

Compartmentalization of Bacteria in Microcapsules

Judith van Wijk¹, Tiaan Heunis², Erika Harmzen³, Leon M.T. Dicks², Jan Meuldijk¹, Bert Klumperman^{3,*}

Supporting information

Experimental

Materials

All chemicals were used as received, unless indicated otherwise. Tetraethyl orthosilicate (TEOS), octadecyltrichlorosilane (OTC) ($\geq 90\%$), dimethyldichlorosilane (DMDCS) ($\geq 99\%$), Trizma[®] base and trimethoxysilyl propyl methyl methacrylate (MPTS) ($\geq 98\%$) were from Sigma-Aldrich (PO Box 10434, Aston Manor 1630, South Africa). Ammonia (32%), *Tris* hydrochloride (molecular biology grade) and *n*-heptane ($\geq 99\%$) were from Merck-Chemicals (1, Friesland Drive, Longmeadow Business Estate, Modderfontein, 1645, South Africa). Ethanol (dehydrated AR) was from Biosolve (P.O. Box 53402, Kenilworth, Cape Town, 7745 South Africa). Double de-ionized water from an Elix Millipore purification system was used. *Lactobacillus plantarum* 423 was cultured in De Man, Rogosa, and Sharpe (MRS) broth (Biolab Diagnostics, Midrand, South Africa).

The membrane-permeable DNA fluorescent stain 4',6-diamidino-2-phenylindole (DAPI), the LIVE/DEAD[®] BacLight[™] Bacterial Viability Kit and DNA probes were from Life Technologies (Kwartsweg 2, 2665 NN Bleiswijk, Netherlands).

Methods

The silica microparticles were synthesized in a two-step process. In the first step, the silica microparticles were synthesized using the Stöber technique.^{26,27} In brief, ethanol (100 g), water (5 mL) and TEOS (7 g) were stirred with a magnetic stirrer bar at 30 °C in a three-neck round bottom flask. After 15 min, ammonia (15 mL, 25%, v/v) was added and the reaction allowed to proceed for 6 h. In the second step, 5 aliquots of TEOS (2 g) were added to the microparticles with time intervals of at least 6 h. A particle diameter of 800 nm was achieved assuming a density of 2.15 g mL⁻¹. This method allows for the synthesis of silica particles in the micron-size range while keeping them monodisperse.²⁸

The surface of the silica microparticles was modified to make them hydrophobic. The reaction with MPTS proceeded in a three-neck round bottom flask under continuous magnetic stirring at 25 °C. The silica microparticles (0.5 g) were suspended in *n*-heptane (10 g). Microparticles were separated from the ethanol solution by centrifugation (15 min, 1500 RPM) and dried at room temperature (25 °C). To this suspension 21 mg (73 μmol) MPTS was added (equivalent to 42 μmol m⁻²_{particle}) and the reaction was allowed to proceed for 24 h. As the surface of the microparticles changed from hydrophilic to hydrophobic, the microparticles dispersed into the *n*-heptane phase.

The inverse Pickering emulsions were produced by adding 2 mL water to 0.25 g modified microparticles dispersed in 15 g *n*-heptane. The droplets were calculated to have a diameter of 50 μm (equation S1). The Pickering emulsion was produced by vigorous shaking of the mixture for approximately 30 seconds.

$$R_D = \frac{3V_D}{N_A 2\sqrt{3}R^2} \quad (\text{S1})$$

Lactobacillus plantarum 423 was cultured, without aeration, in MRS broth at 37 °C for 24 h. Cells were harvested by centrifugation (8 000 x g, 10 min, 25 °C) and re-suspended in MRS broth or Tris-HCl buffer (pH = 8.0) to an Optical Density_{600nm} of 0.01 (equivalent to 1·10⁶ Colony Forming Units ml⁻¹). 2 mL bacterial suspension was added to the dispersed phase of the inverse Pickering emulsion, prepared as previously described.

Microcapsules were produced by the addition of the alkylchlorosilanes to the Pickering emulsion. First, OTC (0.4 mL, 1 μmol) was added to DMDCS (0.22 mL, 1.6 μmol) dissolved in *n*-heptane (4.4 mL). Two thirds of the obtained solution was added to the inverse Pickering emulsion to ensure proper mixing of the monomers before initiation of the interfacial reaction. No microcapsules were formed when only OTC or DMDCS was added. The interfacial reaction proceeded for 1 h under continuous overhead stirring at 37 °C in a three-neck round-bottom flask of 50 mL.

