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Isolation, biocharacterization, and wastewater treatment potential of purple photosynthetic bacterial strain from Lap An Lagoon, Vietnam

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ABSTRACT

The growth of the seafood industry, particularly aquaculture, will play a crucial role in enhancing global food security and driving economic growth, especially in nations such as Vietnam, where it is supporting the livelihoods of millions. However, the rapid expansion of aquaculture has brought about environmental issues, notably the discharge of nutrient-rich wastewater. While microbial treatments are well-established in wastewater management, the application of purple photosynthetic bacteria (PPB) in saline aquaculture systems remains underexplored. This study isolated and characterized a novel purple photosynthetic bacterial strain, LA5.1, from Lap An Lagoon in Vietnam. Strain LA5.1 exhibited robust growth across a salinity range of 0 to 30‰ and reduced the chemical oxygen demand (COD) by 60–80% within six days, even when the initial COD concentration was as high as 800 mg/L. Additionally, the strain efficiently removed up to 80% of total ammonia nitrogen (TAN) in saline environments, demonstrating its robust potential in bioremediation. A phylogenetic analysis of the 16S rRNA gene confirmed the identity of the strain as *Rhodopseudomonas julia*. These findings indicate that *R. julia* LA5.1 is a promising candidate for sustainable aquaculture wastewater treatment systems, offering significant potential for large-scale applications in addressing the environmental challenges associated with intensive aquaculture practices.

Keywords: purple photosynthetic bacteria, *Rhodopseudomonas julia*, biocharacterization, bioremediation, aquaculture, wastewater treatment.

INTRODUCTION

Aquaculture is one of the fastest-growing sectors in global food production, and is significantly contributing to food security and economic development, particularly in countries such as Vietnam, where the seafood industry supports millions of livelihoods. However, this rapid expansion has led to environmental challenges, particularly the discharge of nutrient-rich wastewater (Nguyen, 2017). This wastewater typically contains high concentrations of organic matter, nitrogenous and phosphorus compounds, biological contaminants, and heavy metals, all of which collectively degrade water quality. The environmental consequences of contaminated wastewater include the toxic effects of chemical pollutants, eutrophication, bacterial resistance, and soil degradation. (Nguyen, 2017, Huang et al., 2018; Ogunfowora et al., 2021; Liu et al., 2024). Therefore, it is crucial to develop cost-effective and sustainable wastewater treatment techniques that

can mitigate the environmental impact of aquaculture practices (Liu et al., 2024).

PPB have gained increasing attention as potential solutions to wastewater treatment (Liu et al., 2024). PPB, particularly anoxygenic phototrophic microorganisms of the genus *Rhodopseudomonas*, play a crucial role in carbon and nitrogen cycles (Meng et al., 2018; Liu et al., 2024). Their ability to metabolize organic compounds and fix nitrogen under anaerobic conditions makes them highly effective in treating nutrient-rich environments (Meng et al., 2018; George et al., 2020; Liu et al., 2024). Furthermore, PPB possess significant nutritional value owing to their high contents of proteins, essential amino acids, vitamin B12, and carotenoids, offering dual functionality as both bioremediation agents and nutritional supplements (Shapawi et al., 2012; Shaikh et al., 2024). Consequently, there is growing interest in utilizing PPB for sustainable wastewater treatment (Chen et al., 2020).

Several studies have demonstrated the effectiveness of PPB in aquaculture applications. Specifically, *Rhodopseudomonas* and *Rhodobacter* species have proven highly effective in improving water quality by removing harmful compounds such as ammonia (NH_3), nitrites (NO_2^-), and nitrates (NO_3^-), which pose risks to aquatic organisms such as shrimp (Azad, 2002; Luo et al., 2012) and fish (Zhang et al., 2014). Their ability to reduce the COD and nitrogen content of aquaculture wastewater makes them invaluable for sustaining a healthy aquatic farming environment. Furthermore, the ability of PPB to metabolize organic and inorganic compounds into biomass contributes to nutrient recycling in ecosystems.

The application of PPB is particularly promising in high-salinity environments, which adversely affect the performance of conventional microbial treatments. The demonstrated effectiveness of PPB in these challenging environments creates new possibilities for wastewater treatment (Hülsen et al., 2019). The dual benefits of PPB, i.e., improving water quality and enhancing aquaculture productivity, highlight their role as a sustainable approach to water management and environmental conservation.

The coastal region of Thua Thien Hue, Vietnam, is rich in diverse ecosystems, including lagoon systems such as the Tam Giang–Cau Hai Lagoon (Cao et al., 2021), Lap An Lagoon (Tan et al., 2021), and mangrove forests, offering a potential reservoir of natural microbial diversity. Isolating and selecting PPB strains from these ecosystems could reveal valuable microbial resources, particularly for aquaculture wastewater treatment and microbial biomass utilization. Although PPB have achieved success in controlled environments, their application in saline aquaculture remains underexplored. As salinity significantly influences microbial activity, the adaptability of PPB to varying salinities is crucial for practical applications. Therefore, this study focused on isolating and characterizing *Rhodopseudomonas* sp. from Vietnam's Lap An Lagoon, where fluctuating salinity provides a unique environment for testing microbial resilience. This study aimed to assess the tolerance of an isolated PPB strain to different salinity levels, its effectiveness in reducing COD and TAN in liquid medium, and its potential to contribute to more sustainable and economically viable aquaculture practices. Given the outlined challenges and potential solutions, this study focused on *Rhodopseudomonas julia* LA5.1, a strain with promising bioremediation capabilities, to evaluate its performance in treating aquaculture wastewater under varying salinity conditions.

