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Poster Presentation

A regulatory acceptable alternative to *E. coli*: high yield recombinant protein production using the *Lactococcus lactis* P170 expression system combined with "Reverse Electro Enhanced Dialysis" (REED) for lactate control

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Background

The increasing demand for production of new recombinant pharmaceutical proteins constantly challenges our efforts to improve existing production systems or to develop new systems. So far the most commonly used expression systems are based on the prokaryotic *E. coli*, which is easy to grow and easy to manipulate, or mammalian cells which are able to produce complex heterologous proteins. However, these as well as other expression systems have drawbacks and do not express all proteins efficiently, which emphasizes the need for development of alternative systems.

Bioneer has developed an alternative recombinant protein expression system, the P170 Expression System [1], which is based on the Gram positive lactic acid bacteria, *Lactococcus lactis*. This host organism has GRAS status due to its long term use in the dairy industry, and proteins produced in the system have already entered clinical trials. The P170 Expression System is induced when a certain threshold of lactate is reached in the culture. This type of auto-induction eliminates the addition of exogenous components to induce gene expression. Furthermore, the protein of interest is fused to a secretion signal facilitating secretion of the gene product into the culture supernatant. The extracellular destination of the protein product reduces the number of steps needed for the subsequent downstream processing, thus helping to reduce the overall cost of the process. Finally, we have composed a fermentation medium devoid of animal derived components which is a strict demand for production of pharmaceutical proteins.

Despite all of these advantages, the production of lactic acid – the primary end product of glucose metabolism – will have a limiting effect on biomass production in *L. lactis*. Lactic acid inhibits growth even when the acid is neutralized by addition of base to keep pH constant. As a consequence the yield in biomass production is below that of other expression systems with cell densities of approximately 20 OD_{600} units. With this limited cell density we have still been able to produce up to 200–300 mg/L of secreted protein, but although this level is acceptable for some high value proteins, in most cases higher production levels are desirable.

JURAG has developed the "Reverse Electro Enhanced Dialysis (REED)" process which is a membrane filtration technology that allows continuous removal of low-molecular weight negative ions like lactate from the fermentation broth [2]. The lactic acid produced by the lactic acid bacteria dissociate in the growth medium to form lactate, which is replaced by alkaline hydroxide ions during the REED separation process. In this way both pH and lactate content in the culture are kept constant. By applying this technology the exponential growth phase could be pro-

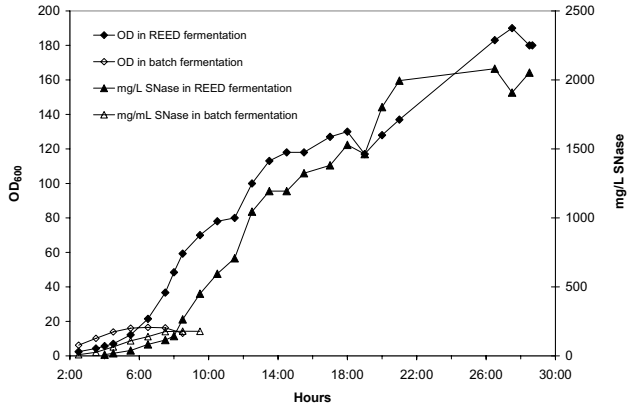

Figure 1
Cell density and yield of secreted heterologous protein (SNase) during continuous fermentation with pH controlled by REED, compared to the batch fermentation where pH was controlled by titration with KOH solution.

longed resulting in a substantial increase in the final biomass yield. The effect of the REED unit was demonstrated by an increase in the biomass yield by more than 10-fold when compared to a standard fermentation.

Results

Here we describe the synergistic effects of the REED technology in combination with the P170 Expression System for increased production and secretion of the model protein *Staphylococcus aureus* nuclease (SNase). In the first phase of the fermentation the REED unit was used to control the lactate concentration below 150 mM to sustain rapid exponential growth until the optical density reached 70 OD_{600} units. The lactate concentration was then increased to 250 mM and kept between 250 and 350 mM for 21 hours. Growth continued at a decreasing rate, while a high specific productivity of SNase was obtained. The culture was continuously fed with concentrated medium, while superfluous volume was drained from the fermentor to keep a constant volume. The figure below shows the yield of biomass and the yield of SNase obtained during the REED fermentation. As can be seen in figure 1 the cell density reached 180 OD_{600} units while the concentration of secreted SNase exceeded 2 g/L, which is approximately 10 fold higher compared to a standard fermentation without lactate control.

Conclusion

The present data demonstrates the enormous potential for increasing the yields of recombinant proteins produced in *L. lactis* by combining the advantages of the P170 Expression System for production of recombinant proteins with the power of the REED separation technology for improving yields. Two other commercially relevant proteins have also been tested in the combined system and 5–10 fold increased production levels were demonstrated.

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References

1. Bredmose L, Madsen SM, Vrang A, Ravn P, Johnsen MG, Glenting J, Arnau J, Israelsen H: **Development of a Heterologous Gene Expression System for use in *Lactococcus lactis*.** In *Recombinant Protein Production with Prokaryotic and Eukaryotic Cells* Edited by: *Merten O-W. A Comparative View on Host Physiology, Kluwer Academic Press, Dordrecht; NL*; 2001:269-275.
2. Garde A Thesis Ph.D.: **Production of lactic acid fromrenewable resources using electrodialysis for product recovery.** 2002. ISBN 87-90142-84-5