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Streptomyces seoulensis sp. nov.

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The taxonomic position of an actinomycete strain isolated from Korean soil was examined by a polyphasic approach. The isolate, designated IMSNU-1, was clearly assigned to the genus *Streptomyces* on the basis of morphological and chemotaxonomic data. The test strain was the subject of a probabilistic identification study using the identification matrices generated by Langham et al. (J. Gen. Microbiol. 135:121–133, 1989) and found to be marginally close to clusters 19 and 39. An almost complete 16S rRNA gene (rDNA) sequence was obtained for the test strain and compared with those of representative streptomycetes. 16S rDNA sequence data not only support the strain's membership in the genus *Streptomyces* but also provide strong evidence that our isolate is genealogically distant from representatives of clusters 19 and 39, forming a separate phyletic line in a clade encompassed by streptomycetes. It is therefore proposed from the polyphasic evidence that strain IMSNU-1 be classified in the genus *Streptomyces* as *Streptomyces seoulensis* sp. nov.

The genus *Streptomyces* was proposed by Waksman and Henrici (34) for aerobic and spore-forming actinomycetes. The taxon currently accommodates aerobic gram-positive bacteria that have DNA with a high guanine-plus-cytosine content (69 to 73 mol%) and that form extensive branching substrates and aerial mycelia (16, 37, 39). Streptomycetes can be also defined by their combination of chemical markers, that is, by the presence of LL-diaminopimelic acid and the absence of characteristic sugars in the cell wall (wall chemotype I according to the work of Lechevalier and Lechevalier [21]). In addition, 16S rRNA sequence data have proved invaluable in streptomycete systematics, in which they have been used to provide the basis for proposals to unify the genera *Kitasatosporia* (35) and *Streptoverticillium* (40) with the genus *Streptomyces*, and in other phylogenetic studies that sought to determine the intrageneric structure of a genus (15, 25, 32, 33).

Streptomycetes are a well-known source of bioactive compounds, such as antibiotics, tumor repressors, and enzymes (4, 16, 39). We have isolated a streptomycete strain, designated IMSNU-1, from Korean soil and reported previously the biochemical characteristics of its catalase-peroxidase (41). In addition, we have isolated and characterized a novel type of superoxide dismutase containing nickel from this organism and from *Streptomyces coelicolor* A3(2) (42). The aim of the present study is to characterize and identify this strain by a polyphasic approach.

MATERIALS AND METHODS

Bacterial strain. Strain IMSNU-1 was isolated from soil collected at Kwanak Mountain, Seoul, Republic of Korea, with Bennett's agar (12) complemented with nystatin (50 mg/ml) at 30°C. The pure culture was maintained as glycerol suspensions (20%, vol/vol) of spores and mycelial fragments at −25°C.

Phenotypic characterization. Morphological properties were examined according to the culture conditions and methods proposed by Williams et al. (37, 39). The microscopes used were an Optiphot-2 phase-contrast microscope (Nikon, Tokyo, Japan) and a Stereoscan 260 scanning electron microscope (Cambridge Ltd., Cambridge, United Kingdom). Phenotypic characters of strain IMSNU-1 were determined according to the methods proposed by Williams et al. (37).

Computer-assisted identification was achieved with Willcox probabilities (36) and the taxon radius model (30). In the taxon radius model, four identification scores were obtained for the following parameters: (i) d, the taxonomic distance from an unknown strain to a given taxon, J; (ii) $s.e.(d)$, the standard error of d, which is expressed as the constant (c) in the equation $d = \overline{d_J} + cSD_J$, where $\overline{d_J}$ is the mean distance from operational taxonomic units (OTUs) in taxon J to its centroid and SD_J is its standard deviation; (iii) $Gauss[s.e.(d)]$, the Gaussian integral of the $s.e.(d)$ score, which is determined by using the algorithm of Hill (10); and (iv) the 95% taxonomic radius, defined as $\overline{d_J} + 1.645SD_J$, which indicates a theoretical radius that encompasses 95% of OTUs in taxon J on the assumption that there is a normal distribution of OTUs in hyperspherical space. All analyses were carried out with the X program (2), which was adopted from the original MATIDEN program (31).

Chemotaxonomy. The test strain was grown in Bennett's broth at 30°C for 7 days. Biomass was harvested by centrifugation, and the pellet was washed twice with aqueous KCl solution (0.85%, wt/vol). The cell wall was partially purified with a Bead-Beater (Biospec Products, Bartlesville, Okla.), and the resultant suspension was collected by centrifugation. Analyses of diaminopimelic acid and sugars were performed by the method of Lechevalier and Lechevalier (21). The fatty acid methyl esters (FAMEs) were prepared by the alkaline methanolysis method (26). The resultant FAMEs were separated with a gas chromatograph (model 5890; Hewlett-Packard Co., Palo Alto, Calif.) fitted with a model HP-5 capillary column (0.53 mm by 30 m; Hewlett-Packard). The column was kept at 150°C for 2 min and then programmed to change from 150 to 280°C at the rate of 10°C/min, with helium as a carrier gas. The resultant peaks were identified with a mixture of standard FAMEs and by gas chromatography-mass spectrometry (VG Quattro; Fisons, Altrincham, United Kingdom). The phospholipids were extracted from frozen cells with chloroform–methanol–0.3% aqueous sodium chloride (9:10:3, vol/vol/vol) and detected by two-dimensional thin-layer chromatography according to the methods proposed by Embley and Wait (5).

DNA base composition. The guanine-plus-cytosine content of DNA was determined by the thermal denaturation method (24).

16S rDNA sequencing. The chromosomal DNA of strain IMSNU-1 was isolated according to the procedure described by Hopwood et al. (11). The PCR, cloning, and sequencing of the 16S rRNA gene (rDNA) were carried out with a *Taq* DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) and an Applied Biosystems 373A DNA sequencer as described elsewhere (3). The resultant 16S rDNA sequence of strain IMSNU-1 was aligned manually against representative sequences of streptomycetes obtained from the Ribosomal Database Project (23) and EMBL databases. Evolutionary trees were inferred by using three tree algorithms, namely, the neighbor-joining (29), Fitch-Margoliash (9), and maximum-parsimony (8) methods. The distance model of Jukes and Cantor (13) was used for generating an evolutionary distance matrix. The tree was rooted with three outgroup sequences, namely, those of *Actinoplanes philippinensis*, *Dactylosporangium aurantiacum*, and *Streptosporangium ro-*

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TABLE 1. Results of the numerical phenetic identification of strain IMSNU-1 based on the probabilistic matrix of Williams et al. (38) as arranged by Langham et al. (19)

Cluster[a] (species)	Identification score				
	Willcox score	95% Taxon radius	*d*	*s.e.*(*d*)	*Gauss*[*s.e.*(*d*)]
Major clusters (26 clusters with 50 unit characters)					
19 (*Streptomyces diastaticus*)	0.48	0.4508	0.4028	0.41	0.3404
3 (*Streptomyces atroolivaceus*)	0.36	0.3621	0.4148	3.33	0.0004
15 (*Streptomyces chromofuscus*)	0.16	0.4259	0.4482	2.25	0.0122
5 (*Streptomyces exofoliatus*)	0.00	0.4463	0.4736	2.35	0.0093
18 (*Streptomyces cyaneus*)	0.00	0.4504	0.4973	2.85	0.0022
1B (*Streptomyces albidoflavus*)	0.00	0.4406	0.5026	3.27	0.0005
37 (*Streptomyces griseoflavus*)	0.00	0.3668	0.4692	4.88	0.0000
1C (*Streptomyces albidoflavus*)	0.00	0.3887	0.4823	4.43	0.0000
23 (*Streptomyces microflavus*)	0.00	0.3931	0.4733	4.01	0.0000
12 (*Streptomyces rochei*)	0.00	0.4185	0.4880	3.56	0.0002
Minor clusters (28 clusters with 39 unit characters)					
39 (*Streptomyces longisporoflavus*)	0.99	0.3562	0.4771	5.18	0.0000
2 ("*Streptoverticillium olivoverticillatum*")	0.01	0.3962	0.5314	5.19	0.0000
38 (*Streptomyces prasinosporus*)	0.00	0.3962	0.5623	6.01	0.0000
34 (*Streptomyces nogalater*)	0.00	0.4056	0.5940	6.48	0.0000
63 (*Streptomyces xanthochromogenes*)	0.00	0.4056	0.6156	7.03	0.0000
25 (*Streptomyces canus*)	0.00	0.3538	0.5785	8.26	0.0000
9 (*Streptomyces californicus*)	0.00	0.3454	0.6137	9.73	0.0000
43 (*Streptomyces griseoluteus*)	0.00	0.3666	0.6111	8.59	0.0000
35 (*Streptomyces chattanoogensis*)	0.00	0.3015	0.5994	11.94	0.0000
24 (*Streptomyces flaveolus*)	0.00	0.3562	0.6436	10.06	0.0000

[a] The first ten clusters with the highest Willcox scores are given. Clusters are numbered according to the system of Williams et al. (37).

seum; these species were found to be close relatives of streptomycetes when all of the representative actinomycete sequences were examined. The topologies of resultant trees were evaluated by the bootstrap analyses (6) of the neighbor-joining method based on 1,000 resamplings. The PHYLIP package (7) was used for all phylogenetic analyses.

Nucleotide sequence accession number. The partial 16S rDNA sequence of strain IMSNU-1 has been deposited in the EMBL database under accession number Z71365.

RESULTS

Morphological characteristics. Strain IMSNU-1 showed the typical morphology of streptomycetes on various agar plates. The color of the substrate mycelium was yellow on glycerol-asparagine agar (International *Streptomyces* Project [ISP] 5), and the color of the aerial spores was gray on inorganic salts-starch agar (ISP 4). The vegetative hyphae grown in Bennett's broth were branched but not fragmented. Verticils were not detected. The test strain produced long-chain rectiflexible spores with smooth surfaces. Diffusible pigment was not detected on ISP 5 medium. Melanin was not produced on peptone-yeast extract-iron agar (ISP 6) and tyrosine agar (ISP 7) plates.

Computer-assisted identification. The results of the probabilistic identification of strain IMSNU-1 against the identification matrices of Langham et al. (19) are summarized in Table 1. The test strain showed its highest Willcox score (0.48) when it was compared with cluster 19 of Williams et al. (37), and its next-highest scores were obtained when it was compared with clusters 3 (0.36) and 15 (0.16), when the probability matrix containing 29 major clusters was employed. In contrast, strain IMSNU-1 was successfully assigned to cluster 19 by the taxon radius model, given its small *s.e.*(*d*) score (0.41) and taxonomic distance ($d = 0.4028$), the latter of which places the test strain within the 95% taxon radius (0.4508) (Table 1). None of the remaining clusters in the identification matrix generated for major streptomycete clusters gave an *s.e.*(*d*) score of less than 2 or a taxonomic distance less than that of the 95% taxon radius. By the same procedure, strain IMSNU-1 was examined against the identification matrix comprising 28 minor clusters (19). The strain was identified as belonging to cluster 39 (38) on the basis of its Willcox score (0.99). However, none of the minor streptomycete clusters, including cluster 39, showed an *s.e.*(*d*) score of less than 5 or a taxonomic distance less than that of the 95% taxon radius.

Chemotaxonomy. Only LL-diaminopimelic acid was detected in whole-cell hydrolysates, and no diagnostic sugars were present, indicating that our isolate has a chemotype I cell wall (21). Phosphatidylethanolamine, phosphatidylinositol, diphosphatidyl glycerol, and phosphatidylinositol mannosides were present in the polar lipid fraction (phospholipid type II according to the work of Lechevalier et al. [22]). Saturated straight-chain and *iso*- and *anteiso*-branched fatty acids were found (fatty acid type 2c according to the work of Kroppenstedt [17]). All of these chemical properties provide strong evidence that strain IMSNU-1 is a member of the genus *Streptomyces.*

16S rDNA sequencing. Strain IMSNU-1 was the subject of a 16S rDNA sequencing study to reveal its phylogenetic relationships with representative streptomycetes. An almost complete 16S rDNA sequence was determined (1,479 nucleotides). Its similarity to nucleotide sequences of representatives of the genus *Streptomyces* is given in Table 2. *Streptomyces subrutilus* showed the highest similarity (98.4%), though *Streptomyces griseus* and *Streptomyces setonii* also exhibited comparable levels of similarity (98.3%). A rooted phylogenetic tree based on the neighbor-joining method is given in Fig. 1. The isolate represented a separate phyletic line among streptomycetes and formed a sister species to a clade containing the type strains of *Streptomyces lavendulae*, *S. subrutilus*, *S. griseus*, and *S. setonii*. The phylogenetic position of strain IMSNU-1 is stable to some extent, though the branch-off points of strain IMSNU-1 and

TABLE 2. 16S rRNA sequence similarity values between strain IMSNU-1 and representatives of the genus *Streptomyces*

Streptomyces species[a]	Similarity to strain IMSNU-1 (%)	No. of nucleotide differences/total no. of nucleotides compared
S. abikoensis	96.6	38/1,102
S. acidiscabies	97.8	32/1,435
S. albus	95.8	49/1,177
S. ambofaciens	97.1	41/1,435
S. baldaccii	96.7	36/1,085
S. bikiniensis	97.4	38/1,435
S. bluensis	96.4	51/1,435
S. bottropensis	97.6	35/1,435
S. brasiliensis	95.8	50/1,177
S. caelestis	96.4	51/1,435
S. cinnamoneus	96.5	41/1,155
S. coelicolor A3(2)	97.0	43/1,435
S. diastaticus	97.0	34/1,127
S. diastatochromogenes	97.6	35/1,435
S. eurythermus	97.6	35/1,435
S. galbus	97.6	35/1,435
S. griseolosporeus	95.1	67/1,364
S. griseus	98.3	25/1,434
S. ladakanum	96.6	38/1,122
S. lavendulae	97.8	25/1,122
S. lincolnensis	97.7	33/1,435
S. macrosporus	95.0	72/1,433
S. mashuensis	97.4	37/1,434
S. megasporus	94.0	86/1,433
S. neyagawaensis	97.4	38/1,435
S. olivoreticuli subsp. *cellulophilus*	97.0	35/1,150
S. phosalacineus	94.6	74/1,370
S. purpureus	97.2	32/1,126
S. salmonis	96.6	36/1,072
S. sampsonii	97.1	41/1,435
S. scabies	97.5	36/1,435
S. setae	95.1	67/1,375
S. setonii	98.3	24/1,435
S. subrutilus	98.4	23/1,435
S. tendae	97.2	40/1,435
S. thermodiastaticus	96.1	56/1,435
S. thermolineatus	95.5	64/1,435
S. thermonitrificans	95.9	59/1,435
S. thermoviolaceus subsp. *apingens*	96.7	47/1,435
S. thermoviolaceus subsp. *thermoviolaceus*	96.3	53/1,435
S. thermovulgaris	96.0	58/1,435

[a] Strains were type cultures unless otherwise indicated.

Streptomyces purpureus were interchanged when the Fitch-Margoliash method was employed (Fig. 1).

DISCUSSION

The results of morphological and chemotaxonomic studies of isolate IMSNU-1 clearly placed this organism in the genus *Streptomyces*. The test strain was then the subject of a computer-assisted identification study using the comprehensive phenetic database generated by Langham et al. (19).

It has been emphasized by several workers (19, 27, 28, 38) that both probabilistic approaches, namely, the use of the Willcox score based on the Baysian model and the use of scores based on the taxon radius model, must be considered to avoid the assignment of fresh isolates to the incorrect taxon in situations where the correct taxon is not represented in the identification matrix. Bascomb et al. (1) adopted a Willcox score of >0.999 as the threshold value for successful identification, and Lapage et al. (20) showed that setting the threshold to less than 0.99 led to incorrect identifications. In contrast, since this level of threshold was thought to be too stringent for the genus *Streptomyces*, in which several species are likely to be heterogeneous, the threshold value of 0.95 was suggested by Williams et al. (38). In the taxon radius model, the most popular criterion is the *s.e.*(d) score, which takes into consideration the standard deviations of the mean distance of OTUs in a cluster (31). A negative score implies a distance closer to the centroid than the mean, and a value of less than 2 or 3 is considered an indication of successful identification (28).

In the present study, we have adopted an additional score, i.e., the 95% taxon radius. This score indicates the envelope that theoretically encompasses 95% of OTUs in a given cluster and can be compared with the taxonomic distance (d) between an isolate and the centroid. It is worth noting that the Willcox score represents the relative relationship of a test strain to the clusters in a given identification matrix but does not indicate absolute closeness to each cluster. In contrast, the scores based on the taxon radius model provide somewhat objective criteria that are independent of the addition or deletion of clusters in the identification matrix.

It is evident from Table 1 that strain IMSNU-1 cannot be assigned to any of the known streptomycete clusters when the results of both the Willcox probability analysis and the taxon radius analysis are considered. It is therefore concluded from a study of numerical phenetic identifications that strain IMSNU-1 shows no apparent phenetic relationship with the clusters defined by Williams et al. (37), though as possible candidates, clusters 19 and 39 gave satisfactory scores based on either Willcox probabilities or the taxon radius model. Nevertheless, strain IMSNU-1 can be readily distinguished from the streptomycete clusters mentioned above on the basis of its phenotypic characters (Table 3).

Small-subunit rRNA sequence data provide strong evidence that our isolate is phylogenetically distinct from representatives streptomycetes. *S. subrutilus*, *S. griseus*, and *S. setonii* exhibited high levels of nucleotide similarities (98.3 to 98.4%). Strain IMSNU-1 was recovered as a sister group to a clade consisting of *S. lavendulae*, *S. subrutilus*, *S. griseus*, and *S. setonii. S. lavendulae* and *S. subrutilus* formed a significant monophyletic clade and showed almost identical 16S rRNA primary structures (99.6%). This apparent phylogenetic relationship was supported by numerical taxonomic studies of Williams et al. (cluster 61 [37]) and Kämpfer et al. (14) but not by a DNA-DNA pairing study of Labeda (18) in which these two strains exhibited a low level of homology (27%). Similarly, *S. griseus* and *S. setonii*, which belong to the phenotypic subcluster 1B (37), showed almost identical 16S rRNA sequences (99.9%) and also formed a well-supported monophyletic clade. Both of the phenotypic clusters 1B and 61 gave insignificant identification scores and therefore illustrate no apparent phenotypic relatedness to strain IMSNU-1 (Table 1 and data not shown). These data suggest that there is good agreement between numerical phenetic and 16S rRNA sequence data, though the degree of chromosomal heterogeneity, as revealed by DNA-DNA pairing studies, may be greater than those estimated from the other two procedures.

It is evident from Fig. 1 that strains which exhibited possible phenotypic relatedness to strain IMSNU-1 based on a probabilistic identification procedure, i.e., *Streptomyces diastaticus* (cluster 19 [37]), *Streptomyces lincolnensis* (cluster 19), *Streptomyces bottropensis* (cluster 19), and *Streptomyces bluensis* (cluster 39), showed no close phylogenetic relationships to our isolate or among themselves.

It is evident from the chemical, molecular systematic, and

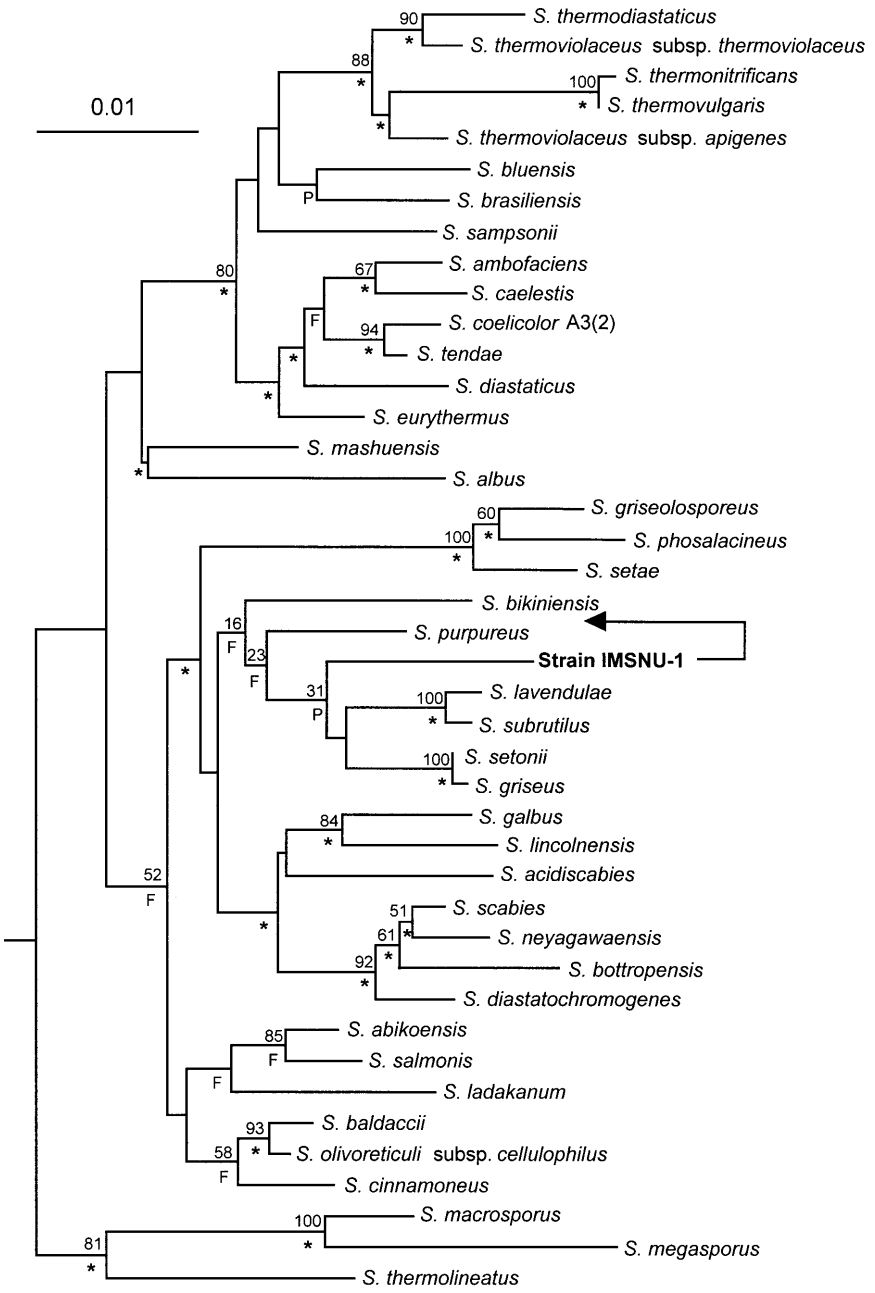

FIG. 1. Rooted neighbor-joining tree (29) based on 1,023 unambiguously aligned nucleotide positions. F and P indicate the branches that were also found when we used the Fitch-Margoliash (9) and maximum-parsimony (8) methods, respectively; the asterisks indicate branches that were recovered by all three methods. The arrowhead indicates the branch-off position of strain IMSNU-1 when the Fitch-Margoliash method was used. The numbers at the nodes exhibit the levels of bootstrap support based on a neighbor-joining analysis of 1,000 resampled datum sets. Test strains are type cultures unless otherwise indicated. The scale bar represents 0.01 nucleotide substitution per position.

phenotypic data that our isolate should be given species status in the genus *Streptomyces* Waksman and Henrici 1943^{AL}. Therefore, the name *Streptomyces seoulensis* is proposed for strain IMSNU-1. The type strain (strain IMSNU-1) was deposited in the Institute of Microbiology, Seoul National University, under accession number IMSNU 21266.

Description of *Streptomyces seoulensis* sp. nov. *Streptomyces seoulensis* (seo.ul.en′sis, M. L. masc. adj. *seoulensis*, indicating Seoul, Republic of Korea, the geographical origin of the species) is an aerobic, gram-positive, non-acid-fast, nonmotile actinomycete which forms a yellow substrate mycelium on glycerol-asparagine agar and a gray aerial mycelium and spores on inorganic salts-starch agar. Verticils are not present. Long-chain rectiflexible spores with smooth surfaces are produced. Diffusible pigments are not produced on ISP 5 medium. Melanin is not produced on peptone-yeast extract-iron agar and tyrosine agar.

