

TAXONOMIC STUDIES ON *STREPTOMYCES AQUACANUS*, ANTIBIOTIC AB-74-PRODUCING ORGANISM

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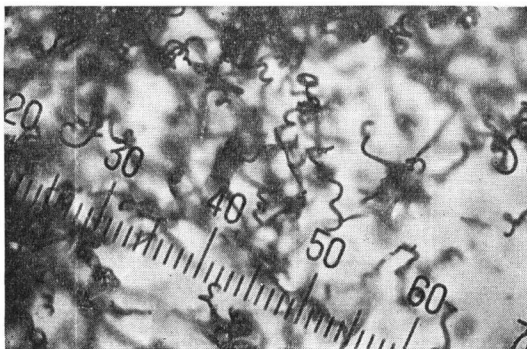
During the course of screening for new antibiotics, a *Streptomyces* strain A-14317 isolated from a soil sample collected at Ito-gun, Wakayama Prefecture was found to produce an antibiotic AB-74,<sup>1)</sup> related to destomycin C, together with neomycins A, B, C and hygromycin B. On the basis of morphological and physiological characteristics of strain A-14317, the organism was considered a new species and designated as *Streptomyces aquacanus* A-14317.

Taxonomic studies were generally carried out in accordance with methods adopted by the International Streptomyces Project.<sup>2)</sup> A detailed characterization of the new taxon is as follows:

### 1. Morphological Characteristics

Spore chain morphology: *Retinaculi-Apertum* (RA) to *Spira*. Flexuous spore chains and chains with terminal hooks or loops were common. Some open spirals were observed (Plate 1). Mature spore chains were long, often with more than 50 spores per chain. This morphology was seen on inorganic salts-starch agar, but sporulating aerial mycelium was usually not formed on glycerol-asparagine agar and yeast

Plate 1. Aerial mycelium of *Streptomyces aquacanus* A-14317 on inorganic salts-starch agar after 21 days.



extract-malt extract agar. Spore surface: smooth (Plate 2).

### 2. Cultural and Physiological Characteristics

Characteristics of the strain A-14317 on various media are listed in Tables 1 and 2. The utilization of carbon sources on PRIDHAM-GOTTLIEB basal medium was as follows: Good growth with D-xylose, D-glucose, D-fructose, maltose and starch; growth with D-galactose; no growth with D-arabinose, sucrose, inositol, L-rhamnose, D-mannitol, inulin,  $\alpha$ -methyl glycoside, salicin, D-sorbitol, L-arabinose, melibiose, meso-erythritol, esculin and no carbon.

From the observation described above, strain A-14317 may be characterized as follows:

Sporophores: *Retinaculi-Apertum* to *Spira*.

Spore surface: smooth.

Aerial mass color: medium gray to pale bluish gray.

Reverse side color: brown series (not distinctive).

Melanin: not produced.

Soluble pigment: not produced.

D-Glucose, D-xylose, D-fructose and D-galactose are utilized for growth.

After comparing the characteristics of those *Streptomyces* species described in the ISP reports by SHIRLING and GOTTLIEB,<sup>3,4,5,6)</sup> BERGEY'S Manual of Determinative Bacteriology (8th Edition, 1974) and other recent literatures, some related strains were selected for further detailed comparison. *Streptomyces avellaneus*, *S. griseoluteus*, *S. murinus* and *S. siyoaensis* were obtained by the key of BERGEY'S Manual. *S. siyoaensis* was obtained by KÜSTER'S key.<sup>7)</sup> *S.*

Plate 2. Electron-micrograph of spores of *Streptomyces aquacanus* A-14317 on inorganic salts-starch agar after 21 days.

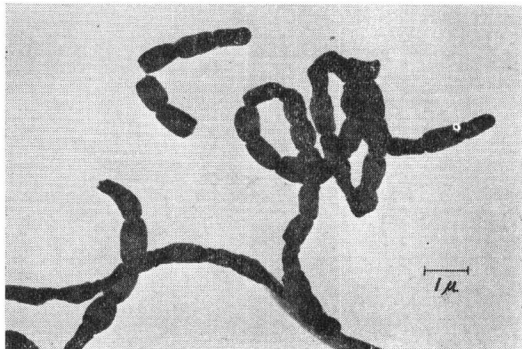


Table 1. Cultural characteristics of strain A-14317

The number in parentheses corresponds to the hue number used in the "Color Harmony Manual, 1958".

