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# Unravelling the structure-function of a tandem pair of glycoside transporters in the marine flavobacterium *Zobellia galactanivorans* Dsij<sup>T</sup>

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Members of the *Bacteroidota* phylum have colonized all types of habitats and substrate niches. They are considered primary degraders of polysaccharides. A unique feature of *Bacteroidota* genomes is the presence of **Polysaccharide Utilization Loci** (PUL) which are clusters of co-regulated genes encoding a complement of cell Surface Glycan-Binding Proteins (SGBPs, including the **SusD-like**), TonB-dependent transporters (**SusC-like**), Carbohydrate Active Enzymes (CAZymes) and carbohydrate sensors/ transcriptional regulators. Among the 68 500 PULs identified by the PULDB<sup>1</sup> tool from the CAZy database, only three SusCD transporters have been structurally characterized so far<sup>2,3,4</sup>. This limited data revealed that the SusCDs mediate substrate uptake via a pedal bin mechanism. The SusC transporter forms the barrel of the bin, while SusD sits on top of the barrel, opening and closing like a lid to facilitate substrate binding. These protein complexes have been also shown to form SusC<sub>2</sub>D<sub>2</sub> homodimers. Nevertheless, many features of SusCD function in glycan uptake remain unclear.

The marine flavobacterium *Zobellia galactanivorans* Dsij<sup>T</sup> is a model organism for the bioconversion of algal polysaccharides<sup>5</sup>, for which genomic and transcriptomic data are available, as well as genetic tools to delete and complement genes. We have identified a PUL (locus tag Zg208-Zg215) whose characterization will contribute to deepen the understanding of the SusCD transport system. First, it contains an adjacent pair of SusCD genes, defined as **tandem repeat SusCD** (trSusCD). While trSusCD are present in only around 3% of the encoded PULs in the phylum *Bacteroidota*, a recent study suggests that they provide a selective advantage in environments where diverse dominant species compete for similar metabolic niches<sup>6</sup>. Nevertheless, the function and structure of trSusCD proteins have never been investigated so far. Second, preliminary data obtained on *Z. galactanivorans* suggest that the two adjacent SusCD could target different substrates, as the PUL is upregulated in presence of laminarin, mixed-linked glucan and xylan<sup>7</sup>. This substrate versatility would expand our perceptions regarding PUL machineries, which so far have demonstrated gene organization that suggests one cognate PUL for each substrate type. Interesting questions arise from these observations, i) do the two SusD-like bind to the same ligand? ii) do the two transporters form a heterodimer? Third, this PUL contains Zg210, a lipoprotein of unknown function. Preliminary bioinformatic analysis tend to show that Zg210 homologues are always found downstream a trSusCD. Is this protein a new CAZyme or a SGBP? Is it part of a protein complex formed by the transporters?

Investigating the structure-function relationships of these proteins by combining different approaches (genetics, biochemistry, crystallography/CryoEM) will provide novelty and a better understanding of PULs functionality.

<sup>1</sup>Terrapon *et al.* (2018) Nucleic Acids Research.

<sup>2</sup>Glenwright *et al.* (2017) Nature.

<sup>3</sup>Gray *et al.* (2021) Nature Communications.

<sup>4</sup>White *et al.* (2023) Nature.

<sup>5</sup>Barbeyron *et al.* (2016) Environmental Microbiology.

<sup>6</sup>Gálvez *et al.* (2020) Cell Host & Microbe

<sup>7</sup>Thomas *et al.* (2017) Frontiers in Microbiology.