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CHARACTERISTIC OF SELECTED SOIL STREPTOMYCETES WITH ANTIMICROBIAL POTENTIAL AGAINST PHYTOPATHOGENIC MICROORGANISMS

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ABSTRACT

The main objective of the present study was isolation and identification of soil streptomycetes, having antibacterial and antifungal activity against 8 selected phytopathogenic microorganisms, which attack crops in Slovakia. Out of 27 morphologically different streptomycete isolates, 14 of them demonstrated antimicrobial activity against at least two tested microorganisms in primary screening. Ethylacetate extract used for secondary screening showed different inhibitory pattern in comparison with primary screening. The tested isolates were mostly active against Gram-positive bacterium *Clavibacter michiganensis subps. sepedonicus*, the causal agent of bacterial canker of tomaten and against fungus *Fusarium poae*, pathogen of cereals, which can also infest stored grains. Only three isolates, namely VY59, VY87 and VY47 showed broad spectrum of inhibition activity and therefore were used for further identification. On the basis of various morphological (color of aerial and substrate mycelium, growth, production of pigments and melanin on ISP media), physiological (optimal pH, temperature, NaCl tolerance and C-utilization) and biochemical (ApiZym and ApiCoryne stripes) methods these strains belonged to the genus *Streptomyces*. In addition to the antimicrobial profile, the strains differed in API ZYM test results, which imply that the selected strains might produce different antimicrobial substances. Throught the comparative analysis of 16S rRNA gene, the most active isolates contained different nucleotide sequences for the 16S rRNA gene. Sequence similarity search by BLAST program revealed that they show sequence similarities to *Streptomyces somaliensis* (VY59), *Streptomyces* sp. (VY87) and *Streptomyces albidoflavus* (VY47). These three isolates with broader spectrum of antimicrobial activity can be used in the development of substances for agriculture purposes.

Keywords: Soil, *Streptomyces* sp., primary and secondary screening, phytopathogenic microorganisms, characterization

INTRODUCTION

The growing human population will call for a significant increase in agricultural production. This challenge is made more difficult by the fact that changes in the environmental conditions under which crops are grown have resulted in the appearance of plant diseases **(Boyd *et al.*, 2013)**. Losses in crop production due to plant disease average 13% worldwide and severely limit production, quality, and safety food **(Oskay, 2009).** The application of fungicides and chemicals can control crop diseases to a certain extent, however, it is expensive and public concern for the environment has led to alternative methods of disease control to be sought, including the use of microorganisms as biological control agents **(Dhanasekaran *et al.*, 2012).** Because of this problem, many researchers are working on isolating actinomycetes which have the ability to degrade these harmful chemicals and also those with ability to act as bio control agents **(Prabhakar *et al.*, 2014).** Actinomycetes are the group of gram positive filamentous bacteria which are ubiquitous various natural and man-made environments. Actinomycetes are the most economically valuable prokaryotes **(Balagurunathan and Radhakrishnan, 2007)** producing antibiotics of agricultural and medical importance (**Tanaka and Omura, 1993).** It is well known that actinomycetes produce 70% to 80% of bioactive secondary metabolites, where approximately 60% of antibiotics development for agricultural use **(Ilic *et al.*, 2007).** Among the genera of actinomycetes, the genus *Streptomyces* is represented in nature by the largest number of species and varieties, which differ greatly in their morphology, physiology and biochemical activities **(Suneetha and Zaved, 2011).** Interestingly, the majority of the antibiotic-producing actinomycetes are found among these species, which led to a growing economic importance for this group of organisms **(Kumar *et al.*, 2012).** Genus *Streptomyces* produces and secrete a wide array of biologically active compounds including antibiotics, hydrolytic enzymes and enzyme inhibitors **(Singh *et al.*, 2006)** enhances soil fertility and have been proved to possess antagonistic activity against wide range of soil-borne plant pathogens **(Aghighi *et al.*, 2004).** Several studies have been focused on identifying biocontrol agents which could be used as an alternative to agrochemicals in plant protection (**Saravanamuthu *et al.*, 2010**).
Therefore, in this study an effort was made to screen soil actinomycetes for inhibition activity against phytopathogenic microorganisms, which can play an important role in plant growth promotion. This study is also an attempt to identify and characterize the most effective isolates by morphological, biochemical, physiological and molecular methods.

