BIOCHEMICAL CHARACTERIZATION OF A SUSD-LIKE PROTEIN INVOLVED IN GLUCOOLIGOSACCHARIDE UTILIZATION BY A COW RUMEN UNCULTURED *BACTEROIDALES*

Xiaoqian LI, TBI, CNRS, INRAE, INSAT, Université de Toulouse, Toulouse, France
xli1@insa-toulouse.fr
Guy Lippens, TBI, CNRS, INRAE, INSAT, Université de Toulouse, Toulouse, France
Gianluca Cioci, TBI, CNRS, INRAE, INSAT, Université de Toulouse, Toulouse, France
Zhi Wang, School of Chemical Engineering, Zhengzhou University, Zhengzhou, Henan, China
Gabrielle Potocki-Veronese, TBI, CNRS, INRAE, INSAT, Université de Toulouse, Toulouse, France
Aurore Labourel, TBI, CNRS, INRAE, INSAT, Université de Toulouse, Toulouse, France

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Mammals harbour widely diverse and immensely active microbial communities. Most intestinal microbes are beneficial and play major metabolic and physiological roles. For example, the rumen microbiome is the most diverse gut ecosystem in the animal kingdom, its composition and activity are essential for the animals' health as well as feed efficiency and emission of environmentally harmful substances [1, 2]. Bacteria is the most abundant, diverse, and metabolically active group among the rumen microbiome [1], the main carbon sources of gut bacteria are glycosides of dietary (plant storage and cell wall polysaccharides), host (glycoproteins) or microbial origin. As the previous studies show, *Firmicutes* and *Bacteroidota* (formerly *Bacteroidetes*) are dominant phylum in the rumen, in particular *Bacteroidota*, which is able to metabolize a wide variety of complex glycans using an arsenal of glycan metabolic systems encoded by polysaccharide utilization loci (PULs). PULs are prevalent in *Bacteroidota* as clusters of colocalized, coregulated genes, the products of which orchestrate the detection, sequestration, enzymatic digestion, and transport of complex carbohydrates. The transporters and carbohydrate-active enzymes (CAZymes) encoded by PULs are necessary to import these substrates into the cells and to degrade them into monosaccharides. Transport is thus a crucial step in the metabolism of glycosides, which is itself a key of microbiota functioning and equilibrium. A noteworthy feature of each PUL is the presence of two genes whose products are homologous to proteins within the starch utilization system (Sus) of *B. thetaiotaomicron*: SusC, a putative TonB-dependent transporter (TBDT) and SusD, a cell surface glycan-binding protein (SGBP) [3]. SGBPs are essential for efficient capture of the glycosides present on the cell surface, providing *Bacteroidota* with a competitive advantage in colonizing their habitats [4-8]. Here, we present the biochemical characterization of a SusD-like protein that is encoded by a glucooligosaccharide utilization locus from clone 41O1, which was issued from an *in vitro* enriched (IVTE, 20,352 clones) cow rumen metagenomic library, screened for endo-acting glycoside hydrolases acting on plant cell wall polysaccharides (Ufarté *et al*., *in prep*.).

In the present study, we showed that the clone 41O1 is able to grow on laminaritriose, cellotriose and a mixture of glucosyl-cellotriose and cellobiosyl-cellobiose as sole carbon sources. It demonstrates the promiscuity of the 41O1_SusC/D transport systems towards β-1,3- and β-1,4-linked glucooligosaccharides. Based on this, we used various *in vitro* analyses to investigate the binding ability of 41O1_SusD towards these oligosaccharides and the corresponding polysaccharides. Saturation transfer difference NMR spectroscopy (STD-NMR) and differential scanning fluorimetry (DSF) revealed that 41O1_SusD can bind to β-(1,3;1,4)- and β-1,3-oligosaccharides but not bind to β-1,4- oligosaccharide (cellotriose). At the same time, STD-NMR and DSF also indicated 41O1_SusD cannot bind to β-(1,3;1,4)- and β-1,4- polysaccharides (Barley β glucan, CM-Cellulose), which is supported by affinity gel electrophoresis (AGE) assays. Strikingly, all methods revealed that 41O1_SusD can bind to β-(1,3;1,6)-linked polysaccharides (Laminarin, Yeast β glucan). We will further investigate the structure-function relationship of 41O1_SusD to understand the molecular determinants of its substrate specificity, thereby providing valuable insights into the critical functions of this protein in the metabolic pathways involved in the breakdown of dietary fibers, which are paramount importance for maintaining mammal health.

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