Actinomadura malachitica and *Microtetraspora viridis* Are Synonyms and Should Be Transferred as *Actinomadura viridis* comb. nov.

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We compared strain SF2461, *Microtetraspora viridis* JCM 3112^T (T = type strain), *Microtetraspora viridis* subsp. *intermedia* JCM 3113^T, and *Actinomadura malachitica* JCM 3297^T by using morphological, cultural, physiological, and chemotaxonomic characteristics, as well as deoxyribonucleic acid homology. All of our results indicated that these four strains belong to the same species and that they are far from the other type strains of *Microtetraspora* species. Therefore, we propose that *Actinomadura malachitica* is a synonym of *Microtetraspora viridis* and that *Microtetraspora viridis* should be transferred to the genus *Actinomadura* as *Actinomadura viridis* comb. nov., with strain JCM 3112 (= ATCC 27103) as the type strain.

During classification studies of an actinomycete designated strain SF2461, which bore greenish aerial masses, we found that this organism was closely related to the descriptions of *Microtetraspora viridis* and *Actinomadura malachitica*. The name *Microtetraspora viridis* was proposed by Nonomura and Ohara in 1971 (16). Independently in 1972, Lavrova et al. (8) published the name *A. malachitica*. Both of these names are valid and appear on the Approved Lists of Bacterial Names (22). It has recently been recognized by some researchers (1, 4, 5, 19) that the genus *Actinomadura* is heterogeneous, and this taxon has therefore been divided into two aggregate groups (group 1, the *Actinomadura madurae*-*A. malachitica*-*Actinomadura citrea* group, and group 2, the *Actinomadura pusilla*-*Actinomadura ferruginea* group). Also, certain species of *Microbispora*, *Microtetraspora*, and *Nocardiopsis* have been transferred to the genus *Actinomadura*. Lechevalier et al. (11), who studied phospholipid compositions, proposed that all *Actinomadura*-like organisms with a type PI phospholipid should be placed in the genus *Actinomadura* and that those organisms with a type PIV phospholipid should be placed in the genus *Microtetraspora*. Chemotaxonomic and deoxyribonucleic acid (DNA) data for sporoactinomycetes with wall chemotype III have not always coincided with the classification system established by using traditional phenotypic characters, especially morphology. We compared strain SF2461 with *A. malachitica* JCM 3297^T (T = type strain), *Microtetraspora viridis* JCM 3112^T, and *Microtetraspora viridis* subsp. *intermedia* JCM 3113^T by using various criteria, including DNA-DNA hybridization, in order to clarify the taxonomic relationships of these organisms. In addition, we studied the taxonomic status of the genus *Microtetraspora* Thiemann et al. (24) by using the type strains of species belonging to the genus *Microtetraspora* and related genera.

MATERIALS AND METHODS

Bacterial strains. Strain SF2461 was isolated in February 1985 in our laboratory by the dry heating method developed by Nonomura and Ohara (15). The soil sample which we used was collected in Yokohama, Japan. The type strains used in this study are listed in Table 1. These strains were

obtained from the Japan Collection of Microorganisms, Saitama, Japan, the Institute for Fermentation, Osaka, Japan, and the American Type Culture Collection, Rockville, Md. The abbreviations used for genus names in Fig. 3 are those proposed by Rogosa et al. (20).

Morphological, cultural, and physiological characteristics. The methods which we used were generally similar to those described previously (13, 14). Briefly, methods adopted by the International *Streptomyces* Project (21) were used for taxonomic characterization and the carbohydrate utilization studies. Cultural and physiological characteristics were determined by using the methods of the International *Streptomyces* Project and of Waksman (26). Observations were made after incubation at 28°C for 2 to 4 weeks. Color determinations were made by comparing the cultures with color chips from the *Color Harmony Manual*, 4th ed. (7). For scanning electron microscopy, a spore-bearing agar block (diameter, 4 mm) 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

