Genes for Uranium Bioremediation in the Anaerobic Sulfate-Reducing Bacteria: *Desulfovibrio* mutants with altered sensitivity to oxidative stress

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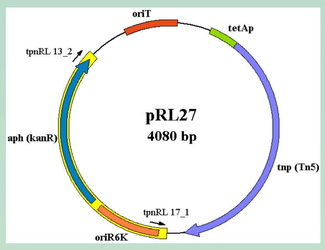
Objective: Sulfate-reducing bacteria of the genus *Desulfovibrio* are ubiquitous in anaerobic environments such as groundwater, sediments, and the gastrointestinal tract of animals. Because of the ability of *Desulfovibrio* to reduce radionuclides and metals through both enzymatic and chemical means, they have been proposed as a means to bioremediate heavy metal contaminated sites. Although classically thought of as strict anaerobes, *Desulfovibrio* species are surprisingly aerotolerant. Our objective is to understand the response of *Desulfovibrio* to oxidative stress so that we may more effectively utilize them in bioremediation of heavy metals in mixed aerobic-anaerobic environments. The enzymes superoxide dismutase, superoxide reductase, catalase, and rubrerythrin have been shown by others to be involved in the detoxification of reactive oxygen species in *Desulfovibrio*. Some members of the genus *Desulfovibrio* can even reduce molecular oxygen to water via a membrane bound electron transport chain with the concomitant production of ATP, although their ability to grow with oxygen as the sole electron acceptor is still questioned.

Experimental approach: We are taking a genetic approach to identify those genes involved in the oxidative response in *Desulfovibrio*. We have recently generated a library of mini Tn*5* transposon mutants in *Desulfovibrio desulfuricans* G20, and *Desulfovibrio vulgaris* and we have begun screening this library for mutants with altered sensitivity to oxygen.

Figure 1. Selection Scheme for Oxygen Resistant Mutants



Desulfovibrio exconjugants from a Tn*5* mutagenesis using the plasmid pRL27 were plated on growth medium with lactate as the carbon and energy source, and sulfate as the electron acceptor, and colonies were allowed to grow anaerobically.

Larsen R. A., et al. (2002) Arch. Microbiol. 178: 193-201.

Approx 700 colonies transferred by replica plating onto LS plates. Plates were then exposed to atmospheric oxygen for 1 or 8 hours.

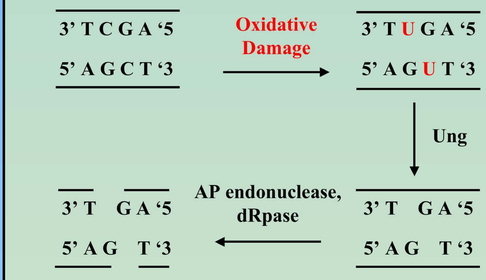
Plates were incubated anaerobically for 1 week. 7 independent colonies survived. One of the survivors had an insertion of the Tn*5* into the gene encoding uracil DNA N-glycosylase (*ung*).

In *Escherichia coli*, Ung is the first enzyme in the pathway of uracil excision and repair in DNA, and *E. coli ung* mutants have previously been shown to be more resistant to some types of DNA damage. We hypothesize that exposure of *Desulfovibrio* to molecular oxygen results in DNA damage, and that the excision and repair of this DNA damage by Ung has a greater potential to create a lethal double stranded break in the chromosome.

Table 1. Tn*5* insertion sites of putative *D. desulfuricans* oxygen resistant mutants

D. desulfuricans strain	Insertion site	VIMSS number
O2R1	<100nts upstream of *cobS* operon	394210
O2R2	Sigma-54 response regulator	395930
O2R3	helicase	394379
O2R5	Lactate permease 2 (*lctP*)	395606
O2R6	1kb downstream of Fe-superoxide dismutase (*sodB*)	395773
O2R7	Uracil DNA N-glycosylase (*ung*)	392942

Figure 2. Model for the role of Uracil DNA N-glycosylase (Ung) in Oxygen sensitivity



We speculate that WT cells create more DSBs during uracil removal from DNA, and therefore are more sensitive to oxidative damage than *ung* mts.

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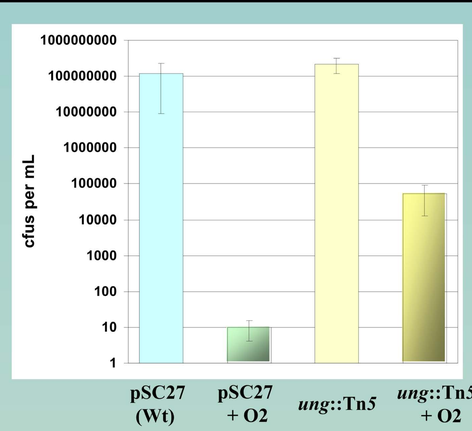
Figure 3. Oxygen Resistance of *D. desulfuricans ung*::Tn*5*



Oxygen Stress

5 mls of late log cultures are exposed to atmospheric oxygen for 8 hours.

↓

Cultures were returned to anaerobic chamber. Serial dilutions were made of all cultures, and they were plated on LS.

↓

Surviving colonies were counted after 1 week.

***D. desulfuricans ung*::Tn*5* is more resistant to oxygen stress than wild type.**

Table 2. Mutator phenotype of *D. desulfuricans ung*::Tn*5*

Strain	Relevant phenotype	Titer (ml^{-1})[a]	Rif^R mutation frequency per 10^9 cells[b]
D. desulfuricans pSC27	Km^R, *ung*$^+$	$9.8 \pm 2.0 \times 10^8$	15 ± 17
D. desulfuricans O2R7	Km^R, *ung*::Tn*5*	$7.1 \pm 1.4 \times 10^8$	264 ± 50

[a]The titer represents the number of colony forming units from a culture that was allowed to rest in stationary phase for 72 hours before plating. This number is the average and standard deviation of four plates from one experiment.
[b]The number of rifampicin resistant colony forming units from a culture that was grown as above. This number is the average and standard deviation of four plates from one experiment.

As expected if Ung functions in DNA repair, the *ung* mutant of *D. desulfuricans* has a spontaneous Rif^R mutation frequency of about 20-times that of wild type.