ORIGINAL ARTICLE

Extraction, characterization, and anticancer potential of extracellular polymeric substances from marine actinobacteria of *Streptomyces* species

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ABSTRACT

To check the ability of the anticancer activity, the extracted extracellular polymeric substances (EPS) from marine actinobacteria were done. Potential of the anticancer activity of EPS which was extracted from marine actinobacteria of *Streptomyces* species through an assay called MTT. Marine actinobacterial isolation, identification and micromorphology of the strain, and biochemical analysis were performed (Shirling and Gottlieb, 1966). The production of EPS from marine actinobacteria was quantified(P. Sivaperumal *et al*., 2018). Carbohydrate content in the EPS was quantified, and MCF-7 cell proliferation was done using an MTT assay. EPS-producing marine *Streptomyces* was isolated and identified. The production of EPS and their protein, carbohydrate, lipid, and other parameters were estimated. Further, the EPS showed more than 50% of inhibition after 72 h using the MTT assay in the MCF-7 cancer cell line. The present study exhibited that EPS from marine *Streptomyces* species has significant anticancer activity.

Key words: Eco-friendly, extracellular polymeric substance, green synthesis, innovative technique, innovative technology, *Streptomyces*

INTRODUCTION

Anticancer is employed against or tending to arrest or prevent cancer. The drugs used for preventing cancer are called anticancer drugs which have an anticancer activity toward cancer-causing stimulation and cancer cells.[1] To check the ability of the anticancer activity of the extracted extracellular polymeric substances (EPS) from marine actinobacteria of *Streptomyces* species through an assay called MTT.[2,3] *In vitro,* the MTT assay was the most abundantly used one to accounting the basic anticancer activity of natural products.[4,5]

Microbial natural polymers such as EPS are huge molecular weight in nature which establish the structural integrity of biofilms and functions. Furthermore, the basic constituent of EPS will define the physical and biochemical properties of biofilm.[6] Protein and polysaccharides are the major constituents of EPS, followed by lipids, nucleic acids, humic substances, and other molecules.[7,8] Microbes are used to produce a wide range of polysaccharides that are

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varied in functional and structural basis. These sugar-based substances from microbes are produced in extracellular and intracellular activity.[9,10] In previous studies, the addition of cyanobacterial EPS can significantly remove heavy metals such as copper, cadmium, and lead and stimulate the biosorption processes in wastewater treatment.[11]

Melanins are termed macromolecules made by oxidative polymerization consisting of indolic or phenolic compounds.[12] Regularly, the next shades are earthy colored or dark in shading; however, numerous different tones have additionally been noticed. Melanins are additionally hydrophobic and contrarily charged.[13] The melanin production is started from L-tyrosine by means of progression an enzymatic as well as nonenzymatic responses through protein tyrosinase. Tyrosinases from various natural sources are used for the amalgamation of (Levodopa and l-3,4-dihydroxyphenylalanine) L-DOPA, and therefore, the expulsion of phenolic compounds from wastewaters.[14,15] There are three sorts of melanins, for instance, eumelanin, pheomelanin, and allomelanins, and their description is also done in microbes.[16-18]

Actinobacteria are foremost biotechnologically and commercially important prokaryotes by the contribution of 50% bioactive metabolites out of 23000 overall microbial secondary metabolites.[19-21] These compounds display an in-depth variety of industrially helpful activities, such as cytotoxic, medicament, antifungal, antiprotozoal, anticancer, medicinal drug, anti-inflammatory drug, anthelmintic, and herbicide.[22] Our team has done research on biomedical aspects and experiences that have been interpreted to a high level of publications.[12,23-41] The present study aimed to analyze the anticancer properties of EPS from marine actinobacterium of *Streptomyces* species.

MATERIALS AND METHODS

Sample processing

The marine sediment collection was done around the Parangipettai coast, Tamilnadu, by Van Veen grab. The collected sediments were carefully transferred into a sterile container and reached to the laboratory. After reaching the laboratory, the sample was air-dried for 48 h then sundried for 12 h. The air-dried samples are macerated through mortar and pestle.

Actinobacterial isolation and identification

Actinobacterial was enumerated on KUA (Kuster's agar medium) supplemented with 10 µg/ml of cycloheximide and nalidixic acid as an antibacterial and antifungal agent.[42] The sediment sample was serially diluted for spread plate on KUA and incubated at ambient temperature for a week. The total population density of actinobacteria from the sediment sample was expressed in colony-forming units per gram. Morphologically, distinct colonies were picked for pure culture and further analysis. The conventional identification of aerial mass color, melanoid pigments,[43] reverse side and soluble pigments, spore chain morphology, and utilization of carbon sources was done by International Streptomyces Project (ISP) method. Further, chemotaxonomical characteristics were also done to identify the marine actinobacteria.

Extracellular polymeric substances production and quantification

The marine actinobacterial EPS production was estimated by the method of Sivaperumal *et al.*,[6] with slight modification.

Estimation of extracellular polymeric substances components

Total carbohydrate in EPS was assessed by the method of phenol sulfuric acid method.[44] Protein content was done by bicinchoninic assay[45], and the nucleic acid content was estimated.[46]

MTT assay

The anti-proliferating activity of marina actinobacteria EPS was analyzed by MTT assay on breast cancer cell line (MSF-7). Morphological observation and statistical analysis also were done, followed by the method of Ciapetti.

