Reclassification of *Streptomyces caeruleus* as a synonym of *Actinoalloteichus cyanogriseus* and reclassification of *Streptomyces spheroides* and *Streptomyces laceyi* as later synonyms of *Streptomyces niveus*

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Previous studies have proposed that *Streptomyces caeruleus* is an earlier heterotypic synonym for *Streptomyces niveus* and *Streptomyces spheroides*. In this study, phylogenetic analysis of the almost complete 16S rRNA gene sequences of the *Streptomyces caeruleus* type strains NBRC 13344^T, JCM 4014^T and NRRL B-2194^T revealed that *S. caeruleus* was closely related to *Actinoalloteichus cyanogriseus* and not to *S. niveus*, *S. spheroides* or any other species of the genus *Streptomyces*. Moreover, the diagnostic cell-wall diamino acid was found to be meso-diaminopimelic acid in *S. caeruleus* and DNA–DNA hybridization studies revealed that *S. caeruleus* NBRC 13344^T was a member of the same species as *A. cyanogriseus* NBRC 14455^T. Based on these chemotaxonomic and phylogenetic data, it is proposed that *Streptomyces caeruleus* (Baldacci 1944) Pridham *et al.* 1958 be reclassified as a heterotypic synonym of *Actinoalloteichus cyanogriseus* Tamura *et al.* 2000. Furthermore, based on phylogenetic, morphological and MALDI-TOF MS analyses, it is proposed that the species *Streptomyces laceyi* Manfio *et al.* 2004 and *Streptomyces spheroides* Wallick *et al.* 1956 are reclassified as later heterotypic synonyms of *Streptomyces niveus* Smith *et al.* 1956.

Streptomyces caeruleus was first described by Baldacci (1944) as '*Actinomyces caeruleus*' and then transferred to the genus *Streptomyces* by Pridham *et al.* (1958). In 2002, Lanoot *et al.* (2002) classified *S. caeruleus* as an earlier heterotypic synonym for both *Streptomyces niveus* (Smith *et al.*, 1956) and *Streptomyces spheroides* (Harris *et al.*, 1956) based on SDS-PAGE patterns of the whole-cell proteins and DNA–DNA relatedness. The International

Streptomyces Project (ISP) studied *Streptomyces caeruleus* ISP 5103^T (Shirling & Gottlieb, 1972), *S. niveus* ISP 5088^T (Shirling & Gottlieb, 1968a) and *S. spheroides* ISP 5292^T (Shirling & Gottlieb, 1968b) and reported on their morphological features and physiological characteristics. *S. caeruleus* ISP 5103^T was observed to develop a flexuous (*rectiflexibles*) spore chain and to produce a distinct blackish-blue to very dark greyish-purple pigment, while *S. niveus* ISP 5088^T and *S. spheroides* ISP 5292^T were observed to develop spiral spore chains and produce no distinctive pigments.

Our preliminary examination revealed that *S. caeruleus* NBRC 13344^T possessed meso-diaminopimelic acid (meso-A₂pm) as the diagnostic diamino acid of the cell-wall peptidoglycan and not LL-A₂pm and we therefore carefully examined the taxonomic position of *S. caeruleus*. Four strains designated as type strains of *S. caeruleus* were obtained from different culture collections, namely NBRC

Abbreviations: A₂pm, diaminopimelic acid; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MS, mass spectroscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Streptomyces caeruleus* NBRC 13344^T and *Streptomyces caeruleus* NRRL B-2194^T are AB184345 and EF178675, respectively.

A supplementary figure showing the MALDI-TOF mass spectra of *Streptomyces niveus* NRRL 2466^T, *Streptomyces spheroides* NRRL 2449^T and *Streptomyces laceyi* NRRL B-24638^T mycelium grown on glucose–proline novobiocin production medium is available with the online version of this paper.

13344^T, JCM 4014^T, NRRL B-2194^T and LMG 19399^T, and were used in this study. PCR amplification and sequencing of the 16S rRNA genes of the strains and phylogenetic analysis were performed as described previously (Tamura & Hatano, 2001). The 16S rRNA gene sequence of *S. caeruleus* NBRC 13344^T was in agreement with those of *S. caeruleus* strains JCM 4014^T and NRRL B-2194^T but was not in agreement with the sequence of *S. caeruleus* LMG 19399^T. Phylogenetic analysis revealed that *S. caeruleus* NBRC 13344^T was found in a monophyletic cluster with members of the genus *Actinoalloteichus* (data not shown) and was closely related to *Actinoalloteichus cyanogriseus*. In contrast, *S. caeruleus* LMG 19399^T was closely related to *S. niveus* and *S. spheroides* as reported by Lanoot *et al.* (2002) (data not shown). The binary similarity values of the 16S rRNA gene sequences of *S. caeruleus* NBRC 13344^T were 100 % to *A. cyanogriseus* NBRC 14455^T, 99.6 % to *Actinoalloteichus spitiensis* MTCC 6194^T and 96.7 % to *Actinoalloteichus hymeniacidonis* HPA177^T. The diagnostic diamino acid of the cell-wall peptidoglycan of *S. caeruleus* NBRC 13344^T was reconfirmed as *meso*-A₂pm by TLC (Hasegawa *et al.*, 1983). The microplate hybridization method developed by Ezaki *et al.* (1988, 1989) was applied to determine DNA–DNA relatedness. The DNA–DNA relatedness values between *S. caeruleus* NBRC 13344^T and *A. cyanogriseus* NBRC 14455^T were 87.7 and 88.1 % and were less than 24 % between *S. caeruleus* NBRC 13344^T and *A. spitiensis* NBRC 102582^T.

