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Protein Expression of *Saccharomyces cerevisiae* in Response to Uranium Exposure

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Protein expression of *Saccharomyces cerevisiae* grown in the medium containing ^{238}U(VI) and ^{233}U(VI) was examined by two-dimensional gel electrophoresis. *Saccharomyces cerevisiae* of BY4743 was grown in yeast nitrogen base medium containing glucose and glycerol 2-phosphate and ^{238}U of 0, 2.0, and 5.0×10^{-4} M or ^{233}U of 2.5×10^{-6} M (radioactivity was higher by 350 times than 2.0×10^{-4} M ^{238}U) and 5.0×10^{-6} M for 112 h at 30 °C. The growth of *Saccharomyces cerevisiae* was monitored by measuring OD_{600} at 112 h after the inoculation. Uranium concentrations in the media also were measured by radiometry using a liquid scintillation counter. The growths of the yeast grown in the above media were in the following order: control > 2.5×10^{-6} M ^{233}U > 2.0×10^{-4} M ^{238}U > 5.0×10^{-6} M ^{233}U > 5.0×10^{-4} M ^{238}U. This result indicated that not only radiological but also chemical effect of U reduced the growth of the yeast. The concentrations of U in the medium containing ^{238}U or ^{233}U decreased, suggesting U accumulation by the yeast cells. The 2-D gel electrophoresis analysis showed the appearance of several spots after exposure to ^{238}U or to ^{233}U but not in the control containing no uranium. These results show that the yeast cells exposed to U express several specific proteins.

1. Introduction

Yeast is known to accumulate U(VI).[1,2] This suggests that yeast may be utilized as bisorbents for removing U from U mine tailings wastewater and from radioactive waste generated at nuclear facilities. Since U is toxic for microorganisms,[3,4] yeast may be affected by exposure to U solution. Previously, we showed that U(VI) retarded the growth of yeast in the medium containing uranium(VI).[5] These results suggest that the exposure of yeast cells to U(VI) solution lead to a response by change of metabolic pathways. However, to our knowledge, little is known about response of yeast to U(VI) solution.

The use of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) have provided powerful tools for studying protein expression during exposure of yeast cells to U(VI) solution. Two-D PAGE separates and allows visualization of most proteins.[6]

In the present study, therefore, we examined the expression of proteins in yeast exposed to U(VI). Protein expression was studied by 2-D gel electrophoresis, and expression pattern of proteins was compared between U(VI)-exposed and non-exposed yeasts.

2. Materials and Methods

2.1. Strain and culture conditions. The yeast strain *Saccharomyces cerevisiae* (*S. cerevisiae*) BY4743 (Funakoshi, Inc.) was grown in yeast nitrogen base (YNB) (with ammonium sulfate, without sulfates, without sodium chloride; Q-BIOgene, Inc.) medium. The YNB medium was prepared as follows: 5.7 g of YNB, 20 g of glucose, 100 mg of glycerol 2-phosphate disodium salt hydrate, 35 mg of DL-histidine, 30 mg of DL-lysine, 25 mg of uracil and 1 g of casamino acids. The mixture was adjusted to 1 L with deionized water, and it was autoclaved at 121 °C for 20 minutes. Uranium nitrate was dissolved in deionized water and the concentration was adjusted at 1.0×10^{-1} M. We used ^{233}U and ^{238}U. Uranium-238 and U-233 emit α particles of 4.20 MeV (77%) and 4.15 MeV (23%) for ^{238}U and 4.82 MeV (84%), 4.78 MeV (13.2%), and 4.73 MeV (1.6%) for ^{233}U. Half-lives of ^{238}U and ^{233}U are 4.5×10^{9} y and 1.6×10^{5} y, respectively. The growth of the yeast was stopped about 100 h under the culture condition. The effect of U for the yeast was not observed when the concentration of U(VI) was 1×10^{-4} M and the yeast did not grow when the concentration of U(VI) was 8×10^{-4} M under the culture condition. Thus the yeast was cultured in the YNB media containing 2×10^{-4} and 5×10^{-4} M U(VI) for 112 h at 30 °C. The initial amount of yeast in the media was adjusted to 0.2 at the optical density at 600 nm (OD_{600}).

