Reclassification of *Streptomyces flavidofuscus* as a synonym of *Nocardiopsis dassonvillei* subsp. *dassonvillei*

Tomohiko Tamura, Yuumi Ishida, Misa Otoguro, Kazunori Hatano and Ken-ichiro Suzuki

Correspondence

Tomohiko Tamura
tamura-tomohiko@nite.go.jp

NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

During the course of quality control studies of the collection of the NITE Biological Resource Center (NBRC), phylogenetic analysis based on 16S rRNA gene sequences of actinomycetes revealed that *Streptomyces flavidofuscus* NBRC 15404^T formed a cluster with *Nocardiopsis dassonvillei* and *Nocardiopsis synnemataformans*. Strain NBRC 15404^T contained meso-diaminopimelic acid as a cell-wall diamino acid and DNA–DNA hybridization studies also showed that *S. flavidofuscus* NBRC 15404^T was a close relative of *N. dassonvillei* subsp. *dassonvillei* NBRC 14626^T. Based on chemotaxonomic, phenotypic and genetic analysis of the type strain, *Streptomyces flavidofuscus* should be reclassified as a later heterotypic synonym of *Nocardiopsis dassonvillei* subsp. *dassonvillei*.

The genus *Streptomyces* is the largest genus of actinomycetes, containing more than 500 species with validly published names. They are characterized phenotypically by morphology and the presence of a type I cell wall. The genus *Nocardiopsis* was established by Meyer (1976) on the basis of morphological characteristics and the presence of cell-wall type III/C. The genus currently comprises more than 20 species. Members of this taxon are characterized chemotaxonomically by possessing phosphatidylcholine and phosphatidylmethylethanolamine as diagnostic phospholipids, menaquinone (MK)-10 with variable degrees of saturation (Collins *et al.*, 1977), iso-, anteiso- and 10-methyl-branched fatty acids (fatty acid type 3d *sensu* Kroppenstedt, 1985) and meso-diaminopimelic acid (*meso*-A₂pm). They lack diagnostic sugars (cell-wall chemotype III/C *sensu* Lechevalier & Lechevalier, 1970) and mycolic acids. They contain the acetyl type of muramic acid and have a G+C content of 64–69 mol% (Grund & Kroppenstedt, 1990).

During the course of quality control of the collection of the NITE Biological Resource Center (NBRC), it was revealed that NBRC 15404^T, the type strain of *Streptomyces flavidofuscus* (Gause *et al.*, 1983), was classified phylogenetically within the genus *Nocardiopsis*. Therefore, the taxonomic position of *S. flavidofuscus* was studied further.

PCR amplification and 16S rRNA gene sequencing and phylogenetic analysis were performed as described previously (Tamura & Hatano, 2001). 16S rRNA gene

sequence analysis revealed that *S. flavidofuscus* NBRC 15404^T was incorporated in a monophyletic cluster with members of the genus *Nocardiopsis* (Fig. 1). The binary similarity value of the 16S rRNA gene sequence of *S. flavidofuscus* NBRC 15404^T was 99.5 % with *Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111^T, 99.7 % with *N. dassonvillei* subsp. *albirubida* NBRC 13392^T and 99.8 % with *Nocardiopsis synnemataformans* IMMIB D-1215^T. The sequence of *S. flavidofuscus* NBRC 15404^T was 100 % similar to the sequence submitted to GenBank as AY999914, derived from *S. flavidofuscus* NRRL B-16366^T. This means the phylogenetic position of the type strain of *S. flavidofuscus* has been confirmed by using multiple strains preserved in different culture collections.

Cell-wall amino acids, whole-cell sugars, cellular fatty acids, isoprenoid quinones and DNA base composition were analysed as described previously (Tamura *et al.*, 1994). Freeze-dried cells for chemotaxonomic analyses were prepared from cultures grown in yeast extract-glucose broth [containing (l⁻¹) 10 g yeast extract and 10 g D-glucose, pH 7.2] on a rotary shaker at 28 °C for 3 days. The predominant menaquinones were MK-10(H₆) and MK-10(H₈), with MK-10(H₄), MK-9(H₆) and MK-9(H₈) as minor components. The strain contained *meso*-A₂pm, alanine and glutamic acid in the cell wall and ribose and mannose as whole-cell sugars. The major cellular fatty acids were iso-C_{16:0} (50 %), 10-methyl C_{18:0} (12 %) and anteiso-C_{17:0} (10 %). The DNA G+C content was 71 mol%. The chemotaxonomic characteristics of *S. flavidofuscus* NBRC 15404^T were consistent with those of the genus *Nocardiopsis*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NBRC 15404^T is AB184655.

Fig. 1. Phylogenetic tree derived from the 16S rRNA gene sequences of *S. flavidofuscus* NBRC 15404^T and members of the genus *Nocardiopsis*. *N. dassonvillei* subsp. *dassonvillei* DSM 43884 is the type strain of *Nocardiopsis antarctica*. The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987). The sequence of *Streptomyces ambofaciens* ATCC 23877^T was used as an outgroup. Bar, 0.01 K_{nuc} in nucleotide sequences. Numbers on branches are confidence limits estimated by bootstrap analysis with 1000 replicates (only values above 500 are presented).

