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# Dextranase from Actinobacteria Streptomyces and their Potential Prevention against Oral Pathogens

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This study focuses on the isolation and identification of marine actinobacteria, specifically identified as Streptomyces sp. Morphological analysis reveals distinctive features, including a white coloration and the absence of pigments. Chemotaxonomic studies further characterize the strain, highlighting a type I cell wall with specific amino acids and a spiral spore morphology. The investigation extends to the antibacterial activity of Streptomyces-derived dextranase against Gram-positive (Streptococcus mutans) and Gram-negative bacteria (Klebsiella sp. & Salmonella typhii) using the agar disc diffusion method. At concentrations of 50 and 100 µg/ml, notable inhibitory effects are observed. Streptococcus mutans exhibit the highest susceptibility, with zones of inhibition measuring 9±0.8 and 10±1 mm, respectively. Salmonella typhii follows with zones at 8±0.5 and 9±0.6 mm, while Klebsiella sp. displays the least susceptibility with inhibition zones of 7.5±0.5 mm and 8±1 mm. Streptomycin, serving as a positive control, validates bacterial susceptibility. This study underscores the potential of Streptomyces-derived dextranase in combating bacterial infections, emphasizing its broad-spectrum antibacterial activity.

**Keywords:** Marine actinobacteria, Streptomyces sp, Dextranase, Morphological analysis, Antibacterial activity.

## 1. Introduction

Actinobacteria, a phylum that predates the Great Oxidation Event by 2.7 billion years, played a role in Earth's early history. This gram-positive bacteria group, characterized by a high G+C content, exhibits diverse morphological features, including rod or cocci shapes. Notably, Actinobacteria possesses fragmenting hyphae that develop into mycelium, akin to fungal characteristics. Actinobacteria serve various ecological functions. These bacteria contribute to carbon and nitrogen fixation in plant roots, participate in bioremediation processes, act as probiotics, and are prolific producers of enzymes and secondary metabolites [1]. Actinobacteria, notably the genus Streptomyces, exhibit the capability to generate a broad spectrum of bioactive compounds within biomolecules, encompassing antimicrobials [2]. Among these compounds are widely recognized substances like gentamycin, chloramphenicol, erythromycin, rifamycin, and streptomycin [3].Belonging to the Actinobacteria phylum and classified as Gram-positive bacteria, the Streptomyces genus holds the distinction of being the largest within its phylum. It is recognized for intricate growth patterns and the production of diverse secondary metabolites [4].Since its identification as a rich source of antibiotics in 1943, the Streptomyces genus has been continually explored, resulting in the documentation of over 800 distinct species with validly published names [5].

Microbes or oral pathogens demonstrate specific adherent mechanisms, leading to their colonization of the oral cavity and contributing to the onset of various diseases [6]. The oral cavity serves as a reservoir for bacterial pathogens, potentially leading to focal infections. While pathogenic strains may initially relate to the oral cavity, the toxins they secrete can circulate through the bloodstream, reaching organs or tissues and causing potential metastatic injuries [7]. Dextran, found in dental plaque or biofilm within the oral cavity, is a byproduct of dental caries pathogens like Streptococcus mutans and Streptococcus sobrinus[8].

Dextran, a water-soluble glucan with a molecular weight ranging from 105 to 107 Da and predominantly characterized by α-1,6-glycosidic linkages, is typically synthesized from sucrose by dental caries pathogens and microorganisms found in contaminated sugarcane and beet juice [9]. It is widely acknowledged that the enzymatic action of dextranase effectively disintegrates the biofilm structure [10]. In the medical context, the partial hydrolysis of native dextran by dextranase yields specific molecular weight fractions utilized in the formulation of blood substitutes. These substitutes play a crucial role in restoring blood volume for patients in shock due to severe blood loss [11].Dextranase has garnered significant attention in the food, medical, and dental sectors in recent years. Nevertheless, its utilization on an industrial scale comes with challenges. Specifically, the enzyme exhibits limited stability in harsh environments, leading to adverse impacts on its enzymatic activity [12].

## 2. Materials and methods

### 2.1 Sample collection

Sediment samples were collected using a Van Veen grab from the Rameshwaram coast of Tamil Nadu. These sediments were carefully put in a sterile container before being transported to the Marine Biomedical Lab & Environmental Toxicology Unit (MBT). Upon arrival, the

samples were air-dried for 24 hours before being sun-dried for an additional 12 hours. The air-dried samples were then thoroughly ground up with a mortar and pestle.

