

TAXONOMIC STUDIES ON  
*KITASATOSPORIA CYSTARGINEA* SP. NOV., WHICH PRODUCES  
A NEW ANTIFUNGAL ANTIBIOTIC CYSTARGIN

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(Received for publication July 29, 1988)

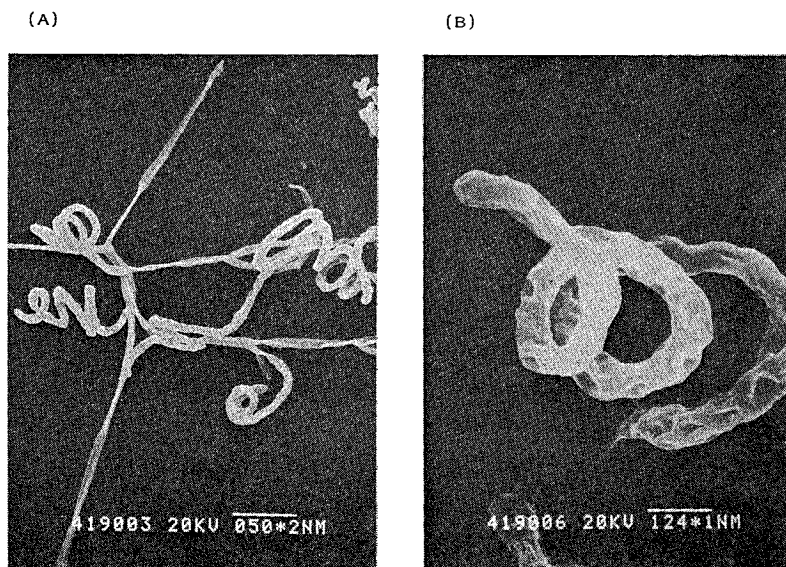
Taxonomic studies on a new species, *Kitasatosporia cystarginea* are presented. Among the several species already described in this genus, this strain is characteristic in forming distinct spirals of spore chains. A significant properties of the species is the production of a new antifungal antibiotic, cystargin.

An actinomycete, strain RK-419 was isolated from a soil sample collected in Susa-machi, Yamaguchi Prefecture, Japan and it was found to produce a new antifungal antibiotic cystargin. The strain which has both L,L- and meso-2,6-diaminopimelic acids as cell wall components was considered to belong to the genus *Kitasatosporia*. Taxonomic studies described herein have led to the conclusion that it is a new species and the name was proposed for the strain as *Kitasatosporia cystarginea*. Taxonomic studies were carried out in accordance with the procedure by LECHEVALIER, and LECHEVALIER *et al.*<sup>1,2)</sup>, and UCHIDA and AIDA<sup>3)</sup>.

Microscopic Characteristics

A mature spore chain on aerial mycelium had 10 to 30 or more spores per chain. The spore chain formed spirals with 3 to 5 turns on oatmeal nitrate medium as shown in Fig. 1(A). The spores were cylindrical and 0.5~1.0  $\mu\text{m}$  in size with a smooth but rugose surface and a definite spore length,

Fig. 1. Electron micrograph of sporophores of strain RK-419.



as shown in Fig. 1(B). Sporangia, zoospores, sclerotia and fragmentation of vegetable mycelium were not observed.

#### Cultural and Physiological Characteristics

The organism was cultivated on various agar media at 27°C, and cultural characteristics were observed after 7, 14 and 21 days cultivation. Results are summarized in Table 1. It forms white to gray aerial mycelium and produces no soluble pigment.

No growth was observed on sucrose - nitrate agar. The physiological properties were examined according to the method described by SHIRLING and GOTTLIEB<sup>4)</sup>. As summarized in Table 2, it hydrolyzed starch but did not reduce nitrate. Utilization of carbon sources was examined on PRIDHAM and GOTTLIEB's inorganic medium. The results were shown in Table 3. D-Glucose and inositol were utilized by the strain. D-Fructose and L-arabinose were doubtfully utilized, and D-mannitol, D-xylose,

Table 1. Cultural characteristics of strain RK-419.

	Growth	Aerial mycelium	Reverse color	Soluble pigment
Starch - yeast extract agar <sup>a</sup>	Moderate	Moderate Natural string (3dc)	Mustard gold (2ne)	None
Yeast extract - malt extract agar (ISP No. 2)	Moderate	Abundant Light gray (c)	Camel (3ie)	None
Oatmeal agar (ISP No. 3)	Good	Abundant Gray (h)	Covert gray (2fe)	None
Inorganic salts - starch agar (ISP No. 4)	Moderate	Moderate Shell (3ca)	Shell (3ca)	None
Tyrosine agar <sup>b</sup>	Good	Moderate Rosewood (5ge)	Maple sugar (3ie)	None
Glucose - asparagine agar	Good	Abundant Silver gray (5fc)	Bamboo (2gc)	None
Glycerol - asparagine agar (ISP No. 5)	Good	Abundant Ashes (5fe)	Camel (3ie)	None
Sucrose - nitrate agar	None			
Nutrient agar	Moderate	Moderate White (a)	Shell (3ca)	None
Peptone - yeast extract - iron agar	Poor	None	Shell (3ca)	None

<sup>a</sup> Starch 1%, yeast extract 0.1%, NZ Amine A 0.1%, agar 1.5% and water 1,000 ml, pH 7.2.

<sup>b</sup> L-Tyrosine 0.05%, glucose 0.2%, yeast extract 1%, agar 2% and water 1,000 ml, pH 7.0.

The color scheme used was Color Harmony Manual (Container Corporation of America).

Table 2. Physiological properties of strain RK-419.

