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Taxonomic re-evaluation of whorl-forming *Streptomyces* (formerly *Streptoverticillium*) species by using phenotypes, DNA–DNA hybridization and sequences of *gyrB*, and proposal of *Streptomyces luteireticuli* (*ex* Katoh and Arai 1957) corrig., sp. nov., nom. rev.

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The taxonomic status of 64 strains of whorl-forming *Streptomyces* (formerly *Streptoverticillium*) species was re-evaluated and strains were reclassified on the basis of their phenotypes, DNA–DNA hybridization data and partial sequences of *gyrB*, the structural gene of the B subunit of DNA gyrase. These strains, which consisted of 46 species and eight subspecies with validly published names and 13 species whose names have not been validly published [including 10 strains examined by the International *Streptomyces* Project (ISP)], were divided into two groups, namely typical and atypical whorl-forming *Streptomyces* species, based on their phenotypes and *gyrB* gene sequences. The typical whorl-forming species (59 strains) were divided into six major clusters of three or more species, seven minor clusters of two species and five single-member clusters, based on the threshold value of 97 % *gyrB* sequence similarity. Major clusters were typified by *Streptomyces abikoensis*, *Streptomyces cinnamoneus*, *Streptomyces distallicus*, *Streptomyces griseocarneus*, *Streptomyces hiroshimensis* and *Streptomyces netropsis*. Phenotypically, members of each cluster resembled each other closely except for the *S. distallicus* cluster, which was divided phenotypically into the *S. distallicus* and *Streptomyces stramineus* subclusters, and the *S. netropsis* cluster, which was divided into the *S. netropsis* and *Streptomyces eurocidicus* subclusters. Strains in each minor cluster closely resembled each other phenotypically. DNA–DNA relatedness between the representative species and others in each major cluster and/or subcluster, and between strains in the minor clusters, was >70 %, indicating that the major clusters and/or subclusters and the minor clusters each comprise a single species. It was concluded that 59 strains of typical whorl-forming *Streptomyces* species consisted of the following 18 species, including subjective synonym(s): *S. abikoensis*, *Streptomyces ardus*, *Streptomyces blastmyceticus*, *S. cinnamoneus*, *S. eurocidicus*, *S. griseocarneus*, *S. hiroshimensis*, *Streptomyces lilacinus*, '*Streptomyces luteoreticuli*', *Streptomyces luteosporeus*, *Streptomyces mashuensis*, *Streptomyces mobaraensis*, *Streptomyces morookaense*, *S. netropsis*, *Streptomyces orinoci*, *S. stramineus*, *Streptomyces thioluteus* and *Streptomyces viridiflavus*.

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Abbreviations: ISP, International *Streptomyces* Project; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the *gyrB* gene sequences are shown in Fig. 1.

INTRODUCTION

The genus *Streptoverticillium* was proposed by Baldacci (1958) for a distinctive group of whorl-forming *Streptomyces* species, in which 40 species have been classified into 12 groups based on aerial and substrate mycelium colour (Baldacci & Locci, 1974). Furthermore, Locci & Schofield (1989) classified 64 *Streptoverticillium* species in 10 major clusters and 14 single-member species by numerical taxonomy based on phenotypes. Witt & Stackebrandt (1990), however, unified the genera *Streptoverticillium* and *Streptomyces*, based on phylogenetic similarities of partial 16S rRNA gene sequences. At present, 46 species and eight subspecies with validly published names comprise the whorl-forming *Streptomyces* species.

Classification of whorl-forming *Streptomyces* species by using DNA–DNA hybridization has been examined by several researchers. Toyama *et al.* (1974) examined DNA–DNA relatedness of 34 *Streptoverticillium* strains and found that *Streptoverticillium netropsis* ISP 5259^T and *Streptoverticillium flavopersicum* ISP 5053^T, and *Streptoverticillium ehimense* ISP 5253^T, *Streptoverticillium luteoverticillatum* ISP 5038^T and '*Streptomyces reticuli* var. *latumcidicus*' At-79 are closely related by DNA–DNA homology, indicating that they are synonyms. Recently, Labeda (1996) also examined DNA–DNA relatedness of 32 species and three subspecies of whorl-forming *Streptomyces* and revealed that *Streptomyces biverticillatus* ISP 5272^T, *Streptomyces fervens* NRRL 2755^T, *Streptomyces roseoverticillatus* NRRL B-1993^T and '*S. rubrochlorinus*' NRRL B-12558 are subjective synonyms of *Streptomyces baldaccii* NRRL B-3500^T; *Streptomyces kentuckensis* NRRL B-1831^T and *Streptomyces flavopersicus* NRRL 2820^T are subjective synonyms of *S. netropsis* NRRL 2268^T; and *Streptomyces distallicus* NRRL 2886^T is a marginal subjective synonym of *S. netropsis* NRRL 2268^T.

Determination of exact taxonomic status of *Streptomyces* species by using traditional methods such as phenotypes and DNA–DNA relatedness is laborious and time-consuming; we have therefore searched for a simple and precise method for classification and identification of *Streptomyces* species. Kataoka *et al.* (1997) analysed 89 strains of the genus *Streptomyces* that belonged to eight major clusters of category I in *Bergey's Manual of Systematic Bacteriology* (Williams *et al.*, 1989) by using phylogenetic analysis of a 120 bp 16S rDNA fragment that contains a highly variable region between positions 158 and 277 in the numbering system of *Streptomyces ambofaciens* (Pernodet *et al.*, 1989); the authors concluded that these 120 bp nucleotide sequences are useful for rapid identification of *Streptomyces* species. However, more comparative data, including DNA–DNA hybridization and phenetic data, are needed to evaluate whether this method is a useful tool for discrimination at species level in *Streptomyces*. Furthermore, phylogenetic analysis of partial sequences of the *gyrB* gene, which encodes the B subunit of DNA gyrase, has been used for classification of several types of bacteria and has been shown to be a useful tool for discrimination at species level (Yamamoto & Harayama 1995, 1996, 1998; Yamamoto *et al.*, 1999; Kasai *et al.*, 2000). Therefore, we undertook phylogenetic analysis based on partial *gyrB* gene sequences for reclassification of the whorl-forming *Streptomyces* species, which are suspected to include many synonyms because of their similar morphology and narrow range of sugars utilized for growth.

This paper deals with reclassification of the whorl-forming *Streptomyces* species by using their phenotypes, DNA–DNA relatedness and phylogenetic analysis of *gyrB* gene sequences, and also assesses whether phylogenetic analysis of *gyrB* is a useful tool for classification and identification of whorl-forming *Streptomyces* species. This paper also deals with the proposal of *Streptomyces luteireticuli* corrig., sp. nov., nom. rev.

METHODS

Organisms used and culture conditions. Sixty-four strains of whorl-forming *Streptomyces* species were used in this study. Strains were cultured in 5 ml YG medium (1 % glucose, 1 % yeast extract, pH 7·0) in a test tube at 28 °C for 2 days on a reciprocal shaker.

