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Antibacterial and Antioxidant Property of Protease from Marine Actinobacteriummicrobispora Sp.

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Actinobacteria, Gram-positive bacteria are the largest phyla among the major species in the bacteria domain. Microbispora sp. is one of the secondary metabolites-producing Actinobacteria, and it has a comprehensive spectrum of antibacterial, antifungal, antitumor, antiviral, antiparasitic, diabetogenic anti-inflammatory, insecticidal, inhibitory of enzyme, antioxidant, and other biological activities. The objective of the study is to assess the antioxidant activity of the ActinobacteriumMicrobispora sp. producing protease enzymes. The marine Actinobacteria, Microbispora sp. are identified by chemo taxonomic characteristics. Protease enzyme exhibited concentration-dependent antimicrobial activity against Streptococcus mutans (7 ± 0.5 mm and 5.2 ± 0.8 mm at 100 and 50 µg/ml, respectively) and Klebsiellapneumoniae (18 mm and 23.5 mm at 100 and 50 µg/ml, respectively), compared to Ampicillin's inhibition zones of 18 mm for both pathogens.The antioxidant activity of the ascorbic acid equivalent which was observed in the Hydrogen peroxide scavenging activity of Microbispora sp. 72.6± 2.6%, DPPH antioxidant activity 75.2± 2.7% and total antioxidant activity 78.5± 1.8% at the concentration level of 100µg/ml. The role of different antioxidants and the action in different diseases were challenged since they could act as many mechanisms such as reducing power, providing hydrogen to radicals, and scavenging activity. To conclude, the potent antioxidant activity was obtained from ActinobacteriaMicrobispora sp. producing Protease enzymes.

Keywords: Marine, Actinobacteria, Microbispora sp., Protease, Antibacterial,DPPH, H2O2,

TAA, Enzymes.

1. Introduction

Actinomycetes, which are filamentous bacteria with a fungal-like appearance, belong to the phylum Actinobacteria[1]. Marine Actinobacteria are a fascinating group of microorganisms that inhabit diverse marine environments, ranging from shallow coastal waters to deep-sea sediments [2]. In soil ecosystems, they serve a significant function by breaking down intricate polymeric compounds in deceased plant and animal matter, contributing to the recycling of industrial byproducts and organic materials [3]. They are known for their ability to produce a wide range of bioactive compounds, including enzymes and secondary metabolites with various industrial and biotechnological applications [4]. Proteases are enzymes that exist across various biological domains, including prokaryotes, fungi, plants, and animals, and they are indispensable for the survival of these organisms [5]. In many pathogenic parasites, serine,cysteine, and metalloproteases are frequently encountered, and they assume crucial functions in immune system evasion, acquiring nutrients for growth and reproduction, facilitating the spread of the infection, and causing tissue damage during the course of an infection [6].Our marine actinobacterium, Microbispora sp., has shown remarkable potential in producing protease enzymes with exceptional properties, such as high stability, broad pH range, and compatibility with diverse substrates [7]. In addition to protease enzymes, our Microbispora sp. also exhibits remarkable antioxidant activity. Antioxidants are essential in combating oxidative stress and preventing damage caused by free radicals [8]. With the growing importance of antioxidants in the food, pharmaceutical, and cosmetic industries, our marine actinobacterium offers a promising source of natural antioxidants with potential health benefits [9].Protease enzymes have been studied for their potential to combat oral pathogens, which play a role in dental plaque formation and oral diseases like gum disease and tooth decay [10]. These enzymes break down proteins in biofilms formed by oral pathogens, potentially reducing their population and aiding in oral health [11]. In this study, a marine actinobacterium, Microbispora sp., was isolated from sediment samples for its protease enzyme production, and its antibacterial andantioxidant properties were evaluated.

2. Materials and Methods

2.1 Sample collection

The marine sediment sample was collected from the Tuticorin area, Tamil Nadu. The collected sediments were carefully transferred into the Marine Biomedical Laboratory and Environmental Toxicology Unit, Saveetha Institute of Medical and Technical Sciences. After reaching the lab, sample was air-dried for 48 hrs and turned into fine powder by mortar and pestle.

