Classification of acidophilic, neutrotolerant and neutrophilic streptomycetes by nucleotide sequencing of 5S ribosomal RNA

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Complete 5S ribosomal RNA sequences were obtained for four acidophilic actinomycetes, seven neutrophilic streptomycetes and a strain of Streptovorticilliurn balducii. All of the organisms contained RNAs belonging to the 120 nucleotide type. An evolutionary tree was generated after combining the test data with results from similar studies on representative Gram-positive bacteria. The acidophilic, neutrotolerant and neutrophilic actinomycetes were recovered in a distinct cluster that was equated with the genus Streptomycyes. The sequence data support the view that the genera Chainia, Elytrosporangium, Kitasatoa and Microellobosporia should be considered as synonyms of the genus Streptomycyes. The recovery of the Streptovorticilliurn balducii strain on the fringe of the Streptomycyes cluster is also consistent with current trends in the taxonomy of these organisms. Further work is needed to determine the taxonomic status of the two streptomycete subgroups that comprised the streptomycete cluster.

Introduction

The genus Streptomycyes is currently defined using a combination of chemical, morphological and physiological properties (Williams et al., 1989). The genera Actinopycnidium, Actinosporangium, Chainia, Elytrosporangium, Kitasatoa and Microellobosporia have been distinguished from Streptomycyes by morphological criteria, but they share many other phenotypic properties with Streptomycyes and have therefore been proposed as synonyms of this genus (Goodfellow et al., 1986, a–d). The genus Streptovorticilliurn can be distinguished from Streptomycyes by its verticillate sporophores but in other respects has many characters in common with streptomycetes. In an extensive numerical taxonomic survey (Williams et al., 1983), numerous type strains of Streptomycyes and related taxa were assigned to 23 major (six or more strains), 20 minor (two to five strains) and 25 single-membered clusters. The minor and single-membered clusters were considered as species and the major clusters regarded as species-groups.

Members of validly described Streptomycyes species behave as neutrophiles in culture, growing between pH 5.0 and 9.0 with an optimum close to neutrality. Acidophilic and neutrotolerant actinomycetes with phenotypic properties characteristic of Streptomycyes have been isolated from acidic habitats, notably soil (Williams et al., 1971; Khan & Williams, 1975; Goodfellow & O'Donnell, 1989). Acidophilic isolates grow in the range from about pH 3.5 to 6.5, with optimum rates at pH 4.5 to 5.5, and their neutrotolerant counterparts between pH 3.5 and 7.5, but optimally around pH 5.5. Acidophilic and neutrophilic actinomycetes have not been the subject of objective comparative studies though members of the two groups have been separated using numerical taxonomic procedures (Khan & Williams, 1975; Williams et al., 1983).

5S ribosomal RNA sequencing has been used to establish evolutionary relationships between diverse prokaryotes including Micrococcus and Staphylococcus spp. (Dekio et al., 1984), Mycoplasma spp. (Rogers et al., 1985), Thiobacillus and Thiomicrospora (Lane et al., 1985), the Vibrionaceae (MacDonell & Colwell, 1985), and coryneform actinomycetes (Park et al., 1987a, b). In the present work representative strains of acidophilic actino-
Table 1. Source and taxonomic histories of test strains

