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16S rDNA Sequences Used for Phylogenetic Classification of *Micromonospora* and Three Bacterial Isolates from Liquid Compost of Dairy Cattle Slurry

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Introduction

In the composting of livestock excrement, desirable maturity depends on microbial function. Early on, Poincelot[6) reported that mesophilic bacteria are largely responsible for degrading decomposable materials in the initial stage of composting, and that thermophilic actinomyces and filamentous fungi, which degrade hemicellulose and cellulose, become predominant in a later stage. Golueke[2) showed that various species of the actinomycete genera *Micromonospora, Streptomyces* and *Actinomyces* are commonly at work in compost materials. More recently, we found the predominance of actinomycetes in the later stage of liquid compost of livestock waste, and described the bacterial utilization of carbohydrate.[4,5) Micromonosporal bacteria are the major microorganisms prevalent in liquid compost in our studies.

The genus *Micromonospora* is the type genus of the family Micromonosporacaea, comprising *Micromonospora, Actinoplanes, Dactylosporagium, Pilimelia, Catenuloplanes, Couchioplanes, Catellatospora* and *Spirilliplanes*.[10) A new genus, *Verrucosispora*, has been proposed on the basis of a recent phylogenetic analysis.[7) The sequencing, or phylogenetic analysis, of the ribosomal RNA gene (rDNA), in this case 16S rDNA, is required for the proposition of new genera of specific taxonomy. Once the sequence is determined, comparative analysis using 16S rDNA sequence databases is an effective method of rapidly identifying the bacteria. The aims of the present study were to classify three Micromonosporal isolates from liquid compost of cattle slurry on the basis of 16S rDNA sequences and to clarify the phylogenetic relationships of the isolates.

Materials and Methods

Bacterial strains

The 16S rDNA sequences for 36 strains of the various species of genus *Micromonospora*, listed in Table 1, were retrieved from the DNA Data Bank of Japan (DDBJ). Two of the Deutsche Sammlung von Mikroorgasmen und Zellkulturen Gmbh (DSM) strains, namely, DSM 43895 and DSM 802, were obtained from the Japan Collection of Microoganisms and from the Institute for Fermentation, Osaka. To determine the missing 16S rDNA sequences of these two DSM strains, as well as that of the three *Micromonospora* isolates taken from liquid compost, we conducted 16S rDNA sequencing analysis and compared the results with the other 36 sequences.

16S rDNA sequencing

Cells from each strain were cultured in ISP No. 2 medium. For extracting genomic DNA, bacteria from a single colony were grown on a plate culture and lysed by ultrasonification or by treatment with acetylmuramidase. The 16S rDNA was amplified from the extracted DNA by polymerase chain reaction (PCR) by using Takara Taq (Takara Shuzo, Kyoto, Japan) and a pair of primers, 8F (5'-AGAGTTTGATCCTGGCTCAG)

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and 1510R (5'-GGTTACCTTGTTACGACTT). The DNA was denatured at 93℃ for 3 min, then submitted to 36 cycles of denaturation at 93℃ for 1 min each, primer annealing at 55℃ for 2 min and primer extension at 72℃ for 3 min. At the end of cycling, the reaction mixture was maintained at 72℃ for 7 min, then cooled to 4℃. The PCR products were sequenced directly with the Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems). The sequence reaction mixtures were electrophoresed with a model-373A DNA sequencer (Applied Biosystems).

Table 1 The various species of *Micromonospora* compared according to their 16S rDNA sequences.

