OM-704 A, A NEW ANTIBIOTIC ACTIVE AGAINST GRAM-POSITIVE BACTERIA PRODUCED BY *STREPTOMYCES* SP.

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A new antibiotic OM-704 A has been isolated from a fermentation broth of a *Streptomyces* sp. OM-704, a soil isolate. It exhibits antibacterial activities against Gram-positive bacteria. The antibiotic (mp 291~295°C, $C_{20}H_{22}N_2O_4$) is a neutral quinoid compound.

In the course of screening for antibacterial antibiotics from actinomycetes, a new antibiotic, OM-704 A, has been isolated from the culture broth of strain OM-704, a soil isolate identified as *Streptomyces* sp. This antibiotic was active against Gram-positive bacteria.

The present paper deals with the taxonomy of strain OM-704 and the fermentation, isolation and biological and physicochemical properties of antibiotic OM-704 A.

Taxonomy of the Producing Strain

Morphology

The vegetative mycelium grows abundantly on both synthetic and complex agar media, and does not show fragmentation into coccoid or bacillary elements. Moderate or good growth of aerial mycelium was observed on various media.

The electronmicrographs of strain OM-704 were taken with a scanning electron microscope (Model S-430, Hitachi). Its sporophores show type *Rectus-Flexibilis* (Plate 1). Mature spore chains on inorganic salts-starch agar have more than ten spores per chain, which are cylindrical in shape, 0.57×1.0 μm in size and have a smooth surface

Plate 1. Scanning electronmicrograph of aerial hyphae of strain OM-704 (×2,300).

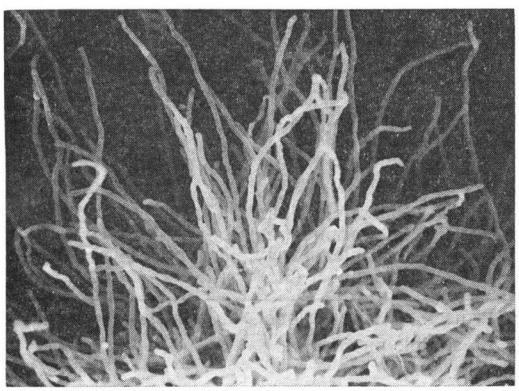
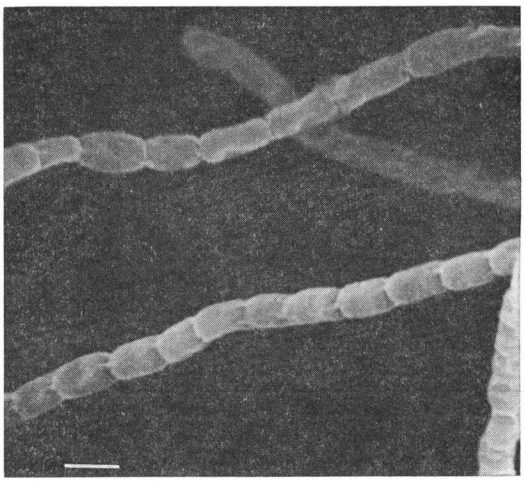


Plate 2. Scanning electronmicrograph of spore chains of strain OM-704. Bar represents 1.0 μm.



(Plate 2). Sclerotic granules, sporangia and flagellated spores were not observed.

Chemical Compositions

The chemical analyses of sugars in whole cells and amino acids in cell walls were carried out by the methods of BECKER *et al.*[1] and LECHEVALIER & LECHEVALIER,[2] respectively. Strain OM-704 shows no characteristic sugar pattern and LL-diaminopimelic acid (DAP) is present.

Table 1. Cultural characteristics of strain OM-704.

