KITASATOSPORIA, A NEW GENUS OF THE ORDER *ACTINOMYCETALES*

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The morphological, cultural, physiological and biochemical characteristics of a new actinomycete strain producing a new antibiotic, setamycin are described. The strain forms aerial mycelia. There is no fragmentation of vegetative mycelia. Since the cell wall type is a new one containing both LL- and *meso*-2,6-diaminopimelic acid, glycine and galactose, strain KM-6054 could not be classified in any previously named genera of the order *Actinomycetales*. Thus, it is considered to be a member of a new genus, for which the name *Kitasatosporia* is proposed. The type species (monotype) of this genus is *K. setalba*. The type strain of *K. setalba* is strain KM-6054 (ATCC 33774).

During a taxonomic study of a soil isolate, strain KM-6054, which produces a new antibiotic, setamycin[1], inhibiting the growth of some fungi, trichomonads and Gram-positive bacteria, it was found that the mycelia of the strain contain similar amounts of both LL-2,6-diaminopimelic acid (DAP) and *meso*-DAP as preliminarily reported[2]. It was also found that when DAP analysis is carried out separately on aerial and vegetative mycelia grown on an agar medium, most of the DAP contained in aerial mycelia is LL-isomer, while that in vegetative mycelia is *meso*-isomer.[2]

The morphology and cultural characteristics resembled those of strains of the genus *Streptomyces*, but because strain KM-6054 possesses a cell wall of a new type (major constituents; LL-DAP, *meso*-DAP, glycine and galactose) it cannot be placed in any of the known genera of the order *Actinomycetales*. The strain has been fully characterized and is described as belonging to a new genus, for which the name *Kitasatosporia* (Ki.ta.sa.to.spo'ri.a: Kitasato, a Japanese bacteriologist; Gr. n. *sporus* a seed; M. L. ferm. n. *sporia* spore) is proposed. The type species of *Kitasatosporia* is *K. setalba* sp. nov.

Materials and Methods

Bacterial Strain

The new organism, strain KM-6054, was isolated by plating a water suspension of a soil sample collected at Setagaya-ku, Tokyo, on a synthetic medium (1% starch, 1% glycerol, 0.2% $(NH_4)_2SO_4$, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.1% NaCl, 0.2% $CaCO_3$ and 1% agar) and by incubating it at 27°C for 2 weeks. The culture was maintained on inorganic salts-starch agar.

Microscopy

The morphology of strain KM-6054 was observed with an Olympus Vanox photomicroscope and a scanning electron microscope (model S-430, Hitachi).

Observation for Cultural and Physiological Characteristics

The media recommended by WAKSMAN[3] and the International Streptomyces Project (ISP) media

recommended by SHIRLING and GOTTLIEB[4] were used. The cultures were observed after incubation at 27°C for two weeks. Color names and hue numbers indicated are those of the Color Harmony Mannual (4th edition) published by Container Corporation of America. Utilization of carbon sources was tested by growth on Pridham and Gottlieb medium[5] containing 1% each carbon source.

Whole Cell and Cell Wall Preparations

A loopful spores and mycelia of strain KM-6054 were transferred to a 500-ml Sakaguchi flask containing 100 ml of a medium consisting of 1% yeast extract and 1% glucose and the flask was incubated on a reciprocal shaker at 27°C for 72 hours. The mycelia were harvested by centrifugation and thoroughly washed with distilled water. The washed mycelia were used as whole cell preparation after treating with ethanol and drying at room temperature. The cell wall preparation was obtained from the washed mycelia by the method of YAMAGUCHI[6].

Chemical Analyses

Amino acids and sugars in cell wall and whole cell preparations were analyzed as described by BECKER *et al.*[7] Analyses of the cellular phospholipids and glycolic acid in cell wall were carried out by the methods of LECHEVALIER *et al.*[8] and of UCHIDA and AIDA[9], respectively.

