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Investigation of the Isolation *Streptomyces* from Marine Mucilage

Ayşenil Bayizit[1*], Sevdan Yılmaz[2*]

[1] *Department of Aquaculture, School of Graduate Studies, Canakkale Onsekiz Mart University, Canakkale 17100, Türkiye*
[2] *Department of Aquaculture, Faculty of Marine Sciences and Technology, Canakkale Onsekiz Mart University, Canakkale 17100, Türkiye*

Ayşenil BAYİZİT: Aysenilbayizit@outlook.com, https://orcid.org/0009-0006-4049-8490
Sevdan YILMAZ: sevdanyilmaz@comu.edu.tr, https://orcid.org/0000-0002-4809-5809

**Corresponding authors: Ayşenil BAYİZİT: Aysenilbayizit@outlook.com, phone, +90-5307885651*
Sevdan YILMAZ: sevdanyilmaz@comu.edu.tr, +90-5452924403

Abstract

This study aims to isolate of *Streptomyces* from mucilage samples collected from the Çanakkale Strait (Dardanelles). Mucilage is a highly viscous structure produced by various microorganisms (diatoms, dinoflagellates, cyanobacteria, and bacteria) under suitable environmental conditions. Although the literature states that mucilage may contain different microorganisms, no studies have specifically examined *Streptomyces* species within mucilage structures. This study cultivated *Streptomyces* species isolated from mucilage in three different growth media (GYM-*Streptomyces*, N-Z-Amine, and ISP 4) with and without calcium carbonate ($CaCO_3$) pretreatment. The results showed that ISP 4 was the most effective medium for *Streptomyces* growth, while $CaCO_3$ pretreatment significantly enhanced *Streptomyces* isolation efficiency by reducing the total bacterial load. 118 *Streptomyces* isolates were obtained, with 62 isolates from ISP 4, 36 from N-Z-Amine, and 20 from GYM. After $CaCO_3$ pretreatment, the total number of *Streptomyces* isolates increased approximately 3.5 times (from 26 to 92) in all media. This suggests that $CaCO_3$ effectively enhances *Streptomyces* recovery from mucilage samples.

This study demonstrated that *Streptomyces* species can be successfully isolated from mucilage structures, and further research should explore their potential applications in biotechnology, particularly in antimicrobial and pharmaceutical studies.

Keywords: Actinobacteria, Probiotic, Drug, Pharmacology, Selection, Media

Introduction

As the world population increases, the demand for industrialization and the amount of industrial and domestic waste are also increasing rapidly. This situation disrupts the ecological balance, leading to environmental pollution, climate change and global warming, thus creating negative effects on ecosystems. Mucilage, one of these changes, was first recorded in the Adriatic Sea in 1729 (Fonda-Umani et al., 1989). In Türkiye, it was seen in the Marmara Sea in 2007-2008 (Aktan et al., 2008; Tüfekçi et al., 2010; Balkis et al., 2011) and the Dardanelles (Yentur et al., 2013).

In the first half of 2021, mucilage density seriously affected fishing, tourism and social life in Türkiye (Yılmaz et al., 2021a; Yılmaz et al., 2021b). Hatchery activities in the Dardanelles Strait in particular have been negatively affected by this situation (Yılmaz et al., 2023). Studies have shown that mucilage hosts a diverse and unique microbial community, including pathogens that are not normally found in the water column (Danovaro et al., 2009; Cozzi et al., 2004). In addition, it has been suggested that mucilage masses may act as biomass and facilitate the spread of these microorganisms in the aquatic environment (Danovaro et al., 2009; Cozzi et al., 2004).

In the articles conducted so far, no research has been found that specifically focuses on the presence of Streptomyces species in marine mucilage structures. It is known that *Streptomyces* species, which are usually isolated from soil, can be found in both terrestrial and aquatic ecosystems (Subramani et al., 2019; Elsayed et al., 2022). Selman Waksman and his student Albert Schatz, who carried out important studies in the field of soil microbiology, discovered the first antibiotic effective against Mycobacterium tuberculosis by isolating the streptomycin metabolite from the bacterium Streptomyces griseus (Schatz & Waksman, 1944). Since the primary and secondary metabolites produced by the *Streptomyces* genus bacteria are considered important in terms of biotechnology, extensive studies have been carried out (Donald et al., 2022; Alam et al., 2022). However, their presence within mucilage structures and their potential applications remain largely unexplored.

To date, no study in the literature has examined the potential use of *Streptomyces* species cultured from mucilage structures in aquaculture. Therefore, this study aims to isolate *Streptomyces* bacteria from marine mucilage samples collected from the Çanakkale Strait (Dardanelles) using different selected nutrient media and to evaluate the potential applications of the isolated *Streptomyces* strains in aquaculture

Material and Method

Study Area

In the marine fish hatchery company (Ida Gıda Tarımsal Üretim İç ve Dış Pazarlama A.Ş.), located at 40°16'45.2"N, 26°35'39.9"E, which sources seawater from a depth of 28–35 meters via intake pipelines positioned approximately at 40°17'16.7"N, 26°35'44.1"E; 40°17'16.1"N, 26°35'55.5"E; 40°17'08.9"N, 26°35'44.3"E; and 40°17'08.4"N, 26°35'53.1"E in the Çanakkale Strait—Kemiklialan region, the first samples were taken from the mucilage structure that reached the facility as of January 2025.

