GLOBOMYCIN, A NEW PEPTIDE ANTIBIOTIC WITH SPHEROPLAST-FORMING ACTIVITY

I. TAXONOMY OF PRODUCING ORGANISMS AND FERMENTATION

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A new peptide antibiotic, globomycin, was found to be produced by four different strains of the actinomycetes. They were identified as *Streptomyces halstedii* No. 13912, *Streptoverticillium cinnamoneum* No. 15037, *Streptomyces neohygroscopicus* subsp. *globomyceticus* No. 15631 and *Streptomyces hagronensis* No. 17834, respectively. Fermentation of globomycin was conducted by conventional submerged culture for antibiotic production, in which 10 μg/ml of globomycin was produced by cultivation of *S. halstedii* No. 13912 for 96 hours at 27°C. Globomycin was named after its activity to form global-shape spheroplasts when *Escherichia coli* was incubated in the presence of this antibiotic.

In the course of screening program directed toward discovery of new antibiotics against Gram-negative bacteria, several strains were found to produce the same compound, a peptide, which was characterized by its selective activity against Gram-negative bacteria with spheroplast formation.

There were several known peptide antibiotics which inhibit bacterial cell wall synthesis, *e.g.* bacitracin, enduracidin, vancomycin, ristocetin, cycloserine, O-carbamyl-D-serine and various β-lactam antibiotics, but none of these is active only against Gram-negative bacteria.

This paper deals with the taxonomy of the producing organisms and the fermentation of this antibiotic, globomycin. Physico-chemical and biological characterization as well as structural elucidation of the antibiotic will be described in the subsequent papers.

1. Taxonomic Studies

The cultural characteristics of four strains of globomycin-producers were determined by the use of conventional media and methods described by SHIRLING and GOTTLIEB.[1] Observation of the cultures was made after cultivation for 2 weeks at 28°C unless otherwise stated. The taxonomic keys of BERGEY'S Manual of Determinative Bacteriology (8th ed.), of WAKSMAN in The Actinomycetes, Vol. 2 and others were used to compare with recognized genera and species of the actinomycetes.

Strain No. 13912

Strain No. 13912 was isolated from a soil sample collected at Sagimori shrine, Kyoto Pref., Japan. The aerial hyphae of strain No. 13912 indicated monopodial branching with sporophores of *Rectus-Flexibilis* on most of the media tested, but many hooks and some irregular coils characteristic to *Retinaculum-Apertum* were also detected on some of the media (Plate 1). The spores were in chains of more than ten, oval to cylindrical in shape, $0.6 \sim 0.8 \times 0.8 \sim 2.0$ μ in size and with smooth surface (Plate 2). The cultural characteristics of strain No. 13912 are summarized in Table 1. On most

Plate 1. *Rectus-Flexibilis* or *Retinaculum-Apertum* sporophores of strain No. 13912 on oatmeal agar, 10 days.

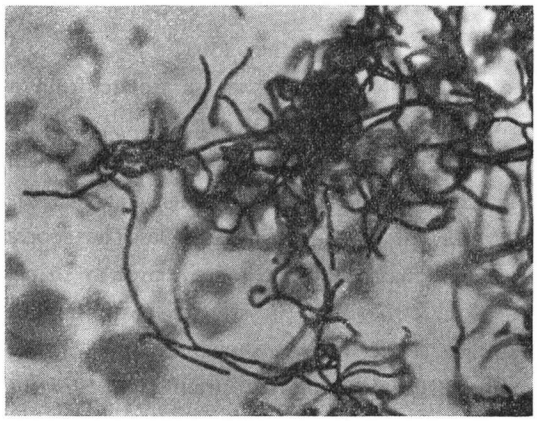


Plate 2. Smooth spores of strain No. 13912, scanning electron micrograph from 10-day culture on soil extract agar. A mark equals 1 μ.

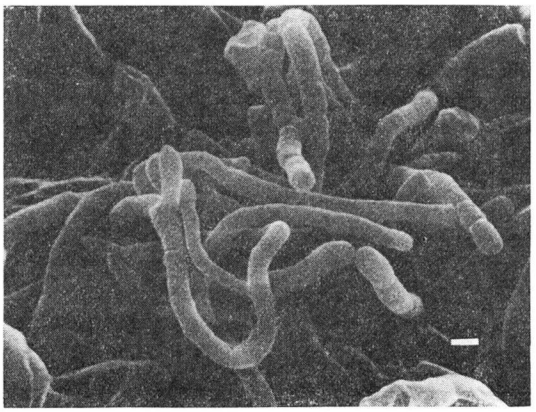


Table 1. Cultural characteristics of strain No. 13912 and *Streptomyces halstedii* ATCC 13499

	Strain No. 13912	*S. halstedii*
Yeast extract-malt extract agar (ISP 2)	G: Abundant AM: Brownish white R: Dark yellowish brown SP: None	G: Abundant AM: Gray R: Yellowish brown SP: None
Oatmeal agar (ISP 3)	G: Abundant AM: Brownish white R: Olive gray SP: None	G: Abundant AM: Gray R: Yellowish gray SP: None
Inorganic salts-starch agar (ISP 4)	G: Abundant AM: Light brownish white R: Pale yellowish brown to brownish gray SP: None	G: Abundant AM: Gray R: Brownish gray SP: None
Glycerol-asparagine agar (ISP 5)	G: Good AM: Light brownish white R: Yellowish brown SP: None	G: Good AM: Grayish white R: Yellowish brown SP: None

G: Growth. AM: Aerial mycelium. R: Reverse. SP: Soluble pigment.
Color names were assigned according to "Guide to Color Standard", a manual published by Nippon Shikisai Kenkyusho, Tokyo, Japan.

of the media, the color of substrate mycelia was yellowish brown and the mass color of aerial mycelia was white to brownish white. Physiological properties and the result of carbon utilization test are shown in Tables 2 and 3, respectively.

From these characteristics strain No. 13912 was classified as a member of genus *Streptomyces* and *S. halstedii*[2] was selected as the most closely related one. The results of simultaneous cultivation of *S. halstedii* ATCC 13499 with strain No. 13912 are shown in Tables 1~3. Morphological as well as physiological properties of these two strains were in good agreement, except for some grayish color of aerial hyphae of *S. halstedii* and non-utilization of D-cellobiose by strain No. 13912. The

Table 2. Physiological properties of strain No. 13912 and *Streptomyces halstedii* ATCC 13499.

	No. 13912	*S. halstedii*
Tyrosinase reaction	negative	negative
Nitrate reduction	negative	negative
Starch hydrolysis	weak	weak
Gelatin liquefaction	positive	positive
Milk peptonization 26°C	negative	positive (pH 7.2)
37°C	positive (pH 4.4)	positive (pH 4.8)
Milk coagulation 26°C	negative	positive
37°C	positive	positive
Melanin formation		
Tryptone-yeast extract broth (ISP 1)	negative	negative
Peptone-yeast extract iron agar (ISP 6)	negative	negative

differences, however, were not sufficient to designate strain No. 13912 as a new species and it was named *Streptomyces halstedii* No. 13912.

Strain No. 15037

Strain No. 15037 was an isolate from a soil sample collected at Kamakura, Kanagawa Pref., Japan. The aerial hyphae of this strain indicated verticillate branching classified as *Biverticillus* as shown in Plate 3. The spores were in chains of three to ten, oval in shape, $0.4 \sim 0.7 \times 0.7 \sim 1.4\ \mu$ in size and with smooth surface (Plate 4). As shown in Table 4, the cultural characteristics of strain No. 15037 in-

Table 3. Carbon utilization patterns of strain No. 13912 and *Streptomyces halstedii* ATCC 13499

	No. 13912	*S. halstedii*		No. 13912	*S. halstedii*
D-Glucose	+	+	D-Mannose	±	+
L-Arabinose	−	±	Dulcitol	−	−
D-Xylose	−	±	Salicin	−	−
i-Inositol	−	−	D-Cellobiose	−	+
D-Mannitol	−	−	Lactose	−	±
L-Rhamnose	−	−	Maltose	+	+
D-Fructose	±	+	Trehalose	±	−
Sucrose	−	−	Melibiose	−	−
Raffinose	−	−	Inulin	−	−
Glycerol	++	+	Dextrin	+	+
Na-acetate	−	−	Soluble starch	+	+
Na-succinate	+	±	Cellulose	−	−
D-Galactose	±	±	Negative control	−	−

++ strongly positive, + positive, ± weakly positive, − negative.

Plate 3. *Biverticillus* sporophores of strain No. 15037 on sucrose-nitrate agar, 7 days.

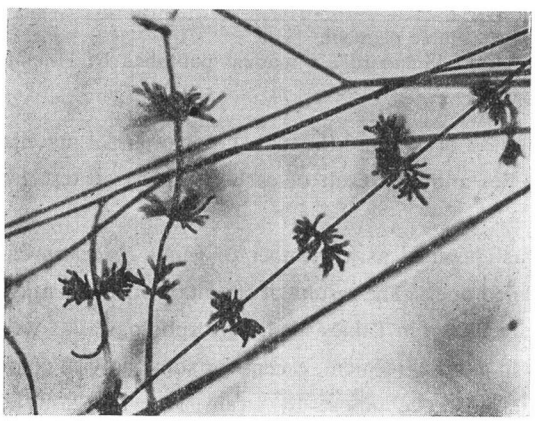


Plate 4. Smooth spores of strain No. 15037, scanning electron micrograph from 10-day culture on chitin agar.[3] A mark equals 1 μ.

