FOREST RESOURCES RESEARCH - TECHNICAL PAPER No. 70

NOTE ON THE ACTINOMYCETES OF THE *STREPTOMYCES HYGROSCOPICUS* - LIKE COMPLEX IN TRADITIONAL TAXONOMY

By L.Gerrettson-Cornell

Forests NSW is a Public Trading Enterprise within the NSW Department of Primary Industries

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FOREST RESOURCES RESEARCH
NSW DEPARTMENT OF PRIMARY INDUSTRIES
SYDNEY
2005

Technical Paper No. 70
February 2005

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Current address: PO Box 563, Baulkham Hills, NSW 2153

Published by:

Forest Resources Research,
Science and Research,
NSW Department of Primary Industries,
121-131 Oratava Avenue, West Pennant Hills 2125
PO Box 100, Beecroft 2119
Australia

ISSN 1324 4345

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ABSTRACT

A *Streptomyces hygroscopicus* complex containing eight species separated on the basis of micromorphological, cultural and biochemical characters is presented. Members of this complex are characterised and distinguished by 'black lysis' of the mycelium in culture in middle and old age. They have often been isolated from forest soils in New South Wales. The work is essentially a literature review of these microorganisms from 1916 to 1990s, with some observations on their association with species of *Allocasuarina* and *Casuarina* in New South Wales.

INTRODUCTION

This chapter deals with the history of the *Streptomyces hygroscopicus* complex and the relationship of the International Streptomyces Project (ISP) with the species of this group.

The *hygroscopicus* complex of streptomycetes encompasses a number of species whose cultures undergo autolysis or lysis of the substrate and/or aerial mycelium. This consists in the appearance, usually within two to three weeks from cultivation (sometimes earlier or later) of brown to black hygroscopic zones which may coalesce into larger areas. When observed under the microscope, this mass appears to be formed of masses of spores and sometimes mycelial fragments in a brownish liquid. This phenomenon is referred to as 'black lysis'. Tresner and Backus (1956) emphasised that this character is one of the chief aids in the initial recognition of possible members of this group. They also reported its occurrence in *S. aureofaciens* but this was probably an unusual event since no mention of it could be found by other authors (Duggar 1948, Duggar *et al.* 1954, Backus *et al.* 1954).

Waksman (1961) established a 'series Hygroscopicus' along with other fifteen series into which he grouped 251 streptomycetes. The series that is now an accepted nomenclatural category for groups of taxa between species and section rank (J. Simpson pers. comm.) had been used in mycology with various genera of fungi (*Penicillium*, *Aspergillus*, *Fusarium* etc.) and was regarded 'to take an intermediate and botanically undefined place between the genus and the species' (Baldacci *et al.* 1954). Besides Waksman (1961), other actinomycetologists (Baldacci *et al.* 1954, Gauze *et al.* 1957, Pridham *et al.* 1958, Falcao de Morais and Maia 1961) had adopted the series for the same purpose. Waksman's 'series Hygroscopicus' included *Streptomyces hygroscopicus*, *S. endus*, *S. platensis*, *S. violaceoniger* (*S. violaceusniger*), *S. limosus* and *S. nigrificans*. The latter (*S. nigrificans*) is nomenclaturally invalid according to the 'Approved Lists of Bacterial Names' (Skerman *et al.* 1980) and DSMZ (2004). Incomprehensible also the inclusion, in this group of *S. limosus*, according to the description of it given by Waksman (1961), Shirling and Gottlieb (1968a) and Bergey (see Pridham and Tresner 1975).

In Gauze *et al.* (1957), a series *Nigrescens* based on the hygroscopic characteristic of the mycelium and encompassing seven isolates of *Actinomyces* ('sensu' Krasil'nikov) (= *Streptomyces) nigrescens* was introduced. While Sveshnikova (the author of this species, in Gauze *et al.* (1957)) regarded *S. nigrescens* as quite distinct from *S. hygroscopicus*, Gauze (Shirling and Gottlieb 1968b) considered the two to be identical. Later on, a number of strains exhibiting the brown-black hygroscopic characteristic were gathered in a group within a series 'Achromogenes' encompassing other actinomycetes (Gauze *et al.* 1983). They were *S. hygroscopicus*, *S.endus*, *S. humidus*, *S. nigrescens*, *S. saraceticus*, *S. platensis*, *S. sioyaensis* and *S. atratus*, all defined as 'close to' *S. hygroscopicus*.

Because some members of the *hygroscopicus* complex were found to be a component of the population of streptomycetes in certain sections of the forest ecosystem in New South Wales (Gerrettson-Cornell 1983-1984, 1987-1988a, unpubl. data), a review of these actinomycetes, based on traditional taxonomic procedure, is presented along with some reference to numerical phenetic taxonomy and DNA relatedness.

Eight species have been included in this group on the basis of characters such as black lysis, grey to brownish grey sporigenous aerial mycelium, spiral type of spore chain, smooth to warty spore surface and negative melanoid pigment production. They are, in alphabetical order *S. endus*, *S. humidus*, *S. hygroscopicus*, *S. melanosporofaciens*, *S. nigrescens*, *S. platensis*, *S. sioyaensis* and *S. violaceusniger*. The first six exhibited black lysis on one or more of the media adopted by the ISP (Shirling and Gottlieb 1966) collaborators. *Streptomyces nigrescens* and *S. humidus* showed this phenomenon only under conditions different from those of the ISP.

