ISOLATION OF A NEW PEPTIDE ANTIBIOTIC TL-119

STUDIES ON ANTIBIOTICS FROM THE GENUS *BACILLUS*. IV

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A new antibiotic TL-119 active against gram-positive bacteria was isolated from a strain resembling *Bacillus subtilis*. The antibiotic is a neutral substance, soluble in a mixture of chloroform and methanol, and is a peptide with an empirical formula of $C_{42}H_{57}N_7O_9$, containing threonine (1), alanine (1), valine (1), leucine (1) and phenylalanine (2).

In the course of our screening program for new antibiotics from *Bacillus* species,[1] a strain TL-119, which was isolated from a soil sample collected in Thailand and similar to *Bacillus subtilis*, was found to produce a new antibiotic active against gram-positive bacteria. The antibiotic, named TL-119, was isolated from the culture broth by *n*-butanol extraction and purified by TLC on silica gel with chloroform - methanol (50 : 1).

The antibiotic TL-119 was obtained as a colorless amorphous powder, which decomposes at above *ca.* 250°C. Elemental analysis and mass spectrometry indicated an empirical formula of $C_{42}H_{57}N_7O_9$. It is a neutral substance soluble in dimethylsulfoxide, dimethylformamide and a mixture of methanol and chloroform, but hardly soluble or insoluble in other organic solvents and water. It shows negative reactions to ninhydrin, DRAGENDORFF, SAKAGUCHI and PAULY reagents, but decolorizes potassium permanganate solution. It is optically active: $[\alpha]_D^{24°}-8.7\pm1.0°$ (*c* 0.482, dimethylsulfoxide).

In the ultraviolet absorption spectrum measured in methonol solution (Fig. 1), a strong end absorption and weak shoulders at 252, 258, 264 and 268 nm, are seen, suggesting the presence of phenylalanine residue. The infrared absorption spectrum (Fig. 2) suggested this substance to be a peptide possibly containing a lactone or ester linkage.

Amino acid analysis with the hydrolyzate of the antibiotic indicated the presence of threonine (1), alanine (1), valine (1), leucine (1) and phenylalanine (2), and a molecular weight of approximately 800.

Structural studies carried out chiefly by mass spectrometry revealed the terminal COOH to be tied up as a lactone, the terminal NH_2 to be acetylated and the presence of an α-amino-dehydrobutyric acid residue. The sequence of the amino acid residues was also determined. However, these data will be reported in the succeeding paper of this series.

Fig. 1. Ultraviolet absorption spectrum of TL-119.

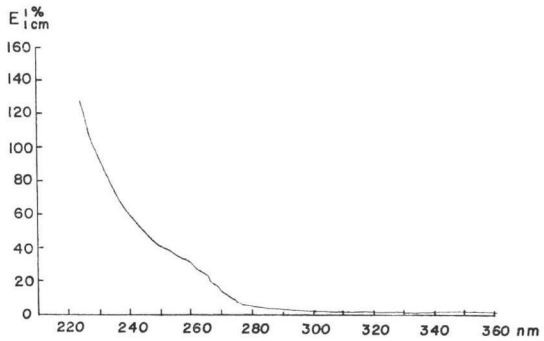

Fig. 2. Infrared absorption spectrum of TL-119.

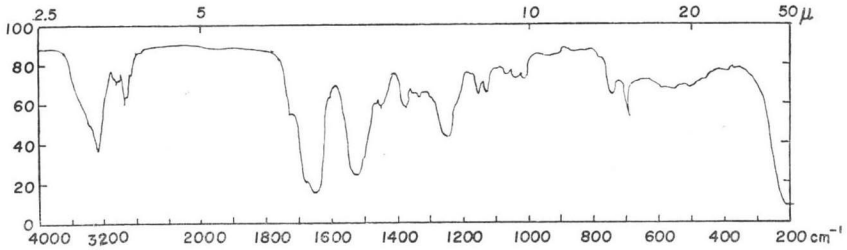

Table 1. Antimicrobial spectrum of TL-119.

