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Isolation and Molecular Identification of *Pseudomonas luteola* from Narayanapuram Lake, Chennai, India

P. Keerthana* and S. Illanjiam

Department of Microbiology, Hindustan College of Arts and Science, Chennai, India

**Corresponding author*

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A B S T R A C T

This study presents the isolation and molecular identification of *Pseudomonas luteola* from Narayanapuram Lake, Chennai. Lake water samples were collected and processed for bacterial isolation. Using molecular techniques, PCR amplification of the 16S rRNA gene, and sequencing, *P. luteola* strains were identified. Phylogenetic analysis was conducted to assess the genetic relatedness of the isolated strains, and a phylogenetic tree was constructed. The obtained sequences were submitted to GenBank for further reference.

Introduction

Narayanapuram Lake, a vital water body situated in Chennai, serves as a crucial ecosystem supporting various ecological functions and human activities. However, concerns regarding water quality and contamination have raised alarms about potential health and environmental risks associated with its usage.

In response to these concerns, this study focuses on the identification and isolation of *Pseudomonas luteola*, a bacterium of interest, from water samples collected from Narayanapuram Lake.

Pseudomonas luteola, formerly known as *Flavimonas* or *Chryseomonas luteola*, is a gram-negative, aerobic, non-spore-forming bacterium belonging to the Pseudomonas genus. It is commonly found in diverse environmental habitats, including soil, water, and hospital settings. *P. luteola* is characterized by its yellow-pigmented colonies, which contribute to its identification on agar plates (Girard *et al.*, 2020).

Pseudomonas luteola include bioremediation, industrial applications for enzyme production, agricultural benefits in plant growth promotion, and research utility in genetics and physiology, as well as potential in water treatment processes (Gayathri *et al.*, 2023). Infections caused by this microorganism are quite rare, with fewer than 25 reported cases. These infections typically manifest as serious conditions like septicemia (bloodstream infection), meningitis (inflammation of the membranes surrounding the brain and spinal cord), peritonitis (inflammation of the abdominal lining),

endocarditis (infection of the heart valves), and ulcer infections. They often occur in connection with surgical procedures or the use of medical devices such as catheters or prostheses (Doublet *et al.,* 2010).

Materials and Methods

Sample Collection

Water sample were collected from Narayanapuram Lake, Chennai, during the hot summer season. Sterilized wide-mouth bottles, each with a capacity of at least 200 ml, were used for sample collection. The bottles were opened and submerged to a depth of 30 cm, positioning their mouths to face the current. Subsequently, the collected sample were transported and were immediately processed in the Microbiology Department of Hindustan College of Arts and Science, Chennai, for further analysis. A sample was stored at 4°C before the examination (Bhumbla *et al.,* 2020).

Isolation and Identification

The water sample from Narayanapuram Lake (in location 12°56'41.3"N 80°12'08.9"E) was diluted in normal saline water from 10-1 to 10-6. These dilutions were then spread onto Nutrient agar media obtained from HiMedia Ltd, Mumbai, and left to incubate at 37°C for 24 hours. Afterward, well-isolated bacterial colony with pigmentation were selected for further morphological and cultural characterization. These isolates were restreaked onto Nutrient agar plates and incubated for purity assessment and gram staining. Pigmented bacteria were identified using biochemical tests and 16s rRNA sequencing to determine their characteristics (Garcha *et al.,* 2016)**.**

Biochemical test

Biochemical test performed were Indole test, Methyl Red (MR), Voges Proskauer (VP), Simmon's citrates test, Oxidase test, Catalase test, Urease test, and Triple Sugar Iron Agar Test (TSI) recommended in the Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994).

Molecular characterization and Phylogenetic tree construction

Analysis of the isolate involved 16S rRNA gene sequencing for taxonomic characterization, conducted by Immugenics Biosciences Pvt Ltd, Chennai. DNA extraction utilized the boiling lysis method, followed by 16S rRNA PCR using broad-range pan Eubacterial primers on a Veriti 96-Well Thermal Cycler (Applied Biosystems, USA). Amplicon purification was achieved with the FavorPrep PCR Purification Mini Kit (Favorgen, Taiwan).

