Researchers track how spores break out of dormant state

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Tapping into the unknown world of awakening dormant bacterial spores, researchers have revealed through atomic force microscopy (AFM) the alterations of spore coat and germ cell wall that accompany the transformation from a spore to a vegetative cell.

When starved of nutrients Bacillus (rod-shaped bacteria) cells initiate a series of genetic, biochemical and structural events that result in the formation of metabolically dormant spores. They can remain dormant for extended periods and, partly because of their tough spore coat, have a significant resistance to extreme environmental factors including heat, radiation and toxic chemicals. However, once in favorable conditions, spores break the dormant state through germination and reenter the vegetative mode of replication.

Although significant progress has been made in understanding the biochemical and genetic bases of the spore germination process, it is still unclear how a spore breaks out of its dormant state.

But a new in vitro study of single germinating Bacillus atrophaeus spores details how the spore coat structures break down, and it shows with unprecedented resolution how the new bacterium emerges from the disintegrating spore. The new research, led by Lawrence Livermore National Laboratory scientists, appears in the May 28-June 1 early online edition of the *Proceedings of the National Academy of Sciences*. The research appears in this week's (June 4) issue of *PNAS*.

“A thorough understanding of spore germination is important for the development of new countermeasures that identify the earliest stages of a wide range of spore mediated diseases, including botulism, gas gangrene and pulmonary anthrax,” said Alexander Malkin, senior author from LLNL’s Biosciences and Biotechnology Division. “But it’s also important to gain fundamental insights into the key events in bacterial cell development.”

The researchers, including Marco Plomp, lead author at LLNL, and those from Children’s Hospital Oakland Research Institute and Northwestern University, used AFM to identify disassembly of the outer spore coat rodlet structures, which appear to be structurally similar to amyloid fibrils that have been associated with neural degenerative diseases, such as Alzheimer’s and prion diseases. “The extreme physical and chemical resistance of Bacillus spores suggests that evolutionary forces have captured the mechanical rigidity and resistance of these amyloid self-assembling biomaterials to structure the protective outer spore surface,” Plomp said.

When exposed to a solution that triggers germination, nanometer sized etch pits were seen developing in the rodlet layer. These etch pits evolved into ever widening fissures, leaving narrow strips of remaining rodlet structure. In the end, 1- to 3- nm-wide fibrils remained. The in vitro AFM imaging also revealed the porous fibrous cell wall structure of newly emerging and mature vegetative cells, consisting of a network of nanometer-wide peptidoglycan fibers. “These results show that dynamic AFM is a promising tool to investigate the formation and evolution of the bacterial cell wall,” Malkin said.

Source: Lawrence Livermore National Laboratory

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