ISOLATION AND CHARACTERIZATION OF KATANOSINS A AND B

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Two peptide antibiotics katanosins A and B were isolated from the culture broth of a strain related to the genus *Cytophaga*. These antibiotics are basic peptides soluble in aqueous alcohols. The molecular formulae $C_{57}H_{95}N_{15}O_{17}$ for A and $C_{58}H_{97}N_{15}O_{17}$ for B were indicated. The constituent amino acids of katanosin A are suggested to be Thr (1), Ser (1), Val (1), Leu (3), Arg (1) and three unusual amino acids. In katanosin B, the Val residue is replaced by Ile. Katanosins A and B are active against Gram-positive bacteria *in vitro* and *in vivo*.

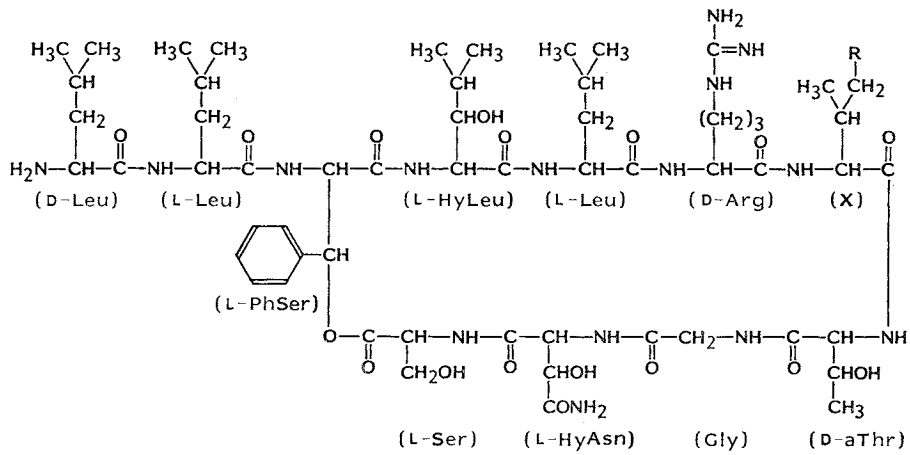
In the course of our screening program for new antibiotics from bacterial strains, a strain numbered PBJ-5356 related to the genus *Cytophaga* was found to produce an antibiotic principle, which proved to be a cell wall synthesis-inhibitor by inhibition of incorporation of radioisotopic diaminopimelic acid into the cell wall peptidoglycan of a *Bacillus* strain[1]. It was isolated as a complex of components A and B, which was then separated by HPLC. These are peptide antibiotics, whose structures (Fig. 1) will be discussed in an accompanying paper[2].

In this paper, the taxonomy of the producing strain, the production and isolation of the antibiotics as well as the physico-chemical and biological properties are presented.

Taxonomy

The producing organism designated PBJ-5356 was isolated from a soil sample collected in Katano-city, Osaka Prefecture, Japan.

Fig. 1. Structures of katanosins A and B.



A R=H (X=L-Val)
B R=CH_3 (X=L-Ile)

Table 1. Physiological characteristics of strain PBJ-5356.

Properties observed	Results	Properties observed	Results
Catalase test	+	Levan formation	+
Oxidase test	+	Indole production	−
OF-Test	Oxidative	H_2S production	−
Peptonization of milk	+	Voges-Proskauer test	−
Coagulation of milk	−	Methyl red test	−
Gelatin liquefaction	+	β-Galactosidase test	+
Esculin hydrolysis	+	Nitrate reduction	−
Chitin degradation	+	Denitrification	−
Starch degradation	−	Citrate utilization	+
Arginine dihydrolase	−	Phosphatase test	+
Lysine decarboxylase	−	Deoxyribonuclease test	+
Ornithine decarboxylase	−		

The organism is an aerobic, Gram-negative, non-sporulating rod (0.3~0.4 μm×1.2~1.7 μm) with tapered ends. It has no flagella and exhibits a spreading growth due to gliding motility. On nutrient agar, it forms circular, entire, slightly convex, wet and smooth colonies with yellowish cream color. Soluble pigments and fruiting bodies are not produced.

