STREPTOALLOTEICHUS, A NEW GENUS OF THE FAMILY *ACTINOPLANACEAE*

KOJI TOMITA, YUMIKO UENOYAMA, KEI-ICHI NUMATA, TAKASHI SASAHIRA, YUTAKA HOSHINO, KEI-ICHI FUJISAWA, HIROSHI TSUKIURA and HIROSHI KAWAGUCHI

Bristol-Banyu Research Institute, Ltd., Meguro, Tokyo, Japan

(Received for publication April 11, 1978)

A new genus *Streptoalloteichus* is proposed in the family *Actinoplanaceae* to distinguish species of actinomycetes which form short or long spore-chains on aerial mycelium, bears oval sporangia with motile spores and has a characteristic cell-wall composition of strain C677–91 type. Strain C677–91 (ATCC 31217, FERM-P No. 4070) was named *Streptoalloteichus hindustanus* gen. nov. and sp. nov.

The actinomycete strain C677–91 produces spore-chain clusters and sclerotia in the aerial mycelium which are morphologically similar to those found in some species of *Streptomyces*. The cultural characteristics of the strain on agar media also resemble those of *Streptomyces* species and the colonies have no distinct color. Strain C677–91 produces sporangia or sporangia-like vesicles which contain one to several spores in the vegetative mycelium. The sporangiospores possess a single long polar flagellum and are motile. The cell wall of strain C677–91 contains *meso*-α,ε-diaminopimelic acid, alanine, glutamic acid, galactose, mannose, rhamnose and glucosamine. Strain C677–91 has several important characteristics in common with *Streptomyces tenebrarius* including the production of nebramycin factors but the latter strain does not produce sporangia.

The significance of the cell-wall composition in the taxonomy of microorganisms has become increasingly important in recent years. Within the order *Actinomycetales*, classification by means of cell-wall composition agrees well with classification by morphological and ecological means. In 1965, BECKER *et al.*[1] classified the various genera of aerobic actinomycetes into four cell-wall types according to the amino acid and sugar composition of the cell-wall. YAMAGUCHI[2] reported five types of cell-wall composition of actinomycetes including microaerophilic organisms. In 1970, LECHEVALIER and LECHEVALIER[3] reviewed 600 actinomycete strains for the main components of cell-wall preparations and whole-cell hydrolyzates, and proposed a classification of this group of microorganisms on the basis of nine cell-wall types.

The actinomycete strain C677–91 described in this paper was isolated from a soil sample collected in Gujarat district, India. Two other strains, C801–104 and D251–1, which were independently isolated from Indian soil samples showed properties identical to strain C677–91*. These microorganisms produced the antibiotics designated Bu-2026 $A_1 \sim A_4$, $B_1 \sim B_4$, C_1 and C_2, among which components A_1, B_1 and B_2 were identified as nebramycin factors V′, II and IV′, respectively. Nebramycin is an aminoglycoside antibiotic complex produced by *Streptomyces tenebrarius*, the name of the strain reportedly originating from its light-sensitive property[4]. The morphological and cultural characteristics of strain C677–91 are similar to those of *S. tenebrarius* except for the production of sporangium in the substrate mycelium. Furthermore, the chemical composition of the cell-wall peptidoglycan of strain C677–91

* The actinomycete strain, E465–94 (ATCC 31158), which produces tallysomycins A and B[31], was found to have mycological properties identical to those of strain C677–91.

was entirely different from that of *Streptomyces* (Type I) and different from any of the known cell-wall types (Types II, III and IV).

This paper reports the morphological, cultural and physiological characteristics of strain C677–91 and the studies on its cell-wall composition. The taxonomic position of the new strain in relation to other genera of the *Actinomycetales* will also be discussed.

Materials and Methods

Microorganisms

The names or culture numbers of the organisms used in the present study are listed in Table 1. They include three strains producing Bu-2026, two species of *Nocardia*, two species of ordinary sporophore-forming *Streptomyces*, four species of cluster-forming *Streptomyces*, *S. tenebrarius* ATCC 17920, *Actinomadura madurae*, and *Streptosporangium rubrum* strain No. C-31751.

Medium and cultural condition for general taxonomic studies

For morphological observations of strain C677–91, C801–104 and D251–1, the organisms were grown at 37°C on malt extract-yeast extract agar, inorganic salts-starch agar and tyrosine agar. For the formation and observation of sporangium, the organisms were grown at 28°C for 3~4 weeks on malt extract agar and glycerol-asparagine agar. The sporangium and other structures in the vegetative mycelium were investigated by microscopic observation of a thin-layer culture prepared by the cover-slip technique described by KAWATO and SHINOBU[5]. The zoospore was liberated from the sporangium into water after immersion for 2~4 hours.

The medium and procedures used for the cultural and physiological characterization of the organisms were those recommended by the International Streptomyces Project (ISP)[6]. Additional culture media described by S. A. WAKSMAN (The Actinomycetes, Vol. 2) and by G. M. LUEDEMANN[10] were also used. For the carbohydrate utilization test, PRIDHAM and GOTTLIEB's basal medium[6] supplemented with 0.01% Difco yeast extract was used.

Medium and cultural condition for cell-wall composition studies

The multiplication medium described by YAMAGUCHI[2] was used for the studies of cell-wall composi-