MATERIAL AND METHODS

Isolation of streptomycetes

Streptomycetes were isolated from arable soil collected in Východná, Slovakia. Soil sample was air dried for 2 days, crushed, and sieved prior to use for isolation purpose. Twenty seven actinomycete strains were isolated as pure cultures by using standard microbiological methods. An aliquot of 0.1 ml of each soil solution (10^{-1}, 10^{-2}, 10^{-3}) was taken and spread evenly over the surface of streptomycete selection Pochon medium **(Korzeniewska *et al.*, 2009)** complemented with nystatin (50 µg/ml). Plates were incubated at 28 ºC for 7 days. Suitable colonies those showed *Streptomyces* like appearance were recultivated several times for purity on yeast-malt extract medium **(Shirling and Gottlieb, 1966)**. The purified actinomycetes were preserved at -20 ºC in the presence of glycerol (30% v/v) for longer period.

Test organisms

Eight test microorganisms were used for detection of streptomycete antibiotic activity. Test organisms used in this study include Gram-positive bacterium

Clavibacter michiganensis subsp. sepedonicus (CCM 7014), Gram-negative bacteria *Xanthomonas campestris* (CCM 22), *Pseudomonas syringae* (CCM 2868), *Erwinia amylovora* (CCM 1114) from Czek Collection of Microorganisms, and fungi *Alternaria tenuissima* (16A6), *Fusarium poae* (12A18), *Penicillium expansum* (KMH5) and *Aspergillus niger* (KMH12) from Microbial Collection of Department of Microbiology, SUA, Slovakia.

Antimicrobial activity of pure cultures

Primary screening

Preliminary screening for antibiotic activity of the isolates was done by using agar plug method on Sabouraud agar (SigmaAldrich, USA) (fungi), tryptic soy agar (Sigma Aldrich, USA) (Gram-negative bacteria) and glucose yeast extract agar agar (Sigma Aldrich, USA) (Gram-positive bacteria). Agar discs were prepared using a sterile cork borer from well grown actinomycetes culture and placed on fresh lawn culture of test microorganisms. Plates were incubated at 25 ºC for fungi and 30 ºC for bacteria. The zones of inhibition were determined after 2-3 days (fungi) or after 1-2 days for bacteria.

Secondary screening

Active isolates in primary screening were subjected to secondary screening using well-dillution method with actinomycete extracts. For preparation of extracts we used liquid cultivation of actinomycetes in medium supporting metabolite production (starch-15g, yeast extract-4g, K2HPO4-1,0g, MgSO4.7H2O-0,5g, destilled water-1000ml, pH-7,0). After five days of incubation we mixed 20 ml of culture with 20 ml of ethyl acetate (Sigma Aldrich, USA). After a 12 min shaking step the sample was centrifuged at 9000 rpm for 10 min and the upper phase was transferred into a round bottom flask. At 40°C the ethyl acetate was evaporated in a rotary evaporator (Stuart, UK). Finally, the extract was solved in 1 ml of ethyl acetate: acetone: methanol (1:1:1) and centrifuged at 14000 rpm for 10 min. We added 50µl of extracts to wells bored into freshly inoculated plates.The plates were incubated and zones of inhibition was recorded like above. For negative control we added 50 µl of ethyl acetate: acetone: methanol (1:1:1) solution to the wells.

Phenotypic characterization

Aerial mass color and reverse side pigments

The mature sporulated aerial and substrate mycelium color was recorded in yeast-malt extract agar (ISP2), oat meal agar (ISP3), inorganic salt starch agar (ISP4), glycerol asparagine agar (ISP5), peptone yeast etract iron agar (ISP6) and tyrosine agar (ISP7) **(Shirling and Gottlieb, 1966)**. The colors were described by the RAL-code. Production of melanoid pigments was tested on ISP6 and ISP7 media.

