RESEARCH PAPER

Eosin Yellow Dye Degradation Using Marine Actinobacteria EPS and Their Characterization

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ABSTRACT

The discharge of synthetic dyes such as Eosin Yellow (EY) into aquatic environments poses significant ecological and health risks due to their toxicity, persistence, and resistance to conventional degradation methods. In this study, a marine Streptomyces sp. was isolated from sediment samples collected along the Tuticorin coast and identified based on morphological and chemotaxonomic characteristics. Extracellular polymeric substances (EPS) produced by this isolate were extracted and characterized using Fourier-transform infrared spectroscopy, which indicated the presence of different functional groups including hydroxyl, carboxyl, sulfate, phosphate, and halogenated moieties. These groups are known to mediate biosorption and possibly catalyze degradation reactions. The EPS was then evaluated for its ability to degrade Eosin Yellow dye at different concentrations (250, 500, and 1000 mg/L) over time intervals up to 48 hours. Spectrophotometric analysis showed a time- and concentration-dependent increase in dye removal efficiency, with maximum degradation (89.07%) observed at 1000 mg/L after 48 hours. The enhanced performance is attributed to the presence of reactive functional groups that facilitate both adsorption and potential redox-based interactions. These findings highlight the potential application of marine actinobacterial EPS as a sustainable, biodegradable, and cost-effective biopolymer for the treatment of dye-contaminated wastewater, contributing to environmentally safe bioremediation strategies.

Keywords: Marine Actinobacteria, Streptomyces sp., Extracellular Polymeric Substances, Eosin Yellow Degradation, Wastewater Bioremediation

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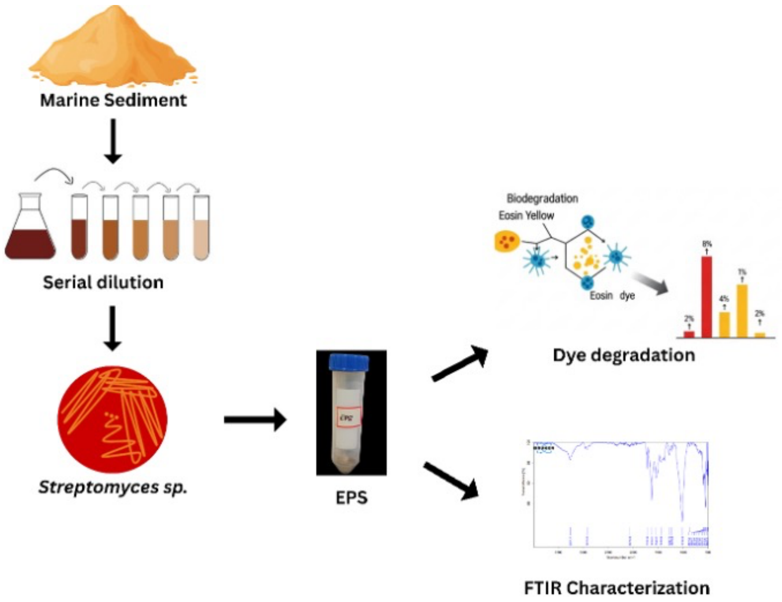

Graphical abstract

Introduction

The widespread use of synthetic dyes in the textile, cosmetic, and pharmaceutical industries has led to significant environmental contamination, particularly in aquatic ecosystems. Eosin Yellow (EY), a xanthene dye widely applied for staining and dyeing purposes, is notably persistent and toxic in nature due to its complex aromatic structure, which renders it resistant to natural degradation pathways (Rani Rosaline et al. 2020). The release of such dyes into water bodies results in reduced light penetration, lower dissolved oxygen, and disruption of photosynthetic activity in aquatic flora, consequently affecting biodiversity (Hoque et al. 2024). Moreover, their carcinogenic and mutagenic potential poses serious health hazards to both wildlife and humans (Dutta et al. 2024). Conventional physicochemical treatment methods such as coagulation-flocculation, membrane filtration, and advanced oxidation, have disadvantages, including high operational costs, partial degradation, and secondary contamination from remaining sludge (Aragaw and Bogale 2023). Biological methods, particularly microbial degradation, have gained prominence due to their ecological sustainability and potential for complete mineralization of pollutants. Microorganisms such as bacteria, fungi, and actinobacteria have shown remarkable potential in degrading or immobilizing synthetic dyes due to their metabolic versatility and extracellular enzymatic systems (Zhang et al. 2019). However, the effectiveness of microbial-based dye removal often depends on the secreted bioactive materials, notably extracellular polymeric substances.

