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Determination of metabolic activity of streptomycetes in soil microcosms

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E.A. KATSIFAS, T.G. KORAKI AND A.D. KARAGOUNI. 2000. Two *Streptomyces griseus* strains were isolated from different soil types. *S. griseus* CAG 17 strain was isolated from an agricultural area with low organic matter but rich in phosphorus content and *S. griseus* 26K strain was isolated from a forest area rich in organic matter with a low phosphorus content. The survival and metabolic activity of these isolates were studied in dynamic sterile soil microcosm systems. The fitness of each isolate was studied by re-inoculation in a soil type different from its origin. Maximum percentage of germination and respiration rates occurred within the first 48 h after each soil turnover (removal and addition of certain soil volumes). Data suggested that *S. griseus* CAG17 survived better independently of the soil type in comparison with *S. griseus* 26K which sporulated within the first 12 h after inoculation. Incubation temperatures did affect the lifecycles in relation to soil type. For example, the lowest temperature tested, 22 °C, was more favourable for extended germination and adaptation in general but revealed lesser spore numbers in the 'foreign' soil environment. Monitoring metabolic activity by estimation of urease, phosphatases and dehydrogenase-specific activities, between 18 and 35 °C incubation temperatures, was a reliable method for studying the survival and growth of streptomycete populations in soil. Results also confirmed that respiration rate and enzyme-specific activity corresponded with spore counts in long-term experiments which were designed for the investigation of survival and growth of *S. griseus* CAG17. Under selective pressure by heavy metals, in soil microcosm systems, metabolic activity proved a useful tool for the investigation of streptomycete activity. These methods could also be applied in agricultural field studies for monitoring microbial populations under conditions where various 'pollutants' are present in soil samples.

INTRODUCTION

Soil is the natural habitat for most streptomycetes, with suitable conditions for growth and proliferation (Williams *et al.* 1984). Streptomycetes are able to degrade relatively complex and recalcitrant plant and animal residues (including polysaccharides, proteins, as well as aromatic compounds). They can use inorganic nitrogen sources, do not require vitamins or growth factors, and find plenty of surfaces to support their mycelial growth in soil (Korn-Wendisch and Kutzner 1992). Moreover, their ability to produce spores contributes to streptomycete survival over long periods of drought, frost, hydrostatic pressure and anaerobic conditions produced by water saturation (Goodfellow and Simpson 1987).

Although it is known that the genus *Streptomyces* is important in decomposition and nutrient cycling, there are very few data on their activity in soil (Wellington *et al.* 1992). In laboratory conditions soil microcosm systems have been used for the study of streptomycete survival and gene transfer under well-defined and varied environmental conditions (Wellington *et al.* 1992; Vionis *et al.* 1998).

The activity of micro-organisms in soil can be estimated by specific activity of different enzymes and by soil respiration rates (Tiwari *et al.* 1989; Jha *et al.* 1992). Soil enzymes catalyse numerous important reactions responsible for maintaining the biological activity of soil and assist soil organisms in their efforts to satisfy their nutritional needs and in their function of degrading and humifying organic material (Sarkar *et al.* 1988). They also serve as useful

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indices of soil fertility (Jha *et al*. 1992). Soil enzymes have been classified into three groups: (a) free enzymes, (b) bound enzymes on cell components, and (c) enzymes in viable but non-proliferating cells (Burns 1981). Some of the most studied enzymes in soil are urease, phosphatases and dehydrogenases. Urease is an important enzyme for the N-economy of soil (Tiwari *et al*. 1989; Vaughan and Ord 1991), whilst the level of phosphatase activity affects the rate of phosphorus mineralization in soil (Pang and Kolenko 1986; Nakas *et al*. 1987). Finally dehydrogenases, a group of intracellular enzymes, have been widely used to measure catabolic activities in soil, and have been shown to be correlated with microbial activity (Cochran *et al*. 1989; Garcia *et al*. 1994). The availability and form of substrates in a soil environment depend, among other factors, on soil structure and composition, which consequently influence the metabolic activity of soil microflora (Van Loosdrecht *et al*. 1990)

An indirect but accurate way of estimating metabolic activity in soil is the estimation of CO_2 released from soil microbiota during a period of 24 h. Soil respiration was one of the earliest, and still one of the most frequently used indices of microbial activity in soils and may reflect the general activity of soil micro-organisms (Edwards *et al*. 1981; Tiwari *et al*. 1989).

