**Relaxed substrate specificity of PglK**

We wanted to examine whether PglK activity required a complete LLO intermediate. Using *E. coli* BL21 containing a plasmid-encoded *pgl* operon with mutated genes, Linton et al (Linton, 2005) showed that incomplete oligosaccharides are transferred to protein. To investigate whether transfer of biosynthetic intermediates depended on *pglK* activity, mutant forms of the *pgl* operon that altered the structure of the glycan were expressed in *E. coli* SCM7 in combination with the AcrA reporter. Glycosylation was analyzed by SDS-PAGE with anti-AcrA and R12 immunodetection. In *pglH* and *pglJ* mutant strains, incomplete oligosaccharides were transferred to AcrA (Fig. S3A, lanes 3 and 5), as indicated by the altered mobility of glycosylated AcrA. In *pglI* mutant strain the electrophoretic mobility of glycosylated AcrA was not affected (Fig. S3A and B, lane 4) because only the branching glucose was missing on the oligosaccharide. The sugar-epimerase mutant, GalE mutant, was previously described to abolish N-glycosylation (Linton, 2005). In our experiment, the R12 antiserum did not recognize AcrA when the protein was expressed in presence of the GalE mutant (Fig. S3 lane 6), meaning that it was not glycosylated.

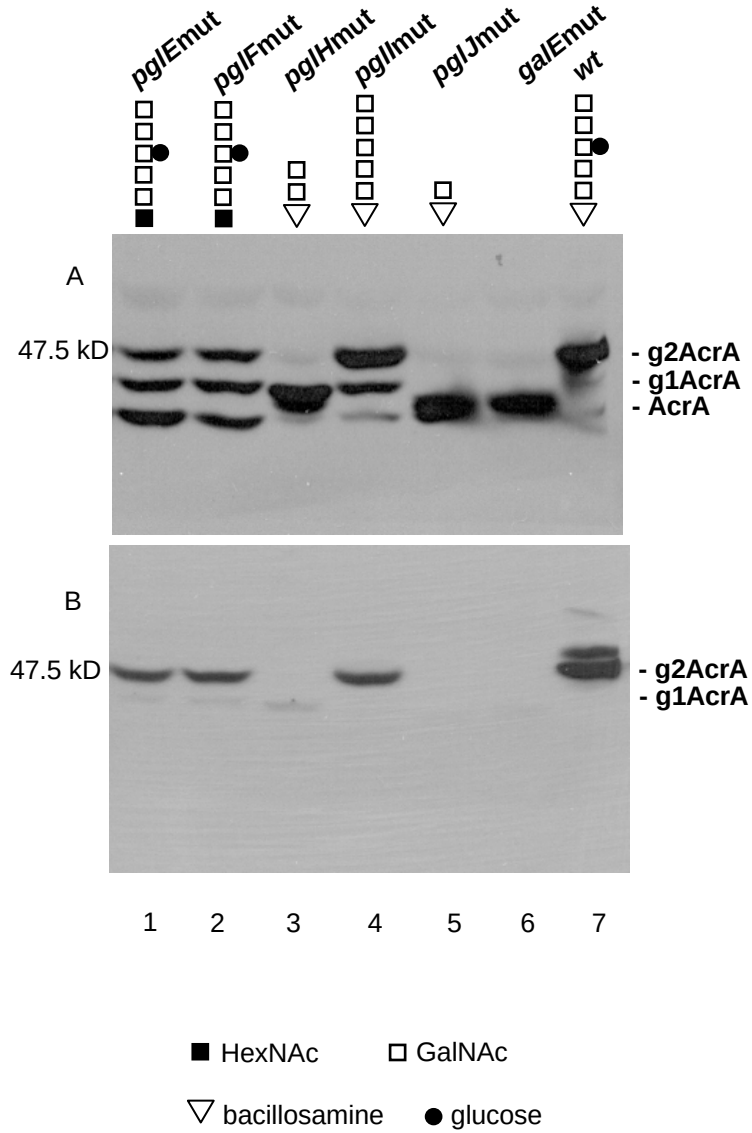
pglEmut
pglFmut
pglHmut
pglImut
pglJmut
galEmut
wt
A
47.5 kD
- g2AcrA
- g1AcrA
- AcrA
B
47.5 kD
- g2AcrA
- g1AcrA
1 2 3 4 5 6 7
HexNAc
GalNAc
bacillosamine
glucose

Fig S3

**Fig. S3. AcrA N-glycosylation in presence of *pgl* mutant plasmids**.
Total extracts prepared from *E. coli* SCM7 cells carrying the AcrA expression plasmid and the *pgl* operon with the deletions indicated (lanes 1-6) or wild-type *pgl* operon (lane 7), were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. AcrA and glycosylated proteins were detected with anti-AcrA (A) and the glycoprotein specific R12 (B) antisera, respectively. The position of bands corresponding to unglycosylated (AcrA), monoglycosylated (g1AcrA), and diglycosylated AcrA (g2AcrA) is indicated. The glycan-structure reported by Linton et al (Linton, 2005) for each of the *pgl* mutant is shown above each lane. Symbols were attributed to the sugar-residues according to the CFG nomenclature, the symbol for bacillosamine was newly introduced.