Discovery of a novel thermophile β-galactosidase, *Tt*bGal1, for the production of prebiotic oligosaccharides from acid whey

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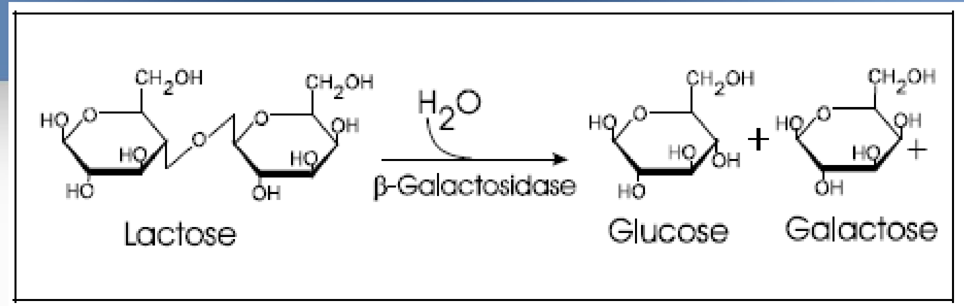
β- galactosidases (E.C. 3.2.1.23)

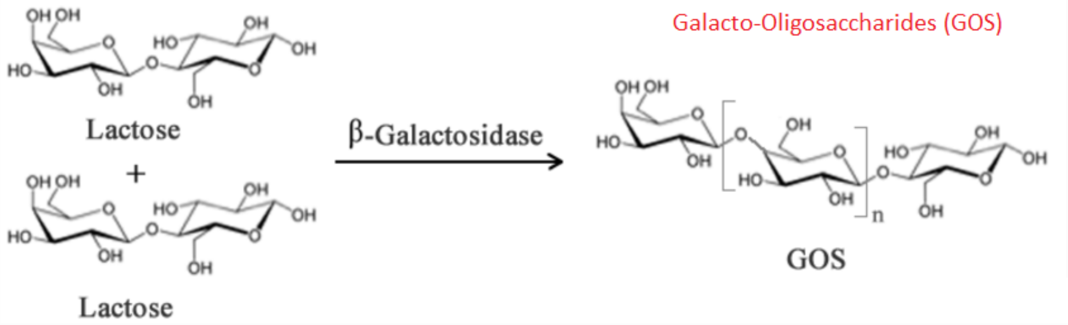
- glycosyl hydrolase → hydrolysis of lactose to glucose and galactose



β-Galactosidases in dairy industry

- lactose hydrolysis in dairy products
- production of lactose-free products, for consumers with lactose intolerance

- β-galactosidases also catalyze the **transgalactosylation** reaction, producing galactooligosaccharides (**GOS**)



GOS are significant prebiotics

- improve the gut health
- promote the growth of the probiotic intestinal bacterial flora.

What about the use of a low-cost material as substrate, which would not compete with food and feed raw materials?

Whey,
a liquid byproduct of the dairy industry

Acid whey
- pH 4.5
- From cottage cheese and Greek yoghurt manufacturing

Sweet whey
- pH 6.5
- From cheese manufacturing

Commercial β-galactosidases for GOS production
- *Aspergillus oryzae*
- *Kluyveromyces lactis,*
- *Bacillus circulans*

High transgalactosylation activity, high GOS yield

Whey as a waste material

Disposal methods

- Spraying in fields
- Discharge in water bodies
- Municipal sewage system
- Animal feed

Issues with current disposal methods

- Smell, salt and heavy polluting load
- High BOD (30.000-35.000 ppm) and COD (60.000-80.000 ppm)

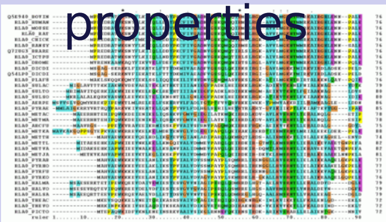
- 100 L of milk used for cheese → 80-90 L of whey
- Annual production of whey: 160 million tons, sweet whey is 22.5 million tons
- Acid whey production is increasing steadily, due to increasing popularity of the Greek strained yoghurt worldwide