Spore chain morphology

The spore bearing hyphae was determined by direct examination of culture under microscope (OLYMPUS CX22LED, Japan) 1000 x magnification using a well grown sporulated culture plates.

Physiological characterization

Utilization of different carbon sources were studied by the method recommended in International Streptomycete Projects using a microplate technique with twelve well plates. Carbon sources like glucose, mannitol, arabinose, inositol, lactose, mannose, fructose, galactose, rhamnose, sucrose and xylose were tested on the carbon utilization agar (ISP9) **(Shirling and Gottlieb, 1966)** supplemented with 1% carbon sources. Sodium chloride tolerance level of the isolates was evaluated on medium (casein peptone – 10.0 g/L, yeast extract - 5.0 g/L, agar – 20.0 g/L, deionized water – 1000 m) supplemented with graded doses of NaCl (0, 2, 5, 5, 7, 5 and 10% of sodium chloride), maximum NaCl tolerance concretation in the medium allowing any growth was recorded. Sodium chloride tolerance was tested on six-well microtiter plates. Physiological characterization such as the effect of pH (5-9) and temperature (25, 30, 45 and 60 ºC) were also tested.

Biochemical characterization with Api stripes

For biochemical identification we used ApiZym and ApiCoryne systems (BioMérieux, USA). After week of actinomycete inbubation in shaking flasks with GYM medium (**Větrovský *et al*., 2014)** were the strains inoculated followed by manufacturer's manual. Incubation time was 24 hours at 30 ºC. After incubation period we added reagents to each cupule and let the colors develop. After five minutes we evaluated stripes according to manual criteria.

16S rRNA sequencing

Molecular taxonomy, sequencing and phylogenetic analysis

The genomic DNA isolation of actinomycetes was done by the method described by **Deininger *et al.* (1989)** and amplified by PCR using primers according to **Cook and Meyers (2003)**. The PCR reaction ran in thermo cycler Biometra T Personal (Germany). (Reaction mixture contained 5 µl of 10 × DreamTaq Green PCR buffer, 5 µl of 2 $mmol.dm^{-3}$dNTP, 2 µl of each 10 $\mu mol.dm^{-3}$ primer, 0,3 µl Taq DNA polymerase and 0,5 µl of template DNA (approximately 20 ng). The PCR reaction ran under the following conditions: 95 °C for 3 min, 40 cycles of 95 °C for 30 sec, 56 °C for 30 sec, 72 °C for 90 sec and final extension at 72 °C for 10 min. Purification of PCR products were done using Exonuclease I and Thermosensitive Alkaline Phosphatase. The sequencing was carried out in both sense and antisense direction in MacroGen Company, South Korea. The similarity and homology of the 16S rRNA partial gene sequence was analyzed with the similar existing sequences available in the data bank of NCBI using BLAST search. The DNA sequences were aligned and phylogenetic tree was constructed with PhyML software using Maximum likehood tree. A bootstrap analysis of 100 replicates was carried out.

RESULTS AND DISCUSSION

A total of 27 morphologically different actinobacterial colonies were isolated from collected soil and made pure culture. According to Bergey´s Manual of Determinative Bacteriology by **Holt *et al.* (1994)** the organisms were identified as *Streptomyces* species based on the morphological characteristics. The colonies were slow growing, aerobic, glabrous or chalky, heaped, folded and with aerial and substrate mycelia of different colors. In addition, all colonies possessed an earthy odour (**Suneetha *et al.*, 2011).** The prevalence of *Streptomyces* species over other actinomycetes was likely due to screening conditions (media and cultivation).

