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In the course of a polyphasic study it was observed that ‘Dactylosporangium variesporum’ NRRL B-16296 is misclassified in the genus Dactylosporangium as it exhibits properties consistent with its assignment to the genus Saccharothrix. Phylogenetic analyses based on 16S rRNA gene sequences show that the strain falls within the evolutionary radiation of the genus Saccharothrix, a result which is supported by corresponding chemotaxonomic and morphological markers. The strain is phylogenetically most closely, albeit loosely, related to Saccharothrix espanaensis, but can be readily distinguished from this and other species of the genus Saccharothrix with validly described names by using a range of phenotypic properties. The combined genotypic and phenotypic data demonstrate conclusively that this strain should be classified as a new species in the genus Saccharothrix for which the name Saccharothrix variisporea sp. nov. is proposed. The type strain is NRRL B-16296T (=ATCC 31203T =DSM 43911T =JCM 3273T =NBRC 14104T).

‘Dactylosporangium variesporum’ sp. nov. was proposed by Tomita et al. (1977) to accommodate a strain isolated from a soil sample collected in India which they considered to have chemical and morphological properties consistent with its assignment to the genus Dactylosporangium. The micro-organism, strain D409-5, produces capreomycin and was reported by Tomita and his colleagues to form finger-shaped sporangia, singly, in pairs or in clusters on substrate hyphae, and to contain meso-diaminopimelic acid in the cell wall peptidoglycan and galactose, mannose and rhamnose in whole-cell hydrolysates. Uchida & Seino (1997), however, questioned the placement of this strain in the genus Dactylosporangium as they were unable to observe sporangia and found that the peptidoglycan contained N-glycolated as opposed to N-acetylated muramic acid moieties.

When ‘D. variesporum’ NRRL B-16296 was included in a study designed to clarify the taxonomy of species of the genus Dactylosporangium with invalidly described names, it was found to have properties consistent with its assignment to the genus Saccharothrix. In the present investigation, strain NRRL B-16296T (=strain D409-5) was the subject of a polyphasic study which showed that it represents a novel species of the genus Saccharothrix for which the name Saccharothrix variisporea sp. nov. is proposed.

‘D. variesporum’ NRRL B-16296 and the equivalent strain DSM 43911 were maintained at −20 °C as glycerol suspensions (20%, v/v). Biomass for 16S rRNA gene sequence analyses of these strains and for the chemotaxonomic analyses of strain NRRL B-16296T was prepared in modified Bennett’s broth (Jones, 1949) at 150 r.p.m. in shake flasks for 21 days at 28 °C. Cells were checked for purity and harvested by centrifugation. Biomass for chemotaxonomic analysis of strain NRRL B-16296T was washed twice in distilled water and freeze-dried. Cells collected for DNA isolation and sequencing of 16S rRNA genes were washed in NaCl/EDTA buffer (0.1M EDTA, 0.1M NaCl, pH 8.0) and stored at −20 °C.

Preparation of genomic DNA, amplification and direct sequencing of the purified PCR products of 16S rRNA genes performed from strains NRRL B-16296T and DSM 43911T were performed after Tan et al. (2006). The resultant, almost complete, 16S rRNA gene sequences (1458 nt) were aligned manually, using the JPHYDIT program (Jeon et al., 2005), against corresponding sequences of the type strains of species of the genus Saccharothrix retrieved from the GenBank database. Phylogenetic trees were inferred using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution and
Saccharothrix australiensis NRRL B-16296T contains the unique oligonucleotide sign.

97.5 % (with

Saccharothrix

ing species of the genus

NRRL B-16296 T and the type strains of the remain-

The 16S rRNA gene sequence similarities between strain

98.8 % (18 nt differences at 1430 locations), respectively.

were 98.5 % (21 nt differences at 1425 locations) and

Saccharothrix espanaensis

of

value of 61 % is formed by this strain and the type strains

by all of the tree-making algorithms and by a bootstrap

root position of the neighbour-joining tree was obtained

using MK-9[H4] as the predominant menaquinone, and
diphosphatidylglycerol, phosphatidylethanolamine, phos-
phatidylinositol and phosphatidylglycerol as major polar
lipids (phospholipid pattern type II sensu; Lechevalier et al. 1977) together with a trace of phosphatidylinositol
mannoside and single unknown aminolipid and phos-
pholipid components. The predominant fatty acids were
13-methyltetradecanoic (iso-C15:0), 14-methylpentadeca-
oc (iso-C16:0) and 14-methylhexadecanoic (anteiso-
C17:0) acids; mycolic acids were not present. All of these
properties are in line with the classification of the strain in
the genus Saccharothrix (Labeleda et al., 1984; Otago et al., 2009).