MATERIALS AND METHODS

Sampling site and conditions

Samples were collected from Lap An Lagoon, Thua Thien Hue Province, Vietnam, on March 10, 2024, during the dry season. At the time of sampling, the water temperature was 29 °C, with a pH of 8.1, reflecting the typical dry-season conditions experienced by the lagoon. The sampling location was 16°13'29.3"N, 108°04'52.5"E (Figure 1), with salinity ranging between 30 and 33‰. Sediment samples were collected from the top 0–5-cm layer over an area of approximately 1 dm² and were placed in sealed Ziplock plastic bags. Water samples were collected in clean 0.5-L PET bottles filled to capacity. Both sediment and water samples were refrigerated in a cooler and transported to the Environmental Laboratory at the University of Sciences, Hue University, on the same day for immediate processing.

Purple photosynthetic bacteria isolation

Water and sediment samples from various locations in Lap An Lagoon underwent a two-stage anaerobic enrichment process (Montiel-Corona et al.,

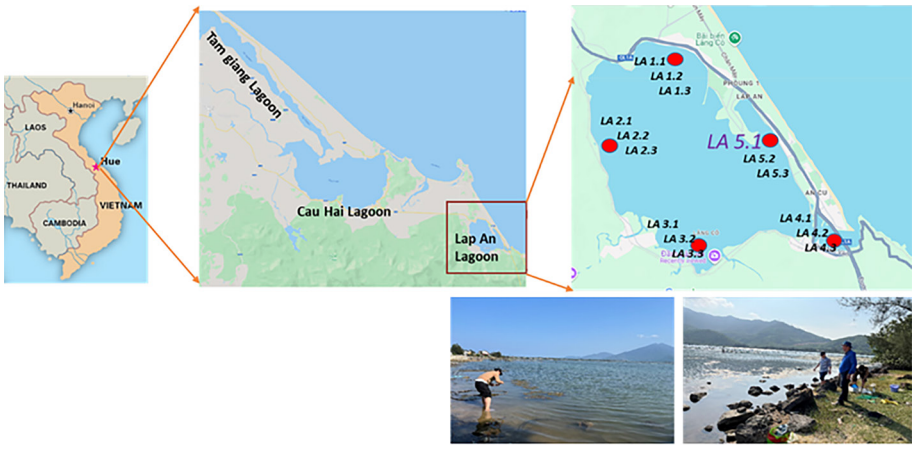

Figure 1. Sampling locations in Lap An (LA) Lagoon, Vietnam

2022). After three weeks of incubation, 50 µL of the enriched culture was streaked onto Rhodospirillaceae Medium (modified) (DSMZ medium 27) agar plates. These plates were incubated under strict anaerobic conditions using an AnaeroPack anaerobic system (Japan) with a light intensity of 2400 lx until colonies appeared (Jiao et al., 2005). Distinctly pigmented colonies were subsequently transferred to DSMZ 27 slant agar tubes and incubated under the same conditions to obtain pure cultures.

The DSMZ medium 27 consisted of disodium succinate (1.0 g/L), KH_2PO_4 (0.5 g/L), ammonium acetate (0.5 g/L), NaCl (0.4 g/L), NH_4Cl (0.4 g/L), $MgSO_4 \cdot 7H_2O$ (0.4 g/L), yeast extract (0.3 g/L), L-cysteine HCl (0.3 g/L), $CaCl_2 \cdot 2H_2O$ (0.05 g/L), Fe(III) citrate (0.005 g/L), resazurin (0.005 g/L), trace elements solution SL6 (1 mL/L), and vitamin B12 solution (0.4 mL/L), all dissolved in 1000 mL of distilled water and adjusted to pH ~6.8. The SL6 trace elements solution contained $ZnSO_4 \cdot 7H_2O$ (0.1 g), $MnCl_2 \cdot 4H_2O$ (0.03 g), H_3BO_3 (0.3 g), $CoCl_2 \cdot 6H_2O$ (0.2 g), $CuCl_2 \cdot 2H_2O$ (0.01 g), $NiCl_2 \cdot 6H_2O$ (0.02 g), and $Na_2MoO_4 \cdot 2H_2O$ (0.03 g), dissolved in 1 L of water. The vitamin B12 solution was prepared by dissolving 10 mg of vitamin B12 in 100 mL of distilled water, which was filtered through a 0.22-µm membrane filter and added to the medium before use. All chemicals used were of analytical grade.