Two actinomycetes in the 'achromogenes' group of Gauze *et al.* (1983), notably *S. saraceticus* and *S. atratus,* have been excluded. *Streptomyces atratus* was not described by the ISP investigators and *S. saraceticus,* which is not listed in Bergey's (see Pridham and Tresner 1975) and is nomenclaturally invalid, does not fit the *hygroscopicus* complex according to the description provided by the ISP (Shirling and Gottlieb 1969). Finally *S.sparsogenes,* which was initially included, has been removed as it does not show black lysis and has coremia and spiny spores (Owen *et al.*1962, Shirling and Gottlieb 1969).

Regarding the second aspect of this work, notably the ISP in relation to the species herein described, the following is noteworthy. Because of the confused state of the taxonomy of streptomycetes due to overspeciation and the lack of uniform descriptive techniques and terminology, the ISP was established in the sixties to redescribe authentique type strains of *Streptomyces* using a relatively small number of tests under standard conditions. A specific methodology was thus established (Shirling and Gottlieb 1966) and about 500 species were redescribed according to these new guidelines. Subspecies were not taken into consideration by the ISP. The results of this international collaboration were published in four major, consecutive works (Shirling and Gottlieb 1968a, 1968b, 1969 and 1972). The descriptions of species in the present study have been extracted from these publications except for some minor additions and citations from different sources. Though not necessarily based on the same experimental conditions, these additional sources represented nonetheless a precious collateral means of information without which it would have been hard to achieve some understanding in certain cases of ambiguity. Such an example is provided by the colour of the sporigenous aerial mycelium in *S. hygroscopicus* and *S. endus*. While for the former the series Grey (Shirling and Gottlieb1972), as well as the tab(s) to which it belongs were reported, for *S. endus* the series alone (Shirling and Gottlieb 1968b) was provided with no further comments.

Finally, a few words need to be said on spore surface and colour determination of the sporigenous aerial mycelium.

The ISP (Shirling and Gottlieb 1966) adopted four types of spore silhouettes, according to Tresner *et al.* (1961), assessed by transmission electron microscope (TEM). They were smooth, warty, spiny, hairy. The present work, like all ISP descriptions (Shirling and Gottlieb 1968a, 1968b, 1969 and 1972) is exclusively based on these. Then a new technique, called carbon repligraphy with TEM, was introduced (Dietz and Mathews 1962) which lead to the discovery of the 'rugose' type of spore ornamentation (Dietz and Mathews 1968). This new type of spore surface was confirmed by scanning electron microscopy (Dietz and Mathews 1969) and was later recognized as an additional type of spore surface (Dietz and Mathews,1971). The ISP did not adopt the 'rugose' type and TEM remained the basis of practically all classification systems prior to Williams *et al.*'s (1983) work.

Regarding the determination of the spore mass colour, all ISP work, including the descriptions of the present study, are based exclusively on the system of colour series of Tresner and Backus (1963). This consisted of seven colour series delineated by colour wheels made with tabs from the Color Harmony Manual (see Shirling and Gottlieb 1966, p. 329-330). Unfortunately, as pointed out by Shirling and Gottlieb (1976) and sadly experienced by this writer a few years later, production of the Color Harmony Manual and tabs was discontinued. Because of this, and the fact that evaluation of colour of the *hygroscopicus* complex does not represent a problem, it is suggested, in dealing with this group of actinomycetes, that spore mass and diffusible pigment colours be described simply as done, for example by Lambert and Loria (1989) with *S. scabies*.

MATERIALS AND METHODS

This chapter presents (a) a brief summary of the methodology adopted by the International Streptomyces Project (ISP) to describe *Streptomyces* species including those of the *hygroscopicus* complex of the present study and (b) a basic technique to isolate, characterise and identify members of this group of microorganisms.

(a) The ISP methodology (Shirling and Gottlieb, 1966)

The ISP work contemplates the assessment of some micromorphological, cultural and biochemical features. The first consist of the spore chain morphology and spore surface. The second is based on colour of the sporigenous aerial mycelium, colour of the reverse and soluble pigment on four standard media (ISP Media 2, 3, 4 and 5). The last tests include the assessment of melanoid pigments production on three media (ISP Media 1, 6 and 7) and the utilisation of nine carbon sources. The morphology of spores and sporophores also refers to ISP Media 2, 3, 4 and 5 even if this may not always be specified as it often happens with the spore surface.

The names of the foregoing media are listed hereunder:

Medium 1 = tryptone-yeast extract broth
" 2 = yeast extract-malt extract agar
" 3 = oatmeal agar
" 4 = inorganic salts-starch agar
" 5 = glycerol asparagine agar
" 6 = peptone – yeast extract iron agar
" 7 = tyrosine agar

The sugars for the carbon utilization test are: D-glucose, sucrose, D-xylose, I-inositol, D-mannitol, D-fructose, rhamnose, raffinose and L-arabinose. Assessment of the degree of utilization of a carbon source was based on comparison with a negative control (basal medium containing no carbon source) and a positive one (basal medium plus D-glucose).

(b) Isolation, characterization and identification of members of the *hygroscopicus* complex

The glycerol (or starch) casein medium of Küster and Williams (1964) with the antibiotics of Williams and Davies (1965) for the isolation of streptomycetes is quite suitable for the recovery of these actinomycetes from soil. The minute, radiating cultures which develop in a few days on this medium, in darkness at 24-26°C, may be transferred onto ISP Medium 2. This substratum promotes abundant sporulation in a wide range of *Streptomyces* and facilitates black lysis in species belonging to the *hygroscopicus* group.

