Research Article

Journal of Diversity Studies

A Study on Diversity and Distribution of Purple Non-sulfur Bacteria in Various Water Bodies

Saraswathi Ramavath* and Rajani Bogarapu

Microbial Physiology Lab, Department of Botany, Osmania University, Hyderabad, Telangana-500 007, India

ABSTRACT

A study was undertaken to explore the potential applications of purple non-sulfur bacteria (PNSB) in the treatment of wastewater. Utilizing PNSB for wastewater treatment has emerged as a particularly promising approach. These microorganisms, widely distributed in nature, exhibit the ability to eliminate organic materials even in the presence of high levels of organic contamination. The investigation identified two strains of PNSB, namely Rhodomicrobium sp RSOU0018, with a focus on the thermos tolerant Rhodopseudomonas Sp(RSOU2020) strain sourced from a water samples collected from the Origin of Jhelum river-Verinag, Kashmir. Wastewater from different rivers such as Arpath river, Lidder river, Neelum river, sind river, kunhar river, pohru river followed by the isolation and identification of Purple Non sulfur Bacteria sp. using traditional bacterial culture techniques and molecular methods. Purple Non sulfur Bacteria sp strains have been identified. The above strains of Rps were found to decrease water pollutants. Furthermore, the strains Rhodomicrobium sp (RSOU0018) & Rhodopseudomonas Sp(RSOU2020) exhibited reductions in COD, BOD, and in TDS. However, there was a noticeable drop in TDS, COD, and BOD. Nevertheless, the PNSB assisting in the treatment had an impact on how much the aforementioned metrics decreased.

Keywords: *Wastewater, Bacteria, Diversity, Rhodomicrobium sp., Rhodopseudomonas Sp.*

Citation: Saraswathi Ramavath and Rajani Bogarapu [2024]. A Study on Diversity and Distribution of Purple Non-sulfur Bacteria in Various Water Bodies. *Journal of Diversity Studies.* **https://doi.org/10.51470/JOD.2024.03.01.08**

Corresponding Author: *Saraswathi Ramavath*

E-mail Address: *saraswathi.ou@gmail.com*

Article History: *Received 20 November 2023 | Revised 29 December 2023 | Accepted 27 January 2024 | Available Online January 29, 2024*

INTRODUCTION

Using phototrophic bacteria, treating waste water is a feasible prospect [1]. Waste treatment and utilization are economically feasible and crucial steps in reducing environmental pollution. However, this method only appears cost-effective when there are sufficient inexpensive waste substrates available. It is economically advantageous to employ industrial, agricultural, and municipal wastes both organic and inorganic for biomass production because it allows for the conversion of unwanted nutrients into biomass that can be used. Apart from employing several organic substances for the purification of waste water, they have the ability to generate H2 [5, 6], indole-3-acetic acid (IAA), and 5-aminolevulinic acid (ALA) [7]. Due to their exceptional efficiency in turning waste materials into valuable products, they are prevalent in places that are contaminated [8, 9]. In order to maintain clean water, the primary function of bacteria and fungi is to break down organic matter in sediments.

The majority of earlier studies on the production of phototrophic biomass were focused on the batch or test-tube breeding of a single strain or specific species of PNSB grown from industrial waste water [10,11,12]. A number of phototrophic treatment methods, such as sequential batch reactors, anaerobic lagoons, stabilization ponds, and anaerobic lagoons, were investigated with the purpose of growing biomass from industrial wastewater [16]. It has been successfully accomplished to extract nitrogen from animal waste, and municipal sewage by using anoxygenic phototrophic bacteria [17, 18]. Purple bacteria have also emerged as extremely promising wastewater purification agents [19]. Various organic waste waters, particularly food industrial wastes with high BOD strength, have been purified utilizing a biological waste water treatment procedure that uses purple non-sulfur bacteria (PNSB) [20]. Because PNSB can grow at a relatively high rate and devour a variety of organic substances, it is thought to be an effective treatment for waste water. PNSB have been utilized to treat a variety of pollutants, including agricultural waste, concentrated latex wastewater, and aquarium wastewater [21,22,23]. These findings motivated researchers to investigate two PNSB strains bioremediation capabilities.

