Microorganisms and Biofilms

LS.1.P024
Ultrastructural characterization of the hyperthermophilic Archaeon *Methanocaldococcus villosus*

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Keywords: Archaea, flagella, FIB-SEM, tomography, 3D reconstruction

The hyperthermophilic Archaeon *Methanocaldococcus villosus* was isolated from a shallow submarine hydrothermal system at Kolbeinsey Ridge, north of Iceland. Cells are regular to irregular cocci with a diameter of 1 µm and possess an S-layer with (pseudo-) sixfold symmetry and up to 50 polar flagella. These cell appendages were shown to be multifunctional organelles which mediate swimming, adhesion to abiotic surfaces, and formation of cell-cell contacts [1]. Using *M. villosus* as a suitable novel model organism, the aim of this study was to gain detailed insights into the whole-cell architecture of Archaea with aid of various microscopic techniques.

To determine the cellular ultrastructure of *M. villosus*, cells were prepared for electron microscopy by high-pressure freezing/freeze substitution or conventional chemical fixation [2]. Specimens were embedded, ultrathin sectioned and analysed by focused ion beam scanning electron microscopy (FIB-SEM) with regard to substructures in the cell and anchoring of flagella in the membrane. In addition, ultrastructural transmission electron microscopy (TEM) investigations were performed using electron tomography of (serial) semi-thin sections. Digital images were then aligned and read into Amira™. For reconstruction of 3D-models, contours of the cells and flagella were colour-coded and movies of rotating cells were recorded with aid of a movie maker.

Electron microscopic analyses of the *M. villosus* cells revealed a densely packed cytoplasm containing distinct globules with a diameter of 80-120 nm (Figure 1). The well-preserved cell envelope consisted of the cytoplasmic membrane, a thin periplasm and the S-layer from which glycoproteins protruded. Some of the cells additionally possessed a submembraneous layer which could be connected with a complex cytoplasmic assembly that closely resembled bacterial chemoreceptor arrays. FIB-SEM allows nearly isotropic imaging of the cells and their 11-nm flagella (Figure 2).

Combining FIB-SEM and electron tomography, three-dimensional analyses of cells and subcellular structures were obtained. The different microscopic techniques used herein confirmed previous ultrastructural findings on flagella and cell envelope construction of the *Methanococcales* and gave new insights into the anchoring of flagella.

Altogether, these findings enhance our general understanding of archaeal cell structures.

1. A. Bellack, H. Huber, R. Rachel, G. Wanner, and R. Wirth, IJSEM 61 (2011), 1239-1245.
2. R. Rachel et al. in “Methods in Cell Biology: Electron microscopy of model systems”, ed. T. Möller-Reichert (Academic Press, New York) (2010), 47-69

