TAXONOMY OF THE ANTIBIOTIC Bu-2313-PRODUCING ORGANISM, *MICROTETRASPORA CAESIA* SP. NOV.

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An aerobic actinomycete strain isolated from an Indian soil sample and designated No. E864-61 was found to produce in submerged fermentation a new antibiotic complex, Bu-2313 (components A and B). Strain E864-61 forms single, pairs or chains of three to eight spores on the aerial mycelium and its aerial mass color is grayish blue-green. The cell wall of strain E864-61 contains *meso*-diaminopimelic acid and galactose. Strain E864-61 has been classified as a new species of the genus *Microtetraspora* and designated as *Microtetraspora caesia* sp. nov.

In the course of screening for anti-anaerobic antibiotics, an oligosporic actinomycete strain No. E864-61=S2 was found to produce a new antibiotic complex which has been designated as Bu-2313[1]. The antibiotic complex was separated into two components, A and B, both of which showed activity against a variety of anaerobic and some aerobic bacteria. The structures of Bu-2313 A and B have been elucidated[2] indicating that they belong to a class of dienoyltetramic acid—containing antibiotics which include streptolydigin[3], tirandamycin[4] and nocamycin[5].

This paper reports the morphological, cultural and physiological characteristics of strain E864-61 and its cell wall composition. The taxonomic position of strain E864-61 is discussed in comparison with related genera of oligosporic actinomycetes.

Materials and Methods

Strain E864-61 was isolated from a soil sample collected at Rajasthan District, India. It was isolated at 42°C on an agar plate containing beef extract-peptone medium supplemented with dihydroxymethylfuratrizine (Panfuran S, Toyama Chemical Co., Ltd., 2 mcg/ml) and nystatin (40 mcg/ml).

Reference microorganisms used for comparative taxonomic studies include the following: *Actinomadura madurae* (VINCENT) LECHEVALIER & LECHEVALIER, 1970; *Micropolyspora angiospora* ZHUKOVA, TSYGANOV & MOROZOV, 1968, strain KCC A-0109[6]; *Micropolyspora caesia* KALAKOUTSKII, 1964, strain KCC A-0098[6]; *Micropolyspora faeni* CROSS, MACIVER & LACEY, 1968, strain KCC A-0099[7]; *Rhodococcus corallius* (BERGEY *et al.*) GOODFELLOW & ALDERSON, 1979, strain NIHJ 300, *Nocardia lutea* CASTELLANI & CHALMERS, 1919, strain NIHJ 303; *Nocardia uniformis* subsp. *tsuyamanensis* AOKI *et al.*, 1976, strain ATCC 21806[8]; *Nocardiopsis dassonvillei* (BROCQ-ROUSSEU) MEYER, 1976, strain ATCC 23218[9]; *Microtetraspora viridis* NONOMURA & OHARA, 1971, strain KCC A-0112[10,11]; *Saccharopolyspora hirsuta* LACEY & GOODFELLOW, 1975, strain KCC A-0170[12] and *Streptomyces sindenensis* NAKAZAWA & FUJII, 1957, strains KCC S-0164 and KCC S-0669[13].

The medium and procedures used for the cultural and physiological characterization of the microorganisms were primarily those recommended by the International Streptomyces Project (ISP)[14]. Media described by S. A. WAKSMAN (The Actinomycetes, Vol. 2) and by G. M. LUEDEMANN[15] also were used. The procedures for chemical analyses of cell wall or whole cell hydrolysate have been described previously[16].

Results

Morphology

Strain E864-61 forms arthrospores only on the aerial mycelium. The aerial spores develop into thick tufts which contains single spores, longitudinal pairs of spores and chains of three to eight spores. The spores are sessile or are borne on monopodially branched short sporophores. Single or paired spores branch out secondarily from the spore-chain downward at an angle of *ca.* 45°C. The mode of sporulation is basipetal budding in the hyphal sheath.

The spores are spherical or oval in shape, 0.5~0.7 by 0.5~1.2 μm in size, and have a smooth surface. The arthrospores and spore-chains are shown in Figs. 1, 2 and 3. Occasionally, single aerial hyphae twist from three to ten turns without envelopment of spores. In the substrate mycelium, spherical or oval translucent capsules (vesicles) are formed which contain single spores or straight chains of two to several spores (Fig. 4). In addition, vesicles enveloping irregularly and tightly coiled structures

Fig. 1. Cluster of conidiospores on aerial mycelium (BENNETT's agar, 37°C, 1 week; scanning electron-micrograph, ×5,000, bar=1 μm).

