ALAHOPCIN, A NEW DIPEPTIDE ANTIBIOTIC PRODUCED BY *STREPTOMYCES ALBULUS* SUBSP. *OCHRAGERUS* SUBSP. NOV.

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An actinomycete strain No. B-52653 was found to produce an antibiotic selectively active against the *in vitro* antibiotic resistant mutant of *Staphylococcus aureus*. Based on taxonomic studies, the name *Streptomyces albulus* subsp. *ochragerus* subsp. nov. is proposed for the strain. The microorganism produced two kinds of antibiotics; one identical with gougerotin, the other an amphoteric water soluble dipeptide containing L-alanine. The latter has the molecular formula $C_9H_{15}N_3O_6$ and is named alahopcin. It has a broad antibacterial spectrum and a synergistic effect with some other antibiotics against some antibiotic resistant staphylococci. Alahopcin has a low toxicity and was effective against experimental infections in mice caused by *Staphylococcus aureus*.

Of the several thousands microbial fermentation products that have been described, only few antibiotics have been reported to be selectively active against antibiotic resistant strains. NISHIKAWA and ISHIDA[1] and ISHIDA *et al.*[2,3] discovered nojirimycin, which is selectively active against drug resistant *Shigella*. MITSUHASHI *et al.*[4] reported that macarbomycin was more active against a resistant strain carrying episomes than against the original strain. In our screening program for new antibiotics, we searched for an antibiotic selectively active against an *in vitro* antibiotic resistant mutant of *Staphylococcus aureus* 209P (*S. aureus* 4R), because such an antibiotic might be active against the bacterial cell surface. We were successful and found several actinomycetes that produced an antibiotic selectively active against *S. aureus* 4R. The actinomycetes produced two kinds of antibiotics. One was found to be a new dipeptide antibiotic and was named alahopcin[5]; the other was identical with gougerotin. In this paper, we describe the taxonomy of one of the producing organism, and the fermentation, isolation, and physico-chemical and biological properties of alahopcin.

Materials and Methods

Screening Method

The modified spot inoculation method[6] was used for screening.

Taxonomic Studies

The morphological and cultural characteristics of the antibiotic producing organism were determined by the media and methods described by SHIRLING and GOTTLIEB[7]. Previously described taxonomic keys and cultural descriptions[8~13] were used to compare the culture with recognized genera and species.

Fermentation Studies

The microorganism was grown on glucose asparagine agar at 24°C for 10 days and a spore suspension (3×10^8 viable cells/ml) was prepared from the culture. Inocula were prepared by transferring 1 ml

of the spore suspension into 2-liter flasks containing 500 ml of the seed culture medium consisting of glucose 2%, soluble starch 3%, soy bean flour 1%, corn steep liquor 1%, Polypepton (Daigo Nutritive Chem., Ltd.) 0.5%, NaCl 0.3%, $CaCO_3$ 0.5% and Actcol (Takeda Chem. Inc.) 0.05%, adjusted to pH 7.0 before sterilization. The flasks were incubated on a reciprocal shaker at 28°C for 40 hours. One liter of the culture was transferred into a 200-liter fermentor containing 100 liters of the seed culture medium. The seed culture was carried out at 28°C for 24 hours under aeration (100 liters/minute) and agitation (170 rpm). Fifty liters of the seed culture were transferred into a 2,000-liter fermentor containing 1,200 liters of the selected fermentation medium (as described in the Results). The fermentation was carried out at 24°C for 66 hours under aeration (1,200 liters/minute) and agitation (180 rpm). Inorganic phosphoric acid and ammonium nitrogen were determined by the modified NAKAMURA's method[14] and the indophenol method[15], respectively. Glucose was determined with the glucose oxidase peroxidase system[16]. The growth in the fermentation broth was determined by packed cell volume.

