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Effect of fertilization on biological activity of community of soil streptomycetes

Vplyv hnojenia na biologickú aktivitu pôdneho spoločenstva streptomycét

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Abstract

The search for new natural mechanisms to inhibit the growth of phytopathogenic microorganisms has become widely widespread. Therefore, the main objective of the present study was determination of antimicrobial activities of actinomycetes isolated from agricultural soil, which was fertilized mainly by organic fertilizers, against 8 selected phytopathogenic strains. Among the actinomycetes, *Streptomyces* species have been extensively studied, because they have been recognized as an important source of secondary metabolites, which can suppress the growth of undesirable pests in crops. The results indicated that the richest source of *Streptomyces* colonies was soil fertilized with compost (103 x10^4 CFU*g^{-1} dry soil). On the basis of morphological signs, total of 65 isolates were selected and examined for antimicrobial activities. Isolates exhibited the best activity against Gram negative bacterium *Clavibacter michiganensis* subsp. *sepedonicus*, disease agent of “ring rot” of potatoes and against fungus *Fusarium poae*, disease agent of *Fusarium* head blight of wheat. Twelve isolates exhibited promising broad-spectrum activity against tested organisms. On the basis of results, six of them were selected for further screening. Comparison of polyphasic studies with available literature led to identification of biological active strains *S. olivochromogenes* (13SC11), *S. avermitilis* (13SC2), *S. rishiriensis* (13SC13), *S. globisporus* (13SC19), *S. sampsonii* (13SPC10) and *S. avidinii* (13SPC4). After quantification analysis of various enzymes, tested isolates produced alkaline phosphatase, leucinearylamidase, valinearylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, glucosidase in high values (>40 nmol) and were positive for nitrate reduction, hydrolysis of gelatin, urease, and esculin. These isolates can be used in the development of new biopesticides anf biofertilizers with antibacterial and antifungal effect.

Keywords: antimicrobial potential, fertilizers, phytopathogenic microorganisms, soil, streptomycetes

Abstrakt

Hľadanie nových prirodzených mechanizmov na potlačenie rastu fytopatogénnych mikroorganizmov sa stalo veľmi rozšíreným. Z tohto dôvodu je hlavným cieľom tejto štúdie detekcia antimikrobiálnej aktivity aktinomycét izolovaných z poľnohospodársky využívanej pôdy hnojenej najmä organickými hnojivami, voči 8 vybraným fytopatogénnym mikroorganizmom. Spomedzi aktinomycét, sme sa v tejto štúdii zamerali na streptomycéty, pretože patria medzi významných producentov sekundárnych metabolitov, ktoré môžu potlačiť rast neželaných škodcov úrody. Výsledky ukázali, že najbohatším zdrojom kolónií streptomycét bola pôda hnojená kompostom (103 x10^4 KTJ*g^{-1} sušiny). Na základe zistených morfologických znakov sme na štúdium antimikrobiálnej aktivity vybrali 65 izolátov. Izoláty vykazovali najlepší účinok voči baktérii *Clavibacter michiganensis* subsp. *sepedonicus*, pôvodcu choroby "kružkovatosti" zamiakov a voči hube *Fusarium poae*, pôvodcu choroby fuzáriovej sneti pšenice. 12 izolátov vykazovalo širokospektrálne účinky voči testovaným organizmom, 6 z nich bolo vybraných na ďalšie analýzy. Porovnanie ich polyfázických znakov s dostupnou literatúrou viedlo k identifikácii produkčných druhov *S. olivochromogenes* (13SC11), *S. avermitilis* (13SC2), *S. rishiriensis* (13SC13), *S. globisporus* (13SC19), *S. sampsonii* (13SPC10) a *S. avidinii* (13SPC4). Tieto izoláty sa vyznačovali tiež vysokou produkciou alkalickej fosfatázy, leucínarylamidázy, valínarylamidázy, kyslej fosfatázy, naftol-AS-BI-fosfohydrolázy a glukozídazy (> 40 nmol) a boli pozitívne pri redukcii dusičnanov, hydrolýzy želatíny, produkcii ureázy a eskulínu. Všetky tieto izoláty môžu byť použité pri vývoji nových biopesticídov a biohnojív s antifungálnymi a antibakteriálnymi účinkami.