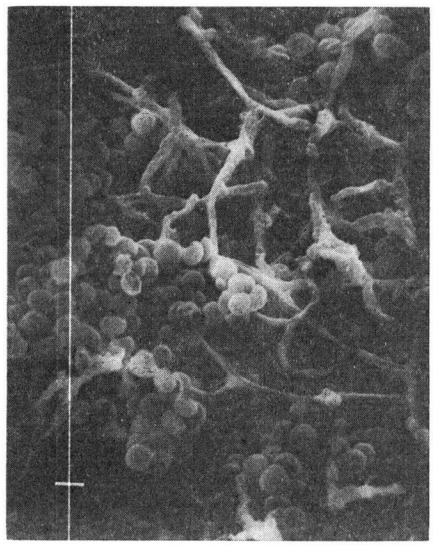


Fig. 2. Branched chain of conidiospores (BENNETT's agar, 37°C, 2 weeks; transmission electronmicrograph, ×14,000, bar=1 μm).

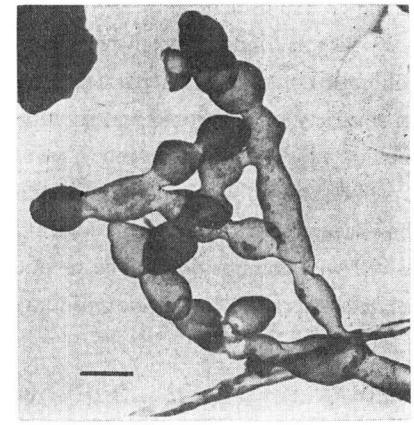


Fig. 3. Thin section of conidiospores (×30,000, bar=1 μm).

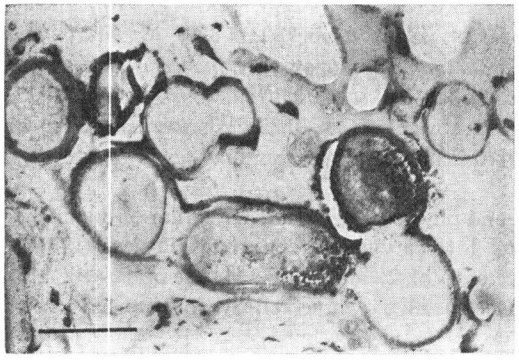
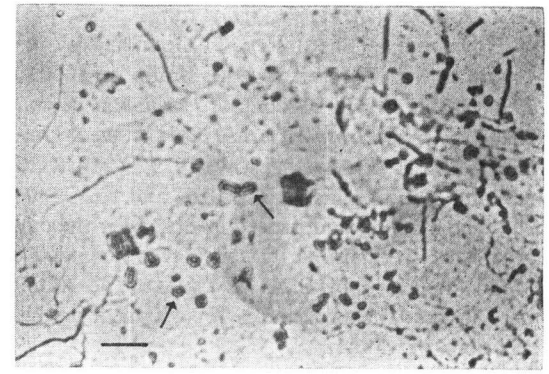
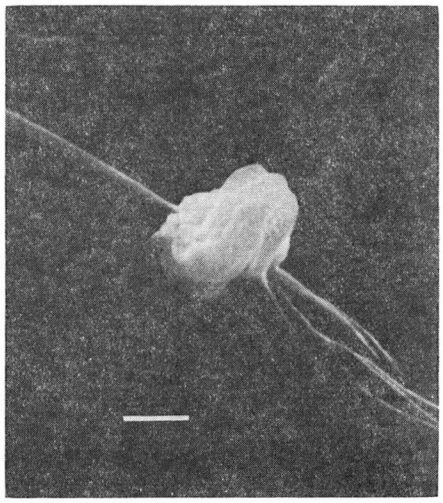


Fig. 4. Translucent capsules enveloping single spores or chains of two to several spores (ISP No. 2 medium, 37°C, 3 weeks; ×950, bar=10 μm).



are occasionally observed (Fig. 5). The above-described two vesicle structures are formed on malt-yeast agar or BENNETT's agar after 3~4 weeks' incubation at 28°C. Oval or banana-shaped spores with a single long polar flagellum are formed capriciously in the aqueous suspension of the substrate mass and produce germ tubes after incubation (Figs. 6 and 7). The substrate mycelium is not fragmented within a week at 37°C, but partially develops into short filaments or rods after 3~4 weeks.

