***Microbispora corallina* sp. nov., a new species of the genus *Microbispora* isolated from Thai soil**

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Two actinomycete strains, DF-28 and DF-32^T, were isolated from soil samples collected in a deciduous dipterocarp forest in Thailand. They produced longitudinally paired spores on the tips of short sporophores alternately branched from aerial hyphae, and the chemotaxonomic properties of the isolates were the same as those of members of the family Streptosporangiaceae. These phenotypic properties, together with the results of a phylogenetic analysis based on 16S rRNA gene sequences, indicated that these isolates should be assigned to the genus *Microbispora*. The two isolates showed more than 93% DNA relatedness to each other, but their relatedness to any previously described species of the genus *Microbispora* was only 45% or less. They were distinguishable from previously described *Microbispora* spp. by a combination of physiological and biochemical properties. Therefore, a new species is proposed for these strains, under the name *Microbispora corallina* sp. nov. The type strain is strain DF-32^T (= JCM 10267^T).

Keywords: *Microbispora corallina* sp. nov., Streptosporangiaceae, Actinomycetales, Actinobacteria, actinomycetes

INTRODUCTION

The genus *Microbispora* was proposed by Nonomura & Ohara (1957) for an actinomycete strain producing longitudinally paired spores on aerial mycelia. In the *Approved Lists of Bacterial Names* (Skerman *et al.*, 1980), 10 species were cited as members of the genus *Microbispora*; later, three others, *Microbispora viridis* (Miyadoh *et al.*, 1985), *Microbispora karnatakensis* and *Microbispora indica* (Rao *et al.*, 1987), were validly proposed as new species. Subsequently, this genus was well defined from a chemotaxonomic point of view, and Kroppenstedt *et al.* (1990) and Miyadoh *et al.* (1990) transferred *Microbispora echinospora* and *M. viridis* to the genus *Actinomadura* as *Actinomadura echinospora* and *Actinomadura rugatobispora*, respectively. On the basis of DNA–DNA hybridization experiments, Miyadoh *et al.* (1990) also proposed that

10 of the remaining species (with the exception of *Microbispora bispora*) should be combined into the type species *Microbispora rosea* with two subspecies, *M. rosea* subsp. *rosea* and *M. rosea* subsp. *aerata*. Analysis of 16S rDNA sequences by Wang *et al.* (1996b) indicated that *M. bispora* was phylogenetically distant from the *M. rosea* cluster and other members of the family Streptosporangiaceae, and this species was transferred to the new genus *Thermobispora* (as *Thermobispora bispora*). Recently, Zhang *et al.* (1998) reclassified the genus *Thermomonospora* and proposed the transfer of *Thermomonospora mesophila*, which produces spores singly borne on aerial hyphae, to the amended genus *Microbispora* (as *Microbispora mesophila*).

While searching for novel actinomycetes from tropical regions of South-East Asia, we isolated two strains (DF-28 and DF-32^T, from soil samples collected in Thailand) showing typical morphological characteristics of the genus *Microbispora*. In this paper we report the taxonomic characterization and classification of these isolates and propose a new species, *Microbispora corallina* sp. nov., for the strains.

Abbreviation: A₂pm, 2,6-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of *Microbispora corallina* DF-32^T (= JCM 10267^T) is AB018046.

METHODS

Bacterial strains. Strains DF-28 and DF-32^T were isolated from soil samples collected in a deciduous dipterocarp forest in Thailand by means of the dilution-agar plating method with humic acid/vitamin agar (Hayakawa & Nonomura, 1987). The following type strains of the named species belonging to the redefined genus *Microbispora* were used for the comparative studies: *M. rosea* subsp. *rosea* JCM 3006^T, JCM 3021 (formerly the type strain of *Microbispora amethystogenes*), JCM 3022 (formerly the type strain of *Microbispora chromogenes*), JCM 3023 (formerly the type strain of *Microbispora diastatica*), JCM 3024 (formerly the type strain of *Microbispora parva*), JCM 8971 (formerly the type strain of *M. indica*), JCM 8972 (formerly the type strain of *M. karnatakensis*), *M. rosea* subsp. *aerata* JCM 3076^T (formerly the type strain of *Microbispora aerata*), JCM 3110 (formerly the type strain of *Microbispora thermodiastatica*) and JCM 3111 (formerly the type strain of *Microbispora thermorosea*). '*M. rosea* subsp. *nonnitritogenes*' JCM 3298 and '*M. amethystogenes* subsp. *nonreducans*' JCM 10122, which do not have standing in nomenclature were also used for reference.

