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Phylogenetic Inter- and Intrarelationships of the Genus *Microbispora* of the Family *Streptosporangiaceae* Based on 16S Ribosomal DNA Sequences

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The 16S rDNA sequences of nine strains, two type strains of validated *Microbispora* species and a strain of invalidated *Microbispora* species, and six soil isolates, were determined and compared with those of representatives of the family *Streptosporangiaceae*. The phylogenetic analysis indicated that all of the validated species of the genus *Microbispora* consistently formed a monophyletic unit and were well separated from the other genera of the family *Streptosporangiaceae*. All the isolates were placed to the genus *Microbispora*, whereas an invalidated *Microbispora* species, *Microbispora griseoalba* IMSNU 22049^T (= KCTC 9314^T), was closely related to members of the genus *Nocardia*.

Key words: *Microbispora*, *Streptosporangiaceae*, 16S ribosomal DNA, phylogenetic analysis

The family *Streptosporangiaceae* was emended by Stackebrandt *et. al.* [1] on the basis of the 16S rDNA sequence-based phylogenetic clustering and the proposal of the family-specific signature nucleotides, and encompasses six genera, *Herbidospora*, *Microbispora*, *Microtetraspora*, *Planobispora*, *Planomonospora*, and *Streptosporangium*. The family *Streptosporangiaceae* phylogenetically forms a distinct lineage within the radius of the class *Actinobacteria* and belongs to the suborder *Streptosporangineae* of the class *Actinobacteria* [1]. Thereafter, the three genera, *Nonomurarea* [2], *Planotetraspora* [2, 3], and *Acrocarpospora* [4] have been placed to the members of the family *Streptosporangiaceae* based on 16S rDNA sequence and phenetic data.

The genus *Microbispora* was originally proposed by Nonomura and Ohara [5] for actinomycetes that produced characteristic paired spores on the aerial mycelium and currently consists of twelve species, *Microbispora aerata*, *M. amethystogenes*, *M. chromogenes*, *M. corallia*, *M. diastatica*, *M. indica*, *M. karnatakensis*, *M. mesophila*, *M. parva*, *M. rosea*, *M. thermodiastatica*, and *M. thermorosea* [2, 6-8]. The other members of the genus *Microbispora* except for *M. corallia* [8] and *M. mesophila* [2] were combined to a single species with two subspecies (*M. rosea* subsp. *rosea* and *M. rosea* subsp. *aerata*) based on DNA-DNA hybridization studies [9]. Although the view of Miyadoh *et. al.* [9] have been validated thereafter, the recent results based on ribosomal protein [10] and 16S rDNA sequence data [2] suggest somewhat uncertainty on this viewpoint. On the other hand, three *Microbispora* species, *M. echinospora*, *M. viridis*, and *M. bispora*, which originally described as members of the genus *Microbispora*, had been transferred to *Actinomadura echinospora*, *Actinomadura rugatobispora*, and *Thermobispora bispora* on the basis of DNA-DNA hybridization data and 16S rDNA sequence data, respectively [9, 11].

The genus *Microbispora* contains actinomycete strains that form one or two spores on aerial hyphae and phylogenetically forms a coherent clade within the radiation of the family *Streptosporangiaceae* [2, 8]. The genus is chemotaxonomically characterized by the presence of a type III/B cell wall, MK-9 ($H_{0,2,4}$) as major menaquinone, a phospholipid pattern including phosphatidylcholine and an unknown glucosamine-containing phospholipid, and DNA base composition of 71.3-73 mol%. In this study, the inter- and intra-generic relationships of the genus *Microbispora* were investigated by 16S rDNA-based phylogenetic analysis. We determined the almost complete 16S rDNA sequences of nine strains of the genus *Microbispora* following the amplification and cloning of

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16S rRNA gene and compared them with the published sequences of type species included in the family *Streptosporangiaceae*.

The origin of isolates and the strains of *Microbispora* species used in this study were listed in Table 1. The isolates were retrieved from several terrestrial sites. The test strains were cultured on YD broth (yeast extract 1% and glucose 1%) with shaking. Genomic DNA was extracted and purified according to the method described previously [12] and used as template for PCR using universal primers, 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525r (5'-AAGGAGGTGWTCCARCC-3') [13], and the product of 16S rDNA was cloned into pGEM-T vector (promega). The sequences of cloned 16S rDNA were determined as described previously [14]. The sequences determined in this study were aligned with those of members of the family *Streptosporangiaceae* by using ClustalX program [15]. The sequence alignment was manually reexamined with groups of pre-aligned sequences [13]. The phylogenetic tree was reconstructed by the neighbor-joining method [16]. Evolutionary distance matrices were calculated by the method of Jukes and Cantor [17]. The stability of tree topologies was evaluated by bootstrap analysis [18] of the neighbor-joining data, using 1000 resamplings.