Characterization of phenotypes. Cultured mycelia were harvested, washed three times with sterile distilled water and suspended in 5 ml sterile water. Washed mycelia were inoculated onto International *Streptomyces* Project (ISP) media: yeast extract/malt extract agar (ISP medium 2), oatmeal agar (ISP medium 3), inorganic salts/starch agar (ISP medium 4), glycerol/asparagine agar (ISP medium 5), yeast extract/iron agar (ISP medium 6), tyrosine agar (ISP medium 7) and Pridham–Gottlieb carbon utilization agar medium (ISP medium 9). After incubation at 28 °C for 14 days, morphological, cultural and physiological characteristics of strains were observed and described according to the method of the ISP (Shirling & Gottlieb, 1966).

Preparation of DNA. Total DNA was extracted from 2-day cultured cells by the method of Saito & Miura (1963) with a minor modification: washed cells were suspended in 2 ml 10 mM Tris/HCl buffer (pH 8·0) that contained 1 mg achromopeptidase ml^{-1} (Wako Pure Chemical Industries) and 5 mg lysozyme ml^{-1} (egg white; Wako Pure Chemical Industries). The suspension was incubated for 2 h at 37 °C, then lysed completely by adding 0·2 ml 20 % (w/v) SDS and heating for 10 min at 65 °C. Total crude DNA was extracted with phenol and precipitated with cold ethanol, rinsed with 70 % ethanol and dried. A solution of dried DNA in 0·2 ml 1 × TE (1 mM EDTA in 10 mM Tris/HCl buffer, pH 8·0) was treated with RNase A (Sigma) and proteinase K (Wako Pure Chemical Industries), extracted with phenol/chloroform/isoamyl alcohol (25 : 24 : 1, v/v) and precipitated with ice-cold ethanol. The precipitate was rinsed three times with 70 % ethanol, dried and dissolved in 0·5 ml distilled water. Purity and concentration of the prepared DNA solution were measured with a Beckman model DU-65 spectrophotometer. Solutions with an A_{260}/A_{280} ratio above 1·9 were used for PCR and DNA–DNA hybridization.

DNA–DNA hybridization. DNA–DNA hybridization was performed by the method of Ezaki *et al.* (1989) at 55 °C in 2 × SSC (1 × SSC: 0·15 M NaCl plus 0·015 M sodium citrate, pH 7·0) that contained 50 % formamide. The experiment was performed at least three times; DNA–DNA relatedness was expressed as a mean percentage of the homologous DNA binding value. Standard deviation (SD) was < 5 %.

PCR amplification of *gyrB*. The *gyrB* gene was amplified by PCR (Saiki *et al.*, 1988) by using TaKaRa Taq LX and one of the following pairs of primers: UP1 (forward: 5′-GAAGTCATCATGACCGTTCTGCAYGCNGGNGGNAARTTYGA-3′) and UP2r (reverse: 5′-AGCAGGGTACGGATGTGCGAGCCRTCNACRTCNGCRTCNGTCAT-3′) (Yamamoto & Harayama, 1995); or PF-1 (forward: 5′-GAGGTCGTGCTGACCGTGCTGCACGCGGGCGGCAAGTTCGGC-3′), complementary to positions 355–396 in the numbering system of *Streptomyces coelicolor* A3(2) *gyrB* sequence (Calcutt, 1994) and PR-2 (reverse: 5′-GTTGATGTGCTGGCCGTCGACGTCGGCGTCCGCCAT-3′), complementary to positions 1624–1659, which were newly designed from conserved regions of the *gyrB* sequence of *S. coelicolor* A3(2) (Calcutt, 1994) and other *Streptomyces* species (H. Kasai, unpublished data). Amplification was performed in a total volume of 50 µl, which contained 10 pmol each primer, 0·1 µg target DNA, 5 µl 10 × buffer and 2·5 U Taq polymerase, in a 0·5 ml microtube. DNA was amplified under the following conditions: 95 °C for 3 min for denaturation of target DNA; 30 cycles of denaturation at 95 °C for 0·5 min, primer annealing at 65 °C for 0·5 min and primer extension at 72 °C for 1 min; 72 °C for 4 min for completion of amplification; and cooling at 4 °C.

Sequencing of *gyrB*. PCR products were run on a 1·5 % (w/v) agarose gel to remove primers; bands of amplified DNA (approx. 1·3 kb) were cut out with a scalpel and purified by using a QIAquick Gel Extraction kit (Qiagen) according to the manufacturer's instructions. Purified PCR products were subjected to cycle sequencing by using a BigDye Terminator Cycle Sequencing kit by Amplitaq FS (PE Applied Biosystems) and a Gene Amp PCR System 9700 (PerkinElmer) according to the manufacturer's protocol with the following seven primers: F-1 (5′-GAGGTCGTGCTGACCGTGCTGCA-3′, positions 355–378), F-352 (5′-TACCACTACGAGGGCGGCATC-3′, positions 779–799), F-701 (5′-AGCCGCAGTTCGAGGGCCAGAC-3′, positions 1128–1149), R-1 (5′-GTTGATGTGCTGGCCGTCGACGT-3′, positions 1637–1659), R-996 (5′-CTCGACGATGAAGATCTCGCAC-3′, positions 1393–1414), R-728 (5′-GTCTTGGTCTGGCCCTCGAACTG-3′, positions 1133–1155) and R-4 (5′-CGCTCCTTGTCCTCGGCCTC-3′, positions 866–885). Conditions for thermal cycling were 25 cycles of denaturation at 96 °C for 10 s, primer annealing at 50 °C for 5 s and primer extension at 60 °C for 4 min. Products were purified as recommended by PE Applied Biosystems and were analysed with a model ABI PRISM 310 Genetic Analyser (PE Applied Biosystems) according to the manufacturer's protocol.

Phylogenetic analysis. GyrB amino acid sequences, translated from *gyrB* gene sequences, were aligned by using CLUSTAL W software, version 1.7 (Thompson *et al.*, 1994) and corrected manually. Sequences of the *gyrB* gene were aligned according to the alignments of GyrB sequences. Phylogenetic analyses were performed by using the neighbour-joining (NJ) (Saitou & Nei, 1987), maximum-parsimony (MP) (Swofford, 2000) and maximum-likelihood (ML) (Adachi & Hasegawa, 1992) methods. Evolutionary distances and similarity values based on Kimura's two-parameter model (Kimura, 1980) were calculated by using CLUSTAL W software, version 1.7. A phylogenetic tree was generated from the NJ method by using NJplot (Perrière & Gouy, 1996). Stability of the tree was assessed by bootstrap analysis with the resampling method of Felsenstein (1993) with 1000 replications, by using CLUSTAL W version 1.7. Nucleotide sequence data reported in this paper are available in DDBJ, EMBL and GenBank under the accession numbers given in Fig. 1.