Fig. 5. Envelope of a coiled tubular mass in substrate mycelium (ISP No. 2 medium, 37°C, 3 weeks; scanning electronmicrograph, ×12,500, bar=1 μm).



Cell Wall Composition

Strain E864-61 contains *meso*-diaminopimelic acid (*meso*-DAP) and galactose as diagnostic cell-wall components. Arabinose or madurose was not detected in whole cell hydrolyzates. The amino acids in the cell wall and the sugars in the whole cell hydrolyzate are shown in Table 1 in comparison with those of other reference microorganisms.

Fig. 6. Motile spore with a single polar flagellum (ISP No. 2 medium, 37°C, 2 months; transmission electronmicrograph shadowed with platinum-palladium, ×17,000, bar=1 μm).

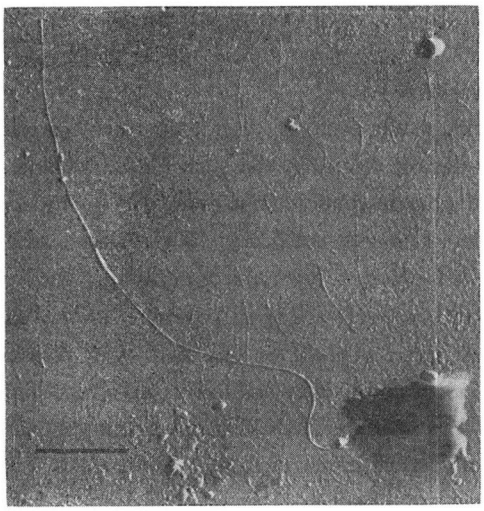


Fig. 7. Germ-tube of a flagellated spore (ISP No. 2 medium, 37°C, 2 months; transmission electronmicrograph shadowed with platinum-palladium, ×17,000, bar=1 μm).

Spontaneous Variants

The original strain E864-61 gave rise to spontaneous variants which differ in their ability to form a blue-green spore mass, white aerial mycelium and violet or green pigment. Some of these variants also show differences in their antibiotic productivity as well as in the ratio of the Bu-2313 A and Bu-

Table 1. Chemical composition of cell wall of strain E864-61 and related organisms.

	Strain No. E 864-61	*Microtetraspora viridis* KCC A-0112	*Micropolyspora caesia* KCC A-0098	*Micropolyspora angiospora* KCC A-0109	*Actinomadura madurae*
Meso-DAP	++	++	++	++	++
LL-DAP	−	−	−	−	−
Glycine	−	−	−	−	−
Galactose	++	−	++	−	++
Glucose	−	+	−	+	−
Mannose	TR	−	−	+	TR
Madurose	−	TR	−	+	++
Arabinose	−	−	++	−	−
Xylose	−	−	−	−	−
Ribose	TR	TR	TR	++	−
Rhamnose	TR	−	TR	TR	−

TR: Trace

Table 2. Properties of variant strains of E864-61.

Strain	Blue-green spore mass	Aerial mycelium	Pigment production		Antibiotic production Ratio of Bu-2313 A/B
			Violet	Green	
E864-61	+	+	+	+	8 : 2
S-26 (variant)	+	+	−	−	1 : 9
S-7 (〃)	−	+	+	−	6 : 4
Y-29 (〃)	−	−	−	+	1 : 9

2313 B components. The properties of the original strain and several of its variants are shown in Table 2.

Cultural Characteristics

Strain E864-61 grows vigorously on nutritionally rich media such as yeast extract-malt extract agar and BENNETT's agar, while the growth is poor on chemically defined media such as starch-mineral salts agar, glucose-asparagine agar, tyrosine agar and glucose-ammonium-salts agar. It produces no growth on CZAPEK-DOX agar suggesting a possible nutritional deficiency. The original strain E864-61 forms abundant aerial mycelia and spore-chains. It produces two types of diffusible pigments: reddish purple to reddish brown pigment on yeast extract-malt extract agar, peptone-yeast extract-iron agar and glucose-ammonium-salts agar, and dark green pigment on oat meal agar, tyrosine agar and BENNETT's agar. Variant No. S-26 shows abundant sporulation and forms aerial mycelium, but lacks the capacity to form pigment. Variant No. S-7 forms white aerial mycelium without a greenish spore mass, and produces abundant red purple pigment but no green pigment. Variant No. Y-29 lacks the ability to form spore-masses and aerial mycelium, and the substrate mycelium fragments easily giving a bacterium-like cultural appearance. It does produce green pigment but this property is lost easily on transfer. The cultural characteristics of the original strain and several of its spontaneous variants are shown in Table 3.

