Scientific Research and Essay Vol.4 (10), pp. 997-1005, October, 2009
Available online at http://www.academicjournals.org/sre
ISSN 1992-2248 © 2009 Academic Journals

Full Length Research Paper

Comparison of *Streptomyces* diversity between agricultural and non-agricultural soils by using various culture media

Mustafa Oskay

Biology Department, Faculty of Sciences and Arts, Celal Bayar University, Manisa, Turkey. E-mail: mustafa.oskay@bayar.edu.tr

Accepted 18 August, 2009

Streptomycetes play a key role in the sustainability of agriculture and indicates the level of health of soil, especially when considering the richness of them that are involved in biological control of soil borne diseases. 20 different soil samples were taken from agricultural (7) and non-agricultural places (13) and populations of streptomycetes were quantified in order to select the general culture media that had better reflect the changes of these bacteria. The most efficient medium for the isolation of *Streptomyces* was starch casein agar by the addition of nystatin. Pretreatment of soil samples with $CaCO_3$ (1%) increased the streptomycetes occurring on the isolation plates. To establish a correlation with soil physico-chemical parameters, such as pH, salt, N, P, K, Na, Fe, Zn and Cu were also determined, most of the correlations being significantly positive on the quantification of *Streptomyces* diversity. Streptomycete counts ranged from a high of 6.7×10^6 to a low of 2.3×10^6 cfu/g dry soil of non-agricultural soils. Streptomycetes constituted 4.8 to 45.8% of the total culturable bacterial community. Higher streptomycete densities were greatest in non-agricultural soils with an average of 14.0% compared to agricultural soils with average of 10.1%. These results suggest that these bacteria may be represent an unexplored resource for pharmaceutical drug discovery but also may provide additional disease control in agriculture.

Key words: Agricultural soil, non-agricultural soils, culturable bacteria, isolation, pretreatment, *Streptomyces* diversity.

INTRODUCTION

The soil microbes perform a wide range of function in the ecosystem. Among soil organisms, bacteria and fungi, actively participate in organic matter decomposition liberating chemical nutrients and furthering plant growth. Bacteria and fungi also play an important role for the stability and productivity of agricultural soils. Therefore for a sustainable and nature saving agriculture with high crop yields the genetic diversity of microbes may play an important role and could be used as an indicator for soil quality, which has to be determined for understanding turnover processes (Schloter et al., 2003; Vargas Gil et al., 2009a). Therefore, soil microbes have an important role to the subsistence on earth, because it has the role on biological and chemical cycling among the flora, fauna and life of microbes itself. Each type of microbes fills as a unique niche, plays a different role in nutrients cycling and soil structure. Microbial communities may be used as indicators of the ecological equilibrium between pathogens and biocontrol agents naturally suppress the incidence of diseases. Since microbial diversity includes the number of different fungal and bacterial species and their relative abundance (Lartey, 2006; Martinez Blanco et al., 2007; Vargas Gil et al., 2009b). Growth of microbial populations and their action on soils are dependent on the interaction between soil type, plant species and its rhizosphere localization. Microorganism numbers vary in and between different soil types and conditions, with bacteria being the most numerous (Vieira and Nahas, 2005).

The isolation and characterization of pure cultures of some microbial species are as important as understanding their objective existence in natural ecosystems. The isolation of diverse and novel pure cultures of actinomycetes provides a theoretical guide for the exploitation and utilization of actinomycete resources (Li et al., 1996). *Streptomyces* are one of the groups of actinomycetes that are widely spread in both terrestrial and aqua-

tic environments (Cross, 1989; Locci, 1989; Williams et al., 1989). They play an important role in the circulation of organic substances in nature. Many representatives of this group have important practical interest as producers of antibiotics and other biologically active substances of high commercial value and are being routinely screened for new bioactive substances, which have wide use in biotechnological production (Anderson and Wellington, 2001; Vijayakumar et al., 2007). Their distribution and predominance depends mainly on several factors, such as nutrient availability, temperature, pH, moisture, soil type, season and climate (Waksman, 1961; Williams et al., 1989; Katsifas et al., 1999; Saadoun and Gharaibeh, 2003). *Streptomyces* have been found in all known soils of the world yet their number, role in biocenosis and biochemical activity vary depending on ecological and geographical conditions (Dolotkeldieva and Totubaeva, 2006). One of the aims of biodiversity studies of action-mycetes is to use effective isolation procedures to study the distribution of actinomycetes in various climatic and ecological environments (Li et al., 1996). Many studies on the ecological distribution of soil streptomycetes and their biotechnological importance in detail has been reported from normal and agricultural soils (Conn and Leci, 1998; Katsifas et al., 1999; Mitra et al., 2008; Zenova et al., 2008; Grishko and Syshchikova, 2009; Vargas Gil et al., 2009b).

Some soil microorganisms have been studied in detail however; studies that are more comprehensive are nee-ded to understand the diversity, distribution and ecology of the large majority of streptomycetes in terrestrial habitats. The objectives of this study were to compare and describe the diversity of *Streptomyces* from different agricultural and non-agricultural fields, which have different crops and vegetation in relation to soil chemical properties. These areas are yet poorly studied and repre-sent diverse and largely unscreened ecosystem for the isolation of *Streptomyces* that potent for pharmaceutical industry and agriculture.

