Open Access Journal [doi: 10.46683/jmvi.2021.42] Research Article

Journal of Molecular Virology and Immunology

Use of *Streptomyces* species as a Biological Agent Against Plant Pathogens

Streptomyces türlerinin Bitki Patojenlerine Karşı Biyolojik Ajan Olarak Kullanılması

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Article Info: Received; 05.11.2022. Accepted; 09.12.2021. Published; 17.12.2021.
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Abstract

Agricultural activities make significant contributions to the national economies around the world. Factors such as rapid population growth and unplanned urbanization cause a decrease in agricultural lands and agricultural crop production over the years with the effect of abiotic and biotic factors. Many studies have been carried out to eliminate factors that reduce crop productivity. Fungal pathogens, one of the leading biotic factors, cause plant diseases and reduce the production efficiency of crops. This study aimed to isolate *Streptomyces* genus bacteria, which can be used as a biological agent against the wheat pathogen *Fusarium culmorum*, and to evaluate their antifungal activities. We found that eight of the 25 *Streptomyces* isolates, which were isolated from soil using the starch-casein agar medium and identified basis on morphological characteristics (colony appearance and microscopic examination results), inhibited the growth of *F. culmorum* on the Mueller-Hinton agar and that *Streptomyces* species could be used as a biological agent against this pathogen. We consider that eight *Streptomyces* isolates having different degrees of antifungal activity is promising for more permanent, economical and efficient solutions against *F. culmorum*, which is one of the fungi that reduces agricultural economic yield in Turkey. This study can also guide further research that will evaluate the potential use secondary metabolites of *Streptomyces* bacteria with antifungal properties for biocontrol or promotion of plant growth in different forms of microbial fertilizers.

Keywords: *Streptomyces*, *Fusarium culmorum*, Biological agent.

Özet

Tarımsal faaliyetler dünya genelinde ülke ekonomilerine önemli katkılar sağlamaktadır. Hızlı nüfus artışı ve plansız kentleşme gibi unsurlar, abiyotik ve biyotik faktörlerin de etkisiyle yıllar içerisinde tarım arazilerinin ve tarımsal ürün üretiminin azalmasına neden olmaktadır. Mahsul verimliliğini düşüren etkenleri ortadan kaldırmak için çok sayıda çalışma gerçekleştirilmiştir. Biyotik faktörlerin başında gelen fungal patojenler sebep oldukları bitki hastalıkları sebebiyle mahsulün üretim verimliliğini düşürmektedir. Bu çalışmada buğday patojeni olan *Fusarium culmorum*'a karşı biyolojik ajan olarak kullanılabilecek *Streptomyces* cinsi bakterilerin izole edilmesi ve antifungal etkinliklerinin değerlendirilmesi hedeflenmiştir. Nişasta kazein agar (*starch casein agar*) besiyeri kullanarak topraktan izole ettiğimiz ve koloni görünümü ve mikroskobik inceleme sonuçları ile morfolojik özellikler temelinde tanımladığımız 25 *Streptomyces* izolatından sekizinin Mueller-Hinton agar üzerinde *F. culmorum* üremesini inhibe ettiğini ve bu patojene karşı biyolojik ajan olarak kullanılabileceğini tespit ettik. Farklı derecelerde antifungal etki gösterdiğini saptadığımız bu sekiz *Streptomyces* izolatının Türkiye'de tarımsal ekonomik verimini düşüren funguslardan birisi olan *F. culmorum'a* karşı daha kalıcı, ekonomik ve verimli çözümler üretmek için umut vaat ettiğini düşünüyoruz. Bu çalışma aynı

zamanda, *Streptomyces* türü bakterilerin sekonder metabolitlerinin biyokontrol amaçlı veya bitki büyümesini teşvik edici farklı mikrobiyal gübre formlarında kullanım potansiyelini değerlendirecek ileri çalışmalara da öncülük edebilir.

Anahtar Kelimeler: *Streptomyces*, *Fusarium culmorum*, Biyolojik ajan.

