ORIGINAL ARTICLE

Melanin pigments from sediment-associated *Nocardiopsis* sp. marine actinobacterium and antibacterial potential

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ABSTRACT

To extract the melanin pigment from marine microbes and their biological potential, the present study was done. Isolation and identification of the melanin-producing *Nocardiopsis* sp. were obtained from the sediment samples. Zone of inhibition and minimal inhibitory concentration test was performed using melanin. Melanin was extracted from sediment-associated marine *Nocardiopsis* sp. In the present study, marine actinobacterium was identified by the conventional method, and the isolate was identified as *Nocardiopsis* sp. Melanin was extracted, and antibacterial activities were performed against different pathogens and the highest zone of inhibition is more in the *E. coli* while related to another two species. From previous observation done by Fu *et al*., they have said that marine actinobacteria have the ability of antimicrobial activity, which is very much helpful in producing the potential antimicrobial drugs this was similar to our study that marine actinobacteria have the capability to produce melanin pigment, and at the same time, it helps as to show the antibacterial activity. We concluded that melanin is produced by the *Nocardiopsis* sp. We also found that melanin extracted from the *Nocardiopsis* sp. of marine actinobacterium also has an antibacterial effect.

Key words: Antibacterial activity, marine actinobacteria, MIC, *Nocardiopsis* sp. novel melanin

INTRODUCTION

Melanin is a natural pigment that is identified in the human skin. The hair, skin, and eye color of a person are typically based on the amount and type of melanin they hold. Melanin is secreted by special skin cells known as melanocytes.[1] Melanin is generally formed through a different stage of a chemical process called melanogenesis, in which the amino acid tyrosine oxidation is followed by polymerization; at the same time, melanin is involved in high attention because of its biological character in technological and photoprotection applications. Melanin present in three different forms such as eumelanin (subdivided further into black and brown color forms), neuromelanin, and pheomelanin.[2] Microorganisms-mediated melanin pigment has high-molecular-weight molecule that is decolorized by oxidizing agents. Melanin is a phenolic compound

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usually containing complex substances such as protein and carbohydrates. Its capability to metal ions chelate and absorb ultraviolet-visible light very strongly. Melanin is not only synthesized in the human and animal skin but it is also synthesized by the marine actinobacterium such as *Nocardia* sp (akalophylic *Nocardiopsis dassonvillei*), *Dietzia,* and *Marinispora*.[3] The antibacterial activity and antifungal activities are also seen in the marine actinobacterium. From recent studies, they have found that the function of melanin is associated with the protection against environmental stress. Melanin-producing bacteria are found to be more resistant to antibiotics.[4] These properties make melanin an important bioactive material with various industrial applications. Studies also revealed the antibacterial and antiviral properties of melanin which opens a new area in the field of research. Melanin derivative products are used as therapeutic representatives for the treatment of neurodegenerative diseases of Alzheimer's disease and dementia.[5] Our research team is involved in high-quality publications and has extensive knowledge to translate the experiments.[6-28] To date to the best of our knowledge, there are no proper reports available on marine actinobacteria *Nocardiopsis* sp. from which the melanin pigment is extracted. The present experiment was to improve melanin pigments that could be extracted from marine actinobacterium *Nocardiopsis* species, and at the same time, it helps to show the antibacterial activity of melanin pigment.

MATERIALS AND METHODS

Collection of the sample and actinobacterial isolation

Marine sediment sample collection was done around Thoothukudi 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.

The isolation of marine actinobacteria was done using Kuster's agar (KUA) medium supplemented with 10 µg/ml of cycloheximide and nalidixic acid as an antibacterial and antifungal agent.[29] The macerated sediment sample was serially diluted, and the samples were spreaded and incubated at ambient temperature for a week in a KUA medium. The population density of actinobacteria from sediment samples was expressed as colony-forming units/g. The distinct morphology of actinobacteria was picked for pure culture and further analysis.[30]

Marine actinobacterial identification

Observation of the aerial mycelium color was done by visual observation and may exhibit two series of colors or white. The spores' color on aerial mycelium was observed in well-grown isolates on yeast extract-malt (YM) agar. Actinobacteria are used to produce melanoid pigment that might be in green, brown, black, or other modified colors 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−).[31]

Reverse side pigments

Pigment production from vegetative mycelium has been noted as reverse side pigment production on the ISP-7 medium. This pigment may be present (1 or +), or absent (0 or −), and sometimes, the shade of pale colors can be produced. The diffusible pigment production of actinobacteria on the ISP-7 medium was reflected positive (+) and not produced (−). It may produce a series of red, orange, green, yellow, blue, and violet colors.

The observation of actinobacterial spore chain morphology on aerial hyphae was done. The loop full of 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 regular time intervals.

Chemotaxonomical characteristics

The chemotaxonomical characteristics of hydrolysis, amino acids, whole-cell sugar patterns, and assimilation of carbon sources are done.

