ORIGINAL ARTICLE

Amylase-producing marine actinobacterium of *Micromonospora* sp. and their potential antibacterial effects against oral pathogens

Rajasri Pradeep, Pitchiah Sivaperumal[1], Anitha Roy

Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, [1]Marine Biomedical Research Lab and Environmental Toxicology Unit, Cellular and Molecular Research Centre, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

J. Adv. Pharm. Technol. Res.

ABSTRACT

Marine actinobacteriological investigation is still in its beginning in India. Earlier, in the 20th century, studies on *Actinobacteria* were started and highly concentrated on diversity, identification, and screening for enzymes, antibiotics, and enzyme inhibitors. With the spurge of infectious diseases requiring antibiotics, novel antibiotics are in search as the prevalent ones have declined uses, due to the antibiotic-resistant microbial growth. Unexploited ecosystems are studied for isolation of rare species such as *Actinobacteria* which are expected to yield newer metabolites. The marine actinobacterial isolation and enumeration were done from sediment samples. The marine *Actinobacteria* were identified by conventional methods. Further amylase enzyme production and their antibacterial activities are also done following the standard methods. The *Micromonospora* sp. was identified by chemotaxonomical characteristics and spore chain morphology. Further, the amylase enzyme production was done and quantification of enzyme also done. The potential antimicrobial activity from the amylase enzyme was done. Zone of inhibition and minimal inhibitory concentration were calculated. It concluded that potent antibacterial activity was obtained from *Actinobacteria Micromonospora* sp. producing amylase enzymes.

Key words: Antibacterial activity, marine *Actinobacteria*, minimal inhibitory concentration, *Micromonospora* sp. novel enzyme

INTRODUCTION

Marine *Actinobacteria* are effective fabricators of new secondary metabolites; it shows a variety of biological activities such as antibacterial, antifungal, insecticidal, enzyme inhibition, and anticancer.[1] Protein acetylation is an important mechanism in *Actinobacteria*.[2] Marine actinobacteriological investigations are still in their beginning in India. Earlier, in the 20th century, studies on *Actinobacteria* were started and highly concentrated on diversity, identification, and screening for enzymes, antibiotics, and enzyme inhibitors.[3] This study uses amylase-producing marine *Actinobacteria* that were identified by the aerial mass color, pigments, and spore chain morphology. *Actinobacteria* are ubiquitous in nature and hence are used as a source for pharmaceutical research.[4] There have been very scarce studies related to marine *Actinobacteria*; however,

Address for correspondence:
Dr. Pitchiah Sivaperumal,
Marine Biomedical Research Lab and Environmental Toxicology Unit, Cellular and Molecular Research Centre, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600 077, Tamil Nadu, India.
E-mail: sivaperumalp.sdc@saveetha.com

Submitted: 09-May-2022 **Revised:** 30-Jun-2022
Accepted: 02-Jul-2022 **Published:** 30-Nov-2022

Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/japtr.japtr_333_22

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Pradeep R, Sivaperumal P, Roy A. Amylase-producing marine actinobacterium of *Micromonospora* sp. and their potential antibacterial effects against oral pathogens. J Adv Pharm Technol Res 2022;13:S76-9.

marine *Actinobacteria* are considered potential producers of important bioactive compounds. With the splurge of infectious diseases requiring antibiotics, novel antibiotics are in search as the prevalent ones have declined uses due to the antibiotic-resistant microbial growth. Hence, unexploited ecosystems are studied for the isolation of rare species such as *Actinobacteria* which are expected to yield newer metabolites.[5]

There have been various researches on the diversity of *Actinobacteria* from unexplored ecological niches of terrestrial ecosystems, which lead to isolate 26 actinobacterial genera in India. The most dominant genus is *Streptomyces* which is followed by *Micromonospora*, the genus used in this study. This is followed by *Actinomadura*, *Rhodococcus*, *Microbispora*, and *Nocardia*. 70% of the earth is covered by oceans, and in terms of microbial diversity, the oceans cover and support the richest ecosystems of the earth.[6] The antimicrobial activities of *Actinobacteria* have been tested in various studies. Velho-Pereira and Kamat reported that, overall 28 *Actinobacteria* were isolated from sediment samples and its ability to show antimicrobial activities.[7]

Amylase is the enzyme that hydrolyzes starch. It converts starch to glucose and this is applied in biotechnology, medicine, nutrition, chemistry, paper, textile, and detergent industries.[8] In general, enzymes that are produced from bacterial and fungal sources are said to have many applications in industries.[9] All the recent advancements in the biotechnological tool led to the utilization of amylase, which has enhanced clinical research. Multipotent application and demand have paved the approach for growing indigenous production of amylase and finding further competent processes for its production.[10]

