M512-02 Structural determinants for the specific recognition of algal cell wall polysaccharides: examples of agarases and alginate lyases. <u>Mirjam Czjzek</u>^{a,b}, François Thomas^c, Etienne Rebuffet^d, Jan-Hendrik Hehemann^e, Murielle Jam^{a,b}, Gaëlle Correc^{a,b}, Tristan Barbeyron^{a,b} and Gurvan Michel^{a,b} ^aUPMC University Paris 6, UMR 7139 Marine Plants and Biomolecules, Station Biologique de Roscoff, F-29682 Roscoff, Bretagne, France ^bCNRS, UMR 7139 Marine Plants and Biomolecules, Station Biologique de Roscoff, F-29682 Roscoff, Bretagne, France ^cBiology Department, Mail Stop 52, Watson Laboratory, Woods Hole Oceanographic Institution Woods Hole, MA 02543, USA ^dDepartment of Chemistry, Biochemistry and Biophysics, University of Gothenburg, Box 462, SE-405 30 Göteborg, Sweden ^eUniversity of Victoria, Dept. of Biochemistry & Microbiology PO Box 3055 STN CSC, Victoria BC V8W 3P6, Canada E-mail: czjzek@sb-roscoff.fr

Marine algal polysaccharides clearly differ from their terrestrial counterparts and their associated enzymes usually constitute novel protein families. We have applied a knowledge-based strategy for the structural and biochemical characterization of candidate 'hypothetical conserved proteins' to identify new enzyme functions, missing in the metabolic pathways by which marine polysaccharides are degraded in the marine environment. The flavobacterium Zobellia galactanivorans has been isolated from the red alga D. sanguinea [2] and extensively studied for its capacity to degrade agars and carrageenans [3]. In collaboration with the MPI for Marine Microbiology (Bremen, Germany) and the Genoscope (Evry, France), we have sequenced and annotated the complete genome of Z. galactanivorans, confirming that this marine bacterium displays a huge potential for the degradation of algal polysaccharides. To characterize new glycoside hydrolases and lyases in the Zobellia genome, we apply different strategies to identify and select relevant target-proteins: phylogenic approaches can be used to detect new subfamilies within polyspecific GH families, as exemplified by the discovery of the first porphyranases (Figure 1) in the family GH16 [4]. Another example concerns the structural and biochemical characterization, of a new family of glycoside hydrolases typical of coastal environment, starting from a 'putative protein with unkown function' [5]. Using a combination of comparative genomic approaches, activity screening and crystallographic methods, we were able to assign the 1,3- α -3,6-anhydro-L-galactosidase activity to a member of the GH117 family (Figure 2). Several alginate lyases cluster together in operon like structures [6]. The biochemical and structural characterization of these enzymes show adapted substrate specific determinants. Several of these examples will be presented, giving insights into structural determinants that appear to relate to 'marine specific' features.

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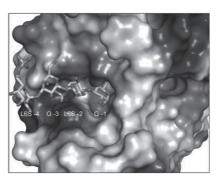


Figure 1. Surface representation of porphyranase A in complex with its substrate.

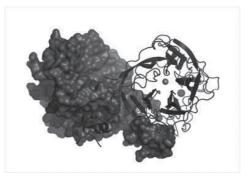


Figure 2. Dimer formation through domain swapping of the $1,3-\alpha-3,6$ -anhydro-L-galactosidase structure.