DNA relatedness among the *Streptomyces fulvissimus* and *Streptomyces griseoviridis* phenotypic cluster groups

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As a continuation of an ongoing study of DNA relatedness among red-spored streptomycetes, the homology between the four species comprising the *Streptomyces fulvissimus* phenotypic cluster (*S. fulvissimus*, *Streptomyces aureovorticillatus*, *Streptomyces longispororuber* and *Streptomyces spectabilis*) and the four species comprising the *Streptomyces griseoviridis* phenotypic cluster (*S. griseoviridis*, *Streptomyces chryseus*, *Streptomyces daghestonicus* and *Streptomyces murinus*) was measured spectrophotometrically from $C_{0t_{0.5}}$ determinations. All strains were also compared to 12 strains representing previously determined DNA-relatedness clusters in the *Streptomyces lavendulae* phenotypic cluster, including the type strain NRRL B-1230^T. The strains segregated into 15 cluster groups at DNA relatedness >80%, including 10 single-membered clusters. *S. griseoviridis* and *S. daghestonicus* were synonymous, as were *S. chryseus* and *S. longispororuber*. *S. spectabilis* exhibited species-level homology with strain NRRL B-2402, which had been received as *S. lavendulae*. *S. aureovorticillatus* exhibited significant DNA relatedness to *Streptomyces flavotricini*. None of the strains from the *S. fulvissimus* or *S. griseoviridis* clusters exhibited significant homology to the type strain of *S. lavendulae*.

Keywords: DNA relatedness, *Streptomyces fulvissimus*, *Streptomyces griseoviridis*

INTRODUCTION

Strains from the *Streptomyces fulvissimus* and *Streptomyces griseoviridis* phenotypic clusters of Williams *et al.* (1983, 1989) were selected for study as a continuation of investigations into DNA relatedness among red-spored streptomycetes. A previous study of DNA relatedness among strains belonging to the *Streptomyces lavendulae* cluster had shown that the other subjective synonyms in this cluster were genetically distinct taxa, other than *Streptomyces columbiensis*, in spite of their morphological and physiological similarities (Labeda, 1993). The *S. fulvissimus* and *S. griseoviridis* phenotypic clusters both contain taxa that are morphologically diverse with regard to spore colour and sporophore morphology, and it was theorized that many of these taxa would also be genetically distinct. DNA relatedness was initially determined among the type strains composing the subjective synonyms of both phenotypic clusters, as well as with the type strain of *S. lavendulae*, NRRL B-1230^T. Subsequently, DNA relatedness was also de-

termined between these strains and selected strains from the DNA-relatedness clusters defined in the previous study of the *S. lavendulae* phenotypic cluster.

METHODS

Strains, cultivation and maintenance. All strains included in this study are held in the ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA. Many of the strains selected for this study, as shown in Table 1, were included in the International *Streptomyces* Project as well as previous phenotypic numerical taxonomic studies. Primary storage of strains was as lyophilized ampoules of mycelial and spore suspensions in sterile beef serum held at 4 °C. Working stock cultures were maintained on slants of yeast extract/malt extract agar (Shirling & Gottlieb, 1966) or synthetic starch agar (Shirling & Gottlieb, 1966) and stored at 4 °C until needed. Biomass for extraction of DNA was grown as shaken cultures in Fernbach flasks containing tryptone/glucose/liver extract/yeast extract broth (Pridham & Lyons, 1980).

Purification of DNA and determination of relatedness. DNA was extracted by the method of Marmur (1961) after cell