RESULTS AND DISCUSSION

Grouping of whorl-forming *Streptomyces* species based on similar phenotypes

Table 1 shows phenotypic characteristics of 64 strains that were newly re-examined in this study. They were classified into 11 groups, based on the similarity of their phenotypes. Five strains failed to show typical whorl-branching hyphae: *Streptomyces aureoverticillatus* NBRC (formerly IFO) 12742^{T}, *Streptomyces lavenduligriseus* NBRC 13405^{T}, '*Streptomyces nobilis*' NBRC 13386, '*S. pallidus*' NBRC 13434 and *Streptomyces spiroverticillatus* NBRC 12821^{T}. *Streptomyces thioluteus* NBRC 13341^{T} was classified as a strain with yellow aerial mass according to the original description, although we could not confirm the whorl formation and aerial mass colour as aerial mycelium was not formed on any of the agar media tested. These groupings by aerial mass and substrate mycelium colour, melanin formation ability and sugar utilization pattern were somewhat different from the numerical classification of the whorl-forming *Streptomyces* (formerly *Streptoverticillium*) based on 41 phenetic characters (Locci & Schofield, 1989).

Phenotypes of 16 strains in groups Ia and Ib closely resembled each other, except for substrate mycelium colour: strains in group Ia were reddish, purplish-brown to dark brownish-red, whereas strains in group Ib were yellowish-brown, brown to dark brown. Phenotypes of strains in each of groups Ib, II, III, IV, V, VI, VIII and IXa, except for *Streptomyces orinoci* NBRC 13466^{T}, and X, except for *Streptomyces stramineus* NBRC 16131^{T}, closely resembled each other. Although strains in group V and VI closely resembled each other phenotypically, they were divided into two groups, *Streptomyces luteoverticillatus* (V) and others (VI) according to the results of Toyama *et al.* (1974). *S. orinoci* NBRC 13466^{T} and *S. stramineus* NBRC 16131^{T} were distinctive in phenotype and DNA–DNA relatedness.

Phylogenetic structure based on *gyrB* sequences and DNA–DNA relatedness

DNA fragments of about 1·3 kb that corresponded to approximately 64 % of the whole *gyrB* gene sequence of *S. coelicolor* A3(2) were amplified by PCR with the primers described in Methods. For phylogenetic analysis, sequences of 1197 nt [positions 397–1623, according to the numbering system of *S. coelicolor* A3(2) (Calcutt, 1994)] of the fragments were used. Sequence similarity values among the typical whorl-forming strains were 90·1–100 %. Phylogenetic tree topologies inferred from NJ, MP and ML were very similar to one another, except that '*Streptomyces luteoreticuli*' NBRC 13422 and *S. thioluteus* NBRC 13341^{T} formed a cluster by MP and ML, but not by NJ. Fig. 1 shows the phylogenetic tree inferred from NJ. The 64 strains formed two independent clusters: typical whorl-forming *Streptomyces* species (59 strains) and atypical whorl-forming ones (five strains), which is the same as the classification of the strains by phenotype. Typical whorl-forming strains formed 18 clusters that consisted of six major clusters, seven minor clusters and five single-member clusters, based on the threshold value of 97 % *gyrB* sequence similarity. These clusters were roughly in accord with phenotypic groups (Table 1). The six major clusters were typified by *S. abikoensis*, *S. cinnamoneus*, *S. distallicus*, *S. griseocarneus*, *S. hiroshimensis* and *S. netropsis*.

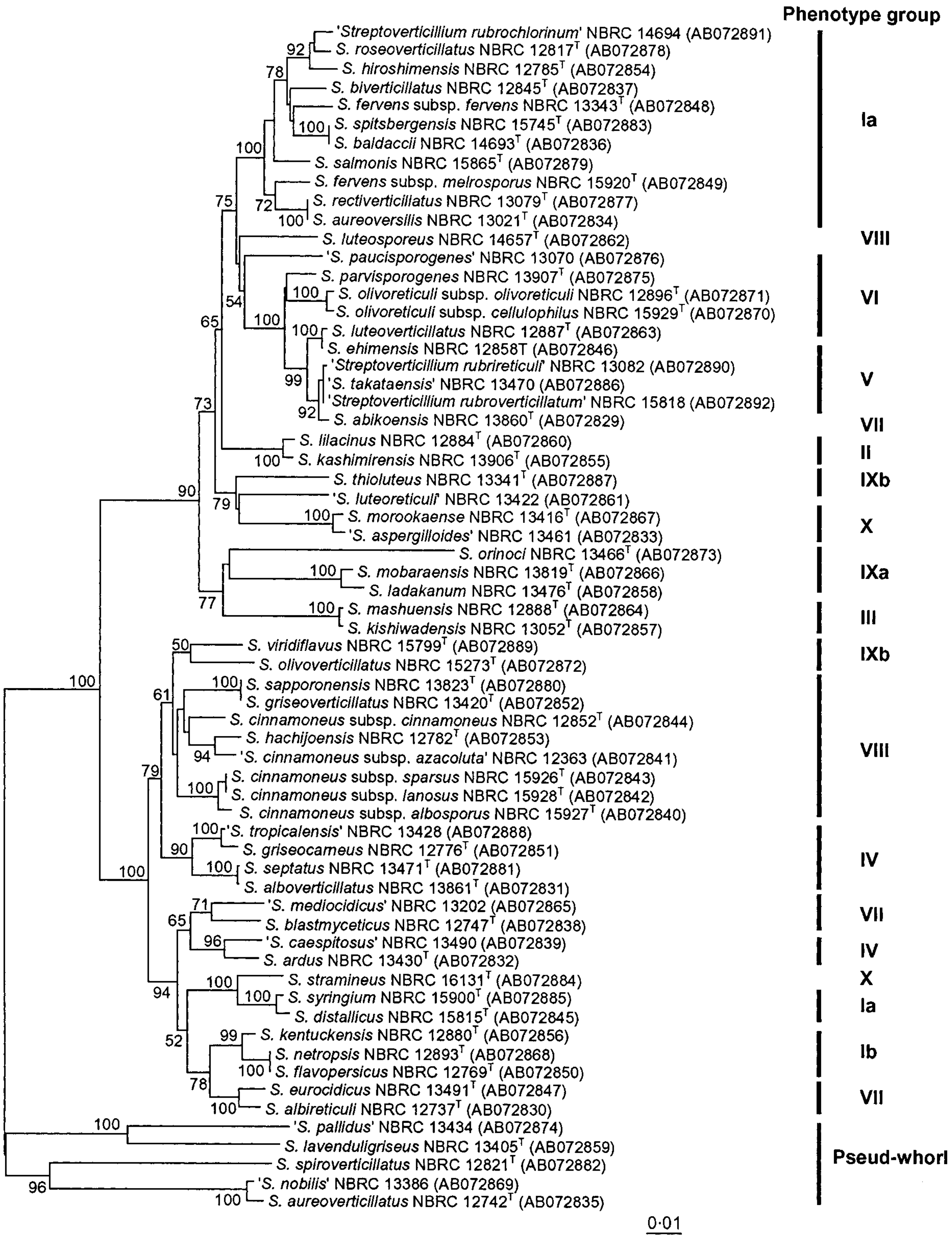

Fig. 1. Phylogenetic tree of whorl-forming *Streptomyces* species inferred by the NJ method (Saitou & Nei, 1987), using Kimura's evolutionary distance (Kimura, 1980) and based on a comparison of 1197 nt of *gyrB* gene sequences. The tree is depicted by NJplot (Perrière & Gouy, 1996). Bootstrap values $>50\,\%$, expressed as percentages of 1000 replications (Felsenstein, 1993), are given at nodes. Accession numbers of *gyrB* sequences are given in parentheses. Bar, 0·01 K_{nuc}.