Morphology. Strains DF-28 and DF-32^T were grown for 2 weeks at 28 °C on oatmeal agar supplemented with 1% (w/v) yeast extract and inorganic salts/starch agar supplemented with 1% (w/v) yeast extract and observed using light microscopy and scanning electron microscopy. Samples for scanning electron microscopy were prepared as described previously (Itoh *et al.*, 1989).

Cultural, physiological and biochemical characteristics. Cultural characteristics were tested by using 14 d cultures grown at 28 °C on various agar media (Table 1). The *Color Harmony Manual* (Jacobson *et al.*, 1958) was used for determining colour designations and names of colonies. The decomposition of various compounds was examined by using the basal media recommended by Gordon *et al.* (1974). The utilization of carbohydrates as sole carbon sources was tested with a neutralized yeast nitrogen base without amino acids (Difco) as a basal medium, according to the method of Stevenson (1967). The utilization of organic acids was

examined on the defined medium of Gordon *et al.* (1974) supplemented with the vitamin B complex solution used in humic acid/vitamin agar. The reduction of nitrate was examined in nutrient broth (Difco) supplemented with 0.1% (w/v) KNO₃. After cultivation of each organism at an optimal temperature, the resulting nitrite was detected as described by Gottlieb (1961). The requirements for vitamins (each at a concentration of 0.5 µg ml⁻¹) were examined as described previously (Kudo *et al.*, 1993). Tolerance of sodium chloride and changes in growth temperature were determined on modified Bennett's agar containing sucrose instead of glucose.

Chemotaxonomy. Freeze-dried cells required for chemotaxonomic analysis were obtained from cultures grown in yeast extract/glucose broth (containing 10 g yeast extract and 10 g D-glucose in 1000 ml distilled water; pH 7.2) on a rotary shaker at an optimal temperature for each organism. Cell-wall peptidoglycan was prepared and hydrolysed by the methods of Kawamoto *et al.* (1981) and the amino acid composition was analysed using an automatic amino acid analyser. The isomers of diaminopimelic acid (A₃pm) in cell walls were determined using the method of Stanek & Roberts (1974), and reducing sugars from whole-cell hydrolysates were analysed using the HPLC method of Mikami & Ishida (1983). The acyl group of muramic acid in peptidoglycan was determined using the method of Uchida & Aida (1984). Phospholipids in cells were extracted and identified using the method of Minnikin *et al.* (1984). Methyl esters of cellular fatty acids were prepared by means of the direct transmethylation method with methanolic hydrochloride (Suzuki & Komagata, 1983) and were analysed by using GLC as described previously (Kudo *et al.*, 1993). The identification of each peak was carried out by comparison with commercially available standards and by using GC-MS. Isoprenoid quinones were extracted according to the method of Collins *et al.* (1977) and were analysed by HPLC with a Cosmosil 5C₁₈ column (4.6 × 150 mm; Nacalai Tesque). The elution solvent was a mixture of methanol and 2-propanol (2:1, v/v).

16S rDNA sequence analysis. The 16S rDNA was amplified using the PCR method with a *Taq* DNA polymerase

Table 1. Cultural characteristics of strains DF-32^T and DF-28

Colour numbers and names (in parentheses) were taken from the *Color Harmony Manual* (Jacobson *et al.*, 1958).