Kľúčové slová: antimikrobiálny potenciál, fytopatogénne mikroorganizmy, hnojivá, pôda, streptomycéty

Introduction

Microorganisms are the main colonizers of the soils, bestowed with inherent physiological and functional diversity and have found applications in many spheres (Balagurunathan and Radhakrishnan, 2010). Composted materials have traditionally been applied to agricultural soils as means of improving soil fertility and crop growth and contribute to improve soil biological characterristics, also often providing an effective control of soil-borne diseases (Vargas-García et al., 2008). Among the various industrially important microorganisms, actinomycetes, particularly *Streptomyces* species, have received considerable attention as biocontrol agents, because they are important producers of secondary metabolites (Sengupta et al., 2015), notably enzymes (Chiani and Mazinani , 2010) and antibiotics (Strohl, 2004). These metabolites are known to possess antibacterial, antifungal, antioxidant, anti-algal, anti-helminthic and anti-malarial activities (Kekuda and Shobha, 2010; Ravikumar et al., 2011). Streptomycetes, represent one of the biggest societies in soil microflora (Hoskisson et al., 2012). Streptomycetes are Gram-positive, free living, saprophytic, filamentous bacteria (Valli et al., 2012) well adapted to this environment because of their high enzymatic activity (Hoskisson et al., 2012). Millions of strains

have been isolated and screened over the decades in research and industrial laboratories, but there are still many explorations needed to discover interesting actinomycetes communities that might be important for agricultural area (Genilloud et al., 2011), because phytopathogens pose serious problems worldwide and cause a number of plants diseases, for example rusts, smuts, rots, and may cause severe damage to crops (Bauman, 2007).

The present work reports the isolation of streptomycetes from soil, and compost-amended soil, which was used for cultivation of medical plants, to perform antimicrobial activity of the cultures and ethyl-acetate extracts against phytopathogenic microorganisms and identification of the most active isolates using polyphasis approach with quantification of their selected enzymes.

Materials and methods

Collection and characterization of isolation material

The potential active streptomycete strains were isolated from soil sample used for cultivation of medical plants by ecological mode of farming, collected at village Východná (geographical coordinates: latitude 49°04´N, longitude 19°54´E) and from soil-amended compost, in autumn of 2013. Soil sample was collected aseptically from a depth of 0.2 m from 6 randomly selected places using clean, dry and sterile polythene bags and was immediately taken to the laboratory and dried at room temperature. Samples used for isolation of streptomycetes were collected from unfertilized soil (variant I and III) and from fertilized soil (variant II and IV). On the basis of pedologic characteristics, the soil was identified as sandy brown soil. After fertilization and cultivation of plants, the samples were differing in amount of nitrates, nitrites and microbial biomass carbon (Table 1).

Table 1. Variants of experiment and agrochemical characteristic of isolation substrates

Tabuľka 1. Varianty pokusu a agrochemická charakteristika izolačných substrátov

No	Variant	DM %	pH H_2O	pH KCl	C_{mic} mg*kg^{-1}	C_{ox} %	N_{tot} %	N-NH4 mg*kg^{-1}	N-NO3 mg*kg^{-1}
I	Soil	91,8	6,2	4,8	288,0	1,7	0,2	6,0	0,4
II	Soil+compost (70 t*ha^{-1})	82,3	6,8	5,3	598,9	1,8	0,2	22,4	2,2
III	Soil+plants	93,5	6,3	4,6	229,5	1,7	0,1	12,4	1,3
IV	Soil+plants+ compost (70 t*ha^{-1})	92,5	6,2	4,4	231,2	1,63	0,1	4,2	0,1

DM - dry matter, pH (H_2O) - active pH, pH (KCl) - replaceable pH, Cmic-microbial biomass carbon, Cox- content of organic carbon, Ntot- total nitrogen

DM – sušina, pH (H_2O) – aktívne pH, pH (KCl) – výmenné pH, Cmic – uhlík mikrobiálnej biomasy, Cox – obsah organického uhlíka, Ntot – celkový dusík

Isolation of actinomycetes

Streptomycetes were isolated using classical plate dilution method. One gram of isolation substrates was suspended in 100 ml of distilled water and incubated in a shaker (200 rpm for 1hr, room temperature). Mixtures were allowed to settle, and then serial dilutions were prepared up to 10^{-4}. From each dilution, 0.1 ml was taken and spread over the surface of Pochon medium (Korzeniewska et al., 2009) complemented with nystatin (50µg.ml^{-1}) and incubated at 30°C for 7 days. After incubation time plates were observed for the appearance of colonies look like streptomycetes. Streptomycete isolates were purified by streak-plate method on GYM medium (Větrovský et al., 2014) several times. Pure strains were maintained as glycerol suspensions (30%, v/v) at - 20 °C.

Phytopathogenic strains

To assess the streptomycete potential antimicrobial activity, 4 phytopathogenic bacteria [(*Xanthomonas campestris* (CCM 22); *Pseudomonas syringae* (CCM 2868); *Erwinia amylovora* (CCM 1114); *Clavibacter michiganensis* subsp. *sepedonicus* (CCM 7014)] obtained from Czech Collection of Microorganisms, Brno, Czech Republic and 4 phytopathogenic fungi [(*Fusarium poae* (Kmi-12A18-ZM), *Penicillium expansum* (Kmi-306-LR); *Aspergillus niger* (Kmi-0146-LR) and *Alternaria tenuissima* (Kmi-16A6-ZM)] obtained from Collection of Microorganisms of Department of Microbiology, Nitra, Slovakia. Bacteria were cultivated on yeast-glucose medium (B8) (Sigma-Aldrich, USA), except for *Clavibacter michiganensis* subsp. *sepedonicus*,

which were cultivated on tryptone-soya medium (Sigma-Aldrich, USA). Microscopic fungi were cultivated on Sabouraud medium (Cazin et al., 1989).

