5TH APPLIED SYNTHETIC BIOLOGY IN EUROPE
2-4 November 2020 | Delft, Netherlands

Universidade do Minho
Escola de Engenharia

CENTRE OF BIOLOGICAL ENGINEERING

MIT Portugal

Topic: Pathway engineering and synthetic metabolism

UDP-glucuronic acid heterologous production in *Escherichia coli* and *Saccharomyces cerevisiae* chassis

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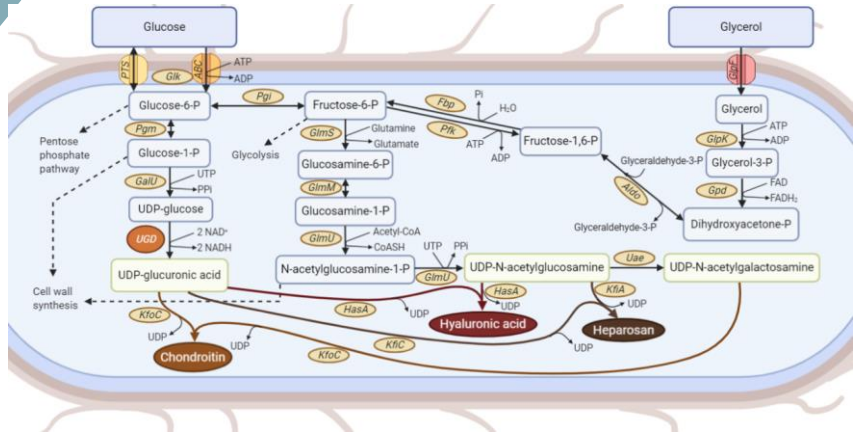
Abstract

Glycosaminoglycans (GAG) such as hyaluronic acid and chondroitin sulfate are **nutraceuticals** with important medical and cosmetic applications. Usually, these compounds are extracted from animal sources, though concerns on animal products use are driving the search for **alternative GAG sources**. Some pathogenic bacterial strains naturally produce GAG-like polysaccharides through identified pathways which have been the basis for their **biotechnological production** using non-pathogenic hosts. Uridine diphosphate **(UDP)-glucose dehydrogenase (UGD)** catalyzes a common step in GAG biosynthetic pathways that **generates UDP-glucuronic acid (GlcA)**, reported as the limiting substrate for GAG production. However, few UGD genes have been evaluated for pathway optimization. In the present work, ***Escherichia coli* and *Saccharomyces cerevisiae* expressing alternative heterologous UGD genes** have been constructed. Three UGD genes originally from different genomic sources (*Capra hircus*, *Zymomonas mobilis*, *Lactobacillus johnsonii*) were expressed in *S. cerevisiae* strains and *in vivo* UDP-GlcA production was achieved up to 7.2 μmol/g_{cells}. The two prokaryotic UGD genes were also expressed in *E. coli* BL21 which resulted in up to 23.6 μmol/g_{cells} UDP-GlcA. The activity of the recombinant UGD enzymes was also evaluated through *in vitro* reactions using cell extracts. This work reveals promising alternative enzymes for industrial GAGs production using heterologous hosts. In particular, the expression of the eukaryotic *Ch*UGD gene and the use of *S. cerevisiae* system were herein explored for the first time.

Introduction



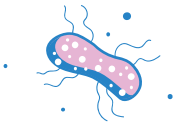
Biosynthetic pathway for glycosaminoglycans (GAG) biotechnological production
UDP-glucose dehydrogenase (UGD) – limiting step in GAGs production

Objective

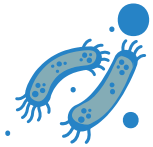
Evaluate new UGD enzymes for GAG production

