PLATOMYCINS A AND B

I TAXONOMY OF THE PRODUCING STRAIN AND PRODUCTION, ISOLATION AND BIOLOGICAL PROPERTIES OF PLATOMYCINS

SEIGO TAKASAWA, ISAO KAWAMOTO, ITARU TAKAHASHI*, MASAHIRO KOHAKURA*, RYO OKACHI, SEIJI SATO, MITSUYOSHI YAMAMOTO, TOMOYASU SATO and TAKASHI NARA

Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.
3-6-6, Asahimachi, Machidashi, Tokyo, Japan
*Fuji Factory, Kyowa Hakko Kogyo Co., Ltd.
Suntogun, Shizuokaken, Japan

(Received for publication May 6, 1975)

Two new antibiotics, platomycins A and B, belonging to the phleomycin-bleomycin family, were isolated from the culture filtrate of *Streptosporangium* sp. MK-78. This strain has been identified as a new variety of *Streptosporangium violaceochromogenes*. Both platomycins A and B are active against a variety of Gram-positive and Gram-negative bacteria, and also inhibit solid Sarcoma 180 and EHRLICH ascites carcinoma.

From a field soil from Ito-shi, Shizuokaken, Japan, the authors isolated a strain (MK-78) capable of producing two new phleomycin-bleomycin-like antibiotics, platomycins A and B. From taxonomical studies, this strain MK-78 is a *Streptosporangium* species and resembles most closely *Streptosporangium violaceochromogenes* nov., sp. KAWAMOTO *et* NARA which was recently reported to produce victomycin, a phleomycin-bleomycin-like antibiotic.[1,2]

This report deals with the taxonomy of *Streptosporangium* sp. MK-78, and the production, purification and biological activities of the platomycins. The physicochemical properties of these antibiotics will be reported in a subsequent paper.[3]

Characteristics of Strain MK-78

The strain (MK-78) has been deposited at American Type Culture Collection as ATCC 21893. Most of the taxonomical studies of this culture were carried out in accordance with the method adapted by the International Streptomyces Project (ISP).[4] Additional media recommended by WAKSMAN[5] were also used.

Morphological Characteristics

Strain MK-78 is very close morphologically to *Streptosporangium violaceochromogenes*.[1] Both aerial and substrate mycelia developed well, branched and seprated, and mycelia were 0.4~0.8μ in width. The spherical sporangia with irregular surfaces were produced on the sporangiophores, 5~10μ in diameter, which were borne only on aerial mycelium. Sporangiospores which arranged in coil form in the sporangium were 0.8~0.9μ by 1.2~1.6μ, oval or cylindrical in shape and non-motile. Flagella were not observed on the surface of spores.

Cultural Characteristics on Various Media

As seen in Table 1, the growth of this strain was generally good on natural nutrient media. The color of the substrate mycelium was gold and the aerial mycelium was white to pinkish

white. The deep reddish-violet pigment was produced in natural nutrient media. The growth of this strain was poor on chemically-defined media such as CZAPEK's agar, glucose-asparagine agar, starch agar and glycerol-asparagine agar, and was not stimulated by the addition of vitamins; biotin, thiamine, pyridoxal, riboflavin, vitamin B_{12}, niacin and *p*-aminobenzoic acid.

Table 1. Cultural characteristics on various media

Medium	Growth	Substrate mycelium	Formation of aerial mycelium	Aerial mycelium	Soluble pigment
CZAPEK's agar	poor	colorless	poor, powderry	white (a)	none
Glucose-asparagine agar	poor	colorless	moderate, powdery	white (a)	none
Nutrient agar	good	yellow maple (3ng)	moderate	white (a) sand (3cb)	cherry wine (7pe)
Egg albumin agar	poor	colorless	none		none
Starch agar	poor	colorless	poor	white (a)	none
Yeast ext.-malt ext. agar	moderate	orange rust (4pc)	moderate	white (a)	none
Oat meal agar	poor	peach pink (5ea)	poor or moderate	white flesh pink (6ca)	none
Glycerol-asparagine agar	poor or moderate	pearl shell tint (2ba)	poor	white (a)	none
BENNETT's agar	good	gold (21c)	good, powdery	white (a)	none
EMERSON's agar	good, granular	amber (3pc)	moderate	white (a) flesh pink (4ca)	none
Glucose-yeast ext. agar	good	mustard gold (2ne)	good, powderly	white (a)	none
HICKEY-TRESNER's agar	moderate	Lt. yellow (1 1/2 ea)	moderate, powderly	white (a) pearl shell tint (3ba)	none
Tyrosine agar	good	amber (3pc)	good	white (a)	amber (3pe)