Analysis

Scanning Electron Microscopy (SEM) was used for imaging of the microcapsules and the microparticles. SEM was performed either on a Zeiss Evo MA15VP scanning electron microscope or on a FEI QuantaTM 3D FEG low vacuum SEM/Focused Ion Beam (FIB) instrument. Samples were prepared by placing a droplet of the sample on a sample holder, which was covered by double-sided carbon tape, and then dried at room temperature (25 °C). The samples were sputter-coated with a thin layer of gold for electrical conductivity.

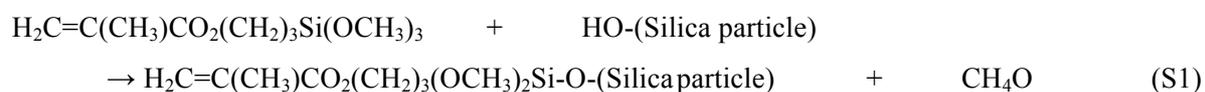
Light microscopy (LM) was used for imaging of the inverse Pickering emulsions and microcapsules. An Olympus CX31 Light Microscope and a Zeiss Axioplan Universal Microscope were used. Samples were prepared by placing a droplet of the inverse Pickering emulsions on a glass slide.

Confocal Fluorescence Microscope (CFM) imaging was conducted on a Carl Zeiss LSM 780 with an Elyra S.1 superresolution platform. The images were acquired with a 561 nm (100 mW) laser (red) and a 488 nm (100 mW) laser (green). Samples were prepared by placing a droplet of the inverse Pickering emulsions on a glass slide.

Thermogravimetric analysis (TGA) was performed on a TA Instruments high resolution TGA Q500 V6.7 apparatus. The measurements were either conducted using a constant temperature, or a heating rate of 10 °C min⁻¹ from 25 °C to 800 °C, in a nitrogen flow of 50 mL/min.

Differential Scanning Calorimetry (DSC) was performed on a DSC Q100 from TA Instruments. Measurements were carried out from -150 to 80 °C, with heating and cooling rates of 20 °C min⁻¹ under a nitrogen flow of 50 mL min⁻¹. Glass transition temperature (T_g) readings were obtained from the inflection point of the curve recorded during the first heating run.

Equation S1:



By reacting the silica microparticles with 3-(trimethoxysilyl)-propyl methacrylate (MPTS) in *n*-heptane it was found that MPTS is a suitable hydrophobizing agent²⁵ and the amount of MPTS agent necessary to provide the proper hydrophobicity to produce a stable inverse Pickering emulsion, was determined to be 1·10⁻² mL·m⁻²_{particle}, which corresponds to 42 μmol·m⁻²_{particle}.

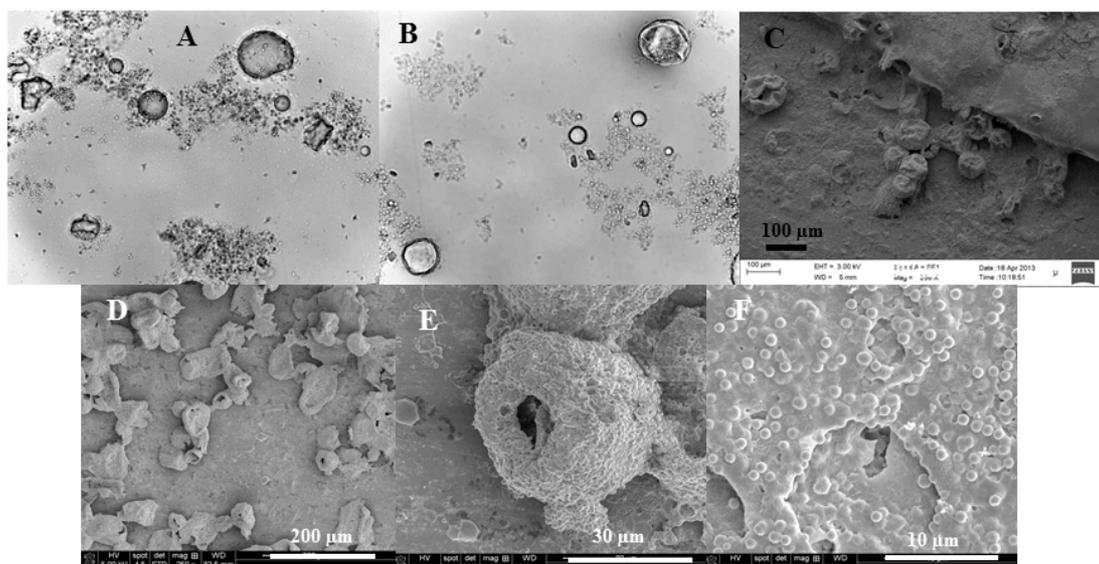


Figure S1. Light microscopy (A and B) and SEM (C - F) images of the produced microcapsules. Microcapsules were synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets, stabilized by hydrophobized silica microparticles. When zooming in on the shell, the newly formed polymer can be distinguished from the primary silica particles (F).