Table 1. Whorl-forming *Streptomyces* species, grouped by their phenotypic characteristics

Abbreviations: Mel, melanin formation on ISP media 1, 6 and 7; Glc, D-glucose; Ara, L-arabinose; Fru, D-fructose; Ino, i-inositol; Man, D-mannitol; Raf, raffinose; Rha, L-rhamnose; Suc, sucrose; Xyl, D-xylose; R, red colour-series (beige, rose-beige or purplish-pink); Y, yellow colour-series (whitish-yellow, light yellow or yellow); W, white colour-series (white or greyish-white); G, grey colour-series (brownish-grey or grey); V, violet colour-series (purple or reddish-purple); P, pink; Br, brown; Or, orange; Pur, purple; Ol, olive; d, dark; y, yellowish; r, reddish; br, brownish; ++, strongly positive; +, positive; +/−, doubtful; −, negative.

Taxon	NBRC no.	Aerial mass colour	Reverse-side colour	Mel	Glc	Ara	Fru	Ino	Man	Raf	Rha	Suc	Xyl
Group I													
S. aureoversilis	13021^{T}	R, Y	r-Or, Br	+	++	−	−	++	−	−	−	−	−
S. baldaccii	14693^{T}	R, P	d-R, r-V	+	++	−	−	++	−	−	−	−	−
S. biverticillatus	12845^{T}	R, V	R, or-R	+	++	−	+	+	−	−	−	−	−
S. distallicus	15815^{T}	R	d-y-Br, d-r-Br	+	++	−	++	++	−	−	−	−	−
S. fervens subsp. *fervens*	13343^{T}	R	R, d-R	+	++	−	−	++	−	−	−	−	−
S. fervens subsp. *melrosporus*	15920^{T}	R	Br, d-r-Br	+	++	−	+	++	−	−	−	−	−
S. flavopersicus	12769^{T}	R	y-Br, Br	+	++	−	+	++	−	−	−	−	−
S. hiroshimensis	12785^{T}	R	r-Y, r-y-Br	+	++	−	+/−	++	−	−	−	−	−
S. kentuckensis	12880^{T}	R, W	y-Br, Br	+*	++	−	−	++	−	−	−	−	−
S. netropsis	12893^{T}	R	y-Br, r-Br	+*	++	−	−	++	−	−	−	−	−
S. rectiverticillatus	13079^{T}	R, W	y-Br, P	+	++	−	++	++	−	−	−	−	−
S. roseoverticillatus	12817^{T}	R	r-Y	+	++	−	−	++	−	−	−	−	−
S. salmonis	15865^{T}	R, Y	d-R, r-V	+	++	−	+/−	++	−	−	−	−	−
S. spitsbergensis	15745^{T}	R	d-R	+	++	−	−	++	−	−	−	−	−
S. syringium	15900^{T}	R	d-y-Br, d-r-Br	+	++	−	−	++	−	−	−	−	−
'*Streptoverticillium rubrochlorinum*'	14694	R, W	r-Br, r-Pur	+	++	−	−	++	−	−	−	−	−
'*Streptoverticillium rubroverticillatum*'	15818	R	y-Br, d-Br	+	++	−	++	++	−	−	−	−	−
Group II													
S. kashimirensis	13906^{T}	R, W, V	d-r-Br, d-r-Pur	+	++	−	−	++	−	−	−	−	−
S. lilacinus	12884^{T}	R, V	d-r-Br, d-r-Pur	+	++	−	−	++	−	−	−	−	−
Group III													
S. kishiwadensis	13052^{T}	R	y-Br, Br	+	++	−	++	++	−	−	−	++	−
S. mashuensis	12888^{T}	R	y-Br, Br	+	++	−	++	++	−	−	−	++	−
Group IV													
S. ehimensis	12858^{T}	R, G, Y	d-Br, Ol	+	++	−	++	++	++	−	−	−	−
'*S. takataensis*'	13470	R, W	y-Br, Br	+	++	−	++	++	++	−	−	−	−
'*Streptoverticillium rubrireticuli*'	13082	R, G, W	y-Br, Br	+	++	−	++	++	++	−	−	−	−
Group V													
S. luteoverticillatus	12887^{T}	Y, W, R	y-Br, Br	+	++	−	+/−	++	−	−	−	−	−
S. olivoreticuli subsp. *olivoreticuli*	12896^{T}	W, R	y-Br, Br	+	++	−	−	++	−	−	−	−	−
S. olivoreticuli subsp. *cellulophilus*	15929^{T}	Y, W, R	y-Br, Br	+	++	−	+/−	+	−	−	−	−	−
S. parvisporogenes	13907^{T}	W, Y, R	y-Br	+	++	−	−	+	−	−	−	−	−
'*S. paucisporogenes*'	13070	G	Br, d-Br	+	++	−	−	++	−	−	−	−	−
Group VI													
S. alboverticillatus	13861^{T}	P, R, G	Br, d-Br	+	++	−	−	+	−	−	−	−	−
S. ardus	13430^{T}	W, G, R	y-Br	+	++	−	+	++	−	−	−	−	−
S. griseocarneus	12776^{T}	R	y-Br, d-Br	+	++	−	−	++	−	−	−	−	−
S. septatus	13471^{T}	R, Y	y-Br, d-Br	+	++	−	+/−	++	−	−	−	−	−
'*S. tropicalensis*'	13428	R, G	y-Br, d-Br	+	++	−	+	++	−	−	−	−	−
Group VII													
S. abikoensis	13860^{T}	Y, W	y-Br	+	++	−	+/−	+	−	−	−	−	−
S. albireticuli	12737^{T}	Y, W	y-Br	+	++	−	+/−	++	−	−	−	−	−
S. blastmyceticus	12747^{T}	Y, Gr	y-Br	+	++	−	+	++	−	−	−	−	−
'*S. caespitosus*'	13490	Y, Gr	y-Br, Br	+	++	−	++	++	−	−	−	−	−
S. eurocidicus	13491^{T}	Y	y-Br, Br	+	++	−	+	++	−	−	−	−	−
'*S. mediocidicus*'	13202	W, G	y-Br	+	++	−	+	++	−	−	−	−	−

Table 1. cont.

