

Orjinal Araştırma Makalesi/ Original Paper

Tıbbi Öneme Sahip *Streptomyces* Bakterilerinin Farklı Besiyerlerinde Çoğalma Oranlarının Belirlenmesi

Determination of the Growth Rate of Medicinally Important *Streptomyces* Bacteria in Different Media

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ÖZET

Amaç: Van Gölü'ne dökülen akarsulardan toplamda 24 sediment örneği alınarak üç farklı besiyerinde çoğalma oranları incelenmiştir. Yapmış olduğumuz çalışmada tıbbi önemi olan *Streptomyces* bakterilerinin farklı besi ortamı kullanılarak en çok hangi besiyerinde çoğaldıklarını araştırmak amaçlanmıştır.

Materyal ve Metot: Çalışmada Van Gölü'ne dökülen akarsulardan toplamda 24 sediment örneği alınmıştır. Daha sonra örneklerin nem ve pH oranları tespit edilmiştir. Alınan örneklerden *Streptomyces* bakterilerinin izolasyonu yapılarak, bakterilerin saf kültürleri elde edilmiştir. İzolasyon işlemi yapılırken modifiye edilmiş Bennett's Agar, Medium 65 ve SM3 Agar olmak üzere üç farklı besiyeri kullanılmıştır.

Bulgular: İzolasyon süreci sonunda süresinden sonra morfolojik duruma göre yapılan sayım işlemine göre modifiye edilmiş Bennett's Agar besiyerinde 145, SM3 besiyerinde 8, Medium 65 besiyerinde ise 26 olmak üzere toplamda 179 *Streptomyces* bakterisi izole edilmiştir.

Sonuç: Sonuç olarak tatlı suların alınan sediment örneklerinden izole edilen *Streptomyces* bakterilerinin modifiye edilmiş Bennett's Agar, SM3 ve Medium 65 besi ortamları içerisinde en çok modifiye edilmiş Bennett's Agar besiyerinde çoğaldığı belirlenmiştir. Sucul ortamlardan sekonder metabolit üreticisi yeni *Streptomyces* türlerinin teşhisi için modifiye edilmiş Bennett's Agar besiyerinin kullanılabilirliği düşünülmektedir.

Anahtar Kelimeler: *Streptomyces*, Bennett's Agar, Medium 65, SM3 Agar, Van Gölü.

ABSTRACT

Objective: A total of 24 sediment samples were taken from the rivers flowing into Van Lake and their growth rates were examined in three different media. In our study, we aimed to investigate which *Streptomyces* (medically important) bacteria reproduce more in different medium.

Material and Method: In the study, a total of 24 sediment samples were taken from the rivers pouring into Van Lake. Then the moisture and pH of the samples were determined. *Streptomyces* bacteria were isolated from the samples taken and pure cultures of the bacteria were obtained. During the isolation process, three different media, Modified Bennett's Agar, Medium 65 and SM3 Agar, were used.

Results: At the end of the isolation process, a total of 179 *Streptomyces* bacteria were isolated, 145 in Modified Bennett's Agar medium, 8 in SM3 medium and 26 in Medium 65 medium, according to the counting process performed according to the morphological condition after the isolation process.

Conclusion: As a result, it was determined that *Streptomyces* bacteria isolated from sediment samples taken from fresh water grew mostly in Modified Bennett's Agar medium among Modified Bennett's Agar, SM3 and Medium 65 media. It is thought that Modified Bennett's Agar medium can be used for the identification of new *Streptomyces* species producing secondary metabolites from aquatic environments.

Keywords: *Streptomyces*, Bennett's Agar, Medium 65, SM3 Agar, Van Lake.

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INTRODUCTION

Correct diagnosis of microorganisms and determination of their metabolic functions is very important in medical and pharmacological terms. However, the isolation of microorganisms constitutes the basic step of new species that can be unearthed.

