Chapter 26
Dysprosium Biomineralization by *Penidiella* sp. Strain T9

Takumi Horiike, Hajime Kiyono, and Mitsuo Yamashita

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 $DyPO_4$. 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 $DyPO_4$. 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

T. Horiike · M. Yamashita (✉)
Shibaura Institute of Technology, Saitama, Japan
e-mail: i030589@shibaura-it.ac.jp; yamashi@shibaura-it.ac.jp

H. Kiyono
Shibaura Institute of Technology, Tokyo, Japan
e-mail: h-kiyono@shibaura-it.ac.jp

K. Endo et al. (eds.), *Biomineralization*,
https://doi.org/10.1007/978-981-13-1002-7_26

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 (15,900× *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′, 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

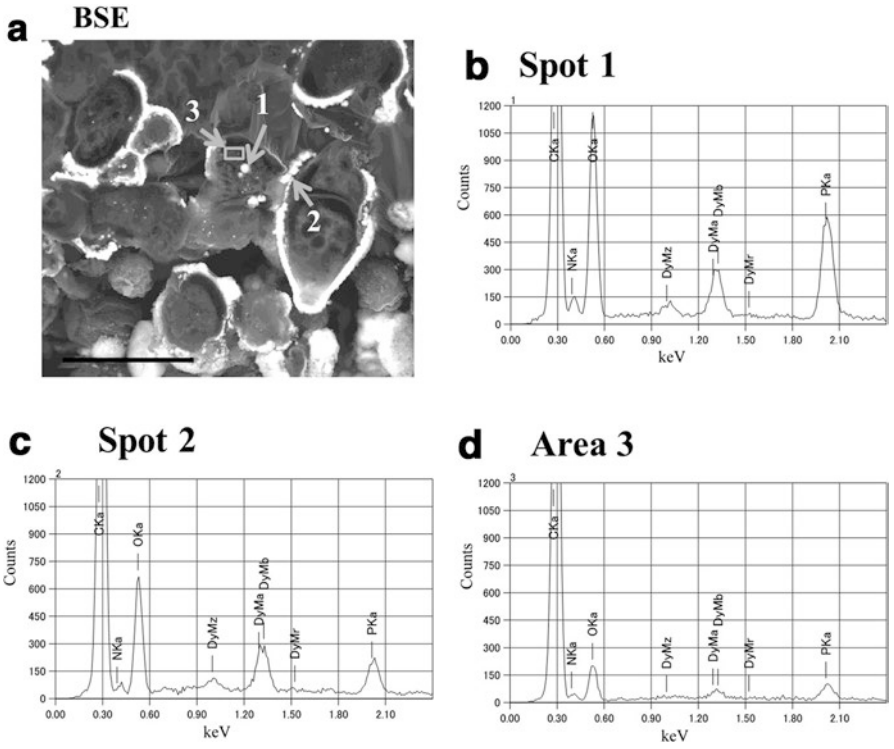

Fig. 26.1 SEM-EDX analyses of a cross section of the Dy compounds from the strain T9. (**a**) Back scattered electron (BSE) image of a cross section of strain T9 with Dy. The image reveals nanoparticles and precipitates in cytoplasmic regions (i.e., Spot 1) and the cell wall (i.e., Spot 2), respectively. Bar, 10 μm. EDX spectra in (**b**) Spot 1, (**c**) Spot 2, and (**d**) Area 3. Area 3 corresponds to a background cytoplasmic region. (Horiike et al. 2016)

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: $DyPO_4 \leqq$ T9-Dy < CMC-Dy $\leqq$ CP-Dy. This indicates that the strength of peak depends on both the

ligand and structure around Dy. The Dy L_I-edge spectrum in XANES is shown (Horiike et al. 2016). The full width at half maximum values of Peak B was as follows: $DyPO_4 \fallingdotseq$ T9-Dy < CMC-Dy $\fallingdotseq$ CP-Dy. A shoulder (C) of Peak B (at ~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_{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 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 $DyPO_4 \cdot 1.5H_2O$ (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.