Taxon	NBRC no.	Aerial mass colour	Reverse-side colour	Mel	Glc	Ara	Fru	Ino	Man	Raf	Rha	Suc	Xyl
Group VIII													
S. cinnamoneus subsp. *albosporus*	15927^T	R, (Y)	br-Y	−	++	−	−	++	−	−	−	−	−
‘*S. cinnamoneus* subsp. *azacoluta*’	12363	R, G, V	y-Br, Br	−	++	−	−	++	−	−	−	−	−
S. cinnamoneus subsp. *cinnamoneus*	12852^T	R	y-Br, Ol-Br	−	++	−	−	++	−	−	−	−	−
S. cinnamoneus subsp. *lanosus*	15928^T	R, (Y)	br-Y	−	++	−	+	++	−	−	−	−	−
S. cinnamoneus subsp. *sparsus*	15926^T	R, (Y)	br-Y	−	++	−	+/−	+	−	−	−	−	−
S. griseoverticillatus	13420^T	R	y-Br, or-Y, Br	−	++	−	−	++	−	−	−	−	−
S. hachijoensis	12782^T	R, V	y-Br, Ol	−	++	−	+/−	++	−	−	−	−	−
S. luteosporeus	14657^T	R, G	y-Br	−	++	−	−	++	−	−	−	−	−
S. sapporonensis	13823^T	W, R	R, Br	−	++	−	−	++	−	−	−	−	−
Group IX													
S. ladakanum	13476^T	W, Y, G	Y, y-Br	−	++	−	+	+	−	−	−	−	−
‘*S. luteoreticuli*’	13422	Y, G	d-Br	−	++	−	+	++	−	−	−	−	−
S. mobaraensis	13819^T	Y, W, G	y-Br, Ol	−	++	−	+	+	−	−	−	−	−
S. olivoverticillatus	15273^T	W, Y, G	y-Br, Br	−	++	−	+/−	+	−	−	−	−	−
S. orinoci	13466^T	G, W, Y	br-Y	−	++	−	+	+	−	−	−	−	−
S. thioluteus	13341^T	None (Y)	Ol, d-y-Br	−	++	−	+/−	++	−	−	−	−	−
S. viridiflavus	15799^T	G, Y	d-y-Br	−	++	−	−	+	−	−	−	−	−
Group X													
‘*S. aspergilloides*’	13461	Y, G	y-Br, Br	−	++	−	++	++	++	−	−	−	−
S. morookaense	13416^T	Y	y-Br, Br	−	++	−	+	++	++	−	−	−	−
S. stramineus	16131^T	Y	Y, y-Br	−	++	−	+	++	++	−	−	−	−
Pseudo-verticillati													
S. aureoverticillatus	12742^T	R, W	y-R	−	++	+	+	++	++	+	−	−	+
‘*S. nobilis*’	13386	R, W	pur-R, r-Br	+	++	++	++	++	++	+	−	−	+
S. lavenduligriseus	13405^T	G, ol-Gr	d-Br, r-Br	+	++	++	++	+	++	−	+	−	+
‘*S. pallidus*’	13434	W, R	y-Br, Br	+	++	+	+	+	−	+	+	++	−
S. spiroverticillatus	12821^T	W, R, Y	r-Br, br-Ol	−	++	+	+	−	−	−	−	−	++

*Positive, but very weak.

The *S. abikoensis* cluster (> 98·1 % *gyrB* sequence similarity) was divided into two subclusters: the *S. abikoensis* subcluster contained six strains with > 99·0 % *gyrB* sequence similarity and the *Streptomyces olivoverticillatus* subcluster contained three strains with > 98·0 % *gyrB* sequence similarity. DNA–DNA relatedness levels between *S. abikoensis* NBRC 13860^T and others in the cluster were > 70 %, except for *Streptomyces parvisporogenes* NBRC 13907^T (65 %) (Table 2). In the *S. abikoensis* cluster, *Streptomyces ehimensis* NBRC 12858^T, ‘*Streptomyces takataensis*’ NBRC 13470 and ‘*Streptoverticillium rubrireticuli*’ NBRC 13082, which exhibited the same phenotype (including sugar utilization pattern; phenotype group IV, Table 1), also exhibited high *gyrB* sequence similarity (99–99·1 %) and high levels of DNA–DNA homology (99–101 %) (data not shown). In phenetic group V, levels of DNA–DNA relatedness among *S. luteoverticillatus* NBRC 12887^T, *Streptomyces olivoreticuli* subsp. *olivoreticuli* NBRC 12896^T, *S. parvisporogenes* NBRC 13907^T and ‘*Streptomyces paucisporogenes*’ NBRC 13070 were 65–83 % (data not shown), in accord with the results reported by Toyama *et al.* (1974).

The *S. cinnamoneus* cluster (> 97·3 % *gyrB* sequence similarity) was divided into four subclusters: the *S. cinnamoneus* subsp. *cinnamoneus* NBRC 12852^T, *Streptomyces griseoverticillatus* (two strains, 100 % *gyrB* similarity), *Streptomyces hachijoensis* (two strains, 98·7 %) and *S. cinnamoneus* subsp. *albosporus* (three strains, 99·4–100 %) subclusters. *S. cinnamoneus* subsp. *lanosus* NBRC 15928^T and *S. cinnamoneus* subsp. *sparsus* NBRC 15926^T, and *S. griseoverticillatus* NBRC 13420^T and *Streptomyces sapporonensis* NBRC 13823^T, had identical *gyrB* sequences and 97 and 100 % DNA–DNA relatedness, respectively. DNA–DNA relatedness between *S. hachijoensis* NBRC 12782^T and ‘*S. cinnamoneus* subsp. *azacoluta*’ NBRC 12363 was 84 %, which is the same as that reported by Labeda (1996). Levels of DNA–DNA relatedness between *S. cinnamoneus* subsp. *cinnamoneus* NBRC 12852^T and *S. cinnamoneus* subsp. *albosporus* NBRC 15927^T, *S. cinnamoneus* subsp. *lanosus* NBRC 15928^T and *S. cinnamoneus* subsp. *sparsus* NBRC 15926^T were 65, 80 and 81 %, respectively, supporting the fact that they belong to the same species. Furthermore, DNA–DNA relatedness and *gyrB* sequence similarity among *S. cinnamoneus* subsp. *cinnamoneus* NBRC 12852^T, *S. hachijoensis*

Table 2. Levels of *gyrB* sequence similarity and DNA–DNA homology between the representative species and others in six major clusters

