STUDIES IN THE ACTINOMYCETACEAE

The nutrition of the streptomycetes.

Summary of a thesis by G.D. Floodgate

Thirty six cultures of streptomycetes taken from a collection gathered from various sources were used in this study, and an attempt made to identify them using well known bacteriological techniques. A description of each organism is given in Appendix I and details of the media used in Appendix II.

The nutrition of the organisms was examined under conditions designed to eliminate unwanted utilisable substances. All glassware was acid washed; chemicals were the finest quality availabl The ability of each organism to assimilate (1) each of 30 carbon substances using ammonium sulphate as nitrogen source, and (2) each of 25 nitrogen substances using sodium pyruvate as carbon source, was investigated. The requirements of 10 of the organisms for 8 growth factors was also studied. All the nutritional experiments were aerated by shaking.

The production of acid from carbohydrates was explored using several techniques.

From these experiments it was concluded that:-

1). Twenty-seven of the cultures were strains of Streptomyces albus species-group; the remaining organisms each belonged to a different species.

2). Simple sugars and some organic acids were well utilised. Alcohols, with the exceptions of glycerol and mannitol, were not

well assimilated, nor were simple fatty acids except acetate.

3). Ammonium and nitrate were good inorganic nitrogen sources. Nitrite supported the growth of many strains providing that the optimum concentration was used, together with a carbon source which was available under these conditions.

Amino acids, except cystine, methionine, valine and tryptophane supported excellent growth. Urea, uric acid and xanthine were also excellent nitrogen sources for these organisms.

4.). None of the organisms examined required any of the growth factors investigated.

5). The carbon assimilation of the Streptomyces albus strains fell into 3 patterns, suggesting that this species-group contains at least 3 nutritional types. The nitrogen assimilation pattern could not be used to differentiate between organisms.

6). No satisfactory method of investigating acid production by streptomycetes was found, and the results obtained were equivocal.

A survey of the relevant literature and a bibliography are included.

STUDIES IN THE ACTINOMYCETACEAE

The nutrition of the streptomycetes

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by

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INTRODUCTION.

The purpose of this study was:-

1). To investigate the carbon, nitrogen and growth factor requirements of a number of streptomycetes.

2). To determine whether any nutritional pattern which might be disclosed can aid in the classification of the genus.

3). To determine whether there is any correlation between the nutritional pattern, the cultural characters and the biochemical reactions of the organisms.

As the work proceeded, it became clear that a wide range of species of Streptomyces could not be obtained. Only a few named species or strains were obtained from Type Culture Collections or other readily available sources, so that most of the organisms examined were obtained by direct isolation. This resulted in a preponderance of S. albus strains which appeared to be the commonest species in the soils examined. Nevertheless, since a standard technique for the examination of the cultures had been developed, it was felt worth while to proceed with the investigation of the available strains; other species and strains could be fitted into the scheme when they became available.

SURVEY OF THE LITERATURE.

1). Mineral metabolism.

The metabolism of inorganic ions by the genus Streptomyces has been rarely investigated. This study does not supply the need, but a survey is included here for completeness.

A. Inorganic content of the mycelium.

Spilsbury (1948) showed by spectrographic analysis that the mycelium of Streptomyces griseus contained calcium, magnesium, silicon, sodium, iron, potassium and phosphorus, together with small quantities of copper, aluminium, barium, strontium, lithium, rubidium, manganese and lead. The presence of zinc was doubtful.

B. The effect of ashed growth stimulants.

Thornberry (1948) showed that the stimulatory effect on streptomycin production of various plant products was retained, and sometimes increased after ashing. Evidence of a similar nature was provided by Stapp & Spicher (1954) who found that an aqueous extract of soil, when added to a synthetic medium, increased the growth of a large number of streptomycetes. None of the growth factors that they examin would substitute for the extract. Spicher (1954) further showed that ashing the extract did not destroy its stimulatory action which was due to inorganic ions.

C. The effect of the nitrogen source.

Spilsbury (1948) found that Streptomyces griseus, grown on a medium containing organic acid salts, was not affected by added copper or manganese up to 64 p.p.m. when glycine was the

nitrogen source; but when either arginine, ammonium nitrate, ammonium chloride or ammonium sulphate replaced glycine, the growth was enhanced.

D. Ions required for growth.

a). Iron and zinc.

Zinc and iron were both essential for the growth of Streptomyces griseus (Thornberry & Anderson 1948; Acker & Lechevalier 1954; Chesters & Rolinson 1954) and for S. fradiae (Nickerson & Mohan 1953b). Spilsbury (1948) found that zinc was inhibitory to his strain of S. griseus, but this was overcome by copper. The sporulation of S. fradiae was suppressed by zinc and iron (Hickey & Tresner 1952).

b). Copper.

Spilsbury (1948) and Chesters & Rolinson (1951) found that copper was essential for the growth of Streptomyces griseus, but Thornberry & Anderson (1948) found that it had no effect, and Acker & Lechevalier (1954) that it was slightly toxic.

c). Manganese.

Streptomyces griseus did not require manganese (Thornberr & Anderson 1948; Acker & Lechevalier 1954; Chesters & Rolinson 1954). Spilsbury (1948), however, found that it was necessary for the developme of the mycelium of that organism, and was adsorbed from the medium.

d). Magnesium.

Streptomyces griseus required magnesium for growth and streptomycin production (Thornberry & Anderson 1948; Acker & Lechevalie 1954).

e). Calcium.

Calcium was required for the healthy growth of

Streptomyces fradiae (Nickerson & Mohan 1953b). It was not necessary for the growth of S. griseus, but calcium carbonate affected the response of this species to magnesium (Thornberry & Anderson 1948). Calcium was reported as the co-factor of the α-amylase of 5 Streptomyces species (Simpson & McCoy 1953).

f). Cobalt.

Cobalt stimulated the production of antibiotic but not the growth of Streptomyces griseus (Principe & Thornberry 1952), and promoted the sporulation of S. fradiae (Hickey & Tresner 1952).

g). Potassium.

Potassium was necessary for the growth of Streptomyces griseus (Thornberry & Anderson 1948; Acker & Lechevalier 1954).

h). Phosphorus.

Phosphate was necessary for the growth of Streptomyces griseus, (Thornberry & Anderson 1948; Acker & Lechevalier 1954), and for its metabolism of glucose (Hockenhull, Fantes, Herbert & Whitehead 1954).

i). Sulphur.

Sulphate sulphur was necessary for the growth of Streptomyces griseus (Acker & Lechevalier 1954).

E. Miscellaneous

a). Selenium and tellurium.

Selenium and tellurium were reduced by some actinomyces. The colonies turned deep red or black due to the deposit of the elemental metal in the mycelium. The phenomenon was intracellular

(Waksman 1927).

b). Arsenic.

The reduction of organic arsenic compounds by actinomyces with the production of a "garlic odour" was claimed by Husz (1914), but this was disputed by Lieske (1921).

2). Growth factors.

Many streptomycetes grow in synthetic media to which growth factors have not been added: neither Streptomyces griseus (Dulaney 1948) nor an unidentified Streptomyces species (Sackman 1956) required any such additions.

Nevertheless, a number of workers have suggested that added vitamins are required by some actinomycetes, particularly those of the genus Nocardia. A soluble, dialysable metabolite, present in tryptic broth, yeast, muscle, serum and wheat embryo, was reported as necessary for the growth of Streptothrix corallina (Nocardia corallina) by Reader (1926; 1928). Peters, Kinnersley, Orr-Ewing & Reader (1928) found that this factor was similar to, but not identical with, vitamin B_1. Mariat (1954a; 1954b), who examined a number of species of Streptomyces and Nocardia, found that two of the latter required either thiamine or the pyrimidine part of the thiamine molecule. McClung (1954) concluded that biotin and thiamine, while not essential, stimulated the growth of his Nocardia strains.

There have also been a few reports that the streptomycet are unable to synthesise all the growth factors that they need. Cochrane (1950) found that Streptomyces griseus required added yeast extract to initiate growth in a medium containing nitrate, with glucose as the carbon source. Eiser & McFarlane (1948) using the same species concluded that the only growth factor required was inositol, which increased both mycelial weight and streptomycin production. Riboflavi and nicotinic acid favoured antibiotic production, but did not affect

growth; folic acid, pyridoxine, pantothenic acid, thiamine and p-aminobenzoic acid had no effect at all. Spilsbury (1948), who also investigated S. griseus, stated that there were certain unidentified factors in extracts of meat, yeast and horse dung which were auxillaries to growth. Creatine, guanidine, inositol, indole-3-acetic acid, indole-3-butyric acid, naphthalene-acetic acid, naphthoxyacetic acid, phenoxyace acid, 2,4,dichlorophenoxyacetic acid and 2,4,dichloropropionic acid had no effect.

The growth of Streptomyces coelicolor was improved by the addition of yeast extract when the organism was grown on nitrate with either glucose or glycerol as carbon source (Cochrane & Conn 1950).

Khambata & Bhat (1954) using a synthetic medium, found that an oxalate decomposing streptomycete did not grow unless yeast extract was added.

Floodgate (unpublished) found that a culture of a thermophilic Micromonospora, probably M. vulgaris, was stimulated both in sporulation and growth by autoclaved rabbit faeces.

3). Carbon sources.

Carbon assimilation by streptomycetes has been frequently investigated, usually with reference to carbohydrates, alcohols and organic acids.

A. Carbohydrates.

a). Pentoses.

i. Arabinose.

Münter (1912) obtained only moderate growth with arabinose as the sole carbon source in a medium containing ammonium nitrate as the nitrogen source. Waksman (1919b; 1919c) using sodium nitrate as the nitrogen source obtained similar results. More recently, Pridham & Gottlieb (1948) using ammonium sulphate as the nitrogen source also, found that arabinose supported the growth of 74% of their streptomycetes. Pridham, Hall & Shekleton (1953) grew all their species on arabinose except Streptomyces lavendulae, S. griseocarnus and possibly S. californicus. Burkholder, Sun, Ehrlich & Anderson (1954) using the medium of Pridham & Gottlieb (1948), found that 96% of their organisms grew on this sugar. Benedict, Pridham, Lindenfelser, Hall & Jackson (1955) using ammonium sulphate with arabinose found that 72% of their streptomycetes grew.

ii. Xylose.

Sixty per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 93% of those examined by Burkholder et al. (1954), and 74% of those examined by Benedict et al. (1955) grew on xylose. Pridham et al. (1953) obtained the same result with xylose as with arabinose.

iii. Rhamnose.

Fifty per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 50% of those examined by Pridham et al. (1953), 35% of those examined by Burkholder et al. (1954) and 48% of those examined by Benedict et al. (1955) grew on rhamnose.

b). Hexoses.

i. Fructose.

Fructose was often well assimilated (Münter 1912), but not all species grew on it (Gottlieb 1953). Eighty seven per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 98% of those examined by Burkholder et al. (1954) and 79% of those examined by Benedict et al. (1955) gave positive results.

ii. Galactose.

Galactose yielded good growth, and was widely used (Münter 1912; Gottlieb 1953). Ninety five per cent of the streptomycetes examined by Pridham & Gottlieb (1948) and all those examined by Burkholder et al. (1954) grew on it.

iii. Glucose.

The actinomycetes readily assimilated glucose (Fousek 1912; Münter 1912; Krainsky 1914; Waksman 1919b;1919c; Pridham & Gottlieb 1948; Gottlieb 1953; Stapp 1953). Some of the Okami's streptomycetes did not grow on this substrate when ammonium was the nitrogen source (Okami 1952).

iv. Mannose.

Mannose was used by all the streptomycetes examined by Pridham & Gottlieb (1948). Pridham et al. (1953) also found this sugar

was widely used.

v. Sorbose.

Sorbose was not utilised by any of the streptomycetes examined by Benedict et al. (1955), or by Streptomyces coelicolor (Cochrane & Conn 1947) or S. aureofaciens (Backus, Duggar & Campbell 1954).

c). Disaccharides.

i. Lactose.

Good growth on lactose was reported by the early workers (Fousek 1912; Münter 1912; Waksman 1919b; 1919c). Seventy-four per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 75% of those examined by Pridham et al. (1953), 83% of those examined by Burkholder et al. (1954) and 66% of those examined by Benedict et al. (1955) grew on this sugar.

ii. Maltose.

Maltose was a good carbon source (Waksman 1919b; 1919c). Eighty per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 97% of those examined by Burkholder et al. (1954) and 97% also of those examined by Benedict et al. (1955) grew on this sugar.

iii. Sucrose.

Münter (1912) reported that sucrose supported good growth of his organisms. Waksman (1919b; 1919c) found it inferior to lactose and maltose. Thirty-five per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 15% of those examined by Pridham et al. (1953), 45% of those examined by Burkholder et al. (1954) and 26% of those examined by Benedict et al. (1955) grew on this sugar.

iv. Cellobiose.

Cellobiose was used by all the streptomycetes examined by Pridham & Gottlieb (1948), by Pridham et al. (1953) and by Burkholder et al. (1954).

v. Melibiose.

Melibiose was assimilated by 37% of the streptomycetes examined by Benedict et al. (1955).

vi. Trehalose.

Trehalose was assimilated by 86% of the streptomycetes examined by Benedict et al. (1955).

d). Trisaccharides.

i. Raffinose.

Thirty-nine per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 58% of those examined by Burkholder et al. (1954) and 29% of those examined by Benedict et al. (1955) grew on raffinose. Gottlieb (1953) found that this sugar was not readily utilised by many of his streptomycetes. This was attributed to the probable absence of a hydrolase for the sucrose portion of the molecule, since almost all the streptomycetes which failed to grow on raffinose also failed to utilise sucrose, although they utilised fructose. On the other hand, raffinose was utilised by the organisms which used sucrose.

ii. Melezitose.

Melezitose was assimilated by 33% of the streptomycetes examined by Benedict et al. (1955).

e). Polysaccharides.

i. Inulin.

Inulin was only of moderate value as a carbon source for the streptomycetes examined by Münter (1912): thirty-four per cent of the organisms examined by Pridham & Gottlieb (1948), 14% of those examined by Pridham et al. (1953) and 25% of those examined by Benedict et al. (1955) grew on it. Gottlieb (1953) considered that since most streptomycetes utilised fructose, the inability of many species to assimilate inulin was due to the lack of inulase.

ii. Dextrin.

Dextrin was used by all the streptomycetes examined by Münter (1912), Pridham & Gottlieb (1948) and Pridham et al. (1953).

iii. Dextran.

Purified dextran from Leuconostoc mesenteroides was assimilated by 40% of the streptomycetes examined by Benedict et al. (1955

iv. Cellulose.

Cellulose supported the growth of streptomycetes (Fousek 1912; Krainsky 1914; Waksman 1919b; 1919c; 1931; Waksman & Hutchings 1937). Seventy-six per cent of the streptomycetes examined by Burkholder et al. (1954) grew on it. Hemicelluloses were also utilised as a carbon source (Waksman & Hutchings 1937).

v. Starch.

Starch was utilised by many of the streptomycetes examined by Fousek (1912), Krainsky (1914) and Waksman (1919b; 1919c). All the organisms examined by Pridham & Gottlieb (1948) and by Pridham et al. (1953) grew on it.

f). Glucosides.

i. Salicin.

Eighty-two per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 20% of those examined by Burkholder et al. (1954) and 72% of those examined by Benedict et al. (1955) grew on salic

B. Alcohols.

a). Ethanol.

Ethanol was an unfavourable carbon source for the members of this genus (Waksman 1950).

b). Glycerol.

Glycerol supported excellent growth of many streptomycete examined by Salzman (1902), Münter (1912) and Waksman (1919b; 1919c). All the organisms examined by Pridham & Gottlieb (1948), Pridham et al. (1953) and Stapp (1953) grew on it.

c). Erythritol.

Erythritol supported the growth of only 7% of the streptomycetes examined by Benedict et al. (1955).

d). Mannitol.

Mannitol was a good carbon source for many of the organisms examined by Münter (1912) and Waksman (1919b; 1919c). Pridha et al. (1953) concluded that this alcohol was used by more streptomycete than were its isomers, sorbitol and dulcitol. Mannitol supported growth of 70% of the streptomycetes examined by Pridham & Gottlieb (1948), 80% of those examined by Burkholder et al. (1954) and 78% of those examined by Benedict et al. (1955).

e). Dulcitol.

Twenty-two per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 74% of those examined by Burkholder et al. (1954) and 2% of those examined by Benedict et al. (1955) grew on dulcitol. Pridham et al. (1953) reported that this substrate was used by a few strains only, including Streptomyces scabies and S. violaceus-ruber.

f). Sorbitol.

Thirty-nine per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 11% of those examined by Pridham et al. (195) 62% of those examined by Burkholder et al. (1954) and 20% of those examined by Benedict et al. (1955) grew on sorbitol.

g). Inositol.

Forty per cent of the streptomycetes examined by Pridham & Gottlieb (1948), 40% of those examined by Pridham et al. (1953), 45% of those examined by Burkholder et al. (1954) and 48% of those examined by Benedict et al. (1948) grew on inositol.

C. Organic acids.

The calcium salts of the monocarboxylic organic acids were considered by Salzman (1902) to be poor carbon sources, but the calcium salts of the dicarboxylic acids were well assimilated. Waksman (1919c) considered that the "organic acids form, with very few exceptions rather poor sources of carbon for the actinomycetes".

a). Formic acid.

Formate was not utilised by any organisms studied by Pridham & Gottlieb (1948). Taylor & Decker (1947) and Stapp (1953),

however, found that it supported the growth of some streptomycetes.

b). Acetic acid.

Mlnter (1912) and Waksman (1919b; 1919c) concluded that acetate was a poor or moderate carbon source for streptomycetes, but thi was not confirmed by more recent work. Many of Stapp's streptomycetes grew on acetate (Stapp 1953), as did 82% of those examined by Pridham & Gottlieb (1948) and 87% of those examined by Benedict et al. (1955).

c). Propionic acid.

Stapp (1953) isolated several streptomycetes which grew on propionate.

d). Butyric acid.

Butyrate was toxic to Streptomyces griseus (Hubbard & Thornberry 1950).

e). Oxalic acid.

Oxalate was usually found to be a poor carbon source for streptomycetes (Mlnter 1912; Waksman 1919b; 1919c; Pridham & Gottlieb 1948; Pridham et al. 1953; Stapp 1953), but several organisms capable of using it were isolated from "scabbed" potatoes by Taylor & Decker (1947), and one oxalate assimilating species was found in the gut of an earthworm by Khambata & Bhat (1954).

f). Malic acid.

Malate wasrarely utilised by streptomycetes, and then only scanty growth was obtained (Mlnter 1912; Waksman 1919b; Pridham et al. 1953). Conversely, some streptomycetes from "scabbed" potatoes grew well on it (Taylor & Decker 1947), as did Streptomyces griseus (Hubbard &

Thornberry 1950) and some of the organisms examined by Stapp (1953).

g). Tartaric acid.

Tartrate was a poor carbon source, and growth in media containing it as the only carbon source was scanty (Münter 1912; Waksman 1919b; Pridham & Gottlieb 1948; Pridham et al. 1953; Stapp 1953).

h). Succinic acid.

Succinate was a good carbon source for streptomycetes (Münter 1912; Taylor & Decker 1947). Sixty eight per cent of the streptomycetes examined by Pridham & Gottlieb (1948) and all those examined by Benedict et al. (1955) grew on it.

i). Citric acid.

Citrate was an excellent carbon source for many streptomycetes (Münter 1912; Taylor & Decker 1947), and 82% of the organisms examined by Pridham & Gottlieb (1948) grew on it. In contrast Stapp (1953) found that this acid was a poor carbon source. Only 19% of the organisms examined by Burkholder et al. (1954) grew on it and Streptomyces coelicolor (Cochrane & Conn 1947) did not grow at all.

D. Miscellaneous.

Among other substances shown to be capable of supplying carbon for streptomycete growth were caffeine (Lieske 1921), lignin and humus (Waksman 1927; 1931; Waksman & Hutchings 1937), rubber (Söhngen & Fol 1914; Rook 1955), agar and alginic acid (Stanier 1942; McDougall, personal communication), chitin (Skinner & Dravis 1937; Jeuniaux 1955) and lipids (Perlman & Wagman 1952; Perlman 1953; Adelson, Schatz & Trelawny 1957). Carbamates (Schatz, Isenberg,

Angrist & Schatz 1954.) and cyanide (Ware & Painter 1955) were used both as carbon and nitrogen source.

There have also been a few reports of actinomycetes which were autotrophic. Kaserer (1906a; 1906b) isolated an organism which he believed was morphologically identical with Bacillus oligocarbophilus, first isolated by Beijerinck & van Delden (1903). He stated that it oxidised carbon monoxide as carbon and energy source. Lantzsch (1922) showed that the organism existed in two forms, as long filaments and as almost coccoid rods. His description resembles Nocardia as defined by Waksman & Henrici (1943). Lantzsch (1922) considered that the filamentou form utilised carbon monoxide.

Facultative autotrophic growth was also claimed for Streptomyces autotrophicus (Takamiya & Tubaki 1956).

4). Nitrogen sources.

Streptomycetes can utilise a wide variety of nitrogenous material. Animal and plant protein, protein breakdown products and other complex nitrogenous mixtures supported satisfactory growth (Winter 1912; 1913; Waksman 1919a; Dulaney 1948; Spilsbury 1948). Both inorganic and organic nitrogen were assimilated, the latter more readily than the former (Waksman 1920; Spilsbury 1948).

A. Inorganic compounds.

a). Nitrogen.

According to Waksman (1950) streptomycetes cannot fix nitrogen. Several authors have, however, shown that an actinomycete was the probable endophyte in the nitrogen fixing nodules of certain non-leguminous plants such as Myrica gale and Hippophae rhamnoides (Uemura 1952; Fletcher 1955).

b). Ammonia.

Many workers have reported on the assimilation of ammonium by actinomycetes (Winter 1912; 1913; Krainsky 1914; Waksman 1919c; 1920; Nickell & Burkholder 1947; Dulaney 1948; Pridham & Gottlieb 1948; Erhlich, Gottlieb, Burkholder, Anderson & Pridham 1948; Waksman 1950; Perlman 1953; Schatz et al. 1954; Burkholder et al. 1954; Benedict et al. 1955). Waksman (1919c; 1920; Waksman & Joffe 1920) considered that, on the whole, ammonium salts were poor nitrogen sources, probably because the removal of the ammonium ion reduced the pH to below the optimum for growth. In the presence of certain favourable carbon sources, however, ammonium salts were assimilated by many species. In contrast, Pridham & Gottlieb (1948), Burkholder et al. (1954), and

Benedict et al. (1955) found that ammonium sulphate supported the growth of many species when combined with a wide variety of carbon sources.

Okami (1952) found a good deal of variation between his organisms from no growth to excellent growth, when they were supplied with ammonium sulphate.

Mariat (1954a) working with aerobic pathogenic cultures obtained only feeble or no growth with ammonium ion as the nitrogen source.

c). Nitrite.

Nitrites are generally believed to be toxic. It was found, however, that streptomycetes could use them as a nitrogen source providing that the concentration was kept low (Waksman 1919c; 1920; Waksman & Joffe 1920). It was also shown that Streptomyces venezuelae (Ehrlich et al. 1948), S. nitrificans (Schatz et al. 1954), S. violaceus-ruber and S. aureus (Gottlieb 1953) grew on nitrite. The preformed mycelium of S. griseus assimilated nitrite (Cochrane 1950).

d). Nitrate.

Many strains of Streptomyces utilised nitrate (Fousek 1912 Minter 1912; Krainsky 1914; Waksman 1919b; 1919c; 1920; von Plotho 1940a; Waksman, Dugie & Schatz 1944; Eiser & McFarlane 1948; Spilsbury 1948; Cochrane 1950; Waksman 1950; Okami 1952; Dulmage 1953; Perlman 1953; Stapp 1953; Schatz et al. 1954; Burkholder et al. 1954). Other streptomycetes did not (Krainsky 1914; Nickell & Burkholder 1947; Dulaney 1948; Thornberry & Anderson 1948).

Variations may exist between the strains of one species. Some strains of Streptomyces griseus used nitrate nitrogen (Waksman 1920;

Waksman et al. 1944; Eiser & McFarlane 1948; Spilsbury 1948; Okami 195 but other strains did not (Dulaney 1948; Thornberry & Anderson 1948). Cochrane (1950) suggested that the inability of some strains of S. griseu to grow on nitrate was due to the lack of hydrogen donors or enzymic moities which were found in yeast extract and in preformed mycelium.

Cochrane & Conn (1950) showed that the growth of Streptomyces coelicolor in a nitrate containing medium was restricted at a low pH, probably because the nitrite formed from the nitrate was mor toxic in acid than in alkaline solutions.

B. Organic compounds.

a). Amino acids.

The amino acids are generally recognised as good nitrogen sources for the streptomycetes.

i. Alanine.

All the streptomycetes examined by Okami (1952) and 97% of those examined by Burkholder et al. (1954) grew on alanine.

ii. Amino-butyric acid.

Fifty seven per cent of the streptomycetes examined by Okami (1952) grew on α-amino-butyric acid.

iii. Arginine.

All the streptomycetes examined by Okami (1952) and 97% of those examined by Burkholder et al. (1954) grew on arginine.

iv. Aspartic acid.

All the streptomycetes examined by Burkholder et al. (1954) grew on aspartic acid.

v. Cysteine.