Streptosporangium MK-78 was cultured at 27°C for 2 weeks. The indications in the parentheses are in accordance with the color classification of Color Harmony Manual (Container Corporation of America).

Physiological Properties

The temperature and pH range for growth were 25~40°C, with optimal growth at 30~37°C and pH 6.0~8.5, with optimal growth at around pH 7.3, respectively, after cultivation for 5 days in a liquid medium consisting of 2% glucose, 0.5% peptone, 0.5% yeast extract and 0.1% $CaCO_3$. This strain utilized D-glucose well, utilized D-galactose, D-levulose, D-mannitol, D-sucrose, soluble starch and xylose, and did not utilize D-arabinose, glycerol, D-lactose, D-inositol, D-raffinose and L-rhamnose as carbon sources. Some other physiological properties were as follows: the liquefaction of gelatin and the peptonization of milk were not observed at 27°C for a month, the hydrolysis of starch was positive, the reduction of nitrate and the formation of tyrosinase were slightly positive, and the decomposition of cellulose and chromogenic action were negative. Amino acids and sugars from cell wall hydrolysate were determined according to the methods of BOONE *et al*,[6] BECKER *et al*[7] and LECHEVALIER *et al*[8] and closely resemble *Streptosporangium violaceochromogenes*.[1] From the results of the morphological characteristics and the cell wall components, strain MK-78 very closely resembled *S. violaceochromogenes* which

can also produce the phleomycin-bleomycin-like antibiotic, victomycin. However, there were some differences between these strains (Table 2) in the state of colonies on an agar medium, the production of soluble pigments, the utilization of glycerol and D-mannitol and some physiological properties. Both strains produced the phleomycin-bleomycin-like antibiotics but these antibiotics are also different.[8] From these results, strain MK-78 was regarded as a subspecies

Table 2. Comparison between MK-78 and MK-49 strains

	MK-78 ATCC 21893	MK-49 ATCC 21807
State of colonies well grown on an agar medium	Forming granular colonies. Irregular in size.	Forming a flat surface colonies. Uniformal in size.
Sporangia formation	Poor	Good
Production of soluble pigments on nutrient agar medium	Poor Cherry wine (7 pe)	Good Raspberry (9 nc)
Utilization of carbon sources:		
Glycerol	Negative	Positive
D-Mannitol	Positive	Negative
Liquefaction of gelatin	Negative	Slightly positive
Action upon milk	Negative	Slightly positive
Tyrosinase	Slightly positive	Negative
Antibiotics produced	Platomycin	Victomycin

of *S. violaceochromogenes* and named *S. violaceochromogenes* subsp. *globophilum* due to its tendency to form granular colonies on nutrient agar medium.

Production of the Platomycins

S. violaceochromogenes subsp. *globophilum* was inoculated into 30 ml of seed medium containing dextrin 1%, glucose 1%, Polypeptone 0.5%, yease extract 0.5% and $CaCO_3$ 0.1%, pH 7.2 (before sterilization) in a 250-ml Erlenmeyer flask and fermented at 30°C for 5 days on a rotary shaker. The volume of the seed medium was increased from 30 ml to 300 ml, from 300 ml to 15 liters, 15 liters to 150 liters and finally from 150 liters to 1,000 liters of the fermentation medium in a 2,000-liter stainless tank. The fermentation medium consisted of glucose 2%, corn steep liquor 3% and $CaCO_3$ 0.1%, pH 7.2 (before sterilization) and fermented at 30°C for 7 to 10 days with 300 liters/min. aeration and 150 r.p.m. agitation. A typical fermentation time course is shown in Fig. 1. The antibiotics' production in culture liquid began at 2 days, and reached a maximum at about 7 days. Cell growth was not good in this medium since maximal cell growth was only 7.8% as packed cell volume.

