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Diversity of streptomycetes among specific Greek terrestrial ecosystems

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E.A. KATSIFAS, E.P. GIANNOUTSOU AND A.D. KARAGOUNI. 1999. The diversity of streptomycetes isolated from different Greek terrestrial ecosystems using phenotypic identification, and the relationship between the number of species and the number of isolates as a diversity index, was studied. A total of 344 *Streptomyces* strains have been isolated and identified from diverse sites in the Greek territory, such as heavily disturbed agricultural areas and preserved forest areas, and from specific rhizosphere ecosystems. According to phenotypic identification, these strains belonged to 19 different cluster groups with a Willcox probability $> 0{\cdot}8$. *Streptomyces cyaneus*, *Strep. albidoflavus*, *Strep. diastaticus* and *Strep. exfoliatus* were the most common cluster groups isolated from at least six different habitats. On the other hand, there were cluster groups that appeared in only one or two habitats, such as *Strep. griseoflavus*, *Strep. rimosus*, *Streptoverticillium blastmyceticum*, *Nocardia mediterranea* and *Strep. fulvissimus*. The diversity indices among the different cluster groups of each sampling area indicated that the different habitats can be sub-divided into two main groups: rhizosphere habitats and non-rhizosphere habitats, showing that the rhizosphere is one of the most important factors which determines the population structure of a specific soil area.

INTRODUCTION

Streptomycetes are natural inhabitants of soil in which they appear to exist predominately as spores (Goodfellow and Simpson 1987). They probably constitute the largest actinomycete group in a number of soils. Members of the genus *Streptomyces* are renowned for the production of an array of industrially important metabolites, including antibiotics, herbicides and insecticides, and this attribute has prompted the isolation of a multitude of streptomycetes in the search for novel metabolites (Korn-Wendisch and Kutzner 1992). To date, taxonomic affinities within the genus have been determined largely on the basis of morphological and biochemical criteria, resulting in the arrangement of strains into cluster groups (Williams *et al*. 1983a).

Streptomyces strains have been isolated from diverse Greek areas and were identified using the probabilistic identification scheme of Williams *et al*. (1983b). Differences in cluster groups diversity among the different areas have been estimated using the relationship between the number of cluster groups and the number of isolates as a diversity index. The aim of the present study was to determine the diversity of *Streptomyces* cluster groups in different soils, and to investigate habitats which might be a rich source of diverse species. This could lead to the isolation of strains with economic interest (strains producing new antibiotics and other metabolites useful in the medicine, food and pharmaceutical industries).

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METHODS

Isolation of indigenous streptomycetes from diverse areas and ecosystems in Greece

Seven diverse habitats in different areas of the Greek territory were selected for the isolation of streptomycete strains. These habitats included the rhizosphere of rare indigenous plants, heavily disturbed agricultural soil and secluded preserved areas. These habitats were selected because there was a possibility that they contained micro-organisms of economic interest. The seven habitats were: (a) soil from a preserved forest

area (sampling area 1A); (b) soil from the rhizosphere of two endemic plants, *Abies cephalonica* (sampling area 1B) and *Ebenus sipthorpii* (sampling area 1C); (c) soil from a heavily disturbed agricultural area (sampling area 2); (d) soil from the rhizosphere of evergreen woody shrubs growing on secluded islands of the Aegean (sampling area 3) and Ionian Sea (sampling area 4); and (e) soil from the rhizosphere of an indigenous plant (*Pinus brutia*) in the island of Crete (sampling area 5). The soil of sampling areas 1A, 1B, 1C and 4 was a sandy loam soil while that of sampling areas 2, 3 and 5 was a sandy silt/sandy silt loam soil. The soil pH was slightly alkaline in all cases (7·7–8·3).

Soil samples (100 g) from each habitat were placed in sterile flasks, mixed with 900 ml sterile Ringers solution (0·25 strength) and shaken on an orbital shaker (Stuart Scientific Co. Ltd., Redhill, Surrey, UK) at maximum speed (500 rev min^{-1}) for 30 min (Wellington *et al*. 1990). Mixtures were allowed to settle before making serial dilutions (up to 10^{-8}) of the supernatant fluids and plating on AGS medium (Herron and Wellington 1990). Plates were incubated at 28 °C until sporulation of *Streptomyces* colonies occurred. *Streptomyces* colonies (where the mycelium remained intact and the aerial mycelium and long spore chains were abundant) were then picked up and purified by further culture.

