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CHARACTERISTIC OF *STREPTOMYCES* SPECIES WITH ANTIMICROBIAL ACTIVITY AGAINST SELECTED PHYTOPATHOGENIC BACTERIA AND FUNGI

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INTRODUCTION

- ***Streptomyces*** sp.

orig.: Kovácsová

- Polyketide synthases (PKS-I) and Nonribosomal peptide synthetases (NRPS)

INTRODUCTION

Pseudomonas syringae

http://lookfordiagnosis.com/mesh_info.php?term=Pseudomonas+Syringae&lang=1

Erwinia amylovora

http://news.cornell.edu/stories/2012/01/strep-resistant-fire-blight-found-new-york-orchards

Clavibacter michiganensis* subsp. *sepedonicus

http://www.bitkisagligi.net/Patates_Clavibacter_michiganensis_sepedonicus.htm

Xanthomonas campestris

http://vegetablemdonline.ppath.cornell.edu/PhotoPages/Crucifers/BlkRot_Cruc/BR_CrucFS5.htm ©M.T. McGrath

Botrytis cinerea

http://www.uoguelph.ca/~gbarron/MISCELLANEOUS/botrytis.htm

Alternaria tenuissima

http://bugwoodcloud.org/images/384x256/5220073.jpg

UGA5220073

Fusarium poae

http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/Fusarium.aspx

Alternaria arborescens

http://passionpepper.altervista.org/parassiti-malattie/?doing_wp_cron=1432279998.3952250480651855468750

commercially produced antifungals obtained from *Streptomyces:*

- by *Streptomyces griseus* used for bacterial diseases of lawn and cherry leaf spot
- from *Streptomyces griseoviridis* and *Streptomyces rimosus* used in biocontrol against *Fusarium* sp., *Botrytis* sp., *Alternaria brassicola*, *Rhizoctonia solani*, *Pythium* sp., *Phomopsis* sp. and *Phytophthora* sp.

THE AIM OF THE STUDY

In this study, streptomycetes isolated from soil, compost and soil amended with compost were screened for their bioactivity against phytopathogenic microorganisms.

MATERIAL AND METHODS

- **Isolation of actinomycetes**- Pochon medium (28 °C, 6 days)
- purification - ISP2 medium

- 57 colonies showed typical features of streptomycetes
- 20 different presumptive streptomycetes selected for further analysis

- Morphological characterization - (ISP) International Streptomyces Project
- production of melanoid pigments - ISP6 medium

Table 1 Isolates of streptomycetes selected for antimicrobial activity analysis

Origin of streptomycete isolates	Labeling of streptomycete isolates
Soil	6 K14, 186 K14
Compost	164 K14, 166 K14, 167 K14, 170 K14, 171 K14, 172 K14, 177 K14, 178 K14
Soil amended with compost	12 VK13, 39 VK13, 51 VK13, 76 K14, 101 K14, 104 K14, 116 K14, 207 K14, 224 K14, 244 K14

MATERIAL AND METHODS

DNA extraction, amplification and sequencing

- DNA extraction according to Sambrook (2001)
- 16S rRNA PCR amplification (Cook and Meyers, 2003):
  - F1: 5´-AGAGTTTGATCITGGCTCAG-3´
  - R5: 5´-ACGGITACCTTGTTACGACTT-3´
- for identification of streptomycetes 16S rRNA gene analysis was used.

Screening genes coding NRPS and PKS-I

	Labeling of primers	Secquences 5´- 3´	References	Expected size of products
NRPS	A3F	GCSTACSYSATSTACACSTCSGG	Ayuso-Sacido, Genilloud, 2005	700 – 800 bp
	A7R	SASGTCVCCSGTSCGGTAS		
PKS-I	PKS-I-A	GCSATGGAYCCSCARCARCGSVT	Schirmer et al., 2005	700 bp
	PKS-I-B	GTSCCSGTSCCRTGSSCYTCSAC		