Antibiotic Susceptibility

The minimum inhibitory concentrations (MICs) were assayed by an agar dilution method. A seed medium (0.1% glucose, 2.4% starch, 0.3% peptone, 0.3% meat extract, 0.5% yeast extract and 0.4% $CaCO_3$) in a 50-ml test tube was inoculated with strain KM-6054 and incubated on a reciprocal shaker at 27°C for 3 days. The culture was streaked onto the surface of yeast extract - malt extract agar containing the antibiotic at various concentrations. After incubation at 27°C for 3 days, the surface growth was compared with that on a control plate without antibiotic.

Results

The salient characteristics of *Kitasatosporia* gen. nov. and of *K. setalba* sp. nov. are as follows: colony, ivory and leathery; fragmentation of vegetative mycelia, none; morphology of aerial mycelia, *Rectus-flexibilis*; aerial mass color, white to gray; spore chains, more than 20 spores ($0.7 \times 0.8 \sim 1.6$ μm) per chain formed from aerial mycelia; sporangium and zoospore, none; cell wall type, a new type containing LL-DAP, *meso*-DAP, glycine and galactose.

Morphology

Strain KM-6054 is well developed on both synthetic and complex media. No fragmentation of the vegetative mycelium is observed (Plate 1). The aerial mycelium, which has more than 20 spores per chain, is classified in the section *Rectus-flexibilis* (Plate 2). The spores are cylindrical in shape and $0.7 \times 0.8 \sim 1.6$ μm in size. The spore surface is smooth with some wrinkled forms. Sporangia, zoospores and sclerotia are not observed. The colony of strain KM-6054 is leathery.

Cultural and Physiological Characteristics

The cultural characteristics of strain KM-6054 are shown in Table 1. White or light gray aerial mycelium is formed on yeast extract - malt extract agar, oatmeal agar, inorganic salts - starch agar and glucose - asparagine agar. However, the strain does not form aerial mycelium on glucose - peptone agar and nutrient agar. It produces a small amount of yellow soluble pigment in inorganic salts - starch agar. Physiological characteristics and utilization of carbon sources of strain KM-6054 are shown in Tables 2 and 3, respectively. Melanin is not produced. Both peptonization and coagulation of milk are positive. The culture grows at $22 \sim 37$°C. The strain utilizes D-glucose, L-arabinose and D-xylose.

Table 1. Cultural characteristics of *Kitasatosporia setalba* gen. nov. and sp. nov.

Medium	Cultural characteristics
Yeast extract - malt extract agar*	G : good, raised, colonial yellow (2ga) R : colonial yellow (2ga) AM: abundant, powdery, white (a) SP : none
Oatmeal agar*	G : moderate, penetrant, pale yellow (1ca) R : antique gold (1½pe) AM: moderate, velvety, white (a)~gray (e) SP : none
Inorganic salts - starch agar*	G : moderate, penetrant, gold (1½pc) R : antique gold (1½ne) AM: moderate, powdery, white (a)~light gray (d) SP : yellow maple (3le)
Glycerol - asparagine agar*	G : poor, penetrant, light ivory (2ca) R : light ivory (2ca) AM: poor, powder, white (a) SP : none
Glucose - asparagine agar	G : moderate, penetrant, raised, gold (1½pc) R : gold (1½pc) AM: moderate, powdery, white (a) light gray (d) SP : none
Peptone - yeast extract - iron agar*	G : good, light ivory (2ca) R : light yellow (1½ea) AM: none SP : none
Tyrosine agar*	G : moderate, penetrant, pearl (2ba) R : pearl (2ba) AM: poor, powdery, oyster white (b) SP : none
Sucrose - nitrate agar**	G : poor, pearl (2ba) R : pearl (2ba) AM: poor, white (a) SP : none
Glucose - nitrate agar**	G : poor, light ivory (2ca) R : light ivory (2ca) AM: none SP : none
Glycerol - calcium malate agar**	G : poor, pearl (2ba) R : pearl (2ba) AM: poor, white (a) SP : none
Glucose - peptone agar**	G : moderate, penetrant, cream (1½ca) R : light ivory (2ca) AM: none SP : none
Nutrient agar**	G : moderate, penetrant, cream (1½ca) R : cream (1½ca) AM: none SP : none

* Medium recommended by International Streptomyces Project.
** Medium recommended by S. A. WAKSMAN.
Abbreviation: G, growth of vegetative mycelium; R, reverse; AM, aerial mycelium; SP, soluble pigment.