Extracellular polymeric substances are high molecular weight biopolymers produced by microorganisms that play essential roles in nutrient capture, surface adhesion, metal ion binding, and pollutant sequestration. Composed mainly of polysaccharides, proteins, nucleic acids, and lipids, EPS exhibits a complex network of functional groups such as hydroxyl, carboxyl, phosphate, sulfate, and amine moieties (Vu et al. 2009). These chemical groups enable EPS to interact with contaminants through hydrogen bonding, electrostatic attraction, and complexation mechanisms (Flemming et al. 2025). The potential of microbial EPS, especially those derived from marine bacteria, in pollutant degradation has gained increasing attention due to their unique structural properties and enhanced chemical stability under saline or extreme environmental conditions (Lo Giudice et al. 2020). Marine actinobacteria, particularly those belonging to the genus *Streptomyces*, are prolific producers of bioactive secondary metabolites and biopolymers, including EPS. Isolates from marine sediments have shown enhanced biosorption potential due to their adaptive mechanisms to saline and metal-rich environments (S. Alharbi 2016). EPS produced by marine *Streptomyces* exhibit diverse functional groups that facilitate the binding of heavy metals and synthetic dyes. For instance, EPS from *Nocardiopsis sp.* 13H was shown to remove over 88% of cesium ions via biosorption, with carboxyl and amide groups playing a critical role in ion binding (Sivaperumal et al. 2018). Marine actinobacteria-derived EPS can be harnessed not only for heavy metal remediation but also for effective dye degradation in wastewater. The chemical characterization of EPS is crucial for understanding its interaction with dye molecules. Fourier-transform infrared spectroscopy is widely used to identify functional groups within EPS that contribute to pollutant binding. Peaks associated with hydroxyl, carbonyl, amide, phosphate, and halogenated groups have been reported in EPS from marine biofilm-forming bacteria and diatoms (Elleuch et al. 2025). These groups not only aid in biosorption but may also mediate redox reactions that destabilize dye structures. Moreover, recent studies suggest that halogenated groups within marine EPS may play a role in enhancing antimicrobial activity and dye decolorization potential (Siddik and Satheesh 2019). This study aims to isolate a marine *Streptomyces* strain, characterizing its extracellular polymeric substances (EPS), and assessing their effectiveness in degrading Eosin Yellow dye as a sustainable solution for wastewater treatment.

Materials and methods

Sample collection

Marine sediment samples were collected from the Tuticorin coast, Tamil Nadu, using a Van Veen grab. The collected sediments were carefully transferred to the laboratory in sterile containers. Upon arrival, the samples were air-dried for 48 hours and then sun-dried for an additional 12 hours. After drying, the samples were finely macerated using a mortar and pestle.

Isolation of actinobacteria

Actinobacteria were counted on KUA medium supplemented with 10 µg/mL of nalidixic acid and cycloheximide, which act as antibacterial and antifungal agents, respectively. Following serial dilution, the sediment samples were spread onto KUA plates and incubated at room temperature for seven days. Distinct colonies were isolated and subcultured to obtain pure strains for further analysis (Fig. 1). Based on the International Streptomyces Project (ISP) guidelines, conventional identification involved assessing aerial mycelium color, spore chain morphology, carbon source utilization, melanoid pigment production, reverse side pigmentation, and soluble pigments. Chemotaxonomic characteristics were used to help identify marine actinobacteria (Noufal et al. 2022).