Table 1. Strains used in this study

<i>Streptomyces fulvissimus</i> cluster	
<i>Streptomyces aureovorticillatus</i>	NRRL B-3326 ^T (= ATCC 15854 ^T = DSM 40080 ^T = IFO 12742 ^T = JCM 4347 ^T)
<i>Streptomyces fulvissimus</i>	NRRL B-1453 ^T (= ATCC 27431 ^T = DSM 40593 ^T = IFO 13482 ^T = JCM 4754 ^T)
<i>Streptomyces longispororuber</i>	NRRL B-2297 (= ATCC 13931)
<i>Streptomyces spectabilis</i>	NRRL 2792 ^T (= ATCC 27741 ^T)
<i>Streptomyces griseoviridis</i> cluster	
<i>Streptomyces chryseus</i>	NRRL B-12347 ^T (= ATCC 19829 ^T = DSM 40420 ^T = IFO13377 ^T = JCM 4737 ^T)
<i>Streptomyces daghestonicus</i>	NRRL B-2710 (= INA 13897/54)
<i>Streptomyces daghestonicus</i>	NRRL B-5418 ^T (= ATCC 19747 ^T = DSM 40149 ^T = IFO 12762 ^T = JCM 4365 ^T)
<i>Streptomyces griseoviridis</i>	NRRL 2427 ^T (= ATCC 23920 ^T = DSM 40229 ^T = IFO 12874 ^T = JCM 4250 ^T)
<i>Streptomyces murinus</i>	NRRL B-2286 ^T (= ATCC 19788 ^T = DSM 40091 ^T = IFO 12799 ^T = JCM 4333 ^T)
<i>Streptomyces lavendulae</i> cluster	
<i>Streptomyces flavotricini</i>	NRRL B-5419 ^T (= ATCC 19757 ^T = DSM 40152 ^T = IFO 12770 ^T = JCM 4371 ^T)
<i>Streptomyces goshikiensis</i>	NRRL B-5428 ^T (= ATCC 23914 ^T = DSM 40190 ^T = IFO 12868 ^T = JCM 4294 ^T)
<i>Streptomyces lavendulae</i>	NRRL B-1230 ^T (= ATCC 8664 ^T = DSM 40069 ^T = IFO12903 ^T = JCM 4664 ^T)
<i>Streptomyces lavendulae</i>	NRRL B-5617 (= ATCC 14158 = DSM 40213 = IFO 12789 = JCM 4055)
<i>Streptomyces lavendulae</i>	NRRL B-2343
<i>Streptomyces lavendulae</i>	NRRL B-2402
<i>Streptomyces lavendulae</i>	NRRL B-3080 (= IFO 3146 = JCM 4263)
<i>Streptomyces lavendulae</i> subsp. <i>brasilicus</i>	NRRL B-2937 ^T
<i>Streptomyces lavendulae</i> subsp. <i>inositophilus</i>	NRRL B-3904 ^T
<i>Streptomyces polychromogenes</i>	NRRL B-12233 ^T (= ATCC 12595 ^T = DSM 40316 ^T = IFO 13072 ^T = JCM 4236 ^T)
<i>Streptomyces toxytricini</i>	NRRL B-5426 ^T (= ATCC 19813 ^T = IFO 12823 ^T = JCM 4421 ^T)
<i>Streptomyces virginiae</i>	NRRL B-1446 ^T (= ATCC 19817 ^T = DSM 40094 ^T = IFO 12827 ^T = JCM 4425 ^T)

disruption by passing through a French pressure cell (SLM Instruments) at 10000 p.s.i. (= 69 MPa). The DNA was further purified by caesium chloride density-gradient centrifugation (Maniatis *et al.*, 1982). Individual strains generally yielded 5–10 mg DNA from 85–100 g biomass. The percentage of DNA relatedness between strains was calculated from $C_{0,t_{0.5}}$ determinations in $5 \times$ SSC ($1 \times$ SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) supplemented with 20% DMSO at 65 °C ($T_m - 23$ °C) by the method of Seidler & Mandel (1971) and Seidler *et al.* (1975) as described in Kurtzman *et al.* (1980). Standard error for this method was determined as $\pm 5\%$.

Data analysis. Statistical calculations of the clustering of DNA-relatedness data were performed using SAS for Windows, Release 6.11 (SAS Institute) on a FoxTech Terminator T5-150 personal computer (Fox Technologies). The data were clustered by mean linkage using the SAS/STAT CLUSTER procedure. The dendrogram was generated with SAS/GRAPH using the SAS macro GRAFTREE written and kindly provided by Dr Dan Jacobs, University of Maryland, MD, USA.

RESULTS AND DISCUSSION

The minimum level of DNA relatedness between strains required to define a species is recommended as being 70% (Wayne *et al.*, 1987), although previous studies of DNA relatedness among *Streptomyces* strains implied that genomic homology greater than 80% may actually equate to species-level relatedness in this genus (Labeda, 1993; Labeda & Lyons, 1992). It

is interesting to note that, from the data shown in Table 2, none of the subjective synonyms of *S. fulvissimus* exhibited species-level DNA relatedness (at even the accepted 70% homology level) with *S. fulvissimus* NRRL B-1453^T or any of the other strains within this phenotypic cluster and, in fact, most formed single-membered clusters indicating that these are separate and distinct taxa. *Streptomyces longispororuber* NRRL B-2297 did, however, exhibit 97% DNA relatedness with *Streptomyces chryseus* NRRL B-12347^T from the *S. griseoviridis* phenotypic cluster, demonstrating that these taxa should be considered synonymous.

