Accumulation and Subcellular Localization of Cesium in Mycelia of *Streptomyces lividans* and a Cs Tolerant Strain, *Streptomyces* sp. TOHO-2

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Cs uptake in *Streptomyces lividans* TK24 and *Streptomyces* sp. TOHO-2, as representatives of actinomycetes, was investigated. Growth of *S. lividans* TK24 was inhibited above 50 mM CsCl in yeast extract-malt extract agar, while that of *Streptomyces* sp. TOHO-2, which was isolated from soil as a Cs tolerant strain, was inhibited above 200 mM CsCl. The amount of Cs incorporated into the mycelia of these strains increased with the concentration of Cs in the medium; *S. lividans* TK24, grown in the presence of 25 mM Cs, accumulated 61.5 mgCs/g dry weight of mycelia, and *Streptomyces* sp. TOHO-2, grown in the presence of 150 mM Cs, accumulated 142.2 mgCs/g dry weight of mycelia. Scanning electron microscopy (SEM) of the substrate mycelia grown on the YM agar plate containing CsCl showed white spots with a similar distribution of mycelia. Elemental analysis using SEM-energy dispersive X-ray analysis showed that there were larger amounts of Cs, P and O in the brilliant spots than in other regions of the cell. *Streptomyces lividans* TK24 and *Streptomyces* sp. TOHO-2 could grow even in the presence of high concentrations of Cs, and accumulated high concentration of Cs in the cells.

Key words —— cesium, accumulation, X-ray microanalysis, *Streptomyces lividans*

INTRODUCTION

After the Chernobyl nuclear power station disaster, widespread contamination of the environment by radionuclides occurred in various areas of Europe and elsewhere.[1] The accumulation of large amounts of radioactive cesium (^{137}Cs) in wild mushrooms was reported in many areas of Europe.[2–4] We also found that the concentration of ^{137}Cs in wild mushrooms was higher than in the higher plants growing around these wild mushrooms in Japan.[5]

Our previous studies on the uptake of Cs by fruit bodies and mycelia of saprophytic *Pleurotus ostreatus* (Fr.) Kummer strain Y-1 showed that the amounts of Cs accumulated in *P. ostreatus* were associated with the Cs concentration in the culture media, and the incorporation of Cs^+ was competed with that of K^+ and Rb^+.[6,7] There have been many reports of the accumulation of cesium by microorganisms such as *Escherichia coli*,[8] *Anabaena variabilis*,[9] *Chlorella salina*,[10] and *Saccaromyces cerevisiae*,[11] and it is suggested that Cs uptake occurs *via* K^+ transport in those microorganisms.

Although a trace amount of Cs is found in most living organisms, Cs is a rare metal and soils generally contain only between 0.3 and 25 μg/g.[12] The higher levels of Cs in wild mushrooms suggests a process whereby Cs is transferred directly from the soil to mushrooms and, possibly, indirect transfer from the cells of soil microorganisms that have accumulated Cs.

We investigated Cs accumulation and made an elementary analysis of mycelia using an electron microscope (SEM)-energy dispersive X-ray microanalyzer (EDX) in *Streptomyces lividans* TK24 and a Cs-tolerant strain, *Streptomyces* sp. TOHO-1, as a representative of actinomycetes, one of the soil microorganisms.

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MATERIALS AND METHODS

Microorganisms and Cultivation —— *Streptomyces lividans* TK24 and *Streptomyces* sp. TOHO-2, which was isolated from soil in Japan as a Cs-resistant strain (200 mM), were used. Each organism was incubated in yeast extract-malt extract medium (yeast extract 4 g, malt extract 10 g, glucose 4 g, pH 7.2). Cultivation of the organisms was carried out at 27°C.

Cs Uptake by the Microorganisms ——Each of the strains was preincubated in R2YE medium for 3 d and cultures were used for inoculation. One milliliter of the culture was inoculated into 30 ml YM broth supplemented with a concentration of CsCl in sets of three, then incubated at 27°C for 4 d with shaking (120 rpm). Mycelia were harvested by centrifugation (4000 rpm, 10 min) and washed once with dist. water. Mycelia were re-suspended in 20–30 ml of dist. water and freeze-dried. The dry weight of mycelia was measured, and the mycelia were digested with HNO_3–H_2O_2 at 180°C for 4–5 h. The Cs and K contents of the mycelia were measured by flame photometry (Hekisa Kagaku FP-3B).

