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Effect of Uranium (VI) on the Growth of Yeast and Influence of Metabolism of Yeast on Adsorption of U (VI)

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We have carried out the growth experiments of 3 strains of yeast in a medium containing uranium (VI) to elucidate the effect of U (VI) on the growth of microorganisms. *Hansenula fabianii* J640 grew in the liquid medium containing 0.1 mM U (VI) at lower rate than the control, but *Saccharomyces cerevisiae* did not grow under this condition. The *H. fabianii* J640 pre-cultured for 21 h in the liquid medium without U (VI) grew even after the exposure to 1 mM U (VI), but did not grow without pre-cultivation. For the pre-cultured *H. fabianii* J640, radioactivity of U in the medium was the same as the initial one for 110 h, and then gradually decreased. TEM-EDS analysis of *H. fabianii* J640 exposed to 1 mM U (VI) for 165 h showed accumulation of U (VI) on the cells. When *H. fabianii* J640 was not pre-cultured, radioactivity of U in the medium was lower than the initial one. These results indicated that U (VI) inhibits the growth of yeast, and that the adsorption of U (VI) by the cells depends on the metabolism of yeast.

1. Introduction

The contamination of the environment by uranium from mine tailing and U mine water is major environmental concern because of α-radioactivity. It is well known that microorganism accumulates U[1–3] and microorganism plays a key role in regulating the mobility of uranium in the underground.[4–6] Many researchers, therefore, have extensively studied the interaction of microorganism with U.[7–10] They suggested that microorganism may be utilized to immobilize U from U mine tailings, U mine wastewater and radioactive waste from nuclear reactors.

Leduc et al.[7] reported that U was twelve times more toxic than copper and nickel for *Thiobacillus ferrooxidans*. Suzuki and Banfield studied the toxicity of U to microorganisms.[11] However, to our knowledge, little is known about effect of U on the growth of microorganisms.

Resting cells of the yeast *Saccharomyces cerevisiae* is known to accumulate U (VI).[9, 10] Although the yeast biomass obtained from brewery waste has been proposed as a treatment process to remove U from waste stream, the effect of U on the growth of yeast in the liquid medium is poorly understood. In this study, we investigated the effect of U (VI) on the growth of several strains of yeast.

2. Experimental

In order to examine the effect of U on growth we tested 31 strains of yeast including *Saccharomyces cerevisiae* X-2180-1B, *Hansenula fabianii* J640, and *Hansenula anomala* J224. These yeasts included 7 types of brewer's yeast for 'sake', 5 types of brewer's yeast for beer, 3 strains of brewer's yeast for wine, 4 strains of brewer's yeast for Japanese spirits, and 2 strains of baker's yeast.

The yeasts were grown in YNB medium consisted of 5.7 g of YNB without Phosphate (Qbiogene, Inc.), 20 g of glucose and 100 mg of β-glycerophosphate per liter. Solid media contained 2% Bacto-agar (BD Biosciences). $UO_2(NO_3)_2$ was dissolved in deionized water and the concentration was adjusted at 100 mM.

The yeasts were cultured on YNB agar media containing 0.1 mM or 1.0 mM U (VI) for 72 h at 30 ˚C. For further investigation, *S. cerevisiae* X-2180-1B and *H. fabianii* J640 were cultured in 100 mL of YNB liquid media containing 0 (control), 0.1, or 1.0 mM U (VI) up to 165 h at 30 ˚C. The initial amount of yeast in the media was adjusted to 0.1 at the optical density at 600 nm (OD_{600}).

In order to examine the effect of pre-culture of yeast, *H. fabianii* J640 was cultured in YNB liquid for 21 h at 30 ˚C following the exposure to U (VI). The growth of the yeast in the liquid media was estimated by measuring OD_{600} at 21, 48, 70, 110, 140, and 168 h after the inoculation. U concentrations in the media were measured by radiometry using liquid scintillation counter at 21, 48, 70, 110, 140, and 165 h. The cells of *H. fabianii* J640 exposed to 1 mM U (VI) were analyzed by transmission electron microscopy (TEM) (Hitachi HF-2000) operating at 200 kV. Elemental analysis was carried out by EDS attached to the TEM using a Kevex Sigma system software package. A small amount of sample of the wet precipitates was dropped on a copper grid sample folder for TEM observation. The sample was dried for 24 h in a desiccator.

3. Results and Discussion

H. fabianii J640 and *H. anomala* J224 showed a high tolerance for U (VI) among yeast strains tested. They grew on YNB agar medium containing 1 mM U (VI), while *S. cerevisiae* X-

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2180-1B did not.