Species	Strain	Accession number*
M. aurantiaca	DSM43813^T	X92064
M. burunnea	DSM43814^T	X92605
M. brunnenscens	DSM43902	X92616
M. carbonacea subsp. *aurantiaca*	DSM43815^T	X92606
M. carbonacea subes. *carbonacea*	DSM43168^T	X92599
M. chalcea	DSM43026^T	X92549
M. chalcea subsp. *izumensis*	DSM43895	NA
M. chersina	DSM44151^T	X92628
M. citrea	DSM43903	X92617
M. coerulea	DSM43143^T	X92598
M. echinoaurantiaca	DSM43904	X92618
M. echinobrunnea	DSM43913	X92625
M. echinospora subsp. *echinospora*	DSM43816^T	X92607
M. echinospora subsp. *ferruginea*	DSM43141^T	X92597
M. echinospora subsp. *pallida*	DSM43817^T	X92608
M. floridensis	DSM43907	X92621
M. fulvopurpurea	DSM43918	X92627
M. fulvoviolacea	DSM43905	X92619
M. fulvoviridis	DSM43906	X92620
M. globosa	DSM43170	X92600
M. halophytica subsp. *halophytica*	DSM43171^T	X92601
M. halophytica subsp. *nigra*	DSM43818^T	X92609
M. inositola	DSM43819^T	X92610
M. inyoensis	DSM46123	X92629
M. lacustris	DSM43908	X92622
M. megaromicea subsp. *megaromicea*	DSM43892	X92614
M. megaromicea subsp. *nigra*	DSM802	NA
M. melanosporea	DSM43126	X92596
M. narashino	DSM43172	X92602
M. olivasterospora	DSM43868^T	X92613
M. peucetica	DSM43363	X92603
M. purpurea	DSM43036^T	X92595
M. purpureochromogenes	DSM43821^T	X92611
M. rhodorangea	DSM1039^T	X92612
M. rosaria	DSM803^T	X92631
M. sagamiensis	DSM43912	X92624
M. viridifaciens	DSM43909	X92623
M. yulongensis	DSM43915	X92626
Micoromonospora sp.	SA-08	NA
Micoromonospora sp.	SA-09	NA
Micoromonospora sp.	SA-15	NA
Spirilliplanes yamanasiensis	DSM44325^T	D63912

*Retrieved from the DNA Data Bank of Japan; ^{T}: Type strain; NA: not available

Sequence alignment, calculation of similarity values and construction of the phylogenetic tree

The nucleotide sequences of the 16S rDNA, determined in this study, were aligned manually along with the sequences obtained from the DDBJ (Table 1). Evolutionary distance (*Knuc*) values were found by using the Clustal V program package. Percent of similarity between individual sequences (A and B) was calculated by the formula: similarity (A, B) = 100 x sum of the matches / [length - gap residues (A) - gap residues (B)] . The neighbor-joining method of Saitou and Nei[8] was employed to construct a phylogenetic tree with the BioResearch SINCA program (Fujitsu, Japan),[9] and the topology of the tree was evaluated by bootstrapping method.[1]

Results and Discussion

This study resulted in three major contributions to the DNA database, i.e., the identity, except for the primer regions, of the 16S rDNA sequences for the *M. chalcea* subsp. *izumensis* DSM 43895 and *M. megaromicea* subsp. *nigra* DSM 802, and for the three isolates of *Micromonospora* genera from liquid compost of dairy cattle waste. The 16S rDNA sequences of the three isolates were completely identical each other. The 16S rDNA sequences of *Micromonospora megalomicea* subsp. *megalomicea* DSM 43892 differed markedly from those of any other *Micromonospora* species, exhibiting 90% sequence similarity or less (data not shown). This was dramatically lower than the percents of similarity shown between *Micromonospora* species and *Spirilliplanes yamanasiensis*, which was used as an out-group (95.7% to 97.0%) (Table 2). For this reason, the sequence values of the *Micromonospora megalomicea* subsp. *megalomicea* DSM 43892 were omitted from the phylogenetic analysis.

Table 2 shows the percents of similarity and the evolutionary distance values between combinations of the 16S rDNA sequences in the various species of *Micromonospora*. The three isolates exhibited both the highest similarity (99.7%) and the lowest evolutionary distance (0.2) to *Micromonospora yulongensis* DSM 43915. These sequences, depicted in the phylogenetic tree shown in Fig. 1, were more closely related to each other than to the other taxa and they exhibited specific association (bootstrap value 100%). A 98.9% sequence similarity was exhibited between *M. fulvoviolacea* DSM 43905 and the three isolates, with an evolutionary distance of 1.0. The group of *M. yulongensis, M. fulvoviolacea* and the three isolates was recovered in 92% of the bootstrapped trees. With the sole exception of *M. olivasterospora*, the *Micromonospora* species exhibited a sequence similarity of 96.7% or greater, forming a monophyletic group which was recovered in 85% of the bootstrapped tree. Due to the relatively short subline formed by the sequence, it is not clear whether *M. olivasterospora* is a distinct species.