For species characterization, the ISP technique (Shirling and Gottlieb 1966) should be followed scrupolously, especially in relation to (a) preparation of the inoculum and (b) carbon utilization test.

For colour determination, because of what was said at the end of the previous chapter, the species at hand may be described simply.

Finally, a number of additional substrata of cultivation is suggested in case black autolysis had not occurred on ISP media but there is reason to suppose, based on micromorphology, cultural characteristics and melanoid pigment production, that the isolate(s) at hand may fall into the *hygroscopicus* group. They are Mineral agar 1 which enhanced black lysis in *S. nigrescens* (Gauze *et al.* 1957) and potato plugs. The composition of the former is reported:

Mineral agar No. 1

KNO_3	1.0 g
K_2HPO_4	0.5 "
$MgSO_4$	0.5 "
NaCl	0.5 "
$FeS0_4$	10.0 mg
Starch	20.0 g
Agar	30.0 "
Tap water	1 lt (we used distilled water)

Biochemical tests like production of H_2S, diastatic activity, gelatin liquefaction, hydrolysis of casein, nitrate reduction and others of the past were not considered by the ISP group of investigators and may be therefore disregarded.

All tests should be carried out at 25° to 30°C with optimum at 28°C. The spore surface should be assessed by trasmission and, if possible, by scanning electron microscopy. Our key (Gerrettson-Cornell and Gwalter 1985) proved quite suitable in the identification of these actinomycetes even if black lysis was not contemplated in it; this feature was nonetheless useful in the final separation of specie, after using the key. Thus, if the identification of an unknown isolate pointed to a number of species, including one known to belong to the *hygroscopicus* group, the occurrence of black lysis in our strains would have final say. Finally, a scheme (Table 1) for a preliminary identification of species of the *hygroscopicus* complex, based exclusively on the ISP methodology (Shirling and Gottlieb 1966) and descriptions of taxa (Shirling and Gottlieb 1968a, 1968b, 1969 and 1972) is presented.

RESULTS – TAXA STUDIED

The following species of the *hygroscopicus* complex are listed in alphabetical order. They were extracted from the International Streptomyces Project (ISP) works (Shirling and Gottlieb 1968a, 1968b, 1969 and 1972) and reproduced almost identically with only a few, minor modifications and additions reported in 'Remarks', at the end of each species description.

Streptomyces endus Anderson and Gottlieb 1952

ISP description in Shirling and Gottlieb (1968b, p. 316)

Type strain:	NRRL 2339
Nomenclaturally valid:	Approved Lists of Bacterial Names (Skerman *et al.* 1980) and DSMZ (2004).
Spore chain morphology:	Spirales. Tight spirals. Individual spores not easily individualized in the spiral chain (Gottlieb and Carter 1956, Shirling and Gottlieb 1968b). No reference made on number of spores per chain. Coalescence of spiral coils in black, moist masses.
Spore surface:	Warty.
Sporigenous aerial mycelium:	In the Grey colour series on ISP Media 2, 3 and 4; white on Medium 5. Black lysis in mature cultures.
Reverse side of colony:	Greyed yellow to yellowish brown or grey on ISP Media 2, 3, 4 and 5. Black autolysis occasionally observed in the substrate mycelium, in older cultures.
Soluble pigment:	None in ISP Media 2, 3, 4 and 5.
Melanoid pigment production in ISP Media 1, 6 and 7:	None.
Carbon utilization test:	D-glucose, D-xylose, D-mannitol, D-fructose, rhamnose and L-arabinose are utilized for growth. No growth or only traces of growth on sucrose, I-inositol and raffinose.
Antibiotic activity:	Produces endomycin, an antibiotic active against bacteria, fungi and yeasts but particularly against fungi (Gottlieb and Carter 1956, Waksman 1961, Pridham and Tresner 1975).

Remarks:

Pridham and Tresner (1975) place *S. endus* in the 'smooth' type of spore surface. As for the sporigenous aerial mycelium, it was also classified as Grey by Pridham and Tresner (1975) and brownish grey by Tresner and Backus (1956).

Streptomyces humidus Nakazawa and Shibata 1956

Description in Shirling and Gottlieb (1968b, p. 334)

Type strains:	ISP 5263; ATCC 12760
Nomenclaturally valid:	Approved Lists of Bacterial Names (Skerman *et al.* 1980) and DSMZ (2004). Not valid as reported by the ISP: *S. humidus* Nakazawa, Shibata, Tanabe and Yamamoto.
Spore chain morphology:	Spirales (10 to 50 spores per chain) on ISP Media 2, 3, 4 and 5. Many incomplete spirals resembling the Retinaculum Apertum also present.
Spore surface:	Smooth.
Sporigenous aerial mycelium:	In the Grey (brownish grey) or Red (greyish yellowish pink) colour series, presumably (not specified in the ISP description) on each of the ISP Media 2, 3, 4 and 5. Black autolysis not recorded in ISP media but reported in original description as indicated by Shirling and Gottlieb (1968b) and Waksman (1961).
Reverse side of colony:	Colourless to pale greyish yellow or light olive grey on ISP Media 2, 3, 4 and 5.
Soluble pigment:	None or only trace of yellow in ISP Media 2, 3, 4 and 5.
Melanoid pigment production in ISP Media 1, 6 and 7:	None.
Carbon sources utilization:	D-glucose, D-xylose, I-inositol, D-mannitol, D-fructose, rhamnose and L-arabinose utilized for growth. No growth or only traces on sucrose and raffinose.
Antibiotic activity:	Produces dihydrostreptomycin, humidin and cobalamines (Imamura *et al.* 1956, Pridham and Tresner, 1975).