Figures 1 and 2 show the growth curves of *H. fabianii* J640 and *S. cerevisiae* X-2180-1B, respectively, cultured in YNB liquid media containing 0.1 mM and 1 mM U (VI). *H. fabianii* J640 grew in the liquid media containing 0.1 mM U (VI), while *S. cerevisiae* X-2180-1B did not. The OD_{600} of *H. fabianii* J640 grown in the liquid media containing 0.1 mM U (VI) was lower than that of control. The OD_{600} reached a steady value of approximately 5 at 48 h after inoculation in the medium containing 0.1 mM U (VI). In contrast, the OD_{600} of control sample increased with time up to 164 h. These results indicated that U (VI) inhibited the growth of *H. fabianii* J640. Suzuki and Banfield[11] reported that the radioactivity of U was not lethal to microorganisms, but the chemical properties of U caused significant toxic effects.

Neither *H. fabianii* J640 nor *S. cerevisiae* X-2180-1B grew in the liquid medium containing 1 mM U (VI). On the other hand, *H. fabianii* J640 grew up to about 5 of OD_{600} (the upper panel of Figure 3) in the medium containing 1 mM U (VI) after the pre-cultivation in the liquid media without U (VI) for 21 h. *H. fabianii* J640 grew to 2 of OD_{600} for 21 h. Addition of 1 mM U (VI) to the culture at 21 h resulted in an increase in OD_{600} to 5 at 50 h. However, no significant increase in OD_{600} was observed for the culture after 50 h in comparison to the control sample without U (VI) exposure.

Radioactivity of U in the liquid media containing 1 mM U (VI) exposed to *H. fabianii* J640 was approximately constant at 250 cpm (the lower panel of Figure 3). This value was lower by about 0.7 than that of the radioactivity estimated by the initial concentration of U (VI) added. This suggests that some fractions of U (VI) were sorbed by the cells of *H. fabianii* J640. The inhibition of the growth of *H. fabianii* J640 is probably caused by the sorption of U (VI).

On the other hand, the radioactivity in the medium for the pre-cultured sample was approximately 350 cpm and it remained constant up to 72 h, whereas the OD_{600} was reached to its maximum from 2 to 5. The radioactivity then decreased with time (Figure 3). TEM image of whole mounts of *H. fabianii* J640 cells at 164 h (Figure 4) showed clear contrast without further staining, suggesting that U (VI) was accumulated by the J640 cells. Santamaria et al.[12] pointed out that the effect Th on *Bradyrhizobium* growth was reduced by the

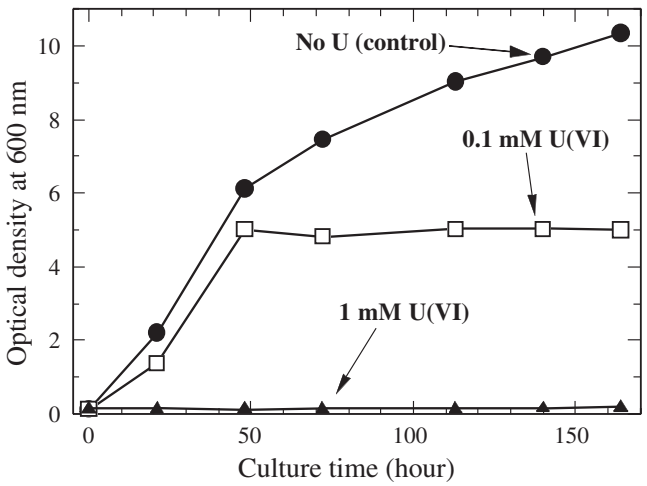

Figure 1. Growth of *H. fabianii* J640 in YNB liquid medium containing 0.1 mM or 1 mM U (VI).

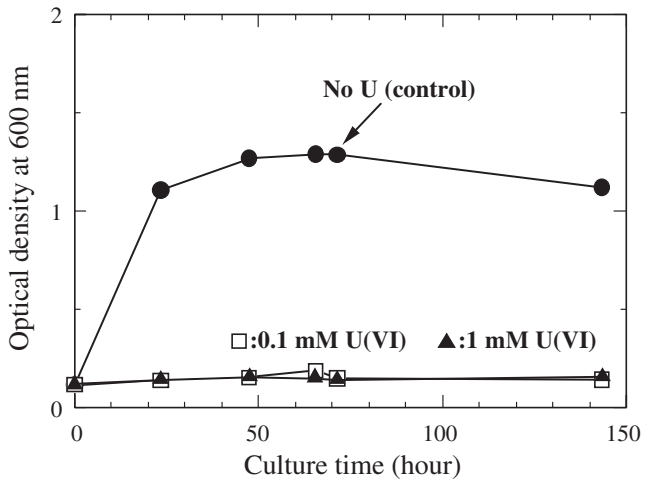

Figure 2. Growth of *S. cerevisiae* X-2180-1B in YNB liquid medium containing 0.1 mM or 1 mM U (VI).