Monitoring microbial populations in soil samples creates several problems particularly in filamentous micro-organisms such as streptomycetes. The aim of our study was to select specific widely distributed *Streptomyces* strains from the natural environments and study their fitness to a given soil type, namely its ability of surviving, colonizing and growing in differing soil types. The selected soils (agricultural and forest) differed in structure, organic matter and phosphorus content. In addition, viable counts coupled with methods for detection of respiration rates and enzyme-specific activities were employed to establish streptomycete population trends and investigate the relationship between the metabolic activity and viable counts of *S. griseus* indigenous inoculants within the dynamic soil microcosm system. Finally the usefulness of enzyme-specific activity as a complementary method for survival and growth when viable counts are under detection limits was investigated under selective pressure.

MATERIALS AND METHODS

Soil

Two soil types, differing markedly in terms of structure, organic matter and phosphorus content, were used in this study. Type A soil was a sandy silt/sandy silt loam soil, taken from an agriculturally developed field in the Marathon area (42 km from the centre of Athens). Analysis of particle size indicated a composition (percent, dry weight) of: sand, 50; silt, 36; clay, 14; and organic carbon, 1·23. The pH of the soil was 7·9. The phosphorus content was 124 mg l^{-1} dry soil. Mineralogy analysis indicated the following results (percent): illite, 65; chlorite, 7; kaolinite, 10; smectite, 12; talc, 6; and calcite < 1. Type B soil was a sandy loam soil and was taken from a forest preserved area Kesariani (4 km from the centre of Athens). Analysis of particle size indicated a composition (percent, dry weight) of: sand, 59; silt, 30; clay, 11; and organic carbon, 2·7. The pH of the soil was 8·2. The phosphorus content was 4 mg l^{-1} dry soil. Mineralogy analysis indicated the following (percent): illite, 76; chlorite, 11; kaolinite, 12; smectite, just detected; talc, not detected; and calcite, 1. The two soil types (A and B) were treated as follows: soil was air-dried in the dark at 22 °C for at least 3 months, then passed through a 2-mm sieve prior to use and autoclaved twice (121 °C, 15 min) on two separate days.

Bacterial strains

Streptomyces griseus CAG17 (wild type) with a chromosomal mutation encoding resistance to rifampicin (100 μg ml^{-1}), sensitive to neomycin (10 $\mu g\,ml^{-1}$), was isolated from soil type A and *Streptomyces griseus* 26K (wild type) with resistance to neomycin (50 $\mu g\,ml^{-1}$) and sensitivity to rifampicin ($\mu g\,ml^{-1}$) was isolated from soil type B. The isolates were maintained on AGS medium (Herron and Wellington 1990). Filtered spore suspensions were prepared as inoculum (Hopwood *et al*. 1985). All plates were incubated at 28 °C for 5–6 d.

Soil microcosms

A dynamic fed batch sterile soil microcosm system which was developed in our laboratory was used (Vionis *et al*. 1998). In the current study this was used to reveal a correlation between lifecycle and enzyme-specific activities in soil. In all experiments the microcosm consisted of 750 g sterile soil in sterile pots (growth chambers). Samples (10% of the total volume) were taken at days 0, 1, 2, 5, 10, 15, 30, 45 and 60 and replaced with an equal volume of sterile uninoculated soil. After soil addition, the whole bulk of soil in the growth chambers was thoroughly mixed, to achieve homogeneity. The microcosms were stirred only on sampling day and when correction of the moisture content was necessary. Microcosms containing noninoculated sterile soil were incubated under the same conditions for estimation of 'background respiration' (CO_2 contamination from air trapped in microcosm growth chambers or contaminating the NaOH solution during sampling and replacement procedures) or enzyme activity due to microbial contamina-

tion or activity of extracellular enzyme stabilized on the sterile soil matrix.

