PRODUCTION OF BACILLIN BY *BACILLUS* SP. STRAIN NO. KM-208 AND ITS IDENTITY WITH TETAINE (BACILYSIN)

KIYOO ATSUMI, RUIKO ŌIWA and SATOSHI ŌMURA

The Kitasato Institute, Minato-ku, Tokyo, Japan

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In the course of screening for new antibiotics, we isolated antibiotic KM-208 possessing an inhibitory activity against certain gram-positive and gram-negative bacteria from fermentation broth of *Bacillus* sp., strain No. KM-208.

Production of KM-208 was carried out using a 100-liter tank fermentor for 40 hours at 27°C, in a medium containing glucose 2.0%, soybean meal 2.0%, dry yeast 0.3%, KCl 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, K_2HPO_4 0.1%, NaCl 0.3% (w/v, pH 7.0). The activity was determined by a paper disc-agar method using *Staphylococcus aureus* FDA 209P as a test organism. The isolation and purification of KM-208 was performed by the following method: Whole broth was adjusted to pH 2.5 with 6 N HCl and centrifuged, and the supernatant was decolorized with activated charcoal. The solution was adsorbed on Amberlite IR-120(H^+), followed by elution with 0.5 N NH_4OH. The active eluate was concentrated *in vacuo* and then lyophilized to give a brown crude powder (6.0 g). Further, the crude powder was chromatographed over carbon (eluted with 50% MeOH), silica gel (EtOH-H_2O, 10:1) and then CM-Sephadex (H_2O) to yield a white powder (75 mg). The IR spectrum of KM-208 showed the presence of peptide linkage at 3500~3000 cm^{-1} and 1700~1500 cm^{-1} (Fig. 1). The result from the amino acid analysis of the hydrolyzate (6 N HCl, 105°C, 18 hours) of KM-208 revealed the presence of alanine and tyrosine, in a molar ratio of 1 : 0.65.

These data suggested a similarity between KM-208 and the antibiotics tetaine[1] (bacilysin) and bacillin[2,3] which was reported by BOROWSKI *et al.* (1952) and WOODRUFF *et al.* (1946) respectively. The structure of bacilysin[4,5,6] has been determined by WALKER *et al.* as shown in Chart 1, but that of bacillin has not been reported. In order to establish the identity of KM-208, bacilysin and bacillin, bacillin was isolated in a similar method to KM-208 from the cultured broth of a bacillin-producing strain (No. MB-155) supplied from the Merck Sharp & Dohme Research Laboratories.

On paper and thin-layer (silica gel and avicel) chromatography, KM-208, tetaine and bacillin gave the same Rf values in various

Chart 1.

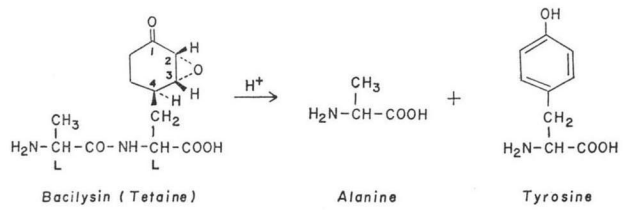
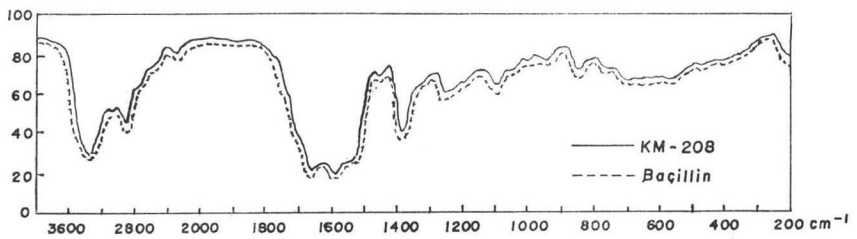


Fig. 1. IR spectra of KM-208 and bacillin (KBr)



solvent systems. As shown in Fig. 1, a comparison of the IR spectra (KBr) of KM-208 and bacillin confirmed their identity. Furthermore, the ORD curves of both KM-208 and bacillin showed a positive COTTON effect at 228 nm and 319 nm, establishing that they have the same absolute configuration. The NMR spectrum (100 MHz, D_2O) of KM-208 was identical with that of bacilysin[5]. Therefore, the structure of KM-208 was identical with that of tetaine[6].

About the mode of action of these antibiotics, it has been reported by BOROWSKI *et al.*[7,8] that tetaine seems to inhibit an incorporation of L-alanine to uridine diphosphate N-acetylmuramic acid (UDP-MurNAc) in murein synthesis, whereas WALTON *et al.*[9] observed that the antibiotic action of bacillin is reversed with N-acetylglucosamine (GlcNAc). Similarly we obtained the result that the inhibition by KM-208 was reversed with GlcNAc, as shown in Table 1.

Taking this into consideration, there is a possibility that the antibiotic also inhibits the step of the synthesis of the murein precursors, GlcNAc.

Table 1. Effect of GlcNAc on antibiotic activity of KM-208

Concentration of GlcNAc (mcg/ml)*	Diameter of inhibitory zone (mm)**
0	22.9
10	22.7
50	19.8
100	(19.3)***
500	—

* GlcNAc was dissolved in aqueous solution of KM-208 in each concentration (KM-208, 50 mcg/ml)

** *Staphylococcus aureus* FDA 209P was used as test organism in nutrient agar medium. Clear zone of inhibition was determined by the paper disc method after incubation of the plates for 16~18 hours at 37°C

*** Hazy zone of inhibition

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