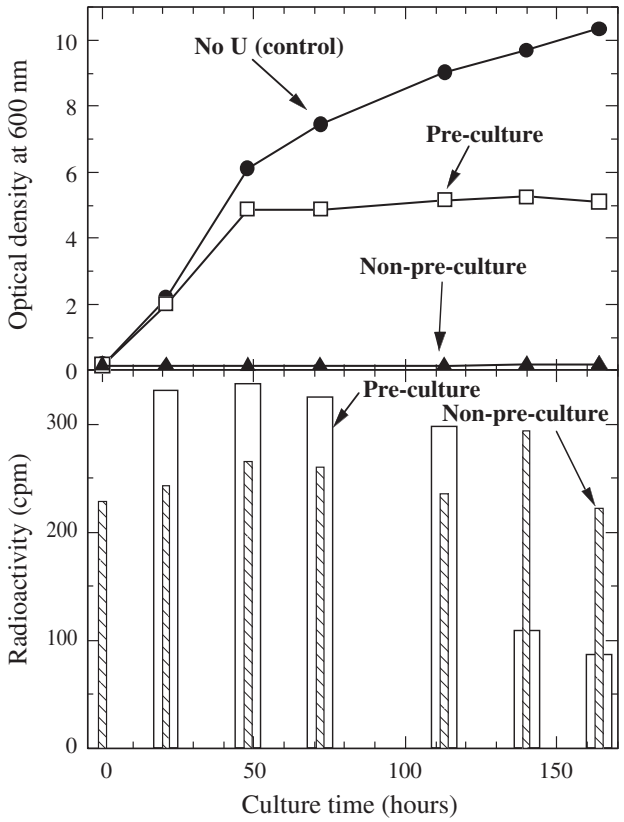

Figure 3. Growth of *H. fabianii* J640 in YNB liquid medium containing 1 mM U (VI) (upper) and radioactivity of U (VI) in the liquid medium (lower).

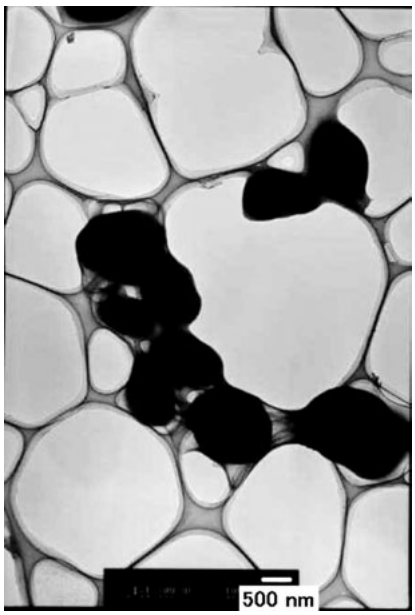

Figure 4. TEM image of 140-h cells of *H. fabianii* J640 pre-cultured for 21 h followed by incubation with 1 mM U (VI).

precipitation of Th. We assume that the accumulation of U (VI) by yeast cells is a different phenomenon from the Th precipitation observed in *Bradyrhizobium*.

Volesky et al.[9] and Strandberg et al.[10] reported the resting cells of *S. cerevisiae* adsorbed U for several hours of incubation. These results are in agreement with the sorption of U (VI) by *H. fabianii* J640 without pre-culture. However, when *H. fabianii* J640 was pre-cultured, the adsorption was not observed up to 72 h. This suggests that the accumulation behavior of U (VI) under the pre-culture condition is different from the resting conditions. Francis et al.[13] indicated that carbonate produced by the metabolism of bacteria dissolved U (VI)-phosphate precipitates. At 21 h, the carbonate concentration should be high in the pre-culture due the growth of *H. fabianii* J640, even though the carbonate concentration was not measured. U (VI)-carbonate complex has low sorption ability on minerals and microorganisms. Thus, the effect of U (VI) on the growth of *H. fabianii* J640 may be masked by carbonate produced at the initial stage of the exposure to U (VI).

However, at the late stage, where OD_{600} was saturated at approximately 5 (Figure 3), metabolic activity of *H. fabianii* J640 probably lower than the initial stage, and the decreased concentration of carbonate resulted in the sorption of U (VI) on the cell surfaces of *H. fabianii* J640.

4. Conclusions

The yeasts *H. fabianii* J640 and *H. anomala* J224 grew in YNB agar medium containing 1 mM U (VI), though *S. cerevisiae* X-2180-1B did not. *H. fabianii* J640 grew in YNB liquid medium containing 0.1 mM U (VI), though *S. cerevisiae* X-2180-1B did not. This indicates that *H. fabianii* J640 has higher tolerance to U (VI) than *S. cerevisiae* X-2180-1B. Neither *H. fabianii* J640 nor *S. cerevisiae* X-2180-1B grew in the liquid media containing 1 mM U (VI). However, after pre-cultivation, *H. fabianii* J640 grew up to about 5 of OD_{600} in the liquid media even containing 1 mM U (VI). According to TEM observation, U was present on the cells of the yeast. These results suggest that the metabolic products of the yeast affect the adsorption of U to the cells.

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