Isolation of Natural Pigment Norbadione A from a Gastromycete fungus in Japanese Pine Woods-Possible Applications to the Environmental Decontamination of Radioactive Cesium

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Abstract

A significant amount of pigment was isolated from a mycorrhizal fungus collected from a pine forest in Japan. The pigment was identified as norbadione A (NBA) by NMR spectrometry. Morphological characterization of the fungus and SEM observation of their basidiospores suggested the fungus was identified as *Pisolithus* sp. After solvent extraction and simple purification steps, NBA made up over 10% of the mushroom's dry body weight. NBA is known to form Cs ion complexes more selectively compared to other alkali metal ions. The authors passed NBA through a Solid-Phase Extraction (SPE) column, and the NBA supported SPE column underwent Cs ion adsorption and desorption experiments. The column was shown to adsorb Cs in diluted aqueous solution, as well as successfully recover the Cs ion with diluted hydrochloric acid. The results suggest that an NBA supported SPE system can be a potential option for decontamination of radioactive pollution caused by nuclear plant accidents.

Keywords: norbadione A, *Pisolithus* sp., cesiun, solid extraction, decontamination

1. Introduction

After the nuclear power plant accidents at Chernobyl in 1986 and Fukushima in 2011, extensive emission of radioactive materials occurred [1]. Among the radioactive pollutants, ^{137}Cs and ^{134}Cs have troubling ecological implications because of the long half-life of each (30.0 years for ^{137}Cs and 2.06 years for ^{134}Cs). Cs is a congener of Na and K, both are common alkali metal elements and abundant in the environment. Therefore, decontamination of the comparatively small amounts of radioactive Cs from the environments is difficult. New Cs selective reagents and novel decontamination processes are required.

Several naphthalenoid pulvinic acid type compounds were isolated from fungi fruit bodies [2-4]. Badione and norbadione A were isolated from bay boletus *(Xerocomus badius)* and the brown pileus of the mushroom was shown to concentrate ^{137}Cs after the Chernobyl accident in Europe. The brown pigments, badione and norbadione A were shown to play a role in concentration of ^{137}Cs by bay boletus [5].

We searched for natural sources producing Cs selective ligand, and found a rich Norbadione A (hereafter noted as NBA) producing fungus growing in sea-side pine forests in Japan.

NBA was reported as the major secondary metabolite of *Pisolithus* sp. collected in Australia [2], and shown to be a selective ligand for K and Cs ions. Another pigment, naphthalenoid pulvinic acid related compound pisoquinone, was found later from *Pisolithus arhizus* in Australia [3]. Recently, Kuad *et al*. [6] reported that the stability of alkali metal complexes with NBA decreased in the order $Cs^+>K^+>Na^+$ for mono and bimetallic complexes. Although many researchers were interested in this type of natural Cs ligand, few reports have mentioned a Cs decontamination process using these compounds. Korovitch *et al*. [7] noted that NBA Cs complex formation in aqueous solution is not particularly strong and that the Cs affinity of NBA in the natural mushroom may be an effect of the particular microenvironment of the mushroom. We supported NBA on a hydrophobic resin surface as a hypothetic model of NBA in nature.

The objective of the present study was to evaluate the potential of an NBA supported solid phase extraction (SPE) column on the Cs exchange reaction.

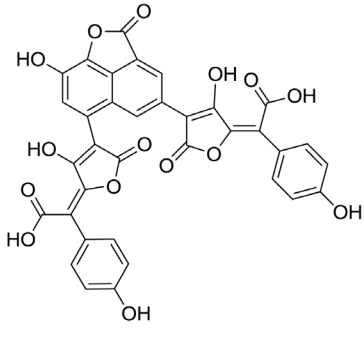

Fig 1 : The structure of norbadione A

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2. Experimental

2.1. Morphological identification of the producing organism

The morphological observation of the fungus fruit body was done by observing not only the outward appearance, but also the cross section of the mushrooms. Bisidiospore of the isolated fungus was examined using scanning electron microscopy (SEM). The basidiospore samples were air dried, held on conductive tape, and spatter coated with gold before examination under a Keyence VE-9800 scanning electron microscope in 3-5KV of accelerating voltage.

2.2. Isolation and identification of NBA

The morphologically characteristic gastromycete fruit bodies were gathered at Nijinomatsubara pine woods, Saga prefecture, Japan in Oct. 2013. The fruit bodies of the fungus were dried both in the air and then in desiccators with silica gel at room temperature until they gained constant weight. These dried fruit bodies were chopped and extracted with acetone acidified by HCl, and the extract was concentrated to give a dark brown solid. The solid material was purified by a Sephadex LH-20 column using 5% DMSO in methanol and the pure fractions were concentrated to a red pigment.