Table 1. Actinomycete strains used in the present study

Name of organisms	Culture collection number	Cluster formation	Antibiotics produced
Unidentified actinomycetes C677–91	ATCC31217 FERM-P No. 4070	Yes	Nebramycin factors
〃 〃 C801–104	ATCC31218 FERM-P No. 4071	Yes	〃
〃 〃 D251–1	ATCC31219 FERM-P No. 4072	Yes	〃
Nocardia lutea CHRISTOPHERSON and ARCHIBALD, 1918	—	No	—
N. corallina WAKSMAN and HENRICI, 1948	—	No	—
Streptomyces fradiae WAKSMAN and HENRICI, 1948	ISP 5063	No	Neomycin
S. kanamyceticus OKAMI and UMEZAWA, 1957	ISP 5500	No	Kanamycins
S. massasporeus SHINOBU and KAWATO, 1959	ISP 5035	Yes	—
S. ramulosus ETTLINGER *et al*, 1958	ISP 5100	Yes	Acetomycin
S. catenulae DAVISSON and FINLAY, 1959	ISP 5258	Yes	Catenulin
S. antimycoticus WAKSMAN, 1957	ISP 5284	Yes	Pentafungin
S. tenebrarius HIGGENS and KASTNER, 1973	ATCC17920	Yes	Nebramycin factors
Streptosporangium rubrum POTEKAHINA	C-31751	No	Anthracyclines
Actinomadura madurae LECHEVALIER and LECHEVALIER	—	No	—

tion. It contained (in g/liter): dextrose, 10; sodium glutamate, 10; Difco yeast extract, 3; K_2HPO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.02; $ZnSO_4 \cdot 7H_2O$, 0.002; $FeSO_4 \cdot 7H_2O$, 0.005. The pH was adjusted to 7.0 before autoclaving. The test organisms were grown in a 500-ml Erlenmeyer flask containing 100 ml of medium on a rotary shaker operated at 200 rpm at 28~30°C for 3~6 days and the mycelium was harvested at maximal growth.

Preparation of cell wall

The mycelium was collected by centrifugation, washed repeatedly with distilled water and disrupted by sonic oscillation (Tomy Seiko Co., model UR-150P) for 30 minutes to 2 hours depending upon the organisms tested. The disrupted cell suspension was centrifuged at $1,500 \times g$ in the cold (5°C) for 20 minutes to remove the unbroken cells. The supernatant was then centrifuged at $8,000 \times g$ for 30 minutes to deposit the crude broken cell walls. The sediment was washed twice with distilled water, followed by 1 M NaCl solution and finally with two washings of water. A portion of the washed solids thus obtained was lyophilized and designated as the "crude cell wall" fraction. The remainder of the solids was defatted with 0.5% KOH-ethanol solution at 37°C for 2 days. After several additional washings with distilled water, the residue was suspended in a freshly prepared solution of trypsin (1 mg/ml, Aldrich Biochemical Comp., U.S.A.) in 0.05 M phosphate buffer of pH 8.0, and digested at 37°C overnight in the presence of chloroform. The mixture was washed twice with distilled water to free it of trypsin, resuspended in 0.02 N HCl with 1 mg/ml of crystalline pepsin (Wako Pure Chemical Ind.) and digested overnight at 37°C. After centrifugation of the reaction mixture, the sediment was washed several times with distilled water, lyophilized, and designated as the "alkaline ethanol-treated purified cell wall" fraction.

Analysis of amino acids

The purified cell wall (10 mg) was hydrolyzed in 1 ml of 6 N HCl in a sealed tube on an oil bath at 120°C for 18 hours. The hydrolyzate was diluted with an equal volume of distilled water, filtered and evaporated to dryness. One-half of the final product was redissolved in 0.1 ml of distilled water and examined by two-dimentional TLC. The other half was dissolved in 2 ml citrate buffer (pH 2.2) and analyzed by liquid chromatography. A 5-μl portion of the hydrolyzate was applied to a silica gel TLC plate ($60F_{254}$, E. Merck AG, Germany), and developed with phenol - water (4: 1) in one direction, subsequently with *n*-butanol - acetic acid - water (2: 1: 1) perpendicular to the first run. The spots were revealed by a spray of 0.2% ethanolic ninhydrin reagent, followed by heating the plate for 5 minutes at 110°C. Reference standard, which is a mixture of diaminopimelic acid (DAP), glutamic acid, glycine, alanine, valine and leucine (20 mg/ml each) was run along with cell wall sample. Satisfactory separation of the amino acids was obtained with the above TLC system.

In order to differentiate *meso*-(or DD-)DAP from LL-DAP, 5 μl of the hydrolyzate was applied to a cellulose powder TLC plate (Avicel SF, Funakoshi Pharmaceutical Co., Japan). The plate was developed with the solvent system described by HOARE and WORK[7], methanol - water - 10 N HCl - pyridine (80: 17.5: 2.5: 10), for 24 hours and then sprayed with 0.2% ninhydrin reagent. *Meso*-α, ε-diaminopimelic acid (Sigma, U.S.A.) was used as a reference standard. In this TLC system, LL-DAP moved faster than *meso*-DAP[7].

Amino acids in the cell wall preparation were also determined by the amino acid analyzer[8] (Hitachi 034 2U Model). The peak of DAP appeared between valine and methionine.

Analysis of carbohydrates in the cell wall

A 50-mg sample of the crude cell wall was dissolved in 3 ml of 2 N H_2SO_4 and hydrolyzed in a sealed tube in an oil bath at 120°C for 2 hours. The hydrolyzate was neutralized with saturated $Ba(OH)_2$ solution, the precipitated $BaSO_4$ removed by centrifugation at 3,000 rpm and the supernatant was lyophilized. The lyophilizate was trimethylsilylated using the method of SWEELEY *et al*[9] and the product was subjected to gas chromatography with a Shimadzu GC4BRT with 3% OV 17 on Shimalite W (80~100 mesh, 0.3×300 cm). Glucose, galactose, mannose, arabinose, xylose, ribose, fucose, rhamnose and glucosamine standards were treated in the same manner prior to gas chromatography.

Determination of whole cell sugar components

Strain C677-91 and *Actinomadura madurae* were grown in the multiplication medium which was

used for the cell-wall analysis. Whole cell hydrolyzates of the organisms were prepared according to the methods described by LECHEVALIER and GERBER[11]. Sugar components in the whole cell hydrolyzates were identified by paper chromatography according to the procedure of LECHEVALIER and LECHEVALIER[12].

