Our research – your profit

Identification, expression and characterization of marine polysaccharide degrading enzymes from novel bacteria isolated from intertidal biotopes.

Varsha Kale[1,2], Elisabet Gudmundsdottir[1], Olafur Fridjonsson[1], Sesselja Omarsdottir[3], Solveig Petursdottir[1], Brynjar Orn Ellertsson[1] , Bryndis Bjornsdottir[1], Solveig Olafsdottir[1], Gudmundur Hreggvidsson[1,2].

Introduction

Intertidal areas are highly dynamic environments where temperature, salinity, pH and nutrients fluctuate with tidal rhythms and wave action. Microorganisms inhabiting these areas adapt to these conditions and the available resources. Macroalgae are abundant in most intertidal areas and they contain a variety of polysaccharides, lignocellulose sugars and some rare ones. These are often complex in structure, branched and highly substituted. The adaptation of intertidal microorganisms often involves metabolizing complex sugars and their genomes are therefore expected to encode polysaccharide processing enzymes. Among these are chondroitin lyases that degrade chondroitin sulfate into unsaturated oligo- and disaccharides, sulfatases that catalyse cleavage of sulfate ester from sulfated carbohydrates and fucosidases that degrade fucoidan and remove fucose from complex marine poly-saccharides. All have gained interest in recent years for potential value in production of deoxy- or oligo-saccharides, role in biological processes and as therapeutic agents.

Figure 1. Algae growth in intertidal area in Iceland.

Materials and methods

- Bacteria harvested by *In situ* artificial support colonization; strains purified and grown in enrichment culture in laboratory. Phylogeny by 16S rRNA sequencing and NCBI blast.
- Genome sequencing by 454 FLX+; assembly applying GS deNovoAssembler; annotation using the Rapid Annotation Server; genome screening for carbohydrate enzyme genes by annotation filtering and NCBI blast.
- Cloning using *E.coli* expression system, expression confirmation by SDS-PAGE. Protein purification by affinity chromatography (chondroitin lyase, sulfatase) or hydrophobic interaction chromatography (fucosidase).
- Enzyme activity analysis by TLC and HPLC (sulfatase and α-L-fucosidase) or product detection by modified DNS assay, spectroscopy and HPLC (chondroitin lyase).

	Arthrobacter MAT3885	*Litorilinea MAT4143*
Chondroitin sulfate lyases	1	0
Hyaluronic acid lyases	1	0
Sulfatases	11	22
Fucosidases	3	4
Beta-Galactosidases	4	7
Alpha-galactosidase	1	4
Glucuronyl hydrolyse	0	2
Polysaccharide Deacetylases	7	18
Galacturonate lyase	0	2
Beta-glucosidases	1	6
Alginate lyases	1	0
Galacturonate lyase	0	3
Chitinase	1	2
Mannosidase	1	1
Xylan/arabinosidase	0	3
Sialidase	1	0
Rhamnosidase	0	3

Table 1. The number of carbohydrate enzyme genes observed in two bacteria genomes, isolated from intertidal areas and enriched in chondroitin sulfate medium.

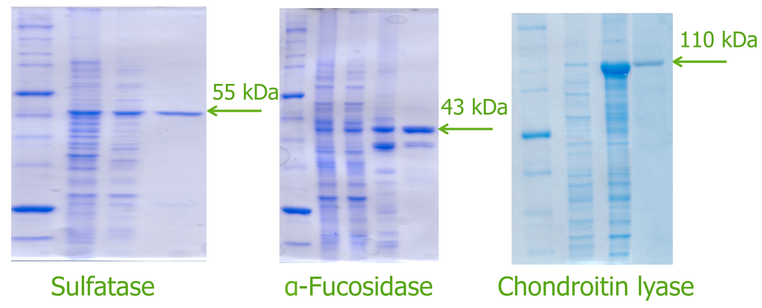

Figure 2. SDS acrylamide gels showing the recombinant marine polysaccharide converting enzymes in crude extracts and following purification.