MATERIALS AND METHODS

Selection of Sampling Station

Water samples were collected from the origin of the Jhelum River in Verinag, Kashmir, India. The Jhelum River is the westernmost of the five rivers of the Punjab region and flows through Kashmir. It is a tributary of the Chenab River and has a total length of approximately 725 kilometers. Some of its tributaries include the Poonch River and the Sukhnag River on the left, and the Arpath River, Lidder River, Neelum River, Sind

River, Kunhar River, and Pohru River on the right. The river is significant for agricultural and drinking purposes, with dams and bridges such as the Mangla Dam and Uri Dam supporting its utilization.Were collected for the current study. Water samples were collected from surrounding areas of rivers, ponds and were carried to laboratory in sterile bottles and stored at 4°C for further processing. Following Kashmir, 's ascension to the state capital, the city experienced unparalleled population expansion and modernization. Utilizing artificial fertilizers and agriculture-related pesticide use increased pollution. Untreated home sewage, solid waste, and industrial effluents inevitably found their way into this Rivers and lake's catchment area.

In addition to the dirt that builders threw in the ground and the chemical and solid waste that industries released, a lot of trash was gathered along the rivers and lake's edge. Later, the burned trash was thrown into the lake, further contaminating it. At a depth of 15 cm, water samples were taken in one-liter bottles from lakes[2,3,4]. The physicochemical parameters, including pH, temperature, chlorides, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, total solids, total dissolved solids, sulfates, and nitrates, were assessed using the standard procedures outlined by the American Public Health Association (APHA).

Table 1: Physico-chemical Analysis of Water Samples

S.NO	TEST PARAMETER	UNIT	SPECIFICATION
1.	pH	------	6.5-7.5
2.	Electrical Conductivity	NTU	500 μmohs/cm
3.	TDS	(umohs/cm)	500-2000
4.	Organic carbon	mg/l	NLT 1 %
5.	Available Nitrogen as N	mg/l	150-420 kg/ha
7.	Available Phosphorus as P	mg/l	13-22 kg/ha
8.	Available Potassium as K	mg/l	200-240 kg/ha
9.	Calcium as Ca	mg/l	----
10.	Magnesium as Mg	mg/l	60-100 mg/kg
11.	**Micronutrients**: Copper as Cu	mg/l	0.3-0.8 mg/kg
12.	Zinc as Zn	mg/l	1-3 mg/kg
13.	Manganese as Mn	mg/l	1.2-3.5 mg/kg
14.	Iron as Fe	mg/l	--
15.	Boron	mg/l	0.5-1 mg/kg
16.	BOD	-----	30-100

Water electrical conductivity (EC) is used to estimate the concentration of soluble salts. The typical procedure is to add water to the soil sample until it is saturated, then remove the water using a vacuum filter before measuring the EC of the saturated paste extract. The outcome is known as EC and is given in deci Siemens per meter, or dS/m, units of measurement.

Identification of *PNSB sp.* by biochemical tests

In accordance with Bergey's Manual for Determinative Bacteriology [7], several biochemical tests [12,13], were carried out to identify PNSB sp. from the isolated bacterial colonies [15].

Results and Discussion

PNSB strains exhibit a remarkable ability to utilize a diverse range of chemical and inorganic compounds as electron donors, although their growth necessitates an external electron source. In this study, we aimed to capitalize on the polluted waters collected from lakes, rich in organic and inorganic chemicals, as a cost-effective raw material for the development and bioremediation of these organisms. The PNSB strains displayed resilience to a wide range of pH values and demonstrated the capability to utilize various organic and inorganic substances present in wastewater as electron donors, thereby supporting their growth and biomass production. Simultaneously, we conducted efforts aimed at bioremediating wastewater.

