The ESF Short Visit Grantee attended the 10th Carbohydrate Bioengineering Meeting (CBM) that was held on April 21-24, 2013 in Prague, Czech Republic. This meeting was part of the ESF Activity entitled Euroglycoscience Forum.

The CBM meeting focused on presentation and discussions on mechanisms and structure-function relationships of carbohydrate modifying enzymes, glycomics, systems glycobiology, and bioinformatics, synthesis, structure and function of carbohydrates and glycoconjugates, carbohydrate and enzyme engineering to name a few. Different scientists around the globe actively participated in the said meeting either by being an oral speaker, poster presenter or attentive listener.

The grantee was privileged to present her PhD research in University of Natural Resources and Life Sciences Vienna, Austria on the said Meeting. Her topic dealt on the “Enzymatic synthesis of prebiotic galacto-and hetero-oligosaccharides by *β*-galactosidase (*β*-gal II) from an infant isolate of *Bifidobacterium breve*” was staged as a poster presentation (a copy of the poster is attached to this report).

During her presentation, some questions were asked either on the method used or results obtained. There were also some information that were suggested on how to further improve the research. Moreover, during the 3-day meeting, the grantee was also able to meet other scientists, senior researchers and fellow students from other institutions that are on the same field. This was a good way as the grantee was able to start build relationships with other researchers in the field.

There were also oral presentations that were really on the same interest of the grantee. Listening to those presentations helped the audience informed what others have done or are doing and inspired the grantee’s research ideas more.

In summary, the 3-day CBM meeting was a fruitful one. Presenting own result research and listening to senior scientists on the research inspired more the younger researcher, like the grantee, to continue and excel on her own research.

Enzymatic synthesis of prebiotic galacto-and hetero-oligosaccharides by β-galactosidase (β-gal II) from an infant isolate of *Bifidobacterium breve*

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INTRODUCTION

The β-gal II gene from *B. breve* DSM 20213, encoding a β-galactosidase, was cloned and heterologously expressed in *Escherichia coli*. The recombinant β-galactosidase was purified, characterized and investigated in detail with respect to its propensity to transfer galactosyl moieties onto lactose, the primary hydrolysis products D-glucose and D-galactose, and certain sugar acceptors such as L-fucose, *N*-acetyl-D-galactosamine and *N*-acetyl-D-glucosamine under defined, initial-velocity conditions. The rate constant ratios determined for these different acceptors can be used as a measure for the ability of a certain substance to act as a galactosyl acceptor, which in turn allows an estimation of the transgalactosylation products obtained.

RESULTS

Molecular Weight Determination

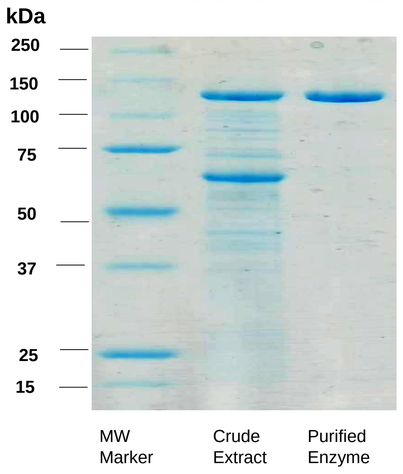


Figure 1. SDS – PAGE of *B. breve* β-gal II

Table 1. Molecular mass of *B. breve* β-gal II

Method		Proposed Molecular structure
SDS- PAGE	Static Light Scattering	
~116 kDa	211kDa	Homodimer

Kinetic constants

Table 2. Kinetic parameters of *B. breve* β-gal II for the hydrolysis of lactose and *o*NPG

Kinetic parameter	Substrates	
	Lactose	*o*NPG
v_{max} (μmol.min^{-1}.mg^{-1})	97 ± 5	188 ± 3
K_m (mM)	7.5 ± 0.9	0.67 ± 0.07
k_{cat} (s^{-1})	188 ± 10	364 ± 6
k_{cat}/K_m (mM^{-1} s^{-1})	25 ± 4	543 ± 65
$K_{i,\ Gal}$ (mM)	27 ± 6	34 ± 5
$K_{i,\ Glc}$ (mM)	-	37 ± 4