Sampling and Isolation

Samples taken into sterile falcon tubes (50 mL) were brought to the laboratory under cold chain conditions and subjected to calcium carbonate ($CaCO_3$) application (Tsao et al., 1960) pretreatments reported in the literature for *Streptomyces* isolation, while an untreated group was

maintained for comparison (Figure 1). For this purpose, samples taken at the beginning of January 2025 were prepared for growth media in three replicates.

After ISP 4, N-Z-Amine media and GYM-*Streptomyces* media were prepared according to previous studies (Shirling and Gottlieb 1966; Öztürk et al., 2004; Singh et al., 2006). The media were autoclaved at 121°C for 15 minutes and cooled to 50°C, then 50 µg/mL potassium dichromate and 5 µg/mL rifampicin were added to prevent competitive fungal and bacterial growth, respectively (Hameş-Kocabaş and Ataç 2012).

After the mucilage samples were subjected to pretreatment (calcium carbonate), 100 microliters of each dilution were inoculated aseptically with a sterile Drigalski spatula into the ISP 4, N-Z-Amine and GYM-*Streptomyces* media using the spread plate method. Additionally, an untreated group was plated under the same conditions to compare the effect of the pretreatment on *Streptomyces* isolation. After incubation at 28°C for 1 to 4 weeks in the media, colonies that developed were considered *Streptomyces* if they had a hard, leather-like texture, a dry or curly appearance, and if they had branched filaments and aerial mycelium or not (Mincer et al., 2002; Jianyou et al., 2011; Chemoh et al., 2021). The 118 pure isolates were produced in N-Z-Amine liquid culture media at 50 rpm in a tube roller mixer (Medispec) at 28°C and stored in cryotubes with 20% glycerol at -20°C and -80°C (Cordovez et al., 2015).

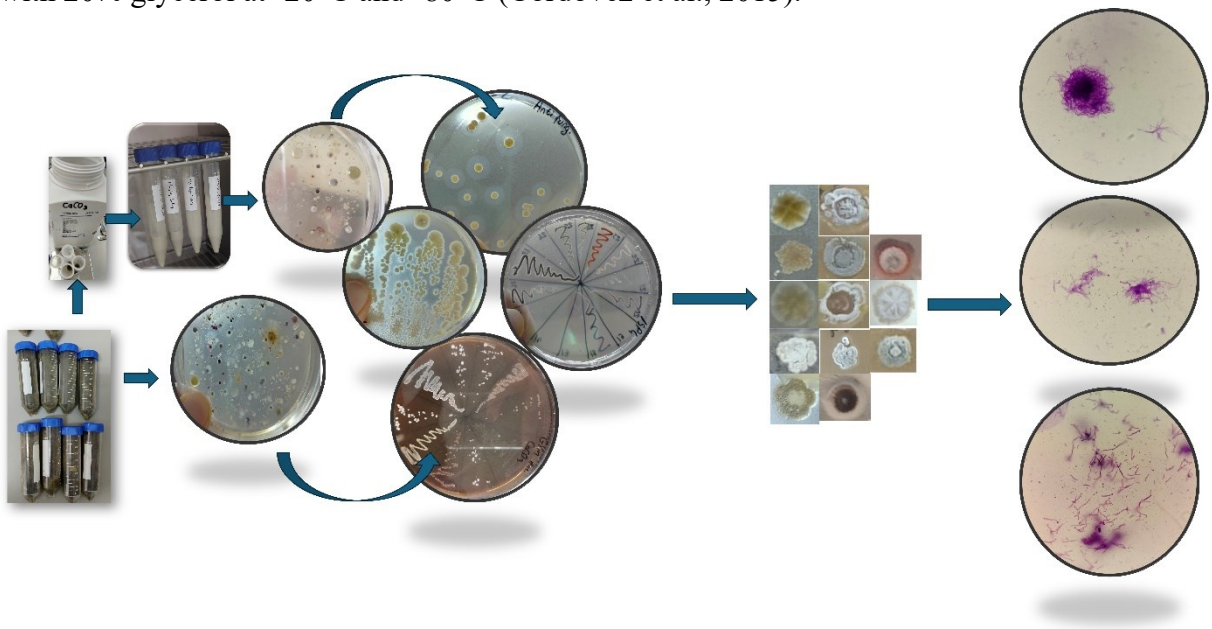

Figure 1. *Streptomyces* isolation process from the mucilage structure (Photos taken by Ayşenil Bayizit and Sevdan Yılmaz)

Statistical analysis

All data are presented as mean ± standard error of the mean (S.E.M.). Normality and variance homogeneity of the data were assessed by Shapiro–Wilk and Levene tests, respectively. Statistical analyses were performed using one-way ANOVA and Tukey's multiple comparison test. Analyses were performed using SPSS 19.0 (SPSS Statistics) program, and differences between groups were considered statistically significant when p value < 0.05.

