RESEARCH
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EVALUATION AND EXTRACTION OF MELANIN PIGMENT FROM MARINE ACTINOBACTERIA AND THEIR INHIBITION OF STREPTOCOCCUS SP BIOFILM

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Abstract
Aim: To evaluate and extract melanin pigment from actinobacteria and their inhibition of streptococcus sp biofilm
Introduction: Melanin is a biopolymeric pigment widely recognized for its role in providing coloration and protection in humans, animals, and microorganisms. Actinobacteria, a diverse group of bacteria, have been identified as a significant source of melanin production. Actinobacteria-derived melanin has garnered attention due to its wide range of biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties.
Materials and methods: Isolation and Culture of the microorganism was done to isolate and analyze the bacteria. The Histamine analysis and the biofilm study helped us recognise the bacteria and isolate melanin pigment from the same. Further study of the melanin pigment was done using FTIR and Confocal imaging.
Results: It was observed that actinobacteria sps derived melanin pigment inhabit biofilms of the microorganism. Melanin pigment from the bacteria had proliferative activity on that of the streptococcus sps denoting it's activity on the harmful bacteria.
Discussion:In contrast to regularly occurring actinobacteria like Streptomyces, unusual actinobacteria are rarely isolated. Due to incomplete knowledge of their growth needs, marine actinobacteria are equally challenging to culture in lab settings.

Keywords: Actinobacteria, Streptococcus, Melanin, Biofilm, Pigment

INTRODUCTION

The human body is constantly exposed to a variety of microorganisms, such as bacteria, which can inhabit different surfaces and create biofilms. These biofilms are intricate communities of microorganisms enclosed in a matrix they produce themselves, offering protection and enhancing bacterial survival. Among the bacteria known for biofilm formation is Streptococcus sp., a common pathogen responsible for several human infections. More than 10,000 bioactive chemicals (enzymes, enzyme inhibitors, antibiotics, pigments, and single cell proteins) have been described from actinobacteria, which are remarkable as secondary metabolite makers due to their structural diversity.

Biofilm-related infections present a considerable challenge in healthcare since they exhibit heightened resistance to antimicrobial agents and host immune responses. As a result, innovative strategies are necessary to effectively combat these biofilm-associated pathogens. Bacterial pigment production is one of the emerging fields of research. One of the newest areas of study is the generation of pigments by bacteria. Because they can create colours, actinobacteria attracted a lot of attention. Production of microbial pigments using fermentation technology is more flexible and practical. Compared to synthetic and plant-based pigments, these colors are more stable, biodegradable, and less harmful. Microbial pigments are used extensively in industry as food coloring, dyes, and medicinal substances. Microorganisms have been used to create pigments including carotenoids, anthraquinone, zeaxanthin, lycopene, and melanin, among others. In recent years, researchers have shifted their focus to natural compounds that possess potential

antibiofilm properties, with melanin pigments emerging as promising candidates.

Melanin is a biopolymeric pigment widely recognized for its role in providing coloration and protection in humans, animals, and microorganisms. A criterion for taxonomical characterisation is the production of the dark-colored pigments melanin or melanoid by actinobacteria. Tyrosinase is a notion used in the biosynthesis of melanin in lower organisms. Tyrosine is oxidized to 3, 4-dihydroxyphenylalanine (DOPA) in the presence of the enzyme tyrosinase and oxygen, and is then further oxidized to DOPA-quinone. DOPA- quinone is changed into an indole derivative, which polymerizes to generate melanin, through a series of processes.

Actinobacteria, a diverse group of bacteria, have been identified as a significant source of melanin production. Actinobacteria-derived melanin has garnered attention due to its wide range of biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties. Recent research has shown that melanin's role in protecting against environmental stress is related to this. Antibiotic resistance has been discovered to be higher in bacteria that produce melanin. These characteristics make melanin a significant bioactive substance with several industrial uses. Studies have also shown that melanin has antibacterial and antiviral characteristics, which expands the topic of study. Neurodegenerative illnesses including Alzheimer's disease, retinitis pigmentosa, schizophrenia, and dementia are treated using medicinal medicines derived from melanin.

Thus, as a biomolecule of biotechnological relevance, we want to investigate the generation of melanin in our study. Although Streptococcus spp. are common makers of melanin, uncommon actinobacteria contribute relatively little to this. This prompts us to investigate the potential role of uncommon actinobacteria in the synthesis of melanin. Anti-cancer, anti-fungal, anti-candidal, and anti-microbial substances may be produced by it. The goal of the study was to identify and isolate melanin-producing actinobacteria from maritime soil sediments. It also included the physical and chemical characterisation of melanin that has been synthesized.