Introduction

Grains are an important economic resource for the entire world, and global grain use is expected to increase by 1.7 percent year-on-year to 2,812 million tons in 2021/22, according to the Food and Agriculture Organization estimates [1]. The data of the Turkish Statistical Institute (TurkStat) in 2016 indicate that grains have the largest share (49%) of 23.9 million hectares of land that can be planted in Turkey [2]. According to the TurkStat data, in the second estimation of 2021, there will be a 12% decrease in cereals and other plant productions compared to the previous year [3]. Today, wheat and barley, which have a high rate of cultivation and production power globally, are unfortunately faced with biotic and abiotic stress factors, which leads to great yield losses [4]. Abiotic stress factors, such as excessive salinity, extreme drought, temperature, toxicity, and oxidative stress have serious effects on agricultural yield [5]. In addition to the direct effects of abiotic stress factors, it has also been found that pathogenic microorganisms as biotic stress factors have increased their spread and negative effects [6]. Fungi, oomycetes, bacteria, and nematodes, which are in the group of pathogenic microorganisms, are known to have harmful effects on plant growth and development [7].

It has been determined that post-harvest losses due to plant diseases in wheat agriculture can reach up to 20% of the wheat produced each year [8]. Fungi, which are among the pathogenic microorganisms, are of great importance as they constitute 70% of plant diseases [9]. One of these pathogenic microorganisms is fungi belonging to the *Fusarium* genus. The genus *Fusarium* has an important place among plant pathogens due to its features such as containing a large number of species and the wide ecological distribution of these species in the world [10,11].

Fusarium culmorum, one of the fungi of the genus *Fusarium* in the *Nectriaceae* family, is an important plant pathogen due to its wide effects on grain and non-grain products [12–14]. *F. culmorum* is a plant pathogen whose habitat is generally soil and is more prevalent in the north, central, and west of Europe [15]. Its pathogenicity range extends from cereals to fruits and has a 150-year history of disease [16]. In addition to having effect on many plants, *F. culmorum* is considered as one of the three dangerous pathogens in the world and contaminated plants are quarantined globally due to the diseases of ear blight and root rot [16,17]. It has been reported that plant diseases caused by *Fusarium* species have become more common in many countries in America, Asia, and Europe [16]. In addition to the economic loss it causes at high rates, *F. culmorum* is also the source of a wide variety of chemical structures called mitotoxins, which it produces through a secondary metabolism [18]. Mitotoxins deoxynivalenol, nivalenol and zearalenone produced by *F. culmorum* threaten not only plant health but also human and animal health [19,20]. It has been observed that one quarter of the crops produced across the world are contaminated by these natural mycotoxins, and this causes significant losses in terms of plant health, while causing losses in economic yield and quality loss [13,18]. Therefore, the fight against this fungal pathogen, which causes many plant diseases, has become very important [21].

Streptomyces species are used as plant control agents due to their potential to control soil and seed-borne plant diseases through the antifungals they produce [22–24]. Taking strength from these properties, *Streptomyces* bacteria have become a part of microbial fertilizer studies, considering that it can show biocontrol and plant growth enhancing effects [25].

The aim of this study was to isolate *Streptomyces* bacteria, which can be used as a biological agent against the wheat pathogen *F. culmorum*, and to evaluate their antifungal effects.

Material and Method

Soil samples were collected from various parts of Hacettepe University Beytepe campus for *Streptomyces* bacteria isolation.

These soil samples were then incubated separately at 30 °C for 24 hours. Then, soil samples were serially diluted and inoculated into the starch-casein agar (SCA) selective media. The samples taken from the diluted liquid during the inoculation stage were dispersed into the medium using glass beads. The media were incubated at 30 °C for five days. After the incubation period was completed, colonies resembling *Streptomyces* bacteria morphology were selected and passaged. Care was taken to select colored colonies while performing this selection. Gram staining was performed on the isolated strains first. Bacteria with gram-positive hyphae were considered probable *Streptomyces* strains. These strains, which are likely to be *Streptomyces* species, were named numerically and the study was continued. The isolated strains were stored in 50% glycerol and resuscitated during the study period.

For stock control and resuscitation, a single colony from 25 different *Streptomyces* species was inoculated on the SCA medium (starch 10 g/L, casein 0,3 g/L, KNO_3 2 g/L, NaCI 2 g/L, $MgSO_4.7H_2O$ 0.01 g/L, $CaCO_3$ 0.02 g/L, $F_2SO_4.7H_2O$ 0,01 g/L, agar 18 g). The cultivated bacteria were incubated at 30 °C for seven days to check existing stocks and make all bacterial samples ready for antifungal effect testing.

