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Reversible pH-dependent curium(III) biosorption by the bentonite yeast isolate *Rhodotorula mucilaginosa* BII-R8

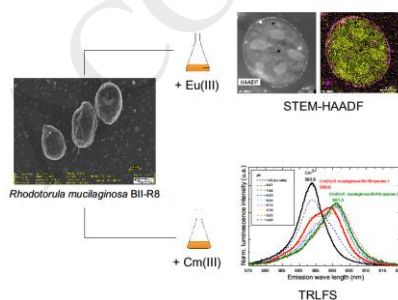
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Graphical abstract



Highlights

- Reversible pH-dependent Cm(III) biosorption by *R. mucilaginosa* BII-R8 cells
- Two different Cm(III)-*R. mucilaginosa* BII-R8 species were identified
- Eu(III)-phosphates were accumulated at the yeast cell membrane
- Eu(III) had a negative effect in the yeast cell viability and metabolic activity
- Yeast-Cm(III) interaction will make Cm more mobile at near neutral and alkaline pH

Abstract

This work describes the molecular characterization of the interaction mechanism of a bentonite yeast isolate, *Rhodotorula mucilaginosa* BII-R8, with curium(III) as representative of trivalent actinides and europium(III) used as inactive analogue of Cm(III). A multidisciplinary approach combining spectroscopy, microscopy and flow cytometry was applied. Time-Resolved Laser Induced Fluorescence Spectroscopy (TRLFS) analyses demonstrated that the biosorption of Cm(III) is a reversible and pH-dependent process for *R. mucilaginosa* BII-R8 cells. Two Cm(III)-*R. mucilaginosa* BII-R8 species were identified having emission maxima at 599.6 and 601.5 nm. They were assigned to Cm(III) species bound to phosphoryl and carboxyl sites from the yeast cell, respectively. Phosphate groups were involved in the sorption of this actinide, as demonstrated by the Eu(III)-phosphate accumulates at the cell membrane shown by microscopy. In addition, cell viability and metabolic potential were assessed to determine the negative effect of Eu(III) in the yeast cells. The results obtained in this work showed that the interaction of Cm(III) with the yeast *R. mucilaginosa* BII-R8 cells at circumneutral and alkaline pH values will make this

radionuclide more mobile to reach the biosphere. Therefore, geochemical conditions in the bentonite engineering barrier need to be carefully adjusted for the safe deep geological disposal of radioactive wastes.

Keywords (5): curium; europium; biosorption; yeast *Rhodotorula mucilaginosa*; Time-Resolved Laser Induced Fluorescence Spectroscopy (TRLFS).

1. Introduction

Nuclear reactors generate waste containing important radionuclides such as uranium, europium and transuranic isotopes (americium, curium and neptunium isotopes). Curium is a high radiotoxic transuranic isotope created by successive neutron captures on plutonium and americium isotopes [1] and is one of the main contributors to the long-term radiotoxicity of the spent fuel wastes [2]. ^{244}Cm and ^{242}Cm isotopes are strong thermal and neutron emitter with long half-life which may be an issue for their long-term storage. In addition, after reprocessing and recovery from the spent fuel curium can be turned into fission products by submitting it to a neutron flux [1]. For example, converting the decay heat from ^{242}Cm into ^{238}Pu which is then converted into electricity by various forms of thermoelectric conversion technology [3]. However, due to the high α activity and long-lived curium isotopes, this actinide must be safely stored for long periods of time. Deep geological repository (DGR) is the international accepted way to store highly radioactive wastes including curium. This concept implies a multi-barrier system where radiotoxic materials will be encapsulated into resistant canisters, surrounded by an engineered barrier and embedded in a host rock [4]. Clays have been selected as engineering barrier for the underground disposal of radioactive wastes within a geological formation [5]. Microbial activity may have a great impact in the stability of this repository system since the presence of microorganisms several kilometers