Effects of temperature and soil type. In order to study the effect of incubation temperature and soil type on the survival and metabolic activity of *S. griseus* isolates, one set of short-term (15 d) experiments with 16 different sterile soil microcosm systems were run in parallel (Table 1). Incubation temperature was maintained constant at the desired temperature throughout each individual experiment. Inoculants (10^5 cfu g^{-1} dry soil) in an adequate quantity of sterile distilled water, were added in each microcosm system, resulting in a final moisture content of 10·97% (w/w) for soil A and 16·7% (w/w) for soil B (equal to −210 kPa in both cases). The given water content was kept constant throughout each experiment.

Additional experiments on 18 °C incubation temperatures were performed for the estimation of the enzyme-specific activity temperature range.

Effects of soil turnover. Long-term experiments were designed in order to achieve repeated cycles of growth for the isolate *S. griseus* CAG17 in soil. Six microcosm systems contained soil type A with water holding capacity equal to −210 kPa, were incubated at 28 °C. Large portions of soil (10, 20, 30, 40, 60 and 75%, respectively, for each microcosm) were removed and replaced with an equal volume of sterile uninoculated soil (soil turnover) at days 15, 30 and 45. Additional samples were taken at days 16, 17, 20, 31, 32, 35, 46, 47 and 50, immediately after each soil turnover for the demonstration of the germination/sporulation cycle of the population.

Table 1 Soil microcosm systems for the effect of temperature and soil type on the survival and metabolic activity of the two *Streptomyces griseus* isolates

	S. griseus CAG17	*S. griseus* 26K
Soil type A		
22 °C	1	9
28 °C	2	10
32 °C	3	11
35 °C	4	12
Soil type B		
22 °C	5	13
28 °C	6	14
32 °C	7	15
35 °C	8	16

Effects of heavy metals. Two sets of four microcosm systems containing soil type A were set up to study the effect of heavy metal concentration on the survival and metabolic activity of *S. griseus* CAG17. In both set of microcosms chloride salts of Cu^{2+} and Hg^{2+} were added, respectively, in desired concentrations (0, 25, 50 and 100 mg of heavy metal g^{-1} soil). Incubation temperature (22 °C) and water holding capacity (−210 kPa) were kept constant throughout the experiments.

Enumeration of inoculants – germination efficiency

Spores and mycelia were estimated from three 1·0-g aliquots, with 0·25-strength Ringer's solution (Wellington *et al.* 1990) and spores were extracted from 10·0 g soil (Herron and Wellington 1990). The drop in viable counts between days 0 and 1 was equivalent to the number of spores that germinated and became undetectable by the spore extraction method. The germination efficiency can be expressed as the percent drop in viable counts between day 0 (X_0) and subsequent sample day (X_t): germination efficiency $= [(X_0 - X_t)\ X_0^{-1}]\cdot 100$ (Herron and Wellington 1990). All points on graphs are: (a) the means of three replicate samples counted in triplicate for total counts, and (b) the use of a 10·0-g sample for spore extraction, plated out in triplicate.

Analytical procedures (determination of metabolic activity)

Determination of the respiration rates of micro-organisms in soil was based on the method described by Kibble (1966) modified as follows: small sterile pots, containing 20 ml, 1 mol l^{-1} NaOH solution (Merck, Darmstadt, Germany) were placed in the growth chambers of inoculated microcosms. The NaOH solution was replaced daily. To estimate the CO_2 produced by micro-organisms, the samples were titrated with 1 mol l^{-1} HCl (Merck), using phenolophthalein as indicator (Vionis *et al.* 1998). All points on graphs are the means of the samples for respiration rates from triplicate parallel microcosm systems.