Within the *S. griseoviridis* cluster, as can be observed from the data in Table 2, *Streptomyces daghestonicus* NRRL B-2710 is clearly a subjective synonym of *S. griseoviridis* NRRL 2427^T, with 95% DNA relatedness between these strains. *S. daghestonicus* NRRL B-5418^T, the type strain, was subsequently found to exhibit 100% DNA relatedness with both *S. daghestonicus* NRRL B-2710 and *S. griseoviridis* NRRL 2427^T and thus was not evaluated further. All three of these strains share similar morphology with smooth surfaces and red-coloured spores borne on spiral sporophores. Although, as mentioned above, *S. chryseus* NRRL B-12347^T did exhibit significant DNA relatedness with *S. longispororuber* NRRL B-2297, none of the other strains showed any close genetic relatedness to other members of this phenotypic cluster. All formed single-membered clusters, and thus

Table 2. DNA relatedness among strains

		DNA relatedness (%)							
	NRRL	2792*	B-2710	2427 ^T	B-3326 ^T	B-1453 ^T	B-1230 ^T	B-2286 ^T	B-12347 ^T
<i>Streptomyces daghestonicus</i>	B-2710	19							
<i>Streptomyces griseoviridis</i>	2427 ^T	9	95						
<i>Streptomyces aureovorticillatus</i>	B-3326 ^T	13	0	14					
<i>Streptomyces fulvissimus</i>	B-1453 ^T	16	19	11	3				
<i>Streptomyces lavendulae</i>	B-1230 ^T	21	23	19	19	40			
<i>Streptomyces murinus</i>	B-2286 ^T	14	5	21	14	8	9		
<i>Streptomyces chryseus</i>	B-12347 ^T	0	7	50	17	36	16	32	
<i>Streptomyces longispororuber</i>	B-2297	12	0	56	9	22	34	34	97

* NRRL 2792, *Streptomyces spectabilis*.

Fig. 1. Dendrogram calculated from mean linkage clustering of DNA relatedness between the *S. fulvissimus* and *S. griseoviridis* strains and selected representative strains from the genetic clusters identified from the previous study of the *S. lavendulae* phenotypic cluster. Species clusters are formed at greater than 80% DNA relatedness.

should not be considered subjective synonyms of *S. griseoviridis*.

It can also be seen from the data in Table 2 that none

of the strains from these two phenotypic clusters have any significant DNA relatedness to the type strain of *S. lavendulae*, NRRL B-1230^T. An evaluation of DNA relatedness between the strains in the present study and a subset of strains from the *S. lavendulae* phenotypic cluster is shown graphically in the mean linkage dendrogram in Fig. 1. Note that *Streptomyces spectabilis* NRRL 2792^T exhibits significant (81%) DNA relatedness to strain NRRL B-2402, which was deposited in the ARS Culture Collection as a strain of *S. lavendulae*. The latter strain showed very low DNA relatedness (26%) to authentic strains of this species (Labeda, 1993), indicating that it was a member of some other species. *Streptomyces aureovorticillatus* NRRL B-3326^T can be seen in Fig. 1 to exhibit significant (70%), but marginally less than species-level, DNA relatedness with *Streptomyces flavotricini* NRRL B-5419^T, which had formed a single-membered DNA relatedness cluster when compared to other members of the *S. lavendulae* phenotypic cluster (Labeda, 1993). The taxonomic level of the relationship between strains NRRL B-3326 and NRRL B-5419 is not clear at this time, but they may represent subspecies.

The DNA relatedness data presented support the separation of the *S. fulvissimus* and *S. griseoviridis* phenotypic clusters into six genetically distinct species: *S. aureovorticillatus*, *S. fulvissimus*, *S. griseoviridis* (with *S. daghestonicus* as a subjective synonym), *S. longispororuber* (with *S. chryseus* as a subjective synonym), *Streptomyces murinus* and *S. spectabilis*.

A careful evaluation of the physiological and morphological properties of these taxa will be necessary to determine those characteristics that can discriminate between the species that have been defined by > 80% DNA relatedness.

ACKNOWLEDGEMENTS

The able technical assistance of E. N. Hoekstra in the isolation and purification of DNA, and performance of the DNA hybridizations required for this study is gratefully acknowledged.

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