ASPICULAMYCIN, A NEW CYTOSINE NUCLEOSIDE ANTIBIOTIC

I. PRODUCING ORGANISM, FERMENTATION AND ISOLATION

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Aspiculamycin, a new cytosine nucleoside with versatile antibiotic activity, was produced by a strain of *Streptomyces* designated as *S. toyocaensis* var. *aspiculamyceticus*. The antibiotic was produced in submerged fermentation together with two other antifungal antibiotics, toyocamycin and an unidentified tetraene antibiotic. Isolation of aspiculamycin was proceeded by ion-exchange column chromatography of the culture filtrate on IRC-50 followed by rechromatography on CG-50.

Several antibiotics with cytosine nucleoside in their molecules are known in the literature. They are gougerotin[1], blasticidin S[2], amicetin B[3,4], bamicetin[4], plicacetin[4], hikizimycin[5], ezomycin[6] and oxamicetin[7]. Although their structures are not elucidated yet, physico-chemical properties of anthelmycin[8] and moroyamycin[9] strongly suggest that they also have a cytosine chromophore in their molecules. These antibiotics are all elaborated by the *Streptomyces* and the interests are directed toward their versatile antibiotic activity, such as anthelmintic, acaricidal, antimycoplasma and antitumor as well as antibacterial activities.

From the viewpoint of its chemical structure, aspiculamycin was found to be closely related to gougerotin, having the characteristic biological activities of cytosine nucleoside antibiotics mentioned above.

This paper deals with taxonomy of the antibiotic-producing organism and production and isolation of the antibiotic. Structural elucidation and biological activities of aspiculamycin will be presented in the succeeding papers.

Characterization of Strain No. 1040

Strain No. 1040 was isolated from a soil sample collected at Hiraizumi, Iwate Prefecture, Japan. Its morphological and physiological characteristics are as follows:

(1) Morphology.

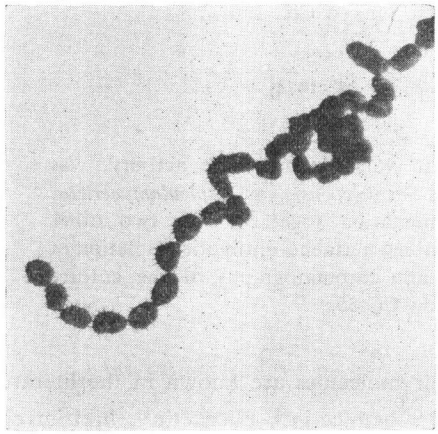
The aerial mycelium of strain No. 1040 was composed of numerous cluster-like branches and the chains of spores formed spirals of 5~20 turns. Usually a spore chain possessed 50 or more spores which were elliptical to spherical in shape and 0.4~0.6 μ×0.7~1.0 μ in size. The surface of the spore was spiny as shown in Plate 1.

(2) Cultural characteristics.

The cultural characteristics of strain No. 1040 on various media are presented in Table 1. In general, the aerial mycelium was white to yellowish gray and the spores were powdery in mass. The reverse of the culture was pale yellowish brown to yellowish gray and no soluble pigment was formed on all of the media tested. Color names were assigned according to

"Guide to Color Standard" (a manual published by Nippon Shikisai Kenkyusho, Tokyo, Japan). The growth of strain No. 1040 was noted between 10~40°C with an optimum temperature at 28°C. Physiological properties of strain No. 1040 observed on various media are summarized in Table 2. In utilization of carbon sources by strain No. 1040 according to the method of PRIDHAM and GOTTLIEB, good growth was observed with D-glucose, D-fructose, *i*-inositol and D-mannitol as shown in Table 3.

Plate 1. Electron micrograph of spores of strain No. 1040 on yeast extract-malt extract agar, 14 days (× 2,000 × 10)



On the basis of taxonomic characters described above, strain No. 1040 was classified as a member of *Streptomyces albus* group according to the keys of BERGEY's Manual (7th ed.) and of WAKSMAN in The Actinomycetes, vol. 2. Among known species of *Streptomyces* in this group, many characteristics of *Streptomyces toyocaensis* were in common with strain No. 1040.

A comparison of the characteristics of

Table 1. Cultural characteristics of strain No. 1040

Medium	Growth	Aerial mycelium	Substrate mycelium	Reverse	Soluble pigment
Sucrose-nitrate agar	Moderate	Pale brown	Colorless	Pale brown	None
Glucose-asparagine agar	Abundant	Pale brown	Brownish white	Yellowish gray	None
Glycerol-asparagine agar	Abundant	Brownish gray	Brownish white	Yellowish gray	None
Inorganic salts-starch agar	Abundant	Brownish gray	Brownish white	Yellowish gray	None
Tyrosine agar	Abundant	Bright brownish gray	Yellowish gray	Pale yellowish brown	None
Nutrient agar	Good	White	Yellowish gray	Pale yellowish brown	None
Yeast extract-malt agar	Abundant	Grayish brown	Pale yellowish brown	Pale yellowish brown	None
Oatmeal agar	Abundant	Brownish gray	Yellowish gray	Yellowish gray	None

Table 2. Physiological properties of strain No. 1040

Temperature range for growth	10~40°C
Gelatin liquefaction	Good
Hydrolysis of starch	Positive
Coagulation of milk at 25° and 37°C	Positive
Peptonization of milk	Positive at 25° and 37°C
Nitrate reduction	Negative
Melanin formation, on tyrosine agar	Negative
on peptone-yeast iron agar	Negative

of strain No. 1040 with those of *S. toyocaensis* in the published description was made as shown in Table 4. It is likely that strain No. 1040 is closely related to *S. toyocaensis*, because one of the two antibiotics produced simultaneously with aspiculamycin by strain No. 1040 was toyocamycin, which was known to be produced by *S. toyocaensis*. Differences were noted, however, between these two strains in several respects. In contrast to the numerous cluster-like long branching of the aerial mycelium of strain No. 1040, only a few and short branches were formed by *S. toyocaensis*. Open spirals were formed on the inorganic salts-starch agar by strain No. 1040, but straight spore-bearing aerial hyphae by *S. toyocaensis*. Generally, good growth on various

