OM-173, NEW NANAOMYCIN-TYPE ANTIBIOTICS PRODUCED BY A STRAIN OF STREPTOMYCES

TAXONOMY, PRODUCTION, ISOLATION AND BIOLOGICAL PROPERTIES

YUZURU IWAI, KEIKO KIMURA, YŌKO TAKAHASHI, KIYOIZUMI HINOTOZAWA, HIDEKI SHIMIZU, HARUO TANAKA and SATOSHI ŌMURA

The Kitasato Institute and School of Pharmaceutical Science, Kitasato University, Minato-ku, Tokyo 108, Japan

(Received for publication June 23, 1983)

Actinomycete strain OM-173, a new soil isolate, was found to produce five nanaomycin-type antibiotics. Antibiotic OM-173 components αA, αE, αB, βA and βE were isolated from the fermentation broth of strain OM-173 by solvent extraction, silica gel chromatography and preparative thin-layer chromatography. The components are active against mycoplasmas and to a lesser extent against fungi. Strain OM-173 was identified as a strain of genus *Streptomyces* and differed apparently from *Streptomyces rosa* var. *notoensis*, the nanaomycin-producing strain, in cultural characteristics.

In previous screening for new antimycoplasmal antibiotics of actinomycetes origin, we discovered the nanaomycins[1~6], frenolicin B[7], 2′-amino-2′-deoxyadenosine[8] and cervinomycins[9]. In the subsequent screening program, *Streptomyces* sp. OM-173, a soil isolate, was found to produce five quinone antibiotics, OM-173 αA, αE, αB, βA and βE. It was later shown that antibiotics OM-173 αE, αB, βA and βE are new antibiotics structurally related to the nanaomycins[1~6] which possess a benzoisochromane-quinone skeleton and have been designated nanaomycins αE, αB, βA and βE, while antibiotic OM-173 αA is identified as nanaomycin A methyl ester[3,4] which had been chemically prepared from nanaomycin A produced by *Streptomyces rosa* var. *notoensis*.

The present paper deals with the taxonomy of strain OM-173, and the production, isolation, and biological properties of antibiotics OM-173.

Taxonomy of the Producing Organism

Antibiotic OM-173 producing strain was isolated from a soil sample collected in Yonago City, Tottori Prefecture, Japan.

Morphology

The vegetative mycelia grow abundantly on both synthetic and complex media, and septa were not observed. The velvety aerial mycelia grow abundantly on yeast extract - malt extract agar, oatmeal agar, inorganic salts - starch agar, glycerol - asparagine agar and glucose - nitrate agar.

The spore chains are of the *Spira* and have more than ten spores per chain. The spores are cylindrical in shape, 1.0×1.3 μm in size, and have a smooth surface (Plate 1). Sclerotic granules, sporangia and flagellated spores were not observed.

Cultural and Physiological Characteristics

The International Streptomyces Project (ISP) media recommended by SHIRLING and GOTTLIEB[10]

Table 1. Cultural characteristics of strain OM-173.

