BACILLUS SUBTILIS investigated by bio- and nanotechnological methods

O. Hekele[1], C.G. Goesselsberger[1], M. Brandstetter[1], M. Aumayr[1], S. Radel[1], R. Sommer[2] and I.C. Gebeshuber[1]

[1]Institut für Allgemeine Physik, Vienna University of Technology,
Wiedner Hauptstraße 8-10, 1040 Vienna, Austria

[2] Clinical Institute of Hygiene and Medical Microbiology - Water Hygiene, Medical University of Vienna
Kinderspitalgasse 15, 1095 Vienna, Austria

ABSTRACT

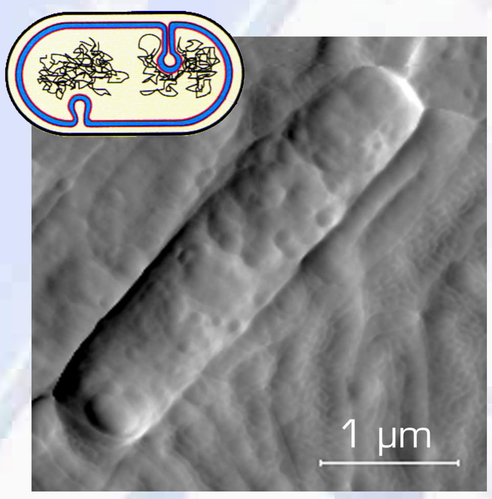
Bacillus subtilis is a single celled bacterium commonly found in soil. It can sporulate, i.e. reversibly form a tough and protective endospore that allows the organism to tolerate extreme environmental conditions. *B. subtilis* is not harmful to human health and its robust spores may therefore serve as safe model organisms for pathogenic microorganisms in drinking water. Thus, this organism can be used to monitor the quality of water disinfection devices that utilize UV radiation. One type of *B. subtilis* spores is highly resistant to UV irradiation, whereas the other type shows a low UV resistance.

Biotechnological methods used are ultrasonic treatment for separating cells from nutrient solution and inducing sporulation in vegetative *B. subtilis* by induction of adverse environmental conditions, such as shortage of certain nutrients.

Intermittent contact atomic force microscopy mode (using an MFP-3D from Asylum research, Santa Barbara, CA, USA) was used to discern vegetative bacteria cells from spores [1]. The living cells were imaged on days 2, 3, 4 and 7 after induction of adverse conditions. Different sporulation stages (stage 0 to stage VII) are revealed by this nanotechnological method.

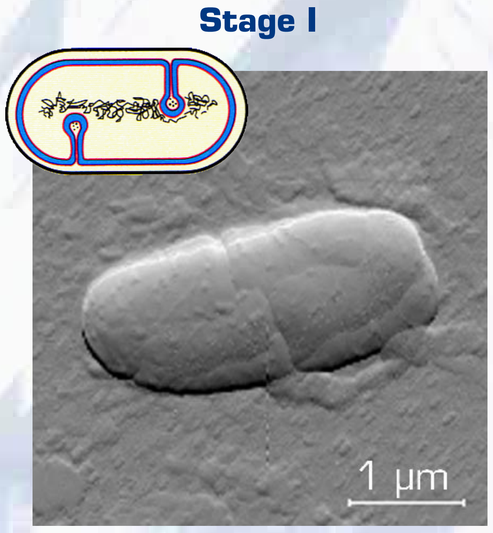
Detailed scientific understanding of the sporulation of this organism shall provide information regarding the development of novel biomimetic UV resistant materials.

Stage 0



During endospore formation, a vegetative cell is converted to a nongrowing, heat-resistant structure by several cellular differentiations. Inserts from (2)

Stage I



DNA becomes more dense as the Bacillus starts sporulating.

PREPARATION

Calcium chloride - Columbia Agar

By adding a sterile 1% calcium chloride solution to the plates of Columbia agar, the salt concentration rises, which stimulates endospore formation (3, 4). Calcium is an essential ion in the sporulation process, as it combines with dipicolinic acid and forms a complex. This complex reduces water availability within the endospore and therefore dehydrates it and stabilizes the endospore to heat denaturation by inserting bases of the DNA. After 2, 3, 4 and 7 days the bacteria and spores were removed from the agar plates, centrifuged, washed and imaged with AFM

References

[1] Hekele O., Goesselsberger C.G., Brandstetter M., Aumayr M., Sommer R. and Gebeshuber I.C. (2007) "Nanobiological atomic force microscopy study of the sporulation of Bacillus subtilis.", Abstract Ninth Yugoslav Materials Research Society Conference YUCOMAT 2007, Herceg Novi, Montenegro, September 10-14, 2007

[2] Madigan M.T. and Martinko J.M. (2006) Brock, Biology of Microorganisms, Pearson Prentice Hall, Upper Saddle River, New Jersey, USA, 11th Edition, p. 91.

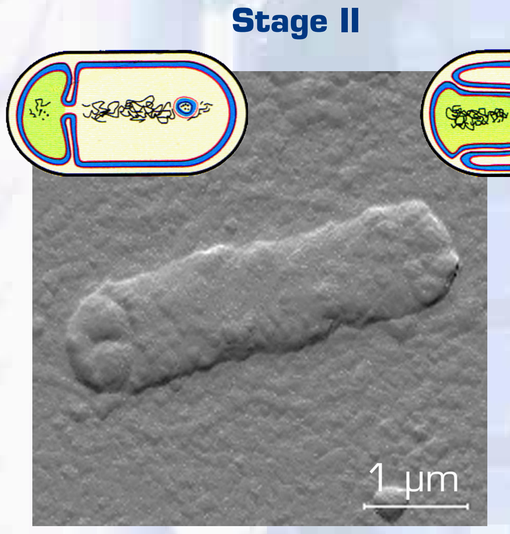
[3] Schlegel H.G. and Schmidt K. 1981 Allgemeine Mikrobiologie, Georg Thieme Verlag, Stuttgard - New York, 5th Edition.