Planting the correct media from the samples taken allows many new organisms to be brought to the scientific world. It makes the isolation of *Streptomyces* bacteria, which is a secondary metabolite producer, which has a serious potential in the field of health,

especially. Although *Streptomyces* species are isolated from the soil, they are widely distributed in both aquatic and terrestrial habitats. In addition to being an important component of the microbial community involved in the degradation and cycle of natural polymers such as cellulose, lignin, and chitin, *Streptomyces* bacteria also have an important biotech potential as the source of many biologically active compounds (Williams et al., 1989; Semêdo et al., 2004; Mokraneet, 2013). Some of the characteristics of *Streptomyces* bacteria are to produce bioactive secondary metabolites such as antifungal, antiviral, antitumoral, antihypertensive, immunosuppressant and especially antibiotics (Araújo et al., 2012). *Streptomyces* bacteria are the source of many bioactive compounds used in medical treatment and produce approximately 75% of known antibiotics (Demain, 2014; Cheng et al., 2016). *Streptomyces* bacteria provide the production of antibiotics in many important drugs that contribute to human health (Lewin et al., 2016). Members of the *Streptomyces* genus are a great resource for new antibiotics, alkaline enzymes, and enzyme inhibitors (Sharma et al., 2016). Bennett's Agar medium was used for the isolation of *Streptomyces* strains from soil samples taken from the Çukurova region (Sarigüllü Önalın, 2020). *Streptomyces* species were isolated from soil samples of volcanic sediments collected from Longwan city in Jilin province in northern China (Jia et al., 2015). *Streptomyces* bacteria were isolated from soil samples taken from the Sakarya region using Medium 65 medium (Özok and Özdemir, 2019). 26 *Streptomyces* species were isolated from samples taken from garden soil as a result of planting on Bennett's Agar medium (Chellapandi and Jani, 2008).

In our study, *Streptomyces* bacteria were isolated using three different media from 24 samples taken from 8 main streams pouring into Van Lake. A comparison of the media was made considering the growth results. It was examined in which medium the samples taken from the aquatic environment grow the most.

MATERIAL and METHODS

Collection of sediment samples, determination of pH and humidity rates

Sediment samples taken from different parts of 8 rivers pouring into Van Lake in sterile containers with the help of spoons were brought to the laboratory. In order to determine the moisture content of the collected sediment samples, 1 g of each was weighed and placed in porcelain crucibles, and then placed in an oven set at 105 °C. It was weighed every 24 hours and this process was continued until the weight stabilized. The average % of the weight lost as a result of these measurements was calculated as the moisture content of the samples. In order to determine the pH of the sediment samples, the samples were brought to the laboratory, 20 g of soil was placed in beakers from each sample, pure water was added and mixed for 3 hours until the soil surface was covered, and pH was measured (Özdemir, 2008; Ertas et al., 2013).

Isolation of *Streptomyces* species and media used

Classical dilution and spreading method was used for the isolation of *Streptomyces* bacteria. 25 g of each sample was weighed and put into 500 mL sterile glass bottles containing 250 mL of Ringer's solution. In this way, 10^{-1} solutions prepared for each soil sample were shaken for 1 hour to separate microorganism spores and micelles attached to soil colloids. These 10^{-1} solutions were then kept in a hot water bath set at 65 °C for 10 minutes to reduce contamination caused by vegetative forms. Each soil sample was made homogeneous and 1 mL was taken under aseptic conditions with an automatic pipette and placed in sterile glass tubes containing 9 ml of Ringer's solution. In this way, a 10^{-2} soil solution was obtained and the same procedures were repeated to obtain 10^{-3} , 10^{-4} dilutions. In this way, dilution series were continued until dilution of 10^{-4} . Later, it was inoculated on Modified Bennett's Agar (Table 1.), Medium 65 agar (Table 2.) and SM3 (Table 3.) (Jones, 1949; Atalan, 1995; Tan et al., 2006; Awla et al., 2017).

Table 1. Modified Bennett's Agar medium content and rates.

