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Isolation and Extraction of Secondary Metabolites of Marine Actinomadura Species and its use Against Oral Pathogens

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Marine actinomadura, secondary metabolites, oral pathogens

Abstract:
Introduction:Due to the various opportunities as novel physiologically active bio compounds, marine microorganisms have drawn much attention lately. They can offer a variety of uses in many industries like pharmaceuticals, food and detergents. It is now of growing interest in the dental and medical spectrum too. Marine bacteria can be used to combat the growing trend of antibiotic resistance. In this study the isolation and extraction of secondary metabolites of marine *Actinomadura species* and its use against oral pathogens. Materials and methods: Samples were collected followed by the identification and spore chain formation. The secondary metabolites were isolated and tested at concentrations of 100, 200, 300, 400, 500µg/ml of *Streptococcus mutans*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *P. aeroginosa* with tetracycline as the control. Results: The extrcation of secondary metabolites and their antimicrobial potential was observed from the different oral pathogens and the zone of inhibition. Conclusion: Isolated samples of *Actinomadura species* shows effective results at various concentrations.

Introduction

Researchers have recently paid a lot of attention to marine microorganisms as potential sources of novel physiologically active compounds. This is because marine microorganisms live in such a wide range of environments and because we don't understand them well enough(1)]. Numerous marine bacteria are of significant biotechnological interest, such as those used in bioremediation, the creation of biosurfactants, and the biochemistry of "psychrophilic" enzymes produced by microbes suited to low temperatures(2) .These compounds offer great promise for biotechnological uses in the detergent and food industries, as well as in the manufacture of chemical reagents and pharmaceuticals.(3) Additionally, a significant number of fresh antibiotics derived from marine bacteria have been discovered. Marine bacteria in antibiotics can prove to be a breakthrough in developing countries especially where the unsolicited prescription has led to development of the multi-drug resistant bacteria strains(4). Actinomycetes are considered to be the most significant category of microbes in the field of biotechnology because they create bioactive secondary metabolites that have uses in agriculture, industry, and medicine(4,5). Less than 1% of the actinomycetes have, however, so far been identified, studied, and recorded(6).

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Out of the 500,000 natural chemicals identified from biological sources worldwide, about 70,000 are generated from microorganisms (including bacteria and fungi), with actinomycetes accounting for 29% of them(6). Rare actinomycetes that are produced from the sea are thought to be a potentially abundant source of different chemicals, secondary metabolites with unique structural characteristics, and novel medicinal molecules(7)]. By 1970, there were only 11 unique actinomycetes genera known, then 100 genera by 2005, and 220 genera by 2010(8) . Our understanding has grown as a result of the use of high-throughput metagenome sequencing techniques, which have also identified numerous novel actinomycetes that have not yet been identified in cultivation experiments(8,9) . In traditional cultivation trials, the recovery of unusual actinomycetes is typically lower than that of the streptomycete strains(9)]. However, recent advances in our comprehension of the physiological, chemical, and structural characteristics of marine actinomycetes have made it possible to construct selective isolation mediums(10). This study was hence conducted to ascertain the use after the isolation and extraction of secondary metabolites from the marine *Actinomadura* species against oral pathogens after the isolation and extraction.

Materials and Methods

Collection of sediment samples and isolation of *Actinomadura* sp

The sediment samples in marine environment were collected from east coasts region of Chennai. The samples was dried inside a laminar air flow for about 10 hrs and kept in a petri dish for 20 days which was further used for isolation of *Actinomadura* sp. The Actinomycetes Isolation Agar (AIA) media with 100% sea water, 25% sediment extract was innoculated for the isolation of actinomycetes. Plates were incubated at 28°C for 12-14 days. Then the isolated *Actinomadura sp* was sub-cultured and the slant culture was been stored at 4°C. And 20% glycerol stock was maintained at -80 °C(11).

Spore chain morphology and identification of *Actinomadura* sp.

The morphology and biochemical characterization of the potential isolate was recorded. By using light microscope, the spore chain that is composed of hyphae which bears spores in them with aerial and substrate mycelium of the strain(12)(13).

Extraction of secondary metabolites:

The isolated bacterium spores were inoculated in 1000 ml Erlenmeyer flasks having 200 ml of Yeast-malt broth. The inoculated broth was incubated for 5-7 days in an orbital incubator shaker at 200 rpm at room temperature. The culture broth was then centrifuged at 10000 rpm for 10 minutes and the supernatant and mycelium was been separated. the supernatant was added equal volume of ethyl acetate in a separating funnel and shaken well. The layer of organic metabolites was extracted and the excess solvent was evaporated. And further it was lyophilized (14),(13).