In the present study, agar plug method was used for primary screening of antimicrobial activity. This method allowed utilizing of very small amount of medium for the culturing and production of bioactive compounds and also for the detection of antimicrobial activity of more number of actinobacterial isolates against wide range of microorganisms with less investment costs **(Mohanraj *et al.*, 2011).** The results of primary testing indicated that 14 of the total isolates demonstrated antimicrobial activities against two or more tested microorganisms, the remaining 13 isolates showed meagre activity. Antibacterial activity of soil actinomycetes against various phytopathogenic bacteria was determined by many researchers (**Muangham *et al.*, 2015 and Encheva-Malinova *et al.*, 2014).** The highest inhibition activity was measured against gram-positive bacterium *Clavibacter michiganensis subps. sepedonicus* in comparison with Gram-negative bacteria. Similar findings concur with the findings by various researchers, where they observed that antagonistic reaction against the Gram positive bacteria were much higher than the Gram negative (**Basilio *et al.*, 2003; Kumar *et al.*, 2012; Sacramento *et al.*, 2004**).

In case of antifungal activity, the highest inhibited fungus was *Fusarium poae*. The mechanism of antifungal antagonists can be due to the secretion of hydrolytic enzymes which degrade the fungal cell wall, or the secretion of antifungal compounds **(Yuan *et al.*, 1995)**. But it is not known whether the zone of inhibition, caused by the our *Streptomyces* strains occurs as a result of hydrolytic enzymes or antifungal metabolites. To determine whether our strains produced antifungal metabolites, crude extracts were prepared. Using a well diffusion method inhibition of mycelial growth was clearly observed in the presence of extracts and therefore it is possible that these strains are producing antifungal metabolites.

Further work carried by the leading 14 isolates. After cultivation, the ethylacetate extracts from these positive isolates were prepared and subjected to secondary screening using the same test pathogens. Solvent extraction is usually employed for the extraction of antibiotics from the culture filtrates. Organic solvents with different polarities have been used by many researchers for the extraction of antimicrobial compounds from actinomycetes (**Selvameenal *et al.*, 2009**). This result clearly indicated that the antimicrobial activity of potential strains is due to the production of extracellular bioactive compounds. The published literature stated that most of the antibiotics from actinomycetes are extracellular in nature **(Valanarasu *et al.*, 2008**). In well diffusion method, extracts of active actinobacterial strains showed different activity from that of primary screening; some of the active isolates didn't show the activity (2 isolates) in the secondary screening, perhaps due to the inconvenient liquid growth medium, while some showed only little activity (8 isolates) and some showed similar activity (VY59, VY47, VY87). Such results had been reported from other scientists too, which had found the activity reducing in comparison with that showed by the method of agar plug method. Actinomycete isolates namely VY59, VY47 and VY87 showed broad spectrum of antimicrobial activity against test phytopathogens (Table 1) and therefore, they were selected and characterized for further study.

Table 1 Size of inhibition zones (mm) of the most active isolates

Strain	*Xanthomonas campestris*	*Pseudomonas syringae*	*Erwinia amylovora*	*Clavibacter michiganensis*	*Alternaria tenuissima*	*Fusarium poae*	*Penicillium expansum*	*Aspergillus niger*
VY59	0*/0**	12/0	0/0	20/19	11/0	18/14	12/12	10/10
VY9	0/0	0/0	0/0	28/26	18/12	0/0	20/20	0/0
VY11	0/0	0/0	0/0	16/14	12/11	12/11	0/0	0/0
VY31	11/0	0/0	0/0	12/11	14/0	0/0	0/0	11/0
VY87	12/12	13/11	11/10	20/22	10/8	24/16	12/0	0/0
VY3	0/0	11/0	11/0	15/17	0/0	0/0	0/0	11/0
VY7	0/0	0/0	0/0	24/24	0/0	11/12	0/0	0/0
VY53	12/12	0/0	12/0	26/24	0/0	12/0	0/0	0/0
VY47	12/12	11/0	14/14	30/26	22/14	12/0	12/11	12/12
VY26	0/0	14/12	0/0	12/11	12/12	11/0	0/0	28/20
VY53	0/0	0/0	0/0	12/0	0/0	0/0	0/0	12/11
VY43	0/0	0/0	0/0	22/16	0/0	12/12	0/0	0/0
VY28	11/0	0/0	0/0	18/18	14/11	0/0	11/0	0/0
VY14	0/0	11/0	0/0	30/22	0/0	14/12	0/0	0/0