Remarks:

Regarding the spore chain type, Pridham and Tresner (1975) apportioned *S. humidus* to the Spirales but later, in the description only referred to it as atypical Retinaculum Apertum. Pridham and Tresner (1975) also apportioned the spore mass of this actinomycete to the Grey colour series. Imamura *et al.* (1956), Waksman (1961) and Gauze *et al.* (1983) regarded *S. humidus* to be closely related to *S. hygroscopicus*.

Streptomyces hygroscopicus (Jensen 1931) Waksman and Henrici 1948

ISP description in Shirling and Gottlieb (1972, p. 307).

Type strain:	ATCC 27438.
Nomenclaturally valid:	Approved Lists of Bacterial Names (Skerman *et al.* 1980).
Spore chain morphology:	Spirales (10 to 50 spores per chain), on ISP Media 2, 3, 4 and 5. Spirals are compact, in dense clusters often coalescing in dark, moist masses of spores.
Spore surface:	Warty, with individual spores not clearly delineated.
Sporigenous aerial mycelium:	Brownish grey or light greyish reddish brown (Grey color series) on ISP Media 2, 3, 4, and 5. Medium to dark grey areas also observed on Media 3 and 4. Black autolysis observed, especially on Media 3 and 4.
Reverse side of colony:	Colourless to greyish yellow, pale yellow, light olive brown or grey on ISP Media 2, 3, 4 and 5. Dark discoloration below hygroscopic areas may be observed.
Soluble pigment:	None to traces of yellow in ISP Media 2, 3, 4 and 5.
Melanoid pigments production in ISP Media 1, 6 and 7:	None.
Carbon sources utilization:	D-glucose, D-mannitol, D-fructose and rhamnose utilized for growth. Utilisation of D-xylose, I-inositol and L-arabinose doubtful due to the fact that growth on carbon free medium (negative control) was good; this made comparison with it difficult. Sucrose and raffinose 'probably' not utilised (definitely not utilised, according to Labeda and Lyons 1991). In the same work, these two authors also reported positive utilisation of D-xylose, I-inositol and L-arabinose.
Antibiotic activity:	Produces the hygromycin complex, active against mycobacteria and roundworms (Waksman 1961, Pridham and Tresner 1975). Other antibiotics such as geldanamycin, rapamycin and nigericin have been recently reported (Google Search 2004).

Remarks:

Streptomyces hygroscopicus was first isolated from soil samples of the University of Sydney collection (origin unspecified) by Jensen (1931). He observed black autolysis on dextrose (or glycerin) asparagine agar. Jensen referred to this microorganism as a 'species-group' because of its great variability, thus adopting a concept established by Waksman (1919) for the same reason. Jensen defined the species-group as 'a broad group of strains agreeing in certain morphological and biological features'. He went on to say that 'the opposite practice adopted by Millard and Burr (1926) of establishing species differentiation on the basis of every observed constant difference is certainly logical but hardly practicable, since nearly every observed strain of actinomycetes isolated from plating from an ordinary soil could be raised to the rank of species'.

Additional information on spore chain type, spore surface and sporigenous aerial mycelium are included hereinafter.

Jensen reported: 'Spirals are numerous, sinistrorse, narrow, sometimes long but mostly quite short, only one to two turns, closed, typically situated as dense clusters on the main stem of the aerial hyphae." Tresner and Backus (1956) spoke of short sporophores arising as side branches of main hyphae and terminating in tight one to two turns or more.

The spore surface was smooth according to Waksman (1961). Smooth according to Tresner *et al.* (1967). These authors examined many strains of *S. hygroscopicus* and *S. platensis* including their respective holotypes by direct electron microscopy and found them all smooth. However, while the former species exhibited short-cylindrical, phalangiform spores, the *S. platensis* spores were elliptical or (a minority) half-moon or reniform in shape. The distinction, as shown by the photo attached in these authors' article is quite marked. Spore surface smooth, at times roughened, according to Pridham and Tresner (1975); on the other hand these authors placed this species in the group with 'smooth' spores. Rugose according to Williams *et al.* (1983) and Williams *et al.* (1989). Rough according to Labeda and Lyons (1991).

As for the sporigenous aerial mycelium, Tresner and Backus (1956) also reported, besides the brown-black hygroscopic patches, a brownish grey spore mass on media promoting abundant sporulation including potato plugs, at 28°C. Pridham and Tresner (1975) placed this species in the Grey series without further specification. On the other hand Grey in their script meant 'grey to brownish' (p. 748). Labeda and Lyons (1991), in their emended description of *S. hygoscopicus* simply referred to the spore mass as 'Grey'. They also observed black autolysis.

Streptomyces melanosporofaciens Arcamone, Bertazzoli, Ghione and Scotti 1959

ISP description in Shirling and Gottlieb (1969, p. 452).

