Microbial Cell Factories

Poster Presentation

Production of proteins in *Bacillus subtilis* can be improved by engineering components affecting posttranslocational protein folding and degradation

Marika Vitikainen*, Hanne-Leena Hyyryläinen, Anna Kivimäki, Vesa P Kontinen and Matti Sarvas

Address: Laboratory of Infection Pathogenesis, National Public Health Institute, Mannerheimintie 166, FIN-00300 Helsinki, Finland
* Corresponding author

Background

Bacillus species have a high capacity to secrete their own proteins into the extracellular medium and are therefore considered as attractive hosts for producing heterologous proteins. However, the secretion is often inefficient suggesting that there are bottlenecks in the secretion pathway. This research focuses on the posttranslocational events in protein secretion. Three putative bottleneck components (see Results) affecting the extracytoplasmic protein folding and degradation were genetically engineered and the effects on the protein secretion were studied [1].

Currently we are participating in EuroSCOPE program on Science of Protein Production for Functional and Structural Analysis. The project will continue to further develop and exploit *B. subtilis* as a host especially for the production of protein complexes and membrane proteins. The aim is to find out balanced sets of quality control and protein folding factors to be able to optimise protein production. In the EuroSCOPE program membrane proteins of *B. subtilis* as well as gram-positive pathogen representing potential vaccines and targets for novel antimicrobial drugs are used as model proteins. The project has been initiated by producing a membrane protein *B. subtilis*, histidine kinase YxdK.

Results

The first component modulated is the amount of PrsA lipoprotein on the outer surface of the cytoplasmic membrane. PrsA is an essential membrane-bound folding factor with peptidyl prolyl isomerase activity. Previous studies have identified that secretion of some exoproteins is dependent on the PrsA lipoprotein. Dependency of model proteins on the PrsA was studied by lowering the PrsA level below the wild type level. The effect of PrsA overproduction was also investigated. The second component is the increase of the net negative charge of the cell wall generated by mutating the *dlt* operon and consequently preventing the D-alanine substitution of anionic cell wall polymers. The third component modulated is the level of HtrA-type quality control proteases at the membrane-cell wall interface that serve as cleaning proteases to degrade accumulated and misfolded proteins. The effect on the secretion of 11 industrially interesting heterologous proteins was studied by western blotting and enzymatic assays [1]. The results are shown in table 1. The secretion of four of proteins was dependent on the PrsA lipoprotein and the overproduction of PrsA enhanced the secretion of two of them, α-amylase of *B. stearothermophilus* (4-fold) and pneumolysin (1.5-fold). The mutation in the dlt operon enhances the secretion of one protein, pneumolysin, about 1.5-fold. Decreasing the level of HtrA proteases caused harmful effects on growth and did not enhance secretion. Pertussis toxin subunit S1 was found to be a substrate for HtrA-type proteases and its secretion was dependent on these proteases.

Table 1: Effect of PrsA, *dlt* mutation and depletion of HtrA proteases on secretion of heterologous proteins studied.

Protein and origin	PrsA dependency	PrsA Over- expression	*Dlt* mutation	Depletion of HtrA protease
α-amylase (*B. stearothetmophilus*)	yes(+)	↑	no effect	↓
β-glucanase (*B. licheniformis*)	no		no effect	
Penicillinase (*B. licheniformis*)	no		no effect	no effect
Pneumolysin (*S. pneumoniae*)	yes(+)	↑	↑	↓
Diphteria toksoid (*C. diphteriae*)	no	no effect		
Staphylokinase (*S. aureus*)	yes(-)			
Pectinase (*E. carotovora*)	yes(+)	↓	↓	no effect
Pectin methylesterase (*E. chrysanthemi*)	no		no effect	no effect
β-lactamase (*E. coli*)	yes(+)	no effect	↓	
Pertussis toxin S1 (*B. pertussis*)	no	↓	↓	↓
Pertussis toxin S4 (*B. pertussis*)	no			

yes(+), positive dependency, yes(-) negative dependency, ↑, increase in secretion and ↓, decrease in secretion.

Conclusion

In this research a fairly large number of model proteins were used allowing us to make more general conclusions on how secretion of heterologous protein in *B. subtilis* can be improved. Results indicate that some heterologous proteins are secreted at enhanced levels when components involved in the late stages of protein secretion are modulated. PrsA lipoprotein and its overproduction are good candidates to be tested when optimising the secretion of an exoprotein in *B. subtilis*. Modulation of the net charge of the cell wall may also have role in biotechnological applications. However lowering the level of HtrA proteases does not seem to be a means to enhance protein secretion.

References

1. Vitikainen M, Hyyryläinen HL, Kivimäki A, Kontinen VP, Sarvas M: **Secretion of heterologous proteins in *Bacillus subtilis* can be improved by engineering cell components affecting post-translocational protein folding and degradation.** *J Appl Microbiol* 2005, **99**:363-375.