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004 EPA STAR Graduate Fellowship Conference *Next Generation Scientists—Next Opportunities*

ntification of Genes from *Mycobacterium smegmatis* Involved in the Decolorization of the Triarylmethane Dye Malachite Green

Overview

nvironmental Issue:

alachite green:
is used extensively in commercial fisheries as an tifungal agent and may bioaccumulate in aquatic osystems.
is used in textile industries for dyeing nylon, wool, k, leather and cotton.
causes organ damage & developmental abnor- alities in mammals & is mutagenic & carcinogenic.

ycobacteria

are saprophytic, acid fast, soil-dwelling bacilli.
can degrade malachite green & pollutants such as e BTEX group of compounds.
belong to the Order Actinomycetales and thus are ated to the bacteria in the genus *Rhodococcus*, own bioremediators.

e genomes of many mycobacterial species have en sequenced or are in the process of being quenced since many species in this genus, such *M. tuberculosis* (Mtb), are medically important.
smegmatis is used as a model organism for Mtb. onsequently, genetic tools such as transposon utant libraries and expression vectors are available.

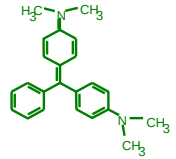
bjectives:

) screen the transposon mutant library of *ycobacterium smegmatis* for mutants unable to colorize malachite green.
) identify the genes disrupted in mutants unable to colorize malachite green, confirm identity by mplementation, and determine if the identified gene induced by malachite green.

Research Highlights

Preliminary Results:

- 3000 transposon mutants have been screened to date
- 3 mutants, 28F6, 28F9 and 31B4 are impaired in their ability to decolorize malachite green



Malachite green

Table 1. Average Minimum Decolorization Concentration (MDC) & Minimum Inhibitory Concentration (MIC) for wild-type and 3 mutants in liquid media supplemented with malachite green.

	MDC (mg/ml)	MIC (mg/ml)
Wild-type	0.437	0.437
28F6	0.328	0.437
28F9	0.082	0.164
31B4	0.082	0.164

Figure 1. Growth of wild-type *M. smegmatis* in solid media supplemented with (from Clockwise top left) 0, 0.01, 0.1, 1.0 mg/ml malachite green.

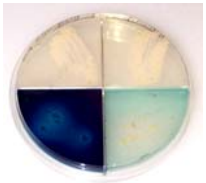


Figure 3. Growth of wild-type (left) and mutant 28F9 in solid media supplemented with malachite green.

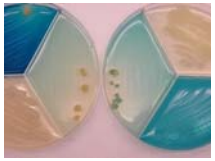


Figure 2. Growth of wild-type & 3 mutants & decolorization in liquid media supplemented with malachite green in concentrations ranging from 0 to 3.0 mg/ml.

Table 2. Characterization of mutants impaired in their ability to decolorize malachite green

Transposon Mutants	Similar Protein[a] (% similarity with *Mycobacterium tuberculosis* homolog)	Open Reading Frame	Putative Function
28F6	lpqW (92%)	Rv1166	Probable conserved lipoprotein
28F9	fbiC (96%)	Rv1173	Probable coenzyme F420 biosynthesis protein
31B4	Rv2983 (63%)	Rv2983	Conserved hypothetical alanine-rich protein

[a] similarity based on BLAST program of Tuberculist against the genome of *Mycobacterium tuberculosis* H37Rv

Impact

Bioremediation is the transformation of pollutants by organisms to products that are no longer hazardous to human health or the environment. By utilizing genetic tools such as mutant libraries, we are identifying genes involved in the degradation of the pollutant malachite green. Identification of these genes will aid in the development of strategies for the bioremediation of pollutants such as malachite green.

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