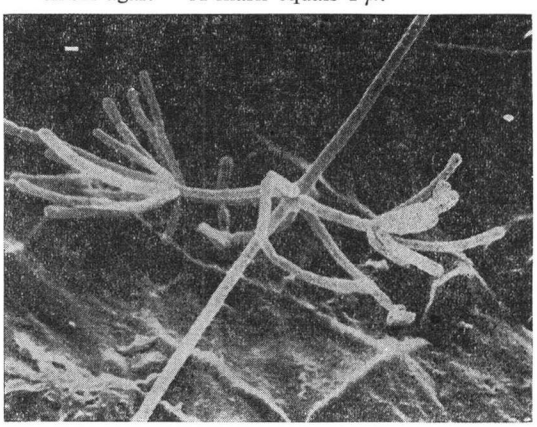


Table 4. Cultural characteristics of strain No. 15037 and *Streptoverticillium cinnamoneum* ATCC 11874

	Strain No. 15037	*S. cinnamoneum*
Yeast extract-malt extract agar (ISP 2)	G: Abundant AM: Pale yellowish orange R: Yellowish brown SP: None	G: Abundant AM: Pale brown R: Yellowish brown SP: None
Oatmeal agar (ISP 3)	G: Abundant AM: Yellowish gray R: Pale olive SP: None	G: Abundant AM: Pale orange to pale brown R: Pale yellowish brown SP: None
Inorganic salts-starch agar (ISP 4)	G: Abundant AM: White and light brownish gray R: Pale yellowish brown SP: None	G: Abundant AM: Brownish white R: Pale yellow SP: None
Glycerol-asparagine agar (ISP 5)	G: Good AM: Yellowish gray R: Pale yellow SP: None	G: Moderate AM: Pale orange R: Pale yellowish brown SP: None

G: Growth. AM: Aerial mycelium. R: Reverse. SP: Soluble pigment.

dicated yellowish brown color of the substrate mycelia and yellowish gray color of the aerial mycelia on most of the media.

On the basis of these characteristics as well as of the result of physiological properties in Table 5 and carbon utilization test in Table 6, strain No. 15037 was classified as a member of *Streptoverticillium*. Among known species of this genus, *S. cinnamoneum*[4] was selected as being most related and the results of simultaneous cultivation of *S. cinnamoneum* ATCC 11874 with strain No. 15037 are comparatively shown in Tables 4~6. Some differences observed between these two strains were as follows: More grayish mass color of the aerial hyphae of strain No. 15037 than that of *S. cinnamoneum*. Utilization of D-galactose and liquefaction of gelatin were only observed in *S. cinnamoneum*. These differences, however, were not sufficient to differentiate strain No. 15037 from *S. cinnamoneum* and the former was named *Streptoverticillium cinnamoneum* No. 15037.

Table 5. Physiological properties of strain No. 15037 and *Streptoverticillium cinnamoneum* ATCC 11874

	No. 15037	*S. cinnamoneum*
Tyrosinase reaction	negative	negative
Nitrate reduction	negative	negative
Starch hydrolysis	positive	positive
Gelatin liquefaction	negative	positive
Milk peptonization 26°C	positive (pH 6.2)	
37°C	positive (pH 6.2)	positive
Milk coagulation 26°C	positive	
37°C	positive	positive
Melanin formation		
Tryptone-yeast extract broth (ISP 1)	negative	negative
Peptone-yeast extract iron agar (ISP 6)	negative	negative

Strain No. 15631

Strain No. 15631 was isolated from a soil sample collected at Ohta, Tokyo, Japan. The aerial hyphae of this strain showed monopodial branching with sporophores of *Spira* as indicated in Plate 5.

Table 6. Carbon utilization patterns of strain No. 15037 and *Streptoverticillium cinnamoneum* ATCC 11874

	No. 15037	*S. cinnamoneum*		No. 15037	*S. cinnamoneum*
D-Glucose	+	+	D-Mannose	+	+
L-Arabinose	−	−	Dulcitol	−	−
D-Xylose	−	−	Salicin	−	N.T.
i-Inositol	+	±	D-Cellobiose	−	±
D-Mannitol	−	−	Lactose	−	−
L-Rhamnose	−	±	Maltose	+	+
D-Fructose	±	−	Trehalose	+	+
Sucrose	−	−	Melibiose	−	N.T.
Raffinose	−	−	Inulin	−	N.T.
Glycerol	+	N.T.	Dextrin	++	+
Na-acetate	−	N.T.	Soluble starch	++	N.T.
Na-succinate	−	N.T.	Cellulose	−	N.T.
D-Galactose	−	+	Negative control	−	−

++ strongly positive, + positive, ± weakly positive, − negative, N.T. not tested.