Growth and cultivation conditions of purple photosynthetic bacteria

A single colony of isolated PPB was inoculated in 40 mL of DSMZ 27 liquid medium supplemented with 10 mg/L Na_2S to maintain the reducing conditions. The inoculum (0.5×10^7 cells/mL) to DSMZ 27 liquid medium was prepared at 1:9 (v/v) ratio. To ensure anaerobic conditions, a layer of paraffin oil was added to the culture medium's surface. The culture was incubated at 28 °C with continuous shaking at 120 rpm under a light intensity of 2400 lx. After 7 days of incubation, bacterial growth was assessed by measuring the absorbance at 570 nm (Nguyen et al., 2020) using a spectrophotometer (Cary 60 UV-Vis, Agilent, USA). The growth rate was monitored daily by measuring absorbance at specified intervals during the incubation period.

Phenotypic characteristics

The phenotypic characteristics of the isolated PPB strain were assessed after growth on DSMZ 27 agar plates at 28 °C for 7 days. Colony morphology, including size, shape, and pigmentation, was recorded through direct visual observation. Cellular morphology was determined by Gram staining, which was performed using the HiMedia Gram staining kit (Cat No: K001) according to the manufacturer's instructions. The stained cells were then observed under a phase-contrast Olympus BX51 microscope (Olympus, Japan) to assess the cell shape and arrangement.

Bacteriochlorophyll was extracted from the dense cell biomass obtained by centrifugation of the culture suspension. Pigment extraction was performed using an acetone/methanol (7:2, v/v) solvent mixture. The absorption spectrum of the bacteriochlorophyll in the cell extract was measured using a spectrophotometer (Cary 60 UV-Vis, Agilent, USA), allowing for the identification of specific absorption peaks characteristic of bacteriochlorophyll.

16S rRNA gene sequencing and phylogenetic analysis

A sterile culture of the bacterial strain was grown in Luria–Bertani (LB) broth (Wako Chemical Co. Ltd., Japan) and shaken for 16 h at 30 °C and 180 rpm. Cells were harvested by centrifugation at 13.000 rpm for 1 min at 4 °C. Genomic DNA was extracted using the Top-PURE® Genomic DNA Extraction Kit (Hi-112, ABT, Vietnam), according to the manufacturer's instructions, and stored at 4 °C. DNA quality was assessed using 1% agarose gel electrophoresis. The genomic DNA was diluted to a final concentration of 50 ng/µL for PCR amplification.

The 16S rRNA gene was amplified using the primer pair 27F and 1492R (Hoang et al., 2020) with the following sequences: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The PCR reaction mixture consisted of 15 µL of 2 × GoTaq® Green Master Mix (Promega, USA), 2.5 µL of forward primer (10 pmol), 2.5 µL of reverse primer (10 pmol), 2.5 µL of template DNA (50 ng), and 7.5 µL of sterile distilled water. PCR amplification was performed using a SimpliAmp™ Thermal Cycler (Applied Biosystems, Europe) under the following cycling conditions: initial denaturation at 95 °C for 5 min, followed by 30 cycles at 95 °C for 1 min, 55 °C for 50 s, and 72 °C for 1 min, with a final extension at 72 °C for 7 min. The PCR products were examined by electrophoresis on 1% agarose gel at 70 V in 1X TAE buffer containing SafeView™ dye (20:1 ratio of 1X TAE to SafeView™). Gel images were obtained using an ultraslim LED illuminator (Miulab, China).

Following amplification, the PCR products were sequenced using Sanger sequencing. The resulting nucleotide sequences were analyzed using the BioEdit software and compared with sequences in the GenBank database using the NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/). This comparison of 16S rRNA gene sequence similarities with available sequences enabled the identification of the purple photosynthetic bacteria.

The 16S rDNA sequences of the isolated strain and type strain reference sequences within the *Rhodopseudomonas* genus were retrieved from the LPSN database (https://lpsn.dsmz.de/) and GenBank (https://ncbi.nlm.nih.gov/). Sequence alignment was performed using the MUSCLE tool in the MEGA v.11. A phylogenetic tree was constructed using MEGA v.11 based on the maximum likelihood method, and bootstrap analysis was performed using 1000 replicates to evaluate the robustness of the tree.

Experimental setup for salinity tolerance and wastewater treatment potential of the isolated PPB

The salinity tolerance and wastewater treatment potential of the isolated PPB strain were evaluated in experiments using 100-mL glass bottles under controlled conditions. The bottles were placed in a clean, illuminated environment with a light intensity of 4200 ±250 lx provided by LED lights, and the ambient temperature was maintained between 25 and 30 °C (Figure 2).

The growth medium used for the experiments contained 3.0 g/L CH_3COONa (as a carbon source), 1 g/L yeast extract, 0.5 g/L $MgSO_4 \cdot 7H_2O$, and 1 g/L K_2HPO_4. High-purity $(NH_4)_2SO_4$, $NaNO_2$, and $NaNO_3$ were used as inorganic nitrogen sources for NH_4-N (10 mg/L), NO_2-N (5 mg/L), and NO_3-N (50 mg/L), respectively. The salinity of the medium was adjusted using pure NaCl to achieve concentrations of 0‰, 5‰, 15‰, 20‰, and 30‰. The initial inoculum was prepared by adding 10 mL of bacterial culture to 90 mL of the prepared medium (10% inoculum ratio).

