SELECTION OF ANTAGONISTIC ACTINOMYCETE ISOLATES AS BIOCONTROL AGENTS AGAINST ROOT-ROT FUNGI

Gul Erginbas[1], Mustafa Yamac[2]*, M. Julia Amoroso[3,4] and S. Antonio Cuozzo[3,4]

[1] CIMMYT Turkey Office, International Maize and Wheat Improvement Centre, Wheat Program, Ankara, Turkey
[2] Eskisehir Osmangazi University, Faculty of Arts and Science, Department of Biology, Eskisehir, Turkey
[3] Universidad Nacional de Tucumán. Argentina.
[4] Planta Piloto de Procesos Industriales y Microbiológicos (PROIMI-CONICET) Av. Belgrano y Pasaje Caseros 4000, Tucumán, Argentina

ABSTRACT

In this study, actinomycetes isolates, isolated from rhizosphere of wheat (*Triticum aestivum* L.), were screened for antagonistic activities on certain root rot fungi (*Fusarium culmorum*, *Fusarium graminearum*, *Fusarium verticilloides* and *Bipolaris sorokiniana*). The *in vitro* antagonistic effects of actinomycetes isolates were determined on solid media against fungal pathogens. The inhibition mechanism, effect of application time and pH on inhibition was investigated. The actinomycete isolate 129.01 exhibited a high inhibition ratio of more than 60 % against all fungi. The activity of the isolate 129.01 against root rot fungi was tested under greenhouse conditions. The root rot score (1-10), mean plant height (cm) and mean weight of green part of plant (g) were determined after an incubation period. The root rot score of the infected plants was decreased significantly by this isolate, even if the plants were inoculated with all of the pathogen fungi together ($P<0.05$). The results indicate that isolate 129.01 could be useful as a biocontrol agent. The assignment of the isolate 129.01 to the genus *Streptomyces* was supported by 16S rRNA analysis.

KEYWORDS:
actinomycetes, biocontrol, root rot fungi, streptomyces

INTRODUCTION

Root rots are among the major diseases of wheat resulting in important yield losses throughout the world. The damage from root rot pathogens varies from year to year as well as from field to field depending upon the amount of inoculum present, cultural practices, and soil and climatic factors [1]. Root rots on cereals occur in countries worldwide including France 15-75% [2], USA 40-50% [2,3], Canada 5-28% [4-7], Australia 26.6% [3, 8], Brazil 15-38% [9], Hungary, Morocco 4-6% [3], India, England, Italy [8], Poland, Syria, Russia, China, South Africa, Tunisia and Turkey [10]. Records show that root rots cause an average 34% yield reduction on a range of cereals [10]. Many different fungal species are known to be associated with root rot on wheat, including *Pythium, Rhizoctonia, Gaeumannomyces, Fusarium,* and the *Bipolaris* genera. The predominant *Fusarium* species responsible for the root rot diseases are *Fusarium graminearum* and *F. culmorum* [11].

To overcome root rot diseases, screening and/or improvement of resistant cultivar(s) is one of the most important research areas worldwide. Since there is no highly resistant wheat cultivar, control of these diseases depends mainly on fungicide application. Before planting, soil sterilization, especially with methyl bromide, was used for the management of *Fusarium* wilt. Higher rates of fungicide treatments are needed to control root diseases. Furthermore, the use of methyl bromide for soil sterilization has been precluded in Europe by legislation on ozone-depleting chemicals since 1 January 2005 [12].

Pesticides are widely distributed environmental contaminants that have hazardous biological effects, including acute and chronic toxicity, mutagenity, and carcinogenity. The excessive use of pesticides belonging to different biological classes (herbicides, insecticides, fungicides) causes food contamination, environmental pollution and toxicity. All ecosystem components are affected and almost all animals have detectable amounts of pesticides in their tissues. Furthermore, the development of pathogen resistance to pesticide is a worsening problem in disease control.

