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Department für Lebensmittelwissen-
schaften und Lebensmitteltechnologie

Production of Prebiotic Galacto- Oligosaccharides Using Novel β -Galactosidases from Lactobacilli

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Prebiotics – Developments

- despite a range of commercially available oligosaccharides there is plenty of room to develop new, functionally enhanced prebiotics
 - current generation of oligosaccharides was not rationally developed
 - better understanding of factors determining the prebiotic activity of a particular oligosaccharide
 - new developments in the manufacturing biotechnology of oligosaccharides





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Prebiotics – Potential Future Developments

- functionally enhanced prebiotics
- novel prebiotics
- persistent prebiotics
- anti-adhesive oligosaccharides
- synbiotics

Rastall & Maitin (2002) *Current Opinion in Biotechnology*, 13:490



Functionally Enhanced Prebiotics

- to date very little research into the possibility of rationally targeting prebiotics at specific groups of bacteria
- possible target groups include
 - **infants**, significant differences between the gut microflora of breast-fed and formula-fed infants, colons of breast-fed infants tend to be dominated by bifidobacteria and lactobacilli → it might be possible to identify prebiotics specifically targetting these bifidobacteria and lactobacilli
 - **elderly**, number of bifidobacteria decline significantly after the age of 55

Functionally Enhanced Prebiotics

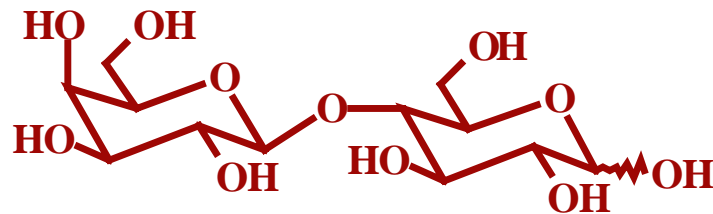
- possible target groups include
 - **probiotics**, design of prebiotics targeting at specific probiotics of proven utility; e.g., the use of enzymes from probiotics was used to produce oligosaccharides, several of the strains showed enhanced growth on the oligosaccharide synthesised by the homologous enzyme

(Rabio et al. (2001) Applied Environmental Microbiology 67:2526)



Galacto-Oligosaccharides

- Starting material is lactose, derived from whey which contains approx. 70–75% lactose
- Availability approx. 3.2 million tons per year worldwide, largest producer is Europe