*Primary screening / ** secondary screening

It was possible to identify these actinomycete isolates based on pigment production, morphological and physiological characteristics and biochemical properties, which can provide more details that can be used for identification purposes as reported by **Oskay *et al.* (2004),** but other advanced methods such as gene analysis of 16S rRNA are more reliable **(You *et al.*, 2005).**

Morphological characterization of the isolates

Many characteristics of actinomycetes have been employed for the purpose of easy classification and ideally, these should be constant under the same cultural conditions **(Sathi *et al.*, 2001).** All selected strains were Gram-positive, very long, rod shaped and possessing an earthy odour characteristic for actinomycetes. Morphological characteristics of the active strains on different ISP specific media are shown in table 2.

Table 2 Morphological identification of the selected active strains

Medium	Color of Aerial mycelium			Color of Substrate mycelium			Production of soluble pigments		
	VY59	**VY87**	**VY47**	**VY59**	**VY87**	**VY47**	**VY59**	**VY87**	**VY47**
ISP2	Lemon yellow	Curry	Sand yellow	Oyster white	Platinum grey	Oyster white	Lemon yellow	Sandy yellow	-
ISP3	No growth	Pale brown	Brown beige	No growth	Platinum grey	No growth	-	-	Brown beige
ISP4	Ivory	Green beige	Ivory	No growth	Pure white	No growth	-	-	-
ISP5	Ivory	Yellow grey	Sand yellow	Oyster white	Granite grey	Light ivory	-	-	-
ISP6	No growth	Brown beige	No growth	No growth	Pearl light grey	No growth	-	Ochre yellow	Broom yellow
ISP7	Honey yellow	Sepia brown	Sand yellow	Stone grey	Light ivory	Oyster white	-	Black brown	Sandy yellow

Under microscopic observation all strains showed the presence of substrate and aerial mycelium. Using an identification guide by the International Steptomyces Project **(Getha *et al.*,)**, the characteristic of the sore bearing hyphae of the isolates VY59 and VY87 was found to be rectus flexibilis, whereas that of VY47 was spira. The different media did not have an effect on micro-morphological characteristics of the isolates but it had an effect on the melanin production. Only isolate VY87 showed melanin production and this was determined on ISP7 agar medium. **Mutitu *et al.* (2008)** also found that a variety of pigments and colony types are produced by the same organism on different media.

Table 3 Physiological characteristics of active isolates

Parameter	Carbon sources			Parameter	pH range		
	VY59	**VY87**	**VY47**		**VY59**	**VY87**	**VY47**
Arabinose	+	+	+	**2**	-	-	-
Cellulose	+	+	+	**3**	-	-	-
Fructose	(+)	+	(+)	**4**	-	-	-
Glucose	+	+	+	**5**	-	-	-
Inositol	(+)	-	-	**6**	-	(+)	-
Mannitol	+	+	+	**7**	+	+	(+)
Raffinose	+	+	+	**8**	(+)	+	+
Rhamnose	-	+	(+)	**9**	-	-	-
Sucrose	(+)	+	(+)	**10**	-	-	-
Xylose	(+)	-	(+)				
NaCl concrentation (%)				**Growth at (ºC)**			
0	**(+)**	**+**	**(+)**	**25**	(+)	+	+
2,5	+	+	+	**28**	+	+	+
5	-	(+)	(+)	**35**	(+)	-	(+)
7,5	-	-	-	**45**	-	-	-
10	-	-	-	**60**	-	-	-

+ good growth, (+) moderate growth, - no growth

Physiological characterization

In general, biochemical and physiological characteristics of the actinomycetes vary from isolate to isolate depending on the growth conditions. The present investigation concluded that the physiological characteristics of actinomycetes varied depending on the avaliable nutrients in the medium and the physical conditions. Thus, it was concluded on the basis of the present and previous studies that the nutrient composition of the medium greatly influence the growth and morphology of organisms **(Gesheva *et al.*, 1993).** Tested isolates showed variable results, in the utilization of the carbon sources tested, isolate VY59 utilized all the carbon sources except rhamnose, isolate VY47 did not utilize inositol and VY51 cellulose, raffinose and sucrose (Table

