

SOME PLANT GROWTH PROMOTING TRAITS OF *STREPTOMYCES* SPECIES ISOLATED FROM VARIOUS CROP RHIZOSPHERES WITH HIGH ROOT COLONIZATION ABILITY OF SPINACH (*SPINACIA OLERACEA* L.)

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(Received 7th Feb 2021; accepted 14th May 2021)

Abstract. *Streptomyces* species a genus in *Actinobacteria* phylum were isolated from the rhizosphere of some plants in Los Angeles. Some plant growth promotion properties of isolates were determined, such as: indole acetic acid, siderophore production and phosphate solubilization. The colonization ability of *Streptomyces* species was investigated under in vitro conditions on spinach seeds. Maximum likelihood approach was applied to 16S rRNA gene sequences to draw a phylogenetic tree of the best identified isolates, according to their measured properties. A total of ten isolates were characterized as *Actinobacteria* according to their morphological properties. Of those, four isolates possessing some traits related to plant growth promotion were identified as *Streptomyces griseus* strain 52-1 (Step1) and *Streptomyces albogriseolus* strain DSM 4 (Step-2) from tomatoes, *Streptomyces aurantiacus* strain 4683 (Step-5) and *Streptomyces kanamyceticus* strain 22-5 (Step-8) from sunflowers' rhizosphere. The identified isolates showed the maximum indole acetic acid production, 95.67, 110.14, 94.12 and 105.04 mg L⁻¹, respectively. The maximum siderophore production (22.23, 19.34, 10.12 and 33.24 mg mL⁻¹) and mineral phosphate solubilization (61.8, 69.32, 61.6 and 68.4 mg 100 mL⁻¹) were obtained using Strep-1, Strep-2, Strep-5 and Strep-8 isolates, respectively. None of the isolates could grow on nitrogen free media. The results of spinach root colonization test with the isolates indicated the potential of Strep-1, Strep-2, Strep-5 and Strep-8 isolates to colonize spinach roots.

Keywords: *Actinobacteria, auxin, fertilizer, phosphate solubilization, siderophore*

Introduction

In the recent decades, the negative impacts of chemical fertilizers on the environment have resulted in a search for new ways to increase productivity in the agricultural sector while decreasing the eradication of natural resources. One way to address this issue is taking advantage of the vast variety of beneficial microorganisms that are already present in soil and rhizosphere of plants. They can be applied as bio-fertilizers rather than using chemical fertilizers to promote plant growth (Chandini et al., 2019). Plant growth promoting rhizobacteria (PGPR) have various modes of actions such as altering plant hormones, secretion of organic compounds, increasing abiotic stress tolerance in plants, and enhancing nutrient availability and uptake (Wang et al., 2020). Based on the

need for desirable PGP (plant growth promoting) traits, different rhizobacteria could be selected to be applied as bio-fertilizers. The most common desirable traits that we are looking for in PGPR include the production of auxin and siderophores, solubilization of phosphate and nitrogen fixation. Vast majorities of PGPR have been identified and exploited so far (Lamizadeh et al., 2016; Wang et al., 2020; Pourjasem et al., 2020). *Actinomycetes* comprise 10 to 50% of the soil microflora (Olanrewaju and Babalola, 2019) and produce a wide range of effective compounds. The researchers have documented them due to secondary metabolites produced as antifungal, insecticidal, antibacterial and anthelmintic compounds (Tyc et al., 2017).

Streptomyces is the largest genus of *Actinobacteria* in soil and widely recognized due to its ability to produce antibiotics and its biocontrol and pharmaceutical exploitation (Quinn et al., 2020; Newitt et al., 2019; Kumari et al., 2017; Franco-Correa et al., 2010). However, there has not been proper attention paid to the agricultural aspect of this genus, in regards to its use as biofertilizer and PGP traits, whereas there has been more focus on the biocontrol activity of this genus (Hamedi and Mohammadipanah, 2015; Adegboye and Babalola, 2012; Tyc et al., 2017).