Extraction of EPS from actinobacteria

EPS extraction from *Streptomyces sp.* was carried out following the method described by (Homero et al. 2021) with slight modifications. The culture was grown under standard conditions for 7 days, after which the broth was centrifuged at 5000 rpm for 15 minutes at 20 °C to remove bacterial cells. The supernatant containing extracellular EPS was collected and subjected to sonication to enhance EPS release. The sonicated supernatant was centrifuged again, and the EPS was precipitated by adding three volumes of cold 95% ethanol, followed by incubation at 4 °C for 24 hours. The resulting precipitate was collected by centrifugation at 15,000 rpm for 30 minutes, washed with ethanol, lyophilized, and stored at 4 °C (Fig. 2).

Characterization of EPS

The extracellular polymeric substance (EPS) was characterized using Fourier-transform infrared (FT-IR) spectroscopy. The IR spectrum was obtained with a Bruker Alpha II spectrophotometer, scanning in the range of 1000 to 3500 cm^{-1} at a scanning speed of 1 µm/min.

Results and discussion

Isolation and identification of actinobacteria

The marine actinobacterial isolate was identified as *Streptomyces sp.* based on its morphological and chemotaxonomic characteristics. On ISP medium, the strain shows grey aerial mycelium. The strain did not produce melanin, soluble pigments, but reverse pigmentation was observed on the reverse side of the colony (Table1). The colony surface was dry and showed filamentous growth with aerial hyphae. These phenotypic features are consistent with classical descriptions of *Streptomyces sp.,* and support preliminary genus-level identification (Silva et al. 2016). Chemotaxonomic analysis revealed the presence of LL-diaminopimelic acid (LL-DAP) and glycine in the peptidoglycan layer, while DL-diaminopimelic acid (DL-DAP), lysine, alanine, and ornithine were absent. This amino acid composition indicates a type I cell wall. Whole-cell sugar analysis indicated that arabinose, galactose, xylose, madurose, and ribose were not detected, and the sugar pattern was categorized as non-characteristic (N.C.) (Table 2). Such sugar profiles, although variable among *Streptomyces*, are commonly reported in isolates from marine environments and may reflect adaptation to specific ecological conditions (Buangrab et al. 2022).

FTIR characterization of EPS

Fourier-transform infrared spectroscopy was used to determine the functional groups present in the extracellular polymeric substances (EPS) extracted from marine *Streptomyces sp.* (Fig 3). The spectrum revealed a strong, broad absorption at 3267.11 cm^{-1}, corresponding to C-H stretching in alkynes and overlapping with N-H and O-H groups, indicating the presence of alcohols and primary amines. These hydrophilic groups are functionally important in water retention, hydrogen bonding, and biosorption activities of microbial EPS (Elnahas et al. 2017; Mahmoud et al. 2023). A strong absorption at 2927.83 cm^{-1}, attributed to N–H stretching in amine salts, further confirms the nitrogen-rich nature of the EPS, which may enhance metal binding and antimicrobial potential (Chakraborty et al. 2023). A distinct peak at 2074.83 cm^{-1} was assigned to N=C=S stretching, indicating the presence of isothiocyanate groups, which are often associated with bioactive sulfur-containing secondary metabolites in marine actinomycetes (Alam et al. 2021). The band at 1723.36 cm^{-1} corresponded to C=O stretching in α,β-unsaturated esters, which likely originates from uronic acids or acetylated polysaccharides. These groups are known to impart a negative charge to the EPS matrix, facilitating cation exchange, flocculation, and gel formation (Sivaperumal et al. 2018). The peak at 1633.01 cm^{-1}, assigned to C=C stretching, confirmed the presence of unsaturated carbon groups, while the strong peak at 1544.86 cm^{-1} was attributed to nitro compounds (N–O stretching), which have also been linked to antimicrobial and redox-active molecules in EPS (Wang et al. 2021). C–O stretching vibrations detected at 1280.76 cm^{-1} and 1227.62 cm^{-1}, associated with aromatic esters and alkyl aryl ethers respectively, suggest the presence of glycosidic linkages and substituted sugar units typical of complex polysaccharides (Yu et al. 2020). The band at 1019.25 cm^{-1} was consistent with C–F stretching, a rare but reported feature in marine microbial metabolites. A peak at 867.34 cm^{-1}, assigned to C–H bending in 1,3-disubstituted aromatics, indicates the presence of aromatic ring structures, which can enhance the structural rigidity and functional stability of EPS (Wang et al. 2016). A unique feature of this EPS spectrum was the appearance of multiple peaks in the halogen fingerprint region: 599.47 cm^{-1} (C-Br), 587.54 cm^{-1} (C-Cl), and 557.40, 538.15, 514.30 cm^{-1} (C-I). The presence of halogenated functional groups in EPS is uncommon in terrestrial bacteria but has been frequently reported in marine actinobacteria, particularly *Streptomyces* strains, as an adaptive response to mineral-rich and high-salinity environments (Al-Dhabi et al. 2018).