MATERIAL AND METHODS

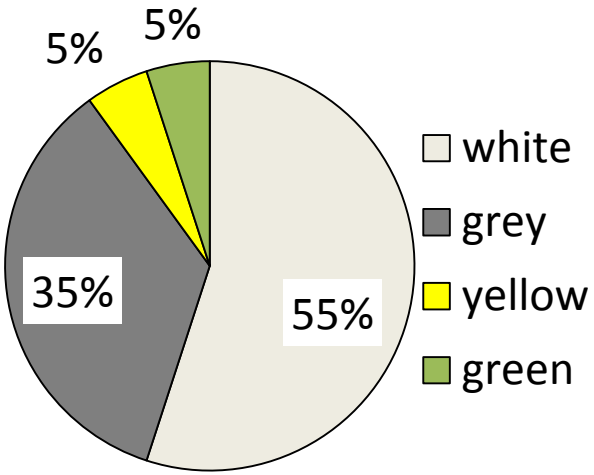
Detection of antimicrobial activity

- agar plug method

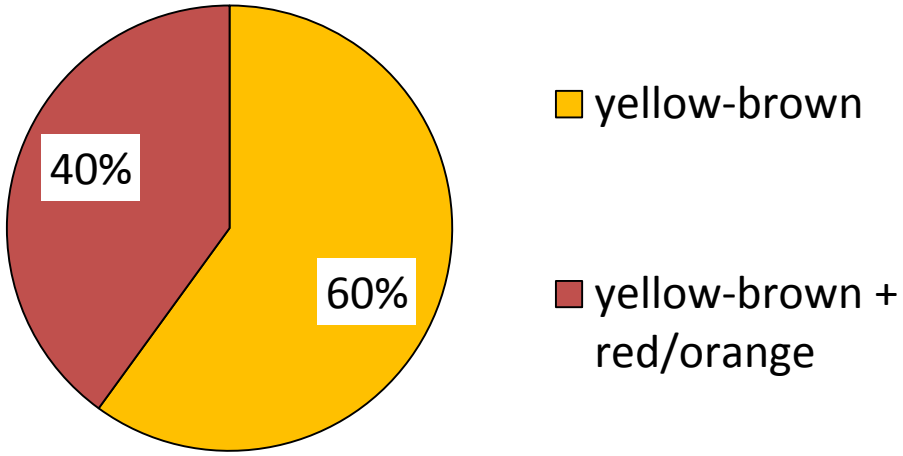
Test-microorganisms		Medium and conditions of cultivation
Bacteria	*Xanthomonas campestris* (CCM 22), *Pseudomonas syringae* (CCM 2868), *Erwinia amylovora* (CCM 1114)	Yeast-glucose agar 24 h 30 °C
	Clavibacter michiganensis subsp. *sepedonicus* (CCM 7014)	Tryptone-soya agar 72 h, 25 °C
Fungi	*Botrytis cinerea* (29B11), *Alternaria tenuissima* (16A6), *Fusarium poae* (12A18), *Alternaria arborescens* (15H6)	Malt extract agar 72 h, 25 °C