Results

Morphology

The actinomycete strain C677-91 produces sporangia singly or collectively in the vegetative hyphae on yeast extract-malt extract agar and glycerol-asparagine agar. The sporangia are subspherical or occasionally spherical in shape; the surface is often uneven (Figs. 1 and 2) and the size of sporangia ranges from 1.5~4.5 by 2.7~7.0 μ. A peanut shell-shaped sporangium is also formed (Fig. 2). Sporangiophores are formed along the surface of agar medium often with branching and are 5~20 μ in length. Each sporangium contains one to four (or more) spores which are arranged in a rod or a V-shaped line. The spores are oval or rod shaped, and the rod-shaped spores are usually curved and possess one or two swellings similar to a soybean shell. The spores are 0.9~1.5 by 1.2~4.0 μ in size and are motile with a single polar flagellum which measures 50 μm or longer (Fig. 3).

Strain C677-91 also produces spore-chain clusters and sclerotia (Fig. 4) in the aerial mycelium on yeast extract-malt extract agar (ISP No. 2), inorganic salts-starch agar (ISP No. 4) and other solid media. The cluster is the dominant conidiophore-forming structure and the formation of sclerotia is somewhat

Fig. 1. Sporulated sporangia
(Glycerol-asparagine agar, 28 days. ×600)

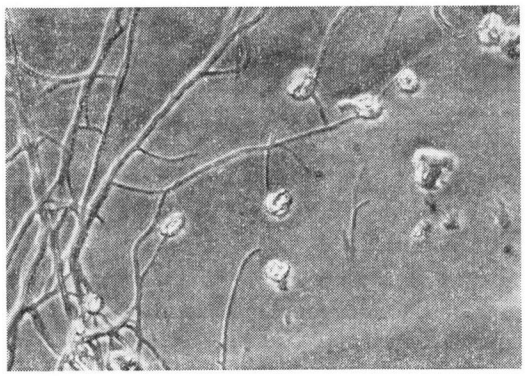


Fig. 2. Peanut shell-shaped sporangia
(Glycerol-asparagine agar, 28 days. ×400)

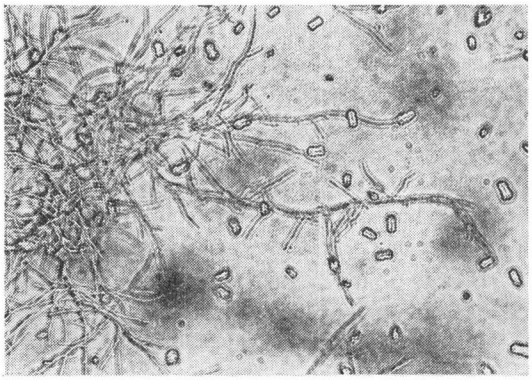


Fig. 3. Flagellated sporangiospores
(Glycerol-asparagine agar, 28 days. ×1,500)

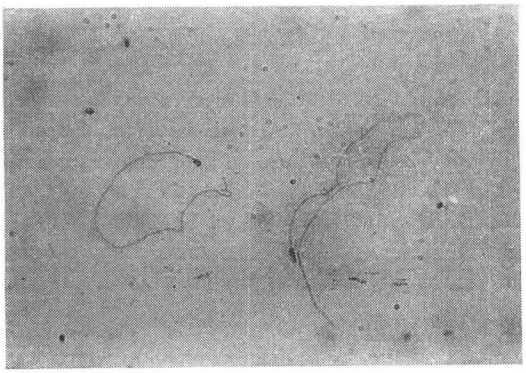


Fig. 4. Sclerotia in aerial mycelium
(Yeast extract-malt extract agar, 14 days. ×600)

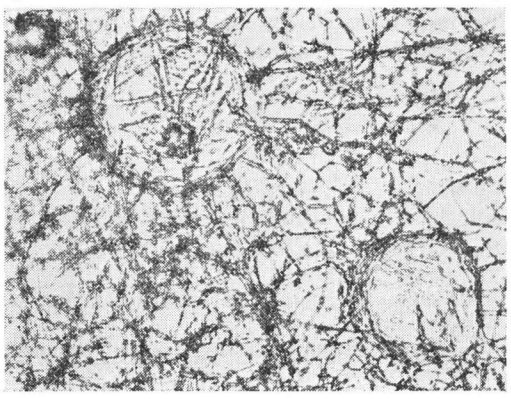
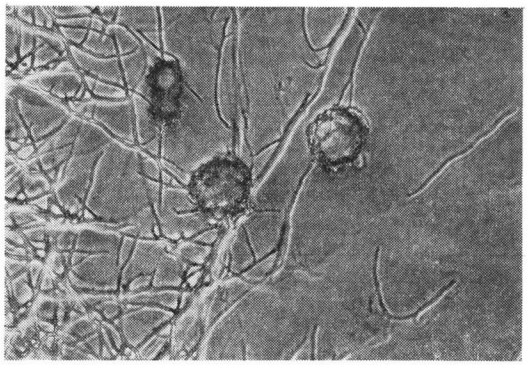


Fig. 5. Electronmicrograph of conidiospore and chain
(Yeast extract-malt extract agar)

Fig. 6. Globose thick bodies in substrate mycelium
(Glycerol-asparagine agar, 28 days. ×600)



capricious. The spore-chain cluster consists of curved or L-shaped short conidiospore-chains with many branches and often develops into a thick mass. The short spore-chains contain 10~20 spores in a chain. The conidiospore has a smooth surface and is oval to short-cylindrical in shape. The electronmicrograph of the conidiospore and spore-chain is shown in Fig. 5. Some spore-chains protrude from the cluster structure and often form open spirals, which contain 30~50 spores in a chain. The shape of the sclerotium is oval or occasionally irregular. The aerial mycelial mass consisting of mature spores is easily scraped off from the agar surface. The substrate mycelium is branched, occasionally twisted and coiled, not septated and not fragmented into coccoid elements. A globose thick body, 3~20 μm in diameter (Fig. 6) covered with mycelia is produced, and is similar to that reported for the species of Genus *Kitasatoa.*

Cultural and Physiological Characteristics

Strain C677-91 produces abundant aerial mycelia on most of the agar media tested. The color of mature aerial mycelium is light yellowish beige or pale pinkish yellow. The substrate mycelium does not have any characteristic color. When the aerial mycelium is not formed, the substrate mycelium penetrates into the agar. Diffusible pigment is not produced. The substrate mycelium mass of the colony is thin especially in chemically defined media, wrinkles radially, and is covered with white or beige thick mass of the aerial mycelium and spore-chains. Tyrosinase reaction is negative. Strain C677-91 is thermoduric and grows abundantly at 50°C. The growth is restricted in the agar medium containing 5% NaCl and no growth occurs at 7% NaCl. It gives normal growth on the LUEDEMANN's potato plug acidity test[10]. The cultural and physiological characteristics, and the carbohydrate utilization pattern of strain C677-91 are shown in Tables 2, 3 and 4, respectively.