2.2 Isolation of Actinobacteria from sediment sample

Marine Actinobacteria were isolated through a cultivation process on Yeast Malt Agar (YM) medium, to which 10µg/ml of nalidixic acid was added to prevent the growth of bacteria and fungi. Sediment samples were macerated and then subjected to serial dilution. The diluted

samples were spread on YM agar plates and incubated at a mild temperature for one week. The population density of Actinobacteria in the sediment samples was quantified as colony-forming units per gram. Unique colonies with distinct morphologies were identified, and these were chosen for pure culture isolation and subsequent analysis. The confirmation of marine Actinobacteria identity was achieved by observing their characteristic features.

2.3 Collection of bacterial stains

The bacterial strains (Streptococcus mutans andKlebsiellapneumoniae) were collected from the Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai.

2.4 Antibacterial activity

The antibacterial efficacy of protease enzyme was evaluated using the disc diffusion method. 6mm diameter disc was used for the assay, and various concentrations (50 and 100 µg/mL) of protease samples were tested, along with ampicillin as the positive control. The agar plates were then incubated for one day at room temperature. The results were assessed by measuring the diameter of the zone of inhibition against the pathogens. A larger zone indicated more significant antimicrobial activity. The diameter of the inhibited pathogenic growth zone was measured, and the antibacterial activities against the oral pathogens were calculated based on these results [11].

2.5 Antioxidant activity

The total antioxidant activity, DPPH and Hydrogen peroxide scavenging activity of the Actinobacterial protease enzyme was done by [24].

3. Results & Discussion

In this study, marine Actinobacteria were isolated from sediment samples, and a genus of Microbispora was identified based on specific bacterial characteristics (Figure 1). The isolate displayed pink aerial mycelium with paired longitudinal spore chains. In this species, aerial mycelium differentiates into axial hyphae and spore-bearing side branches. Spiny spores are formed in clusters and the aerial mycelium is yellowish pink and the substrate mycelium yellowish brown on inorganic salts-starch agar v. But in Actinomycetes isolation agar pink color of the aerial mycelium produced. Optimal temperatures for growth are 35 to 40 °C with slightly alkaline pH 7.0-8.0. Biochemical analysis revealed the presence of meso-diaminopimelic acid, indicating a cell wall type III and glycogen type B. The substrate mycelium was orange and there were no melanoid or soluble pigments. The bacterium could utilize madurose.Further, commonly biosynthesized compounds from the marine actinobacterium showed potential antioxidant properties.

Figure 1 shows the pure culture of Actinobacteria and spore chain morphology of Microbispora sp.

3.1 Antibacterial activity against oral pathogens

The antimicrobial activity of the protease enzyme against oral pathogens (Streptococcus mutansand Klebsiellapneumoniae) was investigated using the agar disc diffusion method. The results, presented in Fig. 2, indicate that the reactions of oral pathogens to the protease enzyme are concentration dependent. Notably, Streptococcus mutans exhibited the highest susceptibility, with inhibition zones of 7 ± 0.5 and 5.2 ± 0.8 mm at concentration of 100 and 50 µg/ml. Klebsiellapneumoniae displayed the lowest susceptibility. Ampicillin served as a positive control, confirming bacterial susceptibility. The inhibition zones for S. mutans and Klebsiella sp. were 18 mm and 23.5 mm.

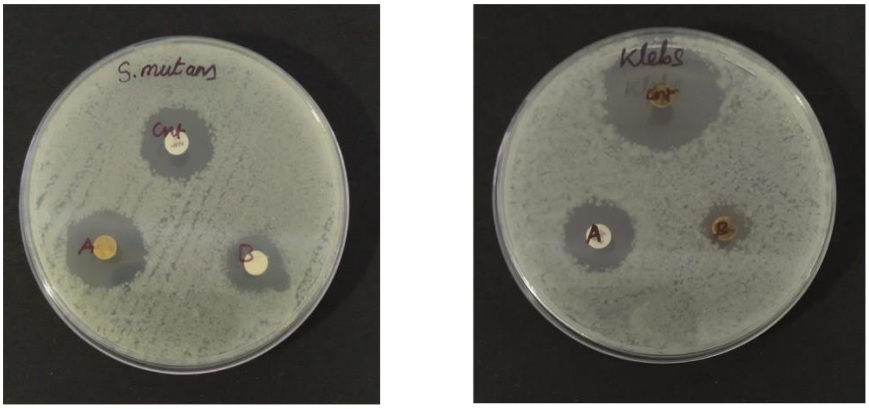

Figure 2 shows the Antibacterial activity against oral pathogens using protease enzyme fromMicrobispora sp.

