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Use of a Cell Wall-less Mutant Strain to Assess the Role of the Cell Wall in Cadmium and Mercury Tolerance by *Chlamydomonas reinhardtii*

Jerome R. Cain and Renee K. Allen
Department of Biological Sciences, Illinois State University, Normal, IL 61761

In order for heavy metals to be toxic to algal cells, they must enter the cell protoplast. For most algal cells, this means that the metals must first penetrate through a cell wall. Plant cell walls are normally considered to be highly permeable to compounds of low molecular weight. However, if materials present in the algal cell wall show a high affinity for environmental contaminants, particularly heavy metals, entry into the protoplast, and thus toxicity, could be affected.

The cell wall of *Chlamydomonas reinhardtii*, a common freshwater green alga, has been well characterized, both biochemically and ultrastructurally (ROBERTS *et al.* 1972, HILLS *et al.* 1973). The wall of this alga is known to lack cellulose, but contains significant amounts of glycoproteins -- compounds known to have high affinity for heavy metals (GURD and WILCOX 1956). BUTTON and HOSTETTER (1977) have shown that cells of *C. reinhardtii*, when exposed to exogenous copper, accumulate this metal, much of which remains bound to the cell wall. However, demonstration of cell wall affinity for heavy metals provides no direct evidence if, or to what degree, presence of the cell wall affects toxicity of the metals to the algal cells. The only way such direct evidence may be obtained is to compare the relative tolerance for heavy metals of algal cells which possess cell walls and those which lack them.

DAVIES and PLASKITT (1971) have isolated and characterized a series of *C. reinhardtii* mutant strains which display defective cell walls. In most strains the mutation involves a single gene, and in some strains the cell wall is totally lacking. The existence of cell wall-less mutant strains provides a unique opportunity to study experimentally the role of the cell wall in tolerance of environmental contaminants by *C. reinhardtii*. This current study was undertaken to determine the feasibility of using a cell wall-less mutant strain for the aforementioned purpose, and in particular, to demonstrate the role of the cell wall in Cd and Hg tolerance by *C. reinhardtii*.

MATERIALS AND METHODS

A culture of the *C. reinhardtii* wild-type strain (strain no. 89) was obtained from the Culture Collection of Algae at the

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University of Texas (STARR 1978). A culture of the cell wall-less mutant strain (strain CW-15 of DAVIES and PLASKITT 1971) was kindly supplied by Prof. Michel Dron. This latter strain is also maintained and available from the Culture Centre of Algae and Protozoa, Cambridge, England (GEORGE 1976).

The minimal medium employed for both stock and experimental cultures was a modification of the high-salt medium successfully employed by LIEN and KNUTSEN (1976) for culture of the wall-less mutant strain. This medium (hereafter referred to as modified HSM) contains the following macronutrients (g/l):NH_4Cl, 0.50; $MgSO_4 \cdot 7H_2O$, 0.20; K_2HPO_4, 1.44; KH_2PO_4, 0.72; Na_3 citrate$\cdot 2H_2O$, 0.50. The trace element mix of WATT and FOGG (1966) was also employed in the medium to provide the following (mg/l): $FeCl_3 \cdot 6H_2O$, 5.00; Na_2EDTA, 6.89; $CaCl_2 \cdot 2H_2O$, 26.55; $MnCl_2 \cdot 4H_2O$, 0.30; $CoCl_2 \cdot 6H_2O$, 0.02; $CuSO_4 \cdot 5H_2O$, 0.06; $ZnSO_4 \cdot 7H_2O$, 0.04; $Na_2MoO_4 \cdot 2H_2O$, 0.02.

Axenic stock cultures of both strains were maintained on agar plants of modified HSM and incubated at 21 ± 1°C under continuous flourescent illumination of 5000 lx. These conditions are hereafter referred to as standard culture conditions. Cells from week-old stock cultures were used to establish liquid inoculum cultures in 500 ml Erlenmeyer flasks containing 250 ml of modified HSM. After incubation for one week under standard culture conditions, the cell densities of the inoculum cultures were determined by direct cell counts taken on a haemocytometer. The cell densities were then adjusted to 2.5 x 10^6 cells ml^{-1} by appropriate dilution with modified HSM.