Yeast extract	1.0 g
Lab lemco	0.8 g
Bacto casitone	2.0 g
Gliserol	10.0 g
Agar	18.0 g
Distilled water	1 liter

Table 2. Medium 65 agar medium content and rates

Glucose	4.0 g
Yeast extract	4.0 g
Malt extract	10.0 g
CaCO ₃	2.0 g
Agar	12.0 g
Distilled water	1 liter

Table 3. SM3 medium content and rates

Glucose	10.0 g
Pepton	5.0 g
Tryptone	3.0 g
NaCl	5.0 g
Agar	15.0 g
Distilled water	1 liter

RESULTS

Isolation Study Results from Samples

Streptomyces bacteria were isolated from sediment samples taken from 24 different regions. Modified Bennett's Agar, SM3 Agar and Medium 65, to which cycloheximide (50 µg/ml) and nystatin (50 µg/ml) were added, were isolated according to *Streptomyces* bacteria, air mycelium and color pigmentation. After the incubation period, a total of 179 *Streptomyces* bac-

teria were isolated, including 145 in Modified Bennett's Agar medium, 8 in SM3 medium, and 26 in Medium 65 medium, according to the counting process performed according to the morphological condition. The media images in which *Streptomyces* bacteria grow in other microorganisms including *Streptomyces* bacteria are given in Figure 1 and the ratio of total colonies to *Streptomyces* bacteria occurring in the media as a result of the isolation studies are given in Figure 2 for Modified Bennett's Agar, in Figure 3 for SM3 Agar and in Figure 4 for Medium 65.