Plate 5. *Spira* sporophores of strain No. 15631 on sucrose-nitrate agar, 7 days.

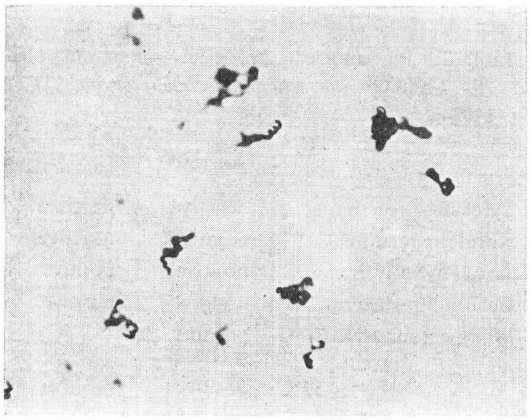
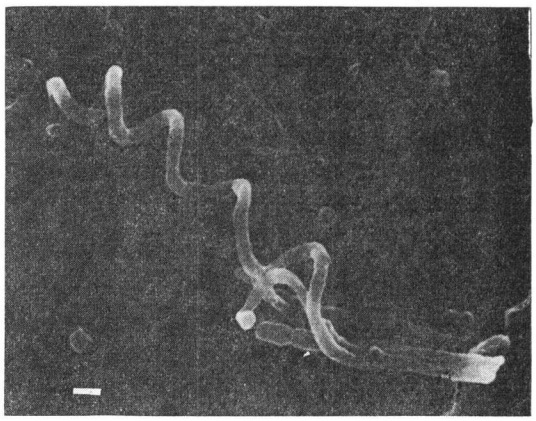


Plate 6. Smooth spores of strain No. 15631, scanning electron micrograph from 7-day culture on sucrose-nitrate agar. A mark equals 1 μ.



The spores were in chains of more than ten, oval in shape, $0.4\sim0.8\times0.7\sim1.1$ μ in size and with smooth surface as shown in Plate 6. As shown in Table 7, the color of substrate mycelia was yellow to yellowish brown and that of aerial mycelia was yellowish to brownish gray, the latter of which, however, later changed into moist black on some of the media, such as inorganic salts-starch, yeast extract-malt extract and oatmeal agars. This hygroscopic feature of the mass color of the aerial mycelia is a well-known characteristic property of *S. hygroscopicus*.[5] Studies were thus undertaken to compare morphological and physiological properties of strain No. 15631 with those of *S. hygroscopicus* ATCC 13810 as shown in Tables 7~9. More abundant growth of *S. hygroscopicus* than that of strain No. 15631 was observed on some of the media, such as sucrose-nitrate agar, glycerol-asparagine agar and tyrosine agar. Soluble pigment was produced by strain No. 15631 but not by *S. hygroscopicus*. Distinct differences between these two strains were also noted in their physiological properties as well as carbon utilization patterns.

Table 7. Cultural characteristics of strain No. 15631 and *Streptomyces hygroscopicus* ATCC 13810

	Strain No. 15631	*S. hygroscopicus*
Sucrose-nitrate agar	G: Poor AM: Light brownish gray (2–7–5)* R: Pale brown (2–8–5) SP: None	G: Good AM: Light brownish gray (2–7–5) R: Brownish white (1–8–6) SP: None
Glucose-asparagine agar	G: Abundant AM: Brownish gray (2–6–4) R: Pale yellow (6–8–10) SP: Pale yellow	G: Good AM: Brownish white (1–6–6) R: Light brownish gray (2–8–7) SP: None
Glycerol-asparagine agar (ISP 5)	G: Poor AM: Yellowish gray (1–9–10) R: Yellowish gray (2–9–10) SP: None	G: Good AM: Pale brown (2–8–9) R: Pale yellowish brown (4–8–9) SP: None
Inorganic salts-starch agar (ISP 4)	G: Abundant AM: Pale pink (2–7–4), later moist black (hygroscopic) R: Pale yellow (6–8–10) SP: None	G: Abundant AM: Brownish white (1–6–6), later moist black (hygroscopic) R: Pale yellowish brown (2–7–9) SP: None
Nutrient agar (Difco)	G: Good AM: Yellowish gray (1–9–10) R: Pale yellow (12–8–10) SP: Yellow (distinct)	G: Good AM: White R: Pale yellowish brown (4–8–9) SP: None
Tyrosine agar (ISP 7)	G: Poor AM: Yellowish gray (1–9–10) R: Pale yellow (3–9–10) SP: None	G: Abundant AM: White R: Pale yellowish brown (4–6–8) SP: None
Yeast extract-malt extract agar (ISP 2)	G: Abundant AM: Brownish gray (2–6–4), later moist black (hygroscopic) R: Yellowish brown (10–6–8) SP: Yellow (distinct)	G: Abundant AM: Grayish white to brownish white (N-7 ~ 1–6–6) R: Yellowish brown (6–6–8) SP: None
Oatmeal agar (ISP 3)	G: Abundant AM: Brownish gray (2–6–4), later moist black (hygroscopic) R: Yellowish brown (8–7–9) SP: None	G: Abundant AM: White, later moist black (hygroscopic) R: Pale yellowish brown (6–8–9) SP: None

G: Growth. AM: Aerial mycelium. R: Reverse. SP: Soluble pigment.
* Coloration code number according to "Guide to Color Standard".

