

This document has been scanned from hard-copy archives for research and study purposes. Please note not all information may be current. We have tried, in preparing this copy, to make the content accessible to the widest possible audience but in some cases we recognise that the automatic text recognition maybe inadequate and we apologise in advance for any inconvenience this may cause.

**Notes on the
ACTINOMYCETES
of a Forest Soil in
New South Wales**

**1. Isolation & Preliminary
Characterization**

AUTHORS

L. Gerrettson-Cornell

&

J. Kelly



FORESTRY COMMISSION OF N.S.W.

Research Note No. 46

Sydney 1981

**Notes on the
ACTINOMYCETES
of a Forest Soil in
New South Wales**

**1. Isolation & Preliminary
Characterization**

AUTHORS

L. Gerrettson-Cornell

&

J. Kelly



FORESTRY COMMISSION OF N.S.W.

Research Note No. 46

Sydney 1981

RESEARCH NOTE No. 46

NOTES ON THE ACTINOMYCETES OF A FOREST

SOIL IN NEW SOUTH WALES

1. ISOLATION AND PRELIMINARY

CHARACTERIZATION

AUTHORS

L. GERRETSON-CORNELL

J. KELLY

FORESTRY COMMISSION OF N.S.W.

SYDNEY 1981

Forestry Commission of N.S.W.
95 - 99 York Street,
SYDNEY. 2000
AUSTRALIA

The Authors —

L. Gerretson-Cornell, Research Officer, Biology Group
J. Kelly, Research Officer, Chemistry Group.
Wood Technology and Forest Research Division,
P.O. Box 100, Beecroft 2119, Australia.

National Library of Australia Card Number

ISBN 07240 6264 5
ISSN 0085 — 3984

SUMMARY

Actinomycetes were found to be present in large numbers in a soil under a mixed forest of *Eucalyptus*, *Angophora* and understorey species. 126 of these organisms were examined and belonged to 25 groups of streptomycetes. A description is given of their morphology and certain biochemical properties.

INTRODUCTION

Actinomycetes are an important, physiologically active group of microorganisms which play a number of vital roles in the soil. These include the decomposition of organic matter and a direct effect on soil structure (Sykes and Skinner, 1973), but perhaps most important is their capacity to produce antibiotics which are inhibitory to other soil microorganisms. As producers of antibiotics, actinomycetes occupy the highest position in the microbial world (Waksman, 1959).

The abundance of actinomycetes in soil is controlled by a number of factors including (a) the nature and abundance of the organic matter, (b) soil pH, (c) relative moisture content, (d) temperature, (e) soil aeration and (f) vegetation type (Waksman, 1959).

In Australia in recent years there have been few studies on actinomycetes in forest soils (Broadbent and Baker, 1974; Weste and Vithanage, 1977; Sivasithamparam and Parker, 1978; Malajczuk and Comb, 1979). Broadbent and Baker (1974) found that in soils suppressive to the root rot fungus *Phytophthora cinnamomi* Rands, the populations of bacteria and actinomycetes were higher than in soils conducive to root rot. Weste and Vithanage (1977) established that areas with severe disease caused by *P. cinnamomi* had a small microbial population, particularly of actinomycetes, compared with soil from areas with moderate disease. In Western Australia Malajczuk and Comb (1979) found that the more fertile loam soils had higher populations of bacteria, actinomycetes and fungi than lateritic soils.

The aim of the present study was to assess the presence of actinomycetes in a soil supporting sclerophyllous forest, their number and distribution in the soil profile, and their characterization. This study is part of a long term research program devised to establish the role played by these organisms in the transformation of organic matter and their suppressive effects on other organisms.

MATERIALS AND METHODS

a) Site

The study was carried out at Cumberland National Forest (33°45'S, 151°02'E) which is managed by the Forestry Commission of N.S.W. and is located 40 km N.W. of central Sydney. The elevation is 125 m and rainfall averages 1180 mm per year, the driest month being September (60 mm average rainfall) and the wettest February (160 mm average rainfall). The mean daily maximum temperature is 22.8°C ranging from 15.5°C in July to 28.5°C in January while the mean daily minimum temperature is 11.0°C ranging from 5.2°C in July to 16.2°C in February.

The site is located within a forest stand dominated by Sydney blue gum (*Eucalyptus saligna* Sm.) with associated blackbutt (*Eucalyptus pilularis* Sm.) and smooth-barked apple (*Angophora costata* (Gaertn.) Druce). The understorey is composed mainly of shrubs less than 4 m in height dominated by *Pittosporum* spp. and *Lantana* spp. with various smaller shrubs and grasses. The stand was logged at various times prior to 1930, mainly by removing *E. saligna* and *E. pilularis*. The *E. saligna* was allowed to regenerate and the site was restocked in 1932 with *E. pilularis*, and is now relatively uniform except for the presence of some overmature *A. costata*.