In the second stage of the study, *Streptomyces* strains were tested to determine whether they had antifungal effects against *F. culmorum*. Twenty-five *Streptomyces* isolates were inoculated on the Mueller-Hinton agar medium (34 g/L) using the line inoculation technique (Figure 1) and incubated at 30° C for seven days. After the incubation period was completed and the bacteria had achieved the necessary growth, the *F. culmorum* strain obtained from Hacettepe University Biology Department stocks was inoculated (using sterile forceps) immediately opposite the sowing end of the petri dish (Figure 1). After this process, the medium plate was incubated at 37 °C for 14 days.

At the last stage, at the end of the 14th day, the presence of growth and inhibition was evaluated and the observed results were recorded. The sowing and evaluation processes were repeated on the plates where contamination was observed. The results were evaluated as positive in growth-inhibited plaques around the *Streptomyces* colonies, and the positive seedlings were noted with their zone diameters. The plates in which *F. culmorum* grew to completely cover the culture plate and covered *Streptomyces* colonies without a measurable distance were considered negative. The study was carried out in three parallels with the same procedures.

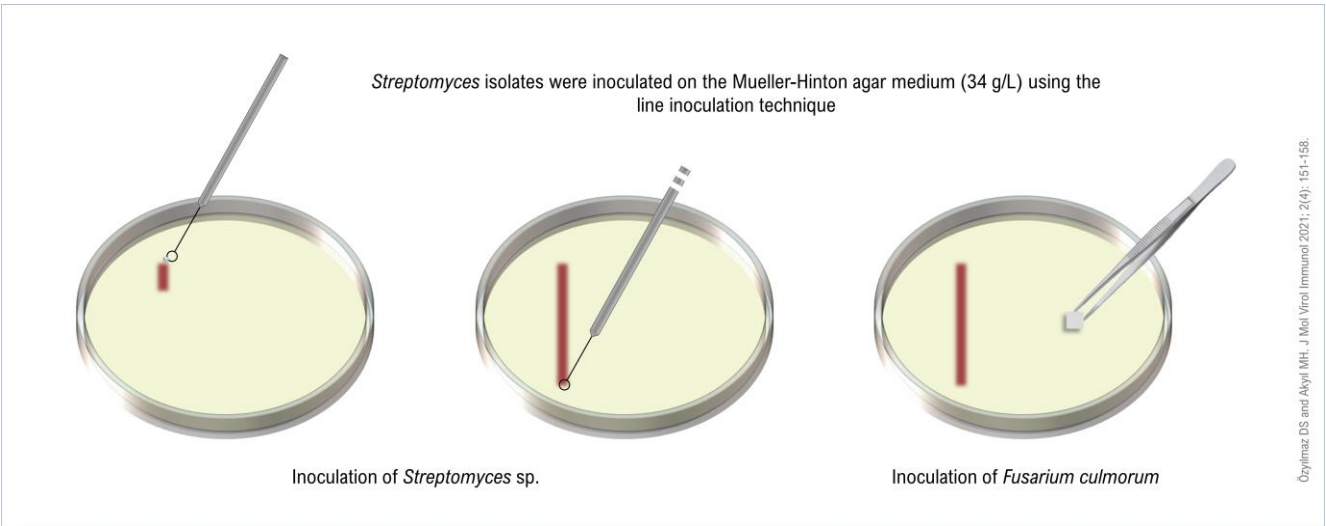

Figure 1. Schematic representation of the cultivation of *Streptomyces* species effective against *Fusarium* sp.

Results

It was observed that eight of the 25 *Streptomyces* isolates inoculated in the same medium with *F. culmorum* inhibited the growth of *F. culmorum* and prevented the distribution of fungal growth throughout the medium. The remaining 17 isolates could not stop the growth of *F. culmorum,* and fungal growth covered the entire medium. The positive and negative results, along with zone diameters, are shown in Table 1.