Production of GOS from whey

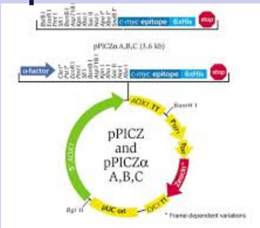
- Good yield with β-galactosidases from *A. oryzae* and *K. lactis* in sweet whey (32.5%)
- Most known β-galactosidases are active in neutral pH
- For valorization of acid whey, ***thermophile, acidic*** β-galactosidases are needed

Discovery and characterization of novel enzymes

Database mining of genes with desired properties



Cloning in proper vectors



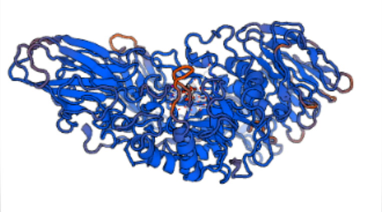
Transformation in the yeast *Pichia pastoris*

Screening and selection of recombinant clones

Production of heterologous enzymes

Purification of recombinant enzymes with chromatographic methods

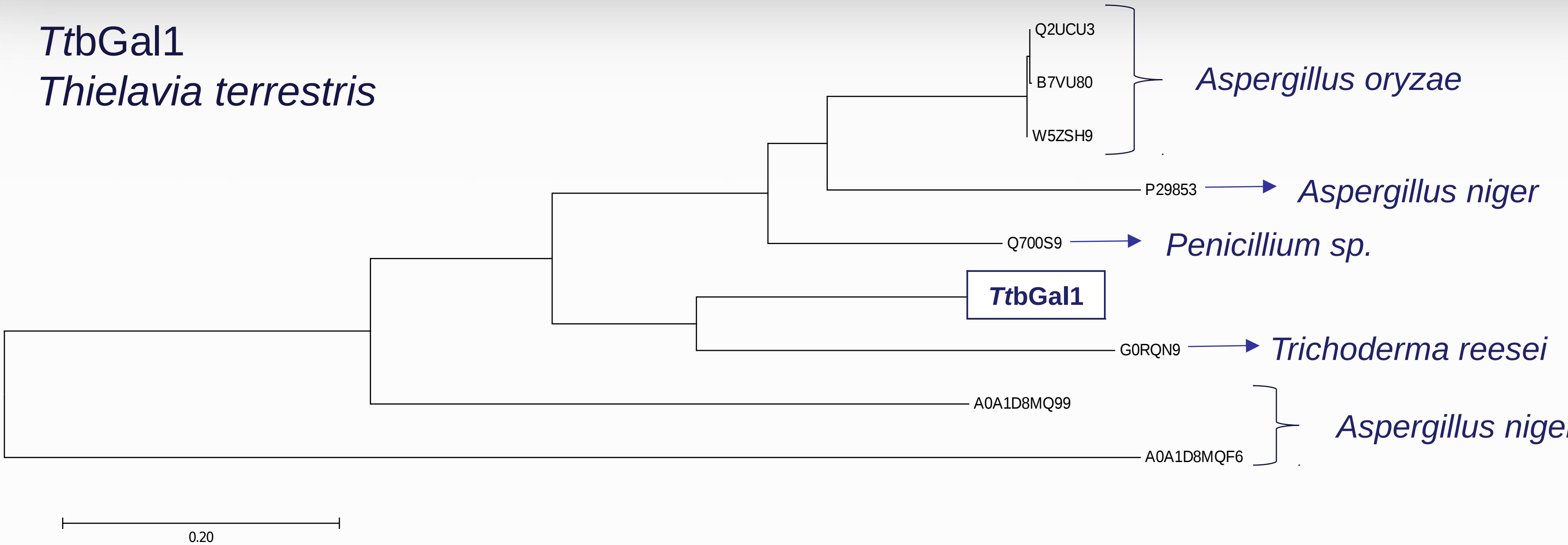
Biochemical and physicochemical characterizati