Streptomyces effectively colonize the rhizoplane and rhizosphere. Due to their endophytic ability, they are capable of controlling gene expression and impacting the production of siderophores, phytohormones and some other traits (de Jesus Sousa and Olivares, 2016). Plant growth promoting traits by *Streptomyces* have been reported in various studies (Suarez-Moreno et al., 2019). *Streptomyces* have their own filaments which enable them to absorb nutrients easily in the soil and rhizosphere (Liu et al., 2019) and through degradation of biological polymer or dissolution of mineral compounds they can provide nutrients for plants (Khamna et al., 2009). High production of indole acetic acid (IAA) (136 mg/l) has been recorded for *Streptomyces* mhcr0816 (Ghosh et al., 2013) which was comparable to the reported values of standard strains *Rhizobium* sp. and *Bacillus* sp. with the amount of 142 and 55 mg/l, respectively. Chouyia et al. (2020) reported *Streptomyces roseocinereus* as an isolate capable of solubilization of phosphate through secretion of malic acid. Also, production of secondary metabolite such as siderophore by *Streptomyces griseus* has been studied by Fiebig et al. (2018). *Streptomyces*, being useful for functions such as biocontrol, biofertilizers, and volatile compound secretion is an excellent candidate for investigation (Jones and Elliot, 2017). Therefore, finding a good PGP *Streptomyces* (PGPS) and choosing the best root colonizer is the ultimate goal. The aim of this study was to isolate *Streptomyces* from the rhizosphere of some different plants and trees at different locations of San Fernando Valley, Los Angeles, and to evaluate some PGP traits of the isolates. The second goal was to investigate the isolates' abilities to colonize spinach (*Spinacia oleracea* L.) roots, as spinach is a highly consumed vegetable with high nutritional values. The best PGPS strains will be considered as potential strains for future field study.

Materials and methods

Isolation of Streptomyces from collected samples

Soil samples were collected from 4 different locations of San Fernando Valley Los Angeles, California (Table 1). The soil samples were gathered from the rhizosphere along with the roots from fields of tomatoes, sunflowers and orange trees. The samples were transferred to the laboratory and kept in a refrigerator at 4 °C until the analysis started.

Table 1. Coordination and location of sampling for each identified isolate as *Streptomyces*

Isolate code	Plant's rhizosphere	Field location	Latitude	Longitude
Strep-1	Tomatoes	West Hills	34°10'54"N	118°34'16"W
Strep-2	Tomatoes	San Fernando	34°16'45"N	118°27'47"W
Strep-3	Cactus	Woodland Hills	34°10'59"N	118°34'14"W
Strep-4	Nectarines	West Hills	34°21'78"N	118°64'04"W
Strep-5	Sunflowers	San Fernando	34°16'40"N	118°27'51"W
Strep-6	Lettuce	San Fernando	34°16'56"N	118°27'38"W
Strep-7	Cactus	Woodland Hills	34°10'51"N	118°34'20"W
Strep-8	Sunflowers	San Fernando	34°16'42"N	118°27'49"W
Strep-9	Nectarines	West hills	34°21'71"N	118°64'16"W
Strep-10	Lettuce	West hills	34°21'61"N	118°64'25"W

Serial dilutions of each sample up to 10^{-5} were made using one gram of soil. Then, 100 μ l of each dilution was spread evenly on plates containing ISP-2 (Yeast Extract-Malt Agar) medium supplemented with streptomycin to avoid the growth of unwanted microorganisms. After 7 days candidate colonies were streaked on ISP-2 media to achieve single colonies for further investigations (Priyadarshini et al., 2016). The carbohydrate utilization of purified *Streptomyces* candidates was determined by growth on carbon utilization medium (ISP-2), Tryptone yeast extract agar (ISP-1) and Starch casein agar (ISP-4). Gram staining and catalase tests were performed on the isolates.

