Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Are bacteria claustrophobic? The problem of micrometric spatial confinement for the culture of micro-organisms

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1. VAHeat heating stage

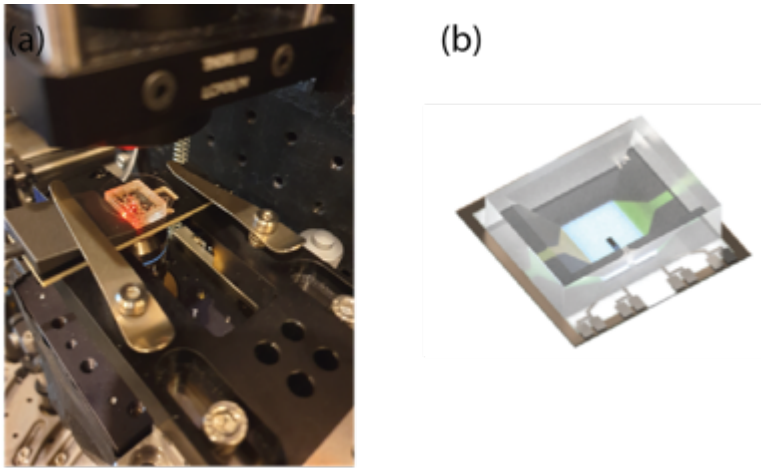

Fig. S1: VAHeat heating stage. (a) Experimental set-up with the substrate holder and the reservoir. (b) PDMS reservoir (outer dimension 16 x12 x 5 mm3 - extracted from the VAHeat user manual).

Experimental procedure:
0.2 µL of bacterial suspension (OD 0.3) was dropped in the bottom of the reservoir and immediately covered by a small coverslip (triangular shape). To observe at the same time bacteria under the coverslip and bacteria in open space, the coverslip has to be half the size of the bottom of the reservoir. Then the reservoir was filled with culture media and closed with a coverslip to prevent evaporation of the liquid. With the microscope stage, observation of growth was done at the border of the coverslip, in order to observe at the same time an area in open space and an area in confined space. After 1h30 of sedimentation, the heating was started manually and gradually to achieve the optimum growth temperature without any heat-shock.

2. Growth in 120 μm liquid thickness close to air bubble

These experiments highlighted a strong growth in 120 μm liquid thickness close to the air for *Geobacillus stearothermophilus* and *Thermus thermophilus*. On the same sample for each bacteria, an area away from air was observed. Concerning *Geobacillus stearothermophilus* (Figure S2), mainly sporulation occurred. For *Thermus thermophilus* (Figure S3) no growth was reported. These experiments argued that the presence of an air bubble or an air layer is mandatory to grow *Geobacillus stearothermophilus* and *Thermus thermophilus* in small liquid thickness.

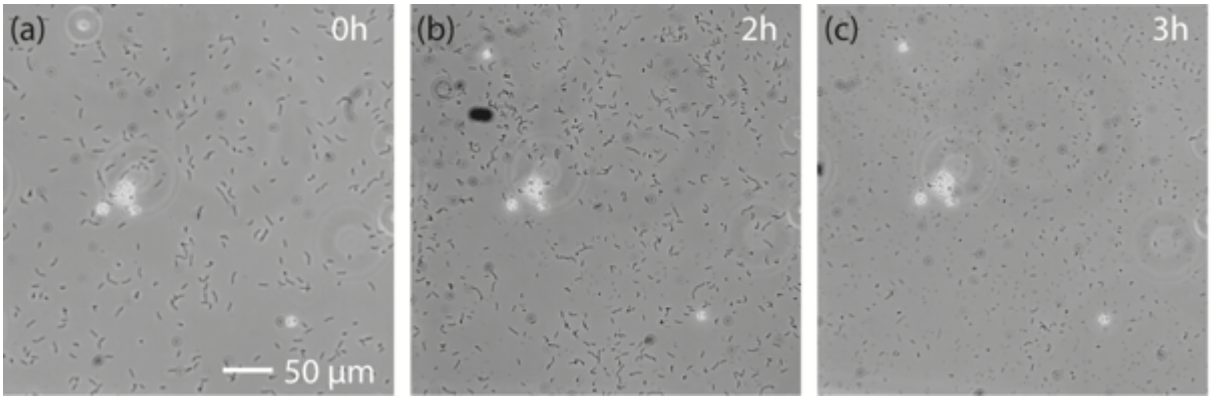

Fig. S2: Phase-contrast images of *Geobacillus stearothermophilus* after (a) 0h, (b) 2h and (c) 3h of incubation at 60°C.