The 16S rDNA sequences of nine strains, two type strains of validated *Microbispora* species and a strain of invalidated species, *Microbispora griseoalba*, and six isolates, were determined and have been deposited to the GenBank database under the accession numbers listed in Table 1. To investigate phylogenetic inter- and intra-relationships of members of the genus *Microbispora*, the sequences obtained were compared with all of the validated *Microbispora* species except for *Microbispora indica* and representatives of the other genera of the family *Streptosporangiaceae*. The 16S rDNA sequence of the type strain of *Microbispora indica* was not determined due to the failure of DNA extraction from the culture broth. A total of 1335 unambiguous nucleotide positions present in all sequences between 57 and 1470 (*Escherichia coli* numbering [19] were used for final tree construction. The phylogenetic tree obtained using neighbor-joining method (Fig. 1) showed a similar topology to the previous results [2, 4]. The family *Streptosporangiaceae* was divided into two subgroups (Fig. 1), which was supported by a high bootstrap value. One subgroup encompasses four genera, the genera *Nonomurarea*, *Planobispora*, *Planomonospora*, and *Streptosporangium*, and was supported by a bootstrap value of 77%. The unique pattern of 16S rRNA consists of U-A (or C-G) nucleotide pair at positions 129-232 (*E. coli* numbering), G-C at positions 657-749, U-A at positions 658-748, A-U at positions 659-746, and U at position 747. The other subgroup encompasses five genera, the genera *Acrocarpospora*, *Herbidospora*, *Microbispora*, *Microtetraspora*, and *Planotetraspora* (A bootstrap value of 88%). A set of the unique 16S rRNA signature nucleotides contained U-G nucleotide pair at positions 129-232, G-U at positions 657-749, C-U at positions 658-748, U-A (or U-G) at positions 659-746, and U at position 747. Most of the *Streptosporangiaceae* genera formed distinct lineage and were well separated to each other, whereas some strains of genus *Streptosporangium* were placed outside the evolutionary radiation of the genus *Streptosporangium* (data not shown). *Acrocarpospora corrugata* (formerly *Streptosporangium corrugatum*) formed a cluster with *Herbidospora cretacea*, which was supported by a moderate bootstrap value (52%). These relationships were also shown on the previous results [2], suggesting the taxonomic status of these species should be further investigated using phenetic and genetic methods.

A phylogenetic analysis based on 16S rDNA sequence studies indicated that all of the strains of the genus *Microbispora* formed a distinct clade and were well

Table 1. The strains used in this study and GenBank accession numbers.

Strain	Source(s) [a]	Accession number
Strain LM 164	Korean cave	AY445640
Strain H886	Korean soil	AY445641
Strain H347	Korean soil	AY445642
Strain S19	Australian soil	AY445643
Strain S20	Australian soil	AY445644
Strain S21	Australian soil	AY445645
Microbispora chromogenes	IMSNU 20074^T (= IFO 14876^T)	AY445646
Microbispora karnatakensis	IMSNU 22065^T (= KCTC 9316^T)	AY445647
Microbispora griseoalba	IMSNU 22049^T (= KCTC 9314^T)	AY445648

[a] IMSNU, Institute of Microbiology, Seoul National University; IFO, Institute for Fermentation, Osaka; KCTC, Korean Collection for Type Cultures.

