

# Exploring the potential of Marine Actinobacteria DMA2 (*Nocardiopsis* sp.); Characterization and Rhodamine B Dye Degradation in Environmental Applications

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In this study, eight bacterial strains were isolated from water and sediment samples from the Ennore Creek area. Morphological and biochemical analyses identified three genera: *Streptomyces* sp., *Nocardia* sp., and *Nocardiopsis* sp. The *Nocardiopsis* DMA2 strain exhibited the highest exopolysaccharide (EPS) production, primarily comprising carbohydrates (68.6%), proteins (12.3%), nucleic acids (10.4%), and other substances (8.7%). Scanning Electron Microscopy (SEM) revealed smooth, porous surface morphology, while Fourier Transform Infrared Spectroscopy (FTIR) identified functional groups including hydroxyl, carbonyl, and carboxyl. The EPS exhibited significant dye degradation capacity, with 1000 mg adsorbing 78.71% of Rhodamine B dye. This study demonstrates the potential of marine actinobacteria-derived EPS in bioremediation applications, highlighting their functional groups and bioactivities. The findings suggest a promising role for *Nocardiopsis* DMA2 in industrial and environmental applications, particularly in dye degradation and biopolymer production. Further research may explore the molecular mechanisms and optimization of EPS production and functionality.

**Keywords:** Marine Actinobacteria, EPS, Carbohydrates, Proteins, FTIR, Rhodamine B, Nocardiosis, Dye degradation.

## 1. Introduction

Marine environments, particularly sediments, harbor a rich diversity of microorganisms that hold significant biotechnological potential (Bech et al., 2020). Among these, marine actinobacteria have garnered considerable attention due to their ability to produce a wide array of bioactive compounds (Kamala et al., 2023; Karthikeyan et al., 2022). One such group, the genus *Nocardiosis*, has been recognized for its diverse metabolic capabilities, making it a valuable resource for environmental and industrial applications (Farda et al., 2022). This study focuses on a specific strain, *Marine ActinobacteriaNocardiosis* sp., exploring its potential for Rhodamine B dye degradation and its broader environmental applications. Rhodamine B is a synthetic dye widely used in textiles, paper, and other industries. It is known for its stability and resistance to degradation, making it a persistent environmental pollutant (Saigl et al., 2021; Khan et al., 2017). *Marine ActinobacteriaNocardiosis* sp. employs enzymatic pathways to break down Rhodamine B into less harmful compounds (Ting et al., 2020; Rando et al., 2022). The degradation process involves adsorption of dye molecules to the bacterial cell surface, followed by enzymatic breakdown by peroxidases and laccases, leading to decolorization and detoxification (Saravanan et al., 2021; Alsukaibi et al., 2022). Additionally, this strain produces extracellular polymeric substances (EPS), which play a crucial role in the biodegradation process by enhancing the stability and adherence of the bacterial cells to the dye molecules, thereby facilitating more efficient degradation (Mahto et al., 2022; Wei et al., 2023). Extracellular Polymeric Substances (EPS) are a term for numerous classes of macromolecules found inside diverse microbial assemblages, including polysaccharides, proteins, nucleic acids, lipids, and other polymeric substances (Decho et al., 2017; Nagar et al., 2021). The bioremediation potential of *Marine ActinobacteriaNocardiosis* sp. extends beyond dye degradation, encompassing a range of environmental applications (Dias et al., 2023). In wastewater treatment, the bacterium's ability to degrade various organic pollutants, including dyes, phenols, and hydrocarbons, can significantly reduce environmental pollution (Aragaw et al., 2021; Chan et al., 2022). In soil remediation, the application of this bacterium can help degrade organic pollutants, restoring soil health and fertility (Rebello et al., 2021). Moreover, in marine environments, this strain can be employed to break down oil spills and other organic pollutants, helping to preserve marine biodiversity. The production of EPS by *Nocardiosis* sp. not only aids in pollutant degradation but also contributes to the formation of biofilms, which can further enhance the bioremediation process by providing a protective environment for the bacterial cells.