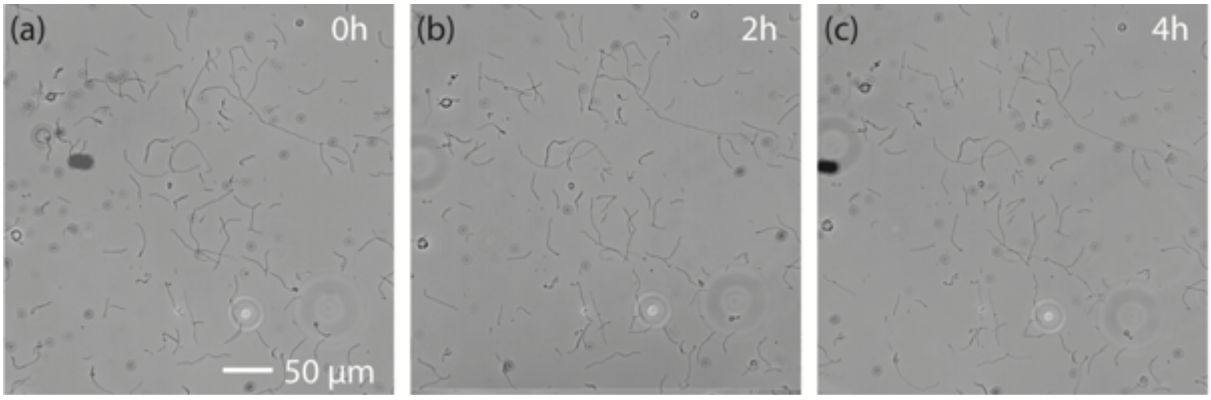

Fig. S3: Phase-contrast images of *Thermus thermophilus* after (a) 0h, (b) 2h and (c) 4h of incubation at 70°C.

3. Movies of the 4 bacterial strains

These movies were recorded in confined space and in open space simultaneously

Ecoli.mov
Growth of *E. coli* in confined space and in open space simultaneously recorded (SID4-sc8, Phasics) during 2h50. The optimal growth temperature (37°C) was addressed with the VAHeat heating stage. Growth was observed on both side, with or without spatial confinement, with a similar generation time.

LactobacillesR.mov
Growth of *Lactobacillus reuteri* in confined space and in open space simultaneously recorded (SID4-sc8, Phasics) during 4h40. The optimal growth temperature (35°C) was addressed with the VAHeat heating stage. Growth was observed on both side, with or without spatial confinement, with a similar generation time.

GeobacillusS.mov
Growth of *Geobacillus stearothermophilus* in confined space and in open space simultaneously recorded (SID4-sc8, Phasics) during 2h00. The optimal growth temperature (60°C) was addressed with the VAHeat heating stage. We observed growth only in open space.

ThermusT.mov
Growth of *Thermus thermophilus* in confined space and in open space simultaneously recorded (Orca C13440, Hamamatsu) during 2h30. The optimal growth temperature (70°C) was addressed with the VAHeat heating stage. We observed growth only in open space.

We provided an additional movie for *Thermus thermophiles* due to the small number of bacteria on each movie.

ThermusT2.mov
Growth of *Thermus thermophilus* in confined space and in open space simultaneously recorded (SID4-sc8, Phasics) during 1h30. The optimal growth temperature (70°C) was addressed with the VAHeat heating stage. We observed growth only in open space.

3. Comsol program

BacteriaClaustrophobia.mph
Comsol 5.6 program used for the production of the data of Figure 5.