Evolutionary and functional analysis of the two copies of heat shock proteins GroEL and GroES in *Vibrio vulnificus*

J. J. Kingston* and E.F. Boyd

Department of Biological Sciences, University of Delaware, Newark, DE 19716, USA

*** Corresponding author**

Vibrio vulnificus contains a copy of heat-shock proteins GroEL and GroES on both chromosomes. Heat-shock proteins, or molecular chaperones, are essential cellular components required for protein folding, degradation, and trafficking. GroEL interacts with GroES to assist in the proper folding of proteins, and in *Escherichia coli,* these proteins were shown to be essential at all temperatures. Increased expression of *groEL* is known to occur under a variety of stress conditions. Genomic sequence analysis has shown that among some Gram-positive and a limited number of Gram-negative bacteria, duplication of *groEL* has occurred. In Gram-positive bacteria, usually only *groEL* is copied, whereas in Gram-negative bacteria duplication is bicistronic including both *groEL* and *groES*. In *V. vulnificus* the duplicated *groEL* gene is accompanied by the *groES* gene. Whether both copies of *groEL* and *groES* are functional or if functional divergence has occurred is unknown and this will be addressed in this study. Using phylogenetic approaches and molecular genetics, we determined the evolutionary history and function of both copies in *V. vulnificus*. Among the 24 Vibrionaceae genome sequences in the database, only 7 species contain the duplication, which is conserved within a species. Phylogenetic analysis suggests that the *groEL* and *groES* genes on chromosome 1 are the ancestral copy and those on chromosome 2 of *Vibrio* species arose by horizontal gene transfer and not gene duplication. We examined the expression patterns of both *groEl-1* and *groEL-2* under various temperature, NaCl concentrations, and pH conditions to determine whether there are differences in expression patterns. We evaluated mRNA transcript levels of both *groEl-1* and *groEL-2* in both exponential and stationary phase growth relative to transcript levels in cells grown in LB 2% NaCl at pH 7. We determined that each copy is differentially expressed.