NOTES

AMPHOMYCIN ACYLASE

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The amphomycin family[1] of antibiotics have been characterized by BODANSZKY *et al.*[2] as antibiotic peptides containing an 11 member peptide esterified with isotridecanoic acid. Other antibiotics which are apparently chemically related include aspartocin, crystallomycin, glumamycin, laspartomycin, tsushimycin, and zaomycin[3]. Of this group, only amphomycin has found therapeutic application as an antibacterial agent against Gram-positive organisms in ointments and lotions used in human and veterinary medicine.

In a study of microbial transformation of amphomycin we have isolated a *Corynebacterium* species from garden soil which inactivates the antibiotic by deacylation. The acylase differs from that described by KIMURA and HIRAI[4] and used by SHOJI *et al.*[5] to remove the fatty acid from polymyxin-related antibiotics.

The *Corynebacterium* cells contain the amphomycin acylase constitutively, and the acylase level is increased at least 6-fold when the bacteria are grown in nutrient media supplemented with low levels of calcium amphomycin. Resting cells, growing cells, and lyophilized cells are all active in degrading the amphomycin, while boiled cells and acetone-dried cells are not. The pH optimum for acylase activity was found to be pH 7.0 and the optimum temperature for inactivation was about 45°C. Ten mg of lyophilized cells inactivated 1 mg of calcium amphomycin in about 60 minutes (inactivation measured by agar diffusion bioassay with *Bacillus subtilis* as test organism).

The inactivated amphomycin-cell mixture was extracted with ethyl acetate and the extract examined for the character of the fatty acid(s) present in comparison with the fatty acids extracted from a chemically-hydrolyzed sample of amphomycin[6] using NMR spectrometry, mass spectrometry, gas chromatography, and thin-layer chromatography. The measurements with the material obtained by the biological degradation of amphomycin agreed with those obtained with the fatty acids obtained by chemical hydrolysis of amphomycin.

The peptide present in the aqueous phase of the inactivated amphomycin-cell mixture was recovered and purified by preparative paper electrophoresis (pH 1.9). When hydrolyzed by 6 N HCl *in vacuo* at 110°C for 18 hours this material was found to contain aspartic acid, glycine, proline, and valine in the same molar ratio as expected for amphomycin, *e.g.* 4: 2: 1: 1, and 2,3-diaminobutyric acid was also present. Preliminary experiments with this peptide showed that it was not hydrolyzed by chymotrypsin, subtilopeptidase, *Streptomyces griseus* protease, *Bacillus subtilis* protease, or Nagarse®.

As the lyophilized cell preparations did not inactivate polymyxin B or the octapeptin EM-49 (as measured by agar diffusion bioassay), it would seem that the enzyme system in this *Corynebacterium* differs from the system reported by KIMURA and HIRAI[4] from *Pseudomonas* which hydrolyses polymyxins.

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