Strain	*gyrB* sequence similarity (%)	DNA–DNA homology (%)
***S. abikoensis* cluster**		
S. abikoensis NBRC 13860^T	100	100
'*Streptoverticillium rubroverticillatum*' NBRC 15818	99·6	81*
'*S. takataensis*' NBRC 13470	99·6	81
'*Streptoverticillium rubrireticuli*' NBRC 13082	99·5	81
S. ehimensis NBRC 12858^T	99·2	83
S. luteoverticillatus NBRC 12887^T	99	85
S. parvisporgenes NBRC 13907^T	98	65
S. olivoreticuli subsp. *olivoreticuli* NBRC 12896^T	97·7	71
'*S. paucisporogenes*' NBRC 13070	95·8	59
***S. cinnamoneus* cluster**		
S. cinnamoneus subsp. *cinnamoneus* NBRC 12852^T	100	100
S. hachijoensis NBRC 12782^T	97·8	80
S. griseoverticillatus NBRC 13420^T	97·6	79
S. cinnamoneus subsp. *albosporus* NBRC 15927^T	97·3	65
S. cinnamoneus subsp. *lanosus* NBRC 15928^T	97·3	80
S. cinnamoneus subsp. *sparsus* NBRC 15926^T	97·3	81
S. viridiflavus NBRC 15799^T	97·3	32
***S. distallicus* cluster**		
S. distallicus NBRC 15815^T	100	100
S. syringium NBRC 15900^T	99·5	97
S. stramineus NBRC 16131^T	97·5	45
***S. griseocarneus* cluster**		
S. griseocarneus NBRC 12776^T	100	100
'*S. tropicalensis*' NBRC 13428	99·5	96
S. alboverticillatus NBRC 13861^T	97·8	71
S. septatus NBRC 13471^T	97·7	69
***S. hiroshimensis* cluster**		
S. hiroshimensis NBRC 12785^T	100	100
S. roseoverticillatus NBRC 12817^T	98·8	88
'*Streptoverticillium rubrochlorinum*' NBRC 14694	98·7	73
S. baldaccii NBRC 14693^T	97·7	91
S. biverticillatus NBRC 12845^T	97·7	81
S. spitsbergensis NBRC 15745^T	97·7	72
S. aureoversilis NBRC 13021^T	97·6	72
S. rectiverticillatus NBRC 13079^T	97·6	76
S. fervens subsp. *fervens* NBRC 13343^T	97·4	73
S. salmonis NBRC 15865^T	97·1	68
***S. netropsis* cluster**		
S. netropsis NBRC 12893^T	100	100
S. flavopersicus NBRC 12769^T	100	93
S. kentuckensis NBRC 12880^T	98·8	75
S. albireticuli NBRC 12737^T	97·4	32
S. eurocidicus NBRC 13491^T	97·2	38
S. distallicus NBRC 15815^T	95·9	73

*Data from Labeda (1996).

NBRC 12782^T and *S. griseoverticillatus* NBRC 13420^T were 79–80 and 97·6–97·8 %, respectively (Table 2), suggesting that the three species are synonymous.

The *S. distallicus* cluster ($>97·5$ % *gyrB* sequence similarity) was divided into two subclusters according to distinctive phenotypes: the *S. distallicus* subcluster, which consisted of

S. distallicus NBRC 15815^T and *Streptomyces syringium* NBRC 15900^T, and the *S. stramineus* subcluster. Sequence similarity in *gyrB* and DNA–DNA relatedness between *S. distallicus* NBRC 15815^T and *S. syringium* NBRC 15900^T were 99·5 and 97 %, respectively, suggesting that they are synonymous. On the other hand, DNA–DNA relatedness between *S. distallicus* NBRC 15815^T and *S. stramineus* NBRC 16131^T was 42–48 %, indicating that these subclusters are independent despite their comparatively high *gyrB* sequence similarity (97·5 %).

The *S. griseocarneus* cluster (> 97·7 % *gyrB* sequence similarity) was also divided into two subclusters: *S. griseocarneus* (two strains, 99·5 % similarity) and *Streptomyces septatus* (two strains, 99·5 % similarity). DNA–DNA relatedness levels between *S. griseocarneus* NBRC 12776^T and '*S. tropicalensis*' NBRC 13428, and between *S. septatus* NBRC 13471^T and *Streptomyces alboverticillatus* NBRC 13861^T were 96 and 91–96 %, respectively. In addition, DNA–DNA relatedness between *S. griseocarneus* NBRC 12776^T and *S. septatus* NBRC 13471^T was 69–71 %, indicating that the *S. griseocarneus* cluster consists of a single species. On the other hand, DNA–DNA relatedness and *gyrB* sequence similarity between *S. griseocarneus* NBRC 12776^T and *Streptomyces ardus* NBRC 13430^T, and between *S. alboverticillatus* NBRC 13861^T and *S. ardus* NBRC 13430^T, which belong to the same group phenetically (Table 1), were < 40 % and approximately 94 %, respectively (data not shown).

The *S. hiroshimensis* cluster (> 97 % *gyrB* sequence similarity) was divided into four subclusters: *S. baldaccii* (four strains, 98–100 % *gyrB* similarity), *S. hiroshimensis* (three strains, 98·7–98·8 % similarity), *Streptomyces rectiverticillatus* (three strains, 98·2–100 % similarity) and *Streptomyces salmonis*. *Streptomyces aureoversilis* NBRC 13021^T and *S. rectiverticillatus* NBRC 13079^T, and *S. baldaccii* NBRC 14693^T and *Streptomyces spitsbergensis* NBRC 15745^T, had identical *gyrB* sequences and high levels of DNA–DNA relatedness (97–100 and 94 %, respectively). As shown in Table 2, DNA–DNA relatedness between *S. hiroshimensis* NBRC 12785^T (the representative species) and others was 68–91 %. Furthermore, levels of DNA–DNA relatedness between two representative species, *S. baldaccii* NBRC 14693^T and *S. roseoverticillatus* NBRC 12817^T, and others were 78–99 and 67–92 %, respectively (data not shown). These results indicate that the *S. hiroshimensis* cluster consists of a single species. On the other hand, DNA–DNA relatedness and *gyrB* sequence similarity among *S. baldaccii* NBRC 14693^T, *S. distallicus* NBRC 15815^T and *S. syringium* NBRC 15900^T, which belong to the same group phenetically, were 46–55 and 90·8–91·0 %, respectively (data not shown), suggesting that *S. distallicus* NBRC 15815^T and *S. syringium* NBRC 15900^T did not belong genetically to the *S. baldaccii* subcluster, as shown in Fig. 1.

The *S. netropsis* cluster (> 97·1 % *gyrB* sequence similarity) was divided into two subclusters by phenotype: the *S. netropsis* subcluster (three strains, 98·9–100 % *gyrB* similarity) and the *Streptomyces eurocidicus* subcluster (two strains, 98·7 % similarity). *S. netropsis* NBRC 12893^T and *S. flavopersicus* NBRC 12769^T exhibited identical *gyrB* sequences. DNA–DNA relatedness of three strains in the *S. netropsis* subcluster was 75–93 %, which agrees with the results of Labeda (1996). DNA–DNA relatedness and *gyrB* sequence similarity between *S. eurocidicus* NBRC 13491^T and *Streptomyces albireticuli* NBRC 12737^T were 96–97 and 99·1 %, respectively. On the other hand, DNA–DNA relatedness between *S. netropsis* NBRC 12893^T and *S. eurocidicus* NBRC 13491^T was 32–44 %, indicating that these two subclusters are independent of each other.

