A taxonomic review of the genus *Microbispora* and a proposal to transfer two species to the genus *Actinomadura* and to combine ten species into *Microbispora rosea*

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We conducted a taxonomic review of the genus *Microbispora* using chemotaxonomic and DNA–DNA hybridization techniques, and reached the following conclusions: *Microbispora viridis* should be transferred to the genus *Actinomadura* as *Actinomadura rugatobispora* comb. nov., nom. nov. (type strain SF2240 = IFO 14382 = JCM 3366) and *Microbispora echinospora* should be transferred to the genus *Actinomadura* as *Actinomadura echinospora* comb. nov. (type strain JCM 3148 = ATCC 27300). We also propose that *Microbispora rosea*, *Microbispora amethystogenes*, *Microbispora chromogenes*, *Microbispora diastatica*, *Microbispora indica*, *Microbispora karnatakensis* and *Microbispora parva* should be combined into the species *Microbispora rosea* subsp. *rosea* (type strain JCM 3006 = ATCC 12950), and that *Microbispora aerata*, *Microbispora thermodiastatica* and *Microbispora thermorosea* should be combined and transferred to the new subspecies *Microbispora rosea* subsp. *aerata* comb. nov. (type strain IFO 12581 = ATCC 15448). *Microbispora bispora* clearly differs from these ten strains at the species level.

Introduction

As originally described by Nonomura & Ohara (1957), the genus *Microbispora* (type species *M. rosea*) of the order *Actinomycetales* is characterized by the formation of longitudinally paired spores on the aerial mycelium. In the same year, the genus *Waksmania* (type species *W. rosea*) was independently proposed by Lechevalier & Lechevalier (1957). Henssen (1957) also described a new species, *Thermopolyspora bispora* (= *M. bispora*). These two genera are now regarded as synonyms of *Microbispora* (Lechevalier, 1965). Nonomura & Ohara (1960, 1969a, b) isolated several new species from Japanese soils (*M. amethystogenes*, *M. chromogenes*, *M. diastatica*, *M. parva*, *M. thermodiastatica* and *M. thermorosea*) by developing the dry heat method. Gerber & Lechevalier (1964) created a new species, *M. aerata*, to accommodate the producers of phenazines and phenoxazinones. Subsequently, Nonomura & Ohara (1971) proposed the name *M. echinospora* for organisms forming spiny ornamented spores. These authors stated that the spore-bearing characteristics of the species resembled those of the genus *Actinomadura*. In 1985, we reported a new taxon, *M. viridis*, for organisms bearing rugose ornamented spores and having a green aerial mycelium (Miyadoh *et al.*, 1985). Rao *et al.* (1987)

isolated two new species, *M. karnatakensis* and *M. indica*, from Indian soils. There are now 13 valid species in the genus *Microbispora*.

Several groups (Fischer *et al.*, 1983; Athalye *et al.*, 1985; Fowler *et al.*, 1985; Poschner *et al.*, 1985) have recently recognized that sporoactinomycetes with wall chemotype IIIB (a cell wall having meso-diaminopimelic acid as its major constituent and whole-cell hydrolysates containing madurose as a diagnostic sugar), such as the genera *Actinomadura*, *Microbispora* and *Microtetraspora*, could be divided into two aggregate groups using chemotaxonomic, molecular genetic and numerical phenetic methods. Goodfellow *et al.* (1988) proposed, unofficially, a new genus *Nonomuria* and recommended the transfer of the *Actinomadura pusilla* group to the new taxon. In 1989, we stated that the genus *Microtetraspora* (with the exception of *Microtetraspora viridis* and '*Microtetraspora caesia*') and the *Actinomadura pusilla* group should be classified in a single genus (Miyadoh *et al.*, 1989). Goodfellow (1989), in his introduction to the Maduromycetes in *Bergey's Manual of Systematic Bacteriology*, vol. 4, took account of these more recent findings in recommending that the genus *Actinomadura* be restricted to *Actinomadura madurae* and related

species (*A. madurae* group) and that *Actinomadura pusilla* and related taxa (*A. pusilla* group) merited separate generic status.