TABLE 1. Strains used in this study

Species or subspecies	Strain designation
Our isolates	
" <i>Actinomadura viridis</i> "	SF2461 (= IFO 14771)
<i>Actinomadura atramentaria</i>	SF2197 ^T (= JCM 6250 ^T)
<i>Microbispora viridis</i>	SF2240 ^T (= IFO 14328 ^T)
Type strains	
<i>Actinomadura citrea</i>	ATCC 27887 ^T
<i>Actinomadura ferruginea</i>	JCM 3283 ^T
<i>Actinomadura helvata</i>	JCM 3143 ^T
<i>Actinomadura macra</i>	ATCC 31286 ^T
<i>Actinomadura malachitica</i>	JCM 3297 ^T
<i>Actinomadura pusilla</i>	ATCC 27296 ^T
<i>Actinomadura verrucosospora</i>	JCM 3147 ^T
<i>Microbispora rosea</i>	JCM 3006 ^T
<i>Microtetraspora glauca</i>	JCM 3300 ^T
<i>Microtetraspora fusca</i>	ATCC 23058 ^T
<i>Microtetraspora niveoalba</i>	JCM 3149 ^T
<i>Microtetraspora viridis</i>	JCM 3112 ^T
<i>Microtetraspora viridis</i> subsp.	
<i>intermedia</i>	JCM 3113 ^T
" <i>Microtetraspora caesia</i> "	ATCC 31295 ^T
<i>Streptosporangium roseum</i>	IFO 3776 ^T
<i>Streptomyces griseus</i>	IFO 12875 ^T

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TABLE 2. Morphological, cultural, and chemotaxonomic characteristics of strain SF2461 and related strains^a

Strain	Aerial mass color	Spore chain morphology ^b		Spore surfaces	Fragmentation of mycelia ^c	Wall chemo-type	Guanine-plus-cytosine content (mol%)	Phospholipid type	Menaquinone composition (%)			
		Four spores	Long straight chains						MK-9 (H ₂)	MK-9 (H ₄)	MK-9 (H ₆)	MK-9 (H ₈)
SF2461	Green (22ec-24ge)	+	+	Smooth	—	IIIB	72	PI	2	9	60	29
<i>A. malachitica</i> JCM 3297 ^T	Green (24ec-24ge)	+	+	Smooth	+	IIIB	72	PI	2	20	56	22
<i>Microtetraspora viridis</i> JCM 3112 ^T	Green (24ec)	+	—	Smooth	—	IIIB	72	PI	1	12	60	27
<i>Microtetraspora viridis</i> subsp. <i>intermedia</i> JCM 3113 ^T	Green (24ec-24ig)	+	+	Smooth	—	IIIB	72	PI	6	32	51	8

^a For fatty acid compositions see Table 5.^b Strains SF2461, JCM 3297^T, and JCM 3113^T had two types of spore chains, as shown in Fig. 1A.^c Fragmentation of vegetative mycelia was observed under submerged growth conditions.

ion sputter instrument (model JFC-1100; Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan) and was then observed with an electron microscope (model JEM 100C-ASID; Japan Electron Optics Laboratory Co., Ltd.).

Chemical analysis of cells. The presence and form of diaminopimelic acid and the presence of sugars in purified cell wall and whole-cell hydrolysates were determined by the methods of Becker et al. (2) and Lechevalier (9). Phospholipids were analyzed by the procedure of Lechevalier et al. (10, 11). Menaquinone samples were prepared by extraction with chloroform-methanol (2:1) from dried cells and subsequent purification by thin-layer chromatography. They were analyzed by using a mass spectrometer (model M-80B; Hitachi, Ltd., Tokyo, Japan) and by high-performance liquid chromatography (model LC-6A; Shimadzu Corp., Kyoto, Japan) (3, 23). The compositions of the methyl esters of cellular fatty acids (18) were determined by gas chromatography (model HP-5880A; Hewlett-Packard Co., Avondale, Pa.), using SPB-1 and Supelcowax 10 capillary columns with a bacterial fatty acid methyl ester mixture (type 4-7080; Supelco, Inc., Bellefonte, Pa.) as the standard. The guanine-plus-cytosine contents of the DNAs were calculated from

their thermal denaturation temperatures (12) by spectrophotometry (model Response TM; Gilford Instrument Laboratories, Inc., Oberlin, Ohio) in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0), using DNA from *Micrococcus luteus* JCM 1464^T as the standard (72 mol%).