A variety of methods have been studied for use in control of root rots including crop rotation [3], stubble burning [9], and integrated control [12]. However, biological control as either an environmentally friendly alternative or a supplement to other methods may be a viable alternative not only for root rot disease [11, 13] but also for other plant diseases [14, 15]. Actinomycetes of the genus *Streptomyces* are well known for their ability to suppress growth of a wide variety of fungal pathogens both *in vitro* and *in planta* [16-20]. Antagonistic actinomycetes have been isolated from different plant rhizospheres by numerous researchers [19, 21-24]. The action modes of tested Streptomycetes in rhizosphere include antibiosis, lysis of fungal

cell walls, competition and hyperparasitism [25]. Although many species of *Streptomyces* with a broad spectrum of antifungal activities are known to be antagonist organisms, only a limited number could be used for commercial preparations, such as Mycostop prepared from *Streptomyces griseoviridis* and Actino-Iron prepared from *Streptomyces lydicus* [24]. However, the number of commercial biofungicides prepared from actinomycetes is still insufficient. The objective of the present work is to determine if actinomycetes isolates could control root rot fungi both *in vitro* and *in planta*. To our knowledge this is the first report on *in vivo* and *in planta* selection of antagonistic *Streptomyces* isolates against any plant pathogen fungi from Turkey.

MATERIALS AND METHODS

Origin of the Isolates and Plant Material

The actinomycetes isolates tested in this study were isolated from 77 different wheat fields in Turkey by the serial dilution-spread plate technique [21]. Wheat roots and adhering soil were used as a source of rhizosphere actinomycetes. Dilutions were plated onto selected actinobacterial isolation media (Yeast-extract malt extract agar, Gliserol yeast extract agar and Tryptone yeast extract agar) and were incubated at 25 °C for 2–4 weeks. In total, 180 actinomycetes isolates were selected from isolation plates based on colony morphology.

Virulent plant pathogenic fungi isolates used as test organisms (*Fusarium graminearum*, *Fusarium verticilloides*, *Fusarium culmorum*, *Bipolaris sorokiniana*) were kindly donated by Dr. Berna Tunalı, Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, Samsun, Turkey. All microorganism cultures were stored at 4 °C until used.

Susceptible wheat (*Triticum aestivum*) variety Bezostaja 1 seeds were obtained from the Anatolian Agricultural Research Institute, Eskişehir, Turkey.

In vitro Antagonism Testing between Actinomycetes and Root Rot Fungi

To screen *in vitro* antagonistic activities of isolates, the method suggested by Yuan and Crawford was used [26]. Briefly, each actinobacterial isolate was streaked onto one third of the area of a Potato Dextrose Agar (PDA) plate (pH 7.0). The plates were incubated at 27 °C before an agar plug (0.5 cm) of fungal mycelium was transferred onto the center of the actinobacteria-free area of the plate. Fungal discs were also grown on PDA plates that were not inoculated with the actinobacteria as a negative control. The plates were examined for any inhibition of growth after 2 and 5 days. The level of inhibition ($\Delta\gamma$ (cm)) was defined as the subtraction of the fungal growth radius (γ_o (cm)) of a control culture from the distance of the growth direction of the actinomycete isolates (γ (cm)), where $\Delta\gamma : \gamma_o - \gamma$ [26]. After 5 days of paired incubation, mycelial plugs from the fungal colony edge in the peryphery of actinomycete colonies were transferred to a fresh medium to check for the viability of the mycelia in that area [26]. The fungal pathogens examined were *Fusarium graminearum*, *Fusarium verticilloides*, *Fusarium culmorum*, *Bipolaris sorokiniana*. Each experiment was repeated at least twice and each of the actinomycete isolates were tested against fungal pathogens, with the obtained results evaluated as mean.

To determine the effect of media pH on the inhibition of fungal growth during incubation, a cellophane overlay technique was used. A cellophane membrane was placed on the agar in each petri plate and dried in a laminar flow cabinet. Actively growing actinomycete spores were smeared over the entire surface of cellophane on a PDA plate (pH 7.0). After incubation at 27°C for 7 days, the cellophane membrane with adhering bacterial culture was removed. The medium was homogenized and the media pH changing was then determined. The results were compared with the control.

To test the effects of the application time of the actinomycete isolates against plant pathogens, actinomycete cultures were streaked on the edge of the dishes and, at the same time, each of the different plant pathogenic fungus discs (0.5 cm) were transferred across the center of the plate at 2 cm intervals.

To determine the ability of the extracellular hydrolytic enzymes production, selected isolate was grown on colloidal chitin agar and was examined for visible clear zone around the colony during the incubation period.

