Bull Environ Contam Toxicol (1984) 32:597–601

Bulletin of Environmental Contamination and Toxicology

Heavy Metal Tolerance in a Cadmium-resistant Population of *Euglena gracilis*

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Resistance to metal ions has been observed for some microorganisms (Chopra 1971, Mitra *et al.* 1975, De Filippis and Pallaghy 1976, Butler *et al.* 1979) as well as for human and animal cells *in vitro* (Rugstad and Norseth 1975, 1977, Hildebrand *et al.* 1979). Most of the reported data were obtained using individual metal, but an investigation of an increasing tolerance to various heavy metals by the cells which had accomodated to one metal was rarely published.

We have previously described some aspects of the cadmium toxic action on *Euglena gracilis* cells *in vitro* cultured (Bonaly *et al.* 1978, 1980). We showed the acquisition by the *Euglena* populations of a Cd^{2+} resistance to toxic concentrations.

In this paper, the growth of a Cd-resistant and a non-resistant strain of *Euglena gracilis* in media containing Hg^{2+}, Ni^{2+}, Se^{4+}, Cu^{2+}, Zn^{2+} or Co^{2+} is compared, in order to ascertain the mechanism to tolerance in this alga.

MATERIALS AND METHODS

The two strains of *Euglena gracilis* used in these studies are the wild type (Eg - Z) and a Cd-resistant strain (Eg - ZR) adapted from the first one. Fresh cultures were inoculated weekly from the previous culture. Cells were grown for 6 months in the presence of 5. 10^{-4} M $CdCl_2$ (Eg - ZR) or in a cadmium free medium (Eg - Z). Then, both strains were grown for 6 months in the same cadmium free medium containing 33 mM lactate at pH 3, 5 (Bonaly *et al.*, 1978).

To investigate the action of heavy metal on cell growth, 2. 10^6 exponentially growing cells (Eg Z and Eg - ZR) were incubated with 50 ml of medium containing phenyl-mercuric acetate, sodium selenite, nickel chloride, copper sulfate, zinc sulfate or cobalt chloride, for 7 days.

Toxicity experiments were performed at different concentrations to determine the minimal inhibitory concentration (mic) and the critical concentration (cc). **mic** was determined as the lowest concentration of inhibitory preventing growth. **cc** was the concentration at which no growth occured after 15 days. Cell counting was carried out daily in a model B coulter using 100 μm aperture.

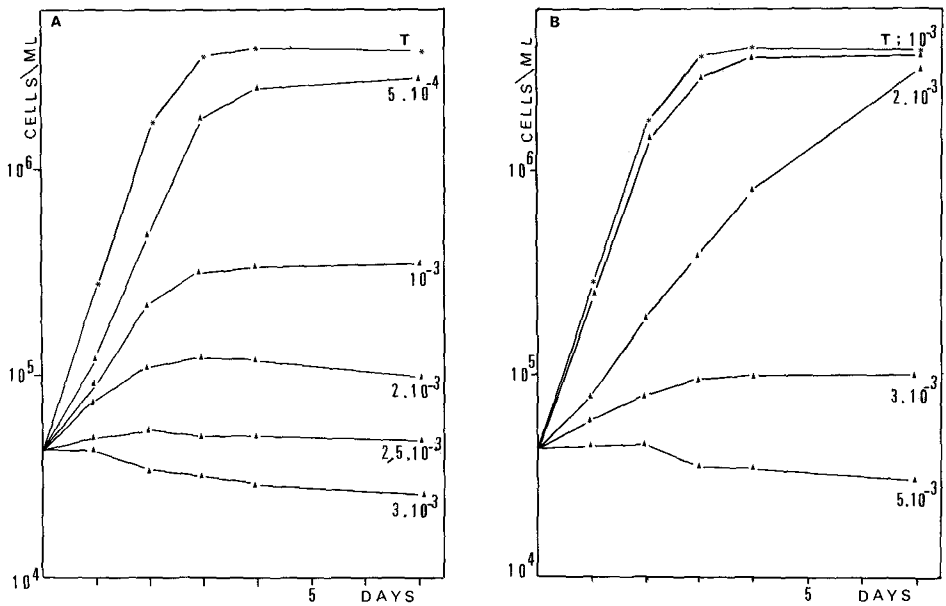

Figure 1.- The effect of Co^{2+} on the growth of non-resistant (A) and Cd-resistant (B) strains of *Euglena*. (★) : control ; (▲) : media with Co^{2+}. Results are mean of 5 duplicates. Variation about the point is less than 5 %.

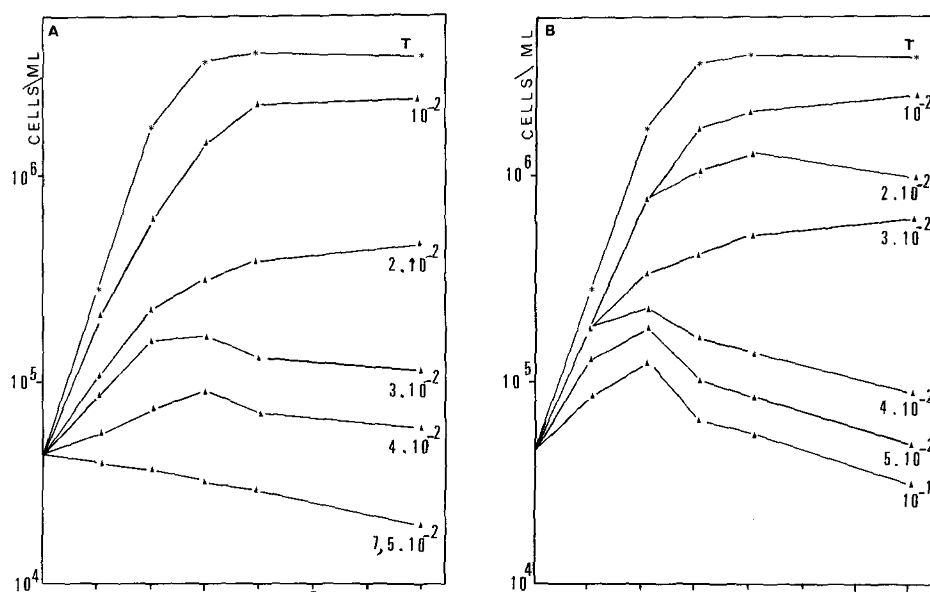

Figure 2.- The effect of Zn^{2+} on the growth of non-resistant (A) and Cd-resistant (B) strains of *Euglena*. (★) : control ; (▲) : media with Zn^{2+}. Results are mean of 5 duplicates. Variation about the point is less than 5 %.

RESULTS AND DISCUSSION

Figures 1 and 2 show the growth of the two strains of *Euglena gracilis* in different external cobalt and zinc concentrations. They show that the growth of the Cd-resistant stain is greater than that of the non-resistant strain in cobalt and in zinc.

Table 1.- Toxic levels of various heavy metals for algae samples from a non-resistant (Eg - Z) and a Cd-resistant (Eg - ZR) strain of *Euglena gracilis.*

Metal	mic (M)* Eg - Z	mic (M)* Eg - ZR	cc (M) Eg - Z	cc (M) Eg - ZR
Hg	5×10^{-7}		3×10^{-6}	
Ni	1×10^{-4}		1×10^{-3}	
Se	7.5×10^{-5}		1×10^{-3}	
Cu	7.5×10^{-5}		1×10^{-2}	
Co	5×10^{-4}	2×10^{-3}	3×10^{-3}	5×10^{-3}
Zn	1×10^{-2}		7.5×10^{-2}	$> 10^{-1}$

*mic, minimal inhibitory concentration and cc, critical concentration, expressed in Mole.

For both strains, the growth rate in the exponential phase and the algae concentration in the stationnary phase decrease as Co^{2+} (fig. 1) and Zn^{2+} (fig. 2) in the culture media increase ; the effect being more pronounced for the non-resistant strain. The Cd-resistant strain is not inhibited by a concentration of cobalt (10^{-3} M) which inhibited the growth of the non-resistant strain by 50 %.

A similar, but less pronounced effect, is observed for algae grown in zinc. The difference of toxicity between the strain, in zinc, is only visible for the greatest concentrations ($> 2.10^{-2}$ M). There is no difference in the growth of the two populations in mercury, nickel, selenium and copper (table 1).

Heavy metal resistance mechanisms which conferred tolerance to other metals have been described by some authors. For examples, the presence of increased tolerance to copper in a cadmium resistant population of *Pseudomonas aeruginosa* and the presence of increased tolerance to cadmium, chromium and copper in a mercury-resistant population of *Pseudomonas oleoronans* have been shown by Horitsu *et al.* (1978, 1979) ; and tolerance to zinc and cobalt has been reported for a copper-tolerant population of *Ectocarpus siliculosus* (Hall, 1980). These mechanisms can be explained by R plasmid *(Pseudomonas)* or physiological adaptation *(Ectocarpus).* In addition to antibiotic resistance, R plasmids are known to provide resistance to heavy metals (Chopra 1971, 1975, Kondo *et al.* 1974, Tynecka *et al.* 1975, Weiss *et al.* 1977, 1978) ; and multiple resistance determined by genes on bacterial plasmids has been described (Smith and Novick 1972, Meargeay *et al.* 1978 a et b).

In copper-resistant algae, the resistance mechanism is based on cellular exclusion which seems to be related to the release of organic material ; the organic material produced by the tolerant cells binds copper more readily than the material produced by the non-tolerant strain (Foster 1977, Hall *et al.* 1979). The organic material produced by the tolerant cells could detoxify cobalt and zinc externally (Hall, 1980).

In *Euglena gracilis,* the tolerance mechanism may not be explained by such a detoxification ; we showed previously that Cd-resistance is not related to a decrease of the external cadmium toxicity, but is associated with a lowered level of uptake of Cd^{2+} by the resistant cells (Bariaud, 1982).

The modification of membrane permeability to cadmium ions could also explain the increased tolerance to cobalt and zinc in the Cd-resistant cells. Common uptake mechanisms for divalent cations have been demonstrated in different organisms (Webb, 1970, Silver *et al.,* 1972, Weiss *et al.,* 1978), and it was suggested that a common uptake mechanism of Cd^{2+}, Zn^{2+} and Co^{2+} (but not of Hg^{2+}, Cu^{2+}, Se^{2+}, Ni^{2+}) may partly be responsible for the metal tolerance observed in the *Euglena* ; thus the decreased affinity of the permeases to Cd ions would reduce the uptake of Zn and Co in this cells.

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Received september 10, 1983 ; accepted october 5, 1983.