Reclassification of *Nonomuraea flexuosa* (Meyer 1989) Zhang *et al.* 1998 as *Thermopolyspora flexuosa* gen. nov., comb. nov., nom. rev.

Michael Goodfellow, Luis A. Maldonado† and Erika T. Quintana‡

Correspondence

Michael Goodfellow
m.goodfellow@ncl.ac.uk

School of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

A polyphasic study was undertaken to clarify the taxonomic position of *Nonomuraea flexuosa* DSM 41386^T. The distinct 16S rRNA gene sequence phyletic branch formed by this strain was equated with nine related monophyletic clades composed of representatives of the genera classified in the family *Streptosporangiaceae*. The organism produced a PCR product characteristic of this taxon when examined using a set of oligonucleotide primers specific for members of the family *Streptosporangiaceae*. Strain DSM 41386^T could also be distinguished from representatives of the nine genera assigned to this family using a combination of chemotaxonomic, morphological and physiological properties. It is evident from the genotypic and phenotypic data that strain DSM 41386^T is misclassified in the genus *Nonomuraea* and merits recognition as a monospecific genus within the family *Streptosporangiaceae*. It is proposed that the name *Thermopolyspora flexuosa* gen. nov., comb. nov., nom. rev. be used for this purpose, with the type strain DSM 41386^T (=NRRL B-24348^T).

Thermopolyspora flexuosa was proposed by Krassilnikov & Agre (1964) for a group of thermophilic actinomycetes that formed short chains of spores on short sporophores. The species was subsequently associated with a number of other genera, including *Nocardia* (Becker *et al.*, 1965; Lechevalier *et al.*, 1966), *Actinomadura* (Cross & Goodfellow, 1973; Lacey *et al.*, 1978), *Micropolyspora* (Krassilnikov *et al.*, 1968), *Microtetraspora* (Kroppenstedt *et al.*, 1990) and latterly *Nonomuraea* (Zhang *et al.*, 1998). The present polyphasic study was undertaken to clarify the taxonomic position of *Nonomuraea flexuosa* DSM 41386^T. The resultant data show that the strain should be classified in a new genus within the family *Streptosporangiaceae* Goodfellow *et al.* 1990, emend. Ward-Rainey *et al.* 1997. It is proposed that the name *Thermopolyspora flexuosa* be used for this taxon, a proposal advanced by Greiner-Mai *et al.* (1987) on the basis of chemotaxonomic and morphological data.

Nonomuraea flexuosa DSM 41386^T was maintained on glucose-yeast extract-malt extract agar plates (ISP2; Shirling & Gottlieb, 1966) at 45 °C and as a glycerol suspension

(20 %, v/v) at –20 °C. Biomass for the molecular systematic studies was prepared from shake flasks of ISP2 broth incubated at 45 °C for 15 days and then washed in NaCl/EDTA buffer (0.1 M EDTA, pH 8.0, 0.1 M NaCl) and stored at –20 °C until needed.

Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified products of the test strain were carried out after Quintana *et al.* (2003). An almost full-length 16S rRNA gene sequence was aligned manually with corresponding sequences of representatives of genera classified in the suborder *Streptosporangineae* retrieved from the DDBJ/EMBL/GenBank databases using the PHYDIT program (Chun, 1995). Evolutionary trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices for the least-squares and neighbour-joining methods were generated after Jukes & Cantor (1969). The robustness of the resultant trees was evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining dataset on 1000 resamplings using the SEQBOOT and CONSENSE options from the PHYLIP package.

DNA from the organism was also examined using the primers Sm6F and Sm5R which yield a product that is specific for members of the family *Streptosporangiaceae* (Monciardini *et al.*, 2002).

†Present address: Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (UNAM), CP 04510, México DF, México.

‡Present address: Centro Interdisciplinario de Investigaciones y Estudios en Medio Ambiente y Desarrollo (CIEMAD), Instituto Politécnico Nacional (IPN), CP 07700, México DF, México.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Thermopolyspora flexuosa* DSM 41386^T is AY039253.

Strain DSM 41386^T formed the PCR product characteristic of members of the family *Streptosporangiaceae* when examined with the family-specific set of oligonucleotide primers developed by Monciardini *et al.* (2002) for the recognition of members of this taxon (data not shown). Comparison of the almost complete 16S rRNA gene sequence (1493 nt) of the tested strain with corresponding sequences of representatives of taxa classified in the suborder *Streptosporangineae* showed that the organism forms a distinct phyletic line within the evolutionary radiation occupied by the family *Streptosporangiaceae* (Fig. 1). The organism showed 16S rRNA gene sequence similarities to other members of the family within the range 92.7 to 94.7 %, values consistent with the assignment of the strain to a novel genus. It is apparent from Table 1 that strain DSM 41386^T shows a range of phenotypic properties that distinguish it from representatives of the established genera classified in the family *Streptosporangiaceae*.

It is evident from present and previous data that strain DSM 41386^T can be distinguished from representatives of all of the genera classified in the family *Streptosporangiaceae* by using a combination of genotypic and phenotypic properties. It is, therefore, proposed that this organism be assigned to this taxon as *Thermopolyspora flexuosa* comb. nov.