<table>
<thead>
<tr>
<th>Laboratory number</th>
<th>Name and cluster</th>
<th>Source†</th>
</tr>
</thead>
<tbody>
<tr>
<td>*KCTC 9080</td>
<td>S. griseus subsp. griseus</td>
<td>JCM 4047; KCC-S 0047; Y. Okami, NIHJ 106; IMRU 3463</td>
</tr>
<tr>
<td>*KCTC 9079</td>
<td>S. griseus subsp. cretensis</td>
<td>JCM 4742; KCC-S 0742; IFO 13457; SAJ; ISP 5561; CBS 137.21</td>
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<tr>
<td>*KCTC 9065</td>
<td>S. sclerotialus (syn. Chainia antibiotica)</td>
<td>JCM 3039; KCC A-0039; H. A. Lechevalier, 3750; M. J. Thirumalachar; soil, Poona, India</td>
</tr>
<tr>
<td>*KCTC 9066</td>
<td>S. cinereus (syn. Microellobosporia cinerea)</td>
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<td>S. purpureus (syn. Kitasatoa kanaiensis)</td>
<td>JCM 3177; KCC A-0177; A. Matsusume, KI-100027; T. Hata, KAC 281; soil, Kauai Island, Hawaii, USA</td>
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<tr>
<td>KCTC 9142</td>
<td>S. diastaticus</td>
<td>NCIMB 9603; NRR 2650</td>
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<tr>
<td>*KCTC 9071</td>
<td>S. brasiliensis (syn. Elytrosporangium brasiliense)</td>
<td>JCM 3086; KCC A-0086; IMUR 2572; J. C. Falcão de Morais, CCIB 71; soil, Alcan, North of Pernambuco, Brazil</td>
</tr>
<tr>
<td>KCTC 9091</td>
<td>Acidophilic actinomycete (cluster 28)</td>
<td>JL 46, Hamsterley Forest, County Durham, UK, A. horizon</td>
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<tr>
<td>KCTC 9092</td>
<td>Acidophilic actinomycete (cluster 4)</td>
<td>JL 85, Hamsterley Forest, F. horizon</td>
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<td>KCTC 9093</td>
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<td>JL 338, East Cramlington Colliery, Northumberland, UK, coal waste</td>
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<tr>
<td>KCTC 9094</td>
<td>Acidophilic actinomycete (cluster 10)</td>
<td>JL 445, Woodhorn Colliery, Northumberland, UK, coal waste</td>
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<tr>
<td>*KCTC 9081</td>
<td>Streptoverticillium baldacci</td>
<td>JCM 4272; KCC S-0272; IPV 174; soil, Arcinazzo, Italy</td>
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</tbody>
</table>

* Type strain.
† Clusters defined by Lonsdale (1985).
‡ CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; IFO, Institute for Fermentation, Yodogawa-ku, Osaka, Japan; IMRU, Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, Piscataway, NJ, USA; IPV, Institute of Plant Pathology, University of Milan, Milano, Italy; ISP, International Cooperative Project for Description and Deposition of Type Cultures of Streptomyces; JCM, Japan Collection of Micro-organisms, RIKEN, Wako-shi, Saitama, Japan; JL, John Lonsdale, Department of Microbiology, University of Newcastle, Newcastle upon Tyne, UK; KCC, KCC Culture Collection of Actinomycetes, Kaken Pharmaceutical Co. Ltd, Tokyo, Japan; KCTC, Korean Collection for Type Cultures, Genetic Engineering Research Institute, Korea Institute of Science and Technology, Daejon, Korea; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NIHJ, Department of Antibiotics, National Institute of Health of Japan, Tokyo, Japan; NRRL, ARS Culture Collection, Northern Regional Research Center, Peoria, IL, USA.

mycetes (Lonsdale, 1985) were compared with marker neutrophilic streptomyces using data derived from SS rRNA sequencing studies.

**Methods**

**Bacterial strains and culture conditions.** The sources of the test strains are given in Table 1. The strains of *Streptomyces brasiliensis*, *S. griseus*, *S. purpureus* and *S. sclerotialus* were grown in yeast-starch medium (yeast extract, 0.2%, w/v; soluble starch, 1.0%, w/v) at pH 7.3. The acidophilic and neutrotolerant actinomycetes and *S. diastaticus* KCTC 9142 were grown in Bennett’s broth (Jones, 1949), and *S. cinereus* KCTC 9066 and *Streptoverticillium baldacci* KCTC 9081 in ISP media 3 and 4 (Difco), respectively. The organisms were grown in shake flasks at 30°C for 2 d, then checked for purity and harvested by centrifugation.

**Isolation and sequencing of SS rRNA.** Wet biomass was homogenized with aluminium oxide, mixed with Tris/HCl and DNAase, and the subsequent phenolized lysate treated by polyacrylamide gel electrophoresis, as previously described (Park et al., 1987.a). The sequences were determined using both the chemical and enzymic methods (Peattie, 1979; Donis-Keller, 1980).

**Phylogenetic analysis.** Evolutionary distances (K\textit{sub} values) were calculated after Kimura (1980). An evolutionary tree was generated using published procedures and data from earlier studies on representative Gram-positive bacteria (Park et al., 1987.a). SS rRNA secondary structure models were constructed using the method of Tinoco et al. (1971) as adapted by Hori & Osawa (1986).