MATERIALS AND METHODS

Microorganism and culture:

The clinical isolates were obtained from Saveetha medical college and stored in airtight storage containers with proper label which has name of isolate, date of storage, name of culture medium and details of preservation solution. All microbes were identified based on Bergey’s manual of determinative bacteriology (1923).

Chemicals:

Acridine orange, propidium iodide- Dyes

Shiga toxin producing isolates were grown on chromogenic ESBL agar medium then enterobacteria enrichment broth has been used for the formation. Staphylococcus was grown on Mannitol salt agar. Biofilm on coverslip has been formed on Mannitol salt broth Luria-Bertani agar medium has been used for the growth of E. coli, Biofilm on coverslip has been formed on Luria-Bertani broth.

Staphylococcus sp. was grown on Baird Parker Agar and the biofilm on coverslip has been formed on Baird Parker broth base.

Klebsiella sp. was grown on MacConkey agar and the biofilm on coverslip has been formed on MacConkey broth.

Pseudomonas sp. was grown on Pseudomonas agar and the biofilm on coverslip has been formed on pseudomonas selective broth

Methicillin resistant Staphylococcus was grown on mannitol salt agar and biofilm on coverslip has been formed on Mannitol salt broth

Methods:

Cell viability has been analyzed with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay by the method of Peter and Toshima, 2023.

(Peter, L. and Z.P.Toshima, 2023. Optimization of. Cell viability assay for drug sensitivity screens. Methods Mol.Biol., 2644:287-302)

Haemolysis activity:

Haemolysis analysis was with whole blood samples were collected from volunteers. The assay was performed by the method of Imran et al., (2020).

Imran, M., S.Riaz, S.M.H.Shah, T.Batool, H.N.Knan, A.N.Sabri and S.Naseem, 2020. In vitro hemolytic activity and free radical scavenging by sol gel synthesized Fe3O4 stabilized ZrO2 nanoparticles. Arabian J.Chem., 13(11): 7598-7608.

Histamine analysis:

Histamine production was measured by the method of Gerrit et al., (2022).

Gerrit, S., D. Brendan, A.Polycronnis and R.Gregor, 2022. In vitro assessment of histamine and lactate production by a multi stain symbiotic. J.food Sci.Technol., 59(9): 3419-3427.

β-Hexosaminidase:

β-Hexosaminidase was measured by the method of Oeystein et al., (2020).

Oeystein, R.B., J.A.Korecka, C.C.Crapart, M.Huebecker, Z.K.Macbin, S.A.Rosenthal, M.S.Esteves, D.A.Priestman, F.M.Platt, O.Isacson and P.J.Hallett, 2020. Upregulating β-Hexosaminidase activity in rodents prevents α-synuclein lipid associations and protects dopaminergic neurons from α-synuclein-mediated neurotoxicity. Acta Neuropathol commun 8, 127.

Biofilm study:

Biofilm formation was studied by the method of Rawaf, (2023). The pathogens (109 CFU/ml) were inoculated in microtiter plate along with various concentration of antibiotics. The plates were stored ate room temperature 24hrs and the biofilm was measured using acridine orange and propidium iodide. Coverslip method also followed for the observation of biofilm inhibition. The pathogens were cultivated in conical flask with coverslip and incubated for 24hrs. After incubation coverslip was stained with acridine orange and propidium iodide.

Rawaf, A., 2023. Antimicrobial activities and biofilm inhibition Properties of Trigonella foenumgraecum Methanol Extracts against Multidrug-Resistant Staphylococcus aureus and Escherichia coli. Life, 113(3): 703.

Actinobacterial Identification:

Actinobacteria was identified based on Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST: Bergey's Manual of Determinative Bacteriology. 1994, Baltimore: Williams & Wilkins.

Goodfellow M, O'Donnell AG. Roots of bacterial systematics. In: Goodfellow M, O'Donnell AG. (Eds) Handbook of New Bacterial Systematics 1993; pp. 3-54. Academic Press Ltd., London.

