ASPARENOMYCINS A, B AND C, NEW CARBAPENEM ANTIBIOTICS

I. TAXONOMIC STUDIES ON THE PRODUCING MICROORGANISMS

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Two streptomycete isolates, PA-31088 and PA-39504, were found to produce new carbapenem antibiotics, asparenomycins A, B and C. Strain PA-31088 was identified as a new species of *Streptomyces* and the name *Streptomyces tokunonensis* sp. nov. proposed. Strain PA-39504 was identified as *Streptomyces argenteolus*.

Two streptomycete isolates, PA-31088 and PA-39504, were found to produce new carbapenem antibiotics, asparenomycins A, B and C[1,2]. This paper deals with the taxonomic studies on these two strains.

Materials and Methods

Morphology

The morphologies of the two strains were studied on cultures grown on BENNETT's agar at 28°C for 2 weeks.

Cultural Characteristics

Spores of the strains were collected from 14-day-old cultures grown on BENNETT's agar and suspended in sterile water. One drop of the suspension was inoculated on the various media according to the description of SHIRLING and GOTTLIEB[3] and incubated at 28°C. Cultural characteristics were observed after 2 weeks.

Utilization of Carbon Sources

Utilization of carbon sources was investigated by the method of PRIDHAM and GOTTLIEB[4].

Results and Discussion

Morphological Characteristics

(1) Strain PA-31088

The vegetative mycelium develops well on most of the media used. On some media such as yeast extract - malt extract agar and BENNETT's agar, it forms abundant aerial mycelia which develop into many spirals (Fig. 1).

The spores are cylindrical and surfaces of the spores are smooth under electron microscope (Fig. 2). Mature spore-chains are moderately long with 10~50 spores per chain on the tip of serial mycelia; sporangium and flagellated spores are not observed. In vegetative mycelium, fragmentation and sclerotia are not observed.

(2) Strain PA-39504

The vegetative mycelium develops well on some media such as tyrosine agar and BENNETT's agar.

Fig. 1. Aerial mycelium of strain PA-31088.

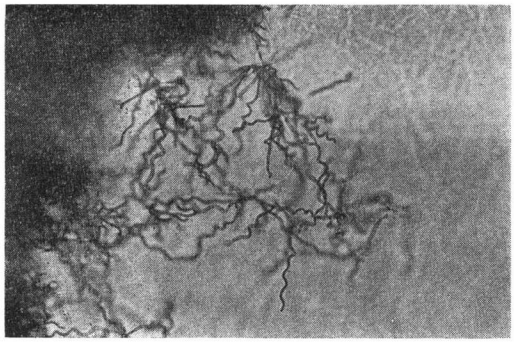

Fig. 2. Electron micrograph of spores of strain PA-31088.

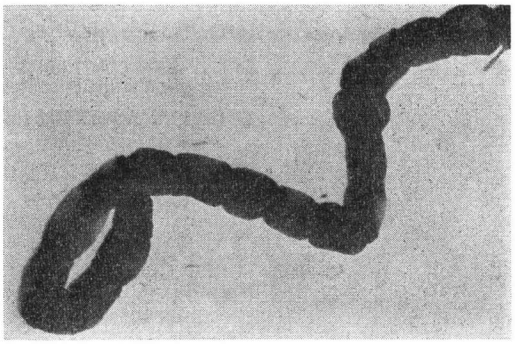

Table 1. Cultural characteristics of strain PA-31088.

Media	Growth	Sporulation	Color of colony	Reverse color	Soluble pigment
Sucrose - nitrate agar	Fair	Fair	Pale brown	Pale yellowish brown	None
Glucose - asparagine agar	Fair	No	—	Pale yellowish brown	None
Glycerol - asparagine agar	Fair	Poor	White	Pale yellowish brown	None
Inorganic salts - starch agar	Good	Good	Pale reddish orange	Pale yellowish brown	None
Tyrosine agar	Good	Fair	Pale brown	Pale yellowish brown	None
Nutrient agar	Fair	No	—	Pale yellowish brown	None
Yeast extract - malt extract agar	Good	Good	Pale reddish orange	Pale yellowish brown	None
Oatmeal agar	Good	Good	Pale reddish orange	Pale yellowish brown	None
BENNETT's agar	Good	Good	Pale reddish orange	Pale yellowish brown	None

Table 2. Cultural characteristics of strain PA-39504.