The growth of the PPB strain was monitored by measuring the absorbance at 570 nm and turbidity over time, with cell counts used

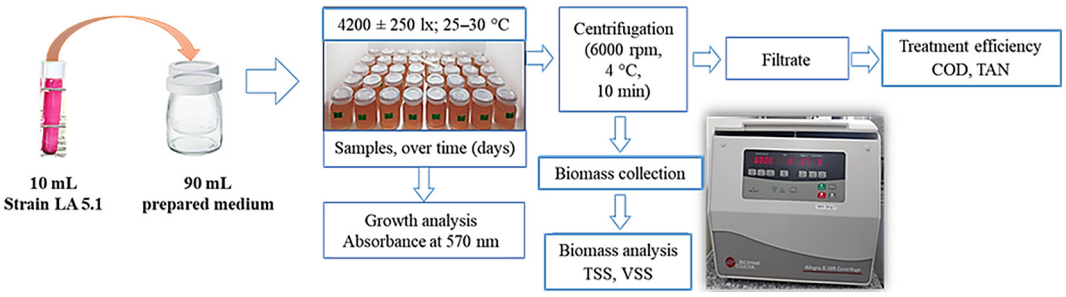

Figure 2. Experimental setup for salinity tolerance and wastewater treatment evaluation of the isolated PPB strain

for secondary verification. Biomass production was quantified in terms of total suspended solids (TSS) and volatile suspended solids (VSS). The potential of the strain for wastewater treatment was assessed by monitoring COD and TAN at regular intervals. The samples were centrifuged for 10 min at 6.000 rpm and 4 °C using a refrigerated centrifuge (Allegra X-30R, Beckman Coulter, Inc., Japan) to separate the biomass. The supernatant was used for COD and TAN analyses, whereas the biomass was dried and analyzed for TSS and VSS. Standard methods (APHA, 2012) were used to analyze the COD, TAN, TSS, and VSS. The COD and TAN concentrations were measured spectrophotometrically using a UV-1800 spectrophotometer (Shimadzu, Japan). Turbidity was determined by optical absorbance measurement following the program 95 instructions using a multiparameter colorimeter (DR/890 Colorimeter, Hach, USA). All experiments were performed in triplicates to calculate the sample mean values.

Statistical analysis

Statistical analyses were conducted using R software (version 4.3.3, accessible at http://cran.R-project.org). Pearson's correlation coefficients were calculated to evaluate the relationships between OD570 and cell concentration, with significance determined at the 5% level ($p < 0.05$).

RESULTS AND DISCUSSION

Isolation and growth of PPB strain LA5.1

In this study, we isolated purple non-sulfur bacteria (PNSB) from the Lap An Lagoon ecosystem to investigate their potential applications for treating aquaculture wastewater and utilizing microbial biomass. After an initial screening of 11 distinct PPB cultures, strain LA5.1 was selected for further investigation because of its robust growth and characteristic pink–purple pigmentation (Figure 3a). This strain exhibited characteristic PPB features and maintained stable growth across multiple subcultures, unlike other isolates, which exhibited diminished growth over time.

Strain LA5.1 was isolated through the anaerobic enrichment of water and sediment samples collected from Lap An Lagoon. These samples were enriched in DSMZ 27 medium exposed to 2400-lx light intensity, using Na_2S (10 mg/L) as the electron donor. After 14 days of incubation, pink–purple colonies were visible on the agar plates, consistent with the characteristics of *Rhodopseudomonas* species. Further purification was achieved by repeated sub-culturing on DSMZ 27 slant agar, resulting in a pure culture of strain LA5.1.

The inoculum (0.5×10^7 cells/mL) was added to DSMZ 27 medium at a 1:9 (v/v) ratio. The strain exhibited a characteristic growth pattern with a short initial delay followed by rapid proliferation,

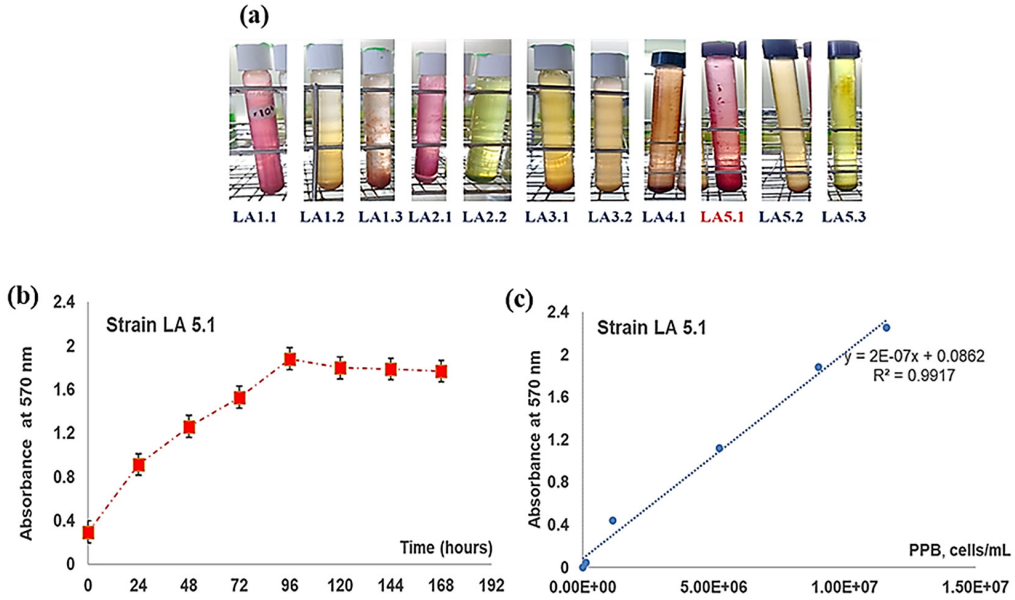

Figure 3. (a) Phenotypic diversity of PNSB strains isolated from Lap An Lagoon in DSMZ 27 medium, (b) growth curve of strain LA5.1 over time, as measured by absorbance at 570 nm, (c) linear correlation between absorbance at 570 nm and bacterial cell density (cells/mL)