We analyzed two or three subspecies of *M. carbonacea, M. chalcea, M. halophytica* and of *M. echinospora*. The *M. carbonacea* subsp. *carbonacea* and *M. carbonacea* subsp. *aurantiaca* exhibited a specific association (99.9% similarity of sequence, with a bootstrap value of 95%). However, the other subspecies either formed individual sublines or appeared in close genealogical affinity to different species. For example, the *M. chalcea* subsp. *izumensis* formed a distant subgroup with the two subspecies of *M. carbonacea*, which were recovered in 100% of the bootstrapped trees, as did the *M. echinospora* subsp. *furriginea* and *M. rhodorangea*. Further comparison and analysis of the physiological and chemotaxinomical features are necessary, in addition to DNA pairing experiments, to define the true phylogenetic positions of these organisms. Koch *et al.*[3] reported that the *M. echinospora* subsp. *pallida* is characterized by the presence of the major menaquinone MK-12(H_4, H_6, H_8). However, The MK-12 is not present in other type strains of the *Micromonospora* species, which contain mainly MK-9(H_4, H_6) or MK-10(H_4, H_6). This phenomenon gives rise to the argument between the chemotaxonomy and the phylogenetic classification based on 16S rDNA sequencing. To verify the relationships between the results of the present analyses, we are currently analyzing the menaquinone patterns of the

Table 2 Percent similarity (upper right) and evolutionary distance (lower left) between pairs of the various *Micromonospora* species, as compared by their 16S rDNA sequences.

Evolutionary distance (per 100 bases)