Yeast extract - malt extract agar*	G:	Good, dark brown (5pn)
	R:	Dark brown (3pn)
	AM:	Abundant, velvety, olive gray (lig)
	SP:	Mustard brown (2ni)
Oatmeal agar*	G:	Good, golden olive (1½lg)
	R:	Golden olive (1½lg)
	AM:	Abundant, velvety, olive gray (lig)
	SP:	Golden olive (1½lg)
Inorganic salts - starch agar*	G:	Good, clove brown (3ni)
	R:	Dark brown (3pn)
	AM:	Abundant, velvety, olive gray (lig)
	SP:	Rose beige (4ge)
Glycerol - asparagine agar*	G:	Good, mustard brown (2ni)
	R:	Mustard brown (2ni)
	AM:	Abundant, velvety, olive gray (lig)
	SP:	Light mustard tan (2ie)
Glucose - asparagine agar	G:	Moderate, clove brown (3ni)
	R:	Mustard tan (2lg)
	AM:	Moderate, velvety, olive gray (lig)
	SP:	Dusty yellow (1½gc)
Peptone - yeast extract - iron agar*	G:	Moderate, light ivory (2ca)
	R:	Light ivory (2ca)
	AM:	None
	SP:	None
Tyrosine agar*	G:	Poor, light spice brown (4lg)
	R:	Chestnut brown (4ni)
	AM:	Poor, velvety, white (a) and covert tan (2ge)
	SP:	Light spice brown (41g)
Sucrose - nitrate agar*	G:	Thin, colorless
	R:	Covert tan (2ge)
	AM:	Moderate, velvety, slate tan (2ig)
	SP:	Covert tan (2ge)
Glucose - nitrate agar**	G:	Good, light brown (3lg)
	R:	Light brown (4ng) and light mustard tan (2ie)
	AM:	Abundant, velvety, white (a) and light olive gray (1½ge)
	SP:	Bamboo (2gc)
Glycerol - calcium malate agar**	G:	Good, colorless
	R:	Cream (1½ca)
	AM:	Abundant, velvety, white (a) and light olive gray (1½ge)
	SP:	Covert tan (2ge)
Glucose - peptone agar**	G:	Good, light ivory (2ca)
	R:	Gold (2lc)
	AM:	Moderate, velvety, white (a)
	SP:	Bamboo (2gc)
Nutrient agar**	G:	Moderate, cream (1½ca)
	R:	Cream (1½ca)
	AM:	None
	SP:	None

* Medium recommended by International Streptomyces Project.
** Medium recommended by WAKSMAN.
Abbreviation: G, growth of vegetative mycelium; R, reverse; AM, aerial mycelium; SP, soluble pigment.

Plate 1. Scanning electron micrograph of aerial mycelia of *Streptomyces* sp. OM-173.
Bar represents 1 μm.

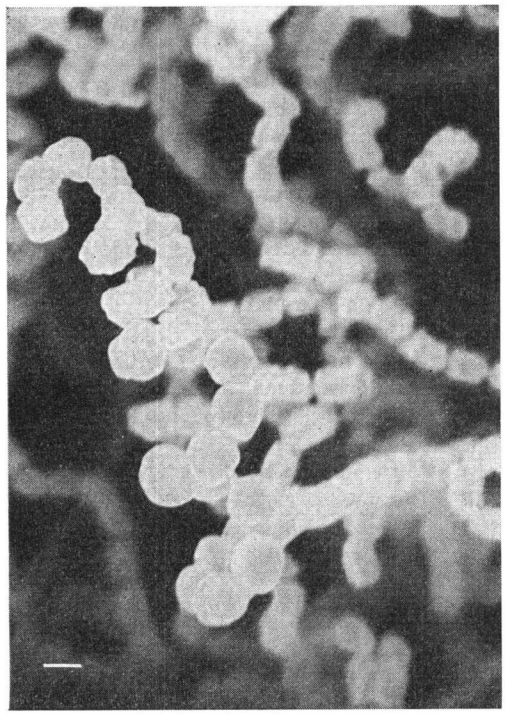


Table 2. Physiological properties of strain OM-173.

Melanin formation	Positive*
Tyrosinase reaction	Positive
H_2S production	Negative
Liquefaction of gelatin (21~23°C)	Positive
Peptonization of milk (37°C)	Positive
Coagulation of milk (37°C)	Negative
Hydrolysis of starch	Positive
Cellulolytic activity	Weakly positive
Temperature range for growth	15~45°C

* Positive on tyrosine agar, but negative on peptone - yeast extract - iron agar, glucose - peptone gelatin and Tryptone yeast.

Table 3. Utilization of carbon sources by strain OM-173.

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

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

and those recommended by WAKSMAN[11] were used for these experiments. Cultures were observed after incubation at 27°C for two weeks and compared with Color Harmony Manual (4th edition) published by the Container Corporation of America. The utilization of carbon sources was tested by growth on PRIDHAM and GOTTLIEB's medium containing 1% carbon source each. The cultural and physiological characteristics and the utilization of carbon sources of strain OM-173 are shown in Tables 1, 2 and 3, respectively.