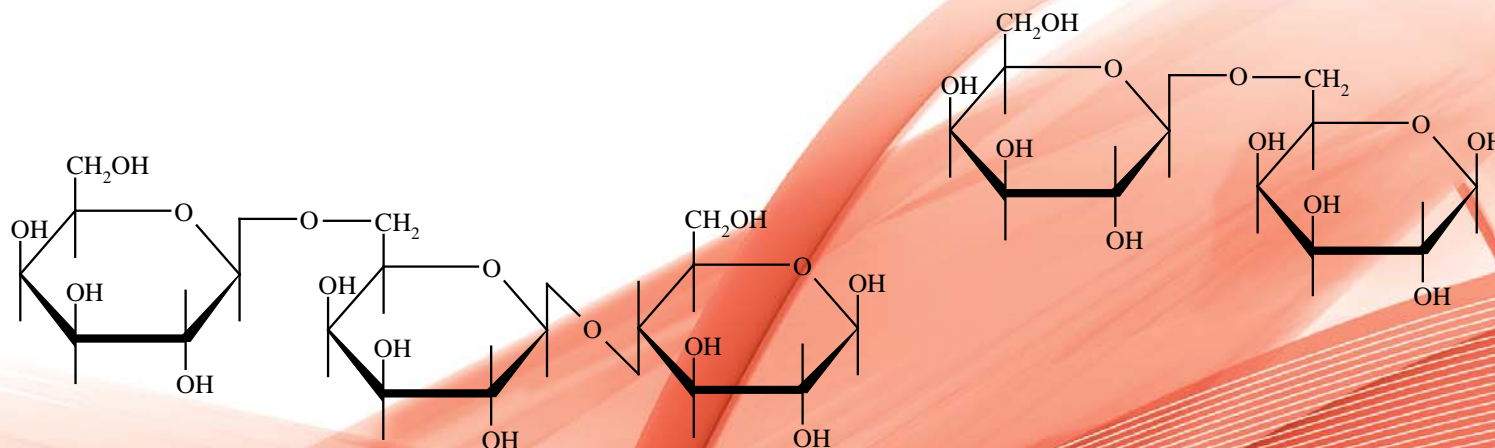


β -D-galactopyranosyl-(1 \rightarrow 4)-
 D-glucopyranose



Galacto-Oligosaccharides

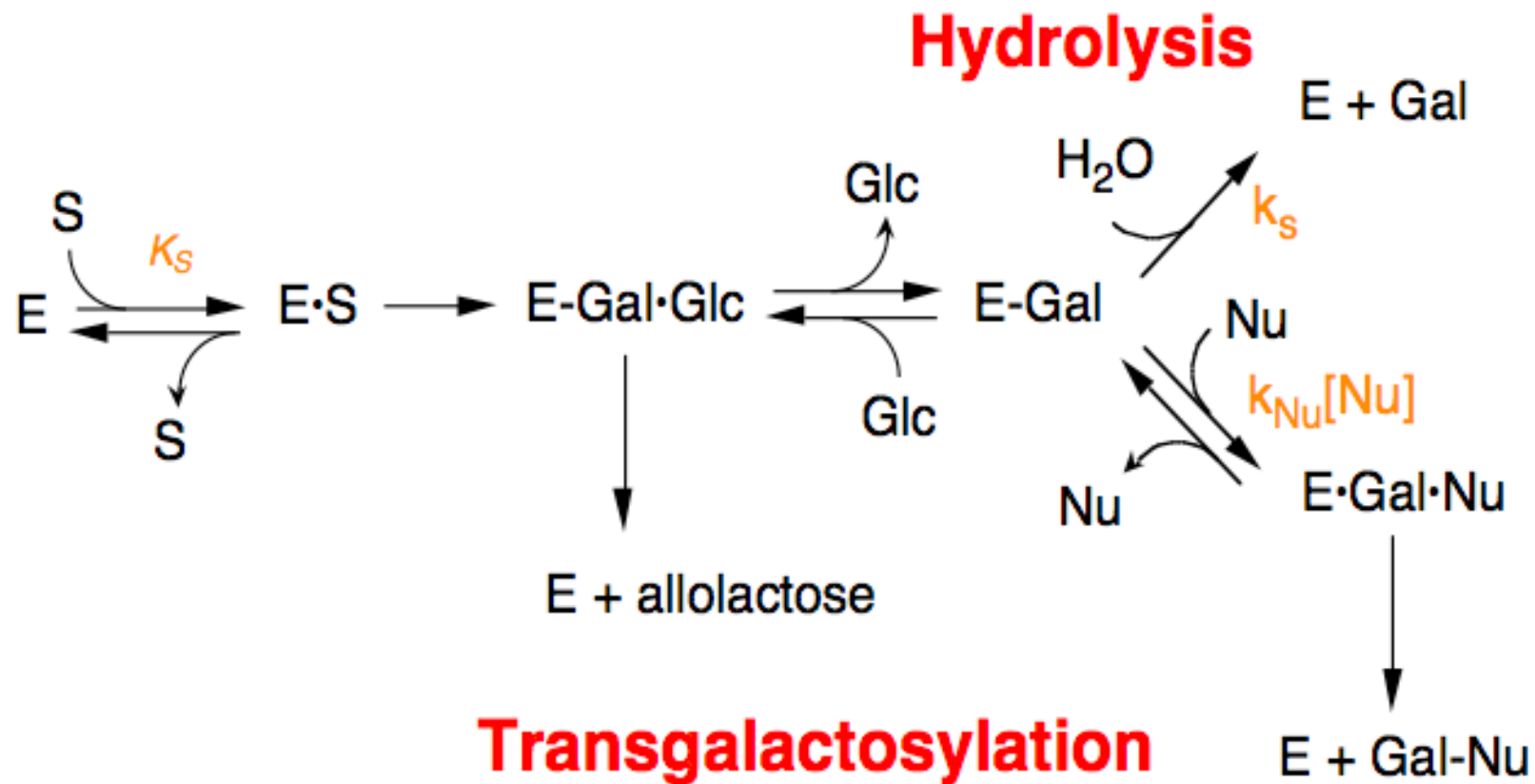
- Galacto-oligosaccharides (GOS) are derived from lactose by the **transgalactosylation reaction** catalyzed by β -galactosidases.
- GOS are **galactose-rich oligosaccharides** consisting of 2-10 monosaccharides joined by different glycosidic linkages, often contain one glucose molecule



Catalytic Mechanism of β -Galactosidases



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Factors Influencing GOS Production



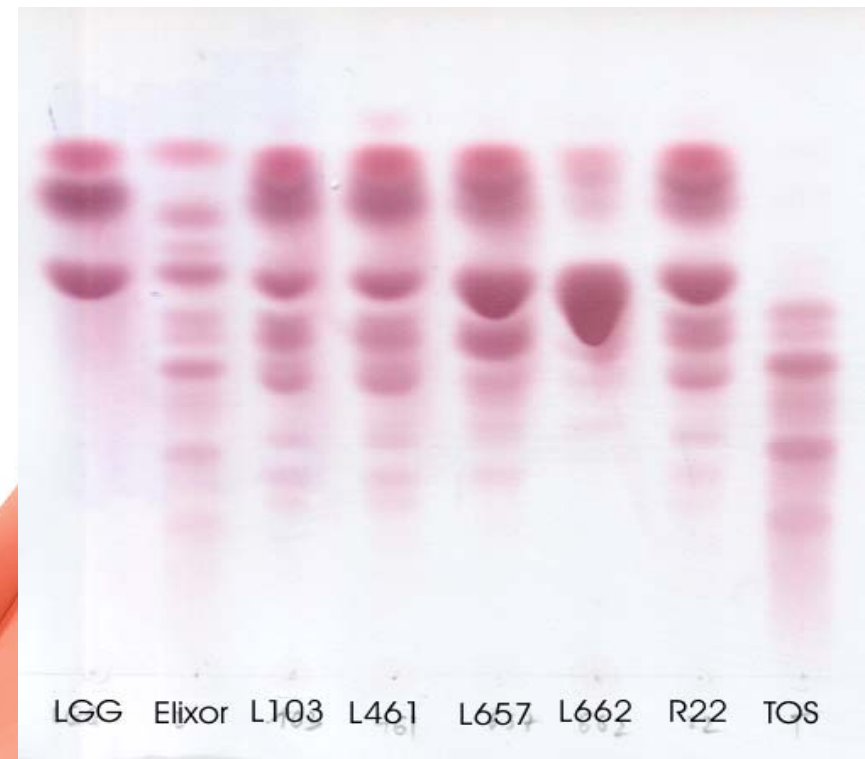
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- Enzyme source
- Concentration of substrate
- Extent of lactose conversion
- Process conditions / reactor type
- Temperature, pH-value