Type strains:	ISP 5318; ATCC 25473.
Nomenclaturally valid:	Approved Lists of Bacterial Names (Skerman *et al.* 1980) and DSMZ (2004).
Spore chain morphology:	Spirales (loops, incomplete spirals or true spirals); sporophores often of 3 to 10 spores per chain on ISP Media 2, 3, 4 and 5.
Spore surface:	Smooth, sometimes warty.
Sporigenous aerial mycelium:	In the Grey colour series on ISP Media 2 and 3. Poor sporulation or Yellow series on ISP Media 4 and 5. Strong black autolysis.
Reverse side of colony:	Dark greyish yellow to orange yellow on ISP Medium 2; light yellow to light greyish olive on ISP Medium 3; colourless to strong yellow on ISP Media 4 and 5. These pigments are not pH indicator.
Soluble pigment:	None in ISP Media 2, 3, 4 and 5.
Melanoid pigment production in ISP Media 1, 6 and 7:	None. In Medium 7, a reddish brown, rose or yellow non-melanoid colour may be formed.
Carbon sources utilization:	D-glucose, D-xylose, I-inositol, D-mannitol, D-fructose, rhamnose, raffinose and L-arabinose are utilised for growth. Only traces of growth with sucrose.
Antibiotic activity:	Produces melanosporin which inhibits bacteria and fungi including (to some extent) *Candida albicans* in mouse and elaiophylin which is active against gram positive bacteria and tubercle bacilli (Arcamone *et al.* 1959, Pridham and Tresner 1975).

Remarks:

Spores smooth, according to Pridham and Tresner (1975). The same authors apportioned the colour of the sporigenous aerial mycelium of this actinomycete to the Grey series.

Streptomyces nigrescens (Sveshnikova 1957) Pridham, Hesseltine and Benedict 1958

(Actinomyces nigrescens Sveshnikova in Gauze *et al.* 1957).

Description in Shirling and Gottlieb (1968b, p. 353).

Type strains:	ISP 5276, ATCC 23941.
Nomenclaturally valid:	Approved Lists of Bacterial Names (Skerman *et al.* 1980) and DSMZ (2004).
Spore chain morphology:	Spirales; 10 to 50 spores and more per chain on ISP Media 2, 3, 4 and 5.
Spore surface:	Smooth to warty.
Sporigenous aerial mycelium:	In the Grey colour series on ISP Media 2, 3, 4 and 5. No black autolysis reported by ISP investigators but this phenomenon was very marked on Mineral agar 1 (Gauze *et al.* 1957).
Reverse side of colony on ISP Media 2, 3, 4 and 5:	Colourless to greyish yellow.
Soluble pigment:	None in ISP Media 2, 3, 4 and 5.
Melanoid pigment production in ISP Media 1, 6 and 7:	None.
Carbon utilization test:	D-glucose, sucrose, D-xylose, I-inositol, D-mannitol, D-fructose and raffinose are utilized for growth. No growth or only trace with L-arabinose and rhamnose.
Antibiotic activity:	Strongly inhibits staphylococci, *Bacterium subtilis* and *B. mesentericus* but not *B. coli* (Gauze *et al.* 1957). To a lesser extent, it also inhibits *Candida albicans*.

Remarks:

Pridham and Tresner (1975) reported a smooth spore surface and a Grey sporigenous aerial mycelium.

Streptomyces platensis Tresner and Backus 1956

ISP description in Shirling and Gottlieb (1968b, p.360).

Type strains:	NRRL 2364.
Nomenclaturally valid:	Approved Lists of Bacterial Names (Skerman *et al.* 1980) and DSMZ (2004). The name *S. platensis* Pittenger and Gottlieb 1954 used in the ISP descriptions is invalid according to the above Lists of Bacterial Names.
Spore chain morphology:	Spirales; 10 to 50 spores and over per chain on ISP Media 2, 3, 4 and 5.
Spore surface:	Smooth.
Sporigenous aerial mycelium:	In the Grey colour series on ISP Media 2, 3, 4 and 5. Strong black autolysis.
Reverse side of colony:	Greyish yellow on ISP Media 3 and 4; greyish yellowish pink or light reddish brown on ISP Media 2 and 5. The reddish pigment turns to colourless or pale yellow with 0.05 N HCl.
Soluble pigment:	Traces of red and yellow in ISP Media 2, 3, 4 and 5. The reddish pigment is pH sensitive changing to nearly colourless with 0.05N HCl.
Melanoid pigment production in ISP Media 1, 6 and 7:	Negative.
Carbon sources utilization:	D-glucose, sucrose, I-inositol, D-mannitol, D-fructose and raffinose are utilized for growth. Utilization of L-arabinose and D-xylose is doubtful. No growth or only trace with rhamnose.
Antibiotic activity:	Produces terramycin, a valuable antibacterial agent (McGuire 1954). Pridham and Tresner (1975) report oxytetracycline and 7-chlortetracycline.

Remarks:

Tresner *et al.* (1967) also reported a smooth spore surface for *S. platensis*. Pridham and Tresner (1975) placed it in the 'smooth' category. Dietz (1976) reported *S. platensis* NRRL 2364 as being characterised by smooth spores silhouette and spore surface with basket-weave ornamentation. Tresner and Backus (1956) reported a brownish-grey spore mass colour and Pridham and Tresner (1975) placed it in the Grey series.

Streptomyces sioyaensis Nishimura, Okamoto, Mayama, Ohtsuka, Nakajima, Tawara, Shimohira and Shimaoka 1961

ISP description in Shirling and Gottlieb (1968a, p.170).

