

Introduction

Functional genomics and proteomics have been fields of intense research over recent years. Structure-based drug design, antibody production and screening of protein libraries have all set an urgent demand for reliable and efficient technologies, suitable for parallel or automated screening of protein samples. In these fields of research small scale cultivation methods are commonly used for optimising the expression of recombinant proteins. Shake flask cultivations are known to be easy and low cost, but generally their reliability and final product yield is poor.

The small-scale cultivations described above are performed in shake flasks and in micro-well formats as a batch process which often leads to oxygen limitation, medium acidification, and overflow metabolism. High cell densities are not routinely reached in this batch process and consequently low product yield rates occur. By contrast in industrial processes, the fed-batch process is used to avoid inhibitive effects and the controlled substrate addition in fed-batch cultivation ensures reproducibility, high cell densities and high product yields.

Here we describe the application of EnBase™ a recently developed innovative technology where substrate controlled cultivation occurs in the fed-batch mode, at small scale, which provides a simple, highly beneficial, time space saving, & economical solution to high-throughput protein production in *E. coli* [1].

Cultivation with EnBase™

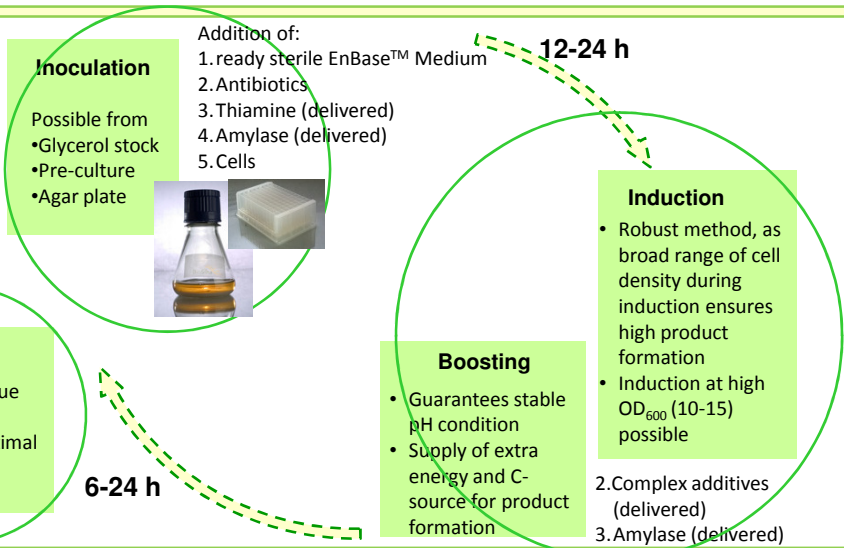
Recombinant protein expression with EnBase™ is performed in a two phase cultivation (Fig.1):

In the first step cells grow in fed-batch like mode controlled by addition of amylase.

In the second phase medium is optimised for protein expression by supplying medium boosters.

Balanced addition of C and N-source during induction increases the final volumetric product yield. The benefit of this method of having optimal induction cell density and improved conditions for protein production, can be seen in Fig.2 (blue lines) and Fig. 3, where the method was applied.

Fig. 1



Using EnBase™ for high-throughput protein production within the Human Protein Atlas project

In the atlas project nearly 300 different recombinant proteins (25–150 amino acids) are produced per week with the present setup [2]. The protein fragments are fused with an N-terminal hexa-histidine albumin binding protein tag and expressed in *E. coli* Rosetta (DE3) cells. When using the present setup the cells are cultivated in 100 ml complex medium in 1 L shake flasks. In an attempt to reduce the manual handling steps and increase the throughput, 15 randomly

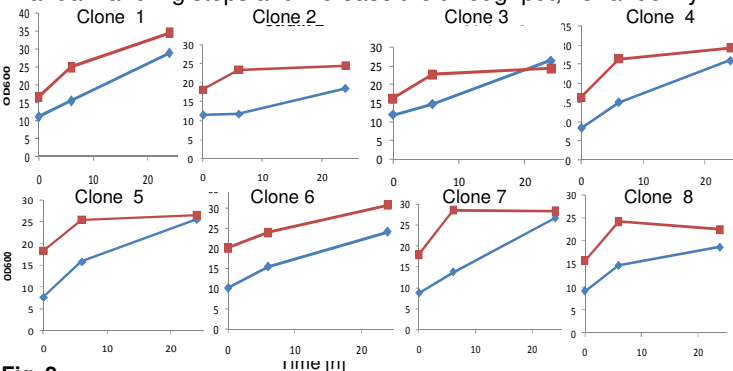


Fig. 2

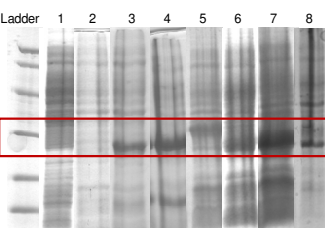


Fig. 3

selected clones have been tested in the 24 DWPs using two different EnBase™ media. Using the EnBase™ DWPs the final cell densities reached about OD₆₀₀=25 (Fig. 2) compared to OD₆₀₀=6 which was the average cell density in the shake flasks. Regarding the protein yields (Fig. 3) obtained with the “blue” medium the preliminary data looks very promising and indicates that one 24DWP could substitute 6 shake flasks.

Improved recombinant production of *Pseudozyma (Candida) antarctica* lipase B (PalB) with EnBase™ mineral salt medium (MSM)

Recombinant expression of *Pseudozyma antarctica* lipase B was investigated by comparison of different expression systems in *Pichia pastoris* and *E. coli* strains [3]. The demonstrated control expression (Fig. 4) was performed in periplasmic space of *E. coli* Rosetta (DE3) using 1 L shake flask with superbroth medium. The highest enzyme activity [mg/L] value was obtained, when

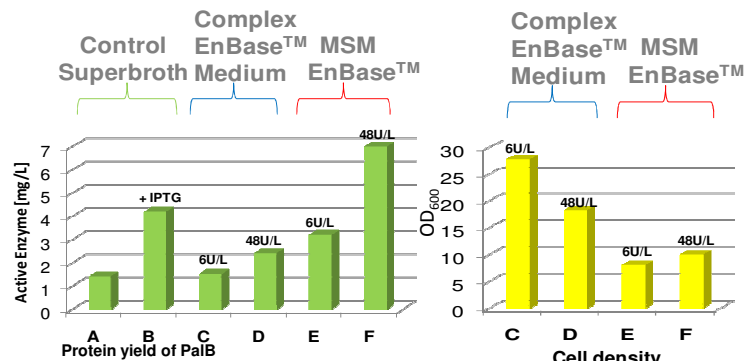


Fig. 4

Fig. 5

EnBase™ MSM was used for cultivation. Highest cell density did not guarantee highest protein yield, instead the EnBase™ MSM with 48 U/L amylase showed a yield of 7.0 mg/L (Fig.4), but had a low cell density of only OD=10 (Fig. 5). The high yield of active enzyme was thus reached due to higher amount of soluble protein per cell.

Conclusions:

⇒ Enbase™ is a new fed-batch related cultivation technique which can be applied for recombinant protein production

⇒ Enbase™ saves time and space. Preliminary data shows that

References:

1. Panula-Perälä et al. Enzyme controlled glucose auto-delivery for high cell