Urease (EC 3.5.1.5. urea aminohydrolase) enzyme-specific activity was determined by the amount of hydrolized urea according to the method of Burns (1982). Acid and alkaline phosphatases (EC 3.1.3.2. and EC 3.1.3.1., orthophosphoric monoester phosphohydrolases) enzyme-specific activity was determined by a colourimetric method, based on the estimation of p-nitrophenol according to the method of Tabatabai (1982). Activity of the enzyme group of dehydrogenases was determined using a colourimetric method, by estimation of produced triphenyl formazan (TPF) (Tabatabai *et al.* 1982). All points on graphs are: (a) the means of four replicate samples for urease, acid and alka-

line phosphatase, and (b) the means of three replicate samples for dehydrogenase activity.

Statistical analysis

Statistical analyses were accomplished using the MINITAB statistical package (Minitab Statistical Software, State College, PA, USA). Minimum significant differences (MSD) were calculated from analysis of variance using the Tukey–Kramer method (Petersen 1985; Fry 1989).

RESULTS

Effect of temperature and soil type on survival and metabolic activity of *S. griseus* isolates

Survival. In short-term experiments, one cycle of germination/sporulation events in the streptomycete population was chosen to study the effect of temperature and soil type on the survival and metabolic activity of the two indigenous isolates (Table 1).

When *S. griseus* CAG17 was inoculated into the microcosm with the soil of its origin and incubated at 28 and 32 °C, 97% of inoculum germinated within the first day of incubation. At 22 °C incubation temperature, 87% of inoculum germinated and germination continued until day 2, whilst at 35 °C a profuse sporulation was observed within the first 12 h of incubation (data not shown). In all incubation temperatures spore counts reached a maximum of about 10^7 cfu g^{-1} dry soil which remained constant unless enrichment with fresh uninoculated soil was employed. When the isolate *S. griseus* CAG17 was inoculated in microcosms containing soil type B, a similar pattern of germination/sporulation was observed only at 22 °C. In all other incubation temperatures (28, 32 and 35 °C) a profuse sporulation occurred. Total spore counts, with an exception of temperature 22 °C, were about two logs higher in soil type B compared with soil type A on the 5th day of incubation and remained constant for the rest of incubation. At 22 °C incubation temperature total spore counts reached only a value of about 10^6 cfu g^{-1} dry soil.

S. griseus 26K showed the germination/sporulation pattern only at 22 °C and only in the soil of its origin (soil type B). At all other incubation temperatures no germination was observed on day 1 of sampling; instead the inoculant sporulated profusely shortly after its introduction in soil.

Respiration rates. S. griseus CAG17 exhibited higher respiration rates in both soil types within the first 24 h of incubation in all temperatures except 22 °C where the highest rate was observed within the second 24 h of incubation. The peaks in the respiration rate correspond precisely to the drops in spore numbers, which represent the germination of spores and emergence of germination tubes (Herron and Wellington 1990). Zero respiration rate corresponded in all cases to maximum spore counts under the studied conditions, marking the cessation of growth and metabolic activity.

S. griseus 26K showed higher respiration rates at all incubation temperatures in both soils during the first 24 h. Respiration rates were approximately doubled for *S. griseus* 26K in soil type B than in soil type A.

Enzyme activity. Enzyme-specific activity was estimated on all sampling days.

Urease. In the temperature range examined, urease showed an optimum specific activity for *S. griseus* CAG17 in both soil types at 28 °C (1·5-fold higher for soil type A). The optimum temperature for urease-specific activity of *S. griseus* 26K was at 32 °C. Temperature seemed to be an important factor for urease-specific activity of *S. griseus* CAG17 whilst there were no significant differences of urease-specific activities between the examined temperatures for *S. griseus* 26K. Both strains showed higher rates in the soil of their origin under all examined temperatures.