Preparation of DNA. Cells for DNA studies were grown in YD broth (1% yeast extract, 1% glucose, pH 7.0) on a rotary shaker (220 rpm) at 28°C for 2 to 3 days, harvested by centrifugation, and washed with distilled water. The frozen cells were then ground into fine powder under liquid nitrogen by using a blender (model Homogenizer AM-3; Nihon Seiki Kaisha, Ltd., Tokyo, Japan) and suspended in TES [0.2 M tris(hydroxymethyl)aminomethane hydrochloride, 0.02 M ethylenediaminetetraacetic acid, 0.05 M NaCl, pH 8.0] containing 1% sodium dodecyl sulfate at 40°C. The total DNA was extracted twice with phenol saturated with TES, precipitated with 2 volumes of ethanol, and then spooled with a glass rod. The total DNA was dissolved in 0.05× TES and then treated with ribonuclease A (Sigma Chemical Co., St. Louis, Mo.) and pronase K (Kaken Seiyaku Co. Ltd., Tokyo, Japan) according to the directions of the suppliers. When this procedure was used, DNA fragments that were 5

TABLE 3. Physiological characteristics of strain SF2461 and related strains

Characteristic	Strain SF2461	<i>A. malachitica</i> JCM 3297 ^T	<i>Microtetraspora viridis</i> JCM 3112 ^T	<i>Microtetraspora viridis</i> subsp. <i>intermedia</i> JCM 3113 ^T
Liquefaction of gelatin	+	—	+	+
Hydrolysis of starch	+	—	+	+
Reduction of nitrate	—	—	—	—
Peptonization of milk	+	+	+	+
Coagulation of milk	—	—	—	—
Growth at 42°C	+	+	+	+
5% NaCl tolerance	+	+	+	+
Formation of melanoid pigment	—	—	—	—
Utilization of:				
D-Glucose	+	+	+	+
Glycerol	(+)	(+)	(+)	(+)
D-Fructose	(+)	(+)	(+)	(+)
D-Xylose	(+)	(+)	(+)	(+)
L-Arabinose	(+)	(+)	(+)	(+)
D-Mannitol	+	+	+	+
myo-Inositol	—	—	—	—
L-Rhamnose	+	+	+	+
Raffinose	—	—	—	—
Sucrose	(+)	(+)	(+)	(+)

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

TABLE 4. DNA homologies of strain SF2461 and related strains

Source of unlabeled DNA	% Hybridization with ³² P-labeled DNA from:			
	Strain SF2461	<i>A. malachitica</i> JCM 3297 ^T	<i>Microtetraspora viridis</i> JCM 3112 ^T	<i>Microtetraspora viridis</i> subsp. <i>intermedia</i> JCM 3113 ^T
Strain SF2461	100	72	71	69
<i>A. malachitica</i> JCM 3297 ^T	69	100	59	58
<i>Microtetraspora viridis</i> JCM 3112 ^T	81	68	100	93
<i>Microtetraspora viridis</i> subsp. <i>intermedia</i> JCM 3113 ^T	84	71	96	100
<i>Actinomadura citrea</i> ATCC 27887 ^T	16	20	16	17
<i>Actinomadura verrucospora</i> JCM 3147 ^T	18	18	17	16
<i>Actinomadura atramentaria</i> SF2197 ^T	20	22	23	22
<i>Microbispora viridis</i> SF2240 ^{Ta}	15	17	16	15
<i>Microtetraspora glauca</i> JCM 3300 ^T	7	9	7	8
<i>Microtetraspora fusca</i> ATCC 23058 ^T	8	9	9	8
<i>Microtetraspora niveoalba</i> JCM 3149 ^T	8	7	6	6
" <i>Microtetraspora caesia</i> " ATCC 31295 ^T	11	12	9	10
<i>Microbispora rosea</i> JCM 3006 ^T	9	10	7	7
<i>Streptomyces griseus</i> IFO 12875 ^T	6	7	5	6

^a We are planning to propose the transfer of *Microbispora viridis* to the genus *Actinomadura*.

to 23 kilobase pairs long or longer were obtained, as measured by 0.8% agarose gel electrophoresis, using fragments of λ phage DNA digested with *Hind*III as the standard.

