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Proposal to reclassify the *Streptomyces albidoflavus* clade on the basis of multilocus sequence analysis and DNA–DNA hybridization, and taxonomic elucidation of *Streptomyces griseus* subsp. *solvifaciens*

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Abstract

The *Streptomyces albidoflavus* 16S rRNA gene clade contains 10 species and subspecies with identical 16S rRNA gene sequences and very similar numerical taxonomic data, including *Streptomyces griseus* subsp. *solvifaciens*. Type strains of this clade, as well as three CGMCC strains which were received as *Streptomyces galilaeus*, *Streptomyces sioyaensis* and *Streptomyces vinaceus*, respectively, that shared the same 16S rRNA gene sequences with the clade, were subjected to multilocus sequence analysis (MLSA), DNA–DNA hybridization (DDH) and phenotypic characterization for a comprehensive reevaluation. The 13 strains still formed a distinct, albeit loosely related, clade in the phylogenetic tree based on concatenated sequences of *aptD*, *gyrB*, *recA*, *rpoB* and *trpB* genes, supported by a high bootstrap value and different tree-making algorithms, with MLSA evolutionary distances ranging from 0 to 0.003. DDH values among these strains were well above the 70% cut-off point for species delineation. Based on the genotypic data of MLSA and DDH, combined with key phenotypic properties in common, it is proposed that the 10 species and subspecies of the *S. albidoflavus* clade, namely *S. albidoflavus*, *S. canescens*, *S. champavatii*, *S. coelicolor*, *S. felleus*, *S. globisporus* subsp. *caucasicus*, *S. griseus* subsp. *solvifaciens*, *S. limosus*, *S. odorifer* and *S. sampsonii*, should be merged into a single genomic species, for which the name *S. albidoflavus* is retained, and that the three strains *S. galilaeus* CGMCC 4.1320, *S. sioyaensis* CGMCC 4.1306 and *S. vinaceus* CGMCC 4.1305 should be assigned to *S. albidoflavus* as well. The results also indicated that MLSA could be the procedure of choice for distinguishing between species within *Streptomyces* 16S rRNA gene clades.
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Keywords: *Streptomyces albidoflavus* clade; *Streptomyces griseus* subsp. *solvifaciens*; 16S rRNA; MLSA; DDH; Reevaluation

Introduction

The application of modern taxonomic procedures has led to considerable improvements in the classification of the genus *Streptomyces* [1,15,18,26,27]. The genus currently encompasses over 550 validly described species (list of prokaryotic names with standing in nomenclature, http://www.bacterio.cict.fr/s/streptomycesa.html),

Abbreviations: MLSA, multilocus sequence analysis; DDH, DNA–DNA hybridization; NJ, neighbour-joining; MP, maximum parsimony; ML, maximum likelihood.

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most of which have been delineated using a combination of genotypic and phenotypic methods. There is also evidence that closely related *Streptomyces* species can be classified into species-groups, as exemplified by the *S. albidoflavus* [27], *S. griseus* [13,29], *S. violaceoruber* [4], and *S. violaceusniger* [12] 16S rRNA gene clades. However, it is becoming apparent from such studies that the resolution of 16S rRNA gene sequences is not sufficient to distinguish between closely related species. The circumscription of species within such clades requires the use of other molecular techniques, notably DNA–DNA hybridization (DDH) [40,41]. DDH procedures are known to be laborious, time-consuming and do not allow the generation of cumulative databases [10,35], consequently, rapid and reliable methods are urgently needed to distinguish between closely related *Streptomyces* species.

There is evidence that multilocus sequence analyses (MLSA) are providing valuable data for the delineation of closely related species, as illustrated by studies on the bacterial genera *Ensifer* [31,32], *Enterococcus* [34], *Mycobacterium* [33], *Pseudomonas* [22] and *Xanthomonas* [45]. This approach offers considerable advantages over other molecular techniques, such as DDH and DNA-fingerprinting, since the procedure is reproducible, allows the generation of cumulative databases [30] and can give a level of resolution comparable to DDH, as shown by the study on members of the *S. griseus* 16S rRNA gene clade [13]. In the comprehensive analysis of Guo et al. [13], representatives of more than 50 species were reevaluated and several reduced to members of established species, in addition, the type strain of *S. griseus* subsp. *solvifaciens* and the two strains *S. galilaeus* AS (now CGMCC) 4.1320 and *S. vinaceus* AS (now CGMCC) 4.1305 were shown to be misclassified as they belonged to the *S. albidoflavus* 16S rRNA gene clade.