Screening of Antimicrobial Activity

Primary screening of the streptomycete isolates was done by agar plug method. The test organisms were inoculated (200 µl) over the surface of cultivating media. Agar discs were prepared using a sterile cork borer from well grown streptomycetes cultures and placed on fresh lawn cultures of the test microorganisms. Plates were incubated at 25 ºC for fungi and *Clavibacter michiganensis* subsp. *sepedonicus* (72 hr) and at 30 ºC for bacteria (24 hr). The zones of inhibition were measured by Haloes Caliper (IUL Instruments, USA).

Active isolates in primary screening were subjected to secondary screening. The active isolates were inoculated to 5333 medium (Wink, 2014) supporting production of secondary metabolites and incubated at 30 °C for 5-7 days. Antimicrobial compounds were recovered from the filtrate by solvent extraction with ethyl acetate. Ethyl acetate (Sigma Aldrich, USA) was added to the cultures in the ratio of 1:1 (v/v) and centrifuged. The ethylacetate phase that contained secondary metabolites, was transferred into a round bottom flask. At 40 °C the ethylacetate was evaporated in a rotary evaporator (Stuart, UK). The dried precipitate was dissolved in 1 ml of ethylacetate: acetone:methanol in ratio of 1:1:1 (v/v) and used to determine the antimicrobial activity. Secondary screening was done by well-diffusion method. 100 µl of crude extract was placed into the wells in Petri dishes, prepared with cork borer, that were previously seeded with different phytopathogenic microorganism. The plates were incubated at the same conditions mentioned in primary screening.

The inhibitory effect was evaluated according to Gopinath et al. (2013). Strains were divided into three groups, i.e., negative, group one, two and three. The negative inhibition shows passive nature with l0 mm, group one shows 11-20 mm with slightly active, group two shows 2l-33 mm with moderately active while group three shows 33 mm with highly active inhibition.

Polyphasis characterization

Only streptomycetes isolates that gave the best results in the antimicrobial screening were characterized by morphological and physiological approach. Morphological characteristic as color of aerial and substrate mycelium, morphology of spore chain, production of melanin and other soluble pigments were recorded according to International Streptomycetes Project (ISP) (Shirling and Gottlieb, 1966) on IPS2 - ISP7 media. The colors were done with RAL-code. The spore bearing hyphae was determined by direct examination using 7 days old cultures under light microscope (OLYMPUS CX22LED, Japan) using 1000 x magnification.

Physiological characterization was done by utilization of 10 different carbon sources (glucose, arabinose, inositol, cellulose, mannose, fructose, galactose, rhamnose, sucrose, xylose) using a 12 well plates on ISP9 medium (Shirling and Gotllieb, 1966), sodium chloride tolerance was evaluated on ISP9 medium supplemented with 2.5; 5; 7.5 and 10 % of sodium chloride using 6-well microtiter plates. Effect of pH (range 2-

9) and temperature (4, 25, 28, 30, 34 and 45 °C) were observed on ISP2 medium. The observed structures were compared with Compendium of Actinobacteria written by Dr. Joachim Wink and with Bergey´s Manual of Systematic bacteriology (Vos et al., 2011), and the organisms were identified.

Detection of enzymatic activities using Api Zym® and Api Coryne® stripes

For quantification of various enzymes and fermentation tests were used two different Api® systems – ApiCoryne® and ApiZym®. After 7 days of incubation in shaking flasks with 100 ml of GYM medium, cultures were inoculated followed by manufacturer´s manual and stripes were incubated 24 hr at 30 °C. After incubation period was added one drop of ZYM A and one drop of ZYM B reagents to each cupule of ApiZym® stripes. To ApiCoryne® stripes was added one drop of NT1+NT2 reagents to the first cupule, PYZ reagent to the second cupule and one drop of ZYM A and ZYM B to the next six cupules. The rest of cupules were not filling with any reagents. The stripes were evaluated according to manual criteria.

Statistical analysis

Difference among variants in numbers of CFU (colony forming units) was analyzed using one factorial analysis of variance in STATGRAPHICS Centurion XV. The number of CFU was transformed to ln (x+1) prior analysis. All means were compared by the Tukey test, with the level of significance set at 95%.

