NEW ANTIBIOTICS, ALGACIDINS A AND B*

Sir:

New antibiotics, algacidins A and B were isolated from a culture of a *Streptomyces* sp. RK-1339. The antibiotics are active against algae and fungi, and cytotoxic to Yoshida sarcoma cells in culture. The antibiotic producing strain was isolated from a soil sample collected in Miyako City, Iwate Prefecture, Japan.

The strain was cultured at 28°C for 66 hours in a jar fermenter containing 18 liters of a medium composed of glucose 2%, soluble starch 1%, meat extract 0.1%, dry yeast 0.4%, soybean flour 2.5%, NaCl 0.2% and K_2HPO_4 0.005%. The fermentation broth (pH 7.4) was filtered and the filtrate (15 liters) was extracted with ethyl acetate and the mycelium was extracted with 80% acetone. The acetone extract was concentrated *in vacuo* to give an aqueous solution, which was then extracted with ethyl acetate. Both the ethyl acetate extracts were combined and concentrated *in vacuo* to dryness. The syrupy residue was chromatographed on a silica gel column with a gradient, benzene - acetone, 10: 1→5: 1→1: 1. The algacidins were eluted from this column, first A then B. Each component was further purified by preparative silica gel TLC using the solvent system, chloroform - methanol, 6: 1. Active zones (Rf 0.59 for algacidin A and 0.48 for algacidin B) were extracted with acetone and concentrated *in vacuo* to dryness, suspended in water and lyophilized to give colorless powder of algacidin A (200 mg) and algacidin B (20 mg).

Algacidin A is a colorless powder with a melting point of 147~155°C. It is a basic compound with *pKa'* 7.3 (70% methyl cellosolve) and is optically active, $[\alpha]_D^{25}$ −16.7° (*c* 0.5, methanol). The molecular formula was determined to be $C_{50}H_{87}NO_{14}$ on the basis of FD mass spectroscopy [$(M+H)^+$ *m/z* 926] and elementary analysis. *Anal.* Calcd. for $C_{50}H_{87}NO_{14}$: C 64.86, H 9.40, N 1.51. Found: C 64.58, H 9.37, N 1.48. The UV absorption spectrum showed a shoulder at 210 nm (ε 13,800) in 90% methanol and alkaline methanol, which showed an irreversible shift to 256 nm (ε 20,400) in acidic methanol (Fig. 1).

Algacidin B is colorless powder with a melting point of 88~91°C. It is a basic compound with

Fig. 1. UV spectra of algacidins.
Algacidin A —— 90% MeOH and 1 N NaOH-MeOH (1: 9)
---- 1 N HCl - MeOH (1: 9)
Algacidin B —— in all solvents

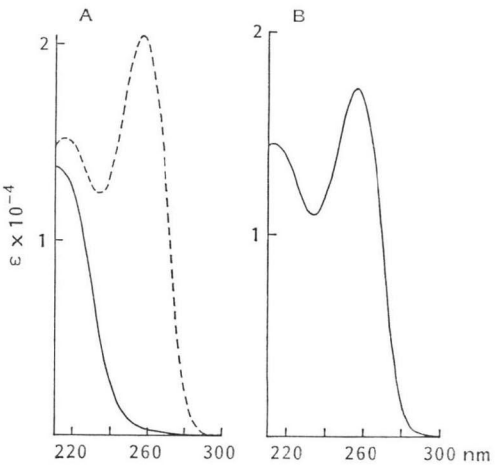

Table 1. Antimicrobial activity of algacidins.

Test organism	MIC (μg/ml)	
	Algacidin A	Algacidin B
Glomerella cingulata IFO 9767	6.25	100
Colletotrichum lagenarium IFO 7513	0.4	25
Botrytis cinerea IFO 5365	1.6	25
Pyricularia oryzae IFO 5994	0.05	25
Fusarium oxysporum IFO 9761	12.5	100
Alternaria mali IFO 8984	12.5	50
Cochliobolus miyabeanus IFO 5277	12.5	50
Rhizoctonia solani IFO 6258	0.012	25
Aspergillus oryzae	6.25	>100
Penicillium chrysogenum	50	>100
Chlorella vulgaris	25	25
Staphylococcus aureus FDA 209P	>100	100
Bacillus subtilis NIHJ PCI 219	50	100
Escherichia coli K-12	>100	>100

Conventional agar dilution method was employed. Medium: potato - sucrose for fungi, glucose - peptone - yeast extract for *Chlorella*, bouillon for bacteria.

