Sujet de M2

Title : Structure-function characterization of a Carbohydrate Binding Module (CBM) 4 and genetic modification of the marine flavobacterium *Z. galactanivorans*

Key words : marine bacterium, carbohydrates, genetics, protein production and purification, functional and structural characterization.

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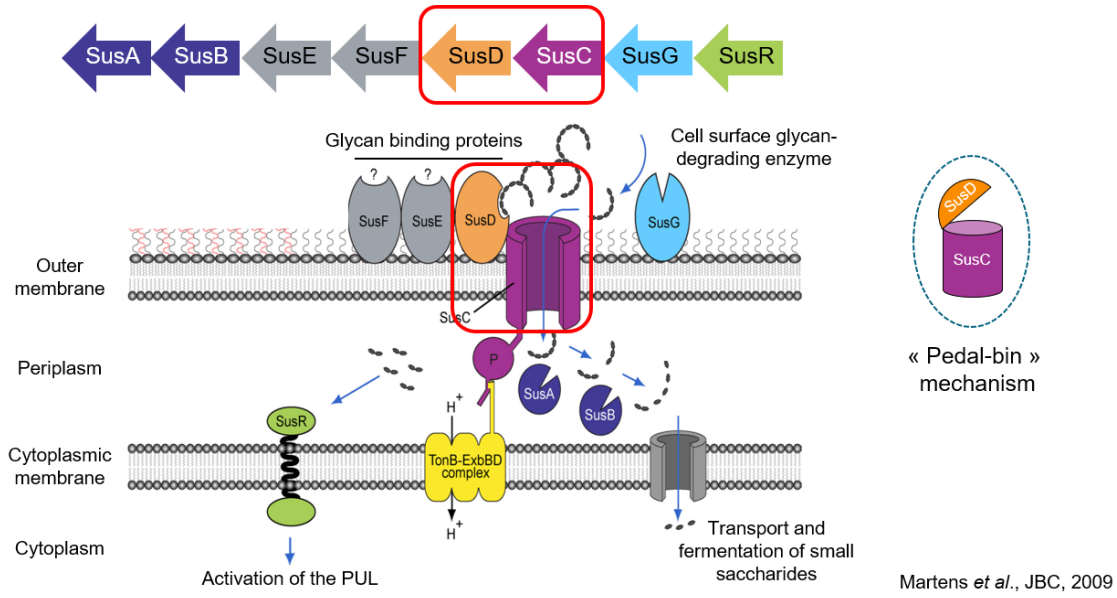
The M2 internship will takes at the Toulouse Biotechnology Institute (TBI) in the Catalysis and Enzyme Molecular Engineering (CIMEs) team.

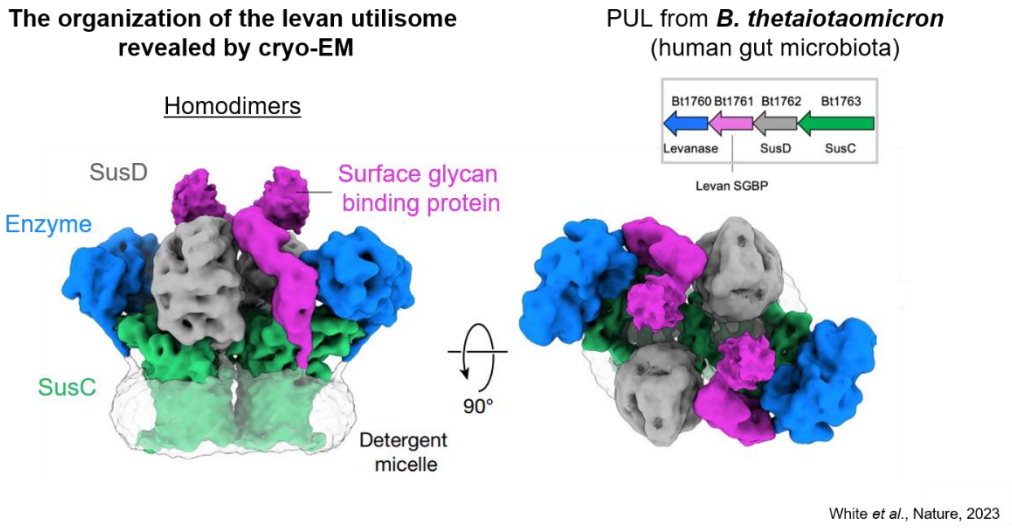
Context : Members of the Bacteroidota phylum have colonized various environments (from the human gut microbiota to the marine microbiota). *Zobellia galactanivorans* is a marine flavobacterium considered as a model organism for the bioconversion of algal polysaccharides. Its genome has been sequenced and revealed the presence of numerous Polysaccharide Utilization Loci (PULs). PULs are set of physically-linked genes encoding for different proteins involved in the binding, degradation and uptake of polysaccharides. A hallmark of PULs is the presence of the SusCD genes which encode for the transporter and its associated protein involved in the active uptake of the oligosaccharides.