We conducted comprehensive assessments of various physicochemical parameters, including pH, temperature, total solids, total dissolved solids, chlorides, sulfate (SO4), nitrate (NO3), chemical oxygen demand (COD), and biochemical oxygen demand (BOD), in the contaminated waters of different rivers and lakes. These values were measured both before and after the treatment processes, and Table 2 presents the recorded data.

Table 2: Waste water treatment (polluted waters of different lakes samples) by Rhodomicrobium sp RSOU0018 and Rhodopseudomonas Sp thermotolerance RSOU2020 Parameters

S No.	PARAMETER	UNIT	Result 1	Result 2
1.	pH	------	6.10	7.5
2.	Turbidity	NTU	0.8	0.6
3.	Electrical Conductivity	(umohs/cm)	515	502
4.	Total Dissolved Solids (TDS)	mg/L	340	295
5.	Total Alkalinity as $CaCO_3$	mg/L	112	116
6.	Total Hardness as $CaCO_3$	mg/L	185	196
7.	Sulphates as SO_4	mg/L	21	25
8.	Chlorides as Cl	mg/L	51	56
9.	COD	mg/L	110	125
10.	BOD	mg/L	40	45
11.	Nitrate Nitrogen NO_3	mg/L	0.51	0.58
12.	Iron as Fe	mg/L	0.25	0.20
13.	Calcium as Ca	mg/L	58	55
14.	Magnesium as Mg	mg/L	16	13
15.	Sodium as Na	mg/L	15	17
16.	Potassium as K	mg/L	9	12

The application of *Rhodomicrobium sp*. (RSOU0018) and *Rhodopseudomonas sp.* (RSOU2020) resulted in significant decreases in various parameters. Previous studies have reported the successful utilization of wastewater for the generation of photosynthetic bacteria on various substrates, including industrial wastes, orange processing wastes, raw starch, distillery wastes, organic wastes/pharmaceutical wastes, lactic acid-containing wastes, and industrial wastes [23, 24]. A variety of sources and procedures, including animal wastes, soybean wastes, municipal and purified biogas plant effluents, have been employed to generate biomass of phototrophic bacteria [31,29].

It was previously claimed that immobilized photosynthetic bacteria might be used to treat oil containing sewage waste water. Tributyl phosphate, which is frequently used in the processing and other waste-generating chemical industries, may be degraded by *Rhodomicrobium sp* (RSOU0018) [30]. It was determined whether *Rhodopseudomonas Sp(RSOU2020)* could be used to bioremediate habitats contaminated with heavy metals [31]. Large-scale research may suggest using the isolated strain of photosynthetic purple non-sulfur bacteria in waste water treatment facilities since naturally occurring microorganisms may work better in this regard. The strain might make use of contaminated waters that have greater biomass outputs. This suggests that more large-scale attempts at mass growing these organisms could prove to be profitable. The aforementioned studies may pave the path for the effective application of PNSB.

The analysis of various water quality parameters provides a comprehensive overview of the characteristics of the sample Table.3 The results obtained from the testing reveal key information about the suitability of the water for its intended use. The pH level of 6.10-7.5 falls within a slightly acidic range. While it is essential to consider the specific requirements of the application, this pH value generally falls within acceptable limits for many purposes. Turbidity, measured at 0.8-0.6 NTU, indicates relatively clear water, suggesting minimal suspended particles. This is favorable for applications where clarity is crucial, such as drinking water. Electrical Conductivity and Total Dissolved Solids provide insights into the water's salinity and dissolved substance content. These values, while within acceptable limits for various uses, may require consideration depending on the specific application. Total alkalinity and total hardness, measured as $CaCO_3$, are recorded at 116 mg/L and 125 mg/L, respectively. These values influence the water's buffering capacity and potential to cause scaling, aspects crucial for industrial and domestic purposes. Sulphates (21-25 mg/L) and chlorides (56 & 51) contribute to the assessment of potential corrosiveness and taste of the water. These concentrations generally align with standard guidelines for drinking water quality.