Between the publication of *Bergey's Manual of Determinative Bacteriology*, (8th edn) in 1974 and *Bergey's Manual of Systematic Bacteriology*, vol. 4 in 1989, the suprageneric and generic classification of actinomycetes has been revolutionized by chemical and genetic methods, especially ribosomal RNA sequencing analysis. Nonetheless, species-level classification remains equivocal in several genera. In this paper, we will conduct a taxonomic review of the genus *Microbispora*, using results from chemotaxonomic and DNA-DNA hybridization techniques to clarify the speciation of the genus.

Methods

Bacterial strains. Strains SF2240 and SF2292 were isolated by the dry heat method (Nonomura & Ohara, 1969a) from soil samples collected at Takayama, Gifu Prefecture, and Wakayama, Wakayama Prefecture, Japan, respectively. The type strains used in this study are listed in Table 1. These strains were obtained from the Japan Collection of Micro-organisms (JCM), Saitama, Japan; the Institute for Fermentation (IFO), Osaka, Japan; and the American Type Culture Collection (ATCC), Rockville, Md, USA.

Morphological, cultural and physiological characteristics. The general methods of taxonomic characterization have been described previously (Miyadoh *et al.*, 1987, 1989). For scanning electron microscopy, a spore-bearing agar block was fixed in osmium tetroxide fumes overnight, frozen in liquid nitrogen, and then freeze-dried. Each specimen was coated with gold-palladium by using an ion sputter

instrument and was then observed with an electron microscope (model JEM 100C-ASID; Japan Electron Optics Laboratory Co.). The temperature range for growth was observed on agar media consisting of 1% (w/v) starch, 0.2% yeast extract and 2% (w/v) agar, pH 7.0. Cells for chemical analysis were grown for 1–2 d in YD broth [1% (w/v) yeast extract, 1% (w/v) glucose, pH 7.0] on a rotary shaker (220 r.p.m.) at the temperature indicated below, harvested by centrifugation, and washed with distilled water. The cultivations were done at 55 °C for *M. bispora*, 47 °C and 37 °C for *M. aerata*, *M. thermodiastatica* and *M. thermorosea*, and at 28 °C for the remaining species.

Chemotaxonomic characterization. The methods used were similar to those described previously (Miyadoh *et al.*, 1989). Briefly, isomers of diaminopimelic acid and sugars in purified cell wall and whole-cell hydrolysates were determined by the methods of Becker *et al.* (1965) and Lechevalier (1968). Phospholipids were analysed by the procedure of Lechevalier *et al.* (1981). Menaquinones were analysed using mass spectrometry and high-performance liquid chromatography (Tamaoka *et al.*, 1983). The compositions of methyl-esterified cellular fatty acids were determined by gas chromatography with capillary columns. The mol% G + C values of the DNAs were calculated from their thermal denaturation temperatures (Marmur *et al.*, 1962) by using spectrophotometry in 0.1 × SSC (1 × SSC is 0.15 M-NaCl plus 0.015 M-sodium citrate, pH 7.0).

DNA-DNA hybridization. The DNA isolation procedure and the method of DNA-DNA hybridization were as described previously (Miyadoh *et al.*, 1989). The pelleted wet cells were frozen, ground into fine powder under liquid nitrogen using a blender and suspended in TES (0.2 M-Tris/HCl, 0.02 M-EDTA, 0.05 M-NaCl, pH 8.0) containing 1% (w/v) SDS at 40 °C. Total DNA was extracted with phenol, precipitated with ethanol, then spooled onto a glass rod. This total DNA was dissolved in 0.05 × TES and then treated with ribonuclease and protease. DNA probes (100 ng purified DNA per labelling reaction) were radiolabelled with 20 to 30 µCi [α -³²P]dCTP using a multiprimer DNA labelling kit (Amersham). Hybridizations were done on nylon membranes (Amersham) according to the dot blotting protocol of the supplier. Unlabelled target DNAs (0.6 µg) were spotted onto a membrane in triplicate, denatured with 1.5 M-NaCl/0.5 M-NaOH, neutralized with 1.5 M-NaCl/0.5 M-Tris/HCl/0.001 M-EDTA, pH 7.5, and then fixed to the membrane using an ultraviolet transilluminator. The blotted membranes were prehybridized with 10 to 15 ml of hybridization solution [3 × SSC/0.1% SDS containing denatured non-homologous DNA from calf thymus at a final concentration of 100 µg ml⁻¹ and 4 × Denhardt's solution (0.02% BSA, 0.02% polyvinylpyrrolidone and 0.02% Ficoll)] in a plastic bag at 70 °C for 1 to 2 h. Heat-denatured probe (50 ng) was then added to the bag. The reassociation reaction between the membrane-fixed DNA and ³²P-labelled DNA was done at 70 °C for 16 to 20 h. After hybridization, the membranes were washed once in 2 × SSC/0.1% SDS at 70 °C for 1 h. These membranes were air-dried, exposed to X-ray film at -80 °C overnight, then cut into individual dots in order to measure the radioactivity using a liquid scintillation counter. Hybridization experiments were repeated at least three times.