Sixty eight per cent of the streptomycetes examined

by Okami (1952) and 85% of those examined by Burkholder et al. (1954) grew on cysteine.

vi. Cystine.

Eighty five per cent of the streptomycetes examined by Burkholder et al. (1954) grew on cystine.

vii. Glutamic acid.

Eighty one per cent of the streptomycetes examined by Okami (1952) and all those examined by Burkholder et al. (1954) grew on glutamic acid.

viii. Glycine.

Waksman (1919c) found that glycine was a good nitrogen source for actinomycetes. All the streptomycetes examined by Okami (1952), and 98% of those examined by Burkholder et al. (1954) grew on it.

ix. Histidine.

Eighty six per cent of the streptomycetes examined by Okami (1952) and 96% of those examined by Burkholder et al. (1954) grew on histidine.

x. Hydroxy-proline.

Sixty six per cent of the streptomycetes examined by Burkholder et al. (1954) grew on hydroxy--proline.

xi. Leucine.

Waksman (1919c) found leucine was a good nitrogen source for actinomycetes. Ninety one per cent of the streptomycetes examined by Burkholder et al. (1954) grew on leucine, 86% grew on isoleucine, and 59% grew on nor-leucine.

xii. Lysine.

Fifty seven per cent of the streptomycetes examined by Okami (1952) and 97% of those examined by Burkholder et al. (1954) grew on lysine.

xiii. Methionine.

Sixty five per cent of the streptomycetes examined by Okami (1952) and 70% of those examined by Burkholder et al. (1954) grew on methionine.

xiv. Phenyl-alanine.

Seventy three per cent of the streptomycetes examined by Okami (1952) and 85% of those examined by Burkholder et al. (1954) grew on phenyl-alanine.

xv. Proline.

All the streptomycetes examined by Okami (1952) and 95% of those examined by Burkholder et al. (1954) grew on proline.

xvi. Serine.

Ninety three per cent of the streptomycetes examined by Burkholder et al. (1954) grew on serine.

xvii. Threonine.

Ninety eight per cent of the streptomycetes examined by Burkholder et al. (1954) grew on threonine.

xviii. Tryptophane.

Forty six per cent of the streptomycetes examined by Okami (1952) and 52% of those examined by Burkholder et al. (1954) grew on tryptophane.

xix. Tyrosine.

Waksman (1919c) obtained good growth with tyrosine as sole nitrogen source. Seventy eight per cent of the streptomycetes examined by Okami (1952) and 55% of those examined by Burkholder et al. (1954) grew on tyrosine.

xx. Valine.

Seventy three per cent of the streptomycetes examined by Okami (1952) and 52% of those examined by Burkholder et al. (1954) grew on valine.

xxi. Pairs of amino acids.

Nickerson & Mohan (1953b) investigated the utilisation by Streptomyces fradiae of pairs of amino acids with glucose as the carbon source. Their results indicated that glutamate plus aspartate, glycine or arginine produced more growth, while glutamate plus asparaginat or casein hydrolysate produced less growth, than glutamate alone. They concluded that "the nutritional selectivity of S. fradiae centers, mainly around the carbon requirements of the organism".

xxii. Amino acids as carbon and nitrogen source.

Nickerson & Mohan (1953a) showed that glutamate supported good growth of Streptomyces fradiae as both carbon and nitrogen source. Arginine, lysine and histidine supported only moderate growth in both roles; alanine and proline would do so only under certain conditions, the most important of which was the inorganic salt composition of the medium. Certain other amino acids provided carbon as well as nitrogen, providing that another carbon source such as glucose or glutamate was

present. Most of the common amino acids provided nitrogen only.

xxiii. Humic acid formation.

The production of humic acid from 23 amino acids was an important factor in the division of 20 streptomycetes into 6 groups by Flaig, Beutelspacher, Kuster & Segler-Hozwessig (1952). Spilsbury (1948) noted that Streptomyces griseus produced brown pigments when grown on certain amino acids.

b). Amides.

i. Urea.

Winter (1912) and Waksman (1919c; 1920) found that urea was used only to a limited extent as a nitrogen source by streptomycetes. Later, Waksman (1950) revised his opinion, and stated that urea was readily utilised. The organisms studied by Lieske (1921), Stapp (1953) and Stapp & Spicher (1954) grew well on it as did Streptomyces coelicolor (Cochrane & Conn 1947) and S. nitrificans (Schatz et al. 1954). Eighty five per cent of the streptomycetes examined by Burkholder et al. (1954) grew on this substrate.

ii. Asparagine.

Ninety two per cent of the organisms examined by Okami (1952), and 85% of those examined by Burkholder et al. (1954) grew on asparagine. Waksman (1919c) found that it provided both carbon and nitrogen for growth.

c). Purines.

Lieske (1921) could not demonstrate growth of streptomycetes on either uric or hippuric acids, but later workers showed that uric acid, xanthine, hypoxanthine and adenine supported good growth

provided that the optimal concentrations were used (Stapp 1953; Stapp & Spicher 1954). It was also the opinion of Waksman (1950) that uric acid was readily utilised and converted to complex organic compounds.

The majority of Mariat's species grew only feebly or not at all on a mixture of purines and pyrimidines. A few, however, grew well (Mariat 1954a).

Streptomyces nitrificans utilised purines either alone or with glucose (Schatz et al. 1954).

5). The effect of physical factors on nutrition.

Hendlin (1954) pointed out the importance of environmental factors on the nutrition of bacteria in general, and Erikson (1955) showed that these factors apply to Streptomyces species also.

A. The effect of pH.

Various authors concluded that streptomycetes grew best at a neutral or slightly alkaline pH and were inhibited below pH 5.0 (Gillespie 1918; Waksman 1922; Skinner, Emmons & Tsuchiya 1947; Waksman 1950).

As already noted (page 17), it was suggested that ammonium sulphate was of limited value as a nitrogen source due to the accumulatic of sulphate ions as the ammonium was assimilated, so producing a pH too low for streptomycete growth (Waksman 1919c; 1920; Waksman & Joffe 1920) Minter (1913) obtained better growth on ammonium salts when he used a buffered medium.

Evidence of a similar nature was obtained by Cochrane & Corn (1947). They found that the utilisation of nitrogenous substrates by Streptomyces coelicolor depended upon the nature and concentration of the carbon source, because these factors governed the rate of acid production, and hence the pH.

B. The effect of oxidation-reduction potential.

Another physical factor of importance to the growth of streptomycetes is the oxidation-reduction potential. Streptomycetes are strongly aerobic organisms, and it has become customary to grow them in aerated cultures as this increases growth (Waksman, Schatz & Reilly 194 Skinner, Emmons & Tsuchiya 1947; Waksman 1950; Villemin, Lechevalier

& Waksman 1953).

Woodruff & Foster (1953) working with Actinomyces lavendulae (Streptomyces lavendulae) and A. antibioticus (S. antibiotic showed that aeration (a) reduced variability of the strains, (b) accelerated growth, (c) favoured the production of acid from sugar, and (d) decreased the efficiency of carbon utilisation. They further concluded that washed aerated cultures were suitable for general biochemical studies requiring homogenous physiological conditions.

Aeration also accelerated the rate of acid production by Streptomyces griseus due to an increase in the rate of sugar oxidation, the oxidation rate of the α-amino acids remaining constant. This resulted in sporulation and the inhibition of further development (Sevcik 1952).

C. The carbon-nitrogen ratio.

Eiser & McFarlane (1948) found that Streptomyces griseus produced good growth over a wide range of C/N ratios when glucose and histidine were the carbon and nitrogen sources respectively. Cochrane & Dimmick (1949) and Sevcik (1952) emphasised that the carbon-nitrogen ratio and the environmental conditions should be so adjusted that the metabolism of the organism is not deranged.

6). Relationship of nutrition to taxonomy.

The nutritional characteristics of micro-organisms have been regarded as criteria for classification by Lochhead & Chase (1943), Knight & Froom (1950), Stevenson & Rouatt (1953) and Knight (1955 Stanier (1942) first drew attention to the possibility of using the carbon assimilation pattern of the streptomycetes to this end. He pointed out that while attempts to base a classification on a single compound could not be successful, a general pattern of carbon utilisation might emerge which would be of significance. This was investigated by Taylor & Decker (1947), by Pridham & Gottlieb (1948), by Burkholder et al. (1954), and by Benedict et al. (1955). All concluded that, although there were variations between the strains of the one species, the nutritional pattern as presented by each species was sufficient to distinguish one from another.

Okami (1952) who investigated the utilisation of various nitrogenous materials by streptomycetes, concluded that the nitrogen assimilation pattern was a useful addition to the taxonomic criteria.

DESCRIPTION OF THE TEST ORGANISMS.

DESCRIPTION OF THE TEST ORGANISMS.

1). Sources of the organisms.

The following species were received from the National Collection of Type Cultures:-

Sg as *Streptomyces griseus* N.C.T.C. No. 6961. (R.C.S.T. No. 122)

Sa as *Streptomyces aureofaciens* N.C.T.C. No. 8128.

Ms as *Streptomyces* spp. Maxted strain N.C.T.C. No. 7807.

265 as *Streptomyces listeri* N.C.T.C. No. 434.

266 as *Streptomyces pelletieri* N.C.T.C. No. 3026.

267 as *Streptomyces pelletieri* N.C.T.C. No. 4162.

Of the remaining cultures,

197 was a contaminant on an agar plate;

224 and 228 were obtained from the dust of a hay bin;

259 and 260 were obtained from a liquid medium in which seedlings of sea buckthorn (*Hippophae rhamnoides*) were growing.

All the other organisms were isolated from soil.

2). Isolation technique.

Approximately 1 g. of soil or hay-dust was shaken in about 10 ml. of sterile water, the larger particles allowed to settle, and the supernatant diluted by x10 serial dilution to $1/10^{-9}$. A loopful of each of the last three dilutions was streaked on Conn's agar plates (Appendix II: medium 17) and incubated at 25° for 3 or 4 days. Individual colonies of *Streptomyces* spp. were picked off, grown in nutrient broth and examined for purity.

The organisms from sea buckthorn cultures were isolated by direct plating on glucose agar (Appendix II: medium 4).

From the collection of streptomycetes thus gathered, 36 were examined in this work.

3). Preservation of cultures.

The stock organisms were subcultured on glucose agar slopes (Appendix II: medium 4) every 6-8 weeks, and were also preserved in sterile soil (Jones 1940; 1946; Erikson 1947).

4). Identification of the organisms.

A. Methods.

a). Morphology of the mycelium.

The organism was grown in an enclosed hanging drop of either nutrient broth (Appendix II: medium 1), glucose broth (Appendix II medium 3), Bennett's medium (Jones 1949; Appendix II: medium 18), or cobalt medium (Hickey & Tresner 1952; Appendix II: medium 19). Each was incubated for 3 to 7 days at 25°. The growth was then dried, fixed by ethanol or, preferably, by 10% formalin, stained with approximately 0.1% w/v crystal violet, washed, dried and mounted in D.P.X.. It was found that nutrient broth and glucose broth both induced sporulation as frequently as either Bennett's or cobalt medium, which were reported to be particularly useful for this purpose.

Measurements of the spores and the width of the primary mycelium were made from these preparations by projection on to a screen from a microprojector. The image was measured with a pair of compasses, and the size read off from a scale which had been standardised against the image thrown by a stage micrometer. At least 20 measurements of both primary and secondary mycelium of each organism were made.

b). Cultural characteristics.

i. Colony.

The characters of the isolated colony were described from a culture on a nutrient agar plate (Appendix II; medium 2) incubated at 25°. Observations were made over 21 days.

By "colony" was meant the growth resulting from a single spore or piece of mycelium, without implication as to whether such growth constituted an individual or not.

Differences of opinion exist as to the value of the shape and size of streptomycete colonies as a character of taxonomic validity (Burkholder et al. 1954; Hesseltine, Benedict & Pridham 1954; Jones 1954). Observations made during this study suggested that both characters depend on the shape and size of the piece of mycelium used as inoculum. These features were not therefore, reported.

ii. Streak.

Descriptions were made of streak cultures growing on slopes of the following media, incubated at 25°, during 21 days:-

nutrient agar (Appendix II; medium 2)

glucose agar (Appendix II; medium 4.)

Czapek Dox glucose agar (Appendix II; medium 5)

Czapek Dox sucrose agar (Appendix II; medium 5)

potato plug (Appendix II; medium 6).

As was also noted by Burkholder et al. (1954), it was observed that the "reverse" side of the growth, particularly on nutrient and glucose agar slopes, gave useful information. This character was, therefore recorded.

iii. Liquid media.

Descriptions were made of cultures growing in nutrient broth (Appendix II; medium 1) and glucose broth (Appendix II; medium 3), incubated at 25°, during 21 days.

c). Physiological and biochemical characteristics.

i. Growth temperatures.

Optimum and limiting temperatures for growth were determined by growing the organisms in nutrient broth (Appendix II; medium 1) at 1° (refrigerator), room temperature (approximately 16°), 25°, 30°, 37°, 45° and 55°. The growth was recorded after 3-4 days.

ii. Oxygen relationships.

A nutrient agar shake (Appendix II; medium 7) of each organism was incubated at 25° for 2-5 days, and the distribution of growth in the medium observed.

iii. Starch hydrolysis.

Amylase was detected by growing the organism on starch agar (Appendix II; medium 8) at 25°. After 2-5 days, the surface of the medium was flooded with dilute iodine solution. The production of amylase was inferred from the absence of a blue coloration round the growth.

iv. Proteolytic activity.

The following media were inoculated with each organism and incubated at 25°:-

Dorset's egg slope (Appendix II; medium 9)

gelatine stab (Appendix II; medium 10)

inspissated horse serum slope (Appendix II; medium 11)

B.C.P. milk (Appendix II; medium 12).

The cultures were examined at intervals for at least 21 days. Proteolysis was indicated in the first 3 media by clearing, pitting and liquefaction, and in the last by peptonisation and digestion of any clot formed.

v. Indol production.

The organisms were grown in peptone water (Appendix II; medium 13) at 25° for 2-10 days. Indol was detected by Kovac's reagent.

vi. Acetylmethylcarbinol production.

The organisms were grown in glucose phosphate peptone water (Appendix II; medium 14) at 25° for 2-5 days. Acetylmethylcarbinol was detected by the addition of small amounts of creatine, α-naphthol and concentrated potassium hydroxide (Barritt's modified method).

vii. Nitrate reduction.

The organisms were grown in nitrate broth (Appendix II; medium 15) at 25° for at least 21 days. Nitrite was detected by the addition of α-naphthylamine and sulphanilic acid (Griess-Ilosvay method).

d). Production of antibiotics.

The ability of the organisms to produce antibiotics was examined using the cross streak method of Waksman (1945). Each of the streptomycetes was grown for 3 days on glucose agar (Appendix II; medium 4), and then cross streaked with a 24 hr. broth culture of Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli, Bacillus subtilis, B. cereus and Micrococcus citreus.

The plates were then incubated at 37° for 24 hr. and examined.

B. Results of the tests.

The results of these tests, and a detailed description of each organism is given in Appendix I.

C. Identity of the organisms.

An attempt was made to identify the organisms using data from the above tests. It is, however, generally conceded that the identification of species of Streptomyces is difficult because of (i) the marked variations caused by a high mutation rate, and the great effect on the culture of small changes in the environment. This gives rise to a lack of stable type species. (ii) The lack of adequate and agreed criteria for differentiating between species. (Waksman 1919c; 1957; Jensen 1931; Jones 1940; 1946; 1949; 1954; Schatz & Waksman 1945; Carvajal 1946; Waksman, Reilly & Johnstone 1946; Erikson 1948; Williams & McCoy 1953; Burkholder et al. 1954; Hesseltine et al. 1954; Krassilnikov 1957).

To meet these difficulties, the species-group or series has been proposed to include all the organisms which resemble, but are not necessarily identical with the type species (Waksman 1919c; 1950; 1957; Baldacci 1947; Baldacci, Comaschi, Scotti & Spalla 1953; Waksman & Lechevalier 1953; Baldacci, Spalla & Grein 1954). Unfortunately only a few species-groups of the Streptomyces have been described, or the limits of their variations investigated. Furthermore, these descriptions do not always agree with each other.

It was only possible, therefore, to tentatively assign each organism to its species-group, and where no description of the species-group was found, to include it, where possible, in the species

it most closely resembled in Bergey's manual (1948).

In the light of these considerations and the evidence given in Appendix I, it was judged that the test organisms belonged to the following species or species-groups:-

Organism Sg	Streptomyces griseus
" Sa	" aureofaciens
" Ms	" albus
" 2	" albus
" 7	" albus
" 9	" albus
" 13	" albus
" 33	" albus
" 34	" albus
" 48	" albus
" 59	" albus
" 76	" albus
" 103	" albus
" 131	" albus
" 132	" albus
" 133	" albus
" 190	" albus
" 193	" albus
" 195	" albus
" 196	" albus
" 197	" albus
" 204	" coelicolor
" 214	" flaveolus
" 216	" albus
" 217	" albosporeus
" 224	" albus
" 228	" albus
" 235	" albus
" 242	" albus
" 245	unidentified Streptomyces spp.
" 250	Streptomyces albus
" 259	" albus
" 260	" albus
" 265	" listeri
" 266	" violaceoniger
" 267	" pelletieri

NUTRITIONAL STUDIES

NUTRITIONAL STUDIES

1). Preparation of the materials.

A. Glassware.

All glassware was thoroughly washed with 'teepol', rinsed well with tap water, allowed to drain, and then acid washed in an apparatus based on that of Earle (1943). Sulphuric acid, which contained nitric acid as an oxidising agent, was heated to about 120° for about 6 hours, and then left to cool overnight. The glassware was rinsed at least 6 times in tap water, twice with glass distilled water and dried at 104.°.

Pipettes were washed in acid as above and rinsed thoroughly in an automatic pipette washer with tap water, twice with glass distilled water and finally dried.

Since cotton wool plugs are undesirable for this kind of work, glass wool was at first used to plug the Erhlenmyer flasks used in the nutritional experiments. Several types of glass fibre were tried, but none was satisfactory. Eventually rimless flasks were obtained and covered with a glass cap.

B. Media.

All reagents used in the preparation of the media were of the highest quality available.

The sodium pyruvate was obtained by Robertson's method (Robertson 1942) from freshly prepared pyruvic acid (Vogel 1951).

Glass distilled water only was used.

The complete medium was incubated overnight to test for sterility.

a). Stock solutions.

i. Basal salt solution.

The following salt solution, based on the medium of Pridham & Gottlieb (1948), was used in all the nutritional experiments.

K_2HPO_4	5.5 g.
KH_2PO_4	2.5 g.
$MgSO_4 \cdot 7H_2O$	1.0 g.
$CuSO_4 \cdot 5H_2O$	5.0 mg.
$FeSO_4 \cdot 7H_2O$	1.0 mg.
$MnSO_4 \cdot 4H_2O$	8.0 mg.
$ZnSO_4 \cdot 7H_2O$	2.0 mg.
$CaCl_2 \cdot 6H_2O$	1.0 mg.
Glass distilled water to 1 litre.	

ii. Growth factor mixture.

The following growth factor solution was prepared:-

Thiamine	5.0 mg.
Calcium pantothenate	10.0 mg.
Pyridoxine hydrochloride	2.0 mg.
Nicotinic acid	10.0 mg.
Riboflavin	2.0 mg.
p-Aminobenzoic acid	2.0 mg.
Biotin	2.5 µg.
Folic acid	1.0 mg.
Glass distilled water to 100 ml.	

The solution was sterilised by filtration through a 5/3 sintered glass filter, and stored at 1°.

iii. Solutions of carbon sources.

The following aqueous stock solutions of the carbon sources were prepared:-

L(+)	Arabinose	20% w/v
D(+)	Xylose	20% "
L(+)	Rhamnose	20% "
D(-)	Fructose	20% "
D(+)	Galactose	20% "
D(+)	Glucose	20% "
D(+)	Mannose	20% "
L(-)	Sorbose	20% "
	Lactose	10% "
	Maltose	20% "
	Sucrose	20% "
	Raffinose	10% "
	Salicin	1% "
	Ethanol	20% "
	Glycerol	20% "
	i.-Erythritol	20% "
	Dulcitol	1% "
D(+)	Mannitol	10% "
D(+)	Sorbitol	20% "
	Inositol	1% "
	Sodium formate	20% "
	Sodium acetate	20% "
	Sodium proprionate	20% "

Sodium butyrate	20% w/v
Potassium oxalate	20% "
Sodium malonate	20% "
Sodium lactate	20% "
Sodium pyruvate	20% "
Sodium potassium tartrate	20% "
Potassium citrate	20% "

The solutions were sterilised by filtration through 5/3 sintered glass filters, and stored at room temperature.

iv. Solutions of the nitrogen sources.

The following aqueous solutions of the nitrogen sources were prepared, sufficient hydrochloric acid or sodium hydroxide to effect solution being added where necessary:-

Sodium nitrite	1% w/v
Sodium nitrate	4% "
DL-Alanine	4% "
L-Arginine	4% "
DL-Aspartic acid	4% "
L-Cystine	4% "
L-Glutamic acid	4% "
Glycine	4% "
L-Histidine	4% "
L-Hydroxy-proline	4% "
L-Leucine	4% "
DL-Lysine	4% "
DL-Methionine	4% "

DL-Ornithine	4% w/v
DL-β -Phenylalanine	4% "
DL-Proline	4% "
DL-Serine	4% "
DL-Threonine	4% "
DL-Tryptophane	4% "
L-Tyrosine	4% "
DL-Valine	4% "
Urea	1% "
Uric acid	2% "
Xanthine	2% "

The solutions were sterilised by filtration through 5/3 sintered glass filters and stored at -20°.

A purple pigment formed in the tryptophane solution, probably due to the presence of impurities. (Harvey, Miller & Robson 1941). It was therefore frequently renewed.

b). Carbon assimilation media.

The carbon assimilation media were prepared as follows:- 1 litre of basal salts solution, to which had been added 2.5 g. of ammonium sulphate, was adjusted to pH 7.0-7.2, sterilised by filtration through a 5/3 sintered glass filter, and 9 ml. quantities dispensed into sterile, acid washed Erhlenmyer flasks. Each flask then received aseptically 0.5 ml. of the growth factor mixture, and 0.5 ml. of a carbon source solution. The final concentration of each carbon source was 1% except lactose, raffinose and mannitol where it was 0.5%, and dulcitol, inositol and salicin where it was 0.05%.

c). Nitrogen assimilation media.

The nitrogen assimilation media were prepared as follows:- 8.5 ml. of the basal salts solution were dispensed into acid washed Erhlenmyer flasks. Each flask received 0.5 ml. of one of the nitrogen sources, and the pH was adjusted to 7.0-7.2. The flasks were then steamed for 1.5 hr. When cool, 0.5 ml. of the growth factor mixture, and 0.5 ml. of a sterile 20% solution of sodium pyruvate were added to each flask. The final concentration of each nitrogen source was 0.2%, except uric acid and xanthine where it was 0.1%, and urea and sodium nitrite where it was 0.05%. The concentration of sodium pyruvate was 1%.

d). Growth factor requirement media.

The growth factor requirement media were prepared as follows:-

A volume of basal salts solution was divided, and either glycerol or glucose added to each part to 1% concentration. Each part was then divided into four, and to each part thus formed was added either phenyl-alanine, arginine or glutamic acid to 0.2% concentration, or ammonium sulphate to 0.25% concentration. Each of the eight media

tration, or ammonium sulphate to 0.25% concentration. Each of the ei

The growth factor mixture was aseptically added to one half of each medium to give the same final concentration as in the carbon and nitrogen assimilation media i.e.

Thiamine	2.5 µg/ml
Calcium pantothenate	5.0 µg/ml
Pyridoxine hydrochloride	1.0 µg/ml
Nicotinic acid	5.0 µg/ml

Riboflavin	1.0 µg/ml
p-Aminobenzoic acid	1.0 µg/ml
Biotin	0.001 µg/ml
Folic acid	0.5 µg/ml

Ten ml. of each medium, with and without growth factors, were measured aseptically into sterile, acid washed Erhlenmyer flasks.

C. The inoculum.

a). The type of inoculum.

It was necessary to decide whether to use, as the inoculum for the nutritional experiments, secondary mycelium (aerial spores) washed from an agar slope, or primary mycelium grown in liquid medium. The advantage of aerial spores was that they were easily washed free from the medium on which they were grown; the disadvantages were (i) they were difficult to get into an homogenous suspension because of their lipoidal surface, and it was undesirable to use a surface active agent, (ii) their germination might have been adversely affected by a medium which was otherwise adequate to support growth, (iii) in some species they produced a greater number of variants than did the primary mycelium (Erikson 1949), and (iv) several of the test organisms did not sporulate readily. Primary mycelium, although it was not so easily washed as the spores, suffered from none of these disadvantages. Hence it was judged that primary mycelium was to be preferred.

Furthermore, in liquid media, the primary mycelium formed either large, floating colonies or a mass of short filaments, so that the culture had a "porridge" like consistency. The former type of growth was undesirable because it was extremely difficult to wash free of the

growth medium, and it was almost impossible to add an equal inoculum to each test flask. The "porridge" type of growth, however, was easily washed, and formed an homogenous suspension that was easily distributed. It was found that a heavy inoculum of spores or primary mycelium fragments usually gave rise to the "porridge" type of growth when grown in glucose broth, and this was used for the nutritional experiments.