Type strains:	ISP 5032; ATCC 13989.
Nomenclaturally valid:	Approved Lists of Bacterial Names (Skerman *et al.* 1980) and DSMZ (2004).
Spore chain morphology:	Spirales; 10 to 50 spores or more per chain on ISP Media 3 and 5. Poor aerial mycelium on ISP Media 2 and 4 made assessment impossible.
Spore surface:	Smooth.
Sporigenous aerial mycelium:	In the Grey colour series on ISP Media 2, 3, 4 and 5. However, on ISP Media 2 and 4, growth may be slow or nil. Black autolysis reported by one collaborator.
Reverse side of colony:	Greyed yellow modified or not by green on ISP Media 2, 3, 4 and 5.
Soluble pigment:	Trace of yellow (not pH sensitive) in ISP Media 2, 3, 4 and 5.
Melanoid pigment production in ISP Media 6 and 7:	Negative. No information in ISP Medium 1.
Carbon sources utilization:	No growth or only traces on L-arabinose and rhamnose. D-glucose, sucrose, D-xylose, I-inositol, D-mannitol, D-fructose and raffinose are utilized for growth.
Antibiotic activity:	Produces the antibacterial antibiotic siomycin (Pridham and Tresner 1975).

Remarks:

Spore surface smooth and sporigenous aerial mycelium in the Grey series according to Pridham and Tresner (1975).

Streptomyces violaceusniger (Waksman and Curtis 1916) Pridham, Hesseltine and Benedict 1958

Nomenclaturally valid according to DSMZ (2004).

Type strain:	NRRL B 1476. The ISP named this species *S. violaceoniger* (Waksman and Curtis 1916) Waksman and Henrici 1948, based on type strains ISP 5563 and NRRL B 1476 (Shirling and Gottlieb 1972, p. 364). This was nomenclaturally invalid, according to the Approved Lists of Bacterial Names (Skerman *et al.* 1980) but valid, by the same 'Lists', as *S. violaceoniger* (Waksman and Curtis 1916) Pridham, Hesseltine and Benedict 1958, based on the same description and type strain NRRL B 1476. Following Labeda and Lyons' (1991) dispute over validity of NRRL B 1476 as type strain for the species (see "Discussion and Conclusions"), the DSMZ (2004) retained that strain as valid.
Spore chain morphology:	Spirales, with compact spirals on ISP Media 2, 3, 4 and 5. Spirals often coalescing in black, moist masses. No reference made to number of spores per chain.
Spore surface:	Smooth (though Figure 301, in this writer's opinion, shows some roughness).
Sporigenous aerial mycelium:	In the Grey colour series (yellowish grey, greyish yellowish brown, light greyish brown, brownish grey, light greyish reddish brown, dark grey) on ISP Media 2, 3, 4 and 5. Strong black autolysis on these media.
Reverse side of colony:	Colourless to greyish yellow or light olive green to dark olive or dark greyish green, presumably (not specified in the description) on ISP Media 2, 3, 4 and 5.
Soluble pigment:	None in ISP Media 2, 3, 4 and 5.
Melanoid pigment production in ISP Media 1, 6 and 7:	None.
Carbon sources utilization:	D-glucose, sucrose, D-xylose, I-inositol, D-mannitol, D-fructose, rhamnose, raffinose and L-arabinose are all utilized for growth. The same according to Labeda and Lyons (1991).
Antibiotic actyivity:	Produces the antibiotic nigericin, active against bacteria and fungi (Waksman 1961, Pridham and Tresner 1975).

Remarks:

Pridham and Tresner (1975) also regarded the spore surface as smooth but added that spores may appear roughened. Labeda and Lyons (1991) reported 'smooth to rough' spores.

For the sporigenous aerial mycelium we read in Waksman (1961) that Ettlinger *et al.* (1958) reported carmine red or cinnamom brown colour of the spore mass. Pridham and Tresner (1975) also place it in the Grey series and Labeda and Lyons (1991) refer to it as Grey, turning black and moist when mature.

Table 1. Subdivision of the *Streptomyces hygroscopicus*-complex species based on ISP methodology and descriptions. The melanoid pigment production is not listed being constantly negative.

1. Black lysis observed on ISP media

    2. Spore chain prevalently short (< 10 spores/chain)

        Spore surface smooth to warty. Sporigenous aerial mycelium poor or in the Yellow series on Media 4 and 5. Colourless to strong yellow reverse observed on Media 4 and 5. Sucrose not utilised.

        S. melanosporofaciens

    2. Spore chain not as above (> 10-50 spores/chain)

        3. Spore surface warty. Sporigenous aerial mycelium grey to brownish grey.

            4. Sporigenous aerial mycelium white on Medium 5.

            S. endus

            4. Not as above.

            S. hygroscopicus

        3. Spore surface smooth. Sporigenous aerial mycelium grey to brownish grey.

            4. Poor or no growth on Media 2 and 4. Rhamnose not utilised for growth.

            S. sioyaensis

            4. Growth on Media 2 and 4 not as above.

                5. Traces of yellow and red in reverse and soluble pigment. Rhamnose not utilised.

                S. platensis

                5. Reverse colourless to dark olive or dark greyish green. No soluble pigment. Rhamnose utilised.

                S. violaceusniger

1[1] Black lysis only observed on media other than the ISPs (see 'Materials and Methods').

    2[1] Spore surface smooth to warty. Spore chain 'Spirales'. Sporigenous aerial mycelium in the Grey series on Media 2, 3, 4 and 5. Sucrose and raffinose utilised.