The obtained pigment was analyzed by ^{1}H and ^{13}C NMR spectroscopy using a JEOL JMN-ESC400 spectrometer. Infrared adsorption spectra were recorded on a Perkin Elmer Spectrum 100 spectrometer. The spectra data were compared with previous research [2,8].

2.3. Supporting NBA on SPE column and Cs adsorption-desorption experiment

The solution of NBA in 10% aqueous DMSO was passed through an Oasis HLB column (6 ml of vol., 500 mg of resin wt., obtained from Waters Corp.). The weight of supported NBA was calculated by HPLC analysis of the passed solution.

A CsCl solution in 2mM Tris-buffer (pH 9.0) was passed through the NBA supported column and the Cs concentration of eluent was analyzed by an atomic absorption spectrometer (AAS) using Shimadzu AA-6200 Atomic Absorption Flame Emission Spectrophotometer. After the Cs adsorption, the column was washed with water followed by desorption using 0.1M HCl. Again, the Cs concentration was monitored using AAS analysis.

3. Results and discussion

3.1. Characteristics of the NBA producing fungus

The fresh fruit bodies of the collected gastromycete were cut with a knife and the cross section was observed. A characteristic granular appearance, or periodole structure appeared in the cross section of immature fruit body (Fig.2, lower picture). SEM observation of basidiospore of the fungus is shown in Fig. 3. The visual examination as well as the basidiospore SEM observation confirmed the fungus belongs to genus *Pisolithus* [9], but precise identification requires a molecular-genetic approach.

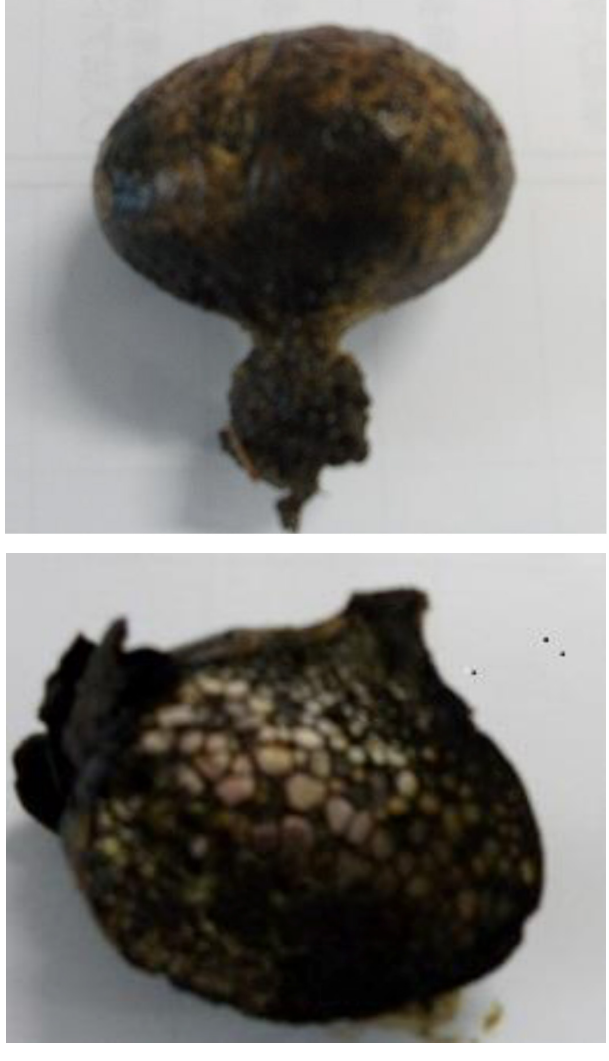

Fig 2 : A fruit body of the collected fungus (the size was 60mm in longitude and 40mm in latitude). Surface (upper) and cross section (lower) observation.

