International Journal of Systematic Bacteriology (1998), **48**, 411–422 Printed in Great Britain

Reclassification of *Thermomonospora* and *Microtetraspora*

Zhenshui Zhang, Yue Wang and Jisheng Ruan

Author for correspondence: Yue Wang. Tel: +65 7723207. Fax: +65 7791117.
e-mail: mcbwangy@imcb.nus.edu.sg

Microbial Collection and Screening Laboratory, Institute of Molecular and Cell Biology, National University of Singapore, 30 Medical Drive, Singapore 117609

Almost complete 16S rRNA sequences from seven *Thermomonospora* strains, *Thermomonospora curvata*, *Thermomonospora formosensis*, *Thermomonospora fusca*, *Thermomonospora mesophila*, *Thermomonospora chromogena*, *Thermomonospora alba* and *Thermomonospora mesouviformis* (a synonym of *Thermomonospora alba*) were determined and subjected to phylogenetic analysis together with the sequences from all the representative members of the suborder *Streptosporangineae*. On the basis of phylogenetic, chemotaxonomic and phenotypic evidence, the transfer is proposed of *Thermomonospora formosensis* to the genus *Actinomadura* as *Actinomadura formosensis* comb. nov., *Thermomonospora mesophila* to the genus *Microbispora* as *Microbispora mesophila* comb. nov., and *Thermomonospora fusca* and *Thermomonospora alba* to a new genus, *Thermobifida* gen. nov., which belongs to the family *Nocardiopsaceae*, as *Thermobifida fusca* comb. nov. and *Thermobifida alba* comb. nov. *Thermobifida alba* is designated the type species of the genus. The transfer is also proposed of all species of the *Microtetraspora pusilla* group, which were transferred from *Actinomadura*, to a new genus, *Nonomuria* gen. nov., as *Nonomuria africana* comb. nov., *Nonomuria angiospora* comb. nov., *Nonomuria fastidiosa* comb. nov., *Nonomuria ferruginea* comb. nov., *Nonomuria flexuosa* comb. nov., *Nonomuria helvata* comb. nov., *Nonomuria polychroma* comb. nov., *Nonomuria pusilla* comb. nov., *Nonomuria recticatena* comb. nov., *Nonomuria roseola* comb. nov., *Nonomuria roseoviolacea* comb. nov., *Nonomuria rubra* comb. nov., *Nonomuria salmonea* comb. nov., *Nonomuria spiralis* comb. nov. and *Nonomuria turkmeniaca* comb. nov. *Nonomuria pusilla* is designated the type species of the genus.

Keywords: *Thermomonospora*, *Microtetraspora*, *Nonomuria* gen. nov., *Thermobifida* gen. nov.

INTRODUCTION

The genus *Thermomonospora* was proposed by Henssen in 1957 for thermophilic actinomycete strains isolated from composted horse manure and characterized by formation of single spores on aerial mycelium (13). Three species, *Thermomonospora curvata*, *Thermomonospora lineata* and *Thermomonospora fusca*, were originally described. Since *Thermomonospora curvata* was the only species isolated and maintained in pure culture, it was designated as the type species of the genus by Henssen & Schnepf in 1967 (14). Nonomura & Ohara later assigned mesophilic monosporic actinomycetes to the genus as *Thermomonospora mesophila* (38). As a result of the rejection of the genus *Actinobifida* (3), the species *Actinobifida alba* (27), characterized by formation of single spores on dichotomously branched sporophores, was relocated in the genus *Thermomonospora* as *Thermomonospora alba* (30), while the taxonomic position of *Actinobifida chromogena* (18) remained uncertain. In 1984, McCarthy & Cross (30) carried out a comprehensive numerical taxonomic study of *Thermomonospora* and related actinomycetes and identified five *Thermomonospora* species, *Thermomonospora curvata*, *Thermomonospora alba*, *Thermomonospora chromogena*, *Thermomonospora fusca* and *Thermo-*

The DDBJ accession numbers for the sequences reported in this paper are listed in Table 1.

Table 1. *Thermomonospora* species used in this study

Organism	Source	16S rDNA sequence GenBank accession no.
Thermomonospora alba	JCM 3077^T	AF002260
Thermomonospora chromogena	JCM 6244^T ATCC 43196^T	AF002261
Thermomonospora curvata	JCM 3096^T	AF002262
Thermomonospora formosensis	JCM 7474^T	AF002263
Thermomonospora fusca	IFO 14071^T ATCC 27730^T JCM 3262^T	AF002264
Thermomonospora mesophila	JCM 3151^T	AF002265
Thermomonospora mesouviformis	JCM 3169^T	AF002266

monospora mesophila. A previously described species, *Thermomonospora mesouviformis* (40), was reduced as a synonym of *Thermomonospora alba*. In 1986, Hasegawa described another species, *Thermomonospora formosensis* (12). All six species are listed in the ninth edition of the *Bergey's Manual of Determinative Bacteriology*.

Several detailed chemical analyses of the members of the genus *Thermomonospora* revealed that the genus was highly heterogeneous and the constituent species could be separated into three distinct groups (11, 20, 22). *Thermomonospora curvata* and *Thermomonospora formosensis* were tentatively assigned to a group which had chemotaxonomic properties similar to the members of the genus *Actinomadura* (25) (cell wall type III, phospholipid type I, menaquinone type 4B2 and fatty acid type 3a); *Thermomonospora chromogena* and *Thermomonospora mesophila* were found sharing almost identical chemotaxonomic characteristics with *Microtetraspora* species (cell wall type III, phospholipid type IV, menaquinone type 4A2 and fatty acid type 3c); and *Thermomonospora alba*, *Thermomonospora mesouviformis* and *Thermomonospora fusca* were characterized by cell wall type III, phospholipid type II, menaquinone type 4D and fatty acid type 3e. Based on the highly heterogeneous chemotaxonomic properties demonstrated by different members of *Thermomonospora*, the possibility was discussed of combining *Thermomonospora curvata* and *Thermomonospora formosensis* with *Actinomadura* and transferring *Thermomonospora chromogena* and *Thermomonospora mesophila* to the newly revised genus *Microtetraspora* (20). Such new classification, if proposed, would leave only *Thermomonospora fusca* and *Thermomonospora alba* in the genus *Thermomonospora*.