Growth temperature range <sup>a</sup>	17~40°C
Optimum temperature range	28~35°C
Nitrate reduction	Negative
Starch hydrolysis	Positive
Gelatin liquefaction	Negative
Milk coagulation	Positive
Milk peptonization	Positive
Melanin formation <sup>b</sup>	Negative

<sup>a</sup> Starch - yeast extract agar.

<sup>b</sup> Tyrosine agar.

Table 3. Utilization of carbohydrates by strain RK-419.

D-Glucose	+
L-Arabinose	±
D-Xylose	-
D-Fructose	±
Sucrose	-
Inositol	+
L-Rhamnose	-
Raffinose	-
D-Mannitol	-
Control	-

+, Growth; ±, growth doubtful; -, no growth.

Table 4. Physiological characteristics of *Kitasatosporia*.

	<i>K.s.</i> KM-6054	<i>K.s.</i> SANK 60684	<i>K.p.</i> KA 338	<i>K.g.</i> AM 9660	<i>Kitasatosporia</i> sp. MF-730-N6	<i>K.k.</i> 9482	<i>K.c.</i> RK-419
Aerial mycelium		Rectiflexible			Rectiflexible	Hook or spirals	Spirals
Spore shape	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Spore surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Growth temperature (°C)	15~37	6~38	15~42	15~37	20~37	25~33	17~40
Optimum temperature (°C)		19~28			27~30	30	28~35
Melanin formation	Negative	Negative	Negative	Negative	Negative	Positive	Negative
Soluble pigment	Yellow maple		Light tan	Pink	Pale yellow	None	None
Nitrate reduction	Negative	Positive	Negative	Negative	Negative	Negative	Negative
Starch hydrolysis	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Gelatin liquefaction	Negative		Negative	Negative	Negative	Negative	Negative
Milk peptonization	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Aerial mycelium color	White	Gray	White	Gray	Gray	Gray	Gray
GC content (%)	73.1		66.6	66.0	70.8	71.2	71.7
Antibiotic produced	Setamycin	Propioxatin	Phosalacin	Setamycin	Terpentecin	RF-900494	Cystargin
Utilization of carbohydrates:							
D-Glucose	+	+	+	+	+	+	+
L-Arabinose	+	±	+	+	+	+	±
D-Xylose	-	-	-	+	+	-	-
D-Fructose	-	-	+	-	-	-	±
Sucrose	-	-	+	-	-	±	-
Inositol	-	-	-	-	-	-	+
L-Rhamnose	-	-	+	-	-	-	-
Raffinose	-	-	+	+	-	-	-
D-Mannitol	-	-	-	-	-	+	-

Abbreviations: *K.s.*, *Kitasatosporia setae*; *K.p.*, *Kitasatosporia phosalacinea*; *K.g.*, *Kitasatosporia griseola*; *K.k.*, *Kitasatosporia kifumense*; *K.c.*, *Kitasatosporia cystarginea*.

+, Growth; ±, growth doubtful; -, no growth.

L-rhamnose, raffinose and sucrose were not utilized.

#### Chemical Analysis

The mycelia from a submerged culture and the aerial mycelia grown on agar plates, were used for analysis<sup>5)</sup>. Both mycelia contained spores. Cell wall fractions were hydrolyzed with 6 N HCl at 110°C, 18 hours, and amino acid components of the hydrolysate were analyzed. Diaminopimelic acid, glutamic acid, glycine and alanine were present. The hydrolysate was dansylated with dansyl chloride and dansyl amino acids were analyzed by HPLC<sup>6)</sup>. Both L,L- and meso-2,6-diaminopimelic acids were detected in almost equal molar ratio. The GC content was determined to be 71.7% by the method described by KANEKO *et al.*<sup>7)</sup>. The analyses of phospholipid show that the strain belongs to type PII as defined by LECHEVALIER *et al.*<sup>8)</sup>, and MINNIKIN *et al.*<sup>9)</sup>. Glycolic acid was not detected.

#### Comparison of the Strain RK-419 with Other Species of *Kitasatosporia*

The data described above clearly show that the strain belongs to the genus *Kitasatosporia*. The comparison of the strain RK-419 with *Kitasatosporia setae* KM-6054<sup>5,10,11)</sup>, *K. setae* SANK 60684<sup>12)</sup>, *Kitasatosporia phosalacines*<sup>11,13)</sup>, *Kitasatosporia griseola*<sup>11,13)</sup>, *Kitasatosporia* sp. MF-730-N6<sup>14)</sup> and *Kitasatosporia kifunense*<sup>15)</sup> are shown in Table 4. The aerial mycelium of the strain RK-419 terminated in distinct spirals, in 3 to 5 turns on various agar media. *K. kifunense* is the only species whose aerial mycelium is described to form spirals. However they are often hooked and straight<sup>14)</sup>. On the other hand, the strain RK-419 is first one which forms distinct spirals. It is also different from *K. kifunense* in producing no melanoid pigment on tyrosine agar. In addition, the utilization of carbon sources significantly different in both strains.

Based on the above properties, the strain RK-419 is considered to be a new species of the genus *Kitasatosporia*, for which the name *K. cystarginea* sp. nov. is proposed on the basis of the productivity of a new antibiotic cystargin. Strain RK-419 is the type strain of *K. cystarginea*; a culture of this strain was deposited in Japan Collection of Microorganisms at RIKEN, the Institute of Physical and Chemical Research under the number JCM 7356 (FERM P-8006).

The significant feature of *K. cystarginea* is the production of a new antifungal peptide antibiotic cystargin. The isolation and characterization of the antibiotic is described in the following paper<sup>16)</sup>.

#### Acknowledgment

The authors wish to thank Dr. A. SEINO of Japan Collection of Microorganisms at this Institute for his valuable advice and electron microscopy.

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