

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

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(Received 25 February 1991; accepted 28 May 1991)

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Complete 5S ribosomal RNA sequences were obtained for four acidophilic actinomycetes, seven neutrophilic streptomycetes and a strain of *Streptovorticillium baldaccii*. 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 *Streptomyces*. The sequence data support the view that the genera *Chainia*, *Elytrosporangium*, *Kitasatoa* and *Microellobosporia* should be considered as synonyms of the genus *Streptomyces*. The recovery of the *Streptovorticillium baldaccii* strain on the fringe of the *Streptomyces* 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.

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### Introduction

The genus *Streptomyces* 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 *Streptomyces* by morphological criteria, but they share many other phenotypic properties with *Streptomyces* and have therefore been proposed as synonyms of this genus (Goodfellow *et al.*, 1986, *a–d*). The genus *Streptovorticillium* can be distinguished from *Streptomyces* 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 *Streptomyces* 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.

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The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers M59335–M59346 inclusive.

Members of validly described *Streptomyces* 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 *Streptomyces* 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.*, 1987*a, b*). In the present work representative strains of acidophilic actino-

Table 1. Source and taxonomic histories of test strains

Laboratory number	Name and cluster†	Source‡
*KCTC 9080	<i>S. griseus</i> subsp. <i>griseus</i>	JCM 4047; KCC-S 0047; Y. Okami, NIHJ 106; IMRU 3463
*KCTC 9079	<i>S. griseus</i> subsp. <i>cretosus</i>	JCM 4742; KCC-S 0742; IFO 13457; SAJ; ISP 5561; CBS 137.21
*KCTC 9065	<i>S. sclerotialis</i> (syn. <i>Chainia antibiotica</i> )	JCM 3039; KCC A-0039; H. A. Lechevalier, 3750; M. J. Thirumalachar; soil, Poona, India
*KCTC 9066	<i>S. cinereus</i> (syn. <i>Microellobosporia cinerea</i> )	JCM 3040; KCC A-0040; IMRU 3855; H. A. Lechevalier, 3855; soil, Piltla, Malta
KCTC 9075	<i>S. purpureus</i> (syn. <i>Kitasatoa kauaiensis</i> )	JCM 3177; KCC A-0177; A. Matsuname, KI-100027; T. Hata, KA281; soil, Kauai Island, Hawaii, USA
KCTC 9142	<i>S. diastaticus</i>	NCIMB 9603; NRRL 2650
*KCTC 9071	<i>S. brasiliensis</i> (syn. <i>Elytosporangium brasiliense</i> )	JCM 3086; KCC A-0086; IMUR 2572; J. C. Falcão de Moraes, CCIB 71; soil, Alianca, North of Pernambuco, Brazil
KCTC 9091	Acidophilic actinomycete (cluster 28)	JL 46, Hamsterley Forest, County Durham, UK, A <sub>2</sub> horizon
KCTC 9092	Acidophilic actinomycete (cluster 4)	JL 85, Hamsterley Forest, F horizon
KCTC 9093	Neutrotolerant actinomycete (cluster 30)	JL 338, East Cramlington Colliery, Northumberland, UK, coal waste
KCTC 9094	Acidophilic actinomycete (cluster 10)	JL 445, Woodhorn Colliery, Northumberland, UK, coal waste
*KCTC 9081	<i>Streptoverticillium baldaccii</i>	JCM 4272; KCC S-0272; IPV 174; soil, Arcinazzo, Italy

\* 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 of University of New Jersey, Piscataway, NJ, USA; IPV, Institute of Plant Pathology, University of Milano, 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, Daejeon, 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 streptomycetes using data derived from 5S 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. sclerotialis* 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 baldaccii* 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 5S 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.*, 1987a). The sequences were determined using both the chemical and enzymic methods (Peattie, 1979; Donis-Keller, 1980).

**Phylogenetic analysis.** Evolutionary distances ( $K_{nuc}$  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.*, 1987a). 5S rRNA secondary structure models were constructed using the method of Tinoco *et al.* (1971) as adapted by Hori & Osawa (1986).