Plant growth promotion traits

Indole-3-acetic acid production

The IAA production ability of the *Streptomyces* candidates was assessed using the colorimetric method (Bano and Musarrat, 2003). The isolates were grown in tryptone yeast extract broth medium containing 2 mg mL⁻¹ L-tryptophan and incubated at 29 °C under shaking at 125 rpm for 7 days. Thereafter cells were collected by centrifugation of inoculum at 11,000 rpm for 15 min. Then 2 ml of Salkowski reagent (2 ml 0.5 M FeCl₃, 98 ml 35% HClO₄) was added to one mL of the collected supernatant and mixed well. The amount of IAA production was calculated after reading the optical density at 535 nm by spectrophotometer (Laxco, Alpha1502. USA).

Phosphate solubilization

The qualitative and quantitative ability of the *Streptomyces* isolates to solubilize phosphate were investigated using methods of Pikovskaya (1948). A spot inoculation of different isolates of *Streptomyces* was applied on solid PVK medium in plates at 3 replications and the plates incubated at 29 °C for 7 days. The plates of PKV without inoculation were used as control.

Creation of clear halo around the colonies was indicated as phosphate solubilization capability of the isolates. For quantitative analysis of phosphate dissolution 2% v/v of 5-day old isolate (10^6 Cfu mL⁻¹) was inoculated in liquid PVK medium containing 1.5 g L⁻¹ of Ca₃(PO₄)₂. After 5 days shaking-incubation of flasks at 28 °C, inside suspensions were centrifuged to remove cells and debris. The phosphorous content of

supernatants was determined by colorimetric method using an ammonium molybdate vanadate reagent and reading absorbance at 470 nm (Jeon et al., 2003) via spectrophotometer (Laxco, Alpha1502. USA).

Siderophore production

The *Streptomyces* candidates were evaluated for quantitative and qualitative siderophore production according to the method of Alexander and Zuberer (1991). The plates containing of Chrome Azurol S agar medium were prepared and inoculated with fresh 5-day culture (10^6 CFU mL⁻¹) of each isolate and incubated for 7 days at 29 °C. The orange and yellow halos around the colonies were considered as positive results for siderophore production test. A siderophore production index was calculated using the ratio of total diameter of the colony plus the halo divided by colony diameter. The quantitative siderophore production was determined by inoculating ISP-2 broth media with 5-day old *Streptomyces* isolates and incubation the inoculated flasks at 29 °C under constant shaking condition (120 rpm) for 5 days. Afterwards, suspensions were centrifuged (11,000 rpm for 14 min) and the amount of produced siderophore was measured following assays of CAS-Shuttle (Milagres et al., 1999). In which 0.5 mL of supernatant was completely mixed with 0.5 mL CAS reagent then absorbance was read at 630 nm using a spectrophotometer (Laxco, Alpha1502. USA).

Nitrogen fixation

Nitrogen fixation ability of the isolates was determined using nitrogen free medium through acetylene reduction assay (ARA) and gas chromatography (Hardy et al., 1968).

In-vitro spinach seed colonization test

The ability of *Streptomyces* isolates to colonize spinach seeds was investigated by the described method of Bulgarelli et al. (2012). For this purpose, Spinach seeds, which were donated by Sakata Seeds America INC, were sterilized by submerging the seeds in 2% sodium hypochlorite solution for 5 min then the seeds were rinsed by sterile water up to six times. The tubes (25×150 mm diameter) were filled with sterilized mixture of soil peat and sand (3:1) and a seed placed at depth of 2 cm of soil surface inside the tubes. The experiment was performed as completely randomized design at three replications including 4 isolates. Seedlings with root system were carefully removed from the tubes. About 1 mL of broth culture (5-day old) of each *Streptomyces* isolate with 10^6 Cfu mL⁻¹ was inoculated on seed. Tubes were kept in growth chamber at 25 °C, 70% humidity and 12 h photo period for 20 days. In order to supply water, the tubes were irrigated with sterilized distilled water. The roots were separated from seedlings and gently washed by sterile water, then air-dried and weighed. The roots were cut and vortexed after soaking for 1 h in sterile solution of 0.9% NaCl. Serial dilutions of soaked roots were prepared and 100 µl of suspension was cultured on ISP-4 solid media for colony counting. After 4 days the population of bacteria were enumerated and recorded as CFU g⁻¹ of root weight.