In 2024 Subash et al.,reported that the responses of oral pathogens to the keratinase enzyme vary with concentration. Streptococcus mutanswas most susceptible, with 9 ± 0.5 mm and 9.6 ± 0.8 mm inhibition zones at 50 µg/ml and 100 µg/ml, respectively. Salmonella typhishowed intermediate susceptibility (8 ± 1 mm and 8.2 ± 0.5 mm), while Klebsiellapneumoniae was

least susceptible [12]. Studies have demonstrated that several probiotic strains, including Streptococcus salivarius K12, Lactobacillus reuteri AN417, and Lactobacillus salivarius subsp. salicinius AP-32, possess notable antibacterial effects against oral pathogens such as Streptococcus mutans, Porphyromonasgingivalis, and Fusobacteriumnucleatum [13, 14].Studies have indicated that these probiotics can impede the growth and formation of biofilms by these pathogens, indicating their promise as natural alternatives to conventional antibacterial agents. Furthermore, protamine, an antimicrobial peptide loaded onto dental materials, has demonstrated effective inhibition of Streptococcus mutans growth, underscoring the potential of protease enzymes in fighting oral pathogens [15].

3.2 Antioxidant activity

Biosynthesized compounds from the marine actinobacteriumMicrobispora sp., were assessed for their antioxidant capabilities using various methods, revealing distinct levels of antioxidant activity across different concentrations (25, 50, 75, and 100 µg/ml).

3.2.1 Hydrogen peroxide scavenging activity

The antioxidant activities of the protease enzymes were compared to that of the standard ascorbic acid equivalents (AAE), and the hydrogen peroxide scavenging activity of marine compounds shows the minimum and maximum absorbance of (38.5±2.6% & 72.6±2.6%) at the concentration level of 25 & 100µg/ml.

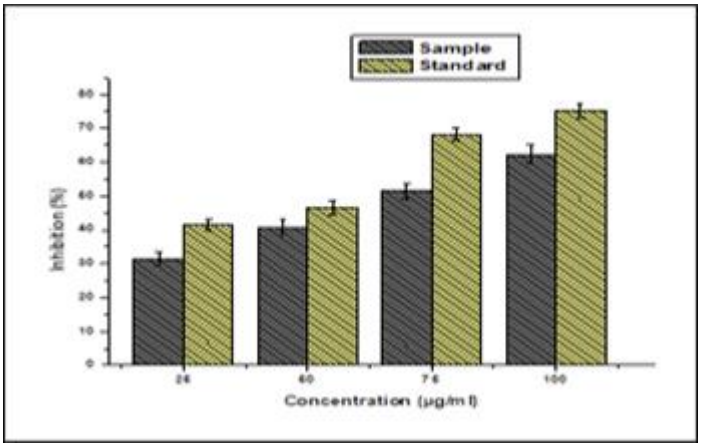

Figure 3 shows the Hydrogen peroxide scavenging activity of protease enzyme derived from the marine actinobacterium ofMicrobispora sp.

In 2022 George et al., reported the antioxidant activity of EPS is compared to the antioxidant activity of standard ascorbic acids (AAEs), and the antioxidant activity of 142.651.286 mg/ml AAE is equivalent to 150 mg/ml of total antioxidant activity produced by EPS [16]. The marine actinobacteriumMicromonosporasp. exhibited remarkable antioxidant potential in comparison to other organisms studied for their antioxidant properties [17]. The H2O2 scavenging activity of the extracts showed a range of 64%, while ascorbic acid demonstrated 74.5% activity at a concentration of 0.05 mg/mL. In terms of H2O2 scavenging activity, the extract from S. variabilis exhibited a nearly equivalent level of activity to that of ascorbic acid [18].

3.2.2 DPPH scavenging activity

In the DPPH radical scavenging assay, the antioxidants within a substance interact with a stable free radical known as 1,1-diphenyl-2-picrylhydrazyl (DPPH), which initially appears deep violet in color[19]. Our results showed the DPPH radical scavenging activity of protease enzyme in Microbispora sp. displayed a maximuminhibition of 75.2 ± 2.7% at the concentration level of 100 µg/ml. This interaction results in the transformation of DPPH into a yellow-colored compound called 1,1-diphenyl-2-picrylhydrazine. The DPPH antioxidant activity of Halococcus Sp. EPS 97.23 ± 0.21% was observed at 100µg/ml of archeal EPS [20]. The bacterial extract (Streptomyces variabilis) demonstrated DPPH free radical scavenging activity ranging from 43.67% to 82.86% at concentrations ranging from 0.05 mg/mL to 5.0 mg/mL.In contrast, ascorbic acid exhibited an 86% scavenging activity at a concentration of 0.05 mg/mL [18].

