Abstract of PhD thesis

Membrane proteins (MPs) are major structural and functional components of cell membranes in all living organisms, where they perform numerous important functions, such as maintenance of structural integrity, signaling or transport of nutrients, ions, and small molecules. Their crucial role is highlighted by the fact that they are encoded by 20-30 % of genes, both within prokaryotes and eukaryotes, while their proper folding and function are involved in a wide variety of diseases such as Alzheimer's disease, cystic fibrosis, or cancer. Due to their multiple and important functions MPs are high-priority targets for drug development, and up to now, more than half of all known pharmaceuticals target such proteins. The detailed understanding of both MP structure and function requires the availability of significant amounts of isolated protein of high quality, to perform all the necessary structural and biochemical studies. Since MP natural abundance is usually very low, the amounts needed are typically obtained through recombinant overexpression in heterologous host.

Escherichia coli is one of the most widely utilized host-organisms for the recombinant production of soluble and membrane proteins. Bacterial MP production, however, is usually accompanied by severe toxicity and low-level volumetric accumulation leading to low levels of final biomass and reduced accumulation of recombinant MPs in the cell membrane. Towards this direction, a bacterial strain, termed SuptoxR, was previously constructed. In this strain, co-expression of RraA, an inhibitor of the mRNA degrading activity of *E. coli* RNase E, can efficiently suppress the MP-induced toxicity, and simultaneously significantly enhance the cellular accumulation of membrane-incorporated recombinant MPs. Based on SuptoxR, a series of novel bacterial strains with further improved properties were developed, following two different strategies.

In the first part, naturally occurring variants of RraA were searched within genomes of proteobacteria and plant chloroplasts. The selected variants were evaluated for their toxicity-suppressing and MP production-promoting effects, compared to the original *E. coli* RraA of SuptoxR. It was found that a number of these homologous RraA proteins also suppressed MP-induced cytotoxicity and enhanced the accumulation of MPs, and more importantly, co-expression of the RraAs from *Proteus mirabilis* and *Providencia stuartii*, frequently outperformed the MP-enhancing capabilities of *E. coli* SuptoxR. Thus, two second-generation *E. coli* SuptoxR strains were developed, termed SuptoxR2.1 and SuptoxR2.2, that overexpress the RraAs from *P. mirabilis* and *P. stuartii*, respectively. These new strains often achieved even further increased accumulation of well-folded, recombinant MPs compared to the original SuptoxR strain.

The second part involved the evaluation of a set of previously generated *E. coli* strains that carry specific deletions in parts of the C-terminal domain of RNase E and exhibit reduced ribonucleolytic activity, for their ability to suppress the toxicity which is caused by MP-overexpression process and to enhance recombinant MP-productivity, in a manner resembling the conditions of RraA overexpression in *E. coli* SuptoxR. Some of these strains that encoded specific RNase E truncation variants, resulted in significantly enhanced levels of recombinant MP production. Among these, the *E. coli* strain expressing RneΔ22 mutation, suppressed efficiently the MP-induced cytotoxicity and achieved greatly enhanced levels of recombinant MP production of both prokaryotic and eukaryotic origin, while it also performed better than the commercially available bacterial strains, which are frequently utilized for recombinant MP production. This specific strain, in analogy to the original SuptoxR, termed SuptoxRNE22. Overall, the new strains presented here may become broadly applicable biotechnological tools for

the production of recombinant MPs in bacteria, as their use enables the production of recombinant MPs of high quality and quantity, suitable for functional and structural studies.

Keywords: *Escherichia coli*, Recombinant membrane protein production, toxicity, RraA, RNase E, SuptoxR, SuptoxR2.1, SuptoxR2.2, SuptoxRNE22