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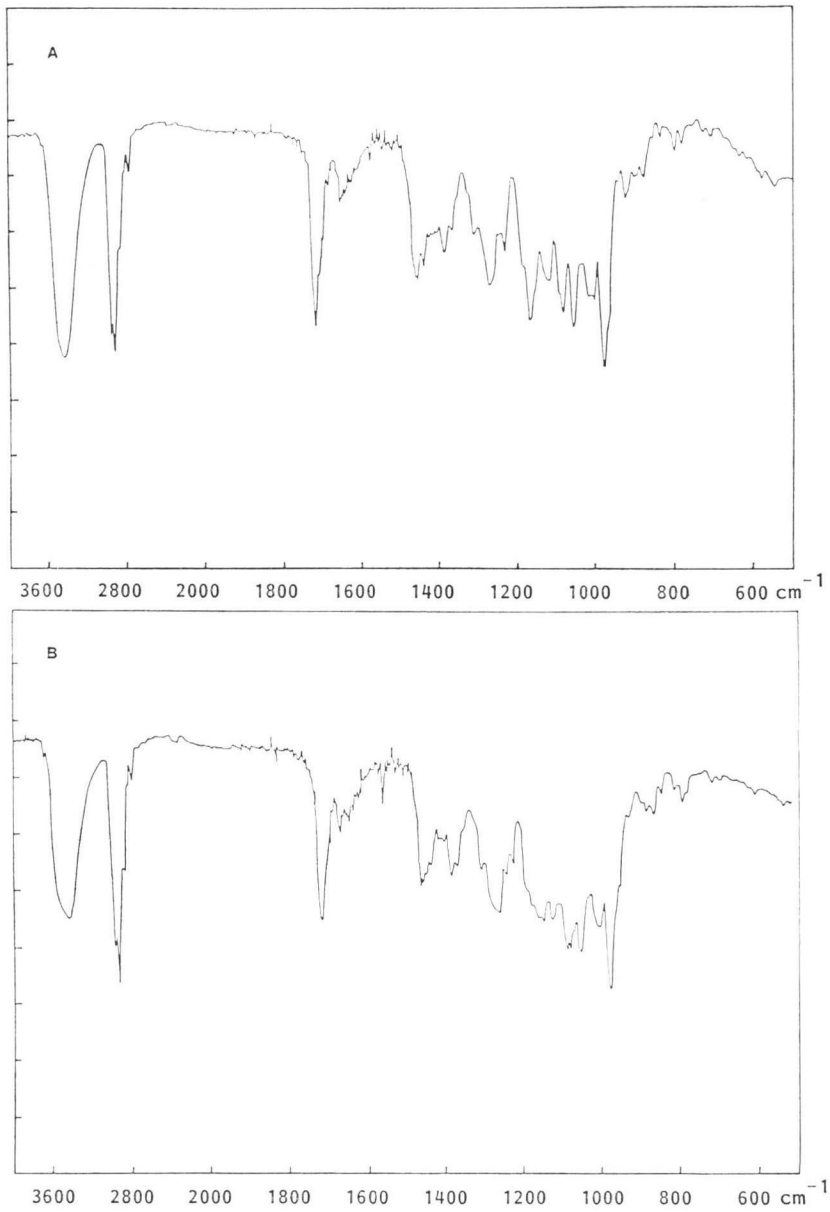
Algacidin B is announced under a code number, RK-1339.

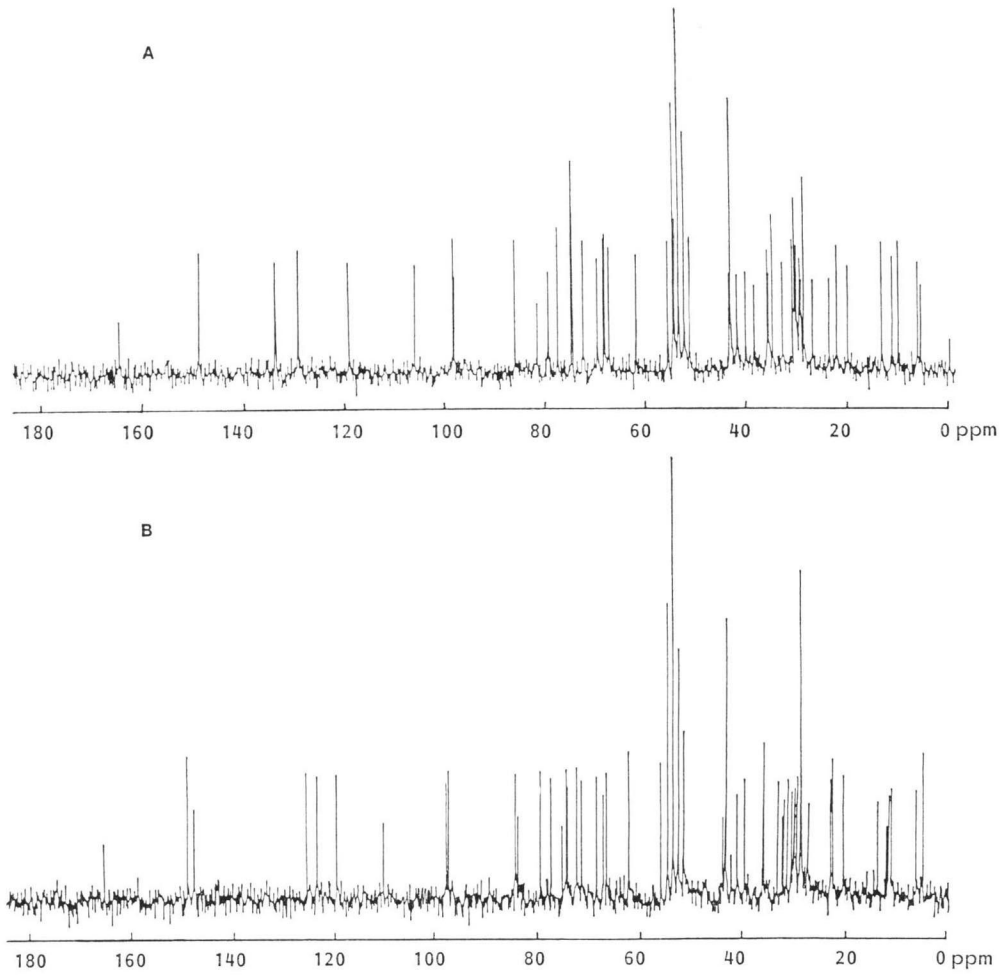
Fig. 2. IR spectra of algacidins A and B (KBr).