Preparation of actinobacterial inoculum and seed coating

Actinomycete isolates which have antagonistic activity were grown on a PDA medium at 27 °C for 14 days until complete sporulation occured. After the incubation period, each plate of actinomycete culture was scraped into a 10 ml saline solution (0.1%). For surface disinfection, the wheat seeds were soaked in 96% ethanol for 6 min, 4.5% NaOCl solution for 10 min and then rinsed three times in SDW (sterile distilled water). Surface-disinfested 60 wheat seed were treated in 30 ml spore suspension for 30 min. [27]. Then the seeds were dried in a laminar flow cabinet. Control treatments included seeds soaked in SDW.

Wheat seeds coated with actinomycetes isolate were vernalized at 2 °C for 5 weeks. Vernalized seeds were transferred to a TYE (Tryptone Yeast Extract) medium and incubated for 7 days to observe whether or not they were coated with actinomycete. Vernalized-coated seed was diluted in sterile saline solution and spread onto PDA to determine the spore count on each seed. Colonies from pure cultures of actinomycete isolates were used as a positive colony morphological to help to ascertain the identity of the isolate colonies retrieved from the seed.

In planta biocontrol assay in greenhouse

The most active actinomycetes isolate against all tested fungal pathogens was selected for *in planta* biocontrol studies. To examine the ability of this actinomycete

isolate to control root rot fungi, greenhouse experiments were performed.

To obtain inoculant, fungal cultures were grown on PDA medium at 25 °C for 7 days. After incubation, five discs (5 mm diameter) were transferred into 200 g autoclaved wheat grains in 500 ml Erlenmayer flasks and incubated at 25 °C for 4 weeks. In this way, wheat grains with fungal pathogen were provided as an inoculum source for plant inoculation. Pathogen fungi inocula were stored at 5 °C until used.

Soil experiments were conducted under greenhouse conditions using 3 cm diameter, 12.5 cm high plastic tubes. Each pathogenic fungus inoculum (0.5 g) was distributed in 2 cm deep of plastic tubes filled with an autoclaved soil mixture (70: 29: 1 soil: sand: organic fertilizer). Then one disinfected wheat seed was sown in each tube. In total there were 12 treatments: (1) *F. culmorum* alone, (2) *F. graminearum* alone, (3) *F. verticilloides* alone, (4) *B. sorokiniana* alone, (5) All pathogens, (6) *F. culmorum* + Antagonist, (7) *F. graminearum* + Antagonist, (8) *F. verticilloides* + Antagonist, (9) *B.sorokiniana* + Antagonist, (10) All pathogens + Antagonist, (11) Antagonist (Isolate 129.01) alone, and (12) Uninoculated control.

To evaluate the effects of the antagonist on pathogen fungi, the tubes were placed in racks in a completely randomized block design with 7 replications. Plants were grown in a greenhouse of 12 h photoperiod of light for 9 weeks. Nine weeks after the seed sowing, the plants were harvested and scored for disease symptoms. Mean root rot score was assessed according to a 1-10 scale according to Nicol et al. [28]. The mean plant height (cm) and mean weight of the green part of the plants (g) were also determined after 9 weeks.

Characterization of the Antagonistic Actinomycete

Selected isolate for *in planta* studies, 129.01, was characterized by chemotaxonomic and morphologic methods suggested by Lechevalier [29]. The morphological properties of isolate were determined using a s x 40 long working distance objective. The isomeric form of diaminopimelic acid in the isolate's cell wall was determined for chemotaxonomic analysis. In addition, a 16S rRNA analysis of the isolate 129.01 was also performed.

DNA extraction. 1 ml of culture was centrifuged and resuspended in 1 ml of TE (tris-HCL, 50 mM; EDTA, 20 mM; pH 8.0). A lysis solution, 0.38 ml, was added, followed by 0.40 ml of sodium perchlorate solution. Phenol-chloroform was added to fill the 2 ml centrifuge tube, and the culture was extracted. The aqueous upper phase was transferred into another tube and extracted using chloroform-isoamyl alcohol. Then 2 ml of 95% ethanol was added to the aqueous phase, and the DNA was spooled out, washed in 80% ethanol, and air dried. The DNA was resuspended in 0.1x SSC (15 mM sodium chloride, 15 mM sodium citrate; pH 7.0). RNase was added to a final concentration of 1 mg/ml. The mixture was extracted once again with chloroform-isoamyl alcohol and centrifuged; the aqueous phase was then transferred to another tube, and SSC was added (1x, final concentration). The DNA was then dissolved in 500 µl of TE.