Assay of Antibiotics

The amount of gougerotin was determined by the cup method on nutrient agar (Difco) using *Escherichia coli* IFO 12734 as the test organism. Sterilized 0.1 M phosphate buffer (pH 8.0) was used for dilution. The amount of alahopcin was determined by the paper disk method on an assay medium (medium A) consisting of glucose 3%, sodium glutamate 0.5%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 0.05%, yeast extract (Difco) 0.05%, Casamino Acids (Difco) 0.02% and Bacto-agar (Difco) 1.5%, adjusted to pH 7.0 before sterilization. Sterilized 0.1 M phosphate buffer (pH 6.0) was used for the dilution. *S. aureus* 4R was used as the test organism. The microorganism is a mutant strain derived from *S. aureus* FDA 209P (IFO 13267) *in vitro* and is resistant to tetracycline, erythromycin, chloramphenicol and streptomycin.

Isolation and Purification

The fermentation broth (980 liters) was adjusted to pH 5.0 with oxalic acid and filtered. The filtrate was adjusted to pH 7.5 with 3 N NaOH and passed through a column containing Amberlite IRC-50 (H^+) (98 liters). Gougerotin was adsorbed on the resin and alahopcin passed through.

Gougerotin: The antibiotic was eluted from the resin with 0.5 N HCl and the active eluate was adjusted to pH 7.0 with 0.5 N NaOH. The eluate was concentrated and chromatographed on activated carbon with water, on alumina with water, and then on activated carbon with water. The active eluate was concentrated, and MeOH - Me_2CO (1 : 1) was added to the concentrate to give colorless crystalline gougerotin.

Alahopcin: The filtrate that passed through the Amberlite IRC-50 column was adsorbed on an Amberlite 200 (H^+) column (350 liters). The column was washed with water and then eluted with 0.5 N aqueous ammonia. The active eluates were collected and concentrated under reduced pressure. The concentrate (20 liters) was adsorbed on an activated carbon column (200 liters) and eluted with 5% aqueous methanol. The fractions containing alahopcin were combined and concentrated to 1.5 liters.

A 750-ml portion of the concentrate was adsorbed on an alumina (Merck, activated alumina 90, neutral, activity I) column (4.5 liters). The column was washed with water and was eluted with 0.2 N aqueous ammonia. The eluate was concentrated to 1 liter under reduced pressure. The concentrate was adsorbed on an Amberlite IRA-68 (OH^-) column (1.5 liters). After the column was washed with water and with 0.1 M acetic acid, elution was carried out with 0.2 M acetic acid. The eluate was concentrated and chromatographed on an activated carbon column (2 liters) with water. The fractions containing alahopcin were combined, concentrated under reduced pressure, and lyophilized to give 17.5 g of a white solid (purity: about 93%).

The white solid (1.0 g) was dissolved in 4 ml of 50% aqueous methanol, diluted with 4 ml of 70% methanol, chromatographed on Sephadex LH-20 (swollen with 70% aqueous methanol), and eluted with 70% aqueous methanol. The eluate was concentrated and lyophilized to give 0.9 g of alahopcin.

Amino Acid Analysis

Alahopcin was hydrolized with 6 N HCl at 100°C for 15 hours and analyzed with an amino acid analyzer (Jeol-JLC BC2).

Antimicrobial Activities

The activities were determined by the agar serial two-fold dilution method. The synergistic effect was calculated by the method of ALLEN *et al.*[17].

Protective Effect in Mice

The protective effect of alahopcin was studied on groups of five mice (male Slc: ICR, 18~22 g) infected intraperitoneally with *S. aureus* 308A-1. The antibiotic was administrated intraperitoneally, subcutaneously, or orally in single dosage regimen immediately after infection (0 hour), or in three times dosage regimens at 0, 1 and 3 hours post-infection. The 50% effective dose (ED_{50}, mg/kg) was calculated from the survival rate after seven days.

Results

Screening

Among about 2,000 strains isolated, four were selectively active against *S. aureus* 4R. The sources and antimicrobial activities of these four are shown in Table 1. On the basis of their taxonomic properties, and antimicrobial spectra, the strains were considered to belong to the same species. Strain No. B-52653 was used for further study because of its high antibiotic productivity.

Taxonomy

The vegetative mycelium develops well on almost all media. The aerial mycelium is simply branch-

Table 1. Source and antibacterial activities of the isolates.

