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 ‘Actinoalloteichus cyanogriseus’ 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 diaminoacid 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.
13344\textsuperscript{T}, JCM 4014\textsuperscript{T}, NRRL B-2194\textsuperscript{T} and LMG 19399\textsuperscript{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 \textit{S. caeruleus} NBRC 13344\textsuperscript{T} was found in a monophyletic cluster with \textit{T. tamurae} strains JCM 4014\textsuperscript{T} and NRRL B-2194\textsuperscript{T} but was not in agreement with the sequence of \textit{S. caeruleus} LMG 19399\textsuperscript{T}. Phylogenetic analysis revealed that \textit{S. caeruleus} NBRC 13344\textsuperscript{T} was found in a monophyletic cluster with members of the genus \textit{Actinoalloteichus} (data not shown) and was closely related to \textit{Actinoalloteichus cyanogriseus}. In contrast, \textit{S. caeruleus} LMG 19399\textsuperscript{T} was closely related to \textit{S. niveus} and \textit{S. spheroides} as reported by Lanoot et al. (2002) (data not shown). The binary similarity values of the 16S rRNA gene sequences of \textit{S. caeruleus} NBRC 13344\textsuperscript{T} were 100\% to \textit{A. cyanogriseus} NBRC 14455\textsuperscript{T}, 99.6\% to \textit{Actinoalloteichus spitensis} MTCC 6194\textsuperscript{T} and 96.7\% to \textit{Actinoalloteichus hymeniacidonis} HPA177\textsuperscript{T}. The diagnostic diamino acid of the cell-wall peptidoglycan of \textit{S. caeruleus} NBRC 13344\textsuperscript{T} was reconfirmed as 

\textit{S. caeruleus} and \textit{A. cyanogriseus} exhibited almost the same phenotypic features in that they developed flexuous spore chains and produced blackish coloured soluble pigments (Tamur et al., 2000). Strains NBRC 13344\textsuperscript{T}, JCM 4014\textsuperscript{T} and NRRL B-2194\textsuperscript{T} are members of the species \textit{S. caeruleus} since they exhibit the same characteristics reported by the ISP (Shirling & Gottlieb, 1972). Further, \textit{S. caeruleus} DSM 40103\textsuperscript{T} has also been found to belong to the genus \textit{Actinoalloteichus} (R. M. Kroppenstedt, personal communication).

Based on the phylogenetic, chemotaxonomic and DNA–DNA relatedness data, strain NBRC 13344\textsuperscript{T}, a type strain of \textit{S. caeruleus}, is conclusively identified as a strain of \textit{A. cyanogriseus}. Therefore, we propose that \textit{Streptomyces caeruleus} (Baldacci 1944) Pridham et al. 1958 should be considered as a synonym of \textit{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 \textit{S. niveus} and \textit{S. spheroides} are later synonyms of \textit{S. caeruleus} as proposed by Lanoot et al. (2002). It is likely that strain LMG 19399\textsuperscript{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 \textit{S. niveus} and \textit{S. spheroides} as suggested by Lanoot et al. (2002). In addition, it was observed that \textit{Streptomyces laceyi} NBRC 100783\textsuperscript{T} (Manfio et al., 2003) was very closely related phylogenetically to \textit{S. niveus} and \textit{S. spheroides} based on 16S rRNA gene sequences. The 16S rRNA gene sequences of \textit{S. laceyi} NBRC 100783\textsuperscript{T} and \textit{S. spheroides} NRRL 2449\textsuperscript{T} exhibited 99.8\% and 99.9\% sequence similarity to \textit{S. niveus} NRRL 2466\textsuperscript{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. \textit{S. niveus} NRRL 2466\textsuperscript{T} and \textit{S. spheroides} NRRL 2449\textsuperscript{T} are both reported to produce the same secondary metabolite, the antibiotic novobiocin, and it was of interest to determine whether \textit{S. laceyi} NRRL B-24638\textsuperscript{T} (=NBRC 100783\textsuperscript{T}) exhibited a similar biosynthetic profile.
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 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* Wallack et al. 1956 which seems to have appeared in print in May 1956. We therefore propose the following circumscription for *Streptomycyes niveus*.

*Streptomycyes niveus* Smith et al. 1956, type strain NRRL 24664T [=ATCC 19793T =CBS 545.68T =BCRC (formerly CCRC) 11514T =CCUG 11108T =DSM 40088T =IFO 1181T =IFO (now NBRC) 12804T =IMET 43503T =JCM 4251T =JCM 4599T =LMG 5980T =LMG 19395T =NCIMB 9219T =NRRL-ISP 5088T =RI A 1072T =UNIQEM 179T], has the following later heterotypic synonyms: *Streptomycyes laceyi* Mannio et al. 2003, type strain C7654T (=AS 4.1832T =DSM 41788T =JCM 12606T =NCIMB 13928T =NRRL B-24638T) and *Streptomycyes spheroides* Wallack et al. 1956, type strain NRRL 2449T [=ATCC 23963T =CBS 491.62T =CBS 948.68T =BCRC (formerly CCRC) 11559T =DSM 4092T =IFO (now NBRC) 12917T =JCM 4252T =JCM 4670T =LMG 19392T =NCIMB 11891T =NRRL-ISP 5292T =RI A 1200T =RI A 700T].

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**References**