β -Galactosidases from Probiotic Bacteria for GOS Production

- Screening for probiotic bacteria with pronounced β -galactosidase activity
- Spectrum of oligosaccharides formed in transgalactosylation
- Evaluation of transgalactosylation potential via the partitioning coefficient
- Stability

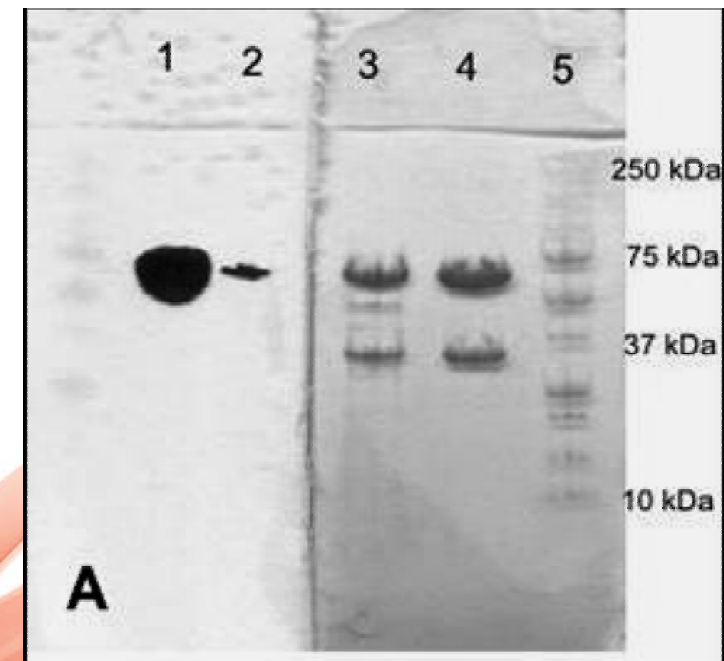


Galacto-Oligosaccharides

- Based on this screening *Lb. reuteri* and *Lb. acidophilus* were selected for further studies
- β -galactosidase was efficiently formed when lactose was used as a growth substrate (up to 2,500 U/L)
- enzymes were purified by hydrophobic interaction (Phenyl-Sepharose) and affinity chromatography (p-aminobenzyl thiogalactoside agarose)
- apparently homogenous enzyme preparations, approx. 160 U/mg specific activity

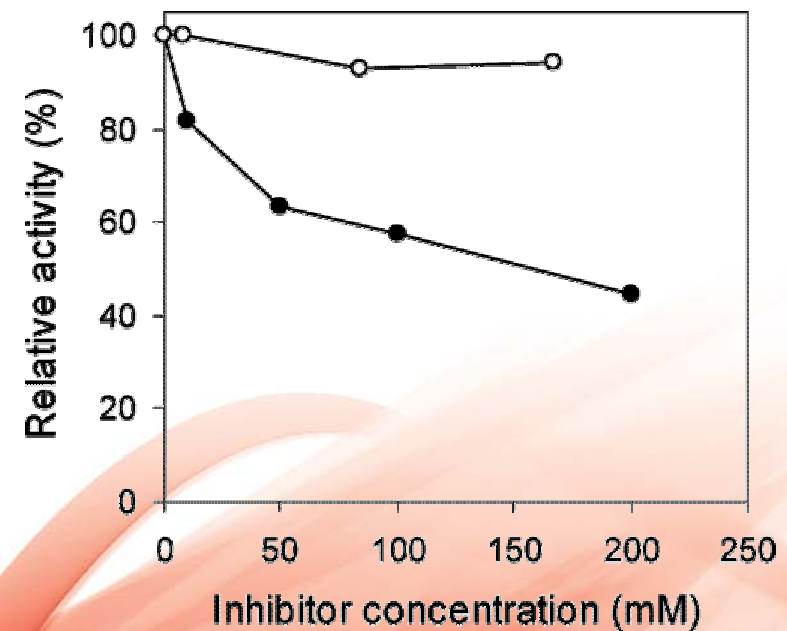
β -Galactosidase from *Lb. reuteri*

- β -Galactosidase from *Lb. reuteri* L103 is a heterodimer, consisting of a larger subunit (72 kDa) and a smaller one (35 kDa)
- on SDS-PAGE (nondenaturing) the larger subunit shows β -galactosidase activity
- isoelectric point of around 4.5