MATERIALS AND METHODS

Soil sampling and processing

7 soil samples were collected from agricultural fields (AG) (Corn, cotton, wheat, barley, vegetable, vineyard and orchard fields) in Manisa Province, Turkey and 13 of non-agricultural soils samples (NAG) were collected from various locations of North Cyprus and its surroundings into sterile plastic bags, to avoid external conta-mination.. Every sample is a mixture of soils from five to ten holes at a depth of from 10 to 30 cm. Soils were air-dried and stored at 4.0℃ until processed.

Calcium carbonate soil treatment

A method (El-Nakeeb and Lechevalier, 1963) with some modify-cations was used according to our laboratory conditions. The air-dried soil (10 g) was mixed in a mortar with 1% of calcium carbon-carbonate ($CaCO_3$) and then incubated for 2 days at 30.0℃ in a closed inverted sterile Petri dish in which a high relative humidity was maintained by water saturated of filter paper. To assess the effect of pretreatment, soils without $CaCO_3$, served as a control.

Isolation and enumeration of *Streptomyces*

10 g of pretreated soil samples were added to 90 ml sterilized water in 250 ml Erlenmeyer flasks. Flasks were shaken on rotary shaker at 200 rpm for 30 min. All samples were diluted (up to 10^{-7}) with sterile distilled water prior to inoculation into the isolation plates. Isolation of streptomycetes were performed by soil dilution plate technique using different media such as starch-casein agar (SCA) (Kuster and Williams, 1964), glycerol asparagine agar (ISP 5) (Shirling and Gottlieb, 1966), potato dextrose agar (PDA) and nutrient agar (NA). All isolation media also contained nystatin at final concentrations of 50 µg/ml, to minimize fungal contamination (Waksman, 1961). The pH of each medium was adjusted to 7.0 - 7.5 to match that of the soil sample. 1 ml of soil of 10^{-6} dilution (in most situations) is plated out and thoroughly mixed with about 20 - 25 ml of melted desired agar medium at around 45.0 - 50.0℃. After gently rotating, the isolation plates were incubated at 28.0 - 30.0℃ for 7 - 14 days to allow sufficient time for fast-growing streptomycetes or longer for the slow-growing ones.

Streptomyces counts

Streptomycetes were quantified on each plate by eye and with the aid of a stereomicroscope (Olympus, magnification: 10 - 90 x); then recognized by the presence of filamentous hyphae; a characteristic that was just within the range of detection at the highest magni-fication used and/or by the formation of floccose, powdery, tough, leathery colonies that adhered to the agar surface and colors of pigmentation including diffusible pigments (Waksman, 1961; Williams et al., 1989; Cross, 1989; Anderson and Wellington, 2001). Colony formation units (cfu) per gram counting the average for each soil sample estimated for densities of total culturable streptomy-cetes and bacteria. The total number of streptomycete colonies observed was counted and representatives with different morpho-logies were obtained in pure culture by repeated transfer from a single colony. Slants of yeast extract-malt extract agar (ISP2) or oatmeal agar (ISP4) containing pure cultures were maintained at 4℃ in culture collection of Biology Department, Celal Bayar University, Manisa Turkey.

Physico-chemical analyses of the soil samples

Samples were also taken from each site for analyzing physico-chemical parameters such as soil structure, lime ($CaCO_3$), satura-tion, pH, salinity, available nitrogen (N), phosphorus (P), potassium (K), sodium (Na), ferrous (Fe), copper (Cu), zinc (Zn), manganese (Mn), calcium (Ca) and magnesium (Mg) (Scheffer and Schachtschabel, 1966; Schlichting and Blume, 1966; Ryan et al., 1996). The air-dried soil samples were ground mixed properly and sieved to remove gravel and debris. Physico-chemical parameters of soils were determined in the Vali Ecemiş Soil Analysis Laboratory of Manisa, Directorate of the Ministry of Agriculture (report number: 3644/63-06; report date: 20.12.2006).

Taxonomic grouping of isolates

Streptomyces colonies were placed in genera and taxonomic groups based on the morphological, cultural characteristics and chemical compositions of cells. Morphological and cultural observa-tion were carried out by using the methods and media proposed as described in the International *Streptomyces* Project (ISP) (Shirling

and Gottlieb, 1966) and the Bergey's Manual of Systematic Bacteriology (Cross, 1989; Williams et al., 1989). The morphology of the spore bearing hyphae with the entire spore chain, the structure and arrangement of the spore chain with aerial mycelium of the streptomycetes were examined using slide culture technique (Cross, 1989). After growth, the slide cultures were examined under light microscope (Magnification, 400 and 1000X). Colors were determined according to the scale adopted by Prauser (1964) and isolates were grouped into separate color series according to the system proposed by Shirling and Gottlieb (1966). For chemotaxonomic studies, aerial and substrate mycelia of streptomycetes were scraped from the ISP2 plates and processed for the isomers of diaminopimelic acids (*LL*-DAP or *meso*-DAP) and whole cell sugar patterns by the method of Lechevalier and Lechevalier (1970). Precoated silica gel plates (20X20, 60 F_{254}, Merck, Darmstadt, Germany) were used for thin layer chromatography.