The soil parent material is predominantly Wianamatta shale with influence from Hawkesbury sandstone. This has given rise to a red podzolic soil with a very clayey subsoil below 80 mm. The nutritional status of the soils (Table 1) is moderate with reasonable drainage.

b) Sampling procedure, isolation, counting and grouping

Soils from horizons A1, A2, and B-C were sampled from five randomly selected points within the study area. Samples from the A1 horizon were bulked as were those from the A2 and B-C. Each soil was passed through a 2 mm sieve and soil moisture (105°C) and pH were determined. Actinomycetes were isolated by a soil dilution-plate method using the starch-casein medium of Küster and Williams (1964) modified by addition of four antibiotics (Williams and Davies, 1965).

The dilution-plate method was carried out as follows: 10 g of soil were agitated in 95 mL of sterile de-ionized water with a mechanical stirrer for 20 minutes. Tenfold dilutions (10^{-2} , 10^{-3} , 10^{-6}) were prepared from the initial suspension and 5 plates (replicates) established for each dilution, making a total of 30 plates. Into each plate 1 mL of soil suspension was poured followed by 1 mL of aqueous solution of antibiotics and finally 13 mL of molten medium at 48°C. To ensure homogeneous distribution of soil and antibiotics within the medium, each plate was hand rotated 15 times clockwise and 15 times anticlockwise. The plates were inverted and incubated for 8 days at 26°C, and examined from the 6th day onward, using an inverted microscope. Counting was done on the 8th day according to the method of Clark (1965) and hence only those dilutions whose numbers of colonies per plate fell between 25 and 300 were considered.

A number of colonies were randomly selected and subcultured on Czapeck agar. This medium produced a scanty growth of aerial mycelium thus enabling a rapid preliminary evaluation of the type of fructification. The number of cultures subcultured and then identified to the genus was 150. Of these 126 were further investigated.

To divide these actinomycetes in groups, certain cultural characteristics on oatmeal agar and yeast extract-malt extract agar (Shirling and Gottlieb, 1966) were determined. These were the spore mass colour, the substrate mycelium colour, soluble pigment other than melanin secreted into the medium, the shape of the spore chain and the appearance of the spore surface. Production of melanoid pigments was also checked, initially only on peptone-yeast extract iron agar (Tresner and Danga, 1958). Representative strains of each group were grown on oatmeal agar and stored at 26°C.

c) Characterization procedure

Representative strains were characterized according to the methodology of the International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966). This included:

- an assessment of morphological characteristics of each isolate on oatmeal agar, yeast extract-malt extract agar, inorganic salts — starch agar and glycerol-asparagine agar. However the types of spore chain and spore surface were determined only with cultures on oatmeal agar, this being the medium on which the Küster (1972) key is particularly based.
- the use of peptone-yeast extract iron agar and tyrosinase agar to assess melanin formation. Tryptone-yeast extract broth was used as a supplementary test.
- a carbon utilization test, based on nine carbon sources, in which comparison was made with two controls, one negative (no carbon sources) and one positive (basal medium plus D-glucose).

The Methuen Handbook of Colour (1963) was used in most cases to delineate colour. However in some instances, those tables were not applicable and the most descriptive colour in the authors' judgement has been used. The 'spore surface' was determined from specimens either unstained or treated according to the flagella staining technique of Harrigan and McCance (1966).

All tests were carried out at 26°C in darkness interrupted by a few minutes exposure to room light every 2-3 days during examinations. Each test consisted of 3 replicates for each actinomycete group.

RESULTS AND DISCUSSION

a) Counting and division into groups

Actinomycetes were found in high numbers in the soils from the study area. They were particularly numerous in the A1 and A2 horizons (Table 2). However there were significantly less numbers of propagules per gram of dry soil in the A2 compared with the A1 horizon and similarly less in the B-C compared with both the other horizons. This trend corresponded with the decrease in organic matter down the profile (Table 1). It has been reported (Waksman, 1967) that at the surface of a soil, the number of actinomycete colonies developing on agar plates was between 743,000 and 933,000 per gram of soil. At a depth of 75 cms, the number declined to 240,000 per gram of soil. The soils were found to be acidic (Table 2), and hence the present study has complemented other data (Waksman, 1959; Williams *et al.*, 1971; Khan and Williams, 1976) regarding the widespread distribution of acidophilic actinomycetes in acidic soils. The critical pH for the growth of the majority of actinomycetes in soil is considered to be 4.8 — 5.0 and the optimum range between 7.0 and 8.0 (Waksman, 1959).

Microscopical examination of the selected 126 cultures in this study (Tables 3 to 8) showed that they all belonged to the genus *Streptomyces*. On the other hand in a previous study of soil from a different plot within the same forest, some *Nocardia* spp., a member of the genus *Streptoverticillium* and a member of the subgenus *Chainia* were also isolated (L. Gerretson-Cornell, unpublished data). These results confirm the selectivity of the modified starch casein medium of Küster and Williams (1964) for streptomycetes. Because the conditions of the experiment were more favourable to streptomycetes than to all other groups of actinomycetes and since only mesophilic actinomycetes have been considered, the results obtained can only be regarded within the context of the experimental conditions used.