Results

Taxonomic features of *M. viridis* and *M. echinospora*

As shown in Tables 2 and 3, the chemotaxonomic features of the two strains (SF2240 and SF2292) of *M. viridis* [wall chemotype IIIB, type PI phospholipid pattern (no nitrogenous phospholipids present), MK-9

Table 1. Strains used in this study

Species	Strain designation
<i>Actinomadura citrea</i>	ATCC 27887
<i>Actinomadura malachitica</i>	JCM 3297
<i>Actinomadura verrucosospira</i>	JCM 3147
<i>Actinomadura pusilla</i>	ATCC 27296
<i>Actinomadura ferruginea</i>	JCM 3283
<i>Microbispora aerata</i>	IFO 12581
<i>Microbispora amethystogenes</i>	JCM 3021
<i>Microbispora bispora</i>	ATCC 19993
<i>Microbispora bispora</i>	JCM 3082*
<i>Microbispora chromogenes</i>	JCM 3022
<i>Microbispora diastatica</i>	JCM 3023
<i>Microbispora echinospora</i>	JCM 3148
<i>Microbispora indica</i>	ATCC 35926
<i>Microbispora karnatakensis</i>	ATCC 35927
<i>Microbispora parva</i>	JCM 3024
<i>Microbispora rosea</i>	JCM 3006
<i>Microbispora thermodiastatica</i>	IFO 14046
<i>Microbispora thermorosea</i>	IFO 14047
<i>Microbispora viridis</i>	SF2240 (IFO 14328)
<i>Microbispora viridis</i>	SF2292* (IFO 14697)

* These two strains are the only ones studied that are not type strains.

Table 2. Chemotaxonomic characteristics and growth temperature requirements of strains of *Microbispora* species

All strains had wall chemotype IIIB; cell walls having *meso*-diaminopimelic acid as a major constituent and whole-cell hydrolysates containing madurose as a diagnostic sugar.

Strain		Chemotaxonomic marker										
		Mol% G + C	Phospholipid*	Menaquinone†, MK-9%					Growth at:			
				(H ₀)	(H ₂)	(H ₄)	(H ₆)	(H ₈)	25 °C	35 °C	55 °C	60 °C
<i>M. rosea</i>	JCM 3006	71	PIV	21	49	30			+	+	—	—
<i>M. amethystogenes</i>	JCM 3021	71	PIV	21	54	21	2		+	+	—	—
<i>M. chromogenes</i>	JCM 3022	71	PIV	11	34	50	3		+	+	—	—
<i>M. diastatica</i>	JCM 3023	71	PIV	26	51	23			+	+	—	—
<i>M. indica</i>	ATCC 35926	71	PIV	15	43	40	1		+	+	—	—
<i>M. karnatakensis</i>	ATCC 35927	71	PIV	15	32	46	5		+	+	—	—
<i>M. parva</i>	JCM 3024	71	PIV	42	45	12			+	+	—	—
<i>M. aerata</i>	IFO 12581	72	PIV	52	36	9			—	+	+	—
				40	36	20	2					
<i>M. thermodiastatica</i>	IFO 14046	72	PIV	8	44	43	3		—	+	+	—
				9	33	47	9					
<i>M. thermorosea</i>	IFO 14047	72	PIV	2	14	57	22		—	+	+	—
				5	20	51	20					
<i>M. bispora</i>	ATCC 19993	71	PIV ^a	75	19	4			—	—	+	+
<i>M. bispora</i>	JCM 3082	71	PIV ^a	55	32	11	2		—	—	+	+
<i>M. viridis</i>	SF2240	73	PI ^b			1	64	35	+	+	—	—
<i>M. viridis</i>	SF2292	73	PI			11	73	15	+	+	—	—
<i>M. echinospora</i>	JCM 3148	74	PI			1	97	2	+	+	—	—