pKa' 7.1 and optical rotation of $[\alpha]_D^{23}$ −28.6° (*c* 0.5, methanol). The molecular formula was determined to be $C_{50}H_{85}NO_{13}$ on the basis of FD mass spectroscopy [$(M+H)^+$ *m/z* 908] and elementary analysis. *Anal.* Calcd. for $C_{50}H_{85}NO_{13}$: C 66.15, H 9.37, N 1.54. Found: C 66.38, H 9.67, N 1.52. The UV spectrum showed maxima at 212 nm (ε 14,400) and 256 nm (ε 17,200) in 90% methanol (Fig. 1). This absorption did not change in acidic or alkaline methanol.

Algacidins are soluble in methanol, ethanol, acetone, ethyl acetate, chloroform and dichloromethane, slightly soluble in water but insoluble in *n*-heptane. They gave a positive reaction to iodine vapor, permanganate and anisaldehyde-sulfuric acid tests, but were negative to ninhydrin. The IR spectra (Fig. 2) indicated the presence of an unsaturated lactone or ester group (1718 cm^{-1}).

Fig. 3. ^{13}C NMR spectra of algacidins A and B (in CD_2Cl_2).



The ^{13}C NMR spectra are shown in Fig. 3, which account for almost all of the 50 carbon atoms.

As indicated in the UV absorption spectra, algacidin A is considered to be dehydrated to give algacidin B on acidification. The anhydro derivative was isolated and identified as algacidin B. The UV absorption (256 nm) may be assigned to α,β-γ,δ-unsaturated lactone or ester. Although algacidin A is easily transformed to algacidin B by acidification, algacidin B is not considered to be an artifact because its presence was detected in the mycelium as well as in the culture filtrate by solvent extraction at neutral pH followed by direct HPLC analysis.

Among known antibiotics, algacidin A resembles ossamycin[1] in many respects. However, side-by-side comparison of both samples by HPLC showed that they are not identical [Nucleosil $5C_{18}$, $6\phi \times 200$ mm, solvent: 1% diethylamine - HCOOH (pH 5.0) - CH_3CN - MeOH, 1 : 6 : 3, R_T: 14.5, 12.2 and 13.0 minutes for algacidins A, B and ossamycin respectively. In addition, ^1H and ^{13}C NMR spectra of both compounds are quite different, especially in an alkyl region.

Algacidins are inhibitory to *Chlorella vulgaris* and fungi but showed only weak activity against bacteria. Growth of *C. vulgaris* was partially inhibited at the concentration of as low as 0.0002 μg/ml of algacidin A. Minimal inhibitory concentrations are shown in Table 1. The antibiotics are toxic to Yoshida sarcoma cells in culture. Partial inhibition of growth was observed for a wide range of concentration (0.001 ~ 10 μg/ml for algacidin A and 0.01 ~ 10 μg/ml for algacidin B).

Complete inhibition was observed at 50 μg/ml. This inhibition pattern is similar to that of cytovaricin previously reported from this laboratory[2]. They are toxic to mice. LD_{50}'s are approximately 1.5 mg/kg for algacidin A and 10 mg/kg for algacidin B by intraperitoneal administration.

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