Chapter 26
Dysprosium Biomineralization by Penidiella sp. Strain T9

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Abstract Biomineralization approaches have gained significant attention as a means to recover rare earth elements from acidic mine drainage and industrial liquid wastes. We isolated an acidophilic fungus, Penidiella sp. strain T9, that accumulates dysprosium (Dy) from acidic model drainage during growth. To develop the application of biomineralization by the strain T9, we elucidated the localization and the chemical structure of biomineralized Dy and performed to establish the labo-scale bioprocess for selective recovery of Dy. High-magnification scanning electron microscopic analysis showed that the strain T9 formed a mineralized Dy (T9-Dy) layer with 1.0 μm thickness over the cell surface, along with some intracellular nano-micro meter-sized Dy particles. X-ray photoelectron spectrometry and X-ray absorption fine structure analyses showed that the chemical composition of T9-Dy corresponded to DyPO₄. X-ray diffraction analysis did not yield any spectrum from T9-Dy. Therefore, we concluded that the strain T9 accumulates and mineralizes Dy as an amorphous DyPO₄. Dysprosium desorption rate from T9-Dy was 100% using 0.3 M hydrochloric acid. Furthermore, after desorption process, the strain T9 grows again in the new medium and retains the Dy accumulation ability. Thus, the strain T9 has a potential as a bioaccumulator for Dy recovery from acidic drainage through biomineralization.

Keywords Biomineralization · Bioaccumulation · Drainage · Dysprosium · Acidophilic microorganism · Metal-biotechnology
26.1 Introduction

Rare earth elements (REE) are indispensable ingredients in the manufacturing of high-tech products (US Geological Survey 2015). In particular, dysprosium (Dy) has increased its global demand because to use in making heat resistant magnets for high-tech products; nevertheless, the supply of Dy is limited. Acidic mine drainage and industrial liquid waste containing low concentration of Dy have attracted attention as a new Dy resource (Protano and Riccobono 2002). Therefore, it is important to develop new recovery techniques from Dy-containing drainage using as a new resource.

Recently, biotechnological approaches for metal resource recycling have been emphasized (Zhuang et al. 2015). Biomineralization and bioaccumulation are studied for recovering targeting metal from mine drainage and industrial waste (Nancharaiah et al. 2016). An REE-bioaccumulating acidophilic fungus, *Penidiella* sp. strain T9 was isolated and able to recover approximately 50% (w/v) soluble Dy from acidic model drainage during growth (Horiike and Yamashita 2015). To exploit this capability on an industrial scale, the rate of accumulation and reaction time must be improved. To start addressing these issues, we have explored the localization and the chemical structure of Dy-containing compounds following their biogenetic solidification by the strain T9. To develop the application of biomineralization by the strain T9, we elucidated the localization and the chemical structure of biomineralized Dy and performed to establish the labo-scale bioprocess for selective recovery of Dy.

26.2 Materials and Methods

26.2.1 Media and Cultivation Conditions

The Basal salt medium (BSM) was prepared in accordance with a recent study (Horiike and Yamashita 2015). For Dy bioaccumulation tests, BSM was prepared at pH 2.5 with 20 mM potassium hydrogen phthalate-hydrochloric acid (HCl) buffer. Cultivation was carried out at 30 °C on a rotary shaker at 120 rpm.

26.2.2 Dy Bioaccumulation by Strain T9

The Dy accumulation test was performed in accordance with a recent study (Horiike and Yamashita 2015). The strain T9 was cultivated in 50 mL of BSM containing 100 mg/L Dy (as DyCl$_3$) and cultivated on a rotary shaker at 120 rpm at 30 °C for 7 days. After 7 days of cultivation, the strain T9 cells were centrifuged (15,900x g, 20 min, 4 °C) and then collected by filtration using an Omnipore membrane filter.
(0.2-μm pore size; Merck Millipore, MA, USA). The cell pellet on the filter was washed twice with sterile saline water (isotonic solution).

### 26.2.3 Analytical Methods

To characterize Dy precipitate prepared by the strain T9, scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX), X-ray absorption fine structure (XAFS) analyses, and X-ray diffractometry (XRD) were conducted with the detail procedure and the standard materials prepared according to reference (Horiike et al. 2016).

### 26.2.4 Purification of Dy from Dy Precipitate

Dy precipitate prepared by the strain T9 after 3 days cultivation was used. Each of HCl and ethylenediamine-N, N', N'-tetraacetic acid (EDTA), as solubilizing reagents of Dy-precipitated compounds in the strain T9, was prepared at 300 mM, 30 mM, and 3.0 mM, respectively. The final concentration of Dy in the solution containing HCl or EDTA was 500 mg/l (3.0 mM Dy) from Dy precipitate, and then the mixture was reacted at 30 °C on a rotary shaker at 120 rpm for 3 h. The concentration of Dy in the solution was determined using inductively coupled plasma-atomic emission spectrometry (ICP-AES).