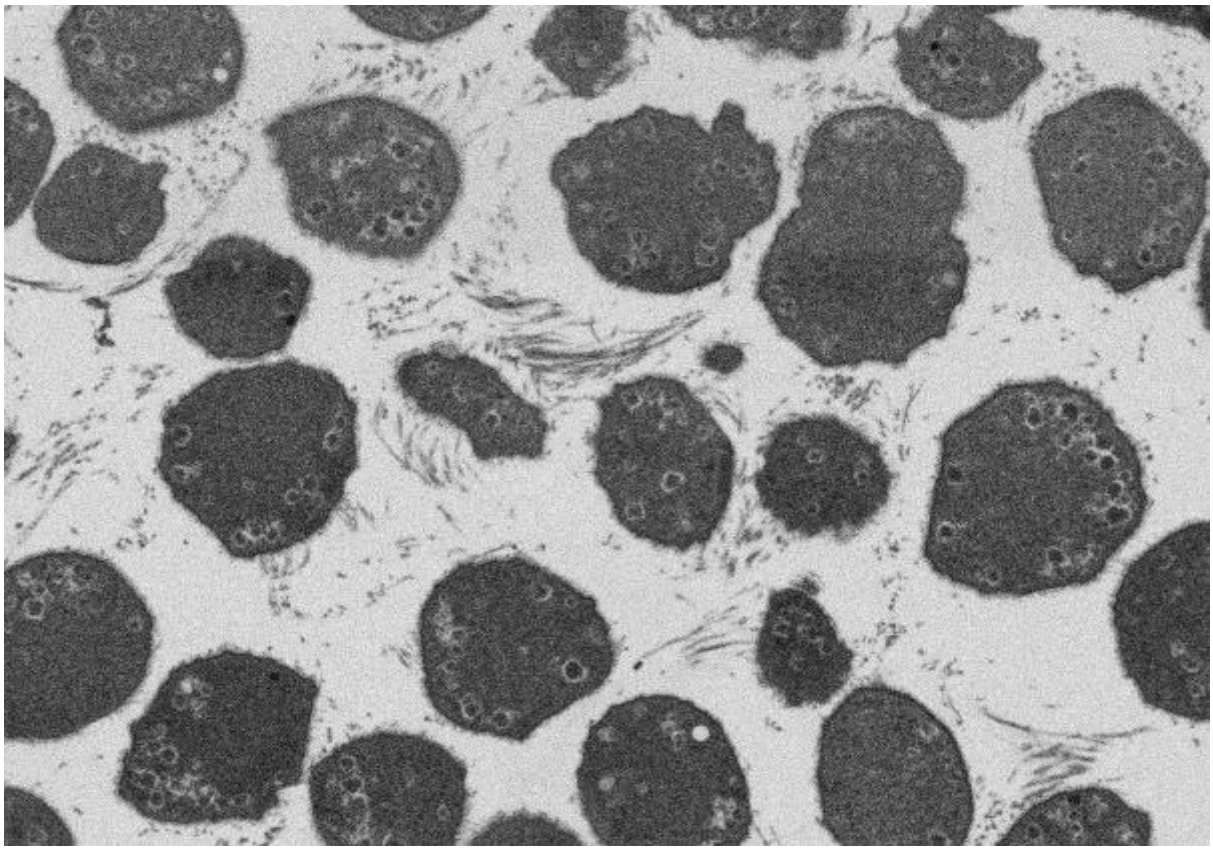

Figure 1. Overview of *M. villosus* cells recorded with FIB-SEM.

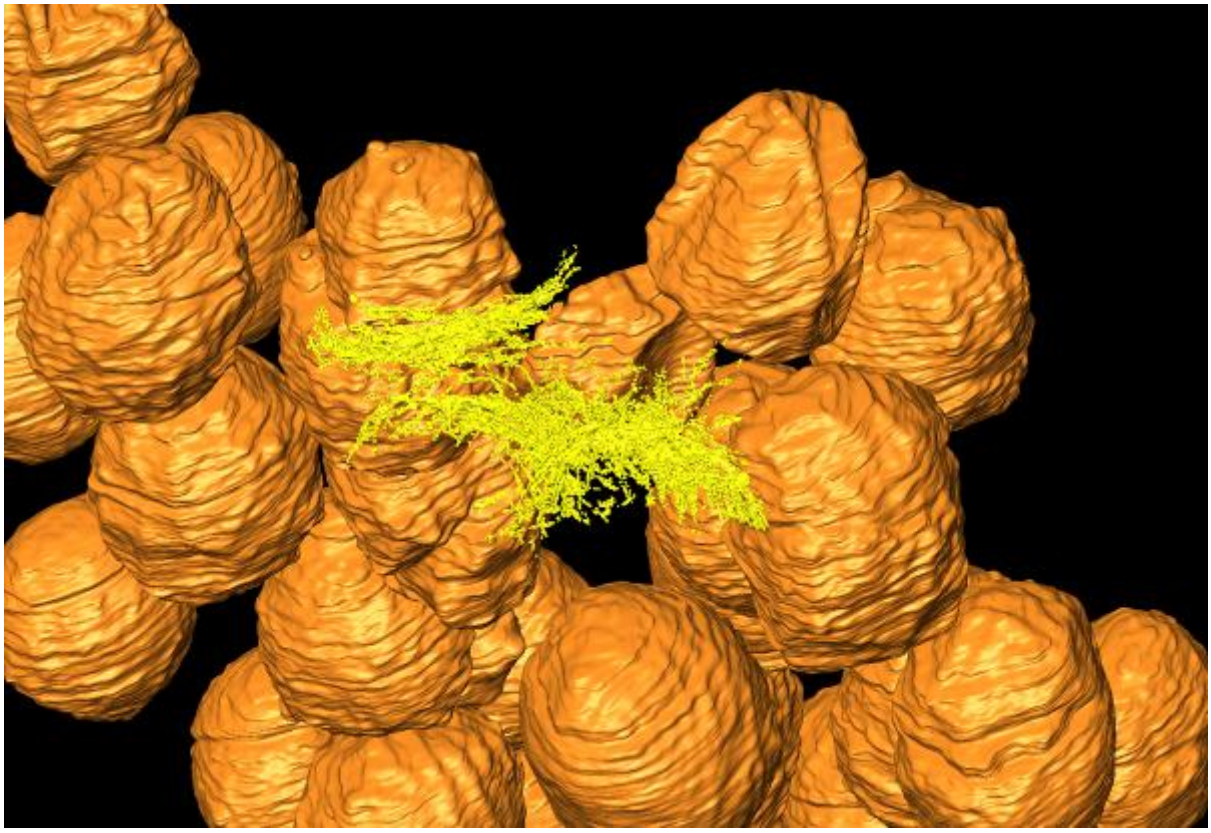

Figure 2. 3D-reconstruction of *M. villosus* cells from a FIB-SEM dataset of around 1100 8-nm slices; S-Layer orange, flagella yellow.