Reclassification of *Streptomyces nigrifaciens* as a later synonym of *Streptomyces flavovirens*; *Streptomyces citreofluorescens*, *Streptomyces chrysomallus* subsp. *chrysomallus* and *Streptomyces fluorescens* as later synonyms of *Streptomyces anulatus*; *Streptomyces chibaensis* as a later synonym of *Streptomyces corchorusii*; *Streptomyces flaviscleroticus* as a later synonym of *Streptomyces minutiscleroticus*; and *Streptomyces lipmanii*, *Streptomyces griseus* subsp. *alpha*, *Streptomyces griseus* subsp. *cretosus* and *Streptomyces willmorei* as later synonyms of *Streptomyces microflavus*

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A DNA–DNA hybridization survey was performed on 13 *Streptomyces* species and two subspecies, dispersed over five genotypically defined clusters as delineated by Lanoot *et al.* [*Syst Appl Microbiol* **27** (2004), 84–92]. Within each of the latter clusters, strains shared DNA–DNA relatedness values above 70 %. On the basis of published recommendations, the following eight *Streptomyces* species with validly published names are considered as later synonyms: *Streptomyces nigrifaciens* as a synonym of *Streptomyces flavovirens*; *Streptomyces citreofluorescens*, *Streptomyces chrysomallus* subsp. *chrysomallus* and *Streptomyces fluorescens* as synonyms of *Streptomyces anulatus*; *Streptomyces chibaensis* as a synonym of *Streptomyces corchorusii*; *Streptomyces flaviscleroticus* as a synonym of *Streptomyces minutiscleroticus*; and *Streptomyces lipmanii*, *Streptomyces griseus* subsp. *alpha*, *Streptomyces griseus* subsp. *cretosus* and *Streptomyces willmorei* as synonyms of *Streptomyces microflavus*. Emended descriptions are proposed.

Since the introduction of the genus *Streptomyces* by Waksman & Henrici (1943), classification systems have basically relied on the use of morphological and phenotypic criteria. As shown in the literature, these classification

schemes often lack taxonomic discriminatory power at the species level. Genotypic approaches have revealed synonymies among several species, as shown for example in the *Streptomyces violaceusniger*, *Streptomyces cyaneus* and *Streptomyces lavendulae* species groups (Labeda & Lyons, 1991a, b; Labeda, 1993). However, the large number of species with validly published names remains a major practical obstacle in an overall genotypic reclassification of streptomycetes.

During a recent study, 451 *Streptomyces* species with validly

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Details of phenotypic properties of the studied strains are available as supplementary material in IJSEM Online.

published names were screened using BOX-PCR. Numerical analysis of patterns resulted in the delineation of 30 clusters, each comprising multiple type strains with nearly identical patterns. For ten of them (clusters 2, 11, 14, 17, 21, 23, 24, 27, 29, 30 in Lanoot *et al.*, 2004), taxonomic reclassifications have been proposed and resulted in the emended descriptions of seven species (Lanoot *et al.*, 2004).

In the present study, the taxonomic relationships between the type strains within clusters 3, 9, 12, 18, 26 (clusters delineated in Lanoot *et al.*, 2004) were investigated by DNA–DNA hybridizations. The strains investigated were *Streptomyces minutiscleroticus* LMG 20062^T and *Streptomyces flaviscleroticus* LMG 19886^T (cluster 3), *Streptomyces nigrifaciens* LMG 20048^T and *Streptomyces flavovirens* LMG 20516^T (cluster 9), *Streptomyces lipmanii* LMG 20047^T, *Streptomyces microflavus* LMG 19327^T, *Streptomyces willmorei* LMG 21046^T, *Streptomyces griseus* subsp. *alpha* LMG 19953^T, *S. griseus* subsp. *cretosus* LMG 19946^T (cluster 12), *Streptomyces citreofluorescens* LMG 20475^T, *Streptomyces chrysomallus* subsp. *chrysomallus* LMG 20459^T, *Streptomyces fluorescens* LMG 8579^T and *Streptomyces anulatus* LMG 19301^T (cluster 18) and *Streptomyces chibaensis* LMG 20456^T and *Streptomyces corchorusii* LMG 20488^T (cluster 26). All strains were grown on Bennett’s agar medium (0·1 % yeast extract, 0·1 % beef extract, 0·2 % casein hydrolysate, 1 % glucose, 2 % agar; final pH 7·3) and incubated at 28 °C for 24 h.

