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TOWARDS A PLATFORM PROCESS FOR THE MANUFACTURE OF GLYCOCONJUGATE VACCINES FOR PNEUMOCOCCAL DISEASE

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Streptococcus pneumoniae is one of the leading causes of invasive bacterial disease in children where infection results in pneumonia and meningitis. The introduction of glycoconjugate vaccines for the bacterium has meant a significant reduction in cases of invasive bacterial diseases in children under the age of 5 worldwide. The current gold standard glycoconjugate vaccine for immunization is Prevenar- 13. However, the combination of a currently complex and long manufacturing process involving chemical conjugation means the final cost per dose of the vaccine is high.

The advent of Protein Glycan Coupling Technology (PGCT) has therefore been an important development. This recombinant process is able to produce glycoconjugate vaccines intracellularly in *E. coli* (1, 2). As a result, this reduces the number of steps required in the manufacturing process, meaning it presents itself as an attractive alternative to the current manufacturing process. This work aims to develop a platform process for glycoconjugate vaccines.

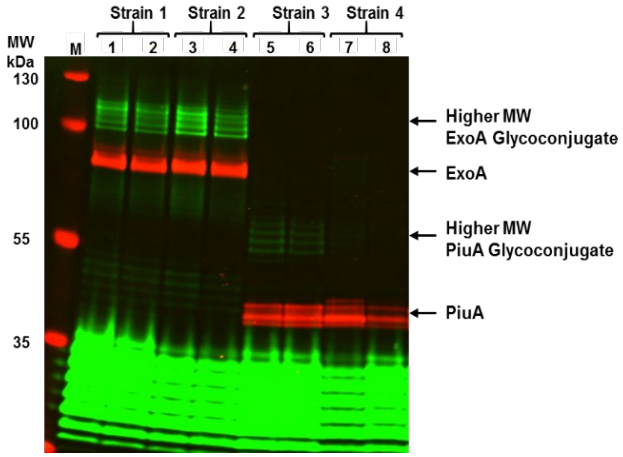

Figure 1- Immunoblot comparing end point samples (28 hours post induction) from fed batch fermentations. 11µL of lysate OD_{600} matched to 2.5 run in each lane. M is the molecular weight marker. From left to right; Strain 1: ExoA acceptor protein PglB chromosomal (lanes 1 and 2), Strain 2: ExoA acceptor protein PglB plasmidic (lanes 3 and 4), Strain 3; PiuA acceptor protein PglB chromosomal (lanes 5 and 6), Strain 4: ExoA acceptor protein PglB plasmidic (lanes 7 and 8).

First, *E.coli* cells have been engineered to produce a glycoconjugate of serotype 4 of *S.pneumoniae*. Fed-batch fermentations with an initial cell line demonstrated the need to further optimize the PGCT system. To this end new strains were engineered which introduced a different acceptor protein (ExoA), or had the glycosyltransferase enzyme PglB, catalyzing the glycoconjugation, either integrated in the chromosome or expressed from a plasmid. Fed-batch fermentations with these four strains found that the introduction of the ExoA acceptor protein had a greater impact in improving the glycoconjugate production compared to the location of the PglB enzyme as shown in Figure 1.

Thus, strain 1 was selected for further process optimization and used in a small Design of Experiment study looking at three factors i.e. pre-induction growth rate, post-induction temperature and feed rate. Here a condition has been identified which improves product formation by 1.9 fold compared to the standard fed-bath process.

In summary, initial optimisation of both the PGCT system and the fermentation process has been performed to improve the glycoconjugate production.

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