RESULTS

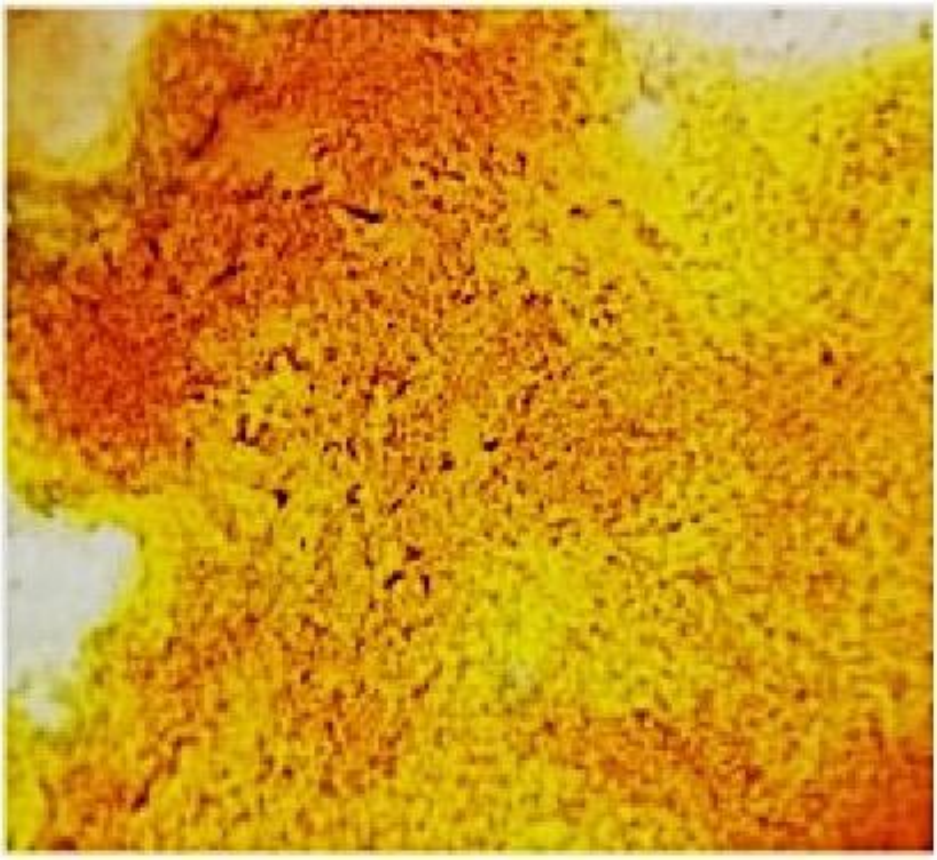

Fig 1: Biofilm of Streptococcus sp - Control

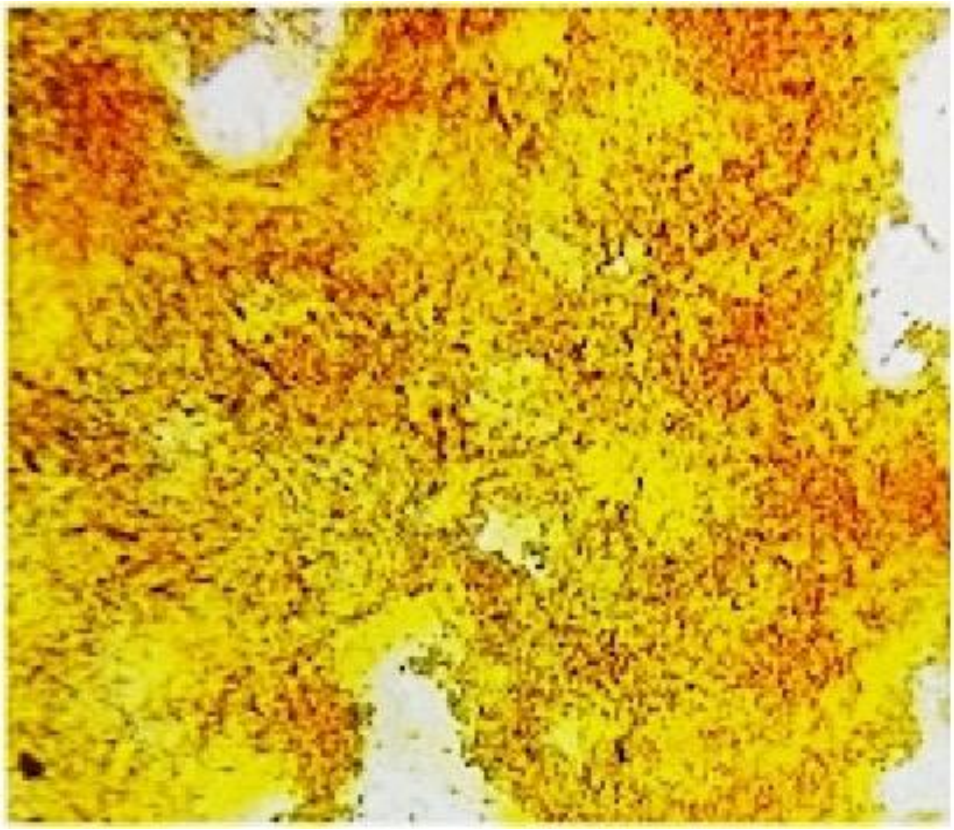

Fig 2: Treated biofilm with melanin pigment- 24 hrs