Medium	Strain DF-32 ^T			Strain DF-28		
	Growth and colour	Aerial mycelium and colour	Soluble pigment	Growth and colour	Aerial mycelium and colour	Soluble pigment
Nonomura's yeast extract/starch agar	Good (dark lacquer red, 6 pe)	Poor (shell pink, 6 ca)	Yellow	Good (dark lacquer red, 6 pe)	Poor (shell pink, 6 ca)	Yellow
Oatmeal agar	Good (persimmon, 5 nc)	Poor (pink tint, 7 ba)	Yellow	Good (bright peach, 5 ia)	Poor (pink tint, 7 ba)	Yellow
Oatmeal agar + 1% yeast extract	Good (light coral pink, 6 la)	Good (pale pink, 7 ca)	None	Good (coral, 6 lc)	Good (pale pink, 7 ca)	None
Inorganic salts/starch agar + 1% yeast extract	Good (coral, 6 lc)	Good (cherry pink, 7 ea)	None	Good (shrimp pink, 6 1/2 ia)	Good (pale pink, 7 ca)	None
Modified Bennett's agar containing sucrose	Good (barn red, 6 pg)	Poor (shell pink, 6 ca)	Yellow	Good (Venetian red, 6 pi)	Poor (shell pink, 6 ca)	Yellow
Dilute (1/20) V8 juice agar	Moderate (amber, 3 pc)	None	None	Moderate (russet orange, 4 pc)	None	None
Glucose/asparagine agar + 1% yeast extract	Good (russet, 5 pg)	Poor (pink tint, 7 ba)	Yellow	Good (russet, 5 pg)	None	Yellow
Yeast extract/malt extract agar	Good (terracotta, 5 pe)	Moderate (shell pink, 6 ca)	None	Good (tile red, 5 ne)	Moderate (pale pink, 7 ca)	None
Yeast extract/starch agar	Good (paprika, 6 pc)	Moderate (shell pink, 6 ca)	Yellow	Moderate (bitter sweet, 5 pc)	None	Yellow
Dilute (1/5) yeast extract/starch agar	Moderate (persimmon, 5 lc)	None	Yellow	Moderate (persimmon, 5 nc)	None	Yellow

Fig. 1. 16S rDNA-based neighbour-joining tree showing the relationship between strain DF-32^T and *Microbispora* species and selected organisms belonging to the family Streptosporangiaceae. The numbers on the branches indicate the percentage bootstrap values of 1000 replicates (greater than 50%). The scientific names in parentheses are the originally proposed names of the strains; the numbers in parentheses are the GenBank/EMBL/DBJ database accession numbers.

(Takara) and primers 20F (positions 10–25, *Escherichia coli* numbering) and 1540R (1542–1525). Amplification was carried out using a DNA thermal cycler (GeneAmp PCR System 9700; Applied Biosystems) according to the following programme: 94 °C for 5 min followed by 30 cycles of denaturation (94 °C for 1 min), primer annealing (55 °C for 1.5 min) and primer extension (72 °C for 2.5 min). At the end of the cycle, the reaction mixture was kept at 72 °C for 10 min and then cooled to 4 °C. The amplified 16S rDNA fragment was purified and subjected directly to cycle sequencing using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems), according to the manufacturer's protocol, with the following primers: 27F, 11–27; 350F, 343–357; 350R, 357–342; 780F, 786–803; 780R, 803–785; 1100F, 1099–1114; 1492R, 1510–1492. The conditions for thermal cycling were as follows: 96 °C for 30 s followed by 25 cycles of denaturation (96 °C for 10 s), primer annealing (50 °C for 5 s) and primer extension (60 °C for 4 min). The products were analysed with an ABI PRISM 310 Genetic Analyser (Applied Biosystems). The sequence was multiply aligned with selected sequences (Fig. 1) obtained from the GenBank/EMBL/DBJ databases by using the CLUSTAL W program package (Thompson *et al.*, 1994); the alignment was manually verified and adjusted prior to the construction of a phylogenetic tree. The phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) in the PHYLIP package (Felsenstein, 1995). The confidence values of branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 re-

samplings. The values for sequence similarity among *Microbispora* strains were calculated manually after pairwise alignments obtained using the CLUSTAL W package. Gaps and ambiguous nucleotides were eliminated from the calculations.

Preparation of DNA, DNA base composition and DNA–DNA hybridization. DNA was isolated from cells grown in yeast extract/glucose broth according to the method of Saito & Miura (1963) but with a minor modification described previously (Kudo *et al.*, 1998). The G + C content of the DNA was determined using the HPLC method of Tamaoka & Komagata (1984). An equimolar mixture of nucleotides for the analysis of DNA base composition (Yamasa Shoyu) was used as the quantitative standard. DNA–DNA relatedness was measured fluorometrically using the microplate hybridization method devised by Ezaki *et al.* (1989).

RESULTS

Morphology

Strains DF-28 and DF-32^T produced branched and unfragmented substrate mycelia and monopodially branching aerial mycelia. The aerial hyphae bore non-motile spores on short sporophores in characteristic longitudinal pairs (Fig. 2). The sporophores with paired spores were usually branched alternately from the main hypha. Each spore was oval and its surface

Fig. 2. Scanning electron micrograph of strain DF-28 grown on inorganic salts/starch agar (ISP medium no. 4) supplemented with 1% yeast extract for 14 d at 28 °C. Bar, 2 µm.

was smooth. Neither sporangium-like bodies nor any other special structures were observed.

Cultural, physiological and biochemical characteristics

The cultural characteristics of our isolates are shown in Table 1. The substrate mycelium of both strains on most media tested was pinkish to brownish red. When produced, the aerial mycelium was pink. Yellowish, soluble pigments were produced in several media. Table 2 summarizes the results of physiological and biochemical testing of our isolates and strains used for comparison.

Chemotaxonomic properties

The chemotaxonomic features of our isolates were alike and were also similar to those of members of the family *Streptosporangiaceae*. The cell wall peptido-

Table 2. Physiological and biochemical characteristics differentiating the isolates from authentic strains of the genus *Microbispora*

Glyc., Glycerol; *myo*-Inos., *myo*-inositol; D-Sorb., D-sorbitol; Hypox., hypoxanthine; Testost., testosterone.

Organism		Growth at:			Utilization of:			Decomposition of:		Utilization of:		Requirement for:		Reduction of nitrate	Production of iodinin
		17 °C	25 °C	55 °C	Glyc.	<i>myo</i> -Inos.	D-Sorb.	Hypox.	Testost.	Lactate	Malate	Biotin	Thiamine		
Isolate															
DF-28		+	+	—	++	++	+	+	—	—	+	—	+	+	—
DF-32 ^T		+	+	—	++	++	+	—	—	+	—	—	+	+	—
<i>M. rosea</i> subsp. <i>rosea</i>															
JCM 3006 ^T	<i>M. rosea</i>	+	+	—	—	—	±	+	+	+	+	+	+	++	—
JCM 8971	<i>M. indica</i>	+	+	—	+	—	+	+	+	+	+	+	+	++	—
JCM 3022	<i>M. chromogenes</i>	+	+	—	++	+	++	+	+	+	—	+	+	++	—
JCM 3023	<i>M. diastatica</i>	+	+	—	++	+	++	+	+	+	—	—	—	++	—
JCM 8972	<i>M. karnatakensis</i>	+	+	—	+	±	+	+	+	+	+	—	+	++	—
JCM 3024	<i>M. parva</i>	+	+	—	++	—	±	—	+	+	+	—	+	—	+
JCM 3021	<i>M. amethystogenes</i>	+	+	—	—	—	—	—	—	+	—	—	+	++	+
<i>M. rosea</i> subsp. <i>aerata</i>															
JCM 3076 ^T	<i>M. aerata</i>	—	+	+	±	±	±	—	—	±	+	+	+	++	+
JCM 3110	<i>M. thermodiastatica</i>	—	—	+	±	—	+	+	+	—	—	+	+	—	—
JCM 3111	<i>M. thermorosea</i>	—	—	+	+	—	+	+	+	—	+	+	+	—	—
<i>Microbispora</i> sp.															
JCM 3298	' <i>M. rosea</i> subsp. <i>nonnitritogenes</i> '	+	+	—	—	—	±	+	+	+	—	+	+	—	—
JCM 10122	' <i>M. amethystogenes</i> subsp. <i>nonreducans</i> '	+	+	—	—	—	—	—	—	—	—	—	+	+	+

Table 3. Cellular fatty acid compositions of the isolates and the strain of *M. rosea* subsp. *rosea*

TR, Trace amount (< 0.5%).