Examination of the morphology of the 126 cultures of streptomycetes combined with tests for the production of melanoid pigments, enabled 25 groups to be distinguished. The frequency of recovery within these groups showed a wide variation (Table 3).

b) Melanin pigment formation and carbon utilization

The melanin reaction was studied on media of which peptone-yeast extract iron agar and tyrosinase agar are the most important. Of these, peptone-yeast extract agar was chosen by Küster (1972) for his identification key. Fifteen of the 25 groups (60%) of isolates gave a positive reaction on peptone-yeast extract iron agar whereas one appeared doubtful. On tyrosinase agar, the number was slightly less (14 positive plus 1 doubtful). Two cultures (E12 and E17) showing good positive melanin reaction on the peptone-yeast extract iron agar failed to do so on tyrosinase agar. This is consistent with the observations of Shirling and Gottlieb (1970). With tryptone-yeast extract broth, the discrepancies were even greater.

The carbon utilization test (Table 8) gave the following observations:

- Approximately 80% of the isolates representing the 25 groups of streptomycetes were capable, either poorly or moderately, to grow on a control medium which had no organic source of food (negative control). Isolates such as E8, E9, E14 and E19, grew very well on this medium, i.e. they appeared at least 'to some' extent, to be autotrophic. Autotrophism in actinomycetes has already been reported. Takamiya and Tubaki (1956) observed an actinomycete, later named *Streptomyces autotrophicus* which could be grown in a pure mineral solution. Fedorov and Kudriasheva (in Waksman, 1959) established that various actinomycetes are capable of fixing atmospheric nitrogen. The reports in this regard appear to be divided. Recently more evidence has been collected that an unidentified soil actinomycete may be involved in nitrogen fixation within the root nodules of *Casuarina cunninghamiana* Miq. (Torrey, 1976).
- Most isolates grew exceptionally well on D-glucose medium.
- Fructose enhanced growth but sucrose did not. With the latter source of carbon, growth was either the same as with the negative control or slightly better.
- More than 50% of the isolates gave very good growth on a medium containing mannitol. On the contrary, when cellulose was added to the basal medium, growth in most cases was very poor. The minus (-) sign (Table 8) indicates growth was inferior to that of the negative control.
- Arabinose and inositol were utilized by a small number of the isolates.

The minimal activity towards sucrose by the group of isolates and the generally more active response on glucose and fructose media suggest that these organisms either have nil or a limited ability to hydrolyse sucrose, i.e. they appear to lack the enzyme sucrase (invertase) found commonly in yeasts.

c) Characterization of isolates

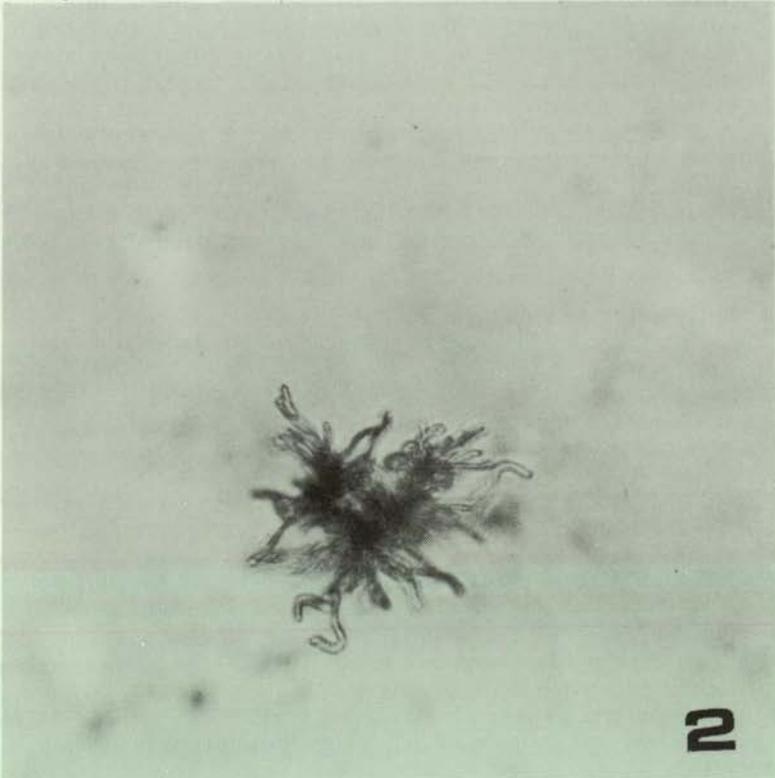
The characterization of all isolates was carried out between the 16th and 21st day of incubation. On oatmeal agar (Table 4), eleven of the twenty five groups of actinomycetes showed a predominant spira type of spore chain. In other groups the spore chain was flexuous or rectus flexibilis. Of the spira chain types, the extended type (Fig. 1) prevailed. The 'flexibilis' spore chain also occurred in tufts (Fig. 2).