DNA-DNA hybridizations. The methods used for DNA-DNA hybridization were generally the same as those described by Hopwood et al. (6). DNA probes (100 ng of purified DNA per labeling reaction) were radiolabeled with 20 to 30 μ Ci of deoxycytidine[α -³²P]triphosphate (type 33004X; ICN Radiochemicals, Irvine, Calif.) by using a multiprime DNA labeling kit (type RPN 1601Y; Amersham International plc., Amersham, United Kingdom) and the rapid protocol. Hybridizations were carried out on nylon membranes (type Hybond-N RPN 1732N; Amersham International plc.) by using the dot blotting protocol of the supplier. Unlabeled target DNAs (0.6 μ g) were spotted onto a membrane in triplicate, denatured by using 1.5 M NaCl-0.5

N NaOH, neutralized with 1.5 M NaCl-0.5 M tris(hydroxymethyl)aminomethane hydrochloride-0.001 M ethylenediaminetetraacetic acid (pH 7.5), and then fixed to the membrane by using an ultraviolet transilluminator. The reassociation reaction between the membrane-fixed DNA and 50 ng of heat-denatured [³²P]DNA was performed with 8 to 12 ml of hybridization solution (3 $\times$ SSC-0.1% sodium dodecyl sulfate containing denatured nonhomologous DNA from calf thymus at a final concentration of 100 μ g/ml and 4 $\times$ Denhardt solution) within a plastic bag at 70°C for 16 to 20 h. After hybridization, the membranes were washed once in 2 $\times$ SSC-0.1% sodium dodecyl sulfate at 70°C for 1 h. These membranes were air-dried, exposed to X-ray film at -80°C overnight, and then cut into individual dots in order to measure the radioactivity by using a liquid scintillation counter (model LS-5800; Beckman Instruments, Inc., Ful-

TABLE 5. Fatty acid compositions of the strains tested

Organism	Fatty acid composition (%) ^a											
	14:0	15:0	16:0	17:0	18:0	i-15	i-16	a-17	10M17	10M18	16:1	18:1
Strain SF2461	2		26	1	16		4			14	3	32
<i>A. malachitica</i> JCM 3297 ^T	1		25	2	7		8			20	5	29
<i>Microtetraspora viridis</i> JCM 3112 ^T	1		24	1	12		5			13	6	36
<i>Microtetraspora viridis</i> subsp. <i>intermedia</i> JCM 3113 ^T	1		24	1	10		3			18	6	35
<i>Actinomadura verrucospora</i> JCM 3147 ^T	1		35	1	7		3			24	5	22
<i>Actinomadura citrea</i> ATCC 27887 ^T	1		36		6		3			24	4	25
<i>Actinomadura macra</i> ATCC 31286 ^T	2	1	38	2	5		2	1	1	26	4	15
<i>Actinomadura atramentaria</i> SF2197 ^T	1	3	17	4	2		6	4	8	39	2	7
<i>Actinomadura pusilla</i> ATCC 27296 ^T	1	5	4	4	1	4	21	10	24	4	3	4
<i>Actinomadura helvata</i> JCM 3143 ^T	2	4	17	4	1	4	19	11	8	2	8	5
<i>Microbispora rosea</i> JCM 3006 ^T	2	10	9	3	2	41	8	12	1	3	3	3
<i>Microtetraspora glauca</i> JCM 3300 ^T	1	6	14	9	3	4	23	9	13	3	3	3
<i>Microtetraspora fusca</i> ATCC 23058 ^T	1	3	2	3	2	1	38	9	19	2	3	6
<i>Microtetraspora niveoalba</i> JCM 3149 ^T	1	2	1	9	3	45	11	8	1	1	1	8
" <i>Microtetraspora caesia</i> " ATCC 31295 ^T	2	2	3		8	15	40	1			4	3

^a Fatty acid abbreviations are exemplified by the following: i-16, 14-methylpentadecanoic acid; 14:0, tetradecanoic acid; a-17, 14-methylhexadecanoic acid; 16:1, hexadecenoic acid; 10M18, 10-methyloctadecanoic acid. The columns and conditions used for gas chromatography were as follows: for the SPB-1 fused silica capillary column (30 m by 0.53 mm [inside diameter]), the column temperature was held for 4 min at 150°C and then increased to 250°C at a rate of 4°C/min, the injection temperature was 250°C, and the detector temperature was 280°C; for the Supelcowax-10 fused silica capillary column (30 m by 0.53 mm [inside diameter]), the column temperature was 160°C, the injection temperature was 200°C, and the detector temperature was 250°C. The detector was a flame ionization detector. The carrier gas was helium (30 to 40 ml/min).