Bacterial Suspension:

The pathogenic bacteria such as *Streptococcus mutans*, *Klebsiella pneumonia, Staphylococcus aureus* and *P. aeroginosa* was collected from the Department of Microbiology, Saveetha Medical College and Hospital, Tamilnadu. These pathogens were inoculated in a Muller Hinton broth for 24 hr at 28ºC. The optical density of bacterial culture was measured at 600 nm and the pathogens were loaded 1ml over a agar plate (Muller Hinton) with 24hrs of incubated at room temperature for 24hrs.

Antibacterial Activity:

The antibacterial assay was performed by disc diffusion method. Whatman filter paper discs (6mm) were impregnated with 5 different concentrations (100, 200, 300, 400, 500µg/ml) of secondary metabolites and the Tetracycline antibiotic was used as standard drug. These agar plates were incubated at ambient temperature for 24 hrs and the zone of inhibition around the discs were evaluated. The results were exhibited from an average of three with standard deviation ((15)).

Results

Identification of marine actinobacterium of *Actinomadura* sp.:

The conventional identification of marine *Actinomadura* sp was done using different techniques. The cell wall amino acids, cell wall sugar and cell wall type were confirmed as the *Actinomadura* species (Table-1). The colour of mycelium was whitish gray (Fig.1) and Melanoid pigment was absent; however, the reverse side pigment was present. The spore chain morphology was hook spiral type (Table-2). The other biochemical characteristic and assimilation of carbon source of Arabinose, Xylose, Fructose, Sucrose, Raffinose and Inositol presence/absence was also

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observed (Table-2). The extraction of secondary metabolites and their antimicrobial potential was observed from the different oral pathogens and the zone of inhibition was mentioned in the Table-3. The tetracycline was used as a standard and standard deviation was also analyzed.

Fig.1 marine *Actinomadura* sp

Table-1: conventional identification of marine actinobacterium

Cell wall amino acids			**Cell wall sugar**		**Cell wall type & Index**
LL-DAP	MesoDAP	Glycine	Arabinose	Madurose	
-	+	-	-	+	III B& *Actinomadura*

Table-2 Biochemical characteristic of marine *Actinomadura* sp

Color of aerial mycelium	Whitish gray
Melanoid pigment	-
Reverse side pigment	+
Soluble pigment	-
Spore chain	Hook spiral
Assimilation of carbon source	
Arabinose	+
Xylose	±
Inositol	-
Mannitol	-
Fructose	±
Rhamnose	+
Sucrose	-
Raffinose	±

Table-3: Antimicrobial activity against different human pathogens

Con µg/ml	*K. pneumoniae*	Std.	*S. aureus*	Std.	*P. aeroginosa*	Std.	*S. mutans*	Std.	*MRSA*	Std.
100	11±2.3	15±2.5	7±2.4	12±2.5	9±2.3	14±2.5	6±2.2	11±2.5	5±2.5	13±2.5

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200	14±2.6	18±2.7	11±2.7	17±2.7	14±2.1	21±2.7	12±2.5	16±2.7	9±2.1	18±2.7
300	19±2.1	24±2.5	15±2.4	25±2.5	18±2.4	29±2.5	16±2.1	20±2.5	12±2.5	22±2.5
400	23±2.2	29±2.4	19±2.3	29±2.4	23±2.2	34±2.4	21±2.6	24±2.4	15±2.2	26±2.4
500	28±2.4	32±2.6	21±2.4	33±2.6	28±2.4	38±2.6	24±2.2	29±2.6	19±2.7	30±2.6

Discussion

Various metabolite compounds produced by actinobacteria play important functions in their diversified and intricate microenvironments. Natural products—also referred to as secondary metabolites—are beneficial substances created by bacteria. Normally, these are not required for the growth of natural cells, but they nonetheless benefit the cells in other ways(16). The compounds that are produced during or just before the stationary stage of an organism's development are known as secondary metabolites(11). They are crucial for nutrition and health, and as a result, they have economic significance(17). Actinobacteria are among the makers of secondary metabolites and are highly valued in both science and business. It may create secondary metabolites with considerable efficiency, including immunomodulators(18), antibiotics(19), anti-cancer medications and, growth factors(20)(21).

