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Ultrastructural details of resistance factors in *Bacillus subtilis* spores revealed by cryo-FIB milling and electron tomography

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Résumé

Bacterial spores are dormant cells that can withstand a wide range of stresses, including antibiotics, detergents, irradiation and high temperatures. Such resilience is an asset when spores are used for the benefit of humans, as in the case of probiotics or spore-based delivery technologies. On the other hand, it represents a major problem in terms of infectious diseases, food safety or biological warfare when it comes to spores of pathogenic bacteria. Despite their importance for public health and industry, the assembly, architecture and composition of the molecular structures contributing to spore resistance are not yet fully elucidated. A major constraint in understanding these features lies in their nanometric dimensions, which require high-resolution observation methods to be studied.

Cryo-electron tomography (Cryo-ET) meets this need and opens wide frontiers towards the three-dimensional visualization of ultrastructures and molecules in the cell. The resolution power of cryo-ET is however limited by the penetration of electrons, thus requiring sample thinning to reach molecular details. In the past decade, cryo-focused ion beam milling monitored by scanning electron microscopy (cryo-FIB/SEM), emerged as a state-of-the-art sectioning technique to generate cell lamellae with nanoscale precision.

In our work, we have implemented cryo-FIB/SEM-ET to access deep cell regions and shed light on ultrastructures of sporulating *Bacillus subtilis* cells. Our tomograms show a wide variety of cellular ultrastructures with unprecedented level of details, including DNA compaction in the forespore, the architecture of the mother cell and forespore envelopes, and early coat layers. Segmentation was performed to get a three-dimensional overview of the cellular content. In parallel, we investigated the nature of the nascent coat layers in various mutant strains using cryo-FIB/SEM/ET and transmission electron microscopy on resin sections of freeze-substituted bacteria. Combining these two cellular electron microscopy approaches, we distinguish seven nascent coat regions with different molecular properties, and propose a model for the contribution of the morphogenetic proteins SpoIVA, SpoVID, SafA and/or CotE.