To determine Cd and Hg toxicity to both strains, experimental cultures were grown in 50 Erlenmeyer flasks containing 25 ml of modified HSM. Experimental media were modified to contain various amounts of Cd or Hg by the addition of appropriate amounts of reagent grade $CdCl_2 \cdot 2\frac{1}{2} H_2O$ or $HgCl_2$. Experimental cultures were inoculated with 0.2 ml of liquid inoculum suspension, thus establishing an initial cell density of 2.0 x 10^4 cells ml^{-1}. Simultaneous control cultures were established in medium containint no added Cd or Hg. The experimental and control cultures were grown under standard culture conditions for one week, after which time the cells were fixed with IKI and final cell densities determined by haemocytometer counts.

RESULTS AND DISCUSSION

In order to accomplish the goals of this study, it was necessary to establish a culture medium and set of laboratory environmental conditions which would promote good growth of both the wild-type and wall-less mutant strains. The data presented in Table 1 show that growth of both strains in modified HSM under standard culture conditions is nearly identical, and averages better than one population doubling per day during a 7 day growth period.

TABLE 1

Growth of *C. reinhardtii* wild-type and wall-less mutant strains in modified HSM

Strain	Growth (doublings/7 days)*
wild-type	7.28 ± 0.07
wall-less mutant	7.29 ± 0.05

*Numbers represent averages (± the standard error of the mean) from six individual experiments run in triplicate.

The effects of Cd on growth of both wild-type and wall-less mutant strains is shown in Table 2. For both strains, growth, expressed as a percentage of controls, decreased when the Cd concentration was increased over a range of 1.0 - 10.0 ppm Cd. There is no striking trend, however, in the overall growth responses when the growth percentages for the two strains are compared at each Cd concentration. Evidently the cell wall plays little or no role in the ability of *C. reinhardtii* to tolerate Cd.

TABLE 2

The effects of cadmium on growth of *C. reinhardtii* wild-type and wall-less mutant strains

	Growth (% control)*	
Cd conc. in medium (ppm)	wild-type	wall-less mutant
1.0	98	94
2.0	93	92
3.0	86	88
4.0	75	82
5.0	73	72
6.0	61	62
7.0	55	49
8.0	44	41
9.0	33	29
10.0	23	26

*Numbers represent averages from 3 individual experiments run in triplicate.

The effects of Hg on growth of both wild-type and mutant strains are shown in Table 3. As with Cd, growth of both strains decreased when the Hg concentration was increased over a range of 0.5-1.4 ppm Hg. When the overall growth responses of both strains are compared, however, growth of the wall-less mutant strain was consistently less than that of the wild-type. This trend indicates that the cell wall presence does affect the ability of *C. reinhardtii* to tolerate Hg. A comparison of the data in Tables 2 and 3 also indicates that Hg is considerably more toxic to *C. reinhardtii* than is Cd.

TABLE 3

The effects of mercury on growth of *C. reinhardtii* wild-type and wall-less mutant strains

	Growth (% control)*	
Hg conc. in medium (ppm)	wild-type	wall-less mutant
0.5	93	93
0.6	86	80
0.7	90	75
0.8	87	70
0.9	78	69
1.0	76	68
1.1	71	68
1.2	68	64
1.3	65	49
1.4	55	47

*Numbers represent averages from 3 individual experiments run in triplicate.

For most algae, determination of how a cell wall presence affects tolerance of environmental contaminants is a difficult problem to approach experimentally. Certain algae normally lack cell walls in the vegetative state (BOLD and WYNNE 1978), and the tolerance of these organisms can be compared to that of related algae which possess walls. This approach was taken by LUARD (1973), who compared the resistance of *Dunaliella* (a cell wall-less alga) and *Scenedesmus* (an alga with walls) to DDT and PCB. However, when one compares data obtained with organisms sufficiently different morphologically (and hence genetically) to be placed in separate taxa, one must not exclude the possibility that any observed similarities or differences in resistance might be a result of major physiological differences between strains, and may or may not be related to the cell wall presence.

For certain algal strains, it may be possible to create wall-less cells for comparative studies from walled cells by removing the cell wall by enzymatic digestion. This approach, though perhaps technically feasible, is experimentally impractical, especially when large numbers of wall-less cells are required.

The approach taken in this study, namely the use of a cell wall-less mutant strain, has not been explored previously. Our studies indicate, however, that this approach is not only feasible, but may be the best means currently available to assess the role of a cell wall in resistance of algal cells to environmental contaminants.

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