The colour of the spore mass of these cultures was generally pale. Seven cultures were white and four were faint pink to pink (including pale red). Two varied from faint cream-grey, greyish-cream or chalky to faint yellow-green colonies. A few cultures were beige and one was greyish-green (light green). The darkest isolates formed either a grey, a beige or a greyish-beige mycelium on this cultural medium. The reverse colour was also prevalently very light except in a few cases such as E2, E16, E24 and E25 which showed either an olive brown, a brownish-orange or a distinctive brown colour. The majority of cultures on oatmeal agar did not produce soluble pigments. There were however a few examples of light beige, brown, grey and even pale red pigments.



Fig. 1. Spira type of spore bearing hyphae in E15. X625. (L1491/5)

Fig. 2. Tuft formation of spore bearing hyphae in E24. X700. (L1491/21)



Examination of the aerial mycelium, reverse colour, and soluble pigments on the other three media showed some variability regarding one or more characteristics. Alternatively a feature could be similar on two to three media and different on the others. For example, culture E2 showed a reverse colour of olive to olive brown on yeast extract-malt extract agar and oatmeal agar, whereas on inorganic salts-starch agar the produced pigment was lemon-yellow and brownish-yellow on glycerol asparagine agar. There was a striking difference in soluble pigment in culture E24 between growth on yeast extract-malt extract agar and that on inorganic salts-starch agar. This actinomycete produced a beautiful brown pigment on the former medium.

Finally an attempt was made to identify these *Streptomyces* to species using Küster's (1972) key. Of the twenty five groups of *Streptomyces* examined, fifteen did not fit this key whereas ten were found to be close to the following species:

- E 1 — *S. pyridomyceticus* Okami and Umezawa
- E 3 — *S. hawaiiensis* Cron, Whitehead, Hooper, Heinemann and Lein
- E 5 — *S. hawaiiensis* Cron, Whitehead, Hooper, Heinemann and Lein
- E 6 — *S. pyridomyceticus* Okami and Umezawa
- E 9 — *S. aureomonopodiales* Krasil'nikov and Yuan
- E 10 — *S. collinus* Lindenbein
- E 11 — *S. filipinensis* Anmann, Gottlieb, Brock, Carter and Whitfield
- E14 — *S. aurantiogriseus* Preobrazhenskaya
- E17 — *S. kursanovii* Preobrazhenskaya, Kudrina, Ryabova and Blinov
- E22 — *S. yokosukanensis* Nakamura

Further comparison of these identifications with the descriptions for each species given by Shirling and Gottlieb (1968a; 1968b; 1969; 1972) showed the existence of some discrepancies.

CONCLUSIONS

This study has shown the presence of a large number of propagules of actinomycetes in a soil under a mixed forest of eucalypt, *Angophora*, and understorey species. Because they were isolated from an acidic environment, there is an indication of a strong capacity for adaptation to low soil pH by these organisms.

Practically all the isolates examined belonged to the genus *Streptomyces*. However this does not exclude the possible existence of other actinomycete genera within the same soil.

The presence of twenty five different groups of streptomycetes within 126 randomly selected isolates indicates the existence of a relatively high degree of speciation. Of these, 44% exhibited a spira type of spore chain. Ten of the twenty five groups were found to be close to but not conspecific with certain described species. This is probably due to the fact that the key (Küster, 1972) which was used as a preliminary attempt at characterisation, was based on only 274 taxa of the approximately 500 which were described by Shirling and Gottlieb (1968a; 1968b; 1969; 1972). Until such time as a final key based on the methodology described in this study is made available, it is not possible to further the identifications.