Biocatalysis applications

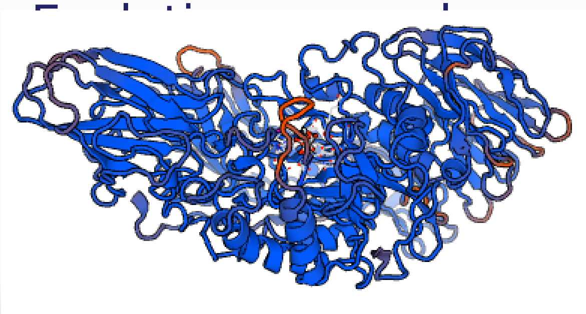
Bioinformatic analysis

*Ttb*Gal1
Thielavia terrestris



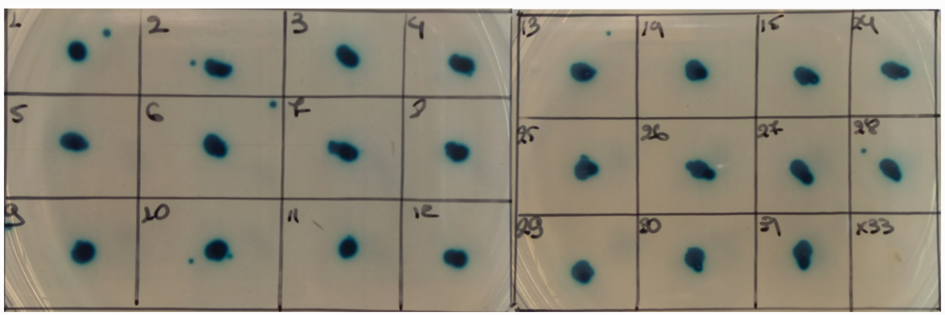
Molecular Phylogenetic analysis by Maximum Likelihood method

were conducted in MEGA7 (Kumar

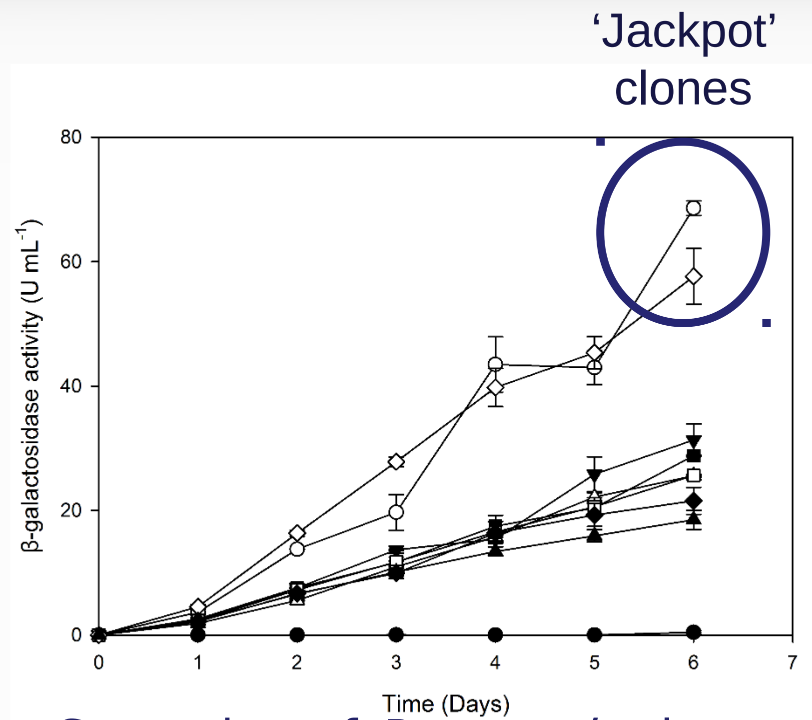


Thielavia terrestris 2047729 structure prediction based on beta-galactosidase from *Aspergillus oryzae* (61.22% identity) with SWISS-MODEL

Screening of recombinant clones

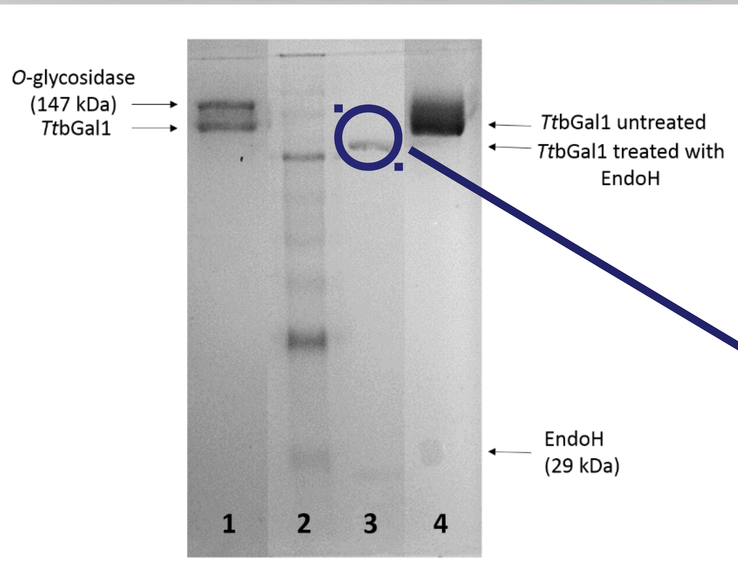


Screening of *P. pastoris* clones for β-galactosidase activity in plate assays with X-GAL as the substrate