In 1990, Kroppenstedt *et al*. revised the genus *Microtetraspora* by transferring to this genus a group of *Actinomadura* species, which was represented by *Actinomadura pusilla*. The species of the newly transferred group share very similar chemotaxonomic characteristics with the three original *Microtetraspora* species, *Microtetraspora glauca*, *Microtetraspora fusca* and *Microtetraspora niveoalba* (21). However, there are several lines of evidence indicating considerable differences between the species of the two groups in properties of taxonomic value. First, a numerical taxonomic analysis revealed that *Microtetraspora glauca* and *Microtetraspora niveoalba* were more closely related to *Microbispora rosea* than to the species of the *Microtetraspora pusilla* group (1). Second, the relative electrophoretic mobilities of the ribosomal AT-L30 proteins of the three original species ranged from $-6{\cdot}5$ to $-5{\cdot}0$, while most of the members of the *Microtetraspora pusilla* group had relative electrophoretic mobilities ranging from $-1{\cdot}5$ to $0{\cdot}0$ (41, 42, 43). Third, the three original *Microtetraspora* species are apparently more closely related to each other than to the species belonging to the *Microtetraspora pusilla* group on the basis of DNA–DNA homology data (34, 35). Furthermore, when the fatty acid profiles of the two groups were compared, the difference between these two groups was also evident (21, 34, 35). In the three original species, the amount of iso-16 branched fatty acid is more than twice that of 10 methyl-17 branched fatty acid, a feature resembling that of *Microbispora*. In the *Microtetraspora pusilla* group, the amounts of iso-16 branched and 10 methyl-17 branched fatty acids are either comparable or there is more of the latter. Recently, we reported that the three original species formed a coherent clade more closely related to the genus *Microbispora* than the species of the *Microtetraspora pusilla* group on the basis of 16S rRNA gene sequence analysis (57).

16S rRNA gene sequence-based phylogenetic analysis has been widely used to resolve phylogenetic relationships between organisms at virtually all taxonomic levels (50, 51, 52, 57–62). To determine the exact phylogenetic positions of *Thermomonospora* species, 16S rRNA genes of all the *Thermomonospora* species listed in the ninth edition of *Bergey's Manual of Determinative Bacteriology* were sequenced and subjected to phylogenetic analysis with many representative members of the suborder *Streptosporangineae* (52). In addition, to resolve the observed heterogeneity of the genus *Microtetraspora*, we included sequences, in the phylogenetic analysis, from representative

members from all the genera of the family *Streptosporangiaceae*. Here, we report the results of these studies.

METHODS

Organisms and culture conditions. The actinomycete strains used in this study were purchased from the IFO (Institute for Fermentation, Osaka, Japan), JCM (Japan Collection of Microorganisms, Wako, Japan) and ATCC (American Type Culture Collection, Rockville, MD, USA). Strain names and GenBank nucleotide sequence accession numbers are listed in Table 1. The purity of the strains purchased from various culture collections were examined first. The cells were then grown in liquid medium for preparation of genomic DNA as described previously (57, 58).

Preparation of genomic DNA and PCR amplification, cloning and sequencing of 16S rRNA genes. Preparation of genomic DNA and PCR amplification, cloning and sequencing of 16S rRNA genes were carried out as described previously (28, 57, 58).

Sequence alignment and phylogenetic analysis. Multiple alignments of sequences and calculations of levels of sequence similarity were carried out using the CLUSTAL method of the DNASTAR program. Phylogenetic trees were reconstructed using the maximum-parsimony method contained in the PAUP package (54) and the neighbour-joining method (48) contained in the CLUSTAL phylogenetic analysis software package (15). The confidence level of the phylogenetic tree topology was determined using the bootstrap programs contained in these packages.

RESULTS

Determination of 16S rDNA sequences

In this study, at least three independent clones of PCR-amplified rDNAs from each organism were analysed and some *Thermomonospora* strains were acquired from different culture collections to confirm their new phylogenetic positions. For example, the same strains of *Thermomonospora chromogena* and *Thermomonospora fusca* were ordered from more than one culture collection (Table 1). Almost complete 16S rRNA sequences [7–1507, *Escherichia coli* numbering (2)] were determined for *Thermomonospora curvata*, *Thermomonospora alba*, *Thermomonospora formosensis*, *Thermomonospora chromogena*, *Thermomonospora fusca*, *Thermomonospora mesophila* and *Thermomonospora mesouviformis*. The 16S rDNA sequence of *Thermomonospora curvata* that we obtained is nearly identical with two sequences from the same species deposited in GenBank by other researchers. The sequences of *Thermomonospora chromogena* and *Thermomonospora fusca* strains ordered from different culture collections were identical.

Pairwise 16S rRNA sequence comparison

After a primary analysis of the *Thermomonospora* sequences together with the 16S rRNA sequences of many representative species from most genera of actinomycetes, we found high levels of sequence similarity between *Thermomonospora* species and members of the genera *Actinomadura*, *Microbispora* and *Nocardiopsis*. We then carried out a more detailed analysis focusing on comparison with members of the these genera and a few other closely related taxons. The levels of 16S rDNA sequence similarity are shown in Table 2.

The sequences of *Thermomonospora curvata*, the type species of the genus, and *Thermomonospora formosensis* are 93·3 % identical but share a lower level of similarity (88·3–90·1 %) with those of other *Thermomonospora* species. A moderate level of sequence similarity (90·1–93 %) was found between *Thermomonospora curvata* and *Actinomadura* species, while higher sequence similarities were scored between the sequence of *Thermomonospora formosensis* and those of the members of *Actinomadura*, as exemplified by a 96·5 % identity between the sequences of *Thermomonospora formosensis* and *Actinomadura madurae*. The sequences of *Thermomonospora alba*, *Thermomonospora mesouviformis* and *Thermomonospora fusca* share a high level of similarity amongst themselves (>96 %), but a much lower level of similarity (<90 %) with other *Thermomonospora* species. Interestingly, *Thermomonospora fusca*, *Thermomonospora alba* and *Thermomonospora mesouviformis* seem more closely related to *Nocardiopsis* species with sequence similarity levels ranging from 91 to 92·6 % than to other *Thermomonospora* species (<90 %). The sequences of *Thermomonospora alba* and *Thermomonospora mesouviformis* are 98·7 % identical, which supports the reduction of *Thermomonospora mesouviformis* as a synonym of *Thermomonospora alba* proposed by McCarthy & Cross (30). *Thermomonospora mesophila* is apparently more closely related to *Microbispora* species (mean similarity value of 94·4 %) than to other members of *Thermomonospora* (mean similarity value of 88 %). *Thermomonospora chromogena* demonstrates generally low levels of sequence similarity with other actinomycete species, with the highest value (91·2 %) scored with two *Microbispora* species.