Medium	Cultural characteristics
Yeast extract-malt extract agar (ISP)*	G : Good, light yellow (1 1/2 ea) R : Light tan (3gc) AM: Abundant, velvety, pearl (3ba) SP : Slightly, camel (3ie)
Oatmeal agar (ISP)*	G : Good, pastel yellow (1 1/2 fb) R : Bamboo (2fb) AM: Abundant, velvety, pastel yellow (1db) SP : Slightly, camel (3ie)
Inorganic salts-starch agar (ISP)*	G : Good, light antique gold (1 1/2 ic) R : Light tan (3gc) AM: Abundant, velvety, ivory (2db) SP : Light orchid pink (9ca)
Glycerol-asparagine agar (ISP)*	G : Moderate, light cherry rose (7ga) R : Salmon pink (5ga), light beige (3ec) AM: Abundant, velvety, white (a) SP : Slightly, light beige (3ec)
Glucose-asparagine agar	G : Good, pearl (2ba) R : Pale pink (7ca), bamboo (2gc) AM: Abundant, velvety, hygroscopic, white (a) SP : None
Peptone-yeast extract iron agar (ISP)*	G : Good, rosewood tan (5ie) R : Light brown (31g) AM: Poor, white (a) SP : None
Tyrosine agar (ISP)*	G : Moderate, light mustard tan (2ie) R : Light mustard tan (2ie) AM: Moderate, velvety, pearl (3ba) SP : None
Sucrose-nitrate agar	G : Moderate, penetrant, coloress R : Pearl (3ba) AM: Moderate, velvety, pearl (3ba) SP : None
Glucose-nitrate agar	G : Good, light beige (3ec) R : Outer; light tan (3gc) Inner; light spice brown (41g) AM: Abundant, velvety, pearl (3ba) SP : None
Glycerol-calcium malate agar	G : Moderate, cream (1 1/2 ca) R : Ivory (2db) AM: Moderate, velvety, light ivory (2ca) SP : Slightly, bamboo (2gc)
Glucose-peptone agar	G : Good, camel (3ie) R : Cork tan (4ie) AM: Moderate, powdery, white (a) SP : Peach tan (5gc)
Nutrient agar	G : Moderate, pale pink (7ca) R : Pastel orange (4ic) AM: Moderate, velvety, pearl pink (4ca) SP : Light amber (3ic)**

* Medium employed by International Streptomyces Project. Abbreviations: G, growth of vegetative mycelium; R, reverse color; AM, aerial mycelium; SP, soluble pigment.

** Light amber soluble pigment was observed after 3 weeks.

Table 2. Physiological properties of strain OM-704.

Melanin formation	Positive on glucose-peptone gelatin (21°C)
Tyrosinase reaction	Negative
Nitrate reduction	Negative
H_2S production	Negative
Liquefaction of gelatin	Positive (21°C)
Hydrolysis of starch	Positive
Coagulation of milk	Positive (37°C)
Peptonization of milk	Positive (37°C)
Cellulolytic activity	Negative
Temperature range of growth	15~40°C

Table 3. Utilization of carbon sources by strain OM-704.

Carbon source	Response*	Carbon source	Response*
D-Glucose	+	*i*-Inositol	±
D-Xylose	+	Raffinose	−
D-Mannitol	+	Sucrose	−
D-Fructose	+	Melibiose	−
L-Arabinose	±		

* +, Utilized,
±, weakly utilized,
−, not utilized.

Cultural and Physiological Characteristics

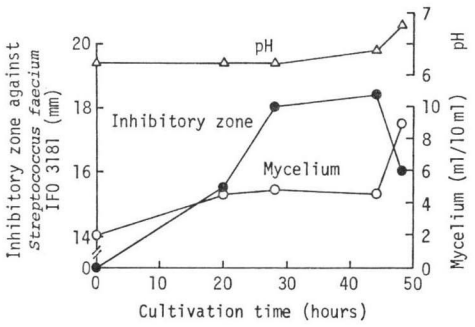
The International Streptomyces Project (ISP) media recommended by SHIRLING and GOTTLIEB[3] and those recommended by WAKSMAN[4] were used for these experiments. Cultures were observed after incubation at 27°C for two weeks. Color names and hue numbers indicated are those of the Color Harmony Manual (4th edition) published by the Container Cooperation of America. The utilization of carbon sources was tested by growth on PRIDHAM and GOTTLIEB's medium containing 1% each carbon source at 27°C. The cultural and physiological characteristics are shown in Tables 1 and 2, respectively. The utilization of carbon sources of strain OM-704 is shown in Table 3.