There is strong evidence of overspeciation in the *S. albidoflavus* 16S rRNA gene clade, since most species representing this clade have identical 16S rRNA sequences [20,24], nearly identical 16S-ITS RFLP fingerprinting patterns [27], similar pyrolysis mass spectrometry [8] and rep-PCR profiles [47], as well as phenotypic consistency [11,19,43,44]. However, no all-inclusive proposition has been made to date. The primary aims of the present study were to use MLSA and DDH procedures to clarify relationships between members of the *S. albidoflavus* 16S rRNA gene clade, including *S. griseus* subsp. *solvifaciens*, and to establish an MLSA scheme that could be the procedure of choice for distinguishing between species within *Streptomyces* 16S rRNA gene clades by comparing the resolution of the MLSA and DDH approaches.

Materials and methods

Strains and culture conditions

The sources of the strains used in this study are shown in Table 1. All of the strains were cultivated on yeast extract–malt extract agar (ISP medium 2 [36]) plates at 28 °C.

Gene amplification and sequencing

Genomic DNA was isolated and purified as described by Chun and Goodfellow [2]. The genes used were the ones studied by Guo et al. [13], namely *atpD* (ATP synthase F1, β-subunit), *gyrB* (DNA gyrase B subunit), *recA* (recombinase A), *rpoB* (RNA polymerase, β-subunit) and *trpB* (tryptophan synthase β-subunit), as were the primers and PCR conditions for *atpD*, *recA*, *rpoB* and *trpB*. The improved *gyrB* primers were redesigned in this study. Briefly, *gyrB* sequences of streptomycetes and related bacteria were aligned in order to identify conserved regions for the development of improved primers. Software packages Primer premier 5.0 (Premier Biosoft International) and DNAMAN 5.2.2 (Molecular Biology Insights) were used to design and evaluate the primers. The primer sets for amplification and sequencing of *gyrB* were *gyrB*PFA 5′-CTCGAGGGTCTGGACGCGGTCCGCAAGCGACCCGGTATGTA-3′ and *gyrB*PRA 5′-GAAGGTCTTCACCTCGGTGTTGCCCAGCTTCGTCTT-3′, and *gyrB*FA 5′-GCAAGCGACCCGGTATGTAC-3′ and *gyrB*RA 5′-GAGGTTGTCGTCCTTCTCGC-3′. The primer positions at the *gyrB* gene of *Streptomyces coelicolor* were 121–161, 1150–1185, 143–162 and 1052–1071, respectively. Amplification was performed in a 50 µl reaction mixture containing 1 µl template DNA (50–200 ng), 5 µl 10 × PCR buffer, 1 µl each PCR primer (20 µM), 1 µl dNTP mix (10 mM), 6 µl $MgCl_2$ (25 mM), 2.5 U *Taq* DNA polymerase, 5 µl DMSO and 29 µl sterile MilliQ water. The reaction conditions consisted of an initial denaturation at 95 °C for 5 min; 5 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 90 s; 30 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, extension at 72 °C for 60 s; and a final extension at 72 °C for 10 min. The PCR products were purified and directly sequenced as described by Guo et al. [13].

Phylogenetic analysis

The almost complete 16S rRNA gene sequence of *S. griseus* subsp. *solvifaciens* AS (now CGMCC) 4.1845^T was subjected to a BLAST search. The organism was found to have an identical sequence to the type strains of *S. albidoflavus*, *S. canescens*, *S. champavatii*,

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Table 1. Sources of strains used in this study, and the numerical subclusters and groups to which they were assigned.