The intrageneric levels of sequence similarities for the members of *Microtetraspora* appeared to vary over a wide range from 91·8 to 98·4 %. The levels of 16S rRNA sequence similarity amongst the three original species, *Microtetraspora fusca*, *Microtetraspora glauca* and *Microtetraspora niveoalba*, range from 97·9 to 98·4 %, but the levels of similarity with the species that were transferred from *Actinomadura* are much lower (91·8–95·5 %); when the intergeneric levels of sequence similarity were determined, we found high levels of similarity (93·7–96 %) amongst the three original *Microtetraspora* species and *Microbispora* species. This observation indicates heterogeneity of the genus *Microtetraspora*.

Phylogenetic analysis

Phylogenetic trees were reconstructed using both the maximum-parsimony (54) and neighbour-joining

Table 2. Levels of 16S rRNA sequence similarity

Organism	Percentage similarity																																				
	Actinomadura citrea	*Actinomadura echinospora*	*Actinomadura madurae*	*Actinomadura fulvescens*	*Microbispora aerata*	*Microbispora amethystogenes*	*Microbispora parva*	*Microbispora thermorosea*	*Microbispora diastatica*	*Microtetraspora glauca*	*Thermobispora bispora* (type II 16S)	*Thermobispora bispora* (type I 16S)	*Microtetraspora pusilla*	*Microtetraspora angiospora*	*Microtetraspora africana*	*Microtetraspora fastidiosa*	*Microtetraspora spiralis*	*Streptosporangium corrugatum*	*Streptosporangium fragile*	*Streptosporangium longisporum*	*Streptosporangium nondiastaticum*	*Streptosporangium roseum*	*Streptosporangium vulgare*	*Streptosporangium violaceochromogenes*	*Nocardiopsis alborubidus*	*Nocardiopsis albus* subsp. *albus*	*Nocardiopsis antarcticus*	*Nocardiopsis dassonvillei*	*Nocardiopsis listeri*	*Thermomonospora chromogena*	*Thermomonospora curvata*	*Thermomonospora fusca*	*Thermomonospora alba*	*Thermomonospora formosensis*	*Thermomonospora mesophila*	*Thermomonospora mesouviformis*
Actinomadura atramentaria	93.3	92.6	93.5	93.1	89.6	90.1	89.9	88.9	89.1	89.3	78.1	78.8	89.6	88.3	89.4	88.4	88.8	88.4	88.9	89.2	88.1	89.1	90.1	88.8	86.4	86.7	86.4	86.4	86.2	86.4	92.0	87.5	87.5	94.0	89.1	88.6
Actinomadura citrea		93.5	96.0	95.2	89.6	89.3	89.1	88.2	88.8	89.6	78.0	77.9	89.6	88.5	89.5	88.7	87.4	88.9	87.7	88.6	87.8	88.3	88.8	87.4	85.7	85.5	85.8	85.8	85.5	86.9	91.2	87.0	87.1	96.0	88.8	87.2
Actinomadura echinospora			94.5	94.1	91.3	91.3	91.3	90.1	91.4	91.4	80.9	78.4	90.9	89.6	91.6	90.4	89.7	91.0	89.6	89.8	90.5	90.8	90.3	87.4	88.2	88.5	88.2	88.3	87.7	84.4	92.6	89.6	88.7	92.9	89.1	89.4
Actinomadura madurae				93.9	90.7	90.6	90.1	89.5	89.5	89.9	83.1	82.5	88.4	88.4	89.9	89.7	88.5	89.4	88.5	89.0	88.3	89.1	88.8	86.2	84.5	86.5	84.6	84.6	86.1	82.9	91.4	87.6	87.2	94.5	88.7	87.7
Actinomadura fulvescens					89.8	90.2	90.5	88.4	90.0	90.7	78.7	82.0	90.4	90.5	91.4	89.9	90.1	89.8	88.7	89.4	89.2	89.5	89.6	88.6	86.1	85.7	86.1	86.1	86.0	82.9	90.3	87.1	86.7	94.2	89.5	87.6
Microbispora aerata						96.8	97.7	97.7	96.3	95.3	86.6	83.8	92.6	92.3	92.6	93.6	91.9	94.5	91.2	91.5	91.5	91.7	92.2	91.3	86.2	86.7	86.3	86.3	87.0	91.2	89.0	87.4	86.7	90.0	94.3	87.6
Microbispora amethystogenes							98.4	95.1	97.8	96.1	85.5	82.8	93.1	92.8	93.4	92.6	93.0	93.7	90.7	90.7	90.7	91.7	91.8	90.9	85.9	86.1	85.9	85.9	86.2	89.8	89.0	87.1	87.1	89.8	94.4	87.6
Microbispora parva								96.1	97.7	95.8	85.9	82.6	93.1	92.8	93.1	92.5	93.0	94.1	91.4	91.3	90.8	91.4	92.1	91.3	86.3	86.8	86.4	86.4	87.1	90.2	89.2	87.5	87.4	90.0	94.6	88.1
Microbispora thermorosea									96.1	94.5	81.1	83.0	92.6	91.8	91.4	93.0	91.8	93.0	91.4	91.0	91.2	90.0	90.9	89.0	85.6	86.4	85.6	85.6	86.6	90.6	89.1	87.0	86.1	88.9	93.8	87.1
Microbispora diastatica										96.2	86.1	83.4	93.7	93.8	93.4	92.6	94.0	93.4	92.1	91.4	91.7	90.7	91.3	91.1	86.3	87.0	86.4	86.4	86.7	90.0	89.6	87.7	87.4	89.6	93.8	87.9
Microtetraspora glauca											80.3	82.8	94.7	93.9	94.3	92.1	93.9	93.5	91.9	92.7	91.9	91.6	92.9	92.3	85.1	85.5	85.2	85.2	85.9	90.3	88.7	86.9	86.1	90.4	92.7	86.6
Thermobispora bispora (type I 16S)												89.8	83.8	79.2	78.8	84.8	78.6	84.1	79.2	78.1	78.9	79.4	78.2	76.5	80.0	79.9	80.1	80.1	79.1	86.9	78.5	82.2	81.6	78.2	80.1	82.1
Thermobispora bispora (type II 16S)													82.2	77.9	78.1	82.6	77.2	81.9	80.5	82.3	81.4	81.7	82.5	81.7	77.1	77.1	77.1	77.1	77.1	85.3	79.4	78.0	77.6	82.8	82.3	77.9
Microtetraspora pusilla														94.6	93.6	93.1	94.5	92.8	93.3	92.3	92.8	91.9	93.1	91.9	85.8	85.9	85.9	85.9	86.0	89.1	88.9	87.1	86.7	89.7	92.0	87.2
Microtetraspora angiospora															96.5	94.3	96.2	91.3	91.6	91.2	91.2	90.1	91.6	91.1	86.0	85.7	86.0	86.0	85.7	83.7	87.4	85.7	86.0	88.9	90.8	86.1
Microtetraspora africana																93.6	96.0	92.2	92.6	93.3	92.6	92.4	93.8	92.4	86.7	87.0	86.8	86.8	86.9	85.5	88.6	87.7	87.0	90.4	90.2	87.3
Microtetraspora fastidiosa																	94.7	91.6	90.2	90.3	90.5	90.0	90.0	88.2	85.6	86.2	85.6	85.6	86.0	89.0	87.9	86.7	86.7	88.7	91.9	86.7
Microtetraspora spiralis																		91.4	91.6	91.2	91.5	90.7	91.5	91.2	85.4	86.5	85.4	85.4	86.1	83.7	88.1	86.4	86.4	88.4	91.1	86.7
Streptosporangium corrugatum																			86.9	92.7	94.2	94.6	93.3	90.6	86.5	86.9	86.6	86.6	86.9	84.5	88.1	87.1	86.2	89.3	92.4	86.7
Streptosporangium fragile																				96.7	96.0	95.5	96.7	95.6	87.3	87.5	87.3	87.3	81.1	87.9	88.4	82.1	86.0	89.2	90.7	81.6
Streptosporangium longisporum																					95.2	96.0	98.2	95.3	86.3	87.0	86.4	86.4	87.1	89.3	88.1	87.0	85.9	89.4	90.7	86.9
Streptosporangium nondiastaticum																						96.9	95.2	92.6	86.7	87.5	86.8	86.8	87.0	88.3	87.6	86.7	86.3	88.6	90.3	86.6
Streptosporangium roseum																							96.9	92.3	87.1	87.4	87.1	87.1	87.1	88.2	87.9	87.4	86.1	88.8	90.3	86.7
Streptosporangium vulgare																								95.3	87.1	87.1	87.2	87.2	87.5	89.6	88.4	87.4	86.3	90.5	91.2	87.4
Streptosporangium violaceochromogenes																									85.7	86.1	85.8	85.8	87.0	88.0	87.5	86.7	85.7	89.0	90.6	86.6
Nocardiopsis alborubidus																										97.5	99.7	99.4	96.7	84.6	88.4	92.5	91.0	86.7	86.4	91.7
Nocardiopsis albus subsp. *albus*																											97.7	97.6	97.5	84.6	88.9	92.6	91.9	86.6	86.7	92.6
Nocardiopsis antarcticus																												99.5	96.9	84.6	88.5	92.6	91.3	86.8	86.5	92.1
Nocardiopsis dassonvillei																													96.8	84.6	88.5	92.6	91.4	86.8	86.5	92.1
Nocardiopsis listeri																														85.0	87.4	91.9	91.4	86.8	86.5	92.0
Thermomonospora chromogena																															87.9	86.2	86.0	88.6	89.5	86.2
Thermomonospora curvata																																89.9	88.9	93.3	90.1	89.4
Thermomonospora fusca																																	96.1	88.5	85.7	96.7
Thermomonospora alba																																		88.3	86.9	98.7
Thermomonospora formosensis																																			89.8	88.8
Thermomonospora mesophila																																				87.1
Thermomonospora mesouviformis																																				