Physiological Characteristics

The optimal growth temperature for strain E864-61 ranges from 32°C to 42°C, and moderate growth is obtained at 20°C and 48°C. No growth is observed at 55°C. Strain E864-61 is resistant to the

Table 3. Cultural characteristics of strain E864-61.

Medium	*	Strain E864-61	Variant No. S-26	Variant No. S-7	Variant No. Y-29
Sucrose-nitrate agar (CZAPEK-DOX agar)	G. VM	No growth.	No growth.	No growth, or growth of colonies. Dark rose.	No growth.
	AM			Moderate. White.	
	D			None	
Yeast extract-malt extract agar (ISP No. 2)	G. VM	Moderate growth. Reddish brown.	Moderate growth. Yellowish brown.	Moderate growth. Deep orange.	Moderate growth. Gold or olive green.
	AM	Abundant. Dull bluish green.	Abundant. Dull yellow to light grayish green.	No or scant. White.	None.
	D	Light reddish brown.	Light reddish brown.	Deep reddish orange.	Gold or olive.
Oat meal agar (ISP No. 3)	G. VM	Moderate growth. Dark green.	Moderate growth. Light brown.	Moderate growth. Dull reddish purple.	Scant growth. Dull yellow.
	AM	Abundant. Dull bluish green.	Scant. White and light grayish green.	No or scant. White.	None.
	D	Light reddish brown.	None.	Dull reddish purple.	None.
Starch-mineral salts agar (ISP No. 4)	G. VM	Scant growth.	Scant growth.	Scant growth. Light yellowish brown.	Scant growth. Colorless.
	AM			No or scant. White.	None.
	D			Pale purplish pink.	None.
Glucose-asparagine agar (ISP No. 5)	G. VM	Scant growth.	Scant growth.	Scant growth. Orange.	Poor growth. Whitish.
	AM		Scant. White.	None.	None.
	D		None.	None.	None.
Peptone-yeast extract-iron agar (ISP No. 6)	G. VM	Poor growth.	Poor growth. Colorless.	Restricted growth. Colorless.	Poor growth. Whitish.
	AM	None.	None.	None.	None.
	D	Dull reddish purple.	None.	None.	None.
Tyrosine agar (ISP No. 7)	G. VM	Restricted growth. Olive green.	Poor growth. Pale yellowish pink.	Poor growth. Light yellowish brown.	Poor growth. Gold.
	AM	Abundant. Pale bluish green.	Scant. White.	Scant. Pale yellow.	None.
	D	None.	None.	Dull reddish purple.	None.
Glucose-ammonium salts agar	G. VM	Poor growth. Light yellowish brown to light reddish brown.	Scant growth. Light brown.	Scant growth. Light yellowish brown.	Scant growth. Gold to yellowish brown.
	AM	Scant. White.	Scant. White.	None.	None.
	D	None.	None.	Deep reddish purple.	None.
BENNETT's agar	G. VM	Moderate growth. Dark green.	Poor growth. Colorless.	Moderate growth. Deep orange.	Poor growth. Whitish.
	AM	Abundant. Light grayish green.	Scant. White and light grayish green.	None.	None.
	D	Olive.	None.	Grayish pink.	None.

* Abbreviation: G—Growth. VM—Vegetative mycelium. AM—Aerial mycelium. D—Diffusible pigment.

Table 4. Physiological reaction.