Original species designation	Strain no. and history	Kämpfer subcluster[a]	Williams group[b]
S. albidoflavus (Rossi Doria 1891) Waksman and Henrici 1948	DSM 40455^T ← ISP 5455^T ← CBS 416.34^T	1-1	A 1A
S. argenteolus Tresner et al. 1961	CGMCC 4.1693^T ← JCM 4229^T ← KCC S-0229^T	1-5	A 15
S. canescens Waksman 1957	CGMCC 4.1681^T ← JCM 4196^T ← KCC S-0196^T	1-1	A 1A
S. champavatii Uma and Narasimha Rao 1959	CGMCC 4.1615^T ← JCM 5066^T ← KCC S-1066^T	1-1	
S. coelicolor (Müller 1908) Waksman and Henrici 1948	DSM 40233^T ← ISP 5233^T ← CBS 210.27^T	1-1	A 1A
S. felleus Lindenbein 1952	CGMCC 4.1677^T ← JCM 4368^T ← KCC S-0368^T	1-1	A 1A
S. galilaeus	CGMCC 4.1320 ← Newcastle University		
S. globisporus subsp. *caucasicus* (Kudrina 1957) Pridham et al. 1958	NBRC 100770^T ← JCM 9867^T ← NRRL B-2593^T	1-1	
S. griseus subsp. *griseus* (Krainsky 1914) Waksman and Henrici 1948	CGMCC 4.1419^T ← JCM 4644^T ← KCC S-0644^T	1-3	A 1B
S. griseus subsp. *solvifaciens* Pridham 1970	CGMCC 4.1845^T ← DSM 40933^T ← NRRL B-1561^T	1-1	
S. limosus Lindenbein 1952	NBRC 12790^T ← SAJ (Society for Actinomycetes, Japan) ← ISP 5131^T	1-1	A 1A
S. odorifer (Rullmann 1895) Waksman 1953	NBRC 13365^T ← SAJ ← ISP 5347^T	1-1	A 1A
S. sampsonii (Millard and Burr 1926) Waksman 1953	NBRC 13083^T ← SAJ ← ISP 5394^T	1-1	A 1A
S. sioyaensis	CGMCC 4.1306 ← Newcastle University		
S. vinaceus	CGMCC 4.1305 ← Newcastle University		

[a]Data from [19].
[b]Data from [44].

S. coelicolor, *S. felleus*, *S. globisporus* subsp. *caucasicus*, *S. limosus*, *S. odorifer*, *S. sampsonii*, and to strains *S. galilaeus* CGMCC 4.1320, *S. sioyaensis* CGMCC 4.1306 and *S. vinaceus* CGMCC 4.1305. The sequences of the 16S rRNA gene and each of the five protein-coding loci of the above strains were aligned, respectively, using MEGA 4.0 software [25], with the sequences of their closest neighbour *Streptomyces argenteolus* CGMCC 4.1693^T [13] and the type strain of *S. griseus* subsp. *griseus*, and trimmed manually at the same position before being used for further analysis and submitted to the *Streptomyces* MLST website database (http://pubmlst.org/streptomyces). Statistics for each locus were calculated as previously described [13]. Trimmed sequences of the five protein-coding loci were joined head-to-tail in-frame to form a concatenated sequence.

Phylogenetic trees based on the almost complete 16S rRNA gene sequences (1386 nucleotides) and on the concatenated protein-coding sequences (2520 nucleotides) were constructed by using three tree-making algorithms, neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML). The NJ and MP methods were from the MEGA 4.0 package [25] with the option of complete deletion of gaps, and the topologies of the resultant trees were evaluated by bootstrap analysis [6] of 1000 resamplings. The K2P model [23] was chosen as a substitution model for NJ tree construction. The ML phylogenetic tree was inferred by using the Dnamal Program from the PHYLIP package version 3.68 [7] with 100 replicates, and the resultant tree was combined to yield a consensus tree using the Consense Program. Nucleotide sequence data used in this paper are given in Supplementary Table S1.

DNA–DNA hybridization

Levels of DNA–DNA relatedness between reference and donor (reciprocal) strains were determined using the fluorometric micro-well method [5], and the modifications described by He et al. [16]. The hybridization value was calculated from triplicate experiments of reciprocal hybridizations and expressed as a mean of the corresponding reciprocal values.