Strain	Fatty acid composition (%)																			
	Normal										Iso					Anteiso			10-Methyl	
	13:0	14:0	15:0	16:0	16:1	17:0	17:1	18:0	18:1	14:0	15:0	16:0	16:1	17:0	18:0	18:1	15:0	17:0	10-Me-16:0	10-Me-17:0
Isolate																				
DF-32 ^T	TR	1	10	8	2	9	6	1	1	2	7	19		2	1		3	3	4	17
DF-28	TR	1	3	14	1	20	1	10	1	1	2	13		3	2		3	7	2	10
<i>Microbispora rosea</i> subsp. <i>rosea</i> JCM 3006 ^T	TR	1	5	8	1	19	3	3	1	TR	3	26	TR	3	2	TR	1	4	3	11

Table 4. Levels of DNA–DNA relatedness among the isolates and authentic strains of the genus *Microbispora*

Organism	G + C content (mol%)	Percentage DNA complementarity with labelled DNA from:													
Current name and strain	Original name	DF-32 ^T	DF-28	JCM 3006 ^T	JCM 8971	JCM 3022	JCM 3023	JCM 8972	JCM 3024	JCM 3298	JCM 3076 ^T	JCM 3110	JCM 3111	JCM 3021	JCM 10122
Isolate															
DF-32 ^T		71.5	100	99	34	32	37	37	31	31	32	31	32	31	36
DF-28		71.2	93	100	32	31	35	36	32	30	31	31	32	32	36
<i>M. rosea</i> subsp. <i>rosea</i>															
JCM 3006 ^T	<i>M. rosea</i>	69.9	45	33	100	112	75	74	65	66	62	71	58	56	53
JCM 8971	<i>M. indica</i>	70.0	41	35	93	100	72	76	66	75	63	63	55	57	48
JCM 3022	<i>M. chromogenes</i>	70.0	35	32	73	70	100	80	67	61	58	60	55	56	49
JCM 3023	<i>M. diastatica</i>	68.9	34	29	73	69	85	100	68	60	53	55	56	55	49
JCM 8972	<i>M. karnatakensis</i>	70.4	36	32	70	65	77	78	100	61	60	63	56	57	52
JCM 3024	<i>M. parva</i>	69.8	31	27	68	65	61	60	57	100	54	52	51	52	41
<i>Microbispora</i> sp. JCM 3298	<i>M. rosea</i> subsp. <i>nonnitritogenes</i> *	70.2	41	30	64	61	61	63	58	51	100	69	57	54	55
<i>M. rosea</i> subsp. <i>aerata</i>															
JCM 3076 ^T	<i>M. aerata</i>	70.8	35	33	62	59	62	62	58	60	58	100	80	69	47
JCM 3110	<i>M. thermodiastatica</i>	70.3	32	31	58	56	60	58	55	55	55	79	100	69	46
JCM 3111	<i>M. thermorosea</i>	70.1	35	33	61	59	61	60	61	58	59	68	69	100	44
<i>M. rosea</i> subsp. <i>rosea</i> JCM 3021	<i>M. amethystogenes</i>	70.5	34	30	49	47	50	48	44	41	48	51	44	43	100
<i>Microbispora</i> sp. JCM 10122	<i>M. amethystogenes</i> subsp. <i>nonreducans</i> *	70.2	32	29	47	44	51	50	46	43	46	46	44	43	71