Test	Response			Method and medium
	E864-61	S-26	Y-29	
Nitrite from nitrate in inorganic medium	−	+	−	CZAPEK-DOX sucrose nitrate broth.
Nitrite from nitrate in organic medium	−	−	−	0.5 % yeast extract, 1.0 % glucose, 0.5 % KNO_3, 0.1 % $CaCO_3$.
Sodium chloride	Moderate growth at 4 % NaCl or less concentration. No growth at 5 % NaCl.			Basal medium: 1 % yeast extract, 2 % soluble starch, 1.5 % agar.
Resistance to lysozyme	Resistant			GORDON's glycerol broth. Lysozyme at 0.001 %.
Casein hydrolysis in agar medium	++	++	−	LUEDEMANN's agar medium.
Reactions in skimmed milk solution				
Coagulation:	++	−	++	
Peptonization:	+	−	−	
Gelatin liquefaction	++	+	−	1.0 % malt extract, 0.4 % yeast extract, 0.4 % glucose, 16 % gelatin.
H_2S production from L-cysteine	++	++	++	L-Cysteine (0.1 %) added to tryptone-yeast extract broth (ISP No. 1 medium) plus agar. H_2S detected with a paper strip containing 10 % aq. leadacetate solution.
Hydrolysis of tyrosine	+	+	+	L-Asparagine was omitted from the tyrosine agar.
Formation of melanoid pigment	−	−	−	Tyrosine agar and peptone-yeast-iron agar.
Effect of pH: Potato plug acidity tolerance test	Normal growth both in acidic and neutral plugs.			LUEDEMANN's potato agar.
Acid from glycerol, fructose & glucose	Negative			Basal medium: 0.1 % $(NH_4)_2HPO_4$, 0.02 % KCl, 0.02 % $MgSO_4$, 0.2 % peptone, 1.5 % agar. Indicator: Brom cresol purple.
Catalase	++	++	++	Overnight growth on nutrient agar. Hydrogen peroxide solution.
Oxidase	−	−	−	Overnight growth on nutrient agar. KOVACS' reagent.
Effect of temperature	Maximal growth at 32~42°C. Moderate growth at 20°C and 48°C. No growth at 55°C.			Yeast extract-malt extract agar.

action of lysozyme. The growth is completely inhibited in the presence of 5 % NaCl. Gelatin is liquefied by the original strain, but not by variant No. Y-29. Melanoid pigments are not produced in ISP medium Nos. 1, 6 and 7. Growth is not inhibited at pH 5.7 in LUEDEMANN's potato plug test, and acid is not produced from glucose, fructose or glycerol. The physiological characteristics of strain E864-61 and variants S-26 and Y-29 are shown in Table 4. The carbohydrate utilization pattern of strain E864-61 is shown in Table 5 in comparison with those of several reference microorganisms.

Taxonomy

Strain E864-61 was compared with six genera of order *Actinomycetales, i.e., Rhodococcus, Micropolyspora, Microtetraspora*[10,11], *Nocardiopsis*[9], *Saccharopolyspora*[12] and *Actinomadura*[17,18,19], all of

Table 5. Utilization of carbon sources.

	Strain No. E864-61	*Micropolyspora caesia* KCC A-0098	*N. uniformis* subsp. *tsuyamanensis* ATCC 21806	*Microtetraspora viridis* KCC A-0112
Glycerol	+	+	+	+
D(−)-Arabinose	+	−	−	−
L(+)-Arabinose	+	+	+	+
D-Xylose	+	+	+	+
D-Ribose	+	+	−	+
L-Rhamnose	+	−	+	+
D-Glucose	+	+	+	+
D-Galactose	+	+	+	+
D-Fructose	+	+	+	+
D-Mannose	+	+	+	+
L(−)-Sorbose	−	−	−	−
Sucrose	−	−	+	+
Lactose	+	−	+	+
Cellobiose	+	+	+	+
Melibiose	+	−	+	−
Trehalose	+	+	+	+
Raffinose	−	−	−	−
D(+)-Melezitose	−	−	−	−
Starch	+	+	+	+
Dulcitol	−	−	−	−
Inositol	−	−	−	−
D-Mannitol	+	+	+	+
D-Sorbitol	−	−	+	−
Salicin	±	−	−	+
Cellulose	−	−	−	+
Chitin	−	−	−	+
Keratin	−	−	−	+

Incubation period prior to observation: 14 days at 28°C.
Basal medium: LUEDEMANN's medium composed of 0.5% yeast extract, 0.1% $CaCO_3$ and 1.5% agar.

which produce spore-chains on the aerial mycelium and contain *meso*-DAP in the cell-wall[13,20].

Strain E864-61 resembles some species of genus *Micropolyspora* in its formation of a greenish-blue mass of short spore chains on the aerial mycelium and in its basipetal budding sporulation, but differs from the latter organism in its lack of spore-chain clusters in the substrate mycelium, its resistance to lysozyme, its sensitivity to sodium chloride as well as in the absence of arabinose and nocardomycolic acid in the cell-wall. The genera, *Nocardiopsis* and *Saccharopolyspora*, bear spores over the entire aerial mycelium, and aerial spores are formed in such a way that hyphae divide into long segments which subsequently further divide into smaller fragments of irregular size. Strain E864-61 is clearly distinguished from these genera on the basis of the above-listed sporulating mechanisms.