## Results and Discussion

The 5S 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 base-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. sclerotialis* KCTC 9065 and *Stv. baldaccii* KCTC 9081, had 5S rRNAs consisting of 121 and 122 nucleotides, respectively. On the basis of primary and secondary structure the 5S 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 5S rRNA sequences of coryneform (pleomorphic) actinomycetes and from a strain of *S. griseus* (Park *et al.*, 1987a, b; Simoncsits, 1980). The 5S rRNAs from *S. diastaticus* KCTC 9142, *S. purpureus* KCTC 9075, the *S. griseus* strains (KCTC 9079

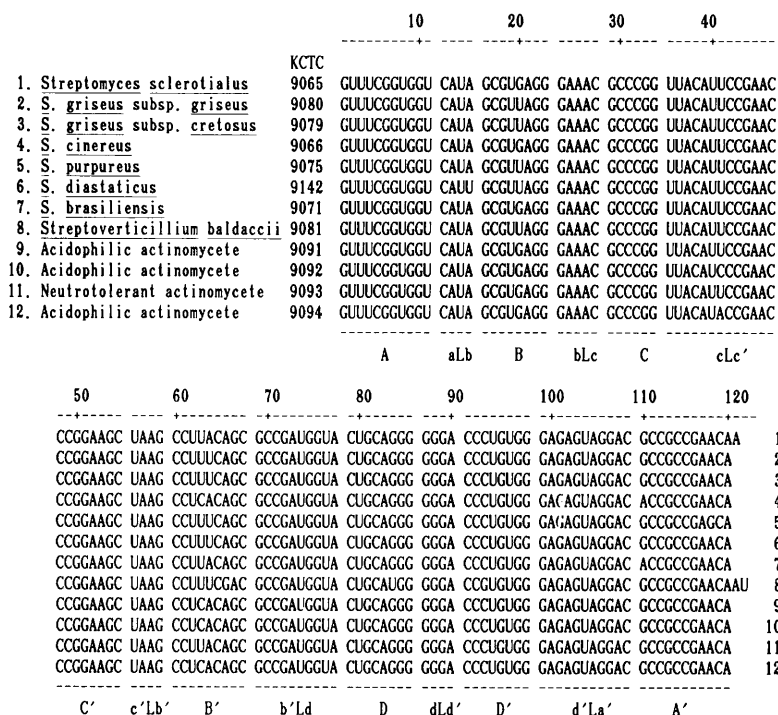


Fig. 1. Sequence alignment of 5S rRNAs from 12 actinomycete strains. A, A', etc., base-paired regions; aLb, bLc, etc., loop regions.

Table 2. Homology percentage matrix of 5S rRNA sequences

	1	2	3	4	5	6	7	8	9	10	11	12	
KCTC													
1. <i>Streptomyces sclerotialis</i>	9065	100											
2. <i>S. griseus</i> subsp. <i>griseus</i>	9080	97	100										
3. <i>S. griseus</i> subsp. <i>cretosus</i>	9079	97	100	100									
4. <i>S. cinereus</i>	9066	97	96	96	100								
5. <i>S. purpureus</i>	9075	96	99	99	95	100							
6. <i>S. diastaticus</i>	9142	96	99	99	95	98	100						
7. <i>S. brasiliensis</i>	9071	98	97	97	99	96	96	100					
8. <i>Streptoverticillium baldaccii</i>	9081	94	95	95	91	94	94	92	100				
9. Acidophilic actinomycete	9091	98	97	97	99	96	96	98	92	100			
10. Acidophilic actinomycete	9092	97	96	96	98	95	95	97	91	99	100		
11. Neutrotolerant actinomycete	9093	99	98	98	98	97	97	99	93	99	98	100	
12. Acidophilic actinomycete	9094	97	96	96	98	95	95	97	91	99	99	98	100

and 9080) and *Stv. baldaccii* KCTC 9081 were characterized by the presence of double U-U mismatches in the B-B' helix.

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 streptomycetes should be assigned to the genus *Streptomyces*.

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 (Gładek *et al.*, 1985).