Chemical Compositions

The chemical analyses of sugars in whole cells and diaminopimelic acid (A_2pm) in cell walls were carried out by the method of LECHEVALIER & LECHEVALIER[12]. Strain OM-173 showed no characteristic sugar pattern. LL-A_2pm was detected in the cell walls.

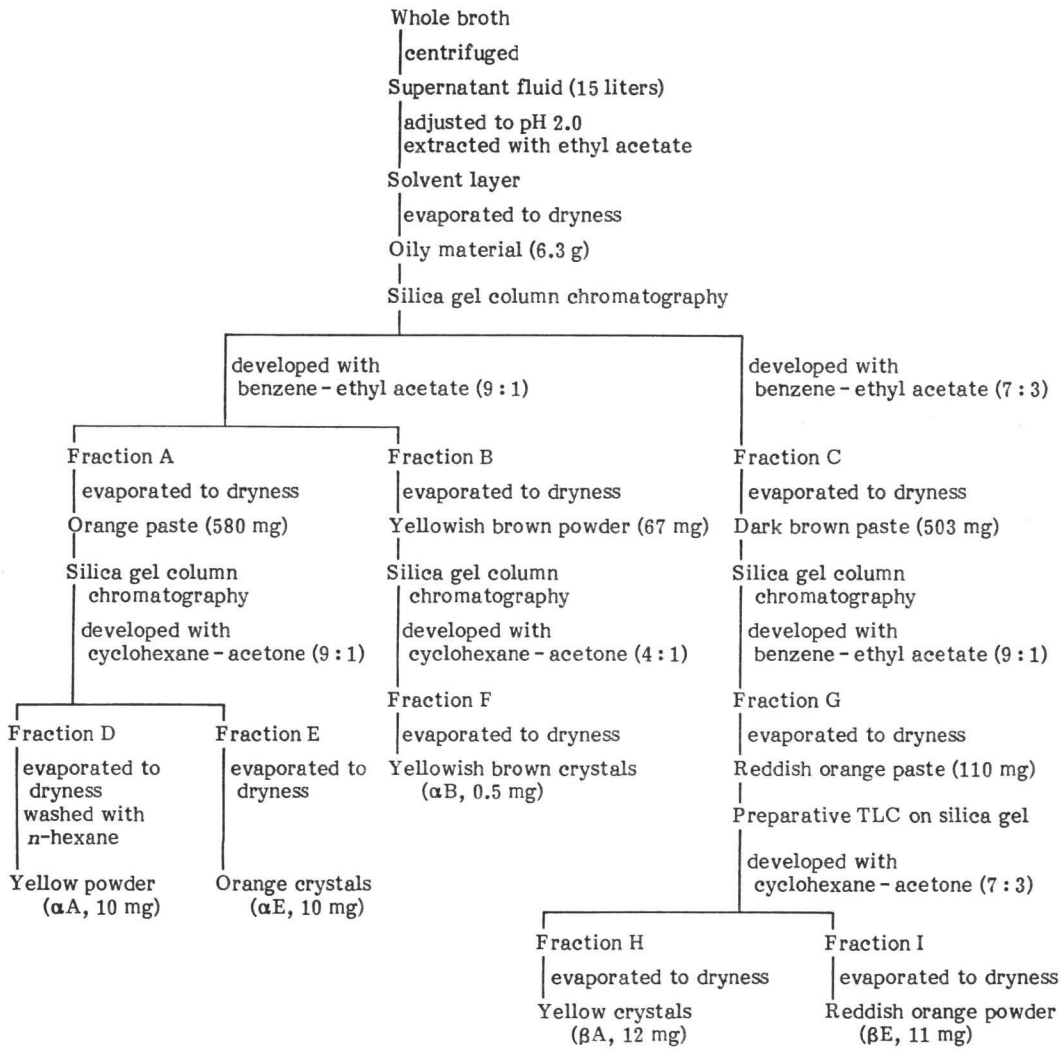
Strain OM-173 exhibits the following properties: sporophore, *Spira*; spore, cylindrical and smooth surface; aerial mass color, olive-gray; vegetative mass color, brown or olive; melanoid pigment, positive on tyrosine agar; soluble pigment, olive or brown; A_2pm in cell wall, LL-isomer.