In addition, strain E864-61 differs from *Nocardiopsis* in its resistance to lysozyme, and from *Saccharopolyspora* in its smooth spore surface, its lack of arabinose in the cell-wall, its resistance to lysozyme and in its sensitivity to sodium chloride. Strain E864-61 resembles some species of the genus *Actinomadura* which form blue-green aerial mycelium and aerial short spore-chains, but differs from the

Table 6. Major characteristics of reference

	Strain E 864-61	*Nocardia*	*Micropolyspora*
Aerial mycelium:			
Formation	Present; scant to abundant	Absent or rudimental	Present; scant to abundant
Color	Greenish blue	White	White, yellow, blue, green, gray
Spore-chain	Clusters consisting of single spores, longitudinal pairs of spores and chains of 2~8 spores.	Short chains (not in cluster)	Clusters consisting of single spores and chains of 2~20 spores.
Mode of sporulation	Basipetal, budding	Segmentation of hyphae	Basipetal, budding
Surface of spore	Smooth	Smooth	Smooth, warty or spiny
Substrate mycelium:			
Degree of fragmentation into rods or coccus	+	++	++
Spore-chains in substrate mycelium	Absent	Absent	Present
Special morphology	Two types of spore-bearing vesicles; motile spores with flagellum.	Fragmentation of substrate mycelium.	*M. angiospora*: translucent capsule enveloping spores.
Cell wall composition:			
Diagnostic amino acid & sugar (Type)	Meso-DAP, galactose	Meso-DAP, arabinose, galactose (IV_A)	Meso-DAP, arabinose, galactose (IV_A)
Nocardomycolic acid (LCN-A)	−	+	+
Resistance to			
Lysozyme (0.001 %)	+	+	−
NaCl (7 %)	−	+	+
Acid from			
Glucose, fructose & glycerol	−	+	−

latter in its basipetal budding-type sporulation, spore-chain cluster and its lack of madurose in the whole cell hydrolyzate. Sporogenic species of genus *Nocardia, i.e. N. mediterranea* and *N. uniformis* subsp. *tsuyamanensis*, bear some similarities to strain E864-61 in the formation of short spore-chains, the lack of acid production from glucose, fructose and glycerol, the resistance to lysozyme and the sensitivity to sodium chloride. However, strain E864-61 differs from the sporogenic *Nocardia* species in its basipetal budding, spherical spores, the distinct spore-chain clusters formed on many branched sporophores, fused chains of one to several spores and dense green-blue aerial mycelium. In addition, the sporogenic species of *Nocardia* bear rod-shaped spores which occur by segmentation of the hyphae, and have the ability to form single spore-chains and rudimentary aerial mycelium of white or pale color. The above described comparisons of strain E864-61 to several related genera are summarized in Table 6.

According to the descriptions reported by THIEMANN *et al.* (1968),[21] the genus *Microtetraspora* (*M. fusca* and *M. glauca*) is characterized by short spore-chains (usually four spores in the chain) on

actinomycetes related to strain E864-61.

Microtetraspora	*Nocardiopsis*	*Saccharopolyspora*	*Actinomadura*
Present; scant to abundant	Present; sparse to thick	Present; sparse	Present; scant to abundant
White, gray, green	White, yellow, gray	White	All color types
Clusters consisting of chains of several spores (mostly 4 spores).	10~50 spores in a chain. Hyphae dividing into long segments, then subdividing into smaller spores of irregular size.	10~50 spores in a chain. Each spore in chain often separated by lengths of empty hyphae.	5~15 spores in a chain.
Basipetal, budding	Segmentation in hyphae	Segmentation in hyphae	Segmentation in hyphae
Smooth	Smooth	Tufts of long or curved hairs	Smooth, warty or spiny
−	+	+	−
Absent	Absent	Absent	Absent
M. viridis var. *intermedia*: segmented aerial hyphae.	Zigzag-shaped mycelium formed at the beginning of aerial sporulation occurring in entire mycelium.	Sporulation in entire aerial mycelium.	Slime enveloping hooks or closed spirals: pseudosporangia.
Meso-DAP, glucose, traces of glycine, aspartic acid, rhamnose (III_C)	Meso-DAP (III_C)	Meso-DAP, arabinose, galactose (IV_A)	Meso-DAP, madurose (III_B)
−	−	−	−
+	−	−	−, +
−	+	+	−
−	−		−