S. caeruleus and *A. cyanogriseus* exhibited almost the same phenotypic features in that they developed flexuous spore chains and produced blackish coloured soluble pigments (Tamura *et al.*, 2000). Strains NBRC 13344^T, JCM 4014^T and NRRL B-2194^T are members of the species *S. caeruleus* since they exhibit the same characteristics reported by the ISP

(Shirling & Gottlieb, 1972). Further, *S. caeruleus* DSM 40103^T has also been found to belong to the genus *Actinoalloteichus* (R. M. Kroppenstedt, personal communication).

Based on the phylogenetic, chemotaxonomic and DNA–DNA relatedness data, strain NBRC 13344^T, a type strain of *S. caeruleus*, is conclusively identified as a strain of *A. cyanogriseus*. Therefore, we propose that *Streptomyces caeruleus* (Baldacci 1944) Pridham *et al.* 1958 should be considered as a synonym of *Actinoalloteichus cyanogriseus* Tamura *et al.* 2000.

The present data and the phenotypic characteristics reported by the ISP (Shirling & Gottlieb, 1968a, b, 1972) do not support the hypothesis that *S. niveus* and *S. spheroides* are later synonyms of *S. caeruleus* as proposed by Lanoot *et al.* (2002). It is likely that strain LMG 19399^T could have been replaced with a streptomycete during deposit or during the subsequent maintenance of the strain. The present study, however, does support the synonymous relationship between *S. niveus* and *S. spheroides* as suggested by Lanoot *et al.* (2002). In addition, it was observed that *Streptomyces laceyi* NBRC 100783^T (Manfio *et al.*, 2003) was very closely related phylogenetically to *S. niveus* and *S. spheroides* based on 16S rRNA gene sequences. The 16S rRNA gene sequences of *S. laceyi* NBRC 100783^T and *S. spheroides* NRRL 2449^T exhibited 99.8 % and 99.9 % sequence similarity to *S. niveus* NRRL 2466^T, respectively, and 99.9 % similarity to each other. Moreover, the morphological appearance of all of these strains on various agar growth media is identical. *S. niveus* NRRL 2466^T and *S. spheroides* NRRL 2449^T are both reported to produce the same secondary metabolite, the antibiotic novobiocin, and it was of interest to determine whether *S. laceyi* NRRL B-24638^T (=NBRC 100783^T) exhibited a similar biosynthetic profile.

Fig. 1. MALDI-TOF mass spectra of (a) *Streptomyces niveus* NRRL 2466^T; (b) *Streptomyces spheroides* NRRL 2449^T, and (c) *Streptomyces laceyi* NRRL B-24638^T mycelium grown for 14 days on N-Z-amine medium (DSM medium 554).

All three strains were grown on N-Z-amine agar (DSMZ, 2001) and colonies were analysed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS of intact cells using a Omniflex MALDI-TOF mass spectrometer (Bruker Daltonics) run in reflectron mode. The MALDI-TOF MS analysis of the three strains showed that these strains produced the same ion profiles (major ions m/z 605 and m/z 621; Fig. 1 and shown in more detail in Supplementary Figure S1, available in IJSEM Online), although none of these matched the mass for the novobiocin standard (data not shown), and can thus be considered as the same species.

The DNA–DNA relatedness values of *S. niveus* NBRC 12804^T were 66 and 90% to *S. spheroides* NBRC 12917^T and *S. laceyi* NBRC 100783^T, respectively. An investigation of the literature found that the species *S. niveus* Smith *et al.* 1956 was effectively published in February 1956 and should take precedence over *S. spheroides* Wallick *et al.* 1956 which seems to have appeared in print in May 1956. We therefore propose the following circumscription for *Streptomyces niveus*.

Streptomyces niveus Smith *et al.* 1956, type strain NRRL 2466^T [=ATCC 19793^T=CBS 545.68^T=BCRC (formerly CCRC) 11514^T=CCUG 11108^T=DSM 40088^T=IFM 1181^T=IFO (now NBRC) 12804^T=IMET 43503^T=JCM 4251^T=JCM 4599^T=LMG 5980^T=LMG 19395^T=NCIMB 9219^T, NRRL-ISP 5088^T=RIA 1072^T=UNIQEM 179^T], has the following later heterotypic synonyms: *Streptomyces laceyi* Manfio *et al.* 2003, type strain C7654^T (=AS 4.1832^T=DSM 41788^T=JCM 12606^T=NBRC 100783^T=NCIMB 13928^T=NRRL B-24638^T) and *Streptomyces spheroides* Wallick *et al.* 1956, type strain NRRL 2449^T [=ATCC 23965^T=CBS 491.62^T=CBS 948.68^T=BCRC (formerly CCRC) 11559^T=DSM 40292^T=IFO (now NBRC) 12917^T=JCM 4252^T=JCM 4670^T=LMG 19392^T=NCIMB 11891^T=NRRL-ISP 5292^T=RIA 1200^T=RIA 700^T].

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