Oral pathogens or microbes exhibit specific adherent mechanisms, and they colonize the oral cavity and cause various diseases.[11] The oral cavity is a reservoir for bacterial pathogens for focal infections. Pathogenic strains may pertain to the oral cavity, but the toxins secreted can regenerate rate and reach an organ or tissue via the bloodstream and cause any sort of metastatic injury.[12,13] Antibacterial activity of various species and nanoparticles has been tested in various studies, and they have proven to be useful for future uses.[14-16]

Apart from antibacterial effects, *Actinobacteria* have also shown anti-inflammatory, anticancer, and antioxidant properties.[17-19] Further, in the present study, potent antibacterial activity was obtained from *Actinobacteria Micromonospora* sp.-producing amylase enzymes.

MATERIALS AND METHODS

The sediment sample was collected around Cuddalore coast, Tamil Nadu. Collected samples were dried in air condition for 1 day and sun-dried for 48 h. After sundry, the sediment sample was macerated into powder. The activity of amylase in the strains was vetted qualitatively in starch agar. The hydrolysis zone observation was done the gram iodine in flooded condition. The clear zone (in diameter) on starch agar measured from actinobacterial colonies and enzyme activity also was done.

Bacterial suspension

The dental pathogens *Escherichia coli*, *Streptococcus mutans*, and *Streptococcus sanguinis* were collected from Saveetha Dental College and Hospital, Tamil Nadu. Collected pathogens were cultured and stock was maintained in Muller–Hinton agar plate. Further, the broth preparation of the bacterial culture was done in the Muller–Hinton broth and kept for 1 day at room temperature condition and used for antibacterial activities.

Antibacterial activity

Antibacterial potential of the enzyme amylase was done by following the method of disc diffusion. The 5 mm diameter discs were used for the disc diffusion assay and different concentrations (50, 100, 150, 200, and 250 μg/ml) of enzyme samples were used and antibiotic tetracycline used as positive control and DMSO used as a negative control. Further, the plates were kept in 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.

Minimal inhibitory concentration

The minimal inhibitory concentration of the enzyme amylase was analyzed using different concentrations such as 50, 100, 150, 200, and 250 μg/ml of enzyme with tetracycline (standard), and DMSO was the negative control. The bacterial suspension (in test tubes) was kept in incubator for 1 day in room temperature and the optical density was analyzed.

RESULTS

The *Micromonospora* sp. was identified by chemo-taxonomical characteristic and spore chain morphology such as cocci shape also done [Table 1]. Further, the Amylase enzyme production was done. The potential antibacterial activity using Amylase enzyme was done against the oral pathogens. Different concentration of amylase enzyme was used and the results are expressed in the zone of inhibition and mentioned in the Table 2. While increasing the concentration of enzyme, the zone of inhibition also increased. Further, MIC test was also calculated and mentioned in the Table 3.

DISCUSSION

Marine actinobacterial *Micromonospora* sp. showed potential

antibacterial activity against all other antibacterial studies when compared to other studied organisms. It can be comprehended from various studies that there is still a very little understanding of diversity in this genus. Generally, the marine actinobacterial genus of *Micromonospora* have morphological traits such as color of vegetative mycelium, aerial hyphae and spores with different shapes. Further, it might be isolated from plant or environment samples also for obtaining bio-active compounds. The results obtained confirmed that *Micromonospora* species were isolated from the *Actinobacteria* [Table 1]. *Micromonospora* species were verified by the gray color of the aerial mycelium. In addition to that there were positive readings for reverse side pigments, inositol, mannitol, sucrose, xylose, and raffinose and there were negative readings for melanoid pigment, soluble pigment, arabinose, and rhamnose. The total antibacterial activity of the amylase enzyme from *Micromonospora* species was discovered. Values of Table 3 consist of values of Minimum inhibitory concentration of *E. coli*, *S. mutans*, and *S. sanginis* compared to the standard tetracycline.

Table 1: Characteristic features of marine Micromonospora species

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

Table 2 : Antibacterial activity of the amylase enzyme from *Micromonospora* spp.

***µg*/ml**	***Escherichia coli***	***Streptococcus mutans***	***Streptococcus sanguinis***
50	8±1.6	6±2.1	5±1.9
100	12±2.1	11±2.6	10±2.3
150	15±2.8	16±2.4	15±2.1
200	19±2.4	20±2.1	21±2.6
250	26±2.1	25±2.6	28±2.4

Table 3: Minimum inhibitory concentration determination of amylase enzyme from *Micromonospora* spp.

