Summer practice: 6.8. – 13.8.2007 and 29.8. – 7.9. 2007

Day	Place	Time
06.08.2007	Hygieneinstitute, TU Vienna	9.30 - 18.30
07.08.2007	TU Vienna	9.30 - 14.30
08.08.2007	Hygieneinstitute, TU Vienna	8.30 - 15.00
09.08.2007	TU Vienna	9.30 - 16.45
10.08.2007	Hygieneinstitute, TU Vienna	8.30 - 15.00
13.08.2007	Hygieneinstitute, TU Vienna	8.30 - 18.00
20.08.2007	TU Vienna	9.30 - 17.00
21.08.2007	TU Vienna	9.30 - 17.30
22.08.2007	TU Vienna	9.30 - 17.00
23.08.2007	Centrum of Pharmacy	9.30 - 16.00
24.08.2007	TU Vienna	11.00 - 17.00
27.08.2007	TU Vienna	9.30 - 16.40
28.08.2007	TU Vienna	9.30 - 17.30
29.08.2007	TU Vienna	10.30 - 18.00
30.08.2007	TU Vienna	9.30 - 18.00
31.08.2007	TU Vienna	9.30 - 15.30
03.09.2007	TU Vienna	9.30 - 17.00
04.09.2007	TU Vienna	9.30 – 17.00
05.09.2007	TU Vienna	9.30 – 17.00
06.09.2007	TU Vienna	9.30 – 17.00

First week: 6.8 – 10.8, 13.8.2007

- Hygieneinstitute:
  - Culture medium (Columbia agar) was prepared on first day (6.8). 1% calcium chloride was added to the agar, as it rises the salt concentration and therefore stimulates endospore formation. The mixed solution was autoclaved for 15 minutes (…bar). In the meantime a tube already containing *Bacilli subtilis* was centrifuged twice, so that only the bacteria remained (The rest was washed away). Then the plates were filled with the autoclaved agar and the bacteria were withdrawn into the petri dishes using a pipetor (see Fig. 1).
  - After 2, 4 and 7 days 4 plates of bacteria were harvested via the same procedure: First about 5ml of pure (sterile) water was added to the skin,

Fig 1. Pouring the plates

Fig 2: Skin grown after 2 days

which had grown on the Petri dishes (see Fig. 2). Then using a spreader the water was mixed with the skin, so that the whole suspension could be taken away and filled in a glass, which was then put into a centrifuge (see Fig. 3). The speed of the centrifuge was chosen to be 5000 revolutions per seconds for 15 minutes and 15°C inside (see Fig. 4). The liquid sample (cells and distilled water) was spun at high speed. Due to the centripetal acceleration the substance of greater and lesser density could be separated. The water was poured off and the process was repeated again. After that procedure only the cells should remain, and the culture medium and water should be washed away. The cells were then again filled in a tube with distilled water and transported to the TU Vienna, where they were imaged with the AFM.

Fig 3: Centrifuge (Hygieneinstitute Vienna)

Fig 4: Settings of centrifuge

- TU Vienna:
  - In the first two days I was introduced to work with the Atomic force microscope. Then on the following days the samples of the

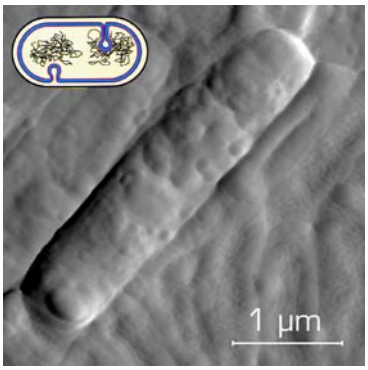

Fig 6: Stage 0 (vegetative bacterium)

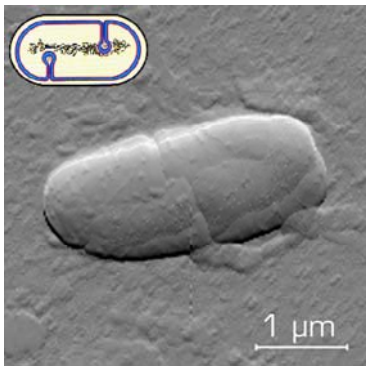

Fig 7: Stage I (Start of sporulation)