The recovery of the streptomycetes 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.*,

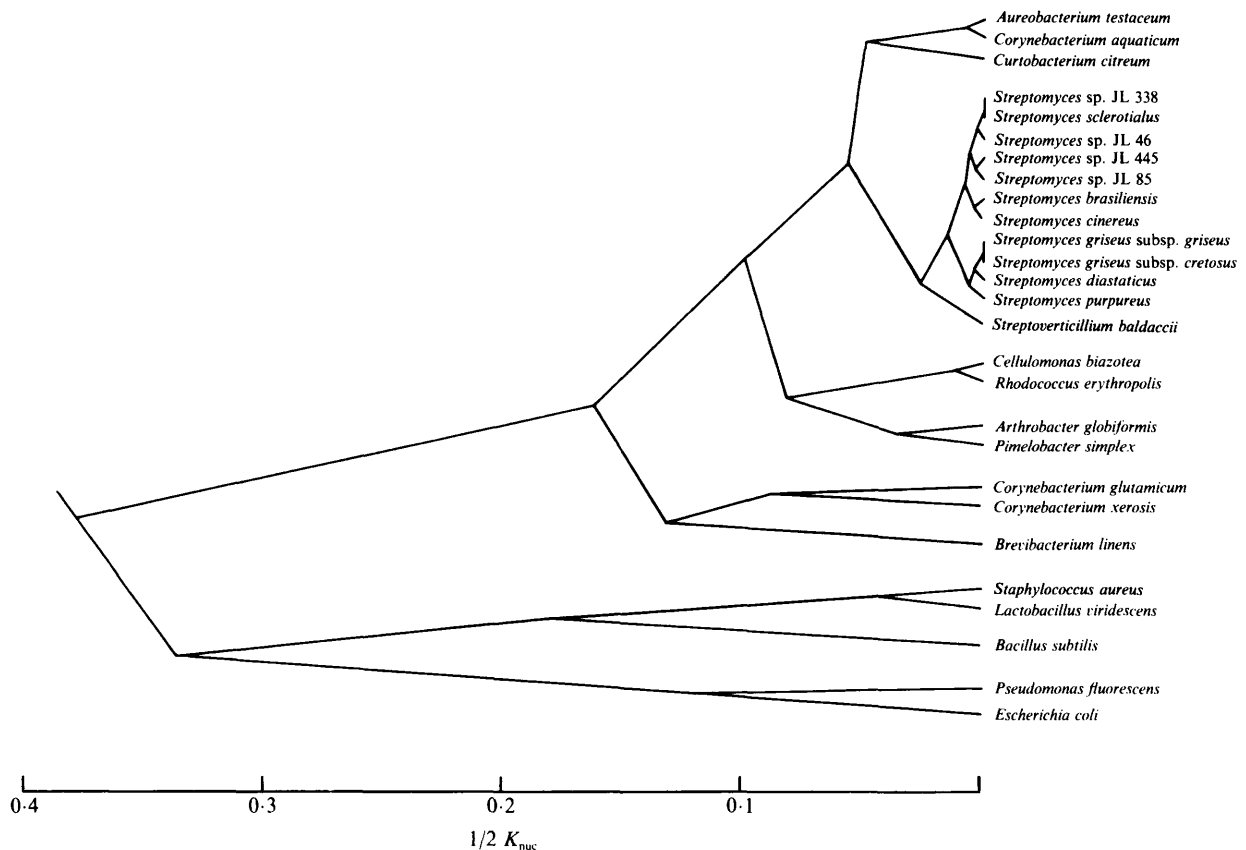


Fig. 2. Phylogenetic relationships among streptomycetes and selected Gram-positive and Gram-negative bacteria based on 5S rRNA sequence data.

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. brasiliensis* KCTC 9071, *S. cinereus* KCTC 9066, *S. sclerotialis* KCTC 9065 and the acidophilic streptomycetes.

This research was supported by grant BSE7029-125-3 from the Ministry of Science & Technology (MOST) of the Republic of Korea and by UNDP, ROK/87/018/A/01/12. One of us (J.L.) gratefully acknowledges receipt of a NERC studentship. We would also like to express our appreciation to Professor Yung-Chil Hah, Professor Kook-Hee Kang and Dr Heui-il Kang for helpful suggestions and discussions. We also thank Kie-Jung Park, Keun-Chul Lee, and Moon-Soo Lee for their excellent computing and technical assistance.

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