Research Article

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Antalya Bölgesinden Soğuğa Dayanıklı Bakteri İzolasyonu ve Büyüme Hızlarının Analizi

Isolation of Cold Tolerant Bacteria from Antalya Region and Analysis of Their Growth Rates

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Öz: Toprağa uygulanan kimyasal gübrelerin ancak küçük bir kısmı bitkiler tarafından kullanılabilmektedir. Çünkü besin maddeleri toprakta alınamaz forma geçmektedir. Biyogübre uygulaması besin maddelerini bitkiler için alınabilir forma sokar. Bu uygulama hem bitki üretimi hem de çevre için yararlı olabilir. Bu çalışmanın amacı Antalya'nın yüksek ve düşük rakımlı iki bölgesinden bakteri izolasyonu yapmak ve izole edilen suşların büyüme hızlarını karşılaştırmak olmuştur. Yüksek rakımdan izole edilen suşların hem oda sıcaklığında (15-25 °C) hem de 2 °C'de düşük rakımdan izole edilen suşa göre daha hızlı ürediği görülmüştür. Bu da 2 °C'de üreyebilen suşların düşük sıcaklıklarda da yüksek enzim aktivitesine sahip olabileceğini öne sürmektedir. Soğuğa dayanıklı biyogübre kullanımı ülkemizin soğuk bölgelerindeki zirai alanlarda bitki büyümesine pozitif etki gösterebilir. Bu tür soğuğa dayanıklı suşların izolasyonu ve biyogübre aktivitelerinin test edilmesi zirai uygulamalarımız için önem taşımaktadır.
Anahtar Kelimeler: Psikrofilik bakteriler, soğuğa dayanıklı bakteriler, bakteri izolasyonu, biyogübre

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Abstract: Only a portion of chemical fertilizers applied to soil can be used by plants, because nutrients can become insoluble in soil. Application of biofertilizers can make the nutrients bio-available for plants. This practice would be beneficial for both plant productivity and the environment. Aim of this study was to isolate and compare growth rates of bacterial strains isolated from low altitude and high altitude regions of Antalya. Our results showed that, bacterial strains isolated from high alitudes have higher growth rates at room temperature (15-25 °C) and temperatures as low as 2 °C, compared to strains isolated from low altitudes. This suggests that strains that can grow at 2 °C also would have higher enzymatic activities at low temperatures which makes them better candidates for development of biofertilizers. Using cold adapted biofertilizer would have a positive effect on plant productivity in agricultural areas located in cold regions of Türkiye. Therefore, isolation of these organisms and testing of their biofertilizer potential is important for our agricultural applications.
Keywords: Psychrophilic bacteria, cold tolerant bacteria, bacterial isolation, biofertilizer

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INTRODUCTION

Nitrogen (N), phosphorous (P) and potassium (K) are the most utilized macroelements by plants. However, P and K that is applied with chemical fertilizers can become insoluble in the soil and cannot be bioavailable for plant uptake (Sharma et al., 2013; Setiawathi and Mutmainnah, 2016). Therefore, only a portion of chemical fertilizers can be taken by plants. pH and type of soil, concentration of cations that are found in soil and microbial activities can affect uptake of nutrients. Even though, P can be abundant in soil, however only 0.1% of total P is bioavailable for plants (Sharma et al., 2013). Organic acids, phosphatase and phytase enzymes that are secreted by soil microorganisms can solubilize and turn P into a form that can be taken by plants. Like phosphate, sulfate (SO_4^{2-}) too can bind to other molecules and can take a form that cannot be taken by plants. Microorganisms secrete sulfatase and solubilize sulfate from other molecules (Knauff et al., 2003). For these reasons, soil microbial activities are very important for plant metabolism.

Biofertilizers may include bacteria, cyanobacteria, fungi and microalgae. But most of the species that are found in the literature are mesophilic species that can grow at room temperature. Literature shows that even in Antalya region soil temperatures can fall below 4 °C (Yiğitbaşıoğlu, 2000). Mesophilic soil microorganisms may have slow growth at these low temperatures. Cold adapted organisms are designated as psychrophilic (cannot grow above 20 °C) and psychrotolerant (can grow at low temperatures and up to around 30 °C). Using species that are adapted to cold temperatures as biofertilizer can have positive effect on plant productivity at low temperature seasons. There are only a few works in the literature on isolation of psychrotolerant bacteria and testing their acitivity as biofertilizer. These studies show that cold adapted strains can a have positive effect on plant growth and also defence (Balcazar et al., 2015; Yarzábal et al., 2018; Torracchi et al., 2020). Psychrophillic fungi have also been isolated from diverse habitats on earth (Hassan et al., 2016). Bacterial strains isolated from high altitude Himalayan regions have been shown to be able to solubilize phosphate at low temperatures (Adikari et al., 2021). In our country, effectes of bacteria isolated from Erzurum has been tested on chickpeas (Kantar et al., 2003; Öğütçü et al., 2008; Öğütçü et al., 2010). But to our knowledge, there is no work on psychrophilic microorganism isolation in Antalya region.

