Summary report for DOE Grant DE-FG02-93ER61701

Paul Blum, PI
University of Nebraska-Lincoln

Project funding under the auspices of DOE’s Ocean Margin program to my laboratory supported research into the development and application of novel molecular methods to measure bacterioplankton growth state in situ. These methods included bulk or population-based studies and single cell studies. Due to the limited duration of support and subsequent termination of the molecular-focused PIs, only the former bulk method was applied to marine samples. In addition, basic laboratory studies were completed which addressed why the selected biomarkers were regulated by bacterial growth state. Three manuscripts were published and two additional manuscript are in preparation. In addition, the funding provided supported one Ph.D. student for most of their degree program.

Studies in my laboratory to improve the method for bacterial growth state determination have been continued with support from other sources. We recently completed methods development and application to bacterial growth state studies in terrestrial water supplies. This immunomicroscopy method is based on the quantitation of highly conserved proteins present in either growing or nongrowing cells. The method is completely compatible with more traditional methods for taxonomic determination of bacterial identity using 16S rDNA fluorescently labeled probes.

Project #1. The highly conserved molecular chaperone, DnaK, was evaluated as an indicator of cellular growth state for the analysis of bacterial populations. Quantitative Western blot analysis of pure cultures of Alteromonas haloplanktis, Vibrio fisheri and Pseudomonas stutzeri demonstrated that DnaK levels increased upon carbon starvation when adjusted for total cell protein. Lipopolysaccharide (LPS) levels were determined in these cultures as a measure of bacterial biomass. DnaK/LPS ratios for all species readily distinguished between the stationary and exponential phase of growth. Marine samples obtained from the Atlantic Ocean near Cape Hatteras were examined directly for DnaK, LPS, total cellular protein, and bacterial rRNA content. Large variations in DnaK levels, LPS and total protein were observed between sample locations at a constant depth of 10 meters. DnaK levels correlated closely with amounts of total sample protein. The ratio between amounts of DnaK and LPS or, DnaK and prokaryotic protein, were examined as potential indicators of cell physiology. Though both ratios were disparate between sample sites, a correlation was observed between these parameters at all sample locations. These results suggest DnaK may be useful as an indicator of marine microbial physiology in situ.

This work was performed in collaboration with S. Giovannoni of Oregon State University and resulted in a manuscript currently in preparation.

Project #2. This project was concerned with determining why molecular chaperones accumulate in starving bacteria and resulted in several publications. Such information was crucial for the development of single cell methods for the determination of bacterial growth state in among a range of gram negative (proteobacteria) bacterial species. Title and abstracts
DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.
DISCLAIMER

 Portions of this document may be illegible electronic image products. Images are produced from the best available original document.
of these three papers are included here.


During a three day period, glucose starvation of wildtype Escherichia coli produced thermotolerant, H₂O₂ resistant, small cells with a round morphology. These cells contained elevated levels of the DnaK protein adjusting either for total protein or on a per cell basis. Immunoprecipitation of ³⁵S-methionine labeled protein produced by such starving cells, demonstrated DnaK underwent continuous synthesis but at decreasing rates throughout this time. Glucose resupplementation of starving cells resulted in rapid loss of thermotolerance, H₂O₂ resistance and the elevated DnaK levels. A dnaK deletion mutant but not an otherwise isogenic wildtype strain, failed to develop starvation-induced thermotolerance or H₂O₂ resistance. The filamentous phenotype associated with DnaK deficiency was suppressed by cultivation in a defined glucose medium. When starved for glucose, the non-filamentous and rod shaped dnaK mutant strain, failed to convert into the small spherical form typical of starving wildtype cells. The dnaK mutant retained the ability to develop adaptive H₂O₂ resistance during growth, but not adaptive resistance to heat. Complementation of DnaK deficiency using Pₗₐₑregulated dnaK⁺ and dnaK⁺ J⁺ expression plasmids, confirmed a specific role for the DnaK molecular chaperone in these starvation-induced phenotypes.


Overproduction of DnaK in E. coli results in a bacteriocidal effect. This affect is most acute in stationary phase cells. A selection was developed to isolate multicopy suppressors from an E. coli plasmid expression library which overcame the stationary phase toxicity of DnaK excess. Two suppressor plasmids were recovered which contained inserts of 1.85 kb and 2.69 kb respectively. Rearranged and deleted plasmid derivatives were constructed and used to further localize the suppressors. DNA sequence analysis demonstrated that one suppressor encoded phosphogluconate dehydratase (Edd) while the other suppressor encoded the N-terminal 237 amino acids of DnaK itself (DnaK⁺). Strains bearing the suppressor plasmids constitutively overproduced proteins with apparent masses of 66 kDa (Edd) and 37 kDa (DnaK⁺) as determined by SDS PAGE. Western blot analysis using polyclonal antisera specific for either Edd or DnaK confirmed the identity of these overproduced proteins. Suppression of DnaK toxicity was eliminated by the introduction of a +1 frameshift mutation early in the respective coding regions of either of the two suppressors. These results suggest that suppressor gene translation plays a role in the mechanism of DnaK suppression.


DnaK is essential for starvation-induced resistance to heat, oxidation and reductive division in Escherichia coli. Studies reported here indicate DnaK also is required for starvation-induced osmotolerance, catalase activity and the production of the RpoS-controlled Dps (PexB) protein. Because these dnaK mutant phenotypes resemble closely those of rpoS (o₃⁵) mutants, the relationship between DnaK and RpoS was evaluated directly during growth and starvation at 30°C in strains with genetically altered DnaK content. A starvation-specific effect of DnaK on
RpoS abundance was observed. During carbon starvation, DnaK deficiency reduced RpoS levels by three-fold while DnaK excess increased RpoS levels nearly two-fold. Complementation of the dnaK mutation restored starvation induced RpoS levels to normal. RpoS deficiency had no effect on the cellular concentration of DnaK revealing an epistatic relationship between DnaK and RpoS. Protein half-life studies conducted at the onset of starvation indicate that DnaK deficiency significantly destabilized RpoS. RpoH (σ32) suppressors of the dnaK mutant with restored levels of RpoS, and dnaK rpoS double mutants were used to show that DnaK plays both an independent and an RpoS-dependent role in starvation induced thermotolerance. The results suggest that DnaK coordinates sigma factor levels in glucose starved E. coli.

Project #3. This is an ongoing project directed to the development and application of our method to determine bacterial growth state in situ, that is in the absence of cultivation. We have recently succeeded in the development of the method and have applied it to studies on bacterial growth state in municipal waste water. A manuscript is in preparation describing these studies.