* Type PI phospholipid, no nitrogenous phospholipids present; type PIV phospholipid, glucosamine-containing phospholipids present. ^aWhile the strains of genus *Microbispora* with type PIV phospholipid pattern contained large quantities of glucosamine-containing phospholipids, the two strains of *M. bispora* contained only small amounts. All of the strains with type PIV phospholipid contained phosphatidylethanolamine, but none contained phosphatidylglycerol. ^bIn a previous paper (Miyadoh *et al.*, 1985), we described incorrectly that *M. viridis* SF2240 had a type PIV phospholipid pattern.

† MK-9 (H₂), menaquinones having two of the nine isoprene units hydrogenated. No mycolic acids were formed by any strains. Menaquinones of *M. aerata*, *M. thermodiastatica* and *M. thermorosea* were analysed at the two cultivation conditions of 37 °C for 2 d (upper line) and 47 °C for 1 d (lower line).

(H₆) (hexahydrogenated menaquinones with nine isoprene units) as the predominant menaquinone, 16:0 (palmitic acid), 18:1 (oleic acid) and 10Me-18 (tuberculoostearic acid) as the major fatty acids, and 73 mol% G + C] were identical to those of the *A. madurae* group (i.e. *A. citrea* ATCC 27887 and *A. verrucosospora* JCM 3147) (Goodfellow, 1989), and were clearly different from those of typical *Microbispora* species. Also, on the basis of the DNA–DNA hybridization data shown in Table 4, these two strains were more closely related to strains of the *A. madurae* group (homology value 18 to 22%; mean 19.5%) than to the other *Microbispora* strains (homology value 4 to 12%; mean 7.1%).

The chemotaxonomic features of *M. echinospora* JCM 3148 are summarized as follows: wall chemotype IIIB, type PI phospholipid pattern, MK-9 (H₆) as predominant menaquinone, 17:0, iso-18, 10Me-17 and 10Me-18 as the major fatty acids, and 74 mol% G + C. These features showed a close similarity to *A. madurae* group strains (ATCC 27887 and JCM 3147) and to *M. viridis* strains (SF2240 and SF2292). The fatty acid

profile of *M. echinospora* JCM 3148 (Table 3) was considered to be intermediate between *Actinomadura* and *Microbispora*, and the DNA homology values indicated a moderately close relationship to strains of the *A. madurae* group and *M. viridis* (homology value 13 to 15%; mean 14.0%).

Taxonomic features of the other *Microbispora* species

As shown in Table 2, the other species of the genus *Microbispora* can be separated into two groups according to their temperature range for growth, either mesophilic or thermophilic (growth at 55 °C). Mesophilic strains, *M. rosea* JCM 3006, *M. amethystogenes* JCM 3021, *M. chromogenes* JCM 3022, *M. diastatica* JCM 3023, *M. indica* ATCC 35926, *M. karnatakensis* ATCC 35927 and *M. parva* JCM 3024, were closely related to each other on the basis of DNA homology (homology value 48 to 93%; mean 64.7%) and also in their chemotaxonomy [wall chemotype IIIB, type PIV phospholipid pattern (glucosamine-containing phospholipids present), MK-9 (H₀), MK-9 (H₂) and MK-9 (H₄) as the predominant

Table 3. Fatty acid composition of the test strains

Abbreviations for fatty acids are illustrated by the following examples: *straight-chain saturated* – hexadecanoic acid (palmitic acid, 16:0); *iso-branched* – 14-methylpentadecanoic acid (i-16); *anteiso-branched* – 14-methylhexadecanoic acid (a-17); *10-methyl-branched* – 10-methyloctadecanoic acid (tuberculostearic acid, 10Me-18); *monounsaturated* – octadecenoic acid (oleic acid, 18:1). Fatty acids of *M. aerata*, *M. thermodiastatica* and *M. thermorosea* were analysed at the two cultivation conditions of 37 °C for 2 d (upper line) and 47 °C for 1 d (lower line).

