RESEARCH PROFILE

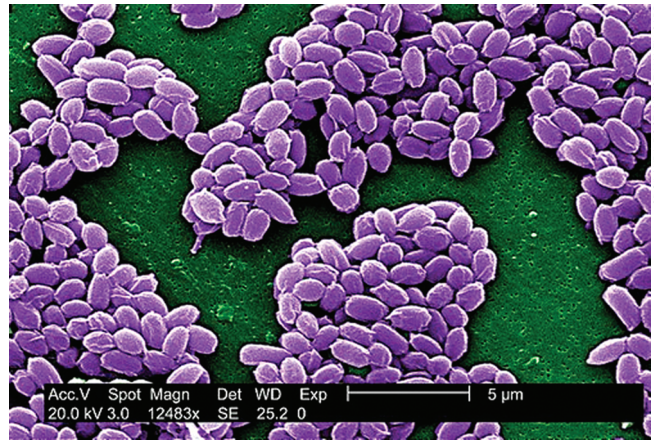
Tracking refractive and molecular changes during bacterial spore germination

Some bacteria including those that cause food spoilage, anthrax, and botulism take on a highly protective dormant structure called a spore. Developing better ways to kill these bacteria requires an understanding of germination, the process during which bacteria emerge from dormancy. In *AC* (DOI 10.1021/ac1003322), Yong-qing Li and colleagues at East Carolina University and the University of Connecticut Health Center report new findings on the germination of single *Bacillus cereus* spores obtained by combining phase contrast microscopy, Raman spectroscopy, and optical tweezers.

Peter Setlow of the University of Connecticut Health Center explains, "While dormant spores are relatively hard to kill, the germinated spore has lost most of the resistance properties that make dormant spores so very hard to kill. Consequently, understanding the mechanism of spore germination could lead to ways to block germination, thus preventing spores from causing food spoilage or disease, and/or stimulate spore germination, thus making it much easier to kill the spores and again prevent food spoilage and disease."

One method used to study germination is phase contrast microscopy. Phase shifts that occur as the illuminating light passes through the specimen translate into dormant spores appearing brighter because of their high refractive index and germinated spores appearing darker. Time-lapse phase contrast microscopy has shown two changes in refractility during germination. It is thought that during the first change (stage I), the small molecule calcium dipicolinic acid (CaDPA) is released and replaced with water, and during stage II, the peptidoglycan cortex degrades and the spore becomes fully hydrated. However, this has not been proven because phase contrast microscopy doesn't provide molecular information.

Combining phase contrast microscopy with Raman spectroscopy, which can analyze molecular components, would allow correlation of spore refractility with specific molecular changes occurring during germination. However, combining these techniques comes with a challenge. In most phase contrast microscopy setups, the phase plate is placed on the back pupil of phase objectives, blocking much of the weak Raman scattering light.



Spores of a *Bacillus* species. Magnification is 12,483×.

"We used an external phase configuration that allows the phase plate to be placed in an intermediate image plane such that high-throughput objectives can be used for Raman spectroscopy and optical tweezers, while performing phase contrast microscopy," says Li of East Carolina University.

By using a highly efficient CCD and spectrograph, the researchers increased the time resolution of Raman spectroscopy by approximately an order of magnitude (to ~2 s) as compared to their previous work. They found that the phase contrast intensity of individual dormant spores was proportional to the spore's CaDPA content. In addition, the end of the first stage of germination as identified by phase contrast microscopy precisely corresponded with complete CaDPA release shown with Raman spectroscopy. Such information might allow phase contrast microscopy to be used alone to determine CaDPA levels.

They also used the setup to study spores adhered on a coverslip rather than optically trapped and were able to pinpoint the likely phase in the spore germination process where there is variability in the process of spore germination, Setlow says.

Because the technique can be used to study the behavior of large numbers of individual microorganisms, Setlow says, it could help reveal differences in the behavior of genetically identical organisms. These differences can be hidden when studying millions of bacteria but are often crucial to understanding how bacterial populations can respond when stressed or attacked.

"This is particularly important in bacterial spore germination, since some spores in populations germinate very quickly (a few minutes), while some small number may germinate only after many hours or even days," Setlow says. "These latter slow germinators are a major concern, since potentially they can come back to life and cause food spoilage or disease long after one might suspect no spores are still present."

Alexander Malkin from Lawrence Livermore National Laboratory says that the approach could be useful for in vitro studies that evaluate the responses of a wide range of microbial and cellular systems to various treatments and environmental changes. "One of the current great scientific challenges at the intersection of life and physical sciences is to define the biophysical pathways of cellular life and to elucidate the molecular mechanisms that carry out cellular and microbial function and propagate the disease," he says. "The capability to simultaneously analyze refractive properties and molecular composition of a single germinating spore with high temporal resolution meets these requirements, and has the capacity to provide significant insights into the fundamental understanding of key cellular processes."

—Nancy D. Lamontagne