glycan of our isolates contained glutamic acid, alanine and A₂pm in a molar ratio of 1.0:1.3:0.9 (calculated by defining the amount of glutamic acid as 1.0); an isomer of A₂pm was in the *meso*- form. These data indicate that their cell wall type (Lechevalier & Lechevalier, 1970) and their peptidoglycan type (Schleifer & Kandler, 1972) are type III and type A1γ, respectively. The *N*-acyl group of muramic acid in the peptidoglycan was of the acetyl type. Glucose, mannose, ribose and a trace amount of madurose (3-*O*-methyl-D-galactose) were detected as whole-cell sugars, but galactose, arabinose, xylose and rhamnose were not [whole-cell sugar pattern B of Lechevalier & Lechevalier (1970)]. The polar lipid profile was characterized by the presence of phosphatidylethanolamine and ninhydrin-positive glycopospholipids. This result suggests that the phospholipid pattern is type PIV of Lechevalier *et al.* (1977). The cellular fatty acid compositions of our isolates and *M. rosea* subsp. *rosea* JCM 3006^T are shown in Table 3. These organisms all contained hexadecanoic (*n*-16:0), heptadecanoic (*n*-17:0), 14-methylpentadecanoic (*i*-16:0) and 10-methylheptadecanoic (10-Me-17:0) acids as major components. The predominant menaquinones were MK-9(III,VIII-H₄), MK-9(H₂) and MK-9(H₀). The G + C content of the DNA from the isolates was 71–72 mol% (Table 4).

16S rDNA sequence

The almost complete 16S rDNA sequence of our isolate (strain DF-32^T), consisting of 1442 nucleotides, was compared with sequences from members of the genus *Microbispora* and the other strains selected from the family *Streptosporangiaceae* (Fig. 1). The data-set used for constructing the phylogenetic tree contained 1379 nucleotide positions for each sequence as a result of the elimination of gaps and ambiguous nucleotides

from the sequences between positions 57 and 1491 (*E. coli* position numbers). As shown in Fig. 1, members of the genus *Microbispora* formed a coherent cluster and the phylogenetic position of strain DF-32^T was entirely located within this cluster. The levels of 16S rDNA sequence similarity between strain DF-32^T and other members of the genus *Microbispora* ranged from 97.3 to 98.4%, whereas the level between this strain and *T. bispora* ATCC 19993^T (GenBank accession no. U58523) was 92.0%.

DNA–DNA hybridization

Table 4 shows results of DNA–DNA hybridization experiments among our isolates and *Microbispora* species. The levels of DNA relatedness between strains DF-28 and DF-32^T were 93–99%, whereas these isolates exhibited only 27–45% DNA relatedness to previously described *Microbispora* species. However, values of DNA relatedness among strains currently designated as *M. rosea*, except for two strains originally named *M. amethystogenes*, ranged from 51% to approximately 100% (mean 67%). *M. amethystogenes* JCM 3021 and *M. amethystogenes* subsp. *nonreducans* JCM 10122 exhibited high levels of DNA relatedness (more than 71%) to each other, but exhibited only 41–55% DNA relatedness (mean 48%) to other *M. rosea* strains.

DISCUSSION

Both strains, DF-28 and DF-32^T, showed morphological and chemotaxonomic features peculiar to the genera *Microbispora* (Kroppenstedt *et al.*, 1990; Miyadoh *et al.*, 1990) and *Thermobispora* (Wang *et al.*, 1996b). Although it is difficult to distinguish these two genera phenotypically, the phylogenetic analysis indicated that one of our isolates (strain DF-32^T) should

be classified as a member of the genus *Microbispora* (Fig. 1).

Comparisons with the descriptions of previously characterized species of the genus *Microbispora* show that the morphological features of our isolates are obviously different from those of *M. mesophila*, which forms a single spore on each sporophore and was recently transferred from the genus *Thermomonospora* (Zhang *et al.*, 1998). We compared our isolates with all of the remaining species in terms of physiological and biochemical properties and DNA–DNA reassociation. Both strains, DF-28 and DF-32^T, showed a similar pattern in physiological and biochemical tests (Table 2). As our isolates could not grow at 50 °C, they were clearly distinguishable from the thermophilic strains belonging to *M. rosea* subsp. *aerata* (previously described as *M. aerata*, *M. thermorosea* and *M. thermodiastatica*), all of which showed good growth at 55 °C. The isolates were also distinguishable from the remaining mesophilic strains belonging to *M. rosea* subsp. *rosea* (including the invalidly proposed subspecies '*M. rosea* subsp. *nonnitritogenes*' and '*M. amethystogenes* subsp. *nonreducans*') by a combination of biochemical properties, in particular the utilization of *myo*-inositol and the decomposition of testosterone, as shown in Table 2. In the DNA–DNA hybridization experiments, our isolates exhibited high levels of relatedness to each other (93–99%), whereas values for relatedness between the isolates and all of the strains used for comparison were only 45% or less (Table 4). Consequently, we propose a new species, *Microbispora corallina*, to accommodate the two strains DF-32^T (= JCM 10267^T) and DF-28 (= JCM 10266).