Species or subspecies	% Similarity with :																																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1 *M. aurantiaca*		98.5	98.3	98.3	98.4	99.0	98.3	97.6	98.5	97.6	98.1	98.3	97.6	98.0	97.9	98.3	97.4	99.8	98.5	99.4	99.0	97.9	97.6	97.6	98.8	98.5	98.7	99.0	97.5	97.5	98.4	99.2	98.0	98.1	97.8	97.9	98.3	98.1	96.1
2 *M. brunnenscens*	1.5		98.3	98.6	98.7	97.9	98.8	98.1	98.3	97.5	97.9	97.7	97.9	98.0	97.9	98.3	97.7	98.6	98.2	98.5	98.5	97.2	97.6	97.6	98.3	98.1	97.6	98.6	97.5	97.7	97.3	99.0	98.0	97.6	97.6	97.8	98.3	98.3	96.3
3 *M. burunnea*	1.7	1.7		97.9	98.0	98.2	97.9	98.3	98.0	97.2	97.5	97.5	97.4	97.7	97.7	98.2	97.6	98.3	97.4	98.2	98.3	97.2	97.9	97.2	98.3	97.6	97.9	98.8	96.7	97.5	97.6	98.8	97.7	97.8	97.4	97.5	97.7	97.4	95.6
4 *M. carbonacea* subsp. *aurantiaca*	1.7	1.4	2.1		99.9	97.7	99.7	97.5	97.8	97.3	98.0	97.7	97.8	98.1	98.1	98.4	97.2	98.4	98.6	98.1	98.5	97.6	97.7	97.4	98.3	98.0	97.4	98.3	98.0	97.2	97.2	98.5	98.1	97.6	97.6	98.2	98.1	97.9	96.3
5 *M. carbonacea* subes. *carbonacea*	1.6	1.3	2.0	0.1		97.8	99.8	97.6	97.9	97.4	98.1	97.8	97.9	98.2	98.2	98.5	97.3	98.5	98.7	98.1	98.6	97.7	97.8	97.4	98.3	98.1	97.5	98.3	98.1	97.3	97.2	98.5	98.2	97.7	97.7	98.3	98.2	98.0	96.3
6 *M. chalcea*	1.0	2.0	1.7	2.2	2.2		97.9	97.9	98.1	97.4	97.6	97.6	97.6	97.9	97.7	98.2	97.8	99.0	97.7	98.9	98.6	97.7	97.7	97.8	98.5	98.2	99.2	99.0	97.0	97.9	99.2	98.4	97.9	98.3	97.7	97.4	97.6	97.5	96.1
7 *M. chalcea* subsp. *izumensis*	1.7	1.2	2.2	0.3	0.2	2.1		97.6	97.7	97.2	97.9	97.6	98.0	98.2	98.1	98.3	97.4	98.4	98.5	98.2	98.5	97.6	97.6	97.6	98.2	98.3	97.6	98.2	97.9	97.3	97.2	98.4	98.2	97.8	97.7	98.1	98.1	98.1	96.5
8 *M. chersina*	2.2	1.8	1.6	2.4	2.3	1.9	2.3		98.8	98.2	98.3	98.5	98.8	99.0	98.4	97.6	98.8	97.7	97.1	97.4	97.7	98.1	99.1	98.5	97.8	98.2	98.6	98.5	97.4	98.3	98.5	98.1	99.0	99.2	98.7	98.2	97.4	97.2	96.5
9 *M. citrea*	1.5	1.7	2.0	2.2	2.2	1.9	2.3	1.0		98.6	98.6	99.0	98.6	99.0	98.8	97.6	98.3	98.5	97.5	98.3	98.6	98.5	98.5	98.5	98.0	98.8	98.8	98.9	98.1	98.5	98.6	98.7	99.0	98.4	98.8	98.6	97.7	97.4	96.8
10 *M. coerulea*	2.3	2.4	2.6	2.6	2.5	2.4	2.6	1.8	1.3		98.3	98.6	98.1	98.4	98.0	97.1	97.4	97.8	97.7	97.4	97.6	97.7	97.9	97.9	97.7	98.1	97.8	97.9	97.8	97.8	97.9	98.1	98.4	97.9	98.1	97.9	97.9	97.7	96.8
11 *M. echinoaurantiaca*	1.7	1.9	2.2	1.7	1.7	2.0	1.8	1.5	1.1	1.5		98.7	98.7	99.0	98.7	97.3	98.3	98.1	97.8	97.9	98.3	98.3	98.7	98.4	97.9	98.8	98.1	98.3	98.2	97.7	97.9	98.3	99.0	98.4	98.8	98.9	97.4	97.4	96.8
12 *M. echinobrunnea*	1.6	2.2	2.4	2.2	2.1	2.2	2.2	1.5	0.8	1.4	1.2		98.5	98.9	98.5	97.6	98.1	98.2	98.1	97.9	98.3	98.5	98.5	98.3	97.9	98.3	98.3	98.4	98.1	98.5	98.2	98.3	98.9	98.3	98.7	98.5	97.7	97.4	96.5
