CLONING AND EXPRESSION OF SPORE CORTEX-LYTIC ENZYMES

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In *Bacillus subtilis* two proteins, CwlJ and SleB, are involved in degradation of the cortical peptidoglycan during spore germination. CwlJ is of particular interest as it is thought to be activated *in vivo* by the efflux of Ca-DPA from the spore core, and offers the potential for signal amplification using the novel holographic platform technology. The objective of this work was to produce recombinant histidine-tagged cortex-lytic enzymes (CwlJ & SleB) for application in a novel holographic spore detection system. Fragments of DNA containing the *cwlJ* and *sleB* open reading frames, amplified from *Bacillus subtilis* genomic DNA using Kod polymerase (Novagen), were digested with Nco1 and Xho1, and ligated with pET24d digested with the same enzymes to create his-tag fusions. These respective plasmids, pET24d:*cwlJ*-His_6 and pET24d:*sleB*-His_6, were then used to transform *E. coli* BL21(DE3) for IPTG induced protein expression. His-tagged proteins were purified from solubilised inclusion bodies using a Novagen His-bind purification system. Bands of the expected size for CwlJ and SleB were observed by PAGE. Proteins were re-folded by dialysis in a variety of re-folding buffers, however, to date, only recombinant SleB has been observed to show some activity against purified cortical peptidoglycan. Experiments aimed at preparing functional CwlJ, and studies into the mode of activation by Ca-DPA, are currently being conducted.

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