Morphological and physiological characterization

Hyphal and spore-chain arrangement of the tested strains were observed on ISP 2 plates after incubation at 28 °C for 10–14 days, by using the cover slip technique [21]. Spore-chain morphology and spore-surface ornamentation were observed by examining gold-coated dehydrated specimens of cultures under an FEI QUANTA 200 scanning electron microscope. Aerial

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spore-mass colour, substrate mycelial pigmentation and the production of diffusible pigments were estimated on a number of standard agar media following incubation at 28 °C for 14 days. The strains were examined for a range of biochemical and physiological characteristics using established procedures [19,44]. Tolerances to temperature were tested at 10, 15, 28, 40 and 45 °C on ISP medium 2 incubated for 7–14 days.

Results

The 10 type strains and three CGMCC strains in the *S. albidoflavus* 16S rRNA gene clade had identical 16S rRNA gene sequences (Fig. 1A). In contrast, variations were found in the sequences of the five protein loci. The features of each of five loci and concatenated sequences are displayed in Supplementary Table S2. Good congruence was found between the phylogenetic trees of concatenated sequences of the five protein-coding genes (MLSA trees) constructed using three tree-making algorithms (Fig. 1B). The 13 strains formed a distinct clade in the MLSA tree, supported by a 95% bootstrap value, with MLSA evolutionary distances ranging from 0 to 0.003. The MLSA distances between *S. albidoflavus* and *S. argenteolus* and *S. griseus* subsp. *griseus* were 0.010 and 0.114, respectively.

The strains of the *S. albidoflavus* clade in the MLSA tree could be assigned to three multi-membered and three single-membered subclades. Subclade I contained type strains of *S. felleus*, *S. limosus*, and *S. sampsonii* and showed a distance of 0.001; this taxon was supported by an 80% bootstrap value and by all of the tree-making algorithms. Type strains of *S. albidoflavus*, *S. globisporus* subsp. *caucasicus*, *S. odorifer*, and *S. coelicolor* formed a more heterogeneous subclade II which was also supported by the ML method, therein the subgroup of *S. albidoflavus* and *S. globisporus* subsp. *caucasicus* was also formed by the MP method. *S. galilaeus* CGMCC 4.1320, *S. sioyaensis* CGMCC 4.1306 and *S. vinaceus* CGMCC 4.1305, which shared identical five-gene sequences, formed monophyletic subclade III; the integrity of this taxon was supported by a 95% bootstrap value and by the other tree-making algorithms. The remaining type strains of *S. canescens*, *S. griseus* subsp. *solvifaciens* and *S. champavatii* formed single-membered subclades, with *S. canescens* being found at the peripheral of subclade I, with *S. griseus* subsp. *solvifaciens* and *S. champavatii* at subclade II and subclade III, respectively.

The DDH data among reference and donor strains of the *S. albidoflavus* clade, *S. argenteolus* and *S. griseus* subsp. *griseus* are shown in Table 2. *S. albidoflavus* and *S. griseus* subsp. *solvifaciens* shared DDH values of 85.7%. It can also be seen that *S. albidoflavus* and all of the other 12 strains belonging to the *S. albidoflavus* clade shared DDH values of between 73.4% and 92.9%, which were well above the 70% cut-off point generally recognized for genomic species [41]. On the other hand, the DDH values between *S. albidoflavus* and *S. griseus* subsp. *griseus* (27.3%) and *S. argenteolus* (43.7%) were very low. In addition, within the *S. albidoflavus* clade, high DDH values were also found between *S. griseus*