Table 3 shows DNA–DNA relatedness levels and *gyrB* sequence similarity in the seven minor clusters: strains in each cluster resembled each other closely in phenotype. Strains in each cluster exhibited species levels of DNA–DNA relatedness with each other (72–100 %) and high *gyrB* sequence similarity (97–99·8 %), suggesting that each cluster consists of a single species. *Streptomyces ladakanum* NBRC 13476^T exhibited species levels of identity with *Streptomyces mobaraensis* NBRC 13819^T, with high DNA–DNA relatedness (97 %) and *gyrB* sequence similarity (99·1 %). This result is consistent with that of Labeda (1996). DNA–DNA relatedness and *gyrB* sequence similarity values between '*S. luteoreticuli*' NBRC 13422 and *S. thioluteus* NBRC 13341^T, which formed a cluster in MP and ML, were 31 and 95·2 %, respectively, suggesting that these two strains are independent taxa. *Streptomyces luteosporeus* NBRC 14657^T and *S. orinoci* NBRC 13466^T are distinct taxa on the basis of *gyrB* sequence similarity and DNA–DNA relatedness. '*S. paucisporogenes*' NBRC 13070 is considered

Table 3. Levels of DNA–DNA relatedness and *gyrB* sequence similarity in seven minor clusters

Cluster	*gyrB* sequence similarity (%)	DNA–DNA homology (%)
S. mashuensis NBRC 12888^T, *S. kishiwadensis* NBRC 13052^T	99·8	90–97
S. morookaense NBRC 13416^T, '*S. aspergilloides*' NBRC 13461	99·4	100
S. lilacinus NBRC 12884^T, *S. kashimirensis* NBRC 13906^T	99·4	90
S. mobaraensis NBRC 13819^T, *S. ladakanum* NBRC 13476^T	99·1	97
S. ardus NBRC 13430^T, '*S. caespitosus*' NBRC 13490	98·2	72–79
S. blastmyceticus NBRC 12747^T, '*S. mediocidicus*' NBRC 13202	97·3	94–95
S. viridiflavus NBRC 15799^T, *S. olivoverticillatus* NBRC 15273^T	97·0	70–80

to be marginally synonymous with *S. abikoensis* NBRC 13860^{T}, as it exhibits 59, 62 and 65 % DNA–DNA relatedness with *S. abikoensis* NBRC 13860^{T}, *S. ehimensis* NBRC 12858^{T} and *S. luteoverticillatus* NBRC 12887^{T}, respectively, which are in accord with the results of Toyama *et al.* (1974). This strain may be related to *S. abikoensis* NBRC 13860^{T} at the subspecies level. *S. stramineus* NBRC 16131^{T} is also a distinct taxon in phenotype and DNA–DNA relatedness, although it has 97·5 % *gyrB* sequence similarity with *S. distallicus* NBRC 15815^{T}. *S. distallicus* NBRC 15815^{T} was considered to be a subjective synonym of *S. netropsis* NBRC 12893^{T} based on DNA–DNA relatedness (73 %), although the *gyrB* sequence similarity value between *S. netropsis* NBRC 12893^{T} and *S. distallicus* NBRC 15815^{T} was 95·9 %. This result is in accord with that of Labeda (1996), who indicated that *S. distallicus* NRRL 2268^{T} is marginally synonymous with *S. netropsis* NRRL 2886^{T}.

According to the study of Labeda (1996), *S. baldaccii* NRRL B-3500^{T} and *S. hiroshimensis* NRRL B-5484^{T}, and *S. griseocarneus* NRRL B-1068^{T} and '*S. tropicalensis*' NRRL B-12481, are distinct taxa based on DNA–DNA relatedness. However, results of *gyrB* sequence similarity and DNA–DNA relatedness from the current study indicated that each cluster consists of a single species (Table 2 and Fig. 1). To confirm these results, we reanalysed the *gyrB* gene sequences of *S. baldaccii* JCM 4272^{T}, *S. hiroshimensis* NBRC 3839^{T}, *S. griseocarneus* NRRL B-1068^{T} and '*S. tropicalensis*' NRRL B-12481. Their *gyrB* sequences were the same as obtained previously.

Among the atypical whorl-forming *Streptomyces* species, *S. aureoverticillatus* NBRC 12742^{T} and '*S. nobilis*' NBRC 13386 closely resembled each other in phenotype, except for the ability to form melanin (Table 1). They also had high DNA–DNA relatedness (73–75 %) and *gyrB* sequence similarity (99·4 %), indicating that they are synonyms.

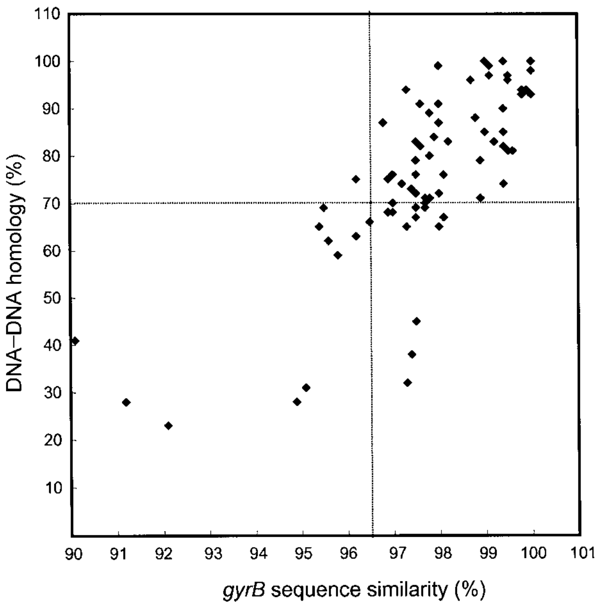

Fig. 2. Correlation between DNA–DNA relatedness and *gyrB* sequence similarity. Horizontal line indicates 70 % DNA–DNA relatedness; vertical line indicates 96·5 % *gyrB* sequence similarity.

Correlation between *gyrB* sequence similarity and DNA–DNA relatedness

Fig. 2 shows the correlation between *gyrB* sequence similarity values and levels of DNA–DNA relatedness in whorl-forming *Streptomyces* species. All strains that exhibited 98·5–100 % *gyrB* sequence similarity showed almost-identical phenotypes and high DNA–DNA relatedness (70–100 %) without exception, suggesting that these strains are synonyms. This result is in good accord with that of Kasai et al. (2000), who reported that a *gyrB* genetic distance of about 0·0014, roughly equivalent to 98·5 % *gyrB* sequence similarity, would correspond to 70 % DNA–DNA relatedness. Furthermore, strains with approximately 97 % or higher *gyrB* sequence similarity and similar phenotypes in the cluster exhibited DNA–DNA relatedness of >65 %, an acceptable value for proposal of a single species. Three exceptional cases were found: the combinations of *S. distallicus* NBRC 15815^{T} and *S. stramineus* NBRC 16131^{T} (97·5 % *gyrB* sequence similarity and 45 % DNA–DNA relatedness), *S. netropsis* NBRC 12893^{T} and *S. albireticuli* NBRC 12737^{T} (97·4 % sequence similarity and 38 % DNA–DNA relatedness), and *S. viridiflavus* NBRC 15799^{T} and *S. cinnamoneus* subsp. *cinnamoneus* NBRC 12852^{T} (97·3 % sequence similarity and 32 % DNA–DNA relatedness); in each combination, the strains are independent species. Strains with 95·5–96·5 % *gyrB* sequence similarity and similar phenotypes in a cluster exhibited DNA–DNA relatedness of 59–75 %, indicating that they are grouped at the species or subspecies level.