Screening of *P. pastoris* clones for β-galactosidase activity in liquid media.

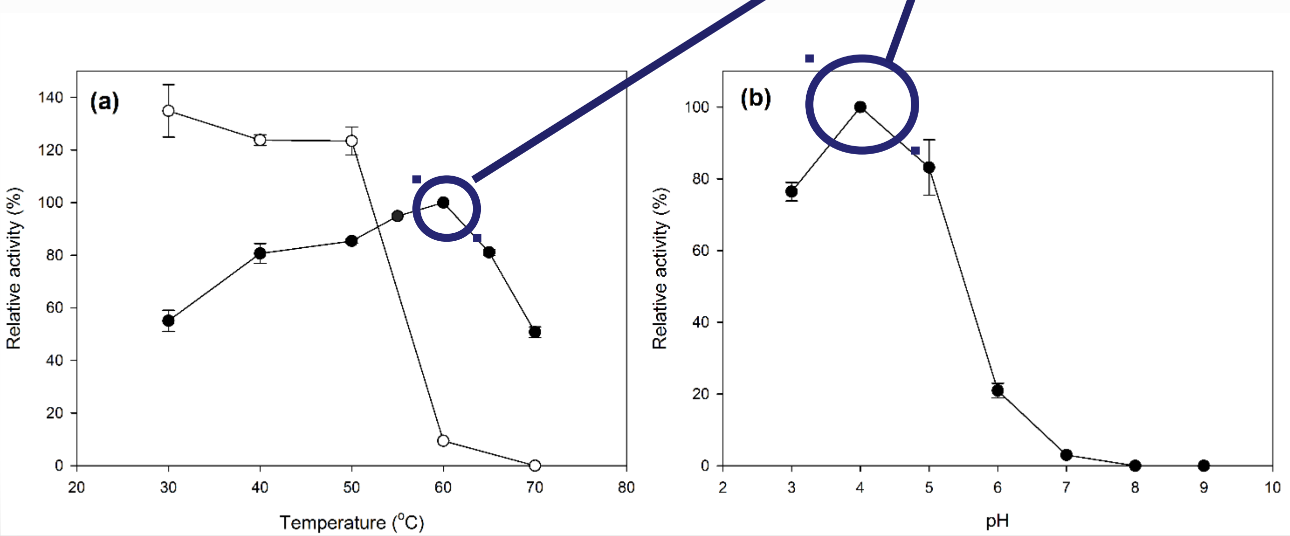
Characterization of purified TtbGal1



T optimum: 60 °C

pH optimum: 4

M.W.: 110 kDa



Characterization of purified TtbGal1

Kinetic parameters

	Km (mM)	Kcat (min^{-1})	k_{cat}/K_m $(mM\ min)^{-1}$	Specific activity ($U\ mg^{-1}$)
oNPhG	0.18 ± 0.02	275280 ± 7932	1522566 ± 187443	1956.5 ± 117.7
lactose	12.4 ± 1.4	24636 ± 759	1981 ± 233	95.3 ± 10.6