Statistical analysis

The average values of the number of cfu g/dry soil were statistically analyzed by Minitab 13.20 (Minitab Inc., 2000) program by the multivariate cluster analysis to find out the similarity (%) of *Streptomyces* diversity between agricultural and non-agricultural soils.

RESULTS AND DISCUSSION

Streptomycetes are best known as soil bacteria and largely occur as dormant spores (Waksman, 1961; Cross, 1989; Williams et al., 1989). The distributions and ecological roles of Streptomycetes in the agricultural environment have remained an unresolved issue in soil biology. In an effort to gain a better understanding of soil streptomycete diversity, a culture-dependant study was undertaken using samples collected from agricultural and non-agricultural soils.

The occurrence of streptomycetes in the 20 soil samples (13 from non-agricultural and 7 from agricultural soil) were extensively investigated using $CaCO_3$ treatment procedure and different media. The distributions of colonies in four different media were expressed by summing up the colonies observed of the 10^{-6} dilution series. Mean of *Streptomyces* colonies were 4.1 x 10^6 (14.0%) and 3.3 x 10^6 (10.1%) for NAG and AG, respectively. However, the total isolated bacterial colonies (adding all the colonies obtained in four different media) were 35.5 x 10^6 and 29.5 x 10^6 for NAG and AG, respectively (Table 1). The influence of culture media on streptomycete diversity was evaluated. Among the four different media used, SCA was the most effective medium supplemented with nystatin (50 µg/ml) in the isolation of streptomycetes from soils (average, 5.4 x 10^6 cfu/g). PDA was the second most effective (3.7 x 10^6 cfu/g) while GAA, was the third (3.3 x 10^6 cfu/g). NA was the least effective medium for the isolation of streptomycetes (2.6 x 10^6 cfu/g) (Figures 1 and 2). Varied nature of growth of the *streptomycetes*, chemical parameters of soils (Table 2) and the type of selective media used may be the three possible reasons for this result. The amount of time for *Streptomyces* colony development was similar in all culture media tested. The use of nystatin in culture media notably reduced fungal contamination; it was effective in the count of streptomycetes. Total numbers of *Streptomyces* in each soil samples of $CaCO_3$ treatment were expressed as the average number of colonies in four different media (Table 1).

In the control experiment, a significantly lower amount of streptomycetes was recorded, compared with $CaCO_3$ treatment. Average of *Streptomyces* densities were recorded 1.9 x 10^6 (5.0%) and 1.6 x 10^6 (3.9%) for NAG and AG, respectively. However, the amount of total culturable bacteria increased as 42.5 x 10^6 and 38.6 x 10^6 for NAG and AG, respectively.

Results revealed that the streptomycetes diversity of the sampling sites was influenced by the chemical nature of the soil. A very typical pattern of soil characteristics of almost all of the samples were observed, that is low in salinity, P and Zn (except the sample site, ST8) (Table 2). Among the exchangeable cations (Fe, Na, K, P, Cu, Zn, Mn, Ca and Mg), Ca^+ (4256 ppm) was present in the highest amount following Na^+, Mg^+ K^+, Fe^+ and Mn^+. The soil pH was ranging from neutral to slightly alkaline (pH range 7.23 to 7.78). Soil of the ST8 contains higher amounts of P, which supports the proliferation of *Streptomyces* (Parfitt et al., 2005). The total N was observed between 1.33 ppm in ST3 and 134.43 ppm in ST14. The highest of *Streptomyces* colonies were observed in site, ST8 (6.7 x 10^6 cfu/g), having rich N, P, Fe, Mn and Ca; clayed-loamy soil and the lowest number in ST15 (1.9 x 10^6 cfu/g), that site containing wheat vegetation. The highest number of total viable bacteria was observed in ST9 (48.1 x 10^6 cfu/g) and the lowest in ST18 (20.4 x 10^6 cfu/g) of vineyard soils.

There was significant variation in total viable bacterial and *Streptomyces* densities between field types *in vitro*. Overall, streptomycete densities were negatively correlated with total bacterial densities and streptomycete densities were positively correlated with vegetations. Similar patterns were found within each soil type. Cornfields had a marginally significantly greater density of streptomycetes (6.2 x 10^6 cfu/g) than other agricultural fields that had streptomycete density ranging from 1.9 to 3.8 x 10^6 cfu/g. However, according to the all-statistical analyses, there was not relationship between the intensity of streptomycete in AG and NAG fields, which were similar percentage at least 98.47% (Figure 3). This result suggest that just a small percentage of soil streptomycete is culturable (1 - 3%) (Watve et al., 2001), even using a set of culture media (Waksman, 1961). Therefore, using a selective media is a highly necessary step, because *Streptomyces* densities are directly affected by culture medium. However, considering the different of culture media and many environmental factors, most streptomycetes can use a wide variety of compounds as energy source such as glucose, starch, amino acids and proteins. The best nitrogen sources for these bacteria are proteins, peptones, amino acids, nitrates and ammonium salts (Waksman, 1961; Shirling and Gottlieb, 1966). In addition, the main factors that determine

Table 1. Comparison of culturable *Streptomyces* diversity and total bacteria between NAG and AG.