Table 2. Physiological properties of *Kitasatosporia setalba* gen. nov. and sp. nov.

Melanin formation	−
Tyrosinase reaction	−
Nitrate reduction	−
Hydrolysis of starch	+
Liquefaction of gelatin	−
Peptonization of milk	+
Coagulation of milk	+
Cellulolytic activity	−
Temperature range for growth	22~37°C

Table 3. Utilization of carbon sources by *Kitasatosporia setalba* gen. nov. and sp. nov.

Carbon source	Utilization
D-Glucose	+
D-Fructose	−
L-Rhamnose	−
D-Mannitol	−
L-Arabinose	+
i-Inositol	−
Raffinose	−
D-Xylose	+
Sucrose	−
Cellulose	−

Plate 1. Photomicrograph of vegetative mycelia of *Kitasatosporia setalba* gen. nov. and sp. nov.

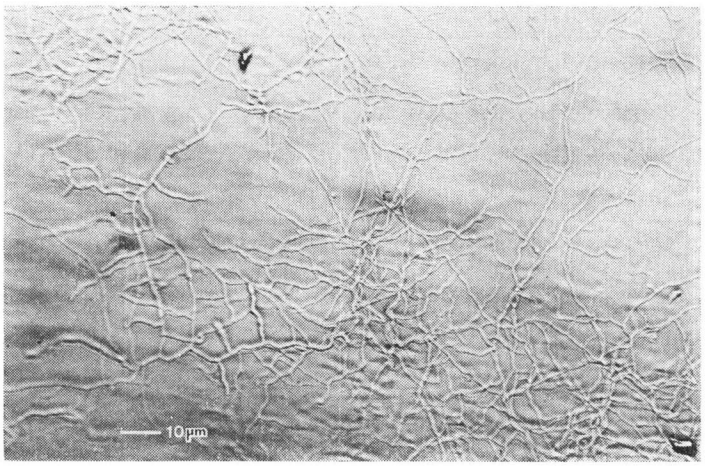
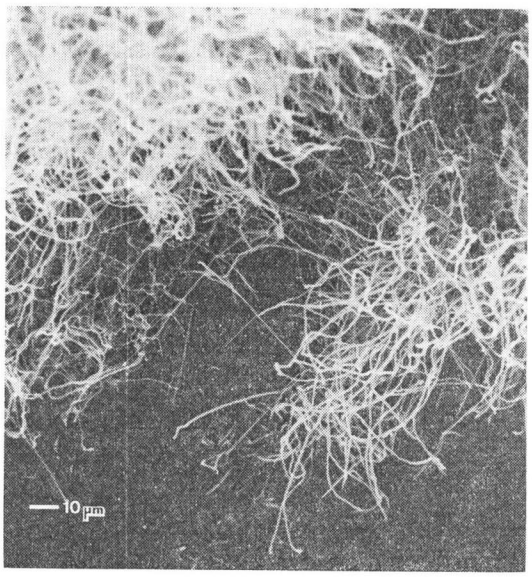
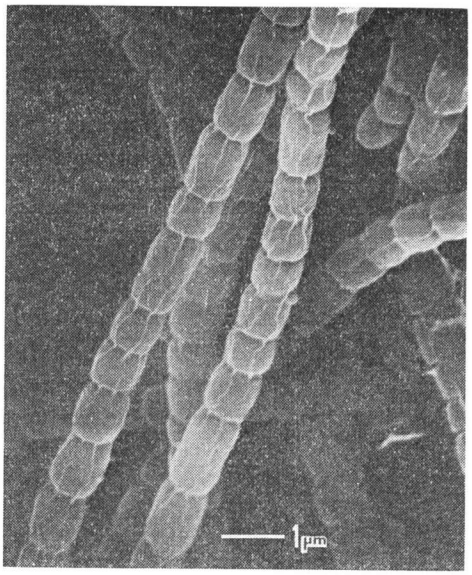


Plate 2. Scanning electronmicrograph of aerial mycelia of *Kitasatosporia setalba* gen. nov. and sp. nov.