below the surface has been demonstrated [6, 7]. A high microbial diversity has been described in clays as host rock [8] and for their use as engineered barriers [9-11]. Microbes are able to control the speciation and mobility of radionuclides [12]. Therefore, understanding the microbial interaction mechanisms with radionuclide will provide insights in the impact of microbial mobilization of these hazardous materials from repositories to the biosphere. Most of the radionuclide interaction studies have been performed between uranium and bacterial isolates from clays considered for deep geological disposal of radioactive wastes [9, 13, 14]. In the case of curium, few studies were performed addressing the interaction mechanisms between this actinide and isolated bacterial strains from natural environments [15, 16], or deep biosphere ecosystems such as Opalinus Clay formation [17] and groundwaters from Äspö Hard Rock Laboratory [18, 19]. However, although yeast are widely distributed in the environment [20] they have been poorly studied in these specific sub-surface ecosystems. Not many indigenous yeast strains have been isolated from clay studied scenarios for the DGR of nuclear wastes [9, 21]. The yeast *Rhodotorula mucilaginosa* BII-R8 was isolated from bentonite deposits (Almeria, Spain) studied as an analogue of engineered barriers for deep geological disposal of radioactive wastes [9]. The uranium biosorption process by these yeast cells has been lately described showing that uranium is bound to organic phosphate containing molecules at the yeast cell wall with a local coordination similar to that of meta-autunite [22]. However, to our knowledge, this is the first study describing curium and europium interaction mechanisms with yeast cells.

The objectives of this work are to determine the molecular characterization of the interaction mechanisms of the natural bentonite yeast isolate *Rhodotorula mucilaginosa* BII-R8 with curium(III) as a stable representative of the trivalent actinides exhibiting excellent luminescence properties; and europium(III) as inactive analogue of Cm(III). By applying a multidisciplinary approach the speciation of curium associated with *R. mucilaginosa* BII-R8

cells was studied by means of Time-Resolved Laser Induced Fluorescence Spectroscopy (TRLFS), which is a very sensitive technique that can be successfully used for direct identification and quantitative estimation of different species of curium complexes in the μM concentration range [17]; moreover, the ability of the yeast cells to deal with the chemical toxicity of europium was tested using flow cytometry and Scanning Transmission Electron Microscopy-High Angle Annular Dark Field (STEM-HAADF) techniques.

2. Experimental

2.1 Yeast strain and growth conditions

Yeast strain *R. mucilaginosa* BII-R8 was isolated from a bentonite sample recovered from Almeria, Spain [9]. Yeast cells were grown in Luria-Bertani medium pH 7, consisting of 1.0 g/l tryptone, 0.5 g/l yeast extract, and 0.5 g/l NaCl at 28 °C on agitation (120 rpm).

2.2. Preparation of curium(III) and europium(III) solutions

A stock solution of the long-lived curium isotope ^{248}Cm (half-life: 3.4×10^5 years) was used. This solution had the following composition: 97.3% ^{248}Cm , 2.6% ^{246}Cm , 0.04% ^{245}Cm , 0.02% ^{247}Cm , and 0.009% ^{244}Cm in 1.0 M HClO_4 . The experiments were performed in a glove box under a N_2 atmosphere at 25° C. As a background electrolyte, analytical grade 0.1 M NaClO_4 (Merck, Darmstadt, Germany) was used. To prevent the carbonate complexation of curium(III) carbonate-free water and NaOH solution were used. The pH was measured using an InLab Solids combination pH puncture electrode (Mettler-Toledo, Giessen, Germany) calibrated with standard buffers. The pH was changed by adding analytical grade NaOH (Merck) or HClO_4 (Merck) with an accuracy of ± 0.02 units.

A europium stock solution was prepared by dissolving $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ (Sigma-Aldrich, trace elements basis, 99.99%) in ultrapure water.

2.3 TRLFS Cm(III)-yeast cell suspension preparation and experimental setup

Two series of experiments were performed at 0.3 μM Cm^{3+} to explore its interaction behavior with the yeast strain *R. mucilaginosa* BII-R8. The biomass concentration was kept constant at 0.2 and 0.45 $\text{g}_{\text{dry weight}}/\text{L}$, while varying pH between 8.0–2.0. The time-resolved luminescence spectra were recorded using a unique pulsed flash lamp pumped Nd:YAG-OPO laser system (Powerlite Precision II 9020 laser equipped with a Green PANTHER EX OPO from Continuum, Santa Clara, CA, USA). The laser pulse energy, which was between 1.5 and 2.5 mJ depending on the excitation wave length used, was monitored using a photodiode. The luminescence spectra were detected using an optical multi-channel analyzer-system, consisting of an Oriel MS 257 monochromator and spectrograph with a 300 or 1200 line mm^{-1} grating and an Andor iStar ICCD camera (Lot-Oriel Group, Darmstadt, Germany). The curium(III) single luminescence emission spectra were recorded in the 570–650 nm (1200 line mm^{-1} grating with 0.2 nm resolution) range. The time-dependent luminescence spectra were detected in the 500–700 nm (300 line mm^{-1} grating: high intensity with a lower resolution >0.6 nm) range. A constant time window of 1 ms length was applied. Due to the high absorption of the F-band usually observed in Cm^{3+} excitation spectra an excitation wavelength of 396 nm was used. More details concerning the TRLFS setup and data evaluation are summarized in Moll et al. [17]. The luminescence spectra were base-line corrected, energy corrected and normalized using the ORIGIN 6.1G (OriginLab Corporation, USA) code. The lifetime of luminescence species was obtained also with the same software. The time-dependent luminescence spectra measured with the 300 line mm^{-1} grating were evaluated using parallel factor analysis (PARAFAC) [23]. As a result, luminescence of both data evaluation procedures lifetimes of the different Cm(III) species were obtained. The

spectra deconvolutions of the pH-dependent Cm(III) luminescence measurements were performed using the factor analysis software HypSpec [24].