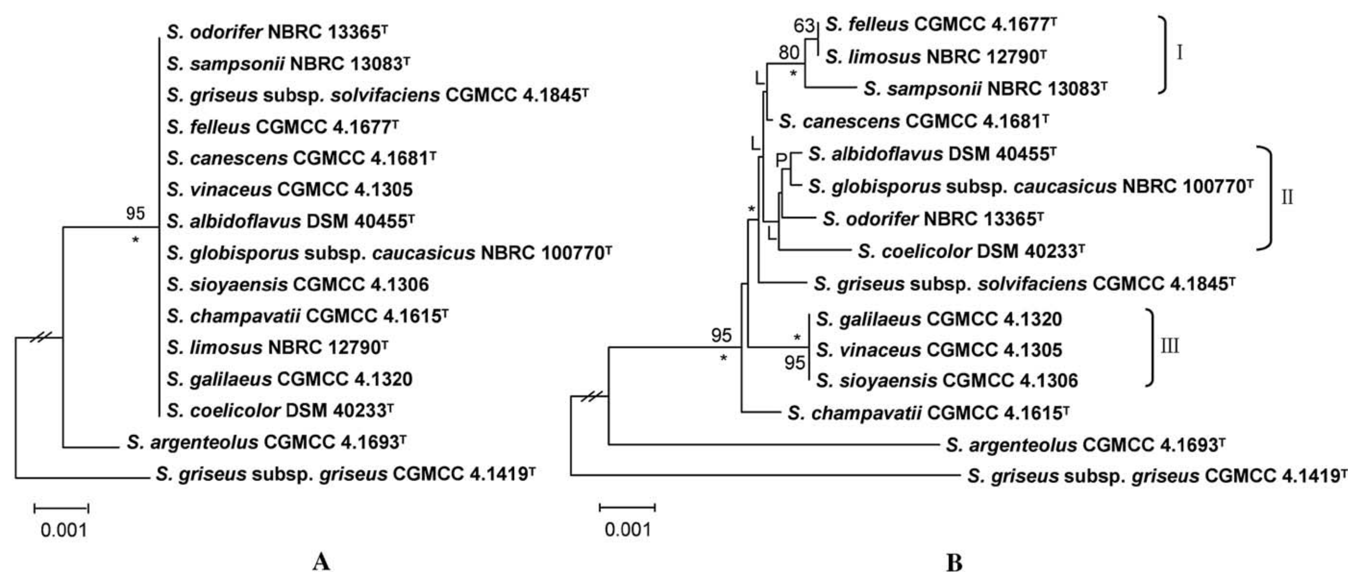

Fig. 1. Neighbour-joining trees based on 16S rRNA gene sequences (A) and five-gene concatenated sequences (B), showing the relationships between the members of the *S. albidoflavus* clade, *S. argenteolus* and *S. griseus* subsp. *griseus*. P and L indicate branches of the tree that were also recovered using MP and ML methods, respectively. The asterisk labels branches that were supported by all three tree-making algorithms. The scale bars indicate 0.001 nucleotide substitutions per nucleotide position.

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Table 2. DDH and MLSA evolutionary distance values among strains of the *S. albidoflavus* clade, *S. argenteolus* and *S. griseus* subsp. *griseus*.

