Aluminum is the most abundant metal in the Earth’s crust, making up 8.2% of the total mass of the crust (Yamamoto et al., 2018). It is mostly present in metal form, which is sparingly soluble at neutral pH conditions in the soil. Decreases in soil pH promote the release of Al ions. Although the insoluble form of Al is not significantly toxic, the soluble ions are toxic to a variety of organisms, including animals, vascular plants, and microbes (Yamamoto et al., 2018). However, the mechanism of Al cytotoxicity is still poorly understood. Due to its toxicity to bacteria, Al-containing compounds like alum are used as additives for food preservation. However, due to its potential detrimental effect on the developing nervous system and the reproductive system in experimental animals, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) advises a very low, tolerable weekly intake of 1 mg/kg body weight (Chemical Hazard Evaluation, 2009).

Extraction of Al from foods treated with Al-containing food additives would thus be beneficial, with emphasis placed on food safety in the process. Recently, microbial biosorption has been harnessed for extracting heavy metals from water. For example, aluminum biosorption from wastewater based on the adsorbability of bacterial surfaces has been reported in aluminum-tolerant Pseudomonas putida (Boeris et al., 2016). Other aluminum-tolerant bacteria, including Klebsiella sp., Enterobacter sp., and Serratia sp., have also been used to improve the growth of ryegrass on volcanic soil land (Mora et al., 2017). However, pharmacological safety is not compromised when bacteria are used in food processing. Brewer’s yeast, Saccharomyces cerevisiae, has the following advantages in biosorption: S. cerevisiae is generally regarded as safe along with its by-products, e.g., alcohol fermentation, and the resulting biosorbents are commercially utilized, especially in food processing (Do et al., 2016; Wang and Chen, 2006). Al-absorbing yeast strains have similar advantages to S. cerevisiae regarding the exclusion of metals from food items because numerous yeast species, excluding a few pathogenic species, have similar characteristics to S. cerevisiae. Moreover, large scale culture of yeast is easy and biomass is easily recovered through filtration or sedimentation. Furthermore, the absorption rate of yeast cells is greater than that of bacterial cells, thus facilitating studies on Mn absorption from contaminated solutions (Do et al., 2016). Yeast cells are easy to recover and have high absorbability, therefore, it is easy to separate Al-absorbing yeast cells from solubilized food or beverages after food processing. Herein, we consider the use
of yeast cells for extracting Al during food processing.

Some toxic metals (like copper and cadmium) have been reported to be absorbed by yeast strains that are tolerant to the toxic metals (Ma et al., 2015, Radić et al., 2017). Therefore, the isolation of an Al-tolerant yeast strain would enable its use for absorbing and extracting Al from processed foods. In addition, the proposed Al-absorbing strain might be able to recover Al from industrial goods and machines. Rhodotorula sp. is a highly Al-tolerant yeast with high surface adsorbability (Hu et al., 2016). However, it is better to use uncolored (near white) strains of Al-absorbing yeast than red strains in solubilized foods or beverages, because staining materials from colored yeast potentially decrease food quality. We thus screened for more Al-tolerant yeast strains used in the food industry, since Al is toxic to S. cerevisiae. This study describes the isolation of an Al-tolerant and Al-absorbing yeast strain.

The Escherichia coli strain DH5α was used for transformation. Saccharomyces cerevisiae BY4741 was used as the standard yeast strain. Two identified Al-tolerant strains, Alt-OF2 and Alt-OF5 (identified as Schizoblastosporion sp.), were used as Al-absorbing strains. Glucose–yeast–peptone (GYP) medium (2.0% glucose, 1.0% peptone, and 0.5% yeast extract) was used for the culture, with 50 mg/mL ampicillin, if required. For solid media, 2.0% agar was added. Various concentrations of aluminum chloride (AlCl₃) were added into the GYP medium to assess growth-inhibition.

The sensitivity of the yeast to AlCl₃ was determined using Saccharomyces cerevisiae BY4741. The yeast, precultured in GYP at 30 °C for 24 h, was inoculated into GYP with 0, 0.5, 1.0, 5.0, and 10.0 mM AlCl₃. After incubation at 30 °C for 72 h, the cell suspensions were diluted and spread onto GYP plates. The AlCl₃ concentration at which S. cerevisiae BY4741 could not grow was used as the cytotoxic concentration.