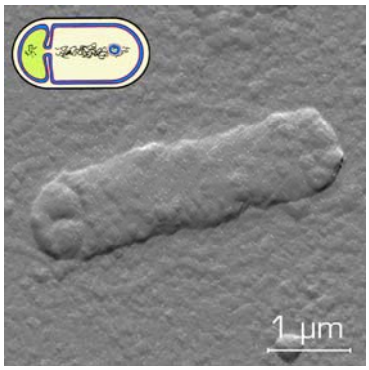

Fig 5: Stage II (Engulfment)

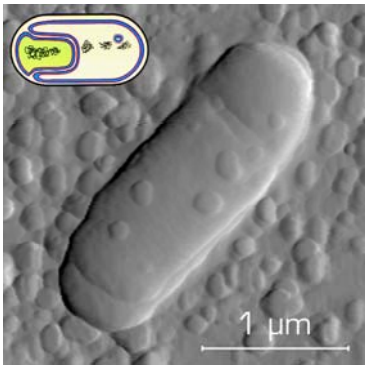

Fig 8: Stage III, IV, V (exosporium)

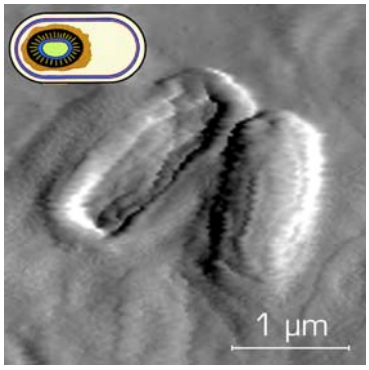

Fig 9: Stage VI (Lysis of mother cell)

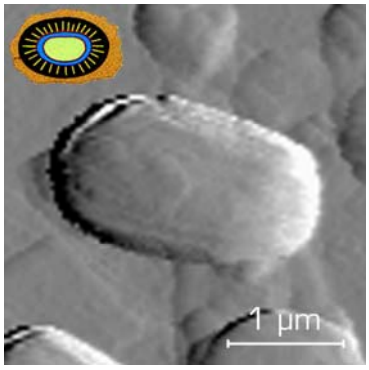

Fig 10: Stage VII (mature endospore)

vegetative cells and spores from the Hygiene institute were imaged. Different stages of the Sporulation process of *Bacilli subtilis* could be imaged with the AFM (see Fig. 5 – 10).

Second week: 20.8 – 24.8.2007

- TU Vienna:
  - Started working on the poster ("*BACILLUS SUBTILIS* investigated by bio- and nanotechnological methods", see attachment)
- Center of pharmacy (23.8.2007):
  - Introduction into the work of the center of pharmacy and about nanoparticles by Prof. Gabor
  - Delivering of stemcells from mice into new growth medium.
  - DNA staining from stemcells
  - Fluorescence microscopy with stemcells of mice (green: active cytoplasm, red: inactive nucleus)

Third week: 27.8 – 31.8.2007

- TU Vienna:
  - Ultrasonic Particle separation (experimental set-up see Fig. 11)
    - Solution of cells and culture medium (and distilled water) was used for the filtration process.

Fig 11: Experimental set-up of ultrasonic separation

    - H – shape resonator was used, which has one entry and two exits (H-shape resonator see Fig. 12). In the entry tube, the solution which should be separated was pulled up. In the H – shape receptor a frequency of about 2.03 MHz was used to create the ultrasound.
    - When the volume is irradiated with ultrasound the initially homogeneously distributed particles are driven into the nodal regions of the standing wave field. Layers of cells of suspended solution collocate to nodes or antinodes

