3164
doi:10.1017/S1431927621010941
Microsc. Microanal. 27 (Suppl 1), 2021

Freeze-fracturing of microbes producing biopolymers at liquid Helium temperature: cryo-SEM application in biotechnology

Kamila Hrubanova[1], Kateřina Mrázová[1], Pavel Urban[1], Vojtěch Krutil[2], Radim Skoupý[1], Stanislav Obruča[3] and Vladislav Krzyzanek[4]

[1]Institute of Scientific Instruments of the CAS, v. v. i., Brno, Czech Republic, [2]Institute of Scientific Instruments of the CAS, v. v. i., Brno, Jihomoravskykraj, Czech Republic, [3]Brno University of Technology, Chemical Faculty, Brno, Czech Republic, [4]Institute of Scientific Instruments of the Czech Academy of Sciences, Czech Republic, Brno, Jihomoravskykraj, Czech Republic

Interesting morphologies can be observed when microbial cells, in our case Cupriavidusnecator H16, containing polymer granules, such as polyhydroxyalkanoates (PHA), are fractured at very low temperature, far below the glass transition temperatures of such polymers. It was described that these particles can be stretched extensively when the cells are broken at -120°C which is standard temperature of freeze-fracturing used in electron microscopy [1]. It is interesting that this property of elasticity at low temperature is specific for polymers in cells. Well our question was specified: Will polymer particles inside the cells still be elastic even at a much lower temperature close to the temperature of liquid Helium, even further away from the glass transition temperature? Therefore our main challenge was to perform an experiment allowing the fracturing at this low temperature and observing by cryo-SEM.

Polyhydroxyalkanoates (PHAs) are polyesters of hydroxyalkanoic acids which are accumulated in a form of intracellular inclusions by numerous prokaryotes [2]. These materials primarily serve as carbon and energy storage; nevertheless, their biological function is far more complex. Generally, presence of PHAs in microbial cells enhances their robustness against various stress factors. Apart from their biological function, PHAs attract attention as ecological alternative to petrochemical plastics which can be biotechnologically produced from waste streams of various industrial processes.

Freeze-fracturing is well described as a separation of a specimen along a line of least resistance parallel to the applied force. In our study the cross fracture of polymer particle inside the cells is most interesting because we can observe their needle or mushroom deformation. With the aim to achieve the sample temperature below -240°C during freeze-fracturing the standard liquid nitrogen cooling can't be used because the temperature of LN2 at atmospheric pressure is -196°C thus the cooling by LHe was required. The novel device was developed at the ISI Brno. New cooling system was mounted onto the sputter coater and cryo-preparation chamber ACE 600 produced by Leica Microsystems. The original Dewar was dismounted and new chamber was used for the connection of the cooling device. Heat exchangers were then insulated by chamber vacuum of the sputter coater. In conclusion we would like to summarize that our new instrumental setup was successfully tested and we were able to achieve the sample temperature up to -248°C during freeze-fracturing. In addition, it was experimentally found that fracturing at this very low temperature resulted in deformation changes of the PHA granules. Moreover, it was showed that the combination of Raman spectroscopy with cryo-SEM technique can provide a deeper insight into the chemical and mechanical properties of intracellular polymeric granules inside the bacterial cells [3]. We believe that this study will be of significant assistance to research group being involved in bacterial strains which accumulate PHA. Presented results are convincing enough to warrant more extensive investigations with larger sets of bacterial strains to evaluate combination of described techniques.