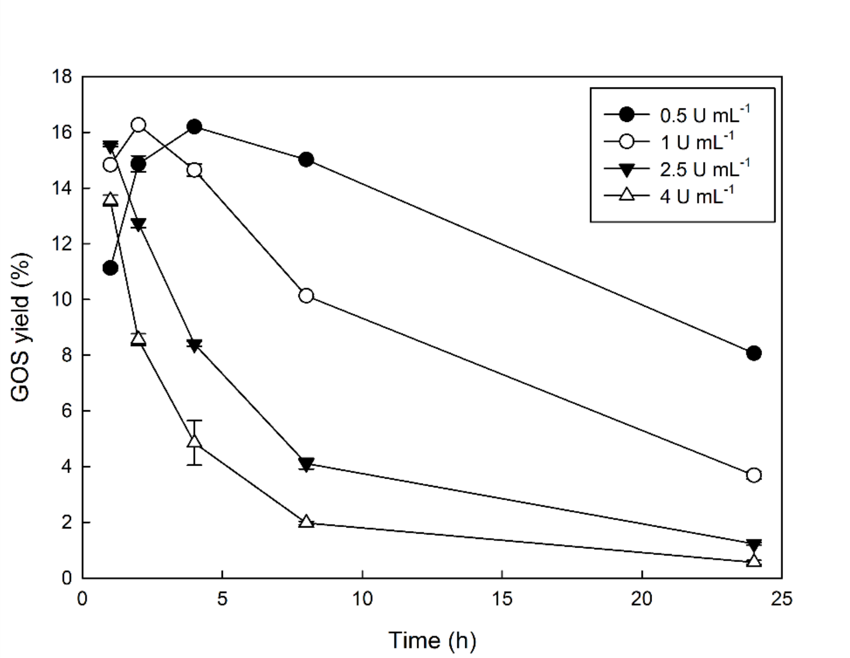
Effect of salts on the activity of TtbGal1.

Salts	*Residual activity (%)*	
	1 mM	**10 mM**
Control	100	100
$MgCl_2$	79.8 ± 13.7	105.9 ± 0.6
$CuCl_2$	93.4 ± 5.5	97.2 ± 2.9
NaCl	86.5 ± 7.2	87.7 ± 2.6
$MnCl_2$	96.2 ± 2.1	-
KCl	86.8 ± 3.1	91.9 ± 3.4
$CaCl_2$	94.0 ± 8.8	-
NaN_3	94.8 ± 10.5	-

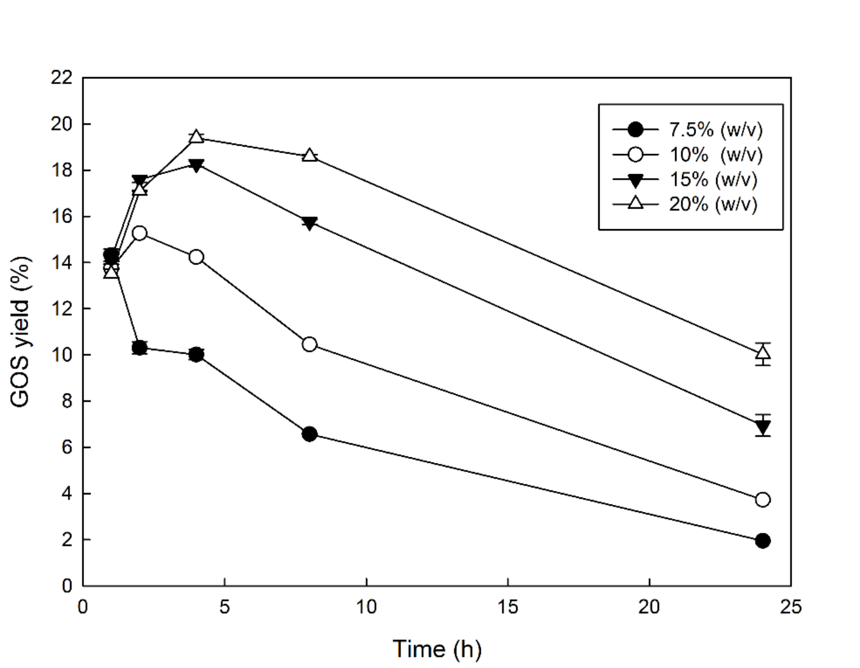
Very satisfactory activity in the presence of a variety of salts → promising property for application in untreated acid whey

