ORIGINAL ARTICLE

Antioxidant activities from melanin pigment produced by marine actinobacterium of *Streptomyces* species

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ABSTRACT

Melanin is macromolecules which have been developed while oxidative polymerization of phenolic compounds. It has been found that the majority of marine organisms produce melanin pigment. To obtain the melanin from marine actinobacteria and their biological properties are studied. Isolation and identification of marine actinobacteria were carried out using the media of ISP-1, ISP-7. Spore chain morphology, chemotaxonomic characteristics were also analyzed by the International *Streptomyces* Project. The antioxidant activities of DPPH, lipid peroxide using actinobacterial melanin were determined. The *Streptomyces* species have the capacity to produce melanin pigments and it shows potential antioxidant properties. When DPPH concentrations were compared with the ascorbic acid standard, the melanin of 150 µg/ml showed 93.47% of scavenging. The present study was concluded that melanin pigment obtained from marine actinobacterium of *Streptomyces* species has potential antioxidant activities and these components might be useful to pharmaceutical industries.

Key words: Antioxidant, DPPH, eco-friendly, green synthesis, marine actinobacteria, melanin, *Streptomyces* sp.

INTRODUCTION

Around 70% of universal surface is roofed by the sea which contains exceptional diversity[1] and living diverse is huge in few marine ecosystems, such as the coral reefs and deep-sea than in the rainforests tropical area. Melanin is a macromolecule that has been developed by oxidative polymerization reaction in phenolic compounds. It also found that the majority of marine organisms produce melanin pigment, but here we have chosen a "*Streptomyces* sp.[2] Melanins are also hydrophobic and negatively charged. Usually, melanin pigments are black or brown in color, but there are many other color pigments produced. Moreover, the species of *Streptomyces* has the capacity to produce an extensive range of bioactive compounds having therapeutic activities such as antimicrobial, antiviral, antimalarial, anticancer, and anti-inflammatory activity.[3] In recent days, the exploration of bioactive natural products from the marine environment has increased due to the unique structure and novelty of the compounds. Around 75% of economically important

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antibiotics in industries were obtained from *Streptomyces* species. Similarly, *Streptomyces* can produce antioxidant compounds which prevent cells from the free radical damage and further adverse health issues like tumors, cancer, etc., The commercially important species of *Streptomyces* is widely explored in various environments worldwide. The biologically active compound from *Streptomyces* has fractionated using chromatography to purify and those fractions showed the potential antioxidant property as compared with ascorbic acid standard.[4] The actinobacterial methanolic extract has exhibited significant antioxidant activity,[5] In general, actinobacteria have been known well to produce bioactive secondary metabolites compressed with beneficial antibiotics that could inhibit multidrug-resistant pathogens.[3] Hence, the promising bioactive source microbe *Streptomyces* was isolated from the coastal sediment sample. The present study is aim to analyze an antioxidant activity from melanin pigment obtained from marine actinobacterium of *Streptomyces* species.

MATERIALS AND METHODS

Sampling and preprocessing

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

Isolation of actinobacteria

The isolation of marine actinobacteria was done using KUA (Kuster's agar medium) supplemented with 10 µg/ml of cycloheximide and nalidixic acid as an antibacterial and antifungal agent.[6] The macerated sediment sample was serially diluted and the samples were spread and incubated at ambient temperature for a week in KUA medium. The population density of actinobacteria from sediment samples was expressed as colony forming units per gram. The distinct morphology of actinobacteria were picked for pure culture and further analysis.[7]

Marine actinobacterial identification

The observation of aerial mycelium color through visual observation may exhibit two series of color or white. The spore's color on aerial mycelium was observed in well-grown isolates on Yeast-Malt (YM) Extract Agar.