reaching its maximum absorbance on the fifth day before entering the stationary phase (Figure 3b). A strong linear relationship was observed between OD570 and the bacterial cell density (Figure 3c). Specifically, when the cell density reached 1.16×10^7 cells/mL, the corresponding OD570 was 2.254, with an R^2 value of 0.992. The robust positive correlation between OD570 and bacterial cell density was further validated by Pearson correlation analysis ($r = 0.996$, $p < 2.2 \times 10^{-16}$), underscoring OD570 as a reliable cell concentration predictor.

The ability of LA5.1 to sustain strong growth through successive transfers in liquid culture media highlights its potential suitability for wastewater treatment applications, where consistent microbial activity is crucial. The observed growth pattern is consistent with that in previous studies on *Rhodopseudomonas* species (Jiao et al., 2005), which also demonstrated a lag phase, followed by exponential growth under anaerobic conditions. The positive correlation between OD570 and bacterial cell density supports OD570's utility as a noninvasive method for monitoring bacterial growth, a widely accepted method in microbiological research.

The capacity of strain LA5.1 to thrive under anaerobic and moderately saline conditions, combined with its consistency across multiple transfers, underscores its potential for practical applications in environments with varying salinity and low oxygen availability. Owing to these characteristics, it is particularly suitable for use in wastewater treatment systems, especially in aquaculture, where rapid microbial growth and metabolic adaptability are crucial for the efficient removal of organic matter and nutrients.

Phenotypic and bacteriochlorophyll analysis

The phenotypic characteristics of strain LA5.1 were examined to confirm its identity as a PNSB. Strain LA5.1 formed smooth, glossy colonies with a reddish-purple coloration and rounded edges when grown on DSMZ 27 agar plate (Figure 4b). In liquid culture, the strain exhibited a distinct pink–purple coloration (Figure 4a). Gram staining revealed that strain LA5.1 was Gram-negative, which is consistent with the typical traits of PPB. Microscopic observations showed that the cells were short rods (Figure 4c), a characteristic feature of this strain.

The bacteriochlorophyll content of strain LA5.1 was extracted using an acetone/methanol (7:2, v/v) solvent mixture. The cell extract from strain LA5.1 exhibited absorption maxima at 494, 577, 684, and 770 nm (Figure 5). The absorption peak at 770 nm indicates the presence of bacteriochlorophyll-a, a characteristic pigment found in purple bacteria, particularly within the genus *Rhodopseudomonas* (Redd et al., 1972; Hajdu et al., 2017).

Although bacteriochlorophyll-a in purple bacteria usually exhibits absorption maxima at 805 and 830–890 nm, in this study, the acetone extract displayed maximum absorption at 770 nm, which corresponded to the lower range of the typical absorption spectrum for bacteriochlorophyll-a (Hajdu et al., 2017). This observation aligns with the findings of Tarabas et al. (2019), who reported a similar absorption peak at 770 nm for bacteriochlorophyll-a in *Rhodopseudomonas yavorovii* IMV B-7620.

Bacteriochlorophyll-a plays a crucial role in energy capture, particularly in low-oxygen environments, such as anaerobic wastewater systems. The presence of this pigment in strain LA5.1 suggests its suitability for environments where oxygen is limited or variable, such as aquaculture wastewater systems, making it a promising candidate for bioremediation and treatment applications. According to Imhoff (2005), PNSB exhibit adaptability in similar environments, where these

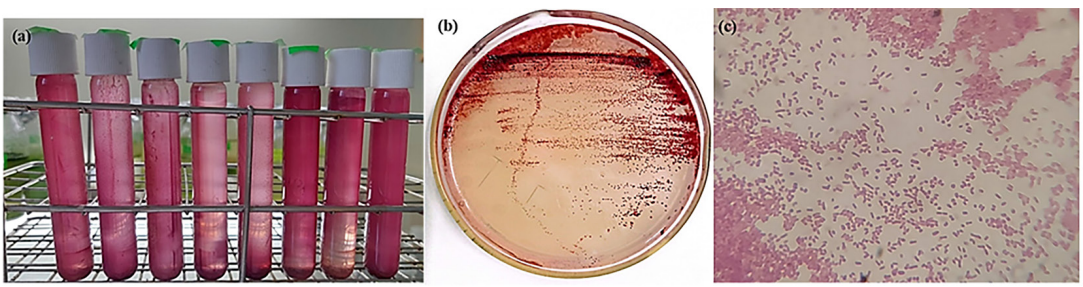

Figure 4. (a) Liquid culture of strain LA5.1 in DSMZ 27 medium, (b) colonies of strain LA5.1 on a DSMZ 27 agar plate, (c) microscopic image of the Gram-stained cells of strain LA5.1, using simple staining under a 100× objective lens