Acid and alkaline phosphatases.

Acid phosphatase enzyme-specific activity was very low in all cases because of the alkaline pH of both soils. Alkaline phosphatase-specific activity of *S. griseus* CAG17 was high and similar in both soil types with an optimum specific activity at 32 °C. *S. griseus* 26K yielded higher enzyme-specific activity in its soil of origin (about two- to fourfold increase) with an optimum at 22 °C. In soil type A, the enzyme-specific activity was low and almost constant at all incubation temperatures.

Dehydrogenases.

The optimum temperature for *S. griseus* CAG17 dehydrogenase-specific activity depended on the soil type; it was higher in the soil of origin with an optimum at 32 °C compared with 22 °C in soil type B. The optimum temperature for *S. griseus* 26K dehydrogenase-specific activity was 22 °C for both soils, but enzyme activity was up to twofold higher for the soil of origin.

The survival and metabolic activity of *S. griseus* CAG17 in long-term soil microcosm experiments

Estimation of respiration rates and enzyme-specific activity were also performed in long-term microcosm experiments in order to monitor the metabolic activity of the population in microcosms after different percentages of soil turnover. The enzyme-specific activity and CO_2 released was correlated with total spore counts. In all cases the results con-

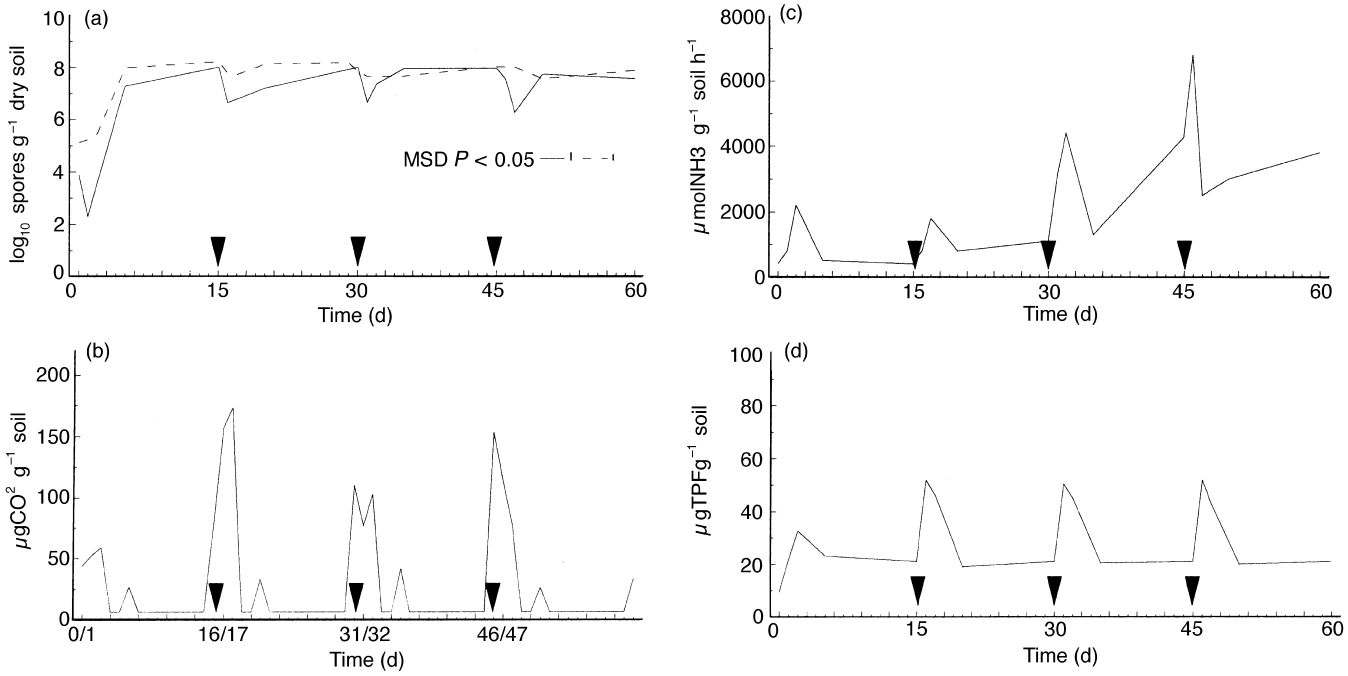

Fig. 1 Repeated life-cycles of the *Streptomyces griseus* CAG17 in soil type A with different methods of metabolic activity of the population in soil, expressed as (a) total viable (- - -) and spore counts (—), (b) respiration rates, (c) urease activity, and (d) dehydrogenase activity. Forty percent of soil volume was removed and replaced every 15 d. Error bars show standard error of estimated parameters. Minimum significant differences $P < 0{\cdot}05$ for b–d

firmed viable counts data. After each soil turnover the decrease of spore counts in the system coincided with peaks in respiration rates and dehydrogenase and urease-specific activity (Fig. 1). This was also the case with alkaline phosphatase-specific activity (data not shown).

Effect of heavy metals on the enzyme-specific activity of *S. griseus* CAG17 isolate in soil type A

In soil microcosm systems the effect of mercury and copper concentrations on the survival and metabolic activity of *S. griseus* CAG17 was studied and the total number of colony forming units was under detection limit in microcosm systems with heavy metal concentrations over $25\,\mu g\ g^{-1}$. Increase of heavy metal concentration corresponded with a significant decrease of enzyme-specific activity. In all cases though, these activities were above detection limit.

