

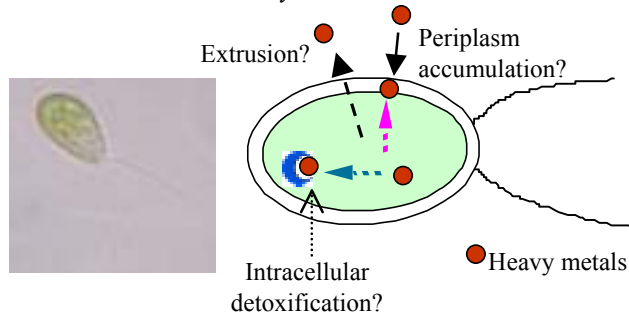
Chlamydomonas W80, a green alga highly tolerant to cadmium: a basic study for its application for phytoremediation

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Discover the cadmium-tolerant

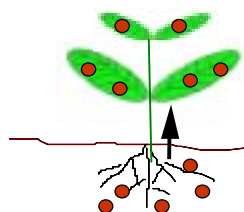
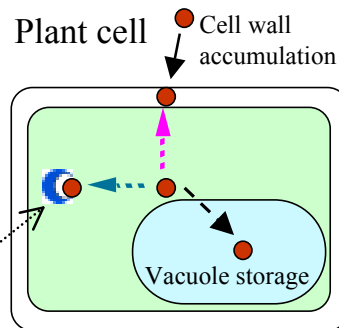
mechanisms of *Chlamydomonas* W80



Future application to higher plants (Introduction of algal genes into higher plants)



Intracellular detoxification



Transgenic plant for phytoextraction of heavy metals

Goal & Contribution to well-being for all humanity

Heavy metal contamination of soil and groundwater is a serious threat to the well-being of all humanity, and phytoremediation is one of the most promising counter measures for this problem. *Chlamydomonas* W80 is a unicellular marine green alga that shows a high tolerance to the cadmium ion (up to 10 mM CdCl₂). In this study, we will discover the cadmium-tolerant mechanisms, such as intracellular detoxification and/or extrusion, of this alga. The mechanism will be applied in the future to generate transgenic plants, which can effectively extract cadmium (and other toxic heavy metals) from the contaminated soil.

What we know about *Chlamydomonas* W80 at present:

Highly tolerant to cadmium ion; cells grow in the medium with 0.5 to 1mM CdCl₂, and tolerate (but no growth) up to 10mM. Unexpectedly low intracellular concentrations of cysteine, glutathione, and phytochelatin (PC) in comparison with another cadmium tolerant green alga *Dunaliella tertiolecta*, suggesting that the thiol (-SH) mediated detoxification system is not the major tolerant mechanism in *C. W80*.

What we are going to discover:

The mechanism of cadmium tolerance of *C. W80*

- (i) Are there any intracellular detoxification mechanisms?
If so:
What are the chelating compounds?
Where is the place of cadmium accumulation? (cellular localization)

- (ii) Are there any extruding mechanisms?

Cloning of the genes related to heavy metal tolerance

How is the response of *C. W80* to other heavy metals?

Method / Approach

Cadmium tolerant mechanism

Examine the changes in the intracellular cadmium concentration of *C. W80* cells cultured in the medium with 10mM CdCl₂ to find out if *C. W80* cells accumulate and/or extrude the cadmium ion. The distribution of cadmium ion in the cells will also be examined by energy dispersive spectroscopy (EDS).

Isolate the cadmium binding cellular molecules by metal affinity chromatography, and analyze them with various methods (e.g. SDS-PAGE, gel filtration, HPLC, etc.).

Cloning of the genes related to heavy metal tolerance

Approaches for the screening of the cDNA library are as follows:

- (i) Screening based on the homology with the previously reported genes (e.g. heavy metal transporters).
- (ii) Functional expression screening based on the acquisition of heavy metal tolerance of the host cells expressing the genes of *C. W80* (host: *E. coli* and yeast).
- (iii) Isolation of heavy metal binding proteins by metal affinity chromatography, and screening the cDNA library of *C. W80* with the antibody raised against the isolated proteins.
- (iv) Cloning of the cadmium-inducible genes by subtractive hybridization and differential display.

Call for collaboration: We focus mainly on the heavy metal detoxifying proteins and peptides, but there could be some other unknown (novel) cadmium-chelating cellular compounds other than proteins and peptides. If this is the case, we need a collaboration with a chemist whose expertise is chelate chemistry.