Analysis of Cs Distribution in the Mycelia by SEM Equipped with an Energy Dispersive X-Ray analysis System (SEM-EDX) —— *S. lividans* TK24 and *Streptomyces* sp. TOHO-2 were inoculated onto membrane filters (pore size; 0.45 μm) placed on the YM agar containing 10 mM CsCl and incubated for 7 d at 27°C. The mycelial growth on the filters was scraped off and smeared onto carbon double-faced tape stuck on a specimen mount. The specimens were sputter-coated with carbon, and viewed with a Hitachi SEM at an acceleration voltage of 15 kV. Elemental analysis of the mycelia was attempted using a Hitach S-3500N Natural SEM (Hitachi Co. Ltd., Japan) equipped with an EDX system (Horiba EMAX-7000, Horiba Co. Ltd., Japan).

RESULTS AND DISCUSSION

Accumulation of Cs in the Mycelia of *S. lividans* TK24 and *Streptomyces* sp. TOHO2

S. lividans TK24 could grow on the YM agar plate containing 25 mM CsCl, but not in the presence of 50 mM CsCl. *Streptomyces* sp. TOHO-2 could grow even in the presence of 200 mM CsCl. The Cs content of the mycelia obtained from the cultivation of both strains in liquid medium containing various concentrations of CsCl is shown in Fig. 1. In *S. lividans* TK24, the presence of 50 mM CsCl in the medium reduced the final growth yield to about 10%, while 5 and 10 mM CsCl produced almost no growth inhibition. The amount of Cs accumulated in the mycelia increased linearly with a slight decrease in mycelial weight. At 25 mM CsCl, the mycelia had accumulated 61.5 ± 6.9 mgCs/g dry wt, or 6.1% Cs.

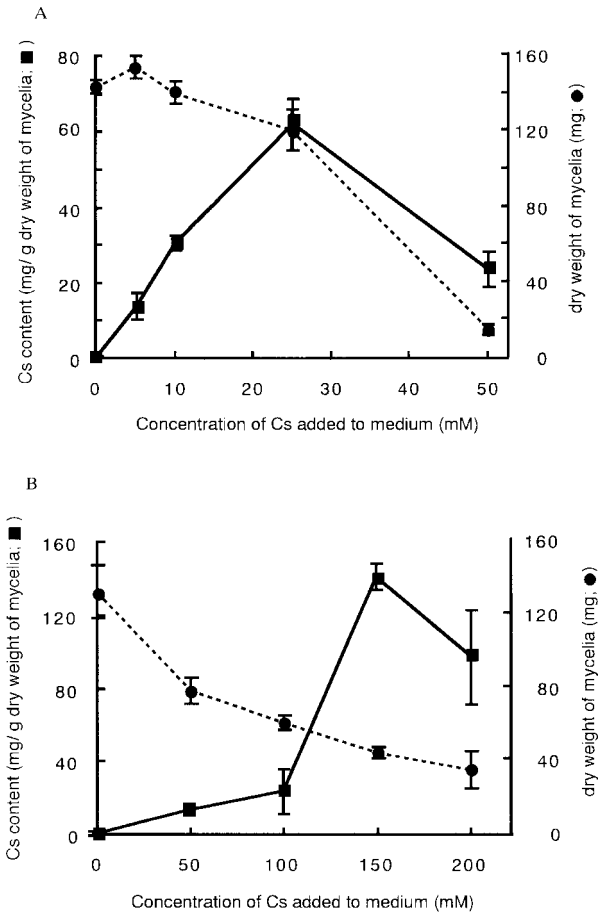

Fig. 1. Growth and Cs uptake in *S. lividans* TK24 (A) and *Streptomyces* sp. TOHO-2 (B)

Cultures of the strains incubated for 3 d in R2YE medium were inoculated into 30 ml YM broth containing various concentrations of CsCl in sets of three, and incubated at 27°C for 4 d. Harvested mycelia were freeze-dried and then digested with HNO_3–H_2O_2. The Cs content of the digest was measured by flame photospectrometry.

In the Cs-tolerant strain, growth inhibition paralleled the CsCl concentration in the medium. The Cs content in the presence of 50 and 100 mM CsCl was 14.8 ± 2.9 and 25.0 ± 12.0 mg/g dry mycelia respectively, lower than that in strain TK24 (61.5 ± 6.9 mg/g dry weight of mycelia in the presence of 25 mM CsCl). In the presence of 150 mM CsCl,

strain TOHO-2 accumulated 142.2 ± 7.1 mg Cs/g dry mycelia and the growth yield was reduced to about 40% that in Cs-free medium. There was a marked increase in Cs accumulation in strain TOHO-2 when it was incubated in the presence of 150 mM CsCl. Derks & Borst-Pauwels (1980) reported the kinetics of concentration-dependent increases in Cs^+ influx in yeast , suggesting the involvement of three sites in the translocation of Cs^+ across the plasma membrane. The marked increase in Cs observed in strain TOHO-2 suggested that there were at least two sites at which Cs^+ was translocated across the membrane, which were dependent on the external Cs^+ concentration. Also the tolerance to Cs in strain TOHO-2 depended on the excretion of the incorporated Cs, a high external Cs concentration resulting in more influx than efflux.

