AMINO ACID COMPOSITION OF BACILLUS LICHENIFORMIS SPORE COAT[1]

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Bernlohr and Novelli (1,2) have postulated that the production of the peptide antibiotic bacitracin in cultures of Bacillus licheniformis is related to the process of sporulation. They have further concluded that the intact bacitracin molecule is incorporated into newly formed spores (3). These conclusions were supported by the experiments of Bernlohr and Sievert (4), who isolated spore coat material and found that it had essentially the same amino acid composition as bacitracin. The tentative conclusion was that bacitracin serves as a structural unit in the spore coat.

Several observations made in this laboratory were inconsistent with the above conclusions, and consequently made it important to determine whether the spore coat composition of the B. licheniformis strain employed by us was the same as that reported by Bernlohr and Sievert. Spores of B. licheniformis (ATCC 10716) were harvested from a four day old culture containing one per cent tryptone grown at 32°. The vegetative cells were removed by treatment with lysozyme and the spores were isolated by differential centrifugation. A "spore coat" fraction was obtained by disrupting the isolated spores in a Mickle disintegrator and collecting the sedimentable

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fraction (5). Spores suspended in water were shaken for 30 minutes with a weight of glass beads (average diameter 0.2 mm) equal to the weight of the suspension. The disintegration was followed by measuring the optical density of a diluted sample of the suspension at 620 mμ. During the 30 minute treatment the optical density decreased from 12.4 to 1.95. Direct count with a phase contrast microscope showed that less than one per cent of intact spores remained. The disrupted spore preparation was centrifuged and the sediment was washed thoroughly with water. The isolated "spore coat" material in two separate preparations was 43.4 and 43.8 per cent of the dry weight of the intact spores. The isolated fractions were hydrolyzed for 24 hrs in 6 N HCl at 105° in an evacuated, sealed tube. The hydrolysate was taken to dryness and the amino acid composition was determined using the Spinco Amino Acid Analyzer Model 120B (6). The determination of ornithine which was present in only trace amounts was carried out on the 50 cm column (Column 3) normally used for the determination of basic substances in physiological fluids. The amount of material applied to the columns was approximately 1 mg except in the case of the ornithine determination for which 5 mg was used. The results on two separate "spore coat" preparations are given in the table below. The number in parenthesis following certain of the amino acids, indicates the number of residues of this amino acid found in bacitracin.

AMINO ACID COMPOSITION OF SPORE COAT

Amino Acid		μmoles/mg	Amino Acid		μmoles/mg
Ornithine	(1)	.005 -	Proline		.32, .31
Lysine	(1)	.56, .71	Glycine		.79, .82
Histidine	(1)	.35, .41	Alanine		.43, .37
Ammonia		.84, .91	Half-Cystine	(1)	.26, .23
Arginine		.14, .14	Valine		.28, .25
Aspartic Acid	(2)	.84, .85	Methionine		.10, .13
Threonine		.26, .24	Isoleucine	(3)	.24, .17
Serine		.29, .30	Leucine	(1)	.27, .24
Glutamic Acid	(1)	.43, .37	Tyrosine		.86, .99
			Phenylalanine	(1)	.42, .32

It is obvious that the amino acid composition of the "spore coat" as determined has no relationship to bacitracin. Not only are the ratios of the amino acids which are constituents of bacitracin far different, but there are present a number of amino acids not found in bacitracin (i.e., tyrosine, glycine, proline, serine, threonine and valine). Indeed the composition would appear to be that of a protein, although of one with unusually high tyrosine content. The determination of ornithine is a particularly valuable measure of the bacitracin content since this amino acid is not found in proteins. Assuming that all of the ornithine found was derived from bacitracin, it can be estimated that nor more than 0.74 per cent of the "spore coat" could contain bacitracin. This determination was also carried out on the Amino Acid Analyzer with a hydrolysate of the soluble fraction obtained after disruption of the spores. In this case the ornithine content was found to be 0.008 μmoles per mg of material. Again this would correspond to a maximum of 1.25 per cent of bacitracin in the soluble fraction.

In order to rule out the possibility that the spore preparation was contaminated by some other organism, the spores were plated out on agar medium to allow for the isolation of individual clones. A total of forty-eight clones were isolated and transferred to flasks containing a medium suitable for growing B. licheniformis. After the cultures had grown for forty-eight hours, the antibiotic in the medium was determined. Every one of the cultures produced antibiotic; the actual amounts varied between the limits of 10-15 units of bacitracin per ml. By this criteria, the spore preparation is free of non-producers of antibiotic.

The results presented in this report are totally unlike those of Bernlohr and Sievert and no ready explanation for the differences can be offered. In both cases the isolated "spore coats" are approximately 40 per cent of the spore weight and the amino acids recovered are about 80 per cent of the "spore coat". Whatever the explanation is for the differences, it is clear that bacitracin is not a structural unit of the spore coat of all organisms which produce the antibiotic.

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