Chemical Analyses

The cell wall was found to contain similar amounts of LL- and *meso*-DAP, glycine and galactose. These analytical data indicate that there is no known genus in which strain KM-6054 should be classified. From the analysis of phospholipids, the strain belongs to PII type. The glycolate test was negative.

Staining Reactions

The mycelium grown under submerged conditions was Gram-positive and was not acid-fast.

Susceptibility to Antibiotics

The susceptibility was evaluated by MICs. Strain KM-6054 is sensitive to streptomycin (5 μg/ml), penicillin G (5 μg/ml) and chloramphenicol (10 μg/ml). It is resistant to colistin (>100 μg/ml) and nystatin (>100 μg/ml).

Table 4. Comparison of *Kitasatosporia* gen. nov. with related genera of the order *Actinomycetales*.

Genera	Cell wall						Whole cell sugar					Aerial mycelium (sporulation)	Vegetative mycelium (fragmentation)
	Type	DAP		Glycine	Arabinose	Galactose	Sugar pattern	Arabinose	Galactose	Xylose	Madurose		
		meso-	LL-										
Kitasatosporia	new type (X)[a]	+	+	+	−	+	new pattern (E)[b]	−	+	−	−	+	−
Streptomyces	I	−	+	+	−	−	NC[c]	−	−	−	−	+	−
Nocardioides	I	−	+	+	−	−	NC[c]	−	−	−	−	+	+
Actinomadura	III	+	−	−	−	−	B	−	−	−	+	+	−
Nocardiopsis	III	+	−	−	−	−	C	−	−	−	−	+	+
Pseudonocardia	IV	+	−	−	+	+	A	+	+	−	−	+	+/−[d]
Nocardia	IV	+	−	−	+	+	A	+	+	−	−	−/+[e]	+

[a]: Cell wall types I~IX are known[10].
[b]: Whole cell sugar patterns A~D are known[10].
[c]: NC, no characteristic sugar pattern.
[d]: Some species of the genus *Pseudonocardia* do not always form fragmentation of vegetative mycelia.
[e]: Some species of the genus *Nocardia* from aerial mycelia.

NaCl Tolerance

Yeast extract - malt extract agar medium was used for NaCl tolerance test. The strain grows on the agar medium containing less than 2% NaCl. No growth was observed at the concentration of 3%.

Type Strain

The type strain is *K. setalba* KM-6054 (American Type Culture Collection 33774). Because the species description is based on a single strain (type strain), the species description given here also serves as the description of the type strain.

Discussion

The morphology and cultural characteristics of the new actinomycetes strain KM-6054 described above resemble those of a strain of the genus *Streptomyces*. However, the cell wall contains LL-DAP, *meso*-DAP, glycine and galactose. The cell wall type does not belong to any cell wall types (I~IX) reported by LECHEVALIER and LECHEVALIER[10]. Thus, it is considered to be a new one, for which the type X is proposed. Since the cell wall type has been one of the most important criteria in the classification of genera of the order *Actinomycetales*[10], this evidence indicates that the strain belongs to a new genus, for which the name *Kitasatosporia* is proposed.

Streptomyces, Nocardioides, Actinomadura, Nocardiopsis, Pseudonocardia and *Nocardia* can be listed up as the known genera which produce aerial mycelia and have no sporangium and no zoospore. The comparison of *Kitasatosporia* with these known genera for the major constituents in cell wall and whole cell is shown in Table 4. It is apparent that *Kitasatosporia* is distinguishable from these six known genera.

As described above, LL- and *meso*-DAP were detected in cell wall of the genus *Kitasatosporia*. Interestingly, we have found that when DAP analysis is carried out separately on the aerial and vegetative mycelia grown on an agar medium, most of the DAP in the aerial mycelium is LL-isomer while that in the vegetative mycelia is *meso*-isomer. The analyses of glycine, galactose and arabinose carried out separately on the aerial and vegetative mycelia are also of interest and more studies are now in progress.

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