FIG. 1. Scanning electron micrographs of strain SF2461 and related strains. (A) Strain SF2461. Note the two types of spore chain morphology. Magnification, $\times 6,000$. (B) *A. malachitica* JCM 3297^T. Magnification, $\times 6,000$. (C) *Microtetraspora viridis* JCM 3112^T. Magnification, $\times 4,000$. (D) *Microtetraspora viridis* subsp. *intermedia* JCM 3113^T. Magnification, $\times 6,000$.

FIG. 2. Scanning electron micrographs of type strains of *Microtetraspora* species. (A) *Microtetraspora glauca* JCM 3300^T. Magnification, $\times 6,000$. (B) *Microtetraspora fusca* ATCC 23058^T. Magnification, $\times 5,000$. (C) *Microtetraspora niveoalba* JCM 3149^T. Magnification, $\times 4,000$.

FIG. 3. Typical autoradiography of DNA-DNA dot hybridization of strain SF2461 and related strains. As indicated on the figure, target DNAs were spotted in triplicate in the following order: *Actinomadura atramentaria* SF2197^T, *A. malachitica* JCM 3297^T, *Microtetrastpora viridis* JCM 3112^T, *Microbispora rosea* JCM 3006^T, *Microbispora viridis* SF2240^T, strain SF2461, *Microtetrastpora niveoalba* JCM 3149^T, *Actinomadura verrucosospora* JCM 3147^T, *Streptomyces griseus* IFO 12875^T, and *Microtetrastpora viridis* subsp. *intermedia* JCM 3113^T. The sources of ³²P-labeled DNA used were *A. malachitica* JCM 3297^T (A), *Microtetrastpora viridis* JCM 3112^T (B), strain SF2461 (C), and *Microtetrastpora viridis* subsp. *intermedia* JCM 3113^T (D). Atma., *Actinomadura*; Mtso., *Microtetrastpora*; Mbso., *Microbispora*; Atma. verrucoso., *Actinomadura verrucosospora*; Stmy., *Streptomyces*.

lerton, Calif.). Hybridization experiments were repeated at least three times.

RESULTS AND DISCUSSION

DNA homology. The most critical point of the hybridization technique was the DNA preparation (cultivation of

microorganisms and extraction and purification of DNAs). Ultraviolet absorption at 260 nm did not always represent the amount of DNA which was effective for DNA-DNA hybridization. To obtain the correct data for DNA relationships, we repeatedly prepared DNAs and experimented with various combinations of probes and targets. In the reassociation reactions, nylon membranes were found to be better than nitrocellulose membranes in both physical strength and binding efficiency, and the technique proved to be simpler than the S1 nuclease method carried out in liquid phase. From the DNA-DNA hybridization results, the DNA homology values were interpreted as follows: strains showing more than 50% homology were considered synonymous, and strains showing 15 to 30% homology were considered different species of the same genus; strains showing less than 10% homology were assigned to different genera.