Several other factors had also to be considered. It was observed by Jones (1946) that the growth was affected by the composition of the previous medium. The medium for growing the inoculum had therefore to be standardised. Glucose broth was chosen for this purpose, as it gave good growth of all the test organisms.

It was also shown that the very active growth phase of streptomycetes may be followed by rapid autolysis (Dulaney & Perlman 1947; Gottlieb & Anderson 1947). As it was undesirable that any autolysed material should be added with the inoculum, only actively growing cultures were used.

b). Preparation of the inoculum.

In view of the above considerations, the following procedure became the standard method of preparing the inoculum for the nutritional experiments.

Ten ml. of glucose broth were heavily inoculated with spores or primary mycelium, and set on a reciprocating shaker with a 3" throw, oscillating at approximately 65 throws per minute, at 25° for 2-4 days. This produced a heavy "porridge" like suspension which was centrifuged to remove the nutrient medium, washed twice with Ringer's

solution diluted 1 in 4, and resuspended in 10-12 ml. of the diluted Ringer's solution.

c). The effect of the inoculum size.

The effect of varying amounts of the inoculum on the total growth was investigated using organism No. 103. Graded amounts of a 2 day washed suspension of the mycelium prepared as above, were inoculated in duplicate into (i) 1% glucose broth and (ii) glycine-pyruvate medium as used in the nitrogen assimilation experiments (page 40) After 4 days growth on the shaker at 25°, the glucose-nutrient broth cultures were filtered through weighed Gooch crucibles, the mycelium washed with glass distilled water and dried at 104° to constant weight. After 10 days, the glycine-pyruvate cultures were harvested and similarly treated. The results are shown in Fig. 1. In glucose broth the weight of harvested mycelium rapidly decreased when the inoculum exceeded 0.2 mg. dry weight. In the synthetic medium, any inoculum greater than approximately 0.07 mg. dry weight produced no additional growth. This suggested that a stringent control on the size of the inoculum was not necessary when using synthetic media providing that its dry weight exceeded this amount.

The dry weight of washed suspensions of 6 organisms prepared as above was found to vary between 3.0 and 4.5 mg/ml. It was also found that a Pasteur pipette of approximately 0.067" external diameter (Morse twist drill gauge No. 51) delivered a drop of approximately 0.03 ml. (27-30 drops/ml). Except for a few preliminary experiments, one drop of the washed suspension from such a pipette was used to seed each flask of the nutritional experiments, so that each

received an inoculum of approximately 0.1-0.16 mg, and it was unlikely that any variation in total growth was due to the inoculum size.

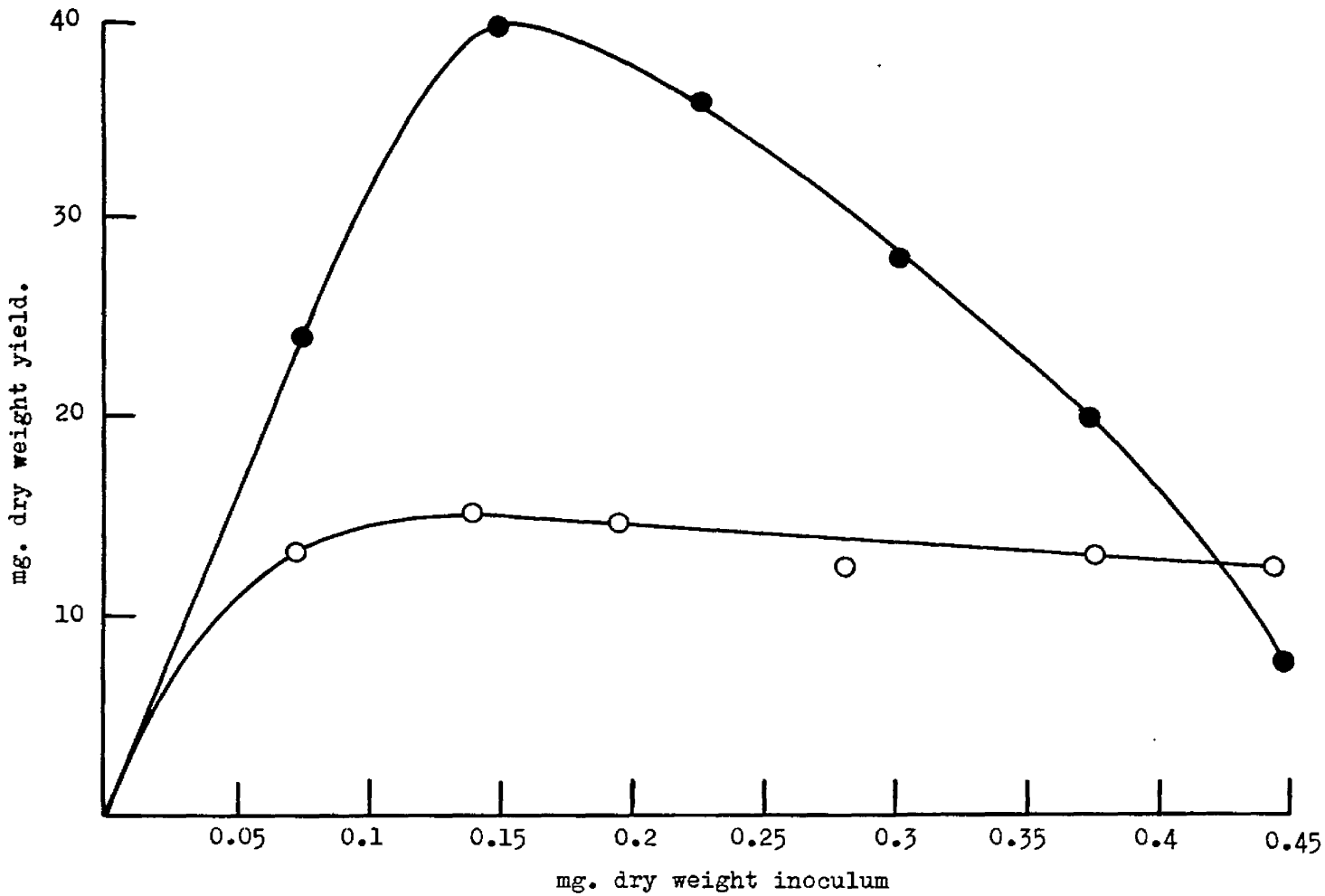

Fig. 1. Effect of the inoculum size on final yield of mycelium of 103.

2). Experimental conditions.

The carbon and nitrogen assimilation and growth factor requirements of the test organisms were investigated by inoculating each strain into the appropriate media prepared as above (page 35). The cultures were incubated at 25° on a reciprocating shaker with a 3" throw oscillating at approximately 65 throws per minute. After 10-12 days, the flasks were examined for growth, and the results recorded. Each experiment was controlled by an inoculated flask which did not contain the substance under test.

3). Growth factors.

Suspensions of the following organisms were prepared as detailed above (page 42), and inoculated into growth factor requirement media (page 40) with and without growth factors:-

Ms, Nos. 34, 48, 59, 76, 103, 196, 216, 224 and 235.

The flasks were incubated on the shaker at 25° for 10 days. The results, which are given in Table 1, showed that these cultures did not require growth factors when grown on the substrates indicated. Since, however, this experiment did not prove conclusively that none of the streptomycetes required growth factors when growing on any of the carbon and nitrogen substances under investigation, growth factors were added to all the media used in the nutritional tests as described above (page 39).

Table 1. The effect of growth factors on the growth of 10 streptomycete strains.

- = no growth, + = slight or moderate growth, ++ = good or abundant growth;

+GF = growth factors present, -GF = no growth factors present.

Organism	GLYCEROL								GLUCOSE							
	NH_4		Arginine		Glutamic		Phenyl-alanine		NH_4		Arginine		Glutamic		Phenyl-alanine	
	+GF	-GF	+GF	-GF	+GF	-GF	+GF	-GF	+GF	-GF	+GF	-GF	+GF	-GF	+GF	-GF
Ms	-	-	++	++	++	++	++	++	-	-	++	++	++	++	++	++
34	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
48	+	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++
59	+	+	++	++	++	++	++	++	+	+	++	++	++	++	++	++
76	-	-	+	+	-	-	++	++	-	-	-	-	-	-	+	+
103	++	++	+	+	++	++	++	++	++	++	++	++	++	++	++	++
196	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
216	+	+	++	++	++	++	++	++	+	+	++	++	++	++	++	++
224	-	-	+	+	++	++	++	++	-	-	++	++	++	++	++	++
235	+	+	++	++	++	++	+	+	+	+	+	+	++	++	+	+

4). Carbon assimilation.

Each of the 36 test organisms was examined for assimilation of each of the 30 carbon sources listed above (page 37). The results are given in Tables 2 and 3. Repetition of the work with several of the strains at an interval of several months showed the same results. Table 4 shows the percentage of organisms growing on each of the carbon substrates.

A. Discussion of the results.

Every substrate examined was used by several of the organisms, as might be expected from the known degradative properties of the streptomycetes.

A substance which was utilised by a large percentage of the organisms did not necessarily produce a heavy growth. Thus galactose supported the growth of 69% of the organisms, but only 8% produced a good crop of mycelium, whereas sucrose, which supported the growth of only 53% of the organisms, produced a large amount of mycelium in 28%.

a). Hexoses.

The percentage of the organisms studied here which utilised fructose, galactose, glucose and mannose was much lower than was found by Pridham & Gottlieb (1948), Okami (1952), Burkholder et al. (1954) and Benedict et al. (1955). The reasons for this discrepancy may be:-

i. Many of the strains studied in this work belonged to the Streptomyces albus species-group, some strains of which did not utilise sugars under the conditions applied here. (Table 2)

ii. The agar which was used by the other workers may have contained sufficient nutrient material to enable the organism to grow. Okami used washed agar, but his controls showed slight growth. As agar was not used here, this source of error was eliminated.

iii. All the other workers used surface growth on solid media, whereas here liquid media, aerated by shaking, were used.

The failure of sorbose to support much growth is also interesting. This sugar is an isomer of fructose which was well utilised. The configuration on carbons 2 and 3 appears to be the deciding factor.

b). Disaccharides.

Lactose was a poor carbon source, although other workers reported to the contrary (Pridham & Gottlieb 1948; Pridham, et al. 1953; Burkholder et al. 1954; Benedict et al. 1955). Both its component sugars, glucose and galactose, were used by many strains.

The strains which broke down maltose also utilised its component glucose. With two exceptions, all the strains which grew on sucrose grew on fructose and glucose. The exceptions were Nos. 224 and 250 which grew poorly on all sugars.

c). Trisaccharides.

Of the strains which assimilated raffinose only two, Nos. 133 and 193, did not grow on all the components of raffinose that were examined, that is fructose, galactose, glucose and sucrose. One of these, No. 133, metabolised the sucrose part of the molecule since it grew on fructose, glucose and sucrose, but not galactose, whereas No. 193 was unable to metabolise sucrose or fructose, and was presumably maintained

on the melibiose or galactose part.

d). Alcohols.

As found by other workers, mannitol was a better carbon source than its stereoisomers, dulcitol and sorbitol, and was the only one of the three to produce a heavy growth of the organisms which utilised it.

e). Organic acids.

Two organisms, Nos. 235 and 267, were able to grow on formic, acetic, proprionic and butyric acids, while another, No. 245, grew on all except formate. The utilisation of formate by some streptomycetes was in accord with the findings of Taylor & Decker (1947) and Stapp (1953), but in contradiction to those of Pridham & Gottlieb (194

Growth on oxalate was more common here than found by other investigators (Minter 1912; Waksman 1919a; Pridham & Gottlieb 1948; Pridham et al. 1953; Stapp 1953).

Lactate, pyruvate and citrate were well utilised.

f). General.

The carbon assimilation pattern of streptomycetes as presented here differs in some respects from that of other workers (Pridham & Gottlieb 1948; Burkholder et al. 1954; Benedict et al. 1955). The most notable differences are i) that the simple carbohydrates and glycerol were not assimilated in these experiments to the extent found by the other workers, and ii) that oxalate and tartrate were able to support the growth of a fair proportion of the organisms. These differences may be due to the different conditions employed, and to the high proportion of Streptomyces albus strains among those studied, some of which have an unusual nutritional pattern.

Table 2. The carbon assimilation of Streptomyces albus strains.

++ = good or very good growth; + = moderate or sparse growth; - = no growth.

Organism	Arabinose	Xylose	Rhamnose	Fructose	Galactose	Glucose	Mannose	Sorbose	Lactose	Maltose	Sucrose	Raffinose	Salicin	Ethanol	Glycerol	Erythritol	Dulcitol	Mannitol	Sorbitol	Inositol	Formate	Acetate	Propionate	Butyrate	Oxalate	Malonate	Lactate	Pyruvate	Tartrate	Citrate	Control	
Ms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	
76	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	
131	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	
132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-	
190	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	+	-	-	-	+	++	++	+	+	-	Sub-group I
197	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	++	++	+	+	-	
224	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	++	-	
228	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	
250	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	++	-	++	-	
259	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	
260	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	
133	+	-	+	+	-	+	+	-	-	-	+	+	-	-	-	-	-	+	+	+	-	+	-	-	+	+	+	+	+	+	-	
193	-	-	-	-	+	+	-	-	-	+	-	+	+	+	+	+	-	-	+	-	+	+	-	-	+	+	+	++	+	++	-	
216	+	+	-	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	+	+	++	++	+	+	-	Sub-group II
235	+	-	-	+	+	+	+	-	-	-	+	-	-	-	+	-	+	+	-	-	+	+	+	+	+	+	+	++	-	++	-	
242	-	+	+	-	+	+	-	+	+	+	-	-	+	-	+	+	-	-	-	-	-	-	+	+	+	+	+	++	+	+	-	
2	++	++	++	++	++	++	++	+	+	++	++	+	-	-	++	+	+	++	+	+	-	-	-	-	-	+	+	+	+	+	-	
7	++	++	+	+	+	++	++	-	-	++	++	+	+	-	++	-	+	-	-	+	-	+	-	-	-	-	+	+	-	+	-	
9	++	+	-	++	+	++	++	+	-	+	+	-	+	-	++	+	-	-	-	-	-	++	-	-	-	-	+	+	-	++	-	
13	-	++	-	++	+	++	++	-	-	+	++	+	++	+	++	-	-	-	-	-	-	++	-	-	-	-	+	++	-	++	-	Sub-group III
33	-	-	-	+	+	++	++	-	-	+	++	-	++	-	++	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	
34	+	+	+	++	++	++	++	-	-	++	++	+	-	-	++	-	-	++	-	-	-	-	-	-	-	-	++	+	-	++	-	
48	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	++	+	+	+	-	
59	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	++	+	+	++	-	
103	++	++	++	++	+	++	++	+	++	++	++	++	++	-	++	+	-	++	++	-	-	+	-	-	-	-	+	++	-	++	-	
195	+	+	++	++	+	++	++	-	-	+	++	-	-	-	++	-	-	++	-	-	-	-	-	-	-	-	+	+	-	+	-	
196	+	+	++	++	+	++	++	-	-	-	++	-	-	-	++	-	-	++	-	-	-	-	-	-	-	-	+	++	-	+	-	

Table 3. The carbon assimilation of species other than Streptomyces albus.

++ = good or very good growth; + = moderate or scanty growth; - = no growth.

Organism	Arabinose	Xylose	Rhamnose	Fructose	Galactose	Glucose	Mannose	Sorbose	Lactose	Maltose	Sucrose	Raffinose	Salicin	Ethanol	Glycerol	Erythritol	Dulcitol	Mannitol	Sorbitol	Inositol	Formate	Acetate	Propionate	Butyrate	Oxalate	Malonate	Lactate	Pyruvate	Tartrate	Citrate	Control
Sg	+	++	-	++	+	+	+	-	-	-	+	-	+	-	++	-	-	+	+	-	-	+	+	-	-	-	++	++	-	++	-
Sa	++	+	-	++	++	++	+	-	-	-	++	-	-	-	++	-	-	-	+	-	-	-	-	-	+	-	+	+	-	+	-
204	++	+	+	++	+	+	+	-	+	-	+	-	+	-	+	+	-	-	+	+	+	+	-	-	-	+	+	++	+	+	-
214	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	++	++	+	++	-
217	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+	-	++	-	+	-
245	-	-	-	++	+	+	+	-	-	-	++	+	-	-	+	-	-	-	-	-	-	++	+	++	-	-	+	+	-	-	-
265	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	+	-
266	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
267	+	-	+	+	+	+	+	+	-	+	-	+	+	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-

Table 4. Percentage of organisms using each of the carbon sources.

Substrate	% giving good growth	% giving moderate growth	% not growing
Arabinose	17	28	55
Xylose	14	32	54
Rhamnose	11	22	67
Fructose	30	28	12
Galactose	8	61	31
Glucose	28	36	36
Mannose	25	30	45
Sorbose	0	16	84
Lactose	3	11	86
Maltose	11	28	61
Sucrose	28	25	47
Raffinose	3	25	72
Salicin	8	20	72
Ethanol	0	8	92
Glycerol	30	33	37
Erythritol	0	20	80
Dulcitol	0	11	89
Mannitol	14	11	75
Sorbitol	3	20	77
Inositol	0	11	89
Formate	0	14	86
Acetate	8	30	62
Propionate	0	14	86
Butyrate	3	8	89
Oxalate	0	50	50
Malonate	0	61	39
Lactate	22	61	17
Pyruvate	42	58	0
Tartrate	0	36	64
Citrate	30	53	17

5). Nitrogen assimilation.

A. Preliminary experiments with nitrite.

To determine the concentration of nitrite which would support growth of streptomycetes, organisms No. 76 and 103 were grown on nitrogen assimilation media (page 40) containing sodium nitrite in concentrations ranging from 0.05% to 1% w/v as nitrogen source and with 1% w/v sodium pyruvate or 1% w/v glucose as the carbon source. No growth was obtained on the glucose-nitrite medium, but the pyruvate-nitrite medium supported the growth of both organisms at concentrations of 0.1% w/v and 0.05% w/v, best growth being obtained at the lower concentration.

The experiment was repeated and extended to 10 organisms. These were grown in nitrogen assimilation media (page 40) containing 0.05% w/v sodium nitrite as the sole nitrogen source, and one of the following as carbon source:-

1% w/v sodium pyruvate

1% w/v glucose

1% w/v glycerol

1% w/v glucose + 1% w/v pyruvate.

Controls without any carbon source were included.
The media were inoculated with a standard inoculum of the following organisms:- Sg, Nos. 2, 7, 33, 48, 76, 103, 131, 133 and 214.
The results were recorded after 12 days incubation at 25° on the shaker, and are shown in Table 5.

No growth was obtained on glycerol or glucose; pyruvate alone supported growth of half the organisms, while all of them grew on

the glucose-pyruvate mixture.

The only flasks in which ammonia, as shown by Nessler's reagent was found, were those containing glucose, although there was no growth. None of the others, whether there was growth or not, were Nessler positive.

The pH of the cultures was recorded. The values observed did not confirm the claim of Waksman & Joffe (1920) that nitrite favoured the production of acid rather than alkali.

It was concluded that, for the strains examined, (i) nitrite was utilised, but with only certain carbon sources, and (ii) a suitable concentration for further investigation was 0.05%.

B. Investigation of nitrogen sources.

Each of 33 test organisms was examined for assimilation of each of the 24 nitrogen sources listed above (page 38). Organisms 8a, 13 and 33 were lost before this section of the work commenced, and are therefore not reported.

The results are shown in Table 6; the growth in pyruvate-ammonium sulphate medium is taken from Tables 2 and 3. Table 7 shows the percentage of organisms growing on each of the nitrogen sources.

C. Discussion of the results.

As with the carbon sources, the nitrogen compounds which supported the growth of many organisms did not necessarily produce a large amount of mycelium. Valine, for example, supported the growth of 75% of the organisms, but never gave rise to more than moderate growth.

a). Inorganic compounds.

Ammonia was the best of the inorganic sources, and was

used by all the organisms. Fifty seven per cent of the organisms utilised nitrate, nitrite and ammonia; 12% used nitrate and ammonia, but not nitrite; 31% used ammonia only. Every strain which used nitrite utilised ammonia and nitrate also.

The effect of the carbon source on the utilisation of nitrite has already been noted (page 50).

b). Amino acids.

The streptomycetes examined utilised α-amino acids very well; only two organisms, No. 266 and 267 did not.

Several α-amino acids, particularly cystine, methionine and tryptophane were less readily assimilated than the rest. The least utilised was tryptophane, although it was a component of the mycelial mat of Streptomyces griseus (Stokes & Gunness 1946). Presumably, many streptomycetes can synthesise tryptophane, but cannot use it as a nitrogen source for growth.

c). Urea.

Urea was utilised by all the organisms examined, except No. 266, 27% producing good growth. This confirmed the findings of other workers (page 23).

d). Purines.

Only two organisms, Nos. 266 and 267, did not grow on uric acid and xanthine. Both these substances were very sparingly soluble in water, so that the media containing them were always cloudy, particularly uric acid which was the more insoluble of the two. Good growth produced clearing of the medium. These results confirmed those of Stapp (1953) and Stapp & Spicher (1954). (page 23).

Table 5. Growth of 10 streptomycete strains on nitrite with various carbon sources.

++ = good growth; + = moderate or scanty growth; - = no growth.

+ = positive reaction to Nessler's reagent; - = no reaction; tr = trace.

Organism	Control			Glucose			Glycerol			Pyruvate			Pyruvate + Glucose		
	Growth	pH	NH_4	Growth	pH	NH_4	Growth	pH	NH_4	Growth	pH	NH_4	Growth	pH	NH_4
Sg	-	7.02	-	-	7.05	+	-	7.09	-	++	8.22	-	++	7.30	-
2	-	7.28	-	-	7.30	+	-	7.28	-	+	7.30	-	++	7.82	-
7	-	7.14	-	-	7.16	+	-	7.10	tr	-	7.12	-	++	7.26	-
33	-	7.06	tr	-	7.00	+	-	6.94	tr	-	7.00	-	+	7.26	-
48	-	6.96	-	-	7.00	+	-	7.20	-	-	7.14	-	+	7.18	-
76	-	7.02	-	-	7.01	+	-	7.03	-	+	7.90	tr	++	7.18	-
103	-	7.12	-	-	7.10	+	-	7.20	-	+	7.28	-	++	8.24	-
131	-	7.26	-	-	7.24	+	-	7.30	tr	+	8.60	tr	++	7.58	-
133	-	7.30	tr	-	7.32	+	-	7.36	tr	+	8.42	-	++	7.43	-
214	-	7.28	-	-	7.28	+	-	7.35	-	+	8.34	-	++	8.22	-

Table 6. The assimilation of nitrogen compounds by streptomycetes.
++ = good or very good growth; + = moderate or sparse growth; - = no growth.

Organism	Ammonia	Nitrite	Nitrate	Alanine	Arginine	Aspartic	Cystine	Glutamic	Glycine	Histidine	Hydroxy-proline	Leucine	Lysine	Methionine	Ornithine	Phenyl-alanine	Proline	Serine	Threonine	Tryptophane	Tyrosine	Valine	Urea	Uric acid	Xanthine	Control
Sg	+	++	++	++	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++	+	++	+	++	++	++	-
Ms	+	-	-	+	++	++	+	+	+	++	+	+	+	+	+	++	++	++	+	-	++	+	+	+	++	-
2	+	+	+	+	++	+	+	++	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	++	-
7	+	-	-	++	++	++	-	++	+	+	++	++	++	+	++	++	++	++	++	-	+	-	+	+	+	-
9	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	++	++	-
34	+	+	+	+	++	++	+	++	+	+	++	+	+	-	+	++	++	+	+	-	++	-	+	+	++	-
48	+	-	-	++	+	+	-	+	+	+	+	+	++	+	+	+	+	++	+	-	-	+	+	+	+	-
59	+	+	+	+	+	+	++	++	++	+	++	++	+	+	+	+	+	+	+	-	++	+	+	+	++	-
76	+	+	+	+	+	++	-	++	+	+	++	+	++	+	+	++	++	+	++	-	+	+	+	++	++	-
103	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++	+	+	++	++	++	-
131	+	+	+	++	++	++	+	++	++	++	++	+	+	+	+	++	++	+	++	-	+	+	+	+	++	-
132	+	++	+	++	++	+	+	++	+	++	++	+	++	+	+	++	++	+	++	-	-	+	++	++	++	-
133	+	+	+	++	++	++	+	++	++	++	++	+	++	+	+	++	++	+	++	-	++	+	+	++	++	-
190	++	-	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	-
193	++	-	+	++	++	+	++	+	++	++	++	+	++	+	++	++	++	+	++	-	++	+	+	+	+	-
195	+	+	+	++	+	+	++	++	+	++	++	+	+	+	+	+	++	++	++	-	++	+	+	+	++	-
196	+	+	+	++	++	+	++	++	+	+	++	+	+	+	+	++	++	++	++	-	++	+	+	+	+	-
197	++	-	+	++	++	++	++	++	++	++	+	++	+	++	+	++	++	++	++	+	++	+	++	++	++	-
204	++	+	+	++	+	+	-	+	++	++	++	+	+	+	+	++	++	+	++	-	++	+	+	+	+	-
214	++	+	++	++	++	++	+	++	+	++	++	+	+	+	+	++	++	++	++	+	++	+	+	+	++	-
216	++	+	+	++	++	+	+	+	+	++	+	+	++	-	+	++	++	+	++	+	+	+	+	++	++	-
217	++	+	+	++	+	+	+	+	+	+	++	+	++	-	++	++	++	+	++	-	++	+	+	+	+	-
224	+	-	-	++	++	++	-	++	++	++	+	+	++	+	++	++	++	+	++	+	++	+	++	++	+	-
228	+	-	-	++	++	++	-	++	++	++	+	+	+	+	+	++	++	+	++	-	+	+	+	++	+	-
235	++	+	+	++	++	++	+	++	++	++	++	+	++	+	+	++	++	++	++	-	-	+	++	++	+	-
242	++	-	+	+	++	++	+	++	+	++	+	+	+	+	+	++	++	+	+	-	++	+	++	++	+	-
245	+	+	+	++	+	+	-	++	++	++	+	++	++	+	++	+	++	++	+	-	+	+	++	++	+	-
250	++	-	+	++	++	++	-	++	++	++	++	+	++	+	++	++	++	++	++	-	+	-	+	+	++	-
259	+	+	+	++	++	++	+	++	++	++	+	+	++	+	+	++	++	++	++	-	-	+	++	+	++	-
260	+	+	+	+	+	+	+	++	+	++	+	+	++	+	+	+	+	+	+	-	-	+	+	++	++	-
265	++	-	-	+	++	++	++	++	++	++	+	+	-	+	+	+	++	+	+	-	++	-	+	+	+	-
266	+	-	-	-	+	-	+	+	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-
267	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-

Table 7. Percentage of organisms using each of the nitrogen sources.