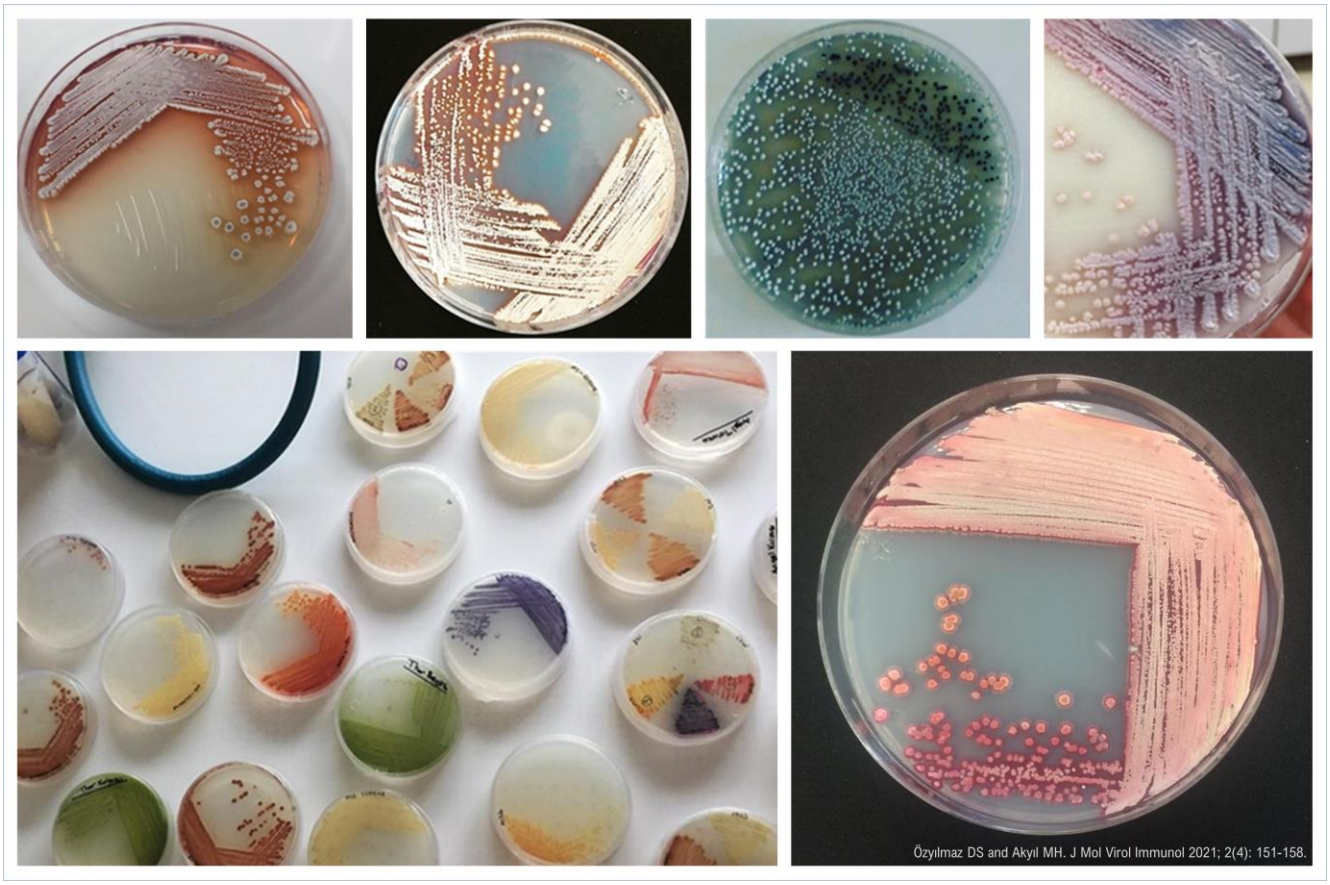

Figure 2. Colony morphology of *Streptomyces* species isolated from soil (starch-casein agar).

Table 1. Effect of *Streptomyces* isolates against *Fusarium culmorum* and zone diameters.

Isolate	Zone diameter	Result	Isolate	Zone diameter	Result	Isolate	Zone diameter	Result
Strept. I-01	0 cm	Negative	Strept. I-10	0 cm	Negative	Strept. I-18	0 cm	Negative
Strept. I-02	**1.2 cm**	**Positive**	Strept. I-11	0 cm	Negative	Strept. I-19	0 cm	Negative
Strept. I-03	0 cm	Negative	Strept. I-12	0 cm	Negative	Strept. I-20	**1.7 cm**	**Positive**
Strept. I-04	0 cm	Negative	Strept. I-13	**1 cm**	**Positive**	Strept. I-21	0 cm	Negative
Strept. I-05	**1.2 cm**	**Positive**	Strept. I-14	**1.2 cm**	**Positive**	Strept. I-22	0 cm	Negative
Strept. I-06	0 cm	Negative	Strept. I-15	0 cm	Negative	Strept. I-23	**1.2 cm**	**Positive**
Strept. I-07	0 cm	Negative	Strept. I-16	0 cm	Negative	Strept. I-24	0 cm	Negative
Strept. I-08	**2.1 cm**	**Positive**	Strept. I-17	0 cm	Negative	Strept. I-25	0 cm	Negative
Strept. I-09	**1.1 cm**	**Positive**						

Strept.: *Streptomyces* sp.