For the development of marine drugs, marine actinobacteria are a rich source of various bioactive substances with biological applications. Nearly 30 actinomycetes strains were identified and isolated from different Streptomyces families. All of the selected strains of Streptomyces that have been shown to have effective antibacterial action against the multidrug-resistant bacteria include M93, W108, W38, M72, M71, and M1 (MDRB)(22). It has been observed that marine Actinobacteria produce antitumor chemicals.

Additionally, marine Actinobacteria have been linked to bioactive substances with antimalarial activity(22–24). Furthermore, 84 Actinobacteria were isolated from freshwater sediments and classified into the common genus Streptomyces as well as eight uncommon genera such as *Micrococcus*, *Kocuria*, *Nocardiopsis*, *Promicromonospora*, *Saccharopolyspora*, *Amycolatopsis*, *Prauserella*, and *Rhodococcus*. All strains inhibited yeast pathogens, Gram-negative bacteria, and Gram-positive bacteria significantly(25). Yücel and Yamaç(26) demonstrated that extracts from *Streptomyces* sp. 1492 have antibacterial activity against MRSA, VRE, and Acinetobacter baumanii with MICs and MBCs of 125 g/mL and 250 to 1000 g/mL, respectively. Atrop-abyssomicin C and proximicins A, B, and C were identified by Goodfellow and Fiedler(27) from Verrucosispora sp. strains obtained from the sediment of the Japan Sea. When tetrahydrofolate is synthesised by Gram-positive bacteria that contain methicillin-resistant Staphylococcus aureus, it prevents the creation of p-aminobenzoate (MRSA)(13). According to Sajid et al(28), the *Streptomyces malachitofuscus* shows anti-fungal effects against *Candida albicans* and *Mucor miehei*. Actinobacterial substances having anti-MRSA activity number 124. Actinobacteria produced a number of bioactive pharmaceutical compounds that were discovered to be efficient against drug-resistant bacterial pathogen strains such Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant Enterococcus (VRE)(29). According to Castillo et al., Kakadumycin A was isolated from Streptomyces sp. NRRL 30566 suppressed the growth of MRSA American Type Culture Collection (ATCC) 33591 at a concentration of 0.5 g/mL compared to the concentration of vancomycin at 2.0 g/mL(30). Nomimicin, a recently identified spirotetronate antibiotic of polyketide origin, was isolated from an *Actinomadura* sp. TP-A0878 culture extract and the compound shown antibacterial effects on *M. luteus* and *C. albicans*. This could also prove to be highly effective against oral microbes in the dental spectrum(31)(32). From the tests that were conducted from the isolated secondary metabolites, the most efficacies was showed against S. mutans when compared to tetracycline. The values obtained were were closed to those displayed by tetracycline which could be used

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against the organism related diseases(12). The secondary metabolites produced by the *Actinomadura* also showed good results when they tested against *Klebsiella pneumoniae*, MRSA and *P. aeroginosa* at all the varying concentrations.

Conclusion

This study could show that the isolated secondary metabolites from the *Actinomadura* sp. proved to be effective against the commonly seen bacteria in the oral cavity. The results show that in the future this could be a useful development in the treatment of diseases which are caused by these bacteria. The formulation of specific antibiotics against these oral microbes would also be beneficial through local drug delivery methods. There is great scope for research in the future.

