Zentrum Angewandte Chemie
Institut für Technische Chemie

Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

Universität Hannover

Development of specific *E.coli* microarrays for the analysis of the protein secretion pathways Sec and Tat

Frank Stahl[1], Michael Reck[1], Bernd Hitzmann[1], Thomas Scheper[1]
Karl Friehs[2] , Benjamin Sommer[2], Sven Aster[2], Erwin Flaschl[2]
[1] Institut für Technische Chemie der Universität Hannover, Callinstr. 3, 30167 Hannover
[2] AG Fermentationstechnik, Technische Fakultät Universität Bielefeld, Universitätsstr. 25, 33615 Bielefeld

Introduction:

E. Coli still remains one of the most important producers for recombinant proteins in science and biotechnological industry. The secretion of proteins to the medium is in most cases of a great advantage for simplifying downstream purification. The mechanisms by which newly synthesized recombinant proteins are translocated across bacterial membranes is a fundamental problem. *E. coli* is enclosed by two cell membranes that need to be passed if enrichment of a specific protein in the cultivation medium is intended. Secretion of recombinant proteins from the cytoplasm into the culture medium is an enormous afford to facilitate downstream processing and often leads to biological more active, correctly fold and soluble proteins. This can be achieved by directing the proteins to the periplasm through the Sec or Tat pathway, followed by bacteriocin release protein (BRP) mediated release to the culture medium. Correct formation of disulfide bonds, for example, is often inhibited in the reducing environment of the cytoplasm. Further aspects are the proteolytic activity and the contamination with host proteins which are significantly reduced if a protein is secreted to the extracelluar space. At least the formation of so called inclusion bodies might be prevented and a conserved N-terminus can be achieved because of the cleavable secretion-signalpeptides.
The aim of this study is to analyse fundamental aspects of protein secretion routes across bacterial membranes, especially the cytoplasmic and outer membranes of E. coli and thereby increase productivity.

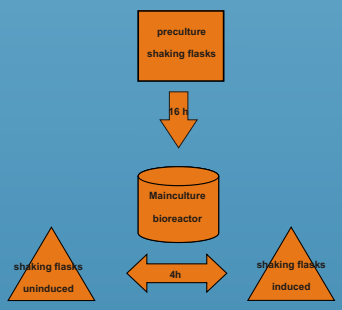


Fig.1: A parallel experiment with shaking flasks

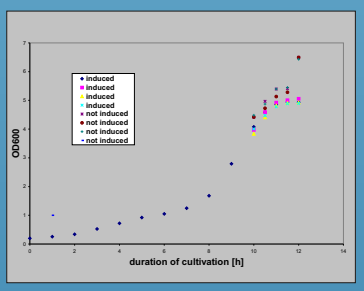


Fig.2: Growthcurve of *E.coli* K12 Mg1655 in shaking flasks

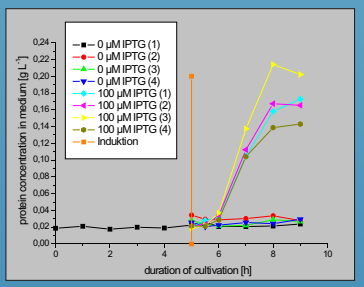


Fig.3: Protein concentration in medium during cultivation

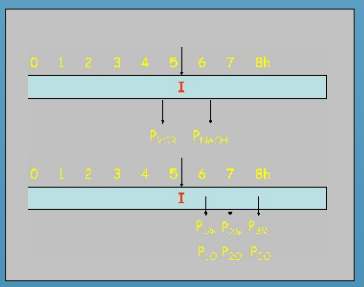


Fig.4: Design of a microarray experiment

Material and Methods:

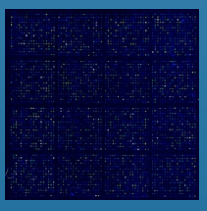
Total RNA was purified by phenol chloroform extraction from $2 \cdot 10^9$ treated and untreated *E. coli* cells. RNA samples were analyzed for integrity in the Agilent 2100 Bioanalyzer using the RNA 6000 Nano LabChip™. During reverse transcription 100 µg of purified total RNA was converted into a Cy3 or Cy5 labeled cDNA by random priming and subsequently hybridized to the same array.
Analyses were based on the use of fully-sequenced *E. coli* wild-type strain K12 MG1655. Utilization of this strain was thought to ensure 100% consistence between the bacterias genomes/transcriptomes and the DNA spotted to the surface of whole-genome DNA-Chips. Moreover, possible uncharacterized mutations of common laboratory strains that may influence the study of membrane translocation processes can be excluded. In a first step we examined the gene regulatory effects caused by expression of different BRPs at different induction levels using the IPTG inducible system of pJL3 and pJL17lpp. New, non-rational correlations between different genes could be analyzed by clustering methods.

In order to optimize the secretion of recombinant proteins we will test:

- Different inducible, constitutive promoters with different induction-concentrations
- Different signalpeptides for the Sec/Tat-Pathway
- Different reporterproteins
- Different synthetic media

Results:

Since *E. coli* secrete only few proteins naturally, there are different attempts to enhance protein secretion of *E. coli* including the utilization of the two main pathways Sec and Tat as well as the release of recombinant proteins from the periplasma by the expression of Bacteriocin release proteins (BRP). This contains very complex processes. In order to get a better picture of the underlying regulatory mechanism we performed a comparative analysis of gene expression in controlled reproducible bioreactor cultivations with plasmid carrying *E.coli* strains that inducibly express BRPs. The data received from whole-genome arrays were used to develop secretion specific low density arrays. With these arrays first kinetic examinations of gene expression in fermentations were performed. First results show clear tendencies and promise a deeper look in the mechanism of secretion. In future experiments, differential gene regulation during translocation of recombinant proteins to the periplasm will be examined and supervised by reporterproteins fused to signal peptides of the Sec and Tat Pathway.



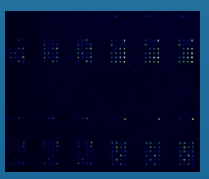


Fig.5: Secretion-specific low-density and whole genome Microarray

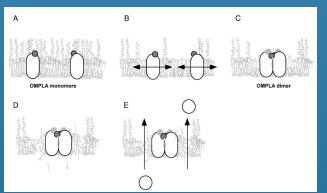


Fig.6: BRP-triggered outer membrane degradation by OMPLA

Fig.7: Affymetrix Arrayer 428