

Fabrication and Characterization of EPS Mediated Nanofiber Production by Marine Actinobacteria 13HB (*Streptomyces* Sp.) and their Antibacterial Potential

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The marine actinobacterial strain 13HB, identified as *Streptomyces* sp., produces extracellular polymeric substances (EPS) with promising applications. Morphological analysis observed yellow aerial mycelium with no melanoid pigment but soluble pigment production. Chemotaxonomical observations indicated the absence of arabinose, galactose, xylose, madurose, and ribose, with the cell wall containing LL-DAP and glycine. Scanning electron microscopy (SEM) showed EPS-mediated nanofibers with smooth surfaces, consistent diameters, and a dense network structure. Fourier-transform infrared spectroscopy showed the functional groups such as hydroxyls, carboxylic acids, and aromatic compounds, enhancing the fibrous structural properties. Antibacterial activity demonstrated significant inhibition of *Escherichia coli* growth by the EPS fibrous membrane, while *Pseudomonas aeruginosa* showed less susceptibility. These findings suggest that EPS-mediated nanofibers from *Streptomyces* sp. 13HB exhibit potential for biomedical and environmental applications due to their structural integrity, functional group diversity, and antibacterial properties.

Keywords: Marine actinobacteria, *Streptomyces* sp., Nanofabrication, Antibacterial, Eco

friendly.

1. Introduction

The electrospinning technique is widely recognized for its ability to produce various polymers matrices and nanofibers, typically with diameters ranging from 50 to 500 nm (Bhagure et al., 2020). Recently, nanofibers are created by applying high electric field to a polymer solution, by meticulously controlling process parameters to achieve specific nanomaterial properties (Chen et al., 2022). The modern advancements in nanotechnology have opened up new avenues for the development of novel bio materials with unique properties. Among these materials, nanofibers have attracted significant attention due to their remarkable characteristics, such as high surface area-to-volume ratio, mechanical strength, and versatility in various applications ranging from biomedicine to environmental remediation (Bhushan, 2017). Marine actinobacteria are a group of Gram +ve bacteria found in marine environments, known for their significant contribution to the production of a wide array of bioactive compounds. These microorganisms are renowned for their ability to thrive in extreme marine environmental conditions, such as extreme salinity, pressure, and temperature fluctuations, which makes them a rich source of unique and diverse secondary metabolites (Rathore et al., 2020; Kamala et al., 2023). There is a wide variety of species in the marine ecosystem that have evolved to survive in extreme environmental conditions. They are excellent biocatalysts that can be used in a variety of biotechnological applications and creative, sustainable industrial processes due to their resistance to changes in salt, temperature pH, and contaminants. These organisms produce distinct therapeutic effects due to the vast diversity and extent of the marine environment. Actinobacteria have historically been characterized by high GC content in their DNA (Barka et al., 2015; Kamala et al 2023; Antony et al, 2024). Marine actinobacteria produce extracellular polymeric substances (EPS), complex polymers, into the environment. Because they provide protection, adhesion, and structural support, these materials are important to the microbial population (Vijayakumar et al., 2022). EPS has been linked to several biological activities, such as immunomodulatory, antiviral, antibacterial, and antioxidant properties (Rodrigues et al. 2020). Extracellular polymeric substances generated from marine microorganisms have demonstrated considerable promise as a source of bioactive compounds with distinctive chemical structures and possible medical uses (Gan et al., 2023). The genus *Streptomyces* is notable for producing an impressive 80% of actinobacterial marine natural products and, therefore demonstrating an unmatched biosynthetic capability in the microbial world (Hu et al., 2015). Marine *Streptomyces* are known to produce wide range of active natural products, including immunosuppressant, antifungal, anticancer, antiparasitic, or antithrombotic properties (Wang et al., 2021). A major advantage of electrospun nanofiber engineering is the capability to fabricate composite polymer fibers with diverse properties by incorporating different drugs to thereby imparting the desired therapeutic effects (Maliszewsha et al., 2022). Electrospun nanofibers can be infused with various agents, such as antimicrobial biopolymers, nanoparticles and carbon nanomaterials, to enhance their antimicrobial properties (Maleki Dizaj et al., 2019). Electrospun nanofibers show great promise in wound dressings. Their large surface area enhances wound healing (Khazaeli et al., 2020). The study focuses on a marine actinobacterium, *Streptomyces* sp., isolated from sediment samples, known for extracellular polymeric substances were utilized for fabrication

and antibacterial potential.