16S ribosomal RNA (rRNA) sequencing. Oligonucleotide primers with specificity for eubacterial 16S rRNA genes (forward primer 27: 5'-AGAGTTTGATCMTGGCTCAG-3' and reverse primer 5'-GGTTACCTTGTTACGACTT-3') were used to amplify 16S rDNA [30]. PCR fragments were purified using Qiaquick Gel Extraction Kit (Qiagen, Valencia, CA) and an ABI 373A sequencer (MACROGENE Inc., Korea). Sequencing data was analyzed by comparison to 16S rRNA genes in the Ribosomal Database Project and EMBL-GeneBank databases, and aligned manually with the MEGA 4.1 Beta software.

PCR amplification. PCR amplification was performed on a Gene Amp PCR System 9700 thermal cycler. The PCR amplification reaction mixtures consisted of template DNA (100 ng), 50 mM KCl, 10 mM Tris-Hcl (pH9.0), 0.1 % (wt/vol), 1.5 mM $MgCl_2$ 100 nM primer, and 1.5 U of Taq polymerase (Promega). Amplification was performed with an initial denaturation step of 4 min at 94ºC and then 30 cycles of 1 min denaturation at 94ºC, 30 s at 57ºC for primer annealing, and 1.5 min at 70ºC for primer extension. A 7-min extension and cooling to 4ºC completed the reaction sequence.

Statistical Methods

Treatment means were separated by the least significant difference test at $P<0.05$ with Statistical Analysis System (SAS) software (SAS Institute, Cary, NC).

RESULTS

In vitro antagonism testing between actinomycetes and root rot fungi

A total of 180 actinomycetes isolates were chosen from isolation plates for *in vitro* antagonism tests against root rot fungi. Among the actinobacterial isolates obtained wheat rhizosphere, significant differences were determined for their antagonistic activity ($P>0.05$). After 2-5 days of incubation in dual cultures, four isolates (03.03, 26.07, 97.03, 129.01) demonstrated stronger activity against all tested fungi (Table 1).

After initial screening studies, the isolate 129.01 was chosen for *in planta* antagonism studies because it has a 60% or higher antagonistic efficiency against all tested fungal isolates *in vitro*.

In planta biocontrol assay in greenhouse

In the absence of pathogens, no significant difference between antagonists inoculated and control treatments in all experimental parameters were detected ($P<0.05$). On the

TABLE 1 - *In vitro* antagonism of actinomycetes isolates against fungal pathogens

	Antagonists											
	03.03			**26.07**			**97.03**			**129.01**		
	Antagonism at [a]:		Viability	Antagonism at:		Viability	Antagonism at:		Viability	Antagonism at:		Viability
Pathogens	Day 2	Day 5	at day 5 [b]	Day 2	Day 5	at day 5	Day 2	Day 5	at day 5	Day 2	Day 5	at day 5
F. culmorum	-	++++	+	-	++++	+	-	+++	+	+++	++++	+
F.graminearum	+	+++	+	-	+++	+	++	+++	+	+++	++++	+
F. verticilloides	-	+++	+	-	+	+	+	++	+	+	+++	+
B. sorokiniana	-	-	+	+	+	+	+	+++	+	+	+++	+

[a] Ratings: ++++, Δγ > 3 cm +++ 3 cm > Δγ > 2 cm; ++, 2 cm > Δγ > 1 cm; +, 1 cm > Δγ > 0.5 cm; -, Δγ < 0.5 cm (see Materials and methods section)
[b] Viability was evaluated as recovery of fungal mycelial plugs on fresh media

TABLE 2 - Root rot score, weight and height values of experimental groups in greenhouse.