Chemical Oxygen Demand (COD) at 110-125mg/L and Biochemical Oxygen Demand (BOD) at 40& 45 mg/L provide information on the water's organic pollutant content. These values are critical indicators of water pollution and its impact on aquatic ecosystems. Nutrient levels, such as nitrate nitrogen (NO3), are crucial for evaluating potential nutrient enrichment or contamination. The recorded value of 0.58 mg/L suggests a relatively low concentration, posing minimal risk to water quality. Trace elements like iron (Fe), calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) are within acceptable ranges for drinking water. These elements contribute to the overall mineral content of the water, influencing its taste and nutritional profile.

Identification and Characterization of *PNSB sp.*

The PNSB sp. that were isolated from the geographical area were coded in the following were further identified initially by their colony morphology (Table 3) and characterized by different staining and Biochemical tests.

Table 3: Biochemical tests for identification of different PNSB strains isolated from different geographical locations in Kashmir.

Sl. No.	**Description of Staining and Biochemical tests**	**Staining and Biochemical test results**	
		Location 1	**Location 2**
1	Staining (Gram stain)	Negative rods	Negative rods
5	Citrate test	Positive	Positive
6	Catalase test	Positive	Positive
7	Nitrate Reduction	Positive	Positive
9	Oxidase test	Positive	Positive
11	Pigment production test	Positive	Positive
16	Motility	Motile	Motile
Carbohydrate Fermentation			
17.1	Glucose	Positive	Positive
17.2	Fructose	Positive	Positive
17.5	Maltose	Positive	Negative
Identified *PNSB sp.*		*Rhodopseudomonas sp*	*Rhodomicrobium sp*

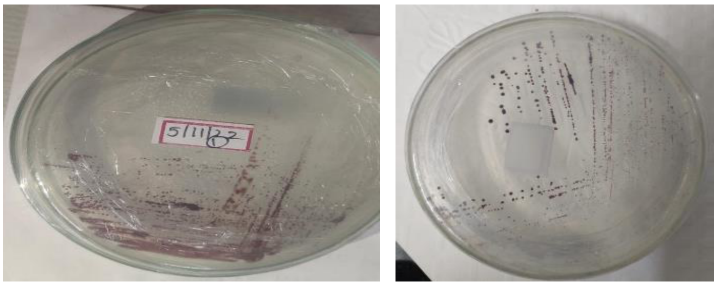

Fig 1: Pure culture of isolated Azotobacter sp. on Ashby Mannitol agar.Rhodopseudomonas sp and Rhodomicrobium sp.

Conclusion

The study's findings reveal the percentages or values of physicochemical parameters of water and soil. Based on the current investigation, it can be inferred that purple non-sulfur bacteria serve as beneficial agents in promoting plant growth. The insights gained from this study will be instrumental in identifying the nature and extent of water-related issues, recommending appropriate remediation measures, and assessing the suitability of land for agricultural purposes. Additionally, further research into the sources of water would be advantageous. Given that the isolates in this study closely resemble *Rhodopseudomonas rosea* and a few other species within the genus, farmers can utilize these findings to estimate the necessary amounts of nutrients and fertilizers required to enhance crop yield.

Acknowledgement

The authors would like to extend their gratitude to the Head of the Department of Botany at Osmania University for providing the necessary resources for this research endeavor. Additionally, the authors express their appreciation to CSIR for the fellowship support provided during the course of this study.

References

1. Ahmad, M. S., Zargar, M. Y., Mir, S. A., Bhat, N. A., Baba, Z. A., & Habib, R. (2019). Isolation and Characterization of Photosynthetic Bacteria from Municipal Waste. *Int. J. Curr. Microbiol. App. Sci,* 8(3), 861-865.

2. Ayyaz, K., Zaheer, A., Rasul, G., & Mirza, M. S. (2016). Isolation and identification by 16S rRNA sequence analysis of plant growth-promoting azospirilla from the rhizosphere of wheat. *Brazilian journal of microbiology* : [publication of the Brazilian Society for Microbiology], 47(3), 542–550. https://doi.org/10.1016/j.bjm.2015.11.035

3. Bellenger, J.; Darnajoux, R.; Zhang, X.; Kraepiel, A.(2020) Biological nitrogen fixation by alternative nitrogenases in terrestrial ecosystems: A review. *Biogeochemistry*

4. Ben-David A, Davidson CE (2014). Estimation method for serial dilution experiments. *J Microbiol Methods*. 2014 Dec; 107:214-21. doi: 10.1016/j.mimet..08.023. Epub Sep 7. PMID: 25205541.

