Selection for proteins that overcome heat-induced lethality of ΔdegP strain

Iris Walker1, Paul Riggs1, Na Ke1, Nicoleen Boyle2, and Mehmet Berkmen1* 1New England Biolabs, Ipswich, MA 01938, USA; 2Suffolk University / correspondence to berkmen@neb.com

INTRODUCTION

The periplasmic protease/chaperone DegP (HtrA) plays a key role in the quality control of many proteins in the periplasm of E. coli. Proteins that fail to fold in the periplasm can be proteolyzed, while others are chaperoned to their native folded state by DegP. In a ΔdegP strain, E. coli is unable to survive the protein folding stress at elevated temperatures. Utilizing this phenotype, we developed a plasmid-based selection of suppression of heat-induced lethality in a ΔdegP strain. Plasmid libraries of various prokaryotic genomes were screened for proteins that overcome heat-induced lethality. Initial hits indicate novel mechanisms of overcoming periplasmic stress, such as the periplasmic expression of a cytoplasmic GrpE homolog and the cytoplasmic expression of an unknown protein.

METHODS

E. Coli ΔdegP + gDNA

Select @ 42°C

42°C survivors

Re-streak @ 42°C

42°C survivors

Colony PCR

Inserts > 0.5 kb?

Discard no yes

Miniprep and re-transform @ 42°C Survivors?

Discard no yes

Sequence DNA & BLAST

RESULTS

Spot titers of confirmed hits

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Size (bp)</th>
<th>Expressed Compartment</th>
<th>Spot Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>GrpE</td>
<td>Geobacillus species</td>
<td>627</td>
<td>Periplasm/cytoplasm</td>
<td>10^5</td>
</tr>
<tr>
<td>Hypothetical transcriptional factor</td>
<td>Citrobacter amalonaticus</td>
<td>501</td>
<td>Cytoplasm</td>
<td>10^6</td>
</tr>
<tr>
<td>DegS</td>
<td>Haemophilus influenza</td>
<td>1020</td>
<td>Periplasm</td>
<td>10^6</td>
</tr>
<tr>
<td>Do/hhoaA</td>
<td>Haemophilus influenza</td>
<td>1392</td>
<td>Periplasm</td>
<td>10^6</td>
</tr>
</tbody>
</table>

ΔdegP E. coli cells were transformed with cytoplasmic expressing (pMER77) or periplasmic expressing (pMER78) plasmids harboring the genes for Geobacillus species GrpE, E. coli GrpE, or Citrobacter amalonaticus hypothetical protein 1. Serial dilutions of these cells plus the wild type and experimental controls were spotted onto agar containing LB + ampicillin and incubated overnight at either 30°C or 42°C.

FUTURE DIRECTIONS

- Continue Library QC & selection process for all available libraries
- Develop an in vivo and in vitro protease and chaperone assay
- Use ΔdnaJ, ΔdnaK, and ΔdnaJ ΔdnaK strains of E. coli to identify Geobacillus GrpE partners
- Knock-out E. coli GrpE in the presence of cytoplasmic Geobacillus GrpE
- Determine active site for GrpE in order to knock-out GrpE activity to verify necessity in complementation

REFERENCES