The organism showed good growth at 28°C. Other physiological characteristics are shown in Table 1. Neither acid nor gas was generated on utilization of D,L-arabinose, D-xylose, D-fructose, D-galactose, D-glucose, D-mannose, L-rhamnose, D-cellobiose, lactose, maltose, sucrose, D-trehalose and D-mannitol.

From the above characteristics in referring to the 8th Ed. of BERGEY's Manual of Determinative Bacteriology[8], the organism is ascribed to the order Cytophagales, and most related to the genus *Cytophaga*, though none of the species of the genus described in the manual is identical with the organism.

Production and Isolation

The cells of the strain PBJ-5356 were suspended in sterilized saline and inoculated into 100 ml of medium 1 or 2 in a 500-ml Sakaguchi flask. Cultivation was carried out at 27°C for 2 days in the usual shaking manner. The composition of medium 1 is: Yeast extract 0.3% and glucose 1.0%, pH 7.0 and medium 2 is: Yeast extract 0.3%, glucose 1.0% and L-valine 0.1%, pH 7.0.

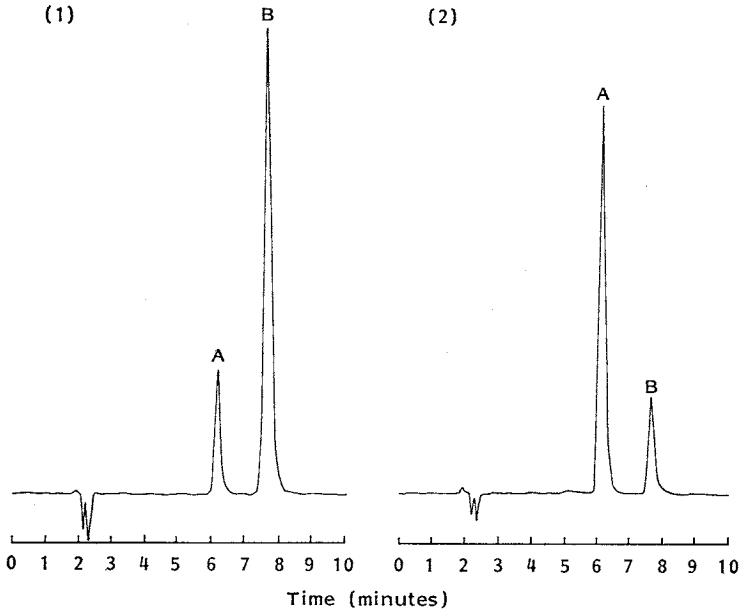
To the culture broth (10 liters) from medium 1, butanol (3 liters) and methanol (300 ml) were added and the mixture was vigorously stirred. The solvent layer separated by centrifugation was decolorlized with active charcoal (20 g) and then concentrated to an oily residue. The residue was washed with petroleum ether and then dissolved in a small amount of methanol. The solution was slightly acidified with 1 N HCl and allowed to stand to yield colorless crystals (280 mg). The crystals were shown to be a complex of katanosins A and B in the form of their hydrochlorides and the content ratio of A and B was estimated to be approximately 2:8 by HPLC experiments as illustrated later.

The culture broth (10 liters) from medium 2 was processed in the same manner as above, yielding a complex of katanosins A and B as crystals of their hydrochlorides (220 mg). The content ratio of A and B in the complex was approximately 7:3.

Some 200 mg of the complex of katanosins A and B derived from medium 1 was subjected to preparative HPLC (column: Nucleosil 30 C_{18}, 2.0×25.0 cm, mobile phase: 35% acetonitrile containing 0.1% trifluoroacetic acid). The fractions of A and B were respectively concentrated to dryness.

Fig. 2. HPLC of katanosins A and B.

(1) The complex from medium 1, (2) the complex from medium 2.



Column: Nucleosil 5 C_{18} (4.6×150 mm). Mobil phase: 35% acetonitrile containing 0.1% TFA. Monitored at 220 nm. Flow rate: 0.9 ml/minute. Chart speed: 1.0 cm/minute.

Table 2. Physico-chemical properties of katanosins A and B hydrochloride.