When examined under microscope, the smooth spore surface of strain No. 15631 was clearly distinguished from the warty one of *S. hygroscopicus* ATCC 13810. According to DIETZ's proposal[6] *S. hygroscopicus* with smooth surface belongs to *S. neohygroscopicus*. Two strains, formerly designated as *S. hygroscopicus* var. *angustmyceticus*[7] and *S. platensis*[4] are involved in this category, but these two strains are also clearly distinguished from strain No. 15631 by their morphological and physiological

Table 8. Physiological properties of strain No. 15631 and *Streptomyces hygroscopicus* ATCC 13810

	No. 15631	*S. hygroscopicus*
Tyrosinase reaction	negative	negative
Nitrate reduction	negative	negative
Starch hydrolysis	weak	weak
Gelatin liquefaction	negative	positive (slow)
Milk peptonization 26°C	negative	positive (pH 6.4)
37°C	positive (pH 6.6)	positive (pH 6.4)
Milk coagulation 26°C	negative	negative
37°C	positive	negative
Melanin formation		
Tryptone-yeast extract broth (ISP 1)	negative	negative
Peptone-yeast extract iron agar (ISP 6)	negative	negative

properties. Therefore, strain No. 15631 has been designated as *Streptomyces neohygroscopicus* subsp. *globomyceticus* No. 15631 ENOKITA *et* ARAI.

Strain No. 17834

The other globomycin producer, strain No. 17834, was isolated from a soil sample collected at Haguro, Yamagata Pref., Japan and also classified as a member of the genus *Streptomyces*. The aerial hyphae of strain No. 17834 indicated monopodial branching with sporophores of *Spira*, forming open or tight spirals as shown in Plate 7. The spores were in chains of more than ten, oval to cylindrical in shape, $0.7\sim0.8\times0.6\sim1.1\ \mu$ in size and with smooth surface as given in Plate 8. As shown in Table

Table 9. Carbon utilization patterns of strain No. 15631 and *Streptomyces hygroscopicus* ATCC 13810

	No. 15631	*S. hygroscopicus*		No. 15631	*S. hygroscopicus*
D-Glucose	+	+	D-Mannose	+	+
L-Arabinose	−	±	Dulcitol	−	−
D-Xylose	+	+	Salicin	−	−
i-Inositol	−	+	D-Cellobiose	+	+
D-Mannitol	+	+	Lactose	−	+
L-Rhamnose	−	+	Maltose	±	+
D-Fructose	+	+	Trehalose	±	+
Sucrose	−	−	Melibiose	−	+
Raffinose	−	−	Inulin	−	−
Glycerol	+	++	Dextrin	+	+
Na-acetate	−	−	Soluble starch	+	+
Na-succinate	−	−	Cellulose	±	−
D-Galactose	+	+	Negative control	−	−

++ strongly positive, + positive, ± weakly positive, − negative.

10, the cultural characteristics revealed yellowish brown color of substrate mycelia and brown to brownish gray mass color of aerial mycelia on most of the media tested. Diffusion of pale to dull yellow soluble pigment was detected in about a half kinds of the media tested. Physiological properties and the result of carbon utilization test of strain No. 17834 are shown in Tables 11 and 12, respectively. Among known species of *Streptomyces*, *S. carnosus*[8] was chosen as the most closely related one. As shown in Tables 10~12, however, some distinct differences between these two strains were noted in the results of a simultaneous comparison of the culture. Good growth of strain No. 17834 was detected on glucose-asparagine agar or nutrient agar but poor one of *S. carnosus* on the same media. On the contrary, good growth of *S. carnosus* but poor one of strain No. 17834 was

Plate 7. *Spira* sporophores of strain No. 17834 on PRIDHAM-GOTTLIEB agar + arabinose (1%), 7 days.

