AN ATP-BINDING CASSETTE (ABC) UPTAKE SYSTEM GOVERNS THE PREFERENCE OF PROBIOTIC BIFIDOBACTERIUM ANIMALIS SUBSP. LACTIS FOR β-GALACTOSIDES

Theilmann M.
Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, Denmark

Introduction:
The human gastrointestinal tract is colonized by a vast amount of microbes forming a diverse and dynamic ecological niche. This microbial community, which is established upon birth, develops rapidly in the first two years of life to a more constant state that persists for most of the adult life of the human host. A diversity of β-galactosides of different monosaccharide composition and glycosidic linkages is abundantly present in the human gut either from dietary intake, e.g. in fruits, vegetables and milk, or from the host mucin glycoprotein layer coating the intestinal epithelial cells. With the exception of lactose, β-galactosides are not hydrolyzed by human digestive enzymes presenting an attractive nutritional resource to human gut adapted bacteria. Probiotic bifidobacteria are preferentially stimulated by various β-galactosides, which is exploited in the manufacturing of galactooligosaccharides (GOS) prebiotics. A degree of species-specific β-galactoside utilization by bifidobacteria is observed, but the molecular basis of this selectivity remains unexplored. The objective of the present study is therefore to evaluate the role of a Bifidobacterium oligosaccharide transport system in the preference for specific β-galactosides.

Methods:
A β-galactoside utilization gene cluster from probiotic Bifidobacterium animalis subsp. lactis Bl-04 (Bl-04) encoding a transcriptional regulator, an ATP-binding cassette (ABC) importer and an intracellular β-galactosidase of glycoside hydrolase family 42 (GH42) has previously been identified from whole genome microarrays transcriptional analysis. The specificity determining solute-binding protein (SBP) of the ABC transporter was recombinantly produced and ligand binding of the SBP with 16 different β-galactosides varying in monosaccharide composition, degrees of polymerization (DP), and glycosidic linkage was evaluated using surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). Selected β-galactosides were evaluated for their ability to support growth of B. animalis subsp. lactis Bl-04 in mono-cultures.

Results:
The SBP showed a strong preference for β-6'-galactosides and the highest affinity binding was observed for β-6'-galactobiose and β-6'-galactotetraose with dissociation constants (KD) in the 100 nM range. The binding of β-6'-galactobiose was enthalpy-driven, whereas the interaction of β-6'-tetraose was driven by both entropy and enthalpy. The SBP interactions with β-3'- and β-4'-galactosides were 300- and 1700-fold weaker, respectively. Growth curves of B. animalis subsp. lactis Bl-04 on three β-galactobiose isomers (β-3'-, β-4'-, and β-6'-galactobiose) reflected the preference of the SBP.

Discussion:
The GH42 enzyme has previously been shown to exhibit broad specificity hydrolyzing β-1,6, β-1,3 and β-1,4 galactosidic bonds with only a modest preference for β-1,6 bonds. The results of the present study represent a unique case allowing the comparison of selectivities of a transport protein and a glycoside hydrolase involved in the metabolism of the same glycan type highlighting the important role of ABC-mediated glycan transport in establishing the metabolic preference of a physiologically important taxon of the gut microbiota. Bioinformatic analyses suggest that the ABC transporter endows B. animalis sp. lactis subspecies with a unique metabolic capability, and hence the presented results provide a methodological framework for the design of highly selective and potent prebiotics that target specific probiotic taxa.
Keywords: Bifidobacterium, Galactooligosaccharides, Oligosaccharide uptake, Prebiotic, Probiotic, Solute-binding protein

Citation: