DNA Relatedness among Verticil-Forming *Streptomyces* Species (Formerly *Streptovercillium* Species)

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Levels of DNA relatedness among 35 strains of *Streptomyces* species originally classified in the genus *Streptovercillium* were determined spectrophotometrically. These strains represent eight of the phenotypic cluster groups described for the genus *Streptovercillium* in *Bergey's Manual of Systematic Bacteriology*. Average linkage clustering of the DNA relatedness data resulted in 20 clusters including 13 single-member clusters, at a level of relatedness of >70%. Several species could be reduced to synonymy on the basis of DNA homology data, but these taxa were not generally equivalent to the clusters suggested by phenotypic numerical taxonomy data.

The genus *Streptovercillium* was described by Baldacci (1) to encompass strains which were previously described as members of the genus *Streptomyces* but had the following morphological property: production of long, straight hyphae containing side branches arranged in verticils bearing terminal umbels of twisted sporogenous hyphae. The taxonomy of the genus *Streptovercillium* has been a subject of discussion for a long time, and the number of species in this genus has ranged from 2, as proposed by Pridham (11), to the 66 nomenspecies grouped by Locci and Schofield (8) in 10 major clusters and 14 single-member clusters on the basis of the results of the numerical taxonomic study of Locci et al. (7).

There is phenetic overlap between the streptovercillia and *Streptomyces* strains, particularly members of the *Streptomyces lavendulae* cluster, but the results of previous rRNA-DNA hybridization studies (2) and differences in specific physiological properties supported the separation of the genus *Streptovercillium* from the genus *Streptomyces*. Recently, however, Witt and Stackebrandt (18) proposed, on the basis of the results of an analysis of 16S rRNA sequences of strains of streptovercillia and streptomycetes, that the two genera should be unified because of the dispersion of the streptovercillia among the streptomycetes in their phylogenetic analysis.

An analysis of the DNA relatedness of the nomenspecies in and between the major phenotypic clusters delineated by Locci and Schofield (8) was performed to determine the genetic integrity of these clusters.

MATERIALS AND METHODS

Strains and cultivation conditions. The strains included in this study are shown in Table 1. These strains represent eight of the phenotypic clusters of Locci and Schofield (8) and include the type strain of each nomenspecies as well as the type strains of other verticillate taxa that have not been validly described yet. The primary method of storage of the strains was as refrigerated ampoules containing freeze-dried spore and vegetative mycelium suspensions in sterile beef serum. Working stock cultures were maintained on slants of inorganic salts-starch agar or yeast extract-malt extract agar (15) and were stored at 4°C until they were needed. Biomass for extraction of DNA was grown in tryptone-glucose-liver extract-yeast extract broth (12) with shaking at 200 rpm at 28°C.

DNA isolation, purification, and hybridization. DNA was extracted from cells by the method of Marmur (10) after they were broken by passage through a French pressure cell (SLM Instruments, Inc., Urbana, Ill.) at 10,000 lb/in². The DNA was purified by chromatography on hydroxyapatite or by cesium chloride density gradient centrifugation (9). The levels of DNA relatedness between strains were calculated from $C_{ot_{0.5}}$ values in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) supplemented with 20% dimethyl sulfoxide at 65°C

($T_m - 23^\circ\text{C}$) by the method of Seidler and Mandel (14) and Seidler et al. (13), as described in Kurtzman et al. (3).

Statistical calculations. Statistical calculations involving the DNA relatedness data were performed by using SAS for Windows, release 6.11 (SAS Institute Inc., Cary, N.C.), and a Zeos Pantera 120-MHz Pentium microcomputer (Zeos Information Systems, Minneapolis, Minn.). The data were clustered by the average-linkage algorithm using the SAS/STAT CLUSTER procedure, and a dendrogram was generated with SAS/GRAPH by using the macro GRAFTREE, which was written and kindly provided by Dan Jacobs, University of Maryland.

RESULTS AND DISCUSSION

The genus *Streptovercillium* was described in *Bergey's Manual of Systematic Bacteriology*, vol. 4 (8), as a taxon that consists of 24 species (10 species groups and 14 single-member clusters), as determined by a numerical taxonomic analysis of 111 *Streptovercillium* and *Streptomyces* strains with pseudovercillate sporophores in which 185 morphological and physiological unit characters were examined. The levels of DNA relatedness for the 35 strains representing eight phenotypic clusters studied in this investigation support the hypothesis that there are 20 distinct species defined by levels of DNA relatedness equal to or greater than 70%. In general, the clusters based on DNA relatedness data are not the same as the cluster groups formed on the basis of numerical taxonomic results. Similar observations were made in DNA relatedness studies involving strains belonging to the *Streptomyces cyaneus* (5), *S. lavendulae* (4), and *Streptomyces violaceusniger* (6) numerical taxonomic cluster groups defined by Williams et al. (17).

Some strains belonging to each phenotypic cluster do exhibit significant levels of chromosomal similarity (level of DNA relatedness, >70%), indicating that they represent synonymous species. This can be clearly seen in the DNA relatedness data in Table 2 and the dendrogram resulting from average clustering of these data (Fig. 1).

All of the strains belonging to the *Streptomyces abikoensis* phenotypic cluster evaluated in this study exhibited more than 81% DNA relatedness to each other, indicating that the species names of these strains, "*Streptomyces takataensis*" NRRL B-12516 and *Streptomyces waksmanii* NRRL B-1707^T (T = type strain), are truly subjective synonyms of *S. abikoensis* NRRL B-2113^T. These data are in good agreement with data obtained in the previous DNA relatedness study of verticillate streptomycetes performed by Toyama et al. (16), although Toyama et al. obtained homology values of 70 to 72% by using the filter binding method. My data also indicate that "*Streptomyces rubroverticillatus*" NRRL B-16433 is misplaced in the *Streptomyces baldaccii* cluster, since it exhibits very high levels of DNA relatedness (81 to 100%) to all of the strains in the *S.*

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TABLE 1. Verticillate *Streptomyces* (*Streptoverticillium*) species included in this study

Cluster group ^a	Species	Strain ^b
<i>Streptomyces abikoensis</i> (formerly <i>Streptoverticillium abikoense</i>)	<i>Streptomyces abikoensis</i> ^c	NRRL B-2113 ^T (= ATCC 12766 ^T = CBS 487.62 ^T = DSM 40831 ^T = IFO 13860 ^T = JCM 4002 ^T)
	“ <i>Streptomyces takataensis</i> ”	NRRL B-12516 (= ATCC 27469 = CBS 771.62 = DSM 40576 = IFO 13476 = JCM 4836)
	<i>Streptomyces waksmanii</i> ^d	NRRL B-1707 ^T (= ATCC 12629 ^T = ATCC 25494 ^T = CBS 373.58 ^T = CBS 954.69 ^T = DSM 40464 ^T = IFO 13082 ^T = JCM 4514 ^T = JCM 4548 ^T)
<i>Streptomyces baldaccii</i> (formerly <i>Streptoverticillium baldaccii</i>)	<i>Streptomyces baldaccii</i> ^c	NRRL B-3500 ^T (= ATCC 23654 ^T = DSM 40845 ^T = IFO 14693 ^T = JCM 4272 ^T = IFO 14693 ^T)
	<i>Streptomyces biverticillatus</i> ^c	NRRL ISP-5272 ^T (= ATCC 23615 ^T = CBS 668.68 ^T = IFO 12845 ^T = JCM 4431 ^T)
	<i>Streptomyces diastallicus</i> ^c	NRRL 2886 ^T (= DSM 40846 ^T = NCIB 8936 ^T = JCM 4544 ^T)
	<i>Streptomyces fervens</i> ^c	NRRL 2755 ^T (= ATCC 27429 ^T = CBS 644.72 ^T = DSM 40086 ^T = IFO 13343 ^T = JCM 4310 ^T = JCM 4750 ^T)
	<i>Streptomyces flavopersicus</i> ^c	NRRL 2820 ^T (= ATCC 19756 ^T = CBS 494.68 ^T = DSM 40093 ^T = IFO 12769 ^T = JCM 4307 ^T = JCM 4370 ^T)
	<i>Streptomyces griseoverticillatus</i> ^c	NRRL B-12432 ^T (= ATCC 27436 ^T = CBS 721.72 ^T = DSM 40507 ^T = IFO 13420 ^T = RIA 1381 ^T = JCM 4202 ^T = JCM 4767 ^T)
	<i>Streptomyces hiroshimensis</i> ^c	NRRL B-5484 ^T (= ATCC 19772 ^T = CBS 510.68 ^T = DSM 40037 ^T = IFO 3839 ^T = IFO 12785 ^T = JCM 4098 ^T = JCM 4586 ^T = NCIB 9838 ^T)
	<i>Streptomyces kentuckensis</i> ^c	NRRL B-1831 ^T (= ATCC 12691 ^T = ATCC 23926 ^T = CBS 910.68 ^T = DSM 40052 ^T = IFO 12880 ^T = JCM 4153 ^T = JCM 4647 ^T = NCIB 9624 ^T)
	<i>Streptomyces netropsis</i> ^c	NRRL 2268 ^T (= ATCC 23940 ^T = CBS 924.68 ^T = DSM 40259 ^T = IFO 12893 ^T = JCM 4063 ^T = JCM 4655 ^T)
	<i>Streptomyces roseoverticillatus</i> ^c	NRRL B-1993 ^T (= ATCC 19807 ^T = CBS 560.68 ^T = DSM 40039 ^T = IFO 3844 ^T = IFO 12817 ^T)
	“ <i>Streptomyces rubrochlorinus</i> ”	NRRL B-12558 (= DSM 40850 = IFO 14694 = JCM 4281)
	“ <i>Streptomyces rubroverticillatus</i> ”	NRRL B-16433 (= DSM 41489 = JCM 4679)
<i>Streptomyces cinnamoneus</i> subsp. <i>cinnamoneus</i> (formerly <i>Streptoverticillium cinnamoneum</i> subsp. <i>cinnamoneum</i>)	<i>Streptomyces cinnamoneus</i> subsp. <i>cinnamoneus</i> ^c	NRRL B-1285 ^T (= ATCC 11874 ^T = ATCC 23897 ^T = CBS 293.64 ^T = CBS 683.68 ^T = DSM 40005 ^T = IFO 12852 ^T = JCM 4152 ^T = JCM 4633 ^T = NCIB 8851 ^T)
	<i>Streptomyces blastomyceticus</i> ^c	NRRL B-5480 ^T (= ATCC 19731 ^T = CBS 470.68 ^T = DSM 40029 ^T = IFO 12747 ^T = JCM 4184 ^T = JCM 4565 ^T = NCIB 9800 ^T)
	<i>Streptomyces cinnamoneus</i> subsp. <i>albosporus</i> ^d	NRRL B-5624 ^T (= ATCC 25186 ^T = DSM 40897 ^T = JCM 4968 ^T)
	<i>Streptomyces eurodicus</i> ^c	NRRL B-1676 ^T (= ATCC 27428 ^T = CBS 792.72 ^T = DSM 40604 ^T = IFO 13491 ^T = JCM 4029 ^T = JCM 4749 ^T)
	<i>Streptomyces lavenduligriseus</i> ^c	NRRL B-3173 ^T (= ATCC 13306 ^T = CBS 706.72 ^T)
<i>Streptomyces griseocarnes</i> (formerly <i>Streptoverticillium griseocarnes</i>)	<i>Streptomyces griseocarnes</i> ^c	NRRL B-1068 ^T (= ATCC 12628 ^T = ATCC 19763 ^T = CBS 501.68 ^T = DSM 40004 ^T = IFO 12776 ^T = JCM 4095 ^T = JCM 4580 ^T)
	<i>Streptomyces ardens</i> ^d	NRRL 2817 ^T (= ATCC 27417 ^T = CBS 731.72 ^T = DSM 40527 ^T = IFO 13430 ^T = JCM 4543 ^T = JCM 4722 ^T)
	<i>Streptomyces cinnamoneus</i> subsp. <i>azacolula</i> ^d	NRRL B-1699 ^T (= ATCC 12686 ^T = CBS 369.58 ^T = IFO 12363 ^T = JCM 4435 ^T)
	<i>Streptomyces mashuensis</i> ^c	NRRL B-8164 ^T (= ATCC 23934 ^T = CBS 279.65 ^T = CBS 918.68 ^T = DSM 40221 ^T = IFO 12888 ^T = JCM 4059 ^T = JCM 4650 ^T)
	<i>Streptomyces olivoverticillatus</i> ^c	NRRL B-1994 ^T (= ATCC 25480 ^T = CBS 890.69 ^T = DSM 40250 ^T = IFO 3842 ^T = IFO 3929 ^T = IFO 13068 ^T = JCM 4100 ^T = JCM 4501 ^T = NCIB 97148 ^T)
	“ <i>Streptomyces pentaticus</i> subsp. <i>genesis</i> ”	NRRL B-12088 ^T (= DSM 40848 ^T = JCM 4211 ^T)
	“ <i>Streptomyces tropicalensis</i> ”	NRRL B-12481 (= ATCC 17963 = CBS 729.72 = DSM 40520 = IFO 13428 = JCM 4845)
	<i>Streptomyces hachijoensis</i> (formerly <i>Streptoverticillium hachijoense</i>)	NRRL B-3106 ^T (= ATCC 19769 ^T = CBS 507.68 ^T = DSM 40114 ^T = IFO 12782 ^T = JCM 4331 ^T = JCM 4583 ^T)
	“ <i>Streptomyces taiuensis</i> ”	NRRL B-16435 (= ATCC 29758 = DSM 41499 = IFO 13808 = JCM 5008)
<i>Streptomyces salmonis</i> (formerly <i>Streptoverticillium salmonis</i>)	<i>Streptomyces salmonis</i> ^c	NRRL B-1472 ^T (= DSM 40895 ^T = IFO 15865 ^T = JCM 4083 ^T)
	<i>Streptomyces aureoversile</i> ^d	NRRL B-3325 ^T (= ATCC 15853 ^T = ATCC 25433 ^T = CBS 664.69 ^T = DSM 40387 ^T = IFO 13021 ^T = JCM 4457 ^T)
<i>Streptomyces ladakanum</i> (formerly <i>Streptoverticillium ladakanum</i>)	“ <i>Streptomyces reticulusruber</i> ”	NRRL B-1484 (= ATCC 25607 = CBS 208.78 = DSM 40893 = NCIB 12857)
	<i>Streptomyces ladakanum</i> ^c	NRRL 3191 ^T (= ATCC 27441 ^T = CBS 777.72 ^T = DSM 40587 ^T = IFO 13476 ^T = JCM 4778 ^T)
<i>Streptomyces mobaraensis</i> (formerly <i>Streptoverticillium mobaraense</i>)	<i>Streptomyces mobaraensis</i> ^c	NRRL B-3729 ^T (= ATCC 29032 ^T = CBS 199.75 ^T = DSM 40847 ^T = IFO 13819 ^T = JCM 4168 ^T = NCIB 11159 ^T)
	“ <i>Streptomyces luteoreticuli</i> ”	NRRL B-12435 ^T (= ATCC 27446 ^T = CBS 723.72 ^T = DSM 40509 ^T = IFO 13422 ^T = JCM 4788 ^T)

^a See reference 7.^b NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Ill.^c See reference 18.^d Former valid *Streptoverticillium* species or subspecies name not validated as a *Streptomyces* taxon by Witt and Stackebrandt (18).

TABLE 2. Levels of DNA relatedness among verticillate streptomycetes

Strain	% DNA relatedness to																																				
<i>S. griseovercillatus</i> NRRL B-12432 ^T	NRRL ^a B-8164 ^T	NRRL B-12432 ^T	NRRL B-12481	NRRL B-1068 ^T	NRRL B-3325 ^T	NRRL B-3106 ^T	NRRL B-16435	NRRL B-1699 ^T	NRRL B-1285 ^T	NRRL B-5624 ^T	NRRL 2886 ^T	NRRL 2268 ^T	NRRL 2820 ^T	NRRL B-1831 ^T	NRRL B-1994 ^T	NRRL B-3173 ^T	NRRL B-5484 ^T	NRRL B-1472 ^T	NRRL B-1484	NRRL B-1993 ^T	NRRL ISP-5272 ^T	NRRL B-12088 ^T	NRRL B-12558	NRRL 2755 ^T	NRRL B-3500 ^T	NRRL 2817 ^T	NRRL B-5480 ^T	NRRL B-12435 ^T	NRRL B-1676 ^T	NRRL 3191 ^T	NRRL B-3729 ^T	NRRL B-16433	NRRL B-12516	NRRL B-1707 ^T	NRRL B-2113 ^T		
" <i>S. tropicalensis</i> " NRRL B-12481	24	36	38	47	32	87	50	85	25	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89		
<i>S. griseocaneus</i> NRRL B-1068 ^T	18	44	38	47	32	87	50	85	25	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89		
<i>S. aureovercille</i> NRRL B-3325 ^T	29	25	35	47	32	87	50	85	25	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89		
<i>S. hachijensis</i> NRRL B-3106 ^T	39	38	52	40	32	87	50	85	25	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89		
" <i>S. taiocensis</i> " NRRL B-16435	24	24	28	48	34	87	50	85	25	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89		
<i>S. cinamomeus</i> subsp. <i>azacoluta</i> NRRL B-1699 ^T	17	26	43	51	25	81	91	50	85	25	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. cinamomeus</i> subsp. <i>cinamomeus</i> NRRL B-1285 ^T	35	48	31	53	42	56	64	50	85	25	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. cinamomeus</i> subsp. <i>albosporeus</i> NRRL B-5624 ^T	27	33	27	13	27	51	41	0	85	25	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. distalicius</i> NRRL 2886 ^T	15	25	21	26	13	27	9	14	35	25	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. netropsis</i> NRRL 2268 ^T	16	31	24	25	30	28	40	23	56	49	70	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. flavopescicus</i> NRRL 2820 ^T	28	30	0	0	37	0	20	43	10	25	61	86	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. kentuckensis</i> NRRL B-1831 ^T	9	11	48	28	46	50	28	32	27	42	73	70	100	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. olivovercillatus</i> NRRL B-1994 ^T	13	12	0	27	35	30	28	25	25	38	16	28	13	25	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89
<i>S. lavendericillatus</i> NRRL B-3173 ^T	13	23	30	17	30	40	19	47	37	20	14	11	25	32	31	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89
<i>S. hiroshimensis</i> NRRL B-5484 ^T	15	39	15	18	10	19	20	51	52	44	20	46	25	10	17	10	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89
<i>S. salmoneus</i> NRRL B-1472 ^T	22	19	34	23	26	22	33	21	34	38	22	39	10	25	55	15	43	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89
" <i>S. reticulustruber</i> " NRRL B-1484	15	16	28	33	18	25	42	20	45	14	27	17	2	14	28	9	65	100	43	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89
<i>S. roseovercillatus</i> NRRL B-1993 ^T	13	19	37	36	34	35	28	64	39	36	21	12	18	24	31	33	46	52	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. biverticillatus</i> NRRL ISP-5272 ^T	26	14	22	15	36	31	27	32	63	31	38	24	0	48	26	50	43	37	40	77	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
" <i>S. penitatus</i> subsp. <i>jeneis</i> " NRRL B-12088 ^T	10	0	25	0	6	8	19	18	0	22	40	14	0	11	27	7	51	47	49	76	76	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
" <i>S. rubrochlorinus</i> " NRRL B-12558	23	23	32	12	12	41	11	36	25	36	16	30	10	26	17	17	42	28	48	73	75	88	68	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. fervens</i> NRRL 2755 ^T	10	0	20	20	9	14	41	15	51	30	7	39	51	32	25	19	45	36	35	61	77	71	79	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. baldacii</i> NRRL B-3500 ^T	15	10	28	40	7	26	23	23	28	19	0	36	17	29	5	39	43	52	45	67	77	71	79	81	55	0	33	28	14	35	25	70	27	89	86	89	
<i>S. ardis</i> NRRL 2817 ^T	26	0	40	30	17	33	4	5	24	19	11	12	24	17	22	26	12	30	38	25	40	27	0	35	34	0	33	28	14	35	25	70	27	89	86	89	
<i>S. blastomyces</i> NRRL B-5480 ^T	12	24	7	37	12	35	16	49	28	41	23	23	27	16	25	28	31	20	27	27	36	30	34	18	25	55	0	33	28	14	35	25	70	27	89	86	89
" <i>S. luteovercilli</i> " NRRL B-12435 ^T	26	19	17	32	11	31	30	31	57	14	27	34	30	2	9	20	7	42	40	9	8	19	23	0	15	12	0	33	28	14	35	25	70	27	89	86	89
<i>S. eurociliatus</i> NRRL B-1676 ^T	9	2	4	27	34	20	25	28	27	17	29	9	19	20	24	25	19	28	17	33	33	22	30	28	11	23	41	39	39	39	39	39	39	39	39	39	
<i>S. ladamum</i> NRRL 3191 ^T	24	23	22	28	0	28	16	47	49	23	0	28	4	53	21	20	0	36	6	15	9	0	24	6	19	31	29	35	18	18	18	18	18	18	18	18	
<i>S. mobuarensis</i> NRRL B-3729 ^T	21	16	30	23	0	19	21	19	33	23	0	21	6	17	13	11	7	20	20	25	26	17	0	0	33	28	14	35	25	70	27	89	86	89	89	89	
" <i>S. rubrovercillatus</i> " NRRL B-16433	29	24	23	21	0	29	55	26	49	20	40	28	11	14	29	10	21	24	23	54	17	11	33	35	24	42	19	35	22	19	27	89	86	89	89	89	
" <i>S. takuensis</i> " NRRL B-12516	16	8	11	33	2	21	0	35	ND ^b	76	10	6	0	5	14	0	18	17	25	20	0	5	0	25	20	31	0	4	18	20	1	89	86	89	89	89	
<i>S. wakamarii</i> NRRL B-1707 ^T	12	13	53	9	53	33	48	71	ND	24	10	19	32	19	16	11	21	18	6	40	20	56	16	11	12	10	7	12	63	24	16	81	86	89	89	89	
<i>S. abikoensis</i> NRRL B-2113 ^T	0	10	9	0	5	49	0	21	ND	25	0	6	0	8	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	81	81	89	89		
<i>S. coelicolor</i> A3(2) (= NRRL B-16638)	ND	ND	ND	ND	ND	32	ND	ND	ND	26	ND	14	ND	ND	ND	ND	ND	7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7	