	MIC
E. coli	30 µg/ml
Tetracycline	10µg/ml
S. mutans	20µg/ml
Tetracycline	10 µg/ml
S.sanginis	20 µg/ml
Tetracycline	10 µg/ml

CONCLUSION

The above-mentioned results concluded that amylase-producing marine *Actinobacteria* have potential antibacterial activity against oral pathogens. Further, it could be useful in pharmaceutical applications.

Acknowledgment

The authors would like to thank Saveetha Dental College and Saveetha Institute of Medical and Technical Sciences for their kind support to utilize the facilities for the study.

Financial support and sponsorship

Golden Sand Property Developers, Chennai, Tamil Nadu, supported the study.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Wang T, Li F, Lu Q, Wu G, Jiang Z, Liu S, *et al*. Diversity, novelty, antimicrobial activity, and new antibiotics of cultivable endophytic *Actinobacteria* isolated from psammophytes collected from Taklamakan Desert. J Pharm Anal 2021;11:241-50.
2. Roy A, Rasheed A, Sleeba AV, Rajagopal P. Molecular docking analysis of capsaicin with apoptotic proteins. Bioinformation 2020;16:555-60.
3. Goel N, Fatima SW, Kumar S, Sinha R, Khare SK. Antimicrobial resistance in biofilms: Exploring marine *Actinobacteria* as a potential source of antibiotics and biofilm inhibitors. Biotechnol Rep (Amst) 2021;30:e00613.
4. Franco-Correa M, Chavarro-Anzola V. *Actinobacteria* as plant growth-promoting rhizobacteria. In: *Actinobacteria*-Basics and Biotechnological Applications. Edited by Dharumadurai Dhanasekaran and Yi Jiang, IntechOpen, UK; 2016.
5. Vaijayanthi G, Vijayakumar R, Dhanasekaran D. *Actinobacteria* – A biofactory of novel enzymes. In: *Actinobacteria* – Basics and Biotechnological Applications. Edited by Dharumadurai Dhanasekaran and Yi Jiang, IntechOpen, UK; 2016.
6. Hamedi J, Poorinmohammad N. The cellular structure of *Actinobacteria*. In: Biology and Biotechnology of *Actinobacteria*. Edited by Dharumadurai Dhanasekaran and Yi Jiang, IntechOpen, UK; 2017. p. 5-28.
7. Velho-Pereira S, Kamat NM. Antimicrobial screening of *Actinobacteria* using a modified cross-streak method. Indian J Pharm Sci 2011;73:223-8.
8. A Rapid and Ultrasensitive Tetraphenylethylene-Based Probe with Aggregation-Induced Emission for Direct Detection of Amylase in Human Body Fluids.
9. Ende N. Amylase activity in body fluids. Cancer 1961;14:1109-14.
10. Street HV. Inhibition of amylase activity of human body fluids by citrate. Biochem J 1960;76:10-3.

11. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. *In silico* analysis of virulence genes in an emerging dental pathogen *A. baumannii* and related species. Arch Oral Biol 2018;94:93-8.
12. Edmisson J. Filifactor alocis, a newly appreciated oral pathogen, fails to induce the respiratory burst response of human neutrophils. College of Arts & Sciences Senior Honors Theses. Paper 109.
13. Kandhan TS, Roy A, Lakshmi T, Rajeshkumar S. Green synthesis of rosemary oleoresin mediated silver nanoparticles and its effect on oral pathogens. Res J Pharm Technol 2019;12:5379.
14. Paul RP, Roy A, Maajida AM, Shanmugam R. Antibacterial activity of white pepper oleoresin mediated silver nanoparticles against oral pathogens. J Evol Med Dent Sci 2020;9:2352-5.
15. Vignesh S, Anitha R, Rajesh Kumar S, Lakshmi T. Evaluation of the antimicrobial activity of cumin oil mediated silver nanoparticles on oral microbes. Res J Pharm Technol 2019;12:3709.
16. Anitha Roy, Akshaya R, Rajeshkumar S, Roy A, Lakshmi T. *Maranta arundinacea* root mediated zinc oxide nanoparticles and its enhanced antibacterial activity. Int J Res Pharm Sci 2020;11:5269-73.
17. Swathy S, Roy A, Rajeshkumar S. Anti-inflammatory activity of Ginger oleoresin mediated Silver nanoparticles. Res J Pharm Technol 2020;13:4591.
18. Ashwini S, Ezhilarasan D, Anitha R. Cytotoxic effect of *Caralluma fimbriata* against human colon cancer cells. Pharmacogn J 2017;9:204-7.
19. Devaraj E, Roy A, Veeraragavan GR, Magesh A, Sleeba AV, Arivarasu L, *et al*. β-Sitosterol attenuates carbon tetrachloride–induced oxidative stress and chronic liver injury in rats. Naunyn Schmiedebergs Arch Pharmacol 2020;393:1067-75.