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Poster Presentation

Bacillus megaterium as a recombinant protein production host

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Background

The gram positive soil bacterium *Bacillus megaterium* is well known for its industrial utilization for production of various extracellular enzymes as amylases. Recently production and secretion of recombinant proteins in *B. megaterium* was also studied [1,2]. In this contribution a homologous model protein (penicillin G amidase (PGA) from *B. megaterium* ATCC14945) and a heterologous protein (a hydrolase from *Thermobifida fusca* DSM43793 (TFH)) were used to further investigate and improve the system. Penicillin amidase is applied in the synthesis of semisynthetic penicillins. TFH is able to degrade specific polyesters such as poly (ethylene terephthalate) (PET) or poly (butylene terephthalate) (PBT), which are hither to regarded as 'non-biodegradable' plastics [3].

Results

We are using the plasmid-based xylose-inducible gene expression system which was optimized via introduction of a multiple cloning site and removing a *cre* element mediating glucose-dependent catabolite repression. In order to improve the induction efficiency a xylose deficient strain was developed by knocking out the xylose isomerase gene (*xyl*A) from MS941 (Δ*npr*M), which improved the PGA production 2 fold. Using the lipA signal peptide instead of the native signal peptide from PGA increased the PGA secretion 1.6 fold in shaking flask cultivation (see Figure 1). N-terminal amino acid sequence results of PGA showed that it has a signal peptide MKTKWLISVIILFVFIFPQNLVFA, a 27,000 Da α subunit which began with G_{25}EDKNEGVKVVR and a 57,000 Da β subunit began with S_{266}NAAIVGSEKSATGN. They matched perfectly with the amino acid sequence derived from the nucleotide sequence of the cloned *pac* gene of pRB49 which came from *B. megaterium* ATCC14945. Further cultivation optimization showed that early induction strategy was better than later induction. 2.5 mM Calcium was

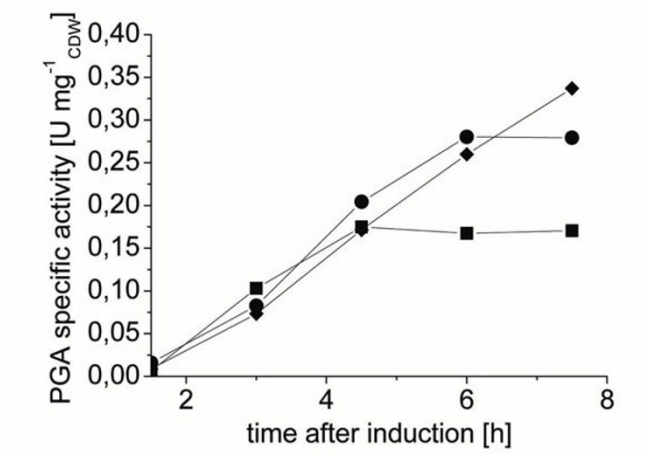

Figure 1
Specific activity curve of PGA after it was secreted into the growth medium by *B. megaterium* MS941 containing pRB23 with native peptide from PGA (●), MS941 containing pRB49 with signal peptide *lip*A (■) and YYBm1 containing pRB23 (◆) in LB complex medium. At $OD_{578\ nm}$ of 0.4 production of TFH was induced by the addition of 0.5 (w/v) % xylose to the growth medium. Samples were taken at various time points after induction.

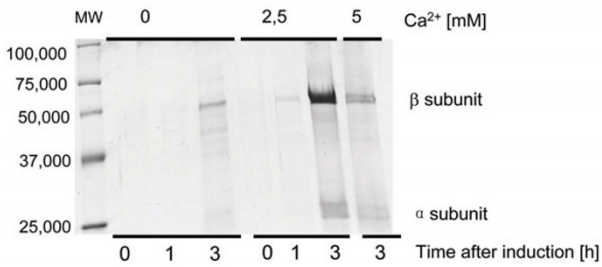

Figure 2
Proteins from 1.5 ml growth medium form samples taken at indicated time points which showed as the lines at the bottom of the graph, precipitated by ammonium sulfate, analyzed by SDS-PAGE and stained by Coomassie Brilliant Blue. G250. The lines at the top of the graph separate the cultivation medium with different Ca^{2+} concentration 0 mM, 2.5 mM and 5 mM. Lane M shows Precision Plus Protein Standard (Bio-Rad, Muechen).

the best concentration for helping PGA binding process (see Figure 2). Currently microtiter plate cultivation was developed for growth medium optimization. Compared to the successful expression of PGA the heterologous TFH gene only expressed after its codon usage was optimized for *B. megaterium* using JCat [4]. Foreign protein production was successfully upscaled from shaking flask cultivation over batch fermentation with control of pH to high cell density cultivation in a 2 L bioreactor.

Conclusion

In this new host system both proteins were secreted directly into supernatant and the good productivity was obtained from fermentation.

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