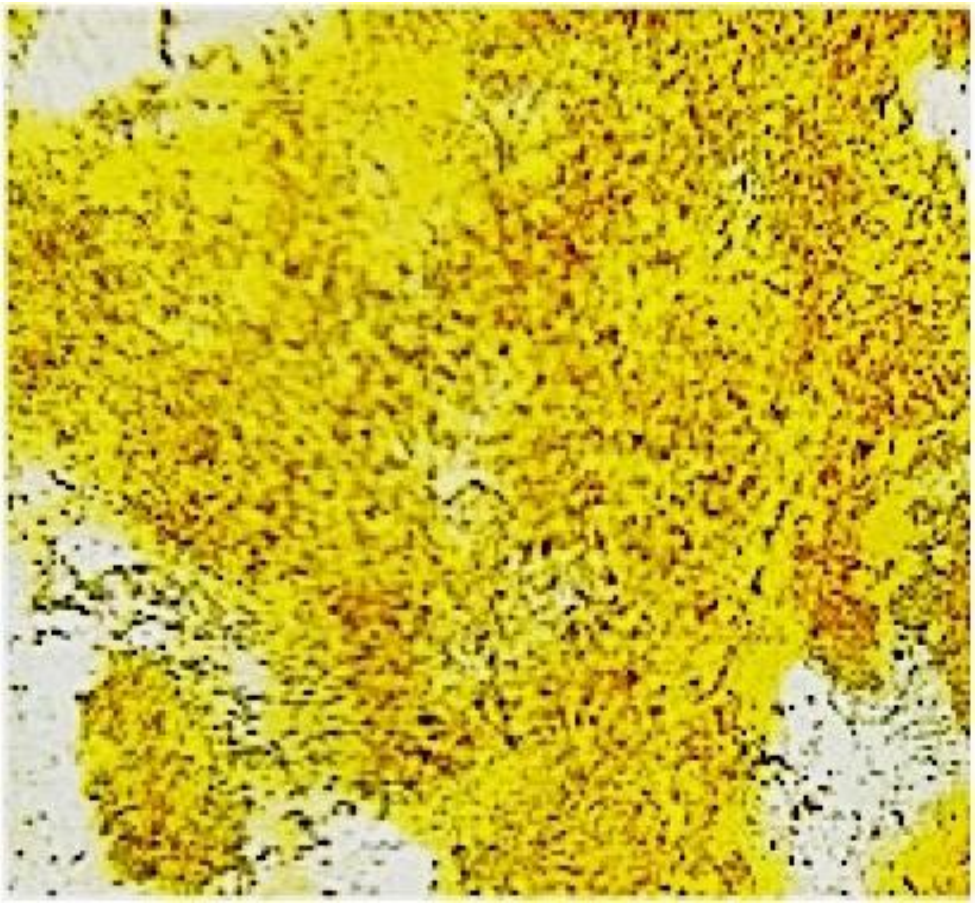

Fig 3: Treated biofilm with melanin pigment- 48 hrs

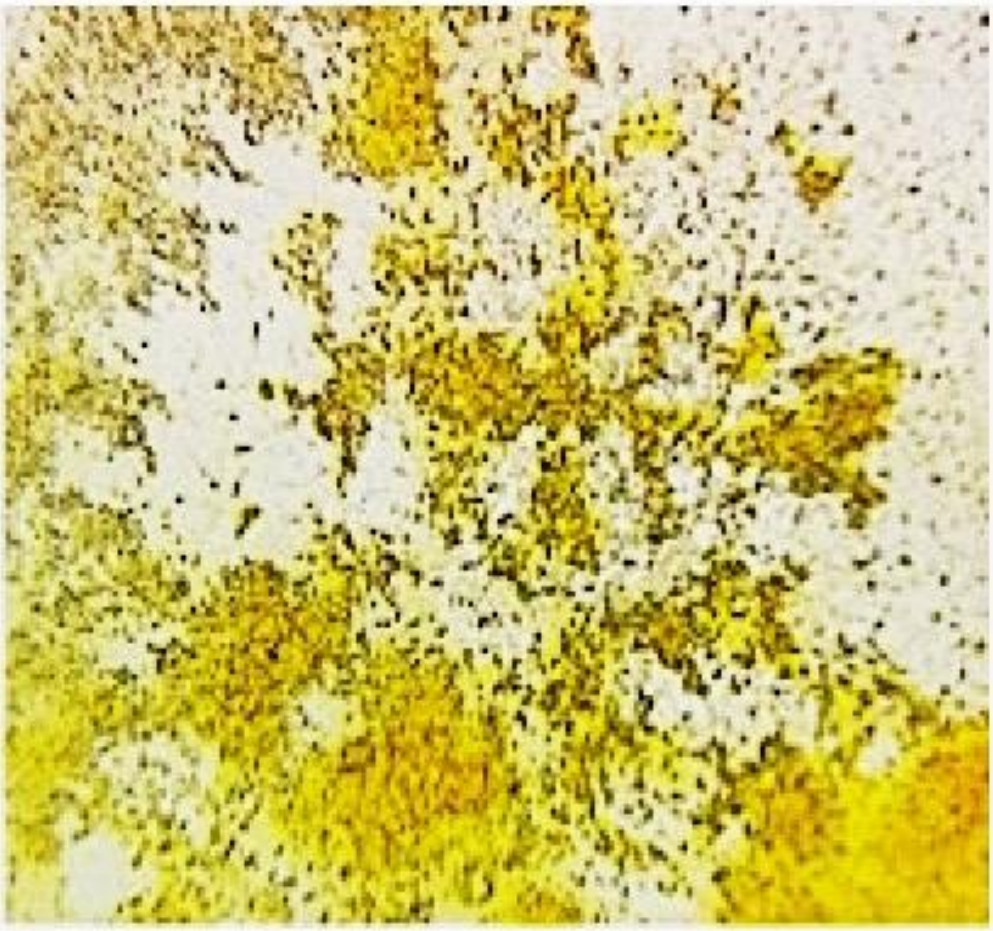

Fig 4: Treated biofilm with melanin pigment- 72 hrs