Melanoid pigments

Actinobacteria are used to produce the melanoid pigment that might be in green, brown, black, or other modified color on the ISP-1 and ISP-7 medium. The presence of melanoid pigment was noted as positive (1 or +) whereas the absence has noted as negative (0 or −).[8]

Table 1: Conventional identification of *Streptomyces* spices

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	-

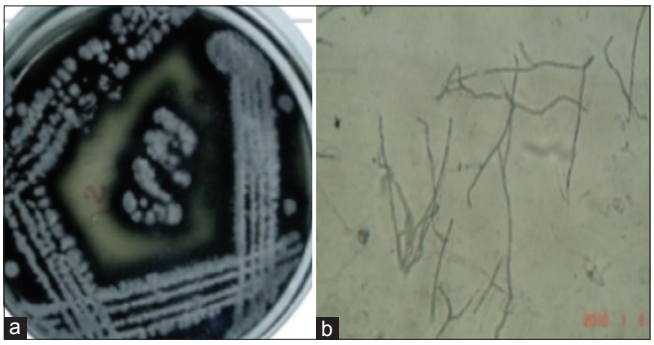

Figure 1: (a) Marine *Streptomyces* species strain and (b) spore chain morphology

Reverse side pigments

Pigment production from vegetative mycelium has been noted as reverse side pigment production on the ISP7 medium. This pigment may be present (1 or +), or absent (0 or −) and sometimes the shade of pale colors can be produced.[9]

Soluble pigments

The diffusible pigment production of actinobacteria on the ISP7 medium was considered positive (+) and not produced (−). It may produce a series of red, orange, green, yellow, blue, and violet colors.[9]

Spore chain morphology

The observation of actinobacterial spore chain morphology on aerial hyphae was done. The loopful well-grown culture was placed on the glass slide to incubate on an agar medium at room temperature. The spore morphology was observed under the microscope at a regular time intervals.[9]

Chemotaxonomical characteristics

Hydrolysis

Well-grown fresh cells (20 mg) were harvested and treated with 1 mL of 6N HCL for amino acid and 0.5N H2SO4 for sugar analysis in ampo bottle and sealed. The sealed

Table 2: The chemotaxonomic characteristics of *Streptomyces* spp.

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

Table 3: Total antioxidant activity using melanin obtained from *Streptomyces* spp.

TAA (µg/ml)	AAE
25	38.58±1.217
50	54.39±1.302
75	76.54±0.812
100	98.37±1.225
125	118.35±1.314
150	142.65±1.286

TAA: Total antioxidant activity, AAE: Ascorbic acid equivalence

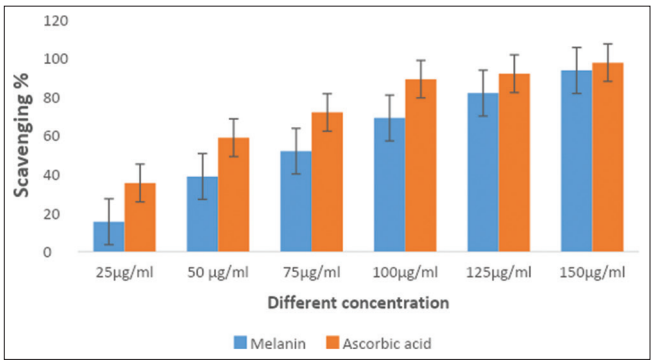

Figure 2: Represents the DPPH Assay of melanin from marine actinobacterium of *Streptomyces* species

acid-treated samples were kept in a sand bath for 20 h at 121°C. Then, the bottles are allowed to cool at ambient temperature.

Thin layer chromatography

Both hydrolyzed samples with standards were placed on a thin layer chromatography plate and allowed to air dry. DL-diaminopimelic acid, lysine, glycine, and ornithine aspartic acid were amino acid standards. Arabinose, xylose, inositol, and fructose were used as sugar standards. The amino acid, cell wall analysis, and sugar patterns were also analyzed.[7,9]

Melanin production and partial purification

The production of melanin was done using ISP-2 medium (prepared in seawater). The YM broth was used for the culture and to obtain the melanin, the acidic condition of pH (2) was used. The partial purification of marine actinobacterial melanin was done by following the method of Sivaperumal *et al*., with slight modification.[9] The chemical analysis of the purified melanin was also done.[10]

Antioxidant activities

The total antioxidant activity (TAA), DPPH assay, and lipid peroxidation assay of the melanin were determined by the method of Kamala *et al*.[11] with slight modification. The scavenging ability of melanin components is expressed as the percentage.[9]

