A NEW ANTIBIOTIC, HONDAMYCIN. II

TAXONOMY OF PRODUCING ORGANISM, PRODUCTION AND BIOLOGICAL PROPERTIES OF HONDAMYCIN

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A strain, *Streptomyces* sp. No. 771, was isolated from a soil sample collected in Chiba Prefecture. The strain produced a new antibiotic which was isolated as colorless hexagonal crystals and named hondamycin. Hondamycin is mainly active against phytopathogenic fungi and *Trichophyton*, but is not active against any bacteria. The LD_{50} was 1.43 mg/kg by intraperitoneal route and greater than 500 mg/kg orally. This strain was recognized to be a variety of *Streptomyces griseochromogenes* and named *Streptomyces griseochromogenes* var. *albicus*.

In a course of screening studies, a colorless hexagonal crystalline antibiotic was obtained from the culture broth and mycelia of a *Streptomyces* strain which had been isolated from a soil sample of Chiba Prefecture in Japan. The antibiotic is mainly active against phytopathogenic fungi and *Trichophyton*, but is not active against bacteria. We have named it hondamycin. In the present paper, the taxonomy of the producing strain, the production and the biological activity of the antibiotic hondamycin are reported.

Taxonomy of St. No. 771

This species belongs to the genus *Streptomyces* and has the following mycological characteristics.

It grows at 27°C on various agar media. At first it has no color, then changes to brown or dark brown, with white aerial mycelium. The aerial mycelium has a closed spiral form on yeast-starch agar and is straight or forms loop on BENNET's agar. Spores are spherical to oval about $1.0\sim1.2\times1.2\sim1.5\,\mu$. The surface of the spores are spiny. The strain produces a brown to dark brown soluble pigment in protein-containing media and therefore is a chromogenic type. This strain is very active in hydrolysing starch and also coagulates and peptonizes milk. It liquefies gelatin slightly but does not decompose cellulose. The carbon source utilization of this strain was investigated by the method of PRIDHAM and GOTTLIEB (Table 2). St. No. 771 utilizes D-glucose, D-fructose, sucrose, raffinose, D-mannitol, rhamnose, L-inositol, D-galactose, maltose, lactose, inulin, salicin, sodium citrate and sodium succinate but does not utilize D-xylose, sodium acetate and L-arabinose.

In view of the above mentioned properties, St. No. 771 closely resembles *St. griseochromogenes*[3,4,5], and parallel studies were therefore done using cultures of St. No. 771 and *St. griseochromogenes* 2A-327 (KCC 39 from Kaken Chemical Co., Ltd.). The

Table 1. Morphological and physiological characteristics of St. No. 771 strain and *St. griseochromogenes* 2A-327

Medium	St. No. 771 strain	*St. griseochromogenes* 2A-327
Morphology	Numerous closed spirals as short branches of hyphae formed on yeast starch agar. On BENNET agar short branches are straight and few spirals. Spores are spherical or oval about 1.0~1.2×1.2~1.5 μ. Surface of spores are spiny.	Sporophores for closed spirals on yeast starch agar, there are no spirals, or only curved tips formed on BENNET agar. Spores spherical or oval about 1.0~1.5 μ.
Nutrient agar	G : good, spreading. R : yellowish brown. AM : abundant, powdery, white with margin natural gray. SP : brown.	G : moderate. R : colorless. AM : moderate, powdery, white. SP : brown.
BENNET agar	G : good, spreading. R : yellowish brown. AM : abundant, velvety, snow white. SP : brown.	G : good, spreading. R : yellowish brown. AM : moderate, cottony, snow white. SP : faint brown.
EMERSON agar	G : good, hard textured. R : yellowish brown. AM : abundant, velvety, snow white. SP : dark brown.	G : moderate. R : bright yellowish brown. AM : moderate, cottony white. SP : dark brown.
Sucrose-nitrate agar	G : moderate, spreading. R : cream. AM : none. SP : pale cream.	G : moderate, spreading. R : light maize. AM : moderate, velvety, pearl pink shell. SP : pearl pink shell.
Inorganic salt-starch agar	G : moderate. R : pearl pink shell. AM : very scant to moderate, white. SP : none. Starch hydrolysis : very strong.	G : moderate. R : ivory buff. AM : moderate, pearl pink shell. SP : pearl pink. Starch hydrolysis : strong.
Ca-Malate agar	G : good. R : light ivory. AM : abundant, powdery, light ivory white. SP : none.	G : moderate, spreading. R : light ivory. AM : moderate, cottony, creamy white. SP : none.
Plain agar	G : very weak. R : colorless. AM : none. SP : none.	G : very weak. R : colorless. AM : very scant, powdery, white. SP : none.
Tyrosine agar	G : very good, wrinkled and hard textured. R : dark brown to black. AM : abundant, cottony, white. SP : slate.	G : very good, hard textured. R : chocolate black. AM : abundant, cottony, neutral gray. SP : brownish black.

(To be continued)

Table 1 (Continued)

Medium	St. No. 771 strain	*St. griseochromogenes* 2A-327
Egg-albumin agar	G : weak. R : cream. AM : scant, cottony, white. SP : none.	G : weak. R : colorless. AM : scant, powdery, white. SP : none.
Potato agar	G : good. R : dark olive bistre. AM : abundant, cottony, light ivory white. SP : brown.	G : good. R : grayish brown. AM : abundant, cottony, light creamy white. SP : mustard tan.
Glucose-asparagine agar	G : moderate. R : colorless to light maize. AM : moderate, cottony, white. SP : none.	G : moderate, spreading. R : light maize. AM : none. SP : none.
Sucrose-nitrate agar	G : moderate, flakey surface. R : white. AM : none. SP : blue gray tint.	G : good, flakey surface and colorless and white flocks on the bottom. R : white. AM : powdery, ivory buff. SP : none.
Tyrosine broth	G : moderate, ring formation. R : olive buff. AM : none.	G : moderate, ring formation and white pellets through media. R : olive buff. AM : none. SP : faint brown.
Tryptone yeast-extract broth	G : ring formation, flocks on bottom. R : light tan. AM : none. SP : dark brown.	G : ring formation and flocks on bottom. R : light tan. AM : none. SP : dark brown.
Bact-nitrate broth	G : moderate, flocks on bottom. R : white. AM : none. SP : brown.	G : moderate, pellicle surface and white little pellets. R : brownish white. AM : none. SP : faint brown.
Potato plug	G : moderate, wrinkled. R : beige gray. AM : cottony, snow white. SP : cocoa brown.	G : moderate, wrinkled and elevated. R : light mustard tan. AM : none. SP : dark brown.
Skim milk	G : ring formation. R : whitish brown. SP : dark brown. Coagulation : positive. Peptonization : positive.	G : ring formation. R : light tan. SP : dark brown. Coagulation : positive. Peptonization : positive.
Litmus milk	G : pellicle surface. R : white. SP : light yellow.	G : good, pellicle surface. R : white to cream. SP : light melon yellow.

(To be continued)

Table 1 (Continued)

Medium	St. No. 771 strain	*St. griseochromogenes* 2A-327
Gelatin stab	G : moderate. R : ivy green. AM : scant, powdery, white. SP : ivy green. Liquefaction : weak.	G : moderate. R : dark olive. AM : light olive. Liquefaction : positive.
Cellulose decomposition*	no growth.	no growth.

* Sucrose-nitrate broth.

Table 2. The utilization of carbon sources of St. No. 771 strain and *St. griseochromogenes* 2A-327.

Carbon sources	St. No. 771 strain	*St. griseochromogenes* 2A-327	Carbon sources	St. No. 771 strain	*St. griseochromogenes* 2A-327
D-Xylose	—	—	D-Galactose	+	+
L-Arabinose	—	—	Maltose	+	+
D-Glucose	+	+	Lactose	++	+
D-Fructose	+	—	Inulin	++	++
Sucrose	++	++	Salicin	++	++
Raffinose	++	++	Na-Citrate	+	+
D-Mannitol	++	++	Na-Succinate	+	+
Rhamnose	+	+	Na-Acetate	—	—
L-Inositol	++	+	Control	—	—

++ : good growth, + : growth, ± : poor growth, — : no growth.

Table 3. Comparison of St. No. 771 strain and *St. albochromogenes* producing orymycin.

		St. No. 771 strain	*St. albochromogenes*
Cultural characteristics	Glucose-asparagine agar	G : moderate. R : colorless to light maize. AM : moderate, cottony, white. SP : none.	G : weak to moderate. R : cream. AM : none. SP : none.
	Sucrose-nitrate agar	G : cream, moderate, spreading. AM : none. SP : pale cream.	G : white. AM : snow white. SP : faint brown.
	Ca-malate agar	G : good. R : light ivory. AM : abundant, powdery, light ivory white. SP : none.	G : weak, restricted. R : colorless or cream. AM : scant or none. SP : none.
	Potato plug	AM : cottony, snow white.	AM : none.
	Milk	Coagulation : positive.	Coagulation : none.
Utilization of carbon sources	Rhamnose Na-Citrate Na-Succinate	+ + +	— — —
Production of antibiotics		Hondamycin	Orymycin

results of a comparison of morphological and biochemical characteristics are shown in Tables 1 and 2. St. No. 771 was also compared with a strain of *St. albochromogenes* producing orymycin[6] and another strain of *St. albochromogenes* which produced raro-

Table 4. Comparison of St. No. 771 strain and *St. albochromogenes* producing raromycin.

		St. No. 771 strain	*St. albochromogenes*
Cultural characteristics	Ca-Malate agar	G : good. AM : abundant, powdery.	G : weak. AM : scant, cottony.
	Sucrose-nitrate agar	G : moderate, spreading. R : cream. AM : none. SP : pale cream.	G : good. R : colorless to brown. AM : cottony, abundant, white to light gray. SP : brown trace.
	Starch agar	G : moderate. R : pearl pink shell. AM : very scant white. SP : none.	G : good. R : colorless to dark olive gray. AM : abundant, cottony, white to light dull gray. SP : none.
	Potato agar	SP : cocoa brown.	SP : none.
	Milk	G : ring formation. Peptonization : strong. Coagulation : positive.	G : good, pellicle surface. Peptonization : trace. Coagulation : trace.
Utilization of carbon sources	D-Xylose	−	+
	L-Arabinose	−	+
	Na-Citrate	+	+
	Na-Succinate	+	+
Production of antibiotics		Hondamycin	Raromycin

mycin[7]. Some differences in morphology and the utilization of carbon sources were seen. The results of the comparison of these strains are shown in Tables 3 and 4.

From these results, the following differences can be noted between St. No. 771 and *St. griseochromogenes* 2A-327.

(1) St. No. 771 strain does not grow with an aerial mycelium and produces pale cream soluble pigment in the sucrose nitrate agar, whereas *St. griseochromogenes* 2A-327 forms a velvety, pearl pink shell aerial mycelium and produces pearl pink shell soluble pigment in the sucrose nitrate agar.

(2) St. No. 771 strain forms a white aerial mycelium and does not produce soluble pigment in the inorganic salts starch agar, while *St. griseochromogenes* 2A-327 forms a pearl pink shell aerial mycelium and produces pearl pink soluble pigment in its medium.

(3) St. No. 771 strain forms a cottony white aerial mycelium on glucose-asparagine agar, whereas *St. griseochromogenes* 2A-327 does not form an aerial mycelium on this medium.

(4) St. No. 771 strain produces a blue tint gray soluble pigment in the sucrose nitrate broth, whereas *St. griseochromogenes* 2A-327 does not produce a soluble pigment in its medium.

(5) St. No. 771 strain grows beige gray and forms a cottony snow white aerial mycelium on the potato plug, whereas *St. griseochromogenes* 2A-327 grows light mustard tan and does not form an aerial mycelium on it.

(6) In the utilization of carbon sources St. No. 771 strain utilizes D-fructose, whereas *St. griseochromogenes* 2A-327 does not utilize this carbon source.