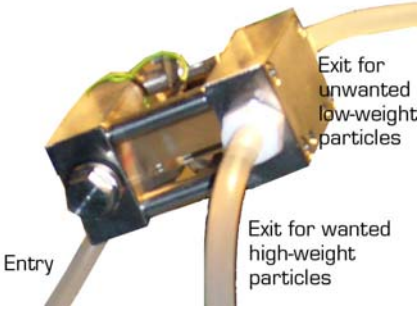

Fig 12: H-shape resonator

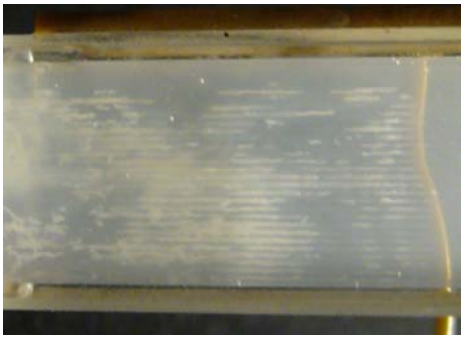

Fig 13: Layers of cells

of the standing ultrasonic wave (see Fig. 13)
    - In the upper exit the low weight particles (such as culture medium) should be extracted. In the lower exit the high weight particles (such as the Bacilli subtilis and the endospores) should sink down and should be extracted down the second tube. Both extracted solutions were imaged under the AFM.
    - Promising results appeared during the separation process as the particles formed segregated layers. But further investigations are necessary, since the bacteria as well as the spores disintegrated during the procedure.
  - The poster was finished (see attachment).

Fourth week: 3.9 – 7.9.2007

- TU Vienna:
  - Investigation how much pressure the spores survive: The spores were put on a glass slide and dried (1st sample: UV resistant spores, 2nd sample: UV sensitive spores). Then they were put into a the vacuum chamber (see Fig. 14). Using the roughing pump only, the chamber was evacuated to a final pressure of about $2 \cdot 10^{-2}$ mbar (or 0.015 Torr) (see Fig. 15).

Fig 14: Vacuum chamber

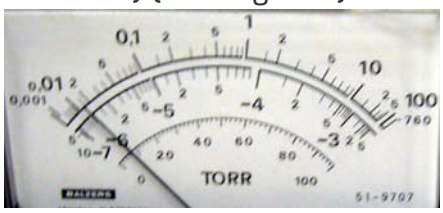

Fig 15: Total pressure of vacuum pump

Then the glass slides were imaged under the AFM. The spores seem to be intact, although deformations visible at certain spores indicate that a certain damage by the vacuum cannot be excluded (see Fig. 16, 17 for the UV sensitive spores and Fig. 18, 19 for the UV resistant spores).

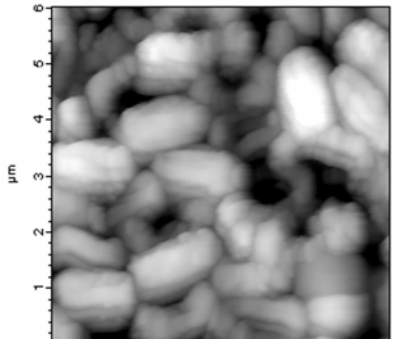

Fig 16: Height trace of UV sensitive spores imaged with the AFM after the vacuum treatment

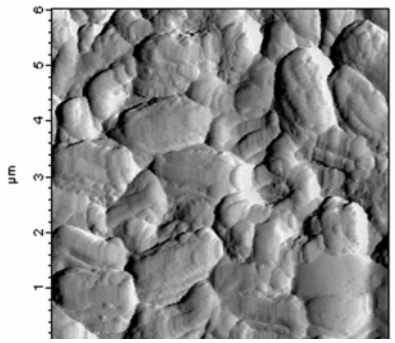

Fig 17: Amplitude trace of UV sensitive spores imaged with the AFM after the vacuum treatment

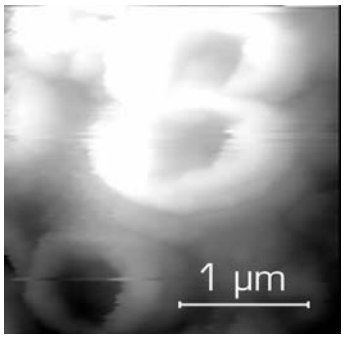

Fig 18: Height trace of UV resistant spores imaged with the AFM after the vacuum treatment

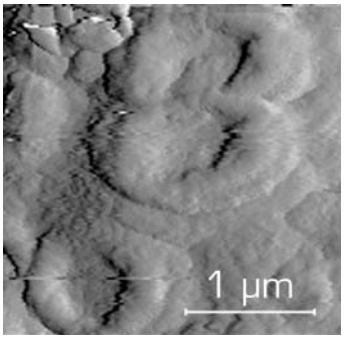

Fig 19: Amplitude trace of UV resistant spores imaged with the AFM after the vacuum treatment