Cell Wall Composition

The amino acid composition of the cell wall preparation was determined by thin-layer chromatography (TLC) for eleven actinomycete species including strain C677-91, 2 species of *Nocardia,* 2 species of *Streptomyces* with ordinary spore-chains, 4 species of typical cluster-forming *Streptomyces, Streptomyces tenebrarius* and *Streptosporangium rubrum.* The amino acids present were expressed in relative amounts, according to the size and intensity of the spots revealed on the TLC plate, and the results are

Table 2. Cultural characteristics of strain C677–91

Yeast extract-malt extract agar (ISP No. 2 medium) PRIDHAM *et al*, 1956–57	G : Abundant R : Pale yellowish brown to light brown A : Thick, velvety, light yellowish beige or pale pinkish beige D : None
Oat meal agar (ISP No. 3 medium) KUSTER, 1959	G : Moderate R : Colorless, partially pale yellowish brown A : Powdery to velvety occasionally with patches, pale pinkish beige D : None
Inorganic salts-starch agar (ISP No. 4 medium) KUSTER, 1959	G : Moderate R : Colorless to pale yellowish brown A : Powdery to velvety, pale pinkish yellow D : None
Glycerol-asparagine agar (ISP No. 5 medium) PRIDHAM and LYONS, 1961	G : Restricted R : Colorless to pale olivaceous yellow A : Powdery with patches, whitish to pale yellowish beige D : None
Peptone-yeast extract-iron agar (ISP No. 6 medium) TRESNER and DANGA, 1958	G : Scant R : Brown A : Scant, white D : Pale brown
Tyrosine agar (ISP No. 7 medium) SHINOBU, 1958	G : Moderate R : Pale yellow to pale greenish yellow A : Velvety to cottony, white later light pinkish yellow D : None
BENNETT's agar	G : Moderate R : Pale olivaceous yellow to light brown A : Velvety, light yellowish beige D : None
Nutrient agar	G : Restricted R : Pale brownish yellow A : Scant, white D : Pale yellow
Soil extract agar	G : Moderate R : Colorless A : Thin, pale yellowish beigh, patches D : None
Tomato paste-oat meal agar	G : Moderate R : Light yellowish brown A : Velvety, pale pinkish yellow D : None

Abbreviation: G=Growth, R=Reverse color, A=Aerial mycelium, D=Diffusible pigment

summarized in Table 5. Alanine and glutamic acid were present in all the actinomycete strains examined in the present study. Glycine was present in all *Streptomyces* strains except *S. tenebrarius*, trace or doubtful in strain C677–91 and in *S. tenebrarius* and negative in *Nocardia* species and in *Streptosporangium rubrum*. *Meso*-DAP was present in strain C677–91, two *Nocardia* strains, *S. tenebrarius* and

Table 3. Physiological reactions of strain C677-91

Gelatin liquefaction	Positive; rapidly liquefied
Starch hydrolysis	Positive
Milk	Remarkable coagulation and slight peptonization. pH alkalinized. Yellowish ring growth.
Melanin from L-tyrosine	Negative tyrosinase
Nitrite from nitrate	Positive
Growth temperature	Abundant growth at 32~50°C, moderate at 25~30°C, restricted at 23°C and 52°C, scant at 20°C and 54°C, no growth at 12°C and 56°C.
Fluorescent light sensitivity	No distinct inhibition of aerial mycelium formation under 15W-fluorescent lamp for 14 days.
NaCl tolerance	Restricted growth and restricted aerial mycelium formation in LUEDEMANN's agar medium[10] in 5% NaCl. No growth at 7% NaCl.
Potato plug acidity tolerance	Normal growth and normal aerial mycelium formation on LUEDEMANN's potato plug test[10].
Catalase reaction	Positive
Oxidase	Negative
Antibiotics produced	Bu-2026 complex (including nebramycin factors II, IV′ and V′).

Table 4. Carbohydrate utilization of strain C677-91

Glycerol	++	D-Glucose	++	Inositol	−
L-Arabinose	−	D-Mannose	++	D-Mannitol	−
D-Xylose	−	Sucrose	±~+	D-Sorbitol	−
L-Rhamnose	−	Lactose	±~+	Cellulose	−
D-Fructose	++	Maltose	++	Inuline	−
D-Galactose	++	D-Raffinose	−	Salicine	+

Basal agar medium: PRIDHAM and GOTTLIEB medium, supplemented with 0.01% yeast extract.
Incubation temperature: 37°C
++: Strongly positive utilization, +: Positive utilization, ±: Utilization doubtful, −: Utilization negative.