BIBLIOGRAPHY

- Broadbent, P. and Baker, K.F. (1974). Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. *Aust. J. Agric. Res.* 25: 121-137.
- Clarke, F.E. (1965). Agar-plate method for total microbial count. In (Ed. C.A. Black) *Methods of Soil Analysis*. 1460-1466. American Society of Agronomy, Madison, Wisconsin. 1572 pp.
- Harrigan, W.F. and McCance, M.E. (1966). *Laboratory Methods in Microbiology*. Academic Press, London and New York. 362 pp.
- Khan, M.R. and Williams, S.T. (1976). Studies on the ecology of actinomycetes in soil. VIII. Distribution and characteristics of acidophilic actinomycetes. *Soil Biol. Biochem.* 7: 345-348.
- Kornerup, A. and Wanscher, J.H. (1963). *Methuen Book of Colour*. Methuen and Co. Ltd. Shenvall Press Ltd., London. 223 pp.
- Küster, E. (1972). Simple working key for the classification and identification of named taxa included in the international *Streptomyces* project. *Int. J. Syst. Bact.* 22: 139-148.
- Küster, E. and Williams, S.T. (1964). Selection of media for isolation of Streptomycetes. *Nature, London*, 202: 928-929.
- Malajczuk, N. and Comb, A.J. (1979). The microflora of unsterilized roots of *Eucalyptus calophylla* R. Br. and *Eucalyptus marginata* Donn ex Sm. seedlings grown in soils suppressive and conducive to *Phytophthora cinnamomi* Rands. I. Rhizosphere bacteria, actinomycetes, and fungi. *Aust. J. Bot.* 27: 235-254.
- Shirling, E.B. and Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bact.* 16: 313-340.
- Shirling, E.B. and Gottlieb, D. (1968a). Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. *Int. J. Syst. Bact.* 18: 69-189.
- Shirling, E.B. and Gottlieb, D. (1968b). Cooperative description of type cultures of *Streptomyces*. III. Additional species descriptions from first and second studies. *Int. J. Syst. Bact.* 18: 270-392.
- Shirling, E.B. and Gottlieb, D. (1969). Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third, and fourth studies. *Int. J. Syst. Bact.* 19: 391-512.
- Shirling, E.B. and Gottlieb, D. (1970). Report of the International *Streptomyces* Project. Five years of collaborative research. In (Ed. H. Prauser) *The Actinomycetales*. 79-89. *Jena Int. Symp. Tax.* (1968) 439 pp.
- Shirling, E.B. and Gottlieb, D. (1972). Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. *Int. J. Syst. Bact.* 22: 265-394.
- Sivasithamparam, K. and Parker, C.A. (1978). Effects of certain isolates of bacteria and actinomycetes on *Gaeumannomyces graminis* var. *tritici* and take-all of wheat. *Aust. J. Bot.* 26: 773-82.
- Sykes, G. and Skinner, F.A. (1973). *Actinomycetales: Characteristics and Practical Importance*. Academic Press. London and New York. 339 pp.
- Takamiya, A. and Tubaki, K. (1956). A new form of *Streptomyces* capable of growing autotrophically. *Arch. Mikrobiol.* 25: 58-64.
- Torrey, J.G. (1976). Initiation and development of root nodules of Casuarina (Casuarinaceae). *Amer. J. Bot.* 63: 335-344.
- Tresner, H.D. and Danga, F. (1958). Hydrogen sulfide produced by Streptomycetes as a criterion for species differentiation. *J. Bacteriol.* 76: 239-244.
- Waksman, S. (1959). *The Actinomycetes*. Vol. I. The Williams and Wilkins Co. - Baltimore, U.S.A.
- Waksman, S. (1967). *The Actinomycetes. A summary of current knowledge*. The Ronald Press Co. - New York. 280 pp.
- Weste, G. and Vithanage, K. (1977). Microbial populations of three forest soils: Seasonal variations and changes associated with *P. cinnamomi*. *Aust. J. Bot.* 25: 377-83.
- Williams, S.T. and Davies, F.L. (1965). Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. *J. Gen. Microb.* 38: 251-261.
- Williams, S.T., Davies, F.L., Mayfield, C.I. and Khan, M.R. (1971). Studies on the ecology of actinomycetes in soil - II. The pH requirements of streptomycetes from two acid soils. *Soil Biol. Biochem.* 3: 187-195.

ACKNOWLEDGEMENTS

We would like to thank Mr. D.W. Edwards, Mrs. M.J. Lambert and Dr. J. Turner for helpful advice and discussions with the work and the manuscript.

APPENDIX

TABLE 1

Chemical analysis of the soils from the study area
within Cumberland National Forest

Depth (mm)	% Stone	pH (1:1 H ₂ O)	N	O.M. ^a	Total P (ppm)	Al	Ca	Mg	K	Na
			%							
0- 80	2.3	5.14	0.34	12.34	271	0.28	7.00	5.56	0.62	0.3
80-150	5.9	4.73	0.19	7.12	191	1.89	1.37	2.27	0.27	0.7
150-300	7.3	4.93	0.07	4.30	188	1.36	0.79	2.01	0.26	0.1
300-350	23.9	4.85	0.06	2.42	170	1.35	0.65	1.78	0.16	0.1

a - Organic matter

Table 2

Number of colonies of actinomycetes (per g of dry soil)

	pH		Dilution factor	No. of colonies per plate					No. of colonies per g of dry soil
	H ₂ O	KCl		1	2	3	4	5	
A1	4.91	4.24	10 ⁻⁴	142	138	149	149	125	1 737 500
A2	4.83	3.81	10 ⁻⁴	40	41	27	62	41	508 433
B-C	4.60	3.77	10 ⁻³	102	93	83	109	99	117 108

