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Cold-induced hibernation of marine vibrios in the Gulf of Mexico: a study of cell-cell communication and dormancy in *Vibrio vulnificus*

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Vibrio vulnificus is a human pathogen commonly found in marine and brackish waters. It is responsible for causing serious life-threatening disease associated with the ingestion of shellfish. Under low temperature conditions, *Vibrio vulnificus* enters an unusual, and poorly understood physiological state called dormancy (also known as viable-but-nonculturable). In this state, cells appear alive but incapable of replication. Aside from being a fascinating biological phenomenon, the dormant state may result in underestimating the contamination hazard of oysters. The focus of this project is to better understand factors that influence entry and exit from dormancy as well as the physiology of dormancy itself. We have made significant progress in this regard over the past four months. Our results have revealed that progression into dormancy is, at a minimum, a two-stage process. The first stage is a pseudodormant state in which cells become ultra-sensitive to oxygen radicals naturally present in plating media. Pseudodormant cells are easily recoverable by plating on media containing catalase or sodium pyruvate. Catalase will consume hydrogen peroxide while sodium pyruvate scavenges oxygen radicals. Annotation of the *V. vulnificus* genome reveals this organism possesses two catalase genes. We are now examining extracts of log phase and dormant cells for the presence of these enzymes. We are also planning experiments to determine if more stable catalases introduced into *V. vulnificus* by genetic engineering will delay or prevent entry into dormancy.

Following the pseudodormant state, *V. vulnificus* enters a second stage where the cells are NOT recoverable by catalase. This occurs approximately 30 days after initial cold induction. These dormant cells still appear alive based on their ability to maintain an energized membrane. We have subjected log phase and dormant cells to SDS PAGE analysis and have identified significant proteomic differences between these two physiological states. Five proteins were observed to decrease or completely disappear in the dormant cells. Preliminary identification of these proteins by mass spectral analysis indicate they are DnaK, an essential chaperone protein, ribosomal proteins S1 and L1, glutamine synthetase, and alanine dehydrogenase. More definitive two-dimensional separations of the proteomes are underway. However, if the loss of two key ribosomal proteins is confirmed, it could explain why dormant cells fail to divide. We will examine the genome for additional proteins that may substitute for these key protein synthesis factors. Why *V. vulnificus* discards these proteins while other organisms that lack a dormancy program do not is a critical question.

We are also investigating whether *V. vulnificus* secretes autoinducer molecules that influence entry or exit from the dormant state. Initial experiments suggest that dormant cells do not produce a factor that triggers dormancy. We are now using a drug

resistant strain of *V. vulnificus* to determine if co-culturing log phase drug sensitive cells with drug-resistant dormant cells will encourage exit from dormancy, a result that would indicate the presence of a secreted rescue molecule.

Another aspect of our work centers on the effect salt concentration has on the synthesis of an apparent autoinducer molecule. As a marine microbe, *V. vulnificus* is a halophile that grows best at high salt concentrations (approximately 1 to 1.5%). We have discovered that this organism produces a factor at low salt concentrations (0.5% or less) that facilitates growth when these cells are diluted to low cell density in low salt media. Experiments are planned to determine if this growth factor will influence the dormant state.

The physiological changes associated with dormancy represent a fascinating biological program that enables cells to resist death under environmental stress. Learning how this program works will impact our general understanding of how marine microbes cope with their unique ecological niche and will aid safety assessment of shellfish contaminated with this pathogen.