The amount of the yeast in the liquid media was estimated by measuring OD_{600} at 112 h after the inoculation. When OD_{600} of the media was beyond 2, it was diluted to 10 times with YNB liquid media. The U concentrations in the media were measured by radiometry using a liquid scintillation counter at 112 h.

To compare proteins expressed by the cells exposed to U(VI) with that of control, yeast was cultured in the U(VI) free medium for 112 h at 30 °C. The effect of α radiation on protein expression by the yeast was examined using the medium containing 1.7×10^{-10} and 3.4×10^{-10} M of ^{233}U but not ^{238}U. Radioactivity of ^{233}U in the medium was higher by 350 times than that of the ^{238}U(VI) containing medium.

2.2. Protein extraction. "ReadyPrep Sequential Extraction Kit" (Bio Rad, Inc.) was used for extraction of the yeast proteins. The yeast cells exposed to ^{238}U of 2×10^{-4} M, ^{233}U of 2.5×10^{-6} M, and U free media for 112 h were centrifuged 6000 × g for 5 min. After removal of the supernatant, 20 mM Tris-HCl (pH 7.4) was added to the cell pellet. The cell pellet was resuspended, centrifuged again and the supernatant was discarded. The wet weight of the precipitation was measured. Five hundreds milliliters of Reagent 1 (including the Kit) and 5

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μL of protease inhibitor cocktail were added to about 100 mg (wet weight) of the cells. The cells were lysed by the crushing machine, "FastPrep FP 120" (Q-Biogene, Inc.). The supernatant was recovered, and its protein content was determined by using the protein assay (Bio Rad, Inc.).

One hundredth of tributylphosphine was added to the Reagent 2 (included in the extraction kit (Bio Rad, Inc.)). About 5 μg of protein solution was adjusted at 125 μL by adding the Reagent 2 mixture. The solution was loaded by rehydration onto a 7-cm-long, pH 3 to 10 or 5 to 8 immobilized pH gradient (IPG) strip (Bio Rad, Inc.) for 15 h at room temperature. Isoelectric focusing was carried out with PROTEAN IEF (Bio Rad, Inc.) for 15 min at 250 V (linear), 1 h at 8000 V (linear) and 1 h and 15 min at 8000 V (rapid) (total about 7500–9500 Vh). After focusing, the gel was reduced for 20 min at room temperature in equilibration buffer 1 (50 mM Tris-HCl (pH 7.4), 6 M urea, 30% glycerol, 2% sodium dodecyl sulfate (SDS), and 10 mg/mL dithiothreitol). After that, the gel was alkylated for 20 min at room temperature in equilibration buffer 2 (50 mM Tris-HCl (pH 7.4), 6 M urea, 30% glycerol, 2% SDS, and 25 mg/mL iodoacetamide). The gel strips was transferred onto a 10–20% ReadyGel (Bio Rad, Inc.), and the second-dimension separation was carried out on the mini PROTEAN system (Bio Rad, Inc.) at constant current (40 mA/gel). The gel was fixed and stained with the silver stain kit (Amersham Pharmacia, Inc.).

3. Results and Discussion

The growth of yeast in YNB medium containing no U (control) and with U of 2.0×10^{-4} M ^{238}U, 5.0×10^{-4} M ^{238}U, 2.5×10^{-6} M ^{233}U, and 5.0×10^{-6} M ^{233}U for 112 h are shown in Table 1. Also shown in the table is the removal of U from solution in the presence of cells. The growth of the yeast was affected by the presence of U at concentrations 2.0×10^{-4} M ^{238}U and 5.0×10^{-4} M ^{238}U. Extents of the growths, however, were smaller than that grown in U free medium by 1.3 and 8, respectively. It is indicated that the U concentration higher than 2.0×10^{-4} M affected the growth of yeast. In the case of ^{233}U, the OD_{600} of yeast grown in the medium containing 2.5×10^{-6} M ^{233}U was slightly lower than U free medium and the OD_{600} of yeast grown in the medium containing 5.0×10^{-6} M ^{233}U was lower by 1.4 than that in U free medium. It is indicated that higher α-radioactivity of U affected more the growth of yeast. These results strongly suggest that inhibition of growth was due to both chemical and radiological effects.

