CONSTRUCTION AND CHARACTERIZATION OF ESCHERICHIA COLI HTPG NULL MUTANT AND STUDY OF HTPG - CLPB INTERACTION

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Background: The heat shock protein HtpG (high temperature protein G) is a member of the Hsp90 protein family. It is well-conserved molecular chaperone involved in the folding and stabilization of diverse proteins. The amino-acid sequence of *E. coli* HtpG is 37% identical to those of human for example Hsp90α/β. HtpG acts as molecular chaperone *in vitro*, but their function in protein folding remains unclear. ClpB is another member of heat shock protein involved in proteolysis.

Objectives:

1. Construction and characterization of *E. coli* htpG deletion mutant.
2. Studying interaction between HtpG and ClpB in two-hybrid system.

Methods: To inactivate *htpG* we used classic gene replacement method [1]. Protein-protein interactions were measured using two-hybrid system. In this system, a lambdoid chimeric operator is recognized by a hybrid repressor formed by two chimeric monomers whose C-terminal domains are composed of heterologous proteins. Dimerization of proteins reconstitutes functional repressor thus inhibiting downstream reporter gene [2].

Results: *E. coli* ΔhtpG mutant is characterized in slower growth at 42°C, impaired osmotic adaptation and biofilm formation in LB, LB+Glc, TSB or TSB+Glc media, especially after relatively short (24 h) time of incubation. We succeed to construct a chimeric operator formed by the two hemi-sites of the phage P22 and phage 434 operators. This operator can be recognized and bound only by a hybrid repressor formed by two chimeric monomers, one with the N-terminal portion of phage 434 and the other with that of phage P22. We identified using these two-hybrid system existence of the interaction between proteins HtpG and ClpB.

Conclusion: Disruption of *htpG* causes severe defects in several cellular functions and ClpB proteins interact, as proved in two-hybrid system.

References:
