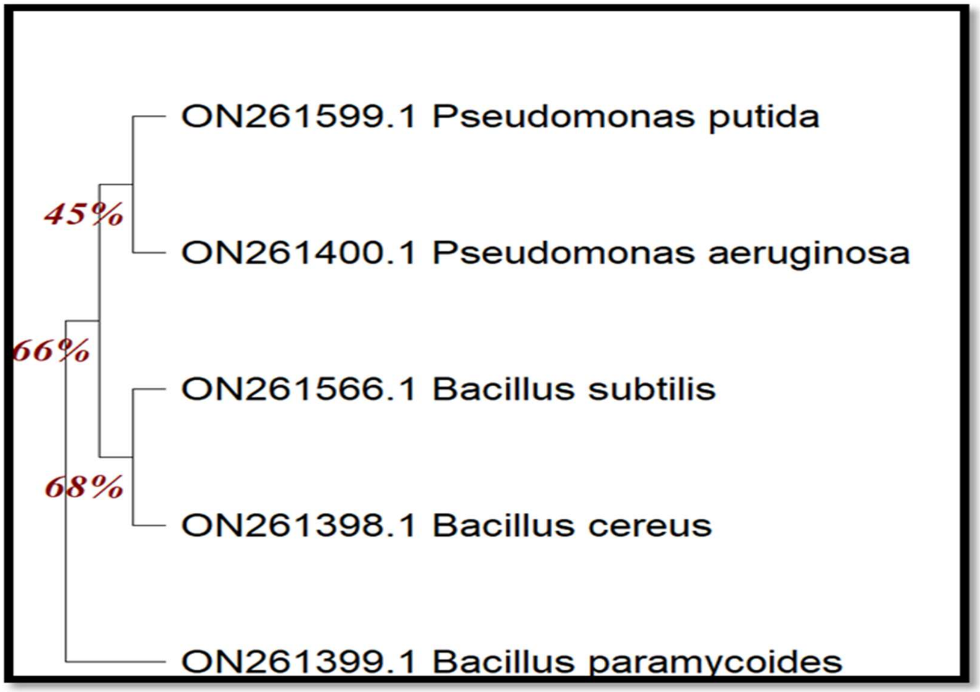

**Figure 1: Phylogenetic Tree of PGPR Bacteria Isolated From *Spinacia oleracea***

## CONCLUSION:

PGPR Bacteria were successfully isolated from the *Spinacia oleracea* soil sample belonging to the regions of the palakarai Area, Tiruchirappalli. From the cultured Bacteria, DNA was isolated, amplified, and sequenced using the 16s rRNA region. The phylogenetic tree obtained by MEGA 11.0 suggests that five PGPR bacteria were isolated from *Spinacia oleracea* Soil samples.

**CONFLICTS OF INTEREST:** Nil.

## REFERENCES:

**[1]** Panda, V., Mistry, K., Sudhamani, S., Nandave, M., & Ojha, S. K. (2017). Amelioration of abnormalities associated with the metabolic syndrome by *Spinacia oleracea* (spinach) consumption and aerobic exercise in rats. Oxidative medicine and cellular longevity, 2017.

**[2]** Ferreira, J. F., Sandhu, D., Liu, X., & Halvorson, J. J. (2018). Spinach (*Spinacea oleracea* L.) response to salinity: Nutritional value, physiological parameters, antioxidant capacity, and gene expression. Agriculture, 8(10), 163.

**[3]** Fahad, S. M., Islam, A. F. M., Ahmed, M., Uddin, N., Alam, M., Khalik, M., & Abedin, M. (2015). Determination of elemental composition of Malabar spinach, lettuce, spinach, hyacinth bean, and cauliflower vegetables using proton induced X-ray emission Technique at Savar Subdistrict in Bangladesh. BioMed Research International, 2015.

**[4]** Stenkula, K. G., Stenblom, E. L., Montelius, C., Egecioglu, E., & Erlanson-Albertsson, C. (2017). Thylakoids reduce body fat and fat cell size by binding to dietary fat making it less available for absorption in high-fat fed mice. Nutrition & metabolism, 14(1), 1-8.

**[5]** Carvajal-Yepes, M., Cardwell, K., Nelson, A., Garrett, K. A., Giovani, B., Saunders, D. G., & Tohme, J. (2019). A global surveillance system for crop diseases. Science, 364(6447), 1237-1239.

**[6]** Cacciola, S. O., & Gullino, M. L. (2019). Emerging and re-emerging fungus and oomycete soil-borne plant diseases in Italy. Phytopathologia Mediterranea, 58(3), 451-472.

**[7]** Wang, X., Li, Y., Song, X., Meng, Q., Zhu, J., Zhao, Y., & Yu, W. (2017). Influence of host tree species on isolation and communities of mycorrhizal and endophytic fungi from roots of a tropical epiphytic orchid, Dendrobium sinense (Orchidaceae). Mycorrhiza, 27(7), 709-718.

**[8]** Zheng, H., Ho, P. Y., Jiang, M., Tang, B., Liu, W., Li, D., & Liu, C. (2016). Interrogating the Escherichia coli cell cycle by cell dimension perturbations. Proceedings of the

National Academy of Sciences, 113(52), 15000-15005.

**[9]** Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017). Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International journal of systematic and evolutionary microbiology, 67(5), 1613.

**[10]** Pikovskaya R.E. (1948) Mobbilization of phosphorus in soil in connection in vital activity of some microbial species. Mikrobiologiya. 17:362-370.

**[11]** Dye D.W. (1962) The inadequacy of the usual determinative tests for identification of Xanthomonas spp. New Zealand J. Sci. 5:69-71.

**[12]** Gordon A.S. and Weber R.P. (1951) Colorimetric estimation of Indole acetic acid. Plant Physiol. 26: 192-195.

**[13]** Brik J.M., Bostock R.M. and Silversone S.E. (1991) Rapid insitu assay of indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl. Environ. Microbiol. 57: 535-538.

**[14]** Bakker A.W. and Schippers B. (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp. mediated plant growth stimulation. Soil Biol. Biochem. 19:451-457.

**[15]** Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

**[16]** Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

**[17]** Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution. https://doi.org/10.1093/molbev/msab120.

**[18]** Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.

**[19]** Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution, 33(7), 1870-1874.