Supplementary material to “Production, characterization and application of an alginate lyase, AMOR_PL7A, from hot vents in the Arctic Mid-Ocean Ridge”

Kiira S. Vuoristo¹, Lasse Fredriksen¹, Maren Oftebro¹, Magnus Ø. Arntzen¹, Olav A. Aarstad³, Runar Stokke², Ida H. Steen², Line Degn Hansen¹, Reidar B. Schüller¹, Finn L. Aachmann³, Svein J. Horn¹, Vincent G.H. Eijsink¹

1. Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), P.O. Box 5003, N-1432 Aas, Norway
2. Centre for Geobiology, University of Bergen, Bergen, Norway, Department of Biology, University of Bergen, Bergen, Norway
3. Department of Biotechnology and Food Science, NTNU Norwegian University of Science and Technology, Sælands vei 6/8, N-7491 Trondheim, Norway
Figure S1. Domain structure and sequence of AMOR_PL7A. A) Domain structure of AMOR_PL7A, drawn to scale. The full-length enzyme contains a signal peptide (SP: residues 1-24) and a polysaccharide lyase family 7 domain (PL7: 66-257). B) Amino acid sequence alignment of the catalytic domain of AMOR_PL7A with the two most related PL7 alginate lyase domains with a known structure, from Sphingomonas sp A1-II (Taxon identifier: 90322; PDB id: 2CWS) and from Pseudomonas aeruginosa (strain ATCC 15692; PDB id: 1VAV) and the two PL7 alginate lyases with the highest sequence identity, from Rhodopirellula sp. SWK7 (EMI43849) and Vibrio hyugaensis (WP_052441931.1). The sequence numbering for AMOR_PL7A is shown above the alignment. Conserved residues appear in white on a red background, and conservatively substituted residues are printed in red. Putative catalytic residues are indicated by black asterisks. Secondary structure assignments are based on the crystal structures of Sphingomonas sp. A1-
II (top; PDB id: 2CWS) and *Pseudomonas aeruginosa* (bottom; PDB id: 1VAV) and defined as follows: $\alpha_1$-3; $\alpha$-helices, $\beta_{1-16}$; $\beta$-sheets, $\eta_{1-2}$; $3_{10}$-helices and TT; strict $\beta$-turns.

Figure S2. SDS-PAGE analysis of purified AMOR_PL7A including a C-terminal His-tag.
Figure S3. Phylogenetic analysis of AMOR_PL7A. Sequences of PL7 were retrieved from dbCAN and domain architecture similarity searches using EMBL-EBI HMMER, aligned using MAFFT-LINSI, trimmed with trimal, and analyzed using IQ-TREE. The numbers at the nodes indicate bootstrap values based on 100 bootstrap replications. Branch support values above 50 are indicated by dots. Collapsed branches are indicated by the number of sequences in parenthesis. PL7 subfamilies are indicated based on the classification in CAZy. The scale bar indicates the number of amino acid substitutions per site.
Figure S4. Steady state kinetics of AMOR_PL7A. The graph shows a non-linear fit for the hydrolysis of sodium alginate by AMOR_PL7A.
Figure S5. Oscillatory measurements. The picture shows the storage modulus, $G'$, versus Shear stress (Pa) at the limit of the Linear Viscoelastic Region (LVR). The viscoelasticity of a material can be characterized by amplitude sweep measurements where the sample is exposed to an oscillatory deformation at a constant frequency. In oscillatory rheology, measurements are non-destructive when operating in the LVR where the applied strain or shear stress does not damage the structure. Amplitude sweeps at a constant frequency and increasing strain are used to characterize the sample. The limit of the LVR reflects the strength of the structure and shows how much strain it can be exposed to without damage. The limit of the linear LVR is here defined by a reduction of the storage modulus, $G'$, by 3%. The storage modulus, $G'$, is an elastic modulus representing how stiff the structure is, so a lower value of $G'$ represents a softer sample.
Figure S6. Oscillatory measurements. The picture shows the storage modulus, $G'$, versus strain % at the limit of the LVR.

References