2.4 Sample preparation for STEM-HAADF and EDX analyses

R. mucilaginosa BII-R8 cells were incubated for 48 h at 28 °C on agitation (120 rpm) in 1 mM Eu(III) solution in Low Phosphate Medium (Table S1), pH 7. Europium treated yeast cells were harvested by centrifugation at 15000 x g for 15 min at 4 °C in an Eppendorf 5804R refrigerated centrifuge and washed twice with 0.9% NaCl to remove the interfering ingredients of the growth medium. Samples for microscopic analyses were prepared as described in Lopez-Fernandez et al. [9]. The samples were examined by using a STEM-HAADF FEI TITAN G2 80-300. TEM specimen holders were cleaned by plasma prior to STEM analyses to minimize contamination. The high resolution STEM is equipped with HAADF detector and EDAX energy dispersive X-ray.

2.5 Flow cytometry sample preparation and experimental setup

R. mucilaginosa BII-R8 cells were loaded with different europium concentrations (0, 0.5, and 1 mM) in 0.1 M NaClO₄ (Merck) for 48 h at 28 °C on agitation (120 rpm). Yeast cells were harvested by centrifuging at 15000 x g for 15 min at 4 °C. Afterwards, cells were suspended and diluted in Phosphate Buffered Saline to adjust the cell concentration to the required one for this experiment (approximately 10⁶ cells/ml). For live/dead staining solutions of 0.1 mg/ml Fluorescein Diacetate (FDA) (Acros Organics) and 1 mg/ml Propidium Iodide (PI) (Invitrogen) were mixed with cell suspensions for staining during 15 min in the darkness, at room temperature. Yeast cell suspensions simultaneously incubated in the presence of both stains were analyzed by flow cytometry. In addition, to study the effect of Eu(III) on the cell

metabolic activity, 10^6 cells/ml cell suspensions were mixed with 10 μ M DiOC₆ (Invitrogen) for 15 min in darkness at room temperature.

Measurements were taken in triplicate using a FACSCantoII cytometer Becton Dickinson (San Jose Palo Alto, California), equipped with three lasers: 488 nm blue, 620 nm red and 405 nm UV. Samples were measured in FL1 (FDA-FITC) and FL2 (PI-PE), in logarithmic scale channels, at medium speed. Filters used were 530 nm and 580 nm Band Pass. Samples were analysed using BD Diva 6.1.

3. Results

3.1 TRLFS analyses of Cm(III) complexes formed by the cells of the yeast *R. mucilaginosa* BII-R8

The interaction between curium(III) and the yeast strain *R. mucilaginosa* BII-R8 was studied at trace Cm(III) concentrations (0.3 μ M) using TRLFS. The assumption was made that the influence of the luminescence properties of the microbial Cm(III)-species dominates over the influence of soluble Cm(III)-species with, for instance, released metabolites. The pH dependent spectroscopic Cm(III) speciation in the cell suspensions is depicted in Figure 1. From the dependencies found in the TRLFS spectra it can be concluded that there are two coordination environments of Cm(III) due to interactions with functional groups of the cell membrane and possibly with released metabolites. Also, the PARAFAC analysis of the time dependent emission spectra measurements revealed two Cm(III) yeast species (Fig. 2). Cm(III)-*R. mucilaginosa* BII-R8 specie 1 is characterized by an emission maximum at 599.6 ± 1 nm and an average luminescence lifetime of 240 ± 50 μ s. Whereas Cm(III)-*R. mucilaginosa* BII-R8 specie 2 shows a more red shifted emission maximum at 601.5 ± 0.5 nm and a shorter average luminescence lifetime of 123 ± 11 μ s. The extracted single component spectra of both species are shown in Figure 1. These are average spectra from both

experimental runs. The spectroscopic species distribution in the Cm(III)-*R. mucilaginosa* BII-R8 system calculated by PARAFAC are shown in Figure 2, revealing how the influence of Cm^{3+} decreased as a function of pH (Fig. 2b). Whereas Cm(III)-*R. mucilaginosa* BII-R8 species 1 dominated between pH 5 and 7, Cm(III)-*R. mucilaginosa* BII-R8 species 2 increased from pH 4 until pH 8. In a relatively large pH region all three species occur together making the determination of their single component spectra challenging.