Strain pair		DDH value (%)[a]	MLSA evolutionary distance
S. albidoflavus DSM 40455^T	*S. canescens* CGMCC 4.1681^T	90.3	0.001
S. albidoflavus DSM 40455^T	*S. champavatii* CGMCC 4.1615^T	80.8	0.002
S. albidoflavus DSM 40455^T	*S. coelicolor* DSM 40233^T	78.8	0.002
S. albidoflavus DSM 40455^T	*S. felleus* CGMCC 4.1677^T	87.2	0.001
S. albidoflavus DSM 40455^T	*S. galilaeus* CGMCC 4.1320	82.4	0.002
S. albidoflavus DSM 40455^T	*S. globisporus* subsp. *caucasicus* NBRC 100770^T	92.9	0.000
S. albidoflavus DSM 40455^T	*S. griseus* subsp. *solvifaciens* CGMCC 4.1845^T	85.7	0.001
S. albidoflavus DSM 40455^T	*S. limosus* NBRC 12790^T	87.7	0.001
S. albidoflavus DSM 40455^T	*S. odorifer* NBRC 13365^T	77.7	0.001
S. albidoflavus DSM 40455^T	*S. sampsonii* NBRC 13083^T	78.9	0.002
S. albidoflavus DSM 40455^T	*S. sioyaensis* CGMCC 4.1306	73.4	0.002
S. albidoflavus DSM 40455^T	*S. vinaceus* CGMCC 4.1305	74.8	0.002
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. canescens* CGMCC 4.1681^T	79.4	0.001
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. champavatii* CGMCC 4.1615^T	83.6	0.002
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. coelicolor* DSM 40233^T	79.8	0.003
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. felleus* CGMCC 4.1677^T	75.9	0.002
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. galilaeus* CGMCC 4.1320	75.7	0.002
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. globisporus* subsp. *caucasicus* NBRC 100770^T	83.6	0.002
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. limosus* NBRC 12790^T	91.3	0.002
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. odorifer* NBRC 13365^T	82.2	0.002
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. sampsonii* NBRC 13083^T	79.3	0.003
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. sioyaensis* CGMCC 4.1306	80.9	0.002
S. griseus subsp. *solvifaciens* CGMCC 4.1845^T	*S. vinaceus* CGMCC 4.1305	84.1	0.002
S. canescens CGMCC 4.1681^T	*S. champavatii* CGMCC 4.1615^T	80.7	0.001
S. canescens CGMCC 4.1681^T	*S. felleus* CGMCC 4.1677^T	92.1	0.001
S. canescens CGMCC 4.1681^T	*S. globisporus* subsp. *caucasicus* NBRC 100770^T	85.0	0.001
S. felleus CGMCC 4.1677^T	*S. limosus* NBRC 12790^T	90.8	0.000
S. argenteolus CGMCC 4.1693^T	*S. albidoflavus* DSM 40455^T	43.7	0.010
S. argenteolus CGMCC 4.1693^T	*S. galilaeus* CGMCC 4.1320	42.1	0.009
S. argenteolus CGMCC 4.1693^T	*S. griseus* subsp. *solvifaciens* CGMCC 4.1845^T	43.1	0.009
S. griseus subsp. *griseus* CGMCC 4.1419^T	*S. albidoflavus* DSM 40455^T	27.3	0.114
S. griseus subsp. *griseus* CGMCC 4.1419^T	*S. canescens* CGMCC 4.1681^T	27.7	0.114
S. griseus subsp. *griseus* CGMCC 4.1419^T	*S. felleus* CGMCC 4.1677^T	20.9	0.113
S. griseus subsp. *griseus* CGMCC 4.1419^T	*S. griseus* subsp. *solvifaciens* CGMCC 4.1845^T	29.2	0.114

[a]The average value of reciprocal hybridizations carried out in triplicate.

Table 3. Cultural characteristics of the 13 strains of the *S. albidoflavus* clade.[a]

Agar medium	Aerial spore mass	Substrate mycelium
Yeast extract–malt extract (ISP 2)	Abundant; light yellow or light greyish yellow	Yellow or light brown
Oatmeal (ISP 3)	Abundant; light yellow or light greyish yellow	Yellow or light greenish yellow
Inorganic salts–starch (ISP 4)	Abundant; light greyish yellow or off-white	Dark yellow or yellow brown
Glycerol–asparagine (ISP 5)	Abundant; greyish white	Ivory-white
Peptone–yeast extract–iron (ISP 6)	None	Brown
Nutrient agar (NA)	Abundant; greyish white	Light yellow

[a]All of the 13 strains produce a reddish purple diffusible pigment on oatmeal agar, but no diffusible pigments on the other media.

subsp. *solvifaciens* and all of the other 12 strains (75.7–91.3%), between *S. canescens* and *S. champavatii* (80.7%), *S. felleus* (92.1%) and *S. globisporus* subsp. *caucasicus* (85.0%), and between *S. felleus* and *S. limosus* (90.8%).

Reassessment of phenotypic attributes showed that all of the 13 strains were consistent with each other in cultural characteristics (Table 3), and presented great unanimous morphological and physiological properties, as given in the description section.

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Discussion

In the present study, the issue of the relationships between members of the *S. albidoflavus* 16S rRNA gene clade and the taxonomic position of *S. griseus* subsp. *solvifaciens* were addressed. *S. griseus* subsp. *solvifaciens* was found to be far from the whole *S. griseus* 16S rRNA gene clade [13]. In this investigation, the low DDH value between *S. griseus* subsp. *griseus* and *S. griseus* subsp. *solvifaciens* (29.2%) doubtlessly indicated that *S. griseus* subsp. *solvifaciens* does merit different species status from the *S. griseus* clade. Moreover, it is apparent from the genotypic and phenotypic data that *S. griseus* subsp. *solvifaciens* is a bona fide member of *S. albidoflavus*.