Sequencing was performed on an ABI 3730XL sequencer (Applied Biosystem, USA) using the ABI PRISM® BigDye™ Terminator. The obtained 16S rRNA gene sequences were compared to NCBI's nucleotide database via BLAST, and a phylogenetic tree was constructed based on the obtained 16S rRNA gene sequences and related sequences (Stewart *et al.,* 2011; Di Sora *et al.,* 2023).

Gen bank submission

The 16S rRNA gene sequences of *Pseudomonas luteola* were prepared and submitted to GenBank. Sequences were formatted, metadata provided, and quality checked before submission. Accession numbers were obtained, allowing public access to the data (Doublet *et al.,* 2010).

Results and Discussion

Isolation and Identification

Upon analysis, various bacterial colonies were observed, including a yellow-pigmented colony in the 10-5 dilution. This yellow-pigmented colony was separately streaked on nutrient agar media and identified as *Pseudomonas luteola* through biochemical and molecular characterization.

Molecular characterization and Phylogenetic tree construction

The amplification of the 16S rRNA gene sequences of *Pseudomonas luteola* strains through PCR. The PCR products had a size of 1021 kilobases (kb), indicating successful amplification of the target gene region.

The Neighbor-Joining method was used to construct a phylogenetic tree based on genetic distances, while Bootstrap analysis assessed the tree's reliability. This approach elucidates evolutionary relationships among *Pseudomonas luteola* strains and related bacteria.

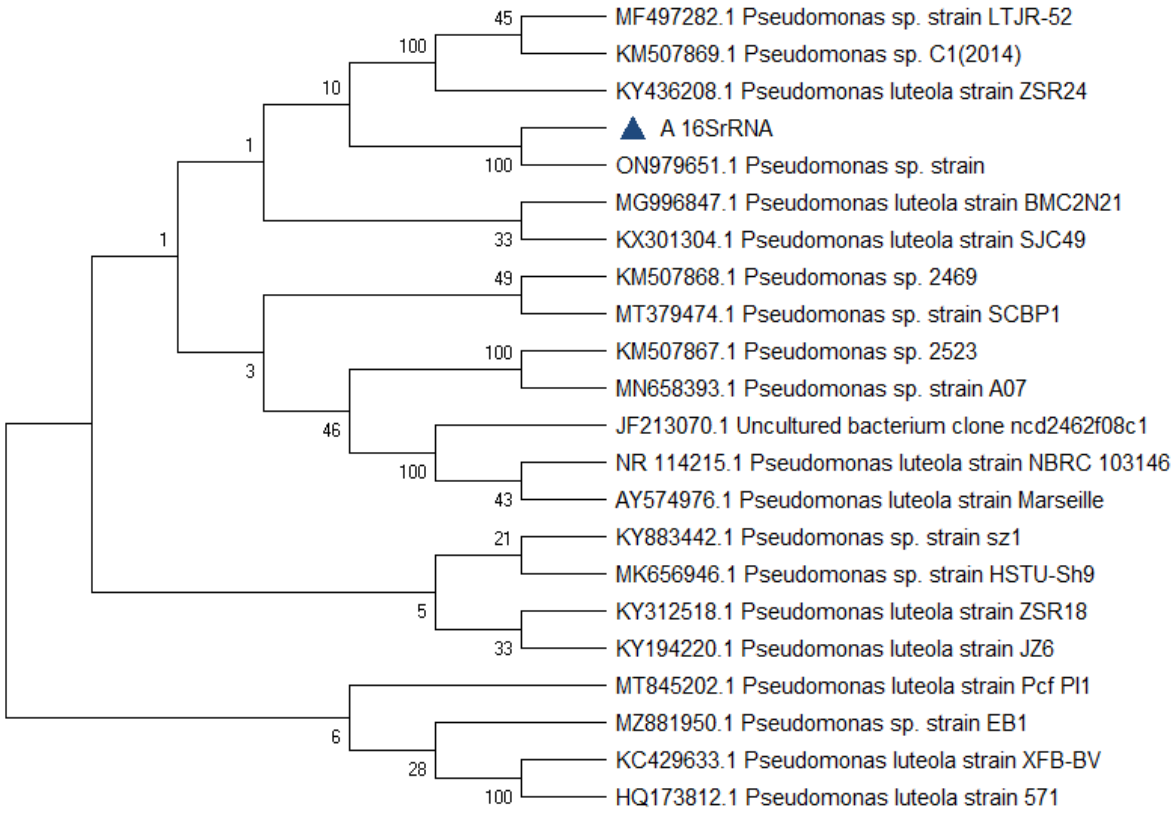
Table.1 Morphological and biochemical characterization of Pseudomonas luteola bacterial colony