Although some differences can be seen between St. No. 771 strain and *St. griseochromogenes* 2A-327, they are similar in many ways. For instance, they are identical hydrolysis of starch, coagulation and peptonization of milk, liquefaction of gelatin,

appearance of spores and color of pigment in many media. Considering the similarities, it was concluded that St. No. 771 strain was a variant of *St. griseochromogenes*.

In many results, *St. griseochromogenes* resembles *St. diastatochromogenes*[8,10], *St. olivochromogenes*[9,10], *St. flavochromogenes*[10,11], *St. mirabilis*[10,11], and *St. resistatomycificus*[10]. However St. No. 771 strain differs from *St. diastatochromogenes* in the color of soluble pigment and in the amount of aerial mycelium observed on sucrose-nitrate agar and nutrient agar. *St. olivochromogenes* differs from St. No. 771 strain in the color of the mycelia on various media and also an abundant white aerial mycelium formed by St. No. 771 strain as *St. olivochromogenes* does not form any aerial mycelium on potato medium. *St. flavochromogenes* differs from St. No. 771 strain in the color of growth on various media. St. No. 771 strain forms closed spirals but *St. mirabilis* does not and the strains differ in the appearance of their growth on several media. *St. resistatomycificus* differs from the St. No. 771 strain in the color of its aerial mycelium observed on various media. *St. resistatomycificus* does not peptonize milk, but St. No. 771 was a rapid peptonizer of milk. Thus St. No. 771 differs from these strains and is not identical to any of these species. Also it is evident that St. No. 771 and *St. albochromogenes*, the orymycin-producing organism, or *St. albochromogenes*, the raromycin-producing organism, are different species, because of the many differences in cultural characteristics and utilization of carbon sources (Tables 3 and 4).

Production

Conditions for producing hondamycin were studied in shake culture using 1 % of glucose, glycerol, starch, dextrin, sucrose, molasses or lactose as the carbon sources, with soybean meal 0.5 %, peptone 0.5 %, NaCl 0.5 %, K_2HPO_4 0.2 % and $CaCO_3$ 0.2 % as a basal medium. Activity against *Piricularia oryzae* was highest, when starch, molasses and dextrine were used.

Suitable nitrogen sources for the production of the antibiotic were studied using 1 % soybean meal, meat extract, Proflo (cotton sead flour), Pharmamedia (cotton sead protein), peptone, yeast and corn steep liquor using starch 0.5 %, molasses 0.5 %, NaCl 0.5 %, K_2HPO_4 0.2 % and $CaCO_3$ 0.2 % for a basal medium. Activity against *Piricularia oryzae* was greatest with 1 % soybean meal or peptone.

Hondamycin was produced in both the broth and mycelium, but was easier to purify from the mycelium. The most effective conditions for promoting growth were also found to be favourable for producing the antibiotic, and 0.5 % of soybean meal and 0.5 % of peptone as the nitrogen source provided the best results.

Using a jar fermenter, the following medium was found to be the most suitable: dextrine 1 %, soybean meal 0.5 %, peptone 0.5 %, K_2HPO_4 0.2 %, NaCl 0.5 % and $CaCO_3$ 0.2 %, and pH 7.0 after sterilization. In order to produce a great amount of mycelia in submerged culture, a temperature of 27~29°C was used. The inoculum was prepared by incubating for 60 hours at 27°C on a reciprocal shaker. Contents of 2 flasks (200 ml) were pooled inoculated into 10 liters of the same medium in a 30-liter stainless steel jar fermenter. After about 90 hours, production of hondamycin reached a maximum and its pH was 7.8.

Biological Properties

The antimicrobial activity of hondamycin was studied by the agar streak dilution method using nutrient agar medium for the bacteria, CZAPEK's agar medium for the fungi, and malt agar medium for the yeasts, glycerine CZAPEK's agar medium for the mycobacterium and potato agar medium for the phytopathogenic fungi. Antibiotic dilutions were made in acetone and added to the medium in a 1% final concentration. Minimal concentration at which complete inhibition was observed against a series of organisms is shown in Table 5. It was evident that hondamycin is inactive against

Table 5. Antimicrobial spectrum of hondamycin.