Treatments[a]	Root Rot Score [b]	Weight (g)	Height (cm)
1	6.40 ± 0.89 a	0.80 ± 0.26 ab	26.86 ± 3.02 d
2	7.12 ± 0.75 a	0.70 ± 0.24 b	32.57 ± 4.08 bcd
3	3.00 ± 1.00 c	0.69 ± 0.11 b	32.57 ± 1.72 bcd
4	4.60 ± 1.52 b	0.96 ± 0.13 ab	39.29 ± 1.98 a
5	6.71 ± 0.49 a	0.93 ± 0.41 ab	36.14 ± 8.17 ab
6	0.71 ± 0.49 ef	0.82 ± 0.16 ab	36.71 ± 4.31 ab
7	1.50 ± 0.58 de	0.90 ± 0.27 ab	34.29 ± 4.89 abc
8	0.67 ± 0.52 ef	0.87 ± 0.33 ab	28.90 ± 12.44 cd
9	2.00 ± 0.00 cd	0.91 ± 0.31 ab	38.86 ± 5.67 ab
10	1.37 ± 0.80 de	1.08 ± 0.45 a	38.86 ± 5.37 ab
11	0.00 ± 0.00 f	1.04 ± 0.28 a	37.71 ± 7.52 ab
12	0.29 ± 0.49 f	0.84 ± 0.21 ab	35.71 ± 6.80 ab

[a] For definition of treatments, see Material and Methods section.
[b] Mean ± S.D., one way ANOVA. Different letters indicate values with significant difference at P ≤0.05.

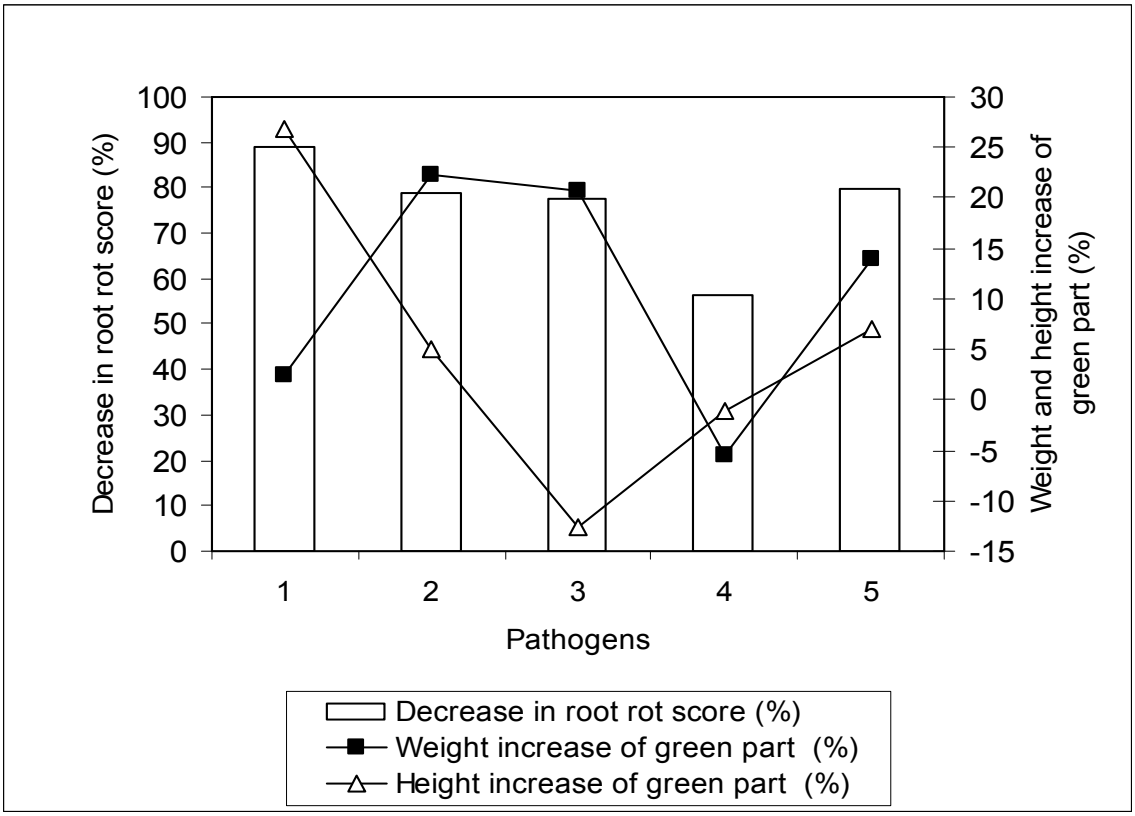

FIGURE 1 - *In planta* effects of isolate 129.01 on fungal pathogens. 1. *Fusarium culmorum*, 2. *Fusarium graminearum*, 3. *Fusarium verticilloides*, 4. *Bipolaris sorokiniana*, 5. All pathogens

other hand, in the presence of pathogens, experimental plants negatively affected in root rot score, mean plant height and weight except *B. sorokiniana* which is the slowest growing fungus *in vitro* and *in vivo* treatments.