PCR amplification and DNA sequencing

Colony PCR (Hou et al., 2016) was applied for molecular identification of 4 selected *Streptomyces* isolates (having best plant growth promoting traits). Fresh cultures of each

isolate were prepared by re-culturing isolates on Starch Casein Agar plates and incubating at 29 °C for 4 days until the colonies reached a diameter of 2 to 3 mm. Colonies were picked up from the plate using a sterile pipette tip and re-suspended in Master Mix (New England Bio labs, USA) solution. PCR amplification was performed using Master Mix and 0.4 µM primer fD1 (5'-GAGTTTGATCCTGGCTCA-3') and Rp2 (5'-CGGCTACCTTGTTACGACTT-3') with final volume of 25 µl in thermal cycler (MJ Research PTC-200, USA) with a program as: 94°C for 5 min as a primary denaturation step, 30 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 105 s and final extension of 72°C for 10 min. The PCR products were closely visualized using gel electrophoresis on 0.8% agarose and compared with 1 kb DNA ladder (New England Bio labs, USA). The PCR products were purified using PCR purification kit (Monarch. USA) according to the manufacturer's instruction then were sequenced by MR DNA Molecular Research, (TX, USA). The products were sequenced and the results were compared with other sequences via BLAST software and the results deposited in NCBI GenBank as 16S rDNA gene of different *Streptomyces* species. Distance matrix was assessed using the Maximum Composite Likelihood approach based on the Kimura 2-parameter model (Kimura, 1980) and phylogenetic tree was drawn using the MGA program (version 6) by bootstrap analysis of 1000 replications.

Statistical analysis

Analyses were done using SAS software, version 9.4. All comparison tests were based on completely randomized design with three replications, submitting to ANOVA and Tukey grouping was done for comparison means at a significant level of $\alpha = 0.05$. Data for root colonization were log-transformed. The Pearson correlations between the P dissolution and siderophore production and pH of each medium were determined using SPSS 16.0 software.

Results

A total of 30 isolates from 6 different plants were isolated. However, 10 isolates with colony similarity to *Streptomyces* standard colony were selected for further analysis. Colonies appearance have been documented in *Table 2*, which were hard with chalky texture and wrinkly appearance (*Fig. 1*). They were gram positive and showed positive results to catalase test. All *Streptomyces* isolates were able to grow and utilize different source of carbon (*Table 2*).

Table 2. The colony color and some biochemical characteristic of isolates and accession number of identified isolates

Isolate code	Colony color	Catalase	C-utilization	Accession number	Species
Strep-1	Yellow	+	+	MK483345	<i>Streptomyces griseus</i> strain 52-1
Strep-2	Gray	+	+	MK483346	<i>Streptomyces albogriseolus</i> strain DSM 4
Strep-3	Light brown	+	+		
Strep-4	Brown	+	+		
Strep-5	Light yellow	+	+	MK483347	<i>Streptomyces aurantiacus</i> strain 4683
Strep-6	Light gray	+	+		
Strep-7	Orange	+	+		
Strep-8	White	+	+	MK490964	<i>Streptomyces kanamyceticus</i> strain 22-5
Strep-9	Gray	+	+		
Strep-10	Yellow	+	+		



Figure 1. Colony morphology of the isolate Strep-2 on Casein yeast extract agar

All the *Streptomyces* isolates were able to produce IAA. Observing different intensity of pink color production by the candidates showed the presence of various amount of IAA (34.69 to $110.14 \mu\text{g mL}^{-1}$) (Table 3). Amount of IAA production by different *Streptomyces* candidates was significantly different by Tukey mean comparison ($p < 0.05$). The maximum amount of auxin production belonged to the isolate of strep-2 which was from the tomato rhizosphere and the least amount belonged to Strep-7 from the cactus rhizosphere.