Results and discussion

Isolation and quantification of streptomycetes

This study was performed with an aim of isolating streptomycete strains from soil used for cultivation of medical plants and detection of their antimicrobial activities against phytopathogenic microorganisms. Most of the colonies that grew on Pochon plates belonged to the genus *Streptomyces*, because colonies were slow growing, aerobic, chalky, heaped, folded and with aerial and substrate mycelia of different colors possessed by earthy odor. Streptomycete strains were isolated from all variants (table 1). The colony forming units (CFU) were determined by counting the colonies on the dilution plates. Total amount of streptomycete colonies was $317x10^4$ $CFU*g^{-1}$ (Figure 1).

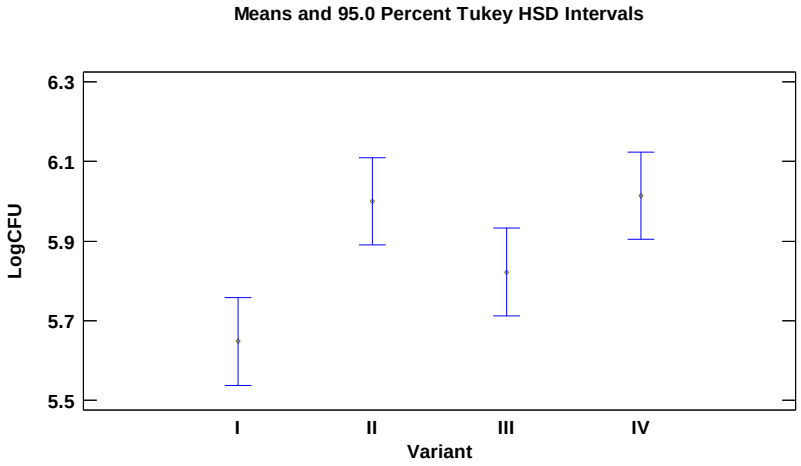

Figure 1. Effect of fertilization on the number of total streptomycete colonies

Obrázok 1. Vplyv hnojenia na počet izolovaných kolónií streptomycét

These data show that population of streptomycetes colonies were not significantly different in variants I and III and in variants II and IV *(*P<0.05). The streptomycete numbers increased in soil treated with compost. It can be due to suitable conditions, which compost offer to microorganisms, which account for most of the decomposition that takes place in compost. Out of the total amount of streptomycete colonies, only different look like isolates were tested for antimicrobial activity (Table 2).

Table 2. Total number of streptomycetes and selected cultures for further screening

Tabuľka 2. Celkové počty streptomycét a vybrané kultúry pre ďalšie testovanie

Variant	NC (CFU)	NMDC	Labeling of selected strains
I	44 x10^4	12	13S1-13S12
II	99 x10^4	24	13SC1-13SC24
III	66 x10^4	10	13SP1- 13SP10
IV	103 x10^4	19	13SPC1-13SPC19

NC-number of colonies, NMDC-number of morphological different colonies

NC – počet kolónií, NMDC – počet morfologicky rozličných kolónií

Antimicrobial activity of streptomycetes

Both the primary and secondary screening were used to screen the streptomycetes for antimicrobial activity and for selection of the best substrate for biological active streptomycete strains. In the present study, 65 streptomycete strains were subjected to preliminary antimicrobial assay by agar plug method. The primary screening was used to select the antimicrobial isolates and determine the range of microorganisms that were sensitive to their potential secondary metabolites. Out of all isolates, 38 strains (58.46%) showed antagonistic activity against phytopathogenic microorganisms. The number of antibiotic-producing isolates varied with the variants. The highest percentage of active isolates came from the variant IV (18 strains) and II (12 strains), while the lowest percentage came from variant I (3 strains) and III (5 strains).

The active isolates exhibited different inhibitory patterns against the test organisms and the bioactivity of the isolates was dissimilar between Gram positive and Gram negative bacterial strains. The isolates showed higher inhibition zones against Gram positive bacteria than Gram negative bacteria. The reason for different sensitivity between gram positive and gram negative bacteria could be explain to the morphological differences between these microorganisms (Shirling and Gottlieb,1966). Similar results obtained also Gebreyohannes et al. 2013; Mohseni et al., 2013 and Pandey et al., 2011 in their studies.

X. campestris and *P. syringeae* were inhibited by 14 strains. *E. amylovora* by 6 strains, *Clavibacter michigenensis* subsp. *sepedonicus* by all 38 strains. In case of antifungal activity, 13 strains inhibited growth of *F.poae*, 11 isolates of *P. expansum* and only 6 isolates *A. niger* and 4 isolates *A. tenuissima*. Several researches have already reported that *Streptomyces* have biocontrol activity against phytopathogenic microorganisms. This has been shown that the principle mechanism of this biological activity involved the production of secondary metabolites (Murray, 2011).

