CALIFORNIA STATE SCIENCE FAIR
2012 PROJECT SUMMARY

Name(s)
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Project Number
S1528

Project Title
Stress-Induced Mutagenesis and Evolution of Competitive Fitness in Shewanella: Applications for Microbial Fuel Cells

Abstract

Objectives/Goals
Bacteria of the genus Shewanella can respire a wide variety of solid-state electron acceptors; they can serve as biocatalysts in microbial fuel cells (MFCs) and as agents for bioremediation of toxic chemicals. To optimize their utility for these applications, it is first necessary to understand the mechanisms that facilitate evolution under conditions of environmental stress, such as nutrient limitation. Some microorganisms including E. coli are known to acquire mutations during long-term stationary phase which confer an ability to outcompete younger cells; this trait is called a growth advantage in stationary phase (GASP) phenotype. My objective was to characterize the effects of extended starvation on the long-term evolution of Shewanella spp. ANA-3 and MR-7 by assaying for changes in mutation frequency and strength of GASP phenotype expression with variable periods of nutrient-limited aging.

Methods/Materials
Str. were differentially marked with mutations conferring resistance to different antibiotics and aged in batch culture for 30 days; samples were frozen every 10 days. Co-culture competitions were performed in which aged cells were inoculated as 1000-fold minorities into overnight cultures of oppositely marked 1-day-old cells; viable counts were monitored over 12 days by serial dilution with plating on marker-selective media. Finally, unmarked 1- and 10-day-old cultures of both spp. were titered and spread on solid media appended with rifampicin, and resistant colonies were enumerated.

Results
All aged str. expressed GASP phenotypes in competition. For both spp., the log ratio of aged to unaged cells in co-culture after 12 days was significantly higher ($p < 0.05$) for 20- and 30-day-old populations than for 1-day-old controls. 10-day-old cultures of both ANA-3 and MR-7 demonstrated significantly elevated rates of spontaneous mutation ($p < 0.05$ and $p < 0.01$, respectively) relative to 1-day-old cells.

Conclusions/Discussion
These results suggest that nutrient limitation during long-term stationary phase may cause a degree of DNA damage sufficient to induce the SOS response, which is known to cause preferential recruitment of low fidelity DNA polymerases that increase the global mutation rate, potentially accelerating the acquisition of beneficial GASP alleles. This finding is of particular importance given the relationship between competitive fitness and capacity for current-production in an MFC.

Summary Statement
I assayed for changes in the relative fitness and mutation frequency of Shewanella spp. with varying periods of starvation, toward the development of specific strategies for optimizing their utility as biocatalysts in microbial fuel cells.

Help Received
Used molecular biology facilities at USC under the supervision of Dr. Steven Finkel, from whom I received technical guidance.