TABLE 3

Frequency of isolation and melanin pigment evaluation of
25 groups of actinomycetes from a forest soil

Group No.	Frequency	Tryptone-yeast extract broth	Peptone-yeast extract iron agar	Tyrosinase agar
E 1	3	-	-	-
E 2	2	-	-	-
E 3	12	-	+	+
E 4	1	-	+	+
E 5	22	±	+	± to +
E 6	4	-	-	-
E 7	2	±	+	+
E 8	2	+	+	+
E 9	3	-	±	+
E 10	1	-	-	-
E 11	12	-	-	-
E 12	16	±	+	-
E 13	5	-	+	+
E 14	1	-	+	+
E 15	5	-	-	-
E 16	7	-	-	-
E 17	4	±	+	-
E 18	3	-	+	+
E 19	4	-	+	+
E 20	3	-	-	-
E 21	2	±	+	+
E 22	2	±	+	+
E 23	8	+	+	+
E 24	1	-	-	±
E 25	1	+	+	+

+ Strong
± Doubtful or weak
- Not formed

TABLE 4

Cultural characteristics on oatmeal agar of 25 groups of actinomycetes from a forest soil

Group No.	Spore chain *	Spore surface	Spore mass colour	Reverse colour	Soluble pigment
E 1	F & RF	Smooth	White	None	None
E 2	F	Smooth	(Aerial mycelium very scanty), whitish	Olive brown (4D3 to 4D4) †	Brownish grey (4D2)
E 3	S (extended)	Warty	Aerial mycelium practically non-existent	Light greyish yellow in the centre, colourless at the periphery	None
E 4	F	Smooth	Pale greyish-green	Greenish grey (1C2) to grey (1D1)	None
E 5	S (extended or short)	Warty	(Aerial mycelium scanty) white to faint pink	None to greyish yellow (4B6)	None
E 6	F (in tufts)	Smooth	White to faint cream-grey	None	None
E 7	S (extended)	Smooth	White	None to greyish yellow (4C3)	None
E 8	RF or F	Smooth	White, with faint purple margin.	Greyish orange (6B6) in the centre, light yellow orange at the periphery	Light greyish orange
E 9	F	Smooth	(Aerial mycelium scanty faint greyish-cream)	None to orange grey (5B2)	Light beige
E 10	S (extended)	Smooth	Brownish grey (5D2)	None to faint lemon Yellow	None
E 11	S	Warty	White with greyish patches	None	None

Group No.	Spore-chain *	Spore surface	Spore mass colour	Reverse colour	Soluble pigment
E 12	S (extended)	Smooth	(Aerial mycelium scanty) white	None to beige	None
E 13	S (extended)	Smooth	White to faint pink	None to greyish yellow (4B5)	None
E 14	RF to F	Smooth	Pale grey	Light grey (1C1) to grey (1D1)	Pale grey
E 15	S (extended or short)	Smooth	Medium grey (1E1) with white margin	None	Pale red (10A3)
E 16	S (short)	Smooth	Beige & grey (1D1)	Brownish orange (5C5) to brownish grey (5D2)	None
E 17	F	Smooth	Pale grey	Light grey (1C1) to grey (1D1)	Pale grey
E 18	S (extended)	Smooth	White	Light beige	None
E 19	RF to F	Smooth	Chalky with patches of faint yellow-green colour	None to greyish orange	None
E 20	F	Smooth	(Aerial mycelium in light greyish beige patches)†	None	None
E 21	F	Smooth	Greyish-green (30B3)	Greyish beige	None
E 22	S (extended or short)	Warty	Pale red (9A3)	Pale beige to greyish orange (6B4)	Faint pink
E 23	F	Smooth	Light pink	Pink (12A5 to 12A6)	Pale yellow- brown

Group No.	Spore chain *	Spore surface	Spore mass colour	Reverse colour	Soluble pigment
E 24	F (in tufts)	Smooth	Pale greyish-beige	Greyish brown (5D3) to yellowish brown (5E4)	Light brown
E 25	S (short)	Warty	(Aerial mycelium scanty) white	Brownish orange (6C4) to brown (6E4)	Light beige

* Spore chain type:—

R = straight, rectus

S = spira

F = flexibilis

RF= rectus-flexibilis

† Kornerup and Wanscher (1963).

‡ Aerial mycelium growing on a brown-black slimy substance which constituted almost a continuous layer on the substrate mycelium.