TRLFS of the supernatants and the Cm(III) loaded biomass after washing with 0.1 M NaClO_4 showed that 30 % of the total Cm(III) luminescence intensity remained in solution at pH 6.2. At pH 8.1 even 82 % of the total Cm(III) luminescence intensity remained in solution. Hence, between pH 6 and 8 approximately 70 % to only 18 % of the Cm(III) was associated with the biomass. This suggests a pH-dependent release of complexing agents, possibly phosphates by the cells. By comparing the luminescence properties of both Cm(III)-*R. mucilaginosa* BII-R8 species with previous data (Table 1), Cm(III)-*R. mucilaginosa* BII-R8 species 1 can be assigned to a Cm(III) species bound to phosphoryl sites and Cm(III)-*R. mucilaginosa* BII-R8 species 2 to a Cm(III) species bound to carboxyl sites of the cell membrane.

3.2 STEM-HAADF analyses

In order to investigate the cellular localization of Cm(III) accumulated by the yeast cells, Eu(III) was used as inactive analogue of this trivalent actinide. Cm(III) and Eu(III) presented similar chemical behaviours, therefore the results obtained on Eu(III)-yeast interactions could be extrapolated to those of Cm(III). *R. mucilaginosa* BII-R8 cells were exposed to 1 mM Eu(III). STEM-HAADF micrographs of thin sections of the yeast are shown in Figure 3, revealing electron-dense accumulates located at the cell surface. Element-distribution mapping showed that the accumulates were mainly composed of europium and phosphorus (Figure 3a and c). These results indicate that biosorption of Eu(III) is the main interaction mechanisms

with the *R. mucilaginosa* R8 cells which in turn shows that Cm(III) would behave similarly in agreement with the results of TRLFS.

3.3 Eu(III) effect on cell viability and metabolic activity by flow cytometry

The effect of Eu(III) on the yeast *R. mucilaginosa* BII-R8 cell viability was tested after 48 h incubation with different europium concentrations. Results showed that europium has an important negative effect on the yeast cell viability (Fig. 4 and Table S2). At 0.5 mM Eu(III) the yeast cell viability was decreased from 97% to 2%. When increasing the europium concentration to 1 mM, the viability was reduced to less than 1%. However, the metabolic activity of the yeast cells was not so affected by this metal (radionuclide) as the cell viability (Fig 5 and Table S3). Concretely, more than 67% of the yeast cells were active at 0.5 mM of europium concentration. Nevertheless, at 1 mM of Eu(III) the yeast metabolic potential is decreased to 37% revealing the negative effect of europium for the yeast cells. Therefore, the cell viability is much more affected by higher concentrations of europium than the cell metabolism.

4. Discussion

This work describes the interaction mechanism of the bentonite yeast isolate *R. mucilaginosa* BII-R8 with Cm(III) as representative of trivalent actinides. In addition, interactions studies were also complemented with Eu(III) as inactive analogue of Cm(III). For this, a multidisciplinary approach combining spectroscopy, microscopy and flow cytometry techniques was applied. Investigating the effect of microbial processes on the fate and behavior of Cm(III) is of great importance since it will be included in the inventory of high radioactive wastes to be disposed in future deep geological repositories.