The results of our DNA–DNA hybridization experiments also provided information regarding the intra-generic classification of the genus *Microbispora*. Miyadoh *et al.* (1990) combined 10 species of this genus into a single species, *M. rosea*, on the basis of DNA relatedness. However, Wang *et al.* (1996a) doubted this conclusion for the following reasons: none of the similarity values for 16S rDNA sequences among *M. rosea* strains calculated by Wang *et al.* exceeded 97%, and 58 of the 90 DNA reassociation values obtained for the 10 strains were less than 60%. We recalculated the sequence-similarity values for sequences from *Microbispora* species obtained in this study and from GenBank/EMBL/DDBJ sequence databases. As a result, the sequence-similarity values among the members classified in *M. rosea* ranged from 97.0 to 99.6%; these results imply that species definition within the genus *Microbispora* should be based on DNA–DNA reassociation results according to the principle of Stackebrandt & Goebel (1994). Certainly, the results of Miyadoh *et al.* (1990) and this study produced homology values of less than 60% among strains of redefined *M. rosea*; this may be correlated to heterogeneity of their phenotypic characteristics. In our results, if strains JCM 3021 (*M. amethystogenes*) and JCM 10122 ('*M. amethystogenes* subsp.

nonreducans') are excluded, the values for DNA relatedness within each subspecies (*M. rosea* subsp. *rosea* or *M. rosea* subsp. *aerata*) and between the subspecies were greater than 57 and 51%, respectively. These values are thought to be rather low for considering them as a single species, but it is also the case that we cannot delineate the species because of the continuity of these values. However, *M. amethystogenes* and the informally proposed '*M. amethystogenes* subsp. *nonreducans*' should be placed in an independent species because of the significantly low values of DNA relatedness with other *M. rosea* strains (41–55%, mean 48%). '*M. amethystogenes* subsp. *nonreducans*' does not merit designation as an independent subspecies of *M. amethystogenes* because of the high values of DNA relatedness between the two strains (71–81%) (Table 4) and the almost identical pattern of their physiological and biochemical characteristics (Table 2).

Description of *Microbispora corallina* sp. nov.

Microbispora corallina (co.ra'l'li.na. L. fem. adj. *corallina* coral-coloured).

Substrate mycelia are coral pink to reddish brown and aerial mycelia are pink on most agar media. Either no pigments or faint, yellow, soluble pigments are produced. Spores arranged in longitudinal pairs are formed on short sporophores alternately branched from aerial hyphae. Spores are oval and have smooth surfaces. Decomposes casein and aesculin, but not adenine, elastin, guanine, hypoxanthine, keratin, testosterone, L-tyrosine, xanthine, xylan or DNA. Utilizes amygdalin, L-arabinose, arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, D-lactose, maltose, D-mannitol, D-mannose, D-melezitose, D-melibiose, methyl α -D-glucoside, D-ribose, salicin, D-sorbitol, L-sorbose, starch, sucrose, D-trehalose and D-xylose as sole carbon sources, but not adonitol, D-arabitol, dulcitol, iso-erythritol, inulin, D-raffinose, L-rhamnose or xylitol. Utilizes fumaric acid and succinic acid, but not benzoic acid, citric acid, mucic acid, oxalic acid or L-tartaric acid. Negative for iodine production. Reduces nitrate to nitrite. No growth occurs in the presence of 3% NaCl. Requires thiamine for growth. Mesophilic. The G+C content of the DNA ranges from 71 to 72 mol%. The type strain is strain DF-32^T (= JCM 10267^T).

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