Relationship between *Microtetrastpora viridis* and *A. malachitica*. The comparative data for the taxonomic characteristics of strain SF2461, *A. malachitica* JCM 3297^T, *Microtetrastpora viridis* JCM 3112^T, and *Microtetrastpora viridis* subsp. *intermedia* JCM 3113^T are summarized in Tables 2 through 5. Morphologically, these organisms were very similar to each other, as shown in Fig. 1, except that strains SF2461, JCM 3297^T, and JCM 3113^T had two types of spore chains (four-spore chains and long straight chains) while *Microtetrastpora viridis* JCM 3112^T did not (no long spore chains). Nonomura and Ohara (16) proposed the name *Microtetrastpora viridis* subsp. *intermedia* to emphasize this morphological characteristic. As shown in Fig. 2, the spore-bearing characteristics of *Microtetrastpora viridis* differed slightly from those of typical *Microtetrastpora* species (*Microtetrastpora glauca*, *Microtetrastpora fusca*, and *Microtetrastpora niveoalba*). These species form linear chains of two to four cylindrical (not spherical) spores on aerial hyphae with short sporophores. In cultural properties, strain SF2461 and related strains were characterized by their greenish aerial masses when they were grown on agar media. However, frequent subculturing onto fresh media tended to produce a faded color. In physiology (Table 3), there were some differences among the strains, such as gelatin liquefaction and starch hydrolysis. On the other hand, the sugar utilization patterns were found to be identical. The chemotaxonomic markers of the strains were substantially identical [wall chemotype IIIB, type PI phospholipid, MK-9(H₆) as the predominant menaquinone, 16:0, 18:1, and 10Me-18 as the major fatty acids, and 72 mol% guanine plus cytosine] and were the same as those of the *A. madurae* group (Tables 2 and 5). According to the results of DNA-DNA hybridization experiments (Table 4 and Fig. 3), *Microtetrastpora viridis* JCM 3112^T was highly related (96 and 93% in reverse combination) to *Microtetrastpora viridis* subsp. *intermedia* JCM 3113^T and was also highly related (59 to 71%) to strain SF2461 and *A. malachitica* JCM 3297^T. However, the two type strains of *Microtetrastpora viridis* showed low homology values (6 to 9%) with the other type strains of *Microtetrastpora* species. These results indicated that strain SF2461 and related strains should be classified in a single species of *Actinomadura sensu stricto*. Accordingly, we propose that *A. malachitica* is a synonym of *Microtetrastpora viridis* and that this taxon should be transferred to the genus *Actinomadura* as *Actinomadura viridis* comb. nov., with strain JCM 3112 (= ATCC 27103) as the type strain.

Taxonomic status of the genus *Microtetrastpora*. In 1968 Thiemann et al. (24) established the genus *Microtetrastpora*, whose members were characterized by the formation of linear chains of four spores borne on short sporophores

TABLE 6. Morphological, cultural, and chemotaxonomic characteristics of type strains of *Microtetraspora* species^a

Strain	Color of aerial mass	Color of reverse side	Spore surfaces	No. of spores per chain	Wall chemotype	Guanine-plus-cytosine content (mol%)	Phospholipid type	Menaquinone composition (%)				
								MK-9(H ₀)	MK-9(H ₂)	MK-9(H ₄)	MK-9(H ₆)	MK-9(H ₈)
<i>Microtetraspora glauca</i> JCM 3300 ^T	White (a) ^b	Green	Smooth	4(2-3)	IIIB/C ^c	70	PIV	12	24	63		
<i>Microtetraspora fusca</i> ATCC 23058 ^T	White (a) ^b	Brown	Smooth	4(2-3)	IIIB/C	71	PIV	15	23	60		
<i>Microtetraspora niveoalba</i> JCM 3149 ^T	White (a)	None	Smooth	4(2-3)	IIIB/C	71	PIV	11	21	67		
<i>Microtetraspora viridis</i> JCM 3112 ^T	Green (24ec)	None	Smooth	4(3-6)	IIIB	72	PI		1	12	60	27

^a For fatty acid compositions see Table 5.^b Thiemann et al. found that the aerial mass color was gray to whitish gray (24).^c The existence of madurose is not a reliable taxonomic criterion because it is present in only trace amounts.

arising from the aerial hyphae at a 45° angle. These authors proposed two species, *Microtetraspora glauca* (type species) and *Microtetraspora fusca*, and reported that their strains had a new cell wall chemotype; however, this was later corrected to wall chemotype III by Nonomura and Ohara (17). In 1971 Nonomura and Ohara (16) proposed the name *Microtetraspora viridis* for organisms forming greenish aerial masses. These authors stated that this species bears a close resemblance to the *Nocardia* (*Actinomadura*) *madurae* group in its cell wall composition. Later, in 1971, they described the new taxon *Microtetraspora niveoalba* (17). In 1980 Tomita et al. (25) proposed a new name, "*Microtetraspora caesia*," for organisms which were characterized by secondary branches from the short spore chains borne downward at an angle of ca. 45°, formation of zoospores, and cell walls containing meso-diaminopimelic acid and galactose but lacking arabinose and madurose. However, this name does not appear on the Approved Lists (22). There are now four valid species (see above) in the genus *Microtetraspora*. The taxonomic data for the type strains of *Microtetraspora* species and related strains are summarized in Tables 5 through 7. The chemotaxonomic markers for *Microtetraspora glauca* JCM 3300^T, *Microtetraspora fusca* ATCC 23058^T, and *Microtetraspora niveoalba* JCM 3149^T were almost identical [wall chemotype IIIB/C, type PIV phospholipid, MK-9(H₄) as the predominant menaquinone, iso-16 and 10Me-17 as the major fatty acids, and 70 to 71 mol%