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1. Sifri Z, Chokshi A, Cennimo D, Horng H. Global contributors to antibiotic resistance [Internet]. Vol. 11, Journal of Global Infectious Diseases. 2019. p. 36. Available from: http://dx.doi.org/10.4103/jgid.jgid_110_18
2. Mondol MAM, Shin HJ, Islam MT. Diversity of secondary metabolites from marine Bacillus species: chemistry and biological activity. Mar Drugs [Internet]. 2013 Aug 12;11(8):2846–72. Available from: http://dx.doi.org/10.3390/md11082846
3. Böhringer N, Fisch KM, Schillo D, Bara R, Hertzer C, Grein F, et al. Antimicrobial Potential of Bacteria Associated with Marine Sea Slugs from North Sulawesi, Indonesia. Front Microbiol [Internet]. 2017 Jun 14;8:1092. Available from: http://dx.doi.org/10.3389/fmicb.2017.01092
4. Manivasagan P, Kang KH, Sivakumar K, Li-Chan ECY, Oh HM, Kim SK. Marine actinobacteria: an important source of bioactive natural products. Environ Toxicol Pharmacol [Internet]. 2014 Jul;38(1):172–88. Available from: http://dx.doi.org/10.1016/j.etap.2014.05.014
5. Subramani R, Sipkema D. Marine Rare Actinomycetes: A Promising Source of Structurally Diverse and Unique Novel Natural Products. Mar Drugs [Internet]. 2019 Apr 26 [cited 2022 Dec 23];17(5):249. Available from: https://www.mdpi.com/1660-3397/17/5/249
6. Bérdy J. Thoughts and facts about antibiotics: Where we are now and where we are heading [Internet]. Vol. 65, The Journal of Antibiotics. 2012. p. 385–95. Available from: http://dx.doi.org/10.1038/ja.2012.27
7. Challinor VL, Bode HB. Bioactive natural products from novel microbial sources [Internet]. Vol. 1354, Annals of the New York Academy of Sciences. 2015. p. 82–97. Available from: http://dx.doi.org/10.1111/nyas.12954
8. Subramani R, Aalbersberg W. Culturable rare Actinomycetes: diversity, isolation and marine natural product discovery. Appl Microbiol Biotechnol [Internet]. 2013 Nov;97(21):9291–321. Available from: http://dx.doi.org/10.1007/s00253-013-5229-7
9. Jose PA, Jha B. Intertidal marine sediment harbours Actinobacteria with promising bioactive and biosynthetic potential [Internet]. Vol. 7, Scientific Reports. 2017. Available from: http://dx.doi.org/10.1038/s41598-017-09672-6
10. Claverías FP, Undabarrena A, González M, Seeger M, Cámara B. Culturable diversity and antimicrobial activity of Actinobacteria from marine sediments in Valparaíso bay, Chile. Front Microbiol [Internet]. 2015 Jul 28;6:737. Available from: http://dx.doi.org/10.3389/fmicb.2015.00737
11. Sheefaa MI, Sivaperumal P. Antioxidant activities from melanin pigment produced by marine actinobacterium of species. J Adv Pharm Technol Res [Internet]. 2022 Nov;13(Suppl 1):S84–7. Available from: http://dx.doi.org/10.4103/japtr.japtr_338_22
12. Melanin from marine Streptomyces sp. (MVCS13) with potential effect against ornamental fish pathogens of Carassius auratus (Linnaeus, 1758). Biocatal Agric Biotechnol [Internet]. 2014 Oct 1 [cited 2023 Sep 20];3(4):134–41. Available from: http://dx.doi.org/10.1016/j.bcab.2014.09.007
13. Isolation and characterization of biologically active alkaloids from marine actinobacteria Nocardiopsis sp. NCS1. Biocatal Agric Biotechnol [Internet]. 2015 Jan 1 [cited 2023 Sep 20];4(1):63–9. Available from: http://dx.doi.org/10.1016/j.bcab.2014.10.005
14. Rajan BM, Kannabiran K. Extraction and Identification of Antibacterial Secondary Metabolites from Marine Streptomyces sp.

VITBRK2. Int J Mol Cell Med [Internet]. 2014 Summer;3(3):130–7. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25317399

