YAZUMYCIN, A NEW ANTIBIOTIC PRODUCED BY *STREPTOMYCES LAVENDULAE*

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An antibiotic was isolated from the culture filtrates of *Streptomyces lavendulae* and named yazumycin. It was isolated from the broth using cation-exchange resin and purified by cellulose chromatography. Yazumycin exhibited inhibitory activities against bacteria, especially Gram-negative bacteria such as *Xanthomonas oryzae*, *Xanthomonas citri* and *Escherichia coli*. No toxicity to mice was shown by intravenous injection of 200 mg/kg.

In the course of screening for antibiotics, a new antibiotic was isolated from the fermentation broth of a *Streptomyces* culture, which resembled to those of *Streptomyces lavendulae*. This antibiotic, which was named yazumycin, is active against bacteria, especially *Xanthomonas oryzae* and *Xanthomonas citri*.

In this paper, the taxonomic studies of the producing strain, and the production, isolation, and properties of yazumycin are described.

Taxonomic Studies of the Strain IN-183-T

The strain IN-183-T was isolated from a soil sample collected in Kahara-machi, Yazu-gun, Tottori Prefecture, Japan.

Most of the general procedures suggested by SHIRLING *et al.*[1], have been followed in this study. Media described by WAKSMAN[2,3] and SHIRLING *et al.*[1] were used in the study of morphological and physiological properties. Either Difco agar or Difco Noble agar was used as the solidifying agent for the various media and for the carbohydrate utilization tests. To avoid coloring of the PRIDHAM-GOTTLIEB basal medium, the phosphate buffer was autoclaved separately. Carbon sources were sterilized in the dry state for 12 minutes at 121°C as suggested by LUEDEMAN *et al.*[4] The inoculum for various solid and liquid media was prepared by washing the mycelium three times. Color comparisons were made using Color Standards (1954) and Tresner-Backus Color Wheel (1963) and description of results were noted the same as in the preceding report[5].

1. Micromorphology.

The vegetative mycelium of strain IN-183-T does not fragment into cocoid or bacillary forms in liquid media. Some of the media, such as inorganic salts-starch agar (ST), glucose-asparagine agar, oat meal agar, yeast extract-starch agar (YS) and yeast

extract-malt extract agar (YM) were useful for morphological observations of this strain.

Spores (electronmicrograph): cylindrical, *ca.* 0.6×1 μ, surface is smooth, phalangiform (Plate 2).

Sporophores: main axis of aerial mycelium is flexible or somewhat wavy and terminate in clusters. The terminal filaments develop into sporophores having both straight and loosely spiralled spore chains. The spirals consist of two or more turns and their average diameter is 15 μ (Plate 1). Sporangia, flagellated spores, coremia, sclerotia or ball-like bodies were not observed.

Plate 1. Aerial mycelium of the strain IN-183-T (Sucrose-nitrate agar, 27°C 14 days, 1 scale=3.3 μ)

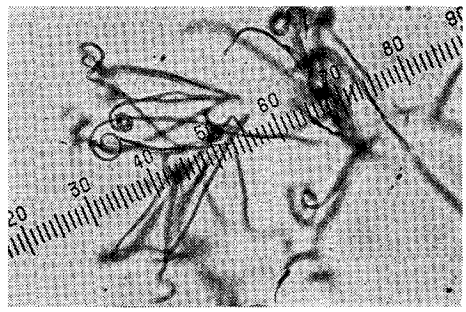
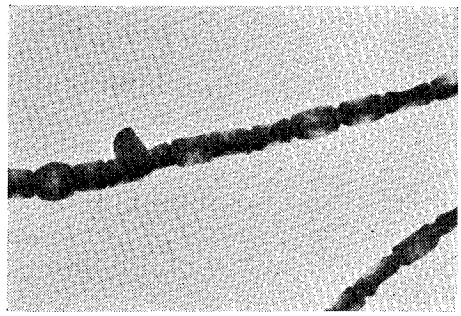


Plate 2. Electron micrograph of conidia of IN-183-T (YS agar, direct magnification ×5,000)



2. Cultural and physiological characteristics.

The cultural and physiological studies are carried out at 28°C and the results read after 14 days unless otherwise noted.

Sucrose-nitrate agar plate: colorless, hyaline, scant spreading.

Aerial mycelium: thin but covered all over surface of growth, powdery light brownish gray (4-18-1) (Grayish Yellowish Pink, ISCC-NBS, 32).

Soluble pigment: none.