Sites	Calcium carbonate treatment			No treatment		
	Culturable *Streptomyces* counts	Total viable bacterial counts	*Streptomyces* %	Culturable *Streptomyces* counts	Total viable bacterial counts	*Streptomyces* %
ST1[b]	5.2[a]	28.6	18.1	2.7	36.7	7.4
ST2	4.7	32.8	14.3	2.2	34.4	6.4
ST3	4.4	36.4	12.0	1.4	48.6	2.9
ST4	3.3	38.7	8.5	2.2	44.3	5.0
ST5	2.6	40.2	6.5	1.3	41.2	3.1
ST6	3.5	41.3	8.5	1.6	46.6	3.4
ST7	4.8	30.4	15.8	2.6	33.9	7.7
ST8	**6.7**[c]	22.3	**45.8**	**3.8**	36.8	**10.3**
ST9	2.3	**48.1**	***4.8***	0.9	**58.4**	***1.5***
ST10	4.2	32.6	12.9	1.3	40.5	3.2
ST11	2.7	39.5	6.8	1.1	45.2	2.4
ST12	2.3	42.2	5.4	0.8	53.7	***1.5***
ST13	6.4	28.3	22.6	3.4	33.4	10.2
ST14	6.2	36.2	17.1	2.8	48.9	5.7
ST15	***1.9***	24.6	7.7	0.9	30.1	3.0
ST16	2.2	33.7	6.5	1.1	33.8	3.2
ST17	3.8	31.3	12.1	2.6	43.7	6.0
ST18	1.8	***20.4***	8.8	***0.7***	***28.6***	2.4
ST19	3.6	29.2	12.3	1.7	40.3	4.2
ST20	3.3	30.6	10.8	1.3	44.8	2.9

[a] Average of 40 plates of four different media, viable counts = $x10^6$ cfu/g dry soil.
[b] Samples of ST1-ST13 are non-agricultural soils, ST14-ST20 are agricultural soils (ST14: Corn, ST15: cotton, ST16: wheat, ST17: barley, ST18: vegetable ST19: vineyard and ST20: orchard fields).
[c] highest values are indicated with **bold**, lowest values are given **bold** and *italic*.

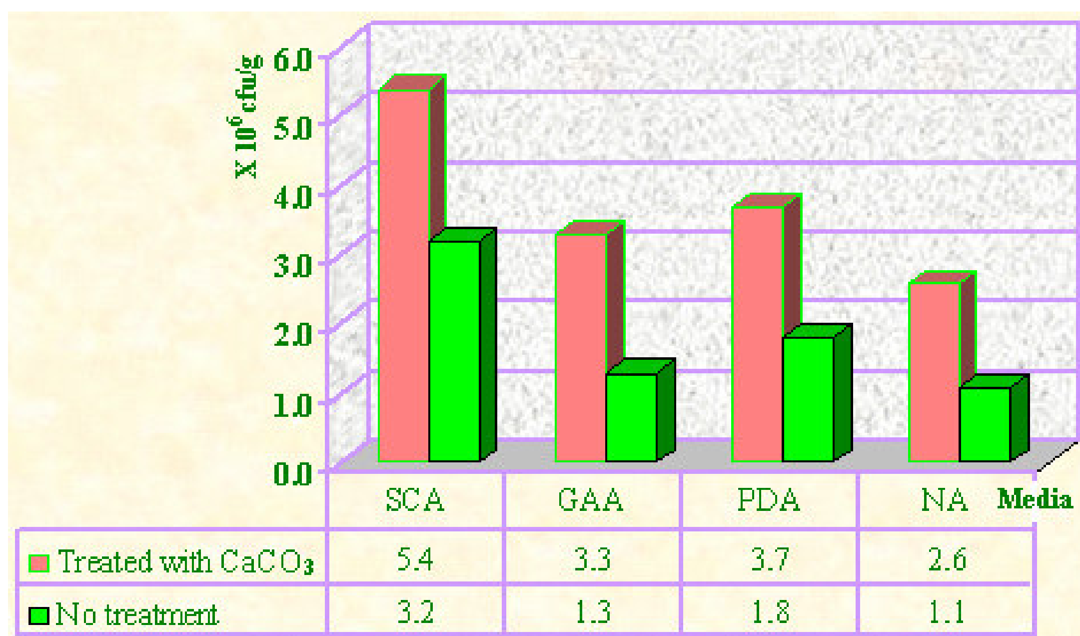


Figure 1. Comparison of the influence of culture media on culturable *Streptomyces* counts (Average of the total *Streptomyces* populations of AG and NAG soils; counts from 200 plates of each medium; cfu: colony-forming units).

