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The cellular location of endo-acting galactanases confers keystone or recipient status to arabinogalactan degrading organisms of the human gut microbiota

Jose Munoz
Northumbria University, UK

Glycans, the major source of energy for the human gut microbiota (HGM), are metabolized primarily by the *Bacteroides* genus. Arabinogalactan proteins (AGPs) are a higher heterogenous group of plant glycans in which the β1, 3-galactan backbone and β1, 6-galactan side chains are conserved features. Diversity is provided by the extensive and highly variable nature of the sugars that decorate both the backbone and side chain galactans. The mechanisms by which nutritionally relevant AGPs are degraded at a cellular and biochemical level are poorly understood, as is the impact of this process on the ecology of the HGM. To address these issues we have explored how the HGM organism *Bacteroides thetaiotaomicron* metabolizes highly complex AGPs. The work provides a degradative model that reveals a repertoire of exo-acting family GH43 β1, 3-galactanases that release backbone galactose units that are attached at O6 to the side chains. The oligosaccharide side chains are depolymerized by the synergistic action of exo-acting enzymes in which catalytic interactions is dependent on whether degradation is initiated by a lyase or glycoside hydrolase. Growth studies of the 20 HGM *Bacteroides* species on a complex AGP revealed three keystone organisms that facilitated utilization of fragments of the glycan by the 17 other bacteria, which thus acted as recipients. The ability to function as a keystone organism was conferred by a surface endo-β1, 3-galactanase, which, when engineered into a recipient enabled the bacterium to also utilize complex AGPs and facilitate the growth of the other *Bacteroides* species. This study underpins future pre- and pro-biotic strategies to exploit AGPs to manipulate the structure of the HGM.

jlmunoz@um.es

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