From the primary screening, all active strains were selected based on their efficiency for secondary screening. In general the antimicrobial activity of crude extracts was fluctuated. This may be associated with disintegration of the crude extracts during preparation and extraction process. Results showed different activity from that of primary screening; some of the active isolates didn't show the activity in the secondary screening while some showed little activity and some showed improved activity. According to Bushell (1997), during the screening of the novel secondary metabolites, streptomycetes isolates are often encountered which show antibiotic activity on agar but not in liquid culture.

On the basis of results only 12 extracts exhibited similar or improved activity during secondary screening (Table 3).

Table 3. Antimicrobial activity of the most active streptomycetes against phytopathogenic microorganisms

Tabuľka 3. Antimikrobiálna aktivity najaktívnejších kmeňov streptomycét voči fytopatogénnym mikroorganizmom

Isolate	variant	X.c.	P.s.	E.a.	C.m.	F.p.	P.e.	A.n.	A.t.
13SC11	II	14/14	12/0	0/0	24/22	22/16	17/11	12/0	0/0
13SC2	II	11/11	18/17	0/0	28/26	14/14	11/0	0/0	0/0
13SC13	II	12/13	11/11	12/14	31/30	16/16	0/0	11/11	12/11
13SC19	II	11/14	12/11	14/15	40/35	12/12	16/14	20/18	12/12
13SC22	II	15/13	16/11	0/0	14/14	0/0	0/0	14/14	12/0
13SPC1	IV	12/11	12/15	12/12	18/17	14/13	0/0	0/0	0/0
13SPC7	IV	0/0	11/11	11/11	12/0	0/0	0/0	15/13	0/0
13SPC10	IV	16/16	0/0	0/0	31/31	12/12	20/20	19/18	0/0
13SPC4	IV	12/11	16/15	0/0	28/24	19/17	0/0	0/0	14/12
13SPC11	IV	14/12	12/11	0/0	16/15	16/16	12/12	0/0	0/0
13SPC12	IV	14/14	0/0	14/0	16/20	0/0	14/14	0/0	0/0
13SPC17	IV	12/12	0/0	16/12	40/38	0/0	18/12	0/0	0/0

X.c.- *Xanthomonas campestris*, P.s.- *Pseudomonas syringeae*, E.a.- *Erwinia amylovora*, C.m. - *Clavibacter michigenensis* subsp. *sepedonicus*, F.p.- *Fusatium poae*, P.e.- *Penicillium expansum*, A.n. - *Aspergillus niger*, A.t.- *Alternaria tenuissima,* primary screening / secondary screening

X.c.- *Xanthomonas campestris*, P.s.- *Pseudomonas syringeae*, E.a.- *Erwinia amylovora*, C.m. - *Clavibacter michigenensis* subsp. *sepedonicus*, F.p.- *Fusatium poae*, P.e.- *Penicillium expansum*, A.n. - *Aspergillus niger*, A.t.- *Alternaria tenuissima,* primárny skríning / sekundárny skríning

Out of all the 12 isolates, six the best antagonistic streptomycetes isolates, which showed the highest inhibition zones against tested microorganisms (13SC11, 13SC2, 13SC13, 13SC19, 13SPC10, 13SPC4) were selected for further studies. Capability of compost microorganisms to suppress soil-borne plant pathogens has become an interesting subject as a strategy for reducing the adverse effects of massive fungicides' applications. Several mechanisms have been proposed to explain plant disease control by compost such as competition, activation of disease-resistance genes, or antibiotic production by beneficial microorganisms (Hoitink and Boehm, 1999).

Characterization of the most active isolates

Six selective media were used to observe the distinct colony morphology of the isolates. Morphology was observed by light microscope, indicating variation among the isolates. Results of morphological characteristics revealed that the growth of the isolates was good in majority of the used media. The aerial mycelium, substrate mycelium growth and pigmentation showed distinct variation based on the culture media in which the isolates were grown. The detailed results of the cultural, morphological and physiological tests have been summarized in Table 4 and Table 5.

Table 4. Morphological characterization of the selected biologically active strains of streptomycetes

Tabuľka 4. Morfologická charakteristika vybraných biologicky aktívnych kmeňov streptomycét

Isolates	Characteristics		
	Aerial mycelium	Substrate mycelium	Soluble pigments
13SC11	none	ISP2-7 beige	none
13SC2	ISP2-5,7 grey; ISP6 none	ISP2-7 brown	ISP2,5,7 ochre brown
13SC13	ISP2-4 grey; ISP5-7 white	ISP2 brown; ISP3-5 yellow; ISP4,6,7 beige	ISP2,5,6 brown
13SC19	ISP2-7 cream	ISP2-4 beige; ISP5-7 yellow	none
13SPC10	ISP2-7 white	ISP2-7 yellow	none
13SPC4	ISP2-7 gray	ISP2,3,6 brown; ISP3-5 yellow	ISP6 brown