Table 3. Phosphate dissolution, siderophore production and pH of broth medium inoculated with rhizospheric *Streptomyces* isolates at in-vitro conditions.

Isolate code	SI ^a	P (mg 100 mL ⁻¹)	pH ^a	SI ^b	Siderophore (mg L ⁻¹)	pH ^b	IAA (mg L ⁻¹)
Strep-1	1.23 ± 0.02	61.8 ± 0.43 ^c	5.5	1.99 ± 0.02	22.23 ± 0.23 ^b	6.6	95.67 ± 0.8 ^c
Strep-2	1.82 ± 0.04	69.32 ± 0.34 ^a	5.1	1.84 ± 0.05	19.34 ± 0.34 ^d	6.7	110.14 ± 0.9 ^a
Strep-3	0.98 ± 0.02	41.53 ± 1.2 ^e	5.5	1.13 ± 0.03	11.31 ± 0.44 ^h	7.0	41.65 ± 0.34 ^g
Strep-4	1.61 ± 0.03	66.3 ± 0.20 ^b	5.4	1.81 ± 0.04	20.3 ± 0.16 ^c	6.6	40.04 ± 0.25 ^h
Strep-5	1.36 ± 0.02	61.6 ± 0.33 ^c	5.4	1.16 ± 0.02	10.12 ± 0.33 ⁱ	7.0	94.12 ± 1.2 ^d
Strep-6	1.27 ± 0.03	59.2 ± 0.24 ^d	5.5	1.26 ± 0.04	16.45 ± 0.19 ^g	6.9	63.93 ± 1.3 ^e
Strep-7	0.88 ± 0.02	39.23 ± 0.65 ^{ef}	5.8	1.44 ± 0.05	17.98 ± 0.45 ^e	6.9	34.67 ± 0.87 ⁱ
Strep-8	1.74 ± 0.04	68.40 ± 0.33 ^a	5.3	2.14 ± 0.06	33.24 ± 0.41 ^a	6.5	105.04 ± 0.98 ^b
Strep-9	0.78 ± 0.04	30.66 ± 0.11 ^g	5.8	1.35 ± 0.01	17.01 ± 0.23 ^f	6.8	41.01 ± 0.87 ^h
Strep-10	0.94 ± 0.05	40.87 ± 0.43 ^{ef}	5.7	1.66 ± 0.07	18.56 ± 0.46 ^e	6.8	52.64 ± 1.32 ^f

Data are means ± standard deviation, n = 3. Columns with the same letter were not significantly different based on Tukey Multiple Test ($\alpha = 0.05$). SI, Solubilization index: (diameter of colony + halo zone)/colony diameter, ^a & ^b the measured SI and pH of media related to the phosphate solubilisation and siderophore production tests, respectively

Among the isolates, six isolates having Solubilization Index (SI) greater than one were able to solubilize tri-calcium phosphate, as exhibited by the appearance of a clear halo around the colonies (Fig. 2). The highest calculated phosphate dissolution index of 1.82 was obtained by isolate Step-2. Quantitative analysis of phosphorus release to the broth medium indicated the maximum ability of isolate Step-2 to dissolve tri-calcium phosphate (Table 3).



Figure 2. Halo around the bacterial colony indicates phosphate solubilization by isolate Strep-2 on PVK medium with Phosphate Solubilization index (SI) of 1.82

There was not a significant difference between Strep-2 and Strep-8 by Tukey test, so both isolates were considered as high phosphate solubilizers. The pH of the culture medium of isolates changed from 6.0 to 5.1. The minimum pH belonged to the inoculated culture medium with the isolate Strep-2 (pH 5.1). There was a negative and significant correlation ($r = -0.88^{**}$) between pH and P dissolution by isolates.

The isolates were able to produce siderophores, as exhibited by the creation of a yellow-orange halo around the colonies (Fig. 3). The maximum index of siderophore production of 2.1 was obtained by isolate Strep-8 from the sunflower's rhizosphere. The highest amount of siderophore production was 33.24 mg mL^{-1} belonged to Strep-8 which was significantly different ($p < 0.05$) among the isolates (Table 3). The highest siderophore content was measured at pH 6.5 and the least content in pH 7.0, but there was not significant correlation between pH of medium and siderophore content.