^a Strain NRRL B-8164^T is a *S. mashuensis* strain.
^b ND, not determined.

FIG. 1. Dendrogram resulting from average clustering of DNA relatedness matrix data. The species or species cluster names indicated on the left were defined at levels of DNA relatedness of >70%.

abikoensis cluster and generally low levels of relatedness (0 to 55%) to all of the other strains included in this study.

Within the *S. baldaccii* cluster, *Streptomyces biverticillatus* NRRL ISP-5272^T, *Streptomyces fervens* NRRL 2755^T, *Streptomyces roseovorticillatus* NRRL B-1993^T, and "*Streptomyces rubrochlorinus*" NRRL B-12558 can validly be considered subjective synonyms of *S. baldaccii* NRRL B-3500^T since the levels of DNA relatedness among these strains are more than 70%. "*Streptomyces pentaticus* subsp. *jenesis*" NRRL B-12088^T, a member of the *Streptomyces griseocarneus* cluster, also appears to be synonymous with *S. baldaccii* on the basis of levels of DNA homology of >70% among all of the strains belonging to this DNA relatedness cluster.

Streptomyces netropsis NRRL 2268^T, *Streptomyces kentuckensis* NRRL B-1831^T, and *Streptomyces flavopersicus* NRRL 2820^T clustered phenotypically with *S. baldaccii* in the study of Locci and Schofield, but these species are clearly subjective synonyms of the distinct taxon *S. netropsis* on the basis of DNA homology data, as shown in Table 2 and Fig. 1. All three of these strains also exhibit low levels of DNA relatedness to *S. baldaccii*. *Streptomyces distallicus* NRRL 2886^T could also be considered marginally synonymous with *S. netropsis*, since it exhibits 70 and 73% DNA relatedness with *S. netropsis* NRRL

2268^T and *S. kentuckensis* NRRL B-1831^T, respectively, but reproducibly exhibits only 61% DNA relatedness with *S. flavopersicus* NRRL 2820^T, which causes it to join the main cluster at a homology level of 62%. This could indicate that this strain is related to *S. netropsis* at the subspecies level.