Based on the mean root rot score of the experimental groups, the antagonist isolate 129.01 decreased significantly the root rot score in all tested pathogenic fungus, even if the plants were inoculated with all of the pathogen fungi together ($P<0.05$). Moreover, similar to the *in vitro* antagonism results, there was no appreciable difference in the root rot score of the isolate 129.01 in *F. culmorum* and *F. verticilloides* groups and that from the control was observed ($P<0.05$, Table 2.). The root rot scores of all pathogens were significantly decreased by the presence of antagonist isolate 129.01 ($P<0.05$).

The weights of the plants were slightly increased in the presence of the antagonist alone. But the increase in weight is not significant statistically ($P<0.05$, Table 2). Similarly the heights of the plants were not increased significantly (Table 2).

The isolate 129.01 had strong antagonistic in vivo activity based on the all the experimental parameters. The antagonist particularly had activity against Fusarium spp. Bipolaris sorokiniana was determined as the most tolerant pathogen to isolate 129.01 (Fig 1).

Phylogenetic analysis of *Streptomyces* sp. 129.01

The isolate 129.01 was isolated from a wheat field near timberland on Turkmenbaba Mountain, Eskisehir, Turkey. The stereochemical form of the diaminopimelic acid in cell wall hydrolysates of isolate 129.01 was the L-form. The isolate 129.01 was confirmed as belonging to the group of cell wall type I. The spore chain of the rectiflexibles type was also observed.

The assignment of the isolate 129.01 to the genus *Streptomyces* was supported by 16S rRNA. A comparison of the almost complete 16S rRNA gene sequence of the tested isolate with corresponding streptomycete sequences from the GenBank database showed that the isolate lay in the evolutionary clade of *Streptomyces* allied taxa (Fig. 2).

DISCUSSION

It is known that endophytic [17] and rhizosphere [7] bacteria are effective in inhibiting soil borne pathogens. Microorganisms that can grow in the rizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front-line defense for roots against attack by pathogens [7]. Actinomycetes are quantitatively and qualitatively important in the rizosphere, where they may influence plant growth and protect plant roots against invasion by root pathogenic fungi. It is reported that rhizosphere associated soils yielded almost twice as many actinomycetes as non-rhizosphere-associated soils [21]. Moreover, culture-undependent methods can increase the number of isolated endophytic actinobacterial isolates in the wheat roots [31]. The use of endophytic actinomycetes as biological control agents of soil-borne root disease is of interest through their ability to colonize healthy plant tissue and produce antibiotics *in situ* [32].

The *in vitro* antagonism tests clearly show that (Table 1) tested actinomycete isolates which were obtained wheat rhizosphere are strong antagonists for root rot fungi. Activities of the tested isolates were very impressive especially against *F. culmorum* and *F. graminearum* which were reported as predominant for root rot diseases [11]. The data obtained from the *in vitro* antagonism tests show that only one isolate, designated 129.01, had a very strong antagonistic activity to all tested fungal pathogens. All fungi were inhibited by isolate 129.01 at least +++ level (see Table 1 for definition) after 5 days of incubation. But, the hyphae of all tested fungi taken from the colony edge in the periphery of the actinomycete colonies were still culturable in fresh media (Table 1). The growth of the pathogens in fresh media was indicative of the fungistatic activity of the isolate 129.01.