## 2. Materials and methods

Isolation and identification of marine actinobacteria

Using both conventional and molecular techniques, 15 marine actinobacterial strains were isolated and identified in the current study from Ennore Creek in Chennai. The diverse colony

morphologies, colour of the aerial and substrate mycelia, and soluble and melanoid pigments of these various isolates were used to select them.

#### Extraction of EPS

Marine actinobacteria were cultured in YM broth (pH 8) at 35°C for 5 days to produce extracellular polymeric substances (EPS). EPS extraction followed the Kamala et al. (2020) method with modifications: cells were separated by centrifugation at 10,000 rpm for 60 minutes at 4°C, and the pellet was suspended in ice-cold ethanol (95%), mixed, and left for 24 hours at 4°C. The EPS precipitate was recovered by centrifugation at 13,000 rpm for 30 minutes at 4°C, purified with two ethanol washes, and dialyzed against double-distilled water for 48 hours at 4°C (Sivaperumal et al., 2018).

#### Characterization of EPS

##### Scanning Electron Microscopy (SEM)

Using a scanning electron microscope, the surface morphology of the marine actinobacterium EPS that had been extracted was examined (SEM). The analysis was conducted using dried EPS samples. A JEOL JSM-630 J scanning electron microscope running at 20 kV was used for the scanning electron microscopy (SEM) (Ahmed et al., 2013).

##### Fourier transform infrared spectroscopy analysis (FT-IR)

The functional groups were determined by characterising the EPS samples, and the groups were verified by FT-IR analysis. Using KBr pellets, the EPS samples were described from 4,000 to 400 cm<sup>-1</sup>. This study examined the IR spectra of the marine actinobacterium's extracted EPS samples (Castro et al., 2014).

##### Dye degradation using EPS

Adsorption experiments were conducted by adding marine actinobacterial EPS adsorbent in three concentrations (0.25g, 0.50g, and 1g) to 50 mL solutions of Rhodamine B in four conical flasks while stirring. The mixtures were kept in an orbital shaker at 450 rpm and room temperature for durations ranging from 12 to 48 hours. To determine the optimal contact time for complete decolorization of the dyes, the effect of contact time between the adsorbent and adsorbate was tested over this period. UV absorption of Rhodamine B dye wavelength is 558nm.

### 3. Results and discussion

#### Isolation and identification of EPS producing marine actinobacteria

In the present study 8 bacterial strains were isolated from water and sediment sample on Ennore creek area (Fig.1). Eight isolates were shown different morphological characteristics and those strains were taken from identification through conventional identification. Those 8 strains belong to 3 genera which are *Streptomyces* sp., *Nocardia* sp., and *Nocardiopsis* sp.,



Figure 1. Pure culture of Actinobacteria- Yeast Malt agar plating *Nocardioopsis* sp.

*Nocardioopsis*: The EPS producing isolates Dye-degradation Marine Actinobacteria) (DMA2 and DMA6 are gram positive, aerobic, non-motile organism has shown indole, vogesproskauer and TSI negative. Methyl red, citrate, oxidase, catalase and urease test positive. Furthermore, lactose, maltose, sucrose, xylose, starch, and inositol can all be used to make acid by this organism. Both isolates produce plentiful to limited amounts of aerial mycelium, and their substrate mycelium is well established. The aerial hypha on the substrate mycelium frequently produces chains of spores. These cultures developed successfully, and the dense aerial mycelium on ISP2 media was whitish grey (Wgy) (DMA2) and white (W) (DMA6) in colour and devoid of melanoid pigments. Both isolates DMA2 and DMA6 has produced yellowish soluble pigment and reverse side pigments on ISP7 medium. It will grow on both solid and liquid media. The aerial mycelium is powdery and entirely broken up into spores of different sizes. There is no definitive sugar pattern, but tiny amounts of ribose and galactose were seen in the cell wall peptidoglycan, which contains significant amounts of meso-diaminopimelic acid and represents the cell wall chemo type III. The isolates DMA7 and DMA8 are believed to belong to the genus *Nocardioopsis* based on the cell wall peptidoglycan and sugar pattern as well as micro morphological examinations. Among the 8 different isolates, the highest EPS production was observed from the *Nocardioopsis* DMA2 and that strain was selected for further studies of EPS production, different characterization and different dye degradation.