Streptomyces griseovorticillatus NRRL B-12432^T and *Streptomyces hiroshimensis* NRRL B-5484^T, the remaining species in the *S. baldaccii* phenotypic cluster, appear to be separate species on the basis of their low levels of DNA relatedness to all of the other species included in this study.

The *Streptomyces cinnamoneus* subsp. *cinnamoneus* phenotypic cluster disintegrated after the levels of DNA relatedness among its members were determined, since the only strains belonging to this phenotypic cluster that exhibited a species level DNA homology value (85%) were type strains *S. cinnamoneus* subsp. *cinnamoneus* NRRL B-1285 and *S. cinnamoneus* subsp. *albosporeus* NRRL B-5624 (Table 2 and Fig. 1). *Streptomyces blastomyceticus* NRRL B-5480^T, *Streptomyces eurocidicus* NRRL B-1676^T, and *Streptomyces lavenduligriseus* NRRL B-3173^T appear to be distinct taxa, again on the basis of the low levels of DNA relatedness to all of the other strains studied.

The relationships among the strains in the remaining phenotypic clusters were also rearranged on the basis of the DNA relatedness data in Table 2 and Fig. 1. The members of the *Streptomyces griseocarneus* phenotypic cluster were separated and placed in five distinct taxa on the basis of low levels of DNA relatedness to the other strains included in this study (*Streptomyces ardens* NRRL 2817^T, *S. griseocarneus* NRRL B-1068^T, *Streptomyces mashuensis* NRRL B-8164^T, *Streptomyces olivovorticillatus* NRRL B-1994^T, and "*Streptomyces tropicalensis*" NRRL B-12481).

Streptomyces cinnamoneus subsp. *azacoluta* NRRL B-1699^T, a member of the *S. griseocarneus* cluster, and "*Streptomyces taitoensis*" NRRL B-16435 are subjective synonyms of *Streptomyces hachijoensis* NRRL B-3106^T (levels of DNA relatedness, >83%). *Streptomyces mobaraensis* NRRL B-3729^T and *Streptomyces ladakanum* NRRL 3191^T, the type strains of their respective phenotypic clusters, merge weakly at a level of DNA relatedness of 70% as synonyms of the species that has priority (i.e., *S. mobaraensis*), but "*Streptomyces luteoreticuli*" NRRL B-12435^T appears to be a genetically distinct taxon. *Streptomyces salmonis* NRRL B-1472^T exhibited 100% DNA homology with "*Streptomyces reticulusruber*" NRRL B-1484, a strain in the Agricultural Research Service Culture Collection which is used as a test microorganism for discoloration of plastics. *Streptomyces aureoversile* NRRL B-3325^T, a member of the *S. salmonis* phenotypic cluster, appears at this time to be a distinct taxon on the basis of its low levels of DNA relatedness to all other strains.

The low levels of DNA relatedness observed among unrelated strains are a reflection of the genus background level of chromosomal homology as determined spectrophotometrically. The levels of DNA relatedness of strains belonging to the major DNA relatedness clusters to a typical streptomycete, *Streptomyces coelicolor* A3(2) (= NRRL B-16638), range from 0 to 32% (Table 1).

The results of the analysis of levels of DNA relatedness among the verticillate strains included in this study clearly indicate that the species groups defined by Locci and Schofield (8), which were based on phenotypic numerical taxonomy data, should be reorganized to reflect the genetic relatedness of the strains. Valid descriptions of the reorganized species will require reevaluation of the morphological and physiological data collected in the previous numerical taxonomic study, as well as an examination of possible new physiological properties, to

determine if differential characteristics which separate and clearly described the taxa defined by molecular systematic methods can be found.

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