Dye degradation using EPS

The efficiency of extracellular polymeric substances (EPS) derived from marine *Streptomyces sp.* in degrading Eosin Yellow dye was evaluated at three concentrations (250 mg/L, 500 mg/L, and 1000 mg/L) over time intervals of 6, 12, 24, and 48 hours (Fig. 4). Dye removal was quantified spectrophotometrically by measuring absorbance reduction at the dye characteristic wavelength. At 250 mg/L, the degradation progressed from 69.17% at the start to 81.87% after 48 hours. Similarly, the 500 mg/L concentration yielded an increase from 70.89% to 83.25%, and the highest efficiency was observed at 1000 mg/L, with dye removal improving from 68.73% initially to 89.07% at 48 hours. This consistent enhancement confirms that dye removal is both concentration and time dependent due to greater availability of active sites and prolonged interaction between dye molecules and EPS components.

The improved degradation efficiency at higher EPS concentrations is attributed to the increased availability of functional groups such as hydroxyl, carboxyl, phosphate, sulfate, and amino groups. These groups, present in the EPS matrix, are known to interact with dye molecules through electrostatic forces, hydrogen bonding, and van der Waals interactions (Sivaperumal et al. 2018). FTIR studies have confirmed the presence of these reactive sites, which promote dye binding and retention. In addition to passive adsorption, EPS may play an active role in the breakdown of dye structures. Several reports suggest that EPS can mediate localized redox activity, potentially destabilizing the dye chromophoric system or facilitating electron transfer processes that contribute to dye decolorization (Gao et al. 2019). Mechanism of Eosin Yellow degradation involves a multi-step interaction. First, the anionic dye binds electrostatically to the cationic regions or protonated amine groups of EPS. Subsequently, hydrophilic and hydrophobic interactions stabilize the dye within the EPS matrix (Salih et al. 2023). At higher EPS concentration, some dye molecules may become encapsulated within polymeric clusters, facilitating sustained degradation or irreversible binding. In some cases, the EPS structure may promote mild redox transformations, further enhancing dye breakdown. Similar EPS-based mechanisms have been documented in marine *Nocardiopsis* and *Klebsiella* systems, which support both adsorption and biotransformation pathways (Wei et al. 2016).

Conclusion

This study highlights the potential of marine-derived *Streptomyces sp.* as a sustainable source of extracellular polymeric substances capable of efficiently degrading synthetic dyes such as Eosin Yellow. The EPS exhibited a chemically diverse structure, as confirmed by FTIR spectroscopy, containing functional groups responsible for strong dye binding and possible redox interactions. The observed degradation efficiency increased with EPS concentration and exposure time, indicating the involvement of multiple mechanisms including electrostatic attraction, hydrogen bonding, and matrix entrapment. With a maximum removal efficiency of 89.07% at 1000 mg/L EPS, the findings underscore the environmental relevance of marine actinobacterial EPS in eco-friendly dye remediation strategies. Further exploration into the scalability, biodegradability, and regeneration of EPS-based systems could contribute significantly to the development of green technologies for industrial wastewater treatment.

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CRediT author statement:

Deepasakthi: Experiment design, Draft making, **Pavithra Thiraviyam:** Draft making, critical revision of the work; **Ragul Gunasekaran:** Critical revision, **Dhanraj Ganapathy:** Data interpretation, Critical Revision, **Pitchiah Sivaperumal:** Revision, Concept, and design of the work, final draft making.

Data Availability declaration: Based on the request, the corresponding will provide the data.

Declaration of Competing Interest

Authors do not have any conflict of interest and all authors are agreed to submit the manuscript to the journal.