DNA was extracted using a glass beads/mutanolysin-based lysis method as described previously (Lanoot *et al.*, 2004). G+C contents were determined via HPLC using the

protocol of Tamaoka & Komagata (1984) and were in the range 70–73 mol% for all strains. Genomic relatedness between the strains of each cluster was determined by DNA–DNA hybridizations using the fluorescence-based microplate method of Ezaki *et al.* (1989). The hybridization temperature was 52 °C. The mean DNA–DNA reassociation value between *S. minutiscleroticus* LMG 20062^T and *S. flaviscleroticus* LMG 19886^T (cluster 3) was 96 %, between *S. nigrifaciens* LMG 20048^T and *S. flavovirens* LMG 20516^T (cluster 9) it was 95 % and between *S. corchorusii* LMG 20456^T and *S. chibaensis* LMG 20488^T (cluster 26) it was 94 %. Strains belonging to cluster 18 (LMG 20475^T, LMG 20459^T, LMG 8579^T and LMG 19301^T) shared DNA–DNA relatedness values in the range 90–97 % (Table 1). Members of cluster 12 (LMG 20047^T, LMG 19327^T, LMG 21046^T, LMG 19953^T, LMG 19946^T) shared DNA–DNA relatedness values in the range 78–99 % (Table 1).

Our study also showed that the species *S. griseus* with its four subspecies is genotypically heterogeneous and its members clustered in BOX-PCR group 12 (*S. griseus* subsp. *cretosus* LMG 19946^T, *S. griseus* subsp. *alpha* LMG 19953^T; Lanoot *et al.*, 2004) or possessed unique patterns (*S. griseus* subsp. *griseus* LMG 19302^T, *S. griseus* subsp. *solivifaciens* LMG 19952^T). In contrast to their phenotypic homogeneity, low DNA–DNA relatedness values in the range 26–53 % (Table 1) were found between the type strain of the species, *S. griseus* subsp. *griseus* LMG 19302^T, and the type strains of the three other subspecies, *S. griseus* subsp. *alpha* LMG 19953^T, *S. griseus* subsp. *cretosus* LMG 19946^T and *S. griseus* subsp. *solivifaciens* LMG 19952^T. Two subspecies, *S. griseus* subsp. *cretosus* and *S. griseus* subsp.

Table 1. DNA–DNA reassociation values among *Streptomyces* species belonging to BOX-PCR clusters 12 and 18 and two subspecies of *S. griseus*

BOX-PCR clusters are given as described by Lanoot *et al.* (2004). –, Not determined. Species for which later synonyms are identified are given in bold.

Strain	G + C content (mol%)	DNA–DNA relatedness (%) [S _D]										
		1	2	3	4	5	6	7	8	9	10	11
BOX-PCR cluster 12												
1. <i>S. microflavus</i> LMG 19327 ^T	71	100										
2. <i>S. lipmanii</i> LMG 20047 ^T	71	92 [8]	100									
3. <i>S. willmorei</i> LMG 21046 ^T	71	91 [3]	85 [12]	100								
4. <i>S. griseus</i> subsp. <i>alpha</i> LMG 19953 ^T	70	100	99 [1]	78 [8]	100							
5. <i>S. griseus</i> subsp. <i>cretosus</i> LMG 19946 ^T	70	100	90 [10]	90 [10]	98 [3]	100						
Strains giving unique patterns												
6. <i>S. griseus</i> subsp. <i>griseus</i> LMG 19302 ^T	72	–	–	–	43 [5]	53 [5]	100					
7. <i>S. griseus</i> subsp. <i>solvifaciens</i> LMG 19952 ^T	73	–	–	–	30 [3]	–	26 [12]	100				
BOX-PCR cluster 18												
8. <i>S. anulatus</i> LMG 19301 ^T	71	–	–	–	–	–	–	–	100			
9. <i>S. fluorescens</i> LMG 8579 ^T	71	–	–	–	–	–	–	–	90 [5]	100		
10. <i>S. citreofluorescens</i> LMG 20475 ^T	72	–	–	–	–	–	–	–	97 [3]	92 [6]	100	
11. <i>S. chrysomallus</i> subsp. <i>chrysomallus</i> LMG 20459 ^T	71	–	–	–	–	–	–	–	94 [6]	94 [6]	93 [7]	100