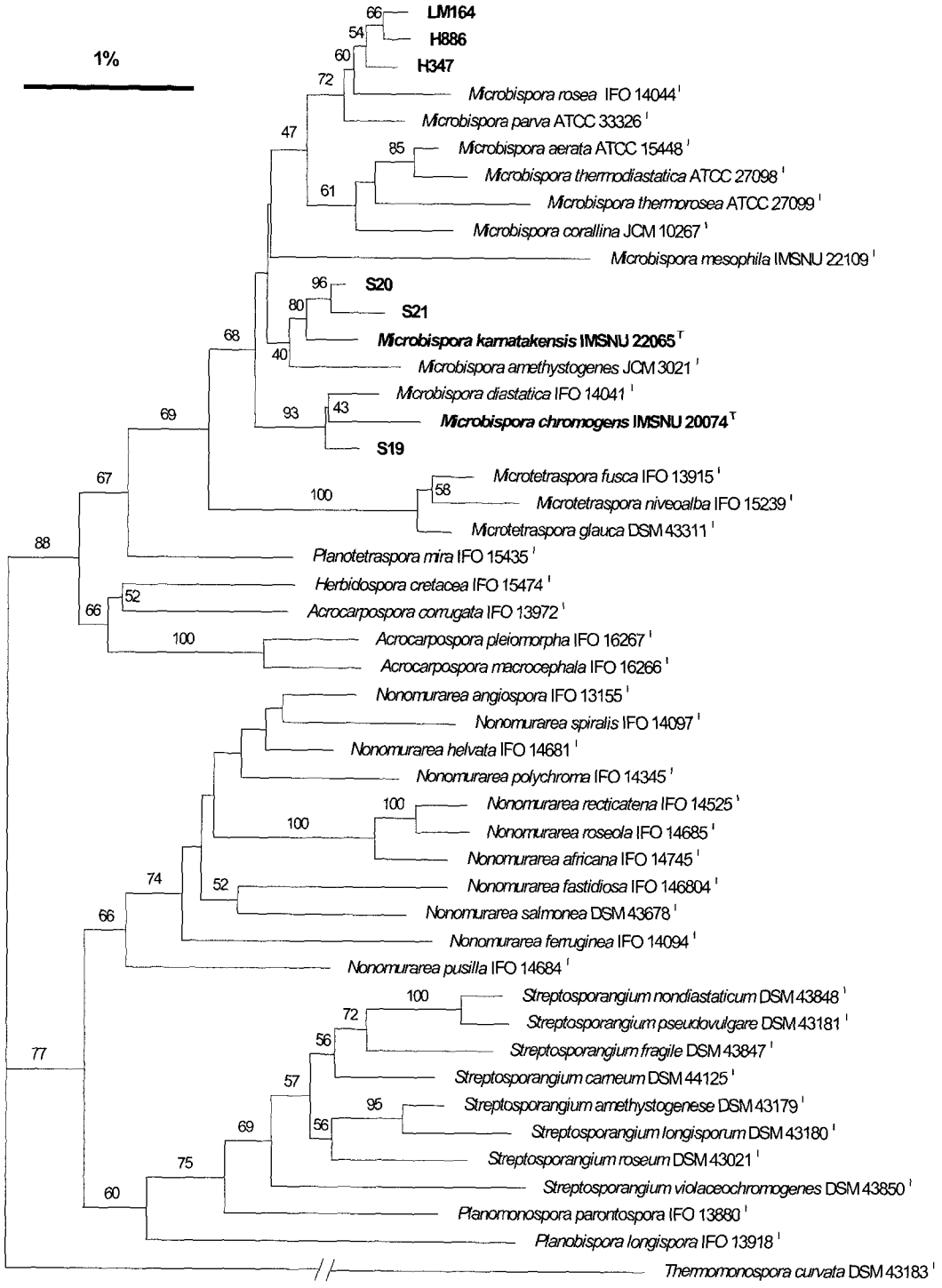

Fig. 1. Phylogenetic tree representing the placement of the *Microbispora* strains within the family *Streptosporangiaceae*, based on 1335 unambiguous nucleotide positions present in all strains. The numbers at the branching points are the percentages of occurrence in 1000 bootstrapped trees (only values greater than 40% were indicated). Bar indicates 1 nucleotide substitution per 100 nucleotides.

separated from members of the other genera of the family *Streptosporangiaceae* (Fig. 1). This relationship was supported by the bootstrap value of 68%. The validated members of the genus *Microbispora* revealed sequence similarity values of 96.9-99.6% to each other, higher than the levels of sequence similarity (94.0-98.1%) found between this genus and the other genera of the family *Streptosporangiaceae*. The monophyletic relationship of

the genus *Microbispora* is also supported by a set of 16S rDNA signature nucleotide that is same for all of the strains belonging to the *Microbispora* lineage. The unique pattern of 16S rRNA for the genus *Microbispora* consists of A-G nucleotide pair at positions 141-222 (*E. coli* numbering), G at position 262, A at position 263, U-A at positions 615-625, G-U at positions 616-624, G-U at positions 834-852, A-G at positions 997-1043, C-U at positions 998-1042, A-G at positions 999-1041, C-U at positions 1000-1040, and U at position 1031 (Table 2). A set of 16S rDNA signature nucleotides proposed in this study is thought to present some valuable information in designing genus-specific primers, which allow rapid detection of members of the genus *Microbispora* from environmental DNA or diverse isolates by means of PCR-specific amplification [20]. One invalidated *Microbispora* species, "*Microbispora griseoalba*" IMSNU 22049^T (= KCTC 9314^T) formed a distinct cluster with members of the genus *Nocardia* of the suborder *Corynebacterineae*, indicating that this strain is phylogenetically related to members of the genus *Nocardia* (data not shown).

