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Root endophytic bacteria of a ^{137}Cs and Mn accumulator plant, *Eleutherococcus sciadophylloides*, increase ^{137}Cs and Mn desorption in the soil

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ABSTRACT

We found that root endophytes of ^{137}Cs accumulator plant produce siderophores, resulting in the desorption of ^{137}Cs from the contaminated soil collected at Fukushima, Japan. We selected an endemic Japanese deciduous tree, *Eleutherococcus sciadophylloides* (Franch. et Sav), that accumulates high concentrations of ^{137}Cs and Mn. Root endophytic bacteria were isolated from *E. sciadophylloides* and microbial siderophore production was evaluated via chrome azurol S (CAS) Fe and CAS Al assays. Of the 463 strains that we isolated, 107 (23.1%) produced the siderophores. Using eight strains that showed high siderophore production in our assays, we examined desorption of ^{137}Cs, Mn, Fe and Al by the bacterial culture filtrates from ^{137}Cs-contaminated soil after decomposing the soil organic matter using hydrogen peroxide. We found ^{137}Cs and Mn desorption concomitant with Al and Fe desorption, as well as a decrease of pH. We also detected succinic acid, a well-known siderophore, in the bacterial culture filtrates of our two root endophytic bacteria. Our results strongly suggest that the root endophytic bacteria of *E. sciadophylloides* produce the siderophores that enhance ^{137}Cs and Mn desorption in the rhizosphere, making the resulting ^{137}Cs and Mn ions easier for *E. sciadophylloides* to absorb from the rhizosphere.
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1. Introduction

Damage to Japan's Fukushima Daiichi Nuclear Power Station caused by a giant tsunami in March 2011 resulted in widespread contamination of the environment via the release of radionuclides. Radiocesium (^{137}Cs) is an especially serious environmental contaminant because of its long half-life (30.2 years) compared with other radionuclides such as ^{131}I (8.04 days) and ^{134}Cs (2.06 years). Because Cs ions are adsorbed on negatively charged sites of soil organic matter and clay minerals (Cornell, 1993; Wahlberg and Fishman, 1962; Yamaguchi et al., 2012; Ohnuki and Kozai, 2013), ^{137}Cs is retained in the surface soil (Fujii et al., 2014; Kato et al., 2012; Tanaka et al., 2012). Although adsorbed ^{137}Cs is not considered to be a bioavailable form in the soil, there are many reports that crops (Harada et al., 2014; Sakai et al., 2014), trees (Kuroda et al., 2013), weeds (Yamashita et al., 2014), and mushrooms (Miyazaki et al., 2013) have absorbed ^{137}Cs from the soil since the Fukushima accident. However, for plants and microbes to absorb ^{137}Cs from soil, the ^{137}Cs must be desorbed from the adsorption sites of clay minerals and organic matter.

Rhizosphere microorganisms, such as those at the root–soil interface, generally enhance nutrient uptake by plant roots via the production of chemical substances such as siderophores (Brimecombe et al., 2001; Nannipieri et al., 2008; Schultz and Boyle, 2006), thereby promoting plant growth. Microbial siderophores, including organic acids, can chelate Fe and other metals and make them bioavailable (Bultreys, 2007). Root endophytes, which are members of the rhizosphere microorganism community, were

Abbreviations: CAS, chrome azurol S; CEC, carbon exchange capacity; GC–MS, gas chromatography–mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; MSTFA, N-methyl-N-(trimethylsilyl) trifluoroacetamide; NBA, nutrient broth agar; PTFE, polytetrafluorethylene; RSM, rhizosphere liquid medium; SIM, selected ion monitoring; TSA, tryptic soy agar.

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isolated from plant species that accumulate metals and found to produce siderophores (Whiting et al., 2001), indicating the possibility of a microbial role in the uptake of metals into plant tissues (Nagata et al., 2015).

Similar microbial support might apply to ^{137}Cs uptake by plant species that accumulate Cs. For example, oxalic acid is probably released from plant roots and microbes (Brimecombe et al., 2001), and can enhance Cs desorption from illite by weathering the illite (Wendling et al., 2004). In addition, Wendling et al. (2005) found that bacterial metabolites of *Bacillus* sp. significantly enhanced Cs desorption from frayed edge sites (FES; strong surface association sites of Cs in illitic minerals) of illite due to the Al-complexing abilities of the metabolites, which enhanced weathering at FES of illite. Chiang et al. (2011) showed that representative siderophores such as acetic, succinic, oxalic, tartaric, and citric acids, which are likely released from microorganisms, weather clay minerals and result in desorption of ^{137}Cs from strongly adsorbed sites, such as interlayer and FES of clay minerals via their abilities to supply H^+ ions to weaken Al–O and Fe–O bonds on clay minerals, and to chelate Al and Fe to weaken the surface structure of clay minerals. Soil is composed of many constituents, including minerals and organic matter. This indicates that the fallout ^{137}Cs in the soil of Fukushima is sorbed not only by illitic minerals, but other minerals (Kozai et al., 2012).