Substrate	% giving good growth	% giving moderate growth	% not growing
Ammonium	45	55	0
Nitrite	9	48	43
Nitrate	9	61	30
Alanine	64	30	6
Arginine	64	33	3
Aspartic acid	51	42	7
Cystine	24	45	31
Glutamic acid	70	27	3
Glycine	45	52	3
Histidine	64	33	3
Hydroxy-proline	51	42	7
Leucine	18	75	7
Lysine	48	45	7
Methionine	3	75	22
Ornithine	24	76	0
Phenyl-alanine	66	27	7
Proline	76	21	3
Serine	39	54	7
Threonine	60	33	7
Tryptophane	3	15	82
Tyrosine	45	30	25
Valine	0	75	25
Urea	27	70	13
Uric acid	42	51	7
Xanthine	54	39	7

ACID PRODUCTION FROM CARBOHYDRATES

ACID PRODUCTION FROM CARBOHYDRATES.

Several authors have shown that the streptomycetes produce organic acids from various carbohydrates (Krainsky 1914; Waksman & Joffe 1920; Plotho 1940a; 1940b; Woodruff & Foster 1943; Schatz & Waksman 1945; Cochrane 1947; Cochrane & Dimmick 1949; Sevcik 1952). Acid production has been suggested as a means for the preliminary differentiation of unknown strains (Cochrane 1947; Buthala & Gilmour 1951).

1. Methods.

A. Carbohydrate peptone water.

Carbohydrate peptone water (Appendix II: medium 16) was prepared and dispensed into ½ oz. Bijou bottles each containing a Durham tube.

The following carbohydrates were used:-

arabinose	maltose
fructose	sucrose
galactose	glycerol
glucose	mannitol
mannose	inositol
lactose	

The cultures were incubated at 25°, and examined periodically over 21 days.

B. "Auxanographic" method.

A medium was prepared consisting of the basal salts solution and growth factors as in the assimilation experiments, but with no carbon or nitrogen source. Bromo-cresol purple was added to give a concentration of 0.16% w/v and agar to 1.5% w/v. Ammonium

sulphate was added to one part to give a concentration of 0.25% w/v and asparagine to the other to give a concentration of 0.2% w/v. The melted agar was cooled to 50°, inoculated with a heavy suspension of washed mycelium and poured into large Petri dishes (diameter 14.5 cm.). Small porcelain cylinders were inserted into the agar before it had set. Into each of these was put one drop of one of the following stock carbon solutions (page 37):-

arabinose	lactose
fructose	sucrose
galactose	glycerol
glucose	mannitol
mannose	inositol

The plates were incubated at 25° for 21 days. Yellow zones round the cylinders indicated acid production.

C. Acid production in the carbon assimilation experiments.

The final pH of the medium of some of the carbon assimilation experiments was measured by adding B.C.P. indicator to the flasks at the end of the experiment.

D. Acid production by resting suspensions.

A solution of 0.1M sodium dihydrogen phosphate and 0.016% w/v bromo-cresol purple was sterilised in the autoclave. Nine ml. quantities were placed in sterile flasks, and 0.5 ml. of one of the following stock carbon source solutions (page 37) was added:-

arabinose	sucrose
glucose	glycerol
mannose	mannitol
lactose	inositol
maltose	

Each flask was then inoculated with a heavy suspension of washed mycelium, and set in the 25° incubator. Observations were made hourly on the first day, and daily thereafter.

2. Results.

A. Carbohydrate peptone water.

The results of testing each test organism in carbohydrate peptone water against 11 sugars are given in Table 8. Each test was repeated at least 4 times with the exceptions indicated in Table 8.

Table 11 shows the percentages of organisms which produced acid from each sugar, and the percentage of those producing acid which gave variable results. This tendency to produce inconsistent results was marked, even when the conditions were carefully standardised. "Alkaline reversal" occurred frequently and rapidly.
Gas was never produced in these tests.

B. "Auxanographic" method.

Thirteen organisms were tested against 10 sugars, using ammonium or asparagine as the nitrogen source. The results are shown in Table 9. Table 11 shows the percentage of organisms which produced acid from each sugar and nitrogen source.

C. Acid production in the carbon assimilation experiments.

The production of acid by the carbohydrates used in the carbon assimilation experiments is shown in Table 10. The percentage of organisms which produced acid from each sugar is shown in Table 11.

D. Acid production from resting suspensions.

Three strains only were investigated by this method, Nos. 9, 131 and 228. No acid appeared in any of the flasks until after several days when growth had taken place, autolysed mycelium probably providing the nitrogen for this.

3. Discussion.

Table 11 shows the percentage of cultures producing acid in carbohydrate peptone water, by the auxanographic method and in carbon assimilation media. Percentages are worked out only on the organisms which grew in a particular medium, and not from those tested, in order to eliminate any variation due to the differences in nutrition.

Unfortunately the results were not satisfactory, so that no firm conclusions could be drawn from them. In general, however, acid was formed more frequently from the hexoses than the other carbohydrates, but less often from the disaccharides and alcohols except glycerol. Clearly the factors governing acid formation by streptomycetes need fuller investigation. Nevertheless, from the experience obtained from this work, it is suggested that among the factors involved are the following:-

a). The nature of the nitrogen source.

Asparagine was a better nitrogen source for the formation of acid than ammonia in the auxographic experiments. This supported the claim of Waksman & Joffe (1920) that the nitrogen source is the determining factor.

In this connection it was thought that incubation of a heavy suspension of mycelium in phosphate solution containing a carbohydrate and an indicator, might have induced the organisms to produce acid rapidly from the sugar, and at the same time, would have eliminated the effect of the nitrogen source. However, the failure of 3 typical organisms to form acid under these conditions suggested that the method was of little value.

b). The physical conditions of the experiments.

The aerated flasks used in the carbon assimilation experiments generally gave rise to acid more frequently than any other method. This suggested that the degree of aeration affected the production of acid by streptomycetes, and confirmed the findings of Sevcik (1952) and Woodruff & Foster (1943).

c). The length of time that the organisms have been kept in the laboratory.

In the course of the experiments, it was noted that the cultures which had been kept in the laboratory for a long period tended to lose the ability to produce acid. This probably accounted for some of the variations observed in the carbohydrate peptone water experiments which were spread over 4 years, whereas the other acid production experiments were done on organisms which had not been under laboratory cultivation for a long period.

Table 8. Production of acid in carbohydrate peptone water.

0 = no acid produced; a = small amount of acid; A = large amount of acid; v = variable results.

Organism	Arabinose	Fructose	Galactose	Glucose	Mannose	Lactose	Maltose	Sucrose	Glycerol	Mannitol	Inositol
Sg	0	0	0	0	0	0	0	0	0	0	0
Sa	0	A	0	A	a	0	a	0	0	0	0
Ms	0	0	0	0	0	0	0	0	0	0	0
2	0	a	0	v	0	0	0	0	0	0	0
7	0	A	A	A	A	v	v	0	A	0	0
9	0	v	0	v	v	0	v	0	v	0	0
13	0	A	0	A	A	0	0	a	A	0	0
33	0	A	0	A	A	0	0	0	A	0	0
34	0	v	0	v	0	0	v	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
59	0	0	v	0	v	0	0	0	0	0	0
76	0	A	A	v	v	0	0	0	0	0	0
103	0	v	0	0	0	0	0	0	0	0	0
131	0	0	v	v	0	0	0	0	v	0	0
132	0	0	A	v	0	0	0	0	0	0	0
133	0	0	v	0	0	0	0	0	0	0	0
190	0	A	0	v	0	0	0	0	0	0	0
193	0	a	v	0	v	0	v	0	0	0	0
195	0	0	0	0	v	0	0	0	0	0	0
196	0	0	0	0	0	0	0	0	0	0	0
197	0	0	0	0	0	0	0	0	0	0	0
204	0	v	a	A	0	0	v	0	0	0	0
214	0	v	0	0	v	0	0	0	0	0	0
216	v	A	v	A	v	0	0	v	v	0	0
217	0	A	A	v	v	0	0	0	0	0	0
224	0	0	v	v	0	0	v	0	0	0	0
228	0	v	0	0	v	0	0	0	0	0	0
235	0	0	A	A	0	0	0	0	0	0	0
242	0	0	0	0	0	0	0	0	0	0	0
245	0	0	0	0	0	0	0	0	0	0	0
250	0	0	0	v	0	0	0	0	v	0	0
259	0	A	0	0	0	0	0	0	0	0	0
260	0	A	0	0	0	0	0	0	0	0	0
265	0	0	0	0	0	0	0	0	0	0	0
266	0	0	0	0	0	0	0	0	0	0	0
267	0	0	0	0	0	0	0	0	0	0	0

Sa, 13 and 33 were tested once only

Table 9. Acid production by the auxanographic method.

Column 1 = NH_4 base; column 2 = Asparagine base. 0 = no acid produced; a = small amounts of acid; A = large amount of acid; v = variable results; NG = no growth.

Organism	Arabinose		Fructose		Galactose		Glucose		Mannose		Lactose		Sucrose		Glycerol		Mannitol		Inositol	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Sg	0	0	0	0	a	A	A	A	a	A	NG	0	0	0	0	0	a	0	NG	0
2	-	A	-	0	-	A	-	A	-	A	-	A	-	0	-	A	-	A	-	0
7	0	0	a	A	0	0	0	A	a	A	NG	0	0	0	A	0	NG	0	0	0
9	0	0	a	A	0	0	0	A	0	A	NG	0	0	0	a	A	NG	0	NG	0
33	NG	0	0	0	0	0	a	A	A	0	NG	0	0	0	0	0	0	0	NG	0
34	0	A	v	A	0	A	0	A	0	A	0	0	0	0	0	A	0	A	0	0
48	0	A	0	A	0	0	a	A	0	0	0	0	0	A	0	v	0	0	0	0
59	0	A	0	0	0	A	0	A	0	0	NG	0	0	0	0	A	0	A	NG	0
76	NG	A	NG	0	NG	A	NG	v	NG	v	NG	v	NG	0	NG	a	NG	A	NG	0
103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
131	NG	A	NG	0	NG	A	NG	A	NG	A	NG	A	NG	0	NG	A	NG	a	NG	0
193	NG	A	0	0	0	0	NG	0	NG	0	NG	0	NG	0	0	0	NG	0	0	0
204	0	A	a	a	a	A	0	0	0	0	0	A	0	0	0	A	0	0	0	0

Table 10. Production of acid in carbon assimilation media.

0 = no acid produced; a = small amount of acid; A = large amount of acid; NG = no growth.

ORGANISM	Arabinose	Xylose	Rhamnose	Fructose	Galactose	Glucose	Mannose	Sorbose	Lactose	Maltose	Sucrose	Raffinose	Salicin	Glycerol	Erythritol	Dulcitol	Mannitol	Sorbitol	Inositol
Sg	0	A	NG	a	a	0	a	NG	NG	NG	0	NG	0	a	NG	NG	a	0	NG
2	A	0	0	A	a	a	a	0	0	a	0	0	NG	a	0	0	a	0	0
7	a	A	0	a	0	A	a	NG	NG	a	0	0	0	A	NG	0	NG	NG	0
9	a	a	NG	a	0	A	A	0	NG	0	0	NG	0	a	0	NG	NG	NG	NG
13	NG	0	NG	a	a	A	A	NG	NG	0	0	0	0	A	NG	NG	NG	NG	NG
33	NG	NG	NG	a	a	A	A	NG	NG	0	0	NG	0	A	NG	NG	NG	NG	NG
34	0	0	0	a	0	a	a	NG	NG	0	0	0	NG	a	NG	NG	a	NG	NG
48	0	0	NG	a	a	0	0	0	NG	a	0	0	NG	0	NG	NG	NG	NG	NG
59	a	0	0	a	a	a	a	NG	NG	0	0	NG	NG	0	NG	NG	a	NG	NG
103	a	A	0	A	0	A	A	0	a	A	0	0	NG	A	0	NG	0	0	NG
193	NG	NG	NG	NG	0	0	NG	NG	NG	0	NG	0	0	0	0	NG	NG	0	NG
204	A	0	0	A	0	0	0	NG	0	NG	0	NG	0	0	0	NG	NG	0	0

Table 11. Percentages of cultures producing acid by various methods.

Carbohydrate	Carbohydrate peptone water		Auxanographic method		Carbon assimilation media
	% producing acid	% of acid producers giving variable results	NH_4 base	Asparagine base	
Arabinose	0	--	0	62	66
Xylose	--	--	--	--	40
Rhamnose	--	--	--	--	0
Fructose	50	33	40	38	100
Galactose	33	50	20	54	50
Glucose	46	59	33	77	66
Mannose	36	69	33	53	82
Lactose	3	100	0	23	33
Maltose	20	86	--	--	40
Sucrose	6	50	0	8	0
Raffinose	--	--	--	--	0
Glycerol	20	57	20	61	66
Erythritol	--	--	--	--	0
Dulcitol	--	--	--	--	0
Mannitol	0	--	14	38	80
Sorbitol	--	--	--	--	0
Inositol	0	--	0	0	0

GENERAL DISCUSSION

GENERAL DISCUSSION.

1). Experimental conditions.

As stated in the introduction, the work was hampered by the failure to obtain a suitably wide range of organisms, but the experimental pattern established is capable of extension to include other species when they become available, and the methods used could be adopted as a standard technique for future research.

In this work it was necessary to use the strictest experimental conditions for the nutritional investigations. Appleby (1948) for example had found that an asporogenous variant of *Streptomyces griseus* was able to grow, though poorly, on a synthetic medium containing washed agar, but no added nitrogen. Cochrane & Conn (1950) also noted that there was sufficient nitrogen in agar to support slight growth, and this was again confirmed by Okami (1952). Cochrane (1950) demonstrated the existence of sufficient nutrient in laboratory reagents and the atmosphere to support slight streptomycete growth. The most stringent precautions were therefore taken to avoid the introduction of spurious nutrients on glassware or in the reagents used in making the media.

Physical factors also play a part in streptomycete nutrition. Conditions of aeration and temperature were therefore carefully controlled throughout the work.

2). Description of the organisms.

It is difficult to classify streptomycetes because of the lack of type cultures, adequate descriptions and differentiating criteria. However, despite this difficulty, it was considered desirable to attempt an estimate of the species-group to which each isolate belonged employing the tests in general use.

A. Streptomyces albus strains.

Four authorities were cited in identifying the Streptomyces albus group of isolates. They were Duché (1934), Baldacci (1939), Bergey* (1948) and Baldacci et al. (1954). The only feature common to all Duché's organisms was the whiteness of the spores. He included in the S. albus species-group many organisms which more recent authors have placed in other species. Baldacci (1939), Bergey (1948) and Baldacci et al. (1954) all placed the main emphasis for identifying streptomycetes on the colour of the primary and secondary mycelia. The description of S. albus

*Since the greater part of this thesis was written, the 7th (1957) edition of Bergey's Manual of Determinative Bacteriology has come into general use. The basic arrangement of the genus Streptomyces has not been greatly changed from the 6th (1948) edition. The main alterations have been that (1) the number of species described has been more than doubled and (2) that greater use has been made of the morphology of the spores and sporophores as a taxonomic criterion. A few other changes which are relevant to the organisms studied in this work are indicated as footnotes or amendments to the text.

in Bergey's manual (1948) includes the modifications by Waksman & Henrici (1943) of the original description by Rossi-Doria (1894). The descriptions of these authors, together with some other relevant papers, are compared below with the strains designated S. albus in this study.

a). Primary mycelium.

As shown by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and as also found here, the primary mycelium of Streptomyces albus strains consists of gram positive, long, branching, colourless filaments of approximately 0.4-1.0 μ diameter. However, Nos. 7, 13 and 33 were exceptional in that the primary mycelium tended to be friable and the filaments short. This was not considered sufficient grounds to place these organisms in a separate group as it was observed in the course of the work that variation in the carbon source altered the length of the filaments of the primary mycelium of these and several other strains.

Baldacci (1939) noted that the reverse of the primary mycelium was sometimes "more or less yellowish". This was confirmed in this work.

b). Secondary mycelium.

All workers agree that this species has white aerial spores which turn grey or brown with age. This was confirmed in this study.

There are considerable differences of opinion with regard to the stability of the arrangement of the spores of streptomycetes, and hence the value of the spore arrangement as a taxonomic criterion. Various workers (Backus et al. 1954; Burkholder et al. 1954; Erikson 1955) have reported considerable variation in the sporophore morphology depending on the conditions under which the organisms were grown. Furthermore, it has also been claimed that a single hypha can bear different types of sporophores, whose morphology may change during development (Skinner et al. 1947). It was observed during this study that a single colony could carry both straight and open spiralled sporophores. In contrast, Pridham, Hesseltine & Benedict (1958) have recently presented a comprehensive scheme of streptomycete classification based on the morphology of the sporophores and the secondary mycelium as a whole, in which they contend that "the morphology of the sporophores of a particular strain does not appreciably change on substrata that support optimal formation of aerial mycelium, sporophores and spores."

Baldacci (1939) described the spores of Streptomyces albus as ovoid, and stated that they were carried on straight filaments which sometimes ended in a "hook" or were spiral, while Bergey's manual (1948) asserted that the spores were carried on coiled chains, but did not indicate whether the

coils were tight or loose, or turned clockwise or anti-clockwise. Fifteen of the organisms assumed to be Streptomyces albus in this study had straight sporophores. They are thus similar to those described by Baldacci (1939). These organisms belonged to the white series of the section called "Rectus-flexibilis" by Pridham et al. (1958). Three of the remaining S. albus strains had sporophores which formed open spirals; the sporophores of two of these organisms had an anticlockwise twist, and the other, a clockwise twist. A further 6 strains showed both straight and open spiralled sporophores usually in the same preparation. In 5 of these cases the spiral was anti-clockwise, and in one case it was clockwise. These 6 strains would fall into the white series of both the "Rectus-flexibilis" and the "Retinaculum-apertum" sections of Pridham et al. (1958). A further two of the S. albus strains had tight spirals, one being anticlockwise and one clockwise. These would fall into the white series of the "Spira" section of Pridham et al. (1958). Only one strain, No. 103, did not answer to the description of the secondary mycelium given by either Baldacci (1939) or in Bergey's manual (1948). In this case the sporophores formed verticils or tufts. Since it resembled the other S. albus strains in many other respects, it was decided to assume that it too was a strain of the same species. This

organism would probably fall into the "Biverticullus" section of Pridham et al. (1958). It would be very useful to know to what extent sporophore morphology can be used taxonomically, and this feature would repay further study.

c). Pigments and odours.

Baldacci (1939) noted that a soluble brown or black pigment was produced by Streptomyces albus which was strain variable. Waksman & Henrici (1943) stated that pigment was not produced on any organic medium. Bergey (1948) does not mention pigments except to state that they were not produced on gelatine. In this work it was found that many strains of S. albus formed a soluble brown pigment on nutrient agar and gelatine, and that the pigment was often lost on prolonged laboratory storage.

Baldacci (1939), Waksman & Henrici (1943) and Bergey (1948) noted the presence of an earthy or musty odour which was also found during this work.

d). Proteolytic and saccharolytic ability.

Bergey* (1948) described Streptomyces albus as having no diastatic activity and Waksman & Lechevalier (1953) reported such activity as "not strong", whereas all strains in the present study broke down starch rapidly. In

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* The 7th (1957) edition of Bergey's Manual states that there is no hydrolysis of starch by some cultures of Streptomyces albus and rapid hydrolysis by others,

addition, Bergey (1948) stated that this species is actively proteolytic which is contrary to the findings of Waksman & Lechevalier (1953), while the results presented in this thesis varied from strongly proteolytic to non-proteolytic. These conflicting observations may have been due to differences in the conditions employed and because various strains of the species have markedly different activities.

In the course of the tests for proteolysis, it was observed that the breakdown of inspissated serum took place in several stages. The first indication of activity was a change in the medium from opaque to translucent after which the streak embedded itself in the medium. Later liquefaction took place. Presumably the "clearing" stage occurred during the breakdown of the proteins to smaller molecules, while during "pitting" the colony was using the breakdown products as fast as they were formed. Only when the breakdown was faster than utilisation was any liquefaction observed.

Milk usually turned alkaline, indicating that proteolysis of casein was in progress. The resulting amino groups and ammonia masked any acid that may have been formed from the lactose as was indicated by the observation that acid sometimes appeared after long incubation; the weakly proteolytic strains often produced a low pH more

quickly than the strongly proteolytic ones.

The ability to attack gelatine also varied from culture to culture, some organisms taking a month to show any sign of liquefaction of the medium. Dorset's egg medium was the least frequently liquefied of all the media examined for proteolysis.

e). Nitrate reduction.

Approximately 60% of the Streptomyces albus strains investigated in this study reduced nitrate to nitrite in nitrate broth. The type culture (Bergey 1948) also carried out this reduction. It is possible that the remaining 40% also reduced nitrate but used the nitrite as fast as it was produced. The problem of the utilisation of nitrate and nitrite is further discussed below under "Nitrogen sources".

f). Other media.

Several other media were examined such as Czapek Dox glucose and sucrose media and potato plug because these were commonly used by the early workers. However, these media were not found to have any particular value in distinguishing one species from another, or the strains within the Streptomyces albus species-group. It was noticed that the potato plug was a good medium for inducing sporulation.

B. Species other than Streptomyces albus.

The identification of the species other than Streptomyces albus was very tentative for the most part, because the descriptions of the species in the literature are inadequate, and the type cultures were not available for direct comparisons. Only 3 organisms were identified with any certainty; 2 of these identifications were confirmations of names given by the National Collection of Type Cultures. The third, No. 204, was identified as S. coelicolor. The peculiar nature of the pigment produced by this species makes identification easy. In 5 other cases the identifications were tentative, and one organism, No. 245, was unidentified.

3.) Carbon sources.

A nutritional study such as the one attempted here is valuable, not only for the knowledge that it provides as to which carbon and nitrogen sources support growth, but also in providing data of possible taxonomic value, and indicating further the metabolic capabilities and biochemistry of the organisms.

A. Relation of nutrition to taxonomy.

Of the 36 organisms studied in this work, 27 were considered to belong to the Streptomyces albus species-group, and the remaining 9 to each represent a different species.

Comparison of the nutrition between the Streptomyces albus strains suggested that the species-group may consist of 3 different types of organism as set out below. Of the 9 cultures each representing a different species, 4 have not, so far as is known, been previously investigated; the remaining 5 have been studied by other workers so that comparisons of their results with the findings of this study were possible and are also set out below.

a). Streptomyces albus strains.

Twenty seven of the organisms examined were considered to be strains of Streptomyces albus. The carbon assimilation of these strains suggested that they can be divided into 3 sub-groups. Their carbon assimilations

and the sub-groups are shown in Table 2.

Sub-group I. Sub-group 1 was characterised by (i) a lack of response to the sugars. Such growth as was obtained was slight, or at the most, moderate. (ii) The response to alcohols was also limited. (iii) Growth on the fatty acids was poor. A good response was obtained to the remaining organic acids except tartrate.

Sub-group II. Sub-group II was characterised by (i) a somewhat better growth on the sugars than that found in sub-group I. Moderate growth was obtained on the hexoses; less vigorous growth on the other sugars. (ii) Moderate growth was obtained on the alcohols. (iii) The fatty acids were more frequently used in this sub-group than in either of the others; 80% of the strains of the group used acetate. All the remaining organic acids were well utilised.

Sub-group III. Sub-group III was characterised by (i) a vigorous assimilation of the sugars. Only sorbose and lactose were not generally attacked. (ii) The growth on the alcohols was limited, except for glycerol which was assimilated by all the strains, and mannitol which supported the growth of 54%. (iii) The growth on fatty acids was very poor; only a few strains grew on acetate. A good response was obtained to the remaining organic acids except tartrate, oxalate and malonate. The lack of

utilisation of malonate and oxalate by the organisms of sub-group III contrasted with sub-groups I and II where the utilisation of these acids was high. The nutrition of sub-group III, in fact, closely resembled that of the genus as a whole as presented by other workers (Pridham & Gottlieb 1948; Pridham et al. 1953; Burkholder et al. 1954; Benedict et al. 1955).

