**CotE and temperature-dependent assembly and function of the *Bacillus cereus* spore surface layers**

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The environmental conditions during sporulation can have a strong impact on the structure, composition, resistance and other functional properties of bacterial spores. In particular, the sporulation temperature is known to affect the spore resistance to heat, the composition of the spore surface layers, and their germination response. The surface layers of *Bacillus cereus* spores are formed by a proteinaceous coat in turn surrounded by a “balloon-like” structure called the exosporium. Assembly of the coat and exosporium rests on a complex protein interaction network that operates in the mother cell during sporulation and involves several morphogenetic proteins, among which CotE.

We have studied the role of the CotE protein in the assembly of the surface layers of *B. cereus* ATCC14579 spores and how it could be affected by the sporulation temperature. We have compared the morphology and functional properties of wild type (WT) and ∆*cotE* spores formed at 37°C or 20°C. Deletion of the *cotE* gene had a limited impact on sporulation efficiency, which was nevertheless lower at 20°C than at 37°C. However, ∆*cotE* spores were more resistant to moist-heat at 90°C than WT spores, regardeless of the sporulation temperature. ∆*cotE* spores also showed higher susceptibility to UV-C, to hydrogen peroxide, and to the peptidoglycan-breaking enzymes lysozyme and mutanolysin. Both ∆*cotE* and WT spores produced at 20°C were more sensitive to those treatments than spores produced at 37°C. Moreover, ∆*cotE* spores were impaired in inosine-triggered germination and were less hydrophobic than WT spores, regardeless of the sporulation temperature. The differences in the resistance and functional properties of the spores were paralleled by extensive modifications in spore morphology as revealed by transmission electron microscopy. ∆*cotE* spores produced at 37°C exhibited a fragmented and detached exosporium and in addition, when produced at 20ºC, a misassembled coat. A proteomic analysis also showed some modifications in the spore protein content caused by *cotE* deletion and by sporulation temperature.

Our results strongly suggest that CotE plays a major role in attachment of the exosporium to the spore coat in *B. cereus* spores and that formation of the spore surface layers is strongly impaired by the absence of CotE, an effect that is exacerbated when decreasing the sporulation temperature.