STUDIES OF THE CLASS A HIGH-MOLECULAR WEIGHT PENICILLIN-BINDING PROTEINS IN *BACILLUS SUBTILIS*

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(ABSTRACT)

The survival of all organisms depends on their ability to perform certain enzymatic activities and the ability to construct certain structures. In prokaryotes, enzymes are required for the final reactions of peptidoglycan (PG) synthesis, the structural element of the bacterial cell wall. These proteins, known as penicillin-binding proteins (PBPs), are identified through the presence of conserved motifs within their functional domains. The Class A high-molecular weight PBPs are bifunctional, performing the penicillin-sensitive transpeptidase activity and the glycosyl transferase (GT) activity required for the polymerization of the glycan strands. The Class A PBPs in *Bacillus subtilis* are PBP1, PBP4, PBP2c, and PBP2d (YwheE) and they are encoded by *ponA, pbpD, pbpF,* and *pbpG* (*ywhE*), respectively. These proteins appear to be somewhat functionally redundant because removal of one or more does not cause any noticeable change in phenotype. However, the loss of PBP1 has previously been demonstrated in *B. subtilis* to cause a decreased growth rate and changes in morphology of vegetative cells, both of which are increased upon the additional loss of PBP4.

Furthermore, the loss of sporulation-expressed Class A PBPs, PBP2c and 2d, causes a 10,000-fold decrease in the production of heat resistant spores. This double mutant is shown to have changes in the structural parameters of cortex PG that appear minor when compared to other strains, but are coupled with a large defect on the deposition of cortex PG, apparently from the synthesis of an abnormal germ cell wall. The Class A PBPs are believed to be the only proteins capable of performing the GT activity and it is therefore believed that cell viability requires the presence of at least one functional Class A PBP. This requirement has been demonstrated in other organisms, but a *B. subtilis* strain lacking all Class A PBPs is viable. The phenotypical changes seen in the PBP1 mutant are exacerbated in this strain. The GT activity remaining in this strain is sensitive to the antibiotic moenomycin *in vitro* whereas it appears resistant *in vivo*. Identification of the protein(s) performing this novel GT activity will rely on the demonstration of the GT activity *in vitro*.