Identification of *Streptomyces* strains

Identification of indigenous soil isolates from the seven different soil samples was carried out using 41 morphological and physiological diagnostic characters contained in the probabilistic identification matrix of Williams *et al*. (1983b). Three identification statistics were used to assess the reliability of the identification of unknown strains: the Willcox probability, taxonomic distance and its standard error (Williams *et al*. 1983b). The Willcox probability was used to assign an identity to an unknown where scores of 0·8 and above indicated a positive identification (Table 1). The study of the taxonomic distance and its standard error confirmed the results using Willcox probability.

Diversity indices – comparison of the different habitats

Differences in species diversity among the different areas were estimated using the relationship between the number of species and the relative importance of individual species, according to Atlas and Bartha (1993) and Germida *et al*. (1998). Species diversity indices relate the number of species and the relative importance of individual species. Two diver-

Table 1 Diversity of streptomycetes associated with seven different soil habitats of the Greek territory

Species	Cluster group	Willcox probability range of scores*	Number of isolates identified†
S. cyaneus	A18	0·854–1·000	81 (7)
S. albidoflavus	A1	0·972–1·000	77 (7)
S. exfoliatus	A5	0·870–0·996	28 (6)
S. violaceus	A6	0·910–0·990	14 (5)
S. atroolivaceus	A3	0·870–0·980	10 (5)
S. diastaticus	A19	0·890–1·000	27 (6)
S. rochei	A12	0·900–1·000	37 (5)
S. griseoruber	A21	0·860–0·920	13 (5)
S. violaceoniger	A32	0·920–0·960	7 (4)
S. phaechromogenes	A40	0·870–0·930	13 (4)
S. chromofuscus	A15	0·900–1·000	21 (6)
S. lavendulae	F61	0·890–0·990	3 (2)
S. griseoviridis	A17	0·930–0·970	2 (2)
S. lydicus	A29	0·950	1 (1)
S. rimosus	B42	0·997	1 (1)
Nocardia mediterranea	E53	0·920	1 (1)
S. fulvissimus	A10	0·920–0·980	5 (1)
S. griseoflavus	A37	0·960	1 (1)
Strepto-verticillium blastmyceticum	F58	0·940	1 (1)
Total	19		344

*According to Williams *et al.* 1983b.
†Numbers in parenthesis indicate the number of habitats where streptomycetes species were detected.

sity indices were used: the Shannon-Weaver diversity index (H) and the Evenness (e). The Shannon-Weaver diversity index is sensitive to both species richness and relative species abundance. Evenness is an estimate of the variance in species abundance over the number of species.

In order to estimate whether the diversities of two populations were equal or not, a t-test was performed (Zar 1984). The similarity between the various sampling areas was studied using cluster and ordination analysis. A Bray-Curtis similarity matrix was calculated on the basis of the quantitative data matrix. The similarity matrix was analysed using the Group Average clustering method, equivalent to the UPGMA clustering method (Pielou 1984). In order to display the relative similarities between the various clusters in higher accuracy, the ordination method of Non Metric Multidimensional Scaling (nMDS) was applied (Clarke and Green 1988). All the multivariate analysis techniques, as well as plotting of the 2-dimensional nMDS solution, were performed using the PRIMER software package (Plymouth Marine Laboratory 1989, Plymouth, UK).

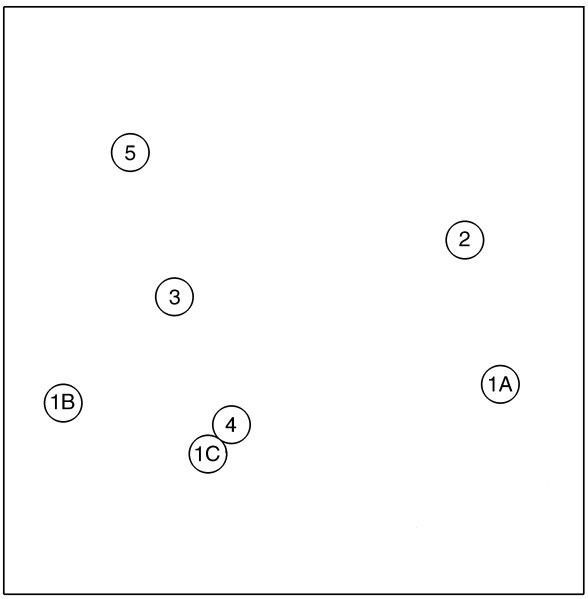

Fig. 1 2-Dimensional MDS solution diagram based on UPGMA method for the seven different habitats