guanine plus cytosine]. DNA homology values (28 and 37%) showed that these three type strains are related to one another. Based on chemotaxonomy and DNA homology results, these organisms clearly differed from *Microtetraspora viridis*, but resembled the *A. pusilla* group. These results are in good agreement with those of Poschner et al. (19). As shown in Table 7, it is interesting that these three strains of *Microtetraspora* spp. are more highly related to *Microbispora rosea* and *Streptosporangium roseum* (homology values of 13 to 20%) than to *Microtetraspora viridis* and *A. citrea* (8 to 10%). "*Microtetraspora caesia*" ATCC 31295^T exhibited low homology values (6 to 10%) with all of the other *Microtetraspora* species as determined by DNA-DNA hybridization. This organism was also clearly different from the other *Microtetraspora* species based on chemotaxonomy. For example, a determination of the menaquinone composition of "*Microtetraspora caesia*" revealed 68% MK-10(H₄) and 26% MK-9(H₄), with other minor components.

Chemotaxonomic and hybridization techniques are more reliable methods than spore chain morphology (whether the chains are composed of two spores, four spores, or more) for the classification of sporoactinomycetes with wall chemotype III. Moreover, as shown in Fig. 1 and 2, the morphological characteristics prescribing the genus *Microtetraspora* are not suitable criteria for the separation of the genus from *Actinomadura*. Accordingly, the *Microtetraspora* species

TABLE 7. DNA homologies of *Microtetraspora* species and related strains

Source of unlabeled DNA	% Hybridization with ³² P-labeled DNA from:			
	<i>Microtetraspora glauca</i> JCM 3300 ^T	<i>Microtetraspora fusca</i> ATCC 23058 ^T	<i>Microtetraspora niveoalba</i> JCM 3149 ^T	" <i>Microtetraspora caesia</i> " ATCC 31295 ^T
<i>Microtetraspora glauca</i> JCM 3300 ^T	100	31	28	6
<i>Microtetraspora fusca</i> ATCC 23058 ^T	30	100	37	7
<i>Microtetraspora niveoalba</i> JCM 3149 ^T	32	36	100	7
<i>Actinomadura ferruginea</i> JCM 3283 ^T	21	15	17	7
<i>Actinomadura helvata</i> JCM 3143 ^T	15	21	19	7
<i>Actinomadura pusilla</i> ATCC 27296 ^T	15	18	18	8
<i>Microbispora rosea</i> JCM 3006 ^T	18	20	16	6
<i>Streptosporangium roseum</i> IFO 3776 ^T	13	17	15	7
" <i>Microtetraspora caesia</i> " ATCC 31295 ^T	8	8	7	100
<i>Microtetraspora viridis</i> JCM 3112 ^T	9	8	9	10
<i>Actinomadura citrea</i> ATCC 27887 ^T	10	8	9	12
<i>Streptomyces griseus</i> IFO 12875 ^T	7	7	6	8

except "*Microtetrastora caesia*" should be combined with the genus *Actinomadura*, which is, however, heterogeneous itself.

ADDENDUM IN PROOF

Recently, Goodfellow et al. (M. Goodfellow, E. Stackebrandt, and R. M. Kroppenstedt, p. 233–238, in Y. Okami, T. Beppu, and H. Ogawara [ed.], *Biology of actinomycetes* '88, 1988) proposed a new genus, "*Nonomuria*," members of which are sporoactinomycetes belonging to the family *Streptosporangiaceae* and are characterized by the following chemical markers: wall chemotype, IIIB; type PIV phospholipid; MK-9(H₄) as the predominant menaquinone; 10Me-17 as the major fatty acid; and 64 to 69 mol% G+C. They also proposed the transfer of *Actinomadura pusilla* and allied species to the new genus. Our results indicate that *Microtetrastora*, with the exception of *Microtetrastora viridis* and "*Microtetrastora caesia*," and the new genus might be united into one genus.

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