Culture media	Growth	Aerial mycelium	Soluble pigment
Sucrose-nitrate agar	Scant, spreading, colorless to white	Scant, powdery, white to gray	None
Glucose-asparagine agar	Moderate, raised, pale yellow green (1ec)	Moderate, powdery, medium gray (g) to pale bluish gray (19fe)	None
Glycerol-asparagine agar	Moderate, raised, light grayish brown (3ie to 4pi)	Moderate, sporulation may be poor, medium gray (g) to pale bluish gray (19fe)	None
Inorganic salts-starch agar	Abundant, raised, dark greenish gray (24 1/2fe)	Abundant, powdery, medium gray (g) to pale bluish gray (19fe)	None
Tyrosine agar	Moderate, raised, light grayish yellowish brown (3ec to 31g)	Moderate, cottony, medium gray (g) to pale bluish gray (19fe)	None
Yeast extract-malt extract agar	Moderate, raised, grayish yellow brown (31e)	Moderate, sporulation may be poor, medium gray (g) to pale bluish gray (19fe)	None
Oatmeal agar	Scant, spreading, pale yellow	Scant, powdery, medium gray (g) to pale bluish gray (19fe)	None
Glycerol-Ca malate agar	Moderate, raised yellowish brown (3ie)	Scant, powdery, medium gray (g) to pale bluish gray (19fe)	None
Nutrient agar	Moderate, spreading, hylaine, colorless to pale yellow	None	None
Glucose-nutrient agar	Moderate, small colonies, spreading, light grayish yellowish brown (21e)	None	None
Glucose-peptone agar	Moderate, spreading, grayish brown (4pe)	None	None
Potato plug	Moderate, lichenoid, light brown (5ne)	None	Plug: grayish brown
Skim milk	Moderate, ring and pellicle attached to glass	None	Pale brown
Cellulose agar	No growth		
Gelatin stab	No growth		

*tharmonitrificans*, *S. naraensis* and *S. sioyaensis* were obtained by NONOMURA's key.<sup>8)</sup> *S. griseus* and *S. aureofaciens* by HÜTTER's key.<sup>9)</sup> It was found, however, that these eight species were differentiated from strain A-14317 in following points.

*S. avellaneus*<sup>6)</sup>: Spore chain: *Rectus Flexibilis*. Inositol, D-mannose and sucrose are utilized for growth.

*S. griseoluteus*<sup>6)</sup>: Spore chain: *Rectus Flexibilis*. L-Arabinose and D-mannitol are utilized for growth.

*S. murinus*<sup>3)</sup>: Aerial mass color: Red color-series on glucose asparagine agar, gray or red color-series on oatmeal agar and glycerol-asparagine agar. D-mannitol is utilized for growth.

*S. sioyaensis*<sup>3)</sup>: Spore chain: compact spirales. Reverse side color: yellow series. Inositol, D-mannitol, raffinose and sucrose are utilized for growth.

*S. tharmonitrificans*<sup>6)</sup>: Spore chain: short (3 ~10 spores per chain). Inositol, D-mannose and sucrose are utilized for growth.

*S. naraensis*<sup>6)</sup>: Aerial mass color: light brownish gray and light grayish reddish brown. Rhamnose, raffinose and sucrose are utilized for growth.

*S. griseus*<sup>4)</sup>: Aerial mass color: yellow color series. Melanin: formed in tyrosine agar. D-Mannitol is utilized for growth.

*S. aureofaciens*<sup>4)</sup>: Spore chain: flexuous. L-Arabinose and sucrose are utilized for growth.

Table 2. Physiological characteristics of strain A-14317

Test	Results
Melanin formation	Negative
Digestion of milk	Peptonized without coagulation
Production of H <sub>2</sub> S	Positive
Reduction of nitrate	Negative
Solubility of Ca malate	Rapidly solved
Hydrolysis of starch	Positive
Durability of NaCl	Optimum growth: 0~1% No growth: 5%
Relation to temperature	Optimum growth: 26~34°C No growth: 40°C
Relation to pH of medium	Optimum growth: pH 7~9 No growth: pH 4 and 11

On the other hand, strain A-14317 was not in accord with hygromycin B-destomycin group producing strains, *S. hygrosopicus*<sup>9)</sup> (Spore surface: warty. Aerial mycelium: moist, black, liquefied areas.), *S. eurocidicum*<sup>9)</sup> (*Verticillati*) and *S. rimofaciens*<sup>10)</sup> (*Verticillati*). Recently, two neomycin-producing strains, *Actinomyces ipomoea* var. 3381 and *A. coeruleoprunus*, with blue aerial mycelia were reported.<sup>11)</sup> But strain A-14317 was not in accord with these strains.

*A. ipomoea* (*S. ipomoea*) var. 3381: Aerial mass color: blue series. Growth: poorly on most synthetic media.

*A. coeruleoprunus*: Aerial mass color: blue series. Spore chain: straight. Soluble pigment and melanoid: produced.

From these considerations, strain A-14317 is reasonably recognized as a new species of *Streptomyces* and designated as *Streptomyces aquacanus* nov. sp. A-14317, referring to pale blue (aqua) gray aerial mass of the organism. Progeny of the type strain of *S. aquacanus* A-14317 have been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Chiba, Japan, as FERM-P No. 2495.

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