**Results and Discussion**

The SS rRNA nucleotide sequences were aligned by juxtaposing the defined secondary structures and were divided into 15 regions (Fig. 1). A and A', B and B', C and C', and D and D' are the sequences that can base-pair with each other. The loop region aLb connects the base-paired regions A and B; other based-paired regions are connected by bLc, cLc', c'Lb', b'Ld, dLd' and d'La as shown. The percentage sequence homology values are given in Table 2.

Ten of the twelve strains contained rRNA molecules 120 nucleotides long. The exceptions, *S. sclerotialus* KCTC 9065 and *St. baldacci* KCTC 9081, had SS rRNAs consisting of 121 and 122 nucleotides, respectively. On the basis of primary and secondary structure the SS rRNAs of the organisms tested belonged to the 120 N type (Hori & Osawa, 1986). However, all of the rRNA sequences were found to have a bulge in the A-A' helix, and unique sequences, such as 5'-CUGCA-3' and 5'-UGUGG-3', in the helix D-D' region. These properties have previously been reported from SS rRNA sequences of coryneform (pleomorphic) actinomycetes and from a strain of *S. griseus* (Park et al., 1987a, b; Simoncsits, 1980). The SS rRNAs from *S. diastaticus* KCTC 9142, *S. purpureus* KCTC 9075, the *S. griseus* strains (KCTC 9079...
Classification of streptomyces by 5S rNAs

The phylogenetic tree (Fig. 2) generated from the 5S rRNA sequence data shows that the acidophilic, neutrotolerant and neutrophilic actinomycetes form a distinct evolutionary group that can readily be distinguished from the marker 'coryneform' actinomycetes and representatives of the genera Bacillus, Lactobacillus and Staphylococcus. It is evident, therefore, that acidophilic and neutrotolerant actinomycetes with a combination of chemical, morphological and physiological properties corresponding to those characteristic of neutrophilic streptomyces should be assigned to the genus Streptomyces.

Table 2. Homology percentage matrix of 5S rRNA sequences

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<td>1. Streptomyces sclerotialus</td>
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<td>8. Streptoverticillium baldaccii</td>
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The proposals that the genera Chainia, Elytrosporangium, Kitasatoa and Microellobosporia become synonyms of the genus Streptomyces (Goodfellow et al., 1986a, b, c) are underlined by the sequence data. Similarly, the recovery of the *Stv. baldaccii* strain on the fringes of the Streptomyces cluster is in line with some previous studies. *Streptomyces* and *Streptoverticillium* can be distinguished on the basis of sporophore morphology (Williams et al., 1989) and by data from numerical taxonomic (Williams et al., 1983) and rRNA : DNA homology studies (Gladek et al., 1985).

The recovery of the streptomyces and the streptoverticillium strain in a phylogenetically distinct taxon is in good agreement with corresponding evidence drawn from 16S rRNA cataloguing studies (Stackebrandt et al.,...
Aureobacterium testaceum
Corynebacterium aquaticum
Curtobacterium citreum
Streptomyces sp. JL 338
Streptomyces sclerotialus
Streptomyces sp. JL 46
Streptomyces sp. JL 445
Streptomyces brassiliensis
Streptomyces cinerius
Streptomyces griseus subsp. griseus
Streptomyces griseus subsp. cretosus
Streptomyces diastaticus
Streptomyces purpurus
Streptosporangium baldaeii
Cellulomonas hiazotea
Rhodococcus erythropolis
Arthrobacter globiformis
Pimelobacter simp1er
Corjnebacterium glutamicum
Corynebacterium xer0si.y
Breribacterium linens
Staphylococcur aureus
Lactobacillus riridescms
Bacillus suhtilis
Pseudomonas puorescens
Escherichia coli

Fig. 2. Phylogenetic relationships among streptomycetes and selected Gram-positive and Gram-negative bacteria based on 5s rRNA sequence data.

1983). The taxon circumscribed using these rRNA data corresponds to the family Streptomycetaceae (Waksman & Henrici, 1943). Further cooperative work, however, needs to be undertaken to determine the taxonomic status of the two streptomycete subgroups that were recognized (Fig. 2). The first subgroup contains the S. griseus strains, S. diastaticus KCTC 9142 and S. purpureus KCTC 9075, and the second subgroup S. brasilienisis KCTC 9071, S. cineretis KCTC 9066, S. sclerotialus KCTC 9065 and the acidophilic streptomycetes.

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References