It is laborious work to determine exact taxonomic status of whorl-forming *Streptomyces* species by using their phenotypes and DNA–DNA relatedness. In particular, the colour of aerial mass and substrate mycelium gives ambiguous results, as colours may vary under different conditions. For classification and identification of *Streptomyces* species, phylogenetic analysis based on the *gyrB* gene sequence gives objective results for clustering of genetically similar strains. For example, *S. abikoensis* NBRC 13860^{T} was classified as a strain in a yellow series (group VII) by phenotypic classification. However, DNA–DNA relatedness between *S. abikoensis* NBRC 13860^{T} and others in the group was <40 %. Phylogenetic analysis based on *gyrB* sequences revealed that this strain formed a cluster with *S. ehimensis* NBRC 12858^{T} (group V) and *S. luteoverticillatus* NBRC 12887^{T} (group VI), which have red aerial masses, and exhibited high levels of DNA–DNA homology with them. As mentioned above, clustering based on *gyrB* sequences was congruent with those of phenotypes and DNA–DNA hybridization.

We conclude that phylogenetic analysis of *gyrB* sequences is the most convenient procedure for classification and/or identification of whorl-forming *Streptomyces* species at the species level. We believe that this method is a useful tool for discrimination at the species level in *Streptomyces*. We are currently constructing a database of *gyrB* sequences for identification and classification of whorl-forming, and other, *Streptomyces* species.

Taxonomic status of whorl-forming *Streptomyces* species

On the basis of similarity of phenotypes, *gyrB* gene sequences and DNA–DNA relatedness, the 59 formerly described whorl-forming *Streptomyces* species actually consist of the following 18 species with subjective synonym(s):

***Streptomyces abikoensis* NBRC 13860^T.** Subjective synonyms: *S. ehimensis* NBRC 12858^T, *S. luteoverticillatus* NBRC 12887^T, *S. olivoreticuli* NBRC 12896^T, *S. parvisporogenes* NBRC 13907^T, '*S. paucisporogenes*' NBRC 13070, *S. olivoreticuli* subsp. *cellulophilus* NBRC 15929^T, '*S. takataensis*' NBRC 13470, '*Streptoverticillium rubrireticuli*' NBRC 13082 and '*Streptoverticillium rubroverticillatum*' NBRC 15818.

***Streptomyces ardus* NBRC 13430^T.** Subjective synonym: '*Streptomyces caespitosus*' NBRC 13490.

***Streptomyces blastmyceticus* NBRC 12747^T.** Subjective synonym: '*Streptomyces mediocidicus*' NBRC 13202.

***Streptomyces cinnamoneus* NBRC 12852^T.** Subjective synonyms: *S. hachijoensis* NBRC 12782^T, *S. griseoverticillatus* NBRC 13420^T, *S. sapporonensis* NBRC 13823^T.

***Streptomyces eurocidicus* NBRC 13491^T.** Subjective synonym: *S. albireticuli* NBRC 12737^T.

***Streptomyces griseocarneus* NBRC 12776^T.** Subjective synonyms: *S. alboverticillatus* NBRC 13861^T, *S. septatus* NBRC 13471^T and '*S. tropicalensis*' NBRC 13428.

***Streptomyces hiroshimensis* NBRC 12785^T.** Subjective synonyms: *S. aureoversilis* NBRC 13021^T, *S. baldaccii* NBRC 14693^T, *S. biverticillatus* NBRC 12845^T, *S. fervens* subsp. *fervens* NBRC 13343^T, *S. fervens* subsp. *melrosporus* NBRC 15920^T, *S. rectiverticillatus* NBRC 13079^T, *S. roseoverticillatus* NBRC 12817^T, *S. salmonis* NBRC 15865^T, *S. spitsbergensis* NBRC 15745^T and '*Streptoverticillium rubrochlorinum*' NBRC 14694.

***Streptomyces lilacinus* NBRC 12884^T.** Subjective synonym: *Streptomyces kashimirensis* NBRC 13906^T.

'*Streptomyces luteoreticuli*' NBRC 13422.

***Streptomyces luteosporeus* NBRC 14657^T.**

***Streptomyces mashuensis* NBRC 12888^T.** Subjective synonym: *Streptomyces kishiwadensis* NBRC 13052^T.

***Streptomyces mobaraensis* NBRC 13819^T.** Subjective synonym: *S. ladakanum* NBRC 13476^T.

***Streptomyces morookaense* NBRC 13416^T.** Subjective synonym: '*Streptomyces aspergilloides*' NBRC 13461.

***Streptomyces netropsis* NBRC 12893^T.** Subjective synonyms: *S. distallicus* NBRC 15815^T, *S. flavopersicus* NBRC 12769^T, *S. kentuckensis* NBRC 12880^T and *S. syringium* NBRC 15900^T.

***Streptomyces orinoci* NBRC 13466^T.**

***Streptomyces stramineus* NBRC 16131^T.**

***Streptomyces thioluteus* NBRC 13341^T.**

***Streptomyces vididiflavus* NBRC 15799^T.** Subjective synonym: *S. olivoverticillatus* NBRC 15273^T.

Description of *Streptomyces luteireticuli* corrig., sp. nov., nom. rev.

Streptomyces luteireticuli (lu.te.i.re.ti′cu.li. L. adj. *luteus* yellow; L. n. *reticulum* net; N.L. gen. n. *luteireticuli* of a yellow net).

The description of *Streptomyces luteireticuli* is the same as that given by Shirling & Gottlieb (1972) for '*S. luteoreticuli*', with some additions from this study. Spore-chain morphology is umbellate monoverticilate. Mature spore-chains are generally short (three to ten or more spores per chain) when grown on oatmeal agar (ISP medium 3) and sometimes on inorganic salts/starch agar (ISP medium 4). Sporulating aerial mycelium is usually thin or absent on yeast/malt agar (ISP medium 2) or glycerol/asparagine agar (ISP medium 5). Spore surface is smooth. Aerial mass colour is in the yellow or grey colour-series on ISP medium 3. Nearest matching colours are pale yellow-green, pale yellow, yellowish-grey and light olive-brown. Reverse-side colour of the colony is not distinctive (olive-brown to dark brown on ISP medium 2 and greyish-yellow or yellowish-brown to olive-brown on ISP media 3, 4 and 5). Melanin pigments are not formed in peptone/yeast/iron agar (ISP medium 6), tyrosine agar (ISP medium 7) or tryptone/yeast broth (ISP medium 1). Yellowish and greenish-yellow pigment is found in ISP media 2, 3, 4 and 5. D-Glucose and i-inositol are utilized for growth. Utilization of L-arabinose, D-xylose, D-mannitol, D-fructose, rhamnose, sucrose and raffinose is doubtful or negative. Utilization of D-fructose is weakly positive in this study.

The type strain is NBRC 13422^T (=ATCC 27446^T=CBS 723.72^T=DSM 40509^T=ISP 5509=JCM 4788^T=RIA 1383^T).

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