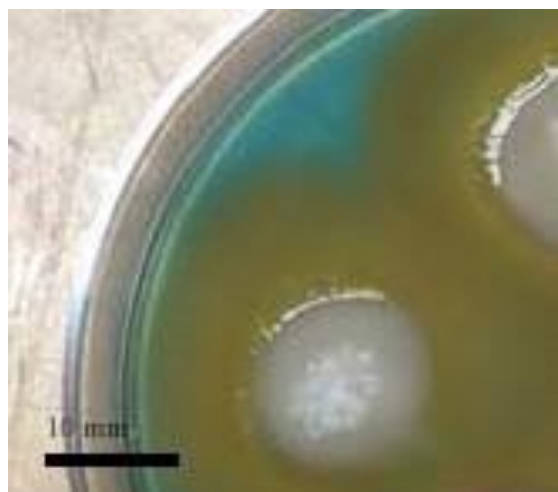


Figure 3. The yellow halo around the bacterial colony indicates siderophore production by isolate Strep-2 on CAS agar with Siderophore production index (SI) 1.84

Streptomyces isolates were not able to grow on nitrogen free medium; therefore, the test did not indicate nitrogen fixing abilities in the isolates.

The ability to colonize spinach roots by *Streptomyces* varied among the isolates, with significant difference shown by Tukey's test (Fig. 4). The isolates of Strep-2, Strep-1 and Strep-8 from tomatoes and sunflower's rhizosphere indicated maximum root colonization with population numbers of 8.4×10^7 , 2.4×10^6 and 6.4×10^6 CFU g⁻¹ fresh weight of root tissue, respectively. The least root colonization ability was shown by Strep-9 from the nectarine tree's rhizosphere with a population number of only 5.3×10^3 CFU g⁻¹ fresh weight of root tissue.

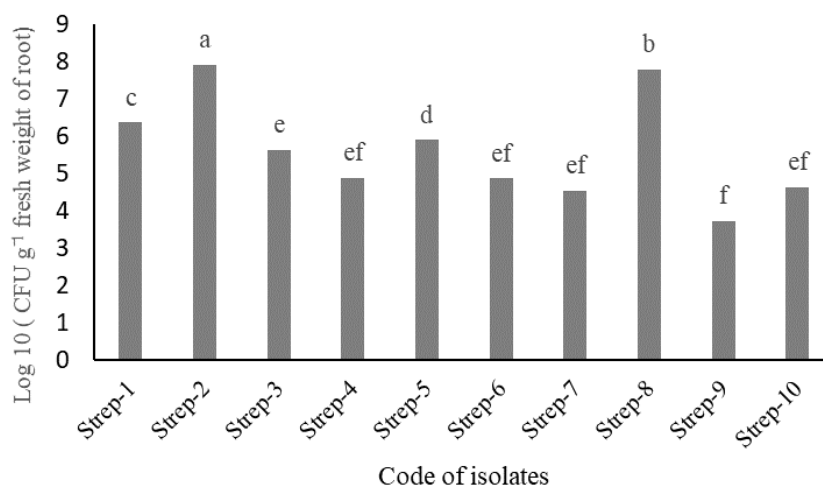


Figure 4. The population number of *Streptomyces* isolates in each treatment indicate the potential of isolates to colonize spinach root. Columns followed by the same letter are not significantly different according to the Tukey test at 5% probability level ($n = 3$)

The sequences of 16S rRNA gene of Strep-1, Strep-2, Strep-5 and Strep-8 isolates having the maximum PGP traits were analyzed through comparison with sequences in GenBank via Nucleotide BLAST on the NCBI website. After comparison, the isolates Strep-1, Strep-2, Strep-5 and Strep-8 showed similarity to *Streptomyces griseus*, *Streptomyces albogriseolus*, *Streptomyces aurantiacus* and *Streptomyces kanamyceticus* with accession number of MK483345, MK483346, MK483347 and MK490964, respectively (Table 2). The tree with the highest log likelihood (-502.05) in Figure 5 indicated that isolates belonged to *Streptomycetaceae*.