13 *M. echinospora* subsp. *echinospora*	2.2	1.9	2.5	2.1	2.0	2.2	1.9	1.3	1.3	1.9	1.2	1.5		99.7	98.8	97.0	98.8	97.7	97.3	97.6	98.2	98.1	98.8	99.1	97.6	98.6	98.3	97.9	97.6	97.9	98.1	97.7	99.7	98.6	99.2	98.1	97.1	97.2	96.6
14 *M. echinospora* subsp. *ferruginea*	1.9	1.9	2.2	1.7	1.7	1.9	1.7	1.0	0.9	1.6	0.8	1.1	0.3		99.2	97.3	98.8	98.1	97.6	97.8	98.5	98.4	99.1	99.2	98.0	98.8	98.5	98.3	97.9	98.1	98.4	98.1	###	98.8	99.5	98.5	97.4	97.4	96.8
15 *M. echinospora* subsp. *pallida*	2.1	2.1	2.3	1.9	1.8	2.2	1.9	1.5	1.3	1.9	1.0	1.4	1.0	0.7		97.2	98.2	98.0	7.6	97.7	98.3	98.5	98.7	98.5	98.2	98.6	98.3	98.3	98.1	97.8	98.1	98.0	99.2	98.4	98.7	98.6	97.3	97.1	96.6
16 *M. floridensis*	1.7	1.7	1.8	1.6	1.5	1.7	1.7	2.2	2.4	2.8	2.4	2.3	2.9	2.6	2.8		97.3	98.3	98.3	98.0	97.9	97.2	97.2	96.8	98.5	97.4	97.9	98.5	97.3	97.9	97.6	98.5	97.3	97.7	97.0	97.2	98.1	97.7	95.7
17 *M. fulvoviridis*	2.5	2.2	2.3	2.7	2.6	2.1	2.5	1.0	1.7	2.4	1.5	1.7	1.0	1.0	1.7	2.6		97.5	96.7	97.4	97.7	98.1	98.7	98.5	97.5	98.1	98.5	98.1	97.8	98.5	98.3	97.8	98.8	98.4	98.5	98.5	96.5	96.5	96.2
18 *M. fulvopurpurea*	0.2	1.4	1.7	1.6	1.5	0.9	1.6	2.2	1.5	2.1	1.6	1.7	2.2	1.8	2.0	1.7	2.4		98.5	99.5	99.1	97.9	97.7	97.6	98.8	98.5	98.8	99.0	97.6	97.6	98.5	99.2	98.1	98.1	97.9	97.9	98.4	98.1	96.1
19 *M. fulvoviolacea*	1.4	1.7	2.4	1.3	1.2	2.1	1.4	2.9	2.4	2.3	2.1	1.9	2.7	2.4	2.3	1.6	3.1	1.3		98.2	98.1	97.4	97.2	96.8	98.6	97.4	97.4	98.0	97.9	97.2	97.2	98.5	97.6	97.3	97.2	97.4	99.1	98.9	96.3
20 *M. globosa*	0.6	1.5	1.8	1.9	1.9	1.0	1.8	2.4	1.7	2.5	1.9	1.9	2.3	2.1	2.3	2.0	2.6	0.5	1.7		99.0	97.6	97.4	97.5	98.5	98.3	98.7	98.8	97.2	97.4	98.3	99.0	97.8	97.8	97.6	97.6	98.1	98.0	96.0
21 *M. halophytica* subsp. *halophytica*	1.0	1.5	1.7	1.5	1.4	1.3	1.5	2.2	1.4	2.2	1.4	1.6	1.7	1.3	1.7	2.1	2.2	0.9	1.7	1.0		97.7	98.0	98.1	98.4	98.4	98.2	99.0	97.4	97.4	98.0	99.0	98.5	97.9	98.3	98.1	98.0	97.7	96.1
22 *M. halophytica* subsp. *nigra*	1.9	2.6	2.6	2.2	2.1	2.0	2.2	1.5	1.3	1.9	1.3	1.2	1.6	1.3	1.3	2.6	1.8	1.9	2.2	2.2	2.1		98.2	97.9	97.8	98.0	98.4	98.0	97.9	98.0	98.3	97.9	98.4	98.1	98.1	98.5	97.2	97.1	96.4
23 *M. inositola*	2.2	2.2	1.9	2.1	2.0	2.0	2.2	0.8	1.3	2.0	1.3	1.4	1.2	0.8	1.1	2.6	1.2	2.1	2.8	2.4	1.8	1.5		98.4	97.6	98.1	98.4	98.2	97.6	97.9	98.3	98.0	99.1	98.7	98.8	98.5	97.2	97.0	96.4
24 *M. inyoensis*	2.3	2.2	2.6	2.5	2.4	2.0	2.3	1.4	1.3	2.0	1.3	1.5	0.8	0.7	1.4	3.1	1.3	2.2	3.1	2.4	1.7	1.7	1.4		97.3	98.5	98.3	97.9	97.4	97.9	98.3	97.5	99.2	98.3	99.3	98.1	96.7	96.7	96.7
25 *M. lacustris*	1.3	1.7	1.7	1.7	1.7	1.5	1.8	2.1	2.0	2.2	1.8	1.9	2.2	1.9	1.8	1.5	2.4	1.2	1.3	1.5	1.6	2.0	2.2	2.6		98.0	98.2	98.8	97.2	97.7	97.9	98.7	98.0	97.7	97.5	97.7	98.5	98.3	95.8