methods (48) and the confidence levels of the tree topology were determined using the bootstrap method (15, 54). The trees reconstructed by the two methods are very similar except that *Thermomonospora chromogena* forms a clade with the two types of 16S rRNA sequences from *Thermobispora bispora* Wang *et al*. 1997 (59) in the maximum-parsimony tree, but forms a single species cluster in the neighbour-joining tree. Here, only the neighbour-joining tree is presented (Fig. 1). Species of the suborder *Streptosporangineae* were separated into three main clades, represented, respectively, by members of three families *Nocardiopsaceae*, *Thermomonosporaceae* and *Streptosporangiaceae* and eight subclades designated I–VIII for the convenience of discussion.

Thermomonospora species are shown to affiliate with several distinct groups of actinomycetes, a result in good agreement with that of the pairwise sequence similarity comparison described above. *Thermomonospora curvata*, the type species, and *Thermomonospora formosensis* are intermixed with *Actinomadura* species, *Thermomonospora curvata* exhibiting the closest relationship with *Actinomadura echinospora* and *Thermomonospora formosensis* forming a tight clade with eight other *Actinomadura* species. *Thermomonospora fusca*, *Thermomonospora alba* and *Thermomonospora mesouviformis* make up a distinct clade with a 100% bootstrap stability. These three species form a supregeneric clade with the clade comprising members of the genus *Nocardiopsis*. The aggregation of these two clades is supported by a high bootstrap value of 1000. *Thermomonospora mesophila* is enclosed in the clade of eight strains of *Microbispora*. The phylogenetic position of *Thermomonospora chromogena* is unclear. Though in the maximum-parsimony tree it aggregated with the two 16S rRNA sequences of *Thermobispora bispora* supported by a bootstrap value of 85% (tree not shown), the level of 16S rRNA sequence similarity ($< 87\%$) between the two species seems too low to substantiate a close relationship.

The genus *Microtetraspora* was separated into two distinct clades, one containing the three original species of *Microtetraspora* and the other containing species of the *Microtetraspora pusilla* group which were transferred from *Actinomadura* (21). This result has been observed before (57) and has apparently remained the same even with the addition of many more sequences from the genera *Microbispora*, *Streptosporangium*, *Planobispora*, *Planomonospora* and *Planotetraspora*. The phylogenetic stability of these two clades is supported by high bootstrap values ($> 80\%$).

DISCUSSION

The results of the pairwise sequence similarity and phylogenetic analyses provide unambiguous evidence for high heterogeneity of *Thermomonospora*. The close relatedness amongst different *Thermomonospora* species and members of several distinct groups of actinomycetes are in excellent agreement with the results of previous numerical and chemotaxonomic studies (11, 20, 22, 29). It is evident that *Thermomonospora* species can be reclassified into at least three phylogenetically distinct groups.