RESULTS AND DISCUSSION

Isolation of marine *Streptomyces* species

The present study, conventional identification was done to identify the marine actinobacteria *Streptomyces* species. The chemotaxonomic characteristics such as cell wall analysis, sugar pattern, and cell wall type also have been done to identify the *Streptomyces* species. The spore chain has shown the actinobacteria rectiflexibles. On the basis of assimilation of carbon source, it has shown positive utilization for arabinose, xylose, inositol, fructose, rhamnose, and sucrose also; it has shown negative

Table 1: Conventional findings of marine actinobacteria *Streptomyces* species

Color of aerial mycelium	**Grey**
Melanoid pigment	-
Reverse side pigment	-
Soluble pigment	-
Spore chain	**RF**
Assimilation of carbon source	
Arabinose	+
Xylose	+
Inositol	+
Mannitol	-
Fructose	+
Rhamnose	+
Sucrose	+
Raffinose	-

Table 2: Chemotaxonomic characteristic of *Streptomyces* sp.

Cell wall amino acids			Cell wall sugar		Cell wall type	Index
LL-DAP	MesoDAP	Glycine	Arabinose	Galactose		
+	-	+	-	-	I	*Streptomyces*

Table 3: Extracellular polymeric substance components of marine actinobacteria *Streptomyces* species

EPS Components	Percentage of composition
Carbohydrate	38
Protein	41
Nucleic acid	11
Unidentified	10

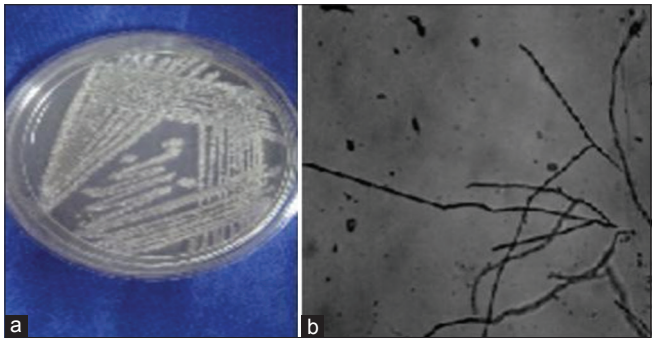

Figure 1: (a and b) Marine *Streptomyces* species strain and their spore chain morphology

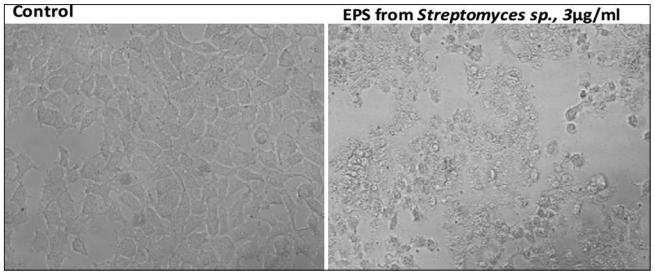

Figure 2: The anticancer potential of EPS obtained from *Streptomyces* sp. against the MCF-7 cell line, the control group used was DMSO. The obtained EPS from *Streptomyces* species was 3 µg/ml. EPS: Extracellular polymeric substances, DMSO: Dimethyl sulfoxide

utilization for mannitol and raffinose [Table 1]. It has been shown positive for LL-DAP and glycine and negative for MesoDAP for cell wall amino acids. In cell wall sugar, both arabinose and galactose were negative. It was found to be a type I cell wall index [Table 2].

Extracellular polymeric substances components

The EPS obtained from *Streptomyces* sp. contains 38% of carbohydrates, 41% of protein, 11% of nucleic acid, and 10% of unidentified compounds [Table 3].

Anticancer potential through MTT assay

Numerous antitumor marine active compounds derived from marine actinobacteria, among them EPS have shown a significant successive part in recognition of bioactive compounds for pharmaceutical use.[47,48] Actinobacteria from marine sources have numerous potential in providing beneficial leads with specific biological and chemical properties such as anticancer, antimicrobial, antimalarial, antiviral, and anti-inflammatory.[49] A novel actinobacteria of *Salinispora* has produced a novel compound salinosporamide A actively hider the growth of cancer cells.[50]

This current original study has clearly shown that the effect of EPS from marine *Streptomyces* species on the culture plate of cancer cells has displayed a reasonable degree of anticancer potential, causing approximately over inhibition of 50% was observed after 72 h. On using the MCF-7 cell line, the capacity of the confirmed compound has shown almost over 50% of inhibition in 72 h using the MTT assay[51] [Figures 1a, b and 2].

In a corresponding clinical study, it has been reported that *Streptomyces* species, which are isolated and contaminated from humus soils, the Western Ghats, have exhibited anticancer potentials against the selected cancer cells. Secondary metabolites compounds from actinobacteria, particularly the *Streptomyces* could be the greatest significant marine source for acting as an anticancer reagent and has high anticancer potential.[52] There are previous studies indicating that the marine actinobacterium of *Streptomyces* species has anticancer potential.[53]

CONCLUSION

In the current study, we thought of finding the anticancer potential of marine actinobacterial (*Streptomyces* species) EPS. It has revealed that the species have over 50% of inhibition after 72 h at 3 µg/ml using the MCF-7. Hence, we concluded that the EPS from marine *Streptomyces* species has potential anticancer properties, and it might be useful for marine drug research in future.

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Conflicts of interest

There are no conflicts of interest.

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