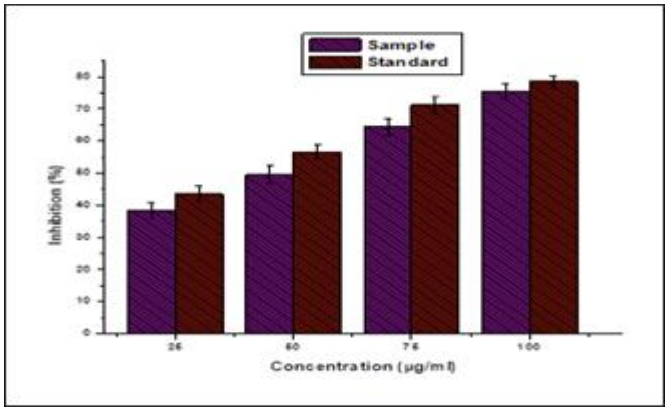

Figure 4 shows the DPPH scavenging activity of protease enzyme from the Microbispora sp.

3.2.3 Total Antioxidant activity (TAA)

The protease enzyme total antioxidant activity was observed a maximum range of 78.5± 1.8% at the concentration level of 100 µg/ml of protease enzyme. In 2021Falade et al., isolated an actinobacterial strain, Streptomyces sp., that showed alpha-amylase inhibitor activity and antioxidant activity, suggesting its potential as an anti-diabetic drug [21].

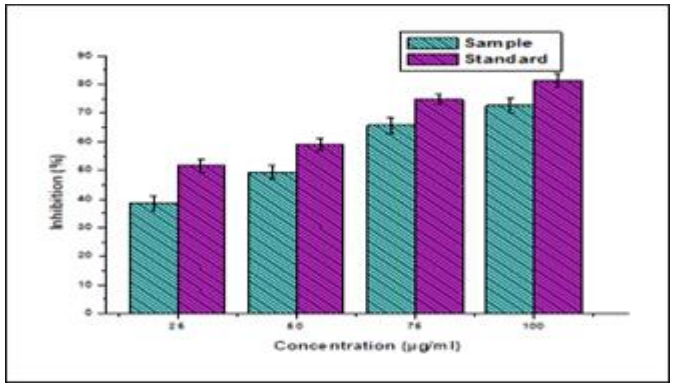

Figure 5 shows the Total antioxidant activity of Microbispora sp. protease enzyme

In 2014, Poongodiet al., conducted a study to assess the antioxidative capabilities of actinobacteria collected from the Gulf of Mannar Biosphere Reserve. Their research revealed that Streptomyces sp., stood out as the most potent strain, exhibiting remarkable antioxidant properties [22]. Additionally, in the same year,Karthik et al.,showcased the antioxidant potential of marine actinobacteria. They observed significant scavenging activity in various assays, further highlighting the antioxidative abilities of these microorganisms [23]. In this research we isolate the Microbispora sp., from marine sediment samples and extract the protease enzyme from marine actinobacteria. TAA, DPPH, Hydrogen peroxide scavenging activity was performed by the protease enzyme from marine actinobacteria.

4. Conclusion

In conclusion, Actinobacteria, particularly Microbispora sp., represents one of the most prominent phyla within the bacterial domain, known for its diverse secondary metabolites. This study focused on assessing the antioxidant potential of Microbispora sp. producing protease enzymes. The findings revealed significant antioxidant properties, including strong hydrogen peroxide scavenging activity (72.6±2.6%), potent DPPH radical scavenging activity (75.2±2.7%), and robust total antioxidant activity (78.5±1.8%) at 100 µg/ml concentration. These results underscore the multifaceted biological activities of Actinobacteria, highlighting their potential applications in various therapeutic and industrial contexts. Thus, Microbispora sp. emerges as a promising source of natural antioxidants, contributing valuable insights into the exploration and utilization of microbial resources for health and environmental benefits.

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Conflict of interest:

All authors state that, we do not have any conflict of interest.

Data availability:

All data collected during this study are included in this manuscript.

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