First, *Thermomonospora formosensis* is virtually indistinguishable from *Actinomadura* in main chemotaxonomic properties (7, 20, 21, 22) and by 16S rRNA sequence-based analysis (this study). The high level of 16S sequence similarity of *Thermomonospora formosensis* to and its intermixing with many members of *Actinomadura* together with their almost identical chemotaxonomic properties should justify the transfer of this species to the genus *Actinomadura*. The taxonomic position of *Thermomonospora curvata*, the type species of *Thermomonospora*, is less certain, mainly because clade VI, where *Thermomonospora curvata* is located, contains species not only from the genus *Actinomadura*, but also from several other genera such as *Actinocorallia herbida*, *Excellospora viridilutea* and *Spirillospora albida*, and their positions in the tree are apparently intermixed with *Actinomadura* species. *Thermomonospora curvata* does not seem to aggregate stably with any other species. Chemotaxonomically, it does not contain the diagnostic sugar madurose which distinguishes it from *Actinomadura* species. Taken together, the exact phylogenetic position of *Thermomonospora curvata* and its relationship with other genera embraced in clade VI should be a subject of further investigation.

Second, *Thermomonospora mesophila* cannot be differentiated from *Microbispora* species either by chemotaxonomic properties (8, 20, 22, 29) or by 16S rRNA sequences. Though *Thermomonospora mesophila* was thought to be related to the revised genus *Microtetraspora* (20), this does not disagree with our result because *Microtetraspora* and *Microbispora* have nearly identical chemotaxonomic properties and a very close phylogenetic relationship (21, 22, 34, 35, 57).

Third, our phylogenetic analysis demonstrates that *Thermomonospora fusca*, *Thermomonospora alba* and *Thermomonospora mesouviformis* form a coherent clade aggregating closely with the clade of *Nocardiopsis* species but distant from other *Thermomonospora* species. This observation may explain the contradictory reports regarding the relationship between *Thermomonospora* and *Nocardiopsis* on the basis of 16S rRNA sequence analysis (9, 46, 50). The close phylogenetic relationship amongst the three *Thermomonospora* species and *Nocardiopsis* is further supported by their sharing of a menaquinone with unusually long partially saturated isoprenyl side chains and a preference for alkaline growth conditions (29, 30, 36).

Although *Thermomonospora chromogena* is phylogenetically distant from other members of the family *Streptosporangiaceae*, they are very similar in chemotaxonomic properties (8, 20). Therefore, the taxonomic position of *Thermomonospora chromogena* remains uncertain.

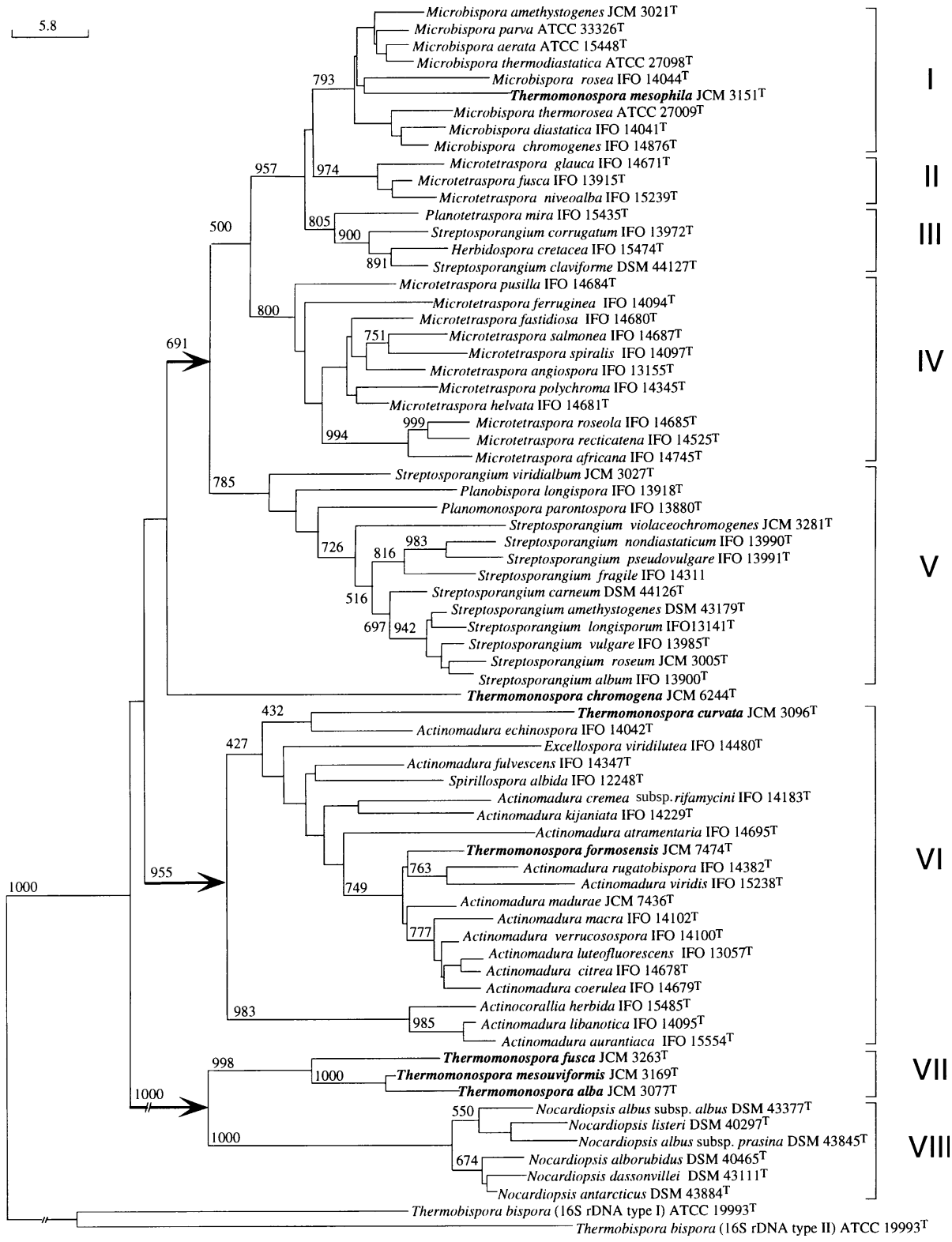

Fig. 1. Unrooted neighbour-joining tree for actinomycetes of the suborder *Streptosporangineae*. The numbers at the nodes are bootstrap values based on 1000 re-samplings. The bar represents the number of inferred substitutions per 1000 nt. The arrows point to the three main clades representing three families *Nocardiopsaceae*, *Thermomonosporaceae* and *Streptosporangiaceae*. The roman numbers denote eight subclades. All the sequences of *Thermomonospora* species were determined in this study and the rest of the sequences were retrieved from the GenBank and EMBL databases. DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

The phylogenetic separation of *Microtetraspora* species into two distinct clades observed in our previous study (57, 58) was again demonstrated here. The three original *Microtetraspora* species *Microtetraspora glauca*, *Microtetraspora fusca* and *Microtetraspora niveoalba* and the species transferred from the previous *Actinomadura pusilla* group form two distinct clades with high bootstrap values. Taken together, all the evidence from this and other studies (as described in the Introduction) suggests that sufficient data are available for reclassification of the genus *Microtetraspora*.

