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Peptidoglycan Differences in Strains of *Bacillus cereus* Constitutive and Inducible for Penicillinase Production

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Strains of Bacillus cereus differing in penicillinase production were shown to possess cell walls of differing muramic acid contents.

Although it is generally agreed (1, 9, 11) that penicillinase (EC 3.5.2.6) is somehow related to a cell wall biosynthetic enzyme, the exact nature of this relation is still obscure. There appear to be two main viewpoints. Pollock (9) envisions an ancient gene duplication, followed by separate evolution, in response to the reputed existence of penicillin as a selective force in nature. In this case, the two enzymes would contain only partially the same amino acid sequence. Alternatively, penicillinase may be a direct, limited step mutation from that biosynthetic enzyme (1). If so, the question arises whether the original biosynthetic capacity has been retained, lost, or altered. The two cell wall biosynthetic enzymes most commonly suggested to be related to penicillinase are D-alanine carboxypeptidase and transpeptidase (9, 11).

We studied cell walls from Bacillus cereus NRRL 569 and B. cereus 569/H (NCTC 9945), inducible and constitutive, respectively, for penicillinase production. These strains differ only by a point mutation (6). Nonconstitutive revertants can be isolated from strain 569/H which again possess basal levels of penicillinase identical to 569. Observed differences in cell wall structure are interpreted in terms of an altered biosynthetic capacity. Ampicillin-resistant strains of Escherichia coli were previously shown (2) to possess "weakened" cell walls.

The amino sugar content of acetone- and ether-washed whole cells is a quantitative measure of cell wall content (7). Two methods were used to make this determination. The average of three Elson-Morgan determinations (7) showed 569/H to possess 40% more amino sugars of all types than did 569. Application of hydrolyzed samples to an amino acid analyzer showed 569/H to possess 37% more glucosamine.

Hydrolysates of mechanically purified cell walls yielded the results shown in Table 1. The purity of the samples is apparent in the absence of any peaks other than those indicated. The walls appear to contain a typical diaminopimelic acid peptidoglycan with the carboxyls of both glutamic acid and diaminopimelic acid present as amides. There is at least one teichoic acid or other glucosamine-galactosamine containing polymer and no attached whole protein.

The most interesting difference between these two strains is the lowered muramic acid value of 569/H. We interpret this to indicate the presence of an enhanced number of extended-chain, peptide cross-links (5, 8,10), in which the L-alanine at the start of one tetrapeptide sequence is no longer attached to muramic acid but is instead attached to the D-alanine at the end of another tetrapeptide. The observed muramic acid difference occurs without a concurrent drop in the amino acids which comprise the peptide cross-links. The lack of such a 1:1 relationship has been observed (3, 4) in many other bacteria and may be a common consequence of extended peptide chain formation.

Significantly, no extended-chain, peptide cross-links have been observed(4) to contain two adjacent D-alanines, indicating that one has been lost in the chain extension process. A transpeptidase has been hypothesized (10) to be the agent of such lengthening. It is not yet clear whether this transpeptidase is identical to the transpeptidase known (11) to form the D-alanyl-diaminopimelic acid cross-links, but the decreased muramic acid content of strain 569/H appears to be due to the enhanced production or activity of a chain-extending transpeptidase. We cannot yet say for certain whether this enhanced activity is due to derepression of the enzymes responsible concomitantly with that of penicillinase in strain 569/H, or whether penicillinase itself still possesses such a biosynthetic function. For reasons presented in

TABLE 1. *Composition of mechanically purified Bacillus cereus cell walls*[a]

Component	Strain[b]	
	569	569/H
Diaminopimelic acid	29	28
Glutamic acid	27	25
Muramic acid	21	10
Ammonia	54	44
Glycine	3	1
Alanine	47	46
Glucosamine	82	84
Galactosamine	24	26

[a] Eighteen-hour cultures were harvested and washed with water. Cells were broken in a 9-kc sonic oscillator. The cell walls were collected by centrifugation and washed repeatedly with phosphate buffer (pH 7.0). They were then incubated at 37 C for 3 hr in phosphate buffer (pH 7.6) with 0.1 mg of trypsin per ml and again washed repeatedly with buffer. Samples were hydrolyzed overnight at 92 C in 6 N HCl and analyzed on a Technicon automatic amino acid analyzer.

[b] Values expressed as micromoles (±1 μmole) per 100 mg of cell wall.

detail elsewhere (Nickerson, Ph.D. Thesis, University of Cincinnati, 1969), we favor the latter view.

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