The speciation of Cm(III) with the cells of the yeast *R. mucilaginosa* BII-R8 under environmental relevant conditions (0.3 μM concentration) was studied by using TRLFS spectroscopy. TRLFS is a useful and very sensitive technique for the determination of the fluorescence parameters and lifetime of actinides complexes formed by different biological samples, even at very low concentrations (μM range) [13, 17]. TRLFS analyses indicated that the biosorption of Cm(III) by *R. mucilaginosa* BII-R8 is a reversible and pH-dependent process where two different species were identified, $(\text{R-O-PO}_3\text{H})\text{-Cm}^{2+}$ and R-COO-Cm^{2+} . These TRLFS results indicated that the organic phosphate groups might be from the phospholipid bilayer of the cytoplasmic membrane of the yeast cells, and carboxylic groups within the polysaccharides of the yeast cell wall. The speciation of Cm(III) with gram positive and gram negative bacteria have been well studied, such as with *Pseudomonas fluorescens* [18, 25], $\text{Cm}^{3+}\text{-Bacillus subtilis}$ complexes [26] or a $\text{Cm}^{3+}\text{-Desulfovibrio äspöensis}$ complex [19]. In addition, the Cm(III) interaction with *Sporomusa* sp. cells is dominated by a major process of reversible biosorption reaction and the speciation is dominated by two complexes, a $\text{R-O-PO}_3\text{H-Cm}^{2+}$ complex and a R-COO-Cm^{2+} complex [27]. Finally, in the case of *Paenibacillus* sp. the $\text{R-O-PO}_3\text{-H-Cm}^{2+}$ complex dominates the speciation up to pH 8 in similar conditions as in this study [27]. Moreover, the involvement of organic phosphates and carboxylic groups in the biosorption of uranium by the yeast cells *R. mucilaginosa* BII-R8 has been recently described [22]. In this study, the uranium biosorption by these yeast cells is described as a time-dependent process forming uranium complexes with a local coordination similar to that of meta-autunite. However, to our knowledge, this is the first study reporting the molecular scale coordination of Cm(III) to yeast cells. The biosorption of Cm(III) by the yeast strain *R. mucilaginosa* BII-R8 seems to be a reversible and pH-dependent process that influences the curium(III) behavior in this environment proposed for future engineering

barrier with indigenous microorganisms. At circumneutral and alkaline pH the Cm(III)-yeast interaction will lead to the mobilization of this hazardous material within the DGR.

The curium biosorption by the yeast cells was supported by the STEM-HAADF analyses with Eu(III) as inactive analogue of Cm(III). *R. mucilaginosa* BII-R8 cells exposed to 1 mM of Eu(III) concentration showed electron-dense accumulates mainly located at the cell surface. The main composition of these accumulates was europium and phosphorus which may be understood as a confirmation of the implication of the phosphate groups from the phospholipids of the yeast cell. In addition, the capacity of the yeast cells to deal with Eu(III) was tested by flow cytometry. The results showed the great negative impact of this lanthanide in the yeast cell viability. After 48 h of incubation at 0.5 mM Eu(III) the majority of the cells were considered as dead cells (98%). In addition, the metabolic potential was studied using the mitochondrial fluorescent marker DiOC₆, which is broadly used for staining mitochondria in living cells [28]. The metabolic potential at 0.5 mM of Eu(III) concentration was 67%, while at 1 mM Eu(III) was 37%. The higher percentage of polarized cells compared to that of viable cells revealed the attempt of the yeast cells to cope with this element. The oxidative stress induced by the europium concentrations may result in reactive oxygen species (ROS) overproduction, such as superoxide anion, hydroxyl radical, and hydrogen peroxide, which can accumulate upon mitochondrial disturbances, endoplasmic reticulum stress or other cellular parts [29]. A recent study showed by proteomics that increased levels of intracellular ROS in the yeast *Saccharomyces cerevisiae* can regulate the global protein synthesis [30]. Several bioremediation mechanisms of different heavy metals by fungal species have been previously described [31]. One example is the biosorption of lead(II) and cadmium(II) by cells of *Amanita rubescens*, also from phylum Basidiomycota [32]. *Rhodotorula mucilaginosa* is a pigmented yeast strain known for their high resistance to different heavy metals, such as lead(II) [33], zinc and silver [34] or copper and chromium [35]. In the case of high copper

concentrations, the response of *R. mucilaginosa* to the induced oxidative stress have been well investigated, revealing the over expression of proteins such as chaperones, superoxide dismutase or catalase [36, 37], or the increased of carotenoid biosynthesis [38]. It is well known that microorganisms can contribute to the speciation and mobility of radionuclides through the geosphere [39]. Microbial activity could also have an impact on the degradation of organic compounds affecting the longevity of the metal container in the future repository [40]. The Spanish bentonite deposits from Almeria have been poorly studied yet as an artificial barrier for the DGR of nuclear wastes in terms of microbial diversity and radionuclide interactions [9, 10, 22, 41]. This is one of the first studies describing the influence of the isolated strain *R. mucilaginosa* BII-R8 in the transport and mobility of curium and europium. Therefore, more evidences are required to show that the presence of microorganisms such as *R. mucilaginosa* BII-R8 in bentonite clays might affect the safety of deep geological disposal of nuclear wastes. In addition, the results of this study are valuable in similar environments representative of repositories.