Herbidospora cretacea, *Planotetraspora mira*, *Streptosporangium claviforme* and *Streptosporangium corrugatum* appear to be closely related to each another as shown by 16S rDNA sequence similarities ranging from 94·3 to 98·2% and by formation of a clade with significant stability (bootstrap value 805). However, the phylogenetic relatedness does not agree with the results of chemotaxonomic analysis. Each of the four species seems to have some distinct chemotaxonomic characteristics (23, 47, 51). Therefore, further taxonomic studies, especially DNA–DNA reassociation, are required to resolve the relationship between these species.

On the basis of phylogenetic, chemotaxonomic and phenotypic evidence, we propose the following reclassification of *Thermomonospora* species: first, transfer of *Thermomonospora formosensis* Hasegawa *et al*. 1986 to the genus *Actinomadura* as *Actinomadura formosensis* (Hasegawa *et al*. 1986) comb. nov.; second, transfer of *Thermomonospora mesophila* Nonomura and Ohara 1971 to the genus *Microbispora* as *Microbispora mesophila* (Nonomura and Ohara 1971) comb. nov.; third, transfer of *Thermomonospora fusca* [(*ex* Henssen 1957) McCarthy and Cross 1984] and *Thermomonospora alba* (Locci *et al*. 1967) Cross and Goodfellow 1973 to the new genus *Thermobifida* gen. nov. as *Thermobifida fusca* (McCarthy and Cross 1984) comb. nov. and *Thermobifida alba* (Locci *et al*. 1967) comb. nov. Since *Thermomonospora alba* was validated before *Thermomonospora fusca* (13), *Thermobifida alba* should be designated the type species of the new genus *Thermobifida*. Now the genus *Thermomonospora* only contains the type species *Thermomonospora curvata*.

We also propose to reclassify the genus *Microtetraspora* by transferring the species of *Microtetraspora pusilla* group to the new genus *Nonomuria* gen. nov. as follows: *Microtetraspora africana* (Preobrazhenskaya and Sveshnikova 1974) Kroppenstedt *et al*. 1990 as *Nonomuria africana* (Preobrazhenskaya and Sveshnikova 1974) comb. nov.; *Microtetraspora angiospora* (Zhukova *et al*. 1968) Kroppenstedt *et al*. 1990 as *Nonomuria angiospora* (Zhukova *et al*. 1968) comb. nov.; *Microtetraspora fastidiosa* (Soina *et al*. 1975) Kroppenstedt *et al*. 1990 as *Nonomuria fastidiosa* (Soina *et al*. 1975) comb. nov.; *Microtetraspora ferruginea* (Meyer 1979) Kroppenstedt *et al*. 1990 as *Nonomuria ferruginea* (Meyer 1979) comb. nov.; *Microtetraspora flexuosa* [(Krassilnikov and Agre 1964) Meyer 1989] Kroppenstedt *et al*. 1990 as *Nonomuria flexuosa* (Krassilnikov and Agre 1964) comb. nov.; *Microtetraspora helvata* (Nonomura and Ohara 1971) Kroppenstedt *et al*. 1990 as *Nonomuria helvata* (Nonomura and Ohara 1971) comb. nov.; *Microtetraspora polychroma* (Galatenko *et al*. 1981) Kroppenstedt *et al*. 1990 as *Nonomuria polychroma* (Galatenko *et al*. 1981) comb. nov.; *Microtetraspora pusilla* (Nonomura and Ohara 1971) Kroppenstedt *et al*. 1990 as *Nonomuria pusilla* (Nonomura and Ohara 1971) comb. nov.; *Microtetraspora recticatena* (Gauze *et al*. 1984) Kroppenstedt *et al*. 1990 as *Nonomuria recticatena* (Gauze *et al*. 1984) comb. nov.; *Microtetraspora roseola* (Lavrova and Preobrazhenskaya 1975) Kroppenstedt *et al*. 1990 as *Nonomuria roseola* (Lavrova and Preobrazhenskaya 1975) comb. nov.; *Microtetraspora roseoviolacea* (Nonomura and Ohara 1971) Kroppenstedt *et al*. 1990 as *Nonomuria roseoviolacea* (Nonomura and Ohara 1971) comb. nov.; *Microtetraspora rubra* (Sveshnikova *et al*. 1969) Kroppenstedt *et al*. 1990 as *Nonomuria rubra* (Sveshnikova *et al*. 1969) comb. nov.; *Microtetraspora salmonea* (Preobrazhenskaya *et al*. 1977) Kroppenstedt *et al*. 1990 as *Nonomuria salmonea* (Preobrazhenskaya *et al*. 1977) comb. nov.; *Microtetraspora spiralis* (Meyer 1979) Kroppenstedt *et al*. 1990 as *Nonomuria spiralis* (Meyer 1979) comb. nov.; *Microtetraspora turkmeniaca* (Terekhova *et al*. 1982) Kroppenstedt *et al*. 1990 as *Nonomuria turkmeniaca* (Terekhova *et al*. 1982) comb. nov. The transfer proposed will leave the three original species, *Microtetraspora glauca*, *Microtetraspora fusca* and *Microtetraspora niveoalba*, in the genus *Microtetraspora*.

Description of *Thermobifida* gen. nov.

Thermobifida [Ther.mo.bi.fi.da. Gr. adj. *thermos* hot, warm; Gr. adj. *bifida* cleft; M.L. fem. n. *Thermobifida* the heat (-loving) cleft (sporophores)].

The emended description of *Thermobifida* is based on the data from previous studies by Henssen (13, 14) and McCarthy & Cross (29, 30).

