Extremophiles

Cryo-electron microscopy of an extremely halophilic microbe: technical aspects

Daniel Bollschweiler, Miroslava Schaffer, C. Martin Lawrence, Harald Engelhardt

Max-Planck-Institut für Biochemie, Martinsried, Germany

engelhar@biochem.mpg.de

Online Resource 1 / Supplementary Figure

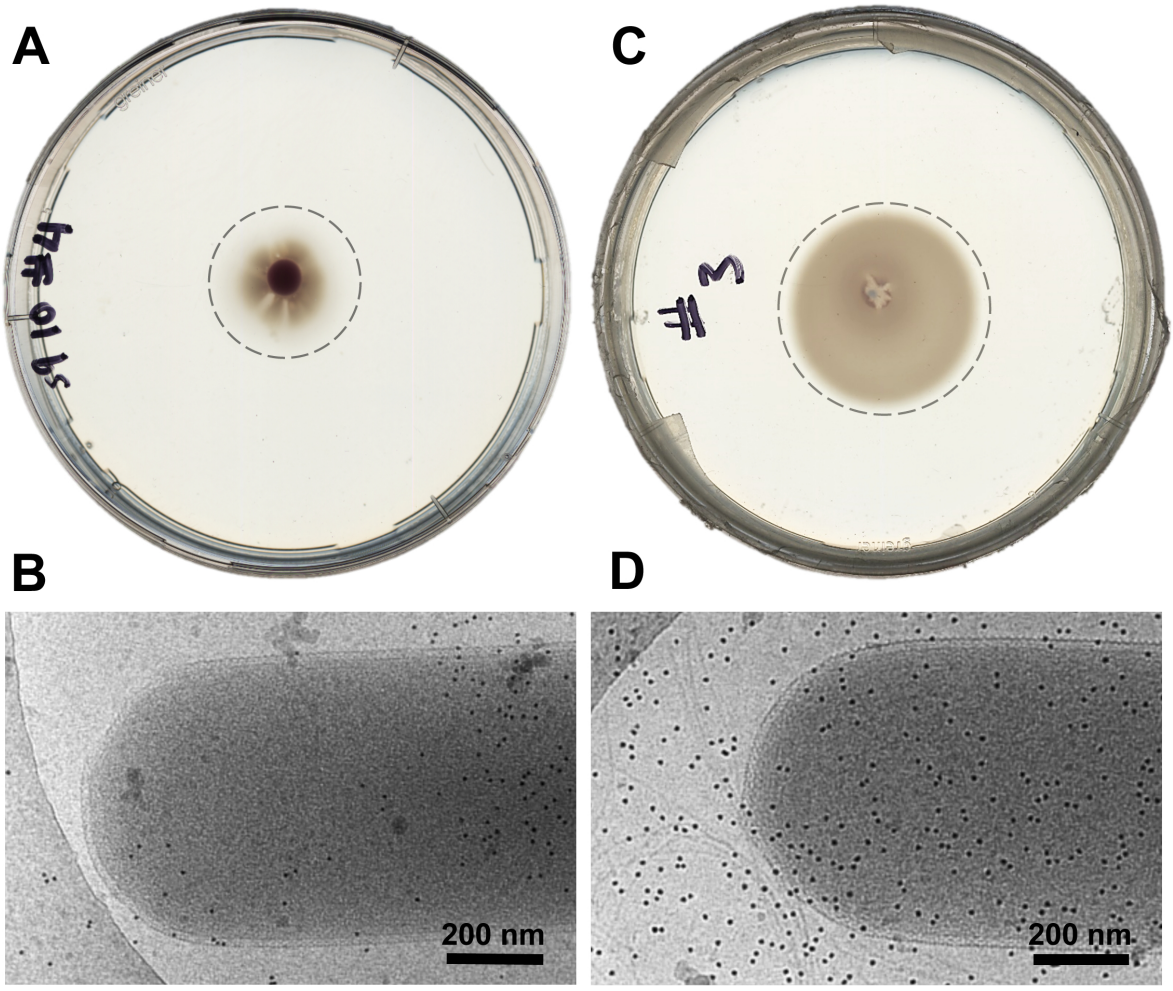

Fig. S1 Motility and flagellation of *Halobacterium salinarum* strains. (A) Swarm agar plate incubated with strain S9 after 9 days of growth. The circle indicates the very faint border of the swarm colony. (B) Cryo-EM image of a representative cell from a sample corresponding to plate A. (C) Swarm agar plate incubated with strain SW5 selected for motile cells after 9 days of growth. (D) Cryo-EM image showing a representative cell with massive flagellation from a sample corresponding to plate C. The black dots in images B and D originate from 10 nm gold particles added to the samples.

Online Resource 2 / Supplementary Table

Droplets of 4 µl cell suspension of *Halobacterium salinarum* in 3 M NaCl plus 81 mM $MgSO_4$ were mixed with 4 µl colloidal gold in the same salt solution and applied to Quantifoil Cu 200 R 2/1 grids for cryo-electron microscopy. The grids were blotted and the samples vitrified by plunge freezing. Tables S1 and S2 compile the preparation parameters and the results of vitrification for manual and Vitrobot-assisted freezing experiments, respectively.

