Reclassification of *Thermomonospora* and *Microtetraspora*

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Almost complete 16S rRNA sequences from seven *Thermomonospora* strains, *Thermomonospora curvata*, *Thermomonospora formosensis*, *Thermomonospora fusca*, *Thermomonospora mesophilica*, *Thermomonospora mesoviformis* (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 hrmosensis* 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 roseovioleacea* 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 mesophilica* (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 re-located 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 *Thermobifida* gen. nov.
Thermomonospora species used in this study

<table>
<thead>
<tr>
<th>Organism</th>
<th>Source</th>
<th>16S rDNA sequence GenBank accession no.</th>
</tr>
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<tbody>
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<td>AF002260</td>
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<tr>
<td>Thermomonospora chromoxygena</td>
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<td>AF002261</td>
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<td></td>
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<td>Thermomonospora curvata</td>
<td>JCM 3096</td>
<td>AF002262</td>
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<tr>
<td>Thermomonospora formosensis</td>
<td>JCM 7474</td>
<td>AF002263</td>
</tr>
<tr>
<td>Thermomonospora fusca</td>
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<td>AF002264</td>
</tr>
<tr>
<td></td>
<td>ATCC 27730</td>
<td></td>
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<td></td>
<td>JCM 3262</td>
<td></td>
</tr>
<tr>
<td>Thermomonospora mesophila</td>
<td>JCM 3151</td>
<td>AF002265</td>
</tr>
<tr>
<td>Thermomonospora mesouviformis</td>
<td>JCM 3169</td>
<td>AF002266</td>
</tr>
</tbody>
</table>

Thermomonospora 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 chromoxygena 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 3e); 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 chromoxygena 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 -6.5 to -5.0, while most of the members of the Microtetraspora pusilla group had relative electrophoretic mobilities ranging from -1.5 to 0.0 (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 Streptosporangiineae (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 rDNA 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 obtained 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 mesophilic and Thermomonospora mesouiformis. 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 Nocardiosis. 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 (90-1-93-9%) 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 mesouiformis 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 mesouiformis seem more closely related to Nocardiosis species with sequence similarity levels ranging from 91 to 92-6% than to other Thermomonospora species (<90%). The sequences of Thermomonospora alba and Thermomonospora mesouiformis are 98-7% identical, which supports the reduction of Thermomonospora mesouiformis as a synonym of Thermomonospora alba proposed by McCarthy & Cross (30). Thermomonospora mesophilic 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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Percentage similarity</th>
</tr>
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<tbody>
<tr>
<td>Actinomadura aramendia</td>
<td>92.6 93.5 93.9 94.1 94.3 94.7 95.1 95.7 96.1 96.7 97.1 97.4 97.5 98.0 98.3 98.6 98.8 99.0 99.3 99.6 100 100</td>
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<tr>
<td>Actinomadura citrea</td>
<td>93.9 94.3 94.7 95.1 95.3 95.7 96.1 96.3 96.7 97.1 97.4 97.7 98.0 98.3 98.6 98.8 99.0 99.3 99.6 100 100</td>
</tr>
<tr>
<td>Actinomadura ochracea</td>
<td>94.7 95.1 95.5 95.9 96.1 96.3 96.5 96.7 96.9 97.1 97.3 97.5 97.7 97.9 98.1 98.3 98.5 98.7 98.9 100 100</td>
</tr>
<tr>
<td>Actinomadura madurei</td>
<td>96.1 96.3 96.5 96.7 97.0 97.2 97.4 97.6 97.8 98.0 98.2 98.4 98.6 98.8 99.0 99.2 99.4 99.6 99.8 100 100</td>
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<tr>
<td>Actinomadura fulvensensis</td>
<td>96.8 97.0 97.2 97.4 97.6 97.8 98.0 98.2 98.4 98.6 98.8 99.0 99.2 99.4 99.6 99.8 100 100</td>
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<tr>
<td>Microbiopora araea</td>
<td>96.4 96.7 97.0 97.3 97.5 97.8 98.0 98.2 98.4 98.6 98.8 99.0 99.2 99.4 99.6 100 100</td>
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<tr>
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<td>96.7 97.0 97.3 97.5 97.8 98.0 98.2 98.4 98.6 98.8 99.0 99.2 99.4 99.6 100 100</td>
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<tr>
<td>Microbiopora parva</td>
<td>96.9 97.2 97.4 97.6 97.8 98.0 98.2 98.4 98.6 98.8 99.0 99.2 99.4 99.6 100 100</td>
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<td>Microbiopora thermoresorae</td>
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</tr>
<tr>
<td>Microbiopora rubra</td>
<td>97.4 97.6 97.8 98.0 98.2 98.4 98.6 98.8 99.0 99.2 99.4 99.6 100 100</td>
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<td>Thermobacterium saepia (type II 16S)</td>
<td>97.6 97.8 98.0 98.2 98.4 98.6 98.8 99.0 99.2 99.4 99.6 100 100</td>
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</table>