pH and Temperature Optimum

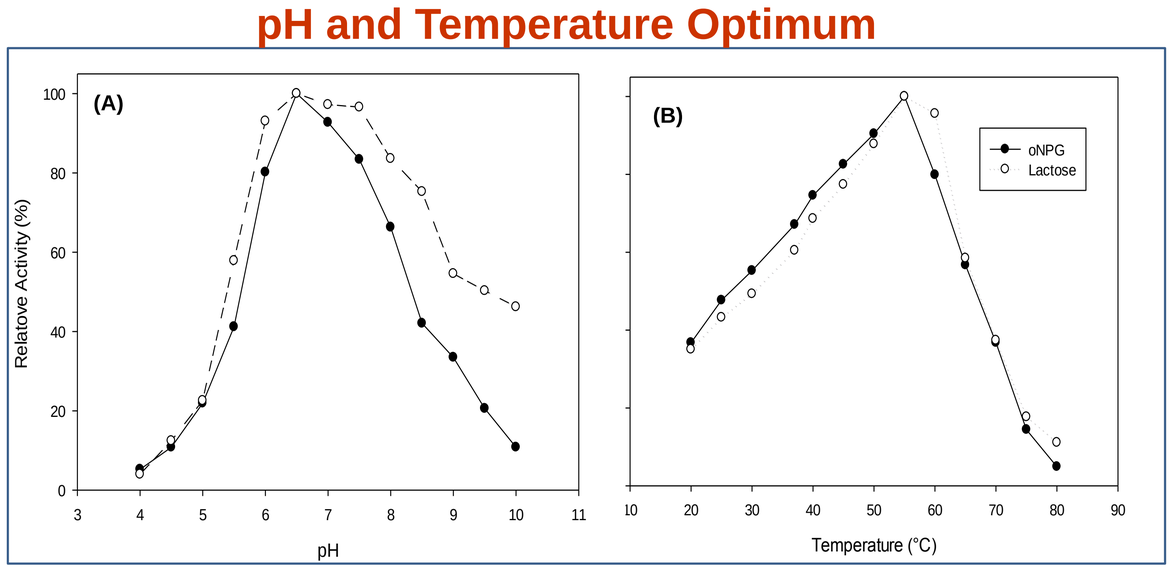


Figure 2. pH (A) and temperature (B) dependence of β-gal II activity, using *o*NPG and lactose.

Temperature Stability

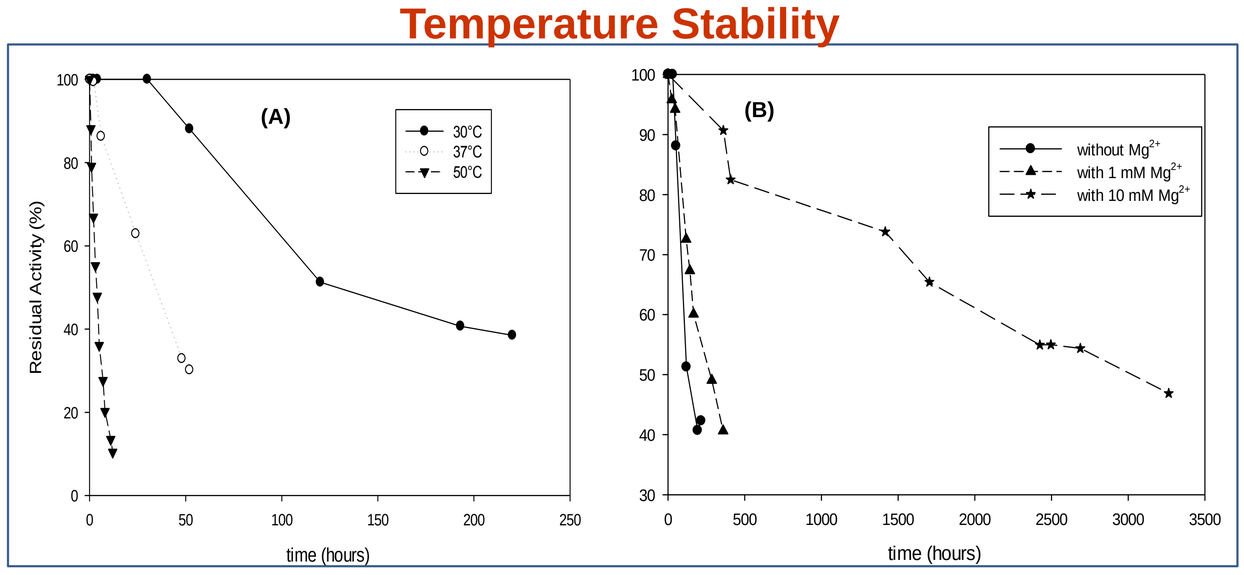
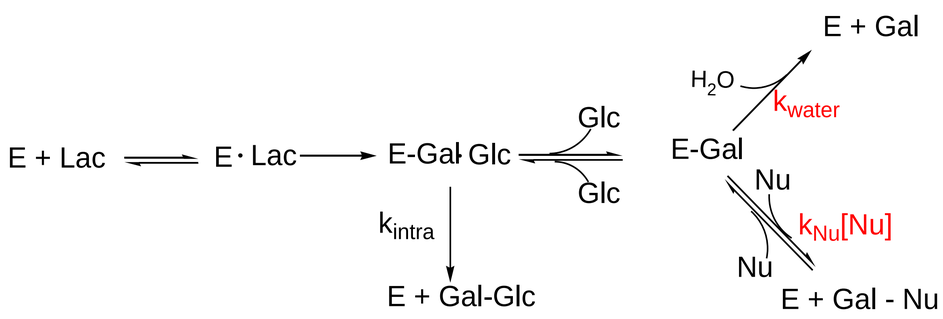


Figure 3. Stability of β-gal II at different temperatures without $MgCl_2$ (A) and with the presence of 1 mM and 10 mM $MgCl_2$ (B) at 30ºC.Experiments were performed in phosphate buffer, pH 6.5.