In this study, we examined the dissolution of ^{137}Cs from the contaminated soil samples collected in Fukushima, Japan by using root endophytes in ^{137}Cs accumulator plants. One of the important problems with radiocesium pollution in Japan is that ^{137}Cs is strongly adsorbed to clay minerals in soil (Yamaguchi et al., 2012), therefore, as a first step, we focused on ^{137}Cs strongly adsorbed to clay minerals. We selected an endemic Japanese deciduous tree, *Eleutherococcus sciadophylloides* (Franch. et Sav), that accumulates high concentrations of ^{137}Cs (Kiyono and Akama, 2013). This plant is also interesting because it is a known hyperaccumulator of Mn (Memon and Yatazawa, 1982, 1984), indicating that specific root endophytes might also enhance Mn desorption. We isolated root endophytic bacteria from *E. sciadophylloides* and examined microbial siderophore production via the chrome azurol S (CAS) Fe and CAS Al assays (Alexander and Zuberer, 1991).

Using eight strains that showed high siderophore production in the CAS assays, we analyzed ^{137}Cs, Fe, Al, and Mn desorption using ^{137}Cs-contaminated soil after decomposing the soil organic matter and obtaining bacterial culture filtrates. Manganese desorption from soil after decomposition of organic matter was also examined. Finally we identified possible siderophores in the bacterial culture filtrates. Our research provides important information on the mechanisms of ^{137}Cs absorption that occur due to plant–microbe interactions.

2. Materials and methods

2.1. Collection of *Eleutherococcus sciadophylloides* and ^{137}Cs analysis

Five individuals of *E. sciadophylloides* (3.4 ± 0.2 years old, confirmed by means of annual ring analysis) were collected in September 2013 from a forest (37°41′N, 140°27′E) at Fukushima University. According to our preliminary analysis, the collection site was not contaminated by metals compared with the data of non-polluted forest soil in Japan (Asami, 2001), and *E. sciadophylloides* contained an extremely high concentration of Mn in its leaves (1280 ± 388 mg/kg dry weight, $n = 6$) in our analysis performed 1 month before the sample collection. Collected plants were used for ^{137}Cs analysis and root-endophyte isolation. Simultaneously, root-zone soil (100 × 100 × 50 mm volume excluding litter was collected from the observed area of the root system, which was shallow) collected from each individual tree. After soil collection, the roots were dug out with trowels so that the whole trees were collected. The collected trees were carefully washed with tap water followed by deionized water, and were then separated into leaves and the root system. In the root system, there were tap roots, which were shallowly sinuous, and few fine roots emerging from tap roots in accordance with Karizumi (1979); we used only tap roots in this study. Leaves and subsamples of tap roots were dried at 80 °C for 48 h and the dried plant samples were then weighed and ground with electric mill (IFM-650D, Iwatani, Tokyo, Japan). Root-zone soil was air-dried and passed through a sieve (<2 mm). Concentrations of ^{137}Cs in the plant powder and soil were analyzed at 662 keV for 3000 seconds using a germanium semiconductor detector (GMX-15190-P, ORTEC, Oak Ridge, Tennessee, USA). The remaining subsamples of tap roots were used for observation and isolation of root endophytes as in Section 2.2.

2.2. Observation and isolation of root endophytes

To measure infection rates by arbuscular mycorrhizal fungi (*Paris* types) (Oba et al., 2006) or fungal endophytes (microsclerotia) (Jumpponen and Trappe, 1998), subsamples of tap roots, which were cut into round slices and stained with trypan blue, were observed under the microscope. Infection percentage of root length colonized was calculated according to the gridline-insect method (Giovannetti and Mosse, 1980; McGonigle et al., 1990). We focused on the bacterial root endophytes because arbuscular mycorrhiza (*Paris* type) and other fungal structures were rarely observed in epidermal cells of the tap roots; the percentage of roots infected by arbuscular mycorrhizal fungi and other fungi was 0.67 ± 0.38% (mean ± SE, $n = 5$). Root bacterial endophytes were isolated from the tap roots of *E. sciadophylloides* by means of the sterile procedure described by Hata (1997). Briefly, subsamples of tap roots were carefully washed with running tap water to remove soil, followed by rinsing with deionized water, then the root surfaces were sterilized in 70% ethyl alcohol for 1 min, in 15% hydrogen peroxide solution for 15 min, and in 70% ethyl alcohol for 1 min. After the surface sterilization, roots were rinsed with sterile deionized water to remove the solvents. The sterilized roots were cut into round slices including epidermal cells (about 10 × 10 × 5 mm pieces), using sterile pruning scissors and a sterile scalpel. We obtained a total of 1193 pieces from the five individuals, and placed half of this sample (597 pieces) on 2% agar plates containing nutrient broth (Difco) (at 1% nutrient broth agar [NBA]) and the remaining 596 pieces on 2% agar plates containing tryptic soy broth (Difco) (at 1% tryptic soy agar [TSA]); thus, about 119 pieces per individual plant were placed on 1% NBA and another 119 were placed on 1% TSA. The agar plates were incubated at 23 °C for 20 days in the dark. After 20 days, root bacterial endophytes were purified by means of serial plating on each medium (191 strains on 1% NBA; 272 strains on 1% TSA).

2.3. Bacterial siderophore detection using the CAS assay

The bacterial siderophores capable of chelating Fe and Al were detected using CAS assay agar plates (90-mm i.d.) using $FeCl_3$ (final concentration, 10 μM) in the CAS Fe assay (Alexander and Zuberer, 1991; Gascoyne et al., 1991) and $AlCl_3$ (final concentration, 10 μM) in the CAS Al assay (Alexander and Zuberer, 1991). Bacterial strains were incubated on CAS Fe and CAS Al assay agar plates for 7 days at 23 °C in the dark. When bacteria produced siderophores, orange halos formed around the bacterial colonies. We measured the diameter of the orange halos and bacterial colonies, and defined

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