Example of a PUL involved in the degradation and the uptake of starch by a human gut bacterium :



Among the thousands of SusCDs identified in PULs from different Bacteroidota, only 3 SusCDs have been structurally and functionally characterized so far. The authors were surprised to find out that the

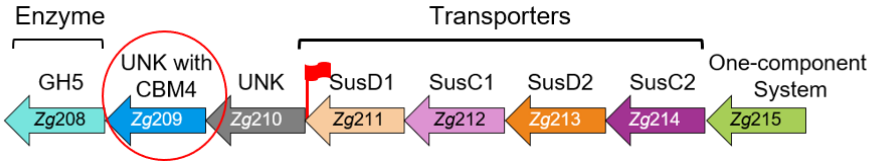
proteins of the studied PUL form homodimers and that they are all organized around the SusCD to produce what they call the « utilisome ».



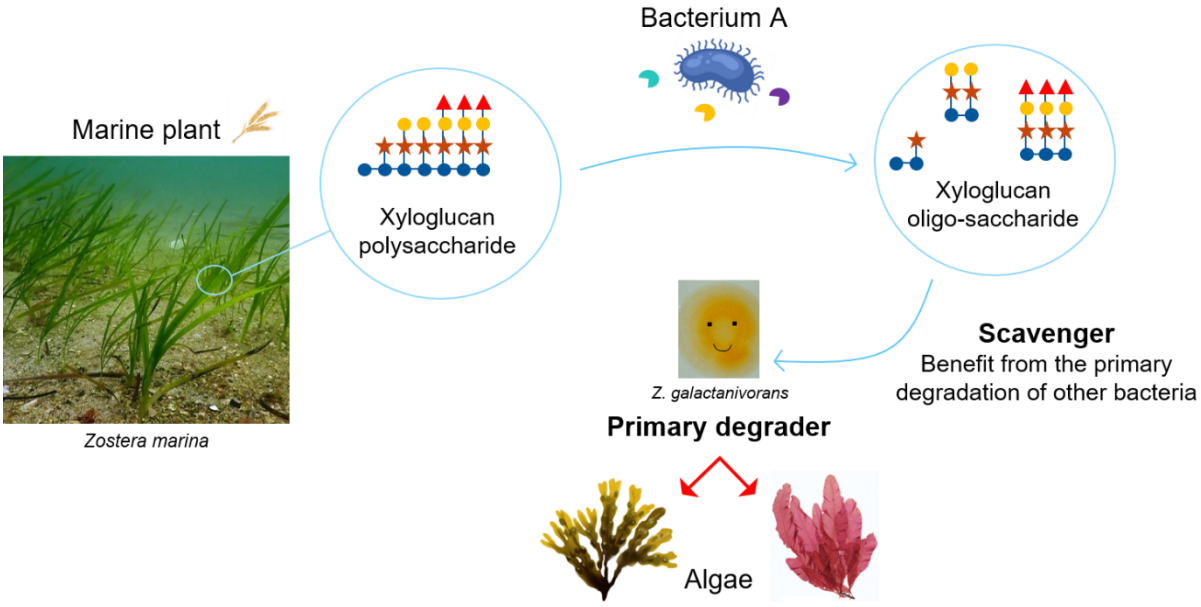
The paucity of structural and functional data about these transporters make it complicated to fully understand their mechanism while they are key elements of the metabolic pathways.

Additionally, we have identified in *Z. galactanivorans* a PUL containing a tandem repeat SusCD. According to the literature one PUL targets one substrate. The presence of two transporter raises several questions : (i) does this PUL target one or at least two substrates ? (ii) what is the quaternary structure of these transporters ? homodimers ? heterodimers ?

The PUL of interest in Z. galactanivorans :



To decipher the substrate targeted by this PUL we work in close collaboration with the « Station Biologique de Roscoff » (Brittany, France). We found out that several proteins of the PUL, including the Carbohydrate Binding Module 4 (CBM4), were able to interact with xyloglucan, which was surprising as *Z. galactanivorans* is not able to grow on this substrate as sole carbon source. We finally showed for the first time that *Z. galactanivorans* is actually able to grow on xyloglucan-oligosaccharides (XG-oligos) as sole carbon source. Knowing that xyloglucan is mostly present in the cell wall of marine plants and not in algae, these preliminary data enabled us to draw a new hypothesis : while *Z. galactanivorans* is considered as a primary degrader on algae, does it behave like a scavenger (benefit from the oligosaccharides released by other bacteria) on marine plant ?



In this context, please find below the different tasks in which the M2 student will be involved :

(1) **Functional and structural characterization of the CBM4 and of its additional domain of unknown function** : we already have preliminary data on the CBM4, like its X-ray 3D structure and binding tests on different polysaccharides. To go deeper in the understanding of this protein we will perform Small Angle X-ray Scattering to investigate to which extend the full-lenght protein (CBM4+UNK) is flexible. We will also perform site-directed mutagenesis to identify the key residues involved in substrate binding. We will also quantify the binding affinity by NMR or ITC.

(2) **Structural characterization of the tandem repeat transporters**: Using genetic tools we will introduce a histag at the C-terminal of the SusD1 to be able to purify by IMAC the transporters directly from *Z. galactanivorans* membrane and we will attempt to get the 3D structure by cryo-EM.

Calendrier : début du stage entre janvier et mars 2026 et pour une durée maximum de 6 mois

Gratification : 610 € par mois

Candidature : CV + Lettre de motivation