Glycerol-nitrate agar plate

Growth: good, not spreading, yellswish brown (6-15-4).

Aerial mycelium: very scant.

Table 1. Physiological characteristics of strain IN-183-T.

	Medium	Response
Melanin formation	Melanin-formation agar stab	Positive
Tyrosinase reaction	Tyrosine agar slant	Positive
Amylosis	ST agar plate	Positive
Proteolytic activity	Skim milk solution	Partially peptonized without coagulation
	Gelatin	Slowly liquefied
	Coagulated serum	Partially liquefied
Cellulolytic activity	PRIDHAM-GOTTLIEB basal medium +cellulose powder	Negative
Production of H_2S	Peptone iron agar+0.1 % yeast extract KLIGLER's iron agar	Positive
Reduction of nitrate	Sucrose nitrate broth Glucose nitrate broth Glycerol nitrate broth Difco nitrate broth	Positive
Solubility of Ca-malate	Glycerol Ca-malate agar plate	Positive
Temperature range	MY agar slant pH 7.0	Mesophilic, no growth at 5°C and 37°C

Table 2. Carbohydrates utilization of strain IN-183-T

Carbohydrates	Response
L-Arabinose	—
d(+)-Cellobiose	++
Glucose (Positive control)	++
D-Galactose	++
Lactose	—
D-Levulose	±
Maltose	++
d(+)-Mannose	++
D(+)-Melelezitose	—
Melibiose	++
Raffinose	±
L-Rhamnose	—
Sucrose	—
Trehalose	++
D-Xylose	—
Salicin	+
Inulin	—
Dulcitol	—
i-Inositol	—
D-Mannitol	—
D-Sorbitol	—
Negative control (No carbon)	—

Table 3. Antimicrobial activity of yazumycin

Test organisms	M.I.C. (mcg/ml)
Staphylococcus aureus FDA 209 P	20
Pseudomonas aeruginosa NRRL B-1000	200
Micrococcus luteus ATCC 398	20
Serratia marcescens IAM-1223	100
Bacillus subtilis PCI 219	10
Proteus vulgaris OX-19	20
Mycobacterium smegmatis ATCC 607	10
Escherichia coli NIHJ	10
Klebsiella pneumoniae ATCC 10031	10
Salmonella typhi-murium	20
Shigella dysenteriae	10
Trichophyton mentagrophytes	100
Trichophyton rubrum	>200
Microsporum gypseum	200
Cryptococcus neoformans	5
Candida albicans YU-1200	>200
Candida albicans 57	>200
Candida tropicalis NI-7495	>200
Aspergillus terreus ATCC 10690	>200
Aspergillus fumigatus NI 5561	>200
Sporotrichum schenckii	200
Hormodendrum pedrosoi	50
Nocardia asteroides ATCC 3308	>200

Aerial mycelium: powdery, white.
Soluble pigment: dark brown to black.
Remarks: tyrosinase reaction positive.

Oatmeal agar plate

Soluble pigment: yellowish brown.

Glycerol-asparagine agar plate

Growth: abundant, spreading, edge myceloid, pale yellow (7-19-2) (Light Orange Yellow ISCC-NBS, 70), later (21 days) changing to light yellowish brown (6-18-3).

Aerial mycelium: abundant, powdery light brown (4-18-2)~light brownish gray (4-18-1) (Grayish Yellowish Pink, 32) at 21 days changing to light brown (4-18-2) ~light brownish gray (5-17-1).

Soluble pigment: none.

Glycerol-calcium malate agar plate

Growth: moderate, light yellowish brown (7-17-2), later (21 days) changing into yellowish brown (7-16-3) with small bluish spots which turn to brown under alkaline conditions.

Aerial mycelium: very good, light brownish gray (4-18-1)~(4-17-1), at 21 days changed to pale reddish brown (3-16-2) with pale bluish spots (Pale Blue ISCC-NBS, 185).

Soluble pigment: pale yellow.

Remarks: calcium malate gradually dissolved accompanied by acid production.

ST agar plate

Growth: abundant, spreading, yellowish gray (8-19-1) later (21 days) becoming light yellowish brown (7-18-2).

Aerial mycelium: abundant, brownish gray (7-16-1) (Light Grayish Reddish Brown ISCC-NBS, 45) later (21 days) turning light brownish gray (3-18-1) to brownish gray (6-16-1).

Soluble pigment: none.

Remarks: diastatic activity positive.

Tyrosine agar slant

Growth: good, not spreading, pale yellow.