Table 3. Utilization of carbon source by strain No. 1040

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

Table 4. Morphological and physiological characteristics of strain No. 1040 and *S. toyocaensis*

		Strain No. 1040	*S. toyocaensis*
Morphological characteristics	Aerial mycelium	Numerous cluster-like branches	Few branches
	Sporophore	Open spirals	Straight
	Surface of spore	Spiny	Spiny
	Spores	Spherical to elliptical	Spherical to elliptical
Cultural characteristics	CZAPEK's agar (27°C)	G*: Moderate AM: Pale brown SM: Colorless SP: None	G: Moderate AM: White to gray SM: Colorless SP: None
	Glucose-asparagine agar (27°C)	G: Abundant AM: Pale brown SM: Brownish white SP: None	G: Abundant AM: Light brownish gray SM: Colorless SP: Pale yellowish brown
	Inorganic salts-starch agar (27°C)	G: Abundant AM: Brownish gray SM: Brownish white SP: None	G: Abundant AM: Light brownish gray SM: Colorless SP: Pale yellow
	Nutrient agar (27°C)	G: Good AM: White SM: Yellowish gray SP: None	G: Good AM: White SM: Pale yellowish gray SP: Yellowish brown
Physiological characteristics	Hydrolysis of starch	Positive	Positive
	Liquefaction of gelatin	Good	Strong
	Nitrate reduction	Negative	Positive
	Coagulation of milk	Positive	Positive
	Peptonization of milk	Positive	Positive

*G: Growth, AM: Aerial mycelium, SM: Substrate mycelium, SP: Soluble pigment.

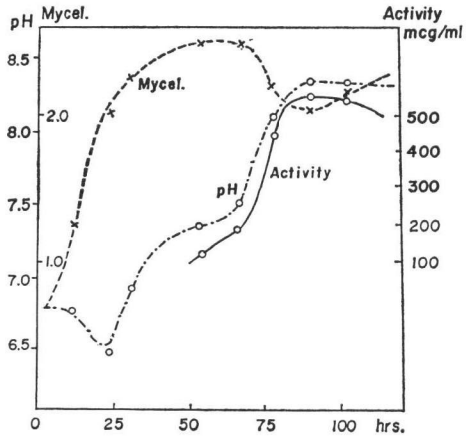
media was exhibited by strain No. 1040 in comparison with that by *S. toyocaensis*. Colors of the growth of strain No. 1040 were slightly different from those of *S. toyocaensis*. Judging from the above-mentioned properties, including the fact of the production of a new antibiotic aspiculamycin, strain No. 1040 was assigned to one of the variants of *S. toyocaensis* and was named *Streptomyces toyocaensis* var. *aspiculamyceticus*.

Production of Aspiculamycin

Aspiculamycin was produced in a medium composed of starch 2.0%, glucose 1.0%, Pharmamedia 1.5%, corn-steep liquor 2.0% and meat extract 1.0%. The pH of the medium was adjusted to 7.4 before sterilization. The fermentation was carried out in 100-liter tank containing 60 liters of the medium at 27°C under aeration of 60 liters per minute and agitated at 150 revolution per minute. The potency of the cultured broth was estimated by cylinder-plate method on 0.5% peptone agar seeded with *Escherichia coli* NIHJ as the test organism. The maximum potency of the antibiotic was obtained after 80~90 hours of cultivation. In the filtrate of a final cultured broth thus obtained, production of two other antibiotics were detected simultaneously with aspiculamycin. One was a tetraene antibiotic with antimicrobial activity against gram-positive bacteria and yeasts and another antifungal antibiotic was identical with toyocamycin. A typical pattern during such a fermentation is presented in Fig. 1.

Fig. 1. Time course of aspiculamycim production by *Streptomyces toyocaensis* var. *aspiculamyceticus*

Mycelium was expressed as packed cell volume (ml) per 10 ml of cultured broth by centrifugation at 3,000 rpm for 15 minutes.



Isolation and Purification of Aspiculamycin

Sixty liters of the cultured broth was filtered together with 6 kg of diatomaceous earth as an aid of filtration. The filtrate was applied on a column packed with 6 liters of Amberlite IRC-50 (H^+ cycle) and washed exhaustively with water to remove tetraene antibiotic. The adsorbed fractions were eluted with 1 N NH_4OH (55 liters). Toyocamycin was recovered in the eluate of pH 5~7 followed by subsequent elution of aspiculamycin at pH 7. Fractions containing aspiculamycin (15 liters) were pooled and concentrated *in vacuo* to two liters and the concentrate was adsorbed on Amberlite CG-50 (NH_4^+ cycle, 2 liters) equilibrated with 0.01 M ammonium phosphate buffer at pH 7.0 and developed with 0.05 M $(NH_4)_2HPO_4$. The active fraction (6.5 liters) was adsorbed on Amberlite CG-50 (NH_4^+ cycle, 2 liters) and developed with water to elute aspiculamycin. After concentration of 40 liters of the eluate *in vacuo* to 100 ml, crude aspiculamycin was precipitated from the concentrate by addition of one liter of acetone. After collection by centrifugation, the precipitate was dissolved in a small amount of water

and lyophilized to yield 2.5 g of white powder of the antibiotic. One gram of the powder thus obtained was dissolved in 10 ml of water and 40 ml of acetone was added to give 200 mg of colorless needle crystals of aspiculamycin.

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