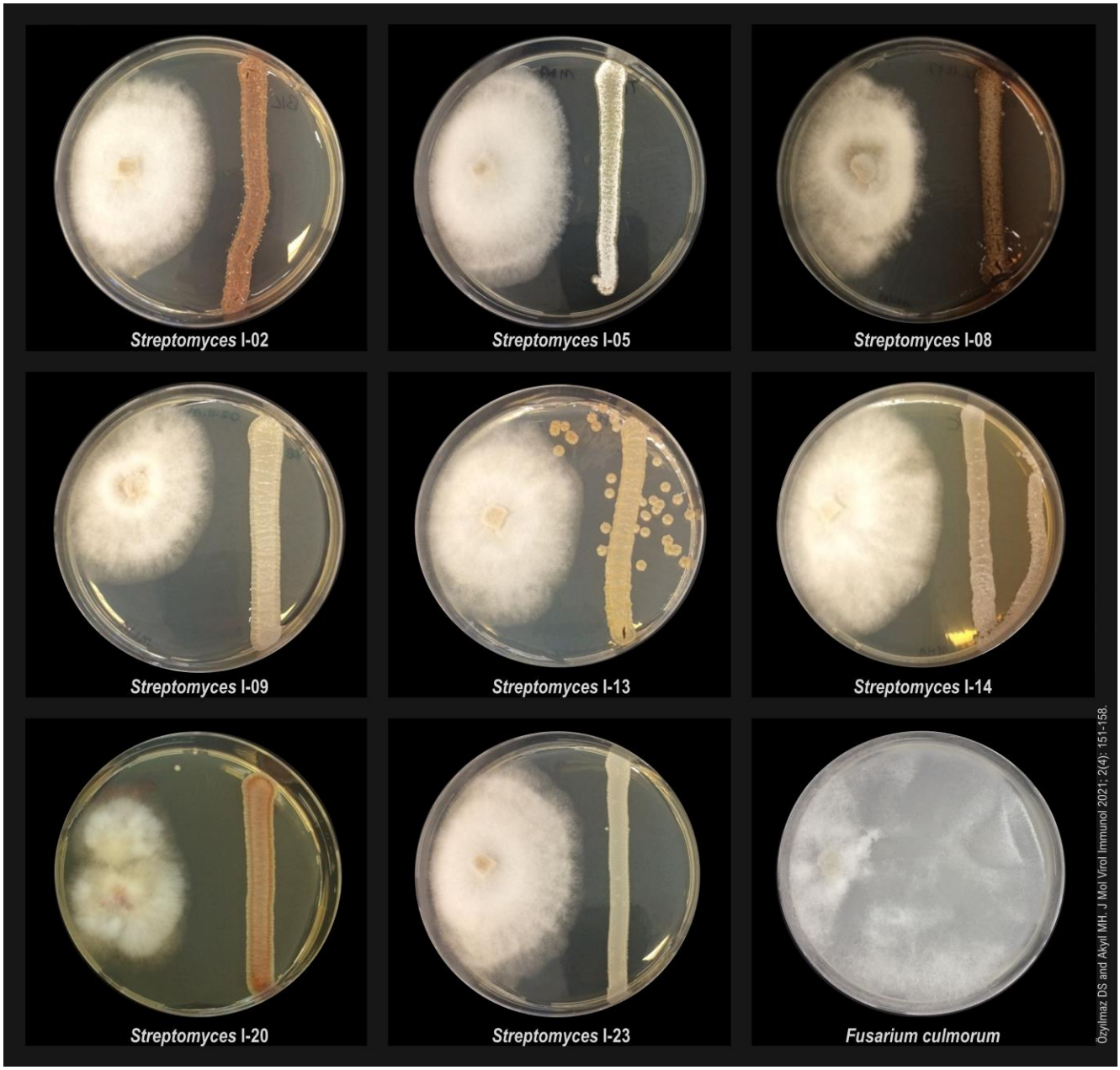

Figure 3. Inhibitory effect of *Streptomyces* isolates against *Fusarium culmorum* and growth of *F. culmorum* covering the entire plaque (*lower right plaque*).