RESULTS

Isolation of marine *Streptomyces* species

The conventional identification of marine actinobacteria was done and the genus of *Streptomyces* sp. was confirmed by the following taxonomical identification Tables 1 and 2: The chemotaxonomic characteristics such as cell wall analysis, sugar pattern, and cell wall type also have been done to identify the *Streptomyces* species Figure 1. In this current original study, we have demonstrated and conducted an experiment based on the DPPH assay. From the current study, the extracted species have shown the gray color of aerial mycelium, it is insolvable in both organic and inorganic solvents. When the assimilation of carbon source was compared, mannitol and raffinose showed negative results, whereas xylose, inositol, fructose, and rhamnose, showed positive results, whereas sucrose and xylose showed mixed results of both positive and negative. It has shown positive for melanoma pigment, for the cell wall amino acids, showed positive for glycine and LLDAP, and negative for Meso DAP. On cell wall sugar, it was negative for both arabinose and galactose. We have identified that it is a type I cell wall index.

Table 3 results show the TAAs using different concentration of melanin pigments obtained from *Streptomyces* species, the results were compared with ascorbic acid equivalence, when melanin TAA was 50 µg/ml, the ascorbic acid equivalence showed the 54.39 ± 1.302. Further, when melanin TAA was increased to 100 µg/ml, the ascorbic acid equivalence was 98.37 ± 1.225. Finally, when melanin TAA was 150 µg/ml, the ascorbic acid equivalence showed 142.65 ± 1.286. Compared to standard, the TAAs showed less concentration of equivalence.

In our study, when DPPH concentrations were compared with the ascorbic acid standard, the melanin of 150 µg/ml showed 93.47% of scavenging when compared with the ascorbic acid scavenging showed 98.6% which shows less percentage of scavenging activity of DPPH. Whereas the melanin concentration (100 µg/ml) was showed 68.95% of scavenging ability, however compared with ascorbic acid

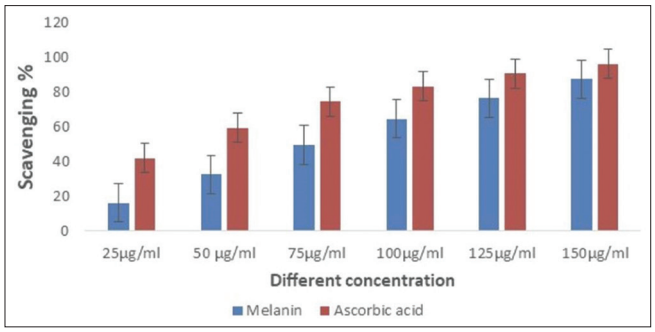

Figure 3: The lipid peroxidation assay of melanin from actinobacterium of *Streptomyces* species

(83.59%), it showed less percentage of scavenging activity for DPPH. When we used 50 µg/ml concentration, it showed 38.76% of scavenging and compared with ascorbic acid (62.7%), it showed less activity in case of DPPH scavenging [Figure 2].

DISCUSSION

In our study, when lipid peroxidase assay concentrations were compared with ascorbic acid standard, melanin of 50 µg/ml, 23.62% of scavenging when compared with the ascorbic acid scavenging showed 51.09% which shows less percentage of scavenging activity of lipid peroxide, melanin of 100, µg/ml showed 48.62% of scavenging when compared with ascorbic acid scavenging showed 72.61% which shows less percentage of scavenging activity of lipid peroxide [Figure 3]. In a corresponding clinical study, it has been reported that *Streptomyces* species, which are isolated and contaminated from humus soils in the Western Ghats, have exhibited antioxidant activities. Previous studies reported that the compounds from actinobacteria have potential DPPH scavenging activity.[12,13] In another study, the actinobacterial extract exhibited 35.02% ± 3.7% of DPPH scavenging ability at a 2 mg/ml concentration of sample additionally it has cytotoxic activity on human neoplastic cell lines.[4] A dose response pattern was evaluated on different extract concentration and it showed more effective against some neoplastic cell lines even at very low concentration of 25 µg/mL.[14]

CONCLUSION

The present study concludes that melanin pigment obtained from marine *Streptomyces* species has potential antioxidant activities. These marine natural products might be useful in drug development-related research and can be used for further clinical studies.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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