Funding Declaration

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Figure

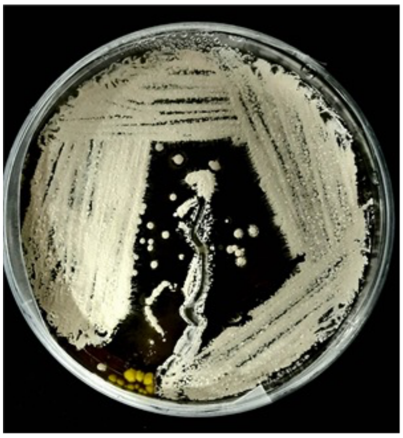

Fig. 1. Isolation of *Streptomyces sp.* from marine sediment on KUA medium.

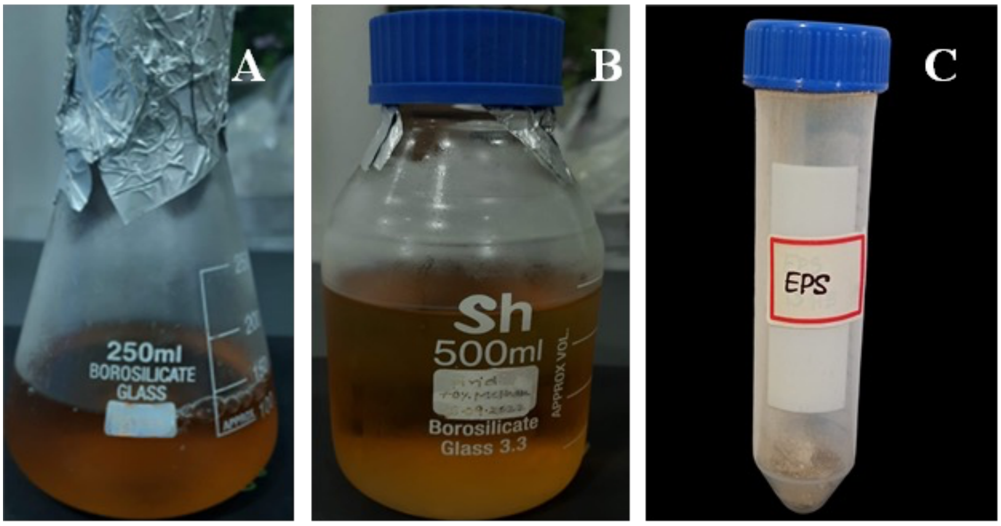

Fig. 2. Extraction of extracellular polymeric substances (EPS) from *Streptomyces sp.* (A) Broth culture after incubation, (B) EPS precipitated by the addition of cold ethanol, and (C) lyophilized EPS obtained following centrifugation and freeze-drying.