The goal of this study was to i) isolate bacterial strains from a high altitude and a low altitude region of Antalya, ii) compare their growth rates at 2 °C and room temperature (15-25 °C).

MATERIAL AND METHOD

Collection of Soil Samples

Soil samples were collected from Akdeniz University Campus and Saklıkent, Antalya. Information about the sampling locations are given in Table 1. Around 500 g of soil was taken 10 cm deep from the surface. Soil samples were put in a plastic bag and transferred to the lab. All the samples were collected on the same day and kept in refrigerator at 4 °C for further use.

Isolation of Bacteria

5 g soil sample was put into 45 ml of autoclaved physiological water (1 g NaCl in 1 litre distilled water) and mixed until large soil particles were all dissolved. From this mixture, a serial dilution was made, 10^{-1} to 10^{-7}, using physiological water as the diluent. 100 µl from each dilution was plated onto nutrient agar plates (Liu et al., 2018). Plates were parafilmed and kept at 10 °C until colonies became visible. From the same soil sample two isolations were made. Second isolation was performed two weeks after the first experiment.

Comparison of Growth Rates

Around 20 colonies were picked from plates that were incubated at 10 °C for each soil sample and transferred to nutrient agar plates. These colonies were grown at 4 °C and their growth was examined visually. A fast growing colony from each soil sample was tested for growth at 2 °C. To do this testing, very small amount of cells were transferred to a nutrient agar plate using a toothpick. A duplicate plate was made from the same colonies. One of the plates was kept at room temperature (15-25 °C), while the other plate was kept at 2 °C. Pictures of plates were taken at 10th day of growth. Diameters of the colonies were measured at different days to compare their growth rates.

Table 1. Soil samples and their locations.
Çizelge 1. Toprak örnekleri ve lokasyonları.

Soil Sample	Location Name	Geographic Location	Altitude (m)
T-1	Akdeniz University Campus	N 36'54.022 E 030'38.998'	34
T-9	Saklıkent	N36'49.259 E038'26.148'	2497
T-10	Saklıkent	N36'49.264' E030'20.159'	2515

RESULTS AND DISCUSSION

Bacterial Colonies Isolated in the Study

It was possible to isolate bacterial colonies from the three soil samples. Number of colonies isolated in two independent experiments are given in Table 2. As shown in Table 2, more than 500 colonies grew at 10^{-1} dilution of T-9 and T-10 samples. However, there were fewer colonies, around 50, in the T-1 sample. Pictures of colonies obtained from 10^{-1} dilution of both experiments are shown in Figure 1. Colonies obtained from the first isolation (Figure 1A) and second isolation (Figure 1B) has similar number of colonies. It was possible to see and count T-9 and T-10 samples by naked eye after 5-6 days growth. In contrast, in both isolations, colonies obtained from T-1 sample grew (visible to naked eye) around five days later than the T-9 and T-10 samples, suggesting bacteria found in T-1 sample grow much slower than the high altitude samples (T-9 and T-10) at 10 °C. This is an expected result, because in low altitude, species are expected to be less acclimated to low temperatures.

Notably, T-9 and T-10 colonies were similar in morphology and color. T-1 colonies were much diverse and different color and morphology of colonies were observed (Figure 1). These results suggest that there could be more diversity in low altitude soil sample. This hypothesis will be checked in future studies by determining the species of the isolated strains.

In summary, we were able to isolate bacterial strains that can grow at 10 °C from low altitude (T-1) and high altitude (T-9 and T-10) soil samples. However, more colonies were isolated from high altitude soil samples.

