Raffinose family oligosaccharide utilisation by probiotic bacteria: insight into substrate recognition, molecular architecture and diversity of GH36 alpha-galactosidases

The organisation of genes conferring utilisation of raffinose family oligosaccharides (RFOs) has been analysed in several probiotic bacteria from the Bifidobacterium and Lactobacillus genera. Glycoside hydrolase family 36 (GH36) alpha-galactosidase encoding genes occur together with sugar transport systems of the glycoside-pentoside-hexuronide cation symporter family (GPH), sugar phosphotransferase systems (PTSs) or ATP-binding cassette systems (ABCs) highlighting the diversity of RFO uptake. The GH36 genes are often clustered together with sucrose hydrolases or phosphorylases ensuring the degradation of RFO to monosaccharides. Differential proteomics and transcriptomics data from our laboratories implicated ABC transporters in the uptake of RFO in both Lactobacillus acidophilus NCFM and Bifidobacterium animalis subsp. lactis BI-04. Interestingly, only one of three GH36 encoding genes in B. animalis subsp. lactis BI-04 was upregulated upon growth on RFO, suggesting that the other two gene products may have different specificities. The structure of the GH36 homotetrameric alpha-galactosidase from L. acidophilus NCFM (LaMel36A) was determined in complex with galactose bound in the active site to 1.58 angstrom. Differences in the N- and C-terminal domains of the LaMel36A monomer distinguished it from the monomeric TmGalA from Thermotoga maritima providing a structural rationale for the observed difference in oligomeric states of the two enzymes. Tetramerisation of LaMel36A creates a narrow and deep active site pocket between three monomers, which explains the preference of tetrameric GH36 enzymes for RFO and their lack of activity on polymeric galacto(gluco) mannan. Finally, GH36 was divided into four subgroups based on active site motifs, which illuminates functional and structural diversity in the family and aids further annotation of emerging sequences.