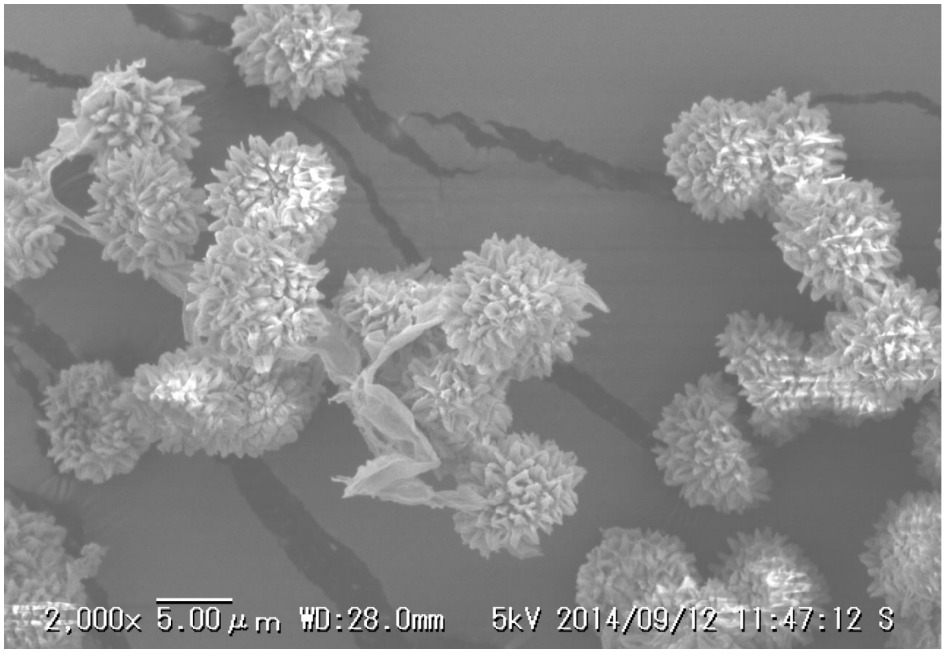

Fig. 3 : SEM observation of basidiospore of the isolated fungus. The scale bar indicates 5.00 μm

3.2. Isolation and identification of the pigment

We extracted and isolated a significant amount of brown pigment from the fruit bodies of a mycorrhizal fungus collected from pine woods. This pigment was confirmed as being norbadione A by ^{1}H and ^{13}C NMR spectrometry as shown in Fig. 4, which is consistent with the previous published data [2,8] . From 420 g of the dried fruit bodies, 44.8 g of NBA was obtained. Surprisingly, a single pigment compound made up over 10% of the dry body weight of the fruit.

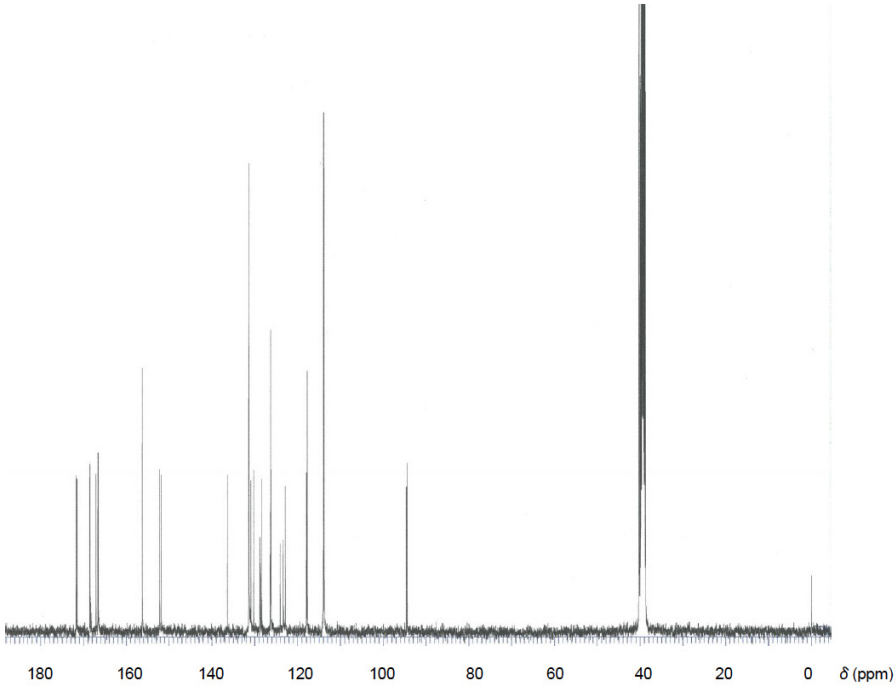

Fig. 4 : ^{13}C NMR spectrum of NBA (100 MHz, DMSO-d6, internal standard TMS)

3.3. Cs ion adsorption and desorption by a NBA supported column

NBA could be supported on an Oasis HLB SPE column by simple adsorption. The amount of supported NBA on a 500mg Oasis HLB column was calculated at 2.3 mg. Aqueous Cs ion was passed through the column in a pH 9.0 buffer solution monitoring Cs^+ concentration of eluent. As shown in Fig. 5, the Cs ion concentration of eluent showed a breakthrough curve suggesting that the NBA supported column could adsorb Cs ions. The amount of adsorbed Cs^+ was calculated as 0.163 mg from the difference of concentration before and after the Cs solution passed through the column. As a reference experiment, same aqueous Cs solution was passed through an Oasis HLB column without NBA support. The SPE column itself did not adsorb any Cs ions.

