Title: Lipid Membrane Remodeling during Ethanol, Isobutanol, and Lignocellulosic Hydrolysate Stress in *Zymomonas mobilis*

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Project Goals: The lipid membrane is the first line of defense for bacteria and evidence shows that microorganisms tend to remodel their lipid membrane in response to stressors in the environment. The response, which aids in the defense, often consists of increasing certain lipid classes and/or changing the composition of the fatty acid chains. Understanding a microorganism's universal lipid membrane remodeling response could lead to the data necessary to engineer strains that are capable of withstanding various stressors.

Abstract Text: *Zymomonas mobilis* is an ethanologenic gram-negative bacterium currently being developed for production of isobutanol. Despite being a proficient alcohol producer, *Z. mobilis* experiences growth inhibition at high ethanol titers and is highly sensitive to isobutanol. It is known that bacteria can modulate lipid membrane composition to increase their tolerance against environmental stressors. In this study, we used LC-MS/MS-based lipidomics to measure changes in lipid membrane composition that occur when *Z. mobilis* is exposed to increasing concentrations of ethanol, isobutanol, or ammonia fiber expansion (AFEX) lignocellulosic hydrolysate. Exposure to ethanol and isobutanol resulted in significant but distinct changes to the lipid and fatty acid composition. Affected lipid classes included ceramides, cardiolipins, phosphatidylcholines, and phosphatidylethanolamines. The fatty acid composition was also significantly affected. Most notably, we observed a substantial increase in C19 cyclopropane fatty acid content when cells were grown at high ethanol concentrations, suggesting that the changes comprise a defense mechanism in response to solvent stress. Previous evidence showed that cyclopropane ringed fatty acids modify membrane fluidity and act as a barrier to prevent detrimental molecules from entering the cell. To test the hypothesis that C19 cyclopropane fatty acids and derived lipids contribute to solvent resistance in *Z. mobilis*, we engineered a strain that overexpressed the Cyclopropane Fatty Acyl Synthase (CFA-Synthase) protein (ZMO1033) responsible for transforming unsaturated fatty acids into cyclopropane fatty acids. Analysis of the lipid membrane composition of the CFA-synthase overexpressing strain showed a significant increase in C19 cyclopropane fatty acid content for all lipid classes. This increase correlated with significantly improved growth rates in the presence of high ethanol and isobutanol concentrations. These data demonstrate the importance of cyclopropane fatty acids to solvent stress resistance in *Z. mobilis* and will allow us to engineer strains that are more resistant to high ethanol and isobutanol concentrations.

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