Experimental report

13/05/2019

Proposal: 8-04-827 **Council:** 4/2017

Title: Biological behavious of bacterial spores

Research area:

This proposal is a new proposal

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Samples: B. subtilis spores (native, FB122 and PS4150 mutants)

Instrument	Requested days	Allocated days	From	To
IN13	9	9	15/06/2018	24/06/2018

Abstract:

Report on June 2018 IN13 experiment 8-04-827
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Biological behavior of bacterial spores

Scientific background

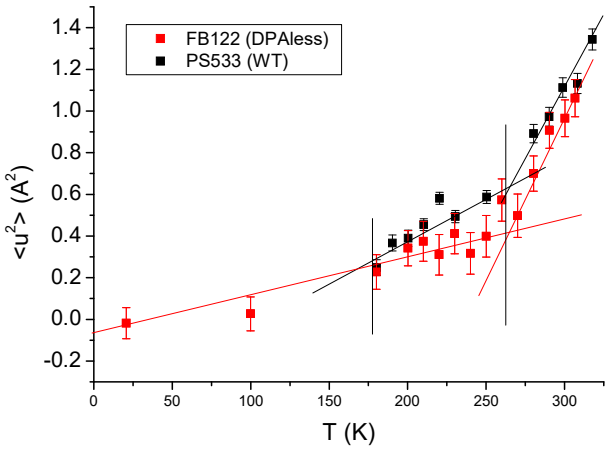
Among food contaminants, bacterial spores represent the main challenge for the decontamination processes because of their exceptional resistance properties to heat, high pressure, dehydration, but also to chemicals. *B. subtilis* is a ubiquitous bacterium that is mostly found in soils. Although it is considered as non-pathogenic, it has been widely studied [1], as it is considered as a model for other sporulating organisms such as *B. cereus* or *B. anthracis* which are important pathogens. An interesting aspect of these organisms is their ability to enter a dormant state under starvation condition, through a differentiation process known as sporulation. This specialized dormant cell, called a spore, has an exceptional resistance to harsh environmental conditions such as extreme heat [2,3].
The sporulation mechanism starts as an asymmetric cell division. The mother cell then engulfs the daughter cell, which synthetizes in the different layers/compartments of the future spore with the help of the mother cell. The mother cell lyses, freeing the endospore in the medium. The spore is composed of 6 different layers: the coat, the outer membrane, the cortex, the cell wall, the inner membrane and the protoplasm. The coat is an important component in the resistance of the spore since it is the first barrier against external aggressions [4]. The protoplasm is characterized by low water content (about 0.6 h, where $h = g_{water}/g_{dry\ matter}$), an elevated concentration of ions and a complex of dipicolinic acid (DPA) and Calcium (Ca^{2+}). The protoplasm is the core structure of the spore, where it stores all the components, such as proteins, enzymes and DNA, necessary to its return to a vegetative cycle. It has been shown that the low water content hinders the denaturation of these macromolecules, and is in part responsible for the spore's heat resistance [5]. The mechanisms involved in the resistance of bacterial spores still remain partly unclear, particularly regarding the protection of core's vital components, but some studies tend to show that it would be related to their mobility.

The germination of dormant spores is the first crucial complex phase in the return of spores to vegetative growth. This process is normally triggered by nutrients through the activation of germination receptors located in the inner membrane. When the germination process starts, various events take place such as: rehydration of the protoplasm and DPA+ions leakage, followed by the lysis of the cortex and partly of the coat. These events are accompanied by the progressive loss of endospores' resistance which makes them more sensitive to heat, pressure, UV light and hydrogen peroxide [6] [7].
In previous experiments performed on IN13 instrument at ILL we observed two interesting dynamical behaviors which could help in understanding the resistance of bacterial spores in relation to their internal dynamics. The first one was the apparent lack of dynamical transition at low temperatures [8], typically observed on proteins at the same hydration level (0.4h), which could reflect a partial immobility of core proteins. The second one concerned the sharp intensity drop that was accompanying the germination of bacterial spores (report 8-04-668). It showed that the structural change occurring along germination can be detected by incoherent neutron scattering.

Results

- Search for the dynamical transition around 220 K

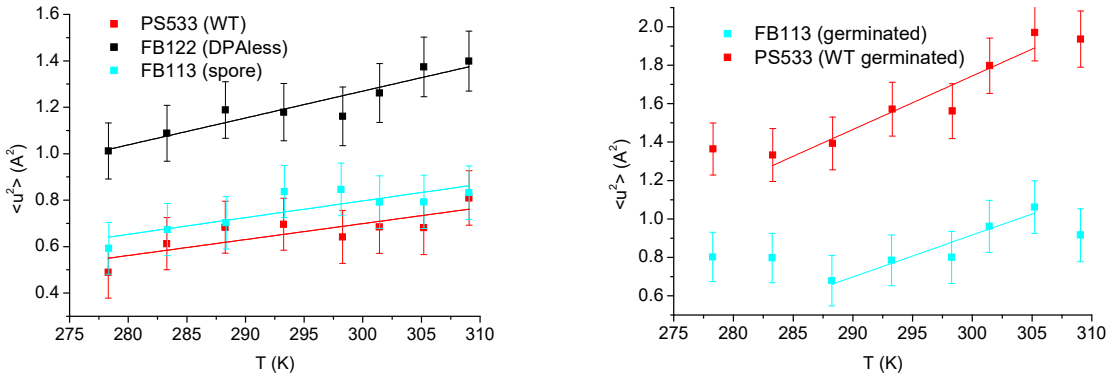
As proteins are the major constituents in microorganisms [9], their dynamics should dominate and we expect the dynamical transition to be measurable.
We performed a temperature scan on the spore mutant FB112 and compared it to the wild type non-germinated spore. The mutant is lacking dipicolinic acid, which is present in large quantity, to evaluate its role in core components mobility. We used the FB122 mutants of *Bacillus subtilis*, depleted for DPA synthesis.



The figure above shows the mean square displacements (MSD) of the two samples, the lines are guides to the eyes. Two changes of slope are apparent for both samples around 180 and around 270 K, but the data does not permit to identify their origin. The behavior of both samples is clearly different.

- Dynamics of fully germinated spores in the powder form:

We performed experiments on fully germinated *B. subtilis* spores (triggered by nutrients and checked offline) in order to extract MSD and to compare them to our previous data on the dynamics of dormant spores to shed light on the resistance of spores related to core mobility in these two distinct states. Experiments were performed on cell pellets at ≈ 1.3 h in D_2O to ensure a maximal loading of water in the core for experiments at positive temperature.



The figure shows MSD of dormant state (left side) and germinated state (right side) samples. The samples are clearly more mobile in the latter state. Our first observations showed that fully germinated spore (Wild type germinated) MSD are higher than the MSD calculated for all other spore samples (i.e. partially germinated FB113 spores, and ungerminated wild-type, DPAless and FB113 spores).

A complete data analysis is under progress and a publication in preparation.

References

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