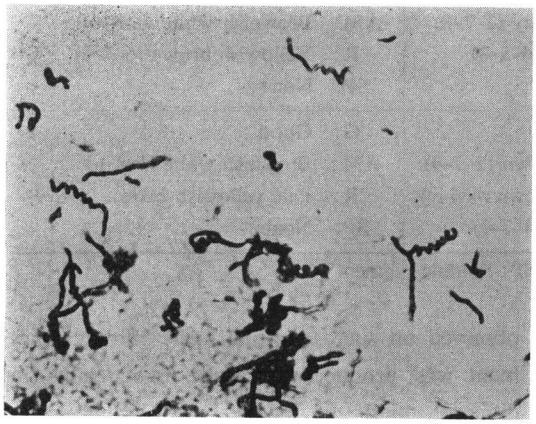


Plate 8. Smooth spores of strain No. 17834, scanning electron micrograph from 7-day culture on PRIDHAM-GOTTLIEB agar+glucose (1%). A mark equals 1 μ.

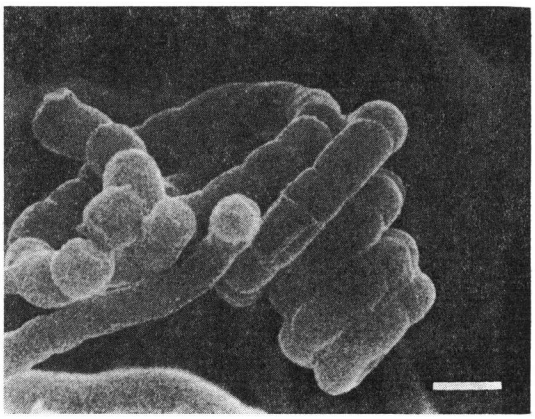


Table 10. Cultural characteristics of strain No. 17834 and *Streptomyces carnosus* ATCC 25437

	Strain No. 17834	*S. carnosus*
Sucrose-nitrate agar	G: Poor AM: Brownish white (2–9–7) R: Brownish white (2–9–7) SP: None	G: Good AM: Brownish white (1–8–6) R: Pale yellowish brown (4–8–9) SP: Pale yellowish brown (6–8–9)
Glucose-asparagine agar	G: Good AM: Light brownish gray (2–7–7) R: Yellowish brown (4–7–9) SP: None	G: Poor AM: Brownish white (1–8–6) R: Light brownish gray (1–8–10) SP: None
Glycerol-asparagine agar (ISP 5)	G: Abundant AM: Light brownish gray (2–7–8) R: Yellowish brown (6–6–9) SP: Pale yellow	G: Good AM: None R: Pale yellowish brown (4–8–9) SP: None
Inorganic salts-starch agar (ISP 4)	G: Abundant AM: Grayish brown (2–6–7) R: Dark yellowish brown (3–3–8) SP: None	G: Good AM: None R: Yellowish brown (4–7–9) SP: None
Nutrient agar (Difco)	G: Good AM: White R: Pale yellowish brown (4–8–9) SP: Dull yellow	G: Poor AM: None R: Yellowish gray (2–8–10) SP: None
Tyrosine agar (ISP 7)	G: Abundant AM: Pale brown (2–8–9) R: Yellowish brown (6–5–8) SP: None	G: Good AM: White R: Pale yellowish brown (4–8–9) SP: None

(to be continued)

Table 10. (continued)

	Strain No. 17834	*S. carnosus*
Yeast extract-malt extract agar (ISP 2)	G: Abundant AM: Light brownish gray (2–7–8) R: Yellowish brown (6–5–8) SP: Dull yellow	G: Good AM: Brownish white (1–8–6) R: Yellowish brown (6–7–8) SP: None
Oatmeal agar (ISP 3)	G: Abundant AM: Pale yellowish brown (2–7–9) R: Dark yellowish brown (4–4–9) SP: Yellowish brown (4–7–9)	G: Good AM: Brownish white (1–8–6) R: Pale yellowish brown (6–8–9) SP: None

G: Growth. AM: Aerial mycelium. R: Reverse. SP: Soluble pigment.

Table 11. Physiological properties of strain No. 17834 and *Streptomyces carnosus* ATCC 25437

	No. 17834	*S. carnosus*
Tyrosinase reaction	negative	negative
Nitrate reduction	negative	positive
Starch hydrolysis	positive	positive
Gelatin liquefaction	positive	negative
Milk peptonization 26°C	positive (pH 7.6)	positive (pH 6.2)
37°C	positive (pH 6.2)	positive (pH 6.2)
Milk coagulation 26°C	negative	positive
37°C	positive	positive
Melanin formation		
Tryptone-yeast extract broth (ISP 1)	negative	negative
Peptone-yeast extract iron agar (ISP 6)	negative	negative

observed on sucrose-nitrate agar. Soluble pigment was produced by these two strains in different kinds of media, such as glycerol-asparagine, nutrient, yeast extract-malt extract and oatmeal agars only in the case of strain No. 17834 but sucrose-nitrate agar only in *S. carnosus*. Strain No. 17834 was also clearly distinguished from *S. carnosus* by its physiological properties, such as nitrate reduction and gelatin liquefaction. Carbon sources utilized by strain No. 17834 but not by *S. carnosus* were D-mannose, D-cellobiose, maltose and soluble starch. On the contrary, L-rhamnose was utilized by *S. carnosus* but not by strain No. 17834. On the basis of these differences,