Test organism	Inhibition diameter (mm)			
	B-29059*	B-39260	B-50788	B-52653
Escherichia coli IFO 12734	0	8	0	10
Proteus vulgaris IFO 3045	0	0	0	0
Staphylococcus aureus IFO 12732	0	0	0	0
S. aureus 4R	25	28	25	35
Bacillus subtilis IFO 3513	0	0	0	0
B. cereus IFO 3514	8	12	8	15
Mycobacterium smegmatis IFO 3038	0	0	0	0

Method : Agar streak method.
Medium : Glucose nutrient agar.
* Soil sources are as follows: B-29059 from Nara, Hyogo; B-39260 from Tsukigase, Nara; B-50788 from Kanzaki, Shiga; B-52653 from Akashi, Hyogo.

Plate 1. Light micrograph of sporophores of strain No. B-52653 on starch agar, 14 days (×700).

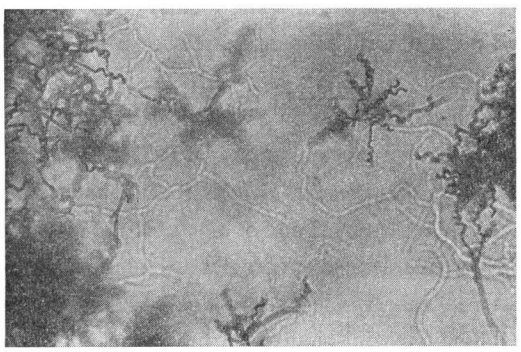


Plate 2. Transmission electron micrograph of spores of strain No. B-52653 on yeast extract - malt extract agar, 10 days (×8,000).

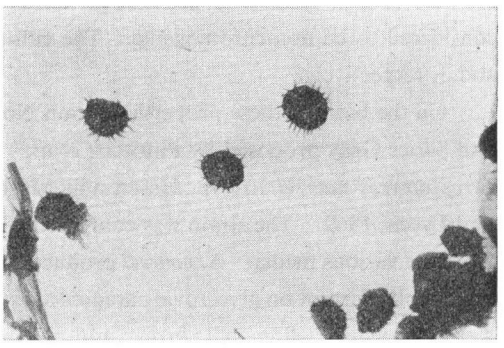


Table 2. Cultural properties of strain No. B-52653.

Medium	Substrate mycelium	Aerial mycelium	Soluble pigment
Sucrose nitrate agar	Moderate, colorless	Moderate, dusty peach (5ec)	None
Glucose nitrate agar	Abundant, pale yellow	Abundant, beige camel (3ge)	Faint yellow
Glycerol nitrate agar	Moderate, colorless	Abundant, natural string (2dc)	Camel (3ie)
Glucose asparagine agar	Moderate, pale yellow	Abundant, ashes (5fe)	None
Glycerol asparagine agar	Moderate, colorless	Abundant, silver gray (3fe)	Light tan (3gc)
Calcium malate agar	Moderate, colorless	Moderate, bisque (3ec)	Rose beige (4ge)
Inorganic salt starch agar	Moderate, colorless	Abundant, silver gray	None
Yeast extract - malt extract agar	Abundant, colorless	Abundant, shadow gray (5ih)	Pale brown
Oatmeal agar	Abundant, colorless	Abundant, silver gray	Honey gold (2ic)

The color was determined with the Color Harmony Manual (4th Ed., Container Corporation of America, 1958).

Table 3. Physiological properties of strain No. B-52653.

