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Thermostable alginate lyases

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Introduction

Alginate lyases are of industrial and scientific importance. They act on glycosidic bonds in alginate, a major macroalgal polysaccharide. Substantial interest has been in the discovery and characteristics of alginate lyases, focusing on their potential use in various processes both in industry and biotechnology. Of particular interest is their application in proposed macroalgal-based biorefineries, where robust enzymes are required.

Alginate forms blocks of linear co-polymers of covalently linked mannuronate (M) and guluronate (G) residues. Alginate lyases are classified by their substrate specificity towards the bonds between these residues as well as their amino acid sequences into polysaccharide lyase (PL) families (CAZy database).

Here we describe the identification and characterisation of the **first thermostable alginate lyases**.

Results

- The four enzymes were termed AlyRm1 to AlyRm4.
- The enzymes were all confirmed as active thermostable alginate lyases (Table 1).
- AlyRm1 and AlyRm2 belong to PL family 6, AlyRm4 belongs to PL17, while AlyRm3 belongs to a hitherto unknown PL family.
- AlyRm1 and 2 were cloned without their C- and N-terminal (ΔC and ΔN) domains, which resulted in changes in enzyme characteristics (AlyRm1) and cleavage specificities (both enzymes, Table 2).
- AlyRm3 produced the highest yield of oligosaccharides.
- Near complete degradation (99%) of alginate into unsaturated mono-uronates by simultaneous incubation with AlyRm3 and AlyRm4 was achieved.

Table 1. Optimum conditions of the alginate lyases.

	T_{opt} (°C)	T_{stab}[1]	pH_{opt}	$NaCl_{opt}$[2] (mM)
AlyRm1	87	5h@70°C >16h@60°C	7.2	0-600
AlyRm1ΔC	68	12h@60°C	8.0	200-1000
AlyRm2ΔN	81	16h@70°C >16h@60°C	6.5	0-800
AlyRm2ΔNC	81	>16h@70°C	6.5	0-800
AlyRm3	75	8h@70°C >16h@60°C	5.5	0-800
AlyRm4	81	>16h@70°C	6.5	0-600

[1]50% residual activity following incubation at 50, 60, 70 or 80°C for up to 16 h. [2]≥ 80% relative activity.

Conclusions

This is the first report of characterised thermostable alginate lyases.

They can be used separately for selective partial degradation of alginate, or in combination for production of mono-uronates.

Their application in industrial processes, where higher processing temperatures are required or preferred, is of substantial interest. Currently, the genes are being integrated into the genome of an ethanologenic mutant of *Thermoanaerobacterium* sp. AK17, along with other necessary alginate and laminarin utilisation genes, for constructing a macroalgal-based bioethanol platform.

Materials and methods

- Four candidate alginate lyase encoding genes were identified in the genome of the marine thermophile *Rhodothermus marinus* str. 378.
- The genes were cloned into an expression vector and their products recombinantly produced in *E. coli* with N-terminal His6-tags.
- Purification of recombinantly expressed enzymes was done using IMAC columns.
- The purified enzymes were characterised, thermostabilty, optimum temperature and pH determined using sodium alginate as substrate and reducing sugars measured using DNS assay.
- The degradation pattern of alginate and blocks of guluronate (G) and mannuronate (M) produced by the enzymes was determined using TLC, MALDI-TOF-MS, HPAEC-PAD and 1D/2D ^{1}H NMR.

Table 2. Preferred activities of the alginate lyases.

	Alginic acid		G-block		M-block	
	Endo	Exo	Endo	Exo	Endo	Exo
AlyRm1	++	+++++	++	+++++	++	+++++
AlyRm1ΔC	+++	+	++++	++	+	-
AlyRm2ΔN	+++	+++	+	+++	+	-
AlyRm2ΔNC	+++	+	+++	+	+	-
AlyRm3	++++	-	++++	-	++++	-
AlyRm4	-	+++++	-	+++	-	+++++

Icelands marine geothermal areas are an abundant source of thermostable enzymes.

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