DISCUSSION

Results reported from many studies concerning the survival and gene transfer of indigenous and laboratory streptomycete strains using soil microcosm systems vary considerably as different soil types were used (Wellington *et al.* 1990). In the current study two *S. griseus* strains were used in order to compare their metabolic adaptation and flexibility in relation to the soil type. Both strains were isolated from environments differing in soil structure and management regimes (type A soil: agricultural soil, high turnover and high sunlight irradiation resulting in higher soil temperatures; type B soil: forest soil, protected and preserved area, low sunlight irradiation because of litter surface layers). *S. griseus* CAG17 was chosen among the agricultural soil isolates, as it belongs to the most dominant species of this streptomycete population (Katsifas *et al.* 1999). Aiming to study similar isolates in matters of genetic background another *S. griseus* strain (26K) was chosen among the forest area isolates.

As expected, the difference of origin is reflected in the strains' behaviour and physiology when re-inoculated in soil. Data suggested that *S. griseus* CAG17, which was isolated from a frequently changing environment, survived better in microcosms independently of the soil type, while *S. griseus* 26K showed comparatively poor survival under non-familiar or non-favourable environmental conditions.

Germination of streptomycete spores in soil seemed mainly to depend on the soil type and to a lesser extent on environmental factors such as temperature. These observa-

tions agree with data obtained by Vionis *et al.* (1998). The range of temperature where both strains were able to follow the typical germination/sporulation pattern was significantly limited when they were re-introduced in 'non-parental soil type' microcosm systems. In all cases under stress conditions the inoculant was forced to shorten the germination period resulting in some cases of profuse sporulation. An interesting observation was made at 22 °C incubation temperature where only *S. griseus* CAG17 showed longer periods of mycelium development (extended germination) in both soil types revealing a better adaptive ability.

In all cases respiration rates confirmed viable count data. The overall rates under all different temperatures were lower for both strains in Marathon agricultural soil. This could be attributed to the low organic carbon content of this soil type (almost 45% less than Kessariani's forest soil content) as the rate of CO_2 released from soil reflects C-mineralization (Anderson 1982).