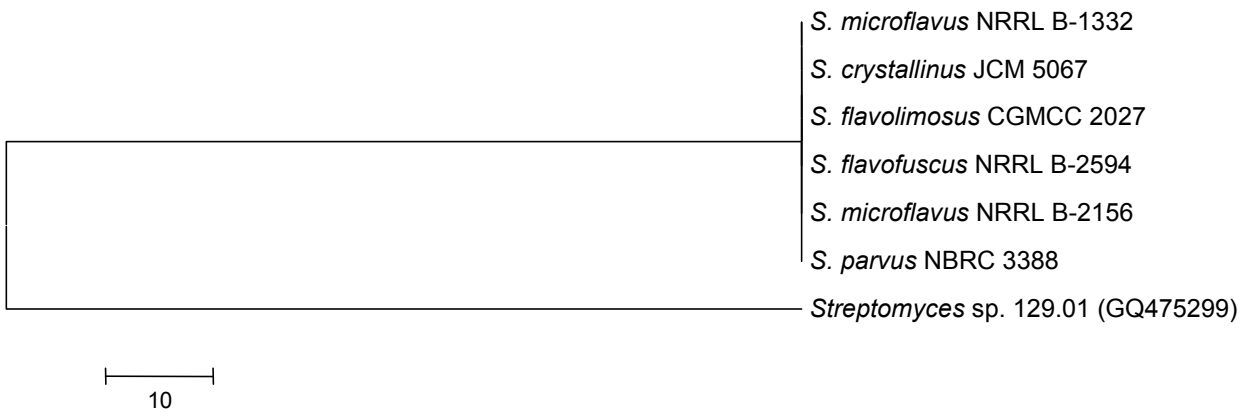

FIGURE 2 - Phylogenetic tree for 16S rRNA gene sequences of the isolate 129.01 and representatives of the *Streptomyces* available in the database.

The antagonistic activity of *Streptomyces* to fungal pathogens is usually related to the production of antifungal compounds and extracellular hydrolytic enzymes [33]. Extracellular hydrolytic enzymes such as chitinase and β-1,3-glucanase are able to lyse fungal cell walls and cause abnormal hyphal morphology [34, 35]. For example, extracellular chitinases produced by *Streptomyces halstedii* AJ-7 [36]. *Streptomyces hygroscopicus* SRA 14 [33] and *Pseudomonas aeruginosa* [37] cause abnormal hyphal morphology including growth aberration, hyphal swelling, distortion and cytoplasm aggregation. In the present study, fungal colonies were examined microscopically at x 10 and x 40 magnifications. There was no abnormal morphology at these magnifications from those of the control treatments. Therefore, it can be asserted that isolate 129.01 do not produce enzymes that can lyse cell walls of the fungi such as chitinase and β-1,3-glucanase. We confirmed this argument with incubation of isolate 129.01 on colloidal chitin agar. There is no visible clear zone around the colony on this medium during an incubation period. From the results, it can be concluded that the fungistatic effect of the isolate 129.01 is not dependent on a hydrolytic enzyme. In this context we can argue that isolate 129.01 excretes a fungistatic secondary metabolite such as antibiotics, toxins, or volatile organic compounds. Therefore, antagonist isolate 129.01 should be alive and should excrete its fungistatic secondary metabolite to inhibit these fungi. Yuan and Crawford [26] also did not observe any abnormal morphology with *S. lydicus* WYEC 108 isolate which is later commercialized. They also conclude that the inhibition of fungal growth by WYEC 108 was due mainly to produced diffusible antifungal compounds.

In media pH studies, pH values of the medium were not significantly changed by actinomycetes isolates ($P > 0.05$). The media pH values of isolates 03.03, 26.07, 97.03, 129.01 and control plates were 7.1, 6.7, 5.5, 6.5 and 7.0, respectively. It can be argued that these media pHs were not changed dramatically by the antagonist isolates. Therefore, we can conclude that inhibition of tested fungus isolates growth were not dependent on the changing of the media pH. It means that inhibition of the growth was caused by the antifungal metabolites of the antagonist isolate.

Contemporaneous inoculation of antagonists and fungi did not give a strong inhibition rate in all treatments, because of a relatively lower growth rate of actinomycetes than most of the fungi on agar plates [26]. In the case of 48 h preinoculation of antagonist, the inhibition rate was more pronounced. Thus, the inhibition rate of the 5th day of paired incubation was determined at higher than the 2nd day. Because of the higher inhibition rate of the 5th day of paired incubation, and normal micromorphology of the fungal colony, it can be claimed that fungistatic effect of the isolate 129.01 depends on a secondary metabolite production not on the hydrolytic enzyme.

To recover isolate 129.01 from the vernalized wheat seeds and to confirm its viability, a PDA medium was used. Colonies of antagonist isolate were counted a comparison of colonies from that of pure stock culture. The spore density of the antagonist isolate 129.01 was determined as 2.2×10^4 spore / seed after the vernalization period. This confirms that seed coating can be used as an effective method for colonization of the antagonists on seeds. In fact, seed coating was also reported as the most effective treatment by El-Abyad et al. [18].

