CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

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Project Number

S1522

Project Title

Site-Directed Mutagenesis of S. oneidensis MR-1: A Novel Strategy for Genetic Engineering in Recalcitrant Microorganisms

Abstract

Objectives/Goals

Due to its respiratory vesatility, Shewanella oneidensis MR-1 is widely employed as a model organism for the study of electron-transport processes including subsurface bioremediation and electricity production in microbial fuel cells. However, MR-1's usefulness as a model organism is limited by its recalcitrance to techniques of genetic engineering developed for Eschericia coli. My objective was to implement a strategy for site-directed mutagenesis of the MR-1 chromosome in the absence of exogenous recombinase functions; to evaluate and optimize its utility for the construction of point substitutions and large deletions by examining strand-, site-, and sequence-specific effects.

Methods/Materials

Flask-cultures of MR-1 were harvested in mid-log phase, made electrocompetent, and snap-frozen for cryostorage. These were thawed and electroporated with DNA oligonucleotides (oligos) that contained the desired sequence alterations (conferring drug-resistance phenotypes) flanked by regions of homology to the target loci. Recombination frequencies were determined by plating on selective media while controlling for basal mutation rate, with subsequent restriction-fragment analysis to confirm presence of the intended mutations.

Results

Point substitutions were achieved at frequencies of 10^-8 to 10^-7. Silent modifications designed to evade methyl-directed mismatch repair (MMR) enhanced this efficiency by ~10-fold ($p < 0.05$) in isolation, but had a negative impact on recombination frequency when in the presence of other similar modifications. Although the antibiotic resistance assay was not sufficiently sensitive to discriminate oligo-mediated deletions from the spontaneous mutation frequency, PCR amplification revealed that a minority of the drug-resistant isolates had incorporated the intended deletion.

Conclusions/Discussion

Ongoing experiments involve the use of ssDNA purified from PCR products to mediate insertional mutagenesis (e.g., of marker cassettes), obviating the need for mutations that confer selectable phenotypes in themselves. Taken together, these results open the door to development of a greatly expanded molecular toolkit for genetic manipulation of Shewanella spp. and provide novel insights into the mechanistic basis of Red-independent recombination.

Summary Statement

I developed and optimized a recombineering strategy, independent of host/exogenous recombinase functions, for genetic manipulation of the bacterium S. oneidensis MR-1; this technique has broad applicability to other recalcitrant microbes.

Help Received

Used laboratory facilities at the University of Southern California under the supervision of Prof. S.E. Finkel.