β -Galactosidase from *Lb. reuteri*

- is inhibited by its reaction products galactose and glucose, but only moderate inhibition

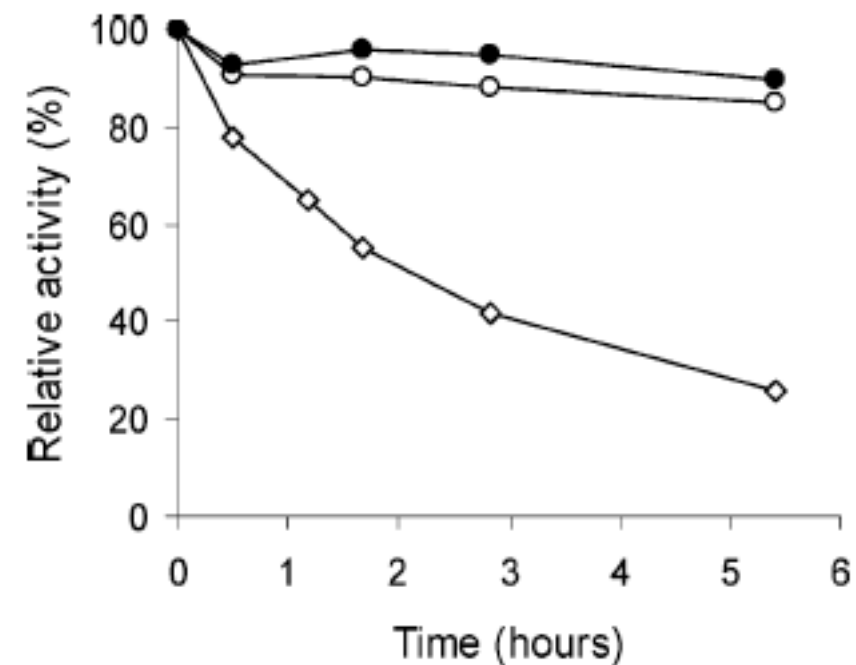


Inhibition by glucose (○) and galactose (●) of oNPG hydrolysis catalyzed by β -galactosidase from *L. reuteri*



β -Galactosidase from *Lb. reuteri*

- is activated and stabilised by mono- and divalent ions (Na^+ , K^+ , Mg^{2+})



Stability of β -galactosidase from *L. reuteri* L461 at 42 °C without MgCl_2 (\diamond) and with the presence of 1 (\circ) and 10 mM (\bullet) MgCl_2 . Experiments were performed in phosphate buffer (pH 6.5).

β -Galactosidase from *Lb. reuteri*

- has been cloned and overexpressed in *E. coli*
- genes for both subunits are slightly overlapping
- expression of both subunits possible, intact heterodimeric recombinant protein obtained in *E. coli*,
- approx. 110,000 units per litre fermentation

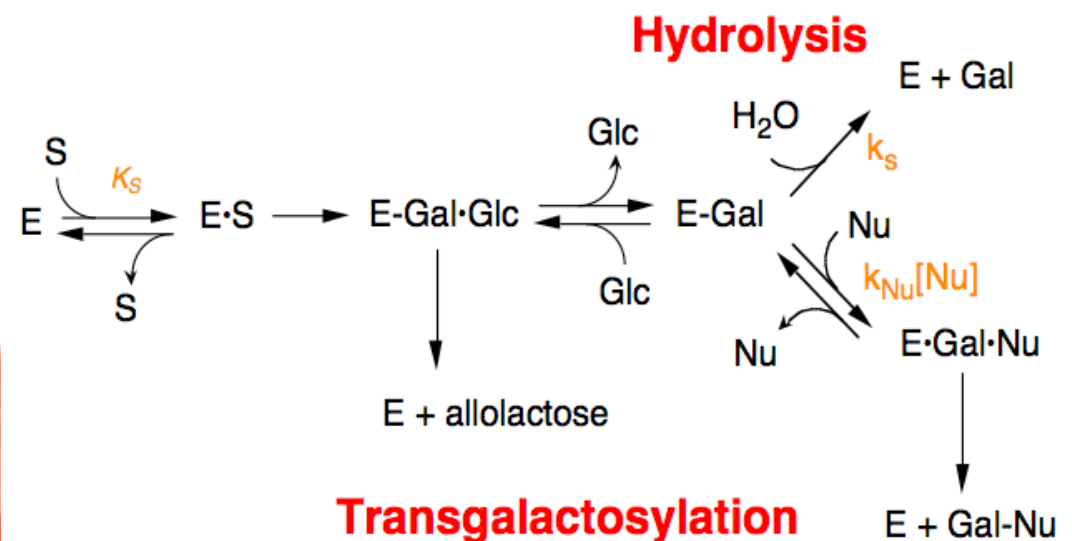


β -Galactosidase from *Lb. reuteri*

- glycosyl hydrolase family 2 according to the structural classification by Henrissat, related to LacZ from *E. coli*
- high sequence similarities to other β -galactosidases from lactobacilli (*Lb. acidophilus*, *Lb. helveticus*, *Lb. plantarum*, etc.)
 - 58–74% similarity for *lacL* (large subunit)
 - 57–71 % similarity for *lacM* (small subunit)