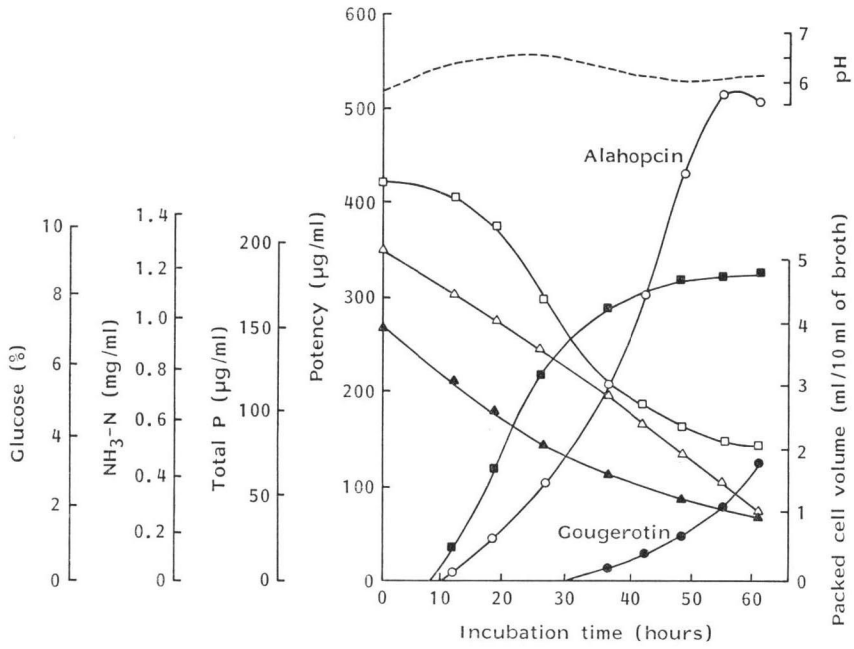
Temperature range for growth*	9~40°C
Optimum temperature for growth*	20~34°C
pH range for growth*	5~9
Melanoid pigment	
Peptone iron agar	Negative
Tyrosine agar	Doubtful
Positive reaction for	Nitrate reduction, liquefaction of gelatin, hydrolysis of starch, coagulation and peptonization of milk.
Carbon source utilization	
Good growth with	D-Fructose, D-glucose, D-galactose, D-mannose, adonitol, D-sorbitol, D-mannitol, *i*-inositol, maltose, sucrose, trehalose, esculin, salicin, erythritol.
No or scarcely any growth with	D-Xylose, L-arabinose, L-sorbose, dulcitol, lactose, melibiose, raffinose, L-rhamnose.

* Medium: Yeast extract - malt extract agar.

ed and the chains of spores form spirals or loops (Plate 1). The spores are spherical to oval, 0.8~1.2 μm×1.0~1.5 μm, with a spiny surface (Plate 2). The aerial mass color is pale gray to brownish gray on various agar media with abundant sporulation. The color of the reverse side is pale yellow to brown. A light yellow to pale brown diffusible pigment was noted in some media. The strain is considered to be non-chromogenic. The cultural and physiological properties are shown in Tables 2 and 3, respectively.

On the basis of these properties, strain No. B-52653 belongs to section *Spirales* or *Retinaculiaperti* and Series Gray proposed by PRIDHAM *et al.*[18]. Among known species, strain No. B-52653 resembles *Streptomyces noursei* Brown, Hasen and Mason, 1953, and *Streptomyces albulus* Routin in Pridham and Lyons, 1969. The strain was compared with *S. noursei* KCC-S0922 and *S. albulus* IFO 13410 (ISP 5492) on various media. *S. noursei* produced a wine color soluble pigment on glycerol nitrate agar and no soluble pigment on glycerol asparagine agar and oat meal agar, whereas strain No. B-52653 produced a pale brownish yellow, pale brown and pale yellow soluble pigment on glycerol nitrate agar, glycerol

Fig. 1. Time course of the production of alahopcin and gougerotin.
Glucose (□), NH_3-N (△), total P (▲), potency (○ and ●), packed cell volume (■) and pH (------).



asparagine agar and oat meal agar, respectively. The former showed poor or no growth with erythritol and salicin, whereas the latter grew well on these carbon sources. *S. albulus* differed from strain No. B-52653 only in the production of soluble pigment on Ca-malate agar and carbon source utilization of sucrose. From these results, strain No. B-52653 resembled *S. albulus*, and was considered to be a new subspecies of it. The name *Streptomyces albulus* subsp. *ochragerus* subsp. nov. is proposed. The name "ochragerus" was chosen because of pale brown pigment produced on Ca-malate agar. The strain has been deposited in the Institute for Fermentation, Osaka, with the accession number IFO 14072.

The gougerotin producing organism, *Streptomyces gougerotii* No. 21544[10], was reexamined taxonomically. The strain produced not only gougerotin, but also alahopcin. The morphology of the spore chain and spore surface was different from those of *S. gougerotii* Waksman and Henrici and taxonomically strain No. 21544 was identical with strain No. B-52653. Therefore, strain No. 21544 should be corrected to be a strain of *S. albulus* subsp. *ochragerus*.