The cell wall contains LL-diaminopimelic acid, and no diagnostic sugars are present in the cell wall fraction (chemotype I). Phosphatidylethanolamine, phosphatidylinositol, diphos-

TABLE 3. Phenotypic characters that differentiate strain IMSNU-1 from the related clusters defined by Williams et al. (37)

Phenotype	Presence or absence of phenotype in IMSNU-1	% of strains with indicated phenotype in cluster:			
		3 (n = 9)[a]	15 (n = 9)	19 (n = 20)	39 (n = 3)
Morphology and pigment production					
Rectiflexibiles spore chains	+	100	22	40	0
Spirales spore chains	−	0	78	60	67
Red spore mass	−	0	0	15	0
Gray spore mass	+	67	33	50	0
Red-orange mycelial pigment	−	0	0	15	0
Diffusible pigment	−	11	11	10	0
Yellow-brown diffusible pigment	−	0	11	5	0
Melanin on peptone-yeast extract-iron agar	−	0	33	50	33
Melanin on tyrosine agar	−	11	22	50	0
Antibiosis to:					
Bacillus subtilis	−	33	11	20	0
Micrococcus luteus	−	33	11	20	0
Candida albicans	−	11	0	0	0
Saccharomyces cerevisiae	−	0	0	0	0
Streptomyces murinus	−	33	22	5	0
Aspergillus niger	−	11	0	10	0
Enzyme activity					
Lecithinase production	−	11	11	0	0
Lipolysis	−	100	89	25	100
Hippurate hydrolysis	−	11	11	25	
Pectin hydrolysis	+	89	22	70	100
Nitrate reduction	−	89	22	45	100
Hydrogen sulfide production	−	100	89	80	100
Degradation of:					
Guanine	−	100	78	65	0
Elastin	+	89	67	35	67
Xanthine	−	100	22	50	33
Xylan	−	33	33	15	67
Urea	+	100	67	100	100
Allantoin	+	44	33	30	33
Arbutin	−	100	100	55	67
Resistance to antibiotics					
Neomycin (50 μg/ml)	−	11	0	0	0
Rifampin (50 μg/ml)	+	89	33	70	100
Oleandomycin (100 μg/ml)	−	78	0	25	33
Penicillin G (10 IU/ml)	+	100	44	50	67
Growth at 45°C	−	0	67	15	0
Growth in the presence of (%, wt/vol):					
Sodium chloride (7)	+	44	44	30	67
Sodium azide (0.01)	−	0	56	5	67
Phenol (0.1)	−	56	22	95	33
Potassium tellurite (0.001)	+	56	67	75	67
Thallous acetate (0.001)	−	0	0	5	0
Growth on sole nitrogen source (0.1%, wt/vol):					
DL-α-Amino-*n*-butyric acid	−	33	67	30	0
Potassium nitrate	−	100	100	90	100
L-Cysteine	−	56	67	75	33
L-Valine	−	0	33	70	0
L-Phenylalanine	−	78	11	20	33
L-Histidine	+	78	78	70	0
L-Hydroxyproline	−	11	0	20	33
Growth on sole carbon source (1%, wt/vol, exceptions noted):					
L-Arabinose	−	78	100	95	100
Sucrose	+	11	33	75	67
D-Xylose	+	67	100	90	100
meso-Inositol	−	0	89	80	100

Continued on following page

TABLE 3—*Continued*

Phenotype	Presence or absence of phenotype in IMSNU-1	% of strains with indicated phenotype in cluster:			
		3 (n = 9)[a]	15 (n = 9)	19 (n = 20)	39 (n = 3)
Mannitol	+	89	100	85	100
D-Fructose	+	78	100	100	100
L-Rhamnose	+	78	67	95	100
Raffinose	+	22	22	85	100
D-Melezitose	−	0	22	25	33
D-Lactose	+	89	100	90	100
Adonitol	−	11	22	15	0
Salicin	+	44	78	90	67
D-Melibiose	+	11	44	95	67
Dextran	−	22	78	15	67
Xylitol	−	0	0	15	0
Sodium acetate (0.1%, wt/vol)	−	33	78	40	0
Sodium citrate (0.1%, wt/vol)	−	44	89	65	33
Sodium propionate (0.1%, wt/vol)	−	44	100	85	0
Sodium pyruvate (0.1%, wt/vol)	+	44	78	65	0

[a] n, number of strains.

phatidyl glycerol, and phosphatidylinositol mannosides are present in the polar lipid fraction (phospholipid type II). The fatty acids are mainly saturated straight-chain as well as *iso*- and *anteiso*-branched fatty acids (fatty acid type 2c).