Samples of soil from vegetable farms in Takarazuka City were screened for yeasts. The samples were suspended in 1 mL of sterile distilled water, and the diluted suspensions were spread on ampicillin-containing GYP plates, with 5 mM AlCl₃ added to achieve the cytotoxic conditions. The microbial colonies growing after incubation at 30 °C for 72 h were examined by microscopy and selected yeast colonies. Only yeast colonies were picked-up and inoculated into GYP with 5 mM AlCl₃ and cultured with shaking at 30 °C for 72 h. The strains that grew were isolated as Al-tolerant yeast strains. Yeast growth was assessed in terms of colony forming units (CFU) determined through culturing. All samples were analyzed at least in triplicate, and data are reported as mean ± standard deviation (SD) values.

The Al-tolerant yeast strains were identified by the analysis of the D1/D2 regions of the large subunit rDNA (Kurtzman and Robnett, 1998). Yeast DNA was extracted from about 2.0 × 10⁶ cells by the fast small-scale isolation protocol (Wach et al., 1994). Yeast cell numbers were counted using hemocytometer. The D1/D2 regions were amplified by polymerase chain reaction (PCR) using Thermal Cycler S1000 (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer’s instruction. The primer sets NL-1 (5’-GCATATCAATAAGCGGAGGAAAAG-3’) and NL-4 (5’-GGTCCGTGTTTCAAGACGG-3’) were used. The PCR consisted of 30 cycles, with DNA denaturation at 94 °C for 1 min, primer annealing at 50 °C for 30 s, and DNA chain extension at 72 °C for 2 min. The PCR-amplified D1/D2 regions were cloned into the pGEM-T Easy Vector (Promega, Madison, WI) and transformed into DH5α. Plasmids were recovered using Monarch Plasmid Miniprep kit (New England Biolabs, Ipswich, MA). The D1/D2 regions were sequenced by the commercial analysis provided by Eurofins Genomics Japan (Tokyo, Japan) and analyzed using the DNA Data Bank of Japan in the National Institute of Genetics (Shizuoka, Japan).

Absorption of Al by yeast cells was evaluated by culturing yeast cells in medium containing 5 mM AlCl₃ for 24 h at 30 °C, with shaking. Subsequently, approximately 5.0 × 10⁷ yeast cells were collected by centrifugation at 5,000 g for 5 min at 4 °C. Whole cells were washed twice by ultrapure water, and the concentration of Al absorbed on yeast cells was measured using an inductivity-coupled plasma optical emission spectrometer (ICP-OES) VISTA-MPX (Hitachi High-Technologies, Tokyo, Japan). The samples subjected to ICP-OES were prepared as follows. Yeast cells were collected and digested by 6 M pure nitric acid for about 1 h at 90 °C. Following dilution using distilled ultrapure water, Al concentration was determined with reference to the appropriate standard Al solution. All samples were analyzed at least in triplicate, and data were reported as

| AlCl₃ concentration (mM) | CFU a |
|-------------------------|-------|
| 0                      | 1.25 ± 0.83 × 10⁷ |
| 0.1                    | 1.12 ± 0.54 × 10⁷ |
| 0.5                    | 8.22 ± 0.28 × 10⁶ |
| 1.0                    | 4.02 ± 0.73 × 10⁶ |
| 5.0                    | N.D. b |
| 10.0                   | N.D. b |

a) Values are mean ± SDs of at least three independent assays.
b) N. D., not detected.
The growth of *S. cerevisiae* BY4741 in GYP medium with various concentrations of AlCl₃ was assessed (Table 1). The yeast could survive concentrations of less than 1 mM of AlCl₃. Its viability gradually decreased with increasing AlCl₃ concentration, and no growth was observed at concentrations above 5 mM AlCl₃. These results suggest that concentrations of above than 5 mM AlCl₃ are toxic to *S. cerevisiae* BY4741.

Two hundred microbial colonies that grew on GYP plates with 5 mM AlCl₃, incubated at 30 °C for 72 h after spreading the soil-suspensions, were inspected by microscopy. Of these, 44 colonies were selected and replica-plated on YPD and incubated at 30 °C for 24 h. All strains were re-inoculated into YPD liquid medium with 5 mM AlCl₃, and all 44 strains (thus referred to as Al-tolerant strains) grew in the AlCl₃-containing medium in which *S. cerevisiae* BY4741 could not grow (Table 2).