the aerial mycelium, and by the presence of *meso*-DAP, glycine, lysine and trace amounts of LL-DAP but no diagnostic sugar in the cell-wall. NONOMURA and OHARA (1971)[10,11] reported on the characteristics of two additional species of *Microtetraspora*, *M. viridis* and *M. niveoala*, which possess only *meso*-DAP as a diagnostic cell-wall constituent. As shown in Table 6, strain E864-61 appears to be closely related to the genus *Microtetraspora* in its major characteristics including spore and spore-chain morphology, mode of sporulation, responses to lysozyme and sodium chloride, and cell wall composition. Thus, strain E864-61 was compared with four known species of *Microtetraspora*. None of the four species grow at 45°C, while strain E864-61 grows at 48°C. The color series of the aerial mass is gray for *M. fusca* and *M. glauca*, green for *M. viridis* and white for *M. niveoalba*, while the aerial mass color of strain E864-61 is placed properly in the blue series.

Strain E864-61 produces two types of vesicles in the substrate mycelium; one is a translucent vesicle which envelops one to several spores in a straight line and the other involves a vesicle which

forms irregularly and tightly coiled tubular mass. Motile spores with a single polar flagellum are also produced in the substrate mycelium. Like strain E864-61, *Micropolyspora angiospora* is reported to have translucent capsules which envelop spores. However, the formation of motile spores has not been reported for any genus classified in the family *Micromonosporaceae* or *Nocardiaceae*[13,20].

On the basis of the described major characteristics of strain E864-61, the strain is considered to be a new species of the genus *Microtetraspora* and, we propose its designation as *Microtetraspora caesia* sp. nov. The type strain is No. E864-61 and has been deposited in the American Type Culture Collection with the accession number ATCC 31295.

Discussion

Strain E864-61 shows a unique spore-chain morphology with single or paired spores branching directly from the spore-chain. The spore observed at the base of branch point is a ghost or abortive spore. In addition, the branched spores make a characteristic turn toward the base of spore-chain at an angle of 45 degrees. This unique structure was not observed among the spores of many diverse genera of actinomycetes including the related species, *Micropolyspora caesia* and *Microtetraspora viridis*. According to the morphological descriptions for the genus *Microtetraspora* by THIEMANN *et al.*[21], the chains of tetraspores on short sporophores branch from the aerial mycelium at an upward angle of 45°, "giving to the sporophores, when examined *en masse* an appearance reminiscent of an ear of wheat". Since the secondary branching of spores or spore-chains of strain E864-61 forms a downward angle of 45° relative to the sporophore, strain E864-61 is different from *Microtetraspora glauca* or *M. fusca* which forms upward spore-chain branching at an angle of 45°. Four species of the genus *Microtetraspora* described by THIEMANN *et al.*[21] and NONOMURA & OHARA[10,11] produce chains of tetraspores, on short sporophores. Strain E864-61 also forms predominantly chains of tetraspores, although single spores and chains of two or six to eight spores are also observed. Secondary branching of single or paired spores are formed usually near the apex of the spore-chains in strain E864-61, and, hence, the arrangement of aerial spores of strain E864-61, as shown in Fig. 1, appears to be similar to those species of the genus *Thermomonospora* which form masses of single spores.

In contrast, the microorganisms which produce the acyltetramic acid group of antibiotics, streptolydigin[3] and tirandamycin[4] have been classified as species of the genus *Streptomyces*. The producing organism of nocamycin has been reported to be *Nocardiopsis syringae*[22,23]. These organisms differ significantly from strain E864-61.

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References

1) a) TSUKIURA, H.; K. TOMITA, M. HANADA, S. KOBARU, M. TSUNAKAWA, K. FUJISAWA & H. KAWAGUCHI: Bu-2313, a new antibiotic complex active against anaerobes. I. Production, isolation and properties of Bu-2313 A and B. J. Antibiotics 33: 157~165, 1980
b) KAWAGUCHI, H.; H. TSUKIURA & K. TOMITA: Production of antibiotics by fermentation of novel strains of *Micropolyspora caesia*. U. S. Patent, 4,181,574, 1980
2) TSUNAKAWA, M.; S. TODA, T. OKITA, M. HANADA, S. NAKAGAWA, H. TSUKIURA, T. NAITO & H. KAWAGUCHI: Bu-2313, a new antibiotic complex active against anaerobes. II. Structure determination of Bu-2313 A and B. J. Antibiotics 33: 166~172, 1980
3) DEBOER, C.; A. DIETZ, W. S. SILVER & G. SAVAGE: Streptolydigin, a new antimicrobial antibiotic. I. Biological studies of streptolydigin. Antibiotics Ann. 1955/1956: 886~892, 1956
4) MEYER, C. E.: Tirandamycin, a new antibiotic. Isolation and characterization. J. Antibiotics 24: 558~560, 1971