7. Conclusions

This work presents the interaction mechanisms of Cm(III) and Eu(III) with cells of a natural yeast isolated from Spanish bentonite formations, considered as analogues of the artificial barriers for the future deep geological repositories of radioactive wastes. The biosorption of Cm(III) by the yeast cells is a reversible and pH-dependent process. Organic carboxyl and phosphates groups were involved in the Cm(III) biosorption, while Eu(III)-phosphate complexes were observed at the yeast cell membrane. The *R. mucilaginosa* BII-R8 cell viability and metabolic potential were highly affected by increasing concentrations of Eu(III). These data indicated that the Cm(III)-yeast interactions can affect the mobility of this radionuclide having a negative effect at circumneutral and alkaline pH in the concept of

radioactive wastes disposal. Therefore, in order to guarantee the safety of the DGR of nuclear wastes, it will be necessary to modify the physical properties of the bentonites. By inhibiting the microbial occurrence and growth it would be possible to prevent the microbial mobilization of Cm(III) from the disposal system to the environment.

Author contributions

MLM and ML-F conceived the study; ML-F prepared the samples, carried out the experiments and interpreted data; HM performed and interpreted the TRLFS analyses; ML-F drafted the manuscript. All authors read and approved the final manuscript.

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Declarations of interest: none.

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Figure legends

Figure 1. Luminescence emission spectra of 0.3 μM Cm(III) in 0.1M NaClO_4 measured as a function of pH at a fixed biomass concentration of 0.45 $\text{g}_{\text{dry weight}}/\text{l}$.

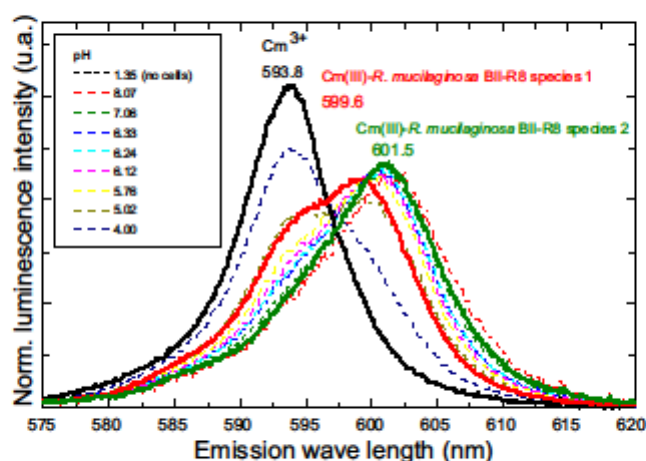


Figure 2. A: Extracted luminescence lifetimes of Cm(III) species and B: their respective pH-dependent distribution in the Cm^{3+} - *R. mucilaginosa* BII-R8 system determined by PARAFAC.

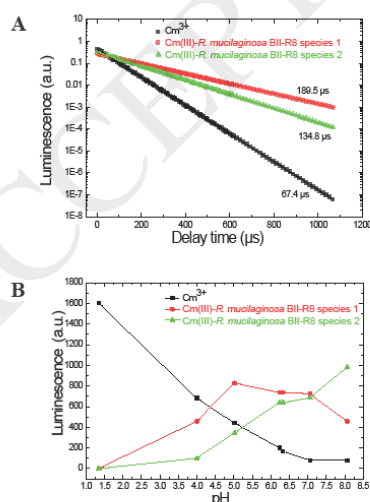


Figure 3. Scanning Transmission Electron Microscopy-High Angle Annular Dark Field (STEM-HAADF) micrographs of thin sections of *R. mucilaginosa* BII-R8 treated with 1 mM Eu (III) for 48 h. A: phosphorus and europium mapping image; B: HAADF image; C: europium mapping image.