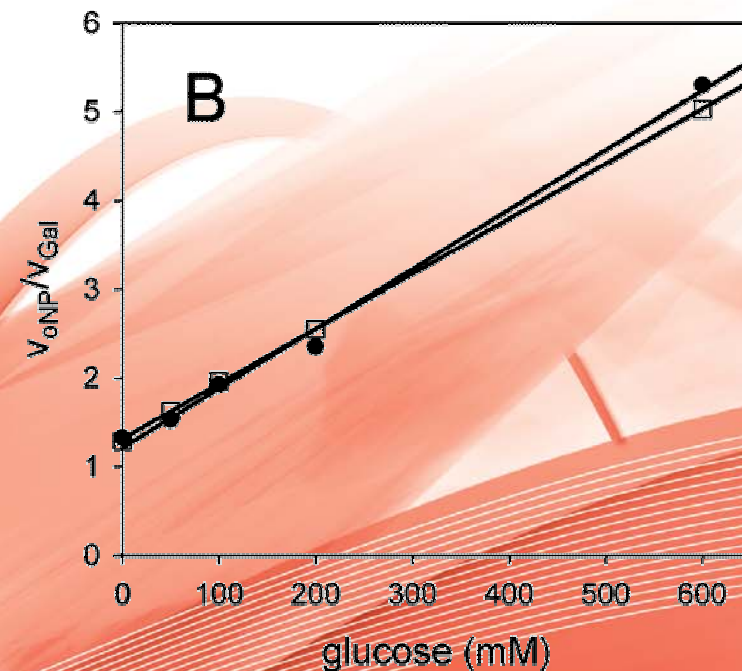
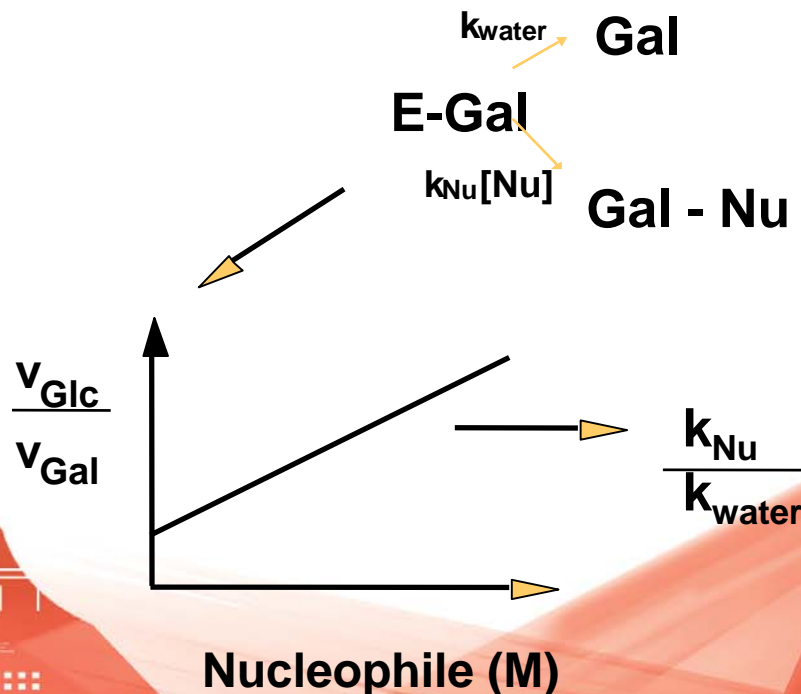
β -Galactosidase from *Lb. reuteri*

- transgalactosylation potential of a galactosidase can be measured by determination of the **partitioning coefficient** for intermolecular transgalactosylation
- measuring the initial velocities at which glucose and galactose are released (v_{Glu} , v_{Gal})



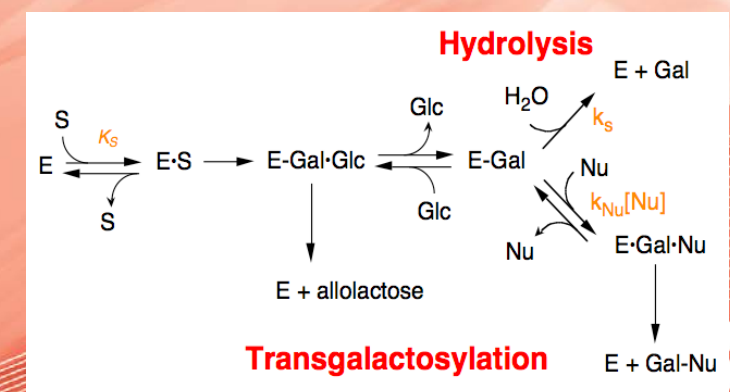
β -Galactosidase from *Lb. reuteri*

- rate constant ratio $k_{\text{Nu}}/k_{\text{water}}$ is obtained as the slope from the linear correlation $v_{\text{Glc}}/v_{\text{Gal}}$ with increasing concentrations of a nucleophile according to equation $v_{\text{Glc}}/v_{\text{Gal}} = 1 + k_{\text{Nu}} [\text{Nu}]/k_{\text{water}}$