Strain		Fatty acid composition (%)											
		Straight chain				Branched chain				10-Methyl-branched chain			Unsaturated
		15:0	16:0	17:0	18:0	i-15	i-16	i-18	a-17	10Me-16	10Me-17	10Me-18	16:1 18:1
<i>M. rosea</i>	JCM 3006	2	9	10	3	2	43		9	2	12	1	3 3
<i>M. amethystogenes</i>	JCM 3021	4	10	5	3	5	18		14	7	15	6	7 5
<i>M. chromogenes</i>	JCM 3022	3	6	7	2	4	35	1	6	9	14	4	3 2
<i>M. diastatica</i>	JCM 3023	4	7	6	2	3	30		10	9	17	3	5 1
<i>M. indica</i>	ATCC 35926	3	7	4	2	2	32		11	8	13	3	5 5
<i>M. karnatakensis</i>	ATCC 35927	3	8	7	2	1	39	4	9	4	10	3	3 3
<i>M. parva</i>	JCM 3024	2	7	2	2	4	20		4	16	15	8	4 5
<i>M. aerata</i>	IFO 12581	1	7	6	1		42		8	5	16	5	
		7	7	16	2	2	38	3	6	1	6	2	
<i>M. thermodiastatica</i>	IFO 14046		7	2	2	1	32	3	21	5	9	6	
		1	8	7	3	1	45	7	10	1	4	2	1 1
<i>M. thermorosea</i>	IFO 14047		7	1	2		32	3	25	5	9	6	
		1	9	9	3	2	23	8	21	1	3	4	1 1
<i>M. bispora</i>	ATCC 19993	1	6	2	3		55	6	8		4	3	2 8
<i>M. bispora</i>	JCM 3082	1	6	3	3		54	7	8	2	3	3	2 6
<i>A. pusilla</i>	ATCC 27296	5	4	4	1	4	21		10	2	24	4	3 4
<i>A. ferruginea</i>	JCM 3283	2	4	8	1	1	18	7	22	1	17	5	3 2
<i>M. viridis</i>	SF2240		28		11		5					18	9 27
<i>M. viridis</i>	SF2292		28		6		5					23	10 27
<i>M. malachitica</i>	JCM 3297		24	2	7		8		1		1	20	5 29
<i>M. verrucosospora</i>	JCM 3147		35	1	7		3			1		24	5 22
<i>M. citrea</i>	ATCC 27887		34	2	6		3		1	1	1	22	4 22
<i>M. echinospora</i>	JCM 3148	5	10	22	7		18	7	2	1	13	13	

Table 4. DNA homologies of strains of *Microbispora* and related strains

Source of unlabelled DNA		Percentage hybridization with ³² P-labelled DNA from:													
		JCM 3006	JCM 3021	JCM 3022	JCM 3023	ATCC 35926	ATCC 35927	JCM 3024	IFO 12581	IFO 14046	IFO 14047	ATCC 19993	SF2240	SF2292	JCM 3148
<i>M. rosea</i>	JCM3006	100	48	61	51	88	69	62	38	42	41	15	6	5	8
<i>M. amethystogenes</i>	JCM 3021	57	100	64	64	60	56	54	44	51	48	14	9	6	11
<i>M. chromogenes</i>	JCM 3022	68	55	100	74	81	71	60	43	45	44	16	9	5	9
<i>M. diastatica</i>	JCM 3023	68	58	93	100	82	91	59	41	57	55	15	8	6	10
<i>M. indica</i>	ATCC 35926	83	48	68	54	100	55	61	40	38	37	16	7	5	9
<i>M. karnatakensis</i>	ATCC 35927	52	54	61	78	60	100	55	39	40	44	14	6	6	8
<i>M. parva</i>	JCM 3024	78	56	74	69	65	54	100	50	60	52	18	10	7	9
<i>M. aerata</i>	IFO 12581	45	47	54	53	51	51	57	100	94	68	18	7	6	10
<i>M. thermodiastatica</i>	IFO 14046	46	40	43	37	40	46	46	68	100	64	19	9	7	7
<i>M. thermorosea</i>	IFO 14047	52	48	48	42	43	45	47	59	80	100	17	8	6	8
<i>M. bispora</i>	ATCC 19993	14	16	17	18	15	15	18	18	22	20	100	6	5	6
<i>M. bispora</i>	JCM 3082	13	14	15	16	17	13	17	17	17	19	55	5	4	6
<i>M. viridis</i>	SF2240	7	12	8	8	9	8	10	8	10	8	6	100	85	14
<i>M. viridis</i>	SF2292	7	8	6	7	8	5	9	5	8	7	5	82	100	13
<i>M. echinospora</i>	JCM 3148	7	5	7	10	6	9	9	10	6	6	6	15	13	100
<i>A. citrea</i>	ATCC 27887	6	7	8	10	8	10	8	7	8	9	6	22	19	14
<i>A. verrucosospora</i>	JCM 3147	7	5	6	8	6	8	6	9	8	9	7	19	18	15
<i>A. pusilla</i>	ATCC 27296	15	16	14	16	13	16	13	15	14	13	12	8	6	8
<i>A. ferruginea</i>	JCM 3283	16	17	15	16	18	15	15	16	17	15	14	12	7	10

menaquinones, iso-16 and 10Me-17 as the major fatty acids, and 71 to 72 mol% G + C]. These chemotaxonomic features were virtually the same as those of the *A. pusilla* group (Goodfellow, 1989). The three thermophilic strains, namely *M. aerata* IFO 12581, *M. thermodiastatica* IFO 14046 and *M. thermorosea* IFO 14047, exhibited higher DNA homologies with one another (homology value 59 to 94%; mean 72.2%) than with the mesophilic strains (homology value 37 to 60%; mean 46.0%).

The two strains of *M. bispora* (ATCC 19993 and JCM 3082) clearly differed from these ten strains. Chemotaxonomically, the *M. bispora* strains had the following characteristics: MK-9 (H_0) as predominant menaquinone, with glucosamine-containing phospholipids and 10-methylfatty acids in low amounts. They were able to grow at 65 °C but not at 35 °C.

Discussion

Eleven species of the genus *Microbispora* are characterized by smooth-surfaced spores (smooth spore species), as shown in Fig. 1(a, b). *M. echinospora* forms spinose spores (Fig. 1c) and *M. viridis* forms rugose spores (Fig. 1d). The aerial mycelia of *M. echinospora* and *M. viridis* differentiate into a non-sporogenous main axis with sporogenous side branches arising from the axis, whereas those of the smooth-spored species bear spores on a wide range of hyphae (Nonomura & Ohara, 1971). The number of spores per chain in *M. echinospora* and *M. viridis* is sometimes three (mostly two); the smooth-spored species never have three spores per chain. Recently, Nonomura (1989) reported that *M. echinospora* formed paired spores in clusters. As described above, *M. echinospora* and *M. viridis* are clearly distinguished from the smooth-spored species in morphology, despite their characteristic formation of paired spores on aerial hyphae. Chemotaxonomically (Table 2), the smooth-spored species have a type PIV phospholipid pattern, with MK-9 (H_0), MK-9 (H_2) and MK-9 (H_4) as the predominant menaquinones, while the spinose- and rugose-spored species have a type PI phospholipid pattern, with MK-9 (H_6) as the major menaquinone.

In *Bergey's Manual of Systematic Bacteriology*, vol. 4 (Nonomura, 1989), the genus *Microbispora* was defined on the basis of a combination of morphology and the following chemical properties: cell walls contain *N*-acetylated muramic acid and major amounts of meso-diaminopimelic acid, but no characteristic sugars; whole-cell hydrolysates contain madurose; menaquinones are partially saturated, with MK-9 (H_4) as major isoprenologue; major phospholipids include

phosphatidylcholine and unknown glucosamine-containing compounds, but no phosphatidylglycerol is present.

Taxonomic status of M. viridis and M. echinospora

The chemotaxonomic data of *M. viridis* are clearly different from those of the genus *Microbispora* mentioned above. Our results indicate that *M. viridis* should be classified as a member of the *A. madurae* group. In the genus *Actinomadura*, there are no previously described species with the same morphology (greenish aerial masses and longitudinally paired spores having rugose surfaces with vertical ridges) as strains SF2240 and SF2292. Accordingly, we propose the transfer of *Microbispora viridis* Miyadoh *et al.* to the genus *Actinomadura* Lechevalier and Lechevalier (1970) as *Actinomadura rugatobispota* (ru.ga.to.bi'spo.ra. L. adj. *rugato* wrinkled; Gr. adj. *bi* paired; Gr. n. *spora* a spore; *rugatobispota* wrinkled paired-spores, referring to spore morphology) comb. nov., nom. nov. with strain SF2240 (= IFO 14328) as the type strain. In a previous paper (Miyadoh *et al.*, 1989), we proposed the transfer of *Microtetrastora viridis* to the genus *Actinomadura* as *A. viridis* comb. nov. According to Rule 41a of the International Code of Nomenclature of Bacteria (1976, revision), authors are obliged to substitute a new specific epithet if the result of the combination would be a new homonym. Since this would be the case here, we have adopted *A. rugatobispota* as the new name.

Also, the chemical data of *Microbispora echinospora* indicate that it should be classified as a member of the *A. madurae* group, although it is not typical of that group. In the genus *Actinomadura*, there are no previously described species such as strain JCM 3148, which was characterized by a pinkish aerial mass and paired spores with a spinose surface. Therefore, we propose the transfer of *Microbispora echinospora* Nonomura and Ohara to the genus *Actinomadura* Lechevalier and Lechevalier as *Actinomadura echinospora* comb. nov., with strain JCM 3148 (= ATCC 27300) as the type strain. Goodfellow & Pirouz (1982) found using numerical classification that three strains of *Microbispora* were recovered in a well-defined cluster, whereas *M. echinospora* and *M. thermodiastatica* showed little affinity to one another or to the *M. rosea* cluster. Poschner *et al.* (1985) reported that *M. echinospora* was more closely associated with the *A. madurae* group than with members of the genus *Microbispora*. With the exception of *M. thermodiastatica*, our studies reconfirmed these results.

With the transfer of *M. viridis* and *M. echinospora*, *Actinomadura* becomes a diverse genus both in spore-chain morphology [ranging from the paired spores shown

Fig. 1. Scanning electron micrographs of type strains of *Microbispora* species. (a) *M. rosea* JCM 3006, (b) *M. aerata* IFO 12581, (c) *M. echinospora* JCM 3148, (d) *M. viridis* SF2240. Bars, 2 µm.

in these species to the long spore chains observed in *A. kijaniata* (Horan & Brodsky, 1982)] and in spore-surface morphology (smooth, warty, spinose and rugose). Other taxonomic properties of these strains are summarized in Table 5. On the other hand, as a result of the transfer of the two species, the genus *Microbispora* becomes a homogeneous and well-defined taxon in morphology (smooth paired-spore species) and in chemotaxonomy.

Relationships among smooth-spored species of the genus Microbispora

It is remarkable that ten type strains of smooth-spored species showed high homology values (mean 56.5%) in every combination of probes and targets (Table 4, Fig. 2), whilst exhibiting very low homology (mean 16.4%) with two strains of *M. bispora*, which also has smooth spores. These results lead us to the conclusion that

smooth-spored species of *Microbispora*, with the exception of *M. bispora*, are so closely related to one another that they should be united into one species. The homology values between the thermophiles and the mesophiles described above are believed to differ at the subspecies level.

In *Bergey's Manual of Systematic Bacteriology*, Johnson (1984) states that DNA homology values in the range of 60 to 70% would appear to be a transitional point for delineating species. According to this guideline, the homology values among these ten species are relatively low for them to be regarded as synonyms. However, we believe that the clear discontinuity in the two mean values (56.5% and 16.4%) for DNA homology must represent a classificatory point at the species level. Accordingly, we propose that *Microbispora rosea* Nonomura and Ohara, *Microbispora amethystogenes* Nonomura and Ohara, *Microbispora chromogenes* Nonomura and Ohara, *Microbispora diastatica* Nonomura and Ohara, *Microbispora indica* Rao *et al.*,

Fig. 2. Typical autoradiography of DNA-DNA dot hybridization of type strains of genus *Microbispora*. Target DNAs were spotted in triplicate in the following order: *M. rosea* JCM 3006, *M. chromogenes* JCM 3022, *M. diastatica* JCM 3023, *M. aerata* IFO 12581, *M. thermodiastatica* IFO 14046, *M. bispora* ATCC 19993, *M. viridis* SF2240, *M. echinospora* JCM 3148, *A. ferruginea* JCM 3282 and *A. verrucosospota* JCM 3147. The sources of ^{32}P -labelled DNA used were *M. rosea* JCM 3006 (a), *M. aerata* IFO 12581 (b), *M. viridis* SF2240 (c) and *M. bispora* ATCC 19993 (d).