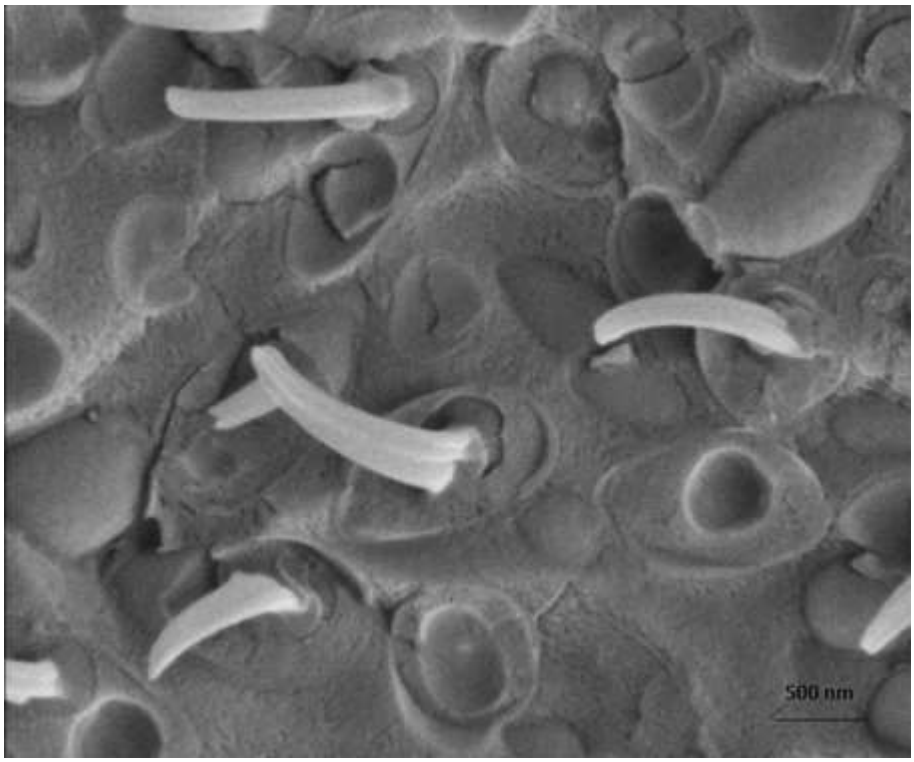
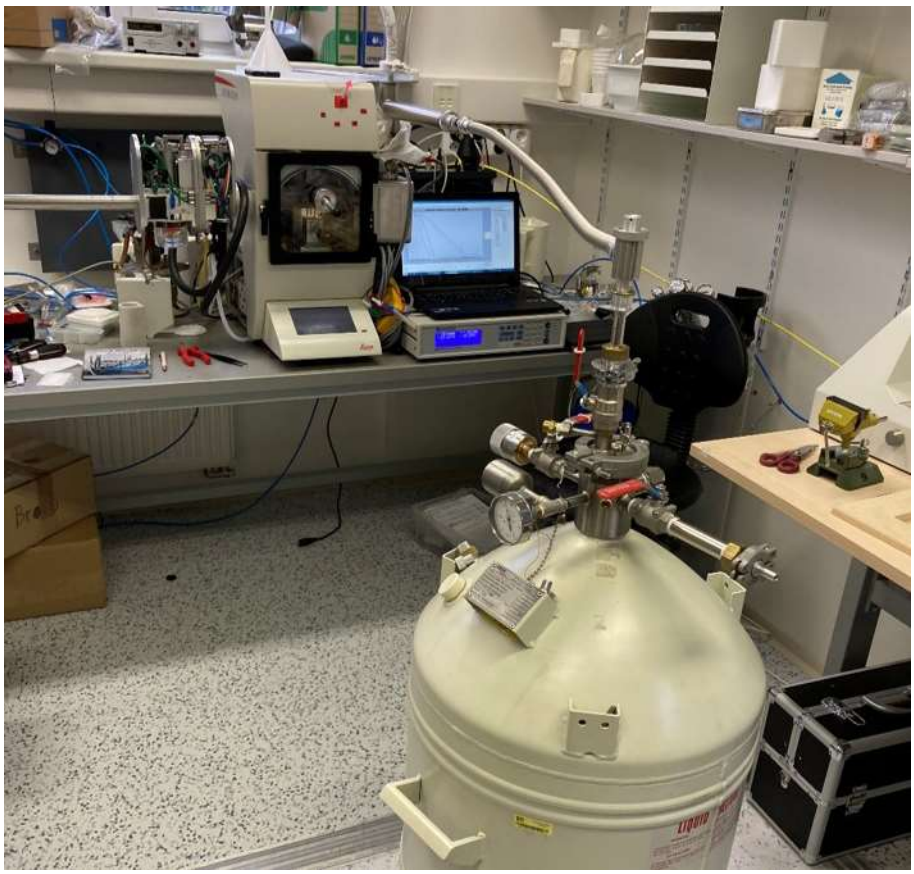


Figure 1. Cryo-SEM image show the interior of non-pathogenic Gram-negative soil bacteria Cupriavidusnecator H16 after freeze-fracture. The culture was exposed to the osmotic stressors.



https://doi.org/10.1017/S1431927621010941 Published online by Cambridge University Press

Figure 2. With the aim to achieve the sample temperature below -240°C during freeze fracturing the standard liquid nitrogen cooling can't be used because its liquid temperature at atmospheric pressure is -190°C. The cooling by liquid helium is required. New cooling system was mounted into the ACE 600.

References

[1] Obruca, S. at al, PLoS ONE, 2016, 11(6), e0157778
[2] Kucera, D. at al, Applied Microbiology and Biotechnology, 2016, 100(3), pp. 1365–1376
[3] Slaninova, E. at al, Applied Microbiology and Biotechnology, 2018, 102(4), pp. 1923–193

The research was supported by the Czech Science Foundation (project GA19-20697S) and the Czech Academy of Sciences (MSM100652102).