Confirmatory evidence. It would obviously be an advantage if this classification based on carbon assimilation could be confirmed by other characteristics. However, the reduction of nitrate to nitrite was the only feature of all the biochemical and morphological characteristics examined which tended to confirm the carbon utilisation groupings. The details are set out in Table 12. From these data it can be shown that 36% of sub-group I, 60% of sub-group II and 91% of sub-group III organisms gave positive results to the nitrate test. It appears that the active nitrate reducing organisms also assimilated the sugars to a high degree.

It can be concluded therefore that the carbon assimilation patterns provides a useful means of distinguishing between the strains of the Streptomyces albus species-group. However, it should be noted that other species exhibited similar carbon assimilation patterns (Table 3)

Table 12. The production of nitrite from nitrate by Streptomyces albus.

+ = NO_2 produced; - = no NO_2 produced; tr = trace amounts.

Organism	NO_2 formed	
Ms	-	Sub-group I
76	+	
131	tr	
132	-	
190	+	
197	+	
224	tr	
228	+	
250	-	
259	tr	
260	tr	
133	+	Sub-group II
193	-	
216	+	
235	-	
242	+	
2	+	Sub-group III
7	+	
9	+	
13	+	
33	+	
34	+	
48	tr	
59	+	
103	+	
195	+	
196	+	

corresponding to each of the S. albus sub-groups, so that these criteria cannot be used to distinguish between species.

b). Organisms also investigated by other workers.

The second category of organisms consisted of 5 species which were represented by only one strain in this study, but which have also been studied by other workers, so that some comparisons have been made. These are set out in Table 13 which shows the carbon assimilation pattern of the 5 species as found by several authors. In each case the nitrogen source was an ammonium salt. Other conditions were not necessarily identical.

Three investigations in which a total of 29 strains of Streptomyces aureofaciens were studied, are summarised in Table 13. Unfortunately of the 21 carbon sources shown in the Table, only 8 are common to all three investigations. The growth on 5 of these substrates was reported to be strain variable. The three remaining compounds are sorbose, sucrose and maltose. Of these, sorbose is a poor carbon source for streptomycete growth (page 9 and Table 4). All the strains used sucrose, and all but Sa, the strain of S. aureofaciens used in this study, assimilated maltose.

A similar position exists with regard to Streptomyces griseus where 3 investigators have examined a total

of 13 strains. Here 12 carbon sources are common to all the investigations, of which 7 are stated to give variable growth. All the strains grew on xylose, fructose, salicin and mannitol. All but Sg, the strain of this species studied in this work, also grew on maltose.

Altogether 4 strains of Streptomyces coelicolor were the subject of 2 investigations. There are 14 common carbon sources of which only raffinose was reported to be strain variable. Of the remaining 13 carbon substrates arabinose, xylose, rhamnose, fructose, sorbose, lactose, salicin and inositol produced the same result in each investigation, and maltose, sucrose, erythritol, mannitol and sorbitol gave differing results.

Lastly, in the cases of Streptomyces albosporeus and S. violaceoniger, it was noticeable that neither species grew as well under the conditions used in this study as under those employed by Benedict et al. (1955).

It is clear that before the knowledge of carbon assimilation in chemically defined media can be of general use in the identification of the streptomycetes, there must be an agreed set of conditions and substrates. Furthermore, the conditions should be arranged so that the lack of growth is due only to the inability of the organisms to metabolise a certain carbon source and not to an unsuitable medium. It is, for instance, possible

that the failure of strain Sg of Streptomyces griseus and strain Sa of S. aureofaciens to grow on maltose, although other workers obtained growth of all their strains of these species on this substrate, may have been due to a lack of coenzymes caused by an inadequate medium. Equally, of course, it is necessary to use carefully defined media in order that the conditions may be exactly reproduced. In addition, a large number of strains must be studied in order that the variations in carbon source utilisation within the one species may be determined. It remains for future research to show whether the variations in the carbon assimilation pattern that exists between cultures of streptomycetes can be utilised to distinguish between species, or whether it can only be used to divide up a species-group as has been suggested above.

c). Remaining single strains.

Four of the organisms investigated were represented by a single strain, and so far as was found, no information on their nutrition has been published.

B. Indications of metabolic activity.

It is known that the streptomycetes possess some of the biochemical mechanisms of bacteria. Thus, it has been shown by various authors (Cochrane & Peck 1953; Cochrane, Peck & Harrison 1953; Gilmour, Butterworth &

Table 13. Comparison of the carbon assimilation of several streptomyces spp. as found by different authors.

\+ = growth; - = no growth; v = variable growth; nd = not done.
NH_4 was the nitrogen source in each case.

ORGANISM	Author	No. of strains	Arabinose	Xylose	Rhamnose	Fructose	Galactose	Glucose	Mannose	Sorbose	Lactose	Maltose	Sucrose	Raffinose	Salicin	Glycerol	Erythritol	Dulcitol	Mannitol	Sorbitol	Inositol	Acetate	Citrate
S. coelicolor	Benedict et al.(1955)	3	+	+	+	+	nd	nd	nd	-	+	+	-	v	+	nd	-	nd	+	-	+	nd	nd
S. coelicolor	204	1	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	-	-	+	+	+	+
S. aureofaciens	Benedict et al.(1955)	4	+	+	-	+	nd	nd	nd	-	v	+	+	-	-	nd	-	nd	-	-	-	nd	nd
S. aureofaciens	Backus et al.(1954)	24	v	v	nd	v	+	+	+	-	v	+	+	nd	nd	+	nd	nd	v	nd	nd	-	v
S. aureofaciens	Sa	1	+	+	-	+	+	+	+	-	-	-	+	-	-	+	-	-	-	+	-	-	+
S. griseus	Benedict et al.(1955)	8	v	+	v	+	nd	nd	nd	-	v	+	v	v	+	nd	v	nd	+	v	v	nd	nd
S. griseus	Pridham & Gottlieb (1948)	4	-	+	-	+	+	+	+	nd	+	+	-	-	+	+	nd	-	+	-	-	+	+
S. griseus	Sg.	1	+	+	-	+	+	+	+	-	-	-	+	-	+	+	-	-	+	+	-	+	+
S. albosporeus	Benedict et al.(1955)	1	+	+	nd	+	nd	nd	nd	nd	+	+	-	nd	+	nd	nd	nd	+	+	-	nd	nd
S. albosporeus	217	1	-	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+
S. violaceoniger	Benedict et al.(1955)	4	+	+	v	+	nd	nd	nd	-	+	+	v	v	+	nd	v	nd	+	v	v	nd	nd
S. violaceoniger	266	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

& Wang 1955; Butterworth, Gilmour & Wang 1955; Cochrane 1955). that Streptomyces coelicolor and S. griseus carry out the reactions of the glycolysis cycle, the hexosemonophosphate shunt and possess many of the enzymes of the tricarboxylic acid cycle. The common intermediate of hexose breakdown by these systems is 3-phosphoglyceraldehyde. If, in addition, it is assumed that streptomycetes also follow the bacterial route for the breakdown of pentoses and glycerol (Lamanna & Mallette 1953) then 3-phosphoglyceraldehyde would be the common intermediate of pentose and glycerol metabolism as well as that of the hexoses. The 3-phosophoglyceraldehyde would then be metabolised to pyruvate by the latter part of the glycolysis cycle. Now if it is further assumed that S. albus possess similar mechanisms, it is reasonable to suppose that, since the organisms of S. albus sub-group I all grew on pyruvate but not on the hexoses, pentoses and glycerol, that in these cases there is a break in the metabolic path between 3-phosophoglyceraldehyde and pyruvate. In this connection, it is interesting to note that Cochrane (1955) suggested that the strict aerobiosis of S. coelicolor was due to incomplete glycolysis brought about by a failure to reoxidise reduced Co I. However, the fault in the case of S. albus sub-group I is unlikely to be a lack of oxidised Co I since the conditions were

strongly aerobic. Again the failure of some of the sub-group I organisms to grow on hexoses, pentoses and glycerol was not complete. Thus Nos. 259 and 260 produced slight growth on xylose and galactose. This suggests that the blockage may only be a partial one, and is due to a limiting rate reaction. Clearly further investigations into carbohydrate metabolism of S. albus would be interesting and rewarding.

Only 38% of the test organisms utilised acetate. It is interesting to note that Cochrane (1952) found that acetate was toxic to Streptomyces coelicolor at concentrations greater than 0.01 M. No. 204, the S. coelicolor strain examined in this study, gave rise to slight growth on acetate at 1% concentration that is 0.166 M. It would be useful to investigate further to see if the comparatively poor growth on this substance was due to toxicity or to the failure of the substrate to penetrate the cell wall.

The inability of 6 organisms to utilise lactate although they used pyruvate indicates that lactic dehydrogenase was probably absent. Nicotinic acid, the precursor of Co I, the coenzyme involved, was present in the medium (page 39).

The close similarity of the chemical structure of

malonate and oxalate may account for the fact that many organisms which assimilated one assimilated the other also.

A number of organisms grew on salicin, the possible mechanism involved being the hydrolysis of salicin to glucose and saligenin, since all the organisms which used salicin also metabolised glucose. The fate of the saligenin is unknown.

4. Nitrogen sources.

A. Inorganic sources.

Nitrite is known to be toxic to many bacteria. At high concentrations it was toxic to the streptomycetes examined. At lower concentrations the toxicity was overcome and the organisms used nitrite as a sole source of nitrogen. When pyruvate was the sole source of carbon, 7 of the 10 organisms examined grew (Table 5). In contrast, nitrite inhibited the metabolism of pyruvate by Fusarium species (Wirth & Nord 1945) and by Vibrio comma (Bernheim 1943). When both pyruvate and glucose were present all the organisms tested grew (Table 5). Glucose may have enhanced the growth because it acted as an hydrogen donator for the reduction of nitrite to ammonia. This hypothesis is supported by the work of Kluyver (1953) who showed that glucose played such a role in the reduction of nitrate to ammonia by Pseudomonas aeruginosa.

The observation that nitrite is more toxic in acid than in alkaline solution (Tarr 1941; Tarr 1941a; Bernheim 1943; Castellani & Niven 1955) may account for the ability of Streptomyces to use nitrite more readily when pyruvate is present in the medium because the utilisation of the pyruvate results in the liberation of sodium ions which tend to keep the pH value high.

Ammonia was not detected as a breakdown product, but this may have been because it was absorbed as rapidly as it was formed. Some ammonia appeared in the flasks containing glucose after 10 days although the streptomycetes had failed to grow (Table 5). The amount of ammonia was not measured, but it was probably very small as indicated by a weak reaction to Nessler's reagent, the sensitivity of which was greater than 0.0002 M. ammonium sulphate. The organisms may have contributed to the ammonia by autolysis.

Several workers (Sacks & Barker 1949; Kluyver 1953) claim that the process of "true dissimilatory nitrate reduction" is by way of nitrite to ammonia and that aeration inhibits the reduction. In this study, it was found that all but 4 of the streptomycetes which grew on nitrate grew also on nitrite and ammonia. Aeration did not inhibit the reduction of nitrate by these organisms since nitrite was found in the nitrate-pyruvate medium in every case after 10 days incubation. It is reasonable to suppose that the path of nitrate metabolism for these organisms is by way of nitrite to ammonia, although since aeration was not inhibitory, probably not by the "true dissimilatory nitrate reduction" process. The 4 exceptions, Nos. 193, 197, 242 and 250, grew on nitrate and ammonia, but not nitrite. They also produced nitrite in the nitrate-pyruvate medium suggesting that the same mechanism was present as in the

majority of the organisms except that for utilising nitrite. But since nitrate supported growth, part of the nitrate must have been assimilated by another path. Whether this alternative path played any part in the nitrate metabolism of the majority of the organism can only be discovered by future research.

It would be interesting to know whether hydroxylamine or nitramide could have been assimilated by the test organisms since both these substances are stated to be involved in several schemes of nitrate and nitrite metabolism (Silver & McElroy 1950; Hofman & Lees 1952; Kluyver 1953; Campbell 1954).

No correlation appears to exist between reduction in peptone-nitrate broth and the ability to use nitrate or nitrite as sole nitrogen source as shown by the nitrogen assimilation experiments. In nitrate-peptone broth the peptone formed a readily available source of nitrogen, so that the production of nitrite was probably an "incidental nitrate dissimilation" rather than a "true dissimilatory nitrate reduction" (Kluyver 1953).

Okami (1952) using solid media and glucose as carbon source found that nitrate supported the growth of more organisms than ammonia. The results presented here show the opposite, probably because different conditions and organisms were employed.

B. Organic sources.

a) Amino acids.

All the amino acids tested were excellent nitrogen sources except tryptophane, methionine, valine, cystine and tyrosine, a finding in agreement with those of Okami (1952) and Burkholder et al. (1954).

Woodruff & Foster (1943) showed that Actinomyces (Streptomyces) lavendulae was able to deaminate many amino acids with the formation of ammonia. Moreover, Gottlieb & Ciferri (1956) found that S. venezuelae deaminated the amino acids which supported growth, but failed to deaminate those which did not provide nitrogen for growth. The amino acids which did not support growth and were not deaminated were tyrosine, leucine, nor-leucine, cysteine and tryptophane. S. lavendulae also failed to deaminate the same group of amino acids with the addition of phenyl alanine. The demonstration of deaminases in the streptomycetes investigated in this work would be of value because if ammonia was formed from all the amino acids which were successful in supporting growth, then since pyruvate supported the growth of all the organisms with ammonium sulphate, the results would be explained. The failure of Nos. 266 and 267 to grow on amino acids would be explained by lack of deaminases.

Other metabolic systems for amino acid metabolism have also been demonstrated in Streptomyces. Romano & Nickerson (1958) found that glutamic acid dehyrogenase was present in S. fradiae and they also found evidence of transamination. They were unable to show an enzyme system in this species by which aspartic acid could be directly deaminated when supplied as sole source of carbon and nitrogen.

The metabolism of tryptophane was investigated by Kawamata, Koyama & Kunita (1956) who found that 36% of streptomycetes grew on tryptophane; their medium contained substantial quantities of yeast extract. They concluded that both resting and proliferating cells broke down tryptophane to kynurenine and anthranilic acid.

It is well known that certain amino acids, particularly the D-isomers, inhibit or modify the growth of bacteria (Gorden & McLeod 1926; Fox, Fling & Bollenback 1944; Fling & Fox 1945; Kohayahi, Fling & Fox 1948; Hodgson, Peterson & Riker 1951). It should be noted that many of the amino acids used in the nutritional experiments were racemic mixtures, and it is at least possible that the nutrition was modified by the presence of the D-isomers.

Castellani & Niven (1955) demonstrated an anaerobic chemical reaction between cystine-cysteine and pyruvate with the removal of the amino acid, and Schubert (1935; 1936;

1939) has shown that the thiol acids react with sugars and sugar breakdown products while a secondary oxidation of cysteine in the presence of pyruvate has been described by Cavallini (1951). Such reactions as these may have interfered with the growth of the organisms on pyruvate-cystine medium.

Okami (1952) claimed that "the utilisation of nitrogen compounds is one support of the classification". A critical examination of his results shows that the support is a frail one on the grounds that the nitrogen assimilation patterns of his species are all very similar, and any differences were largely quantitative rather than qualitative. Furthermore, the variations between the strains of one species was as great as the variation between separate species. In fact the results are very similar to those obtained in this study. An examination of the results given in Table 6 shows that there is no correlation between the carbon assimilation groupings and the nitrogen assimilation of Streptomyces albus nor are any significant differences observed between species.

b). Urea.

Urea was well used. In view of the ability of the streptomycetes examined to use pyruvate-ammonium sulphate medium, the most likely explanation is that urease was present and broke the urea down to ammonia and carbon dioxide.

c). Purines.

The breakdown of purines by streptomycetes raises an interesting biochemical problem. An aerobic attack of uric acid by Pseudomonas species is known (Franke & Hahn 1955) of which the end products were urea and glyoxylic acid, the latter being further oxidised to oxalic acid. If this mechanism was present in the streptomycetes under investigation, presumably the urea provided the nitrogen for growth. Some support for this possibility is provided by the fact that all the organisms which utilised purines utilised urea as well. However, many of the organisms which assimilated the purines did not metabolise oxalic acid.

5). Acid production from carbohydrates.

As already stated (page 57) the failure to obtain reproducible results in acid production was probably due to the difficulty of controlling both the organisms and the conditions. Not the least part of the difficulty is to know which of the variables are important in acid production and should be controlled. It would, for instance, be interesting to know if the greater frequency of acid production which resulted from aeration was due to aeration itself or the enhanced growth that aeration produced. Similarly, the presence of organic nitrogen may have given rise to acid more frequently because it enhanced growth rather than favoured acid production as such. Again, Woodruff & Foster (1943) found that for Actinomyces (Streptomyces) lavendulae the pH was transiently lowered and quickly raised again, but could not tell from their experiments whether this was due to the assimilation of the acid or to the production of extra ammonia. They also obtained some evidence that the carbon-nitrogen ratio was of great importance.

In order to solve the problem of acid production by streptomycetes, it would be necessary to embark on a research programme of some size. Possibly a good series of experiments with which to start would be to follow the fate of several carbohydrates under varying conditions using

different nitrogen sources at varying concentrations along the lines already pioneered by Cochrane & Dimmick (1949) and Cochrane (1952).

SUMMARY and CONCLUSIONS

SUMMARY AND CONCLUSIONS.

The aims of this study are set out on page i. From the data given by the experiments described above, the extent to which each purpose has been fulfilled can be estimated, and some conclusions drawn:-

1). Carbon, nitrogen and growth factor requirements of streptomycetes.

Within the limits of the number of organisms and substrates which were examined, the first object, that of determining the carbon, nitrogen and growth factor requirements of streptomycetes has been fairly well realised. The following conclusions were drawn:-

a). The streptomycetes examined utilised a wide range of common substances as sole carbon source. Simple sugars and some organic acid radicals were the best utilised substances. Alcohols, with the exception of glycerol and mannitol, were not well assimilated, nor were the simple fatty acids with the exception of acetate.

b). Ammonium and nitrate were good nitrogen sources for the streptomycetes examined. Nitrite supported the growth of many strains providing that the optimum concentration was used, together with a carbon source which was available under these conditions.

Amino acids were excellent nitrogen sources for the growth of these streptomycetes. Cystine, methionine and

valine were less readily assimilated than most amino acids, while tryptophane was available to a few species only.

Urea, uric acid and xanthine were excellent nitrogen sources for the growth of these organisms.

c). The 10 streptomycetes examined did not require any of the added growth factors when grown in a synthetic medium.

2). Relation of nutrition to taxonomy.

The second purpose of this study was to determine if the nutritional pattern could aid in the classification of the Streptomyces as has been suggested by other workers (page 27).

From the evidence presented here it is concluded that:-

a). The Streptomyces albus species-group is divided into three nutritional sub-groups, a finding which may well be of taxonomic value.

b). Before the knowledge of the nutritional pattern in chemically defined media can be of general use in the identification of the streptomycetes, there must be an agreed set of conditions and substrates. In addition a large number of strains must be studied in order that the nutritional variation within any one species may be determined.

3). Correlation between nutrition, cultural characteristics and biochemical reactions.

The third aim of this study, which was to discover any correlation between the nutritional pattern, the cultural characteristics and the biochemical reactions, was the least realised. No such correlation was found to exist with the possible exception of a relationship between the nutrition of Streptomyces albus and its breakdown of nitrate. Several of the biochemical properties such as proteolytic ability, breakdown of starch and the reduction of nitrate were common to several species or varied within the one species-group, while others, notably the production of acid from carbohydrates gave equivocal results.

APPENDIX I

Detailed description of the organisms.

Detailed description of the organisms.

The methods used to perform the tests are set out on pages 29-32.

The primary mycelium of each of the organisms was Gram positive.

All were non-acid fast, except No. 245 which was partially acid fast, particularly in young culture.

All the organisms were strongly aerobic, and grew only on the surface of an agar shake.

A strong amylase reaction was given by all the organisms, except Nos. 245, 266 and 267 which showed only weak activity.

No organism produced indol from peptone water.

Acetylmethylcarbinol was not produced in glucose phosphate peptone water by any organism.

Twenty of the streptomycetes produced no antibiotics against the bacteria used. The reaction of the remainder is given in Table 14.

All cultures were incubated at 25° unless otherwise stated.

In the following descriptions, the term "colourless" is used as was suggested by Waksman (1957) to indicate that the mycelium takes on the same colour as the uninoculated medium.

A detailed description of each organism is given below.

Table 14. Antibiotic activity of the organisms.

The remaining 20 streptomycetes did not inhibit growth of the bacteria.

+++ = very good inhibition; ++ = good inhibition; + = slight inhibition; - = no inhibition.

Organism	K. pneumoniae	Ps. fluorescens	E. coli	B. subtilis	B. cereus	M. citreus
Sa	+++	+++	+++	+++	+++	+++
59	-	-	-	+	+	+
76	-	-	-	++	++	-
103	-	-	-	++	+	+
131	-	-	-	+	+	-
132	-	-	-	+	+	-
133	-	-	-	++	++	-
193	-	-	-	++	-	-
195	-	-	-	+	-	+
196	-	-	-	+	+	+
197	-	-	-	+	-	+
214	-	+	-	+++	+++	+++
216	-	-	-	++	++	-
217	-	-	-	++	+	-
259	-	-	+	++	+	+
260	-	-	-	+	-	-

Organism Sg.

Origin. N.C.T.C. No. 6961 (R.C.S.T. 122).

Morphology. Primary mycelium. Very tough, branching filaments. 0.5-1.0 μ. Gram +ve. Non-acid fast.

Secondary mycelium. Fertile hyphae were rarely observed, but occasionally long, straight chains of spores were seen. 0.5-0.6 x 0.7-1.0 μ.

Colonies on nutrient agar. Colourless, opaque, flat colonies, well embedded into the agar (6 days). The entire edge (2 days) became very finely filamentous (6 days), and finally formed a circle of very fine filaments round the colony giving a "halo" effect (14 days). The surface became folded (2 days), then much folded and segmented (10 days). No aerial mycelium developed. A small amount of brown pigment diffused into the medium on prolonged incubation (21 days). No odour.

Streak on nutrient agar. Good, colourless growth (2 days). Surface folded (7 days). The reverse was flat and colourless (10 days). No aerial mycelium was observed. A small amount of brown pigment diffused into the medium (10 days).

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Very slight or no growth (21 days).

Czapek Dox sucrose agar. Very sparse growth (21 days).

Potato plug. Abundant, heaped up, wrinkled, light brown growth. No aerial spores. The plug became light brown in colour (21 days).

Nutrient broth. Growth as a flaky deposit (2 days). Occasional ring formation. Some light brown pigment formed (16 days).

Organism Sg. continued.

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Grew well at 16°, 25°, 30° and 37°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Poor to moderate growth. No proteolysis.

Gelatine. Liquefaction in 10 days.

Serum. Good growth. Deep pitting of the medium (7 days), and liquefaction (10 days).

B.C.P. milk. Alkaline peptonisation beginning at the surface (4 days). A clot formed (10 days) which slowly disappeared (25 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 10 days.

Antibiotics. No streptomycin was detected by Boxer's method (Boxer, Jelinek and Leghorn 1947) in a 7 day culture on glucose broth, nor was any antibiotic action shown by the cross streak method.

Identification. Streptomyces griseus has been described by several authors (Krainsky 1914; Waksman & Curtis 1916; Waksman 1919c; Bergey 1948; Reynolds & Waksman 1948; Waksman, Reilly & Harris 1948; Waksman & Lechevalier 1949; Krassilnikov 1949; 1950). There is some difference of opinion as to the characteristics of this species (Waksman 1957). The failure of Sg. to form spores, whose colour and arrangement were important features of the descriptions of Waksman & Curtis (1916), Krassilnikov (1949; 1950) and Bergey's manual (1948) made comparisons

difficult. The primary mycelium of Sg. was colourless as described by the Russian workers, and not cream coloured as described by the Americans. Sg. further resembled Krainsky's organism in its reduction of nitrate and its action on gelatine and on milk. It appeared advisable then to regard Sg. as a strain of Streptomyces griseus.

Organism Sa.

Origin. N.C.T.C. No. 8128.

Morphology. Primary mycelium. Long, branching filaments. No measurements were made. Gram +ve. Non-acid fast.

Secondary mycelium. The arrangement and size of the spores was not investigated.

Colonies on nutrient agar. Colourless, opaque, flat colonies, well embedded into the agar (4 days). Entire edge (14 days). Slight central depression and segmentation (14 days). No aerial mycelium (14 days). No pigment in the medium (14 days). No odour.

Streak on nutrient agar. Moderate, slow growing, colourless growth. No aerial mycelium, pigment or odour (21 days).

Streak on glucose agar. Good growth, quicker than on nutrient agar, yellow or very light brown, well embedded into the agar and slightly raised (4 days). A few white spores appeared (14 days). A golden brown pigment diffused into the agar (14 days).

Czapek Dox glucose agar. Grew more vigorously than on Czapek Dox sucrose agar (4 days). Colourless (4 days) turned slightly brown (7 days).

Czapek Dox sucrose agar. Good, colourless, sporeless, flat growth (4 days).

Potato plug. Good yellow growth with abundant white spores. The plug became yellow (4 days) and then brown (14 days), and eventually grey or black (25 days). The spores also turned grey (20 days).