Substrate mycelium is extensively branched with non-fragmenting hyphae. Aerial mycelium may be branched and of variable abundance. Single spores, oval to round (0·5–2·0 μm in diameter) are borne on dichotomously branched sporophores, resulting in spore clusters on aerial mycelium and sometimes on substrate mycelium. Surface of spores is smooth. Spores are heat-sensitive. Organisms are Gram-positive, non-acid-fast, chemo-organotrophic and aerobic. Cultures can grow at 35–60 °C and pH 7–9. Cell wall contains *meso*-DAP (cell wall type III). A trace amount of LL-DAP may be detected in whole-cell hydrolysates. Sugar pattern is type C (no diagnostic sugar). Predominant menaquinones are MK-10(H_6), -10(H_8),

-11(H_6). Phospholipid pattern is type II (PE, PME, GL) and the fatty acid pattern is type 3e (10 methyl-17:0- and iso-16:0-branched fatty acids are predominant). Mycolic acids are absent. Habitats are soil, manures, composts and overheated fodders. Phylogenetic analysis reveals that *Thermobifida* is related to the family *Nocardiopsaceae* rather than *Thermomonosporaceae*. Type species of the genus is *Thermobifida alba* (Locci *et al*. 1967) comb. nov.

Description of *Thermobifida alba* (Locci *et al.* 1967) comb. nov.

Pale yellowish substrate mycelium and white aerial mycelium. Single spores on dichotomously branched or unbranched sporophores on aerial mycelium. Optimum temperature for growth and sporulation is 40–45 °C. Chemotaxonomic properties are the same as those given for the genus above. Type strain is JCM 3077^T.

Description of *Thermobifida fusca* (McCarthy and Cross 1984) comb. nov.

Morphological features are the same as those described for *Thermomonospora fusca* (*ex* Henssen 1957) McCarthy and Cross 1984 (13, 30). Chemotaxonomic properties are similar to those given for the genus above. Type strain is JCM 3263^T (= IFO 14071^T, ATCC 27730^T).

Emendation of the family *Nocardiopsaceae* (Rainey *et al.* 1996)

Since phylogenetic evidence alone has been used previously to allocate phenotypically diverse genera of actinomycetes into one family (16, 46), it is appropriate to transfer *Thermobifida* into the family *Nocardiopsaceae* on the same basis. *Nocardiopsis* and *Thermobifida* are morphologically and chemotaxonomically different. However, the 16S rRNA sequence-based phylogenetic analysis showed that they formed a distinct clade in the suborder *Streptosporangineae* (52). The description below is based on the descriptions from previous investigations (4, 19, 31, 46).

Genus *Nocardiopsis* describes aerobic, Gram-positive, non-acid-fast organisms that form fragmenting and branched substrate mycelium 0·5–0·8 μm in diameter. Fragmentation into coccoid and bacillary elements may occur. Aerial mycelium is well developed and abundant; hyphae are long, branched, straight flexuous or irregularly zagged. Hyphae may fragment completely into spores of various length. Spore surface is smooth. Members of genus *Thermobifida* form single, heat-sensitive, non-motile spores on dichotomously branched sporophores resulting in spore clusters on aerial hyphae. Spores may also be produced on substrate mycelium. Substrate mycelium is composed of extensively branched non-fragmenting hyphae. Spores are oval to round and 0·5–2 μm in diameter. Wall peptidoglycan contains *meso*-DAP with no diagnostic sugars. Mycolic acid is absent. Phospholipid types, menaquinone profiles and fatty acid types are heterogeneous. Phospholipid pattern type III (PC, PME, GL), menaquinone pattern type 4c [MK-10(H_2), -10(H_4), -10(H_6)] and fatty acid pattern type 3d are found in members of *Nocardiopsis*, but *Thermobifida* species contain phospholipid pattern type II (PE, PME, GL), menaquinone pattern type 4d [MK-10(H_6), -10(H_8), -11(H_6)] and fatty acid pattern type 3e. Growth temperature is mesophilic, except for *Thermobifida* which can grow in the temperature range 35–60 °C. G + C content is 64–69 mol % (T_m) in strains of *Nocardiopsis*. Type genus is *Nocardiopsis* Meyer 1976 (31).

Description of *Microbispora mesophila* (Nonomura and Ohara 1971) comb. nov.

The description is identical to the *Thermomonospora mesophila* phenotype given by Nonomura & Ohara (1971) (38) and chemotype given by Kroppenstedt & Goodfellow (1992) (20). Type strain is JCM 3151^T.

Emendation of *Microbispora* (Nonomura and Ohara 1957)

All the descriptions are similar to that given by Nonomura & Ohara (1957) (37) and Miyadoh *et al.* (1990) (34) except for a change in the morphology by having one spore in the species of *Microbispora mesophila*.

Description of *Actinomadura formosensis* (Hasegawa *et al.* 1986) comb. nov.

The description of this species is similar to the phenotypic description given by Hasegawa *et al.* (1986) (12) and chemotypic description given by Kroppenstedt & Goodfellow (1992) (20). Type strain is JCM 7474^T.

Emendation of *Thermomonospora* (Henssen 1957)

The description is taken from Henssen (1957) (13), McCarthy & Cross (1984) (29), Kroppenstedt *et al.* (1990) (21) and Kudo (1997) (22). Branched substrate and aerial mycelia are produced. Single spores are borne at the tips of short sporophores branching from aerial or substrate mycelium. Optimum temperature for growth is 45–55 °C. Cell wall contains *meso*-DAP (type III) and sugar pattern C. Predominant menaquinones are MK-9(H_4), -9(H_6) and -9(H_8). Phospholipids are type I (PIM, PI, PG, DPG). Fatty acid pattern is type 3a. Strains of this genus are closely related to strains of the genus *Actinomadura* on the basis of 16S rRNA gene sequence analysis. Type species is *Thermomonospora curvata* and type strain is JCM 3096^T.

Description of *Nonomuria* gen. nov.

Nonomuria (No.no.mu.ri.a. M.L. fem. n. *Nonomuria* after H. Nonomura, a Japanese taxonomist of actinomycetes).

According to chemical criteria and 16S rRNA oligonucleotide sequence data of the *Microtetraspora pusilla* group, Goodfellow, Stackebrandt & Kroppenstedt proposed a new genus, *Nonomuria*, in 1988 to accommodate species of the *Actinomadura pusilla* group (10), but it was not formally published. Based on earlier investigations (1, 21, 34, 35, 41, 42, 43, 57) and our phylogenetic analysis, the *Microtetraspora pusilla* group (clade IV) was found to be distinct from the three original *Microtetraspora* species (clade II), which includes the type species *Microtetraspora glauca*, and should be considered as a new genus. The description of *Nonomuria* presented here is taken from this and previous studies (7, 10, 21, 35).