The DNA G+C content of strain NRRL B-16296T was
determined by using the procedure described by Gonzalez
& Saiz-Jimenez (2005) and found to be 74 mol%. DNA–
DNA relatedness studies were not carried out between
strain NRRL B-16296T and its closest phylogenetic neigh-
bours as type strains of species of the genus Saccharothrix
with similar 16S rRNA gene sequences have DNA–DNA
hybridization values well below the 70 % cut-off point
recommended by Wayne et al. (1987) for the circumscrip-
tion of strains that belong to the same genomic species.
Thus, the type strains of S. espanaensis and S. mutabilis
have a 16S rRNA gene similarity of 99.0 % but a DNA–
DNA relatedness value of 0 % (Labeleda & Lechevalier, 1989)
whereas the type strains of Saccharothrix syringae and

maximum-parsimony algorithms (Takahashi & Nei, 2000)
from the MEGA 3.1 program (Kumar et al., 2004). The
evolutionary distance model of Jukes & Cantor (1969) was
used to generate an evolutionary distance matrix for the
neighbour-joining algorithm. The topologies of the resultant
trees were evaluated by bootstrap analysis (Felsenstein, 1985)
based on 1000 resamplings of the neighbour-joining dataset.
The root position of the neighbour-joining tree was obtained
using Pseudonocardia thermophila IMSNU 20112 as the outgroup.

Identical 16S rRNA gene sequences were obtained for 'D.
variesporum' NRRL B-16296 and DSM 43911; hence only
the sequence for strain NRRL B-16296 was deposited in
GenBank (GQ917213) and is considered further. It can be
seen in Fig. 1 that this strain falls within the evolutionary
radiation of the genus Saccharothrix based on the phylo-
genetic analysis of 16S rRNA genes. A subclade supported
by all of the tree-making algorithms and by a bootstrap
value of 61 % is formed by this strain and the type strains
of Saccharothrix espanaensis, Saccharothrix mutabilis subsp.
capreolus and S. mutabilis subsp. mutabilis. Strain NRRL
B-16296T and S. mutabilis subsp. capreolus NRRL 2773T are
reported to produce the antibiotic capreomycin, though
NRRL B-16296T was most closely related to S. espanaensis
NRRL 15764T; the two strains shared a 16S rRNA gene
similarity of 98.9 %, a value which corresponded to 16 nt
differences at 1446 locations. The corresponding values
between strain 'NRRL B-16296T' and the type strains of S.
mutabilis subsp. capreolus and S. mutabilis subsp. mutabilis
were 98.5 % (21 nt differences at 1425 locations) and
98.8 % (18 nt differences at 1430 locations), respectively.
The 16S rRNA gene sequence similarities between strain
NRRL B-16296T and the type strains of the remaining
species of the genus Saccharothrix ranged from
97.5 % (with Saccharothrix violaceirubra) to 98.5 % (with
Saccharothrix australiensis). The 16S rRNA gene of strain
NRRL B-16296T contains the unique oligonucleotide signa-
ture characteristic for members of the genus Saccharothrix
(Labeleda & Kroppenstedt, 2007).

Chemotaxonomic studies were undertaken to confirm that
strain NRRL B-16296T exhibited a chemical profile which
supported its classification in the genus Saccharothrix.

Fig. 1. Neighbour-joining tree based on almost complete
16S rRNA gene sequences showing the position of strain
NRRL B-16296T in relation to members of the genus
Saccharothrix. Numbers on branches indicate
percentage bootstrap values from 1000 replicates
(only values >50 % are shown). Asterisks indicate the branches that were also
recovered in the minimum-evolution and maximum-
parsimony trees. Bar, 0.01 substitutions
per nucleotide position.
Saccharothrix xinjiangensis share a 16S rRNA gene similarity of 99.1% but have a DNA–DNA relatedness value of only 50% (Hu et al., 2004). Similarly, Saccharothrix algeriensis NRRL B-24137T and S. australiensis NRRL 11239T have a 16S rRNA gene similarity of 98.8% and a DNA–DNA relatedness of 55.9% (Zitouni et al., 2004).

The gross morphological properties of strain NRRL B-16296T were observed on plates of tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic salts-starch, glycerol-asparagine, peptone-yeast extract-iron and tyrosine agar plates (ISP media 1–7, respectively; Shirling & Gottlieb, 1966) which were incubated for 21 days at 28°C. The strain grew well on all of these media, apart from peptone-yeast extract-iron agar (PYEA), producing a light yellow to light reddish-brown substrate mycelium and rudimentary aerial hyphae on glycerol-asparagine, oatmeal and tyrosine agars. Melanin pigments were formed on tyrosine agar, but not on PYEA.