Discussion

Similar in vitro studies have shown that *Streptomyces* bacteria inhibit the growth of fungi with their antifungal effect [26,27]. The excessive use of chemical fungicides used to protect agricultural lands and plants has led to various problems, such as the deterioration of human health, environmental pollution, and the development of pathogen resistance to fungicide, and it has become necessary to seek new solutions for serious problems. Such solutions should be less chemical-dependent and more environmentally friendly, while exhibiting antifungal effects against fungi. Based on their properties, microbial antagonists have been on the forefront in the search for new products and solutions [28].

Streptomyces species are gram-positive, aerobic and filamentous soil bacteria [29]. Many organic compounds can be used as carbon sources for growth and energy. *Streptomyces* species can be distinguished from other filamentous actinomycetes based on morphological features, mainly the mycelium and arthrospores they produce [29]. *Streptomyces* species form two types of mycelium: submerged

mycelium and aerial mycelium (usually colored due to the pigments produced). Colonies may be flat and soft at first, but after a while they may become hard, firm, and velvety [29]. Under normal conditions, they exist in the spore form, but when conditions improve, the spores germinate and begin to form the submerged mycelium [30]. The ratio of guanine + cytosine in the DNA of *Streptomyces* varies between 69 and 78%. Cell walls contain high amounts of L-diaminopimelic acid (L-DAP) and are peptidoglycan in structure. Optimum growth temperatures are between 25 and 35 °C, and some species may be psychrophilic or thermophilic. Optimum pH demands vary between 6.5 and 8.0 [30].

Most *Streptomyces* species are saprophytes, but some have parasitic relationships with plants and animals, also a few species can be pathogenic in humans, animals or plants [31]. It has been revealed that *Streptomyces* play a role in the substance cycle by producing enzymes responsible for the destruction of organic substances, such as cellulose, starch, and chitin in soil [32]. The distribution, development and activity of *Streptomyces* species in soil are determined by factors such as nutrients, humidity, temperature, and pH [33]. They can also multiply at low oxygen concentrations in soil, but when the carbon dioxide rate exceeds 10%, the growth of these bacteria is inhibited [34,35]. Since *Streptomyces* spores are resistant to factors such as drought, their number is much higher in soil compared to the vegetative forms of other microorganisms [34,35]. When *Streptomyces* bacteria are viewed under a microscope, a hyphal structure is seen, which is an indication of how *Streptomyces* species can withstand environments that are not suitable for reproduction [34].

In a study examining *Streptomyces* species, Abbasi et al. found that these bacteria had antifungal activities against *Fusarium oxysporum* species [36]. Bubici suggested that *Streptomyces* spp. were effective against *F. fujikuroi*, *F. graminearum*, *F. solani* and *F. oxysporum* species [37]. Wei et al. showed that the newly isolated *Streptomyces* strains were effective against *F. oxysporum* [38]. Colombo et al. reported that they isolated *Streptomyces* species that had an effect against *F. graminearum* [39]. Winter et al. found that *Streptomyces* isolates exhibited antifungal activity against *F. culmorum* [40]. Due to these features possibly associated with secondary metabolites, studies to produce new metabolites synthetically continue, as well as the discovery of new metabolites [41].

Conclusion

One of the most important features of *Streptomyces* bacteria is their ability to produce secondary metabolites. There is also the possibility that secondary metabolites produced by the newly isolated *Streptomyces*, which were found to be effective against *F. culmorum* in this study, may be new metabolites. Therefore, based on the results we obtained in our study, the effects of the antifungal properties of *Streptomyces* species determined in the field can be elucidated by further studies that will evaluate their potential as a microbial fertilizer in different forms to promote biocontrol and plant growth by partially purifying secondary metabolites with antifungal effects.

Conflict of interest: The authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of the paper.

Financial disclosure: This work has been financially supported by the Scientific and Technological Research Council of Turkey (TUBITAK), Project No: 1919B011701682.

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