Description of *Thermopolyspora* gen. nov.

Thermopolyspora [Ther.mo.po'ly.spo.ra. Gr. n. *thermos* heat; Gr. adj. *poly* many; Gr. n. *spora* a seed; N.L. fem. n. *Thermopolyspora* the heat (-loving) many-spored organism].

The description is taken from this and earlier studies (Krassilnikov & Agre, 1964; Greiner-Mai *et al.*, 1987; Meyer, 1989; Kroppenstedt *et al.*, 1990). Aerobic, Gram-positive, non-acid-alcohol-fast, thermophilic actinomycetes which form non-fragmenting substrate and aerial mycelia. Hooked or irregular spiral chains of 4 to 10 warty to spiny ornamented spores (1.2–1.5 µm in diameter) are arranged in clusters on long, moderately branched aerial hyphae on potato carrot agar; the organism forms a light blue aerial mycelium and a brown soluble pigment on this medium. White to yellowish white aerial hyphae are borne on a light-brown substrate mycelium on oatmeal agar, but diffusible pigments are not produced. Growth occurs from pH 6.0 to 9.0 and from 40 to 60 °C; particularly good growth occurs between 45 and 55 °C. The wall peptidoglycan contains *meso*-diaminopimelic acid and *N*-acetylated muramic acid. Glucose and ribose are found in whole-organism hydrolysates from the type species. The type species contains MK9, MK9(H₂) and MK9(H₄) as predominant isoprenologues

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences showing relationships between *Nonomuraea flexuosa* DSM 41386^T and representatives of genera classified in the family *Streptosporangiaceae*. Asterisks indicate branches of the tree that were also found using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. Numbers at nodes indicate the levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50% are shown. F and P indicate branches that were recovered using the least-squares and maximum-parsimony methods, respectively. Bar, 0.01 substitutions per site.

Table 1. Chemotaxonomic, morphological and physiological characteristics of *Nonomuraea flexuosa* DSM 41386^T and members of the genera classified in the family *Streptosporangiaceae*

Taxa are indicated as: 1, *Nonomuraea flexuosa* DSM 41386^T; 2, *Acrocarpospora*; 3, *Herbidospora*; 4, *Microbispora*; 5, *Microtetraspora*; 6, *Nonomuraea*; 7, *Planobispora*; 8, *Planomonospora*; 9, *Planotetraspora*; 10, *Streptosporangium*. Data were taken from this and previous studies (Greiner-Mai *et al.*, 1987; Goodfellow *et al.*, 1990; Kudo *et al.*, 1993; Tamura *et al.*, 2000; Stackebrandt *et al.*, 2001; Tamura & Sakane, 2004). The major constituents of the cell wall were alanine, glutamic acid, glucosamine and *meso*-A₂pm (Lechevalier & Lechevalier, 1970a). Diagnostic phospholipids: II, phosphatidylethanolamine; IV, glucosamine (with phosphatidylethanolamine and phosphatidylmethylethanolamine variable) (Lechevalier *et al.*, 1977). Whole-organism sugar patterns of actinomycetes containing *meso*-A₂pm: A, arabinose and galactose; B, madurose; C, no diagnostic sugar; D, arabinose and xylose (Lechevalier & Lechevalier, 1970b). For all taxa, the fatty acid type [saturated fatty acids, unsaturated fatty acids, iso-fatty acids, variable and methyl-branched fatty acids (Kroppenstedt, 1985)] was 3c. +, Present; –, absent; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10
Morphology	Hooked or irregular spiral chains of four to ten warty to spiny ornamented spores on aerial hyphae	Club or globose spore vesicles on aerial hyphae	Straight chains of smooth-surfaced spores on aerial hyphae	Smooth-surfaced spores in characteristic longitudinal pairs on aerial hyphae	Spore chains typically containing four smooth-surfaced spores on short aerial hyphae	Spore chains or pseudo-sporangia formed on aerial hyphae	Cylindrical to clavate spore vesicles containing longitudinal pairs of spores on aerial hyphae	Cylindrical to clavate spore vesicles containing single spores on aerial hyphae	Spore vesicles containing four spores on aerial hyphae	Globose spore vesicles on aerial hyphae
Motile spores	–	–	–	–	–	–	+	+	+	–
Cell wall chemotype	III	III	III	III	III	III	III	III	III	III
Major menaquinones*	MK9(H ₂ , H ₄)	MK9(H ₂ , H ₄ , H ₆)	MK10(H ₄ , H ₆ , H ₈)	MK9(H ₂ , H ₄)	MK9(H ₂ , H ₄)	MK9(H ₂ , H ₄)	MK9(H ₂ , H ₄)	MK9(H ₂)	MK9(H ₄)	MK9(H ₂ , H ₄)
Muramic acid type	Acetylated	Acetylated	Acetylated	Acetylated	Acetylated	Acetylated	ND	ND	Acetylated	Acetylated
Phospholipid type	IV	IV, II	IV	IV	IV	IV	IV	IV	ND	IV
Whole-organism sugar pattern	C	B,C	B	B,C	B,C	B,C	B	B	A,D	B
DNA G + C content (mol%)	77	68–69	69–71	71–73	69–71	64–69	70–71	72	71	69–71
Growth temperature range (°C)	37–60	15–30	ND	25–55	20–45	20–45	28–40	28–37	28–37	20–50