Fermentation

During the fermentation studies, it was found that the strain produced two kinds of antibiotics. One was active against *E. coli* and the other was selectively active against *S. aureus* 4R.

After investigating various media and conditions for obtaining a high yield of the latter antibiotic, the following composition of a medium was selected: $FeSO_4$ 1 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, KH_2PO_4 1.3 g, Proflo (Traders Protein Co.) 25 g, soy bean meal 5 g, NH_4Cl 5 g, $CaCO_3$ 12.5 g, glucose 100 g and Actcol 0.5 g per 1 liter of tap water. Using this medium, a high yield of the antibiotic was obtained with regulation of growth and the metabolism of ammonium nitrogen and inorganic phosphoric acid as shown in Fig. 1.

Fig. 2. IR spectrum of alahopcin (KBr).

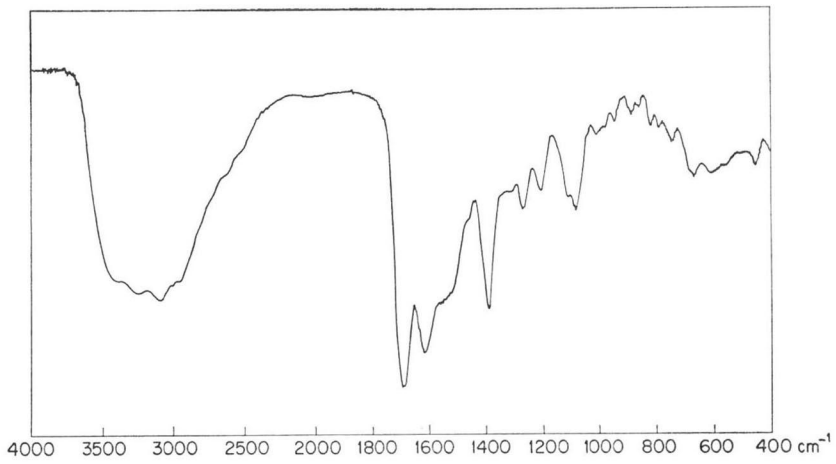
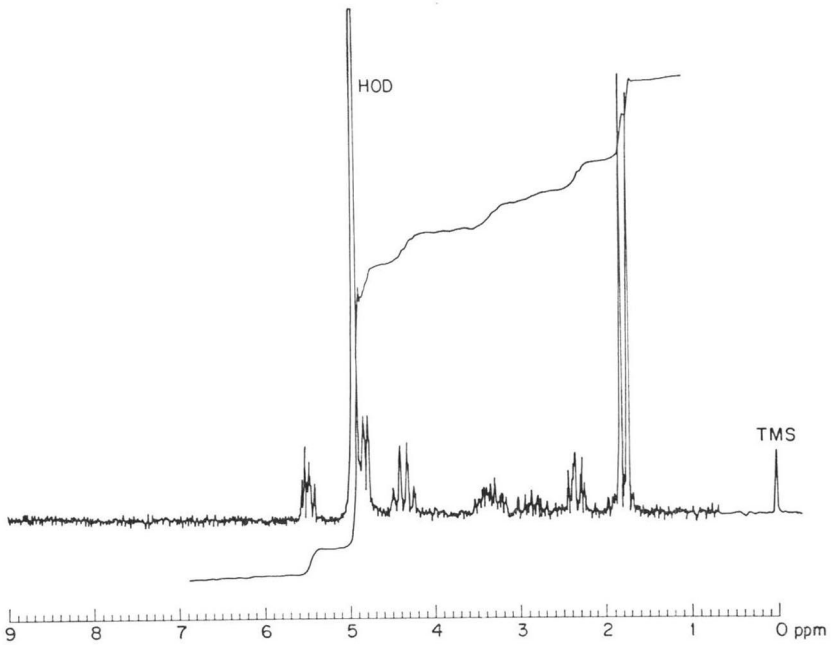


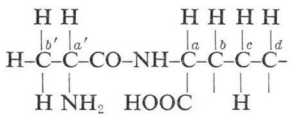
Fig. 3. ^{1}H NMR spectrum of alahopcin (90 MHz, D_2O).