Nutrient broth. Moderate growth as small flakes (4 days).

Organism Sa. continued.

Glucose broth. As for nutrient broth, except that growth was more abundant.

Growth temperatures. Not examined.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Good growth (4 days). The medium was pitted, but not liquefied (21 days).

Gelatine. Slight liquefaction in 14 days.

Serum. Slow growth. Some clearing of the medium around each colony, but no liquefaction.

B.C.P. milk. The milk was peptonised and a clot formed. There was little change of pH (7 days). The clot disappeared (14 days), and the final pH was slightly acid (21 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was not reduced to nitrite.

Antibiotics. An antibiotic was formed which was active against all the test bacteria (Table 14).

Identification. This strain was lost before its description was completed or repeated. However, it closely resembled Streptomyces aureofaciens as described by Duggar (1948; 1949b) and Backus, et al. (1954), and it was assumed to be of that species. Waksman & Lechevalier (1949) placed this species in the S. flavus species-group.

Origin. N.C.T.C. No. 7807.

Morphology. Primary mycelium. Long, branching filaments. 0.4-0.9μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight chains. 0.5-0.8 x 0.8-1.1 μ.

Colonies on nutrient agar. Colourless, opaque, flat, well embedded colonies (3 days). Edge entire (2 days), became finely filamentous (4 days). The surface was smooth (2 days), became folded and contoured (12 days). White spores (6 days), turned grey (10 days). No pigments or odour. (12 days).

Streak on nutrient agar. Very good, colourless growth (4 days). Surface was folded (6 days). The reverse was colourless, yellow or light brown and sometimes raised. White aerial mycelium (12 days). No pigment.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Growth was poor and slow. No spores were formed (27 days).

Czapek Dox sucrose agar. Moderate, slow, colourless growth (12 days). White aerial mycelium which turned grey or brown (19 days).

Potato plug. Good, bright yellow growth. Abundant, white aerial mycelium (3 days) turned brown (12 days). The plug turned slowly black (21 days).

Nutrient broth. Good growth as a flaky deposit (3 days), or surface growth (12 days). White aerial mycelium on the surface growth.

Glucose broth. As for nutrient broth, except that growth was more abundant.

Organism Ms. continued.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16°. Good growth at 30°. Very good growth at 37°. Optimum temperature 37°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Abundant growth (3 days). A small amount of pigment diffused into the medium turning it grey, or black in some cultures. No proteolysis (25 days).

Gelatine. Liquefaction variable and slow.

Serum. Abundant growth (3 days). The medium became clear, pitted (6 days) and was usually liquefied (10 days).

B.C.P. milk. Peptonisation (3 days). The pH slowly turned very alkaline (23 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was not reduced to nitrite.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 2.

Origin. Botanic gardens, Glasgow. Soil No. 1.

Morphology. Primary mycelium. Long, branching filaments. 0.5-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight chains. 0.5-0.8 x 0.5-0.8 μ.

Colonies on nutrient agar. Colourless, translucent colonies (2 days), became opaque (6 days). Flat, well embedded into the medium (2 days). The edge was entire (2 days), became very finely filamentous (9 days). The colonies were smooth, but became segmented (9 days). Plentiful, white aerial mycelium when the culture was first isolated but it became asporogenous on repeated subculture. No soluble pigment formed. Earthy odour.

Streak on nutrient agar. Good, colourless, flat growth (2 days). The surface was smooth or slightly folded (9 days). The reverse was colourless and very slightly raised (9 days). White aerial mycelium formed (4 days). There was no soluble pigment.

Streak on glucose agar. As for nutrient broth except that the reverse was brown (6 days), and the medium became slightly greenish brown (9 days).

Czapek Dox glucose agar. Good, flat, colourless growth (6 days).

Czapek Dox sucrose agar. Poor or moderate, flat, colourless growth (21 days).

Potato plug. Good, brown growth with white aerial mycelium (5 days) which was lost after repeated subculture. No pigment diffused into the plug (21 days).

Organism No. 2 continued.

Nutrient broth. Good growth as flakes (3 days).

Glucose broth. Very good growth as flakes (3 days), with occasional pellicle formation.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16° and 37°. Good growth at 25° and 30°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (3 days).

Dorset's egg medium. Moderate, slow growth. Very slow proteolysis (29 days).

Gelatine. Liquefaction in about 21 days.

Serum. Good, light brown growth. The medium was pitted (5 days) and then liquefied (9 days). A light brown pigment diffused into the medium (9 days).

B.C.P. milk. Alkaline peptonisation with the formation of a clot (8 days), which disappeared (29 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 10 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954) and was assumed to be of that species.

Organism No. 7.

Origin. Botanic gardens, Glasgow. Soil No. 1.

Morphology. Primary mycelium. Branching filaments which were shorter than is usual among the streptomycetes. 0.5-1.1μ. Gram +ve. Non-acid fast.

Secondary mycelium. This organism produced mostly short, thick, infertile hyphae. A few, fertile hyphae formed conidia in long, straight chains, or in open anticlockwise spirals. 0.6-1.1 x 0.6-1.5μ.

Colonies on nutrient agar. Brown, opaque, raised colonies which were friable, probably due to the shortness of the filaments (2 days). Entire edge (2 days) became very finely filamentous (5 days). The colonies were often contoured. There was marked segmentation usually beginning with a central depression near the crown. No spores. A deep brown, soluble pigment was present when the organism was first isolated, but this was lost on continued subculture. No odours.

Streak on nutrient agar. Good, brown, flat, shiny growth with a wrinkled surface (5 days). Reverse brown and flat (5 days). No spores. Brown, soluble pigment diffused into the agar.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. No growth.

Czapek Dox sucrose agar. Moderate, flat, slow growth (12 days). Occasional white spores were formed.

Potato plug. Abundant, yellow growth (2 days), turned brown (13 days). Surface was convoluted forming a spongy texture (13 days). No pigment diffused into the plug (21 days).

Organism No. 7 continued.

Nutrient broth. Growth as a powdery deposit (3 days), became turbid (5 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 37°, 45° and 55°. Moderate growth at 16°. Good growth at 25° and 30°. Optimum growth 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch quickly hydrolysed (3 days).

Dorset's egg medium. Moderate, convoluted growth (5 days). No proteolysis (24 days).

Gelatine. Liquefaction in about 1 month.

Serum. Slow, moderate growth (13 days). No proteolysis (21 days).

B.C.P. milk. Alkaline peptonisation (3 days), but no clot was formed (21 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 3 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism was unusual in that the primary mycelium filaments were short compared with most streptomycetes, and in this respect it resembled a Nocardia species. However, in other respects it resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 9.

Origin. Botanic gardens, Glasgow. Soil No. 1.

Morphology. Primary mycelium. Long, branching filaments. 0.5-1.0μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed straight, branching chains, with a few, open, anticlockwise spirals. 0.6-1.0 x 0.9-1.8μ.

Colonies on nutrient agar. Colourless, opaque, flat or slightly raised, well embedded colonies (5 days). Entire edge (2 days), became very finely filamentous (5 days). A central depression was formed in many colonies which then showed segmentation (12 days). White aerial mycelium was plentiful. A deep brown, soluble pigment was formed in the freshly isolated organism, but this was lost on continued subculture. Marked earthy odour.

Streak on nutrient agar. Good, flat, colourless growth. Folded surface. The reverse was colourless, and sometimes raised (10 days). Plentiful white aerial spores formed. A deep brown pigment which was present in the freshly isolated culture disappeared on long laboratory cultivation.

Streak on glucose agar. As for nutrient agar, except that the reverse was brown.

Czapek Dox glucose agar. Growth very poor (21 days).

Czapek Dox sucrose agar. Growth poor (21 days).

Potato plug. Grew very poorly or not at all.

Nutrient broth. Growth as small flakes. Occasional ring formation.

Organism No. 9 continued.

Soluble brown pigment which disappeared on continuous sub-culture.

Glucose broth. As for nutrient broth, except that the brown pigment was still formed in this medium when it was no longer formed in nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16° and 37°. Very good growth at 25° and 30°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Good growth. The primary mycelium was colourless (5 days), then turned grey (12 days) and eventually black (21 days). The spores were white (5 days) turning grey or light brown (12 days). The medium also became grey (12 days) and then black (21 days), but there was no proteolysis.

Gelatine. Liquefaction slow (14-32 days).

Serum. Good growth. The medium was deeply pitted, and liquefied (12 days).

B.C.P. milk. Alkaline peptonisation with the formation of a clot (5 days) which disappeared (12 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylemthylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 4 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 13.

Origin. Botanic gardens, Glasgow. Soil No. 1.

Morphology. Primary mycelium. Branching filaments which were shorter than is usual among the streptomycetes, although long, more typical filaments were occasionally seen. 0.5-1.1 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, open, anti-clockwise spirals. 0.7-1.0 x 0.9-1.3 μ.

Colonies on nutrient agar. Brown, opaque, raised, friable colonies. Entire edge (2 days), became finely filamentous (5 days). No spores were formed. This organism produced a deep brown, soluble pigment when first isolated, but this was lost after prolonged subculture. No odours.

Streak on nutrient agar. Good, brown growth with folded surface (5 days). Reverse flat and brown. No aerial spores. A soluble, dark brown pigment was present in the freshly isolated culture, but was lost after long laboratory cultivation.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Not examined.

Czapek Dox sucrose agar. Not examined.

Potato plug. Not examined.

Nutrient broth. Growth as small flakes (5 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16°, 25°, 30° and 37°. Optimum temperature 25°-30°.

Organism No. 13 continued.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Good growth but no proteolysis (21 days).

Gelatine. No liquefaction in 30 days.

Serum. Light brown growth, but no proteolysis (21 days).

B.C.P. milk. Alkaline peptonisation (5 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 5 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. The loss of this strain early in the work prevented these tests from being completed and repeated. This organism was, however, very like No. 7, and like it, was included in the Streptomyces albus species.

Organism No. 33.

Origin. Botanic gardens, Glasgow. Soil No. 1.

Morphology. Primary mycelium. Branching filaments which were shorter than is usual among the streptomycetes, though longer branches were occasionally seen. 0.5-1.0 μ. Gram +ve. Non-acid fast.

Secondary mycelium. Most of the aerial hyphae were sterile, but some conidia formed long, straight chains or open, anti-clockwise spirals. 0.6-0.9 x 0.6-1.1 μ.

Colonies on nutrient agar. Brown, opaque, raised, friable colonies. Entire edge (2 days), became very finely filamentous (5 days). Spores were very rarely observed, but when seen, they were white. Dark brown pigment diffused into the medium when the organism was first isolated, but this was lost on repeated subculture. No odours.

Streak on nutrient agar. Good, brown growth, with a folded surface (5 days). The reverse was not raised. Spores were not observed. A dark brown pigment which was present when the organism was first isolated was lost on prolonged laboratory cultivation.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Not examined.

Czapek Dox sucrose agar. Good, colourless growth (5 days). Aerial mycelium was once produced on this medium.

Potato plug. Not examined.

Nutrient broth. Growth as flaky deposit (2 days), became turbid (5 days).

Glucose broth. As for nutrient broth.

Organism No. 33 continued.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16° and 37°. Good growth at 25° and 30°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Good growth, but no proteolysis (21 days).

Gelatine. Liquefaction in about 21 days.

Serum. Moderate growth (5 days). The medium was pitted but not liquefied.

B.C.P. milk. Alkaline peptonisation (5 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 5 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. The loss of this organism early in the work prevented these tests from being completed and repeated. This organism was, however, very like Nos. 7 and 13, and was included with them in the Streptomyces albus species.

Organism No. 34.

Origin. Botanic gardens, Glasgow. Soil No. 1.

Morphology. Primary mycelium. Much branched, tough filaments 0.5-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight chains. 0.6-1.0 x 0.7-1.2 μ.

Colonies on nutrient agar. Colourless, opaque, flat or slightly raised, well embedded colonies. The edge was finely filamentous (4 days). Surface was folded with central depression, followed by segmentation (11 days). Abundant white spores. No pigments were formed. Strong, earthy odour.

Streak on nutrient agar. Good, colourless growth (4 days). Folded surface (4 days). The reverse was sometimes raised, and was colourless. White aerial mycelium was present. No pigments.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. No growth.

Czapek Dox sucrose agar. Moderate to good growth, with white spores (15 days).

Potato plug. Abundant, light brown growth, covered with white spores (4 days). The surface became much folded, and the reverse turned greenish-brown (15 days). The plug slowly turned grey.

Nutrient broth. Growth as a flaky deposit, as a ring or pellicle (4 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 37°, 45° or 55°. Good growth

Organism No. 34 continued.

at 16°, 25° and 30°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch quickly hydrolysed (4 days).

Dorset's egg medium. Moderate growth, but no proteolysis (21 days).

Gelatine. Liquefaction in about 14 days.

Serum. Good growth. The medium became clear and pitted (4 days), and then liquefied (10 days).

B.C.P. milk. Peptonisation without much pH change (6 days). The final pH was sometimes acid (26 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 7 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 48.

Origin. Botanic gardens, Glasgow. Soil No. 2.

Morphology. Primary mycelium. Tough, much branched filaments. 0.5-1.0 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed tight, clockwise spirals. 0.5-1.0 x 0.7-1.2 μ.

Colonies on nutrient agar. Colourless, opaque, raised, well embedded colonies. Entire edge (2 days), became very finely filamentous (4 days). Segmentation was frequently observed. Sparse, white sporulation (7 days). No pigment or earthy odour.

Streak on nutrient agar. Good, colourless, raised growth. Surface folded. The reverse was colourless and often raised. Sparse, white aerial mycelium (5 days). No pigment.

Streak on glucose agar. As for nutrient agar, except that the reverse was brown, and a small amount of pigment diffused into the medium particularly when the strain was freshly isolated.

Czapek Dox glucose agar. Slow, colourless growth (21 days).

Czapek Dox sucrose agar. Good growth, brown in the centre with a colourless periphery. The reverse was raised and dark brown (4 days). The white spores (4 days) turned grey (7 days), and eventually black (4 months).

Potato plug. Moderate, yellow shiny growth (4 days). The plug was not discoloured (21 days).

Nutrient broth. Deposit of flaky colonies (4 days).

Glucose broth. As for nutrient broth.

Organism No. 48 continued.

Growth temperatures. No growth at 1°, 45° or 55°. Poor growth at 37°. Moderate growth at 16° and 25°. Good growth at 30°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch quickly hydrolysed (4 days).

Dorset's egg medium. Yellow, convoluted growth (4 days), turned brown (7 days) and then dark brown (14 days). No pigment in the medium (14 days). No proteolysis (21 days).

Gelatine. Liquefaction in 21-30 days.

Serum. Moderate, colourless or light brown growth (4 days). The medium was pitted, but there was no liquefaction (21 days).

B.C.P. milk. Strongly alkaline peptonisation with a clot (7 days). The clot was later digested (21 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrite was not produced from nitrate, or in only trace amounts.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 59.

Origin. Leaf mould, Dawsholm Park, Glasgow. Soil No. 3.

Morphology. Primary mycelium. Tough, much branched filaments. 0.5-1.0 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, branching chains. 0.5-1.0 x 0.8-1.2 μ.

Colonies on nutrient agar. Colourless, opaque, flat or raised, well embedded colonies. Entire edge, became very finely filamentous (3 days). Segmentation developed (5 days). White spores formed (5 days) No soluble pigment or odour.

Streak on nutrient agar. Colourless, folded growth. Surface convoluted. Reverse raised and colourless. Plentiful white aerial mycelium (5 days). No pigments (21 days).

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Good, flat, colourless growth (5 days).

Czapek Dox sucrose agar. Moderate or poor growth (21 days). Some patches of spores (5 days) which turned brown (10 days).

Potato plug. Grew slowly or not at all. The brownish-white mycelium became white with spores. No pigment diffused into the medium (21 days).

Nutrient broth. Typical flaky deposit (3 days). Surface growth (5 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 37°, 45° or 55°. Moderate growth at 16°. Good growth at 25° and 30°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Organism No. 59 continued.

Starch hydrolysis. Starch quickly hydrolysed (4 days).

Dorset's egg medium. Abundant, colourless growth which attacked the medium (17 days), and sometimes liquefied it (30 days).

Gelatine. Liquefaction in about 21 days.

Serum. Abundant growth and rapid liquefaction (5 days).

B.C.P. milk. Alkaline peptonisation with a clot (5 days), which slowly disappeared (21 days). After about 30 days incubation, the pH tended to become acid.

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 5 days.

Antibiotics. This organism formed an antibiotic which was weakly active against Bacillus subtilis, B. cereus, and Micrococcus citreus, but not against Klebsiella pneumoniae, Pseudomonas fluorescens or Escherichia coli (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954) and it was assumed to be of that species.

Organism No. 76.

Origin. Botanic gardens, Glasgow. Soil No. 2.

Morphology. Primary mycelium. Long, branching filaments. 0.5-1.0 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight chains. 0.5-1.0 x 0.8-1.3 μ.

Colonies on nutrient agar. Colourless, opaque, flat, well embedded colonies. Finely filamentous edge (3 days). Smooth surface (3 days). Sparse, white aerial mycelium (11 days). No soluble pigments. Earthy odour.

Streak on nutrient agar. Good, colourless, flat growth (4 days). Surface smooth or slightly folded (5 days). The reverse was raised in some cases, and light brown in colour (11 days), particularly when freshly isolated. White spores. No pigment.

Streak on glucose agar. As for nutrient agar, except that the growth was more profuse.

Czapek Dox glucose agar. Good, colourless, raised growth (4 days).

Czapek Dox sucrose agar. Moderate growth (4 days). White spores, turned brown.

Potato plug. Good but slow growth (18 days). The white spores turned purple-brown in about 30 days. The plug itself also became grey-brown.

Nutrient broth. Flaky deposit with occasional ring formation (5 days).

Glucose broth. As for nutrient broth.

Organism No. 76 continued.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16°, 25°, 30° and 37°. Optimum growth temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (5 days).

Dorset's egg medium. Abundant growth but no proteolysis (21 days).

Gelatine. Liquefaction in about 21 days.

Serum. Good, light brown growth (4 days). The medium was deeply pitted, but there was no liquefaction (21 days).

B.C.P. milk. Neutral or acid peptonisation (5 days) with the formation of a clot.

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 18 days.

Antibiotics. An antibiotic was produced which was active against Bacillus subtilis and B. cereus, but not against Micrococcus citreus, Klebsiella pneumoniae, Pseudomonas fluorescens or Escherichia coli (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 103.

Origin. Botanic gardens, Glasgow. Soil No. 2.

Morphology. Primary mycelium. Tough, well branched filaments. 0.5-0.8μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidida formed long, branching chains which were grouped in tufts. 0.5-1.0 x 0.5-1.1μ.

Colonies on nutrient agar. Colourless, translucent (2 days), became opaque (4 days), flat, well embedded colonies. Finely filamentous edge (2 days). Smooth surface (10 days). White aerial mycelium. No pigment in the medium. Earthy odour.

Streak on nutrient agar. Good, colourless growth. Surface slightly folded. Reverse flat and colourless (4 days). White aerial mycelium. No pigment.

Streak on glucose agar. Abundant, colourless growth (4 days). The surface was convoluted. The reverse was raised and brown (11 days). White spores turned brownish-white (11 days). No pigment.

Czapek Dox glucose agar. Abundant growth (4 days). White spores (4 days), turned grey (11 days). The reverse was raised, and was colourless (4 days), became dark green (11 days) and then brown or black (21 days). The medium also turned purple-brown (36 days).

Czapek Dox sucrose agar. Poor growth (18 days).

Potato plug. Abundant, much convoluted, brown growth (18 days). Plentiful white spores, turned greyish brown (18 days). The plug turned light brown (8 days) and then black (36 days).

Nutrient broth. Growth as flaky deposit, or as tough, convoluted

Organism No. 103 continued.

pellicle (4 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16°. Good growth at 25°, 30° and 37°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Good growth, but proteolysis was weak and variable.

Gelatine. Liquefaction in about 21 days.

Serum. Good growth. The medium was deeply pitted (7 days), became clear and was liquefied (10 days).

B.C.P. milk. Alkaline peptonisation (10 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 10 days.

Antibiotics. This organism produced an antibiotic which was active against Bacillus subtilis, slightly active against B. cereus and Micrococcus citreus, but inactive against Klebsiella pneumoniae, Pseudomonas fluorescens and Escherichia coli (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954) and it was assumed to be of that species.

Organism No. 131.

Origin. Leaf mould, Dawsholm Park, Glasgow. Soil No. 3.

Morphology. Primary mycelium. Much branched, long filaments. 0.4-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight, branching chains 0.5-0.9 x 0.6-1.3μ.

Colonies on nutrient agar. Colourless, opaque, flat or slightly raised, well embedded colonies. The edge was very finely filamentous (4 days). The surface was sometimes folded and segmented (6 days). White aerial mycelium (6 days), turned brown (10 days). No soluble pigments. Earthy odour.

Streak on nutrient agar. Good, colourless, slightly raised or flat growth (3 days). Surface folded (7 days). Reverse was colourless, became brown (15 days), and was sometimes raised. White aerial spores (6 days), turned brown or grey (10 days). Traces of brown pigment diffused into the medium (20 days).

Streak on glucose agar. Abundant, colourless, flat growth with a much folded surface (7 days). Reverse raised and colourless. Spores white (6 days), turned brown (14 days). No pigment was observed in this medium.

Czapek Dox glucose agar. Good, colourless growth (3 days).

Czapek Dox sucrose agar. Good, colourless growth (3 days), turned brown (7 days). Reverse raised and colourless (3 days), turned brown (7 days) and then dark grey (15 days).

Potato plug. Very good, yellow-brown, convoluted growth (3 days).

Organism No. 131 continued.

White spores turned grey or purple-brown (15 days). The plug became dark brown (15 days).

Nutrient broth. Good growth as flakes, ring or pellicle (3 days). Slight pigment formation (30 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16°, 25°, 30° and 37°. Optimum growth temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (3 days).

Dorset's egg medium. Very good growth with a much convoluted surface (7 days). The medium was pitted (15 days), with a small amount of liquefaction (27 days).

Gelatine. Liquefaction in 7 days.

Serum. Good growth and liquefaction in 12 days.

B.C.P. milk. Neutral or slightly alkaline peptonisation (7 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Trace amounts only of nitrite formed from nitrate in 12 days.

Antibiotics. This organism produced an antibiotic which was weakly active against Bacillus subtilis and B. cereus, but inactive against Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli and Micrococcus citreus (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 132.

Origin. Leaf mould, Dawsholm Park, Glasgow. Soil No. 3.

Morphology. Primary mycelium. Tough, branching, long filaments. 0.4-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed open, clockwise spirals. 0.5-0.8 x 0.8-1.3 μ.

Colonies on nutrient agar. Colourless, opaque, flat or raised, well embedded colonies (3 days). Entire edge (3 days), became very finely filamentous (10 days). Central depression and segmentation (5 days). White aerial mycelium (3 days), turned grey (6 days) and purple-grey (10 days). No pigment. Earthy odour.

Streak on nutrient agar. Good, colourless growth with a folded surface (3 days). The reverse was raised, and turned brown (5 days). White aerial mycelium (3 days) turned grey or brown (7 days). Some brown pigment diffused into the medium on long incubation (20 days).

Streak on glucose agar. As for nutrient agar, except that the pigment was not formed.

Czapek Dox glucose agar. Very good, colourless or light brown growth (5 days). The surface was convoluted. The aerial mycelium white, turned brown (7 days). A purple-brown pigment diffused into the medium (13 days).

Czapek Dox sucrose agar. Moderate to good, grey-brown growth (5 days). Reverse was dark brown. White aerial mycelium (5 days), turned dark brown (13 days).

Potato plug. Dark-yellow abundant growth (5 days), which became much

Organism No. 132 continued.

convoluted (13 days). White aerial mycelium turned light-brown. The plug turned grey or black (21 days).

Nutrient broth. Growth as flakes (5 days) or a ring (13 days). A slight amount of brown pigment diffused into the medium (26 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16° and 37°. Very good growth at 25° and 30°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (5 days).

Dorset's egg medium. Poor growth with no proteolysis (26 days).

Gelatine. No liquefaction.

Serum. Good growth which deeply pitted the medium (13 days). A slight amount of liquefaction took place in some cultures after 21 days.

B.C.P. milk. Alkaline peptonisation (5 days). The final pH was acid.

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was not reduced to nitrite.

Antibiotics. This organism produced an antibiotic which was weakly active against Bacillus subtilis, B. cereus, but inactive against Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli and Micrococcus citreus (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 133.

Origin. Leaf mould, Dawsholm Park, Glasgow. Soil No. 3.

Morphology. Primary mycelium. Long, branching filaments. 0.4-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight, branching chains. 0.5-0.9 x 0.9-1.3 μ.

Colonies on nutrient agar. Colourless, opaque, flat or slightly raised, well embedded colonies (4 days). Entire edge (4 days), became very finely filamentous (6 days). Surface smooth or slightly folded (6 days). White aerial mycelium (3 days) turned grey (6 days). No pigments. Earthy odour.

Streak on nutrient agar. Good, colourless, raised growth (4 days). Surface smooth or folded (6 days). Reverse colourless, and raised in some cultures (4 days). White aerial mycelium. No pigment.

Streak on glucose agar. Good, colourless growth (4 days). The reverse was raised (4 days), turned brown (6 days) and black (8 days). White spores developed (4 days), turned brown (8 days). Some purple-brown pigment diffused into the agar (13 days).