2. Materials and methods

2.1. Sample collection and preparation

The Marine sediment samples were collected around the Thoothukudi coast in Tamil Nadu. The samples were carefully transferred into sterile containers and brought to the Marine Biomedical Research Lab and Environmental toxicology unit. The samples were air-dried for 48 hours and then sun-dried for 12 hours. The air-dried samples were then ground using a mortar and pestle.

2.2. Isolation and identification of extra polymeric substances from actinobacteria

Isolation and enumeration of actinobacteria were carried out in Kuster's medium supplemented with 0.5% NaCl. To minimize the fungal and bacterial contamination, KUA medium was supplemented with 10 μ g/ml cycloheximide and nalidixic acid. The sediment samples were serially diluted, spread on a KUA medium, and incubated at 37 °C for one week. Distinctly morphologically colonies of Actinobacteria were selected for pure culture and further extraction of EPS. The isolates were identified by poly phasic taxonomy which includes morphological observation, analysis of carbon sources and cell wall analysis was confirmed by observing their distinctive characteristics (Fig 1).



Fig.1 Isolation and production of marine actinobacteria 13HB (*Streptomyces* sp.,) producing extracellular polymeric substances

2.3. Nanofiber Fabrication Preparation of EPS Solution

A polystyrene solution (15%) was prepared in dimethylformamide, incorporating extracellular polymeric substances EPS at a concentration of 1%. EPS-embedded nanofibers were then fabricated using the electrospinning process. This procedure was based on the protocol of Mostafa et al. (2022), with slightly modified to accommodate the inclusion of EPS. Stir the solution until the EPS is completely dissolved. Now, load the EPS solution into a syringe equipped with a blunt-ended needle. Set the electrospinning parameters voltage, flow rate, and distance between the needle and collector. Collect the nanofibers on an aluminum foil-covered collector. For bacteriological and cell culture experiments, the samples were decontaminated by soaking in 70% ethanol for 10 minutes. This was followed by two washes in sterile D.H₂O, and then exposed to UV light at 254 nm for 30 min.

2.4. Characterization of Nanofiber

The EPS-mediated nanofiber was analyzed using FT-IR spectroscopy. The IR spectrum of the nanofiber was observed using a Bruker Alpha II spectrophotometer. The IR spectrum of the nanofiber was recorded over the range of 1000 to 3500 cm⁻¹ at a scanning speed of 1µm/min. The size, and shape of the EPS-mediated nanofiber were analyzed using Scanning electron Microscopy (SEM) outlined by (Pitchaiah et al., 2023).

2.5. Bacteriological experiment

Escherichia coli and Pseudomonas aeruginosa were selected to assess the antibacterial activity of EPS fibrous membranes was assessed by culturing the broth at room temperature for 24 h. Initially, 20 ml of sterilized nutrient agar was poured into petri plates, after solidification, 120 µl of bacterial culture were evenly spread across the plates using a spreader. The fibrous membrane was then placed on the surface of the agar medium. Subsequently, the culture plate was incubated at 37 °C for 24 hrs, after which the antibacterial activity was observed.

3. Results and discussion

3.1. Isolation and identification of extracellular polymeric substances

The morphological observations of the EPS-producing marine actinobacterial strain 13HB, identified as Streptomyces sp., are as follows: the aerial mycelium is yellow in color (Table 1). The strain does not produce melanoid pigment but does produce soluble pigment. There is no reversible pigment observed. In the chemotaxonomical study of the Streptomyces sp. strain, whole-cell sugar analysis revealed the absence of arabinose, galactose, xylose, madurose, and ribose. The cell wall amino acid composition included the presence of LL-DAP and glycine, while DL-DAP, alanine, lysine, and ornithine were absent. The sugar pattern was noted as not characterized (N.C). The cell wall type was identified as type I, and the spore morphology was classified as Rectiflexibiles- Atrovirens (Table 2). In 2023, Lertcanawanichakul observed that Streptomyces sp. KB1 is a gram-positive bacterium with long filamentous capable of producing aerial mycelia and spores. It metabolizes mannose, glucose, fructose, and lactose as carbon sources with acid production. However, it does not utilize ribose, rhamnose, arabinose, galactose, inositol, maltose sucrose, glycerol and arabinose shows no growth on MacConkey agar.

Table 1.Morphological observation of Streptomyces sp.

Morphological observation	
Aerial mycelium color	Y
Melanoid pigment	-
Soluble pigment	.+
Reversible pigment	-
Index	<i>Streptomyces sp.</i>

Table 2.Chemotaxonomical study of Streptomyces sp.