    S. nigrescens

    2[1] Spore surface smooth. Spore chain of the 'Retinaculum Apertum' type very frequently observed along with 'Spirales'. Sporigenous aerial mycelium brownish grey to reddish on Media 2, 3, 4 and 5. Sucrose and raffinose not utilized.

    S. humidus

DISCUSSION AND CONCLUSIONS

In a study of *Streptomyces hygroscopicus*, *S. platensis* and *S. endus*, Tresner and Backus (1956) established what they regarded to be the three most common characteristics of the *S. hygroscopicus* group, notably:

(a) Sporophores as short side branches of a subtending hypha, ending in tight spirals of two to more turns and often clustering.

(b) The brownish-grey colour (Mouse Grey to Benzo Brown (Ridgway 1912) of the sporigenous aerial mycelium on media promoting abundant sporulation including potato plugs.

(c) Strong black lysis also on media promoting abundant sporulation. This however varied in intensity according to the medium of cultivation and sometimes on the same medium. Great variability in soluble pigment production, colour of vegetative mycelium and some biochemical reactions was also observed.

Streptomyces platensis and *S. endus* did not appear to be sufficiently distinct from *S. hygroscopicus* to be granted separate status and were reduced to synonymy with it (Tresner and Backus 1956). Waksman (1961) reported these authors conclusion as well as the closeness of *S. violaceusniger* with *S. hygroscopicus* according to other authors. Tresner *et al.* (1967) concluded that *S. hygroscopicus* and *S. platensis* are distinguishable species, based on spore micromorphology and certain physiological reactions.

In a study based on 139 character states (later reduced to 41), Williams *et al.* (1983) apportioned the following species besides *S. violaceusniger* to cluster 32 (see Table 1, p. 1753) of these authors study, named after it instead of after *S. hygroscopicus* because of the priority of *S. violaceusniger:*

S. hygroscopicus (ISP 5578)
S. hygroscopicus (N 736)
S. endus (ISP 5187)
S. melanosporofaciens (ISP 5318)
S. sparsogenes (ISP 5356)
S. violaceoniger (ISP 5563)

This cluster was defined at the 77.5% S_{SM} level. Because of its higher degree of homogeneicity (the presence of spiny spores in *S. sparsogenes* would not represent a problem since, in numerical taxonomy characters have equal weight), the cluster is considered a 'species'. Conversely, more heterogeneus clusters are regarded as 'species groups'. Williams *et al.* (1983) characterised this cluster as having rugose spores in tight spirals which autolized to a black slime.

In Williams *et al.* (1989), the type strains of *Streptomyces* are distributed into 23 major clusters, 20 minor clusters and 25 single members clusters on the basis of a reduced number of characters states (from 139 to 41). The minor (containing two to three strains) and single members clusters are considered 'species' and the major clusters (containing four or more strains) are regarded as 'species-groups'. Again, clusters are named after the earliest validly described species they contain. The other species within the cluster are listed as 'subjective synonyms'. Finally, species regarded as not being entirely typical of their cluster, despite their high degree of similarity are listed as 'allied species'. So we have the species ('species-group') *S. violaceusniger*, defined at the 77.5% S_{SM} (similarity level) and encompassing the following 'subjective synonyms':

S. endus (NRRL 2339)
S. hygroscopicus (ATCC 27438)
S. melanosporofaciens (ATCC 25473)
S. sparsogenes Owen, Dietz and Camiener 1962 (NRRL 2940)

Its characteristics are defined as follows:

Spore surface rugose.
Spore mass becoming black and slimy after maturity.
Reverse yellow brown.
Diffusable pigment not produced.
Melanin pigments not produced.

Finally Labeda and Lyons (1991), on the basis of DNA relatedness, established that:

(a) Strain NRRL B-1478 was the one used by the ISP to describe *S. violaceusniger*, not NRRL B 1476 as reported. Therefore, they concluded, it should be designated the type strain of that species instead of the latter.

(b) Strains NRRL B-1476 and NRRL B-1477, considered to be *S. violaceusniger*, which exhibited a high level of DNA relatedness and phenotipic similarity with the type strain of *S. hygroscopicus* (for instance, they did not utilize raffinose and sucrose) should be transferred to *S. hygroscopicus*. Conversely, DSMZ (2004) regarded NRRL B 1476 as a type strain of *S. violaceusniger* thus validating the nomenclature *S. violaceusniger* (Waksman and Curtis 1916) Pridham, Hesseltine and Benedict 1958, as reported in this study.

(c) *Streptomyces endus* is definitely a subjective synonym of *S. hygroscopicus*.

(d) On the base of DNA relatedness, *S. melanosporofaciens* and *S. sparsogenes* should be considered distinct species, not subjective synonyms of *S. violaceusniger* as suggested by Williams *et al.* (1989).

Labeda and Lyons (1991) also referred to a study of the taxonomic status of a number of strains of *S. violaceusniger*, *S. hygroscopicus*, *S. sparsogenes* and *S. melanosporofaciens*, based on DNA relatedness. This work led to the separation of *S. violaceusniger* strains 'into seven DNA homology groups, including four single-member clusters, at a level of DNA relatedness of 70%. *Streptomyces hygroscopicus* NRRL 2387 (type strain) clustered with the type strain of *S. endus* and several *S. violaceusniger* strains. The other strains of *S. hygroscopicus*, *S. sparsogenes* and *S. melanosporofaciens* formed single-membered homology groups'.