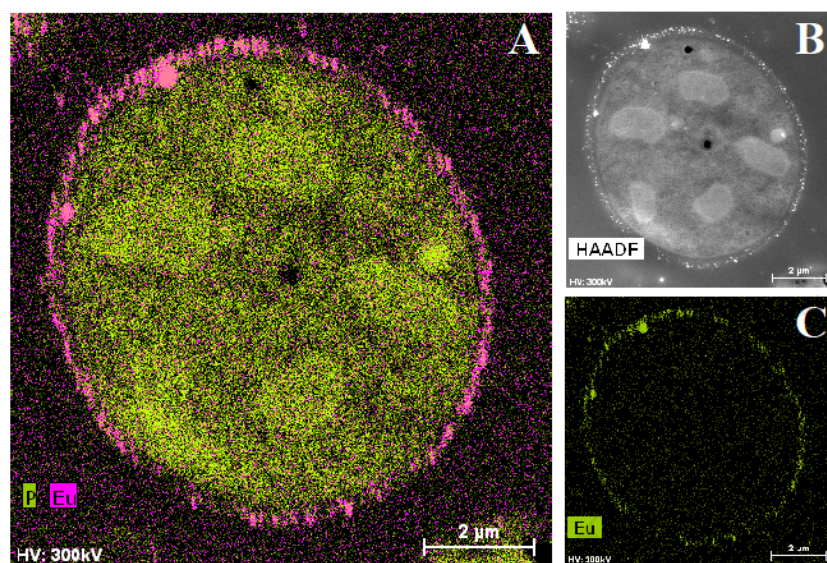


Figure 4. Flow cytometry scatterplots of *R. mucilaginosa* BII-R8 cell viability after 48 h of incubation at europium concentration ranging from 0 to 1 mM. A: 0 mM Eu(III) replicates; b: 0.5 mM Eu(III) replicates; C: 1 mM Eu(III) replicates. PI+, FDA-: dead cells; PI+, FDA+: damaged cells, considered as dead cells; PI-, FDA-: cellular rest, not considered in this study; PI-, FDA+: alive cells.

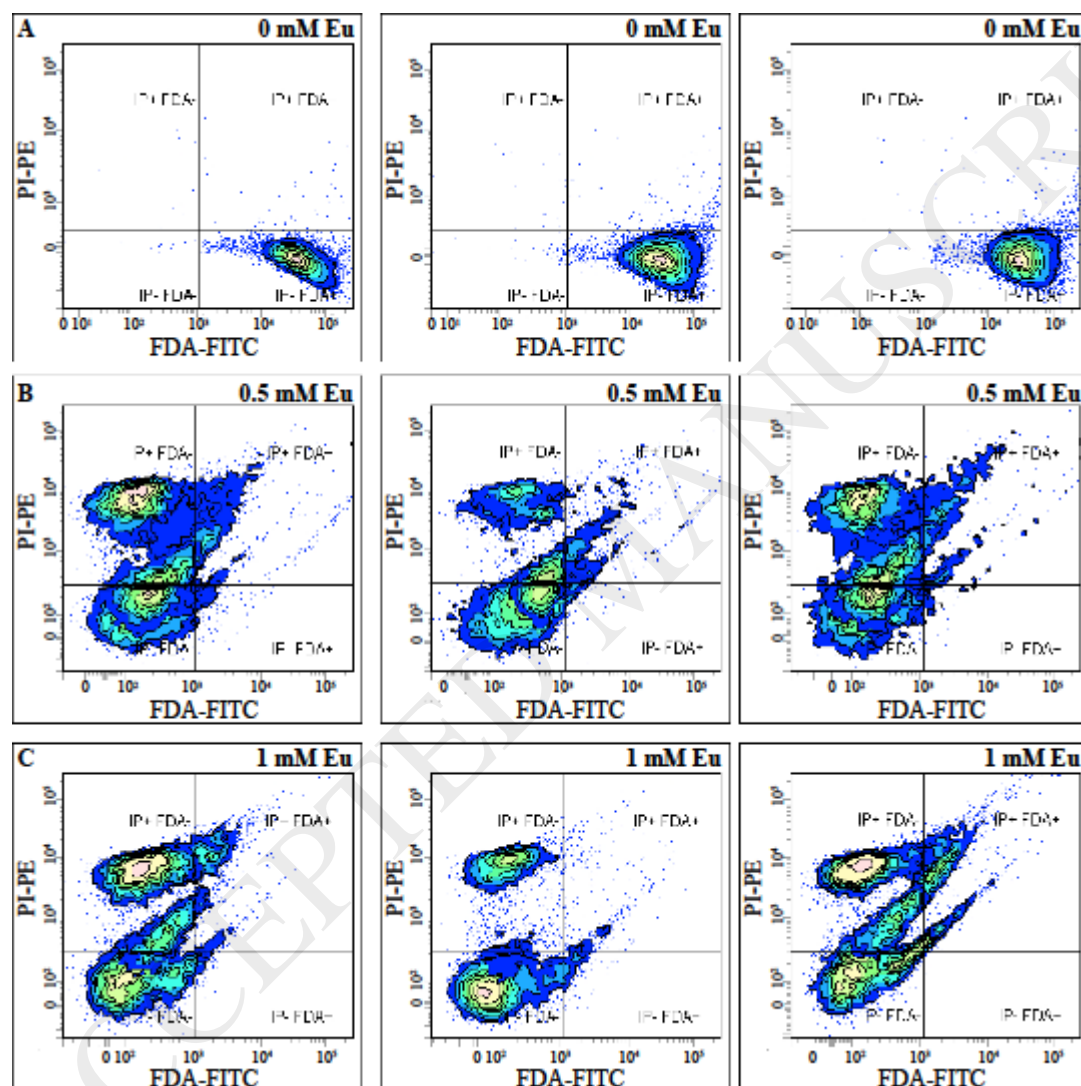


Figure 5. Flow cytometry scatterplots of *R. mucilaginosa* BII-R8 membrane potential after 48 h at different europium concentrations. A: 0 mM Eu(III) replicates; B: 0.5 mM Eu(III) replicates; C: 1 mM Eu(III) replicates. P2: metabolically active cells; rest: metabolically inactive cells.