	A	B
MP (°C, dec)	240~245	240~250
Anal Found	C 48.97, H 7.54, N 15.01, Cl 5.24.	C 48.84, H 7.68, N 14.76, Cl 5.28.
Calculation	C 48.68, H 7.45, N 14.94, Cl 5.04.	C 49.00, H 7.59, N 14.78, Cl 4.99.
	$C_{57}H_{95}N_{15}O_{17}\cdot 2HCl\cdot 4H_2O$	$C_{58}H_{97}N_{15}O_{17}\cdot 2HCl\cdot 4H_2O$
SI-MS (*m/z*, MH^+)	1,262	1,276
$[\alpha]_D^{23}$	−86.0±4.1° (*c* 0.307, MeOH)	−82.2±24° (*c* 0.505, MeOH)
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	End absorption, 257 (1.6), 263 (1.5), 268 (1.0)	End absorption, 257 (1.7), 263 (1.5), 268 (1.0)

The residues were dissolved into methanol and passed through a Sephadex LH-20 column with methanol. Concentration of the eluates afforded crystals of katanosin A hydrochloride (15 mg) and katanosin B hydrochloride (110 mg). A similar procedure on some 200 mg of the complex from medium 2 afforded crystals of katanosin A hydrochloride (110 mg) and katanosin B hydrochloride (30 mg).

Physico-chemical Properties

Katanosins A and B are basic substances. Their hydrochlorides are obtained as colorless crystals. They were not separated by TLC experiments so far as tested, but clearly distinguished by HPLC. The typical chromatograms are illustrated in Fig. 2.

Hydrochlorides of katanosins A and B are soluble in aqueous methanol, aqueous ethanol, aqueous butanol and dimethyl sulfoxide, slightly soluble in methanol, ethanol and water, but insoluble in acetone, ethyl acetate, chloroform and water of pH 7.0. They show positive reactions to ninhydrin and

Fig. 3. IR spectra of katanosins A and B hydrochloride (KBr).

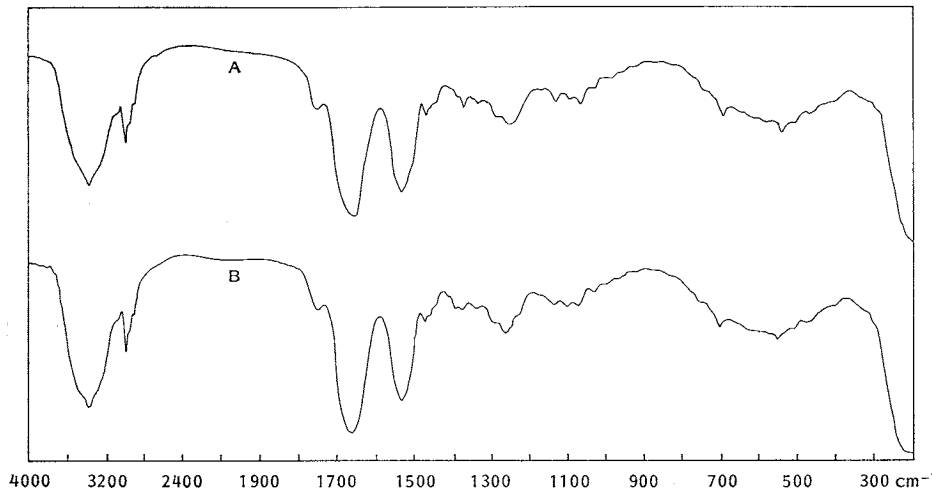
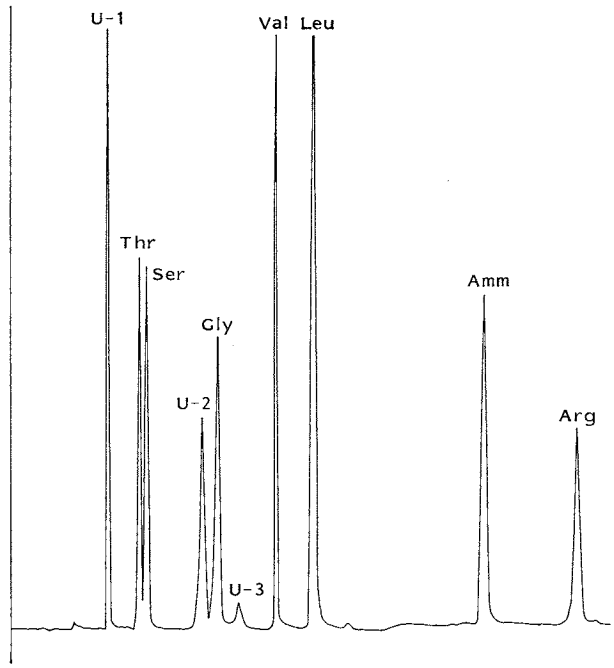