Microscopically, it was observed that the morphology of the spore chain varied depending on the species, where the majority spore chains were *Rectus flexibilis*. It was also observed that some of the strains produced melanoid pigment. Confirmatory identification to species was based on degradation of carbohydrates where all *Streptomyces* strains have variable degradation ability of carbohydrates

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Table 5. Physiological characteristic of the biologically active strains of streptomycetes

Tabuľka 5. Fyziologická charakteristika produkčných kmeňov streptomycét

Observed parametres	Isolates					
	13SC11	13SC2	13SC13	13SC19	13SPC10	13SPC4
Spore chain	RF	SP	RF	RF	RF	RF
Melanin	none	ISP6-7	ISP7	none	ISP7	ISP6
Utilization of carbon sources:						
Glucose	+	+	+	+	+	+
Arabinose	+	+	+	-	+	-
Sucrose	+	-	+	+	-	-
Xylose	-	-	+	-	-	-
Inositol	+	-	+	-	-	-
Mannose	+	+	+	+	+	-
Fructose	+	+	+	+	+	-
Rhamnose	+	+	+	-	-	-
Raffinose	-	+	-	-	-	-
Celulose	-	-	-	+	-	-
NaCl tolerance	2.5	5	2,5	2,5	7,5	2,5
Temperature	28	28	28	28	30	28
pH	7	7	6	7	7	7

RF – *Rectus flexibilis*, SP – *Spira,* + positive growth, - negative growth, (+) – positive growth, (-) – negative growth

RF – *Rectus flexibilis*, SP – *Spira,* + pozitívny rast, - negatívny rast

Morphological examination of these 6 isolates clearly indicated that they belong to the *Streptomyces* genera and *Streptomycetaceae* family (spore chain with branching mycelium). Further comparison of morphological and physiological characteristics of strains with reference to the Bergey´s Manual of Systematic Bacteriology and with Compendium of Actinobacteria from Dr. J. Wink showed the closest streptomycete

strains. Strain 13SC11 was the most similar to *Streptomyces albidoflavus* (database number: FH1932), strain 13SC2 to *Streptomyces avermitilis* (database number: FH1532), 13SC13 to *Streptomyces rishiriensis* (database numer: FH1704), 13SC19 to *Streptomyces globisporus* (database numer: FH8103), 13SPC10 to *Streptomyces sampsonii* (database number: FH 2213) and strain 13SPC4 to *Streptomyces avidinii* (database numer: FH 2494).

In recent years enzymes gained considerable attention in industrial process. Also from terrestrial soil samples various enzymes have been reported by various workers (Sharma et al. 2005). Using ApiZym® and ApiCoryne® stripes is providing the advantage of easy and fast determination between two isolates showing significant appearance by means of the differences in their enzyme profiles (Vítězová, 2013). A huge enzymatic variability was discovered within the range of isolated streptomycetes (Table 6). It was found that each tested strain was possessing alkaline phosphatase, leucinearylamidase, valinearylamidase, acid phosphatase and glucosidase activity in high amounts (>40 nmol). Contrary, the least occurring enzyme was lipase, galactosidase, glucuronidase and fucosidase. Similar results obtained Jiang et al. (2013), who found out, that the tested isolates showed alkaline phosphatase, acid phosphatase, leucinearylamidase, naphtol-AS-BI-phosphatase, β-glucosidase and α-glucosides activity and none strains showed β-glucuronidase activity.

Table 6. Enzymatic potential of selected streptomycetes using Api Zym® stripes

Tabuľka 6. Enzymatický potenciál vybraných streptomycét použitím Api Zym® prúžkov

No.	Isolates					
	13SC11	13SC2	13SC13	13SC19	13SPC10	13SPC4
2	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)
3	+ (3)	(+) (2)	(+) (2)	+ (3)	+ (4)	(+) (2)
4	(+) (2)	+ (3)	(+) (2)	+ (3)	+ (3)	+ (3)
5	- (0)	(+) (2)	- (0)	+ (4)	(+) (1)	- (0)
6	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)
7	+ (5)	+ (4)	+ (5)	+ (4)	+ (3)	+ (5)
8	+ (3)	(+) (2)	+ (3)	+ (4)	(+) (1)	+ (4)
9	+ (5)	+ (3)	(+) (1)	+ (5)	(+) (1)	+ (4)
10	(+) (1)	+ (5)	- (0)	+ (5)	(+) (2)	+ (5)
11	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)
12	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)	+ (5)
13	- (0)	- (0)	- (0)	- (0)	- (0)	+ (3)
14	(+) (1)	(+) (2)	+ (5)	(+) (2)	- (0)	+ (3)
15	- (0)	- (0)	- (0)	- (0)	- (0)	- (0)
16	+ (3)	+ (4)	+ (5)	+ (5)	+ (4)	+ (5)
17	+ (5)	+ (4)	+ (5)	+ (5)	+ (4)	+ (4)
18	+ (5)	- (0)	- (0)	- (0)	+ (5)	(+) (1)
19	+ (5)	- (0)	- (0)	(+) (2)	- (0)	(+) (2)
20	(+) (2)	- (0)	- (0)	(+) (1)	- (0)	- (0)