Test organisms	MIC (mcg/ml)
Bacillus subtilis PCI 219	12.5
Staphylococcus aureus FDA 209P JC-1	1.56
Staphylococcus aureus Smith	3.13
Diplococcus pneumoniae type I	>50
Streptococcus pyogenes C-203	>50
Escherichia coli NIHJ JC-2	>50
Klebsiella pneumoniae	>50
Salmonella typhimurium	>50
Pseudomonas aeruginosa Ps-24	>50
Mycobacterium tuberculosis $H_{37}RV$	>50

Obtained by the usual agar dilution method.

As shown in the antimicrobial spectrum (Table 1), the antibiotic TL-119 is preferentially active against *Staphylococcus aureus*. Intraperitoneal administration of this antibiotic at a dose of 50 mg/kg showed no toxic symptom to mice. Curative effect was observed against mice infected with *St. aureus* by intraperitoneal administration of this antibiotic, but not when administered subcutaneously.

From the above properties, it is concluded that the antibiotic TL-119 is a new antibiotic.

Experimental

Characterization of Strain TL-119

The strain TL-119 is a gram-positive, rod-shaped bacillus ($0.8\times2\sim4\,\mu$) which forms cylindrical spores ($0.7\times1.5\,\mu$) in sporangia of not definitely swollen. It grew rapidly on nutrient agar and glucose-bouillon agar as a grayish-cream, opaque, dull or not shiny, wrinkled film. The appearance is similar to standard cultures of *Bacillus subtilis*. The strain is aerobic and does not produce both acid and gas on O-F test.[2] Hydrolysis of starch and VP-test are both positive. This culture grows well on 7 % NaCl broth.

The above data are not enough for identifying TL-119 as *B. subtilis*.[3] However, the data suggest strongly that TL-119 could be a variety of *B. subtilis*.

Fermentation

Spores of the strain TL-119 were inoculated into 120 ml of a medium consisting of glucose 1.0 %, glycerin 0.25 %, peptone 1.0 %, meat extract 0.5 % and sodium chloride 0.3 %, pH 7.0 in a 500-ml shake flask, and shake-cultured for 24 hours at 28°C. About 4 ml of the culture was then seeded into 120 ml of a medium made of starch 1.0 %, glycerin 0.5 %, Soytone (Difco) 0.5 %, corn steep liquor 0.25 %, yeast extract 0.1 % and sodium chloride 0.3 %, pH 7.0 in the flask, which was shake-cultured for 3 days at 28°C before harvest.

Isolation and Purification

The culture broth (*ca.* 5 liters) was admixed with an equal volume of a mixture of methanol and *n*-butanol (1:1) and filtered at pH 3.0. The filtrate was evaporated to nearly aqueous solution and extracted with *n*-butanol. The *n*-butanol extract was washed with

water and concentrated to a syrup, from which the antibiotic was extracted with ethyl acetate. The extract was dehydrated with anhydrous sodium sulfate and concentrated to dryness to give a crude material (250 mg).

The crude material was applied to two Silica gel GF plates (thickness, 750 μ; 20×100 cm) and continuously developed for 4 hours with chloroform - methanol (50:1). The zone of the antibiotic was detected with a UV lamp and extracted with chloroform - methanol (1:1). This TLC procedure was repeated twice and the extract was concentrated to a small volume, to which ethyl ether was added to precipitate a colorless amorphous powder (170 mg).

Anal. Found:	C, 62.37; H, 7.11; O, 17.68; N, 12.09; M^+, 803.
Calcd. for $C_{42}H_{57}O_9N_7$:	C, 62.76; H, 7.10; O, 17.93; N, 12.20; MW, 803.

Amino Acid Analysis

The antibiotic TL-119 was not completely hydrolyzed by the usual way, because of its insolubility in hydrochloric acid. The sample to be hydrolyzed was dissolved in a mixture of formic acid and conc. hydrochloric acid (1:1) and allowed to stand for 24 hours at 38°C. After that, it was concentrated to dryness and the partially hydrolyzed sample was then hydrolyzed with constant-boiling hydrochloric acid at 110°C for 48 hours.

An automatic amino acid analyzer, Hitachi Model KLA-5, was used for the analysis. The amino acids found which are expressed in μ moles per mg of the antibiotic, were as follows: threonine (1.09), alanine (1.19), valine (1.19), leucine (1.20) and phenylalanine (2.54).

Acknowledgement

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References

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