The species and subspecies phylogenetically related to *S. griseus* subsp. *solvifaciens*, represented here, are all known former members of the numerical taxonomic subcluster *S. albidoflavus* [11,19,44] (Table 1). In our MLSA scheme, these taxa were regenerated and reassured as forming a distinct clade supported by excellent bootstrap values and all three tree-making algorithms. The low MLSA distances (0–0.003) and the high DDH values (73.4–92.9%) among strains of this clade suggest that all of them belong to a single genomic species, where the 10 species and subspecies, as represented by the corresponding type strains, should be merged as one. The low DDH values between the *S. albidoflavus* clade and *S. argenteolus* (42.1–43.7%) and *S. griseus* subsp. *griseus* (20.9–29.2%) further support this. Therefore, on the basis of MLSA and DDH data, combined with the shared cultural, micro-morphological and physiological attributes which are in line with the results of numerical analyses [19,44], it is proposed that *S. albidoflavus*, *S. canescens*, *S. champavatii*, *S. coelicolor*, *S. felleus*, *S. globisporus* subsp. *caucasicus*, *S. griseus* subsp. *solvifaciens*, *S. limosus*, *S. odorifer* and *S. sampsoni* belong to the same genomic species, which should also encompass *S. galilaeus* CGMCC 4.1320, *S. sioyaensis* CGMCC 4.1306 and *S. vinaceus* CGMCC 4.1305. According to Rule 38 of the Bacteriological Code [28], the oldest legitimate species name, *S. albidoflavus*, has priority over the names of the other taxa, and should be retained for the merged species.

The evidence that all the members of the *S. albidoflavus* 16S rRNA gene clade form a monophyletic lineage in the 16S rRNA gene phylogenetic tree, and that the 16S rRNA sequences of *S. griseus* subsp. *solvifaciens*, *S. galilaeus* CGMCC 4.1320, *S. sioyaensis* CGMCC 4.1306 and *S. vinaceus* CGMCC 4.1305 are obviously different from those of the type strains of the *S. griseus* clade [13], *S. galilaeus* (GenBank no. AB184378 = AB045878), *S. sioyaensis* (AB184171 = DQ026654) and *S. vinaceus* (AY999740 = AB184394), further indicate that the 16S rRNA gene is still a significant phylogenetic marker for *Streptomyces* taxonomy. MLSA, which uses housekeeping genes that possess more variable sites than the 16S rRNA gene and are sufficiently conserved to retain the phylogenetic signal, has proven to be a valuable alternative approach [13,32]. Using this technique, it was found in this study that all the strains within the *S. albidoflavus* 16S rRNA gene clade still formed a distinct phylogenetic clade with most strains clearly discriminated in the five-gene tree, which demonstrates again that MLSA is reliable and has good discriminatory power even at the intra-species level [13,17].

S. albidoflavus strains can also be discriminated from each other based on the size and number of the 16S-23S rDNA ITS [14]. However, it is evident that the 16S-23S rDNA ITS difference is a character of the strain itself and is not useful in phylogenetic analysis of *Streptomyces*, since this region exhibits various lengths and highly variable sequence similarities within strains as well as intra- and inter-species [37,42]. In contrast, the MLSA scheme delineates the *S. albidoflavus* clade into three multi-membered and three single-membered subclades, with higher five-gene sequence similarities (99.8–100%) within each multi-membered subclade than between the subclades (99.7–99.9%), indicating that the MLSA scheme is more suitable than 16S-23S rDNA ITS for the study of phylogenetic and taxonomic relationships, even at the strain level in the *S. albidoflavus* clade.