More than 80% of U(VI) from solution were removed after exposure of cells to the media containing U of 2.0×10^{-4} M ^{238}U, 5.0×10^{-4} M ^{238}U, 2.5×10^{-6} M ^{233}U, and 5.0×10^{-6} M ^{233}U, indicating that most of the U in the media was accumulated by the cells. Soares et al.[7] reported that *S. cerevisiae* NCNY 1190 releases P when it is exposed to Cd, Cu, and Pb. This release is attributed to U- or heavy-metal toxicity.[7] Chemical toxicity of U to bacteria was also suggested by Suzuki and Banfield.[4] Our results showed the effect of U on the cell growth depended on the concentration of U. The fraction of U accumulated on the cells was the same in the ^{238}U concentrations of 2.0×10^{-4} M and 5.0×10^{-4} M.

Silver stained 2-D gel electrophoresis patterns of proteins from cell lysate of the yeast grown in 2.0×10^{-4} M ^{233}U are shown in Figure 1. Proteins extracted by the Reagent 1 were isoelectrically focused on the IPG gel of pH range from 3 to 10

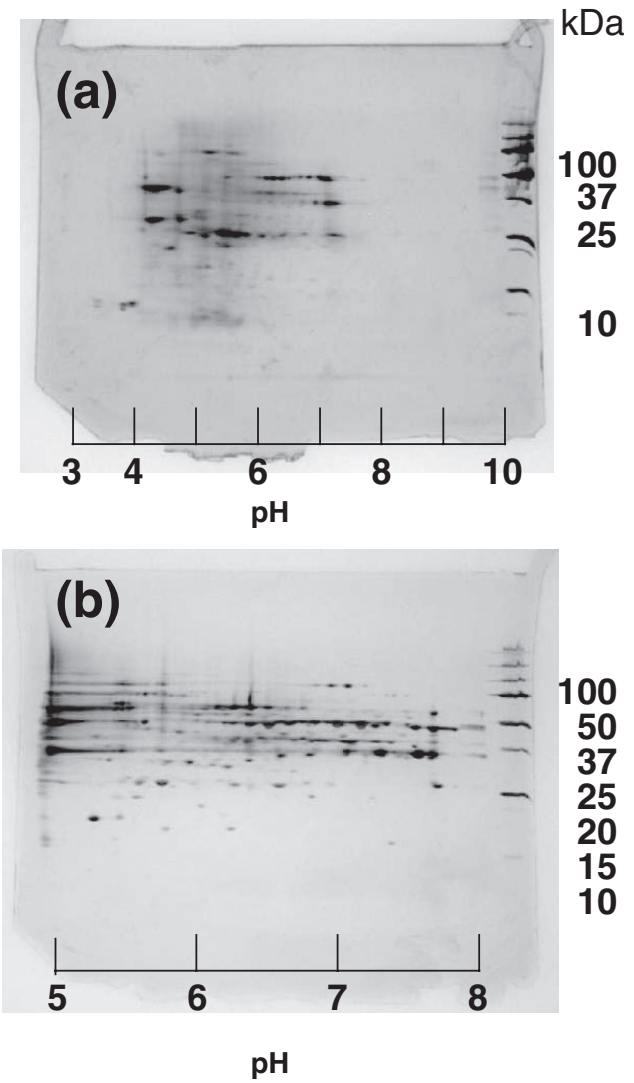

Figure 1. Silver stained 2-D gel electrophoresis patterns of proteins from cell lysate of the yeast grown in 2.0×10^{-4} M U containing medium. Proteins extracted by the Reagent 1 were isoelectrically focused (a) on the IPG gel of pH range from 3 to 10, and (b) on the IPG gel of pH range from 5 to 8.