Aerobic, Gram-positive, non-acid-fast, extensively branched substrate and aerial mycelium. Aerial mycelia bear chains of spores which are hooked, spiral or straight. Spore surface folded, irregular, smooth or warty. Growth temperature ranges from 20 to 45 °C, in some cases up to 55 °C. Cell wall contains *meso*-DAP and the whole-cell hydrolysate contains madurose as the diagnostic sugar (cell wall type III/B). Predominant menaquinones are MK-9(H_4), -9(H_2) and -9(H_0). Phospholipids are type IV (PE, DPG, NPG, OH-PE). Major types of fatty acids are 10 methyl-17- and iso-16-branched fatty acids (in some cases the amount of the former is more than the latter). G+C content ranges from 64 to 69 mol%. Analysis of 16S rRNA gene sequences showed that this genus belongs to the family *Streptosporangiaceae*. Type species is *Nonomuria pusilla* (Nonomura and Ohara 1971) comb. nov.

Description of *Nonomuria pusilla* (Nonomura and Ohara 1971) comb. nov.

The description of *Nonomuria pusilla* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Nonomura and Ohara 1971) is the same as that given by Nonomura & Ohara (1971) (39) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14684^T.

Description of *Nonomuria africana* (Preobrazhenskaya and Sveshnikova 1974) comb. nov.

The description of *Nonomuria africana* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Preobrazhenskaya and Sveshnikova 1974) is the same as that given by Preobrazhenskaya & Sveshnikova (1974) (44) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14745^T.

Description of *Nonomuria angiospora* (Zhukova *et al.* 1968) comb. nov.

The description of *Nonomuria angiospora* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Micropolyspora* Zhukova *et al.* 1968) is the same as that given by Zhukova *et al.* (1968) (63) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 13155^T.

Description of *Nonomuria fastidiosa* (Soina *et al.* 1975) comb. nov.

The description of *Nonomuria fastidiosa* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Soina *et al.* 1975) is the same as that given by Soina *et al.* (1975) (49) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14680^T.

Description of *Nonomuria ferruginea* (Meyer 1979) comb. nov.

The description of *Nonomuria ferruginea* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Meyer 1979) is the same as that given by Meyer (1979) (32) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14094^T.

Description of *Nonomuria helvata* (Nonomura and Ohara 1971) comb. nov.

The description of *Nonomuria helvata* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Nonomura and Ohara 1971) is the same as that given by Nonomura & Ohara (1971) (39) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14681^T.

Description of *Nonomuria polychroma* (Galatenko *et al.* 1981) comb. nov.

The description of *Nonomuria polychroma* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Galatenko *et al.* 1981) is the same as that given by Galatenko *et al.* (1981) (5) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14345^T.

Description of *Nonomuria recticatena* (Gauze *et al.* 1984) comb. nov.

The description of *Nonomuria recticatena* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Gauze *et al.* 1984) is the same as that given by Gauze *et al.* (1984) (6) for phenotypic characteristics and by

Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14525^T.

Description of *Nonomuria roseola* (Lavrova and Preobrazhenskaya 1975) comb. nov.

The description of *Nonomuria roseola* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Lavrova and Preobrazhenskaya 1975) is the same as that given by Lavrova & Preobrazhenskaya (1975) (24) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14685^T.

Description of *Nonomuria salmonea* (Preobrazhenskaya *et al.* 1977) comb. nov.

The description of *Nonomuria salmonea* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Preobrazhenskaya *et al.* 1977) is the same as that given by Preobrazhenskaya *et al.* (1977) (45) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14687^T.

Description of *Nonomuria spiralis* (Meyer 1979) comb. nov.

The description of *Nonomuria spiralis* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Meyer 1979) is the same as that given by Meyer (1979) (32) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is IFO 14097^T.

Although the 16S rRNA sequences of the following species were not determined in this study, on the basis of chemotaxonomic characteristics reported previously, these species should also be transferred to the genus *Nonomuria*.

Description of *Nonomuria flexuosa* (Krassilnikov and Agre 1964) comb. nov.

The description of *Nonomuria flexuosa* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Thermopolyspora* Krassilnikov and Agre 1964) is the same as that given by Krassilnikov & Agre (1964) (17) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is DSM 43186^T.

Description of *Nonomuria roseoviolacea* (Nonomura and Ohara 1971) comb. nov.

The description of *Nonomuria roseoviolacea* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Nonomura and Ohara 1971) is the same as that given by Nonomura & Ohara (1971) (39) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is DSM 43144^T.

Description of *Nonomuria rubra* (Sveshnikova *et al.* 1969) comb. nov.

The description of *Nonomuria rubra* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Mayer and Sveshnikova 1974; *Micromonospora* Sveshnikova *et al.* 1969) is the same as that given by Sveshnikova *et al.* (1969) (53) and Meyer & Sveshnikova (1974) (33) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is DSM 43768^T.

Description of *Nonomuria turkmeniaca* (Terekhova *et al.* 1982) comb. nov.

The description of *Nonomuria turkmeniaca* (*Microtetraspora* Kroppenstedt *et al.* 1990; *Actinomadura* Terekhova *et al.* 1982) is the same as that given by Terekhova *et al.* (1982) (55) for phenotypic characteristics and by Kroppenstedt *et al.* (1990) (21) for chemotaxonomic properties. Type strain is DSM 43926^T.

Emendation of *Microtetraspora* (Thiemann *et al.* 1968) Kroppenstedt *et al.* 1990

The description given below is taken from several investigations (7, 21, 35, 56). Aerobic, Gram-positive, non-acid-fast, alcohol-fast, mesophilic actinomycete that forms a stable, highly branched substrate mycelium. Short aerial hyphae typically contain chains of four spores; in some instances chains with only two or three spores are formed and very rarely chains of five spores. Spores are smooth, spherical to slightly oval and non-motile. Optimum temperature for growth is 20–37 °C, but not 40 °C. Cell wall is type III/B or C. Predominant menaquinone is MK-9(H_4). Phospholipid pattern is type IV. Both iso-16:0- and 10 methyl-17:0-branched fatty acids are presented as predominant fatty acids, but iso-16:0 fatty acids are much more abundant than 10 methyl-17:0 fatty acids in the cell. G+C content is 66 mol%. 16S rRNA sequence analysis indicates that *Microtetraspora* clusters with *Microbispora* belonging to the family *Streptosporangiaceae*.

ACKNOWLEDGEMENTS

This work was supported by the Institute of Molecular and Cell Biology, National University of Singapore. We thank Dr Shijin Miyadoh for critically reading the manuscript and suggestions.

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