Optimization of GOS production in defined lactose solutions

Enzyme load



Substrate concentration

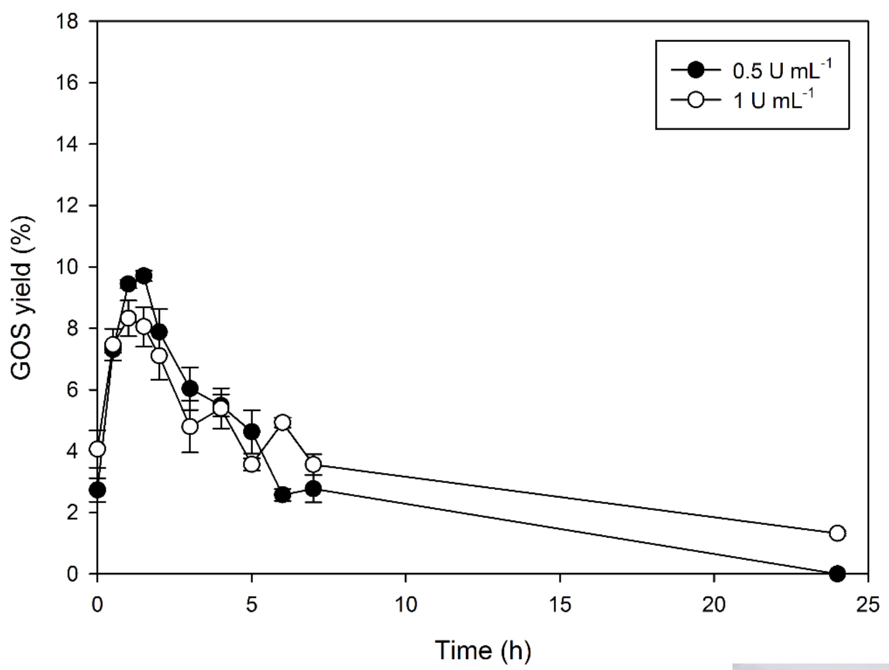


Maximum GOS concentration:
1.46 ± 0.02 % (w/v)

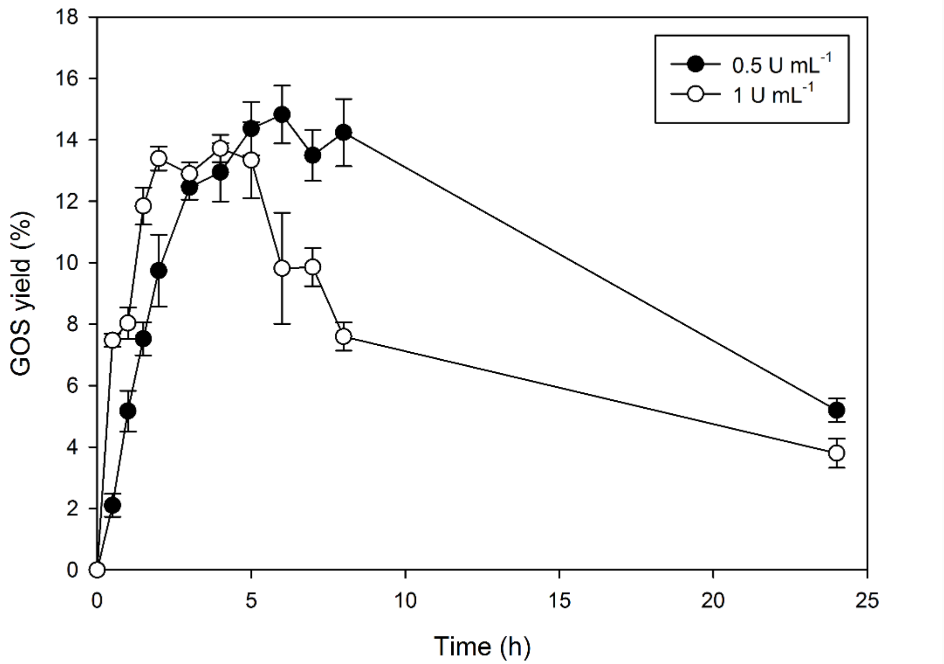
Maximum GOS concentration:
3.26 ± 0.04 % (w/v)