RESULTS – Morphology of isolates

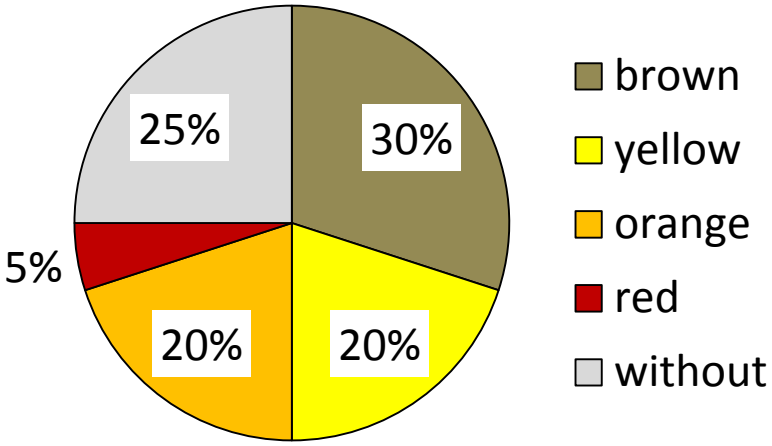
Aerial mycelium



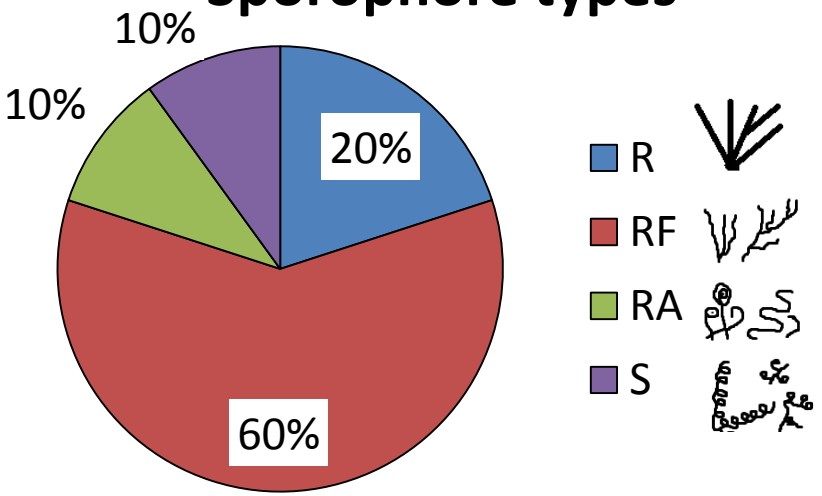
Substrate mycelium



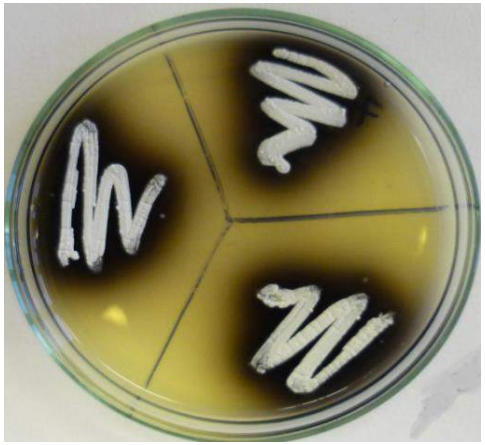
Production of pigments