Fig. 4. Amino acid analysis of the hydrolysate of katanosin A.



SAKAGUCHI's reagents. Some other physico-chemical properties are shown in Table 2. Their IR spectra (Fig. 3) show characteristic absorptions for peptide bond (1650 and 1530 cm^{-1}) and for a lactone linkage (1745 cm^{-1}). Amino acid analysis on the acid hydrolysates of katanosin A suggested that the amino acid residues were Thr (1), Ser (1), Gly (1), Val (1), Leu (3), Arg (1) and three unusual amino acids, two of which (U-1 and U-2) appeared as distinct peaks and the other one (U-3) as a low peak (Fig. 4). The only difference between katanosins A and B in the analysis was the presence of Ile residue in B in place of Val in A.

Table 3. Antimicrobial spectra of katanosins A and B (MIC: μg/ml).

Test organism	A	B
Staphylococcus aureus FDA JC-1	0.78	0.78
S. aureus Smith	0.78	0.78
S. aureus SR 2030	0.78	0.39
Streptococcus pyogenes C-203	0.39	0.39
S. pneumoniae Type 1	0.20	0.39
S. faecalis SR 700	0.78	0.39
Escherichia coli NIHJ JC-2	>100	>100
E. coli EC-14	>100	>100
Klebsiella pneumoniae SRL1	50	25
Proteus vulgaris CN-329	>100	>100
Enterobacter cloacae ATCC 13047	>100	>100
Serratia marcescens ATCC 13880	>100	>100

Table 4. Therapeutic efficacy of katanosins A and B (ED_{50}: mg/kg×2).

	A	B
Staphylococcus aureus Smith	1.20	0.67
S. aureus SR 2030	1.90	0.77
Streptococcus pyogenes C-203	2.86	1.55
S. faecalis SR 700	2.10	1.80

Mice were infected intraperitoneally with test organisms.

Compounds were administered subcutaneously at 1 and 5 hours after infection.

Biological Properties

Katanosins A and B exhibit *in vitro* activity against Gram-positive bacteria as shown in Table 3. They show curative effects in mice infected with pathogenic bacteria by subcutaneous administration. The ED_{50} values are listed in Table 4. An approximate acute toxicity to mice by intraperitoneal route was estimated as follows: Katanosin A hydrochloride, LD_{50} 100~200 mg/kg; katanosin B hydrochloride, LD_{50} 200~300 mg/kg.

Discussion

Comparison of katanosins A and B with the known antibiotics of bacterial origin is discussed. Antibiotic BN-165[4] produced from *Pseudomonas* species has been reported to have the same amino acid residues as those of katanosin B with respect to the usual amino acids. However, in addition to the usual amino acid residues, BN-165 contains one unidentified amino acid, whereas katanosin B contains three unidentified amino acids. A recently reported antibiotic, EM 5587[5], produced from *Lysobacter* species seems to be similar to katanosin B in UV absorption, ^{1}H and ^{13}C NMR spectra and MS spectrum. EM 5587 has been reported to be active against Gram-positive bacteria and to a lesser extent against Gram-negative bacteria. Since the amino acid composition has not been reported, it is difficult to compare exhaustively.

None of these antibiotics is suspected to be identical with katanosin A.

Experimental

The UV absorption spectra were measured with a Hitachi 323 spectrophotometer, IR absorption spectra with a Jasco DS-403G spectrometer, $[\alpha]_D$ with a Perkin-Elmer 241 polarimeter and secondary ion (SI)-MS with a Hitachi M-68 mass spectrometer. Amino acid analysis was carried out with a Hitachi amino acid autoanalyzer 835.

Acknowledgments

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