2 – alkaline phosphatase, 3 – esterase (C4), 4 – esterase lipase (C8), 5 – lipase (C14), 6 – leucinearylamidase, 7 – valinearylamidase, 8 – cystinearylamidase, 9 – trypsin, 10 – chymotrypsin, 11 – acid phosphatase, 12 – Naphtol – AS-BI-phosphohydrolase, 13, 14 – galactosidase, 15 – glucuronidase, 16, 17 – glucosidase, 18 – N-acetyl-glucoseamidase, 19 – mannosidase, 20 – fucosidase, + good, (+) – moderate, - no activity, 5 - >40 nmol, 4 – 20 nmol, 3 – 10 nmol, 2- 5 nmol, 1 – 2,5 nmol, 0 – no activity

2 – alkalická fosfatáza, 3 – esteráza (C4), 4 – esteráza-lipáza (C8), 5 – lipáza (C14), 6 – leucínarylamidáza, 7 – valínarylamidáza, 8 – cystínarylamidáza, 9 – trypsin, 10 – chymotrypsin, 11 – kyslá fosfatáza, 12 – naftol-AS-BI-fosfohydroláza, 13, 14 – galaktozidáza, 15 – glukuronidáza, 16, 17 – glukozidáza, 18 – N-acetyl-glukozamidáza, 19 – manozidáza, 20 – fukozidáza, + dobrá, (+) stredná, - žiadna aktivita

In case of Api Coryne® stripes, positive reaction were confirmed in case of nitrate reduction (strains 13SC2, 13SC19, 13SC10), pyrrolidonylarylamidase (13SC19), α-glucosidase (13SC2, 13SPC10, 13SPC4), β-galactosidase (13SC2, 13SC19) and all strains were positive for production of alkaline phosphatase, urease, esculin and hydrolysis of gelatine. A number of research workers in earlier investigation have also reported that streptomycete from soil possess high number of enzymatically active actinomycetes. Results indicate 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.

Conclusions

The search for interesting metabolites especially from streptomycetes requires screening of large number of isolates. The present study reported the antimicrobial activities exhibited by different isolates of streptomycetes isolated from soil amended with plants and compost. This research is based on the hypothesis that streptomycete diversity may be influenced by the diversity of plants species and with addition of compost. However, adaptation has in turn leaded the streptomycetes to produce their own secondary metabolites. Out of many isolates, six of them showed interesting antimicrobial activity against used microorganisms. Therefore, these isolates can be further studied for its applications in producing important agricultural compounds and also as a biocontrol agent against plant pathogenic microorganisms.

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References

Balagurunathan, R., Radhakrishnan, M. (2010) Biotechnological, genetic engineering and nanotechnological potential of actinomycetes. In: Maheshwari, D.K., Dubey, R.C., Saravanamuthu eds. Industrial Exploitation of Microorganisms, New Delhi: I. K. International Publishing House Pvt. Ltd., 302-321.

Bauman, R. (2007) Microbiology with diseases by taxonomy. San Francisco, Calif.: Pearson/Benjamin Cummings.

Bushell, M.E., Smith, J., Lynch, H.C. (1997) A physiological model for the control of erythromycin production in batch and cyclic fed batch culture. Microbiology, 143 (2), 475-480. DOI: 10.1099/00221287-143-2-475

Cazin, J., Wiemer, D.F., Howard, J.J. (1989) Isolation, growth characteristics, and long-term storage of fungi cultivated by attine ants. Applied and Environmental Microbiology, *55* (6), 1346-1350.

Chiani, M., Mazinani, M. (2010) Desferrioxamine B (Desferal) in Streptomyces pilosus. Pakistan Journal of Biological Sciences, 13 (11), 546-550. DOI: 10.3923/pjbs.2010.1151.1155

García, M.C.V., Estrella, F.S., Lopes, M.J., Moreno, J. (2008) Influence of compost amendment on soil biological properties and plants. Dynamic Soil, Dynamic Plant, 1, 1-9.

Gebreyohannes, G., Moges, F., Sahile, S., Raja, N. (2013) Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia. Asian Pacific Journal of Tropical Biomedicine, 3 (6), 426-435. DOI: 10.1016/s2221-1691(13)60092-1

Genilloud, O., González, I., Salazar, O., Martín, J., Tormo, J. R., Vicente, F. (2011) Current approaches to exploit actinomycetes as a source of novel natural products. Journal of Industrial Microbiology & Biotechnology, 38 (3), 375-389. DOI: 10.1007/s10295-010-0882-7

Gopinath, B.V., Vootla, P.K., Jyothi, R., Sadashiva Reddy, K. (2013) Antimicrobial activity of actinomycetes isolated from coal mine soils of godavari belt region, AP, India. Asian Journal of Experimental Biological Sciences, 4, 518-523.