3). Studies on the requirement of carbon sources for growth showed that cellulose, arabinose, glucose, manitol and raffinose are needed as carbon sources for abundant growth of the isolates. Slight or poor growth is an indication that, the particular carbon source is not an adequate source of carbon or the material may contain traces of other compounds **(Sathi *et al.*, 2001).** Optimal growth of all strains was observed at 2.5% NaCl, but maximum tolerance of chlorid concentration was exhibited up to 5% in case of strain VY47. All the isolates could grow at pH 7. **Park *et al.* (1991)** rewieved that neutrophiles *Streptomycecs* species are able to grow between pH 5 and 9 with optimum growth close to neutrality. Temparature range of isolates was from 25 to 28 ºC, with the optimum conditions at 28 ºC. Isolates VY59 and VY47 exhibited moderate growth at 35 ºC.

Biochemical tests with Api-stripes

Activity of the extracellular enzymes was quantified using API ZYM. During the incubation period, the products of the end metabolism produced and detected as color reaction **(Aljassim, 2015).** It was found that all isolates showed good phosphatase alcaline, leucinearylamidase, phosphatase acid, and N-acetyl-glucoseamidase activity and glucosidase. Contrary, the least occurring enzyme was galactosidase and glucuronidase (Table 4).

Table 4 Detection of various enzymes using ApiZym tests

Enzyme	VY59	VY87	VY47
Phosphatase alcaline	(5) +	(5) +	(5) +
Esterase (C4)	(3) +	(3) +	(2) +
Esterase lipase (C8)	(4) +	(2) +	(3) +
Lipase(C 14)	(0) -	(0) -	(2) +
Leucinearylamidase	(5) +	(5) +	(5) +
Valinearylamidase	(3) +	(5) +	(5) +
Cystinearylamidase	(1) +	(3) +	(1) +
Trypsin	(0) -	(5) +	(0) -
Chymotrypsin	(1) +	(1) +	(3) +
Phosphatase acid	(5) +	(5) +	(5) +
Naphtol-AS-BI-phosposfohydrolase	(5) +	(5) +	(3) +
Galactosidase	(0) -	(0) -	(0) -
Galactosidase	(0) -	(1) +	(0) -
Glucuronidase	(0) -	(0) -	(0) -
Glucosidase	(1) +	(3) +	(0) -
Glucosidase	(4) +	(5) +	(5) +
N-acetyl-glucoseamidase	(5) +	(5) +	(5) +
Mannosidase	(0) -	(5) +	(0) -
Fucosidase	(0) -	(2) +	(0) -

Numbers indicate colour intensity which is proportional to concentration of respective enzyme presence, + enzymatic activity was detected, - enzymatic activity was non-detected

Similar results obtained **Jiang *et al.* (2013)**, who found out, that the tested isolates showed alkaline phosphatase, acid phosphatase, leucinearylamidase, naphtol-AS-BI-phosphatase and B-glucosidase and α-glucosides activity and none strains showed β-glucuronidase activity. According to API CORYNE system we detected nitrate reduction, production of esculin, gelatin hydrolysis, production of alkaline phosphatase and N-acetyl-β- glucosamidase and urease activity. The rest of tested activities were not positive (Table 5)

Table 5 Enzymatic and fermentation tests using Api Coryne system

Parameter	VY59	VY87	VY47
Nitrate reduction	+	-	-
pyrrolidonyl arylamidase	-	-	-
β – glucuronidase	-	-	-
α- glucosidase	-	-	-
esculin	-	+	+
gelatine (hydrolysis)	+	+	+
Ribose fermentation	-	-	-
Mannitol fermentation	-	-	-
Sucrose fermentation	-	-	-
pyrazinamidase	-	-	-
Alkaline phosphatase	+	+	+
β –galactosidase	-	-	-
N-acetyl-β- glucosamidase	-	+	-
Urease	-	-	+
Glucose fermentation	-	-	-
Xylose fermentation	-	-	-
Lactose fermentation	-	-	-
Glycogen fermentation	-	-	-

Results indicates that actinomycetes possess the potential to secrete broad range enzymes, which maybe the results from natural selection of the microorganisms in order to survive in a competing environment.

