Reclassification of '*Dactylosporangium variesporum*' as *Saccharothrix variisporea* corrig. (ex Tomita *et al.* 1977) sp. nov., nom. rev.

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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-16296^T (=ATCC 31203^T =DSM 43911^T =JCM 3273^T =NBRC 14104^T).

'*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-16296^T (=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-16296^T 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-16296^T 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 prepared from strains NRRL B-16296^T and DSM 43911^T 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

The GenBank/EMBL/DBJ accession number for the 16S rRNA sequence of strain NRRL B-16296^T is GQ917213.

A supplementary figure is available with the online version of this paper.

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 phylogenetic 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-16296^T and *S. mutabilis* subsp. *capreolus* NRRL 2773^T are reported to produce the antibiotic capreomycin, though NRRL B-16296^T was most closely related to *S. espanaensis* NRRL 15764^T; 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-16296^T' 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-16296^T 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-16296^T contains the unique oligonucleotide signature characteristic for members of the genus *Saccharothrix* (Labeda & Kroppenstedt, 2007).

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

Standard procedures were used to extract and analyse the isomers of diaminopimelic acid (Staneck & Roberts, 1974), isoprenoid quinones (Collins, 1994), muramic acid type (Uchida *et al.*, 1999), polar lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Schaal, 1985), using appropriate controls. Cellular fatty acids were extracted, methylated and analysed by GC using the standard Sherlock MIDI (Microbial Identification) system (Sasser, 1990) and mycolic acids sought using the TLC procedure described by Minnikin *et al.* (1975).

The strain contained *meso*-diaminopimelic acid (wall chemotype III *sensu*; Lechevalier & Lechevalier 1970), *N*-acetylated muramic acid moieties, galactose, mannose and rhamnose as diagnostic whole-organism sugars, tetrahydrogenated menaquinones with nine isoprene units (MK-9[H₄]) as the predominant menaquinone, and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol 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 phospholipid components. The predominant fatty acids were 13-methyltetradecanoic (iso-C_{15:0}), 14-methylpentadecanoic (iso-C_{16:0}) and 14-methylhexadecanoic (anteiso-C_{17:0}) acids; mycolic acids were not present. All of these properties are in line with the classification of the strain in the genus *Saccharothrix* (Labeda *et al.*, 1984; Otoguro *et al.*, 2009).

The DNA G+C content of strain NRRL B-16296^T 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-16296^T and its closest phylogenetic neighbours 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 circumscription 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 % (Labeda & Lechevalier, 1989) whereas the type strains of *Saccharothrix syringae* and

Fig. 1. Neighbour-joining tree based on almost complete 16S rRNA gene sequences showing the position of strain NRRL B-16296^T 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-24137^T and *S. australiensis* NRRL 11239^T 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-16296^T 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-16296^T 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 synnemata-like structures, but there was no evidence of spore vesicles (Supplementary Fig. S1 available in IJSEM online).

Strain NRRL B-16296^T was examined for a broad range of phenotypic properties using standard media and methods described by Goodfellow *et al.* (1997). It can be seen from Table 1 that the strain can be clearly distinguished from the type strains of species of the genus *Saccharothrix*, including its nearest neighbours, *S. espanaensis* NRRL 15764^T and the type strains of the two subspecies of *S. mutabilis*, by using a combination of phenotypic properties. Strain B-16296^T, unlike the type strain of *S. espanaensis*, grows at 45 °C and

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

Taxa: 1, strain NRRL B-16296^T; 2, *Saccharothrix algeriensis* NRRL B-24137^T; 3, *S. australiensis* NRRL 11239^T; 4, *S. coeruleofusca* DSM 43679^T; 5, *S. espanaensis* NRRL 15764^T; 6, *S. longispora* DSM 43749^T; 7, *S. mutabilis* subsp. *capreolus* DSM 40225^T; 8, *S. mutabilis* subsp. *mutabilis* NRRL B-16077^T; 9, *S. syringae* DSM 43886^T; 10, *S. texasensis* NRRL B-16134^T; 11, *S. violaceirubra* KCTC 19326^T; 12, *S. xinjiangensis* NBRC 101911^T. Data for the reference strains were taken from Labeda (2002), Zitouni *et al.* (2004), Hu *et al.* (2004) and Otoguro *et al.* (2009). +, Positive; –, negative; ND, no data available; w, weakly positive; v, variable.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Decomposition of:												
Adenine	–	–	–	–	–	+	–	–	–	–	–	ND
Hypoxanthine	+	–	–	–	+	–	+	+	–	+	–	ND
Starch	+	–	–	+	–	+	+	+	+	+	–	+
Tyrosine	+	+	+	+	–	+	–	+	+	+	+	+
Nitrate reduction	–	+	+	–	w	+	–	+	–	+	+	–
Assimilation of:												
Citrate	–	+	–	–	v	+	+	+	–	–	–	–
Lactate	–	ND	w	–	+	+	–	+	–	+	–	–
Malate	+	ND	+	–	+	+	+	+	+	+	–	ND
Utilization of:												
Arabinose	+	–	–	+	–	+	+	+	+	+	–	+
Dextrin	+	–	+	+	–	+	+	+	+	+	–	ND
Inositol	+	–	–	–	–	–	+	+	–	+	–	ND
Lactose	+	–	–	+	–	+	–	+	+	+	–	+
Melibiose	+	–	–	–	–	–	+	+	+	+	–	+
Methyl α -D-glucoside	+	–	–	+	–	–	–	+	–	+	–	+
Raffinose	+	–	–	+	–	–	–	+	+	–	–	+
Rhamnose	+	–	–	+	–	+	–	–	+	+	–	+
Salicin	+	ND	–	+	–	ND	+	+	w	+	–	ND
Sorbitol	–	–	+	+	–	–	–	–	–	–	–	–
Sucrose	+	–	–	+	+	+	–	+	+	+	+	+
D-Xylose	+	–	–	+	v	+	+	+	+	+	–	+
Growth in presence of:												
4 % NaCl (w/v)	+	+	+	+	+	+	+	–	+	–	–	–
5 % NaCl (w/v)	–	–	–	+	–	+	+	–	+	–	–	–
37 °C	+	+	+	+	+	+	+	+	+	+	–	+
45 °C	w	+	+	+	–	–	+	+	+	–	–	+

degrades starch and tyrosine, and utilizes sugars such as (+)-L-arabinose, dextrin, *myo*-inositol, lactose, melibiose, (+)-D-raffinose 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-16296^T 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-16296^T be classified in the genus *Saccharothrix* as a new species, *Saccharothrix variisporea* sp. nov.

Description of *Saccharothrix variisporea* sp. nov.

Saccharothrix variisporea (va.ri.i.spo're.a. 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 peptonization of milk, and for hydrogen sulfide and urease production, but negative for nitrate reduction. Degrades arbutin, casein, DNA, elastin, gelatin, Tweens 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), tylosin (8) and vancomycin (2), but sensitive to ciprofloxacin (2), chloramphenicol (8), novobiocin (8), rifampicin (16), streptomycin (4) and tetracycline (8). Growth occurs in the presence of 4 % (w/v) NaCl and 0.05 % lysozyme, 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-C_{16:0}, anteiso-C_{17:0}, iso-C_{15:0}, 9-methyl C_{16:0}, iso-C_{17:0}, iso-C_{16:1} H, C_{17:1}ω6c, C_{16:1} 2-OH, C_{16:1}ω7c, anteiso-C_{15:0}, C_{17:1}ω8c and anteiso-C_{17:1} C. The DNA G + C content of the type strain is 74 mol%.

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

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