Media	Growth	Sporulation	Color of colony	Reverse color	Soluble pigment
Sucrose - nitrate agar	Poor	Poor	Light brownish gray	Pale yellowish brown	None
Glucose - asparagine agar	Poor	No	—	Pale yellowish brown	None
Glycerol - asparagine agar	Fair	Fair	Light brownish gray	Pale yellowish brown	None
Inorganic salts - starch agar	Good	Good	Brownish gray	Pale yellowish brown	None
Tyrosine agar	Good	Good	Light brownish gray	Pale yellowish brown	None
Nutrient agar	Fair	No	—	Pale yellowish brown	None
Yeast extract - malt extract agar	Good	Good	Brownish gray	Yellowish brown	None
Oatmeal agar	Good	Good	Light brownish gray	Pale yellowish brown	None
BENNETT's agar	Good	Good	Brownish gray	Yellowish brown	None

It forms abundant aerial mycelia which develop into loops and short spirals. The spores are cylindrical and surfaces of the spores are smooth under electron microscope. Mature spore-chains are rather short with 10~20 spores per chain on the tip of aerial mycelia; sporangium and flagellated spores are not observed. In vegetative mycelium, fragmentation and sclerotia are not observed.

Cultural and Physiological Characteristics

The cultural characteristics of PA-31088 and PA-39504 grown on various media for 2 weeks are

Table 3. Physiological characteristics of strain PA-31088.

Growth temperature	10°C: No growth 28°C: Good growth and good sporulation 37°C: Fair growth but no aerial mycelium 42°C: No growth 45°C: No growth 50°C: No growth
Production of melanoid pigment	Negative
Tyrosinase reaction	Negative
Milk coagulation	Negative
Milk peptonization	Positive
Starch hydrolysis	Positive
Liquefaction of gelatin	No growth

Table 4. Physiological characteristics of strain PA-39504.

Growth temperature	10°C: Good growth but poor aerial mycelium 28°C: Good growth and good sporulation 37°C: No growth 45°C: No growth 50°C: No growth
Production of melanoid pigment	Negative
Tyrosinase reaction	Negative
Milk coagulation	Negative
Milk peptonization	Positive
Starch hydrolysis	Positive
Liquefaction of gelatin	Negative

Table 5. Utilization of carbon sources by strain PA-31088 and strain PA-39504.

Carbon source	Growth	
	PA-31088	PA-39504
L-Arabinose	−	++
D-Xylose	−	++
D-Glucose	++	++
D-Fructose	−	++
Sucrose	−	−
Inositol	++	++
L-Rhamnose	−	++
Raffinose	−	−
D-Mannitol	−	−
None (Control)	−	−

++: Abundant growth, −: No growth.

shown in Tables 1 and 2. The colors of sporulated aerial mycelia and vegetative mycelia were designated mainly on the basis of the color table in "Guide to Color Standard", published by Nippon Shikisai Kenkyusho, Tokyo, Japan.

Physiological characteristics of PA-31088 and PA-39504 are shown in Tables 3 and 4.

Utilization of carbon sources by PA-31088 and PA-39504 is summarized in Table 5. Inositol and D-glucose are utilized. But L-arabinose, D-xylose, D-fructose, sucrose, L-rhamnose, raffinose and D-mannitol are not utilized for growth by PA-31088. L-Arabinose, D-xylose, D-glucose, D-fructose and L-rhamnose are utilized, but sucrose, inositol, raffinose and D-mannitol are not utilized for growth by PA-39504.