2.2 Isolation of Actinobacteria

Actinobacteria were counted on KUA treated with 10 g/ml of cycloheximide and nalidixic acid, which serve as antibacterial and antifungal agents, respectively. After serial dilution, the sediment sample was spread over KUA and cultured for seven days at the ambient temperature. The overall actinobacterial population density in the sediment sample was calculated and reported in colony-forming units per gram. Selected individual colonies were selected for pure culture, which commenced a more thorough investigation. According to the International Streptomyces Project (ISP) technique, conventional identification processes included analyzing aerial mass color, spore chain shape, carbon source utilization, melanoid pigments, reverse side, and soluble pigments. Furthermore, chemotaxonomical features were used to identify marine actinobacteria[13].

2.3 Antibacterial activity

The disc diffusion method was used to evaluate the antibacterial effectiveness of melanin. The assay used circular discs with a 6 mm diameter, and samples of melanin at different concentrations (50 and 100 µg/mL) were examined. The positive control was the oral antibiotic streptomycin. After that, the plates were incubated for one day at room temperature in a controlled setting. The zone of inhibition was measured to assess the quality of the data, and the activities were then computed [14].

## 3. Results and Discussion

3.1 Isolation and identification of Actinobacteria on KUA

In this study marine actinobacteria was isolated and identified as Streptomyces sp. The isolate has produced white colour of the aerial mycelium.The spore surface is smooth spiral in shape at 0.5-2.0 µm in diameter and it appears to be nonmotile. The optimal growth temperature was observed at 25º C with slight acidic (pH 6.5) condition. Biochemical analysis has shown the presence of saturated, iso and anteiso-fatty acids. The colour of the aerial mycelium is white at KUA with the formation of spore and substrate mycelium as shown in Figure.1. The melanoid and soluble pigment was absent. It can utilize D-Glucose, D-mannitol, and D-fructose as a carbon source for growth. Chemotaxonomical studiesshows, It has a type I cell wall and contains the cell wall amino acid LL-DAP and Glycine. The shape of the spore is spiral, as shown in Figure. 2.

Figure 1. Marine Actinobacteria Streptomyces sp.

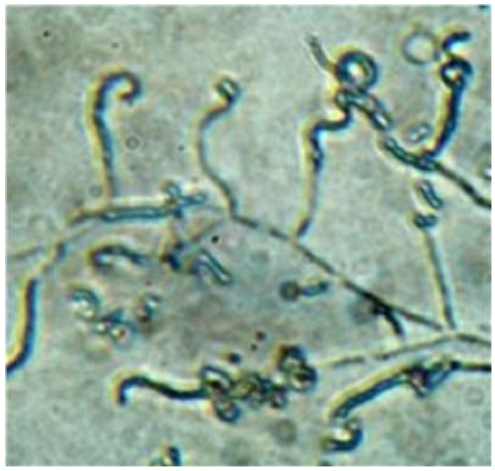

Figure 2. Spore chain morphology

3.2Antibacterial activity

In the current investigation, it was determined that dextranase derived from Streptomyces actinobacteria exhibits antibacterial activity against both Gram-positive bacteria (Streptococcus mutans) and Gram-negative bacteria (Klebsiella sp.&Salmonella typhii) using the agar disc diffusion method. The diameters of the inhibitory zones were measured and are presented in Figure. 3, Table. 1.The findings reveal distinct responses of various bacterial species to the dextranase from Streptomyces actinobacteria at concentrations of 50 and 100 µg/ml. The most significant inhibition occurred against Streptococcus mutans at concentrations of 50 and 100 µg/ml, resulting in zones of inhibition measuring 9±0.8 and 10±1 mm, respectively, through the agar disc diffusion method. This represents the highest level of inhibition observed among all the tested strains. Salmonella typhii demonstrated the second-highest susceptibility, with corresponding zones of inhibition measuring 8±0.5 and 9±0.6 mm,

respectively. Klebsiellasp exhibited the least susceptibility, displaying the lowest sensitivity when tested against 50 and 100 µg/ml, with inhibition zones measuring 7.5±0.5 mm and 8±1 mm, respectively, using the agar disc diffusion method. Streptomycin was employed as a positive control, confirming the susceptibility of the bacteria to Streptomycin.