The cultural and physiological characteristics of strain OM-704 are summarized as follows: sporophore is *Rectus-Flexibilis*; spore is cylindrical and has a smooth surface; color of aerial mycelium is white to pearl; melanoid pigment is produced on glucose-peptone gelatin; soluble pigment is slightly yellowish brown; DAP in cell wall is LL-type. Based on the taxonomic properties described above, strain OM-704 is considered to belong to the genus *Streptomyces* being a strain of the white or red series of PRIDHAM and TRENSNER's grouping.[5] The type strain has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, as *Streptomyces* sp. OM-704-KA333 with the accession number FERM-P 6520.

Fermentation and Isolation

The stock culture of strain OM-704 was inoculated into 100 ml of a seed medium consisting of 1.0% glucose, 2.0% starch, 0.5% yeast extract, 0.5% peptone, and 0.4% $CaCO_3$ in a 500-ml Sakaguchi flask and incubated at 27°C for 48 hours. The seed culture thus obtained (200 ml) was transferred to a production medium (20 liters) in a 30-liter jar fermentor and the fermentation was carried out at 27°C for 48 hours with 10 liters of air per minute and agitation of 250 rpm. The composition of production medium was 0.5% glucose, 1.0% corn steep liquor, 1.0% oatmeal, 1.0% Pharmamedia, 0.5% K_2HPO_4, 0.5% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.001% $MnCl_2 \cdot 4H_2O$, 0.001% $ZnSO_4 \cdot 7H_2O$, 0.001%

Fig. 1. Time course of antibiotic OM-704 A production in a 30-liter jar fermentor.



$CuSO_4 \cdot 5H_2O$, and 0.001% $CoCl_2 \cdot 2H_2O$ (pH 7.0 before sterilization). A typical time course of the production of antibiotic OM-704 A by the strain OM-704 is shown in Fig. 1. The antibiotic activity was monitored by paper disk assay using *Streptococcus faecium* IFO 3181 as test organism.

A 48-hour culture (20 liters) was clarified with a Sharples centrifuge to give a supernatant (16 liters). The antibiotic in the supernatant was extracted with ethyl acetate (12 liters). After evaporation of the extract, the residue (510 mg, a reddish orange powder) was chromatographed on silica gel column (Merck, Kieselgel 60, 20 g) using a eluent of chloroform - methanol (20: 1, v/v). The active fractions were combined and then concentrated *in vacuo* to afford a red powder (120 mg). Red needles of antibiotic OM-704 A (96 mg) were obtained by crystallization from chloroform - methanol (9: 1, v/v).

Physicochemical Properties

Antibiotic OM-704 A is a red crystalline substance, mp 291~295°C. The molecular formula was deduced to be $C_{20}H_{22}N_2O_4$ from its elemental analysis (*Anal.* Found: C 68.19, H 6.24, N 7.93%; Calcd. for $C_{20}H_{22}N_2O_4$: C 67.78, H 6.26, N 7.91%) and high resolution mass spectrum [M^+, m/z 354.157; Calcd. for $C_{20}H_{22}N_2O_4$, m/z 354.157]. As shown in Fig. 2, the UV spectrum in methanol shows characteristic absorption maxima, at 250 nm (ε, 11,800, sh), 260 (13,600, sh), 278 (20,100, sh), 286 (21,700), 309 (9,760), 321 (8,950), 367 (4,130) and 490 (1,150). The IR spectrum (in KBr) exhibited characteristic bands based on methyl or methylene at 2960 cm^{-1} and quinone carbonyl group at 1670 cm^{-1} and 1625 cm^{-1}, as shown in Fig. 3. The ^1H NMR spectrum in $CDCl_3$ - CD_3OD indicates the presence of two methyl groups [δ 1.10 (t) and 2.27 (s)] and two methylenes [δ 1.57 (m) and 3.03 (t)], suggesting the presence of a symmetrical skeleton for the structure of the antibiotic OM-704 A. It is very slightly soluble in chloroform, ethyl acetate, acetone, dioxane, dimethylsulfoxide, ethanol and methanol, and practically insoluble in benzene, ethyl ether, *n*-hexane and water.

