Molecular Mechanisms of Uranium Reduction by Clostridia

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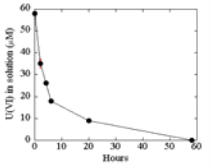
Background

- Clostridia are widespread in soils, sediments, acidic uranium mine water and radioactive wastes. Clostridia reduce U(VI) as well as many other metals.
- Although the phenomenon of uranium reduction by Clostridia has been fully established, the molecular mechanisms underlying such a reaction are not very clear.
- Fundamental knowledge of molecular assessment of radionuclide and metal reduction will allow us to exploit the naturally occurring processes to attenuate radionuclide and metal contaminants in situ in the subsurface dominated by low and high pH, high nitrate, and / or organic matter where the dissimilatory metal reducing bacterial activity will be limited.

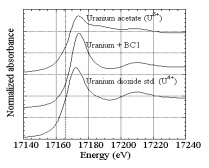
Objective

- The overall objective of this research is to elucidate systematically the molecular mechanisms involved in the reduction of uranium by Clostridia.

Reduction of Uranium by *Clostridium* sp.

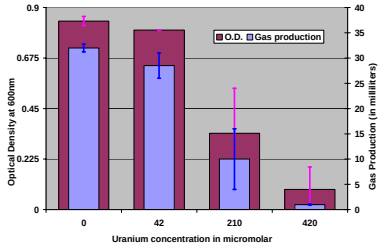


Growing culture of *Clostridium* sp. rapidly reduced U(VI) to U(IV).



XANES analysis shows reduction of U(VI) to U(IV) by shift in absorption spectrum from 17171 eV to 17166 eV.

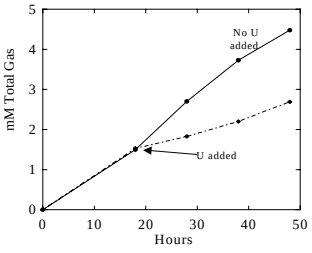
Effect of Uranium Addition on Growth and Gas Production by *Clostridium* sp.



Uranium at higher concentrations affected the growth of bacteria and is toxic. Growth was determined after 45 h.

Error bars represent one standard error of the mean (1 ± SEM).

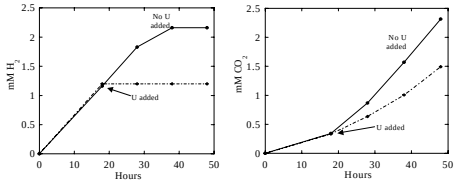
Total Gas Production by *Clostridium* sp.



Total Gas production with and without addition of 100 μM uranium

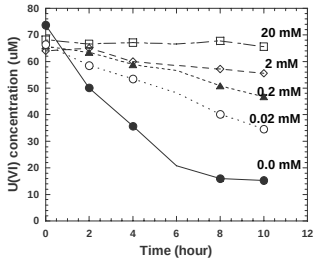
Addition of uranium to an 18 h old culture inhibited total gas production

Hydrogen and Carbon dioxide Production by *Clostridium* sp.



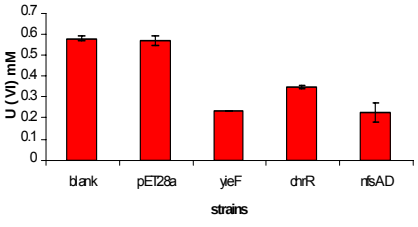
Addition of 100 μM uranium to an 18 h old culture resulted in complete inhibition of hydrogen production. However, carbon dioxide production continued at a slower rate after uranium addition.

Effect of Addition of Copper on Uranium Reduction



Addition of Cu (II) to an 18h old culture inhibited uranium (VI) reduction. 20mM Cu (II) completely inhibited uranium reduction.

U(VI) reduction by chromate reductases



Chromate reductases have been found to reduce uranyl species. Uranium was added as uranyl acetate. 90-95% of U(VI) that was reduced was transformed to U(IV) (data not shown).

U(VI) kinetics by YieF and improved derivative Y6

Strain	V_{max} (nMol U(VI) mg protein^{-1}min^{-1})	K_m (μM)	K_{cat}*(S^{-1})	K_{cat}/K_m
YieF	194 ± 17	373 ± 49	20 ± 11	$1.6x10^4$ ± $1.7x10^3$
Y6	2511 ± 421	335 ± 40	167 ± 39	$5x10^5$ ± $2x10^4$

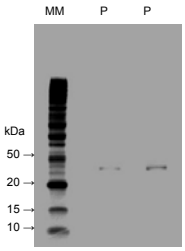
Improved enzymes: One of our objectives is to improve bacterial remediation capacity by evolving enzymes more efficient in remediating multiple pollutants. After three rounds of shuffling, we have achieved 11-fold increase in V_{max} for uranyl reduction by the evolved enzyme (Y6) which has four substitutions: V120A, Y128N, T160N and Q175L. Kinetics of evolved purified protein compared to wild type are shown in this Table. Both YieF and Y6 are also chromate reductases, with Y6 being 30-fold more efficient in this respect. Further improvements are underway but Y6 is clearly useful in remediation for sites such as the DOE.

H_2O_2 Production during Cr(VI) or U(VI) reduction

Strain	H_2O_2 Production (μM) Cr(VI)	H_2O_2 Production (μM) U(VI)
YieF	30.5 ± 2.7 (24%)	40.8 ± 5.0 (32%)
Y6	15.5 ± 1.2 (12%)	20.5 ± 3.7 (16%)

Improved enzymes: Reactive oxygen species (ROS) generation in the form of H_2O_2. Y6 is improved also in terms of "safe" chromate reduction mechanism.

Endogenous *Clostridium acetobutylicum* NAD(P)H oxidoreductase overexpressed in *E. coli*



SDS-PAGE of a purified recombinant NAPH-quinone reductase from *C. acetobutylicum* expressed in *E. coli*. MM (Molecular marker – BenchMark Protein Ladder; P) Purified recombinant *Clostridium acetobutylicum* quinone reductase (in duplicate). This clostridial enzyme has chromate reductase activity and is likely also to be uranyl reductase.

Summary

- Addition of 100 μM uranium to an 18 h old culture of *Clostridium* sp. showed complete reduction of U(VI) to U(IV), partial reduction in total gas and CO_2 production and complete inhibition of H_2 production.
- Addition of varying amounts of Cu(II) inhibited uranium reduction.
- Chromate reductases isolated from *Pseudomonas putida* reduced uranyl acetate to varying degree.
- 11-fold increase in Vmax for uranyl reduction was achieved by the evolved enzyme (Y6) which has four substitutions: V120A, Y128N, T160N and Q175L.
- Y6 is clearly useful in remediation for uranium contaminated sites.

Proposed Studies

- Determine the rate and extent of reduction of uranium complexed with organic and inorganic ligands by *C. acetobutylicum.*
- Isolate mutants impaired in U(VI) reduction.
- Determine the role of hydrogenases and soluble reductases in U(VI) reduction.
- Elucidate the mechanisms of electron transfer by purified proteins.
- Screen for high-activity uranium reducing enzymes.

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