In other bacteria, such as *E. coli* and *B. subtilis*, Cs is incorporated into the cells of these bacteria in the presence of 25 μM Cs and accumulated at a concentration of 0.12–0.53 mg/g dry weight, 0.01–0.05% Cs in the dried cells.[13] Tomioka *et al.* reported that *Rhodococcus* sp. strain CS98 and strain CS402 accumulated 6.9 and 2.5 mg Cs/g dry weight of cells, respectively, when incubated in a medium containing 10 μM Cs/l.[14] Although the concentration of Cs in the medium was different, *S. lividans* TK 24 and *Streptomyces* sp. TOHO-2 accumulated more Cs in the cells than *E. coli*, *B. subtilis* and strains of *Rhodococcus* sp.

SEM-EDX Analysis

Substrate mycelia of *S. lividans* TK24 and *Streptomyces* sp. TOHO-2 grown on agar plates containing 10 mM and 50 mM CsCl, respectively, were observed (Fig. 2). We could see brilliant spots in the mycelia of both strains as shown in Fig. 2, but no such spots were observed in the mycelia grown on Cs-free medium. Most of these brilliant spots were located at intervals of 1.2 to 1.5 μm, suggesting that there was one per cell. It appeared that the Cs incorporated into the cells was assembled in a particular region of the cytoplasm, and irradiation by the electron beam resulted in the brilliant spots in the mycelia.

We analyzed the elements in the brilliant spots by SEM-EDX. As shown in Fig. 3, a clear difference between the elemental composition in areas 1 and 2 was observed. In area 1 containing the spot, distinctive peaks of P and Cs were observed. This result indicated that the Cs incorporated into the mycelia of *S. lividans* was condensed and accumulated in particular regions where the P concentration was high. The mass concentration (%) of the four elements, O, P, K and Cs in area 1 was 65.5, 17.1, 0.5 and 16.9, respectively. Supposing that the concentration of K in the cells remained constant, the ratio of the mass concentration of each element, O, P, K and Cs in area 1 was 131 : 34 : 1 : 34. The corresponding ratio was 48 : 5 : 1 : 8 in area 2. These results indicated that a higher concentration of Cs was accumulated with P and O in the area containing the brilliant spots.

A map of the elements (Cs, P and O) in the mycelia of *S. lividans* TK24 is shown in Fig. 4. Brilliant spots were observed in the scanning electron micrograph (4A), and spots corresponding to the spots in Fig. 4A were observed in the distribution images of

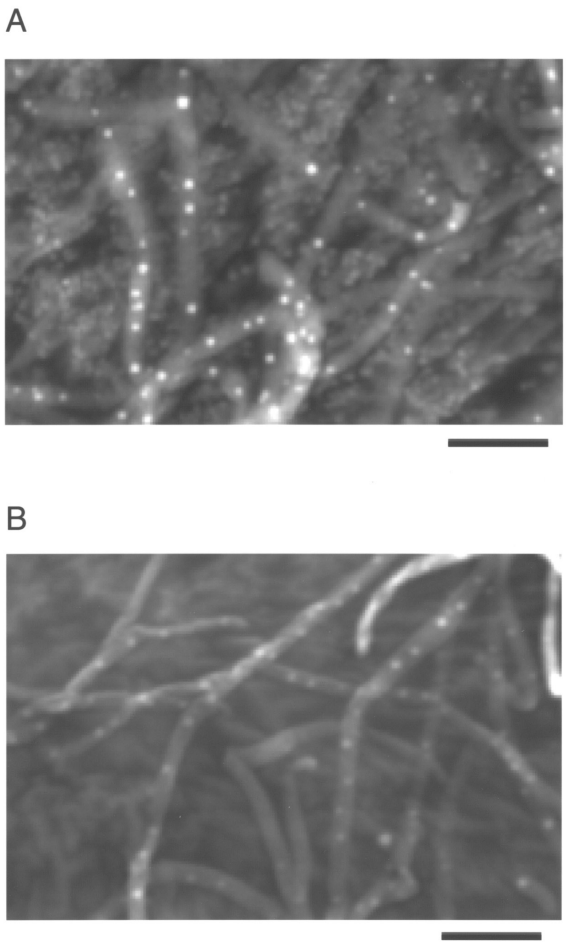

Fig. 2. Scanning Electron Micrographs of the Mycelia of *S. lividans* TK24 (A) and *Streptomyces* sp. TOHO-2 (B)
The organism was inoculated onto membrane filters on YM agar plates containing 10 mM (A) or 50 mM CsCl (B) and incubated at 27°C for 10 d. The mycelia on the membrane filter were subjected to SEM. Bar indicates 5 μm.