Activity is not exhibited against *Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Micrococcus luteus*, *Saccharomyces cerevisiae*, or *Streptomyces murinus*. The strain grows in the presence of potassium tellurite, rifampin, and penicillin G but not at 45°C or in the presence of sodium chloride, sodium azide, thallous acetate, neomycin, or oleandomycin. It utilizes L-histidine as a sole nitrogen source but not DL-α-amino-*n*-butyric acid, L-cysteine, L-valine, L-phenylalanine, L-hydroxyproline, or potassium nitrate. It uses D-fructose, lactose, mannitol, D-melibiose, raffinose, L-rhamnose, salicin, sodium pyruvate, sucrose, and D-xylose as sole sources of carbon but not adonitol, L-arabinose, dextran, *meso*-inositol, D-melezitose, sodium acetate, sodium citrate, sodium propionate, or xylitol. Tests for lecithinase, lipolysis, pectin hydrolysis, and H_2S production are positive but not those for hippurate hydrolysis or nitrate reduction. The organism degrades allantoin, arbutin, and elastin but not guanine, xanthine, or xylan.

The guanine-plus-cytosine ratio of the DNA is 68 mol%.

The type strain is *Streptomyces seoulensis* IMSNU 21266^T.

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REFERENCES

1. **Bascomb, S., S. P. Lapage, M. A. Curtis, and W. R. Willcox.** 1973. Identification of bacteria by computer: identification of reference strains. J. Gen. Microbiol. **77:**291–315.
2. **Chun, J.** 1995. Computer assisted classification and identification of actinomycetes. Ph.D. thesis. University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom.
3. **Chun, J., and M. Goodfellow.** 1995. A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. Int. J. Syst. Bacteriol. **45:**240–245.
4. **Crandall, L. W., and R. L. Hamill.** 1986. Antibiotics produced by *Streptomyces*: major structural classes, p. 355–401. *In* S. W. Queener and L. E. Day (ed.), The bacteria, vol. 9. Academic Press, Orlando, Fla.
5. **Embley, T. M., and R. Wait.** 1994. Structural lipids of eubacteria, p. 121–161. *In* M. Goodfellow and A. G. O'Donnell (ed.), Chemical methods in prokaryotic systematics. John Wiley and Sons, Chichester, United Kingdom.
6. **Felsenstein, J.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791.
7. **Felsenstein, J.** 1993. PHYLIP (phylogenetic inference package) version 3.5c. Department of Genetics, University of Washington, Seattle.
8. **Fitch, W. M.** 1972. Toward defining the course of evolution: minimum change for a specific tree topology. Syst. Zool. **20:**406–416.
9. **Fitch, W. M., and E. Margoliash.** 1967. Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome c sequences is of general applicability. Science **155:**279–284.
10. **Hill, I. D.** 1985. Algorithm AS66: the normal integral, p. 126–129. *In* P. Griffiths and I. D. Hill (ed.), Applied statistics algorithms. Royal Statistical Society, Ellis Horwood Ltd., Chichester, United Kingdom.
11. **Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, J. M. Ward, and H. Schrempf.** 1985. Genetic manipulation of streptomycetes: a laboratory manual. John Innes Foundation, Norwich, United Kingdom.
12. **Jones, K. L.** 1949. Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. J. Bacteriol. **57:** 141–145.
13. **Jukes, T. H., and C. R. Cantor.** 1969. Evolution of protein molecules, p. 21–132. *In* H. N. Munro (ed.), Mammalian protein metabolism. Academic Press, New York, N.Y.
14. **Kämpfer, P., R. M. Kroppenstedt, and W. Dott.** 1991. A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tests. J. Gen. Microbiol. **137:**1831–1891.
15. **Kim, D., J. Chun, N. Sahin, Y. C. Hah, and M. Goodfellow.** 1996. Analysis of thermophilic clades within the genus *Streptomyces* by 16S ribosomal DNA sequence comparisons. Int. J. Syst. Bacteriol. **46:**581–587.
16. **Korn-Wendisch, F., and H. J. Kutzner.** 1992. The family *Streptomycetaceae*, p. 921–995. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York, N.Y.
17. **Kroppenstedt, R. M.** 1985. Fatty acid and menaquinone analysis of actinomycetes and related organisms, p. 173–199. *In* M. Goodfellow and D. E. Minnikin (ed.), Chemical methods in bacterial systematics. Academic Press, London, United Kingdom.
18. **Labeda, D. P.** 1993. DNA relatedness among strains of the *Streptomyces lavendulae* phenotypic cluster group. Int. J. Syst. Bacteriol. **43:**822–825.
19. **Langham, C. D., S. T. Williams, P. H. A. Sneath, and A. M. Mortimer.** 1989. New probability matrices for identification of *Streptomyces*. J. Gen. Microbiol. **135:**121–133.
20. **Lapage, S. P., S. Bascomb, W. R. Willcox, and M. A. Curtis.** 1973. Identification of bacteria by computer: general aspects and perspectives. J. Gen. Microbiol. **77:**273–290.
21. **Lechevalier, M. P., and H. A. Lechevalier.** 1970. Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol. **20:**435–443.
22. **Lechevalier, M. P., C. De Biévre, and H. A. Lechevalier.** 1977. Chemotaxonomy of aerobic actinomycetes: phospholipid composition. Biochem. Syst. Ecol. **5:**249–260.
23. **Maidak, B. L., N. Larsen, M. J. McCaughey, R. Overbeek, G. J. Olsen, K. Fogel, J. Blandy, and C. R. Woese.** 1994. The ribosomal database project. Nucleic Acids Res. **22:**3485–3487.
24. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5:**109–118.
25. **Mehling, A., U. F. Wehmeier, and W. Piepersberg.** 1995. Nucleotide se-