TABLE 5

Cultural characteristics on yeast extract – malt extract agar
of 25 groups of actinomycetes from a forest soil

Group No.	Spore mass colour	Reverse colour	Soluble pigment
E 1	White	Between greyish orange (6B3)* and brownish orange (6C3)	None
E 2†	White	Olive (1E3–1F3)	None
E 3	White	Brownish orange (5C4–5C3)	None
E 4	White to faint green	Brownish grey (6D2)	None
E 5	Faint pink	None	None
E 6	Faint greyish-green	Olive brown (4D4–4E4)	None
E 7	Light purple	Beige	None
E 8	Faint yellowish-green	Brown	None
E 9	No aerial mycelium formed	None	None
E 10	Light grey (1C1) to platinum grey (1D1)	Greyish orange (6B4)	None
E 11	(Aerial mycelium scanty), pale grey	None	None
E 12	White	None to light greyish yellow	None
E 13	Light purple	Brownish orange (5C4)	None
E 14	White	None	None
E 15	Between grey (6B1) and orange grey (6B2) with white margin	Light beige to beige	None
E 16	Beigish-grey (5D2) with white margin	Beige	None
E 17	Pale beige-grey	None to beige	None
E 18	(Aerial mycelium scanty) white	None	None
E 19	Faint yellowish-green	Greyish-brown to brown	None
E 20	Beige ‡	Light beige	Light beige
E 21	(Aerial mycelium scanty), white	Beige	None
E 22	Greyish red (7B3)	Greyish orange (6B3–6B4)	None
E 23	Light pink	Pinkish beige (red marking line close to the margin)	None
E 24	Pale grey (1B1)	Beige to greyish brown (7F3)	Brown (6E6)
E 25	White	Brown to dark brown (6F4)	Brown

* Kornerup and Wanscher (1963).

† The aerial mycelium was so thin as to be visible only under a seteromicroscope.

‡ The same brown-black substance produced as on oatmeal agar.

TABLE 6

Cultural characteristics on Inorganic salts – starch agar
of 25 groups of actinomycetes from a forest soil

Group No.	Spore Mass colour	Reverse colour	Soluble pigment
E 1	White	None	None
E 2	(Aerial mycelium very scanty) white	None to lemon yellow	None
E 3	Between violet white (16A2) ** and pale violet (16A3)	None	None
E 4	White with a yellowish green shade	None to greyish beige	None
E 5	Between violet white (16A2) and pale violet (16A3)	None	None
E 6	Chalky	Greyish beige	None
E 7	Between violet white (16A2) and pale violet (16A3)	None to faint beige	None
E 8	White with yellow-green shade	None to light yellow (4A4) or greyish yellow (4B3)	None
E 9	White	Yellowish grey (2D2)	Light greyish beige
E 10	Greyish brown (5E3)	None	None
E 11	Brownish-grey (5D2)	None	None
E 12	White to faint pink	None to greyish orange (6B5)	None
E 13	Between violet white (16A2) and pale violet (16A3)	None to faint beige	None
E 14	Pale greyish-yellow	Between yellowish grey (2B2) and olive grey (2D2)	None
E 15	Between grey (2C1) & yellowish-grey (2C2)	None to light greyish yellow	None
E 16	Brownish grey (5D2) with white margin	None	None
E 17	Pale greyish-yellow	None to faint greyish beige	None
E 18	(Aerial mycelium scanty) pale pink	None to faint yellow orange	None
E 19	White with a yellowish shade	None	None
E 20	Light beigish grey *	Light grey beige to dark grey	None
E 21	White with greyish green shades	Light grey (1C1 to 1D1)	None
E 22	Pale red (9A3)	Pale beige to greyish-orange	None
E 23	Light pink	None to light pink	None

Group No.	Spore mass colour	Reverse colour	Soluble pigment
E 24	Between grey (2B1) and yellowish-grey (2B2)	Light beige	Light pink
E 25	White	Greyish orange	None

* The same as on oatmeal agar but in patches.

** Kornerup and Wanscher (1963)

TABLE 7

Cultural characteristics on Glycerol Asparagine agar
of 25 groups of actinomycetes from a forest soil

Group No.	Spore Mass colour	Reverse colour	Soluble pigment
E 1	White	Faint beige to greyish orange (5B4)*	None
E 2	Faint white-cream	Between brownish yellow (5C7) & yellowish-brown (5E4)	None
E 3	(Aerial mycelium very scanty) white to pale beige	None to between faint yellow (4A3) and greyish yellow (4B3)	None
E 4	Yellowish white to light grey	Between olive brown (4E3) & greyish brown (5D3)	None
E 5	White	Faint greyish-yellow	None
E 6	Faint grey	None to light beige	None
E 7	Between white & pinkish white (11A2)	None to pale greyish yellow	None
E 8	Pale grey with light green shades	Between orange grey (5B2) & greyish orange (5B3)	None
E 9	Between grey (1C1-1D1) & brownish-grey (8E2)	Between brown (7D2) & dark brown (7F5)	Between Pompeian yellow (5C6) & brownish orange (5C3)
E 10	Between greyish white (1C1) & platinum grey (1D1)	None to faint beige	None
E 11	Greyish white (1C1)	None	None
E 12	(Aerial mycelium very scanty) pale beige	None to greyish yellow (4C5)	None
E 13	White	Light orange beige	None
E 14	Faint cream	Between greyish orange (5D3) & greyish brown (5E3)	None
E 15	Between grey (4B1) & yellowish grey (4B2)	Between pale yellow (4A3) & greyish yellow (4B3)	None
E 16	Between light grey (1C1) & grey (1D1)	Between olive green (1F2) & ivy green (1F3)	None
E 17	White to greyish white (1C1) with yellowish shades	Between brownish grey (6D2) & greyish brown (6D3)	None
E 18	(Aerial mycelium very scanty), white	Beige	None
E 19	Pale grey with faint green shades	Between orange grey (5B2) & greyish orange (5B3)	None