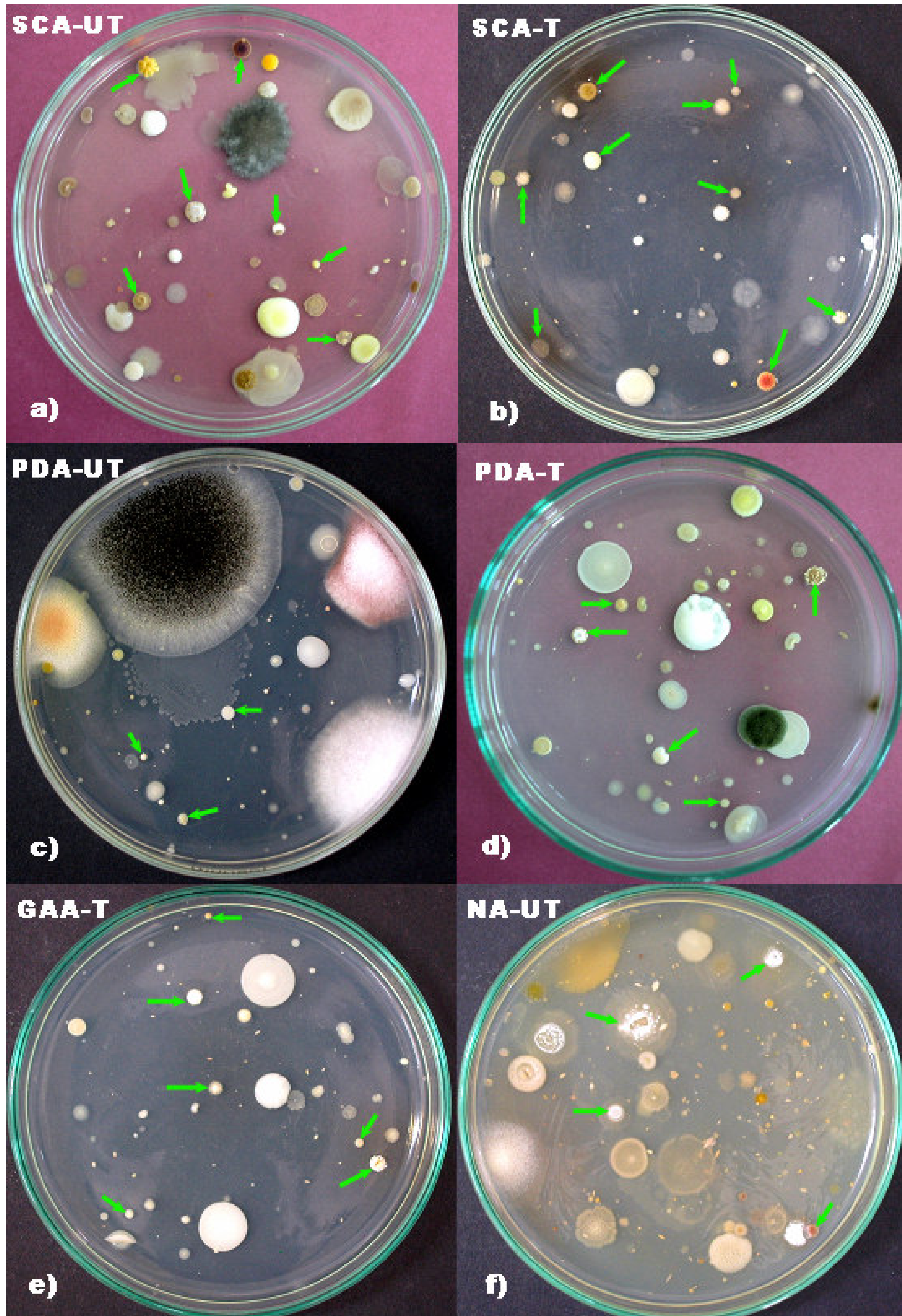


Figure 2. Streptomycete colonies on four different media, SCA; starch casein agar (a-b), PDA; potato dextrose agar (c-d), GAA; glycerol asparagine agar (e), NA; nutrient agar (f), in addition –UT; untreated, –T; treated with $CaCO_3$, dilution; 10^{-6}. Note that the majority of colonies on these media are *Streptomyces* bacteria that are easily recognized by their spherical and wrinkled shape, diffusible pigments and colonies in different color (here white, grey, yellow, cream and pink or red; examples of Streptomycete colonies are shown by green arrows). Photographs were taken after plates had been incubated for 2 weeks at 28.0°C.

Table 2. Physico-chemical properties of agricultural and non-agricultural soil samples (10 - 30 cm).