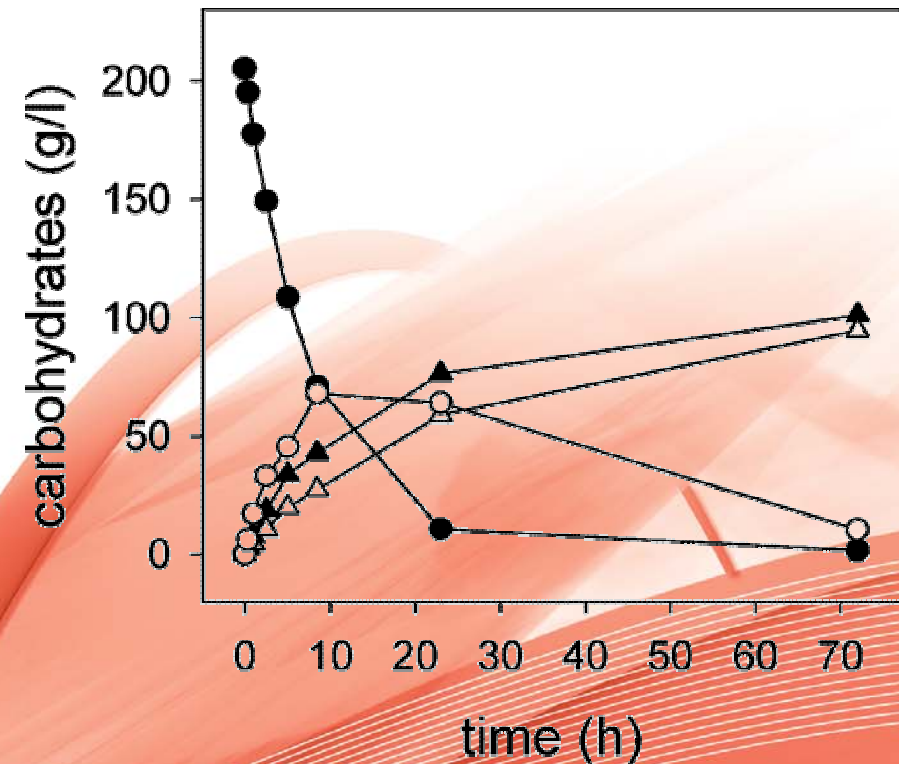
β -Galactosidase from *Lb. reuteri*

- rate constants ratios determined:
 - lactose $k_{\text{Lac}}/k_{\text{water}}$ **1.6**
 - glucose $k_{\text{Glu}}/k_{\text{water}}$ **6.7**
- these values indicate that both lactose and glucose (as well as other sugars) are good acceptors for galactose
- glucose is a much better acceptor than lactose, hence we can expect significant formation of disaccharides other than lactose