Regarding the position of *S. platensis*, *S. nigrescens* and *S. humidus*, it is to be noted that, according to Williams *et al.* (1983), cluster 29, named after *S. lydicus*, encompasses eleven species including *S. platensis*, *S. nigrescens* and *S. sioyaensis*. In Williams *et al.* (1989) the species *S. lydicus* is close to *S. violaceusniger* and includes six subjective synonyms,including *S. nigrescens*, *S. platensis* and *S. sioyaensis* all characterized by spiral spore chain, smooth spore surface, grey spore mass, yellow brown reverse, no soluble pigment and no melanoid pigment. This homogeneous group was defined at the 77.5% S_{SM} level. With one exception represented by *S. albulus* which separated from the rest of the cluster at the 63% similarity level, the other species are considered synonyms of the type species (*S. lydicus*).

Finally, *S. humidus* would fall into the *S. diastaticus* cluster and is regarded as a subjective synonym of it (Williams *et al.* 1983, Williams *et al.* 1989). This rather heterogeneous species-group was defined at the 77.5% S_{SM} level but split into several subgroups at the 63% S_j similarity level (allied species). The type strain and its synonyms were characterised by spiral spore chains, grey or sparse aerial sporigenous mycelium, smooth spores, yellow brown reverse, no soluble pigment and negative melanin reaction.

What has been discussed does not completely infringe the validity of the traditional taxonomic method. After all, we started with the species-group and degree of affinity and we ended up with the cluster (= species-group) and similarity level. What changes is the direction and nature of the relationship but the old method can still provide a good prediction. Besides, so many antibiotics were discovered with it. Occasionally, as experienced by this writer with strains that do not appear to fit anywhere, numerical phenetic taxonomy and genetical relatedness are the only alternatives.

Finally, a few lines on our investigation on actinomycetes in New South Wales forest soils, which also included some members of the *hygroscopicus* complex are included. These studies lead to the isolation of a wide range of *Streptomyces* species from the rhizoplane-rhizosphere of *Casuarina cunnonghamiana*, *C. glauca*, *C. littoralis*, *C. torulosa* and *Allocasuarina verticillata.* Strains affinis to *S. platensis*, *S. violaceusniger*, and *S. hygroscopicus* (Gerrettson-Cornell 1983-1984, 1987-1988a, unpubl. data) were isolated. They showed marked *in vitro* antibiotic activity against *Phytophthora cinnamomi* (Gerrettson-Cornell 1983-1984, unpubl. data), the root rot fungus once quite desctructive in the eucalypt forests of Western Australia and Victoria. Though the presence of antibiotics in soil has been denied by Gray and Williams (1971) and Williams *et al.* (1983a), there is on the other hand abundant literature on direct microbial antagonism in soil, starting from the early work of Krasil'nikov (1958). Our findings appeared to support the hypothesis that these actinomycetes may contribute to the resistance of most members of *Casuarinaceae* to *Phytophthora cinnamomi* in the field. In 1985-1986, studies on mineralisation of organic nitrogen by streptomycetes were also established and given great impetus by the then Chief, Wood Technology and Forest Research Division, Dr Ian Bevege. Tests were performed *in vitro* (Gerrettson-Cornell 1987-1988a, 1987-1988b, unpubl. data) but a few, very encouraging tests, were also carried out in soil (Gerrettson-Cornell 1987-1988c). Many actinomycetes from the rhizoplane of Casuarinas, including the foregoing three species, proved to be strong mineralizers of organic nitrogen (Gerrettson-Cornell 1987-1988a, unpubl. data) thus supporting the idea that they may play an important role in the transformation of organic matter in soil immediately around the roots of these trees.

ACKNOWLEDGEMENTS

Forests NSW and Mr Robert Eldridge, Forest Resources Research, are kindly acknowledged for supporting this work.

Mr Jack Simpson, Forests Resources Research, and Mr John Walker, formerly Research Scientist with the Biological and Chemical Research Institute, NSW Agriculture, Rydalmere, are kindly acknowledged for restructuring the paper and many helpful suggestions.

Mr Narendra Anuj, Senior Librarian, Forests NSW, is acknowledged for providing important literature for this study.

Mrs Joy Gardner is acknowledged for her very good editoral work.

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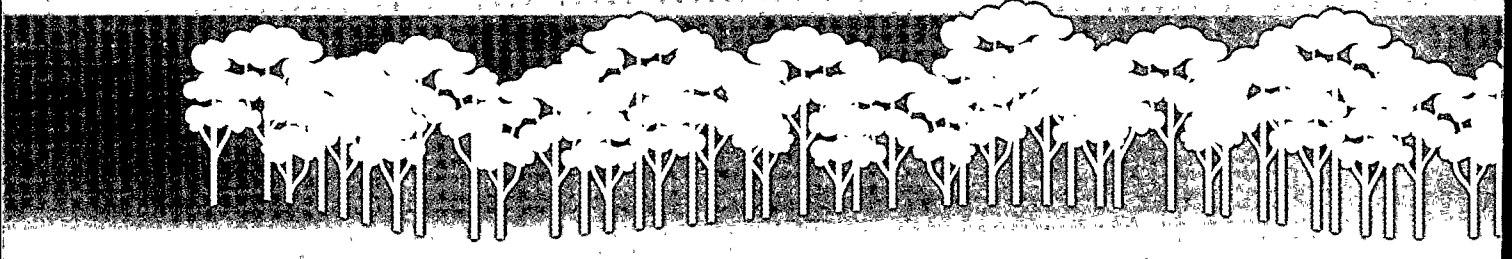

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