26 *M. megaromicea* subsp. *nigra*	1.5	1.9	2.4	2.0	1.9	1.7	1.7	1.7	1.2	1.8	0.9	1.6	1.3	1.0	1.4	2.6	1.9	1.5	2.4	1.7	1.6	1.8	1.7	1.4	2.0		98.6	98.4	98.1	97.9	98.4	98.3	98.8	98.8	98.5	98.5	97.4	97.4	97.0
27 *M. melanosporea*	1.3	2.4	2.1	2.6	2.5	0.8	2.4	1.3	1.3	2.1	1.7	1.5	1.5	1.3	1.7	2.1	1.5	1.3	2.4	1.3	1.8	1.4	1.4	1.5	1.8	1.4		98.8	97.6	98.5	99.5	98.1	98.5	98.8	98.4	97.9	97.4	97.2	96.5
28 *M. narashino*	1.0	1.4	1.3	1.7	1.7	0.9	1.8	1.3	1.1	2.0	1.4	1.5	1.9	1.6	1.7	1.5	1.9	1.0	1.9	1.3	1.0	1.8	1.6	1.9	1.2	1.6	1.3		97.6	98.1	98.5	99.0	98.3	98.3	98.1	98.3	98.0	97.7	96.5
29 *M. olivasterospora*	2.4	2.4	3.2	1.9	1.9	2.9	2.1	2.4	1.9	2.0	1.6	1.7	2.2	1.9	1.9	2.6	2.2	2.4	1.9	2.7	2.5	1.9	2.2	2.4	2.8	1.8	2.4	2.4		98.1	97.4	97.9	97.9	97.6	97.6	98.5	97.2	97.2	97.3
30 *M. peucetica*	2.4	2.2	2.4	2.7	2.6	2.0	2.6	1.5	1.4	2.0	2.1	1.3	1.9	1.7	2.2	2.0	1.5	2.4	2.6	2.5	2.5	1.9	1.9	1.9	2.2	2.1	1.4	1.8	1.9		98.3	97.9	98.1	98.1	97.9	98.1	97.4	97.4	96.6
31 *M. purpurea*	1.6	2.7	2.4	2.9	2.8	0.8	2.8	1.4	1.4	1.9	1.8	1.7	1.7	1.5	1.9	2.4	1.7	1.5	2.7	1.7	2.0	1.5	1.5	1.6	2.1	1.6	0.5	1.5	2.5	1.6		97.9	98.4	98.7	98.3	97.7	97.1	96.9	96.4
32 *M. purpureochromogenes*	0.8	1.0	1.1	1.5	1.4	1.5	1.5	1.7	1.3	1.7	1.5	1.5	2.1	1.7	1.9	1.4	2.2	0.7	1.3	1.0	1.0	1.9	1.9	2.3	1.3	1.7	1.8	0.9	2.1	2.2	2.1		98.1	98.0	97.7	98.2	98.7	98.5	96.5
33 *M. rhodorangea*	1.9	1.9	2.2	1.7	1.7	1.9	1.7	1.0	0.9	1.6	0.8	1.1	0.3	0.0	0.7	2.6	1.0	0.8	2.4	2.1	1.3	1.3	0.8	0.7	1.9	1.0	1.3	1.6	1.9	1.7	1.5	1.7		98.8	99.5	98.5	97.4	97.4	96.8
34 *M. rosaria*	1.8	2.3	2.1	2.2	2.2	1.5	2.1	0.8	1.5	2.1	1.5	1.7	1.4	1.2	1.5	2.2	1.4	1.7	2.7	2.1	2.0	1.6	1.3	1.5	2.2	1.1	1.0	1.5	2.2	1.7	1.2	1.8	1.2		98.5	98.0	97.4	97.2	96.7
35 *M. sagamiensis*	2.1	2.3	2.4	2.2	2.2	2.1	2.2	1.3	1.1	1.9	1.1	1.3	0.8	0.5	1.2	2.9	1.3	2.0	2.9	2.3	1.5	1.5	1.2	0.6	2.4	1.3	1.5	1.7	2.2	1.9	1.6	2.1	0.5	1.5		98.3	97.0	96.9	96.5
36 *M. viridifaciens*	2.0	2.1	2.4	1.7	1.6	2.4	1.7	1.5	1.3	1.9	0.8	1.3	1.6	1.3	1.3	2.7	1.5	1.9	2.3	2.2	1.7	1.3	1.3	1.7	2.2	1.4	2.0	1.5	1.4	1.8	2.2	1.7	1.3	1.7	1.5		97.2	97.1	96.5
37 *M. yulongensis*	1.5	1.5	2.2	1.7	1.7	2.2	1.8	2.6	2.2	2.1	2.4	2.3	2.9	2.6	2.6	1.8	3.3	1.5	0.9	1.7	1.9	2.5	2.8	3.2	1.4	2.5	2.5	1.9	2.6	2.4	2.8	1.1	2.6	2.6	3.0	2.6		99.7	96.4
38 SA-08, SA-09, SA-15	1.7	1.5	2.4	1.8	1.7	2.2	1.6	2.6	2.3	2.2	2.5	2.4	2.7	2.5	2.6	2.0	3.3	1.6	1.0	1.7	2.0	2.6	3.0	3.1	1.5	2.3	2.5	2.0	2.6	2.4	2.9	1.3	2.5	2.6	3.0	2.6	0.2		96.7
39 *Spirilliplanes yamanasiensis*	3.4	3.1	3.9	3.2	3.1	3.3	3.0	3.1	2.7	2.8	2.8	3.1	0.3	2.8	2.9	3.8	3.3	3.3	3.3	3.5	3.3	3.0	3.3	2.9	3.6	2.4	3.0	3.1	2.2	2.9	3.1	3.1	2.8	2.9	3.1	2.9	3.2	3.0	