Results

The study observed a significant difference when the total bacterial load was compared between the untreated groups and the $CaCO_3$ pretreatment groups ($p<0.05$). In addition, in the $CaCO_3$ pretreatment groups, total bacterial load decreased significantly in all growth media ($p < 0.05$).

A total of 118 *Streptomyces* isolates were obtained. The highest number of isolates was obtained from ISP 4 media (62 isolates), followed by N-Z-Amine (36 isolates) and GYM-*Streptomyces* media (20 isolates). Strikingly, *Streptomyces* was not isolated from the untreated groups in GYM and N-Z-Amine media, indicating that these media alone are insufficient for isolating *Streptomyces*. After $CaCO_3$ pretreatment, the total number of *Streptomyces* isolates increased approximately 3.5 times (from 26 to 92) in all media. This suggests that $CaCO_3$ effectively enhances *Streptomyces* recovery from mucilage samples.

Table 1. Effect of different culture media and $CaCO_3$ pretreatment on *Streptomyces* isolation from mucilage samples (Log_{10} CFU/g)

Group	**Media**		
	GYM-*Streptomyces* media	**N-Z-Amine**	**ISP 4**
Untreated group (Total)	6.08 ± 0.08^{Aa}	5.55 ± 0.07^{Ab}	5.32 ± 0.16^{Ab}
Untreated group *Streptomyces*	-	-	2.13±0.43
Stored Streptomyces counts (Untreated group)	*Not isolated*	*Not isolated*	*26 isolates*
$CaCO_3$ pretreatment (Total)	2.77 ± 0.23^{Bb}	3.10 ± 0.10^{Bab}	3.64 ± 0.17^{Ba}
$CaCO_3$ pretreatment *Streptomyces*	2.05 ± 0.13^{a}	2.34 ± 0.07^{a}	2.40 ± 0.10^{a}
Stored Streptomyces counts (Pretreatment)	*20 isolates*	*36 isolates*	*36 isolates*

Different lowercase superscript letters within the same row indicate significant differences in bacterial load among culture media ($p < 0.05$). Different uppercase superscript letters within the same column indicate significant differences between untreated and $CaCO_3$ pretreated groups ($p < 0.05$). Values are presented as mean ± standard deviation ($n = 3$).

Discussion

Streptomyces genus members generally produce extracellular enzymes for the degradation of polymolecules, including polysaccharides, proteins, aromatic compounds, and lignocellulose, and thus contribute to the nutrient cycle (Özdemir and Ünal, 2019). *Streptomyces* genus bacteria, an important group among actinomycetes, are at the forefront of secondary metabolite producers. Up to now, 42% of the more than 23 thousand microbial secondary metabolites known, and 2/3 of antibiotics are produced by actinobacteria. 75% of these are produced by *Streptomyces* genus bacteria (Newman et al., 2003). Other metabolites it produces include antifungal, antiviral, anticancer, antiparasitic, antihypertensive, and pesticide (Özdemir and Ünal, 2019).

In this study, *Streptomyces* was isolated from mucilage samples collected from the Çanakkale Strait (Dardanelles), and the effects of different media (GYM-*Streptomyces*, N-Z-Amine, and ISP 4) and $CaCO_3$ pretreatment on bacterial growth and isolation efficiency were evaluated. Our findings indicate that $CaCO_3$ significantly increased the isolation of Streptomyces from mucilage samples. Similarly, El-Nakeeb and Lechevalier (1963) reported that $CaCO_3$ application reduced bacterial and fungal contamination in soil samples, thus increasing the efficiency by selectively isolating actinobacteria. Furthermore, Sheikh et al. (2019) reported that $CaCO_3$ pretreatment was used to increase actinobacteria isolation in sediment samples from the Alang Sea. This effect of $CaCO_3$ was reported to occur by promoting the formation of aerial mycelium in various actinobacteria and increasing their visibility in the medium (Natsume et al., 1989).

In conclusion

This study is the first to demonstrate that *Streptomyces* species can be successfully isolated from marine mucilage samples. The findings support that $CaCO_3$ selectively isolates *Streptomyces*, facilitating the emergence of *Streptomyces* species from mucilage by reducing the total bacterial load and promoting the formation of aerial mycelium. Future studies should further investigate the biotechnological potential of *Streptomyces* species isolated from marine mucilage.

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Ethical approval

For this type of study, formal consent is not required.

Informed consent

Not available

Data availability statement

The authors declare that data are available from authors upon reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

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Contribution of authors

Ayşenil Bayizit: Formal analysis, Data curation, Writing original draft, writing - review & editing

Sevdan Yılmaz: Conceptualization, Investigation, Visualisation, Formal analysis, Methodology, Writing original draft, Writing - review & editing

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