[4] Sommer R., Cabay A., Sandu T., Lhotsky M., (1999) "Measurement of UV radiation using suspensions of microorganisms", J. Photochem. Photobiol. B: Biol. 53 (1999) 1 - 6

(5) Gröschl M. (1998) " Ultrasonic separation of suspended particles - Part II: Design and operation of separation devices", Acustica acta acustica Vol. 84 (1998) 1 - 1, Hirzel Verlag, EAA

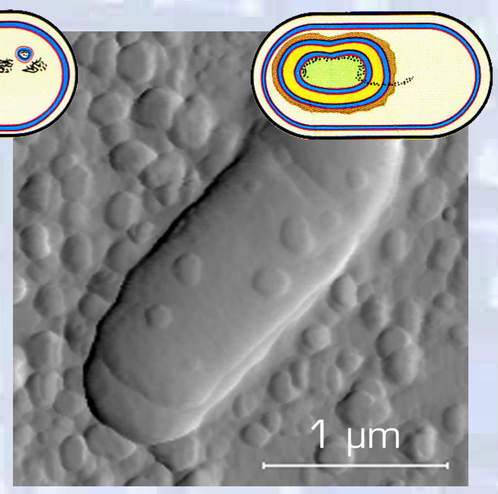
PROCESS OF SPORULATION UNDER THE AFM

Stage II



Endospore septum grows around protoplast (engulfment) and a forespore, containing a core, inner spore membrane and outer spore membrane, is formed.

Stage III/IV/V



As dehydration starts, the exosporium appears and the primordial cortex is formed between the two membranes.

ULTRASONIC PARTICLE SEPARATION

Ultrasonic resonator

Layers of cells of suspended solution collocate to nodes or antinodes of the standing ultrasonic wave.

Principle

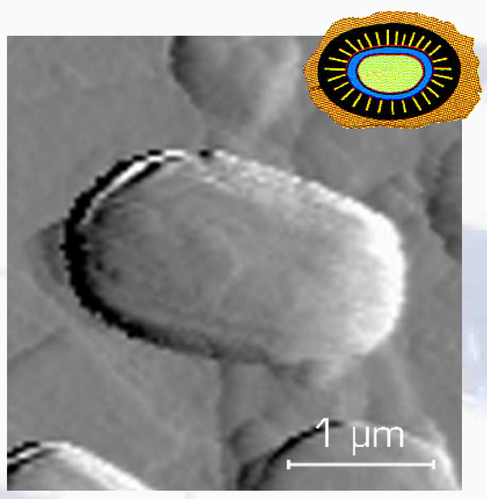
The effect of separating cells arises from forces acting on the particles when a dispersion is irradiated with ultrasound within a plane-wave resonator. The relation between the sound wavelength and the particle diameter is of great importance, the phenomenon is size dependent. When the volume is irradiated with ultrasound the initially homogeneously distributed particles are driven into the nodal regions of the standing wave field. The principle is used for filtration (AppliSens) (5)

Results

Promising results, during our first attempt to separate bacteria and spores from the culture medium via ultrasound, were obtained, as the particles formed segregated layers.

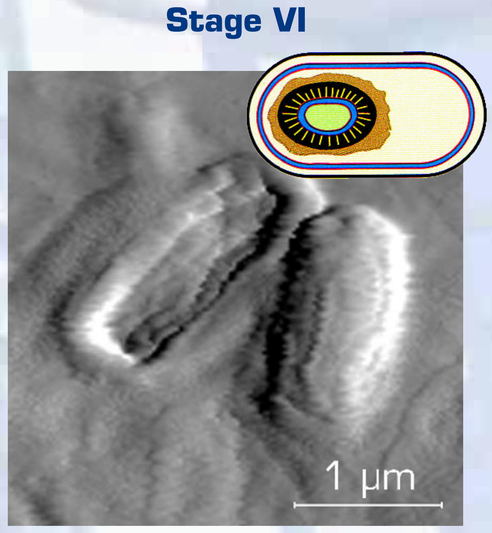
Further investigations are necessary, since the bacteria as well as the spores disintegrated during the procedure.

Stage VII



After the development of resistance to heat and chemicals the cell lyses and releases the mature endospore.

Stage VI



Incorporation of Ca^{2+} and further dehydration results in the production of SASP's (small acid soluble proteins) and dipicolinic acid, which are necessary for protection against ultraviolet radiation, desiccation and dry heat.

DISCUSSION AND OUTLOOK

Here, for the very first time, an atomic force microscopy investigation has revealed the different stages of sporulation at ultra-high resolution on living *B. subtilis cells and spores.* AFM techniques can also be used for manipulating samples and measuring of mechanical properties of cell membranes. In future it shall be investigated if there is a difference in the structure and stiffness of UV resistant and UV sensitive *B. subtilis* spores. Force mapping and viscoselasticity techniques shall be used to examine the spore coat properties. Ultrasonic particle separation seems to be a good method to destroy UV sensitive spores. Further investigations are necessary.

TU WIEN

Institut für Allgemeine Physik - Vienna University of Technology

poster design by F. A.