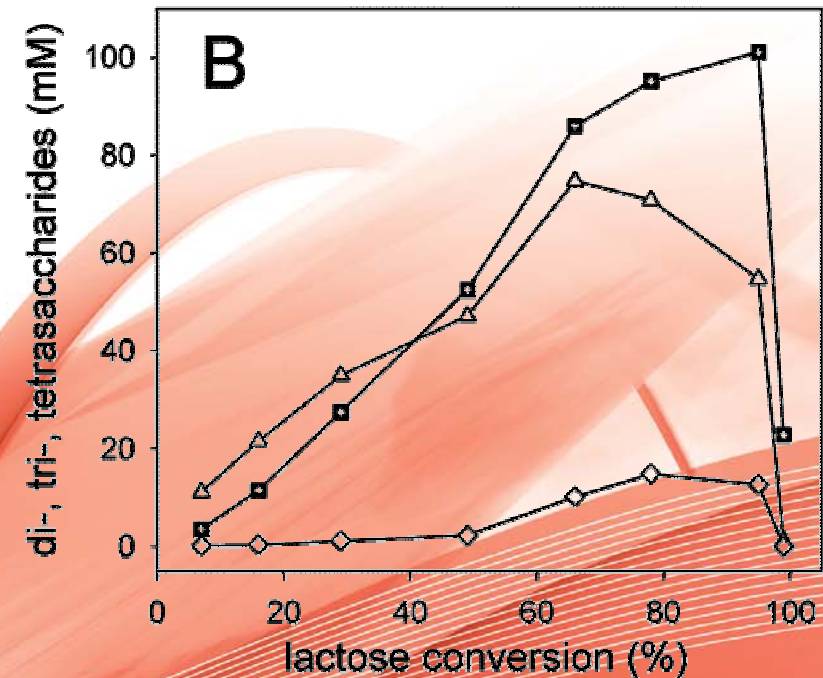
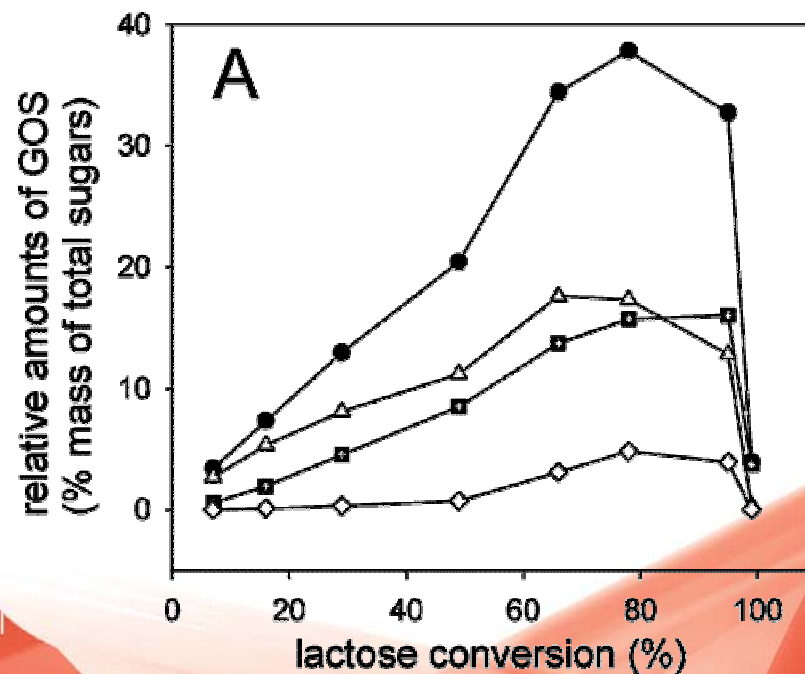
β -Galactosidase from *Lb. reuteri*

- employed for the production of GOS in a batch process
- Conditions:
 - 205 g/L lactose (600 mM)
 - 30°C
 - pH 6.5, 1 mM Mg²⁺
 - 0.8 U β -Gal per mL
- maximum GOS yield of approx. 70 g/L
- GOS are only transiently formed



β -Galactosidase from *Lb. reuteri*

- GOS only transiently formed; composition and concentration of products changes dramatically with degree of substrate conversion
 - , disaccharides; Δ , trisaccharides; \diamond , tetrasaccharides



β -Galactosidase from *Lb. reuteri*

HPAEC with Dionex Carbopac PA1,
identification of products:

1 galactose

2 glucose

3 Gal β -(1,6)-Gal

4 Gal β -(1,6)-Glc (allolactose)

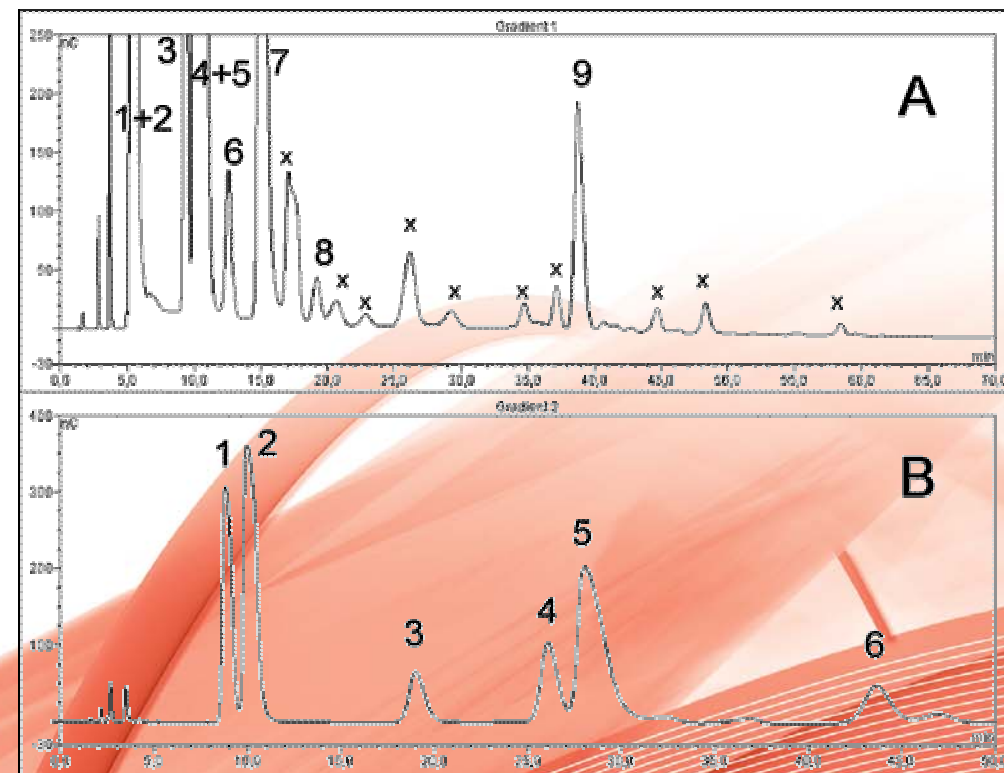
5 lactose

6 Gal β -(1,3)-Gal

7 Gal β -(1,6)-Lac

8 Gal β -(1,3)-Glc

9 Gal β -(1,3)-Lac

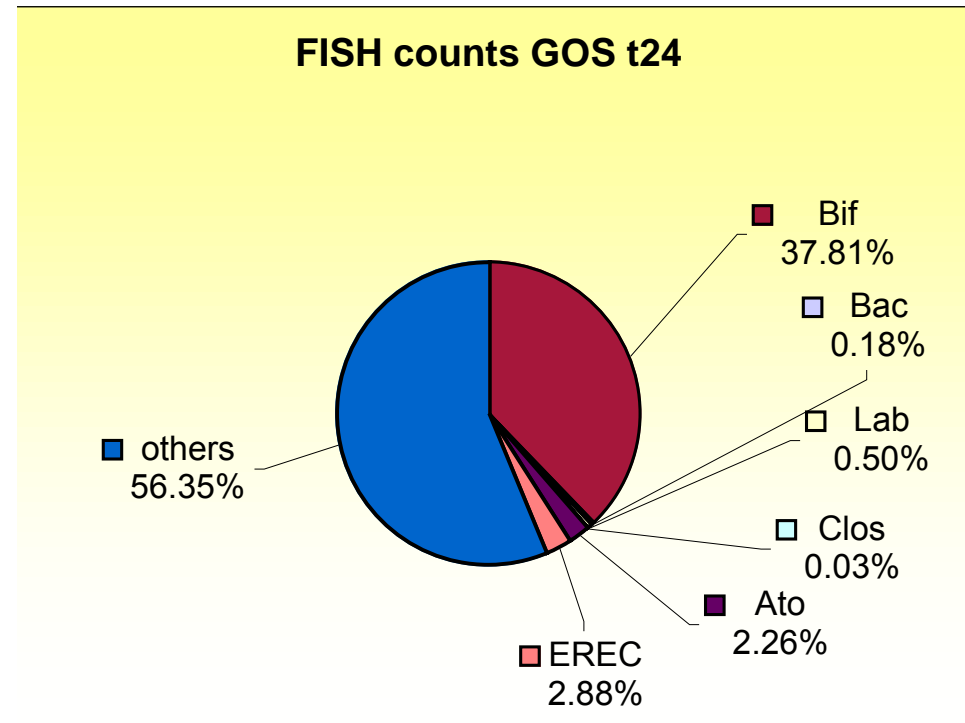
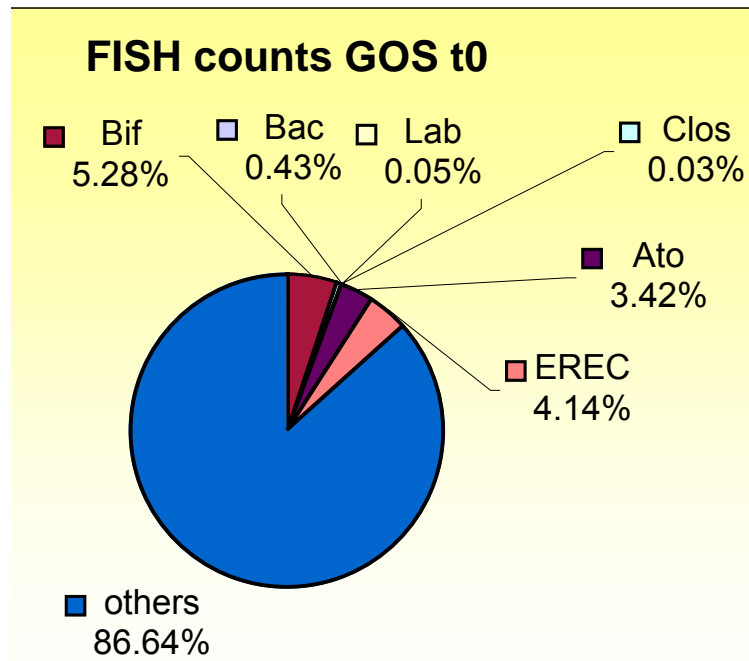


β -Galactosidase from *Lb. reuteri*

- main reaction products during lactose conversion:
 - Gal β (1,6)-Glc (allolactose)
 - Gal β (1,6)-Gal
 - Gal β (1,3)-Glc
 - Gal β (1,3)-Gal
 - Gal β (1,6)-Lac (galactosyl-lactose)
 - Gal β (1,3)-Lac
- composition of individual components changes with degree of lactose conversion
- high specificity for forming β -1,6 linkage, at least 60% of GOS contain this linkage

β -Galactosidase from *Lb. reuteri*

Test for prebiotic effect





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