Table S1 Manual plunge-freezing: parameters and sample quality[1)]

Cells[2)] at RT[3)] prior to dialysis	1 min to 1 h	<10 min / 1 h	<1 min	1 h	1 h
Temperature of dialysis bath	RT / 37 °C	RT	37 °C	RT	RT
Blotting from	sample side	back side	back side	back side	back side
Blotting duration	≈ 3 s / ≈ 5 s	≈ 3 s	≈ 3 s / ≈ 5 s	≈ 1 s	>10 s
Ice quality	varying thickness	varying thickness	varying thickness	very thick ice	carbon film damaged
Sample quality	very few cells on grid	medium to thick cell layer	few cells on grid	cells almost invisible	no cells

1) Result of 4 to 8 independent tests for each condition
2) Cell growth 72 hours troughout
3) Room temperature

Online Resource 3 / Supplementary Table

Table S2 Vitrobot-assisted plunge-freezing: parameters and sample quality[1)]

Chamber humidity[2)]	0 %	0 %	100 %	100 %	100 %	100 %	100 %	100 %
Blotting strength[3)]	3 / 5	10 / 15	3	5	10 / 15	5	5	5
Blotting duration	3 s	3 s	3 s	3 s	3 s	5 s	7 s	9 s / 11 s
Ice quality	very thick ice	thick ice	very thick ice	thick ice	thick ice	medium ice thickness	thin ice	thin to very thin ice
Sample quality	good cell distribution on grid	cells often on carbon and damaged	cells invisible	good cell distribution on grid	cells mostly on carbon and damaged	very good cell distribution on grid[4)]	good cell distribution on grid	few cells on grid, cells compressed

1) Result of 4 to 8 independent tests for each condition
2) Chamber at room temperature throughout
3) Numeric scale assigned to increasing angular range limit of the blotting arms
4) Best experimental parameter setting

Online Resource 4 / Supplementary Figure

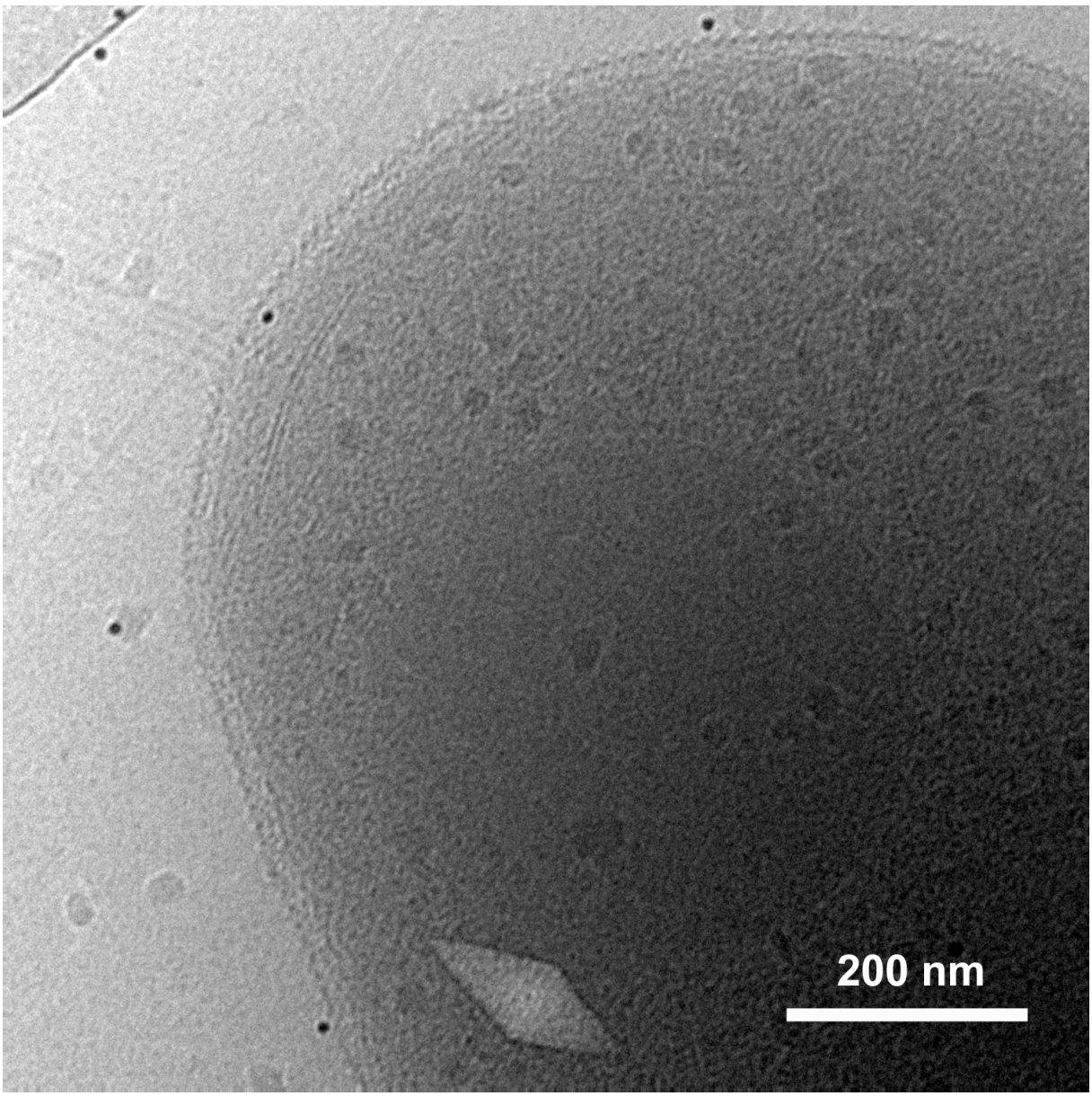

Fig. S2 Cryo-electron micrograph of a partly lysed (flattened) cell of *Halobacterium salinarum* vitrified in a solution of 2.5 M NaCl plus 81 mM $MgSO_4$. The S-layer, cellular membrane, a bundle of flagella and the putative polar cap structure close to the cellular origin of flagella (top left of the cell) together with a small gas vesicle below a globular granule are visible. The black dots orginate from 10 nm gold particles added to the sample, the irregular gray "flakes" are ice contaminations. Projection recorded at 9 µm underfocus.