Table 5. Amino acid composition of cell wall as determined by thin-layer chromatography

	Meso-DAP	LL-DAP	Glycine	Alanine	Glutamic acid	Valine region	Leucine region
Strain C677-91	++	−	±	+++	++	±	±
Nocardia lutea	++	−	−	+++	++	−	−
N. corallina	++	−	−	+++	++	−	−
Streptomyces fradiae	−	++	++	+++	++	−	−
S. kanamyceticus	−	++	++	+++	++	±	±
S. massasporeus	−	++	++	+++	++	±	±
S. ramulosus	−	++	++	+++	++	±	±
S. catenulae	−	++	++	+++	++	±	±
S. antimycoticus	−	++	++	+++	++	±	±
S. tenebrarius	++	−	±	+++	++	±	±
Streptosporangium rubrum	++	−	±	+++	++	±	±

Table 6. Relative amino acid composition of cell wall determined by amino acid analysis

	Strain C677–91	*Nocardia lutea*	*Streptomyces fradiae*
Alanine	213	179	142
DAP	100	100	100
Glutamic acid	97	136	88
Glycine	9	<4.0	97

Table 7. Carbohydrate composition of cell wall

	Arabinose	Galactose	Glucose	Mannose	Rhamnose	Glucosamine
Strain C677–91	−	+++	TR*	+++	+	+
Nocardia corallina	+++	+++	−	+	−	+
Streptomyces fradiae	−	TR	TR	TR	−	+++
S. antimycoticus	−	TR	TR	TR	−	+++
S. tenebrarius	−	+++	TR	+	+	+++
Streptosporangium rubrum	−	−	−	−	−	+

* TR: trace

Table 8. Paper chromatography of sugar components in whole cell hydrolyzate of strain C677–91 and *Actinomadura madurae*

	Sugar	$R_{rib.}$*2 (color-B or P)*3		
		Reference	Strain C677–91	*A. madurae*
System 1*1	D-Galactose	0.23 (B)	0.22 (B)	0.23 (B)
	D-Mannose	0.44 (B)	0.43 (B)	0.43 (B)
	L-Arabinose	0.52 (P)	—	—
	Madurose	0.62 (B)*4	—	0.62 (B)
	D-Xylose	0.71 (P)	—	—
	Unknown	—	0.94 (B)	0.95 (B)
	D-Ribose	1.00 (P)	—	—
	L-Rhamnose	1.20 (B)	1.21 (B)	—
System 2*1	D-Galactose	0.46 (B)	0.47 (B)	0.46 (B)
	D-Mannose	0.60 (B)	0.61 (B)	0.60 (B)
	L-Arabinose	0.71 (P)	—	—
	D-Xylose	0.81 (P)	—	—
	Madurose	0.85 (B)*4	—	0.85 (B)
	D-Ribose	1.00 (P)	—	—
	L-Rhamnose	1.26 (B)	1.25 (B)	—

*1 System 1: *n*-Butanol - water - pyridine - toluene (5: 3: 3: 4)
System 2: Ethyl acetate - acetic acid - water (3: 1: 3)

*2 $R_{rib.}$: Mobility relative to D-ribose

*3 Color developed with acid aniline phthalate (B: brown, P: pink)

*4 Data from reference 11) and 12).

Streptosporangium rubrum. LL-DAP was found in all of the reference strains of *Streptomyces* regardless of whether they were of the ordinary spore chain-forming or the cluster-forming type.

The amino acid composition of the cell wall preparation of strain C677–91 was also examined quantitatively with an amino acid analyzer, and compared with the two reference actinomycete strains,

N. lutea and *S. fradiae*. The results shown in Table 6 confirmed the previous findings obtained by the TLC study.

The carbohydrate composition of the cell wall was determined by gas chromatography of the trimethylsilylated sugar fractions obtained from strain C677–91, *N. corallina, S. fradiae, S. antimycoticus, S. tenebrarius* and *Streptosporangium rubrum* (Table 7). Arabinose was found only in *Nocardia* species. Galactose and mannose were present in strain C677–91, *N. corallina* and *S. tenebrarius*, but not in the other *Streptomyces* strains and *Streptosporangium rubrum*. Rhamnose was found in strain C677–91 and *S. tenebrarius* but not in *Nocardia, Streptosporangium* and two other *Streptomyces* strains.

Whole Cell Sugar Components

The diagnostic sugars present in the whole cell hydrolyzates of strain C677–91 and *Actinomadura madurae* were determined by two descending paper chromatographic systems, and the location of each sugar component is expressed in mobility relative to that of D-ribose (Table 8). The paper strips were sprayed with acid aniline phthalate reagent and the color developed is also reported (Table 8).

The whole cell hydrolyzate of strain C677–91 contained D-galactose, D-mannose, L-rhamnose and possibly an unidentified sugar component. The presence of madurose (3-O-methyl-D-galactose) was confirmed in the hydrolyzate of *Actinomadura madurae*[11] which was absent in strain C677–91.

Taxonomy

Comparisons with related genera:

The aerial spore-chain morphology of strain C677–91 resembles that of *Streptomyces massasporeus, S. ramulosus, S. catenulae* and *S. antimycoticus*. However, strain C677–91 is different from these cluster-forming species of *Streptomyces* in its formation of sporangia-like vesicles with motile spores and in the cell-wall composition. Strain C677–91 contains *meso*-DAP, galactose, mannose and rhamnose in its cell wall, while the above-mentioned four species of *Streptomyces* have the Type I cell wall composition, containing LL-DAP and glycine, but no diagnostic sugar component.

A thick mass of aerial spore-chains, which is found in strain C677–91, is also formed by the species of genera *Micropolyspora, Saccharopolyspora*[13] and *Actinomadura*[14~16]. However, *Micropolyspora* and *Saccharopolyspora* have the cell-wall composition of Type IV containing *meso*-DAP, arabinose and galactose. The spore-chain is formed on both aerial and substrate mycelia, and the number of spores in a spore-chain is less than twenty in the species of *Micropolyspora*, while strain C677–91 forms spore-chains only on aerial mycelium, and has long spore-chains which contain 30~50 spores. In addition, the arthrospores of *Micropolyspora* are reported to have thick walls particularly at the site of contact with adjacent spores[29], while the spore-wall of strain C677–91 is thin as shown by the electron micrograph of aerial spore-chain. The substrate mycelium of *Saccharopolyspora*-like *Nocardia, Nocardiopsis*[17] and *Nocardioides*[18], fragments into rod-shaped elements and hence the genus is differentiated from strain C677–91. *Actinomadura* has the cell wall composition of Type III and contains madurose (=3-O-methylgalactose) in the whole cell hydrolyzate, while madurose is not present in strain C677–91.