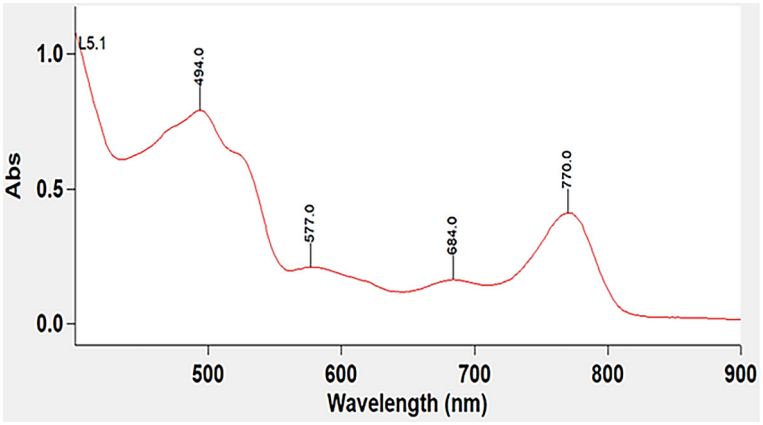

Figure 5. Absorption spectrum of bacteriochlorophyll-a extract from the photosynthetic bacterium strain LA5.1

bacteria flourish using light as an energy source in the absence of oxygen, contributing to their ecological success and industrial applications.

In addition to bacteriochlorophyll-a, the pigmentation and growth characteristics of the strain strongly resemble those of other *Rhodopseudomonas* species. The combination of phenotypic traits and pigment analysis confirmed its classification within this genus, reinforcing its potential in biotechnological applications, particularly in wastewater treatment, where the ability to remove organic matter and nutrients is essential (Rao et al., 2000).

By leveraging its photosynthetic capabilities, strain LA5.1, which can metabolize organic substances in wastewater systems, uses light as an energy source to facilitate these processes under anaerobic conditions. This metabolic versatility, coupled with the ability of the strain to perform efficiently in environments with varying oxygen levels, highlights its significance in wastewater treatment systems. Moreover, the capacity of the strain for sustained growth under anaerobic conditions suggests its viability for long-term use in various environmental and industrial applications (Panwichian et al., 2010).

16S rDNA gene sequencing and phylogenetic analysis

The 16S rDNA gene of strain LA5.1 was sequenced, yielding a 1,260-bp nucleotide sequence. BLAST analysis revealed a 99.84% match with *Rhodopseudomonas julia* (NR_115228.1). The sequence was deposited in GenBank with accession number PP916626 (https://www.ncbi.nlm.nih.gov/search/all/?term=PP916626). Phylogenetic analysis using the neighbor-joining method confirmed the identity of LA5.1. The strain formed a well-supported clade (100% bootstrap value) with *Rhodopseudomonas julia* DSM 11549T and ATCC 51105T (Figure 6). These reference strains were isolated from sulfur springs, further corroborating the close relationship between LA5.1 and other *Rhodopseudomonas julia* strains.

This genetic similarity and phylogenetic placement confirm strain LA5.1's identity as *Rhodopseudomonas julia*, highlighting its evolutionary conservation within the species and its potential for applications in wastewater treatment, particularly in saline environments.

Salinity tolerance of R. julia LA5.1

The growth of *R. julia* LA5.1 was tested in DSMZ 27 medium containing CH_3COONa (3 g/L), yeast extract (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), K_2HPO_4 (1 g/L, equivalent to 174 mg/L P-PO_4), and varying concentrations of NaCl (0‰, 5‰, 15‰, 20‰, and 30‰). Growth was monitored by measuring the absorbance at 570 nm (OD570) and turbidity (NTU), as shown in Figures 7a and 7b. Optimal growth was observed at salinity levels of 5–15‰, while 30‰ salinity reduced growth to less than half of that observed at 0‰. After 6 days at 0‰ salinity, *R. julia* LA5.1 achieved growth equal to an OD570 of 1.787, with lower values in nitrogen-supplemented media.

TSS and VSS analyses showed that the majority of the biomass was composed of volatile solids, with a VSS/TSS ratio of 0.91 ± 0.02, indicating high microbial activity. Biomass production was consistent with the increases in OD570 and turbidity, as shown in Figure 7c.