Absorption of Al by those isolated strains was assayed: the intracellular concentration of Al in those strains is shown in Table 2. The Al concentration of the Al-treated cells was not detectable in *S. cerevisiae* BY4741 because it could not grow in AlCl₃-containing culture. On the other hand, absorption of Al was observed in the Al-tolerant strains. Two strains (no. 2 and no. 5), isolated from the same soil specimen, displayed higher Al absorption (2.08-2.49 and 2.10-2.52 pg/cell, respectively) than the other strains not over 2 pg/cell, when whole cells were cultured in Al-containing medium and subsequently harvested. The absorption level (more than 2 pg/cell) of no. 2 and no. 5 strains may be the similar level advanced in biosorption of metals. However, they cannot compare simply because the various metal absorption values are used. The growth levels of no. 2 and no. 5 in the Al-containing culture were similar to the growth of BY4741 in the

| Strain no. | Growth⁴ | Al concentration⁵ | Strain no. | Growth⁴ | Al concentration⁵ |
|------------|---------|-------------------|------------|---------|-------------------|
| 1          | +       | ±                 | 23         | +       | ±                 |
| 2          | ++      | +                 | 24         | +       | +                 |
| 3          | +       | +                 | 25         | +       | ±                 |
| 4          | +       | ±                 | 26         | +       | +                 |
| 5          | ++      | +                 | 27         | +       | +                 |
| 6          | +       | +                 | 28         | ±       | +                 |
| 7          | +       | +                 | 29         | +       | +                 |
| 8          | +       | +                 | 30         | +       | +                 |
| 9          | +       | +                 | 31         | +       | +                 |
| 10         | ++      | +                 | 32         | +       | +                 |
| 11         | ++      | +                 | 33         | +       | +                 |
| 12         | +       | +                 | 34         | +       | +                 |
| 13         | +       | +                 | 35         | ±       | ±                 |
| 14         | +       | +                 | 36         | ±       | ±                 |
| 15         | +       | +                 | 37         | +       | +                 |
| 16         | +       | +                 | 38         | +       | ±                 |
| 17         | ±       | ±                 | 39         | ±       | ±                 |
| 18         | +       | +                 | 40         | +       | +                 |
| 19         | +       | +                 | 41         | +       | ±                 |
| 20         | +       | +                 | 42         | +       | +                 |
| 21         | +       | +                 | 43         | +       | ±                 |
| 22         | +       | +                 | 44         | +       | +                 |

| BY4741     | –       | N.D.⁶        |

⁴OD₆₀₀ of 1/10 diluted culture was < 0.1 (−), 0.1–0.5 (±), 0.5–1.5 (+), or > 1.5 (++)。

⁵Absorbing Al concentration (pg/cell) was < 1.0 (±), 1.0–2.0 (+), or > 2.0 (++)。

⁶N.D.; Not detected.
Al-free culture.

These two strains were thus selected as AlCl₃-tolerant yeast strains with higher Al absorbability and named Alt-OF2 and Alt-OF5, respectively, even though intracellular location of Al absorption in these strains remains unknown. Our preliminary experiments indicate the possibility that the Al concentration at the cell wall is lower than that of the total Al concentration, using the method of Hu et al. (2016).

The nucleotide sequences of the D1/D2 region of the Alt-OF2 and Alt-OF5 strains were analyzed: the sequences of both strains were identical and showed 99.6% similarity to the yeast strains Schizoblastosporion starkeyi-henricii VKM Y-2535 (accession number JF501534) and S. starkeyi-henricii CBS:2159 (accession number KY109592). These results indicate that both Al-tolerant yeast strains belonged to genus Schizoblastosporion (syn. Nadsonia), not to report the pharmacological properties.

We successfully isolated two Al-tolerant and Al-absorbing yeast strains. These may probably be used to extract Al ions from processed food items if they are regarded as safe. However, they can also be used to recover Al from industrial waste. S. cerevisiae is an ideal model organism (Perego and Howell, 1997) to study the mechanism underlying biosorption for the elimination of metal ions, especially in studies on metal–microbe interactions at the molecular level. Some Al transport-related genes were identified upon screening all yeast deletion mutants displaying an Al-tolerant phenotype (Tun et al., 2014). The yeast strains isolated in this study harbor an Al-transporter gene owing to the low probability of Al accumulation in cell wall. However, Al-absorbing strains do not die upon intracellular Al accumulation. In contrast, Al cytotoxicity is not observed in S. cerevisiae strains with deletions in Al transport-related genes (Tun et al., 2014), indicating the possibility that the isolated Al-absorbing yeast strains have an unknown Al accumulation system.

Preliminary assays of the Al-absorbing strains with regards to the absorption of other metals revealed the absorption of dysprosium, a rare trivalent earth metal ion similar to Al. However, it remains unknown whether transporter-related proteins can act on trivalent ions other than Al. Future studies should focus on identifying the transporter responsible for importing the trivalent Al ions into the cells. The isolated yeast strains have not yet been characterized, and this may present restrictions in their application; nonetheless, we expect the strains will be applicable in biorecycling of valuable metals like Al.

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