Soil property	Non-agricultural soils													Agricultural soils						
	ST1	**ST2**	**ST3**	**ST4**	**ST5**	**ST6**	**ST7**	**ST8**	**ST9**	**ST10**	**ST11**	**ST12**	**ST13**	**ST14**	**ST15**	**ST16**	**ST17**	**ST18**	**ST19**	**ST20**
Soil structure[a]	C-L	C-L	L	C-L	L	L	L	C-L	L	L	C-L	C-L	C-L	C-L	C-L	C-L	C-L	C-L	L	L
Salinity (µS/cm)[b]	686	534	477	1103	199	675	**2570**[c]	**1327**	576	632	475	302	780	**2300**	723	541	308	268	319	238
Lime (%)	14.82	1.95	**17.55**	**17.55**	11.70	2.11	2.73	10.92	17.16	**19.50**	3.51	5.07	**18.33**	14.82	12.48	15.21	3.12	3.51	2.73	4.68
Saturation (ml)	58	59	50	51	44	50	50	56	49	49	51	58	55	69	68	56		53	49	42
pH	**7.78**	7.53	7.37	7.32	7.55	7.28	7.35	7.23	**7.70**	7.20	7.54	**7.77**	7.40	7.64	7.49	7.44	7.47	7.63	7.50	7.44
N (ppm)	1.38	1.52	1.33	1.80	1.62	2.23	1.65	**16.85**	1.73	2.14	2.12	3.27	**12.26**	**134.43**	2.22	2.68	2.05	1.54	2.06	1.59
P (ppm)	0.32	2.61	1.52	8.65	2.69	3.14	0.10	**16.77**	4.10	3.47	1.56	8.21	5.66	9.12	7.86	**15.09**	12.68	7.81	**20.61**	6.69
K (ppm)	263	176	226	263	205	**389**	169	298	2.0	246	186	165	297	**433**	359	**375**	342	214	287	181
Na (ppm)	**310**	119	115	160	42	166	**554**	**527**	33	147	117	119	230	120	81	57	27	45	28	19
Fe (ppm)[d]	5.31	3.47	3.91	4.30	5.07	4.35	4.67	**53.80**	**60.20**	5.19	4.43	**9.35**	5.35	3.71	5.03	7.02	2.51	6.67	8.66	4.30
Cu (ppm)	1.39	3.27	2.51	2.96	2.10	2.38	1.73	2.09	5.32	2.97	3.56	2.71	3.32	2.75	2.66	2.39	2.66	**7.24**	**13.25**	**5.37**
Zn (ppm)	0.14	0.42	0.24	0.89	0.21	0.57	0.12	**2.66**	0.82	0.52	0.71	**2.81**	0.75	0.89	0.69	1.95	0.82	0.70	1.36	**3.73**
Mn (ppm)	2.68	6.10	1.26	2.60	2.98	5.40	1.09	**20.08**	**12.36**	2.49	6.74	2.93	3.29	9.51	2.09	**28.12**	3.76	2.17	6.75	2.77
Ca (ppm)	**4200**	3200	3288	**4256**	2894	2866	3267	4020	2967	3111	2914	3098	**4024**	3867	3714	3024	2916	3214	2866	3024
Mg (ppm)	364	244	248	**428**	255	266	312	**420**	291	314	290	302	288	266	**388**	294	301	299	294	304

[a] Soil structure, C-L: Clayed-loamy soil, L: Loamy soil.
[b] µS/cm: Microsiemens per centimeter, ppm: Parts per million.
[c] First three highest values are indicated with **bold** for parameters of each site.
[d] Fe, Mn, Zn and Cu: extracted with ammonium bicarbonate-diethylenetriamine pentaacetic acid (AB-DTPA).

streptomycetes development are pH and temperature (Williams et al., 1983).

Soil dilution and isolation on culture media have proved to be a useful method; however, it has some limitations: the methodology is slow and laborious and requires a large volume of material. Hence, given the small cultu-rable portion of soil microorganisms, any biodiversity study is limited; furthermore, the nutritional and physio-logical requirements of streptomycetes can be so specific that they restrict the study even more (Waksman, 1999;
Williams et al., 1989; Vargas Gil et al., 2009b).

According to morphological and cultural observations indicated, that taxonomic grouping of these isolates is in the genus *Steptomyces* as reported by other researchers (Shirling and Gottlieb, 1966; Williams et al., 1983; Anderson and Wellington, 2001). The compositions of DAP and sugar components of selected representative's isolates were detected. DAP existed as isomers with LL types. Cells contained no diagnostic sugar components. *Streptomyces* isolates were categorized into six color series according to the color of their mature sporulated aerial mycelium with grey and white color series being the most abundant (Figure 4). Data indicated that 38 and 25% of NAG; 30 and 32% of AG fields have grey and white color series of *Streptomyces*, respectively. Saadoun and Gharaibeh (2003) and Thakur et al. (2007) reported similar results; they showed that grey and white series of *Streptomyces* had the highest occurrence in soils. Microscopic examination of the spore morphology revealed that most of the isolates had rectiflexibile spore type (55 and 45% for NAG and AG, respectively). Spirales spore types that are represented by 40 and 35% for NAG and AG respectively. Retinaculiaperti was less observed (4 and 8%) (Figure 5). The present study is the first report of the diversity and ecological characterization of streptomycetes from agricultural and non-agricultural soils and provides new data on the populations of Streptomyces influenced by soil chemical properties as well as contributes to a better understanding of the dynamic of soil microbial communities. Further studies are needed to elucidate the biocontrol activity of isolates and their role in agricultural fields with dif-