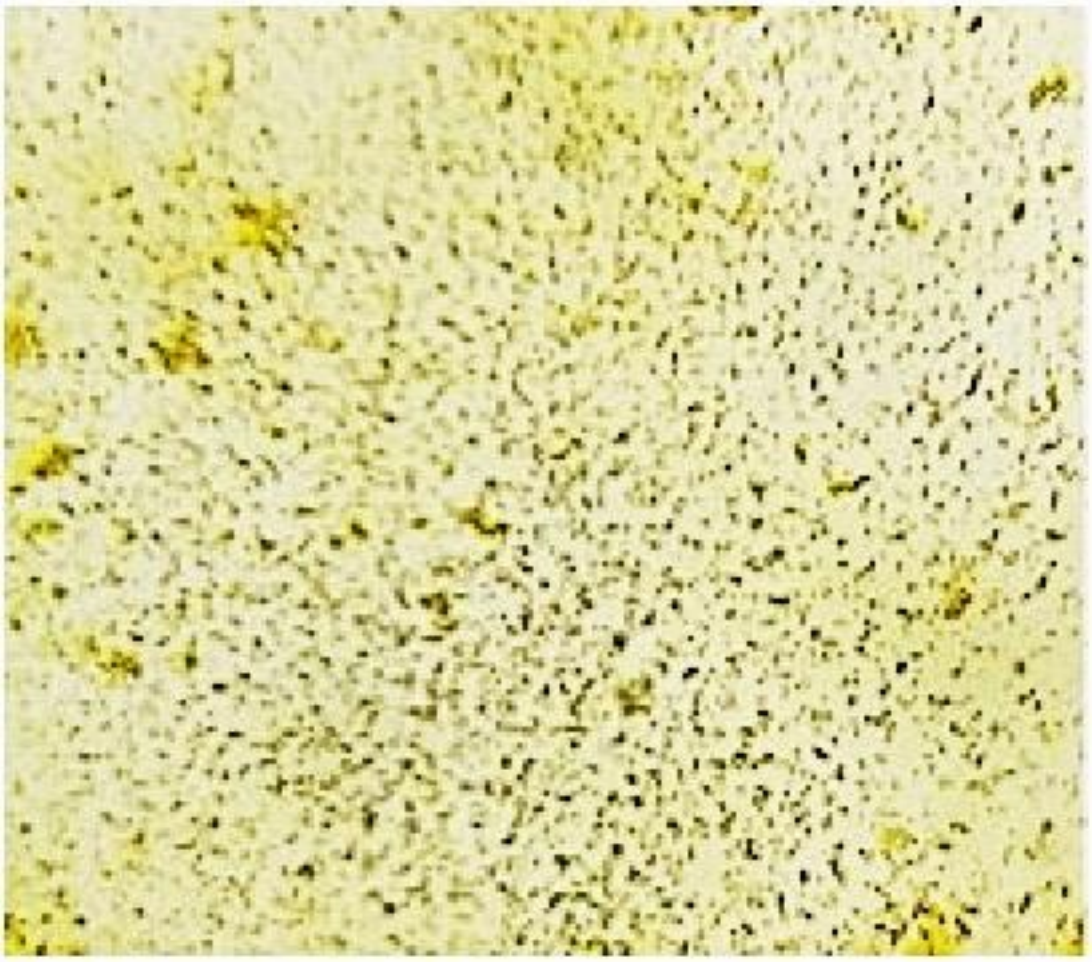

Fig 5: Treated biofilm with melanin pigment- 96 hrs

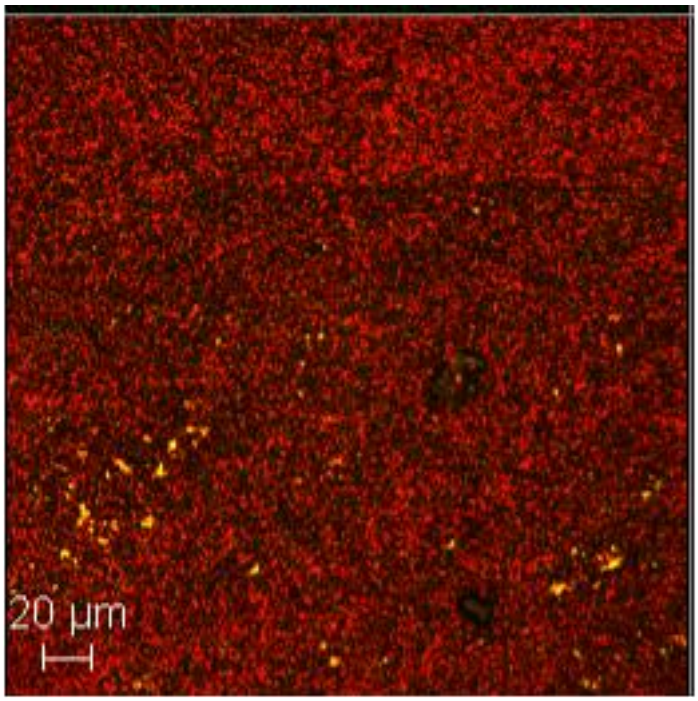

