Regulatory insights into the production of UDP-N-acetylglucosamine by Lactobacillus casei

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UDP-N-acetylglucosamine (UDP-GlcNAc) is an important sugar nucleotide used as a precursor of cell wall components in bacteria, and as a substrate in the synthesis of oligosaccharides in eukaryotes. In bacteria UDP-GlcNAc is synthesized from the glycolytic intermediate d-fructose-6-phosphate (fructose-6P) by four successive reactions catalyzed by three enzymes: glucosamine-6-phosphate synthase (GlmS), phosphoglucosaminemutase (GlmM) and the bi-functional enzyme glucosamine-1-phosphate acetyltransferase/N-acetylglucosamine-1-phosphate uridylyltransferase (GlmU). We have previously reported a metabolic engineering strategy in Lactobacillus casei directed to increase the intracellular levels of UDP-GlcNAc by homologous overexpression of the genes glmS, glmM and glmU.

One of the most remarkable features regarding the production of UDP-GlcNAc in L. casei was to find multiple regulation points on its biosynthetic pathway: (1) regulation by the NagB enzyme, (2) glmS RNA specific degradation through the possible participation of a glmS riboswitch mechanism, (3) regulation of the GlmU activity probably by end product inhibition and (4) transcription of glmU.

Oligosaccharides, which form part of glycoconjugates on the cell surface or are soluble as in the human milk, play active roles in numerous cell-cell interactions. They are also used as anchoring molecules for pathogens, antibodies and hormones. Interestingly, the first recombinant strain that simultaneously overexpressed two of the genes (glmMS), cloned with their intergenic region, was able to accumulate high amounts of UDP-GlcNAc. The other three recombinant strains constructed, PL30 (glmS), PL31 (glmM) and PL32 (glmU), showed no increase or an increase of about 1.5 times on the UDP-GlcNAc level. The measurements of the specific activities of each enzyme in the
recombinant \textit{L. casei} strains as well as the
RT-qPCR results show that the pathway
for the production of UDP-GlcNAc is
very tightly regulated in \textit{L. casei}.

**Regulation by NagB Enzyme**

Cell extracts of strain PL30 ( glmS ) showed
about 12 times more GlmS activity than
the control strain.9 However, this high
increase was not reflected in the UDP-
GlcNAc concentration, which remains
at the same level as that of the control
strain. This result might be explained by
the activity of the enzyme glucosamine-
6-phosphate deaminase (NagB) that
catalyzes the opposite reaction of GlmS,
thus the conversion of glucosamine-6P
into fructose-6P. Accordingly, a gene
LCABL_31070, encoding a presumed
NagB, is present in the \textit{L. casei} BL23
genome.12 Recently, we have assayed the
UDP-GlcNAc production in all the engi-
neered strains described above cultured on
GlcNAc as the carbon source. The UDP-
GlcNAc production in these experimental
conditions was compared with the pro-
duction on glucose (Table 1). The results
showed that the growth on GlcNAc did
not result in an important increment in
the UDP-GlcNAc pool, suggesting that
the NagB activity directed the glucos-
amine-6P obtained from the GlcNAc
catabolism to fructose-6P. Those obser-
vations suggested that the NagB enzyme
plays an important role in the control of
individual genes. However, post-transcriptional modification of such mRNA can modulate the gene’s translational expression under specific environmental conditions. This is the case for the cis-acting regulatory RNAs called riboswitches, including the glmS riboswitch that uses glucosamine-6P as a cofactor and activates self-cleavage of the bacterial ribozyme, glucosamine-6P, which is part of the mRNA coding for GlmS.13 The glmS ribozymes are based in conserved structures more than in conserved sequences and they are highly specific for glucosamine-6P.13-18 The glmS riboswitch was first described in Bacillus subtilis13 but since then it has been found in many other bacterial groups including some Lactobacillus strains.14 In L. casei the glmS transcript levels specific activity respectively.11 Since the glmS strain showed about 12 times more GlmS activity than the control strain, but the same UDP-GlcNAc levels, the GlmM seems to be the key enzyme to increment the UDP-GlcNAc pool in L. casei. The glucosamine-1P produced by the GlmM activity is converted to UDP-GlcNAc-1P. This sugar-P, in a subsequent reaction with UTP, is converted in UDP-GlcNAc. Surprisingly in the glmM strain, but the same UDP-GlcNAc levels, the GlmU enzyme catalyzes the CoA-dependent acetylation of glucosamine-1P to form GlcNAc-1P. This sugar-P, in a subsequent reaction with UTP, is converted in UDP-GlcNAc. Surprisingly in the PL33 (glmMS) strain, which produces about four times more UDP-GlcNAc than the control strain, the GlmU specific activity was reduced to 38% of the control strain.11 This decrease in the GlmU activity might be explained by two possible mechanisms: (1) allosteric inhibition by end product as it has been previously reported to occur with the GlmU enzyme from Escherichia coli15 and (2) transcriptional regulation as we have previously showed that the transcript levels of glmU in the PL33 (glmMS) strain decreased 8-fold compared with the control strain.

### Regulation by glmS Riboswitch

Numerous bacterial genes of related function are organized in operons and transcribed as poly-cistronic mRNA to guarantee the coordinate expression of the individual genes. However, post-transcriptional modification of such mRNA can modulate the gene’s translational expression under specific environmental conditions. This is the case for the cis-acting regulatory RNAs called riboswitches, including the glmS riboswitch that uses glucosamine-6P as a cofactor and activates self-cleavage of the bacterial ribozyme, glucosamine-6P, which is part of the mRNA coding for GlmS.13 The glmS ribozymes are based in conserved structures more than in conserved sequences and they are highly specific for glucosamine-6P.13-18 The glmS riboswitch was first described in Bacillus subtilis13 but since then it has been found in many other bacterial groups including some Lactobacillus strains.14 In L. casei the glmS transcript levels specific activity respectively.11 Since the glmS strain showed about 12 times more GlmS activity than the control strain, but the same UDP-GlcNAc levels, the GlmM seems to be the key enzyme to increment the UDP-GlcNAc pool in L. casei. The glucosamine-1P produced by the GlmM activity is converted to UDP-GlcNAc-1P. This sugar-P, in a subsequent reaction with UTP, is converted in UDP-GlcNAc. Surprisingly in the PL33 (glmMS) strain, which produces about four times more UDP-GlcNAc than the control strain, the GlmU specific activity was reduced to 38% of the control strain.11 This decrease in the GlmU activity might be explained by two possible mechanisms: (1) allosteric inhibition by end product as it has been previously reported to occur with the GlmU enzyme from Escherichia coli15 and (2) transcriptional regulation as we have previously showed that the transcript levels of glmU in the PL33 (glmMS) strain decreased 8-fold compared with the control strain.

### Final Remarks

The production of UDP-GlcNAc is tightly regulated in L. casei BL23. This regulation probably takes place at 4 different levels of the UDP-GlcNAc biosynthetic pathway (Fig. 1). The tight regulation is in agreement with the importance of the production of UDP-GlcNAc to build the cell wall components in L. casei. This addendum has discussed the observations that point to the possible regulation mechanisms but future work should provide additional evidence to confirm these remarkable regulatory events.

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