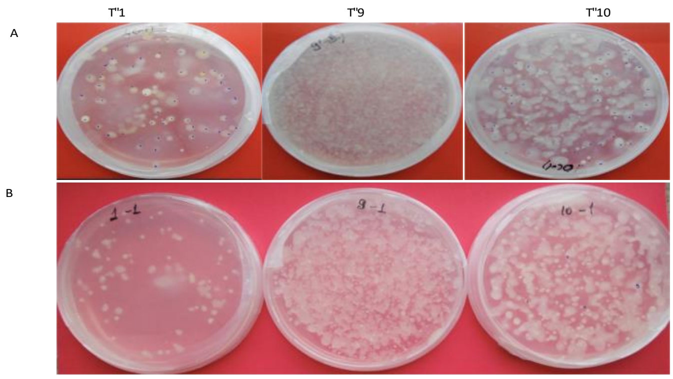

Figure 1. Colonies isolated from 10^{-1} diluted soil samples. A. Colonies of the first isolation experiment. B. Colonies of the second isolation experiment. Descriptions of soil samples are given in Table 1.
Şekil 1: 10^{-1} seyreltilmiş toprak örneklerinden izole edilen koloniler. A. Birinci izolasyon deneyinden izole edilen koloniler. B. İkinci izolasyon deneyinden izole edilen koloniler. Toprak örneklerinin açıklamaları Çizelge 1'de verilmiştir.

Table 2. Number of colonies obtained in the isolation experiments.
Çizelge 2. İzolasyon deneylerinden elde edilen koloni sayıları.

First Isolation					
	Dilutions				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Soil Sample	**# of colonies**				
T-1	50	6	4	1	None
T-9	>500	55	Colonies were mixed	1	1
T-10	>500	30	3	None	None
Second Isolation					
	Dilutions				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Soil Sample	**# of colonies**				
T-1	50	3	None	None	None
T-9	>500	100	25	3	None
T-10	>500	60	5	1	None

Comparison of Growth Rates at Room Temperature (15-25 °C) and 2 °C

After isolating bacterial strains that can grow at 10 °C, next we compared their growth rates at room temperature and 2 °C. As can be seen in Figure 1, there were more than 500 colonies on 10^{-1} dilution in T-9 and T-10 samples. Therefore, most of the colonies were mixed. To pick independent colonies, 10^{-2} dilution plates were used; these plates had less colonies which were apart from each other (Table 2). Around 20 colonies (16 colonies from T-1 and T-9 soil samples and 23 colonies from T-10 soil sample) from each soil sample were transferred to new nutrient agar plates and were grown at 4 °C (Figure 2).

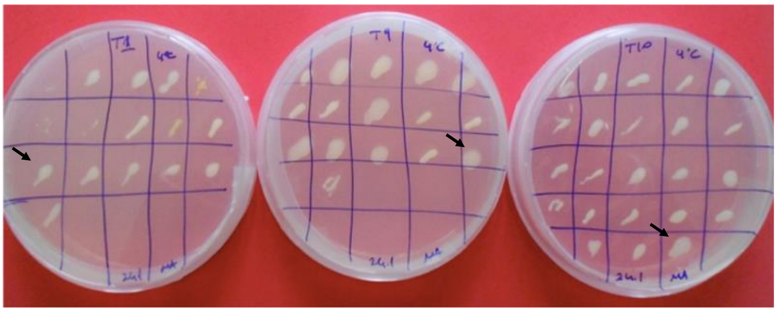

Figure 2. Growth of colonies at 4 °C. Colonies shown with arrows were compared for their growth rates at 2 °C and room temperature (15-25 °C) (see Figure 3).
Şekil 2. Kolonilerin 4 °C'de büyümesi. Ok ile gösterilen kolonilerin 2 °C ve oda sıcaklığında büyümeleri karşılaştırılmıştır (Şekil 3'e bakınız).

As can be seen in Figure 2, all of the T-9 and T-10 colonies were able to grow at 4 °C. However, not all the colonies of T-1 soil sample grew at 4 °C (Figure 2). These results showed that even at 4 °C there was a difference at growth rates of bacterial strains isolated from low altitude and high altitude soil samples.

Next, a single colony from each soil sample (shown with arrows in Figure 2) was tested for growth at 2 °C and room temperature. Colonies that showed similar growth at 4 °C were chosen for this test. Interestingly, the T-1 colony grew slower than the T-9 and T-10 colonies, both at room temperature and 2 °C (Figure 3A). These results show that bacterial strains isolated from high altitude have high growth rates and can grow at a range of temperatures.