The susceptibility of PA-31088 to aminoglycosidic antibiotics were tested by the method of HIGGENS and KASTNER[11], and the MIC values of the aminoglycosidic antibiotics were determined as follows: nebramycin, less than 4 μg/ml; streptomycin, 63 μg/ml; neomycin, 32 μg/ml; kanamycin, 8 μg/ml.

Comparison of Strains PA-31088 and PA-39504 with Other Known *Streptomyces* Species

(1) Strain PA-31088

From the results of the morphological and cultural characteristics described above, strain PA-31088 is assigned to the genus *Streptomyces* and has the following characteristics: Color of mature sporulated aerial mycelium is in the Red color-series; spore-chain morphology shows spirals; spore surface is smooth; production of melanoid pigment is negative; the range of utilization of carbon sources is narrow. According to the taxonomic criteria[5~10] of the genus *Streptomyces*, PA-31088 mostly resembles *Streptomyces tenebrarius*[9~11]. However, it differs from *S. tenebrarius* in formation of sclerotia, utiliza-

tion of D-fructose and sucrose, growth temperature range and resistance to aminoglycosidic antibiotics such as nebramycin, streptomycin and so on.

HIGGENS *et al.*[11] reported that sclerotia of *S. tenebrarius* were produced on several media and most abundantly on BENNETT's agar. On the contrary, no sclerotium of PA-31088 was observed on all media used including BENNETT's agar. They also reported that sucrose utilization by one mutant of *S. tenebrarius* ATCC 17921 was questionable, and this sugar was probably utilized by the type strain ATCC 17920[11]. Consequently, difference in sucrose utilization between *S. tenebrarius* and PA-31088 was not so distinct, but difference in D-fructose utilization was distinct. As to growth temperature range, they emphasized it as a characteristic of *S. tenebrarius*[11]. Both strains ATCC 17920 and 17921 could grow at 26°C to 55°C, although the latter strain showed less growth at high temperatures above 43°C than at 37°C. On the other hand, PA-31088 could not grow at higher temperatures than 42°C. They also reported that resistance to aminoglycosidic antibiotics was one of characteristics of *S. tenebrarius*. MICs of nebramycin, streptomycin, neomycin and kanamycin against *S. tenebrarius* were more than 2000 μg/ml[11]. On the contrary, PA-31088 was susceptible to these antibiotics. Their MICs against PA-31088 were less than 4 μg/ml, 63 μg/ml, 32 μg/ml and 8 μg/ml respectively. Differences between PA-31088 and *S. tenebrarius* are shown in Table 6.

Table 6. Differences between strain PA-31088 and *S. tenebrarius*.

	PA-31088	*S. tenebrarius*
Sclerotia	−	+
Growth temperature		
42°C	−	+
45°C	−	+
55°C	−	+
Carbon utilization		
D-Fructose	−	+
Sucrose	−	Probably utilized
Susceptibility to aminoglycosidic antibiotics	Susceptible	Resistant

From these results, strain PA-31088 was considered to be a new species of *Streptomyces*, and the name *Streptomyces tokunonensis* is proposed. It has been deposited in Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under accession number FERM 4843, and also in American Type Culture Collection under accession number ATCC 31569.

(2) Strain PA-39504

From the results of morphological and cultural characteristics described above, the strain PA-39504 is considered to belong to the genus *Streptomyces* having the following characteristics. Color of mature sporulated aerial mycelium is in the Gray color-series; spore-chain morphology shows spirals; spore surface is smooth; production of melanoid pigment is negative. According to the taxonomic criteria[5~10] of the genus *Streptomyces*, PA-39504 closely resembles *Streptomyces argenteolus*[7,10]. The characteristics of PA-39504 were compared with those of the standard strain of *S. argenteolus*, and good agreements were obtained except utilization of D-mannitol. Therefore, strain PA-39504 was identified as a strain of *S. argenteolus*. It has been deposited in Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under accession number FERM 5265, and also in American Type Culture Collection under accession number ATCC 31589.

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