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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 chromogen* 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 *Streptosporangiaceae*, *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 mesouiformis* make up a distinct clade with a 100% bootstrap stability. These three species form a suprageneric clade with the clade comprising members of the genus *Nocardiosis*. 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 chromogen* 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 viridulenta* and *Spirillopsis 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 mesouiformis* form a coherent clade aggregating closely with the clade of *Nocardiosis* species but distant from other *Thermomonospora* species. This observation may explain the contradictory reports regarding the relationship between *Thermomonospora* and *Nocardiosis* on the basis of 16S rRNA sequence analysis (9, 46, 50). The close phylogenetic relationship amongst the three *Thermomonospora* species and *Nocardiosis* 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 chromogen* 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 chromogen* remains uncertain.
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Fig. 1. Unrooted neighbour-joining tree for actinomycetes of the suborder Streptosporangiineae. 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.
Phylogeny of Thermomonospora

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 pusilla, Microtetraspora fusca and Microtetraspora niveola 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 mesophilia Nonomura and Ohara 1971 to the genus Microbispora as Microbispora mesophilia (Nonomura and Ohara 1971) comb. nov.; third, transfer of Thermomonospora fusca [(ex Hensen 1957) McCarthy and Cross 1984] and Thermomonospora alba (Locci et al. 1967) Cross and Goodfellow 1973 to the new genus Thermobilida gen. nov. as Thermobilida fusca (McCarthy and Cross 1984) comb. nov. and Thermobilida alba (Locci et al. 1967) comb. nov. Since Thermomonospora alba was validated before Thermomonospora fusca (13), Thermobilida alba should be designated the type species of the new genus Thermobilida. Now the genus Thermomonospora only contains the type species Thermomonospora curvata.


Description of Thermobilida gen. nov.

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

The emended description of Thermobilida is based on the data from previous studies by Hensen (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 walls contain meso-DAP (cell wall type III). A trace amount of II-DAP may be detected in whole-cell hydrolysates. Sugar pattern is type C (no diagnostic sugar). Predominant menaquinones are MK-10(H₄), -10(H₄).
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 Thermononosporaceae. 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 3077T.

**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 3263T (=IFO 14071T, ATCC 27730T).

**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 Streptosporangiineae (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 zigzag. 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(H4), -10(H8), -11(H6)] 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(H4), -10(H8), -11(H6)] 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 % (Tm) in strains of Nocardiopsis. Type genus is Nocardiopsis Meyer 1976 (31).

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

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

**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 mesophilia.

**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 7474T.

**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(H4), -9(H8) and -9(H6). 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 3096T.
Phylogeny of Thermomonospora

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, 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(H₂), MK-9(H₄) and MK-9(H₄). 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 14684T.

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 14745T.

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 13155T.

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 14680T.

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 14094T.

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 14681T.

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 14345T.

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 14525T.

**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 14685T.

**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 14687T.

**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 14097T.

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 43186T.

**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 43144T.

**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 43768T.

**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 43926T.

**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(H4). Phospholipid pattern is type IV. Both iso-16:0- and 10 methyl-17:0-branched fatty acids are presented as predomi- nant 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*.

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