BERGEY's Manual of Determinative Bacteriology (8th Ed., 1974) describes ten sporangium-forming genera in family *Actinoplanaceae*. Comparisons of strain C677–91 with the descriptions of important genera of *Actinoplanaceae* are summarized in Table 9. The genus *Actinoplanes* COUCH, 1950[19], which includes seven diverse species, resembles strain C677–91 in the shape of sporangia but differs in the arrangement and number of spores in a sporangium and the type of flagellation. The genus *Spirillospora*

Table 9. Comparison with genera in family *Actinoplanaceae*

	Sporangia		Sporangiospores				Aerial mycelium	Cell wall type
	Shape	Occurrence*	Arrangement	Number in a sporangium	Shape	Flagellation		
Actinoplanes	Spherical, cylindrical	SM	Coils or rows	Several tens to thousands	Globose, some subglobose to rod	Lophotrichous or peritrichous	Mostly none or scant	Type II
Spirillospora	Spherical to vermiform	AM	One or more coils	Several tens to thousands	Short to long rods to spiral	One to three subpolar flagella	White to pale yellow	Type III
Streptosporangium	Spherical to ovoid	AM	A single coil	Several tens to thousands	Spherical to ovoid	No flagellum	Abundant	Type III
Amorphosporangium	Very irregular much lobed	SM		Several tens to thousands	Rod-shaped	Two or three polar	None	Type II
Ampullariella	Bottle- or flask-shaped, digitate or lobate	SM	Parallel chains	Several tens to thousands	Rod-shaped	Polytrichous, polar flagella	None	Type II
Pilimelia	Large, globose or cylindrical		Parallel chains from end to end	*ca.* 1,000	Rod-shaped	Single polar or one to four subpolar or lateral flagella	None	Type IV
Planomonospora	Rod or oval	AM	—	Single	Fusiform	Peritrichous	Moderate	Type III
Planobispora	Finger shape	AM	Longitudinal pair	Two	Rod-shaped	Peritrichous	Abundant	Type III
Dactylosporangium	Finger-shaped usually straight	SM	A single row	Three to five	Rod-shaped	Polytrichous, polar flagella	None or rudimentary	Type II
Kitasatoa	Club-shaped	AM & SM	A single chain	Single, in pair or in parallel	*Diplococcus*-like spherical, elliposoidal	A single polar flagellum	Abundant	Type I
Strain C677–91	Subspherical or spherical	SM	A single rod or V-shaped line	One to four or several	Oval or rod, often bent or swelling	A single polar flagellum	Abundant	New type

* SM: Substrate mycelium, AM: Aerial mycelium.

COUCH, 1963[19] and strain C677–91 have several common characteristics such as the shape of sporangia and sporangiospores, the type of flagellation and the aerial mycelium formation, but they differ in the site of sporangium formation and the motility of arthrospore. The genus *Streptosporangium* COUCH, 1955[19] is similar to C677–91 in the aerial mycelium formation but differs from the latter in many other characteristics listed in Table 9. The genus *Amorphosporangium* COUCH, 1963[19] is similar to C677–91 in the shape of sporangiospores but differs in the flagellation and the aerial mycelium formation. The genus *Ampullariella* COUCH, 1964[19] resembles C677–91 in the shape of sporangiospores but differs in the shape of sporangia, the arrangement of spores in a sporangium, the flagellation and the aerial mycelium formation. The genus *Pilimelia* KANE, 1966[20] resembles C677–91 in the shape of sporangiospores and the flagellation but differs in the arrangement and number of spores in a sporangium and the aerial mycelium formation. The genus *Planomonospora* THIEMANN, PAGANI and BERETTA, 1967[21] forms sporangia of unique palm leaf shape and is different from C677–91 in its formation of sporangia on aerial mycelium and its lack of aerial spore-chain. The genus *Planobispora* THIEMANN and BERETTA, 1968[22] differs from C677–91 in the shape of sporangia and the arrangement of sporangiospores. The genus *Dactylosporangium* THIEMANN, PAGANI and BERETTA, 1967[23] is similar to C677–91 in the arrangement and number of spores in a sporangium, but differs in the shape of sporangia and the type of flagellation. The genus *Kitasatoa* MATSUMAE and HATA, 1968[26] is similar to C677–91 in the flagellation and the aerial mycelium formation, but differs in the shape of sporangia and the arrangement of spores in a sporan-

Table 10. Comparison of major characteristics of strain C677–91 with those of genera *Actinoplanes* and *Spirillospora*

Characters	Strain C677–91	*Actinoplanes*	*Spirillospora*
Sporangia	Formed in substrate mycelium. Subspherical occasionally spherical or peanut shell-shaped. 1.5~7 μ in dia. Contain 1 to 4 or more spores.	Formed in substrate mycelium. Spherical, subspherical or cylindrical. 5~50 μ in dia. Contain several tens to thousands spores.	Formed in aerial mycelium. Spherical, elongated, rarely vermiform. 5~25 μ in dia. Contain one or more coils of spores.
Sporangiospores	Oval to rod or soybean shell-shaped. Motile with single long polar flagellum.	Globose to subglobose in shape. Motile by a tuft of polar flagella.	Short to long rods to spiral in shape. Motile by one to three subpolar flagella.
Conidiospore-chains	Abundantly formed only on aerial mycelium. Morphology similar to some species of *Streptomyces*. Comprise both of long and short spore chains.	Absent. If present, formed in substrate mycelium.	Formed on aerial mycelium. The arthrospores also motile.
Aerial mycelium	Formed thick, and well branched.	Absent or rudimental except for *A. armeniacus*.	Formed thick or rudimentally.
Color of colony	Buff or light brown.	Mostly brilliantly colored: Red orange, yellow, violet and purple.	White, pale yellow, light grey or bright blue.
Diagnostic composition of cell wall	*Meso*-DAP, galactose, mannose and rhamnose. Lacks glycine.	*Meso*-DAP (or hydroxy DAP) and glycine; most species contain galactose, mannose, arabinose and/or xylose.	*Meso*-DAP, galactose. Lacks glycine.
Whole cell sugars	Galactose, mannose and rhamnose.	Arabinose and xylose.	Madurose.
Sodium chloride tolerance	Moderate sensitivity. Growth at 5%.	Sensitive. No growth at 5%.	—
Growth-temperature	Vigorous growth at 50°C.	No growth at 45°C.	No growth at 40°C.