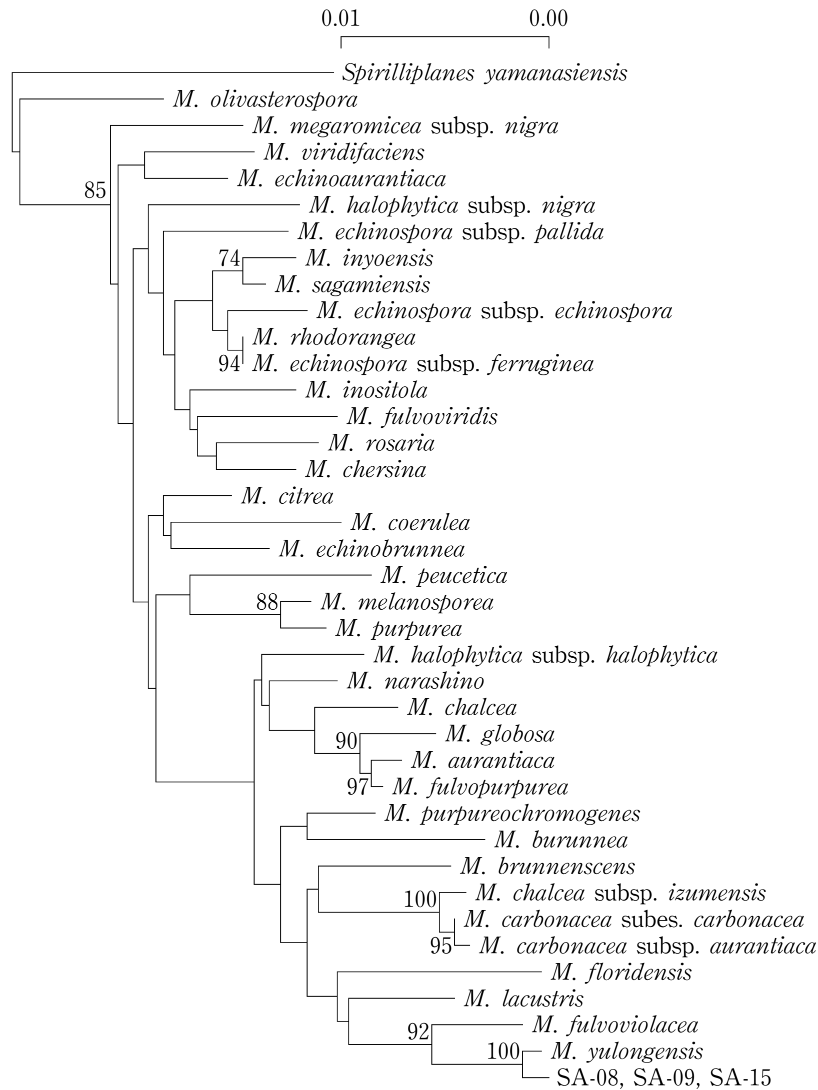

Fig. 1 Unrooted phylogenetic tree for 16S rDNA sequences of various species of *Micromonospora* and of *Spirilliplanes yamanasiensis* compared as an out-group. The scale bar, determined by measuring the horizonal line connecting two strains, indicates the evolutionary distance value (*Knuc*) between sequences. Numeral at the nodes of branching indicate the percent obtained by bootstraping analysis.

organisms.

The 16S rDNA nucleotide stretches possibly specific for a certain species or group of the genus *Micromonspora* were found at residues 589–612, 589–593 and 601–604. Furthermore, highly variable regions of the sequence were detected at residues 178–201, 689–691 and 1000–1003 (data not shown). The specificities of these stretches for the species and for a certain group of the genus *Micromonospora* are still not clear because the information of the 16S rDNA sequences have not been completeness. However, some of the species groups forming monophyletic clades exhibited specific stretches in the domains, indicating that this information is possibly useful for assigning the *Micromonospora* strains. In accordance with recommendations for the species described, any decisions concerning taxonomic status should be made only after extensive DNA-DNA hybridization experiments are completed. In the light of the identical 16S rDNA sequences from different taxa whose phenotypic properties are similar, DNA pairing experiments are thought to shed light on, and possibly clarify, the

taxonomy of such species.

With further efforts to enlarge the sequence database, analysis by 16S rDNA sequences is deemed to be one of the most effective methods currently available for classifying bacteria. Additional analyses such as the sequencing of the intergenic spacer regions of 16-23S rRNA (the rDNA transcript), which is known to discriminate finely between closely related taxa, may be useful for more detailed identification.[12] Furthermore, the nucleotide sequences of *gyrB* (DNA gyrase B subunit) whose more phylogenesis has been described.[11] The sequencing techniques used in the present work are extremely helpful in determining whether such laborious and unstable testing is required.

