



CALIFORNIA STATE SCIENCE FAIR  
2007 PROJECT SUMMARY

<b>Name(s)</b> Shilpa P. Argade	<b>Project Number</b> <b>S0402</b>
<b>Project Title</b> <b>Genetics and Biochemistry of Sialylation in Vibrionaceae</b>	
<p style="text-align: center;"><b>Abstract</b></p> <p><b>Objectives/Goals</b> Sialic acids (Sias) are a family of nine-carbon sugars that play diverse biological roles, including the host-pathogen interaction. Specifically, many bacteria decorate themselves with Sias, a phenomenon related to virulence in several human pathogens. Bacterial #hijacking# of host Sia-binding proteins via #molecular mimicry# may be responsible for the virulence of Sia-decorated pathogens. Vibrionaceae is a family of bacteria that have been shown to possess the gene cluster responsible for Sia biosynthesis. This experiment set out to determine whether Vibrio species express high levels of Sias, explore the complexity of Sias among positive strains, and evaluate the genetic basis for Sia-decoration in Vibrionaceae.</p> <p><b>Methods/Materials</b> The Thiobarbituric-acid (TBA) assay was used to screen through 56 strains of Vibrionaceae representing 14 different species for their total Sia contents. Sia values were normalized to protein content by the Bicinchoninic-acid (BCA) assay. Strains with high levels of Sias were further analyzed by a sensitive DMB-HPLC method with fluorescence detection. To determine the presence of O-acetylation, samples were profiled with and without mild base treatment. A transposon mutant whose transposon landed upstream the hypothesized Sia biosynthetic gene cluster was obtained. DMB-HPLC and LC-MS analysis was used to compare the mutant and WT for Sia expression.</p> <p><b>Results</b> Among all strains of Vibrionaceae, Vibrio Parahaemolyticus 190-2004 had the highest amount of Sia (24.84 pmol Sia/ug protein). The DMB-HPLC profiles showed several peaks, suggesting the presence of Sia derivatives, such as Legionaminic and Pesudaminic (Pse) acid and O-acetylated species. In the WT vs. mutant experiment, the LC-MS analysis of DMB derivatives showed the presence of Pse in the WT, but not in the mutant.</p> <p><b>Conclusions/Discussion</b> This study shows that ten strains of Vibrionaceae had high levels of Sias. DMB-HPLC analysis confirmed the presence of several related species of Sias along with O-acetylation. The mutant showed no Sias by DMB-HPLC and was confirmed by LC-MS analysis, thus supporting the genetic basis of Sia biosynthesis. These experiments document the discovery and genetic basis for Sia-decoration in Vibrionaceae. They begin the process of establishing a biological model that may help us understand the mechanisms of Sia molecular mimicry in host-pathogen interactions.</p>	
<b>Summary Statement</b> This project proves Sia expression in Vibrionaceae, confirms the genetic basis of Sia biosynthesis, and shows the possible role of Sia in host-pathogen interactions.	
<b>Help Received</b> Used lab equipment at UCSD under supervision of Dr. Amanda Lewis and Dr. Victor Nizet.	