alpha, shared a DNA–DNA relatedness value of 98 % (Table 1). In the present study, the latter two subspecies are reclassified as later synonyms of *S. microflavus*.

Emended descriptions

On the basis of the recommendations of Wayne *et al.* (1987) and Stackebrandt & Goebel (1994), from the results of DNA–DNA relatedness studies, the 13 *Streptomyces* species and two subspecies studied consist of the following five species and later synonyms. Phenotypic characteristics are available as supplementary material in IJSEM Online.

S. flavovirens (Waksman 1923) Waksman and Henrici 1948, 382^{AL}, type strain LMG 20516^T (=DSM 40062^T = NRRL B-1329^T), and *S. nigrifaciens* Waksman 1961, 394^{AL}, type strain LMG 20048^T (=DSM 40071^T = NRRL B-2094^T), are heterotypic synonyms. As *S. flavovirens* was described first, its name is given priority over *S. nigrifaciens* for renaming both synonyms.

S. anulatus (Beijerinck 1912) Waksman 1953, 372^{AL}, type strain LMG 19301^T (=AS 4.1421^T = DSM 40361^T) has the following later heterotypic synonyms: *S. chrysomallus* subsp. *chrysomallus* Lindenbein 1952, 376^{AL}, type strain LMG 20459^T (=AS 4.1676^T = DSM 40128^T), *S. citreofluorescens* (Korenyako *et al.* 1960) Pridham 1970, 377^{AL}, type strain LMG 20475^T (=AS 4.1652^T = DSM 40265^T), and *S. fluorescens* (Krasil'nikov 1958) Pridham 1970, 382^{AL}, type strain LMG 8579^T (=DSM 40203^T = NRRL B-2873^T).

S. corchorusii Ahmad and Bhuiyan 1958, 378^{AL}, type strain LMG 20488^T (=AS 4.1592^T = DSM 40340^T) has a later heterotypic synonym *S. chibaensis* Suzuki *et al.* 1958, 376^{AL}, type strain LMG 20456^T (=AS 4.1654^T = DSM 40220^T).

S. minutiscleroticus (Thirumalachar 1965) Pridham 1970, type strain LMG 20062^T (=DSM 40301^T = NRRL B-12202^T), has a later heterotypic synonym *S. flaviscleroticus* (*ex* Pridham 1970) Goodfellow *et al.* 1986, type strain LMG 19886^T (=DSM 40270^T = NRRL B-12173^T).

S. microflavus (Krainky 1914) Waksman and Henrici 1948, 392^{AL}, type strain LMG 19327^T (=AS 4.1428^T = DSM 40331^T), has the following later heterotypic synonyms: *S. griseus* subsp. *alpha* (Ciferri 1927) Pridham 1970, 387^{AL}, type strain LMG 19953^T (=DSM 40937^T = NRRL B-2249^T), *S. griseus* subsp. *cretosus* Pridham 1970, 387^{AL}, type strain LMG 19946^T (=DSM 40561^T = NRRL B-2252^T), *S. lipmanii* (Waksman and Curtis 1916) Waksman and Henrici 1948, 391^{AL}, type strain LMG 20047^T (=DSM 40070^T = NRRL B-1229^T), and *S. willmorei* (Erikson 1935) Waksman and Henrici 1948, 405^{AL}, type strain LMG 21046^T (=DSM 40459^T = NRRL B-1332^T).

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