Sporophore types

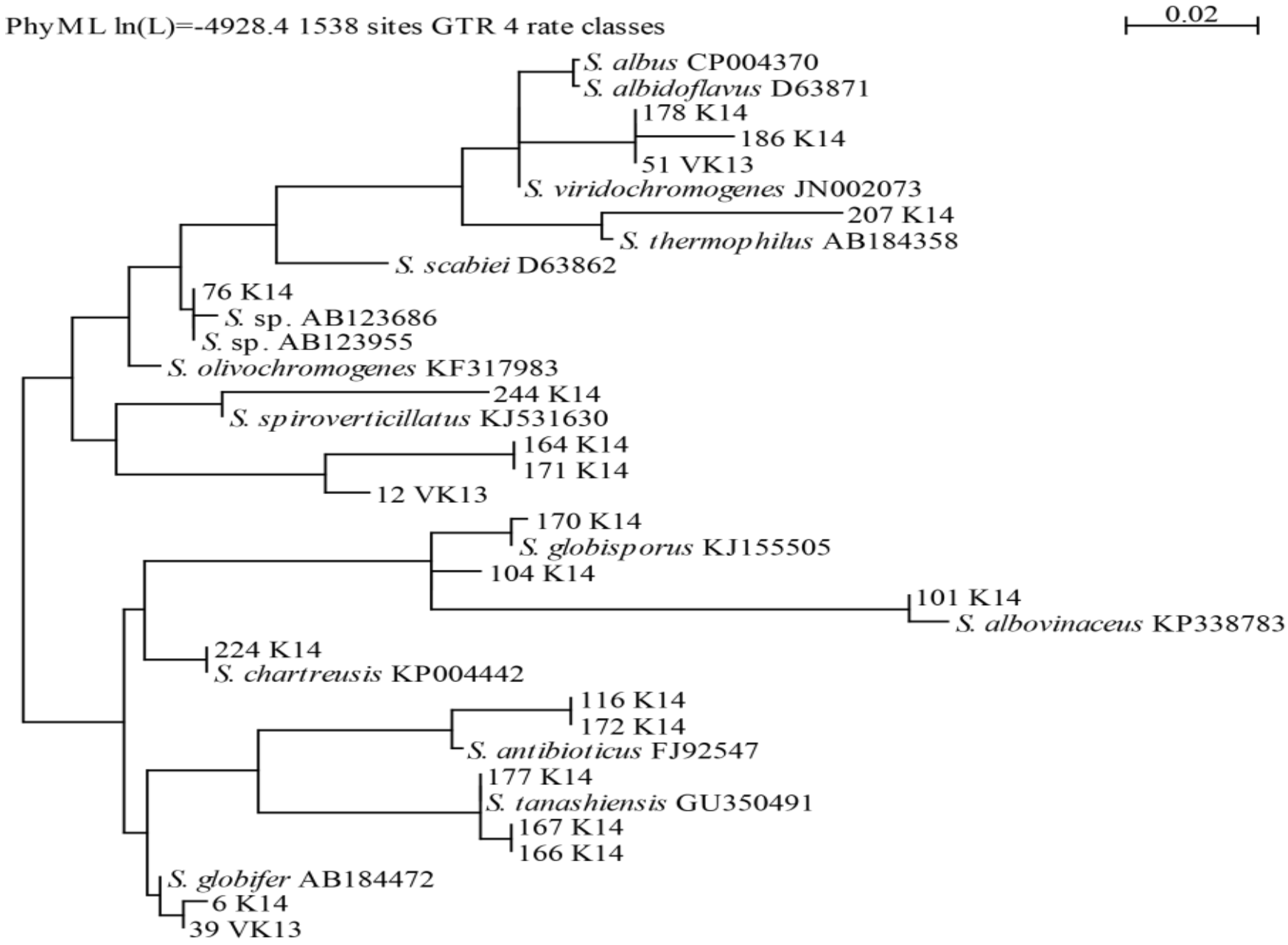


Production of melanin pigment on ISP6 – 45% of streptomycetes

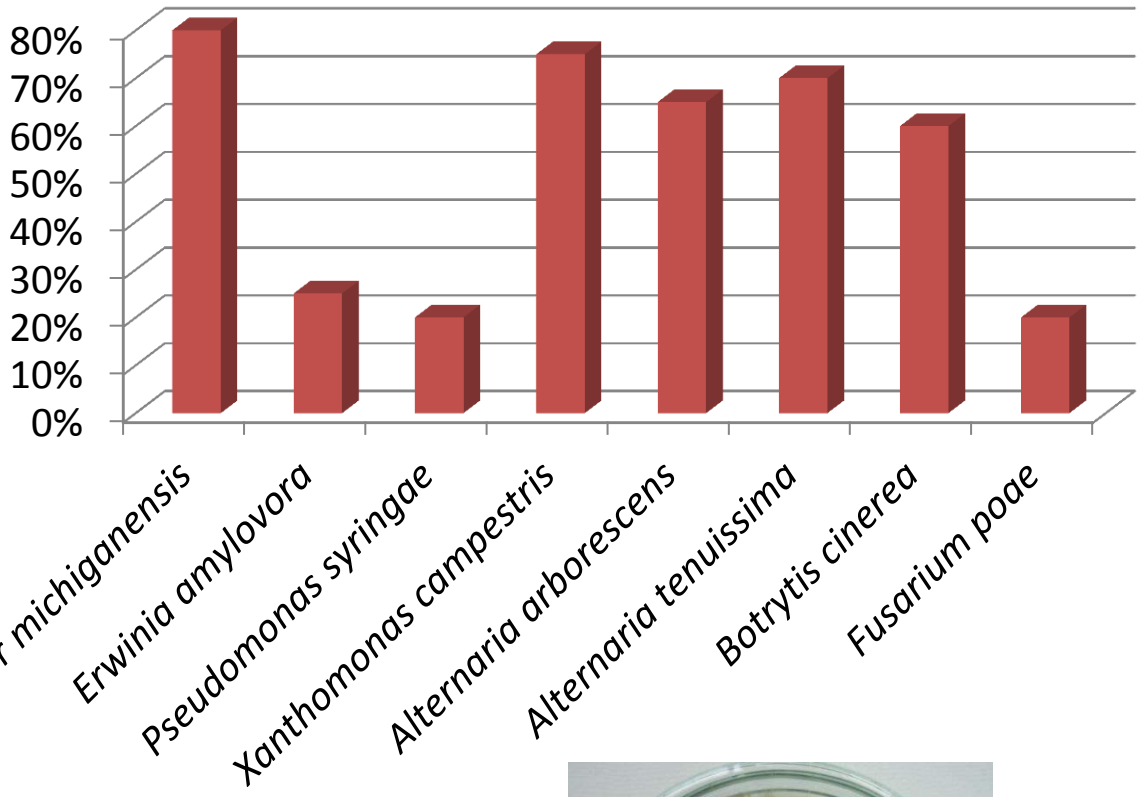


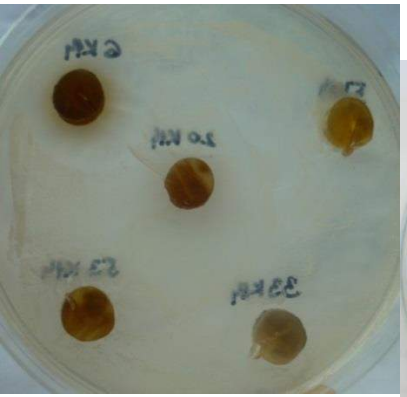
The isolates with the best antimicrobial activity belonged to the white colour series with sporophore type flexibilis

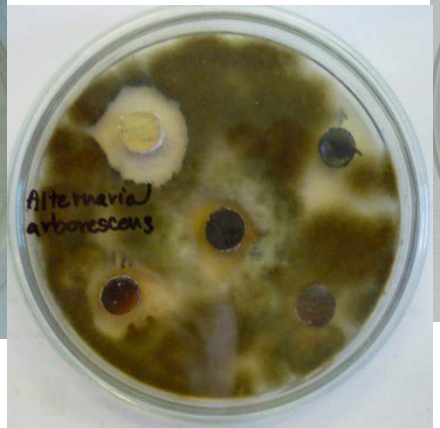
The relatedness to genus *Streptomyces* confirmed
15 different isolates recognized