Properties of the Antibiotics

The antibiotic that was active against *E. coli* was identified as gougerotin[10]. The other antibiotic that was active against *S. aureus* 4R was obtained as an amphoteric white amorphous powder, mp 250~255°C (dec), $[\alpha]_D^{23}$ +52.7° (*c* 1.0, H_2O), +62.6° (*c* 1.0, 1 N HCl). The molecular formula was established to be $C_9H_{15}N_3O_6$ from elemental analysis [Calcd for $C_9H_{15}N_3O_6 \cdot H_2O$: C 38.71, H 6.14, N 15.05, MW 279.25; Found: C 38.55, H 6.12, N 14.78, MW (neutral equiv) 282] and mass spectrum (SIMS) [*m/z* 262 $(M+H)^+$]. It is easily soluble in water and hardly soluble or insoluble in methanol, ethanol, acetone, chloroform, ethyl acetate, benzene, petroleum ether, pyridine, acetic acid and *N,N*-dimethyl-

Table 4. ^1H NMR data (at 400 MHz)* of alahopcin.



Assignment of proton		Chemical shift and multiplicity	
		Major isomer	Minor isomer
b'	CH_3–	1.57 (d, *J*=7.1)	1.57 (d, *J*=7.1)
c	–CH_2–	2.06 (ddd, *J*=2.1, 9.3, 14.2)	1.61 (ddd, *J*=4.6, 7.1, 13.9)
		2.18 (quint, *J*=6.9, 7.1, 14.2)	2.64 (ddd, *J*=7.1, 9.8, 13.9)
b	–CH–	3.18 (ddd, *J*=4.6, 7.1, 9.3)	3.06 (ddd, *J*=4.9, 7.1, 9.8)
a'	–CH–	4.11 (q, *J*=7.1)	4.13 (q, *J*=7.1)
a	–CH–	4.57 (d, *J*=4.6)	4.58 (d, *J*=4.9)
d	–CH–	5.27 (dd, *J*=2.1, 6.9)	5.23 (dd, *J*=4.6, 7.1)

* In D_2O, chemical shift δ in ppm from internal 2,2-dimethyl-2-silapentane-5-sulfate (*J* in Hz), with a Jeol JNM-GX 400FT NMR spectrometer.

Table 5. Antibacterial spectrum of alahopcin.

Test organism	Medium	MIC (μg/ml)
Escherichia coli NIHJ JC2	I	50
Proteus vulgaris IFO 3988	I	25
Bacillus subtilis PCI 219	I	25
B. cereus FDA 5	I	3.13
Micrococcus luteus IFO 12708	I	1.56
Staphylococcus aureus IFO 12732	I	1.56
S. aureus 4R	I	<0.2
Mycobacterium smegmatis IFO 3082	II	100
Penicillium chrysogenum IFO 4626	III	>100
Aspergillus niger IFO 4066	III	>100
Saccharomyces cerevisiae IFO 203	III	>100
Candida albicans IFO 583	III	>100
Alternaria kikuchiana IFO 7515	IV	100
Pyricularia oryzae KHG-1	IV	10
Cochliobolus miyabeanus IFO 5277	IV	100
Botrytis cinerea TKF-12	IV	50
Sclerotinia sclerotiorum IFO 9395	IV	1
Pellicularia sasakii KHG-2	IV	20

Inoculum size of bacteria: 10^6 cfu/ml.

Medium: I; nutrient agar (Difco), II; glycerol nutrient agar, III; glucose nutrient agar, IV; potato sucrose agar.

formamide. It is positive to the ninhydrin and Greig-Leaback peptide tests, but negative to the Sakaguchi, anthrone-H_2SO_4 and orcinol-H_2SO_4 tests. The antibiotic is stable in water at pH 1~13 for 10 minutes at 100°C and unstable in alkaline rather than in acidic solution.

The UV absorption spectrum in water shows end absorption. The IR absorption and the NMR spectrum at 90 MHz are shown in Figs. 2 and 3, respectively. Hydrolysis of the antibiotic gave an acidic amino acid (α-aminotricarballylic acid), L-alanine and ammonia.