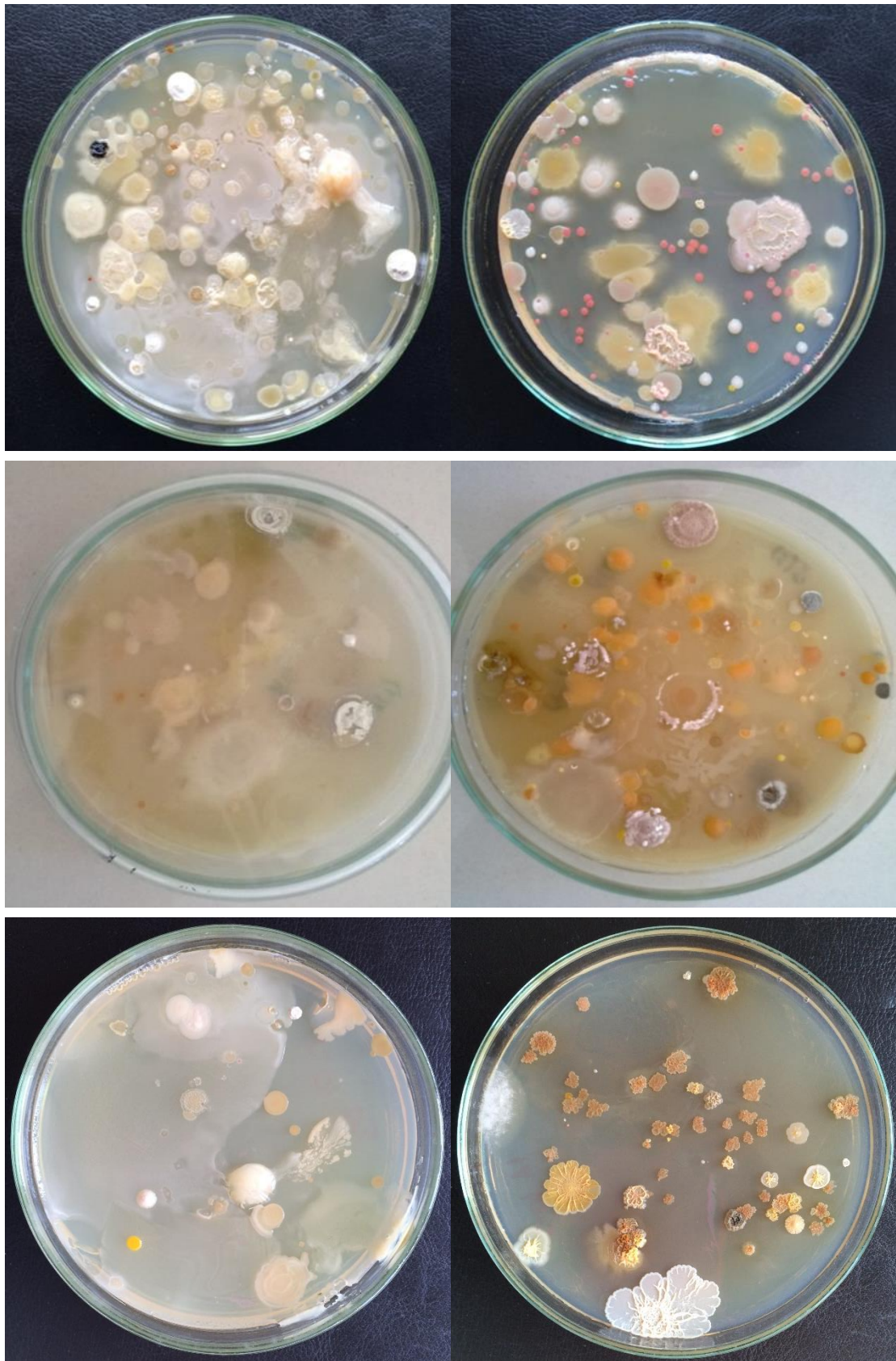


Figure 1. Images of media in which other microorganisms, including *Streptomyces* bacteria, growth

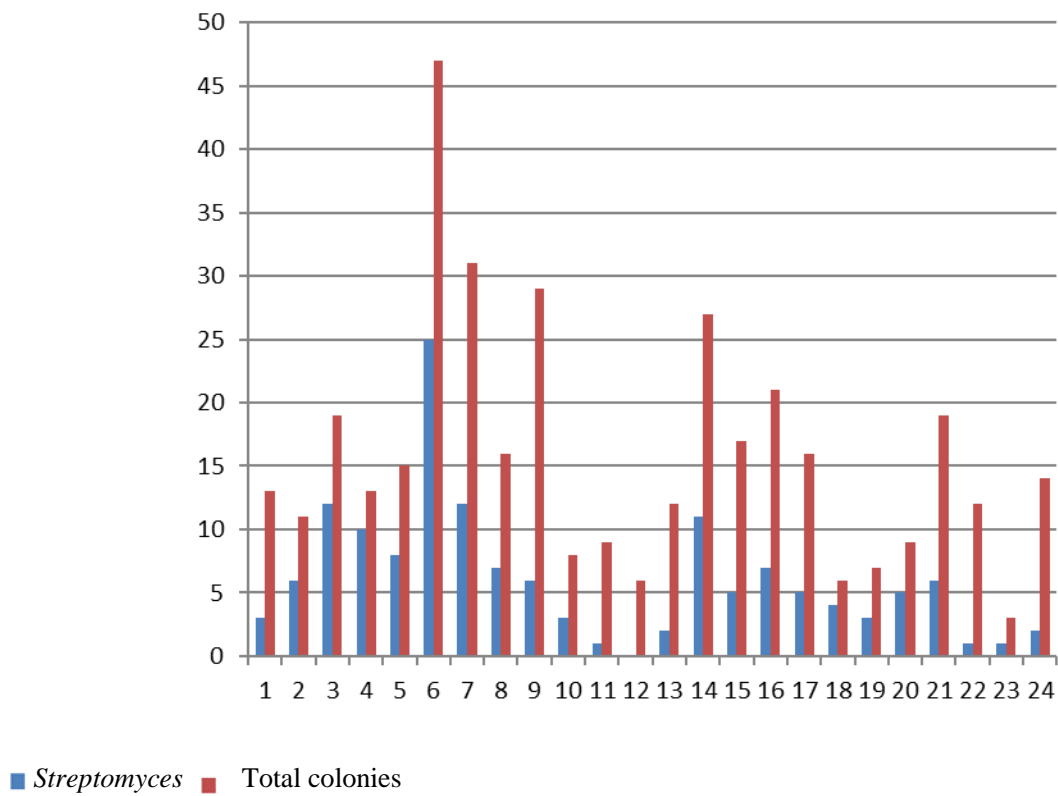


Figure 2. The ratio of total colonies occurring on Modified Bennett's Agar to *Streptomyces* bacteria.