gium and the shape of sporangiospores. Strain C677-91 has *meso*-DAP, galactose, mannose and rhamnose as major distinct components of the cell-wall, but does not have LL-DAP, glycine and arabinose. The genera in family *Actinoplanaceae* with exceptions of *Spirillospora, Streptosporangium* and *Planomonospora* have glycine as one of the diagnostic cell-wall components. The whole cell hydrolyzate composition of strain C677-91 differs from *Spirillospora, Streptosporangium* and *Planomonospora* (cell-wall type IIIB) in the absence of madurose. Thus, among the genera in family *Actinoplanaceae* described above, strain C677-91 appears to be most related to *Actinoplanes* and *Spirillospora*. More detailed comparisons of these genera with strain C677-91 are shown in Table 10.

The sporangia found in several genera of the family *Streptomycetaceae* are generally simpler than those of the family *Actinoplanaceae* in shape and spore-arrangement. A few spores or a short spore-chain are formed within a sporangia-like vesicles in the species of the genera *Microellobosporia*[24], *Elytrosporangium*[25], *Kitasatoa*[26] and *Intrasporangium*[27]. The spore-chains of *Actinosporangium*[28] are covered with viscous material. The predominant aerial spore-chains along with the sporangia are formed in the species of genera *Elytrosporangium, Kitasatoa* and *Microellobosporia*. With respect to a character of the predominant formation of aerial spore-chains as in the genus *Streptomyces* as well as the formation of simple sporangia, strain C677-91 is considered to resemble the genera *Kitasatoa* and *Elytrosporangium*. However, strain C677-91 has an entirely different cell-wall composition from the cell wall Type I of these two genera. Therefore strain C677-91 should not belong to the family *Streptomycetaceae*.

Comparisons with related species:

Streptomyces sclerogranulatus SHIMAZU and YONEHARA, 1969[30], resembles strain C677-91 in the formation of both clusters and sclerotia. Although no description is available concerning its cell-wall composition and sporangium formation, *S. sclerogranulatus* is differentiated from strain C677-91 in its whitish aerial mycelium production on various media, the lack of thermoduric property, and its positive utilization of xylose, raffinose, mannitol and inositol.

The species most similar to strain C677-91 appears to be *Streptomyces tenebrarius* ATCC 17920[4]. They have several important characteristics in common, such as cluster and sclerotium formation, and carbohydrate utilization pattern except for inositol. The thermoduric property and the antibiotics produced are also similar. The cell-wall components of *S. tenebrarius* ATCC 17920 were analyzed and compared to strain C677-91 and revealed a unique cell wall composition similar to that of C677-91, and most unusual for *Streptomyces*. *S. tenebrarius* ATCC 17920, as its species name implies, is light-sensitive and produces no aerial mycelium under fluorescent lamps. Strain C677-91, as well as two other strains of C801-104 and D251-1, grew well with abundant aerial mycelia under the same conditions. Also, strain C677-91 differs from *S. tenebrarius* ATCC 17920 in the lack of red soluble pigment and negative utilization of inositol. HIGGENS and KASTNER[4] described an asporogenous variant of *S. tenebrarius* (ATCC 17921), which is reported to be *Nocardia*-like in morphology showing the fragmentation of vegetative mycelium, and hence the variant ATCC 17921 is also different from strain C677-91. An additional difference was also noted in the tolerance to NaCl: strain C677-91 did not grow at a NaCl concentration of 7% while the two strains of *S. tenebrarius* (ATCC 17920 and ATCC 17921) gave growth in 8% NaCl medium (but not in 10%) in our experiments. Furthermore, the sporangium was not found in the two strains of *S. tenebrarius* and, therefore, should be taxonomically differentiated from strain C677-91.

Proposal of a New Genus

In view of the morphological, cultural and physiological characteristics as well as the cell-wall composition of strain C677–91, it is proposed that a new genus *Streptoalloteichus* be created in the family *Actinoplanaceae* in order to distinguish the sporangium-forming actinomycete strains showing *Streptomyces*-like morphology but with the unusual cell-wall composition of strain C677–91-type: *meso*-DAP, alanine and glutamic acid as major amino acids, and galactose, mannose and rhamnose as diagnostic neutral sugars.

Strain C677–91 has the following major characteristics: (1) production of subspherical sporangia singly or collectively on the vegetative mycelium; (2) 1~4 (or more) sporangiospores arranged in straight or V-shaped chain; (3) sporangiospores oval to bent rod in shape and motile with a long single polar flagellum; (4) production of abundant aerial mycelium, predominant cluster (thick mass of short conidiospore-chains), long conidiospore-chains and sclerotium; (5) vegetative mycelium occasionally twisted and coiled; (6) cell wall composition of *meso*-DAP, galactose, mannose and rhamnose as diagnostic components; (7) growth at 52°C. The genus epithet *Streptoalloteichus* means a *Streptomyces*-like organism with unusual cell-wall-composition (Greek, *allo*=altered, *teichus*=wall).

It is also proposed that strain C677–91 is designated *Streptoalloteichus hindustanus* gen. nov. and sp. nov., because the organism was isolated from a soil which was collected in the west part of India. The type strain is No. C677–91 and has been deposited in the American Type Culture Collection, Rockville, Md., and in Fermentation Research Institute, Ciba, Japan, where it has been assigned the designations ATCC 31217 and FERM-P No. 4070, respectively.