Figure S2 presents the weight loss of the polyalkoxysiloxane capsules from Figure 3B, C, E and F as a function of temperature, determined with Thermogravimetric Analysis (TGA).

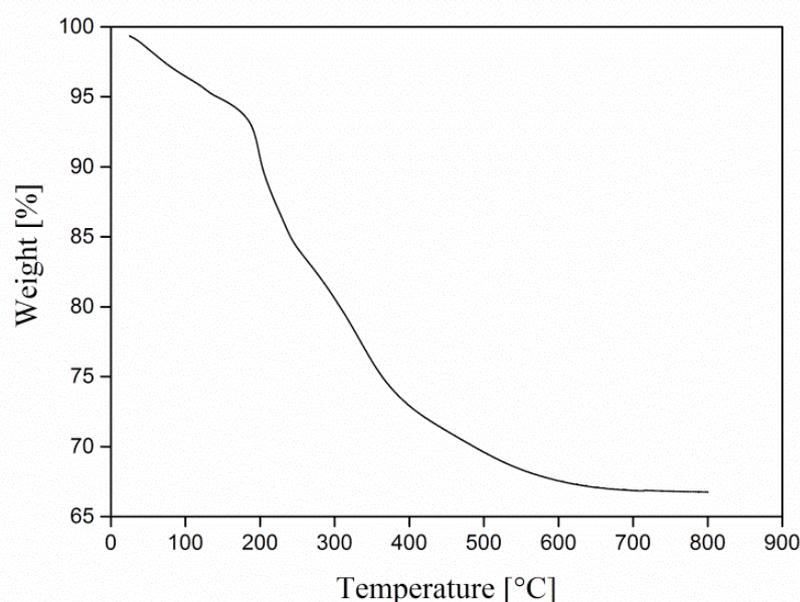


Figure S2. Thermogravimetric Analysis (TGA) curve of microcapsules. Microcapsules were synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets, stabilized by hydrophobized silica microparticles.

In Figure S2, the first weight loss region up to about 200 °C is attributed to physically adsorbed water. The weight loss between 200 and 250 °C is attributed to dehydroxylation, and the third region up to 400 °C is assigned to the degradation of the alkyl moieties. The last step up to 650 °C is assigned to the loss of polymer/copolymer backbone and the residual weight is silica. The glass transition temperature and melting temperature of the poly(organosiloxane) microcapsules were determined with Differential Scanning Calorimetry (DSC), Figure S3. The T_g was -95 °C and the melting temperature was determined to be 62 °C. A T_g of -95 °C is in line with the T_g of DMDCS, which is -122 °C and the melting temperature was expected, since the melting temperature of polyoctadecylsiloxane is 63 °C, probably caused by the octadecyl side chains.^{26,37,38} The melting peak around -30 °C could be of physically adsorbed water, which is known to decrease when adsorbed on silica gel.³⁹

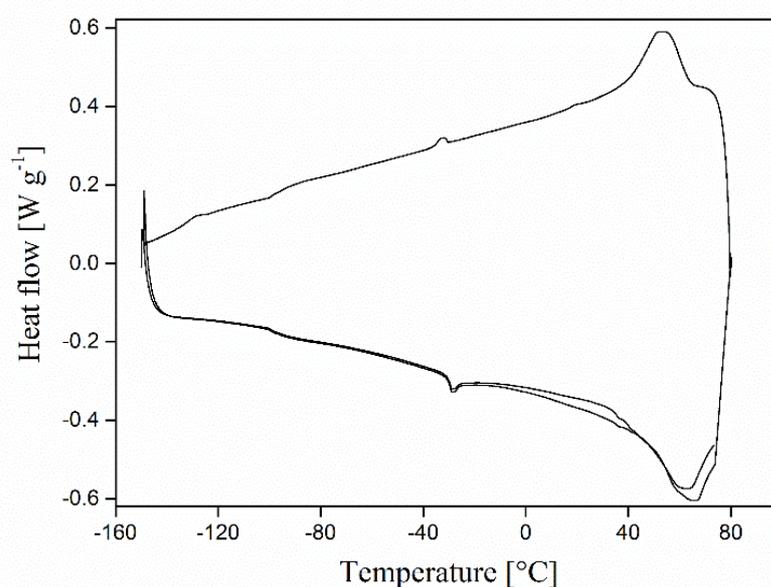


Figure S3. Differential Scanning Calorimetry result of microcapsules synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets, stabilized by hydrophobized silica microparticles. T_g of the polyalkoxysiloxane capsules was determined to be -95 °C.