5. Berne C, David P, Jerome L and Daniel G.(2007). CYP201A2, a Cytochrome P 450 from Rhodopseudomonas palustris, plays a key role in the biodegradation of tributyl phosphate. *Applied Microbiology and Biotechnology*, 77: 1.

6. Chen, J., Wei, J., Ma, C., Yang, Z., Li, Z., Yang, X., ... & Zhang, C. (2020). Photosynthetic bacteria-based technology is a potential alternative to meet sustainable waste water treatment requirement. *Environment international*, 137, 105417.

7. Cowan S.T., (1948).Bergey's Manual of Determinative Bacteriology, Nature 162 833.

8. Dadook M, Mehrabian S, Salehi M, Irian S.(2014). Morphological, biochemical and molecular characterization of twelve nitrogen-fixing bacteria and their response to various zinc concentration. Jundishapur J Microbiol. Apr;7(4):e9415. doi: 10.5812/jjm.9415. Epub 2014 Apr 1. PMID: 25147702; PMCID: PMC4138622.

9. Hameed, S., Hameed, S., Sadia, M., & Malik, S. A. (2012). Genetic diversity analysis of Bemisia tabaci populations in Pakistan using RAPD markers. *Electronic Journal of Biotechnology*, *15*(6). https://doi.org/10.2225/vol15-issue6-fulltext-8

10. Harris, D. F., Lukoyanov, D. A., Kallas, H., Trncik, C., Yang, Z. Y., Compton, P., ... & Seefeldt, L. C. (2019). Mo-, V-, and Fe-nitrogenases use a universal eight-electron reductive-elimination mechanism to achieve N2 reduction. *Biochemistry*, *58*(30), 3293-3301.

11. Hartman D. Perfecting your spread plate technique. J Microbiol Biol Educ. (2011). Dec 1;12(2):204-5. doi: 10.1128/jmbe.v12i2.324. PMID: 23653767; PMCID: PMC3577269.

12. Hiraishi, A., & Ueda, Y. (1995). Isolation and Characterization of Rhodovulum strictum sp. nov. and Some Other Purple Nonsulfur Bacteria from Colored Blooms in Tidal and Seawater Pools. *International Journal of Systematic Bacteriology*, *45*(2), 319–326. https://doi.org/10.1099/00207713-45-2-319

13. Hiraishi, A., Nagao, N., Yonekawa, C., Umekage, S., Kikuchi, Y., Eki, T., & Hirose, Y. (2020). Distribution of Phototrophic Purple Nonsulfur Bacteria in Massive Blooms in Coastal and Wastewater Ditch Environments. *Microorganisms*, *8*(2), 150. https://doi.org/10.3390/microorganisms 8020150

14. Howard, K. S., Hales, B. J., & Socolofsky, M. D. (1983). Nitrogen fixation and ammonia switch-off in the photosynthetic bacterium Rhodopseudomonas viridis. Journal of bacteriology, 155(1), 107–112. https://doi.org/10.1128/jb.155.1.107-112.1983

15. Imhoff JF, Truper HG, Pfennig N.(1984). Rearrangement of the Species and Genera of the Phototrophic "Purple Nonsulfur Bacteria". Int J Syst Bacteriol 1984;34:340–343.

16. Kobayashi M. (1982). The role of phototrophic bacteria in nature and their utilization. In: Advances in Agricultural Microbiology (Ed.N.S.S.Rao). Butterworth Scientific, London.; 643-651.

17. Koh RH and Song HG. (2007). Effects of application of Rhodopseudomonas sp. on seed germination and growth of tomato under axenic conditions. Journal of Microbiology Biotechnology.; 17: 1805–1810.