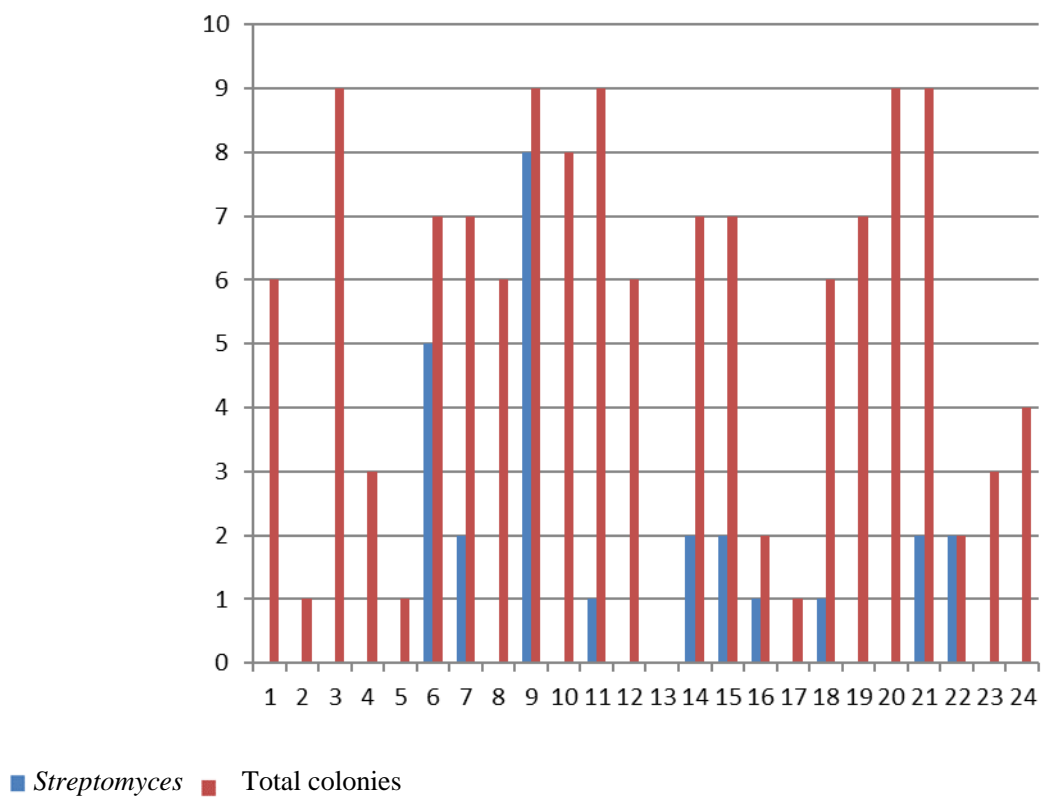
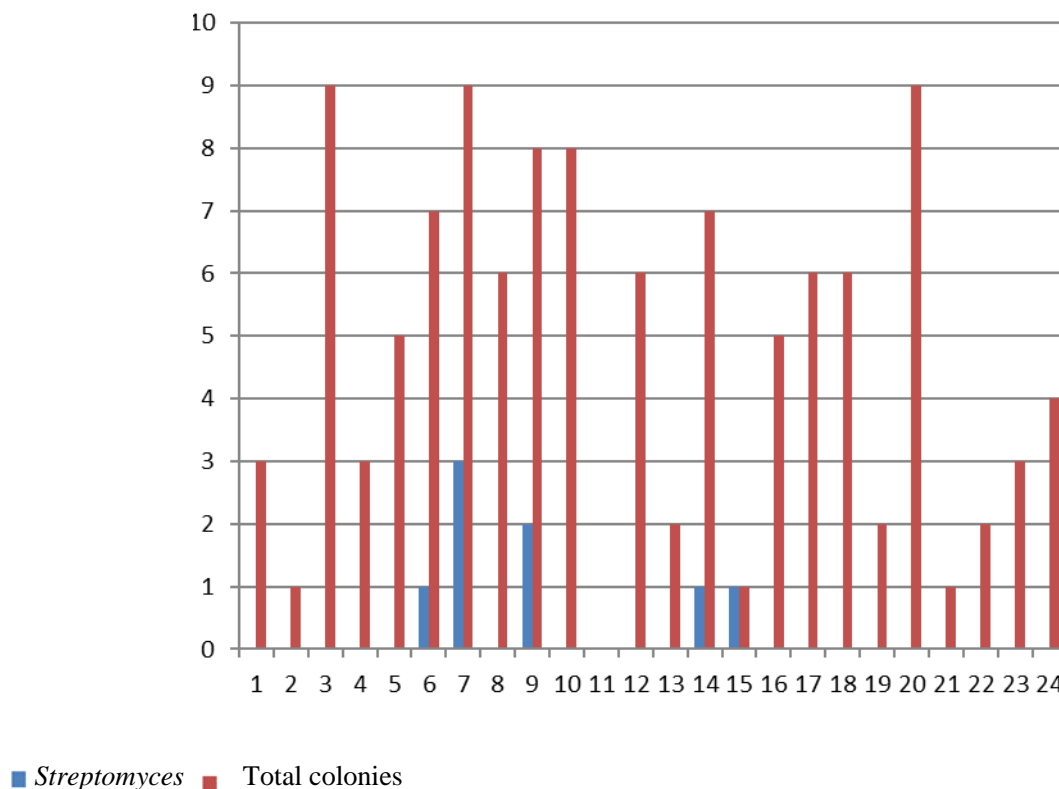