In vivo plant tests are essential to verify the effectiveness of potential antagonists. *In vitro* and *in vivo* biocontrol studies often do not correlate with each other. In the present study, the *in vivo* biocontrol activity of the isolate 129.01 was related to *in vitro* antagonism trends. Studies carried out under greenhouse conditions indicate that selected isolate 129.01 inhibited all tested fungal pathogens except *B. sorokiniana* (Fig. 1). The *F. culmorum* was especially strongly inhibited by the antagonist. Root rot score of this fungal pathogen was distinctly decreased and the weight of the green part of the plant was increased 2.43% by the antagonist (Fig 1). On the other hand, the increase in plant weight reached 22.22% for *F. graminearum*.

Antagonist actinomycete isolates, in addition to their capacity to reduce disease severity, may cause an increase in the fresh weight of plants [38]. This increase may depend on production of bacterial metabolites responsible for plant growth promotion. El-Tarabily et al. [39] reported that endophytic actinomycetes can be used for the biological control of *Pythium aphanidermatum,* and cause growth promotion of cucumber. Thus, actinomycete antibiotics may play an important role in the biocontrol of pathogens and contribute to the enhangement of plant growth [40, 41]. In our study, antagonist isolate 129.01 also causes weight increase when compared with the control (Fig 1).

According to the 16S rRNA analysis, a high similarity value of 1344 bp (higher than 98%) has been observed in 129.01 (accession number: GQ475299) in 16S rRNA gene sequences with *S. microflavus* NRRL B-1332, *S. crystallinus* JCM 5067, *S. flavolimosus* CGMCC 2027, *S. flavofuscus* NRRL B-2594, *S. microflavus* NRRL B-2156 and *S. parvus* NBRC 3388. Among them, only a *S. microflavus* strain was found to have any potent antagonistic activity against a root rot pathogen, *Rhizoctonia solani* [42].

Although some bioactive substances produced by actinomycetes inhibit the root growth of plants [43], in previous studies, *Streptomyces* spp. have been tested as potential biocontrol agents against fungal phytopathogens such as *Pythium ultimum* [21, 26], *Fusarium* spp. [23, 26, 32], *Rhizoctonia solani* [26], *Diaporthe* spp., *Phomopsis* spp. [44], *Curvularia eragrostides* and *Colletotrichum gloeosporioides* [45]. Mycostop (Kemira Agro Oy, Helsinki, Finland), is a commercial formulation of *Streptomyces griseoviridis* that is available in Europe and North America as a wettable powder against soilborne fungal pathogens [24]. If this commercial product colonizes the rhizosphere prior to fungal pathogen, it can control or suppress some root rot and wilt diseases caused by *Pythium*

spp., *Fusarium* spp., *Rhizoctonia* spp. and *Phytophthora* spp. [25]. Similarly *Streptomyces lydicus* isolate WYEC 108 is commercialized to control plant pathogens as a product called Actino-Iron®. Nevertheless, research on the formulation of actinomycetous bacteria as commercial biofungicides or bioinsecticides is limited.

Until the current report, no attempt has been made to select antagonistic actinomycete isolate from Turkey. The present study has demonstrated the *in vivo* potential of *Streptomyces* isolate 129.01 in the biocontrol of root rot fungi especially *Fusarium* spp. The report shows that antagonist isolate 129.01 may be useful in protecting susceptible wheat cultivar from several root rot fungi. The results seem promising in developing a new biocontrol agent for root rot fungi. However, *Streptomyces* isolate 129.01 should be further tested in longer-term field trials in natural conditions, to develop effective formulation type(s), to investigate for food safety, and to purify and characterize secondary metabolites produced by the isolate 129.01 before the commercialization process. Future work will be focused on isolating, purifying, and identifying this metabolite and to determine its effects on the plant pathogen fungi.

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CORRESPONDING AUTHOR

Mustafa Yamac
Eskişehir Osmangazi University,
Faculty of Arts and Science
Department of Biology,
26480 Meşelik, Eskişehir
TURKEY

Phone : + 90. 222. 2393750
Fax: + 90. 222. 2393578
E-mail: myamac@ogu.edu.tr, myamac@gmail.com