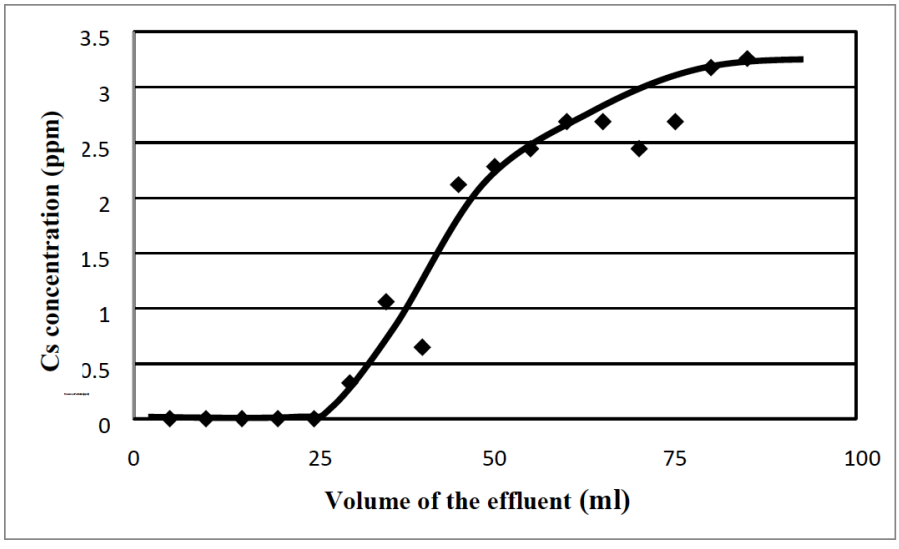

Fig. 5 : The breakthrough curve of Cs ion appeared in the effluent from the NBA supported OASIS HLB column.

After the Cs^+ adsorption, the NBA supported column was washed with water and then with 0.1M HCl, while monitoring Cs^+ concentration. As shown in Fig. 6, the elution pattern clearly demonstrates the quick desorption of Cs^+. The amount calculated from the effluent analysis was 0.160 mg, showing 98% of recovering ratio. This means the NBA supported column has reversible Cs ion exchange ability. The adsorption-desorption experiments were performed several times in reproducible fashion. The re-usability of the NBA supported column was also successfully confirmed.

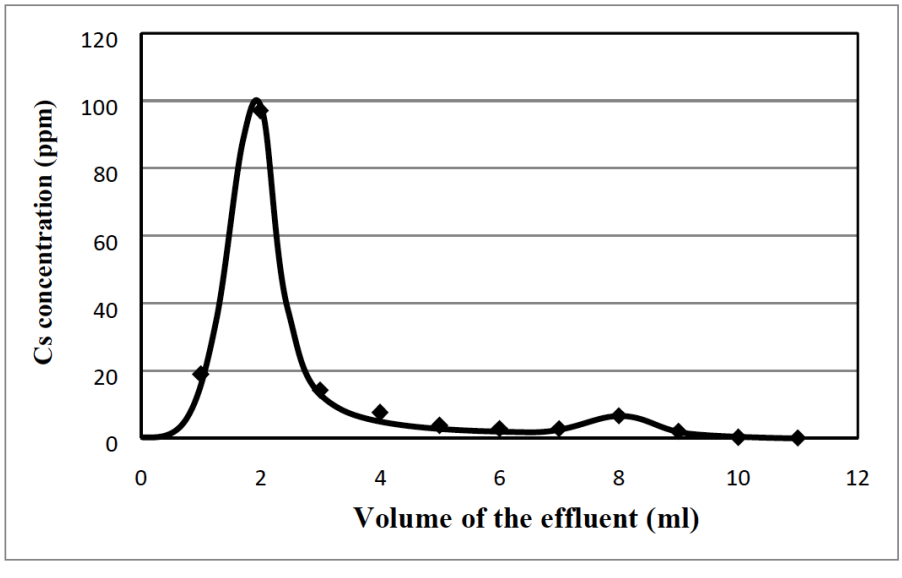

Fig. 6 : The recovery of Cs by 0.1M HCl from the NBA supported OASIS HLB column.

The Cs complex formation at high pH and the immediate complex decomposition in acidic condition is in accordance with the Cs complex formation mechanism as suggested by Kuad, *et al.*[6], where they discussed that NBA conformational change with deprotonation of both carboxylate and enolate functions is essential for the complex formation.

The authors found that the pH dependent adsorption-desorption switching of Cs ions is working in this natural product supported resin. Extensive investigations should be undertaken concerning the proper NBA support materials or conditions to achieve high Cs affinity and high selectivity.

4. Conclusion

The authors found that significant quantities of Cs ligand NBA can be extracted from the fruit bodies of *Pisolithus* sp. found in pine forests in Japan. The NBA supported SPE column showed reversible Cs ion exchange ability, which will be able to be used to capture Cs^+ from diffuse environmental sources and recover enriched Cs^+. The results show a possible new option for decontamination of radioactive pollution caused by nuclear plant accidents.

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