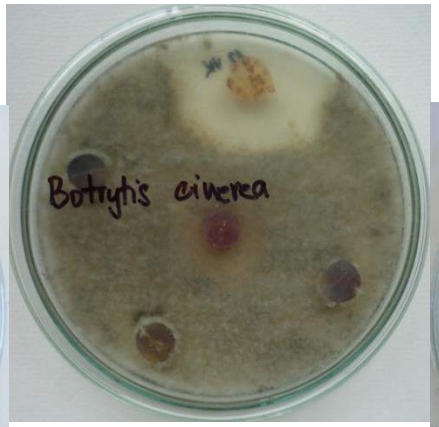


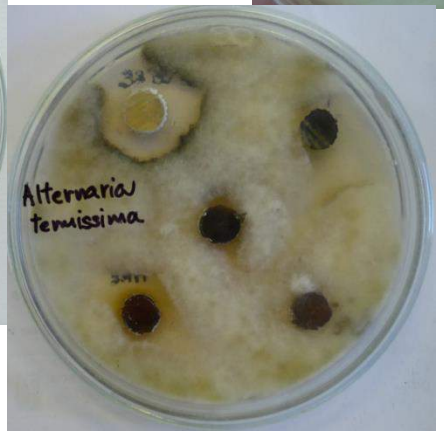
RESULTS – Antimicrobial activity

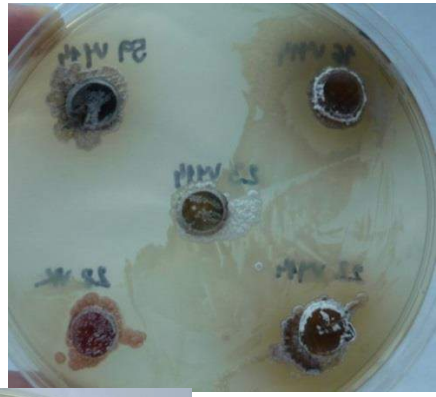








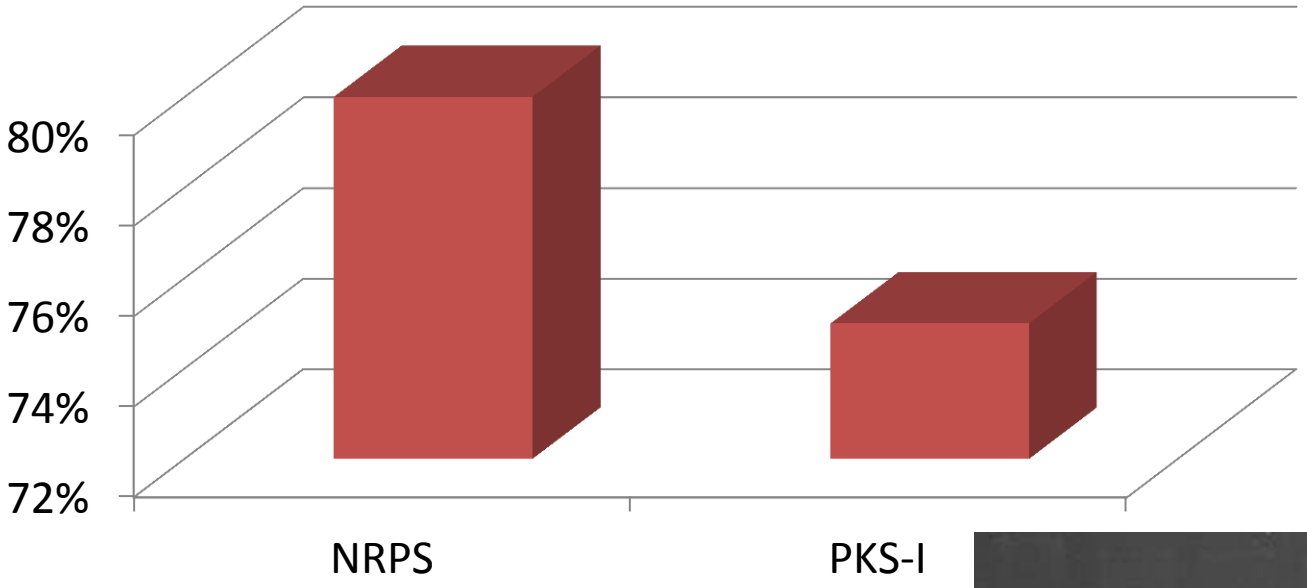




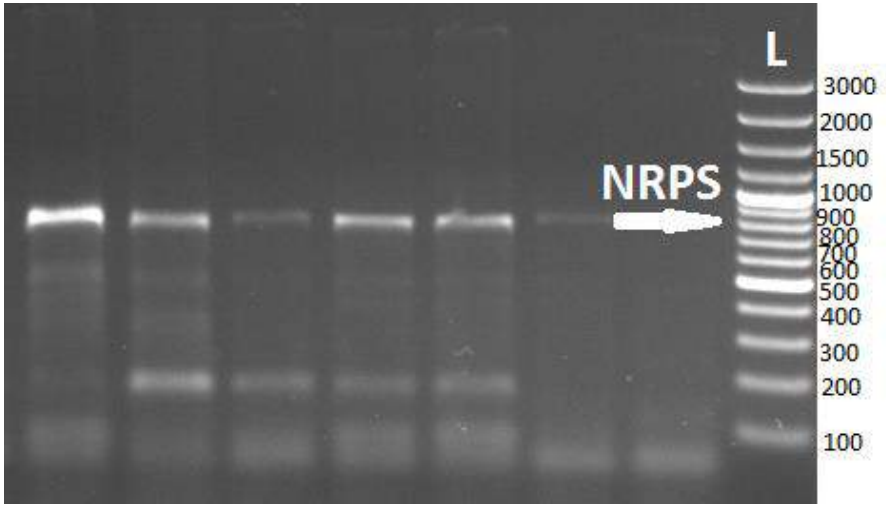
The results of antimicrobial activity measurement and screening genes NRPS, PKS-I