Growth: abundant, spreading, dark yellow (7-17-5) later becoming light yellowish brown (6-17-3) to light brown (5-16-4).
Aerial mycelium: abundant, powdery, light brown (4-16-4) to light reddish brown (3-17-2) (Moderate Yellowish Pink ISCC-NBS, 29), later (21 days) becoming light reddish brown (3-17-3) to grayish brown (4-16-2) (Light Grayish Reddish Brown ISCC-NBS, 45).
Soluble pigment: pale yellow.

YM agar plate
Growth: abundant, spreading, yellowish brown (6-15-4).
Aerial mycelium: very good, powdery, pinkish gray (2-17-1) later turning light brownish gray (4-17-1).
Soluble pigment: dull yellow, no color change observed under alkaline and acidic reactions.

YS agar plate
Growth: abundant, spreading, dark yellowish orange (6-17-5).
Aerial mycelium: abundant, powdery, light brownish gray (3-17-1), later turning light brownish gray (4-17-1).
Soluble pigment: dull yellow, no color change under alkaline and acidic reaction.

Nutrient agar slant (Difco Nutrient agar)
Growth: restricted, medium sized colonies, glistening, pale yellowish brown (7-17-2) (Grayish yellow ISCC-NBS, 90).
Aerial mycelium: very scant or none.
Soluble pigment: light brown.

Gelatin stab
Growth: colonies on the surface of medium, and sparse along stab.
Aerial mycelium: scant.
Soluble pigment: brown.
Remarks: liquefaction weak.

Whole egg
Growth: abundant, pale yellow (7-19-3).
Aerial mycelium: good, powderly, white.
Color of slant: dark purple gray along growth.

Potato plug
Growth: lichenoid, yellowish brown (7-15-3).
Aerial mycelium: powdery, thin, grayish white.
Color of plug: brownish gray.

LÖFFLER'S serum (Difco LÖFFLER blood serum)
Growth: glistening, cream colored.
Aerial mycelium: very scant, powdery white.
Color of coagulated serum: brown to black.
Remarks: coagulated serum partially liquefied.

Table 1 and Table 2 present results obtained in some physiological examinations of the strain IN-183-T.

The characteristics of strain IN-183-T can be summarized as follows: vegetative mycelium not segmenting into bacillary or coccoid elements in submerged cultures. Aerial mycelium forms loose spirals. No whorls were observed. Spores are cylindrical with smooth surfaces and phalangiform electron dense shadows. Vegetative mycelium (reverse color) is colorless to yellowish brown on various media and the representative color of aerial mycelium is light brownish gray (ISCC-NBS, 32~45). Melanoid formation is positive and a light brown to black soluble pigment is produced on

various organic media. Relatively weak proteolytic activities are exhibited on gelatin, milk and coagulated serum. Starch is hydrolyzed and nitrate is reduced to nitrite on glucose-, sucrose-, glycerol-nitrate broth and Difco nitrate broth. Hydrogen sulfide production is positive. This strain exhibits a rather narrow carbohydrates utilization pattern (Table 2).

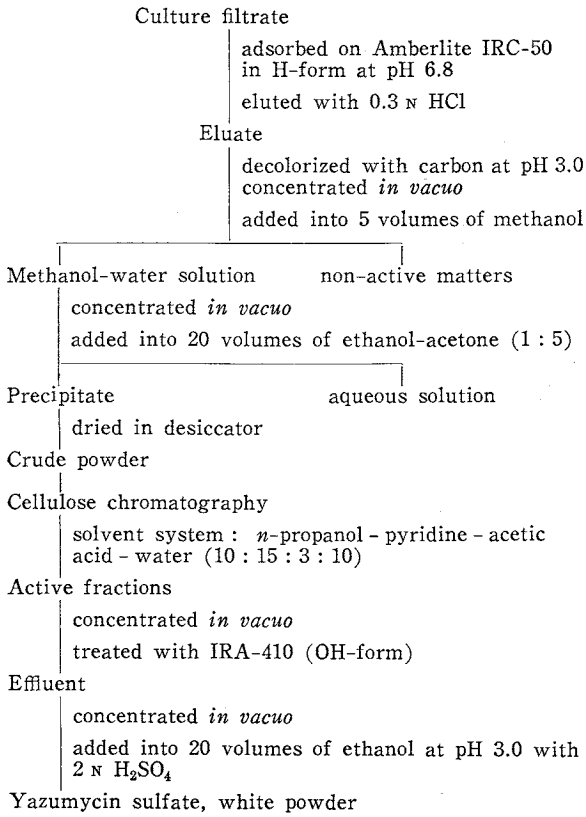
Among the known species of *Streptomyces*, strain IN-183-T resembles most closely *Streptomyces lavendulae* with respect to morphological, cultural and physiological characteristics. Although the optimal temperatue for growth of strain IN-183-T is lower than that for *S. lavendulae*, other characteristics are almost identical with the original description of *S. lavendulae*. Therefore, strain IN-183-T was identified as a strain of *S. lavendulae* (WAKSMAN *et* CURTIS) WAKSMAN *et* HENRICI, 1948.

