POLYMYXIN B: CONTROLLED BIOSYNTHESIS

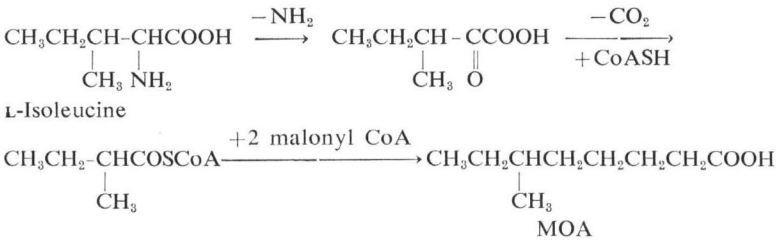
Sir:

The polymyxins form a group of closely related strongly basic cyclicpeptide antibiotics with a rather specific activity against gram-negative bacteria. The most well-known and commercially produced members are polymyxin B and E (colistin). These two consists of polymyxin B_1, B_2, B_3 and E_1, E_2, E_3, the single components of them differing only in the fatty acid moiety of the side chain attached to the cyclic peptides. B_1 contains 6-methyloctanoic acid (MOA), B_2 isooctanoic acid (IOA), B_3 octanoic acid. Corresponding differences exist between the components E_1, E_2 and E_3.

The commercial polymyxin B consists of a mixture with varying contents of B_1, B_2 and B_3, the major constituent being B_1 (above 70%). Little is known about the activity and toxicity of the single components.

The biosynthesis of MOA and IOA is described by ITO *et al.*[1]: L-Isoleucine or L-valine is deaminated, decarboxylated and condensed with CoASH. Further condensation with 2 malonyl CoA leads to MOA, and IOA respectively.

The following experiments describe attempts to change the proportion of B_1, B_2 and B_3 when isoleucine and valine are added to the culture medium of a polymyxin-producing strain of *Bacillus polymyxa*.



Materials and Methods

Culture

Bacillus polymyxa (Wellcome Research Foundation Culture no. 1419) was maintained on Casitone, Soytone, dextrose agar. Seed cultures were prepared in a complex medium containing whey powder 40 g, soybean flower 20 g, $CaCO_3$ 20 g, $(NH_4)_2HPO_4$ 7 g, yeast extract 3 g, $(NH_4)_2SO_4$ 1 g, KCl 1 g, NaCl 1 g per liter tap water; 500-ml flasks containing 100 ml of medium (pH was adjusted to 7.0) were inoculated and incubated on a rotary shaker (250 rpm) for 24 hours at 27°C; 100 ml of such a culture was used as inoculum for 4 liters of the same medium in a 5-liter aerated and stirred laboratory fermentor (vol/vol/min and 250 rpm). pH of the medium was adjusted to 7.0 before inoculation and the fermentation temperature was 27°C.

Isolation of polymyxin

After 48 hours of propagation 9 g of oxalic acid were added to the fermentation broth, the pH was adjusted to 2.0 with conc. H_2SO_4 and heated for ten minutes at 60°C. After centrifugation the supernatant was neutralized using NaOH, and stirred with the ion-exchanger IRC-50 (75 g wet, sodium form). The IRC-50 was collected by decantation, washed with water and eluated with methanol-water (40:60) acidified with HCl to pH 1.8. After neutralization of the eluate the methanol was removed on a rotary evaporator and the polymyxin base precipitated by adjusting the pH to 9.5 with NaOH. The precipitate was washed several times with water and no further attempts were made to purify the material. The average yields were 100 mg/liter, estimated by the agar diffusion method using *Bordetella bronchiseptica* ATCC 4617 as the test organism.

Hydrolysis and isolation of the fatty acid components

Fifty mg of polymyxin were hydrolyzed in 2 ml of 6 N HCl for 16 hours at 120°C. The fatty acids were extracted with ether. The ether was evaporated to a small volume in a stream of dry nitrogen. The acids were

methylated in 5% HCl in dry methanol (4 ml) at 100°C for 2 hours. Three ml of water were added and the methyl esters extracted 3 times with 5 ml of petroleum ether (bp 40~60°C). The petroleum ether was evaporated to a small volume and the esters were taken up in 1 ml of acetone.

Gas chromatography

Gas chromatography was performed using a Perkin Elmer 880 apparatus. The column was packed with 5% carbowax 20 m on diatomite C-AW-DMCS (80~100 mesh). As standards the methyl esters of the pure fatty acids were used kindly supplied by Dumex Ltd., Copenhagen.

Results and Discussion

Table 1 summarizes the most evident results of our experiments. It appears that the commercial polymyxin B and our own preparation contain B_1, B_2 and B_3 components in approximately the same ratio.

Table 1. Ratios of polymyxin B_1, B_2 and B_3 isolated from culture broth of *Bacillus polymyxa*

	%		
	B_1	B_2	B_3
Commercial polymyxin B base (Dumex Ltd., Copenhagen)	81.5	14.4	4.3
No addition of amino-acids to the medium	76.1	19.5	4.4
L-Valine added (1 g/liter medium)	73.0	21.6	5.4
L-Isoleucine added (1 g/liter medium)	94.8	3.0	2.1

Addition of valine, the precursor of isooctanoic acid corresponding to B_2, to the culture medium unexpectedly did not lead to a significant increase of this component.

However, although isoleucine did not stimulate the overall production of the polymyxin B complex, our experiments unambigously show that an addition of this amino acid predominantly leads to the biosynthesis of component B_1.

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References

1) ITO, M.; K. AIDA & T. UEMURA: Studies on the bacterial formation of peptide antibiotic, colistin. Part II. On the biosynthesis of 6-methyloctanoic and isooctanoic acids. Agr. Biol. Chem. 33: 262~269, 1969

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