TABLE 1: The optical densities at 600 nm of yeast and fraction of U eliminated from medium at 112 h after the exposure to the medium containing no U, 2.0×10^{-4} M ^{238}U, 5.0×10^{-4} M ^{238}U, 2.5×10^{-6} M ^{233}U, and 5.0×10^{-6} M ^{233}U

U concentration in the media	Initial optical density of yeast	Optical density of yeast at 112 h after exposure	Fraction of U eliminated from medium at 112 h after exposure
no U (control)	0.2	2.79	
2.0×10^{-4} M ^{238}U	0.2	2.24	91.1
5.0×10^{-4} M ^{238}U	0.2	0.353	84.3
2.5×10^{-6} M ^{233}U	0.2	2.52	84.2
5.0×10^{-6} M ^{233}U	0.2	1.92	80.1

α-radioactivities in the medium are also shown. Initial optical density of yeast is adjusted to 0.2 at initial.

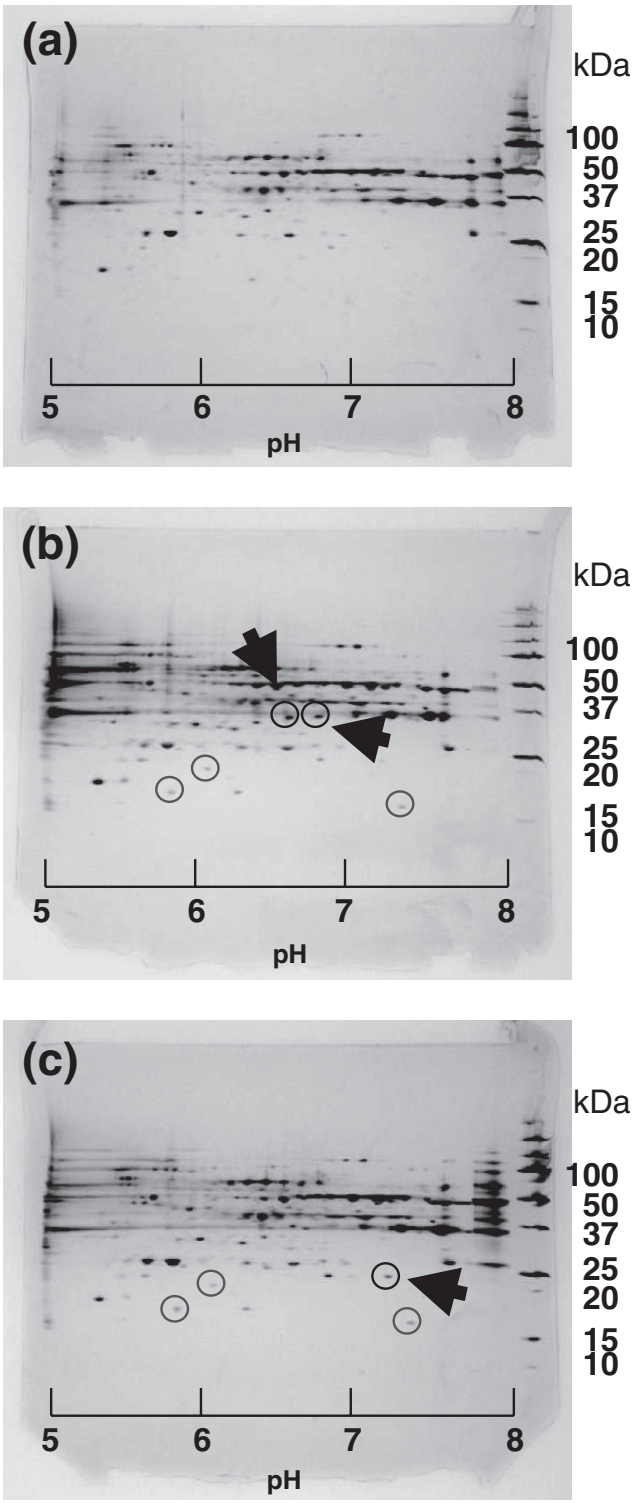

Figure 2. Silver stained 2-D gel electrophoresis patterns of proteins from the yeast grown in (a) U free medium, (b) ^{238}U, or (c) ^{233}U containing medium.