Discussion

In this study, 10 *Streptomyces* isolates were identified from rhizospheric soil of different plants and trees. All of the isolates were investigated for some different plant growth promoting traits, and some of them represented better PGP traits under in-vitro conditions. The *Streptomyces* isolates were able to solubilize tri-calcium phosphate. This was probably by the lowering of pH through organic acid secretion or because of polysaccharide production (Elnahas et al., 2017). Chouyia et al. (2020) reported phosphate solubilization (207.9 mg L^{-1}) by *S. natalensis* through decreasing pH in broth medium from 7 to 6. Similarly, Chaiharn, et al. (2018) reported 290.35 mg L^{-1} phosphate dissolution by *Streptomyces* isolates through decreasing pH from 7 to 4.5 in

the media. Our observation indicated pH reduction in broth media after incubation from pH 6.9 to 5.1 and 6.1 for different isolates. The isolates were able to produce IAA and siderophore which are the other two important factors for plant growth promotion. Siderophores are a low mass chelate made from peptides which form a complex of Fe^{3+} -siderophore for plant uptake under iron deficiency conditions (Vessey, 2003). It has been reported that *Streptomyces* sp. GMKU3100, which is an endophytic bacterium, was capable of producing siderophores and promote the growth and yield of rice (Sadeghi et al., 2012). The amount of produced siderophore by the isolates varied among the *Streptomyces* isolates. Isolate Strep-8, which was from the sunflower's rhizosphere, created the biggest halo zone related to siderophore production, with 33.24 mg L^{-1} production in broth media. This is the same amount as *Actinobacter* strain *mhcr0816* in the study of Elnahas et al. (2017). The reported siderophore and IAA production by *S. roseocinereus* MS1B15 (14.09 ± 1.10 and $6.34 \pm 0.33 \text{ mg/L}$), respectively were comparably lower than the measured amount of those by our studied strains (Suarez-Moreno et al., 2019). All studied isolates grown on tryptophan supplemented ISP-2 media, produced IAA in the range of 37 and 110.4 mg L^{-1} . IAA is a high functional plant-growth hormone for increasing seed germination, hairy roots, root branching and elongation (Farina et al., 2012). Abd-Alla et al. (2013) and Myo et al. (2019) reported IAA production by *Streptomyces* sp. CMU-MH021 ($28.5 \text{ } \mu\text{g mL}^{-1}$) and *Streptomyces* *fradia* ($82.36 \text{ } \mu\text{g mL}^{-1}$). Results of the nitrogen fixation test revealed *Streptomyces* isolates were not able to grow on nitrogen free media. There were several reports which indicated disability of most *Streptomyces* species to fix molecular nitrogen except for *Streptomyces thermoautotrophicus* such that it is still controversial among some scientists to come to an agreement about the ability of this special species to fix nitrogen (Dahal et al., 2017; Zhao et al., 2006). It is a fact that roots of different crop varieties or species might produce different types of exudates, which could support and absorb different kinds of microorganisms with various plant growth promotion traits. Some proteins and chemical substances that are being secreted in the rhizosphere of different plants are crucial for root colonization by *Streptomyces*.