Based on the taxonomic properties described above, strain OM-173 is considered to belong to genus *Streptmyces* and to be classified as the green series of the PRIDHAM and TRESNER grouping[13]. The strain has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan under the name *Streptomyces* sp. OM-173 and the accession No. FERM-P 6509.

Production and Isolation

The stock culture of strain OM-173 on inorganic salts - starch agar was inoculated into 100 ml of

Fig. 1. Isolation procedure of antibiotic OM-173 components.



a seed medium consisting of 1.0% glucose, 1.0% starch, 0.5% yeast extract, 0.5% peptone, 0.4% $CaCO_3$ in a 500-ml Sakaguchi flask and incubated at 27°C for 48 hours on a reciprocal shaker. Two hundred milliliters of the seed culture were transferred to 20 liters of a production medium in a 30-liter jar fermentor and the fermentation was carried out at 27°C for 68 hours with aeration of 10 liters per minute and agitation of 250 rpm. The production medium consisted of 2.0% glycerol, 2.0% starch (Hokuren), 0.2% corn steep liquor (Nihon Shokuhin Kako Co. Ltd.), 1.0% soybean meal (Ogawa Sangyo Co. Ltd.), 0.5% meat extract (Kyokutō Seiyaku Kogyo Co. Ltd.), 0.3% yeast extract (Oriental Kōbo Kogyo Co. Ltd.), 0.3% $CaCO_3$, 1.0% $Mg_3PO_4 \cdot 8H_2O$ (presterile pH 7.0). The antibiotic production started at 30~40 hours after inoculation and reached a maximum at 68 hours.

A flow diagram for the isolation procedure of antibiotic OM-173 compounds is given in Fig. 1. Antibiotics OM-173 αA, αE, αB, βA and βE were isolated from fractions (A~I) by a combination of purification procedures: solvent extraction, silica gel chromatography, preparative thin-layer chro-

matography and crystallization.

The antibiotic activity was assayed by the paper-disc method against *Acholeplasma laidlawii* PG-8 on agar plates. The antibiotics were also monitored by thin-layer chromatography (TLC) on silica gel: the Rf values are presented in Table 4.

Table 4. Rf values of antibiotic OM-173 components on silica gel TLC.

	Solvent system		
	I	II	III
OM-173 αA	0.69	0.18	0.44
αE	0.65	0.12	0.40
αB	0.49	0	0.28
βA	0.27	0	0.26
βE	0.25	0	0.24

Solvent systems:
I. benezne - ethyl acetate (7: 3)
II. cyclohexane - acetone (8: 1)
III. cyclohexane - acetone (7: 3)
Silica gel: Merck, Kieselgel 60 F_{254}, 0.2 mm.
Detection: UV lamp at 3650 Å.

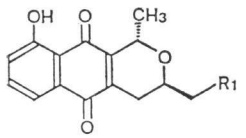
Biological Properties

It was found that antibiotic OM-173 components are analogues of nanaomycins A, E and B, which inhibit mainly fungi and mycoplasmas. The antimicrobial activity of antibiotic OM-173 components was determined by the conventional agar dilution method using Difco PPLO agar for mycoplasmas and glucose - potato agar for fungi. Table 5 shows the comparison of antibiotic OM-173 components with nanaomycins A, E and B as regards antimicrobial activity. Anti-

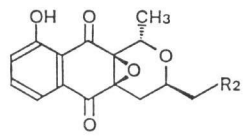
Table 5. Comparison of antibiotic OM-173 compounds with nanaomycin (NNM) A, E and B as regards antimicrobial activity.