15. Website [Internet]. Available from: https://doi.org/10.3390/ jmse10111788
16. Xin T, Su C, Lin Y, Wang S, Xu Z, Song J. Precise species detection of traditional Chinese patent medicine by shotgun metagenomic sequencing [Internet]. Vol. 47, Phytomedicine. 2018. p. 40–7. Available from: http://dx.doi.org/10.1016/j.phymed.2018.04.048
17. Chaudhary AK, Dhakal D, Sohng JK. An Insight into the "-Omics" Based Engineering of Streptomycetes for Secondary Metabolite Overproduction [Internet]. Vol. 2013, BioMed Research International. 2013. p. 1–15. Available from: http://dx.doi.org/10.1155/2013/968518
18. Meklat A, Bouras N, Mokrane S, Zitouni A, Djemouai N, Klenk HP, et al. Isolation, Classification and Antagonistic Properties of Alkalitolerant Actinobacteria from Algerian Saharan Soils [Internet]. Vol. 37, Geomicrobiology Journal. 2020. p. 826–36. Available from: http://dx.doi.org/10.1080/01490451.2020.1786865
19. Hoskisson PA, Fernández-Martínez LT. Regulation of specialised metabolites in Actinobacteria - expanding the paradigms. Environ Microbiol Rep [Internet]. 2018 Jun;10(3):231–8. Available from: http://dx.doi.org/10.1111/1758-2229.12629
20. Bibb MJ. Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol [Internet]. 2005 Apr;8(2):208–15. Available from: http://dx.doi.org/10.1016/j.mib.2005.02.016
21. Sivaperumal P, Sekaran S, Kamala K, Ganapathy D. Marine bioactive compounds as potential therapeutic inhibitor for oral squamous cell carcinoma (OSCC). Oral Oncol [Internet]. 2022 Aug;131:105971. Available from: http://dx.doi.org/10.1016/j.oraloncology.2022.105971
22. Cheema M, Fatima A, Sajid I. Molecular Identification, Bioactivity Screening and Metabolic Fingerprinting of the Actinomycetes of Chenab River Sediments [Internet]. Vol. 17, British Microbiology Research Journal. 2016. p. 1–13. Available from: http://dx.doi.org/10.9734/bmrj/2016/28805
23. Dimri AG, Chauhan A, Aggarwal ML. In vitro antibacterial characterization of Streptosporangium sp. (AI-21) a new soil isolate against food borne bacteria [Internet]. Vol. 01, Science Archives. 2020. p. 25–34. Available from: http://dx.doi.org/10.47587/sa.2020.1103
24. Kim J, Shin D, Kim SH, Park W, Shin Y, Kim WK, et al. Borrelidins C–E: New Antibacterial Macrolides from a Saltern-Derived Halophilic Nocardiopsis sp [Internet]. Vol. 15, Marine Drugs. 2017. p. 166. Available from: http://dx.doi.org/10.3390/md15060166
25. Kandel SL, Joubert PM, Doty SL. Bacterial Endophyte Colonization and Distribution within Plants. Microorganisms [Internet]. 2017 Nov 25;5(4). Available from: http://dx.doi.org/10.3390/microorganisms5040077
26. Yamaç M, Işik K, Şahin N. Numerical classification of streptomycetes isolated from karstic caves in Turkey [Internet]. Turkish Journal of Biology. 2011. Available from: http://dx.doi.org/10.3906/biy-0911-185
27. Goodfellow M, Fiedler HP. A guide to successful bioprospecting: informed by actinobacterial systematics. Antonie Van Leeuwenhoek [Internet]. 2010 Aug;98(2):119–42. Available from: http://dx.doi.org/10.1007/s10482-010-9460-2
28. Sajid I, Shaaban KA, Hasnain S. Identification, isolation and optimization of antifungal metabolites from the Streptomyces Malachitofuscus ctf9. Braz J Microbiol [Internet]. 2011 Apr;42(2):592–604. Available from: http://dx.doi.org/10.1590/S1517-838220110002000024
29. Francis J, Arivarasu L, Sivaperumal P, Thangavelu L. Screening of antibacterial activity from mangrove plants (Rhizophora mucronata) leaf associated Actinobacterium of Streptomyces species. J Pharm Res Int [Internet]. 2021 Dec 28;524–33. Available from: https://journaljpri.com/index.php/JPRI/article/view/5361
30. Castillo U, Harper JK, Strobel GA, Sears J, Alesi K, Ford E, et al. Kakadumycins, novel antibiotics from Streptomyces sp NRRL 30566, an endophyte of Grevillea pteridifolia. FEMS Microbiol Lett [Internet]. 2003 Jul 29;224(2):183–90. Available from: http://dx.doi.org/10.1016/S0378-1097(03)00426-9
31. Igarashi Y, Iida T, Oku N, Watanabe H, Furihata K,

Miyanouchi K. Nomimicin, a new spirotetronate-class polyketide from an actinomycete of the genus Actinomadura. J Antibiot [Internet]. 2012 Jul;65(7):355–9. Available from: http://dx.doi.org/10.1038/ja.2012.30

32. Murugaboopathy V, Saravankumar R, Mangaiyarkarasi R, Kengadaran S, Samuel SR, Rajeshkumar S. Efficacy of marine algal extracts against oral pathogens - A systematic review. Indian J Dent Res [Internet]. 2021 Oct-Dec;32(4):524–7. Available from: http://dx.doi.org/10.4103/ijdr.IJDR_243_20