Dynamic phase microscopy, a new method to detect viable and killed spores and to estimate the heterogeneity of spore populations

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One of the challenging tasks in monitoring studies is to estimate heterogeneity of microbial populations by the physiological state and potential viability of individual cells, especially with regard of their ability to withstand various environmental assaults. Previously, we described some approaches based on electron microscopy methods to discriminate vegetative, dormant, and dead cells in both aged microbial cultures and environmental samples, including permafrost. In this communication, we propose to extend the arsenal of microscopy methods for monitoring studies by a new non-invasive and informative method - dynamic phase microscopy (DPM). The substantial advantage of DPM is that it gives quantitative (digitized) data of un-destroyed (living) microscopic objects, exemplified in our work by Bacillus licheniformis spores. Using DPM made it possible to record interference images of objects (spores) and to produce picture of their “phase thickness” (PT) that is the optical path difference in nm. Thus, it was demonstrated the remarkable difference in the PT of spores at different physiological states: dormant, germinating, and heat-killed spores had PT values of 80 nm, 40-50 nm, and 20 nm, respectively. The other found criterion to distinguish between spores was the PT fluctuations. In contrast to dormant and killed spores, the PT of germinating spores oscillated with amplitude of up to 7 nm, with typical frequencies of 1.3 and 3.4 Hz. A combination of the recorded PT values and PT fluctuations gave a key to detect viable and dead cells. Under the conditions that did not support germination (the lack of nutrients), we were able to follow the response of a single dormant spore and a spore population to heating from 25 °C to 70 °C. Thus, a very small temperature change (from 40 °C to 42 °C) under conditions non-favorable for germination, caused a drastic decrease in the spores’ PT; the second drop in the PT values was observed during heating from 60 °C to 70 °C. These changes were reversible: after cessation of heating, PT values became similar to dormant spores. So,

DPM allowed us to track the first, reversible stage of activation, when a spore maintains the attributes of the dormant state. Under the conditions that favor germination (in the presence of nutrients), irreversible changes in the PT and spore diameter, d, were detectable in a single germinating spore and spore population. In addition, DPM allowed an easy estimation of the heterogeneity of spore populations. It is a great advantage of DPM that it makes possible to reveal the ability of spores to respond to various stimuli with or without further germination and outgrowth - the salient feature of a living cell. Advantages of DPM and its applicability for solving different tasks, especially in monitoring studies, are discussed in this communication.