Microbispora karnatakensis Rao *et al.* and *Microbispora parva* Nonomura and Ohara are combined into the species *Microbispora rosea* subsp. *rosea*, with JCM 3006 (= ATCC 12950) as the type strain, and that *Microbispora aerata* Graber and Lechevalier, *Microbispora thermodiastatica* Nonomura and Ohara and *Microbispora thermorosea* Nonomura and Ohara should be combined and transferred to the new subspecies *Microbispora rosea* subsp. *aerata* comb. nov., with IFO 12581 (= ATCC 15448) as the type strain.

Microbispora species with smooth spores have generally been distinguished mainly by growth temperature requirements and a few physiological tests. In reference to the classification of the genus *Microbispora*, Williams & Wellington (1981) stated that the validity of separating some species on the basis of one or two characteristics is questionable. Goodfellow & Cross (1984) agreed with this comment. The physiological characteristics of *M. rosea*, *M. amethystogenes*, *M. chromogenes*, *M. diastatica*, *M. parva*, *M. aerata*, *M. thermodiastatica* and *M. thermorosea* are similar (Nonomura, 1989), and the differences among these species, such as iodinin production, starch hydrolysis and nitrate reduction, are believed to occur at the strain and not the species level. The emended species *M. rosea* is characterized by its morphology, pinkish aerial mass and requirement for B vitamins (particularly thiamin) for growth (Nonomura & Ohara, 1957). As indicated in Table 2, growth temperature requirements were shown to be a diagnostic criterion for species and subspecies identification within the genus *Microbispora*. The menaquinone compositions of these ten strains showed variation in hydrogenation, but we decided that these variations could be accounted for by the versatility of the species. Also, the menaquinone compositions (Table 2) of *M. aerata*, *M. thermodiastatica* and *M. thermorosea* were not significantly affected by cultivation conditions (incubation at 37 °C for 2 d and 47 °C for 1 d), whereas their fatty acid compositions (Table 3) changed partially, for example the amount of 10-methylfatty acids decreased during growth at the higher temperature.

M. bispora is distinguished from emended *M. rosea* by its growth temperature requirement and whitish aerial mass. Greiner-Mai *et al.* (1987), who studied the morphology and biochemistry of thermophilic actinomycetes, reported that *M. bispora* showed some physiological differences from *M. aerata* and *M. thermodiastatica* (for example, a striking resistance toward lysozyme). Poschner *et al.* (1985), who did DNA-DNA reassociation studies, reported that *M. chromogenes* showed distinctly high homologies with *M. amethystogenes*, *M. thermodiastatica* and *M. aerata*, whilst *M. chromogenes* and *M. bispora* shared a low homology. These results are consistent with our data.

Table 5. Taxonomic characteristics of some strains of *Microbispora*

Test	<i>M. viridis</i> SF2240	<i>M. viridis</i> SF2292	<i>M. echinospora</i> JCM 3148	<i>M. rosea</i> JCM 3006
No. of spores per chain*	2 (1, 3)	2 (1, 3)	2 (1, 3)	2 (1)
Spore-bearing hyphae	Differentiated	Differentiated	Differentiated	Non-differentiated
Spore surface	Rugose	Rugose	Spinose	Smooth
Aerial mass colour†	Green (24ge)	Green (24ge)	Pink (5ca)	Pink (6ca)
Liquefaction of gelatin	+	+	+	—
Hydrolysis of starch	+	+	—	—
Reduction of nitrate	—	+	—	+
Peptonization of milk	+	+	+	—
Coagulation of milk	—	—	—	—
B vitamin requirement	—	—	+	+
Formation of melanoid pigment	—	—	—	—
Utilization of:				
D-Glucose	+	+	+	+
Glycerol	+	+	(+)	+
D-Fructose	+	+	+	+
D-Xylose	—	—	+	(+)
L-Arabinose	(+)	—	+	+
D-Mannitol	+	+	+	+
myo-Inositol	—	—	—	—
L-Rhamnose	+	+	—	+
Raffinose	—	—	—	—
Sucrose	(+)	—	+	(+)

+, Positive; (+), weakly positive; —, negative.

* The number of spores per chain is usually two (rarely one or three).

† The colour codes were taken from the Color Harmony Manual (Jacobson *et al.*, 1958).

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