Czapek Dox glucose agar. Moderate growth. (4 days).

Czapek Dox sucrose agar. Good growth (4 days) which turned grey or black (13 days).

Potato plug. Growth poor (13 days).

Nutrient broth. Growth as flaky deposit or as a ring (4 days).

Glucose broth. As for nutrient broth.

Organism No. 133 continued.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16° and 37°. Good growth at 25° and 30°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Moderate growth, but no proteolysis.

Gelatine. Liquefaction in about 14 days.

Serum. Good growth (4 days). The medium was deeply pitted and clarified (8 days), but not liquefied.

B.C.P. milk. Alkaline peptonisation with a clot (3 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 3 days.

Antibiotics. This organism produced an antibiotic which was active against Bacillus subtilis and B. cereus, but inactive against Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli and Micrococcus citreus. (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954) and it was assumed to be of that species.

Organism No. 190.

Origin. Glasgow garden. Soil No. 5.

Morphology. Primary mycelium. Long, much branched filaments. 0.4-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed either straight chains, or long, open, clockwise spirals. 0.6-1.0 x 0.7-1.5μ.

Colonies on nutrient agar. Colourless, opaque, flat or slightly raised colonies. Very finely filamentous edge (4 days), which finally formed a circle of very fine filaments round the colony giving a 'halo' effect (14 days). Folded surface (9 days). White aerial mycelium. No pigment. Earthy odour.

Streak on nutrient agar. Good, colourless, flat growth. Surface folded. Reverse raised and grey in colour in some cases. White aerial mycelium (9 days). No pigments.

Streak on glucose agar. As for nutrient agar, except that the reverse showed no sign of darkening.

Czapek Dox glucose agar. Very good growth (4 days). The reverse was colourless (4 days), then grey (8 days), and finally black or greenish-black (21 days). White spores were formed (4 days), turned grey or brown (8 days) and finally black (21 days).

Czapek Dox sucrose agar. As for Czapek Dox glucose agar except that the greying of the spores, and the darkening of the medium took place at a quicker rate.

Potato plug. Abundant growth (4 days), which was colourless, but turned greenish-black or black, particularly on the reverse (10 days).

Organism No. 190 continued.

The spores were white (4 days), then dark grey and then black (14 days). Very little pigment diffused into the plug (30 days).

Nutrient broth. Growth as small flakes (4 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 37°, 45° or 55°. Good growth at 16°, 25° and 30°. Optimum growth temperature 25°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Very good growth, but no proteolysis.

Gelatine. Liquefaction slow or not at all.

Serum. Good growth with liquefaction (8 days).

B.C.P. milk. Alkaline peptonisation with a clot (6 days) which was slowly digested (14 days). The final pH was sometimes acidic (21 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 4 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 193

Origin. Glasgow garden. Soil No. 5.

Morphology. Primary mycelium. Tough, branching filaments. 0.4-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed tight, anticlockwise spirals. 0.5-1.0 x 0.7-1.3 μ.

Colonies on nutrient agar. Brown, opaque, flat, well embedded colonies. Very finely filamentous edge (3 days). Contoured and sometimes segmented surface. Sparse, white aerial mycelium (15 days). A copious amount of a dark brown pigment was formed particularly when the organism was freshly isolated. Earthy odour.

Streak on nutrient agar. Good, brown growth (4 days). Folded surface (13 days). Raised, brown reverse (13 days). White, aerial mycelium sparingly produced. Much soluble, dark brown pigment.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Abundant but slow growth (8 days). Growth was colourless at first (4 days), turned greenish yellow (8 days), and then black (20 days). The reverse was raised, and turned black (12 days). Some black pigment diffused into the medium (20 days).

Czapek Dox sucrose agar. Good, colourless growth (4 days) became brown or black (20 days). White aerial mycelium (4 days), turned brown (20 days). Some black pigment diffused into the medium.

Potato plug. Abundant, yellow growth (4 days), turned greenish-black (8 days), and then black (20 days).

Organism No. 193 continued.

Nutrient broth. Growth as flakes or as a ring (4 days). Dark brown pigment produced.

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16°, 25°, 30° and 37°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Good growth with much pigment produced, but no proteolysis.

Gelatine. Liquefaction slow or not at all (20 days).

Serum. Very poor growth with no proteolysis.

B.C.P. milk. Slightly alkaline peptonisation with a clot (4 days). The pH after 21 days incubation was slightly acid.

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was not reduced to nitrite.

Antibiotics. This organism produced an antibiotic which was active against Bacillus subtilis, but inactive against B. cereus, Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli and Micrococcus citreus (Table 14).

Identification. This organism resembled Streptomyces albus as described by Ducho (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954) and it was assumed to be of that species.

Organism No. 195.

Origin. Glasgow garden. Soil No. 5.

Morphology. Primary mycelium. Much branched, long filaments. 0.4-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight chains. 0.5-1.0 x 0.6-1.3 μ.

Colonies on nutrient agar. Colourless, opaque, flat, well embedded colonies (4 days). Entire edge (4 days), became very finely filamentous (6 days). Folded, slightly contoured or segmented surface (13 days). White aerial mycelium (4 days). No pigments. Earthy odour.

Streak on nutrient agar. Very good, colourless growth. Folded surface particularly at the edges (13 days). Reverse colourless and flat. White aerial spores (4 days). No pigment.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Growth variable between poor and good, colourless or slightly yellow (22 days)

Czapek Dox sucrose agar. Abundant, flat, colourless growth with white aerial mycelium (7 days).

Potato plug. Abundant, much folded, brown growth with white aerial mycelium (14 days). The plug turned brown (21 days).

Nutrient broth. Growth as a deposit, as a ring or as colonies floating on the surface (4 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16°, 25° and 30°. Poor growth at 37°. Optimum temperature 25°-30°.

Organism No. 195 continued.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Moderate growth, but no proteolysis.

Gelatine. Liquefaction in about 21 days.

Serum. Very good growth and rapid liquefaction (6 days).

B.C.P. milk. Alkaline peptonisation with a clot (6 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 5 days.

Antibiotics. This organism formed an antibiotic which was weakly active against Bacillus subtilis and Micrococcus citreus but inactive against Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli and B. cereus. (Table 14.).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 196.

Origin. Glasgow garden. Soil No. 5.

Morphology. Primary mycelium. Long, branching filaments. 0.5-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight chains. 0.6-1.1 x 0.6-1.3 μ.

Colonies on nutrient agar. Colourless, opaque, flat, well embedded colonies. Very finely filamentous edge (3 days). Surface smooth or slightly folded (7 days). White aerial mycelium (7 days). A very slight amount of pigment diffused into the medium (14 days). Earthy odour.

Streak on nutrient agar. Good, colourless growth with folded surface (6 days). Reverse was raised and colourless (6 days). White aerial mycelium. No pigment, or only very slight amounts (21 days).

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Very good, colourless or greenish growth (5 days), became brown (7 days) and then greenish black (24 days) particularly on the reverse.

Czapek Dox sucrose agar. Good or moderate, colourless or yellow growth (7 days).

Potato plug. Abundant, grey, raised growth, with white aerial mycelium (3 days) which turned grey (14 days). The plug also turned dark grey in colour (21 days).

Nutrient broth. Growth as flakes and ring (3 days).

Glucose broth. As for nutrient broth.

Organism No. 196 continued.

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16°, 25°, 30° and 37°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Growth poor with no proteolysis.

Gelatine. Liquefaction in 10 days.

Serum. Good growth with liquefaction in 14 days.

B.C.P. milk. Alkaline peptonisation with a clot (5 days) which was digested (22 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 3 days.

Antibiotics. This organism formed an antibiotic which was weakly active against Bacillus subtilis, B. cereus and Micrococcus citreus, but inactive against Klebsiella pneumoniae, Pseudomonas fluorescens and Escherichia coli (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and was assumed to be of that species.

Organism No. 197.

Origin. Air contaminant on an agar plate.

Morphology. Primary mycelium. Long, branching filaments. 0.4-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight, branching chains. 0.5-1.0 x 0.5-1.1 μ.

Colonies on nutrient agar. Colourless, opaque, well embedded colonies (3 days). Very finely filamentous edge (3 days) which formed a circle of fine filaments round the colony producing a "halo" effect (9 days). The surface was folded, and segmentation was often present (9 days). White aerial mycelium. No pigments. Earthy odour.

Streak on nutrient agar. Good, colourless, flat growth (3 days). Folded surface. Reverse raised and colourless (6 days). White aerial mycelium. No pigment.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Good, slightly yellow growth (10 days).

Czapek Dox sucrose agar. Moderate to poor, flat, colourless growth with some aerial spores (16 days).

Potato plug. Good, yellow growth (6 days), turned golden-brown (10 days). White aerial mycelium (6 days), turned grey (10 days). The plug slowly darkened to grey (20 days), and then black (29 days).

Nutrient broth. Deposit of flaky colonies and some surface growth (3 days).

Glucose broth. As for nutrient broth.

Organism No. 197 continued.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16°. Good growth at 25° and 37°. Very good growth at 30°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (3 days).

Dorset's egg medium. Moderate growth but no proteolysis.

Gelatine. Liquefaction in 6 days.

Serum. Moderate growth with slow liquefaction of the medium (20 days).

B.C.P. milk. Slow alkaline peptonisation (20 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 7 days.

Antibiotics. This organism produced an antibiotic which was weakly active against Bacillus subtilis and Micrococcus citreus, but inactive against Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli and B. cereus (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 204.

Origin. Botanic gardens, Glasgow. Soil No. 6.

Morphology. Primary mycelium. Long, much branched filaments. 0.5-1.0 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, open, clockwise spirals. 0.5-1.0 x 0.5-1.1 μ.

Colonies on nutrient agar. Red or blue, opaque, raised, well embedded colonies. Entire edge (3 days), became very finely filamentous (5 days). Surface folded and segmented (5 days). White aerial mycelium (5 days). This organism produced a pigment which coloured the colonies red or blue, and rapidly diffused into the medium. After many subcultures, the pigment producing ability was lost, and some colonies became colourless. No earthy odour.

Streak on nutrient agar. Good growth, pink (3 days), turned blue (7 days). Surface smooth or folded (3 days). Reverse raised and pigmented (4 days). White aerial mycelium (5 days) turned grey (10 days). Red or blue pigment diffused into the agar.

Streak on glucose agar. As for nutrient agar, except that the pink pigment was rapidly produced, and remained pink for about a month before turning blue.

Czapek Dox glucose agar. Good growth (7 days) but no pigment formed (21 days).

Czapek Dox sucrose agar. Good, colourless growth (7 days), which turned blue. Some pigment diffused into the medium (21 days).

Organism No. 204 continued.

Potato plug. Abundant, reddish-grey growth (5 days). Dark blue pigment appeared (7 days), and diffused into the plug (10 days). White aerial mycelium turned grey.

Nutrient broth. Good growth as colourless or blue flakes, with occasional surface growth (3 days).

Glucose broth. As for nutrient broth, except that the pigment was red (10 days).

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16°, 25°, 30° and 37°. Optimum temperature 37°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (3 days).

Dorset's egg medium. Moderate or good growth, but no pigment or proteolysis.

Gelatine. Liquefaction in 7 days.

Serum. Good, yellow growth with deep pitting and clarification of the medium, but only occasional liquefaction.

B.C.P. milk. Neutral or slightly acid peptonisation (5 days). The pH after several weeks incubation was always acid.

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 3 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Blood agar plate. Brownish-grey colonies with white spores. Slow, weak β-haemolysis.

Liquefaction of agar. This organism did not liquefy agar.

Organism No. 204 continued.

Cobalt agar plate. Growth moderate (5 days). The cobalt suppressed the production of pigment. A marked earthy odour which was not otherwise found with this organism was present.

Pigment. It was shown that the pigment produced by this organism acted as an indicator, being red in acid solutions, and blue in alkaline.

The organism was grown on cotton wool in large Petri dishes (Bottcher & Conn 1942) for 10 days, and the pigment extracted using the method of Sanchez-Marroquin & Zapata (1954). The adsorption between 300 mμ and 700 mμ at pH 11.52 was measured. The results appear in Fig. 2. The peak absorption of the pigment of 204 at pH 11.52 was about 630 mμ, whereas the pigment of the Streptomyces coelicolor examined by Conn (1943) had a peak at 580 mμ at pH 10.73. In view of the probability of some impurities still being present in both preparations and the pH shift, these figures are sufficiently alike to indicate that the same pigment, and presumably the same organism, was involved.

Identification. This organism closely resembled Streptomyces coelicolor as described in Bergey's manual (1948), and it was assumed to be of that species.

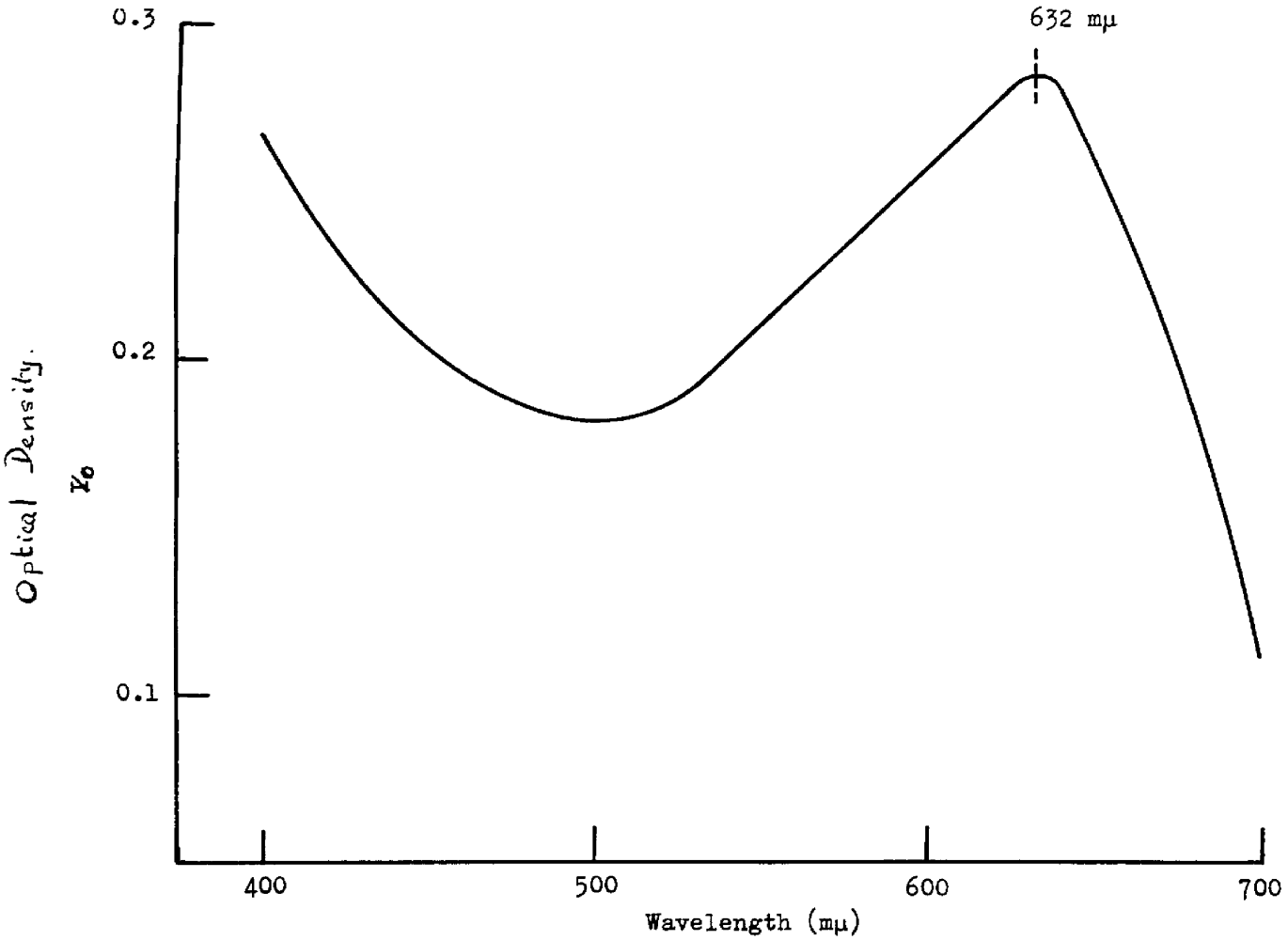

Fig. 2. Absorbtion curve of pigment preparation of 204. Buffered at pH 11.52.

Organism No. 214.

Origin. Botanic Gardens, Glasgow. Soil No. 6.

Morphology. Primary mycelium. Long, branching filaments. 0.4-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed open, anticlockwise spirals. 0.5-1.0 x 1.0-1.9 μ.

Colonies on nutrient agar. Yellow, opaque, flat or slightly raised, well embedded colonies. Very finely filamentous edge (4 days). Segmentation appeared in some colonies (10 days). White aerial mycelium (4 days). A yellow pigment diffused into the agar (6 days). Earthy odour.

Streak on nutrient agar. Good, yellow growth with a folded surface (4 days). The reverse was flat or slightly raised (8 days). White aerial mycelium. Yellow pigment diffused into the medium.

Streak on glucose agar. As for nutrient agar, except that the production of pigment was less marked.

Czapek Dox glucose agar. Good growth which was colourless except for the reverse which was yellow or brown (7 days). No pigment diffused into the medium.

Czapek Dox sucrose agar. Moderate, colourless, sporeless growth (10 days). No pigment diffused into the medium.

Potato plug. Yellow, abundant growth with a much convoluted surface (4 days). Heavy white sporulation turned grey (7 days). The plug turned grey (7 days), and then black (10 days).

Nutrient broth. Growth as flakes or as surface colonies (4 days).

Organism No. 214 continued.

Some pigment diffused into the medium.

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°.

Moderate growth at 16° and 37°. Good growth at 25° and 30°.

Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Very poor growth with no proteolysis.

Gelatine. Liquefaction in about 10 days.

Serum. Moderate, yellow growth (4 days). The medium was deeply pitted, and clarified (10 days), but only a little liquefied.

B.C.P. milk. Alkaline peptonisation with a clot (7 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 5 days.

Antibiotics. This organism produced an antibiotic which was strongly active against Bacillus subtilis, B. cereus and Micrococcus citreus, weakly active against Pseudomonas fluorescens and inactive against Klebsiella pneumoniae and Escherichia coli (Table 14).

Identification. This organism resembled Streptomyces flaveolus as described by Waksman (1919c), Takahashi (1953) and Bergey (1948), and it was assumed to be of that species. Waksman and Lechevalier (1953) placed this species in the S. flavus species group, and suggested that it was closely related to S. aureofaciens. No. 214 differed from organism Sa however in its antibiotic spectrum, its nitrition and the reduction of nitrate.

Organism No. 216.

Origin. Botanic gardens, Glasgow. Soil No. 6.

Morphology. Primary mycelium. Long, branching filaments. 0.4-1.0 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight chains. 0.6-1.0 x 0.6-1.5 μ.

Colonies on nutrient agar. Brown, opaque, flat or slightly raised, well embedded colonies. Very finely filamentous edge (4 days). The surface was slightly folded (15 days). Occasional white spores were produced. A deep brown pigment diffused into the medium. No odour.

Streak on nutrient agar. Good, brown growth with a folded surface. Reverse raised and brown (7 days). Occasional white aerial mycelium. Deep brown pigment diffused into the medium.

Streak on glucose agar. As for nutrient agar, except that less pigment was produced.

Czapek Dox glucose agar. Poor to moderate growth which was either colourless or yellow (4 days). The white aerial mycelium turned grey or brown (12 days).

Czapek Dox sucrose agar. Moderate, colourless growth (4 days) the reverse of which became blackish-brown (12 days). The white spores turned grey or brown (7 days).

Potato plug. Abundant, brown growth with white spores (4 days) which turned grey (7 days). The plug turned black (4 days).

Nutrient broth. Heavy deposit of large flakes (5 days). Much brown pigment.

Organism No. 216 continued.

Glucose broth. As for nutrient broth, except that there was less pigment, and it was yellower in colour.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16°. Good growth at 25°, 30° and 37°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Abundant growth and pigment production but no proteolysis.

Gelatine. No liquefaction.

Serum. Good growth with pitting of the medium, but no liquefaction.

B.C.P. milk. Weak peptonisation only. The final pH was usually acid (21 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 4 days.

Antibiotics. This organism produced an antibiotic which was active against Bacillus subtilis and B. cereus, but was inactive against Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli and Micrococcus citreus. (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 21.7.

Origin. Botanic gardens, Glasgow. Soil No. 6.

Morphology. Primary mycelium. Long, branching filaments. 0.4-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed open, anticlockwise, branched spirals. 0.4-1.0 x 0.6-1.1 μ.

Colonies on nutrient agar. Pink or red, opaque, slightly raised, well embedded colonies. Very finely filamentous edge (5 days). Folded surface (5 days). White aerial mycelium was present (10 days). No pigment diffused into the medium. Earthy odour.

Streak on nutrient agar. Good, pink or red growth (5 days). Surface folded. The reverse was raised, pink, red or colourless (10 days). White aerial spores. No pigment diffused into the medium.

Streak on glucose agar. As for nutrient agar, except that the colour of the growth was more intense.

Czapek Dox glucose agar. Pink, moderate growth (21 days).

Czapek Dox sucrose agar. Poor, colourless growth (21 days).

Potato plug. Moderate or good, pink growth with white aerial mycelium (5 days) which turned grey (10 days). The plug also turned pinkish-grey.

Nutrient broth. Deposit of colourless flakes (5 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16°. Good growth at 25°, 30° and 37°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Organism No. 217 continued.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Good growth with black soluble pigment produced, but no proteolysis.

Gelatine. Liquefaction in 6 days.

Serum. Heavy, pink growth (5 days) with liquefaction in most cultures (21 days).

B.C.P. milk. Neutral or slightly acid peptonisation (6 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 10 days.

Antibiotics. This organism produced an antibiotic which was active against Bacillus subtilis and B. cereus, but inactive against Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli and Micrococcus citreus (Table 14).

Pigment. The pigment of this organism was insoluble, and did not vary in colour with changes of pH.

Identification. This organism resembled Streptomyces albosporeus as described by Bergey (1948), and it was tentatively assumed to be of that species. Waksman and Lechevalier (1953) placed this species in the S. ruber species group.

Organism No. 224.

Origin. Dust of a hay bin.

Morphology. Primary mycelium. Long, branching filaments. 0.4-1.0 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed either long, straight chains, or very open, anticlockwise spirals. 0.6-1.1 x 0.5-1.2 μ.

Colonies on nutrient agar. Colourless or slightly yellow, flat or raised colonies (3 days). Very finely filamentous edge (3 days). Convoluted, sometimes contoured and segmented surface (6 days). White aerial mycelium. Occasionally a small amount of light brown pigment was produced. Earthy odour.

Streak on nutrient agar. Abundant, colourless or yellow growth (6 days). Surface convoluted. The reverse was yellow and raised (6 days). White aerial mycelium (4 days) turned grey (8 days). Little or no brown pigment diffused into the agar.

Streak on glucose agar. As for nutrient agar, except that more pigment appeared on the reverse of the colonies.

Czapek Dox glucose agar. Poor to moderate, colourless growth (13 days), with white or grey aerial mycelium (19 days).

Czapek Dox sucrose agar. As for Czapek Dox glucose agar.

Potato plug. Abundant, yellow, heavily encrusted growth (7 days). Spores white (7 days), turned grey (13 days), and finally nearly black (19 days). The plug turned yellow or brown (7 days) then greenish black (19 days).

Organism No. 224 continued.

Nutrient broth. Deposit of flaky colonies with some surface growth (2 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° and 55°. Good growth at 16° and 25°. Very good growth at 30° and 37°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (5 days).

Dorset's egg medium. Very good growth (6 days) with liquefaction in about 14 days.

Gelatine. Liquefaction in 3 days.

Serum. Very good growth (4 days) with liquefaction of the medium in 19 days.

B.C.P. milk. Alkaline peptonisation (4 days). The final pH was neutral or acidic (21 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrite was produced in only trace amounts from nitrate or not at all.

Antibiotics. No antibiotics were formed against the test organisms.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 228.

Origin. Dust of a hay bin.

Morphology. Primary mycelium. Long, branching filaments. 0.4-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed either long, branching chains, or very open, anticlockwise spirals. 0.5-0.9 x 0.6-1.5 μ.

Colonies on nutrient agar. Colourless, opaque, flat or raised, well embedded colonies (3 days). Finely filamentous edge (3 days). The surface was sometimes contoured and segmented (14 days). White aerial mycelium (7 days). No pigment or odours were present.

Streak on nutrient agar. Good, colourless growth with folded surface (7 days). The reverse was raised or flat, colourless, yellow, or brown (10 days). White aerial mycelium (7 days), turned grey (10 days). No pigment.

Streak on glucose agar. As for nutrient agar, except that some light brown pigment diffused into the medium on long incubation (30 days).

Czapek Dox glucose agar. Poor or moderate, yellow growth with white aerial mycelium (7 days), turned grey (10 days).

Czapek Dox sucrose agar. Poor to moderate, colourless growth (5 days). White aerial mycelium, turned grey (10 days).

Potato plug. Abundant, yellow, wrinkled growth (7 days). White aerial mycelium. The plug turned yellow or yellowish-brown (14 days).

Nutrient broth. Deposit of large flakes and a ring (5 days).

