RHODOBACTER CAPSULATUS AS AN ALTERNATIVE HOST FOR THE EXPRESSION OF MEMBRANE PROTEINS

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Introduction. Membrane proteins (MPs) play a crucial role in the cell biology and physiology of all living organisms. Nearly 30% of all known open reading frames encode MPs. In addition, more than 50% of the currently available pharmaceuticals target MPs (1). Despite their importance, the function of most membrane proteins has not been assigned, yet. This discrepancy is mostly attributed to the hydrophobic nature of MPs that impedes their synthesis in amounts sufficient for characterization. Therefore, it is inevitable to generate novel expression systems that allow the high-level synthesis of functional MPs.
Here, we present a novel expression system based on the phototrophic bacterium *Rhodobacter capsulatus*, which is especially suited for the synthesis of membrane proteins.

Methods. The facultative anaerobic purple bacterium *R. capsulatus* is able to grow either chemotrophically or phototrophically. Under the latter growth conditions it forms an intra-cytoplasmic membrane system (ICM) and vesicles that harbor the enzymes of the host's photosystem as well as the heterologously produced MPs. These vesicles can easily be harvested by ultracentrifugation and can subsequently be used as nanobioreactors. We have described detailed protocols for handling the *R. capsulatus* expression system (2).

Results. Due to its physiological properties, *R. capsulatus* represents a suitable host for MP production. Therefore, we have constructed the *R. capsulatus* expression strain B10S-T7 and a comprehensive set of broad host range expression plasmids, designated as pRho (3). These plasmids allow the constitutive synthesis as well as the inducible high-level production of proteins from different promoters. Different affinity tags allow the immunological detection and/or affinity purification. To characterize the expression properties of this system with respect to MP production, several MP encoding genes – including monooxygenase and GPCR-(like) genes – were cloned into the pRho expression plasmids. Subsequent accumulation and localization studies revealed that the respective gene products mainly assembled into the vesicles of the ICM (Fig. 1). The model membrane protein Bacteriorhodopsin could be incorporated into the ICM vesicles with a yield of more than 2 mg protein per liter culture without any further optimization.
The ICM vesicles can easily be harvested by ultracentrifugation and used in downstream applications.

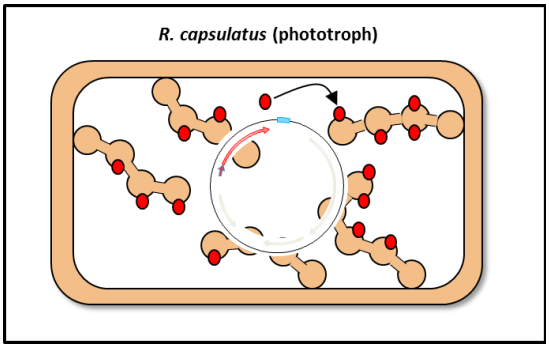

Fig.1 Heterologous MP synthesis in *R. capsulatus*. This phototrophic bacterium forms an ICM system and vesicles that can incorporate foreign MPs (red dots).

Conclusions. With *R. capsulatus* B10S-T7 and the pRho plasmids we have developed a novel, powerful expression system for the synthesis of heterologous MPs. Our system is particularly suited for the production of mono-oxygenases and GPCR-(like) proteins.

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