5) HORVATH, G. & N. P. POTAPOVA: The structure of nocamycin, a new antitumor antibiotic. J. Antibiotics 32: 555~558, 1979
6) CROSS, T.: BERGEY's Manual of Determinative Bacteriology. 8th Edition, pp. 861~865, The Williams & Wilkins Co., Baltimore, 1974
7) CROSS, T.; A. M. MACIVER & J. LACEY: The thermophilic actinomycetes in mouldy hay: *Micropolyspora faeni* sp. nov. J. Gen. Microbiol. 50: 351~359, 1968
8) AOKI, H.; H. SAKAI, M. KOHSAKA, T. KONOMI, J. HOSODA, Y. KUBOCHI, E. EGUCHI & H. IMANAKA: Nocardicin A, a new monocyclic β-lactam antibiotic. I. Discovery, isolation and characterization. J. Antibiotics 29: 492~500, 1976
9) MEYER, J.: *Nocardiopsis*, a new genus of the order *Actinomycetales*. Int. J. Syst. Bacteriol. 26: 487~493, 1976
10) NONOMURA, H. & Y. OHARA: Distribution of actinomycetes in soil. VIII. Green-spore group of *Microtetraspora*, its preferential isolation and taxonomic characteristics. J. Ferment. Technol. 49: 1~7, 1971
11) NONOMURA, H. & Y. OHARA: Distribution of actinomycetes in soil. IX. New species of the genera *Microbispora* and *Microtetraspora*, and their isolation method. J. Ferment. Technol. 49: 887~894, 1971
12) LACEY, J. & M. GOODFELLOW: A novel actinomycete from sugar-cane baggasse: *Saccharopolyspora hirsuta* gen. et sp. nov. J. Gen. Microbiol. 88: 75~85, 1975
13) GOTTLIEB, D.: Order *Actinomycetales* BUCHANAN 1917. BERGEY's Manual of Determinative Bacteriology. 8th Edition, pp. 657~881, The Williams & Wilkins Co., Baltimore, 1974
14) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
15) LUEDEMANN, G. M.: *Micromonospora purpureochromogenes* (WAKSMAN & CURTIS 1916) comb. nov. (subjective synonym: *Micromonospora fusca* JENSEN 1932). Int. J. Syst. Bacteriol. 21: 240~247, 1971
16) TOMITA, K.; Y. UENOYAMA, K. NUMATA, T. SASAHIRA, Y. HOSHINO, K. FUJISAWA, H. TSUKIURA & H. KAWAGUCHI: *Streptoalloteichus*, a new genus of the family *Actinoplanaceae*. J. Antibiotics 31: 497~510, 1978
17) NONOMURA, H. & Y. OHARA: Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* LECHEVALIER *et al.* J. Ferment. Technol. 49: 904~912, 1971
18) PREOBRAZHENSKAYA, T. P.; M. A. SVESHNIKOVA & L. P. TEREKHOVA: Key for identification of the species of the genus *Actinomadura*. *Actinomycetes* and Related Organisms Vol. 12, pp. 30~38, 1977
19) GOODFELLOW, M.; G. ALDERSON & J. LACEY: Numerical taxonomy of *Actinomadura* and related actinomycetes. J. Gen. Microbiol. 112: 95~111, 1979
20) CROSS, T. & M. GOODFELLOW: Taxonomy and classification of the actinomycetes. *In* SYKES and SKINNER (*edit.*), *Actinomycetales*; characteristics and practical importance. Society for Applied Bacteriology Symposium. Series No. 2; pp. 11~112, 1973
21) THIEMANN, J. E.; H. PAGANI & G. BERETTA: A new genus of the *Actinomycetales: Microtetraspora* gen. nov. J. Gen. Microbiol. 50: 295~303, 1968
22) GAUSE, G. F.; M. A. SVESHNIKOVA, R. S. UKHOLINA, G. N. KOMAROVA & V. S. BAZHANOV: Production of nocamycin, a new antibiotic by *Nocardiopsis syringae* sp. nov. Antibiotiki 22: 483~486, 1977
23) BRAZHNIKOVA, M. G.; N. V. KONSTANTINOVA, N. P. POTAPOVA & I. V. TOLSTYKH: Physico-chemical characteristics of nocamycin, a new antitumor antibiotic. Antibiotiki 22: 486~489, 1977