(Figure 1(a)). Many proteins were observed at nearly pH 6. This indicated that the IPG gel of pH range from 5 to 8 gave more appropriate pattern for the protein expression analysis of the yeast.

Silver stained 2-D gel electrophoresis patterns of proteins from the yeast grown in the medium containing ^{238}U or ^{233}U are shown in Figure 2, along with that of U free medium. In these, the protein patterns depended on the culture conditions. The circled protein spots in these patterns (Figure 2) showed the spots appeared after exposure to ^{238}U or ^{233}U, but not in control. The protein spots corresponding to 2.0×10^{-4} M ^{238}U appeared at pH 5.9 and 19 kDa, pH 6.1 and 21 kDa, pH 6.8 and 35 kDa, pH 6.9 and 35 kDa, and pH 7.4 and 16 kDa. The protein spots corresponding to 5.0×10^{-6} M ^{233}U appeared at pH 5.9 and 19 kDa, pH 6.1 and 21 kDa, pH 7.2 and 20 kDa, and pH 7.4 and 16 kDa.

TABLE 2: Isoelectric points (pH) and sizes of expressed proteins of yeast cultured in the medium containing ^{238}U or ^{233}U

U isotope and its concentration in the media	Character of expressed proteins	
	Isoelectric points / pH	Size / kDa
^{238}U 2.0×10^{-4} M	5.9	19
	6.1	21
	6.8	35
	6.9	35
	7.4	16
^{233}U 5.0×10^{-6} M	5.9	19
	6.1	21
	7.2	20
	7.4	16

It is noted that values of pH and protein size obtained were not absolute ones.

Comparison of the protein spots corresponding to 2.0×10^{-4} M ^{238}U (Figure 2(b)) with those corresponding to 5.0×10^{-6} M ^{233}U (Figure 2(c)) showed that the proteins at pH 6.8 and 35 kDa, and pH 6.9 and 35 kDa (shown by arrow head in Figure 2(b)) were only expressed by ^{238}U (Table 2). This result indicated that the proteins at pH 6.8 and 35 kDa, and pH 6.9 and 35 kDa may be originated by response to chemical or radiological effect of U. Similarly, the protein at pH 7.2 and 20 kDa (shown by arrow head in Figure 2(c)) was only expressed by exposure to ^{233}U, indicating that the protein may be originated by the response to α-irradiation. The proteins at pH 5.9 and 19 kDa, pH 6.1 and 21 kDa, and pH 7.4 and 16 kDa were expressed in both conditions, indicating that the proteins were not specific proteins by U and α-irradiation. Additional studies are underway to distinguish the radiological effect on expression of specific proteins.

In this study, the proteins corresponding to exposure to ^{238}U or ^{233}U was not characterized. Characterization of proteins by MALDI-TOF mass spectroscopy is planned.

4. Conclusion

Initial studies of protein expression of yeast exposed to U was examined by two-dimensional (2-D) gel electrophoresis. We confirmed that almost all proteins were observed at nearly pH 6. The 2-D gel electrophoresis patterns of the yeast proteins after exposure to ^{238}U and ^{233}U showed that proteins at pH 6.8 and 35 kDa, and pH 6.9 and 35 kDa were expressed in cells exposed to ^{238}U, but not to ^{233}U. Protein at pH 7.2 and 20 kDa was expressed in cells exposed to ^{233}U, but not to ^{238}U. Further studies are underway to determine the effect of α-irradiation on the expression of proteins in yeast.

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