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

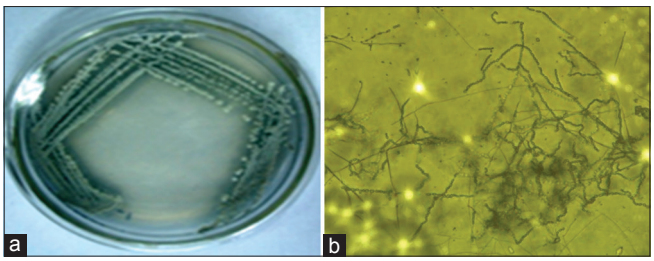

Figure 1: The morphology of *Nocardiopsis* sp. and their spore chain morphology

Table 1: Chemotaxonomical analysis of marine *Nocardiopsis* spp.

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

Table 2: Conventional identification of marine *Nocardiopsis* spp.

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

Table 3: Antibacterial potential against different pathogens using melanin obtained from *Nocardiopsis* spp.

Different concentration (µg/ml)	*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas aeruginosa*
50	8±2.2	5±1.3	6±2.2
100	14±3.4	12±2.6	11±2.6
150	19±2.5	17±2.1	16±2.4
200	24±2.1	21±2.4	20±2.1
250	28±2.7	25±2.7	26±2.7
300	32±2.3	29±2.1	31±2.2

Table 4: Minimum inhibitory concentration against different pathogens using melanin extracted from *Nocardiopsis* spp.

Different concentration (µg/ml)	*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas aeruginosa*
50	100±2.6	100±2.4	100±2.2
100	92.34±3.4	98.64±3.1	94.42±2.5
150	88.61±2.8	94.28±2.5	90.27±2.4
200	83.47±3.1	91.52±3.4	84.82±2.2
250	79.62±2.5	86.07±2.7	78.38±3.4
300	76.4±2.1	82.49±2.1	71.62±2.8

method of Sivaperumal *et al*., with slight modification.[29] The chemical analysis of the purified melanin was also done.[32]

Antibacterial activity

Antibacterial potential of the melanin was done by following the method of disc diffusion. The 5-mm diameter discs were used for the assay and different concentrations such as 50, 100, 150, 200, and 250 µg/mL of melanin samples with oral antibiotic tetracycline and dimethyl sulfoxide (DMSO) as a negative control. Further, the plates were kept in an incubator for 1 day and maintained the room temperature. The zone of inhibition was considered as better results and measured the zone and calculated the activities.

MIC

The Minimum Inhibitory Concentration (MIC) of the melanin was analyzed using different concentrations such as 50, 100, 150, 200, and 250 µg/ml of melanin with tetracycline (Standard), and DMSO was a negative control. The bacterial suspension (in test tubes) was kept in an incubator for 1 day at room temperature, and optical density was analyzed.[33]

RESULTS

Chemotaxonomical characteristics

By analyzing the results of our study, we can say that the cell wall amino acid does not contain LL-di-ammonium phosphate (DAP) and glycine, but it has meso-DAP and at the same time also observe the lack of galactose and arabinose. The whole-cell wall sugar pattern and cell wall chemotype are classified under class 3 variety. From this finding, we can confirm that the actinobacteria extracted from the marine sediments belong to *Nocardiopsis* sp Figure 1. From the chemotaxonomic characteristics, we can say positive control is seen in tetracycline, and negative control is seen in DMSO.

Conventional identification of *Nocardiopsis* sp

The present study results show that the color of aerial mycelium is white, which is produced by the marine actinobacterium Figure 1a and b, we can see the absence of production of melanoid pigments, reverse side pigment, and soluble pigment, and at the same time, we can see the presence and absence of sugar content such as arabinose, xylose, mannitol, sucrose (absent), and rhamnose. Through this conventional method, we can identify that the extracted bacterium species belongs to *Nocardiopsis* sp Tables 1 and 2.

Antibacterial activity

We have found the zone of inhibition for three different species such as *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella* sp., which by testing with different concentrations of melanin (50 µg/ml–300 µg/ml). We observed that *E. coli* showed a zone of inhibition of 32 ± 2.3 mm, which is better than the other two species (*Klebsiella* sp. (29 ± 2.1 mm) and *P. aeruginosa* (31 ± 2.2 mm) Tables 3 and 4.

DISCUSSION

From the previous study done by Fu *et al*.[34]) observed that marine actinobacteria have the ability of antimicrobial activity, which was very much helpful in producing the potential antimicrobial drugs. These findings are similar to our study and marine actinobacteria have the capability to produce melanin pigment, and at the same time, it helps

to show antibacterial activity. The previous research done by Manivasagan *et al*.[35] reported that the extracted melanin showed a high-level zone of inhibition at 18 ± 0.01 mm, which was slightly different from our results. Moreover, the previous study done by Manivasagan *et al*.[36] observed that the MIC (minimum inhibitory concentration) of the extracted melanin has shown good activity against bacterial pathogens. This was similar to our studies that the MIC seen in the partially purified melanin of excellent antibacterial activity.

CONCLUSION

The present study concludes that melanin is produced by the *Nocardiopsis* sp. We also found that melanin extracted from the *Nocardiopsis* sp. of marine actinobacterium also has an antibacterial effect. Consequently, melanin is the potential target for antibacterial drugs. Finally, we can say that melanin has the ability to produce an antibacterial effect and which can be further examined clearly using an *in vivo* condition as an animal model experiment.

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Conflicts of interest

There are no conflicts of interest.

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