The structure of alahopcin was determined to be (2*S*,3*R*)-2-[(L-alanyl)amino]-4-formyl-3-(hydroxyaminocarbonyl)butyric acid by chemical and enzymatic degradation studies and spectrometric studies, and the NMR spectrum at 400 MHz in D_2O (Table 4) revealed that alahopcin exists in a mixture of two

Table 6. Effect of media and inocula on the activity.

Test organism	MIC (μg/ml)				
	10^6 cfu/ml				10^8 cfu/ml
	NA	TSA	Med. A	Synth.	Synth.
Bacillus subtilis PCI 219	25	>100	6.25	1.56	6.25
Staphylococcus aureus FDA 209P	0.78	12.5	0.39	<0.2	0.39
S. aureus 308A-1	1.56	50	1.56	<0.2	0.78
S. aureus 1840	1.56	25	0.78	<0.2	0.78
S. aureus E 97	0.78	12.5	0.39	<0.2	0.39
S. aureus 4R	<0.2	0.78	<0.2	<0.2	<0.2
S. epidermidis FS 5010	0.78	6.25	0.39	<0.2	<0.2
Escherichia coli NIHJ JC2	25	>100	6.25	3.13	6.25
E. coli 0-111	100	>100	6.25	6.25	12.5
E. coli 0-139	100	>100	12.5	6.25	12.5
E. coli K-12 W3110	50	>100	6.25	3.13	12.5
E. coli TN 649	25	>100	3.13	3.13	12.5
Proteus vulgaris IFO 3988	25	>100	3.13	1.56	12.5
P. mirabilis IFO 3849	12.5	>100	3.13	1.56	12.5
P. morganii IFO 3848	>100	>100	12.5	3.13	12.5
Klebsiella pneumoniae D 7	50	>100	12.5	3.13	12.5
K. pneumoniae GN 3848	12.5	>100	6.25	6.25	25
K. pneumoniae IFO 3512	100	>100	12.5	12.5	25
Citrobacter freundii TN 457	25	>100	3.13	1.56	6.25
C. freundii TN 564	12.5	>100	3.13	3.13	12.5
Enterobacter cloacae IFO 12937	>100	>100	50	25	>100
E. aerogenes TN 582	50	>100	6.25	6.25	6.25
Salmonella typhimurium LT 7	6.25	>100	1.56	0.78	1.56
Serratia marcescens IFO 12648	12.5	>100	3.13	0.78	6.25
S. marcescens TN 24	3.13	>100	1.56	0.39	3.13
Pseudomonas aeruginosa U 31	>100	>100	>100	>100	>100

Medium: NA; nutrient agar (Difco), TSA; Trypticase Soy Agar (BBL), Med. A; Medium A, see text, Synth.; synthetic agar, see text.

cyclic hemiacetal type tautomeric forms (a major and minor form, which have not been specified yet) which was formed by intermolecular ring closure between the hydroxyamino group and the formyl group of the open chain form of the antibiotic in aqueous solution. The structural elucidation studies will be reported elsewhere in detail[20].

Among known antibiotics, the following related to alahopcin have been reported; fumaryl-*dl*-alanine[21], phosphinothricylalanylalanine[22], fumarylcarboxyamino-L-2,3-diaminopropionyl-L-alanine[23], prumycin[24] and plumbemycin[25]. Since the physico-chemical and biological properties of these antibiotics differ from those of alahopcin, the latter was recognized as a new antibiotic.

However, it was found that nourseimycin, a new antimetabolite of proline, reported recently by NISHIDA *et al.*[26], is identical with alahopcin[5,20].

Antimicrobial Activities

The antimicrobial spectrum of alahopcin is shown in Table 5. The antibiotic was active against Gram-positive and negative bacteria, and strongly active against *S. aureus* 4R, an antibiotic resistant mutant. The antibiotic was highly active against a pathogenic fungus, *Sclerotinia sclerotiorum*.

The activity of the antibiotic was affected by the kind of assay medium and the inoculum size of the test organism. As shown in Table 6, the antibiotic exhibits a broad spectrum of activity on synthetic

Table 7. *In vitro* synergy between alahopcin and other antibiotics.