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Reference

1) Felsenstein, J., 1985. Confidence limits on phylogenenies: an approch using bootstrap., Evolution, 39: 783-791.

2) Golueke, C.G., 1976. Composting, In Biological Reclamation of Solid Waste. pp. 8-17, Radale Press, Emmaus.

3) Koch C., M.R. Kroppenstedt, and E. Stackebrandt, 1996. Intrageneric relationship of the actinomycete genus *Micromonospora*. Int. J. Syst. Bacteriol., 46: 383-387.

4) Okamoto, E., E. Miyagawa, Y. Matsui and J. Matsuda, 1995. Changes of microbial population on liquid composting of dairy cattle slurry. J. Rakuno Gakuen Univ., 20: 81-91.

5) Okamoto, E., S. Tsukada and E. Miyagawa, 1996. Actinomycetes isolated from dairy cattle slurry. Animal Science and agriculture Hokkaido. 38: 64-68 (in Japanese).

6) Poincelot, R.P., 1975. The biochemistry and methodology of composting. Conn. Agric. Exp. Sta. (New Haven) Blletin, 754: 1-18.

7) Rheims, H., P. Schumann, M. Rohde and E. Stackebrandt, 1998. *Verrucosispora gifhornensis* gen. nov., sp. sov., a new member of the actinobacterial family *Micromonosporaceae*. Int. J. Syst. Bacteriol., 48: 1119-1127.

8) Saitou, N. and M. Nei, 1987. The neighbor-joining method:a new method for reconstracting phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

9) Takahashi, T., M. Kaneko, Y. Mori, M. Tsuji, N. Kikuchi and T. Hiramune, 1977. Phylogenetic analyses of *Staphylococcus* based on the 16S rDNA sequences and assignment of clinical isorates from animals. J. Vet. Med. Sci., 59: 775-783.

10) Wakamoto, I. 1989. Genus *Micromonospora* Ø rskov 1923, In Bergey's manual of systematic bacteriology, (S.T.Williams, M.E. Sharoe and J. G. Holt ed.), vol 4. 9. pp. 2442-2450, The Williams and Wilkins Co., Baltimore.

11) Yamamoto, S and S. Harayama, 1998, Phylogenetic relationships of *Pseudomonas putida* strains deduced from the nucleotide sequences of *gyrB*, *rpoD* and 16S rDNA genes. Int. J. Syst. Bacteriol., 48: 813-819

12) Zhang, Z., Y. Wang, and J. Ruan, 1997. A proposal to revive the genus *Kitasatospora*. Int. J. Syst. Bacteriol., 47 (4), 1048-1054

Summary

To classify three bacterial isolates of the genus *Micromonospora*, retrieved from liquid composting of dairy cattle slurry, we conducted 16S rDNA sequencing and compared the results with the 16S rDNA sequences of the various species of the genus *Micromonospora*. In addition, the reference strains were determined for *M. chalcea* subsp. *izumensis* and *M. megaromicea* subsp. *nigra*, which had been missing from the DNA database. The sequences of the isolates were fully identical each other. On comparison of the isolates with all taxa of the genus *Micromonospora*, the sequences of the isolates were exhibited a highest level of similarity (99.7%) to *Micromonospora yulongensis*. Furthermore, these were recovered in 100% of the bootstrapped trees. Therefore, the isolated bacteria were assigned to the species *Micromonospora yulongensis*.

要　　約

液状コンポストから分離された3株の*Micoromonospora*属細菌の菌種を同定するために16S rDNA塩基配列を決定した。また，配列が決められていない2つの*Micoromonospora*属細菌種の基準株（*M. chalcea* subsp. *izumensis* DSM 43895. *M. megaromicea* subsp. *nigra* DSM 802）についても16S rDNA塩基配列を決定し，比較解析した。その結果，3つの分離株は互いに完全に一致した塩基配列を持つことが示された。これらの配列は*M. yulongensis* DSM 43915と99.7%のきわめて高い類似度を示した。系統樹解析の結果からも，分離株と*M. yulongensis*は高い近縁関係にあることが示唆され，ブーツストラップ値は100%であった。これらの値から液状コンポストからの分離株は，*Micromonospora yulongensis*であることが判明した。