DDH has been universally performed for bacterial species delineation [38]. However, this method is not recommendable for evaluating a large number of strains, mainly because the resultant values cannot reflect unitary relationships within a group, which is the biggest deficiency of DDH [9,35]. In our study, MLSA distances of 0, 0.001, 0.002 and 0.003 correspond to DDH values of 90.8–92.9%, 77.7–92.1%, 73.4–91.3% and 79.3–79.8%, respectively. Within this range of divergence, increase of MLSA evolutionary distance does not always (but generally seems to) parallel a proportional decrease of DDH value. Such dissonance cannot be avoided only by excellent manipulation, because a significant number of physico-chemical parameters, as well as genome size and the presence of large plasmids, could all influence the DDH results [39]. On the other hand, prokaryotic species definition based on genome analysis strongly supported that sequence analysis of a small set of housekeeping genes could predict overall genome relatedness with a high degree of precision and accuracy, and could reliably assign novel strains or isolates to bacterial species [3,10,46]. It is evident from our results that the MLSA scheme provides elaborate species, even intra-species, evaluation, indicating that this approach can be another valuable tool for identification and classification of *Streptomyces* species, especially the closely related species-groups. Moreover, as the general MLSA evolutionary distance within the *S. albidoflavus* clade ranges

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from 0 to 0.003, and the DDH values among members of this clade are all above the 70% cut-off, it is deduced that *Streptomyces* strains close to this clade with MLSA distances equal to or less than 0.003 should be considered as members of the *S. albidoflavus* genomic species as well.

Emended description of *Streptomyces albidoflavus* (Rossi Doria 1891) Waksman and Henrici 1948 (Approved Lists 1980)

S. albidoflavus (al.bi.do.flávus. L. adj. *albidus* white; L. adj. *flavus* yellow; N.L. masc. adj. *albidoflavus* whitish yellow).

Later heterotypic synonyms: *S. canescens* Waksman 1957 (Approved Lists 1980); *S. champavatii* Uma and Narasimha Rao 1959 (Approved Lists 1980); *S. coelicolor* (Müller 1908) Waksman and Henrici 1948 (Approved Lists 1980); *S. felleus* Lindenbein 1952 (Approved Lists 1980); *S. globisporus* subsp. *caucasicus* (Kudrina 1957) Pridham et al. 1958 (Approved Lists 1980); *S. griseus* subsp. *solvifaciens* Pridham 1970 (Approved Lists 1980); *S. limosus* Lindenbein 1952 (Approved Lists 1980); *S. odorifer* (Rullmann 1895) Waksman 1953 (Approved Lists 1980); *S. sampsonii* (Millard and Burr 1926) Waksman 1953 (Approved Lists 1980).

The previous description ([Rossi Doria 1891] Waksman and Henrici 1948) is emended with data from this study, molecular data from Kiss et al. [24], and numerical classification data from Williams et al. [44] and Kämpfer et al. [19]. The organisms form extensively branched substrate hyphae and aerial hyphae that carry smooth-surfaced spores in *rectiflexibiles* or *retinaculiaperti* spore chains. Abundant off-white to light yellow aerial spore masses are formed on a whitish yellow to brown substrate mycelium on ISP media 2, 3, 4 and 5. Reddish purple diffusible pigments are produced on ISP medium 3 (oatmeal agar). Additional cultural properties are given in Table 3. Growth occurs between 15 and 40 °C, but with no aerial spore mass at 40 °C. All strains liquefy gelatine rapidly. All strains degrade aesculin, arbutin, hypoxanthine, starch, Tween 20, Tween 80, tyrosine and xanthine, weakly degrade guanine, but do not degrade cellulose. All are positive for assimilation of L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-ribose, D-trehalose, D-xylose, sodium acetate anhydrous and trisodium citrate dehydrate, weakly positive for assimilation of D-sucrose, but negative for assimilation of D-glucitol, D-melezitose, D-raffinose, and L-rhamnose. Some strains possess the C gene factor (*facC*) and produce extracellular pleiotropic autoregulatory protein factor C. The G+C content of genomic DNA is 74–76 mol% (HPLC method).

The type strain is DSM 40455^T (= ATCC 25422^T = CBS 416.34^T = CIP 105122^T = ISP 5455^T = JCM 4446^T = KCTC 9202^T = LMG 19300^T = NBRC 13010^T = NRRL B-1271^T).

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Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.syapm.2009.05.003.

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