The microplate hybridization method developed by Ezaki *et al.* (1988, 1989) was used for the determination of DNA–DNA relatedness. The relatedness values of *S. flavidofuscus* NBRC 15404^T to *N. dassonvillei* subsp. *dassonvillei* NBRC 14626^T, *N. dassonvillei* subsp. *albirubida* NBRC 13392^T and *N. synnemataformans* NBRC 102581^T were 81–87, 55–79 and 55–63 %, respectively. *N. dassonvillei* subsp. *albirubida* NBRC 13392^T exhibited high relatedness (59–73 %) to *N. dassonvillei* subsp. *dassonvillei* NBRC 14626^T, as reported by Evtushenko *et al.* (2000). Further, *N. synnemataformans* NBRC 102581^T exhibited 43–61 % relatedness to *N. dassonvillei* subsp. *dassonvillei* NBRC 14626^T and 53–79 % relatedness to *N. dassonvillei* subsp. *albirubida* NBRC 13392^T. These species and subspecies are genetically closely related to each other.

The cultural characteristics of *S. flavidofuscus* NBRC 15404^T were similar to those of *N. dassonvillei* subsp. *dassonvillei* NBRC 14626^T, i.e. they produced a pale-brown soluble pigment in NBRC medium 229 [containing (1⁻¹) 5 g yeast extract, 50 g glycerol, 1 g CaCO₃ and 20 g agar, pH 7.3; NBRC catalogue] and developed colourless to pale-brown colonies on NBRC medium 229, yeast extract–starch agar [(1⁻¹) 2 g yeast extract, 10 g soluble starch and 18 g agar, pH 7.3], Bennett's maltose agar [(1⁻¹) 1 g yeast extract, 1 g beef extract, 2 g NZ amine type A, 10 g maltose and 20 g agar, pH 7.3] and yeast extract–malt extract agar (ISP 2; Shirling & Gottlieb, 1966). However, *S. flavidofuscus* NBRC 15404^T differed from *N. dassonvillei* subsp. *dassonvillei* NBRC 14626^T with regard to the production of a pale-brown soluble pigment on yeast extract–starch, Bennett's maltose and yeast extract–malt extract agars.

The results of the present study revealed that strain NBRC 15404^T, the type strain of *S. flavidofuscus*, was identified as

a strain of *N. dassonvillei* subsp. *dassonvillei*. The identity of the 16S rRNA gene sequence of strain NBRC 15404^T to that of NRRL B-16366^T strongly suggests that this is not merely true for this particular strain but is generalized to the concept of the species. Therefore, the name *Streptomyces flavidofuscus* Preobrazhenskaya 1986 should be treated as a later heterotypic synonym of *Nocardiopsis dassonvillei* subsp. *dassonvillei* (Brocq-Rousseau 1904) Meyer 1976.

References

- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- Evtushenko, L. I., Taran, V. V., Akimov, V. N., Kroppenstedt, R. M., Tiedje, J. M. & Stackebrandt, E. (2000). *Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosi* sp. nov., nom. rev. and *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov. *Int J Syst Evol Microbiol* **50**, 73–81.
- Ezaki, T., Hashimoto, Y., Takeuchi, N., Yamamoto, H., Liu, S.-L., Miura, H., Matsui, K. & Yabuuchi, E. (1988). Simple genetic identification method of viridans group streptococci by colorimetric dot hybridization and quantitative fluorometric hybridization in microdilution wells. *J Clin Microbiol* **26**, 1708–1713.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Gause, G. F., Preobrazhenskaya, T. P., Sveshnikova, M. A., Terekhova, L. P. & Maximova, T. S. (1983). *A Guide for the Determination of Actinomycetes. Genera Streptomyces, Streptoverticillium and Chainia*. Moscow: Nauka (in Russian).
- Grund, E. & Kroppenstedt, R. M. (1990). Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. *Int J Syst Bacteriol* **40**, 5–11.

- Kroppenstedt, R. M. (1985).** Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Lechevalier, H. G. & Lechevalier, M. P. (1970).** A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, pp. 393–405. Edited by H. Prauser. Jena: Gustav Fischer.
- Meyer, J. (1976).** *Nocardiopsis*, a new genus of the order Actinomycetales. *Int J Syst Bacteriol* **26**, 487–493.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Tamura, T. & Hatano, K. (2001).** Phylogenetic analysis of the genus *Actinoplanes* and transfer of *Actinoplanes minutisporangius* Ruan *et al.* 1986 and ‘*Actinoplanes aurantiacus*’ to *Cryptosporangium minutisporangium* comb. nov. and *Cryptosporangium aurantiacum* sp. nov. *Int J Syst Evol Microbiol* **51**, 2119–2125.
- Tamura, T., Nakagaito, Y., Nishii, T., Hasegawa, T., Stackebrandt, E. & Yokota, A. (1994).** A new genus of the order Actinomycetales, *Couchioplanes* gen. nov., with descriptions of *Couchioplanes caeruleus* (Horan and Brodsky 1986) comb. nov. and *Couchioplanes caeruleus* subsp. *azureus* subsp. nov. *Int J Syst Bacteriol* **44**, 193–203.