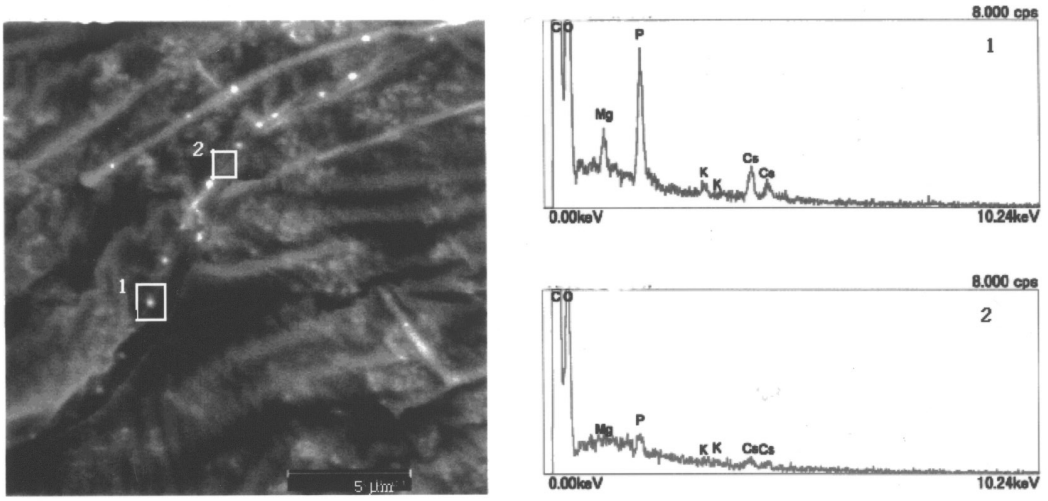

Fig. 3. Element Analysis of the Areas Enclosed by Squares (1, 2)

Elements in the areas of the mycelia of *S. lividans* TK24 grown on YM agar plate containing CsCl were identified by measuring the characteristic energies emitted by electron transitions; C ($K\alpha_1$), 0.277 keV; O ($K\alpha_1$), 0.525 keV; Mg ($K\alpha_1$), 1.253; P ($K\alpha_1$), 2.013 keV; K ($K\alpha_1$), 3.312; Cs ($L\alpha_1$ and $L\beta_1$), 4.286 keV and 4.625 keV.

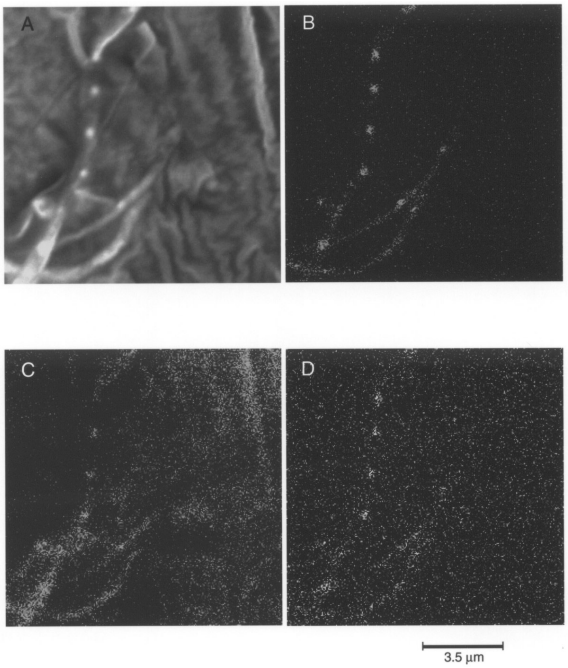

Fig. 4. Element Analysis of the Mycelia of *S. lividans* TK24 Grown on YM Agar Plate Containing 10 mM CsCl

A, Scanning electron micrograph of a mycelium; B, P distribution image; C, O distribution image; D, Cs distribution image. Bar = 3.5 μm.

O (4B), P (4C) and Cs (4D). These results confirmed that the brilliant spots observed in SEM contained higher concentrations of Cs, P and O than other areas of the cell. The fact that Cs accumulated most in a limited area along with P and O in the cells of *S. lividans* TK24 and *Streptomyces* sp. TOHO-2, might be related to the detoxification of Cs in these organisms.

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