Biological Properties

The antibacterial spectrum of antibiotic OM-704 A was determined by conventional agar dilution method using heart infusion agar (Difco). As shown in Table 4, at concentrations of 3.13~50 μg/ml, antibiotic OM-704 A inhibited the growth of Gram-positive bacteria, including *Staphylococcus aureus* FDA 209P, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* FS 1277 (penicillin resistant), *Staphylococcus aureus* KB 199 (erythromycin resistant), and *Streptococcus faecium* IFO 3181, *Micrococcus luteus* ATCC 9341, but was inactive against Gram-negative bacteria and fungi. The acute toxicity

Fig. 2. UV spectrum of antibiotic OM-704 A (in MeOH).

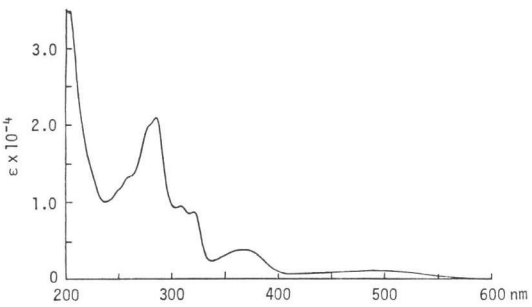


Fig. 3. IR spectrum of antibiotic OM-704 A (KBr).

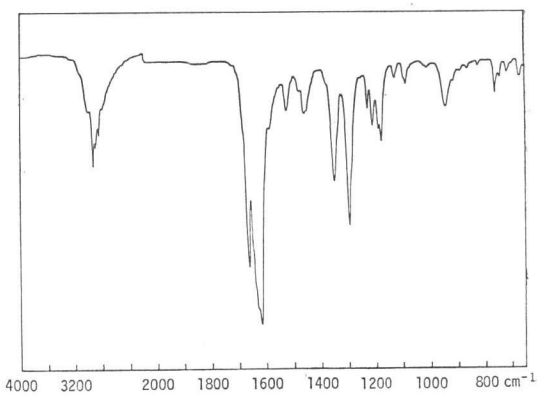


Table 4. Antibacterial spectrum of antibiotic OM-704 A.

Test organism	MIC (μg/ml)*	Test organism	MIC (μg/ml)*
Staphylococcus aureus FDA 209P	6.25	*Bacillus subtilis* ATCC 6633	>100
Staphylococcus aureus ATCC 6538P	6.25	*Bacillus cereus* IFO 3001	>100
Staphylococcus aureus KB 199 (erythromycin resistant)	6.25	*Mycobacterium smegmatis* ATCC 607	100
Staphylococcus aureus FS 1277 (penicillin resistant)	50	*Escherichia coli* NIHJ JC-2	>100
Streptococcus faecium IFO 3181	6.25	*Klebsiella pneumoniae* ATCC 10031	>100
Streptococcus pyogenes C 203	100	*Proteus vulgaris* IFO 3167	>100
Micrococcus luteus ATCC 9341	3.13	*Serratia marcescens* ATCC 8100	>100
		Pseudomonas aeruginosa IFO 3080	>100

* Minimal inhibitory concentrations were assayed by agar dilution method using heart infusion agar (pH 7.0, 37°C, 20 hours).

(LD_{50}) of antibiotic OM-704 A in mice was 100 mg/kg intraperitoneally.

Discussion

From the above results, it was found that antibiotic OM-704 A is a neutral quinoid compound active against Gram-positive bacteria including an erythromycin-resistant strain of *Staphylococcus*. The antibiotic has characteristic UV-visible absorptions (286, 309, 321, 367 and 490 nm). None of the known antibiotics has these absorptions. Consequently, the antibiotic is concluded to be a new compound. The structure elucidation is now in progress.

Acknowledgement

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