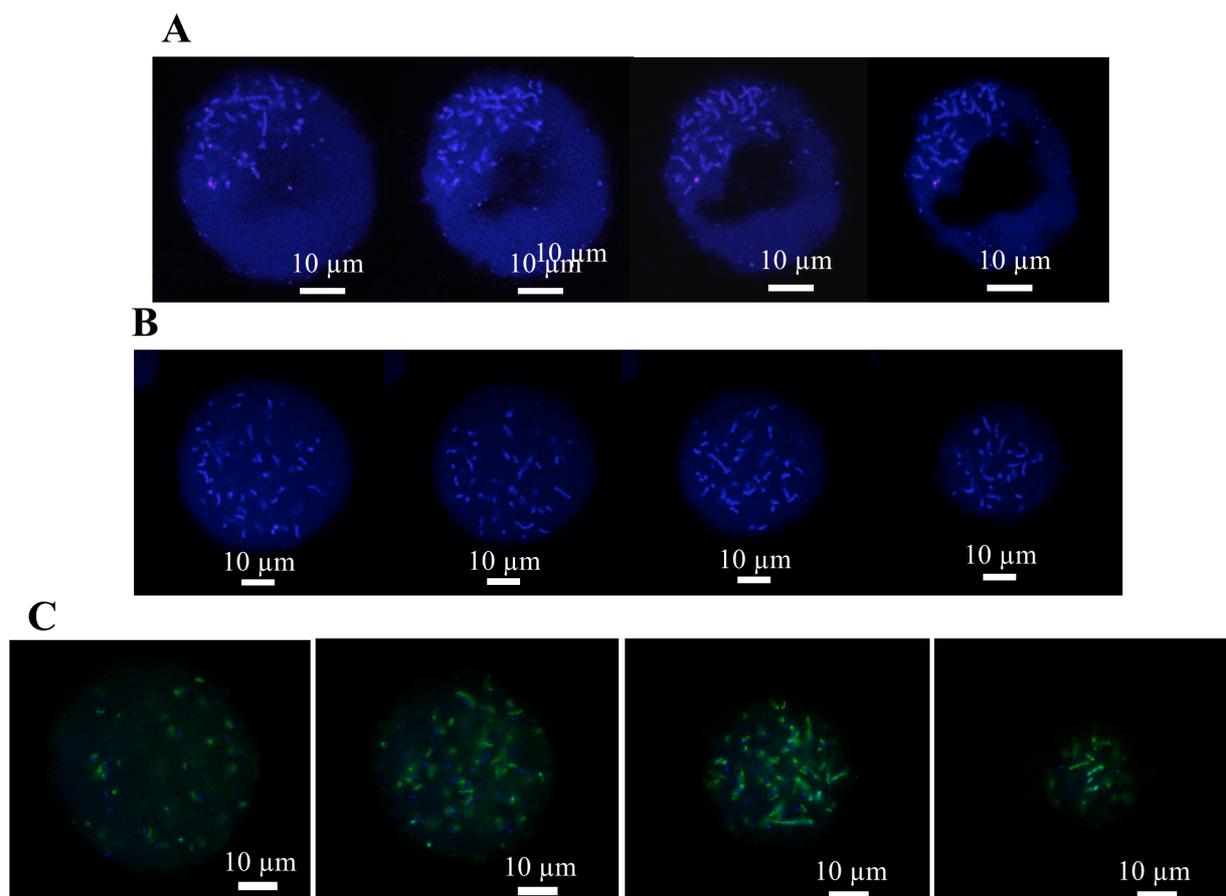


Figure S4. Confocal Fluorescence Microscopy images of isolated *L. plantarum* 423 bacteria in microcapsules. Microcapsules were synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets. In microbiology experiments that contain live bacteria should always be repeated 3 times to determine viability. A-C are independent replicate experiments with different bacterial strains, where only C is stained by SYTO 9. After the interfacial reaction of alkylchlorosilanes, the produced capsules were no longer stained by SYTO 9 as in Figure 4B-D and F. The reason for this different behavior is that the formed polysiloxanes possess very different characteristics from amorphous silica. Therefore, the green dye was not added to the experiments shown in Figures 5A and 5B, but only during the experiment of Figure 5C. Except for the used dyes and the bacteria strains, everything else in the 3 experiments was kept the same. In Figure 5 the deformation of the capsules can be recognized, since the water phase is also mildly stained blue.

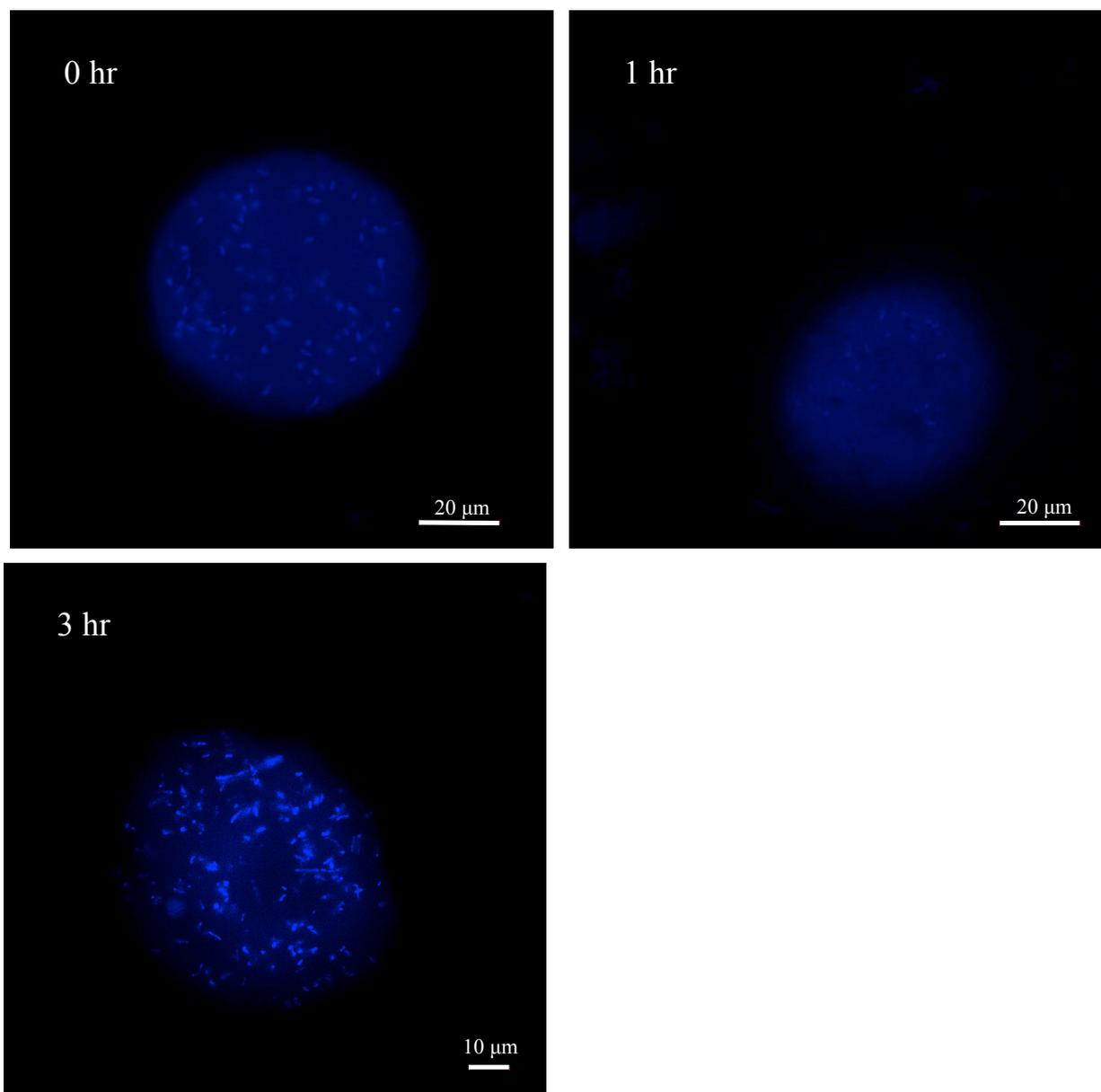


Figure S5. Confocal Fluorescence Microscopy images of isolated *L. plantarum* 423 bacteria in microcapsules. Microcapsules were synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets. All bacteria are stained with DAPI (blue), and dead bacteria are stained with propidium iodide (red). Images were taken immediately after encapsulation (0 hr) and after 1 and 3 hrs standing at room temperature.