Production and Isolation

This antibiotic was produced by shake-cultures in the following medium: glucose 2.4 %, soy bean meal 1.5 %, glycerine 1.0 %, yeast extract 0.5 %, NaCl 0.5 %, $(NH_4)_2SO_4$ 0.5 %, $CaCO_3$ 0.4 %, pH 7.0 before sterilization.

Production reached a maximum after 4 days at 27°C. Yazumycin in the cultured broth was adsorbed on a cation-exchange resin in H-form (Amberlite IRC-50) at pH 6.8 and eluted with aqueous hydrochloric acid (0.3 N HCl). The eluate was adjusted at pH 3.0 and decolorized with active carbon (0.5 %) and concentrated *in vacuo*.

Fig. 1. Isolation of yazumycin



The concentrated solution was added to five volumes of methanol and the inactive precipitate were filtered off. The filtrate was concentrated *in vacuo* and added in to twenty volumes of an ethanol-acetone mixture (1 : 5 in volume), yielding a powdery precipitate containing the yazumycin. After drying *in vacuo* a light brown crude powder was obtained.

The crude powder was further purified on a cellulose column by elution with a solvent system of *n*-propanol · pyridine · acetic acid · water (10 : 15 : 3 : 10).

Active fractions were combined and the solvent evaporated *in vacuo* to dryness. The powder obtained by this method was dissolved in water, and the antibiotic solution was

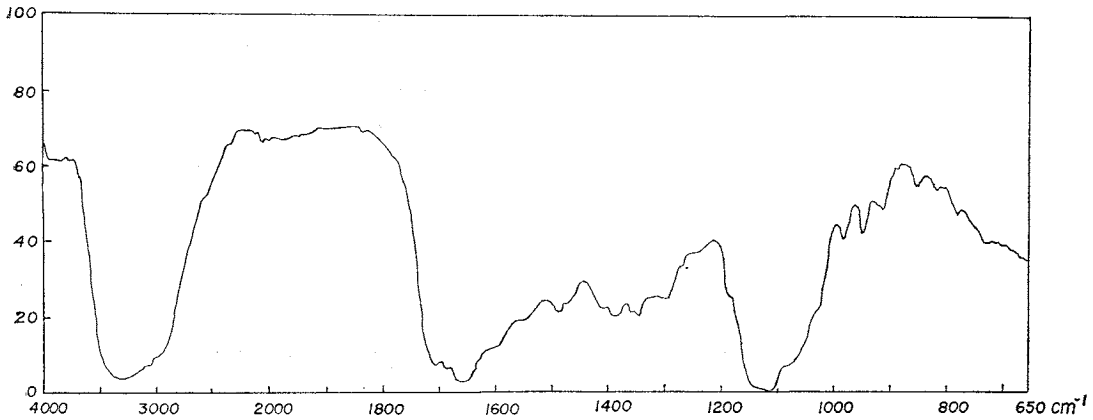
passed over a column of Amberlite IRA-410 (OH-form), then adjusted to pH 3.0 with 2 N sulfuric acid, and added to twenty volumes of ethanol.

As shown in Fig. 1, yazumycin sulfate was isolated as a white powder.

Properties of Yazumycin

Yazumycin sulfate decomposes at 230~236°C. It is soluble in water and insoluble in ethanol, acetone, benzene and ethyl acetate. The water solution shows only end absorption in the ultraviolet spectrum. The infrared absorption spectrum of yazumycin in KBr pellet is shown in Fig. 2.

Fig. 2. Infrared spectrum of yazumycin sulfate in KBr-pellet.



Elemental analysis yields the following:

Calcd. for $C_{15}H_{35}N_6O_{13}S$:
C 33.35, H 6.50, N 15.59, S 5.94
Found: C 33.10, H 6.50, N 15.64, S 5.90

Yazumycin gives positive SAKAGUCHI, ELSON-MORGAN, diazo, ninhydrin, FEHLING, TOLLENS and biuret (light blue tests), but negative maltol, ferric chloride, Benedict, MILLON, BARFOED, xanthoprotein and 2,4-dinitrophenylhydrazine reactions.