RESULTS AND DISCUSSION

In total, 344 strains were isolated and identified from the seven diverse habitats. Marked differences in the phenotype of the 344 strains under study resulted in differing identifications, as shown in Table 1. The strains identified to 19 of the 23 cluster groups within the probabilistic identification matrix (Williams *et al*. 1983b). *Streptomyces cyaneus* and *S. albidoflavus* are common cluster groups which appeared in all seven habitats, while *S. diastaticus* and *S. exfoliatus* appeared in six habitats. These were also the cluster groups with the largest number of isolates. On the other hand, there were rare cluster groups which appeared in only one or two habitats with few isolates, such as *S. griseoflavus* that was found only in sampling area 2, *S. rimosus* and *Streptoverticillium blastmyceticum* found only in sampling area 1C, *Nocardia mediterranea* only in sampling area 1B, and *S. fulvissimus* in sampling area 3. The two islands (sampling areas 3 and 4) showed a relatively common profile on the deriving strains (the 87·5% of the strains in sampling area 3 and the 80·6% of the strains in sampling area 4 belong to common, for the two sampling areas, cluster groups), without the appearance of any exclusive isolates.

It is clearly seen from the 2-dimensional MDS solution diagram (Fig. 1) that the two non-rhizopshere habitats are closely related to each other and totally different from the other five habitats, which form the rhizosphere group. It can also be seen that 1A (non-rhizosphere) bears no resemblance to 1C (rhizosphere), although the soil type is the same. Generally, it could be concluded that the differentiation in the community of streptomycetes surviving on the same soil type, but in different habitats, was due to the rhizosphere of the plant. The production of certain compounds (such as cellulose, sugars, aromatic acids and amino acids) can attract some species and repel others, leading to the structure of different communities. The release of soluble and diffusable material, and mucigel, from the plant is the carbon source for the growth of micro-organisms that are not favoured in the non-rhizosphere habitat (Paul and Clark 1989; Germida *et al*. 1998).

The average dissimilarity between the two groups was 65·2%. Eleven cluster groups accounted for most of this dissimilarity. In order of decreasing importance these were *S. albidoflavus*, *S. cyaneus*, *S. rochei*, *S. chromofuscus*, *S. diastaticus*, *S. exfoliatus*, *S. griseoruber*, *S. violaceus*, *S. phaeochromogenes*, *S. atroolivaceus* and *S. lavendulae*. The rhizosphere group was dominated by *S. albidoflavus* (25·5% or 51/201 identified isolates), *S. cyaneus* (21·5% or 43/201 identified isolates) and *S. rochei* (14·5% or 29/201 identified isolates). In the non-rhizosphere group, the percentage importance of the above cluster groups was significantly lower, i.e. *S. cyaneus* (7·6% or 38/143 identified isolates) and *S. albidoflavus* (5·2% or 26/143 identified isolates), although these were again the dominant species of the group. The distinction between the two groups was also supported by the Evenness diversity index differences. The highest values of Evenness were calculated for areas 3 and 5 while the lowest were for the non-rhizosphere habitats 1A and 2 (Table 2). Respective differences in Evenness values were reported by Germida *et al*. (1998) when studying endophytic and rhizoplane communities. They also reported that the diversity

Table 2 Shannon-Weaver Index of Diversity (H), and Evenness (e) for the seven sampling areas

Sampling areas	1A	1B	1C	2	3	4	5
Shannon-Weaver Index of Diversity (H)	2·838	2·784	3·018	2·971	2·699	2·847	0·898
Evenness (e)	0·767	0·839	0·872	0·829	0·9	2·840	0·947

of a community in a specific environment was depended either on the species of plant or on other abiotic soil parameters. The additional selection pressure exerted on the rhizosphere by the plant could explain the higher Evenness values observed in the rhizosphere communities compared with the non-rhizosphere communities observed in this study. The Evenness value could also suggest that a community with a complex structure, i.e with a low Evenness value, needs a lower amount of energy to maintain such structure.

All isolates were tested for the production of metabolically-active compounds and some were shown to have biotechnological interest (unpublished data). Most metabolically-active strains arose in region 2 (heavily disturbed agricultural area), followed by 1A, 1C and 5. In conclusion, these Greek habitats proved to be a rich source of diverse species with potential economic and industrial interest.

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