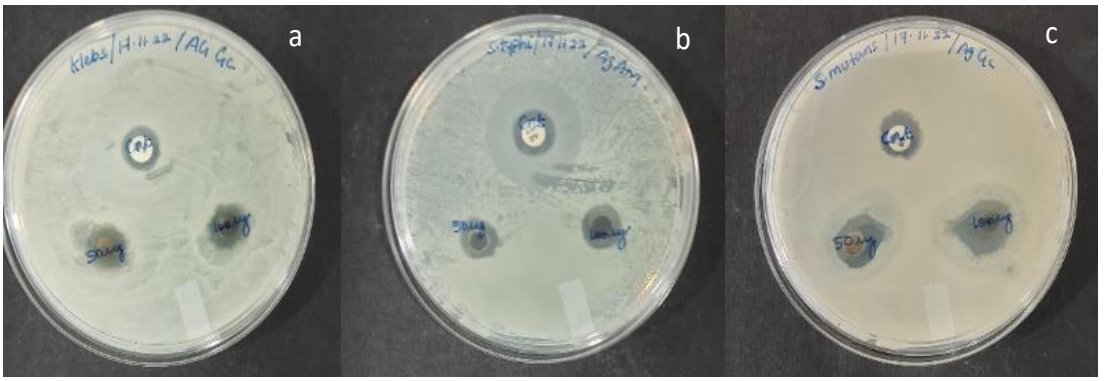


Figure 3. Antibacterial activity against oral pathogens a) Klebsiella pneumoniaeb)Salmonella typhiic)Streptococcus mutans

Cellulosimicrobium sp. PX02, a bacterium producing dextranase, was isolated from the Rizhao seacoast in Shandong, China. The study showcased its effectiveness in both eliminating and inhibiting biofilms formed by Streptococcus mutans[15]. Potent strains of marine actinomycetes, including Streptomyces, displayed bioactivity against multidrug-resistant bacteria. Dextranase enzymes sourced from marine actinobacteria, particularly Streptomyces, are suggested to have significant antimicrobial potential[16]. Streptomycesisolates were identified with notable antibacterial and antifungal activities[17], and various Streptomyces species showed antimicrobial potential against phytopathogenic bacteria[18]. A Streptomyces strain isolated from acidic peatlands demonstrated antibacterial activity against ESBL-producing Escherichia coli[19]. Additionally, dextran purified from Lactobacillus gasseri exhibited antibacterial and anti-virulence effects against Pseudomonas aeruginosa[20].

Table 1: Zone of Inhibition against different pathogens

| STRAINS | SAMPLE CONCENTRATION | |
|---|---|---|
| | 50µ/ml | 100µ/ml |
| Streptococcus mutants | 9±0.8 | 10±1 |
| Salmonella typhii | 8±0.5 | 9±0.6 |
| Klebsiellapneumoniae | 7.5±0.5 | 8±1 |

## 4. Conclusion

In conclusion, Actinobacteria, particularly the Streptomyces genus, emerges as a pivotal phylum with ancient origins and multifaceted ecological functions. The study successfully isolates and identifies marine actinobacteria as Streptomyces sp., characterizing its morphological features and chemotaxonomy. The antibacterial activity of Streptomyces-derived dextranase against both Gram-positive (Streptococcus mutans) and Gram-negative

bacteria (Klebsiella sp. & Salmonella typhii) is demonstrated.The findings highlight substantial inhibitory effects, particularly against Streptococcus mutans, emphasizing the promising application of dextranase in oral health. Despite recognizing challenges in industrial-scale implementation, the research provides valuable insights into the potential applications of enzymes derived from Streptomyces, underscoring their importance across diverse sectors such as food, medicine, and dentistry.

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CRediT authorship contribution statement

G.S.Jaishree: Writing – original draft, Software, Methodology, Formal analysis, Data curation. G Ragul: Writing – original draft, Software, Methodology, Formal analysis. Kamala Kannan: Writing – review & editing, Validation, Software, Formal analysis, Conceptualization. DhanrajGanapathy: Review & editing, Validation,Project administrationSivaperumalPitchiah: Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Disclosure statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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