The *yhbB* gene contributes to the structural organization of the *Bacillus subtilis* spore coat in a temperature-dependent manner

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The spore surface of *Bacillus subtilis* is a highly organized and multilayered structure that plays a crucial role in environmental persistence and stress resistance. The correct assembly of the spore coat depends on a tightly regulated network of sporulation-specific transcription factors (1), yet the function of several coat-associated proteins remains insufficiently understood. Among these, we investigated the role of YhbB in spore coat organization. Transcriptional reporter fusions confirmed the previously reported σ^E-dependent expression of *yhbB* (2) and revealed an additional requirement for SpoIIID, with expression further modulated by temperature. YhbB-GFP fluorescence was clearly detected during sporulation at 25 °C but was strongly reduced at 37 °C, indicating temperature-dependent regulation acting at the transcriptional and/or post-transcriptional level, potentially involving the 5′UTR region. Fluorescence microscopy showed that YhbB localizes around the forespore, forming ring-like structures consistent with coat assembly intermediates. In a *cotH* mutant background, YhbB localization was altered at late sporulation stages, consistent with previous evidence of a functional interaction between YhbB and CotH, a coat protein implicated in coat stability through covalent cross-linking reactions (3). Functional assays revealed that *yhbB* mutant spores produced at 25 °C display reduced germination efficiency, particularly in response to alanine, whereas no differences were observed in spores produced at 37 °C. These results indicate that YhbB-dependent structural features established during sporulation at lower temperature influence spore germination efficiency. Overall, our findings identify YhbB as a σ^E/SpoIIID-dependent factor contributing to temperature-dependent spore coat organization, consistent with a structural role linked to CotH-mediated coat stabilization.

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Oral presentation