Organism No. 228 continued.

Glucose broth. As for nutrient broth, except that some pigment was formed on incubation for 1 month.

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16°, 25°, 30° and 37°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (3 days).

Dorset's egg medium. Good growth (7 days) and liquefaction of the medium (20 days).

Gelatine. Liquefaction in 7 days.

Serum. Good growth with liquefaction (14 days).

B.C.P. milk. Alkaline peptonisation (5 days) with a clot which was digested (21 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 7 days.

Antibiotics. No antibiotics were formed against the test organisms.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 235.

Origin. Glasgow lawn. Soil No. 4.

Morphology. Primary mycelium. Long, branching filaments. 0.5-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight, branching chains. 0.5-0.9 x 0.8-1.2 μ.

Colonies on nutrient agar. Light brown, opaque, raised, well embedded colonies. Very finely filamentous edge (7 days). The surface was folded and segmented (7 days). White aerial mycelium (7 days). Brown pigment diffused into the agar (4 days). No odour.

Streak on nutrient agar. Good, light brown growth with a much convoluted surface (5 days). The reverse was brown and flat. White, sparse aerial mycelium. Considerable amount of dark brown pigment produced (8 days).

Streak on glucose agar. As for nutrient agar, except that less pigment was produced.

Czapek Dox glucose agar. Very sparse, colourless growth (21 days). No pigment formed.

Czapek Dox sucrose agar. Moderate or good, colourless growth (15 days). No pigment formed.

Potato plug. Abundant, grey growth (4 days), turned brown or black. Spores white (7 days), turned grey or purple-brown (21 days). The plug also turned brown or black (12 days).

Nutrient broth. Growth as flakes with much pigment formation (7 days).

Glucose broth. As for nutrient broth, except that no pigment was formed.

Organism No. 235 continued.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16° and 37°. Good growth at 25° and 30°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Very good growth with much soluble pigment present but no proteolysis.

Gelatine. Liquefaction slow or not at all.

Serum. Moderate growth, but no proteolysis.

B.C.P. milk. Slight acid peptonisation (21 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was not reduced to nitrite.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 242.

Origin. Falkirk garden. Soil No. 7.

Morphology. Primary mycelium. Long, branching filaments. 0.5-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, branching chains. 0.6-1.0 x 0.8-1.1 μ.

Colonies on nutrient agar. Colourless, opaque, raised, well embedded colonies. Very finely filamentous edge (4 days), eventually formed a circle of fine filaments which gave the colony a "halo" appearance (12 days). Some colonies showed segmentation. White aerial mycelium (7 days), which turned grey (14 days). A small amount of light brown pigment diffused into the medium after about one month's incubation. Earthy odour.

Streak on nutrient agar. Very good, colourless growth (4 days). Surface smooth or folded (4 days). The reverse was raised, and dark green or grey in colour (14 days). Plentiful white aerial mycelium (4 days), turned grey (14 days). Slight amount of light brown pigment diffused into the medium on long incubation (30 days).

Streak on glucose agar. As for nutrient agar, except that the formation of pigment was more marked.

Czapek Dox glucose agar. Moderate, colourless growth with sparse sporulation (12 days).

Czapek Dox sucrose agar. Moderate or poor, colourless growth. (10 days). The white spores turned grey (14 days).

Organism No. 242 continued.

Potato plug. Abundant, greenish-black growth (4 days). White spores turned grey (7 days) and almost black (14 days). The plug turned greenish-black.

Nutrient broth. Flaky deposit with some surface growth (4 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16°, 25°, 30° and 37°. Optimum temperature 25°-30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Abundant growth (4 days), with liquefaction in 20 days.

Gelatine. Liquefaction in about 21 days.

Serum. Good growth with liquefaction in 14 days.

B.C.P. milk. Neutral or alkaline peptonisation with a clot (5 days). The pH after several weeks incubation was often acidic.

Indol. Indol was not formed.

Acetylmethylcarbinol. No acetylmethylcarbinol was formed.

Nitrate medium. Nitrate was reduced to nitrite in 5 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 245.

Origin. Falkirk garden. Soil No. 7.

Morphology. Primary mycelium. Long, branching filaments which broke into small pieces. 0.5-0.9 μ. Gram +ve. Partially acid fast in young culture.

Secondary mycelium. The conidia formed long, branching chains, or occasional tufts. 0.5-0.8 x 0.8-1.5 μ.

Colonies on nutrient agar. Colourless or orange, opaque, slightly raised, very small colonies. Entire edge (4 days), became very finely filamentous (6 days). Surface smooth. White or pink aerial mycelium (5 days). A slight amount of light brown pigment diffused into the medium on long incubation (24 days). Earthy odour.

Streak on nutrient agar. Very good, colourless, growth (5 days). Smooth surface. Reverse was sometimes raised, and was orange in colour (10 days). Aerial mycelium white or pink (6 days). Slight amount of soluble, light brown pigment diffused into the medium (21 days).

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Good, slow, orange growth (10 days). Aerial mycelium white or pink (10 days).

Czapek Dox sucrose agar. Moderate, orange or colourless growth. White aerial mycelium (15 days).

Potato plug. Abundant, encrusted, orange or pink growth (5 days). White or pink spores (10 days). No change in the plug (25 days).

Nutrient broth. Deposit of tiny flakes (3 days). A pellicle frequently formed which varied considerably in thickness, and carried

Organism No. 245 continued.

a heavy growth of pink or white spores.

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Poor growth at 16° and 37°. Good growth at 25° and 30°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was hydrolysed very slowly, or not at all.

Dorset's egg medium. Growth very poor or absent. No proteolysis.

Gelatine. No liquefaction.

Serum. Moderate growth, but only occasional proteolysis.

B.C.P. milk. White surface growth, but no change in the milk.

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 3 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism was unusual in that it was partially acid fast, and that the primary mycelium broke into fragments. Both these features suggested that it was allied to the genus Nocardia. However, in all other respects it had the features of the genus Streptomyces. No organism similar to No. 245 has been described in the literature as far as was found, although in some respects this organism resembled ~~S. ribogeons~~ Nocardia paraffinae. It was concluded that this organism was a Streptomyces of unknown species.

Organism No. 250.

Origin. Glasgow lawn. Soil No. 4.

Morphology. Primary mycelium. Long, branching filaments. 0.5-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed open, anti-clockwise spirals. 0.6-1.0 x 0.8-1.1 μ.

Colonies on nutrient agar. Brown or colourless, opaque, flat or slightly raised, well embedded colonies (5 days). Very finely filamentou edge (5 days). The surface was folded and segmented (9 days). White aerial spores (5 days), turned grey (9 days). Some brown pigment diffused into the agar (5 days), but this was lost after several subcultures. No odour.

Streak on nutrient agar. Good, brown or colourless growth (5 days). Folded surface. The reverse was raised and brown (9 days). White aerial spores (5 days), turned grey (9 days). Brown pigment diffused into the agar, but this was lost after several subcultures.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Poor, raised growth (5 days). The reverse was raised and faintly yellowish-green in colour (5 days). Spores were white (5 days) or grey (9 days).

Czapek Dox sucrose agar. Abundant, colourless growth with a raised reverse. White or pink spores (5 days), turned reddish-brown (13 days). Brown or purple-brown pigment diffused into the medium (13 days).

Potato plug. Very good, grey-green growth (5 days). White or pink spores (13 days). The plug turned grey-green (5 days), brown and then black (21 days).

Organism No. 250 continued.

Nutrient broth. Deposit of flakes, with occasional surface growth (5 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Good growth at 16°, 25°, 30° and 37°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Good growth but no proteolysis.

Gelatine. Liquefaction very slow (30 days) or not at all.

Serum. Good growth with occasional liquefaction of the medium.

B.C.P. milk. The milk usually became slightly alkaline.

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was not reduced to nitrite.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 259.

Origin. An inorganic salts solution in which seedlings of sea buckthorn (Hippophaë rhamnoides) were growing.

Morphology. Primary mycelium. Long, tough filaments. 0.5-0.8 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed long, straight, branching chains. 0.7-1.1 x 0.7-1.1 μ.

Colonies on nutrient agar. Colourless or faintly yellow, opaque, flat or slightly raised, well embedded colonies (3 days). Entire edge (3 days) became very finely filamentous (7 days). Segmentation was frequently marked. The spores were white (7 days), turned grey (10 days). No pigment. Earthy odour.

Streak on nutrient agar. Moderate to good, colourless or yellow growth (3 days). Surface folded (3 days). The reverse was raised, and yellow to greenish-black (7 days). White aerial spores (3 days), turned grey (7 days). No pigment.

Streak on glucose agar. As for nutrient agar, except that growth was more vigorous.

Czapek Dox glucose agar. Poor to moderate, colourless, yellow or greenish-yellow growth (7 days). White aerial spores which turned grey

Czapek Dox sucrose agar. Moderate, colourless growth with some white spores (3 days), which turned grey.

Potato plug. Abundant, slow, raised, light brown or yellow growth (3 days) with white aerial spores, which turned grey or purple-grey (10 days). The plug became grey (10 days) and eventually black (13 day

Organism No. 259 continued.

Nutrient broth. Flaky deposit (3 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Poor growth at 37°. Good growth at 16°, 25° and 30°. Optimum temperature 25°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (3 days).

Dorset's egg medium. Abundant growth with clarification and pitting of the medium, and occasionally, liquefaction.

Gelatine. Liquefaction in 15 days.

Serum. Good growth, followed by liquefaction (21 days).

B.C.P. milk. Alkaline peptonisation with a clot (7 days) which disappeared (17 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 7 days.

Antibiotics. This organism formed an antibiotic which was active against Bacillus subtilis, B. cereus, Micrococcus citreus and Escherichia coli, but inactive against Klebsiella pneumoniae and Pseudomonas fluorescens (Table 14).

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954) and it was assumed to be of that species.

Organism No. 260.

Origin. An inorganic salts solution in which seedlings of sea-buckthorn (Hippophaë rhamnoides) were growing.

Morphology. Primary mycelium. Long, much branched filaments. 0.5-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The conidia formed straight, branching chains. 0.6-1.1 x 0.6-1.2 μ.

Streak on nutrient agar. Good, colourless or slightly yellow, flat or raised, well embedded colonies (4 days). The edge was entire (4 days), became very finely filamentous (7 days). Marked segmentation was often present (7 days). The spores were white (7 days), turned grey (10 days). No pigments. Earthy odour.

Streak on nutrient agar. Moderate, colourless or slightly yellow growth (5 days). Surface folded (5 days). The reverse was raised, and yellow to greenish-black in colour (7 days). White aerial mycelium turned dark grey. No pigment.

Streak on glucose agar. As for nutrient agar, except that a small amount of purple-brown pigment diffused into the agar (15 days).

Czapek Dox glucose agar. Poor to moderate, yellow growth (5 days). The reverse was yellow or brown (13 days), and sometimes almost black (21 days). The aerial mycelium was white (5 days), and then turned grey or black (22 days).

Czapek Dox sucrose agar. Moderate to good, flat, colourless growth (3 days). White spores (5 days) which turned grey to black.

Organism No. 260 continued.

Potato plug. Abundant, light brown or yellow, encrusted growth (3 days). The spores were white (3 days) then brown or grey (7 days). The plug became purple-brown, and then grey. (13 days).

Nutrient broth. Moderate or good growth as the usual flaky deposit (3 days).

Glucose broth. As for nutrient broth, except that some purple-brown pigment collected in the medium.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 37°. Good growth at 16°, 25° and 30°. Optimum temperature 25°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Abundant growth, with clarification and pitting of the medium and occasional liquefaction (22 days).

Gelatine. Liquefaction in about 14 days.

Serum. Good growth with liquefaction in about 14 days.

B.C.P. milk. Alkaline petonisation with a clot (7 days) which disappeared (22 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. The reduction of nitrate to nitrite was variable; a weak response was obtained in some cultures after 10 days.

Antibiotics. This organism formed an antibiotic which was weakly active against Bacillus subtilis, but inactive against B. cereus, Klebsiella pneumoniae, Pseudomonas fluorescens, Escherichia coli and Micrococcus citreus (Table 14).

Organism No. 260 continued.

Identification. This organism resembled Streptomyces albus as described by Duché (1934), Baldacci (1939), Bergey (1948) and Baldacci et al. (1954), and it was assumed to be of that species.

Organism No. 265.

Origin. N.C.T.C. No. 434.

Morphology. Primary mycelium. Long, much branched filaments. 0.5-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. Fertile, aerial hyphae were never seen in this organism. The secondary mycelium was represented only by a few, very short, thick threads.

Colonies on nutrient agar. Colourless, opaque, flat or slightly raised, well embedded colonies (4 days). Very finely filamentous edge (4 days). Some colonies were segmented. No spores, pigments or odours were produced.

Streak on nutrient agar. Good, colourless growth (4 days). The surface was folded. The reverse was slightly raised and colourless (7 days). No spores or pigments were produced.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Poor or moderate, colourless growth (20 days).

Czapek Dox sucrose agar. Moderate, colourless growth (20 days).

Potato plug. Very poor or no growth.

Nutrient broth. Deposit of colourless flakes (4 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Moderate growth at 16°. Good growth at 25°, 30° and 37°. Optimum temperature 30°.

Oxygen relationships. Aerobic.

Organism No. 265 continued.

Starch hydrolysis. Starch was quickly hydrolysed (4 days).

Dorset's egg medium. Good growth, but no proteolysis.

Gelatine. No liquefaction (30 days).

Serum. Growth moderate to very good (7 days). The medium was clarified (5 days), and sometimes liquefied (21 days).

B.C.P. milk. The milk became very alkaline. Peptonisation was slow (16 days) and variable.

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was not reduced to nitrite.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism differed from Streptomyces listeri N.C.T.C. No. 434 as described by Erikson (1935) in the following respects a) it did not grow on a potato plug, b) it grew well on Dorset's egg medi c) inspissated serum was attacked and d) it produced an alkaline reaction in milk.

Streptomyces listeri was included in the S. albus series by Baldacci et al. (1954), and Organism No. 265 was, in many respects including its nutrition, like a Sub-group I S. albus strain (page 60). However, until both species are better understood it appeared to be wisest to assume that Organism No. 265 belonged to S. listeri species.

Organism No. 266.

Origin. N.C.T.C. No. 3026.

Morphology. Primary mycelium. The filaments were branched, but shorter than in most streptomycetes. 0.5-1.0 μ. Gram +ve. Non-acid fast.

Secondary mycelium. No fertile hyphae were observed, the secondary mycelium being represented only by a few, short, thick threads.

Colonies on nutrient agar. Dark blue-grey or black, opaque, flat, well embedded colonies (3 days). Entire edge (7 days) which did not become filamentous. Much convoluted and folded surface with marked segmentation (7 days). No spores were produced. Brown pigment diffuse into the medium (13 days). No odour.

Streak on nutrient agar. Moderate, blue-black growth (3 days). Smooth or folded surface. Reverse black, but not raised (7 days). No aerial mycelium. Brown pigment diffused into the medium.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Poor growth as tiny, blue-black colonies.

Czapek Dox sucrose agar. As for Czapek Dox glucose agar.

Potato plug. Slow but good, black, convoluted growth (14 days). The plug was not changed.

Nutrient broth. Very small, blue-grey flakes (4 days).

Glucose broth. As for nutrient broth.

Growth temperatures. No growth at 1°, 45° or 55°. Slight growth at 16°. Moderate growth at 25°. Good growth at 30° and 37°.

Organism No. 266 continued.

Optimum temperature 37°.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was hydrolysed very slowly or not at all.

Dorset's egg medium. Good growth with liquefaction in 13 days.

Gelatine. Liquefaction in about 14 days.

Serum. Good, blue growth and liquefaction in 14 days.

B.C.P. milk. Neutral or alkaline peptonisation (5 days).

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was not reduced to nitrite, or only in trace amounts.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. This organism was clearly not that described by Erikson (1935) as Streptomyces pelleteri N.C.T.C. No. 3026. It resembled Streptomyces violaceoniger as described in Bergey's manual (1948) in the colour of its pigment, and in its proteolytic ability, but not in its growth on Czapek Dox agar. Its nutrition also differed from the S. violaceoniger investigated by Benedict et al. (1955), in that it grew very poorly on synthetic media. Hence this organism was only tentatively regarded as S. violaceoniger.

Organism No. 267.

Origin. N.C.T.C. No. 4162.

Morphology. Primary mycelium. The mycelium consisted of small branched pieces. 0.4-0.9 μ. Gram +ve. Non-acid fast.

Secondary mycelium. The secondary mycelium consisted of a few, short, straight, sterile hyphae.

Colonies on nutrient agar. Opaque, flat or slightly raised, well embedded, colourless colonies (5 days), which developed very slowly into pink (15 days), and then red masses (21 days). Entire edge. The surface was convoluted. No aerial mycelium, soluble pigment or odour.

Streak on nutrient agar. Slow, colourless, sporeless, convoluted growth which turned pink and red. Reverse pink and flat (15 days). No pigment diffused into the medium.

Streak on glucose agar. As for nutrient agar.

Czapek Dox glucose agar. Scanty, pink, slow growing, tiny colonies (13 days).

Czapek Dox sucrose agar. As for Czapek Dox glucose agar.

Potato plug. The growth was yellow (12 days), turned brown, and then black (27 days). Some white patches of spores appeared on one occasion on this medium. The plug also turned light brown.

Nutrient broth. Colourless or pink flakes. (10 days).

Glucose broth. As for nutrient broth, except that a pellicle sometimes developed.

Growth temperatures. No growth at 1°, 16° or 55°. Growth at 25°, 30°, 37° and 45°. Optimum temperature 30°-37°.

Organism No. 267 continued.

Oxygen relationships. Aerobic.

Starch hydrolysis. Starch was slowly hydrolysed or not at all.

Dorset's egg medium. Slow but abundant, brown, convoluted growth (10 days). The medium was deeply pitted, clarified (10 days) and finally liquefied in about 2 months.

Gelatine. Little growth. No proteolysis.

Serum. Scanty growth. No proteolysis.

B.C.P. milk. Alkaline peptonisation (7 days). The pH after a couple of months was sometimes very alkaline, and at other times almost neutral.

Indol. Indol was not formed.

Acetylmethylcarbinol. Acetylmethylcarbinol was not formed.

Nitrate medium. Nitrate was reduced to nitrite in 2 days.

Antibiotics. No antibiotics were formed against the test bacteria.

Identification. Organism No. 267 was clearly the same species as was isolated by Smith (1928), and described by Erikson (1935) as Streptomyces pelletieri N.C.T.C. No. 4162. Except for the loss of the ability to liquefy gelatine, the organism was as described in 1935, and was therefore S. pelletieri.

APPENDIX II

Media used in isolating and characterising the organisms

Media used in isolating and characterising the organisms.

Medium 1. Nutrient broth.

Fresh beef, from which the fat had been removed as far as possible, was minced, and 500 g. added to 1 litre of distilled water, together with 10 g. "Bacto" peptone and 5 g. NaCl. The mixture was heated to 67° and held at that temperature for 30 min. It was then brought to the boil and allowed to simmer for 1 hr. After cooling, the mixture was filtered through surgical lint, and the pH adjusted to 7.6-7.8. The filtrate was boiled again for 30 min., filtered and the pH adjusted to 7.5 The medium was then sterilised in the autoclave at 120° for 20 min.

Medium 2. Nutrient agar.

To 1 litre of nutrient broth (medium 1) was added 15 g. Davies New Zealand agar, which was dissolved by heating in the steamer. The pH was checked, and if necessary, adjusted to 7.4-7.6. The medium was then filtered through paper pulp, and sterilised in the autoclave at 120° for 20 min.

Medium 3. Glucose broth.

A concentrated, sterile solution of glucose was aseptically added to nutrient broth (medium 1) until the concentration of the sugar was 1% w/v.

Medium 4. Glucose agar.

A concentrated, sterile solution of glucose was aseptically

added to melted nutrient agar (medium 2) until the concentration of the sugar was 1% w/v.

Medium 5. Czapek-Dox agar.

$NaNO_3$	2.0 g.
KCl	0.5 g.
$MgSO_4.7H_2O$	0.01 g.
$FeSO_4.7H_2O$	1.0 g.
K_2HPO_4	1.0 g.
Dist. water	1 litre.

All the salts, except K_2HPO_4, were dissolved in 500 ml. of the water; the K_2HPO_4 was dissolved in the remaining 500 ml. The solutions were then mixed, filtered, and either glucose or sucrose added to 3% w/v concentration. Fifteen grams of Davies New Zealand agar were added, and dissolved by heating in the steamer. The medium was then filtered, and sterilised in the autoclave at 120° for 20 min.

Medium 6. Potato plug.

A large potato was scrubbed and peeled. Long cylinders were cut from the potato with a cork borer, and then cut diagonally. Each "slope" so formed, was put into a wide necked 1 oz. screw cap bottle, with the butt downwards on a pad of absorbent cotton-wool. Each bottle was filled with distilled water, and stood for 30 min. The water was then poured off, and the plug sterilised in the autoclave at 120° for 20 min.

Medium 7. Nutrient agar shake.

Nutrient agar (medium 2) was melted, cooled to 45°, and left to solidify in an upright position after inoculation.

Medium 8. Starch agar.

Soluble starch (AnalaR) was added to melted nutrient agar (medium 2) to 1% w/v concentration. The medium was sterilised in the autoclave at 120° for 20 min.

Medium 9. Dorset's egg medium.

The medium was prepared aseptically as far as possible, using sterilised apparatus and materials. Four eggs were washed, broken into 25 ml. distilled water, mixed, and strained through muslin. Five ml. amounts were filled out into 1 oz. screw cap bottles and solidified in a sloping position in an inspissator at 75°. The slopes were sterilised by heating at approximately 90° at the top of the steamer for 20 min. on 3 successive days.

Medium 10. Gelatin stab.

Gurr's bacteriological gelatin was added to nutrient broth (medium 1) to 15% w/v concentration, and dissolved by heating in the steamer. The medium was sterilised by steaming for 30 min. on 3 successive days.

Medium 11. Inspissated serum.

Approximately 3 ml. horse serum was filled out aseptically

into sterile test tubes, and solidified in a sloping position in an inspissator at 75°. The slopes were sterilised by heating at approximately 90° at the top of the steamer for 20 min. on 3 successive days.

Medium 12. B.C.P. milk.

One litre of fresh milk was boiled, cooled, and the cream removed. One ml. of 1.6% w/v solution of bromocresol purple in 50% v/v ethanol was added, and the medium sterilised in the autoclave at 120° for 20 min.

Medium 13. Peptone water.

Ten g. "Bacto" peptone and 5 g. NaCl were dissolved in 1 litre distilled water, and the pH adjusted to 7.5-7.6. The medium was heated in the steamer for 30 min., cooled, filtered and sterilised in the autoclave at 120° for 20 min.

Medium 14. Glucose phosphate peptone water.

"Bacto" peptone	5 g.
K_2HPO_4 (anhydr.)	5 g.
Glucose	5 g.
Dist. water	1 litre.

The ingredients were dissolved in the water, and the pH adjusted to 7.5-7.6. The medium was then steamed for 30 min., filtered when cool, and sterilised in the autoclave at 115° for 25 min.

Medium 15. Nitrate broth.

Lab. Lemco (Oxoid)	3 g.
"Bacto" peptone	5 g.
* KNO_3 (AnalaR)	1 g.
Distilled water	1 litre.

The ingredients were dissolved in the water, and the pH adjusted to 7.4-7.5. The medium was then steamed for 30 min., filtered when cool, and sterilised in the autoclave at 120° for 20 min.

* A 0.1% w/v aqueous solution should not give a positive nitrite reaction when tested by the Griess-Ilosvay method.

Medium 16. Carbohydrate peptone water.

The required amount of carbohydrate and 1 ml. of 1.6% w/v solution of bromocresol purple in 50% v/v ethanol was added to 1 litre of peptone water (medium 13). The medium was sterilised by filtration through a Ford SB pad in a glass filter. The carbohydrates were added to give the following final concentrations:-

arabinose	0.5% w/v	maltose	1.0% w/v
fructose	1.0% "	sucrose	1.0% "
galactose	1.0% "	glycerol	1.0% "
glucose	1.0% "	mannitol	1.0% "
mannose	0.5% "	inositol	0.25% "
lactose	1.0% "		

The carbohydrates were the purest available, and were obtained from various suppliers.

Medium 17. Conn's medium (Conn 1921).

Glycerol	10 ml.
Sodium asparaginate	1 g.
K_2HPO_4 (anhydr.)	1 g.
Davies New Zealand agar	15 g.
Dist. water	1 litre

The K_2HPO_4, asparagine and glycerol were dissolved in the water, and the pH adjusted to 7.0-7.2. The agar was added, dissolved by heating in the steamer and filtered. The medium was sterilised in the autoclave at 120° for 20 min.

Medium 18. Bennett's medium (Jones 1949).

Difco yeast extract	1.0 g.
Lab-lemco beef extract	1.0 g.
Hydrolysed casein	2.0 g.
Glucose	10.0 g.
Dist. water	1 litre.

The ingredients were dissolved in the water, the pH adjusted to 7.3 and filtered. The medium was sterilised in the autoclave at 120° for 20 min.

Medium 19. Cobalt medium. (Hickey & Tresner 1952).

Twenty mg. $CoCl_2.6H_2O$ was added to 1 litre of Bennett's medium (medium 18), and the medium sterilised in the autoclave at 120° for 20 min.

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