Methodology

Capra hircus' synthetic *ugd* gene codon-optimized for *S. cerevisiae*



Zymomonas mobilis gDNA



Lactobacillus johnsonii gDNA

Ugd gene amplification

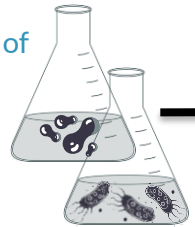
Chugd

Zmugd

Lbjugd

Introduction in an expression vector

Transformation



Production of UDP-GlcA

Lysis

In vitro reactions
2.5 mM UDP-Glc

In vivo analysis

uHPLC

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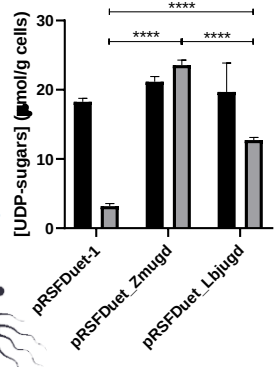
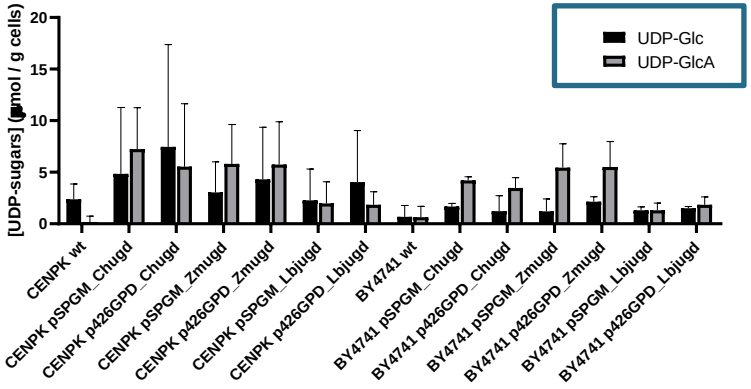
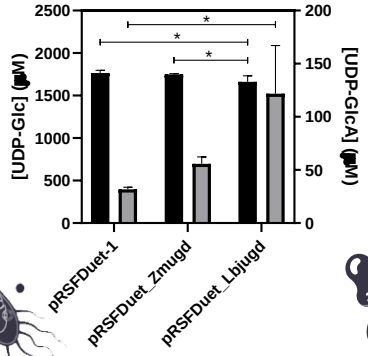
Results

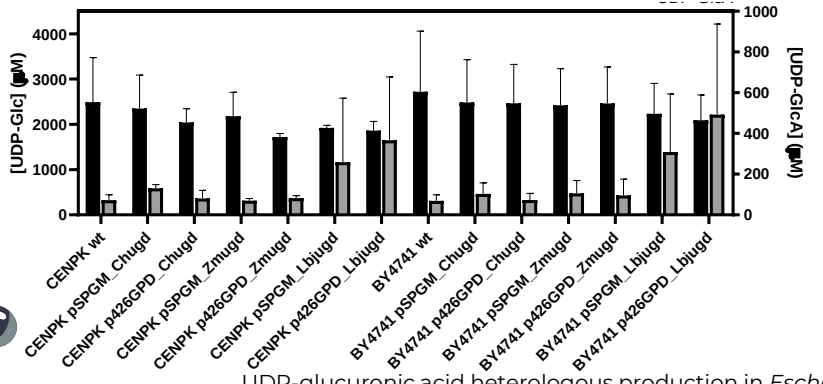
In vivo UDP-glucuronic acid production





In vitro reactions





Conclusions

- UGD from *Z. mobilis* and *L. johnsonii* were sucessfully expressed in *E. coli* and *S. cerevisiae* and resulted in UDP-GlcA production both *in vivo* and *in vitro*
- UGD from *C. hircus* codon-optimized for *S. cerevisiae* allowed the highest UDP-GlcA *in vivo* production reported so far
- While *Chugd* and *Zmugd* expression resulted in higher UDP-GlcA *in vivo*, *Lbjugd* exhibited the highest activity *in vitro*
- Yeast seems a promising alternative host for GAG production

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