Members of the genus *Microbispora* were divided into five clusters (Fig. 1) within the genus, and showed a somewhat different structure from that previously reported [2]. The first cluster comprised two validated species of the genus *Microbispora (M. rosea and M. parva)* and three isolates (LM 164, H347, and H887), and formed a monophyletic clade that was supported by a bootstrap value of 72%. The soil isolates, which were obtained from Korean soils, were closely related to each other (99.6±0.1% sequence similarity), whereas the similarity value of the isolates with the type strains of *M. rosea* and *M. parva* were 99.3-99.4% and 99.1-99.2%, respectively. The second cluster contained four validated *Microbispora* species, *M. aerata*, *M. corallina*, *M. thermodiastatica*, and *M. thermorosea* (a bootstrap value, 61%). The sequence similarity value between the strains ranged from 98.7% to 99.6%. The third cluster contained only one type stain of the genus *Microbispora*, *M. mesophila*, which had been transferred from the genus *Thermomonospora* based on 16S rDNA sequence [2]. The type strain showed sequence similarity values of 96.9-97.8% with other type strains of the *Microbispora* species, and was well separated from all the validated *Microbispora* species except for *Microbispora indica* whose the sequence was not determined yet. The fourth cluster consisted of two *Microbispora* species, *M. karnatakensis* and *M. amethystogenes*, and two soil isolates, S20 and S21. Although this clustering was supported by a relatively low bootstrap value of 40%, The Isolates, S20 and S21, which were obtained from Australian soil, showed sequence similarity values of 99.4±0.1% with *M. karnatakensis* and 99.0% with *M. amethystogenes*. The sequence similarity value between the isolates was 99.6%. The last cluster encompassed two validated *Microbispora* species, *M. diastatica* and *M. chromogenes*, and one isolate, S19 (Australian soil). The relationship was supported by high bootstrap value of 93%. Isolate S19 showed sequence similarity values of 99.5% and 99.3% with the type strain of *M. diastatica* and *M. chromogenes*, respectively.

In this investigation, 16S rDNA sequence analysis resulted in obtaining a molecular characterization of two validated *Microbispora* species, one invalidated *Microbispora* species, and six soil isolates. All the test strains except for an invalidated strain, *Microbispora griseoalba*, belonged to the genus *Microbispora* and formed a distinct clade within the radiation of the family *Streptosporangiaceae*, which

Table 2. 16S rRNA signature nucleotide positions that distinguish the *Microbispora* lineage from other members of the family *Streptosporangiaceae*.

Genera (no. of strains compared)	Position										
	141-222	262	263	615-625	616-624	834-852	997-1043	998-1042	999-1041	1000-1040	1031
Microbispora (17)	A-G	G	A	U-A	G-U	G-U	A-G	C-U	C-U	C-U	U
Microtetraspora (3)	A-A	A	G	G-C	G-C	G-C	A-G	C-U	A-G	C-U	C
Acrocarpospora(3)	A-G	A	G	U-G	G-U	G-C	C-G	A-U	C-G	C-G	C
Nonomuria (11)	A-G	A	G	C-G	A-U	G-C	C-G	A-U	C-G	C-G	C
Streptosporangium (10)	A-A	A	A	G-C	G-U	G-C	C-G	G-U	C-G	C-G	C
Herbidospora (1)	A-G	A	G	U-A	G-U	G-C	C-G	A-U	C-G	C-G	C
Planomonospora (1)	A-A	A	G	G-C	G-U	G-C	C-G	G-U	C-G	C-G	C
Planobispora (1)	A-G	A	G	C-G	A-U	G-C	C-G	A-U	C-G	C-G	C
Planotetraspora (1)	A-G	A	G	U-G	G-U	G-U	C-G	A-U	C-G	C-G	C

genus-specific 16S rDNA signature nucleotides were proposed for the genus *Microbispora*. *Microbispora griseoalba* IMSNU 22049^T (= KCTC 9314^T) was closely related to members of the genus *Nocardia*. In spite of high sequence similarity values, phenetic and genetic characterizations need to be tested to evaluate the taxonomic status of some isolates.

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초 록

16S Ribosomal DNA 염기서열 분석에 근거한 *Streptosporangiaceae*과 *Microbispora* 속의 계통 관계

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Microbispora 속에 소속된 미생물 9주(표준 미생물 3종과 토양 분리주 6주를 포함) 에 대하여 16S ribosomal RNA 유전자 염기서열이 결정되었다. 이들은 *Streptosporangiaceae*과의 대표균주들의 염기서열과 비교하여 *Microbispora* 속에 소속된 미생물들의 진화적 유연 관계가 조사되었다. 계통분석 결과 *Microbispora* 속의 모든 표준종들은 일관성 있게 단계통 단위를 형성하였으며 *Streptosporangiaceae*과의 다른 속들과 잘 구분되었다. 토양 분리주들은 모두 *Microbispora*속에 소속된 반면 비공식적으로 기술되어 온 *Microbispora griseoalba* IMSNU 22049^T (=KCTC 9314^T)는 *Nocardia* 속의 미생물들과 가까운 유연 관계를 보여 주었다.

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