Molecular identification

Although various morphological and biochemical tests were performed, to identify the *Actinomycetes* up to species level, for proper identification of genera and species of *Actinomycetes*, molecular identification is necessary. Identification of using molecular tools proved to be faster and least tedious compared to classical microbiological methods. Initial morphological characterization using light microscope showed that all the 3 isolates belong to the genus *Streptomyces* spp. The results obtained from the direct sequencing of purified PCR products confirm this suggestion. The nucleotide sequences for a section of the 16S rRNA gene from 3 selected strains were subjected to BLAST analysis using NCBI database for identification at the genus level. All three isolates contained different nucleotide sequences for the 16S rRNA gene, indicating that they were different strains. VY59 was most closely related to *Streptomyces somaliensis* (similarity index 99%), VY87 to *Streptomyces* sp. (similarity index 99%) and VY47 to *Streptomyces albidoflavus* with similarity index 99%. The results described above were supported by phylogenetic analysis base on the neighbor-joining tree.

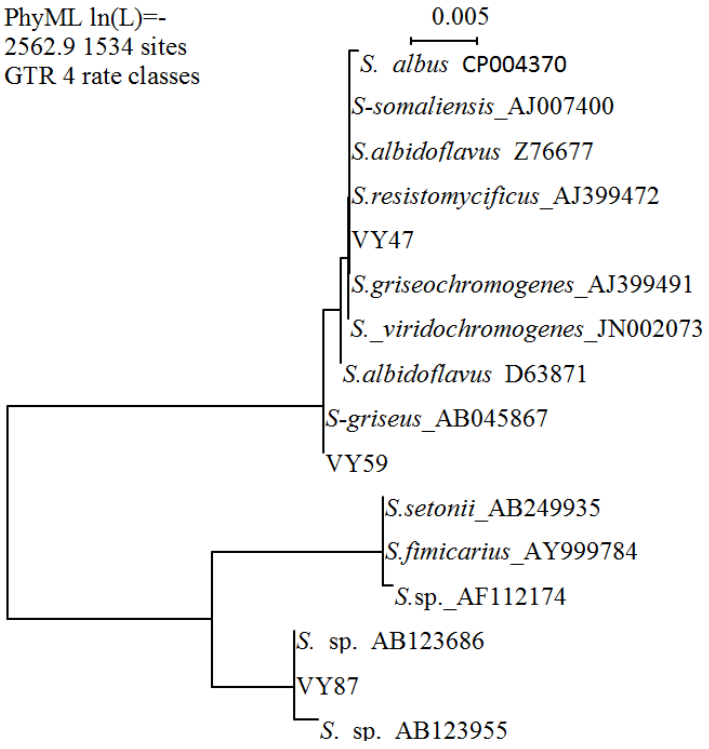

Figure 1 Phylogenetic relationships based on neighbour-joining analysis of 16S rRNA gene sequence of the most active strains and closely related *Streptomyces* species

CONCLUSION

In conclusion, the three streptomycete strains, *S. somaliensis* (VY59), *S.* sp. (VY87) and *S. albidoflavus* (VY47) exhibited interesting antimicrobial activity against phytopathogenic Gram-positive, Gram-negative bacteria and against fungi. This study showed that the test actinomycetes isolates have the potential to act as sources of antimicrobial compounds against phytopathogenic microorganisms which attack crops. It is suggested that these strains of soil streptomycetes be further studied in search for some novel antibiotics, which could be effective in the protection of crop production. Thus our study brings forward a good promise for future drug development and agricultural programs.

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