Isolation and Purification of the Platomycins

The fermentation broth was adjusted to pH 4.0 with HCl, filtered with Radiolite No. 600 (Showa Kagaku Kogyo Co., Ltd.) and adjusted to pH 6.8. The filtrate (900 liters) was passed through a 50-liter column of Amberlite IRC-50 (H^+). The column was washed with 100 liters of water and the antibiotics were eluted with 30 liters of 0.5 N HCl. The eluate was adjusted

to pH 7.0 with Amberlite IR-4B (OH^-), and passed through a 500-ml column of IRC-50 (NH_4^+). After washing with one liter of 0.3 N NH_4OH and 2 liters of water, the platomycins were eluted with 0.5 N HCl. The active fractions were combined, diluted with 5 volumes of water, adjusted to pH 6.8 and passed through a 200-ml column of IRC-50 (H^+) for desalting. After washing with 500 ml of water, the platomycins were eluted with 0.5N HCl, neutralized with Dowex 44 (OH^-) and concentrated to dryness *in vacuo*. The crude preparation was dissolved in 50% methanol, passed through a 500-ml column of Sephadex LH-20 and eluted with 50% methanol. The active fractions were combined, concentrated *in vacuo* and precipitated with 10 volumes of acetone to obtain 2 g of a dark green powder. One gram of this powder was dissolved in 50 ml of 0.05 M aqueous ammonium formate solution and passed through a 50-ml column of CM-Sephadex

Fig. 1. Time course in platomycin fermentation
The antibiotics titer was assayed by the cylinder plate method using *Bacillus subtilis* KY 4273 as a test organism and is shown as a relative activity against the 7-day culture beer (100). The cell growth was measured as the packed cell volume after centrifugation at 3,000 rpm for 15 minutes.

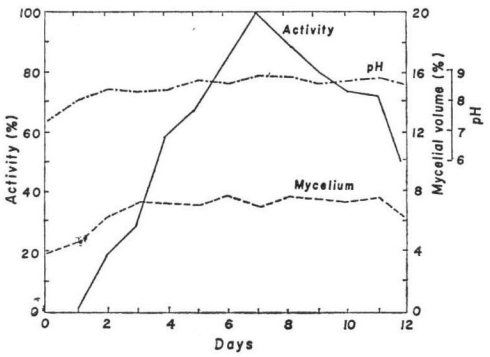

Table 3. Antibacterial spectrum of the platomycins

Organism	MIC* (μg/ml)	
	Platomycin A	Platomycin B
Streptococcus faecalis ATCC 10541	>0.83	>0.83
Staphylococcus aureus ATCC 6538p	0.014	<0.001
S. aureus KY 8942 (R-SM, KM, PM)	0.11	0.013
S. aureus KY 8950 (R-SM, TC, PC, SA)	0.11	0.013
S. aureus KY 8953 (R-SM, KM, NM, TC, EM)	>0.83	>0.42
S. aureus KY 8956 (R-SM, KM, PM, TC, EM)	>0.83	>0.42
S. aureus KY 8957 (R-SM, KM, PM, TC, CP)	>0.83	>0.42
Bacillus subtilis KY 4273	0.007	<0.001
Bacillus cereus ATCC 9634	0.027	0.026
B. cereus var. *mycoides* ATCC 9463	<0.001	<0.001
Escherichia coli ATCC 26	0.007	0.004
E. coli KY 8310 (R-SM, KM, TC, GM, CP)	0.053	0.027
E. coli KY 8302 (R-SM, KM, TC, CP)	0.11	0.027
E. coli KY 8314 (R-SM)	0.053	0.027
E. coli KY 8315 (R-SM, KM, PM, NM)	0.014	0.027
Proteus vulgaris ATCC 6897	>0.83	0.21
Pseudomonas aeruginosa BMH No. 1	>0.83	>0.83
Shigella sonnei ATCC 9290	0.053	0.014
Salmonella typhosa ATCC 9992	0.053	0.027
Klebsiella pneumoniae ATCC 10031	0.027	0.014