quences of streptomycete 16S ribosomal DNA: towards a specific identification system for streptomycetes using PCR. Microbiology **141:**2139–2147.
26. **Minnikin, D. E., and A. G. O'Donnell.** 1984. Actinomycete envelope lipid and peptidoglycan composition, p. 337–388. *In* M. Goodfellow, M. Mordarski, and S. T. Williams (ed.), The biology of the actinomycetes. Academic Press, London, United Kingdom.
27. **Priest, F. G., and B. Alexander.** 1988. A frequency matrix for probabilistic identification of some bacilli. J. Gen. Microbiol. **134:**3011–3018.
28. **Priest, F. G., and S. T. Williams.** 1993. Computer-assisted identification, p. 361–381. *In* M. Goodfellow and A. G. O'Donnell (ed.), Handbook of new bacterial systematics. Academic Press, London, United Kingdom.
29. **Saitou, N., and M. Nei.** 1987. The neighbor joining method: a new method for constructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
30. **Sneath, P. H. A.** 1978. Identification of micro-organisms, p. 10/1–10/32. *In* J. R. Norris and M. H. Richmond (ed.), Essays in microbiology. John Wiley and Sons, Chichester, United Kingdom.
31. **Sneath, P. H. A.** 1979. BASIC programs for identification of an unknown with presence-absence data against an identification matrix of percent positive characters. Comput. Geosci. **5:**195–213.
32. **Stackebrandt, E., D. Witt, C. Kemmerling, R. M. Kroppenstedt, and W. Liesack.** 1991. Designation of streptomycete 16S and 23S rRNA-based target regions of oligonucleotide probes. Appl. Environ. Microbiol. **57:**1468–1477.
33. **Takeuchi, T., H. Sawada, F. Tanaka, and I. Matsuda.** 1996. Phylogenetic analysis of *Streptomyces* spp. causing potato scab based on 16S rRNA sequences. Int. J. Syst. Bacteriol. **46:**476–479.
34. **Waksman, S. A., and A. T. Henrici.** 1943. The nomenclature and classification of the actinomycetes. J. Bacteriol. **46:**337–341.
35. **Wellington, E. M. H., E. Stackebrandt, D. Sanders, J. Wolstrup, and N. O. G. Jorgensen.** 1992. Taxonomic status of *Kitasatosporia*, and proposed unification with *Streptomyces* on the basis of phenotypic and 16S rRNA analysis and emendation of *Streptomyces* Waksman and Henrici 1943, 339^{AL}. Int. J. Syst. Bacteriol. **42:**156–160.
36. **Willcox, W. R., S. P. Lapage, S. Bascomb, and M. A. Curtis.** 1973. Identification of bacteria by computer: theory and programming. J. Gen. Microbiol. **77:**317–330.
37. **Williams, S. T., M. Goodfellow, G. Alderson, E. M. H. Wellington, P. H. A. Sneath, and M. J. Sackin.** 1983. Numerical classification of *Streptomyces* and related genera. J. Gen. Microbiol. **129:**1743–1813.
38. **Williams, S. T., M. Goodfellow, E. M. H. Wellington, J. C. Vicker, G. Alderson, P. H. A. Sneath, M. J. Sackin, and A. M. Mortimer.** 1983. A probability matrix for identification of some streptomycetes. J. Gen. Microbiol. **129:** 1815–1830.
39. **Williams, S. T., M. Goodfellow, and G. Alderson.** 1989. Genus *Streptomyces* Waksman and Henrici 1943, 339^{AL}, p. 2452–2492. *In* S. T. Williams, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 4. Williams & Wilkins, Baltimore, Md.
40. **Witt, D., and E. Stackebrandt.** 1990. Unification of the genera *Streptoverticillium* and *Streptomyces*, and amendation of *Streptomyces* Waksman and Henrici 1943, 339^{AL}. Syst. Appl. Microbiol. **13:**361–371.
41. **Youn, H.-D., Y.-I. Yang, K. Kim, Y. C. Hah, and S.-O. Kang.** 1995. Spectral characterization and chemical modification of catalase-peroxidase from *Streptomyces* sp. J. Biol. Chem. **270:**13740–13747.
42. **Youn, H.-D., E.-J. Kim, J.-H. Roe, Y. C. Hah, and S.-O. Kang.** 1996. A novel nickel-containing superoxide dismutase from *Streptomyces* spp. Biochem. J. **318:**889–896.