Table 12. Carbon utilization patterns of strain No. 17834 and *Streptomyces carnosus* ATCC 25437

	No. 17834	*S. carnosus*		No. 17834	*S. carnosus*
D-Glucose	+	+	D-Mannose	+	−
L-Arabinose	−	−	Dulcitol	−	−
D-Xylose	−	−	Salicin	±	−
i-Inositol	−	−	D-Cellobiose	+	−
D-Mannitol	−	−	Lactose	−	−
L-Rhamnose	−	+	Maltose	+	−
D-Fructose	±	−	Trehalose	−	−
Sucrose	−	−	Melibiose	±	−
Raffinose	±	−	Inulin	−	−
Glycerol	+	++	Dextrin	++	+
Na-acetate	−	−	Soluble starch	++	−
Na-succinate	+	+	Cellulose	−	−
D-Galactose	±	−	Negative control	−	−

++ strongly positive, + positive, ± weakly positive, − negative.

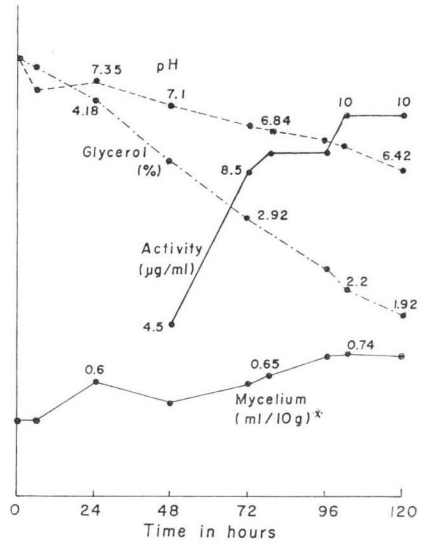
the strain No. 17834 was classified as a new species of *Streptomyces* and designated as *Streptomyces hagronensis* ENOKITA *et* ARAI sp. nov., naming after the place where a soil sample for the isolate was collected.

2. Production of Globomycin

For large scale fermentation of globomycin, the seed medium was inoculated with strain No. 13912 and fermented for 42 hours at 27°C in a 100-liter fermentor containing 50 liters of the medium composed of 1.0% glucose, 2.0% soluble starch, 0.5% soybean oil, 0.5% yeast extract, 0.5% Polypepton (peptone, manufactured by Daigo Eiyo Kagaku Co.), 0.2% $CaCO_3$ and 0.01% Disfoam CB-442 (Anti-foaming agent, manufactured by Nippon Yushi Co.), pH 7.2 before sterilization. A 6-liter aliquot of the seed culture was transferred to a 600-liter fermentor containing 300 liters of the production medium consisting of 4.5% glycerol, 2.0% Render extract (beef extract, manufactured by Mikuni Kagaku Co.), 1.0% feather meal, 0.1% Tachigaren (3-hydroxy-5-methyl isoxazole, manufactured by Sankyo Co., Ltd.), 0.2% $CaCO_3$ and 0.01% CB-442, pH 6.5~6.6 after sterilization. Fermentation was conducted with agitation of 110~150 rev/min and aeration of 150 liters/min for 120 hours at 27°C. An example of time course for the production of globomycin is shown in Fig. 1. The antimicrobial activity of the culture broth was estimated by a cylinder-plate method using *Escherichia coli* SANK 71573 as the test organism.

The maximum potency of globomycin obtained after 96~120 hours of cultivation was 10 μg/ml.

Fig. 1. Fermentation of globomycin



* Mycelium was expressed as packed-cell volume of the culture broth by centrifugation at 3,000 rpm for 15 min.

Discussion

It is of interest to know that four strains belonging to distinctly different species of the actinomycetes produced an identical antibiotic named globomycin. Among them, *S. halstedii, S. cinnamoneum* and *S. neohygroscopicus* have already been known to produce various kinds of antibiotics, but none of these antibiotics is identical to or resembling globomycin.

As will be reported in the succeeding paper,[9] globomycin was determined to be a new cyclic peptide composed of L-serine, glycine, N-methylleucine, L-*allo*-threonine, L-*allo*-isoleucine and 3-hydroxy-2-methylnonaic acid.