References

1) Becker, B.; M. P. Lechevalier & H. A. Lechevalier: Chemical composition of cell-wall preparations from strains of various form—genera of aerobic actinomycetes. Appl. Microbiol. 13: 236~243, 1965
2) Yamaguchi, T.: Comparison of the cell-wall composition of morphologically distinct actinomycetes. J. Bacteriol. 89: 444~453, 1965
3) Lechevalier, M. P. & H. A. Lechevalier: Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol. 20: 435~443, 1970
4) Higgens, C. E. & R. E. Kastner: Nebramycin, a new broad-spectrum antibiotic complex. II. Description of *Streptomyces tenebrarius*. Antimicr. Agents & Chemoth.-1967: 324~331, 1968
5) Kawato, M. & R. Shinobu: On *Streptomyces herbaricolor* nov. sp. Supplement: a simple technique for the microscopical observation. Mem. Osaka Univ. Lib. Arts. Educ. 8: 114, 1959
6) Shirling, E. B. & D. Gottlieb: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
7) Hoare, D. S. & E. Work: The stereoisomers of α,ε-diamino pimelic acid. 2. Their distribution in the bacterial order *Actinomycetales* and in certain *Eubacteriales*. Biochem. J. 65: 441~447, 1957
8) Ganno, G. & T. Satoh: Hitachi liquid chromatogram Type 034, and examples of its practical application. The Hitachi scientific instrument news 10: 505~506, 1967
9) Sweeley, C. C.; R. Bentley, M. Makita & W. W. Wells: Gas liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Am. Chem. Soc. 85: 2497~2507, 1963
10) Luedemann, G. M.: *Micromonospora purpureochromogenes* (Waksman and Curtis 1916) comb. nov. (subjective Synonym: *Micromonospora fusca* Jensen 1932). Int. J. Syst. Bacteriol. 21: 240~247, 1971
11) Lechevalier, M. P. & N. N. Gerber: The identity of madurose with 3-O-methyl-D-galactose. Carbohyd. Res. 13: 451~454, 1970
12) Lechevalier, M. P. & H. Lechevalier: Chemical methods as criteria for the separation of nocardiae from other *Actinomycetes*. Biology of the actinomycetes and related organism 11: 78~92, 1976
13) Lacey, J. & M. Goodfellow: A novel actinomycete from sugar-cane Bagasse: *Saccharopolyspora hirsuta* gen. et sp. nov.. J. Gen. Microbiol. 88: 75~85, 1975
14) Lechevalier, M. P. & H. Lechevalier: A critical evaluation of the genera of aerobic actinomycetes. *In*

H. PRAUSER (*ed.*), The Actinomycetales. G. Fisher, Jena, pp. 393～405, 1968

15) 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

16) PREOBRAZHENSKAYA, T. P.; M. A. SVESHNIKOVA & L. P. TEREKHOVA: A key for identification of the species of the genus *Actinomadura*. Actinomycetes and Related Organisms 12(1): 30～38, 1977

17) MEYER, J.: *Nocardiopsis*, a new genus of the order *Actinomycetales*. Int. J. Syst. Bacteriol. 26: 487～493, 1976

18) PRAUSER, H.: *Nocardioides*, a new genus of the order *Actinomycetales*. Int. J. Syst. Bacteriol. 26: 58～65, 1976

19) COUCH, J. N.: Some new genera and species of the *Actinoplanaceae*. J. Elisha Mitchell Sci. Soc. 79: 53～70, 1963

20) KANE, W. D.: A new genus of the *Actinoplanaceae, Pilimelia*, with a description of two species, *Pilimelia terevasa* and *Pilimelia anulata*. J. Elisha Mitchell Sci. Soc. 82: 220～230, 1966

21) THIEMANN, J. E.; H. PAGANI & G. BERETTA: A new genus of the *Actinoplanaceae*: *Planomonospora*, gen. nov.. G. Microbiol. 15: 27～38, 1967

22) THIEMANN, J. E. & G. BERETTA: A new genus of the *Actinoplanaceae*: *Planobispora* gen. nov.. Arch. Mikrobiol. 62: 157～166, 1968

23) THIEMANN, J. E.; H. PAGANI & G. BERETTA: A new genus of the *Actinoplanaceae*: *Dactylosporangium*, gen. nov.. Arch. Mikrobiol. 58: 42～52, 1967

24) CROSS, T.; M. P. LECHEVALIER & H. LECHEVALIER: A new genus of the *Actinomycetales*: *Microellobosporia* gen. nov.. J. Gen. Microbiol. 31: 421～429, 1963

25) FALCAO DE MORAIS, J. O.: The genus *Elytrosporangium* and its relationship to *Microellobosporia* and *Streptomyces*. Hindustan Antibiot. Bull. 9: 135～137, 1967

26) MATSUMAE, A.; M. OHTANI, H. TAKESHIMA & T. HATA: A new genus of the *Actinomycetales*: *Kitasatoa* gen. nov.. J. Antibiotics 21: 616～625, 1968

27) KALAKOUTSKII, L. V.; I. P. KIRILLOVA & N. A. KRASSILNIKOV: A new genus of the *Actinomycetales* - *Intrasporangium* gen. nov. J. Gen. Microbiol. 48: 79～85, 1967

28) KRASILNIKOV, N. A. & C. S. YUAN: *Actinosporangium*, a new genus of the family *Actinoplanaceae*. IZV. Akad. Nauk. SSSR Ser. Biol. 8: 113～116, 1961

29) CROSS, T. & M. GOODFELLOW: Taxonomy and classification of the actinomycetes. *In* SYKES and SKINNER (*edit.*). Actinomycetales. Characteristics and practical importance. Society of Applied Bacteriology Symposium Series No. 2; 11～112, 1973

30) SHIMAZU, A.; T. HIDAKA, S. OTSUKA, M. NISHIYAMA & H. YONEHARA: *Streptomyces sclerogranulatus* sp. nov., the producer of sclerothricin. J. Antibiotics 22: 590～596, 1969

31) KAWAGUCHI, H.; H. TSUKIURA, K. TOMITA, M. KONISHI, K. SAITO, S. KOBARU, K. NUMATA, K. FUJISAWA, T. MIYAKI, M. HATORI & H. KOSHIYAMA: Tallysomycin, a new antitumor antibiotic complex related to bleomycin. I. Production, isolation and properties. J. Antibiotics 30: 779～788, 1977