18. Luxem KL, Kraepiel AML, Zhang L, Waldbauer J, Zhang X. (2020). Carbon substrate re-orders relative growth of a bacterium using Mo-, V-, or Fe-nitrogenase for nitrogen fixation. Environ Mirobiol. https://doi.org/10.1111/1462-2920.14955 (Luxem et al. 2020b)

19. Madigan, M., Cox, S. S., & Stegeman, R. A. (1984). Nitrogen fixation and nitrogenase activities in members of the family Rhodospirillaceae. Journal of bacteriology, 157(1), 73–78. https://doi.org/10.1128/jb.157.1.73-78.1984

20. Maeda I. (2022). Potential of Phototrophic Purple Nonsulfur Bacteria to Fix Nitrogen in RiceFields. *Microorganisms.*;10(1):28.https://doi.org/10.3390/microorganisms10010028

21. Maeda, I. (2021). Potential of phototrophic purple nonsulfur bacteria to fix nitrogen in rice fields. Microorganisms, 10(1), 28.

22. Mustafa, S., Bhatti, H. N., Maqbool, M., & Iqbal, M. (2021). Microalgae biosorption, bioaccumulation and biodegradation efficiency for the remediation of wastewater and carbon dioxide mitigation: Prospects, challenges and opportunities. Journal of Water Process Engineering, 41, 102009. https://doi.org/10.1016/j.jwpe.2021.102009

23. Nalvothula, R., Challa, S., Peddireddy, V., Merugu, R., Pratap Rudra, M. P., Alataway, A., Dewidar, A. Z., & Elansary, H. O. (2022). Isolation, Molecular Identification and Amino Acid Profiling of Single-Cell-Protein-Producing Phototrophic Bacteria Isolated from Oil-Contaminated Soil Samples. MDPI. https://doi.org/10.3390/molecules27196265

24. Oda Y, Samanta SK, Rey FE, Wu L, Liu X, Yan T, Zhou J, Harwood CS .(2005). Functional genomic analysis of three nitrogenase isozymes in the photosynthetic bacterium Rhodopseudomonas palustris. J Bacteriol 187:7784–7794.

25. Okubo Y and Hiraishi A. (2007). Population dynamics and acetate utilization kinetics of two different species of phototrophic purple non-sulfur bacteria in a continuous co-culture system. Microbes Environment,; 22: 82–87.

26. Prawan, K., & Kumar, B. K. (2023). Isolation and characterization of Azotobacter sp. for plant growth promotion and abiotic stress tolerance in Telangana's agro-climatic regions: Towards sustainable development. South Asian Journal of Agricultural Sciences, *3*(1), 125–132. https://doi.org/10.22271/27889289.2023.v3.i1b.79

27. Sehar, S., & Nasser, H. A. A. (2019). Wastewater treatment of food industries through constructed wetland: a review. *International Journal of Environmental Science and Technology*, *16*(10), 6453–6472. https://doi.org/10.1007/s13762-019-02472-7

28. Singh, R. L., & Singh, R. P. (Eds.). (2019). Advances in Biological Treatment of Industrial Waste Water and their Recycling for a Sustainable Future. Applied Environmental Science and Engineering for a Sustainable Future. https://doi.org/10.1007/978-981-13-1468-1

29. Sousa AM, Machado I, Nicolau A, Pereira MO.(2013) Improvements on colony morphology identification towards bacterial profiling. J Microbiol Methods. Dec;95(3):327-35. doi: 10.1016/j.mimet.2013.09.020. Epub 2013 Oct 9. PMID: 24121049.

30. Srinivas A, Vinay Kumar B, Divya Sree B, Tushar L, Sasikala C. (2014). Rhodovulum salis sp. nov. and Rhodovulum viride sp. nov., phototrophic Alphaproteobacteria isolated from marine habitats. Int J Syst Evol Microbiol; 64:957–962.

31. Takeno K, Yamaoka Y and Sasaki K. (2005). Treatment of oil-containing sewage wastewater using immobilized photosynthetic bacteria. World Journal of Microbiology and Biotechnology, 21: 1385-1391.