

## **TOWARDS EXTRACELLULAR SECRETION OF RECOMBINANT PROTEINS USING ANTISENSE TECHNOLOGY**

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Biopharmaceutical industries have been exploiting microbial organisms such as *Escherichia coli* to manufacture recombinant proteins that mostly are intended for therapeutic applications. For extraction of accumulated proteins in the periplasm, a method of cell lysis must take place which has a number of significant drawbacks such as numerous recovery and purification steps which leads to further reduction of the overall yield. Also, as well as the product, contaminants such as DNA and HCP are released which may be difficult to remove. Antisense technology offers a platform that once optimized can reduce the named drawbacks. Antisense RNA can target and inhibit the synthesis of proteins made by the cell, particularly outer membrane proteins in order to facilitate secretion of the recombinant product out of the cell during fermentation. This project aims to investigate the impact of inhibition of synthesis of selected outer membrane proteins on the secretion levels, thus having an effective release system. Various products such as alpha-amylase, Fab fragments have been investigated. In case of high secretion yields, the methodology can be adapted by the industry to eliminate cell lysis steps and the overall number of recovery and purification steps in the manufacturing of recombinant proteins can be reduced. The success of this project will be significantly attractive for the industry and it can lead to a new bioprocess strategy within the industry. Yields of 60% has been achieved, an increase from 10%. Yield defined as % of total product in the supernatant. Potential significance for the industry include elimination of cell disruption steps in a bioprocess, significant reduction in levels of contaminants such as HCP, DNA, proteolytic activity is greatly reduced in the culture medium, reduction in number of unit operations in a bioprocess and reduction in process running cost and time.