#### EPS production and Characterization

EPS were removed from *Nocardioopsis* DMA2 strain using physical and chemical techniques (Fig. 2). Based on the lyophilized weight yield produced by the *Nocardioopsis* sp., DMA2 strain, the total amount of EPS was estimated. Yeast extract, malt extract, and glucose were included in the basic growth medium to stimulate the formation of EPS (Fig.2). In YM broth, the prospective strain of DMA2 produced EPS primarily made up of carbohydrates (68.6%), followed by proteins (12.3%), nucleic acids (10.4%), and unknown substances (8.7%).



Figure 2. Culture of Nocardiopsis Actinobacteria for EPS production & Process of EPS and Separation of EPS

### Characterization

SEM analysis: The surface morphology of the EPS producing *NocardiopsisDMA2* was exposed by the SEM (Scanning Electron Microscopy) technique (Fig 3). The morphology was appeared mostly in smooth and porous structures are present.

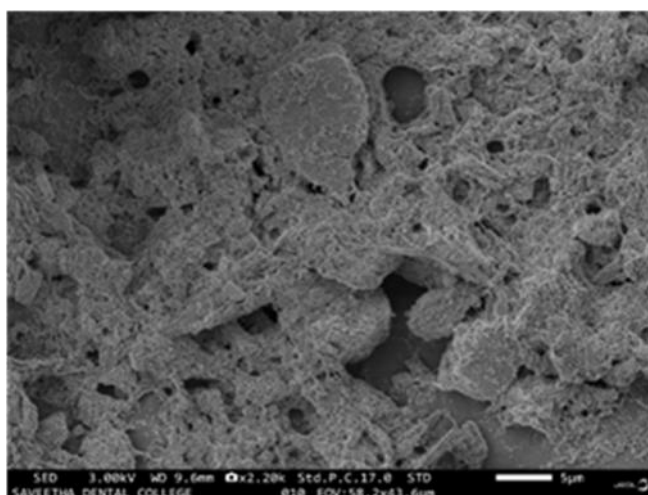


Figure 3. SEM image of EPS from *Nocardiopsis* sp.

Fourier Transform Infrared Spectrophotometer (FT-IR) Analysis of EPS from marine actinobacterium of *Nocardiopsis DMA2*:

The formed EPS were examined to analyse the functional groups present in the cellular metabolites found in the *NocardiopsisDMA2* (Fig. 4). In the marine actinobacterial mediated EPS metabolites from the bacteria acting a pivotal role in EPS production. The FTIR spectrum of the compound reveals several distinctive absorption peaks corresponding to various functional groups. A strong, broad peak at 3546  $\text{cm}^{-1}$  indicates the presence of O-H stretching,