TtbGal1-mediated GOS synthesis from acid whey

Untreated whey
3.4% (w/v) lactose



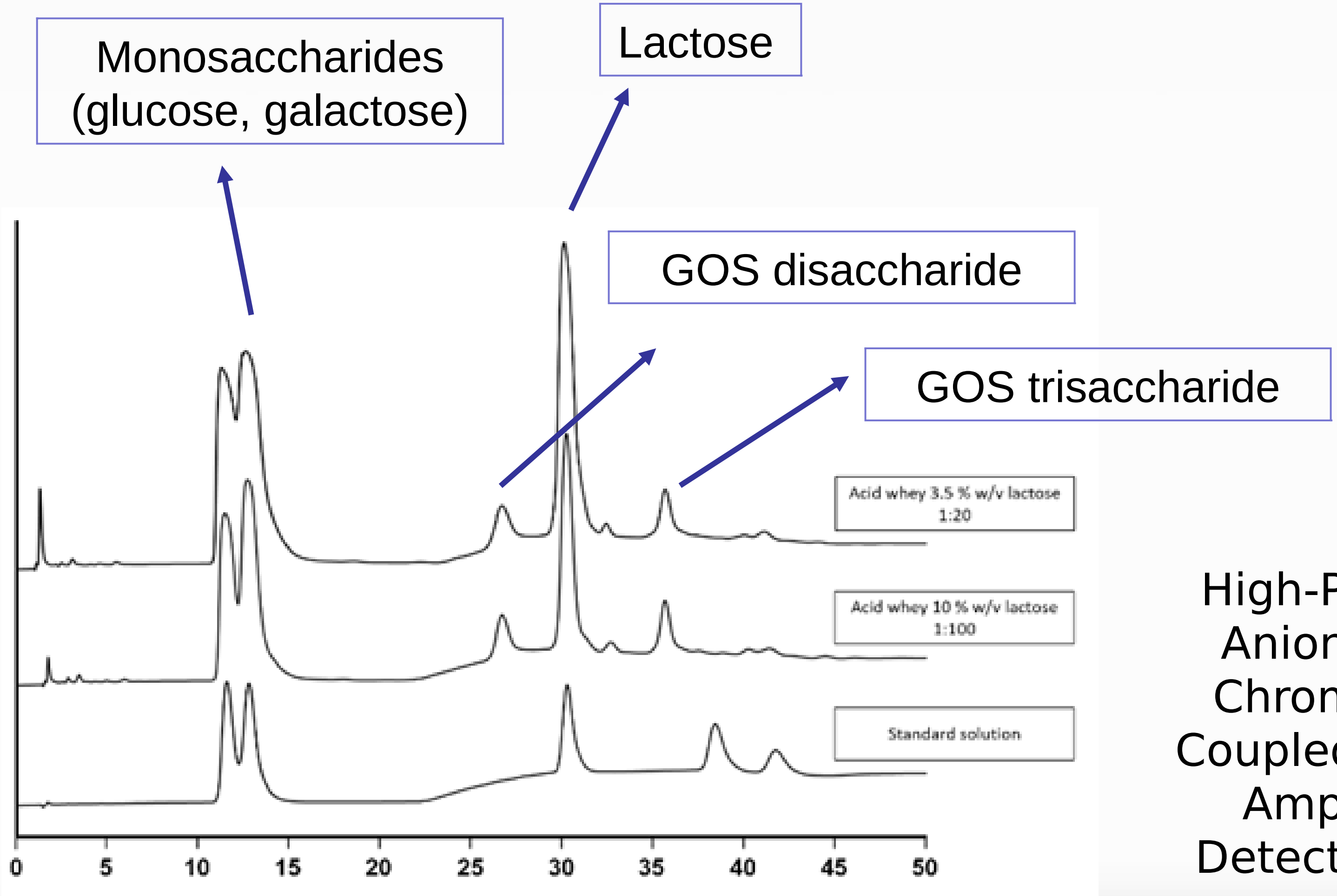
Concentrated whey
9.28% (w/v) lactose



Maximum GOS concentration:
0.35 ± 0.05 % (w/v)

Maximum GOS concentration:
1.49 ± 0.08 % (w/v)

TtbGal1-mediated GOS synthesis from acid whey



High-Performance Anion-Exchange Chromatography Coupled with Pulsed Amperometric Detection (HPAEC-PAD)

Conclusions

- A novel fungal β-galactosidase, *Tt*bGal1, was heterologously expressed, purified and characterized
- *Tt*bGal1 is ***thermostable*** and is optimally active in ***acidic pH***
- Satisfactory activity in the presence of salts
- GOS production with yields up to 19.4%
- Valorization of acid whey as a substrate to produce GOS with prebiotic activity

Work in progress...

- Further optimization is needed
- LC-MS analyses to determine the chemical nature of the produced GOS
- Scale-up of the process

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http://www.chemeng.ntua.gr/indubiocat/index.html

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Thank you for your attention!