Fig 6: Confocal image of dead cells after 96 hrs of treatment

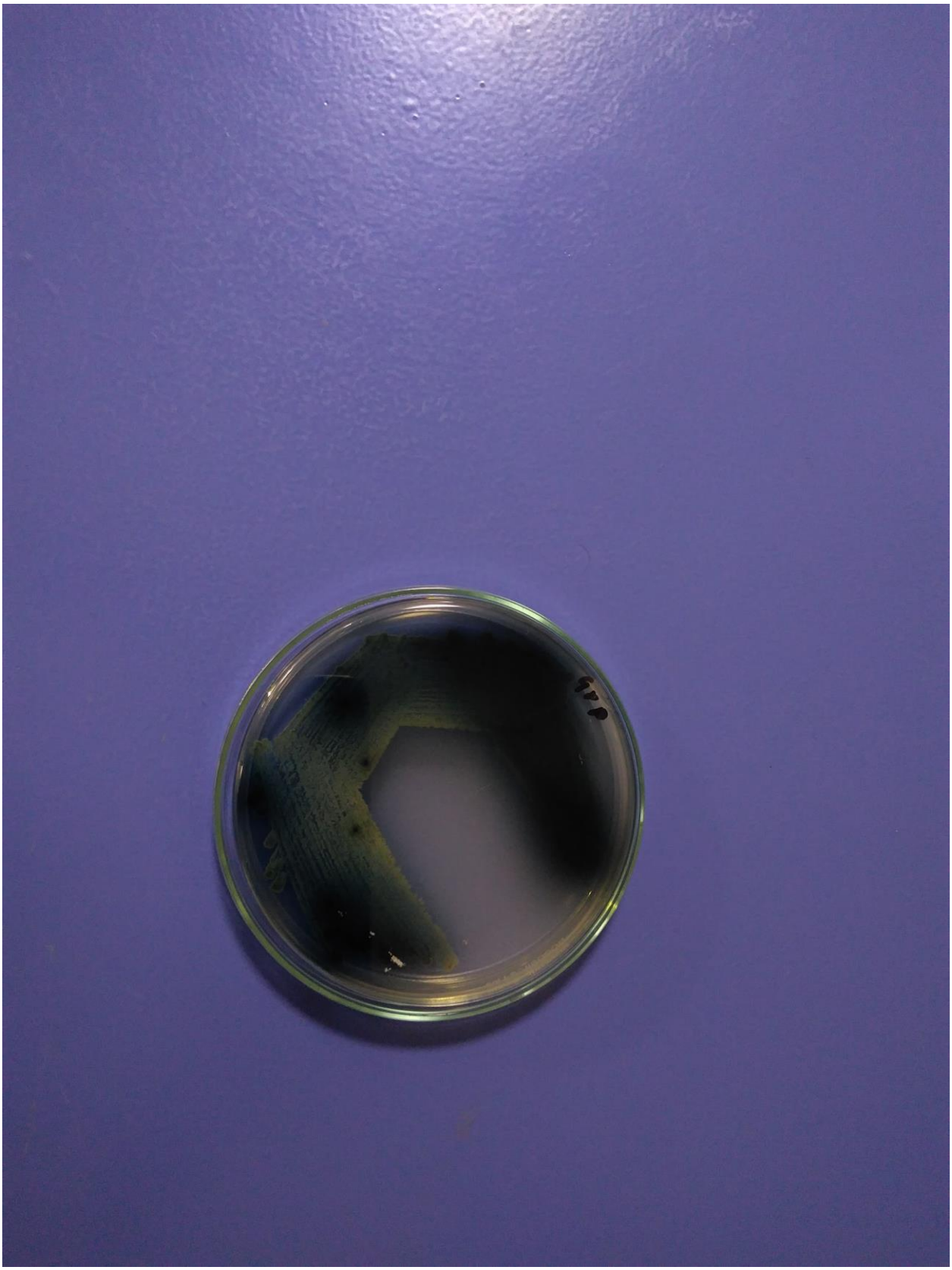

Fig 7: Melanin producing actinobacteria Streptomyces species isolated from mangrove sediment samples

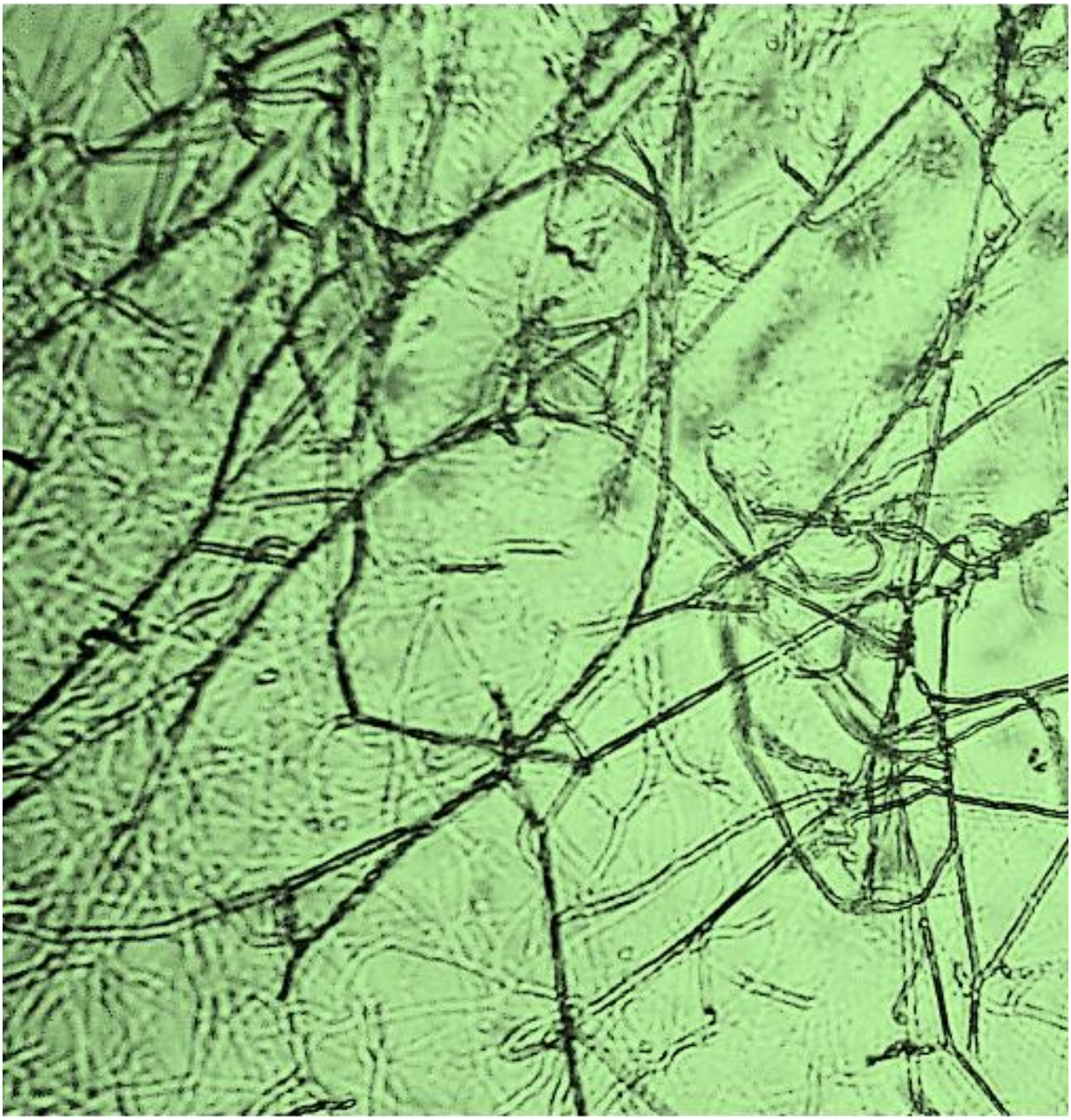

Fig 8: Rectifilexibile spore morphology

From the above results, we can concur that from figure 1

Table 1: Identification of pathogen

Gram stain	+
Shape	**Cocci**
Motility	
Indole	+
MR	
VP	
Citrate	
TSI	+

Oxidase	
Catalase	
Urease	+
Lactose	+
Maltose	+
Sucrose	+
Xylose	
Starch	+
Inositol	
Genus	***Streptococcus***

Table 2: Biochemical characteristics
Isolate

Cell wall	Aminoacid
LL-DAP	Present
DL-DAP	Absent
Glycine	Present
Alanine	Absent

Cell wall sugar

Arabinose	Absent
Galactose	Absent
Xylose	Absent
Madurose	Absent
Ribose	Absent
Inference	I/ N.C
Spore morphology	RF
Index	Streptomyces sp.

Table 3: Morphological Character Studied (As per Nonomura key)

Colour	Ash blue
Melanoid pigment	+
Reverse side	-
Soluble pigment	+
Spore chain	RF

Carbon source assimilation

Arabinose	-
Xylose	+
Inositol	±
Manitol	+
Rhamnose	-
Sucrose	±
Raffinose	+

DISCUSSION

In contrast to regularly occurring actinobacteria like Streptomyces, unusual actinobacteria are rarely isolated. Due to incomplete knowledge of their growth needs, marine actinobacteria are equally challenging to culture in lab settings. Actinobacteria have recently been grown under laboratory settings that can resemble their natural environment. For instance, numerous actinobacteria that were previously impossible to cultivate in labs have been isolated using an isolation chip (ichip). In addition, difficult microbiology methods are being replaced by methods like genomics, proteomics, metagenomics, and transcriptomics for the identification and description of the variety of marine microbes with distinctive properties utilizing bioinformatics tools.