Molecular Identification	***Pseudomonas luteola***
Colony Morphology	yellow-pigmented, circular, rough, dry, wrinkled, adherent colony
Grams Reaction	Gram-Negative
Cell Shape	Rod
Motility	Positive
Biochemicals Test	
Indole	Negative
Methyl Red	Positive
Voges Proskauer	Negative
Citrate	Positive
Urease	Negative
Catalase	Positive
Oxidase	Negative
Triple Sugar Iron Agar Test (TSI)	
Slant	Alkaline
Butt	Alkaline
Gas Production	No
H_2s Production	No

Figure.1 Narayanapuram Lake, Chennai

Figure.2 *Pseudomonas luteola* on Nutrient agar

Figure.3 Neighbor-Joining Bootstrap's Phylogenetic tree of *Pseudomonas luteola* (A 16SrRNA)



Gen bank submission

The nucleotide sequences obtained in this study have been submitted to GenBank, and their accession number is OQ550179.

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Author Contribution

P. Keerthana: Investigation, formal analysis, writing—original draft. S. Illanjiam: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Bhumbla U, Majumdar S, Jain S, Dalal A S. A study of isolation and identification of bacteria from lake water in and around Udaipur, Rajasthan. *J Family Med Prim Care*. 2020 Feb 28;9(2):751-754. PMID: 32318414; PMCID: PMC7113958. https://doi.org/10.4103/jfmpc.jfmpc_1032_19

Di Sora N, Turco S, Brugneti F, Rossini L, Mazzaglia A, Contarini M, Speranza S. Molecular Characterization and Phylogenetic Analysis of the Pine Tortoise Scale Insect *Toumeyella parvicornis* (Cockerell) (Hemiptera: Coccidae). *Forests*. 2023; 14(8):1585. https://doi.org/10.3390/f14081585

Doublet B, Robin F, Casin I, Fabre L, Le Fleche A, Bonnet R, Weill F X. Molecular and biochemical characterization of the natural chromosome-encoded class A beta-lactamase from *Pseudomonas luteola*. *Antimicrob Agents Chemother*. 2010 Jan;54(1):45-51. https://doi.org/10.1128/AAC.00427-09

Garcha, S., Verma, N., and Brar, S. (2016). Isolation, characterization and identification of microorganisms from unorganized dairy sector wastewater and sludge samples and evaluation of their biodegradability. *Water Resources and Industry*, *16*, 19-28. https://doi.org/10.1016/j.wri.2016.10.002

Gayathri V and Krishnaprema K. Bioremediation of Crude Oil using Hydrocarbon degrading *Pseudomonas luteola* by Immobilization methods. *Eco. Env. and Cons.* 29 (January Suppl. Issue) : 2023; pp. (S109-S116), http://doi.org/10.53550/EEC.2023.v29i01s.017

Girard L, Lood C, Rokni-Zadeh H, van Noort V, Lavigne R, De Mot R. Reliable Identification of Environmental *Pseudomonas* Isolates Using the *rpoD* Gene. *Microorganisms*. 2020; 8(8):1166. https://doi.org/10.3390/microorganisms8081166

Holt G, Krieg R, Sneath H, Staley T, and Williams T, *Bergey's Manual of Determinative Bacteriology*, Williams and Wilkins, Baltimore, Md, USA, 1994.

Stewart R M, Wiehlmann L, Ashelford K E, Preston S J, Frimmersdorf E, Campbell B J, Neal T J, Hall N, Tuft S, Kaye S B, Winstanley C. Genetic characterization indicates that a specific subpopulation of *Pseudomonas aeruginosa* is associated with keratitis infections. *J ClinMicrobiol*. 2011 Mar;49(3):993-1003. Epub 2011 Jan 12.
https://doi.org/10.1128/jcm.02036-10

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