*Abbreviations exemplified by MK-9(H₄), menaquinone having four of the nine isoprene units hydrogenated.

and minor amounts of MK9(H₆), phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylethanolamine, phosphatidylinositolmannosides, ninhydrin- and sugar-positive phospholipids and uncharacterized glycolipids as major polar lipids. It also contains fatty acids rich in saturated, unsaturated and branched chain components, but lacks mycolic acids. The G + C content of the DNA of the type species is 77 mol%. The type and only species is *Thermopolyspora flexuosa*. 16S rRNA gene sequence data show that the organism belongs to the family *Streptosporangiaceae* Goodfellow *et al.* 1990, emend. Ward-Rainey *et al.* 1997.

Description of *Thermopolyspora flexuosa* comb. nov., nom. rev.

Thermopolyspora flexuosa (flex.u' o.sa. L. fem. adj. *flexuosa* full of turns or windings, tortuous, flexuous referring to the morphology of the spore chains).

Basonym: *Actinomadura flexuosa* (ex Krasil'nikov and Agre 1964) Meyer 1989.

The description is based on the results of this and previous studies (Krasilnikov & Agre, 1964; McCarthy & Cross, 1984; Greiner-Mai *et al.*, 1987; Meyer, 1989; Kroppenstedt *et al.*, 1990; Stackebrandt *et al.*, 2001).

General chemotaxonomic, growth and morphological properties are included in the genus description. Grows well on peptone glucose agar, producing a brown substrate mycelium but no aerial hyphae. Melanin pigments are formed from tyrosine. Growth poor on Bennett's sucrose and glycerol asparagine agars. Degrades agar, casein, DNA, elastin, gelatin, keratin, starch, testosterone, tyrosine, Tweens 20 and 80, xanthine and xylan, but not carboxymethylcellulose, chitin, guanine, hypoxanthine or pectin. Aesculin, allantoin and arbutin are hydrolysed but not urea. Produces arginine dihydrolase, catalase, citrate lyase, β -galactosidase, β -glucosidase and oxidase, but not acetoin, hydrogen sulphide, lysine decarboxylase, ornithine decarboxylase or phosphatase. (+)-L-Arabinose, (+)-D-fructose, (+)-D-galactose, (+)-D-maltose, (+)-D-glucose, (+)-D-glycerol, (+)-D-mannitol, (+)-D-mannose, (+)-D-sucrose, (+)-D-trehalose and (+)-D-xylose are used as sole carbon sources for energy and growth, but not dulcitol, inulin, (+)-D-lactose, (+)-D-melezitose, (+)-D-melibiose, *myo*-inositol, (+)-D-raffinose, (+)-D-rhamnose, (+)-D-ribose, (+)-D-sorbitol or (+)-D-sorbose (all at 1%, w/v). Sodium citrate is used as a sole carbon source, but not sodium oxalate (both at 0.1%, w/v). Grows well on alanine, asparagine, arginine, glutamic acid, histidine, tyrosine and tryptophan as sole nitrogen sources. Nitrates and ammonia salts are not utilized. Resistant (μ g per filter paper disc) to chloramphenicol (25), cephaloridine (5), cephalixin (30), cefoxin (30), gentamicin sulphate (2), kanamycin sulphate (30), tobramycin sulphate (10) and penicillin (5 U), but sensitive to cephalozin (30) and gentamicin sulphate (10). Grows in the presence of bile salts (0.02% w/v), crystal

violet (0.00002%, w/v), potassium tellurite (0.02%, w/v), sodium azide (0.01%, w/v) and thallous acetate (0.001%, w/v), but is sensitive to bile salts (0.5%, w/v), brilliant green (0.0005%, w/v), crystal violet (0.00005%, w/v), lysozyme (0.0025%, w/v), sodium azide (0.02%, w/v), sodium chloride (3%, w/v), tetrazolium chloride (0.002%, w/v), thallous acetate (0.005%, w/v) and novobiocin (50 μ g ml⁻¹). Produces major proportions of MK-9, MK-9(H₂) and MK-9(H₄) (points of saturation: III and VIII) and minor proportions of MK-9(H₆) (points of saturation: II, III, VIII). Major fatty acids are iso-15:0 (12.5%), 15:0 (5.7%), iso-16:0 (13.8%), iso-17:0 (18.7%), anteiso-17:0 (12.4%) and 10-methyl-branched 17:0 (6.9%) components; minor peaks include anteiso-15:0 (4.3%), *cis*-16:1 (2.8%), iso-2-OH 15:0 (1.4%), 16:0 (3.4%), 10-methyl 16:0 (2.4%), iso-2-OH 16:0 (2.5%), 17:0 (2.0%), iso-2-OH 17:0 (3.0%) and 18:0 (2.5%).

The type strain, DSM 43186^T (=NRRL B-24348^T), was isolated from soil from the Pamir mountains.

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