Test organism	Minimum inhibitory concentration (μg/ml)							
	αA	βA	NNM-A	αE	βE	NNM-E	αB	NNM-B*
Candida albicans	100	50	3.12	>100	50	>100	50	31.2
Saccharomyces sake	25	12.5	3.12	100	>50	>100	50	62.5
Piricularia oryzae	3.12	3.12	0.4	3.12	25	100	6.25	15.6
Aspergillus niger	>100	>100	12.5	100	>50	>100	>50	62.5
Trichophyton interdigitale	12.5	6.25	0.4	6.25	25	100	12.5	12.5
T. mentagrophytes	25	12.5	1.56	25	50	>100	25	25
T. rubrum	12.5	12.5	1.56	25	50	>100	6.25	3.1
T. violaceum	12.5	12.5	0.4	12.5	50	100	12.5	3.1
T. asteroides	25	12.5	1.56	50	50	>100	25	12.5
Mycoplasma gallisepticum KP-13	3.12	6.25	0.10	12.5	25	3.12	1.56	0.1
M. gallisepticum S-6	0.78	3.12	<0.01	12.5	25	6.25	0.39	0.1
M. gallisepticum 333P**	0.39	1.56	<0.01	12.5	12.5	3.12	0.39	0.05
M. pneumoniae	1.56	3.12	0.05	12.5	25	6.25	0.78	3.12
Acholeplasma laidlawii (A) PG-8	1.56	1.56	0.20	6.25	12.5	3.12	0.78	
A. laidlawii (B) Bm-1	1.56	1.56	0.20	6.25	12.5	6.25	0.78	

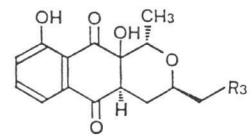
* Data from reference 2.
** Spiramycin resistance.



R_1=$COOCH_3$ OM-173 αA
R_1=CH_2OH OM-173 βA
R_1=COOH NNM-A



R_2=$COOCH_3$ OM-173 αE
R_2=CH_2OH OM-173 βE
R_2=COOH NNM-E



R_3=$COOCH_3$ OM-173 αB
R_3=COOH NNM-B

biotic OM-173 components are active against mycoplasmas and to a lesser extent against fungi. However, the antimicrobial activities of antibiotic OM-173 compounds are weaker than that of nanaomycin A.

Discussion

ŌMURA *et al.* discovered previously the quinone antibiotics, nanaomycins A, B, C, D and E, isolated from the culture broth of *Streptomyces rosa* var. *notoensis*[1~6], and elucidated the structures[3,5,6], biosynthesis[8,14] and mode of action of these antibiotics[15,16]. In the subsequent screening for new antimycoplasmic antibiotics, strain OM-173, a new soil isolate, was found to produce five nanaomycin-type antibiotics. Strain OM-173, which was identified as a strain of genus *Streptomyces*, was different from *S. rosa* var. *notoensis*, the nanaomycin-producing strain: the former belongs to the green series, while the latter belongs to the red series of the PRIDHAM and TRESNER grouping[13].

The structures and antimicrobial activity of antibiotic OM-173 components are shown in Table 5. All components are nanaomycin-type antibiotics. Components αE, αB, βA and βE are new and named nanaomycins αE, αB, βA and βE, while component αA was identified as nanaomycin methyl ester which had been chemically prepared by TANAKA *et al.*[4] Components αA and βA are analogues of nanaomycin A, and have weaker activity against both mycoplasmas and fungi than nanaomycin A. The antimicrobial activity of nanaomycin C, an amide of nanaomycin A, is known to be less than that of nanaomycin A[4]. These results indicate that modification of the carboxy group of nanaomycin A may lead to low antimicrobial activity.

The biosynthetic relationship of the nanaomycin components was studied by a bioconversion method using cerulenin, a specific inhibitor of fatty acid and polyketide biosynthesis. As the biosynthetic sequence for nanaomycins has been proposed to be: D→A→E→B[14], the sequence for the antibiotic OM-173 compounds is speculated to be: αA (βA)→αE (βE)→αB.

A paper describing structure elucidation is now in preparation.

Acknowledgment

The authors wish to thank Asahi Chemical Industry Co., Ltd., for production of antibiotic OM-173 and Mr. K. OKADA and Miss R. SATŌ of Kitasato University for their assistance.

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