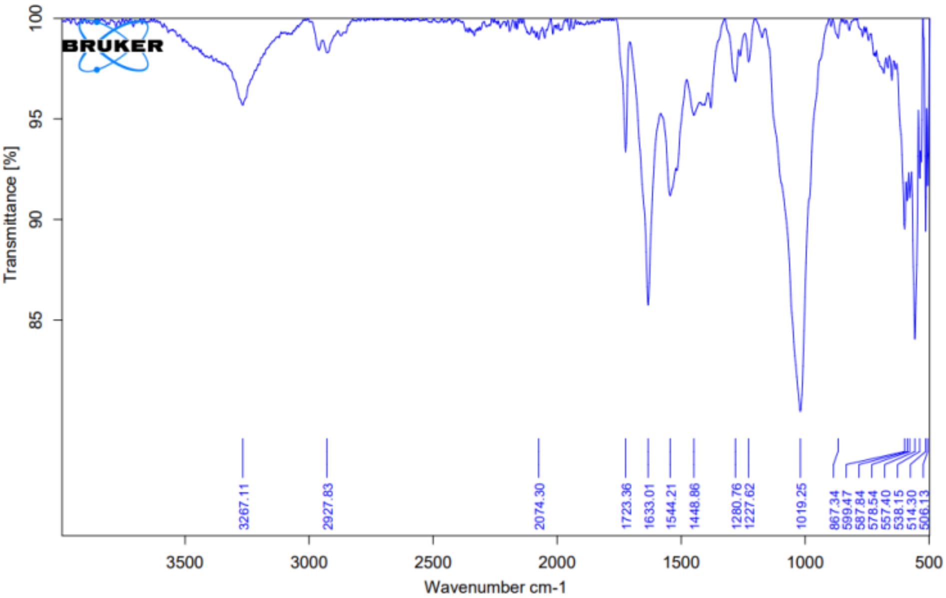

Fig. 3. FTIR spectrum of extracellular polymeric substances (EPS) extracted from *Streptomyces sp.*

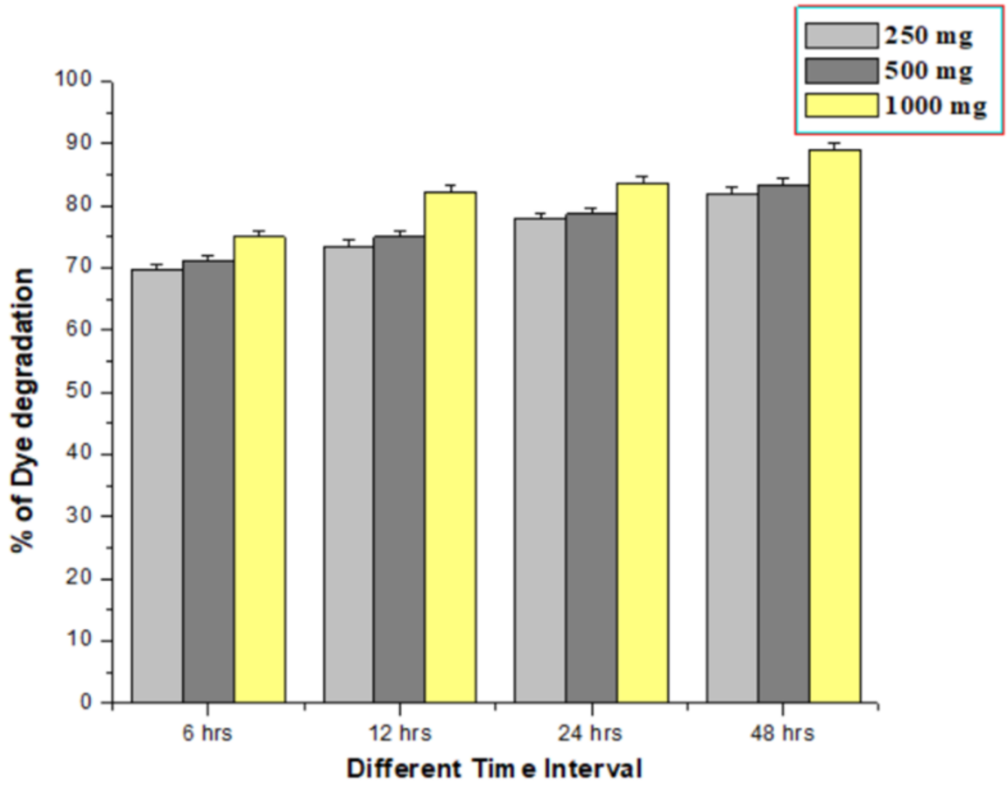

Fig. 4. Degradation of Eosin Yellow dye by EPS from *Streptomyces sp.* at varying concentrations (250, 500, and 1000 mg/L) over different time intervals.

Table 1 Morphological characteristics of marine *Streptomyces sp.* observed on ISP medium.

Aerial mycelium color	White
Melanoid pigment	-
Soluble pigment	-
Reverse side pigment	-

Table 2

Chemotaxonomic characteristics of marine *Streptomyces sp.* based on cell wall amino acid and sugar composition.

Cell wall amino acid		**Whole cell sugar**	
LL-DAP	+	**Arabinose**	**-**
DL-DAP	**-**	**Galactose**	**-**
Glycine	+	**xylose**	**-**
Alanine	**-**	**Madurose**	**-**
Lysine	**-**	**Ribose**	**-**
Ornithine	**-**	**Sugar pattern**	**N.C**