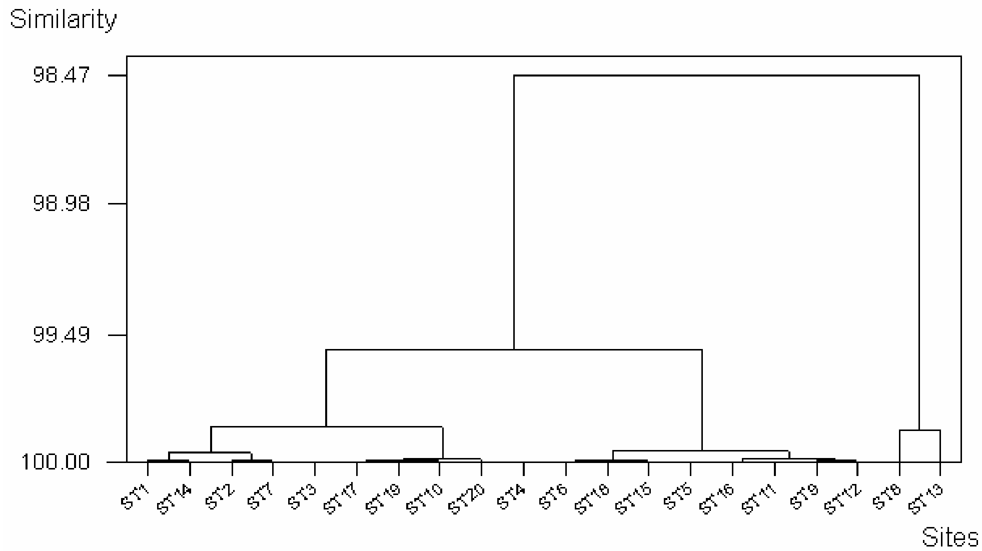
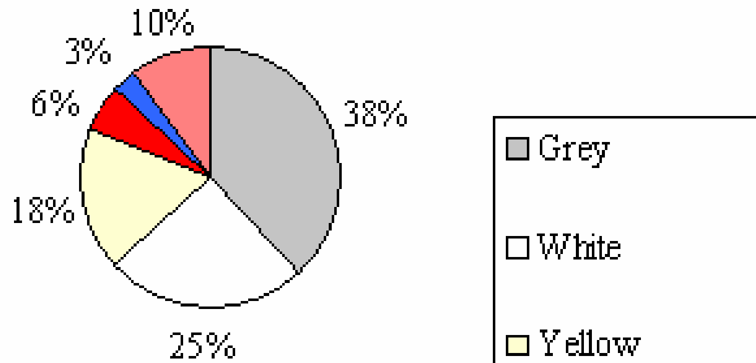


Figure 3. Similarities (in percentage) of *Streptomyces* diversity between AG and NAG soil samples.



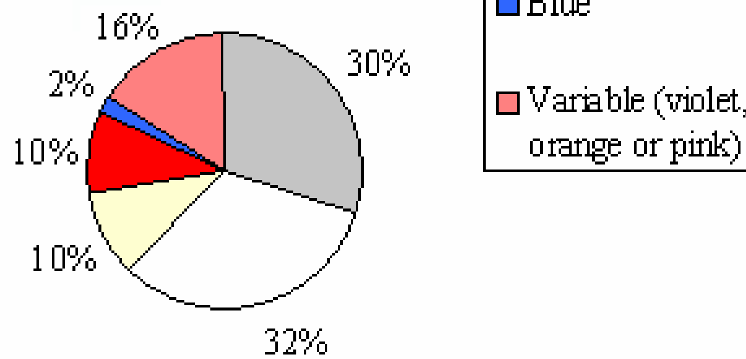


Figure 4. Color series (in percentage) of *Streptomyces* isolated from AG and NAG soil samples.

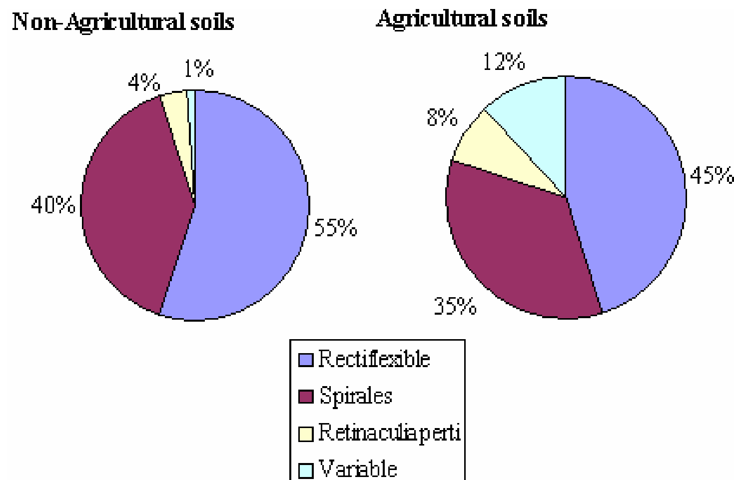


Figure 5. Spore bearing hyphae types (in percentage) of *Streptomyces* isolated from AG and NAG soil samples (Representative of total 50 different streptomycete isolates were analyzed using cover clip method with ISP2 and ISP4).

ferent crop species.

REFERENCES

Anderson AS, Wellington MHE (2001). The taxonomy of *Streptomyces* and related genera. Int. J. Syst. Evol. Microbiol. 51: 797-814.

Conn KL, Leci E (1998). A Quantitative Method for Determining Soil Populations of *Streptomyces* and Differentiating Potential Potato Scab–Inducing Strains. Plant Dis. 82: 631-638.

Cross T (1989). Growth and Examination of *Actinomycetes* Some Guidelines. In Bergey's Manual of Systematic Bacteriology. Williams and Wilkins Company, Baltimore 4: 2340-2343.