Cell wall amino acid		Whole cell sugar	
LL-DAP	.+	Arabinose	-
DL-DAP	-	Galactose	-
Glycine	.+	xylose	-
Alanine	-	Madurose	-

Lysine	-	Ribose	-
Ornithine	-	Spore morphology	RA
Cell wall type	I		

3.2. Characterization of scanning electron microscopy image of EPS mediated nanofiber of 13 HB from streptomyces sp.

The scanning electron microscopy (SEM) image of the EPS mediated nanofibers produced from streptomyces sp., captured at an accelerating voltage of 5.00 kV with a magnification of 20,000X, reveals a uniform, and interconnected network of smooth, continuous fibers. The nanofibers exhibit consistent diameters, ranging from nanometers to a few micrometers, indicating a controlled synthesis process. The surface of the fibers is smooth and free from significant defects, suggesting high-quality production. The dense network formation, with fibers overlapping and intersecting, contributes to enhanced mechanical properties and a high surface area, beneficial for applications in filtration, tissue engineering, and nanocomposites. The porous structure within the network, with visible voids and inter-fiber spaces, further highlights the suitability of these nanofibers for applications requiring permeability and diffusion, such as filtration membranes and drug delivery systems. Overall, the SEM analysis confirms the effectiveness of the EPS mediation method in producing well-defined nanofibers with desirable morphological and structural characteristics for various advanced applications. Similarly, the 5 micrometer scale fibers are smaller in diameter compared to the 10 μm fibers (Fig 2 a.). Similar to the 10 μm fibers, these also exhibit smooth surfaces without significant irregularities or defects (Fig 2 b). These fibers also appear randomly oriented without specific alignment. The network structure appears denser compared to the 10 μm fibers, with smaller spaces between the fibers, suggesting higher porosity at the smaller scale. No surface features such as embedded nanoparticles or significant roughness indicate that these fibers are also pure and uncontaminated. Similar mat-like formation in 10 μm but with a denser network due to the smaller scale. In 2023, Yevhen et al., observed the inclusion of PEG resulted in thinner fibers, with the sample containing PEG exhibited a higher potential of nanometer-sized fibers, particularly those below 100 nm in diameter. Moreover, the PEG-containing sample exhibited an increased no. of pores with sizes ranging below 50 nm^2 . SEM images illustrated the formation of uniform nanofibers, with an average nanofiber diameter ranging from 50–200 nm, as reported by Mehwish et al., 2022. Similarly, the average fiber diameter was determined through manual measurement of randomly chosen fibers in scanning electron microscope. The resulting nanofibers are amorphous, approximately 500 nm in diameter, with a specific surface area of about. 8 m^2g^{-1} , and 5093 km cm^{-3} . Heshem et al. (2022) observed SEM morphologies showing defect-free and uniform nanofibers in PLA, PCL, and PLA/PCL mats. These fibers exhibited diameters ranging from 200 to 500 nm. The comparison between the 5 μm and 10 μm fibers reveals that smaller-diameter fibers exhibit a denser network and higher porosity. This finding is supported by the research of Patel et al. (2017), who observed that reducing fiber diameter generally leads to increased surface area and porosity, enhancing the material's function. The visible voids and inter-fiber spaces within the network highlight the porous structure of the nanofibers, which is crucial for permeability and diffusion applications. This observation aligns with the work of Kumar et al. (2018), who found that nanofibers with controlled porosity are highly effective in filtration membranes and drug delivery systems due to their sustainability to facilitate mass transfer and fluid flow. The dense network formation with overlapping and intersecting fibers is indicative of enhanced mechanical properties, as

noted by Li et al. (2019).

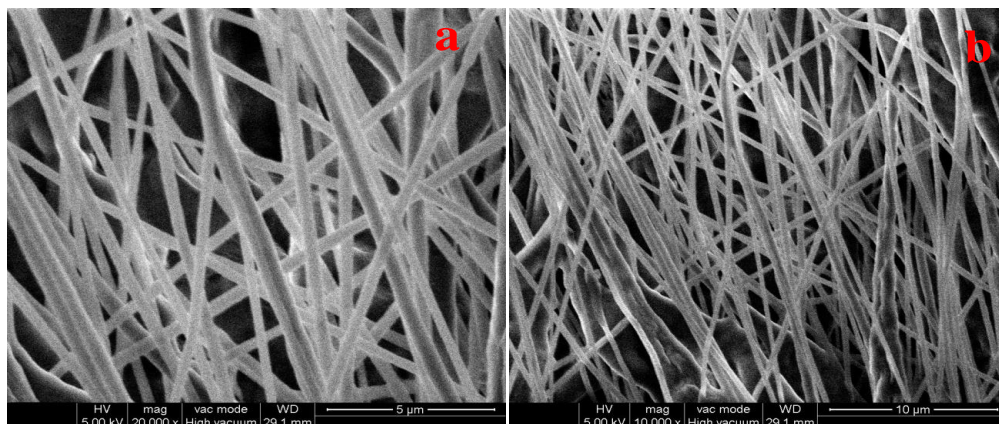


Fig 2. SEM analysis of EPS mediated nanofiber a) 5 μm , b) 10 μm

3.3. Characterization of Fourier transform infrared spectroscopy analysis of EPS mediated nanofiber of 13 HB from streptomycessp.