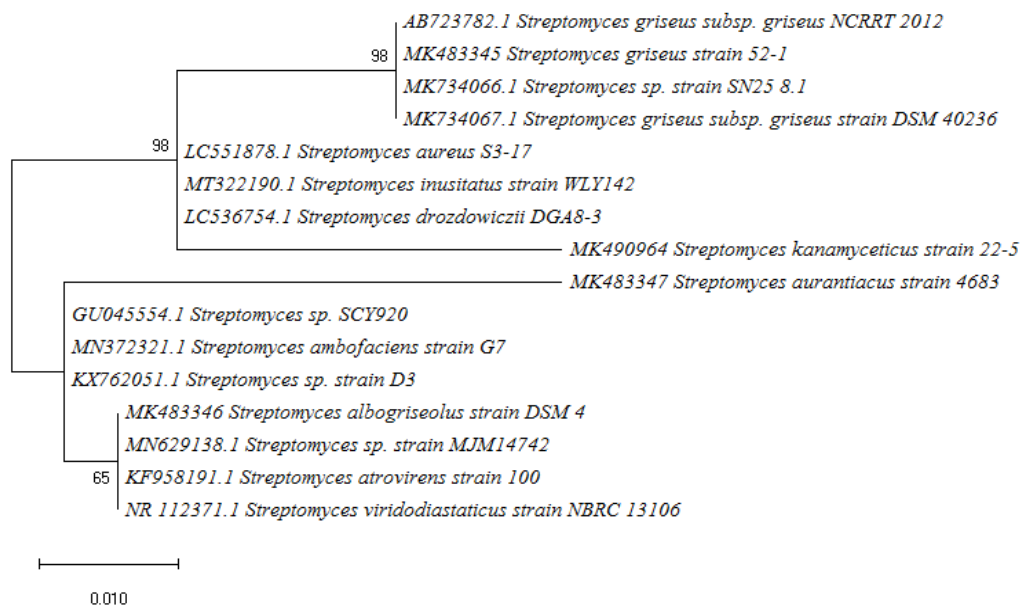


Figure 5. Phylogenetic tree of identified isolates based on 16S rRNA gene sequences.

Kourenblum et al. (2020) reported the effect of rhizosphere and root exudates on the proportion of the microbial community close to the roots. Specific microbes thrive in the plant rhizosphere based on the chemical types of root exudates (Zhalnina et al., 2018). In vitro assessment of the *Streptomyces* isolates on spinach root colonization demonstrated the ability of these strains to colonize spinach roots with significant difference ($p < 0.05$). In our study, the *Streptomyces* isolates from the cactus and nectarine rhizosphere showed lesser root colonization compared to the isolates from the tomato and sunflower rhizosphere, which shows the colonization might depend on the composition of root exudates. Acyl sugar metabolites have been represented as one of the main chemical root exudates in tomato root exudation, which correlated with Bacillales order (Kuorenblum et al., 2020). The higher root colonization by *Streptomyces albogriseolus* and *Streptomyces kanamyceticus* also with higher plant growth promoting traits from the rhizosphere of tomato and sunflower, may indicate the early influence of these isolates on spinach growth.

Conclusion

In sustainable agriculture, it is necessary to look for environmentally friendly ways to increase yield production without damaging the soil and water. Damage can result due to the application of different chemical fertilizers. Using bio-fertilizers as an alternative to chemical fertilizer should be investigated further in sustainable agriculture. *Streptomyces*, with all the evidence supporting its beneficial traits, can be considered as a bio-fertilizer. Up until now, it has been seldom applied as an inoculant to increase yield in agricultural products, despite the excellent potential shown in a vast number of scientific publications. We have found some beneficial *Streptomyces* strains with the ability to colonize spinach roots, and also with the ability of siderophore and IAA production, as well as phosphate dissolution from the San Fernando Valley, Los Angeles. These species are being maintained for future potential research in the field. The plant growth promotion and root colonization ability by strains *S. albogriseolus* and *S. kanamyceticus* suggest that these strains could be applied to plant growth promotion in spinach. More experiments are necessary to further verify the effects on spinach plants under natural growth conditions which is the goal of next study by the authors.

Acknowledgements. The authors appreciate the support of Deputy of Research and Technology of Agricultural Science and Natural Resources University of Khuzestan, Mollasani, Iran. Authors would like to Thank Agriculture and Life Science Departments of Pierce College for providing facilities and support. Thank you to Sakata Seeds. Special thanks to Karin Steinhauer and Amgen Biotech Experience.

Conflict of interests. The authors have declared no conflict of interests.

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