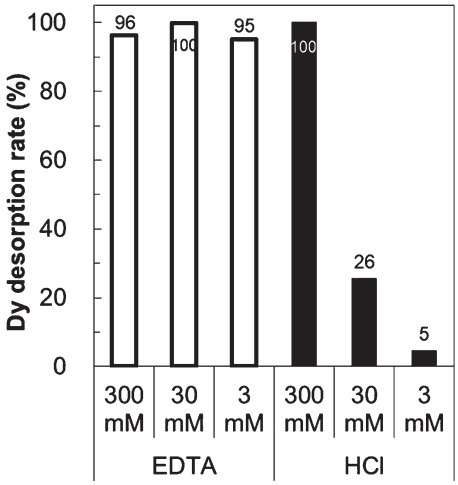

Fig. 26.2 Dy desorption rate from T9-Dy using EDTA or HCl. White bars, using EDTA; black bars, using HCl

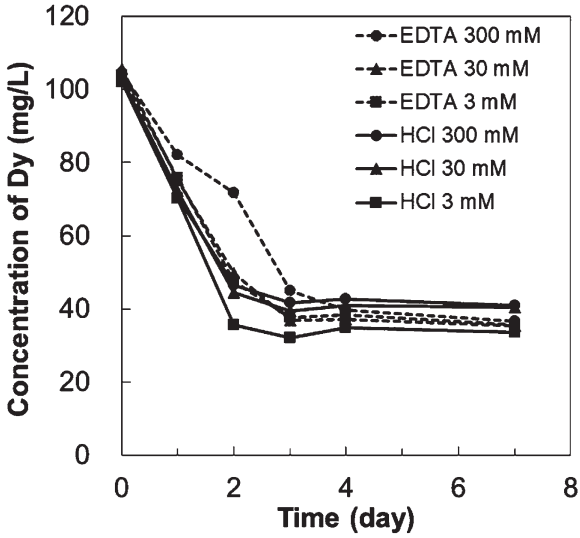

Fig. 26.3 Time course of the Dy concentration during cultivation in BSM using the strain T9 after desorption. Broken lines, cells after desorption using EDTA; solid lines, cells after desorption using HCl. Concentrations of desorption reagents; 300 mM (solid circle), 30 mM (solid triangle), 3.0 mM (solid square)

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, HNO_3, and H_2SO_4, 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.

Acknowledgments XAFS analysis was performed under the approval of the Photon Factory Program Advisory Committee. This study was supported partially by the Japan Oil, Gas and Metals National Corporation and the Kurita Water and Environment Foundation.

References

Horiike T, Yamashita Y (2015) A new fungal isolate, *Penidiella* sp. strain T9, accumulates the rare earth element dysprosium. Appl Environ Microbiol 81:3062–3068

Horiike T, Kiyono H, Yamashita M (2016) *Penidiella* sp. strain T9 is an effective dysprosium accumulator, incorporating dysprosium dysprosium phosphate compounds. Hydrometallurgy 166:260–265

Nancharaiah YV, Mohan SV, Lens PNL (2016) Biological and bioelectrochemical recovery of critical and scarce metals. Trends Biotechnol 34:137–155

Protano G, Riccobono F (2002) High contents of rare earth elements (REEs) in stream waters of a Cu–Pb–Zn mining area. Environ Pollut 117:499–514

Tsezos M (1984) Recovery of uranium from biological adsorbents-desorption equilibrium. Biotechnol Bioeng 26:973–981

U. S. Geological Survey (2015) Mineral commodity summaries 2015. U.S. Geological Survey, Virginia

Zhuang WQ, Fitts JP, Ajo-Franklin CM, Maes S, Alvarez-Cohen L, Hennebel T (2015) Recovery of critical metals using biometallurgy. Curr Opin Biotechnol 33:327–335