Data on specific enzyme activity obtained from five different temperatures suggested that dehydrogenases are reliable indicators of microbial activity in soil. Dehydrogenase-specific activity has been widely used to measure the catabolic activity of total microbial populations in soil (Garcia *et al.* 1994) and appeared to be closely related to microbial biomass (Engelen *et al.* 1998). This was in agreement with our results where, independently of the soil type, dehydrogenase-specific activity reflected the metabolic state of all streptomycete populations. These results compare with data obtained from long-term experiments where enzyme specific activity and respiration rate peaks coincided with spore decreases, allow us to conclude that within the temperature range of 18–35 °C it is possible to monitor the metabolic activity of the streptomycete population during repeated lifecycles using enzyme-specific activity estimation.

This was also the case for alkaline phosphatase which showed a constant behaviour during the spore/mycelium phase in long-term experiments. In all five different temperatures studied, alkaline phosphatase-specific activity was higher in soil type B, although total phosphorus content was significantly lower in comparison with soil type A. This was not surprising since alkaline phosphatase-specific activity is correlated with organically bound phosphorus (Nakas *et al.* 1987) which has been reported to be abundant in forest soils (Pang and Kolenko 1986).

Previously researchers suggested that urease-specific activity could be used as an indicator for total biological activity (Tiwari *et al.* 1989). In our case long-term experiments revealed variations of urease-specific activity (Fig. 1c) which could not be related to variations in microbial populations. Instead a constant cumulative increase of urease-specific activity was observed supporting our suggestion that this is not a suitable indicator of the microbial activity in soil microcosm systems.

S. griseus CAG17 was also selected due to its better adaptive ability and flexibility for the study of heavy metal selective pressure. In all soil microcosm systems that have been amended with various amounts of heavy metals (common soil pollutants) no viable counts were detected. An interesting result was that even in high concentrations of the two heavy metals the streptomycete populations remained metabolically active as indicated by the relatively high dehydrogenase-specific activity. This is a very important finding which establishes these methods as valuable tools for providing data about the behaviour of microorganisms in extreme conditions where the estimation of viable counts is not possible.

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REFERENCES

Anderson, J.P.E. (1982) Soil respiration. In *Methods of Soil Analysis Part 2 Chemical and Microbiological Properties* ed. Page, A.L., Miller, R.H. and Keeney, D.R. pp. 831–859. Basel: American Society of Agronomy, Inc.

Burns, R.G. (1981) Enzyme activity in soil: location and a possible role in microbial ecology. *Soil Biology and Biochemistry* **14**, 423–427.

Burns, R.G. (1982) Enzyme activities in soil. In *Sourcebook of Experiments for the Teaching of Microbiology* ed. Primrose, S.B. and Wardlow, A.C. pp. 430–437. Basel: Academic Press.

Cochran, V.L., Elliot, L.F. and Lewis, C.E. (1989) Soil microbial biomass and enzyme activity in subarctic agricultural and forest soils. *Biology and Fertility of Soils* **7**, 283–288.

Edwards, N.T., Stugart, H.H. Jr, McLaugtin, S.B., Harris, W.F. and Reichle, D.E. (1981) Carbon metabolism in terrestrial ecosystems. In *Dynamic Properties of Forest Ecosystems* ed. Reichle, D.E. p. IBP23, Cambridge: Cambridge University Press.

Engelen, B., Meinken, K., von Wintzingerode, F., Heuer, H., Malkomes, H.P. and Backhaus, H. (1998) Monitoring impact of a pesticide treatment on bacterial soil communities by metabolic and genetic fingerprinting in addition to conventional testing procedures. *Applied and Environmental Microbiology* **64**, 2814–2821.

Fry, J.C. (1989) Analysis of variance and regression in aquatic bacteriology. *Binary* **1**, 83–88.

Garcia, C., Hernandez, T. and Costa, F. (1994) Microbial activity in soils under Mediterranean environmental conditions. *Soil Biology and Biochemistry* **26**, 1185–1191.

