Experimental Report

05/03/2014

Proposal: **8-04-686** **Council:** 10/2012

Title: Physical behaviour of bacterial spores and water: implications for spore resistance

This proposal is a new proposal

Researh Area: Biology

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Samples: Bacillus subtilis spore

Instrument	Req. Days	All. Days	From	To
IN6	0	3	22/07/2013	25/07/2013
IN16B	8	5	12/07/2013	17/07/2013
IN13	5	5	05/06/2013	10/06/2013

Abstract:

Bacillus subtilis cells have the capacity to form spores which are metabolically dormant in response to starvation. In this form, bacterial spores are highly resistant to stress and can survive for millions years [1].They even represent the most resistant model alive. Interestingly, when the conditions become favorable, bacterial spores can readily break their dormancy and return to their previous state of vegetative cells, the so-called germination process [2,3]. The mechanisms involved in the resistance and germination of bacterial spores still remain only partly clear.

One of the main specificity of bacterial spore is its ability to maintain the spore core in a relatively constant hydration state (around 8.5 and 15 % spore water content over dry weight) whatever the external conditions are. This low, but constant hydration seems essential to maintain the functionality of vital biomolecules (ribosomes, enzymes, membrane receptors,…) which are required for spore germination. Quantity, localization, mobility and state of core water are up to now poorly known. Comprehension of core hydration would certainly help to optimize drying methods and spore inactivation in food sterilization

Report on experiment 8-04-686 on IN13 (June 2013) and on IN6 and IN16B (July 2013)

Physical behavior of bacterial spores and water: implications for spore resistance

Bacillus subtilis cells have the capacity to form spores which are metabolically dormant in response to starvation. In this form, bacterial spores are highly resistant to stress and can survive for millions year [1].They even represent the most resistant model alive. Interestingly, when the conditions become favorable, bacterial spores can readily break their dormancy and return to their previous state of vegetative cells, the so-called germination process [2, 3]. The mechanisms involved in the resistance and germination of bacterial spores still remain only partly clear. This lack of understanding is an obstacle for food preservation when the harsh conditions of sterilisation can destroy the sensorial qualities of food products.

In a recent experiment on IN13 (ILL exp. report CRG 1910), we studied spores in the wild type (PS533) and a mutant form that lacks most of the outer coat layers (PS4150) by performing elastic incoherent temperature scans. We clearly saw a change in the slope of the mean square displacements close to 370 K, where an endothermic peak appeared for DSC measurements. In addition we got beam time on IN5 (ILL exp. report 8-04-668) what permitted to measure at an energy resolution around 75 µeV to see particularly the effect of hydration. However, the previous samples were prepared in D_2O, so that we studied mainly the spores themselves. Now we would like to apply a similar approach as Tehei et al. [4] to shed light specifically on bound and free water populations within the samples, which are particularly visible when measuring quasi-elastically and in H_2O. To get an as complete picture as possible, we measured elastically on IN13 (energy resolution 8 µeV), and quasi-elastically on IN6 (energy resolution of 90 µeV) to see free and confined water populations, and on IN16B (energy resolution of 1 µeV) to investigate the tightly bound water. In this way we would like to compare our results found on the physical state of water in bacterial spores with those reported by Sunde et al. using NMR [5].

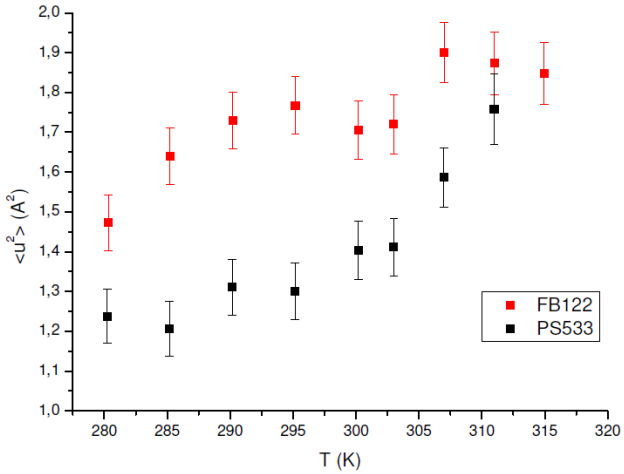

Figure 1: Mean square displacements (MSD) of the wild type spore PS533 and of the mutant FB122 as function of temperature. The samples were hydrated to about > 1 g H_2O/g dry sample.

Figure 1 shows an elastic scan taken in June 2013 on IN13. It permitted to see that the wild type sample underwent a change in the MSD slope above 305 K whereas the mutant did not. This change might be correlated to the DPA leakage from the wild type spore, triggering the germination process and thus, some rehydration of the spore core. This event has been probably hampered for the mutant strain due to the absence of DPA in the core.

For the experiments on IN6 and IN16B, we used the same types of samples. For the wild type PS533 and the mutant FB122 we had now "dry samples" (equilibrated over 75% relative humidity water atmosphere corresponding to 0,20 and 0,26 g H_20/ g dry matter), wet samples (> 1g H_2O/g sample) and decoated wet samples, where the proteinaceous outer layer (i.e. coat) was chemically removed.

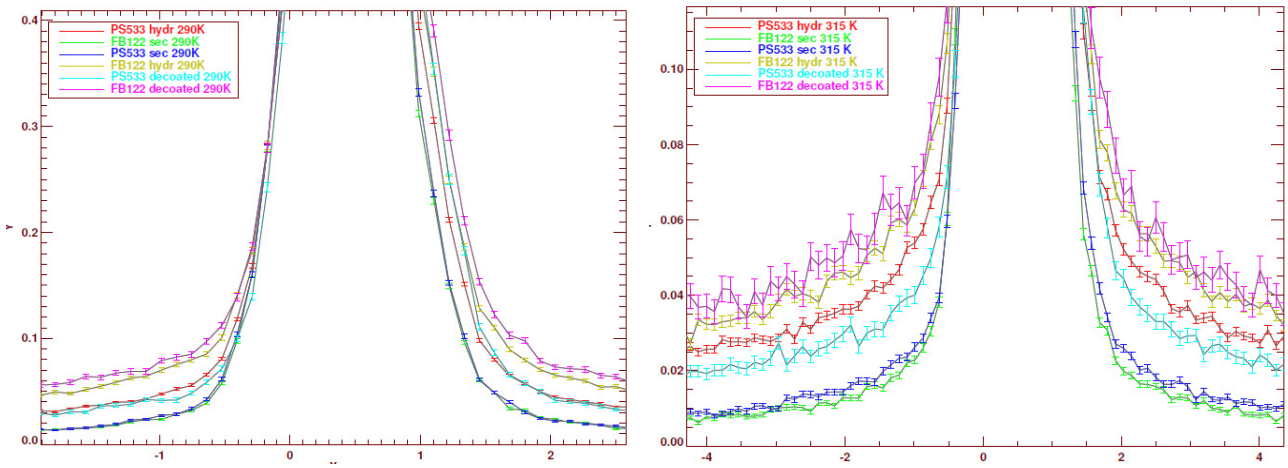

Figure 2: Quasi-elastic scans summed over all available scattering angles taken on IN16B with the same time ramps as on IN13.

The "dry samples" (75% hydration) were the less flexible ones, as expected. The wild type PS533 was less flexible than the mutant FB122, in accordance with the results obtained on IN13.

More detailed analysis of the QENS data taken on IN16B and on IN6 are actually under progress.

[1] R.H. Vreeland, W.D. Rosenzweig, D.W. Powers, Nature 407, 897 (2000)
[2] S.J. Foster, K. Johnstone, in Regulation of procaryotic development, ed. by I. Smith, R. Slepecky, P. Setlow (ASM, Washington, 1989), p. 89
[3] A. Moir, B.M. Corfe, J. Behravan, Cell. Mol. Life Sci. 59, 403 (2002)
[4] Tehei M, Franzetti B, Wood K, Gabel F, Fabiani E, Jasnin M, Zamponi M, Oesterhelt D, Zaccai G, Ginzburg M, Ginzburg BZ. 2007. Proc Natl Acad Sci USA. 2007, 104(3):766-71
[5] E. P. Sunde, P. Setlow, L. Hederstedt and B. Halle, Proc Natl Acad Sci USA 106, 19334 (2009).