Dolotkeldieva T, Totubaeva N (2006). Biodiversity of *Streptomyces* of high-mountainous ecosystems of Kyrgyzstan and its biotechnological potential. Anton. van Leeuwen. 89: 325-328.

El-Nakeeb MA, Lechevalier HA (1963). Selective Isolation of Aerobic Actinomycetes Appl. Microbiol. 11: 75-77.

Grishko VN, Syshchikova OV (2009). *Streptomyces* Communities in Soils Polluted with Heavy Metals. Eurasian Soil Sci. 42: 217-224.

Katsifas EA, Giannoutsou EP, Karagouni AD (1999). Diversity of *streptomycetes* among specific Greek terrestrial Ecosystems. Let. Appl. Microbiol. 29: 48-51.

Kuster E, Williams ST (1964). Selection of media for isolation of Streptomycetes. Nature 202: 928–929.

Lartey RT (2006). Dynamics of Soil Flora and Fauna in Biological Control of Soil Inhabiting Plant Pathogens. Plant Pathol. J. 5: 125-142.

Lechevalier MP, Lechevalier HA (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol. 20: 435–443.

Li HX, Li QR, Jiang CL (1996). Diversity of Soil Actinomycetes in Yunnan, China. Appl. Environ. Microbiol. 62: 244-248.

Locci R (1989). *Streptomyces* and related Genera. In: Bergey's Manual of Systematic Bacteriology, Williams and Wilkins Company, Baltimore 4: 2451-2508.

Martinez Blanco E, Little CR, Davelos Baines AL (2007). Variation in antibiotic inhibitory abilities among streptomycetes from south Texas agricultural soils. Soil Biol. Biochem. 39: 268-275.

Mitra A, Chandra Santra S, Mukherjee J (2008). Distribution of actinomycetes, their antagonistic behaviour and the physico-chemical characteristics of the world's largest tidal mangrove forest. Appl. Microbiol. Biotechnol. 80: 685-695.

Parfitt RL, Yeates GW, Ross DJ, Mackay AD, Budding PJ (2005). Relationships between soil biota, nitrogen and phosphorus availability and pasture growth under organic and conventional management. Appl. Soil Ecol. 28: 1-13.

Prauser H (1964). Aptness and application of color for exact description of colors of *Streptomyces*. Z. Allg. Mikrobiol. 4: 95-98.

Ryan J, Garabet S, Harmsen K, Rashid A (1996). A Soil and Plant Analysis Manual Adapted for the West Asia and North Africa Region. Icarda, Aleppo, Syria. 140: 87-90.

Saadoun I, Gharaibeh R (2003). The *Streptomyces* flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-resistant bacteria. J. Arid Environ. 53: 365-371.

Scheffer F, Schachtschabel P (1966). Lehrbuch der Boden kunde Ferdinand Enke Verlag: Stuttgard p. 473.

Schlichting E, Blume HP (1966). Bodenkundliches praktikum. Verlag Paul Paney, Hamburg und Berlin. pp. 121-125.

Schloter M, Bach H.J, Metz S, Sehy U, Munch JC (2003). Influence of precision farming on the microbial community structure and functions in nitrogen turnover. Agric. Ecosys. Environ. 98: 295-304.

Shirling EB, Gottlieb D (1966). Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313-340.

Thakur D, Yadav A, Gogoi BK, Bora TC (2007). Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. Mycmed. 17: 242-249.

Vargas Gil S, Meriles J, Conforto C, Figoni G, Basanta M, Lovera E, March GJ (2009a). Field assessment of soil biological and chemical quality in response to crop management practices. World J. Microbiol. Biotechnol. 25: 439-448.

Vargas Gil S, Pastor S, March GJ (2009b). Quantitative isolation of biocontrol agents *Trichoderma* spp., *Gliocladium* spp. and actionmycetes from soil with culture media. Microbiol. Res. 164: 196-205.

Vieira FCS, Nahas E (2005). Comparison of microbial numbers in soils by using various culture media and temperatures. Microbiol. Res.160:197-202.

Vijayakumar R, Muthukumar C, Thajuddin N, Panneerselvam A, Saravanamuthu R (2007). Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India. Actinomycetologica 21: 59-65.

Waksman SA (1961). The Actinomycetes: Classification, identification and descriptions of genera and species. The Williams and Wilkins Company, Baltimore 2: 61-292.

Watve MG, Tickoo R, Maithili M, Jog B, Bhole D (2001). How many antibiotics are produced by the genus *Streptomyces*? Arch. Microbiol. 176: 386-390.

Williams ST, Goodfellow M, Alderson G (1989). Genus *Streptomyces* Waksman and Henrici 1943, 339[AL]. In: Williams ST, Sharpe ME, Holt JG (ed), Bergey's Manual of Systematic Bacteriology, Williams and Wilkins, Baltimore 4: 2452-2492.
Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ (1983). Numerical classification of *Streptomyces* and related genera. J. General Microbiol. 129: 1743-1813.
Zenova GM, Gryadunova AA, Pozdnyakov AI, Zvyagintsev DG (2008). Aerobic and Microaerophilic Actinomycetes of Typical Agropeat and Peat Soils. Eurasian Soil Sci. 41: 210-214.