Group No.	Spore mass colour	Reverse colour	Soluble pigment
E 20	Between pale grey (1B1) & medium grey (1E1)	Between rust brown (6E8) & brown chocolate (6F4)	Between light brown (6D8 & dark brown (6F7)
E 21	Between white & greyish white (1C1) with yellowish shades	Between brownish grey (6D2) and greyish brown (6D3)	None
E 22	Between pastel red (7A4) & greyish red (7B4)	Light beige with reddish shades	None
E 23	Between purplish white (14A2) & purplish pink (14A3)	Between pale red (9A3) & dull red (9B3)	Between light yellow (4A4) & greyish yellow (4B3)
E 24	Between pale grey (1B1) & platinum grey (1D1)	Between rust brown (6E8) & brown chocolate (6F4)	Between light brown (6D8) & dark brown (6F7)
E 25	White to faint grey	Between yellowish brown (5E4) & greyish brown (5E3)	Faint brown

* Kornerup and Wanscher (1963)

TABLE 8

Carbon utilization test of 25 groups of actinomycetes from a forest soil

Group	Negative control	D-glucose	Sucrose	D-xylose	I-inositol	D-Mannitol	D-fructose	Rhamnose	Raffinose	Cellulose	L-arabinose
E 1	moderate	very good	±	±	+	++	+	±	± to +	- to ±	-
E 2	poor to moderate	good	- to ±	± to +	±	+ to ++	± to +	- to ±	+	- to ±	+
E 3	moderate	moderate	-	-	±	+	+	±	-	-	+
E 4	moderate to good	good	-	+ to ++	± to +	-	+ to ++	±	±	-	± to +
E 5	moderate	good	± to +	- to ±	±	++	+	± to +	+ to ++	-	++
E 6	poor to moderate	good	-	+	+	++	+	-	-	-	-
E 7	moderate	good	-	-	-	++	+	-	-	-	+
E 8	good	very good	±	+	± to +	+ to ++	++	±	±	- to ±	±
E 9	good to very good	very good	- to ±	±	+	±	+	±	-	- to ±	-
E 10	moderate	very good	+	± to +	++	++	+	+	+	± to +	+
E 11	poor to moderate	very good	±	- to ±	±	- to ±	+	±	+	- to ±	±
E 12	moderate	very good	- to ±	+	±	+ to ++	+ to ++	-	±	-	±
E 13	moderate	very good	-	-	-	+	+	-	-	-	+
E 14	good	very good	± to +	+	+	± to +	±	± to +	±	±	+
E 15	moderate	very good	±	±	+ to ++	++	+	-	+	-	±
E 16	moderate	very good	±	±	- to ±	-	+	++	+	-	+ to ++
E 17	very good	very good	±	+	+	-	± to +	- to ±	± to +	- to ±	± to +
E 18			Undetermined			Undetermined			Undetermined		

Group	Negative	D-glucose	Sucrose	D-xylose	I-inositol	D-Mannitol	D-fructose	Rhamnose	Raffinose	Cellulose	L-arabinose
E 19	good	very good	- to ±	+	+	++	+	±	+	-	- to ±
E 20	moderate	very good	-	±	- to ±	+	± to +	± to +	±	-	±
E 21	moderate	very good	±	±	+	-	±	±	±	-	±
E 22	moderate	good	±	±	- to ±	++	+	±	±	-	±
E 23	moderate	good	±	±	+	- to ±	+	- to ±	± to +	-	+
E 24	poor to moderate	good to very good	-	-	- to ±	-	+	±	+	-	-
E 25	poor to moderate	very good	-	-	-	-	+	-	±	-	-

Strongly positive utilization (++) , when growth on tested carbon in basal medium is *equal to* or greater than growth on basal medium plus glucose.

Positive utilization (+) , when growth on tested carbon is *significantly better than* on basal medium without carbon, but somewhat less than on glucose.

Utilization doubtful (±) , when growth on tested carbon is *only slightly better than* on the basal medium without carbon and significantly less than with glucose.

Utilization negative (-) , when growth is *similar to* or less than growth on basal medium without carbon.

National Library of Australia Card Number
ISBN 07240 6264 5
ISSN 0085 - 3984

D. West, Government Printer, New South Wales - 1981