Test organism	Other antibiotic	MIC with other antibiotic (μg/ml)	MIC with alahopcin (μg/ml)	MIC with combination (μg/ml)	FICI*
S. aureus FDA 209P	Benzylpenicillin	0.013	1,000	0.013+0.013	1
S. aureus 4R		0.013	50	0.013+0.013	1
S. aureus No. 87		2,000	100	35+35	0.367
S. aureus FDA 209P	Chloramphenicol	5	1,000	5+5	1
S. aureus 4R		50	50	10+10	0.4
S. aureus No. 87		10	100	5+5	0.55
S. aureus FDA 209P	Chlortetracycline	0.5	1,000	0.5+0.5	1
S. aureus 4R		5	50	1.3+1.3	0.286
S. aureus No. 87		50	100	10+10	0.3
S. aureus FDA 209P	Streptomycin	2	1,000	2+2	1
S. aureus 4R		500	50	25+25	0.502
S. aureus No. 87		100	100	15+15	0.3
S. aureus FDA 209P	Maridomycin	1	1,000	1+1	1
S. aureus 4R		75	50	4+4	0.13
S. aureus No. 87		2,000	100	20+20	0.22
S. aureus FS 4084		1.56	400	1.56+1.56	1
S. aureus FS 4074		0.78	400	0.78+0.78	1
S. aureus FDA 209P	Erythromycin	0.2	1,000	0.2+0.2	1
S. aureus 4R		200	50	10+10	0.25
S. aureus No. 87		2,000	100	50+50	0.52
S. aureus FS 4084		400	400	6.25+6.25	0.03
S. aureus FS 4074		400	400	6.25+6.25	0.03

Inoculum size: 10^5 cfu/ml.

Medium: Trypticase Soy Agar (BBL).

* Fractional inhibitory concentration index (FICI) were calculated from the equation: FICI=(MIC of potentiator in combination/MIC of antibiotic alone)+(MIC of antibiotic in combination/MIC of antibiotic alone)[17].

media or nutrient agar, but the activity decreased on nutrient rich medium, Trypticase Soy Agar (BBL) and by using larger amount of the inoculum.

As shown in Table 7, when *S. aureus* 4R, and *S. aureus* No. 87, a drug resistant strain from a clinical isolate, were used as test organisms, the antibiotic showed some synergistic effect with streptomycin, erythromycin, maridomycin, chloramphenicol and tetracycline. The antibiotic showed a strong synergistic effect with erythromycin on *S. aureus* FS 4084 and 4074, constitutive-type macrolide resistant strains. However, no synergistic effect was detected with an antibiotic sensitive test organism.

Biological Properties

The intravenous and intraperitoneal administration of 1,000 mg/kg of alahopcin into mice did not result in any toxic symptoms for two weeks after the injection. The protective effects of alahopcin against experimental infection caused by *S. aureus* 308A-1 in mice were as follows: the ED_{50} in single dosage regimen by the intraperitoneal, subcutaneous or oral route was 17.7, 70 and more than 600 mg/kg, respectively; the ED_{50} in the three times dosage regimen by the subcutaneous route was 27.5 mg/kg.

Discussion

Alahopcin was found to be an antibiotic that is selectively active against an antibiotic resistant mutant and it is considered to be a kind of antimetabolite on the basis of its activities in various assay

media. It had no activity (MIC: 800 μg/ml) against *S. aureus* 308A-1 (inoculum: 10^5 cfu/ml) on nutrient rich assay medium such as Trypticase Soy Agar (BBL), though it was effective against experimental infection of this test organism in mice. It showed synergistic activity with erythromycin against drug resistant bacteria, especially constitutive-type macrolide resistant strains even on the nutrient rich assay medium.

Its mechanism of action is assumed to be related to the inhibition of bacterial cell wall synthesis, because it inhibited the incorporation of [1-^{3}H]-*N*-acetylglucosamine and [^{3}H]diaminopimeric acid into the mucopeptide of *S. aureus* and *E. coli*, respectively (unpublished data). In addition, the antibiotic inhibits collagen prolylhydroxylase *in vitro* and *in vivo* and has a similar degree of activity as that of *N*-acetylmuramyl-L-alanyl-D-isoglutamine on the production of humoral antibody to bacterial α-amylase (unpublished data).

Since alahopcin has such characteristic biological and chemical properties, it is assumed that the compound will be a starting material for various semisynthetic derivatives.

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