Test organisms	Minimum inhibitory concentration (mcg/ml)	Test organisms	Minimum inhibitory concentration (mcg/ml)
Bacillus subtilis PCI 219	>100	*Aspergillus niger*	0.1
Bacillus anthracis	>100	*Saccharomyces sake*	>100
Bacillus agri	>100	*Saccharomyces cerevisiae*	>100
Staphylococcus aureus 209 P	>100	*Candida albicans*	>100
Staphylococcus aureus Terajima	>100	*Trichophyton asteroides*	1
Micrococcus flavus	>100	*Trichophyton pomph*	0.01
Sarcina lutea PCI 1001	>100	*Trichophyton beigelii*	>100
Escherichia coli	>100	*Trichophyton gypseum*	1
Salmonella typhi 63 T_3	>100	*Trichophyton rubrum*	0.1
Salmonella pullorum 3H-2	>100	*Trichophyton interdigitable*	0.1
Salmonella enteritidis	>100	*Trichophyton fujii*	0.5
Shigella flexneri NIHJ B 55	>100	*Trichophyton mentagrophytes*	0.5
Corynebacterium xerosis	>100	*Xanthomonas oryzae*	>100
Proteus vulgaris	>100	*Xanthomonas citri*	>100
Klebsiella pneumoniae	>100	*Piricularia oryzae*	0.1
Pseudomonas aeruginosa	>100	*Botrytis cinerea*	1
Pseudomonas aureofaciens	>100	*Alternaria citri*	0.01
Pseudomonas fluorescens	>100	*Alternaria kikuchiana*	0.5
Pseudomonas tabaci	>100	*Glomerella cingulata* G-24	0.5
Mycobacterium tuberculosis 607	>100	*Glomerella cingulata* G-34	0.1
Mycobacterium phlei	>100	*Phytophthora citophthora*	>100
Penicillium chrysogenum Q 176	0.1	*Cerocospora beticola*	0.01
Aspergillus oryzae	>100		

Fig. 1. Microscopic photograph of St. No. 771 strain.

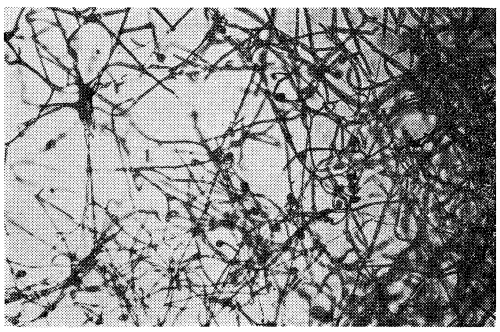
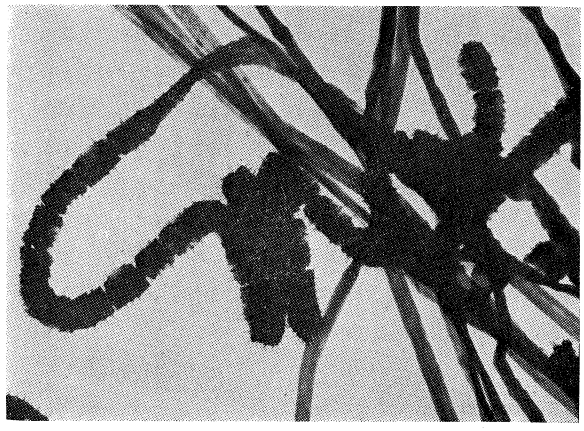


Fig. 2. Electron microscopic photograph of St. No. 771 strain.



Gram positive and negative bacteria, *Mycobacterium tuberculosis* 607, *M. phlei* and also against yeasts. On the other hand, the antibiotic is active against a wide range of fungi, especially *Trichophyton*. The phytopathogenic fungi were also sensitive to hondamycin.

Acute toxicity of hondamycin was determined with mice weighing 19~20 g. The LD_{50} for *DD* mice was found to be 1.43 mg/kg intraperitoneally. Administered orally, mice tolerated 500 mg/kg of the antibiotic without any toxic sign for 14 days.

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