Goodfellow, M. and Simpson, K.E. (1987) Ecology of streptomycetes. *Frontiers of Applied Microbiology* **2**, 97–125.

Herron, P.R. and Wellington, E.M.H. (1990) A new method for the extraction of *Streptomyces* spores from soil applied to the

study of lysogeny in sterile and nonsterile soil. *Applied and Environmental Microbiology* **56**, 1406–1412.

Hopwood, D.A., Bibb, M.J., Chater, K.F. *et al.* (1985) *Genetic Manipulation of Streptomyces. A Laboratory Manual.* pp. 3–6 Norwich: John Innes Foundation.

Jha, D.K., Sharma, G.D. and Mishra, R.R. (1992) Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. *Soil Biology and Biochemistry* **24**, 761–767.

Katsifas, E.A., Gianoutsou, E. and Karagouni, A.D. (1999) Diversity of Streptomycetes among specific Greek terrestrial ecosystems. *Letters in Applied Microbiology* **29**, 48–51.

Kibble, R.A. (1966) Physiological Activity in a Pinewood Soil. PhD Thesis. Liverpool: University of Liverpool.

Korn-Wendisch, F. and Kutzner, H.J. (1992) The Family Streptomycetaceae. In *The Prokaryotes* 2nd edn. Balows, A., Trueper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H. pp. 921–995. New York, NY: Springer Verlag.

Nakas, P.J., Gould, W.D. and Klein, D.A. (1987) Origin and expression of phosphatase activity in semi-arid grassland soil. *Soil Biology and Biochemistry* **19**, 13–18.

Pang, P.C.K. and Kolenko, H. (1986) Phosphomonoesterase activity in forest soils. *Soil Biology and Biochemistry* **18**, 35–40.

Petersen, R.G. (1985) Separation of means. In *Design and Analysis of Experiments: Statistics Vol. 66* ed. Petresren, R.G. pp. 72–111. New York, NY: Dekker

Sarkar, J.M., Leonowicz, A. and Bollag, J.M. (1988) Immobilization of enzymes on clays and soils. *Soil Biology and Biochemistry* **21**, 223–230.

Tabatabai, M.A. (1982) Soil Enzymes. In *Methods of Soil Analysis Part 2: Chemical and Microbiological Properties* ed. Page, A.L., Miller, R.H. and Keeney, D.R. pp. 922–928, 937–940. Basel: American Society of Agronomy, Inc.

Tiwari, M.B., Tiwari, B.K. and Mishra, R.R. (1989) Enzyme activity and carbon dioxide evolution from upland and wetland rice soils under three agricultural practices in hilly regions. *Biology and Fertility of Soils* **7**, 359–364.

Van Loosdrecht, M.C.M., Luklema, J., Norde, W. and Zehnder, A.J.B. (1990) Influence of interfaces on microbial activity. *Microbiological Reviews* **54**, 75–87.

Vaughan, D. and Ord, B.G. (1991) Influence of natural and synthetic humic substances on the activity of urease. *Journal of Soil Science* **42**, 17–23.

Vionis, A.P., Katsifas, E.A. and Karagouni, A.D. (1998) Survival, metabolic activity and conjugative interactions of indigenous and introduce streptomycete strains in soil microcosms. *Antonie Van Leeuwenhoek* **73**, 103–115.

Wellington, E.M.H., Cresswell, N. and Herron, P.R. (1992) Gene transfer between streptomycetes in soil. *Gene* **115**, 193–198.

Wellington, E.M.H., Cresswell, N. and Saunders, V.A. (1990) Growth and survival of streptomycete inoculants and extent of plasmid transfer in sterile and non-sterile soil. *Applied and Environmental Microbiology* **56**, 1413–1419.

Williams, S.T., Lanning, S. and Wellington, E.M.H. (1984) Ecology of Actinomycetes. In *The Biology of the Actinomycetes* ed. Goodfellow, M., Modarski, M. and Williams, S.T. pp. 481–528. London: Academic Press.