Diameters of the colonies were measured at indicated times and a plot was made (Figure 3B). Colony isolated from T-1 soil reached 6 mm and 14 mm at 2 °C and room temperature, respectively. Colonies isolated from T-9 and T-10 soils reached around 25 mm and 17 mm at 2 °C and room temperature, respectively. These results show that, bacterial strains isolated from high altitudes have higher growth rates at all the temperatures tested and it is possible to isolate bacterial strains that can grow at temperatures as low as 2 °C from high altitude regions of Antalya. These results suggest that in high altitudes bacterial strains may also be growing in soil at low temperatures; they may not in dormant stages at low temperatures in soil also.

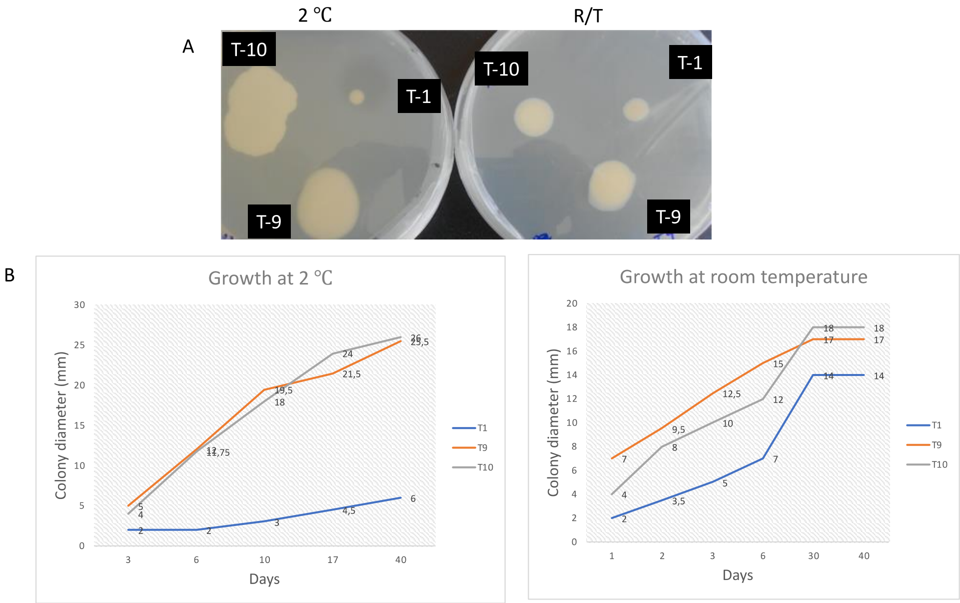

Figure 3. Comparison of growth rates at 2 °C and room temperature (15-25 °C). A. Pictures of plates were taken after 10 days of growth at 2 °C or room temperature. B. Diameter of the colonies (mm) at indicated days.
Şekil 3. Büyüme hızlarının 2 ℃ ve oda sıcaklığında karşılaştırılması. A. Plakların fotoğrafları 10 gün 2 ℃ veya oda sıcaklığında büyümeden sonra çekilmiştir. B. Belirtilen günlerde koloni çapları (mm).

CONCLUSION

Soils of our country are a source of microbial diversity that should be explored for the potential applications in biotechnology and conservation. Many works of study show that application of plant-beneficial microorganisms in agricultural lands increases the crop productivity (Kantar et al., 2003; Öğütçü et al., 2008; Öğütçü et al., 2010). Additionally, it is an ecofriendly approach that can help the economy of local farmers. Cold tolerant biofertilizers can have beneficial effects for plant productivity at low temperatures which mesophilic strains wouldn't be active (Balcazar et al., 2015; Yarzábal et al., 2018; Torracchi et al., 2020). Aim of this study was to isolate bacterial strains from Antalya and test their growth abilities at temperatures as low as 2 °C. Our results show that the strains isolated from high altitudes (T-9 and T-10 soil samples) can grow at temperatures as low as 2 °C. However, bacterial strains isolated from low altitudes (T-1 soil sample) grow slower at low temperature. Interestingly, high altitude strains have higher growth both at low temperatures and also at room temperature.

Growth at low temperature implies that these strains might have higher enzymatic activities at low temperatures, such as phytase activity which makes phosphate bioavailable for plant uptake. Determination of the species and enzymatic activities of these strains will be the subject of our future studies. Also, it is important to find out which cold adapted species are found in high altitude soils of our country.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

DECLARATION OF AUTHOR CONTRIBUTION

MA and FK collected the samples, MA has done the experiments with help of RY, all the authors have analyzed the data, MA has written the manuscript with contribution from all the authors.

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