Production of C-5 epimerase in a laboratory scale bio-reactor with *Pichia pastoris*

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**Abstract**
The enzyme C-5 epimerase was successfully expressed in several *Pichia pastoris* strains. Laboratory scale fermentations with the transformed wild type X33 strain were optimized. The C-5 epimerase enzyme was produced in higher levels than the former expression system.

**Introduction and purpose**
The methylotrophic yeast *Pichia pastoris* has become an interesting host organism for production of recombinant proteins, by challenging insect and mammalian cells, which are difficult and expensive to cultivate in a fermenter. The C-5 epimerase enzyme is utilized in biosynthesis of the anti-coagulant drug bioheparin, converting D-glucuronic acid into L-iduronic acid units in a linear polysaccharide. The C-5 epimerase gene isolated from mouse and bovine organs has, prior to this research, been recombinantly expressed in insect cells. The gene sequence for C-5 epimerase was expressed in different strains of *Pichia pastoris* and the aim of this study was to optimize the C-5 epimerase production in a

**Methods**
The tools and techniques for optimizing C-5 epimerase production in laboratory scale included:
- Transformed *Pichia pastoris* strains with the gene sequence of C-5 epimerase inserted behind the AOX1 promoter
- Chemap CF3000 3.5 L laboratory fermenter
- Fison Gaslab 300 mass spectrometer
- Waters HPLC, FAM-PAK column, IR detector
- *Pichia* Fermentation Process Guidelines from Invitrogen, Carlsbad, CA, USA
- Measurements
- Control algorithms
- Mathematical modeling

**Results**
The C-5 epimerase enzyme could initially be produced extracellularly at levels between 5000 and 7000 cpmp/ul in a laboratory fermenter with Mut+ and Mut- strains of *Pichia pastoris*. The production level of insect cells in shake flask cultivations was approximately 8000 cpmp/ul. Because of its promising production rate, the X33 strain of Mut+ phenotype was selected for further optimization. The productivity could directly be related to the methanol feed rate during the induction phase, where a higher feed rate led to a higher enzyme activity. The highest enzyme activity produced was 10800 cpmp/ul. There was, however, a maximum methanol feed rate, above which the enzyme activity was reduced rather than enhanced.

A degradation of the C-5 epimerase enzyme was observed, coinciding with a decrease in the total protein concentration. Secretion of proteases and subsequent proteolysis was considered to be triggered, possibly due to depletion of magnesium, ammonium or phosphate ions. These ions precipitated when the pH was raised above 5.3. During the induction phase, the pH was kept at 6.5 in order to maintain an active C-5

**Conclusions**
The C-5 epimerase enzyme could successfully be produced in a laboratory fermenter with the host organism *Pichia pastoris*. The production levels were higher than in shake flask cultivations of insect cells. The main difficulty in the fermentations was the degradation of the product by proteolysis. The only successful action for retaining C-5 epimerase during a fermentation run was to end the run before the