Fourier-transform infrared spectroscopy analysis of EPS-mediated nanofibers provides valuable insights into their chemical structure and functional groups (Fig 3). The FTIR spectrum shows a strong, broad peak at 3251.04cm^{-1} corresponding to the O-H stretching vibration, typical of alcohols. This suggests the presence of hydroxyl groups, likely from polysaccharides or other carbohydrate components in the EPS. A strong peak at 2357.01cm^{-1} is associated with the asymmetric stretching of CO, indicating adsorbed carbon dioxide, a common occurrence during sample preparation. The medium peak at 1400.80cm^{-1} corresponds to the O-H bending vibration of carboxylic acids, indicating the presence of acidic polysaccharides or other carboxylated compounds. A strong peak at 1032.90cm^{-1} is attributed to C-F stretching, suggesting the presence of fluorine-containing compounds, possible. The peak observed at 869.81cm^{-1} is attributed to C-H bending in 1, 2, 4-trisubstituted aromatic compounds, indicating aromatic constituents in the EPS. Finally, the strong peak at 703.39cm^{-1} is associated with C=C bending vibrations of alkenes, suggesting the presence of unsaturated bonds. Similar, the peak in the range of $2,975\text{--}2,880\text{cm}^{-1}$ corresponds to the vibration frequency of -CH groups in alkyl chains. The distinct sharp band at $1765\text{--}1,685\text{cm}^{-1}$ is attributed to the carbonyl group of acetylated groups of CTS. The peak observed in the range of $1,120\text{--}990\text{cm}^{-1}$ indicated the C-O-C group of CTS and siloxane linkage Si-O-C and Si-O-Si in the cross linked nanofiber (Wang et al., 2016). According to Xue et al. (2020), the presence of hydroxyl groups indicates that the EPS is rich in polysaccharides, which enhance its hydrophilicity and biocompatibility, making it suitable for biomedical applications. The strong peak at 2357.01cm^{-1} , associated with the asymmetric stretching of CO, suggests the presence of adsorbed carbon dioxide. Smith et al. (2018) reported that CO_2 adsorption from the atmosphere is common during sample preparation, especially in samples with high surface areas like nanofibers. Wu et al. (2019) highlighted that the presence of carboxyl groups indicates uronic acids in the EPS, enhancing the nanofibers' metal-binding capacity and environmental remediation potential. The strong peak at 1032.90cm^{-1} , attributed to C-F stretching, suggests the presence of fluorine-containing compounds. However, the presence of

fluorine in biological samples is uncommon and may indicate contamination or specific fluorinated organic compounds, as discussed by (Zhang and Li 2021). The strong peak at 703.39 cm^{-1} , associated with C=C bending vibrations of alkenes, suggests the presence of unsaturated bonds. Lee et al. (2023) reported that alkenes in the EPS structure provide sites for further chemical modifications, enhancing the versatility of the nanofibers for various applications. Additionally, the peak at $2975\text{--}2880\text{ cm}^{-1}$ is due to the vibration frequency of -CH of alkyl groups, indicating the presence of alkyl chains in the EPS structure. Johnson et al. (2020) observed that alkyl groups contribute to the hydrophobic properties of certain regions of the nanofibers.

