Note

Effects of Zinc on Metal Metabolism of the Zinc Tolerant Chlorotic Mutant of *Euglena gracilis* Z

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In our previous paper,¹⁾ we reported that a zinc tolerant chlorotic mutant strain of *Euglena gracilis* Z was induced when the wild strain was cultured in a zinc-enriched medium. The content of chlorophyll of the mutant strain was quite low, about 1/70 that of the wild one. On the contrary, the carotenoid content was about 1/4 that of the wild strain, therefore, the mutant strain retained a yellowish color. It could grow normally in 300 $\mu\text{g Zn/ml}$ medium and fairly well even in 700 $\mu\text{g Zn/ml}$ medium.

In this study, the effects of external zinc concentration on metal metabolism in the mutant strain were compared with that in the wild one.

The wild and mutant strains of *Euglena gracilis* Z were cultured under the growth conditions described in our previous paper.¹⁾ Cells were harvested at early stationary phase by centrifugation at 3,000 rpm for 5 min and washed with deionized water four times. After lyophilization, 20 mg of dried cells were digested with 1 ml of nitric acid and the digest was made up to 10 ml with deionized water for elemental analysis. Zinc, iron, magnesium, calcium, and phosphorus were determined with an IL PLASMA-100 inductively coupled plasma emission spectrophotometer (Instrumentation Laboratory Inc., Ltd., MA., U.S.A.) and potassium was determined with a SEIKO SAS-725 atomic absorption spectrophotometer (Seiko Instruments Inc., Tokyo, Japan) in the flame emission mode.

Figure 1 shows the effects of the external zinc concentration on the cellular zinc content of the wild and mutant strains at early stationary phase. The zinc contents of the wild and mutant cells in the basal medium were 100 and 50 $\mu\text{g Zn/g}$ dry weight, respectively. In the wild cells

the zinc content reached a maximum (about 5,000 $\mu\text{g Zn/g}$ dry weight) when cells were cultured in the medium of 50 $\mu\text{g Zn/ml}$, while it decreased to about 2,000 $\mu\text{g Zn/g}$ dry weight or below in higher external zinc concentrations. On the contrary, in the mutant cells the zinc content was maximized (about 7,500 $\mu\text{g Zn/g}$ dry weight) when cells were cultured in the medium of 200 $\mu\text{g Zn/ml}$. The higher cellular zinc content in the level of $6,000 \pm 1,000$ $\mu\text{g Zn/g}$ dry weight was retained in the mutant cells cultured in the zinc concentrations from 50 to 500 $\mu\text{g Zn/ml}$. In the medium above 700 $\mu\text{g Zn/ml}$, however, the zinc content abruptly decreased (to 500 $\mu\text{g Zn/g}$ dry weight).

Figure 2 shows the cellular magnesium and potassium contents in the wild and mutant cells. Magnesium and potassium contents in the wild cells cultured in the basal medium were about 900 and 2,400 $\mu\text{g/g}$ dry weight, respectively. Both elements in the wild cells decreased ab-

FIG. 1. Effects of External Zn Concentration on Cellular Zn Content.

○, wild strain; ●, mutant strain.

FIG. 2. Effects of External Zn Concentration on Cellular Mg and K Contents.

Wild strain: ○, Mg; △, K. Mutant strain: ●, Mg; ▲, K.

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ruptly in the medium above 100 $\mu\text{g Zn/ml}$, like the decrease of zinc content shown in Fig. 1. The contents of these elements in the mutant cells cultured in the basal medium were almost the same as those in the wild cells. With increasing external zinc concentration they decreased less steeply than those in the wild cells. More magnesium and potassium were retained in the mutant cells cultured from 50 to 500 $\mu\text{g Zn/ml}$. The external zinc concentrations cited above coincided with those in which the higher zinc content was observed as shown in Fig. 1.

The contents of iron, calcium, and phosphorus in both strains were not as greatly affected as were magnesium and potassium by the external zinc concentrations (data not shown).

Some of the known copper tolerant microorganisms retain a consistently high content of copper in a wide range of concentrations.^{2,3)} Okamoto *et al.* reported that *Penicillium ochro-chloron* maintained a constant copper content (about 1,000 $\mu\text{g Cu/g}$ dry weight) in external copper concentrations from 1,000 to 100,000 $\mu\text{g/ml}$.²⁾

Effluxes of nutritional cations such as magnesium and potassium were also observed in many microorganisms such as *P. ochro-chloron*,²⁾ *Aerobacter aerogenes*,⁴⁾ and *Escherichia coli*⁵⁾ when toxic cations were added to the medium. Silver *et al.* reported that magnesium in the manganese resistant *E. coli* was much less decreased than in the sensitive one.⁶⁾

P. ochro-chloron retained the high copper content by deposition of copper in the cell surface system.⁷⁾ Harada *et*

al. reported that zinc was accumulated in globular substances having 0.5 μm in diameter in the cytoplasm of the mutant strain but not of the wild one observed by a scanning electron microscope with an energy dispersive X-ray microanalyzer (SEM/EDX).⁸⁾

From these observations the zinc tolerance of the mutant strain could be ascribed both to trapping the element as the less toxic globular substances and to suppressing the efflux of nutritional cations.

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