Figure 3. The ratio of total colonies occurring in Medium 65 to *Streptomyces* bacteria**Figure 4.** The ratio of total colonies occurring on SM3 Agar to *Streptomyces* bacteria

DISCUSSION

The bacterial diversity in the world has necessitated the discovery of new antibiotics or the creation of systems that inhibit the resistance mechanism in bacteria, especially after the increase in the number of bacteria that develop antibiotic resistance. The place of *Streptomyces* species in the scientific world makes the isolation of these bacteria important. It is possible to isolate *Streptomyces* bacteria from aquatic and terrestrial environments (Korn-Wendish and Kutzner, 1992). Soil is the best habitat for *Streptomyces* bacteria to grow and survive (Yousif et al., 2015). In our study, *Streptomyces* bacteria were isolated from sediment samples taken from fresh water. In our study, the number of isolates we obtained from sediments taken from the stream environment was determined as 179. As a result, the low number of *Streptomyces* isolates in the aquatic environment is due to the fact

that the main habitat of these bacteria is soil. However, the fact that Veyisoğlu and Şahin (2014) identified many new species from the samples he took from the Black Sea bottom sediment in his study was one of the reasons for our persistence in this study. *Streptomyces* bacteria were grown on Modified Bennett's Agar, Medium 65, and SM3 media. During the preparation of the media, especially fungi that cause mold formation caused contamination during the isolation process. Cycloheximid antibiotic was used at certain rates to overcome this problem. Likewise, Nystatin and Novobiocin antibiotics were used at certain rates in order to prevent the proliferation of gram (-) bacteria. Recent studies have shown that new *Streptomyces* species have been identified from sediment samples taken from aquatic environments. The media contents used in this process enable new species to be introduced to the scientific world. SM3 medium was used for the growth of *Streptomyces*

bacteria from marine sediments (Veyisoğlu and Şahin, 2014). New *Streptomyces* species were isolated from hot water sources of Pakistan using Starch Agar medium (Amin et al., 2016). Bennett's Agar medium was used in tests to identify new *Streptomyces* species from the Lonar saltwater lake in India (Sharma et al., 2016). *Streptomyces* bacteria were isolated from the soil and sediment samples taken from the Sapanca Lake basin using Medium 65 and SM3 media (Ünal and Özdemir, 2019). The distribution of the number of *Streptomyces* colonies isolated in the study we conducted was determined as 145 in Modified Bennett's Agar medium, 8 in SM3 medium, and 26 in Medium 65 medium. As a result, it was determined that *Streptomyces* bacteria isolated from samples taken from 24 different points multiplied mostly in Modified Bennett's Agar medium. For samples taken from fresh waters, it has been observed that Modified Bennett's Agar medium can be used to obtain medical and pharmacologically important *Streptomyces* bacteria. It is thought that it would be appropriate to use Modified Bennett's Agar medium in diagnostic studies of these bacteria in terms of obtaining healthier results.

Conflict of interest

The authors declare that there is no conflict of interest.

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