

A NEW PEPTIDE ANTIBIOTIC, ALBOLEUTIN

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(Received for publication March 27, 1980)

In the course of a screening program for new antifungal antibiotics, *Bacillus* sp. AF-8 was found to produce a new peptide antibiotic, alboleutin, which shows selective activity against plant-pathogenic fungi. The antibiotic producing organism was identified as *Bacillus subtilis*¹⁾ by the taxonomic comparison with type strains of *Bacillus* sp. as well as by its morphological, cultural and physiological characteristics.

A 48-hour culture of *B. subtilis* AF-8 was transferred into 20 liters of medium in a 30-liter fermentor and the fermentation was carried out under the following conditions: Time, 44 hours; temperature, 27°C; agitation, 250 rpm; aeration, 10 liters/min.; pressure, 0.5 kg/cm².

Alboleutin titers were monitored by the paper disc method using *Alternaria kikuchiana* as a test organism and by thin-layer chromatography (TLC) on silica gel plate developed with CHCl₃ - MeOH - AcOH (15: 1: 1, v/v) and detected with DRAGENDORFF reagent. Antibiotic in the broth filtrate was adsorbed on activated carbon (220 g) and eluted with 3 liters of 70% aqueous acetone. The eluate was evaporated to aqueous solution and extracted twice with 500 ml each of *n*-butanol. After washing with 0.1 N HCl and water, the solvent layer was dried to yield a yellowish brown powder (22 g). The crude material was dissolved in chloroform and then chromatographed on silica gel (800 g) eluting with a mixed solvent of chloroform and methanol (7: 1, v/v). The active fractions were concentrated to dryness to yield 4 g of a pale yellowish powder. An ethanol solution of the powder (1 g) was applied on the top of Sephadex LH-20 column (600 ml)

equilibrated with ethanol and the antibiotic was eluted with the same solvent. The active fractions were concentrated *in vacuo* to dryness to give 80 mg of pure alboleutin as a white powder.

Alboleutin is an acidic compound with m.p. 145~147°C and $[\alpha]_D^{25} + 12.0$ (*c* 1.0, CHCl₃). It is soluble in benzene, ethyl acetate, chloroform and, to a lesser extent, methanol, ethanol and ethyl ether, and sparingly soluble in *n*-hexane, acetone and water. The elemental analysis of the antibiotic shows C 58.52%, H 8.64% and N 9.04%. No characteristic UV spectrum was observed with alboleutin at 1,000 μg/ml in methanol. The IR spectrum exhibits amide bonds ($\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1640, 1530) as shown in Fig. 1. The R_f values of alboleutin after TLC on silica gel are as follows; 0.50 with CHCl₃ - MeOH - AcOH (15: 1: 1), 0.40 with *n*-BuOH - acetone - water (4: 5: 1) and 0.72 with *n*-BuOH - AcOH - water (3: 1: 1).

The antibiotic is hydrolyzed with 6 N HCl within 15 hours at 110°C. The amino acid analysis of its acid hydrolyzate with an automatic amino acid analyzer shows the existence of leucine, aspartic acid, glutamic acid and valine (molar ratio; 4.1: 1.0: 1.1: 0.9).

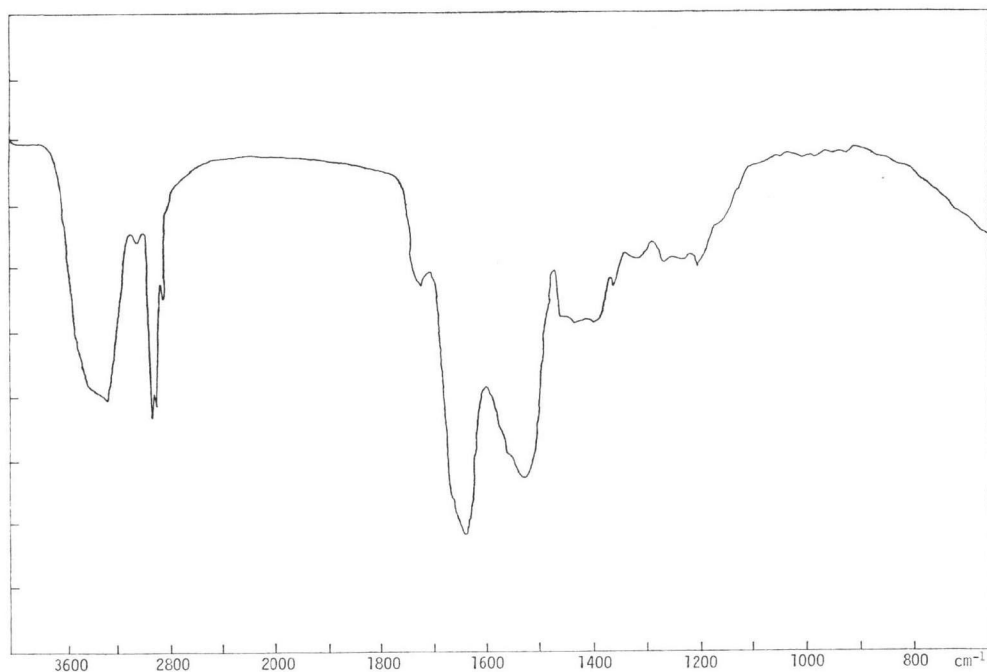
Alboleutin showed activity against *Piricularia oryzae* and *Alternaria kikuchiana* by the paper disc method (Table 1) but no activity by the agar dilution method. No acute toxicity of the antibiotic was observed in mice after 100 mg/kg of intraperitoneal injection.

Table 1. Antimicrobial spectrum of alboleutin by paper disc method.

Test organism	Inhibition zone (mm)*
<i>Staphylococcus aureus</i> FDA 209P	—
<i>Bacillus subtilis</i> PCI 219	—
<i>Mycobacterium smegmatis</i> ATCC 607	—
<i>Sarcina lutea</i> PCI 1001	10.8
<i>Escherichia coli</i> NIHJ	—
<i>Xanthomonas oryzae</i>	—
<i>Pseudomonas aeruginosa</i> P-3	—
<i>Candida albicans</i>	—
<i>Saccharomyces cerevisiae</i>	—
<i>Piricularia oryzae</i>	33.5
<i>Aspergillus niger</i>	—
<i>Alternaria kikuchiana</i>	25.7

* paper disc, 8 mm in diameter

Fig. 1. IR spectrum of alboleutin (KBr).



Many organisms belonging to the genus *Bacillus* are known to produce peptide antibiotics²⁾. However, from the amino acid composition, antifungal activity and properties such as elemental analysis, UV and IR spectra, it can reasonably be concluded that alboleutin is new.

Acknowledgements

The authors wish to thank the late Mr. T. YAMANAKA and Mr. N. KAWADA for their technical assistance.

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