Production of Galacto- and Hetero-Oligosaccharides



Transgalactosylation potential of a β-galactosidase can be *measured* by determination of the **partition coefficient.**

$$\frac{v_{Glc}}{v_{Gal}} = 1 + \frac{k_{Nu}}{k_{water}}[Nu]$$

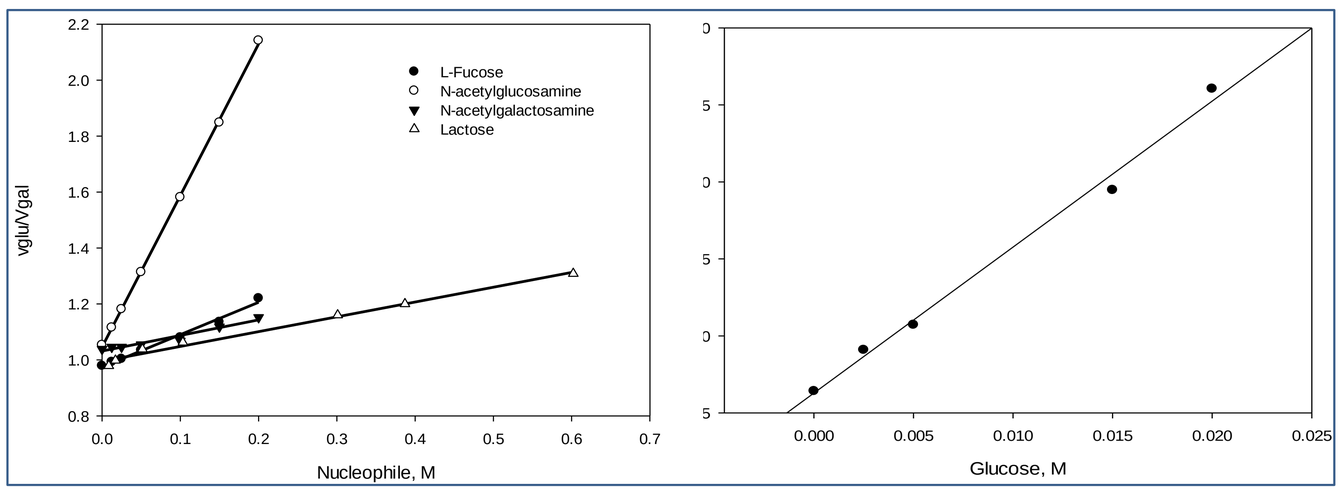


Figure 4. Rate constant ratios of *B. breve* β-gal II for different acceptors using lactose (A) and *o*NPG (B) as galactosyl donor.

Table 3. Transgalactosylation activitiy of β-gal II from *B. breve*

Nucleophile (Galactosyl Acceptor)	k_{Nu}/k_{water} (M^{-1})
D-glucose	9.64 ± 1.07
Lactose	0.53 ± 0.05
L-Fucose	1.16 ± 0.05
N-acetyl-D-glucosamine	5.31 ± 0.13
N-acetyl-D- galactosamine	0.55 ± 0.05

Preferred galactosyl acceptor:

D-glucose **>** *N*-acetyl-D-glucosamine **>** L-Fucose **>** Lactose, *N*-acetyl-D-galactosamine

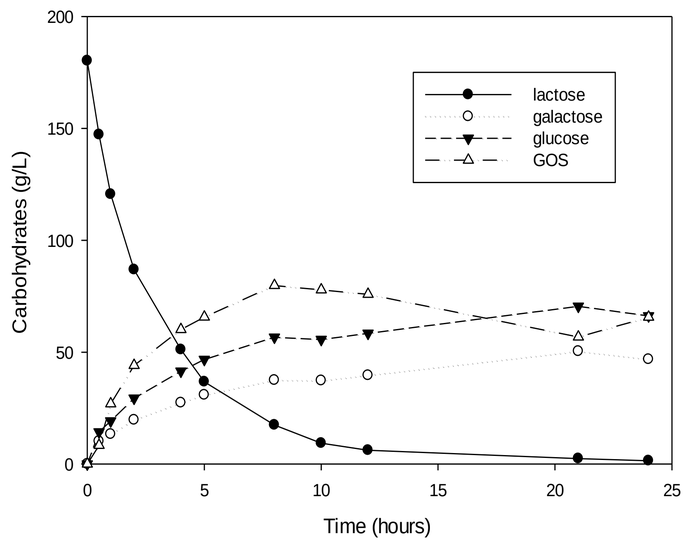


Figure 5. GOS production by *B. breve* β-gal II. Reaction was done at 30 C at 180gL^{-1} initial lactose concentration in 50 mM sodium phosphate buffer, pH 6.5 and 1 mM Mg^{2+}.

CONCLUSION

The β-gal II from *B. breve* was found to have 40% transgalactosylation activity and with high preference to constituents of HMO. This enzyme can be therefore be of interest for the production novel HOS with potentially extended functionality in addition to GOS.

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