Results

- A number of novel bacteria originating in intertidal areas were isolated and their genomes sequenced, including the genome of a novel thermophilic bacterial strain, annotated *Litorilinea aerophila*, which was isolated from an intertidal hot spring. Several genes encoding carbohydrate enzymes of different classes were identified.
- Selected genes were cloned and expressed, including α-L-fucosidase (AfuL2) from *Litorilinea aerophila* strain MAT4131 and sulfatase (SulA2) and chondroitin lyase (ChoA1) from *Arthrobacter* strain MAT3885.
- Functional analysis showed that α-L-fucosidase (Aful2) actively degraded polysaccharides from both sea cucumber and macroalgae and this activity was increased by adding sulfatase and chondroitin lyase into the reaction.
- Sulfatase (SulA2) was shown to desulfate chondroitin sulfate polysaccharides (fig.4).

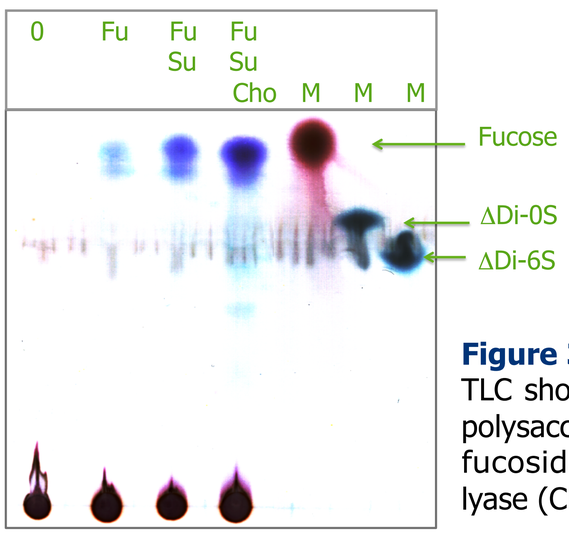

Figure 3. TLC showing the degradation products of natural polysaccharides following incubation with α-L-fucosidase (Fu), sulfatase (Su), chondroitin lyase (Cho). Markers indicated with M.

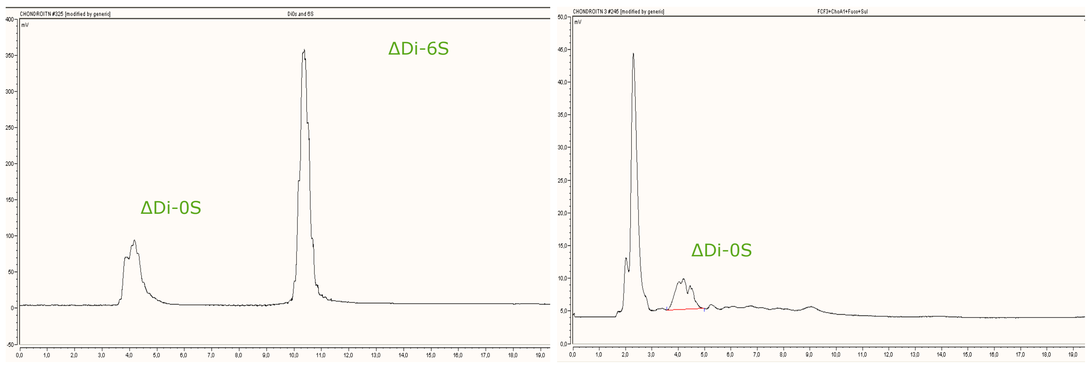

Figure 4. Chromatogram showing product of chondroitin sulfate incubated with SulA2, along with purified chondroitin lyase (right); sulfated (ΔDi-6S) and desulfated (ΔDi-0S) chondroitin sulfate disaccharide markers (left).

Conclusions

Microorganisms adapted to life in intertidal areas are a source of novel carbohydrate enzymes with potential scientific, industrial and medical value. Marine enzymes discovered in intertidal areas of Iceland are active on complex sulfated polysaccharides of natural marine sources and their activity is increased when used in a concerted action.

Varsha Kale (varsha@matis.is; http://www.matis.is)

[1] **Matís Ltd**, Vinlandsleid 12, IS-113 Reykjavik, Iceland; [2] **The University of Iceland**, Life- and Environmental Sciences, Hjarðarhagi 2-6, IS-107 Reykjavik, Iceland; [3] **The University of Iceland**, Pharmaceutical Sciences, Vatnsmýrarvegi 16, IS-101 Reykjavik, Iceland