Although *R. julia* LA5.1 was isolated from Lap An Lagoon's saline water, salinity had a

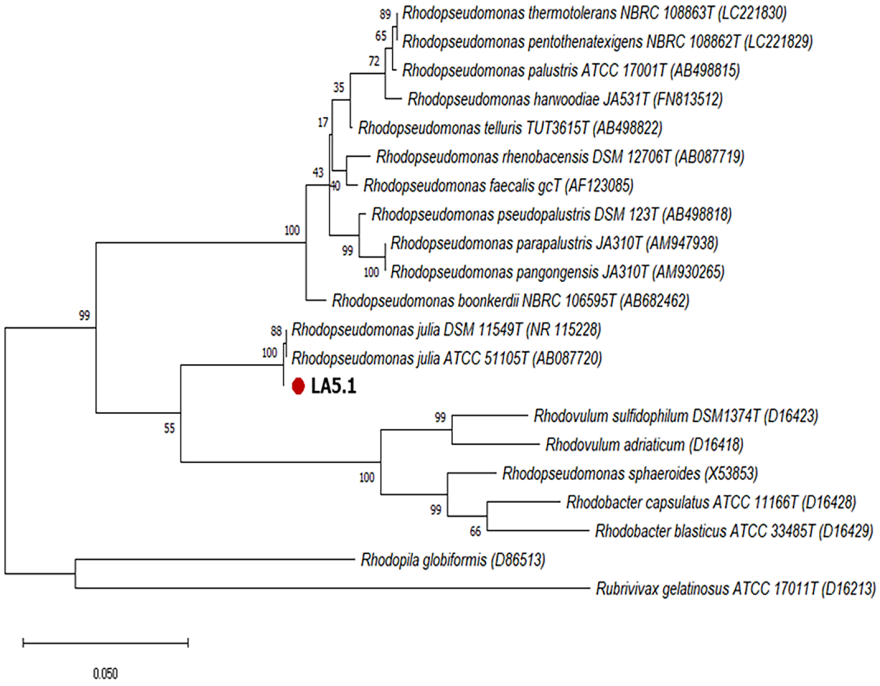

Figure 6. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain LA5.1 with other members of the genus *Rhodopseudomonas*

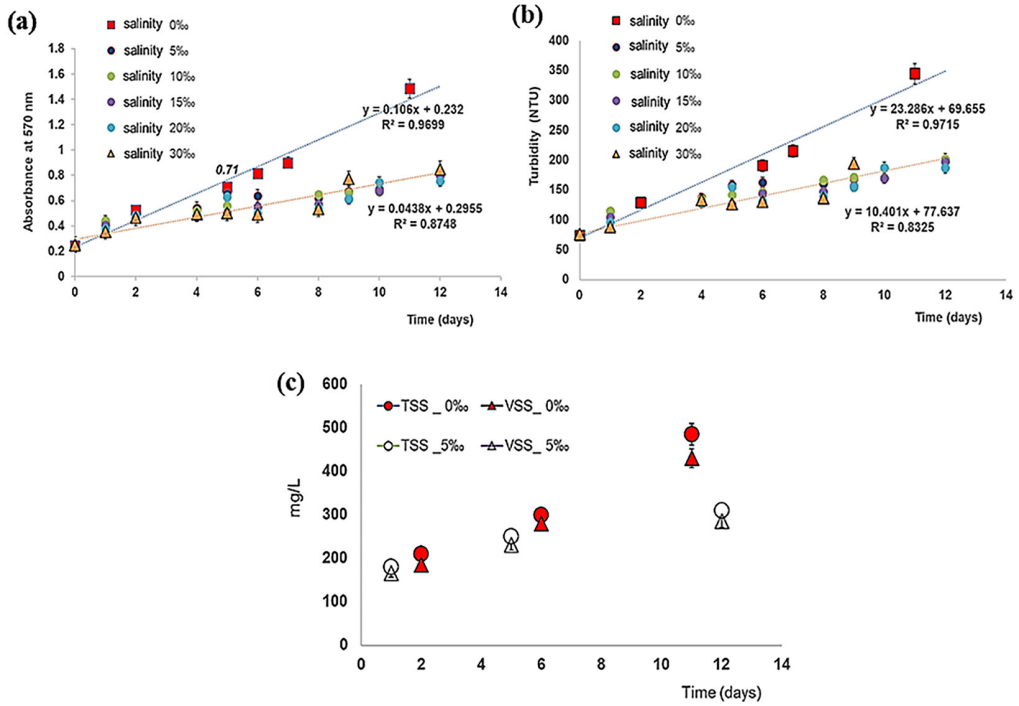

Figure 7. Growth of *Rhodopseudomonas julia* LA5.1 at different salinity levels (0‰, 5‰, 10‰, 15‰, 20‰, and 30‰) over 12 days, measured as: (a) absorbance at 570 nm, (b) turbidity (NTU), (c) TSS and VSS of strain LA5.1 at 0‰ and 5‰ salinity

significant impact on its growth. Optimal growth was observed at moderate salinity levels (5–15‰), while high salinity (30‰) greatly reduced its growth. This indicates that further adaptation to high salinity is necessary for potential industrial applications, regardless of origin.

The salinity tolerance of *R. julia* LA5.1 aligns with the findings of Panwichian et al. (2010) for

Rhodopseudomonas palustris, which also showed reduced growth at higher salinity. Imhoff (2005) emphasized the need to optimize salinity levels for optimal growth performance, noting that elevated salinity can cause osmotic stress and inhibit bacterial metabolism. Similar trends in biomass production and volatile suspended solids were reported for *Rhodopseudomonas* strains by Rao et al. (2000).

Organic matter and nutrient removal by R. julia LA5.1

R. julia LA5.1 demonstrated remarkable adaptability to salinity levels ranging from 0‰ to 30‰, efficiently degrading organic contaminants (COD) and eliminating nutrients (TAN) within a twelve-day retention time (Figure 8). The strain achieved a 60–80% reduction in COD after six days when the initial COD concentration was 800 mg/L (Figure 8a), highlighting its strong potential for wastewater treatment. Additionally, *R. julia* LA5.1 reduced TAN by 80% within a five-day period (Figure 8b).