### 26.3 Results and Discussion

### 26.3.1 Localization of Dy-Precipitated Compounds in Penidiella sp. Strain T9

To clarify the localization of Dy-precipitated compounds in the strain T9, sections of T9 cells were observed using SEM-EDX after the Dy bioaccumulation test (Fig. 26.1). Bright dots and bright regions were observed in the cell (1.0 μm diameter) and on the cell surface (1.0 μm thickness), respectively. Three points, bright dot (Spot 1), bright region (Spot 2), and cytoplasm as background (Area 3), were further analyzed using EDX (Fig. 26.1b–d). Specific high peaks corresponding to Dy, P, and O at spots 1 and 2 were observed; these peaks were absented from area 3 (background). Peaks of C and N were found at all three points. From these results, we conclude that the strain T9 mainly incorporates a layer of Dy on the cell surface and nano-micro sized particles (bright dots) of Dy in the cytoplasm. Furthermore, since P and O were also detected in Dy-precipitated material, we infer that the Dy
compounds also contain phosphate. High-magnification SEM-EDX analysis showed that the strain T9 formed a precipitated Dy (T9-Dy) layer with 1.0-μm thickness over the cell surface and some nanometer-sized particles of Dy in the cytoplasm (data not shown). The T9-Dy layer consisted of coagulated nanometer-sized particles.

26.3.2 Chemical Structure of Dy-Precipitated Compounds

To determine the chemical state of T9-Dy, Dy L_{III}-edge spectrum in XANES of T9-Dy, DyPO_4 (chemical precipitate), CMC-Dy (Dy-binding carboxymethyl cellulose), and CP-Dy (Dy-binding cellulose phosphate) was carried out (Horiike et al. 2016). The strength of the peak across the samples was as follows: DyPO_4 ≦ T9-Dy < CMC-Dy ≦ CP-Dy. This indicates that the strength of peak depends on both the
ligand and structure around Dy. The Dy L_{\text{I}}-edge spectrum in XANES is shown (Horiike et al. 2016). The full width at half maximum values of Peak B was as follows: DyPO_{4} \approx T9-Dy < CMC-Dy \approx CP-Dy. A shoulder (C) of Peak B (at \sim 9072 eV) was observed in the spectra of T9-Dy and DyPO_{4}. From the results, we conclude that the chemical bond and the local structure around Dy in the T9-Dy are closer to that of DyPO_{4} than that of either CMC-Dy or CP-Dy. To identify the ligand-bonding environment in T9-Dy, Dy L_{\text{III}}-edge spectrum in FT-EXAFS was carried out (Horiike et al. 2016). Peak E corresponds to P and C, and Peak F corresponds to Dy or P around Dy. From the FT-EXAFS results, the ligand-bonding environment in the T9-Dy appears to be very similar to that observed in DyPO_{4}.

To characterize the crystalline phase of T9-Dy, XRD analysis was performed. XRD patterns of wet and dry T9-Dy indicated a halo peak at \sim 30° and indistinct peaks, respectively (data not shown), showing that they are amorphous. The positions of the distinct peaks are the same as those reported for DyPO_{4}\cdot 1.5H_{2}O (ICDD 20-0385). Taken together, these data indicate that the crystalline phase of the T9-Dy is clearly different from that of chemically precipitated DyPO_{4}.

The acidophilic fungus *Penidiella* sp. strain T9 accumulates and incorporates Dy in a form that corresponds to DyPO_{4}. The precipitated Dy formed from nano- to micro-meter sized particles in the cytoplasm and aggregates on the cell surface. Therefore, we suggest that the strain T9 must have an active uptake mechanism for Dy into the cells and an ability to sorb Dy into phosphate groups present in the cell wall. These findings highlight the importance of phosphoric acid to improve Dy recovery by the strain T9, which has the available capacity in the cells to accumulate Dy.

### 26.3.3 Purification of Dy from Dy-Precipitated Compounds

To purify Dy from T9-Dy, an examination of Dy solubilization from T9-Dy prepared by 3 days cultivation was performed using a mineral acid (HCl) or EDTA (Fig. 26.2). One hundred percent of Dy contained in T9-Dy was dissolved by 300 mM HCl at 30 min incubation, but 26% and 5% of Dy were dissolved by 30 mM and 3.0 mM HCl, respectively, at 3 h. The results suggested that the purification of Dy from microbial Dy precipitant using the strain T9 was easier than chemical Dy precipitant, which was not dissolved by HCl. Ninety six percent, 100%, and 95% of Dy from T9-Dy were dissolved in 300 mM, 30 mM, and 3.0 mM of EDTA, respectively, at 3 h. Thus, 300 mM HCl and 30 mM EDTA were more effective chemicals to purify Dy from T9-Dy.
26.3.4 Reuse of the Strain T9 to Recover Dissolved Dy

The reusability of the strain T9 in the REE accumulation-desorption cycle is important to develop an economical REE recovery process. The cells of T9 strain after the Dy desorption were cultivated in a new BSM, and then Dy accumulation test was carried out reusing the T9 cells. The dissolved Dy in the culture were decreased less than 50% after 3 days of cultivation using the cells treated with EDTA or HCl in desorption (Fig. 26.3). These results indicated that the cells of the strain T9 grew again in the new medium and retained the Dy accumulation ability after desorption.
process using HCl or EDTA. Taken together, the strain T9 has a great potential as a bioaccumulator to develop a continuous recycling system of Dy. Mineral acids, such as HCl, HNO₃, and H₂SO₄, and chelating agents, such as EDTA and NTA (nitrilotriacetic acid), have lethal effects on most of microbial cells (Tsezos 1984). Since the strain T9 has ability to tolerate chemicals like HCl and EDTA, the strain has strong potential to be developed as the REE-accumulator for REE recovery cycle.

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