S. halstedii produces carbomycins,[10] encaline group[11] and cephamycins A and B.[12] *S. cinnamoneum* is reported to produce fungichromin,[13] HA-106, 145 and 176[14] and neginamycin.[15] *S. neohygroscopicus* (formerly *S. platensis* and *S. hygroscopicus* var. *angustmyceticus*) is known to produce a number of antibiotics, such as oxytetracycline,[16] FS-351 A and B,[17] AH-272 α_2 and β_2,[18] SF-689[19] and SF-689 B,[20] YL-704,[21] robigocidins A-D[22] and angustmycins A and C.[23] Among these antibiotics, FS-351 A and B and neginamycin are peptide antibiotics, but they are clearly differentiated from globomycin by their amino-acid composition. The others are also distinguished from

globomycin by their physico-chemical as well as biological properties.

References

1) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Internat. J. Syst. Bacteriol. 16: 313~340, 1966
2) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. Internat. J. Syst. Bacteriol. 18: 69~189, 1968
3) LINGAPPA, Y. & J. L. LOCKWOOD: Chitin media for selective isolation and culture of actinomycetes. Phytopathology 52: 317~323, 1962
4) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces*. III. Additional species descriptions from first and second studies. Internat. J. Syst. Bacteriol. 18: 279~392, 1968
5) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. Internat. J. Syst. Bacteriol. 22: 265~394, 1972
6) DIETZ, A.: The actinomycetes. The boundary microorganisms. The Toppan Co., Tokyo, 1976
7) SAKAI, H.; H. YÜNTSEN & F. ISHIKAWA: Studies on a new antibiotic, angustmycin. II. J. Antibiotics, Ser. A 7: 116~119, 1954
8) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third and fourth studies. Internat. J. Syst. Bacteriol. 19: 391~512, 1969
9) NAKAJIMA, M.; M. INUKAI, T. HANEISHI, A. TERAHARA, M. ARAI, T. KINOSHITA & C. TAMURA: Globomycin, a new peptide antibiotic with spheroplast-forming activity. III. Structural determination of globomycin. J. Antibiotics 31: 426~432, 1978
10) TANNER, F. W., Jr.; A. R. ENGLISH, T. M. LEES & J. B. ROUTIEN: Some properties of magnamycin, a new antibiotic. Antibiot. & Chemoth. 2: 441~443, 1952
11) MAKSIMOVA, T. S.; E. G. TOROPOVA, V. K. KOVALENKOVA & G. F. GAUZE: Production of antitumor antibiotics of encaline group by actinomycetes. Antibiotiki 10: 201~207, 1965
12) MILLER, T. W.; R. T. GOEGELMAN, R. G. WESTON, I. PUTTER & F. J. WOLF: Cephamycins, a new family of β-lactam antibiotics. II. Isolation and chemical characterization. Antimicr. Agents & Chemoth. 2: 132~135, 1972
13) TYTELL, A. A.; F. J. MCCARTHY, W. P. FISHER, W. A. BOLHOFER & J. CHARNEY: Fungichromin and fungichromatin. Antibiot. Annual 1954/1955: 716~718, 1955
14) THIRUMALACHAR, M. J. & P. W. RAHALKAR: Pentaene antibiotics from some *Streptoverticillium* in India. Prog. Antimicr. Anticancer Chemoth. I: 70~73, 1970
15) MATSUSHIMA, H.; Y. TAKIGUCHI, R. ENOKITA & M. ARAI: Neginamycin, a new peptide antibiotic. Ann. Sankyo Res. Lab. 26: 64~74, 1974
16) MCGUIRE, J. M.: Improvements in or relating to terramycin. Brit. Patent 713,795, Aug. 18, 1954
17) HAYASHI, T.: Method for production of a new antibiotic FS-351. Japan Kokai 74-7, 495, Jan. 23, 1974
18) EVANS, R. H., Jr.; N. J. R. VALE & S. O. THOMAS: Antibiotics AH 272α_2 and AH 272β_2 and process for producing same. U. S. Patent 3,592,925, July 13, 1971
19) TSURUOKA, T.; T. SHOMURA, N. EZAKI, T. NIWA, A. AKITA & T. NIIDA: Production of a new antibiotic SF-689. Japan Patent 70-6,076, Feb. 28, 1970
20) TSURUOKA, T.: Production of a new antibiotic SF-689 B. Japan Kokai 72-43,349, Dec. 19, 1972
21) KINUMAKI, A.; I. TAKAMORI, Y. SUGAWARA, N. NAGAHAMA, M. SUZUKI, Y. EGAWA, M. SAKURAZAWA & T. OKUDA: Studies on the macrolide antibiotic YL-704 complex. II. Isolation and physicochemical properties of YL-704 components. J. Antibiotics 27: 102~106, 1974
22) SATOMI, A.; S. OH, S. SHIRATO, A. SEINO, M. SHIBUYA & S. AIZAWA: Production of a new antibiotic robigocidin A. Japan Patent 70-17,598, June 17, 1970
23) YÜNTSEN, H.; H. YONEHARA & H. UI: Studies on a new antibiotic, angustmycin. I. J. Antibiotics, Ser. A 7: 113~115, 1954