Other *Rhodopseudomonas* species have shown similar COD removal capabilities. For example, *R. palustris* achieved 50–90% COD removal from odorous swine wastewater (Kim et al., 2004), while *R. sphaeroides* (Z08) demonstrated 70–94% COD removal efficiency in synthetic soybean wastewater (Prachanurak et al., 2014). However, the capacity of *R. julia* LA5.1 to function effectively across a wide range of salinity levels provides a distinct advantage, particularly in challenging environments such as aquaculture wastewater.

Additional evidence for this adaptability is provided in studies on other PPB that have demonstrated comparable effectiveness under high-salinity conditions. For example, saline-adapted PPB have been found to thrive in salinity ranges between 30 and 70 mS/cm and efficiently remove COD, nitrogen, and phosphorus with high biomass yields and minimal H_2S production under sulfate-rich conditions (Hülsen et al., 2014). This capability to operate in both saline- and sulfate-rich environments without any significant biological or operational issues, such as sulfide toxicity, aligns with the demonstrated resilience of LA5.1. Such characteristics are critical in wastewater treatment applications where high salinity and sulfate levels can pose significant challenges.

The ability of *R. julia* LA5.1 to withstand fluctuating salinities makes it especially well-suited for treating saline wastewater, where traditional microbial treatments often struggle. Mixed PPB cultures have demonstrated up to 90% COD removal from domestic and poultry wastewaters (Hülsen et al., 2014; Hülsen et al., 2018). *R. julia* LA5.1's capacity to flourish in both freshwater and saline environments offers wider applicability. This adaptability is crucial for sustainable treatment systems in aquaculture, where the salinity can vary significantly.

Consistent with findings from other *Rhodopseudomonas* strains used for biogas slurry treatment (Yang et al., 2017), *R. julia* LA5.1 demonstrated an 80% reduction in TAN, reinforcing its robust performance across different salinities. This makes it a promising candidate for widespread use in various wastewater systems, including industrial, domestic, and agricultural applications. Although *R. julia* LA5.1 has proven effective, its diminished efficiency at higher salinities indicates the need for further optimization, potentially through adaptive evolution or genetic engineering.

To implement this strain on a large scale, it is crucial to develop efficient bioreactors that can

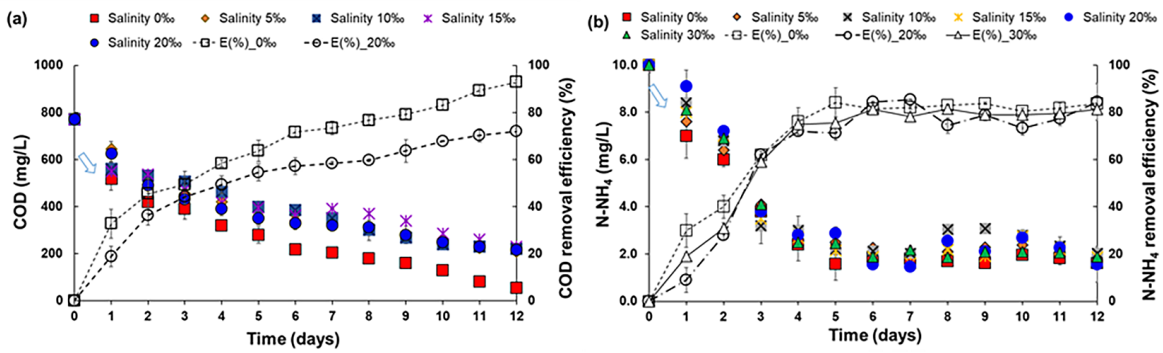

Figure 8. (a) COD removal efficiency of *Rhodopseudomonas julia* LA5.1 at different salinities. (b) N-NH_4 removal by *Rhodopseudomonas julia* LA5.1 at different salinities

sustain high biomass concentrations and operate cost-effectively. Integrating *R. julia* LA5.1 into existing systems, such as anaerobic lagoons or bioreactors, could significantly enhance wastewater treatment efficiency. To assess its viability for widespread industrial applications, a comprehensive cost-benefit analysis is necessary in comparison with conventional methods.

CONCLUSIONS

This study successfully isolated and characterized *R. julia* LA5.1 from Lap An Lagoon, demonstrating its adaptability across a range of salinities (0–30‰). The strain exhibited robust COD and TAN removal efficiencies, particularly at 0‰ salinity, achieving up to 80% COD removal within six days, even at high initial COD concentrations (~800 mg/L). Its nutrient-removal capacity, especially in brackish water, highlights its potential for sustainable wastewater treatment in aquaculture and nutrient-rich industries. However, the decline in performance at higher salinities (20‰ and above) suggests that further optimization is necessary for saline environments. Overall, *R. julia* LA5.1 is a promising approach for wastewater treatment applications, particularly in nutrient-rich effluents. Future research should focus on scaling up the applications and investigating the function of isolated strains in mixed-culture systems to maximize treatment efficiency.

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