No reduction of the antibiotic activity was observed after heating at 100°C for 100 minutes in pH 2.0~9.0 aqueous solutions.

The Rf values on paper chromatograms were as follows:

0.42 in *n*-propanol - pyridine - acetic acid - water (10 : 15 : 3 : 10)
0.53 in 80 % methanol 100 ml, piperidine 10 ml, pH 9.3 with acetic acid
0.09 in water-saturated *n*-butanol, containing 2% *p*-toluenesulfonic acid
0.16 in *n*-butanol - acetic acid - water (2 : 1 : 1)

Fig. 3. Comparison of yazumycin and dihydrostreptomycin.
Solvent system : 80 % MeOH 100 ml, piperidine 10 ml, pH 9.3 with AcOH
Bioautogram against *B. subtilis*

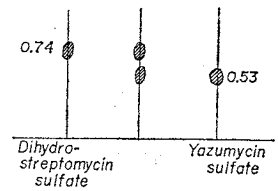


Table 4. Antimicrobial spectrum of yazumycin by the agar dillution method.

Test organisms	Inhibitory concentration (mcg/ml)
Xanthomonas oryzae	5
Xanthomonas citri	5
Cochliobolus miybeanus	>100
Diaporthe citri	>100
Corticium rolfsii	>100
Gloeosporum laeticolor	>100
Piricularia oryzae	>100
Cladosporum carpophilum	>100
Alternaria kikuchiana	>100
Fusarium oxysporum f. *niveum*	>100
Corynebacterium sepedonicum	>100

Table 5. Comparison of yazumycin with other known antibiotics.

Antibiotics	Color reactions			m. p.	Rf values*
	Maltol	Ninhydrin	SAKAGUCHI		
Yazumycin	−	+	+	230～236°C (decomp.)	0.3～0.33
Streptomycin	+		+		
Mannosido-streptomycin	+				
Hydroxy-streptomycin	+ (kojic acid, isomer)				
Streptothricin			−	213～217°C[8] (sulfate, decomp.)	0.26[6]
Neomycin B					0.51[6]
Catenulin					0.60[6]
Zygomycin A					0.62[6]
Kanamycin					0.65[6]
Viomycin					0.11[6]
Paromomycin					0.68[6]
Aminosidin					0.68[6]
Glebomycin		−[7]			

* Rf value by silica gel T.L.C.
solvent : the upper layer of $CHCl_3$: MeOH : 17% NH_4OH (2 : 1 : 1)

Table 6. Acute toxicity of yazumycin to DDN female mice (intravenous injection)

Amount injected	1 day	7 days	14 days
100 mg/kg	0/10	0/10	1/10
200 mg/kg	0/5	0/5	0/5

On thin-layer chromatograms with silica-gel, the following Rf values are observed :

0.3～0.33 in the upper layer of chloroform - methanol - 17 % NH_4OH (2 : 1 : 1)

0.6 in *n*-propanol - pyridine - acetic acid - water (15 : 10 : 3 : 10)

As shown in Table 6, no lethal effect was observed when 100 mg and 200 mg per kg doses were injected to mice intravenously.

The antimicrobial spectrum was tested by the agar dilution streak method and the results are shown in Table 3. As shown in Table 4, yazumycin is active against *Xanthomonas oryzae* and *Xanthomonas citri*.

Yazumycin is a water-soluble, basic antibiotic. It is differentiated from known antibiotics as follows : the Rf value on silica gel thin-layer chromatograms developed with the upper layer of $CHCl_3$ - MeOH - 17 % NH_4OH (2 : 1 : 1) is 0.3～0.33 which is far lower than those of neomycins, paromomycins and kanamycins (Table 5). Yazumycin inhibits not only *Mycobacterium smegmatis* but also Gram-positive and Gram-negative bacteria (Table 3); thus it is differentiated from viomycin[9], capreomycin[10], alboverticillins[11] and triculamin[12]. The negative maltol reaction of yazumycin differentiates it from streptomycins (Table 5). Yazumycin gives positive SAKAGUCHI reaction test differentiating it from streptothricins (Table 5). A comparison of yazumycin and dihydrostreptomycin by paper chromatography shows their different Rf values (Fig. 3).

In elemental analysis, color reactions, Rf value of paper or thin-layer chromatography, and antimicrobial spectrum, no antibiotic so far published coincides with yazumycin.

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