Microbial Cell Factories

Poster Presentation

Protein expression for structural studies

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from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

Microbial Cell Factories 2006, **5**(Suppl 1):P40 doi:10.1186/1475-2859-5-S1-P40

Background

Protein structure determination is an essential tool for studying protein function, and assists the design of novel drugs. The goal of the Israel Structural Proteomics Center (ISPC) is to determine the structures of proteins related to human health in their functional context http://www.weizmann.ac.il/ISPC[1]. One of the bottlenecks encountered, primarily for eukaryotic proteins, is the production of soluble and correctly folded proteins suitable for crystallization trials. In order to overcome this obstacle, we have applied various expression strategies. *E. coli* is the expression system of choice, in which different parameters are tested in parallel. In cases in which post-translational modification is essential for obtaining a functional protein, eukaryotic expression systems such as *Pichia pastoris* or baculovirus are being employed.

Results

Each target is expressed in parallel in several expression vectors in *E. coli*. Design of vectors, which harbor the same restriction sites and code for a cleavable N-terminal His-tag facilitate DNA cloning and purification, respectively. When necessary, a target protein is co-expressed with its natural binding partners or with molecular chaperones. Results of a representative protein expression experiment are shown below. Figure 1 demonstrates the effect of co-expression of molecular chaperones in *E. coli* on protein solubility. Levels of soluble protein were significantly increased when the protein was co-expressed with certain combinations of molecular chaperones (Figure 1, C and 1D).

Conclusion

Rapid evaluation of protein expression under small-scale culture conditions is essential for implementing an efficient high-throughput protein production process. Parallel utilization of a repertoire of protein expression strategies resulted in a significant increase in the number of soluble proteins obtained.

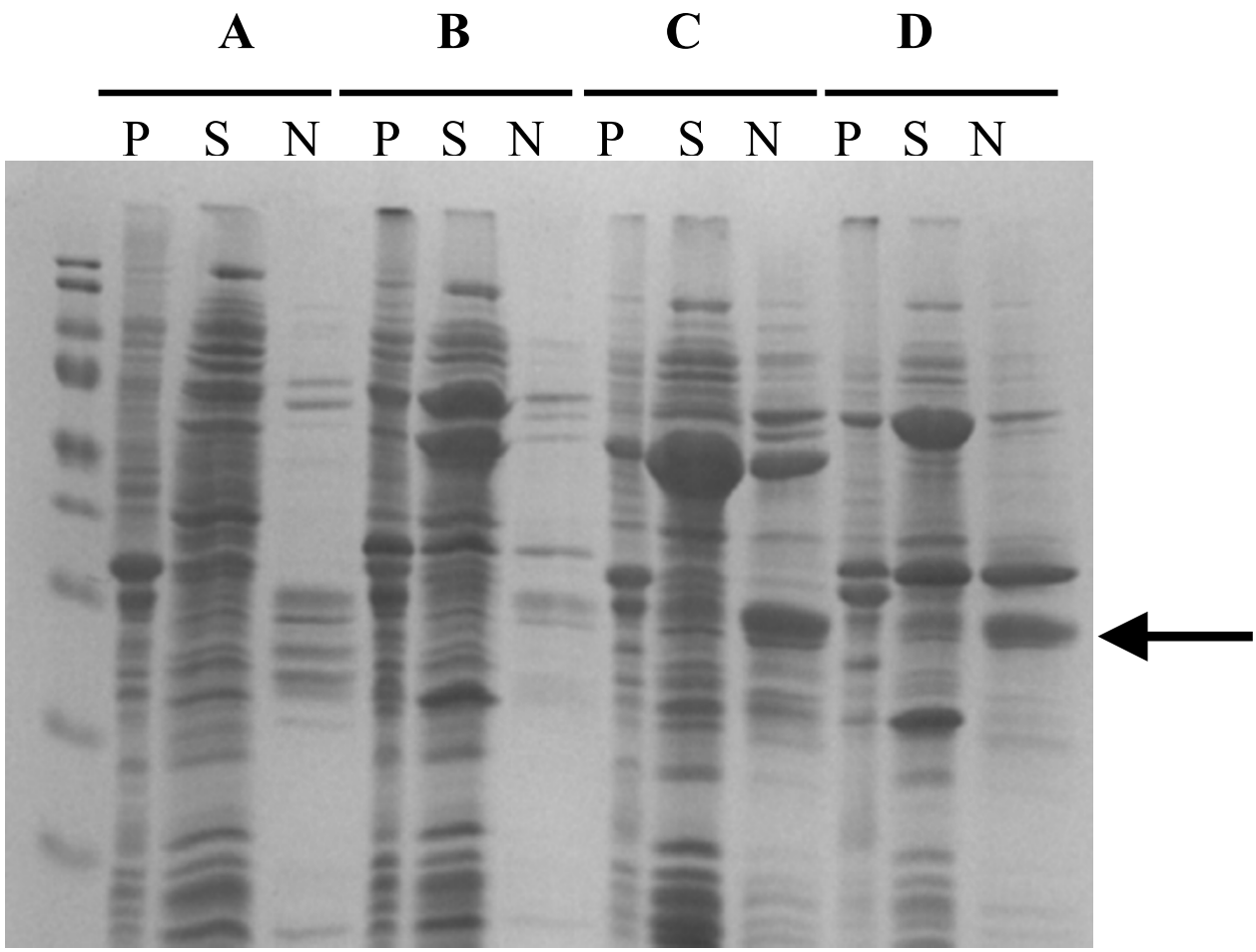

Figure 1
Protein co-expression with molecular chaperones in *E. coli*. Arrow indicates the position of the protein. A- Expression without chaperones; B–D: Expression with various combinations of molecular chaperones; P, Pellet; S, Soluble fraction; N, Protein following capture on Ni-beads.

References

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