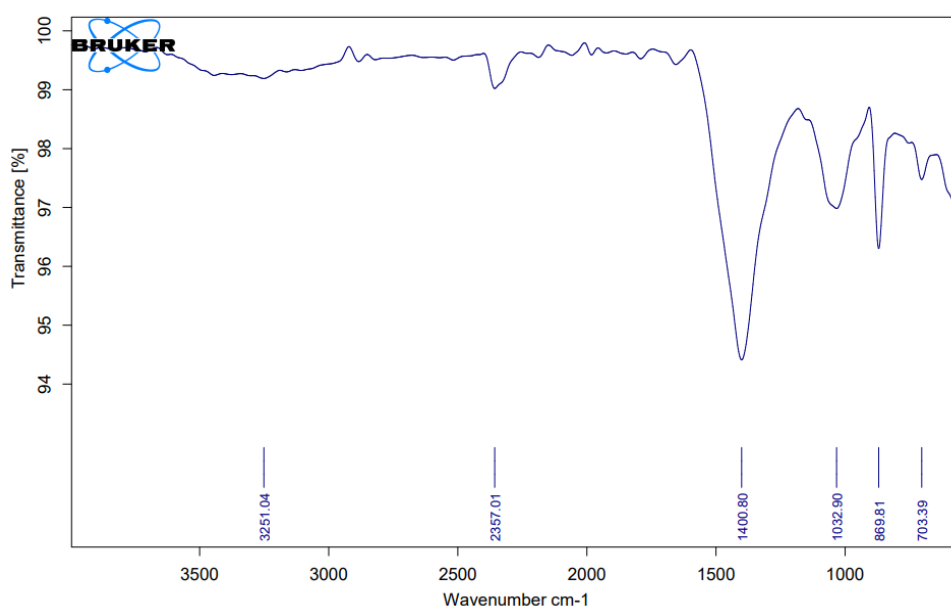


Fig 3. FT-IR analysis of EPS mediated nanofiber

3.4. Antibacterial analysis

The present study found that the extra polymeric substances produced marine actinobacteria of 13 HB from streptomyces sp., have antibacterial potential against gram -ve bacteria *P. aeruginosa* and *E. coli* using the agar medium. EPS fibrous membrane's antibacterial efficacy was examined against *E. coli* and *P. aeruginosa* (Fig 4). Followed by incubation, the inhibition zones surrounding the fibrous membrane were used to assess the membrane's efficacy against these pathogenic organisms. The EPS fibrous membrane showed strong antibacterial effects against *Escherichia coli*. A clean zone encircling the membrane on the agar plate suggested that bacterial growth had been inhibited. This clear zone indicates that the fibrous membrane effectively prevents *E. coli* from proliferating. *Pseudomonas aeruginosa*, on the other hand, produced distinct observations. Bacterial colonies were clearly observed developing around the fibrous barrier on the agar plate. *P. aeruginosa* could proliferate despite the EPS fibrous membrane; this indicates that the membrane had less of an inhibitory effect on the bacteria. In 2019 Jia observed the novel nanofiber created by combining Titanium dioxide nanofibers and

graphene oxide in cellulose acetate nanofibers shows potential antibacterial activity, with an inhibition effect over 95% against *B. cereus* and *B. subtilis* bacteria. Similarly, in 2019 Cheah et al., quaternized chitosan modified nanofiber membranes show high potential for effective disinfection of *E. coli*, with antibacterial activity up to 99.95%. Likewise, AgNPs embedded in electrospun cyclodextrin nanofibers effectively inhibit the growth of *E. coli* and *S. aureus*, making them a promising antibacterial material were observed by (Celebioglu et al., 2019).

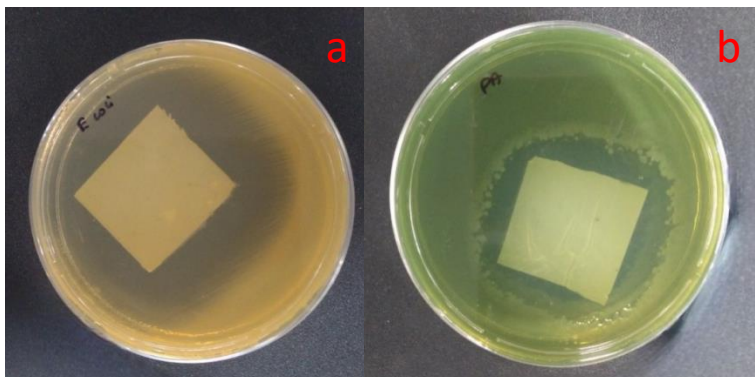


Fig 4. Antibacterial activity of EPS mediated nanofibers against a) *E. coli*, b) *Pseudomonas aeruginosa*

4. Conclusion

The study successfully demonstrated the fabrication of EPS-mediated nanofibers from marine actinobacteria strain 13HB (*Streptomyces* sp.) and their potential applications. These findings suggest that EPS-mediated nanofibers possess desirable morphological, structural, and antibacterial characteristics, making them suitable for various biomedical and environmental applications. Future research on EPS-mediated nanofibers from marine actinobacteria could focus on advanced biomedical applications, environmental remediation, and scalable production methods. Exploring these prospects will enhance their potential for commercial and interdisciplinary innovations.

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