Morphological characteristics were observed by examining gold-coated, dehydrated preparations from a 14-day-old culture of strain NRRL B-16296T grown on inorganic salts-starch agar at 28°C, using a Cambridge Stereoscan 240 scanning electron microscope and the procedure described by O’Donnell et al. (1993). The organism produced a branched substrate mycelium and spores on synnematelike structures, but there was no evidence of spore vesicles (Supplementary Fig. S1 available in IJSEM online).

Table 1. Phenotypic properties which separate strain NRRL B-16296T from the type strains of species of the genus Saccharothrix

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degrades starch and tyrosine, and utilizes sugars such as (+)-L-arabinose, dextrin, myo-inositol, lactose, melibiose, (+)-D-rafhnose and (+)-D-xylose as sole sources of carbon for energy and growth. Additional phenotypic properties of the strain are given in Table 1 and in the species description.

The genotypic and phenotypic data clearly demonstrate that strain NRRL B-16296T is misclassified in the genus Dactylosporangium as its phylogenetic position and chemotaxonomic profile are typical of members of the genus Saccharothrix. Moreover, the phylogenetic data show convincingly that the strain forms a new centre of taxonomic variation within this genus. It is, therefore, proposed that strain NRRL B-16296T be classified in the genus Saccharothrix as a new species, Saccharothrix variisporea sp. nov.

**Description of Saccharothrix variisporea sp. nov.**

Saccharothrix variisporea (var.i.i.spo’r.ea. L. adj. varius diverse, different, various; Gr. n. spora a seed, in microbiology, a spore; L. suff. eus, -a, -um suffix with various meanings, but in general, made of or belongs to; N.L. fem. adj. variisporea with different spores).

The description is based on data taken from the present study and from the earlier work of Tomita et al. (1977). Aerobic, Gram-positive actinomycete which forms branched, light orange coloured mycelia on inorganic salts-starch and oatmeal agars. Grows well between 30 and 37 °C, from pH 4 to 10, and optimally around pH 7. Positive for aesculin and allantoin hydrolysis, catalase, coagulation and peptization of milk, and for hydrogen sulfide and urease production, but negative for nitrate reduction. Degrades arbutin, casein, DNA, elastin, gelatin, Tween 40, 60 and 80, uric acid and xylan, but not cellulose, chitin, guanine, pectin, RNA, tributyrin, Tween 20 or xanthine. Cellobiose, (+)-D-galactose, (-)-D-glucose, glycerol, glycogen, inulin, mannitol, maltose, (+)-D-mannose, (-)-D-ribose, starch and trehalose (at 1.0 %, w/v), and (+)-L-tartaric acid, (-)-L-malic acid, propionic acid, pyruvic acid and uric acid (at 0.1 %, w/v) are used as sole carbon sources, but not adonitol, (+)-D-arabitol, dulcitol, (-)-D-sorbitol, (-)-L-sorbose or xylitol (at 1.0 %, w/v) or (+)-L-lactic acid, oxalic acid or urea (at 0.1 %, w/v). Resistant (μg ml⁻¹) to ampicillin (4), cephaloridine (2), clindamycin (8), gentamicin (8), kanamycin (8), lincomycin (8), oxytetracycline (8), penicillin G (2), tylolin (8) and vancomycin (2), but sensitive to ciprofloxacin (2), chloramphenicol (8), novobioacin (8), rifampicin (16), streptomycin (4) and tetracycline (8). Growth occurs in the presence of 4 % (w/v) NaCl and 0.05 % lysosome, but not in the presence of 5 % NaCl. Additional phenotypic properties are shown in Table 1 and in the main text. The predominant fatty acids are iso-C₁₆:₀, anteiso-C₁₇:₀, iso-C₁₅:₀, 9-methyl C₁₆:₀, iso-C₁₇:₀, iso-C₁₆:₁ H, C₁₇:₁9·C₁₆:₁ 2·OH, C₁₆:₁0·7c, anteiso-C₁₅:₀, C₁₇:₀9·c and anteiso-C₁₇:₁ C. The DNA G+C content of the type strain is 74 mol%.

The type strain, NRRL B-16296T (=ATCC 31203 T =DSM 43911 T =JCM 3273 T =NBRC 14104 T), was isolated from a soil sample from India.

**Acknowledgements**

We are indebted to Professor Jean Euzéby for providing the correct etymology for the species epithet.

**References**


