Natural Gene Transfer to Develop Resistance to Metal Toxicity in Microbial Communities at the Oak Ridge Reservation Field Research Center

Safiyh Taghavi[1], Craig Carafola[1], Daniel van der Lelie[1], and Jeffrey P. Fitts[2]

[1]Biology Department, [2]Environmental Research and Technology Division of the Environmental Sciences Department, Brookhaven National Laboratory, Upton, NY

Our research addresses the need to understand how natural gene transfer can be used to help naturally occurring microbial communities adopt resistance to specific environmental stresses such as heavy metals that inhibit their ability to reduce and immobilize metals and radionuclides. Nickel is being used as a model system to demonstrate how a metal resistance marker can be introduced into both single species and naturally occurring microbial communities in contaminated sediments collected from the Oak Ridge Reservation Field Research Center (ORR FRC). The overall objective of this work is to demonstrate the feasibility of applying natural gene transfer to improve the performance of natural microbial communities under conditions imposed by metal stress, using Ni toxicity and resistance as a model system.

Using natural gene transfer, the nickel resistance operon (*ncc-nre*) was introduced in the following nitrate-reducing bacteria, which were obtained from the fluidized bed reactor that is being used at the ORR FRC to condition groundwater: *Enterobacter* M-53, *Enterobacter* DM-S, *Klebsiella* DM-C3, *Pseudomonas* DM-Y2, *Janthinobacterium* M-A11, *Idobacter* DM-K3, *Stenotrophomonas* M-A15, *Chromobacterium* DM-N3, and *Shewanella oneidensis* MR-1 as a reference strain. The *ncc-nre* operon was introduced either on the broad host-range IncQ plasmid pMOL222 or on a single hopper mini transposon. We had also planned to introduce the *ncc-nre* operon into *Pseudomonas* DM-H2 and *Pseudomonas* M-16, but these strains were found to have a natural high resistance to Ni (MIC of 6 mM).

The integrity of the Ni-resistant transconjugants is being tested by BOX-PCR in comparison with the recipients to check their genetic background and by PCR with *ncc-nre* specific primers to confirm the presence of the *ncc-nre* operon. We are also testing the stability of the Ni-resistance phenotypes by growing the transconjugants for 100 generations under nonselective conditions and then verifying the presence of *ncc-nre*.

Once the Ni-resistance transconjugants have been characterized, we will test them in batch experiments to examine their efficiency in reducing nitrate in the presence of increasing concentrations of Ni. We will also use these strains in column mesocosm experiments to evaluate the possibilities of natural gene transfer to improve the activity of a nitrate reducing microbial community when it is being challenged with high levels of Ni-contamination.

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