A study conducted under the pretense of "Streptomyces derived bio active pigments….compounds" by Laura silva et al, helps us understand the substantial difference of this study with our current study. studied the activity of purified dissolved melanin (PDM), acid-based precipitation of melanin (AM), and synthetic melanin standard (SM) against *Alivibrio fischeri*, determining that the quorum-sensing activity of *A. fischeri* was interrupted more clearly by PDM and SM. Additionally, that was the first report of this activity on melanin and it proposed that the melanin from *S. cavourensis SV 21* may have an important function for the microbe–host and/or microbe–microbe interaction. In the same way, Wang et al. reported that insoluble and soluble melanin pigments could reduce biofilm formation against the Gram-positive *M. smegmatis* ATCC 10231 and the Gram-negative *P. aeruginosa* ATCC 9027 in a dose-dependent manner.

CONCLUSION:

To conclude, we can say that the melanin pigment that was extracted from the marine actinobacteria had proliferative activity on that of streptococcus sps biofilm under the electron microscope denoting it's advantages of further inhibition of the harmful bacteria.

FUTURE SCOPE:

Further research on this study could assist us in developing a viable natural antibacterial agent with distinct redox chemistry and the capacity to produce reactive oxygen species.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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AUTHOR CONTRIBUTION

Jeesha Soni : Literature search, Data collection analysis, Original manuscript draft

Dr. Abirami Arthanari : Data verification, Manuscript verification, Conceptualisation, Project administration

Dr. Kamala : Data collection analysis, Data verification, Conception or design of the work, Supervision of the findings

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