Isolates	*Xanthomonas campestris*	*Pseudomonas syringae*	*Erwinia amylovora*	*Clavibacter michiganensis subsp. sepedonicus*	*Fusarium poae*	*Alternaria tenuissima*	*Alternaria arborescens*	*Botrytis cinerea*	NRPS	PKS-I
104 K14	16	18	22	50	30	16	21	30	-	+
51 VK13	16	13	9	48	21	44	34	32	+	W
167 K14	20	9	16	26	9	15	9	13	+	W
170 K14	17	12	9	40	9	20	26	27	+	W
207 K14	16	20	9	9	14	9	14	9	-	-
164 K14	9	9	17	26	9	28	27	20	+	-
171 K14	14	9	13	9	9	15	22	18	+	W
178 K14	9	9	9	16	9	12	24	18	+	W
12 VK13	9	9	9	50	18	36	32	38	+	+
166 K14	22	9	9	32	9	15	9	9	+	W
6 K14	24	9	9	70	9	9	21	17	+	W
101 K14	32	9	9	9	9	13	9	9	+	+
116 K14	17	9	9	28	9	21	9	9	+	W
172 K14	44	9	14	50	9	9	12	9	-	W
177 K14	9	9	9	30	9	18	17	20	+	-
186 K14	16	9	9	16	9	9	9	9	+	+
224 K14	14	9	9	21	9	9	9	15	+	+
76 K14	9	9	9	12	9	20	13	9	+	-
244 K14	30	9	9	9	9	20	12	16	+	+
39 VK13	13	9	9	26	9	9	9	9	-	-

RESULTS - genes coding NRPS and PKS-I



The percentage of representation of NRPS and PKS-I in genome of isolates



L – GeneRulerTM 100 bp Plus DNA Ladder, ready-to-use (Fermentas)

The results of antimicrobial activity measurement and screening genes NRPS, PKS-I

Isolates	*Xanthomonas campestris*	*Pseudomonas syringae*	*Erwinia amylovora*	*Clavibacter michiganensis subsp. sepedonicus*	*Fusarium poae*	*Alternaria tenuissima*	*Alternaria arborescens*	*Botrytis cinerea*	NRPS	PKS-I
104 K14	16	18	22	50	30	16	21	30	-	+
51 VK13	16	13	9	48	21	44	34	32	+	W
167 K14	20	9	16	26	9	15	9	13	+	W
170 K14	17	12	9	40	9	20	26	27	+	W
207 K14	16	20	9	9	14	9	14	9	-	-
164 K14	9	9	17	26	9	28	27	20	+	-
171 K14	14	9	13	9	9	15	22	18	+	W
178 K14	9	9	9	16	9	12	24	18	+	W
12 VK13	9	9	9	50	18	36	32	38	+	+
166 K14	22	9	9	32	9	15	9	9	+	W
6 K14	24	9	9	70	9	9	21	17	+	W
101 K14	32	9	9	9	9	13	9	9	+	+
116 K14	17	9	9	28	9	21	9	9	+	W
172 K14	44	9	14	50	9	9	12	9	-	W
177 K14	9	9	9	30	9	18	17	20	+	-
186 K14	16	9	9	16	9	9	9	9	+	+
224 K14	14	9	9	21	9	9	9	15	+	+
76 K14	9	9	9	12	9	20	13	9	+	-
244 K14	30	9	9	9	9	20	12	16	+	+
39 VK13	13	9	9	26	9	9	9	9	-	-

CONCLUSIONS

- There were no substantial differences in antimicrobial activity patterns and in the presence of genes coding NRPS, PKS-I among the isolates obtained from soil, compost or soil amended with compost.

- Each of our streptomycete isolates was active against at least two phytopathogenic microorganisms.

- The majority of isolates belonged to white colour series with sporophore type *flexibilis*.

- Our most active isolates, 104 K14 and 51 VK13, were isolated from the sample of soil amended with compost.

THANK YOU FOR YOUR ATTENTION