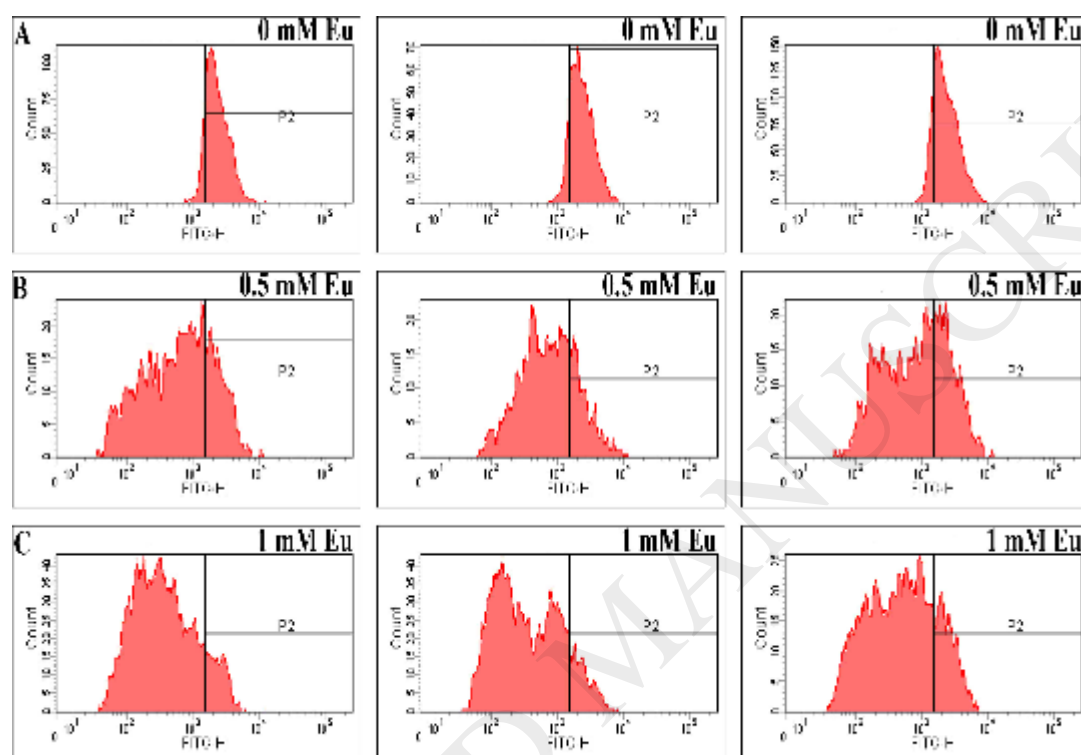


Table 1. Luminescence emission data of the Cm^{3+} -*Rhodotorula mucilaginosa* BII-R8 system including those of relevant model systems for comparison.

Species	Emission peak maximum (nm)	Lifetime (μs)	Reference
Cm^{3+} (aq)	593.8	67 ± 2	This work
Cm^{3+} - <i>R. mucilaginosa</i> BII-R8 species 1	599.6	240 ± 50^a	This work
Cm^{3+} - <i>R. mucilaginosa</i> BII-R8 species 2	601.5	123 ± 11^a	This work
Cm^{3+} - <i>Sporomusa</i> sp. MT-2.99 complexes			[17]
R-O- $\text{PO}_3\text{H-Cm}^{2+}$	599.8	252 ± 46	
R-COO- Cm^{2+}	601.6	108 ± 15	
Cm^{3+} - <i>Pseudomonas fluorescens</i> complexes			[18]
R-O- $\text{PO}_3\text{H-Cm}^{2+}$	599.6	390 ± 78	
R-COO- Cm^{2+}	601.9	121 ± 10	
Cm^{3+} - <i>Paenibacillus</i> sp. complex			[42]
R-O- $\text{PO}_3\text{H-Cm}^{2+}$	598.8	477 ± 73	

^aLifetimes were averaged from the ORIGIN and the PARAFAC evaluations.