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 13344T, JCM 4014T and NRRL B-2194T 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 13344T was a member of the same species as *A. cyanogriseus* NBRC 14455T. 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 ‘*Actinoomyces caeruleus*’ and then transferred to the genus *Streptomyces* by Pridham et al. (1958). In 2002, Lanoo 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 5103T (Shirling & Gottlieb, 1972), *S. niveus* ISP 5088T (Shirling & Gottlieb, 1968a) and *S. spheroides* ISP 5292T (Shirling & Gottlieb, 1968b) and reported on their morphological features and physiological characteristics. *S. caeruleus* ISP 5103T 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 5088T and *S. spheroides* ISP 5292T were observed to develop spiral spore chains and produce no distinctive pigments.

Our preliminary examination revealed that *S. caeruleus* NBRC 13344T possessed meso-diaminopimelic acid (meso-A2pm) as the diagnostic diamino acid of the cell-wall peptidoglycan and not l-l-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...
13344T, JCM 4014T, NRRL B-2194T and LMG 19399T, 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 13344T was in agreement with those of S. caeruleus strains JCM 4014T and NRRL B-2194T but was not in agreement with the sequence of S. caeruleus LMG 19399T. Phylogenetic analysis revealed that S. caeruleus NBRC 13344T 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 19399T 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 13344T were 100% to Actinoalloteichus spithienis MITCC 6194T and 96.7% to Actinoalloteichus hymeniacidonis HPA177T. The diagnostic diamino acid of the cell-wall peptidoglycan of S. caeruleus NBRC 13344T was reconfirmed as meso-Apm 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 13344T and A. cyanogriseus NBRC 14455T were 87.7 and 88.1% and were less than 24% between S. caeruleus NBRC 13344T and A. spheroides NBRC 102582T.

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 13344T, JCM 4014T and NRRL B-2194T are members of the species S. caeruleus since they exhibit the same characteristics reported by the ISP (Shirling & Gottlieb, 1972). Further, S. caeruleus DSM 40103T 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 13344T, 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 19399T 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 100783T (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 100783T and S. spheroides NRRL 2449T exhibited 99.8% and 99.9% sequence similarity to S. niveus NRRL 2466T, 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 2466T and S. spheroides NRRL 2449T are both reported to produce the same secondary metabolite, the antibiotic novobiocin, and it was of interest to determine whether S. laceyi NRRL B-24638T (=NBRC 100783T) exhibited a similar biosynthetic profile.

**Fig. 1.** MALDI-TOF mass spectra of (a) Streptomyces niveus NRRL 2466T; (b) Streptomyces spheroides NRRL 2449T, and (c) Streptomyces laceyi NRRL B-24638T mycelium grown for 14 days on N-Z-amine medium (DSM medium 554).
All three strains were grown on N-Z-amino 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 Daltonic) 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 12804T were 66 and 90% to S. spheroides NBRC 12917T and S. laceyi NBRC 100783T, 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 2466T [=ATCC 19793T=CBS 545.68T=BCRC (formerly CCRC) 11514T=CCUG 11108T=DSM 40088T=IFO 1181T=IFO (now NBRC) 12084T=IMET 43503T=JCM 4251T=JCM 4599T=LMG 5980T=LMG 19395T=NCIMB 9219T, NRRL-ISP 5088T=RIA 1072T=UNIQEM 179T], has the following later heterotypic synonyms: Streptomyces laceyi Manfio et al. 2003, type strain C7654T [=AS 4.1832T=DSM 41788T=JCM 12606T=NBRC 100783T=NCIMB 13928T=NRRL B-24638T] and Streptomyces spheroides Wallick et al. 1956, type strain NRRL 2449T [=ATCC 23965T= CBS 491.62T=CBS 948.68T=BCRC (formerly CCRC) 11559T=DSM 40292T=IFO (now NBRC) 12917T=JCM 4252T=JCM 4670T=LMG 19392T=NCIMB 11891T=NRRL-ISP 5292T=RIA 1200T=RIA 700T].

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References