* Assayed with agar dilution mothod at pH 8.0. R: Resistant, SM: Streptomycin, KM: Kanamycin, PM: Paromomycin, NM: Neomycin, GM: Gentamicin, TC: Tetracycline, CP: Chloramphenicol, SA: Sulfonamide.

C-25. The column was washed with 500 ml of 0.1 M aqueous ammonium formate and eluted gradiently with 0.1~1.0 M aqueous ammonium formate of which the total volume was one liter. The CM-Sephadex column chromatography separated 2 compounds designated platomycin A and platomycin B, which were eluted at about 0.3 M and about 0.4 M aqueous ammonium formate, respectively. Both active fractions could be further purified in the same manner. Each active fraction was diluted with 5 volumes of water and passed through a 50-ml column of Amberlite CG-50 (H^+). After washing with 200 ml of water, the antibiotic was eluted with 0.5 N HCl, adjusted to pH 6.0 with Dowex 44 (OH^-) and concentrated to dryness *in vacuo*. Each preparation was dissolved in a small amount of 50% aqueous methanol and applied to the top of a Sephadex LH-20 column. The column was eluted with 50% aqueous methanol. The active fractions were adjusted to pH 5.0 with HCl, concentrated and precipitated with 10 volumes of acetone. Sixty mg of platomycin A and 20 mg of platomycin B were obtained.

Table 4. Activity against Sarcoma 180 (solid)

	Dosage (ip) (mg/kg)×days	Average tumor weight (g)
Platomycin A	3×8	0.07
	2×8	0.20±0.14
Platomycin B	2×8	0.19±0.17
	1×8	0.38±0.17
None		2.08±0.62

At 24 hours after the subcutaneous trasplantation of about 2 mm^3 fragments of tumors solid into the axillary region of mice (*dd* strain, male, 19±1 g of body weight), a solution of platomycin was administrated intraperitoneally once daily for 8 days.

Tumor solids were taken out and weighed at 10 days after transplantation.

Biological Properties of the Platomycins

As seen in Table 3, both platomycins A and B have potent antibacterial activity against Gram-positive and Gram-negative bacteria including many bacteria resistant to some antibiotics. Furthermore, platomycins A and B, like victomycin,[2] have potent antitumor activity against solid Sarcoma 180 and EHRLICH ascites carcinoma as shown in Tables 4 and 5, respectively.

Table 5. Activity against EHRLICH ascites carcinoma

	Dosage (ip) (mg/kg)×days	Average survival days	Survival for 60 days
Platomycin A	2×6	>28.1	1/10
	1×6	>30.1	1/10
Platomycin B	1×6	>31.8	2/10
	0.5×6	>30.4	1/10
None		15.6	0/10

The daily intraperitoneal injection of platomycin for 6 days to mice (*dd* strain, male, bodyweight; 22±1 g) was started 24 hours after the intraperitoneal transplantation 5×10^6 tumor cells.

However they were inactive against Leukemia L 1210.

The LD_{50} of platomycin A in mice was about 25 mg/kg, intravenously and 40 mg/kg, intraperitoneally. The LD_{50} of platomycin B was about 12.5 mg/kg, intravenously and 35 mg/kg, intraperitoneally.

Acknowledgements

The authors are grateful to Dr. A.C. SINCLAIR and his associates of Abbott Laboratories, North Chicago, Illinois, U.S.A., for their kind advice and encouragement. They are also thankful to Dr. H. NONOMURA, Faculty of Engineering, Yamanashi University, Kofu, for the gift of type cultures of *Streptosporangim* and his advice.

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