Hoitink, H.A.J., Boehm, M.J. (1999) Biocontrol within the context of soil microbial communities: a substrate dependent phenomenon. Annual Review of Phytopathology, 37, 427-446.

Hoskisson, P.A., Jones, A.L., Van- Wezel, G.P., Barona-Gómez, F. (2012) The evolution of actinomycetes: papers from the 16th International Symposium on the Biology of Actinomycetes. Antonie van Leeuwenhoek, 1-2. DOI: 10.1007/s10482-012-9805-0

Jiang, Y., Han, L., Chen, X., Yin, M., Zheng, D., Wang, Y., Huang, X. (2013) Diversity and bioactivity of cultivable animal fecal actinobacteria. Advances in Microbiology, 3 (1), 1-13. DOI: 10.4236/aim.2013.31001

Kekuda, T.P., Shobha, K.S. (2010) Fascinating diversity and potent biological activities of Actinomycete metabolites. Journal of Pharmacy Research, 3, 250–6.

Korzeniewska, E., Filipkowska, Z., Gotkowska-Płachta, A., Janczukowicz, W., Dixon, B., Czułowska, M. (2009) Determination of emitted airborne microorganisms from a BIO-PAK wastewater treatment plant. Water research, 43 (11), 2841-2851. DOI: 10.1016/j.watres.2009.03.050

Mohseni, M., Norouzi, H., Hamedi, J., Roohi, A. (2013) Screening of antibacterial producing actinomycetes from sediments of the Caspian Sea. International Journal of Molecular and Cellular medicine, 2 (2), 64.

Murray, M. Y. (2011) Microbial Secondary Metabolites: Comprehensive Biotechnology. Burlington: Academic Press.

Pandey, A., Ali, I., Butola, K. S., Chatterji, T., Singh, V. (2011) Isolation and characterization of Actinomycetes from soil and evaluation of antibacterial activities of Actinomycetes against pathogens. International Journal of Applied Biology and Pharmaceutical technology, 2, 384-392.

Ravikumar, S., Inbaneson, S. J., Uthiraselvam, M., Priya, S. R., Banerjee, M. B. (2011) Diversity of endophytic actinomycetes from Karangkadu mangrove ecosystem and its antibacterial potential against bacterial pathogens. Journal of Pharmacy Research, 1(4), 294-296.

Sengupta, S., Pramanik, A., Ghosh, A., Bhattacharyya, M. (2015) Antimicrobial activities of actinomycetes isolated from unexplored regions of Sundarbans mangrove ecosystem, BMC Microbiology, 15 (1), 1. DOI: 10.1186/s12866-015-0495-4

Sharma A.D., Kainth S., Gill P.K. (2005) Inulinase production using garlic (*Allium sativum*) powder as a potential substrate in *Streptomyces* sp. Journal of Food Engineering, 77, 1–6. DOI: 10.1016/j.jfoodeng.2005.06.072

Shirling, E.T., Gottlieb, D. (1966) Methods for characterization of Streptomyces species1. International Journal of Systematic and Evolutionary Microbiology, 16 (3), 313-340. DOI: 10.1099/00207713-16-3-313

Strohl, W.R. (2004) Antimicrobials. In: Bull, A.T. ed. Microbial diversity and bioprospecting, Washington DC: ASM press.

Valli, S., Suvathi, S.S., Aysha, O.S., Nirmala, P., Vinoth, K.P., Reena, A. (2012) Antimicrobial potential of Actinomycetes species isolated from marine environment. Asian Pacific Journal of Tropical Biomedicine, 2 (6), 469-473. DOI: 10.1016/s2221-1691(12)60078-1

Větrovský, T., Steffen, K.T., Baldrian, P. (2014) Potential of cometabolic transformation of polysaccharides and lignin in lignocellulose by soil Actinobacteria. PloS One, 9 (2), e89108. DOI: 10.1371/journal.pone.0089108

Vítězová, M. (2013) Characterisation of actinomycetes community from the heavy metals-pollute soil. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 61, 1471-1478. DOI: 10.11118/actaun201361051471

Vos, P., Garrity, G., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Whitman, W. (2011) Bergey's Manual of Systematic Bacteriology, The Firmicutes, New York: Springer Science & Business Media.

Wink, J. Compendium of Actinobacteria. (2014) [Online] Available at: http://www.dsmz.de/bacterial-diversity/compendium-of-actinobacteria.html [Accessed 10 June 2016].