

## Quantitative enzymatic production of sialylated galactooligosaccharides with an engineered sialidase from *Trypanosoma rangeli* - DTU Orbit (08/11/2017)

### Quantitative enzymatic production of sialylated galactooligosaccharides with an engineered sialidase from *Trypanosoma rangeli*

Sialylated galactooligosaccharides (GOS) represent a potential infant formula ingredient, which is believed to contribute with a combination of the beneficial properties of the prebiotic GOS as well as of sialylated human milk oligosaccharides. Sialylated GOS do not exist in natural milk, but can be produced from  $\kappa$ (kappa)-casein glycomacropeptide (CGMP), a sialylated side stream component from cheese-making, by sialidase-catalyzed transsialylation. Using a rationally designed mutant of the sialidase from *Trypanosoma rangeli*, Tr13, with enhanced transsialylation activity, six different GOS preparations with a varying degree of polymerization (DP) were effectively sialylated with molar yields of 20-30% on the CGMP sialyl in batch reactions. The rate of sialylation of the individual DPs was largely dependent on the DP distribution in each GOS preparation, and Tr13 catalysis did not discriminate against large GOS molecules, providing the novelty point that GOS molecules are sialylated independently of their size by Tr13. Using CGMP, GOS, and Tr13, the production of gram-scale quantities of sialyl-GOS was achieved in 20L volume reactions. Compared to the benchmark transsialidase from pathogenic *Trypanosoma cruzi*, the Tr13 was significantly more thermostable. By employing an enzymatic membrane reactor, Tr13 could be recycled and after seven consecutive 1-h reaction cycles, the biocatalytic productivity of the enzyme was increased 7-fold compared to the batch reaction. Assuming that the enzyme may be specific for  $\alpha$ -2,3-bound sialyl moieties only, and that only 50% of sialyl linkages in CGMP are  $\alpha$ -2,3-linked, the molar yield of sialyl-GOS on the available  $\alpha$ -2,3-bound sialyl moieties in CGMP reached 80% in the enzymatic membrane reactor system.

#### General information

State: Published

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Pages: 42-50

Publication date: 2016

Main Research Area: Technical/natural sciences

#### Publication information

Journal: Enzyme and Microbial Technology

Volume: 82

ISSN (Print): 0141-0229

Ratings:

BFI (2017): BFI-level 1

Web of Science (2017): Indexed yes

BFI (2016): BFI-level 1

Scopus rating (2016): CiteScore 2.83 SJR 0.759 SNIP 1.025

Web of Science (2016): Indexed yes

BFI (2015): BFI-level 1

Scopus rating (2015): SJR 0.85 SNIP 0.969 CiteScore 2.63

Web of Science (2015): Indexed yes

BFI (2014): BFI-level 1

Scopus rating (2014): SJR 1.061 SNIP 1.214 CiteScore 3.12

Web of Science (2014): Indexed yes

BFI (2013): BFI-level 2

Scopus rating (2013): SJR 1.165 SNIP 1.376 CiteScore 3.2

ISI indexed (2013): ISI indexed yes

Web of Science (2013): Indexed yes

BFI (2012): BFI-level 2

Scopus rating (2012): SJR 1.204 SNIP 1.281 CiteScore 2.78

ISI indexed (2012): ISI indexed yes

Web of Science (2012): Indexed yes

BFI (2011): BFI-level 2

Scopus rating (2011): SJR 1.062 SNIP 1.27 CiteScore 2.74

ISI indexed (2011): ISI indexed yes

Web of Science (2011): Indexed yes

BFI (2010): BFI-level 2

Scopus rating (2010): SJR 1.201 SNIP 1.565

Web of Science (2010): Indexed yes

BFI (2009): BFI-level 2

Scopus rating (2009): SJR 1.305 SNIP 1.504

Web of Science (2009): Indexed yes

BFI (2008): BFI-level 1

Scopus rating (2008): SJR 1.208 SNIP 1.34

Web of Science (2008): Indexed yes

Scopus rating (2007): SJR 0.976 SNIP 1.257

Web of Science (2007): Indexed yes

Scopus rating (2006): SJR 0.907 SNIP 1.433

Web of Science (2006): Indexed yes

Scopus rating (2005): SJR 0.915 SNIP 1.429

Web of Science (2005): Indexed yes

Scopus rating (2004): SJR 0.847 SNIP 1.263

Scopus rating (2003): SJR 0.798 SNIP 1.218

Web of Science (2003): Indexed yes

Scopus rating (2002): SJR 0.89 SNIP 1.238

Web of Science (2002): Indexed yes

Scopus rating (2001): SJR 0.804 SNIP 1.183

Web of Science (2001): Indexed yes

Scopus rating (2000): SJR 0.668 SNIP 1.191

Web of Science (2000): Indexed yes

Scopus rating (1999): SJR 0.925 SNIP 1.202

Original language: English

Enzymatic membrane reactor, Sialylated galactooligosaccharides, Transsialylation, Trypanosoma rangeli  
DOIs:

10.1016/j.enzmictec.2015.08.010

Source: FindIt

Source-ID: 2280666905

Publication: Research - peer-review › Journal article – Annual report year: 2015