characteristic of an alcohol group. Medium intensity peaks at 2925 cm<sup>-1</sup> and 2857 cm<sup>-1</sup> suggest C-H stretching vibrations typical of alkanes. A strong absorption peak at 2016 cm<sup>-1</sup> signifies N=C=S stretching, indicative of an isothiocyanate functional group. Additionally, a strong absorption at 1643 cm<sup>-1</sup> points to C=C stretching vibrations found in alkenes, while a medium intensity peak at 1239 cm<sup>-1</sup> indicates C-N stretching typical of amines. Furthermore, a strong absorption peak at 1078 cm<sup>-1</sup> corresponds to C-O stretching, characteristic of primary alcohols, and a strong peak at 544 cm<sup>-1</sup> suggests C-I stretching, suggesting the presence of a halo compound containing iodine. Marine actinobacteria's EPS composition has been investigated using FTIR analysis. Orhan-Yanikan (2020) discovered that the EPS isolation technique can impact the FTIR analysis outcomes, with liquid medium extraction yielding a greater protein content. With Banerjee (2019) concentrating on the presence of carbohydrate moieties and protein-associated amides, and Caccamo (2019) investigating the thermal characteristics of an EPS from a marine thermotolerant *Bacillus licheniformis*, both researchers utilized FTIR to characterize EPS from marine bacteria. Noufal (2022) provided evidence regarding the ability of EPS derived from marine actinobacteria belonging to *Streptomyces* species to suppress the growth of MCF-7 cancer cells. Exopolysaccharides (EPS) with a variety of functional groups have been discovered to be produced by marine actinobacteria, which suggests that they could find use in a wide range of industrial and biological applications. Functional groups hydroxyl, carbonyl, carboxyl, and sulfate were detected in the extracellular polymer synthesized by *Microbacterium aurantiacum* FSW-25 and *Streptomyces griseorubens* GD5, respectively, by Sran (2019) and Vinothini (2019). These functional groups support the EPS's biofunctional, viscosifying, and antioxidant qualities. Sahana (2019) described the EPS from *Alteromonas* sp. PRIM-28, which also shown bioactivities such as wound healing and cell proliferation. Together, these investigations demonstrate the potential of extracellular polymeric substances (EPS) derived from marine actinobacteria and the significance of FTIR analysis in elucidating their functional groups and bioactivities.

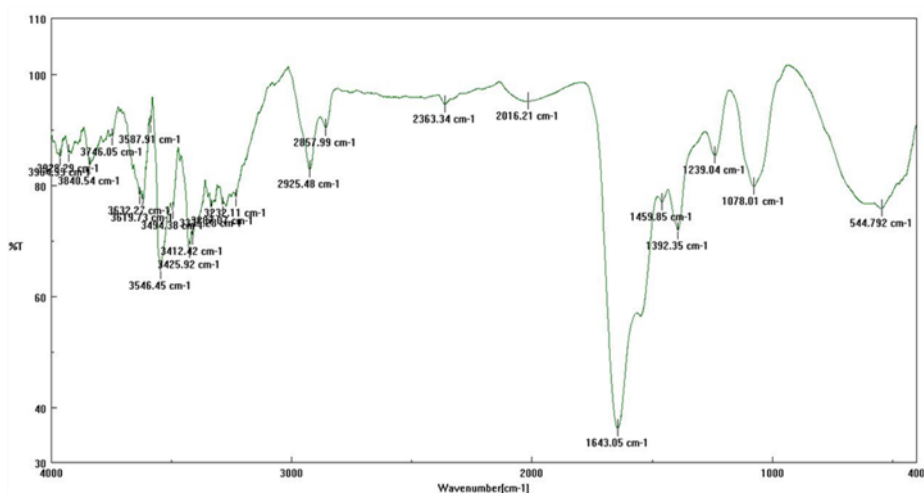


Figure 4. FT-IR Characterization of EPS from *Nocardiopsis* sp.

## Dye degradation

The effect of adsorbent dosage varied from 250mg, 500mg and 1000mg in three concentrations



of EPS were used. The adsorption of Rhodamine B dyes (Fig. 5) percentage removal of 50mL was observed. The UV-Vis absorbance for the colour shift in six experimental beakers containing various amounts of adsorbent was used to measure the adsorption effect of EPS from marine actinobacterium on Rhodamine B. The results are displayed in (Fig. 6). Figure: 7 shows the comparison of colour change in Rhodamine B by EPS adsorbent. It is evident that 1000mg of EPS adsorbing capacity is 78.71% for Rhodamine B adsorbent given better results. The residue pellets of Rhodamine B dye (Fig.8&9) were used for the functional group characterization. Numerous research has investigated how actinobacteria EPS degrades the color rhodamine B. The ability of particular bacterial strains, *Brevundimonas diminuta* and *Coelastrella* sp., respectively, to degrade the dye was shown by Saravanan (2022). Baldev (2013) emphasized the part played by microalgae in this process, whereas Saravanan (2022) went on to identify the breakdown products of Rhodamine B. Ting (2020) gave a more comprehensive summary of actinobacteria's capacity to remove dyes, highlighting their efficiency in this regard. Tahir (2020) elaborated on the function of *Staphylococcus* sp. exopolysaccharides in the uptake of co-contaminants, such as dyes, and offered a possible direction for future study in this area. *Brevundimonas diminuta* has demonstrated the ability to degrade Rhodamine B dye, as evidenced by Saravanan (2022), who identified the resulting breakdown products. Additionally, George (2022) reported that extracellular polymeric substances (EPS) from the actinobacterium *Micromonospora* sp. possess antioxidant properties, suggesting a potential role in dye degradation. Pham (2023) further discussed the application of EPS-producing extremophilic bacteria in dye degradation, highlighting their resilience in harsh environments. Mohod (2023) explored advanced oxidation techniques for Rhodamine B degradation, providing insights into their effectiveness. Collectively, these studies indicate a potential role for actinobacteria-derived EPS in the degradation process of Rhodamine B dye.



Figure 5. Preparation of Dye stock solution



Figure 6. Rhodamine B dye degradation initial stage



Figure 7. Comparison of color change by EPS adsorbent

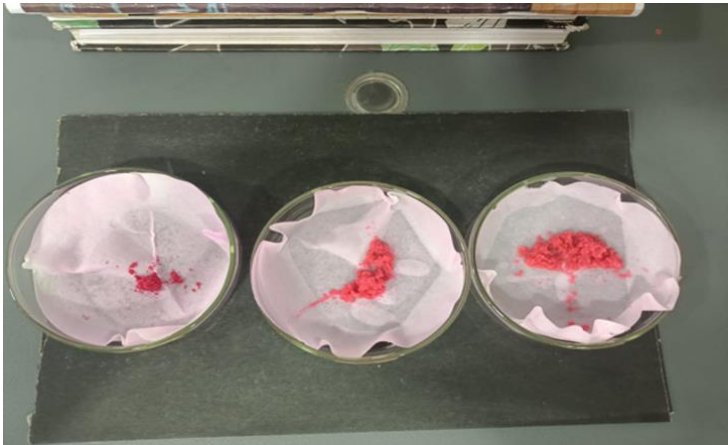


Figure 8. Dried pellets of Rhodamine B dye



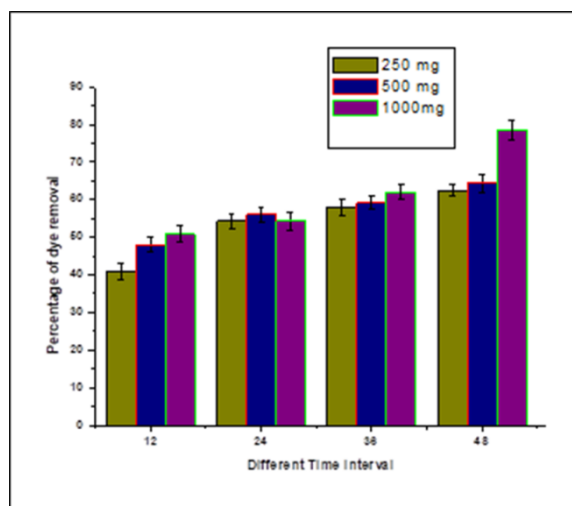


Figure 9. Percentage of dye removal using EPS

#### 4. Conclusion

From the results it was observed that, potential environmental applicable marine actinobacterial EPS were extracted for the dye degradation. Rhodamine b was showed potential adsorption by EPS and the maximum biosorption of dye. While increasing the concentration of EPS, the dye removal capacity also increased. Hence, the present research work concluded that, the Marine actinobacterial EPS can be used as an effective bio adsorbent in the treatment of dye pollutants.

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#### Conflict of interest

All authors state that, we do not have any conflict of interest.

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