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研究課題名（和文）現場調査と研究室実験を組み合わせた原木シイタケのセシウム移行要因解析

研究課題名（英文）Cesium transfer factor analysis in log-grown shiitake mushrooms combining field surveys and laboratory experiments

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：汚染された原木からシイタケへの放射性セシウム（134Csおよび137Cs）の移行を理解するために、安定セシウム（133Cs）を放射性セシウムの代用として活用した。まず、原木とシイタケの迅速サンプリング方法を開発した後、日本全国から原木を収集してシイタケ菌を接種・培養したの後、可食部を収穫し原木からシイタケへの133Csの移行係数（TF）を調べたところ、新鮮重量基準で約4.0であることが判明した。原木中のK濃度が高いと、133CsとKの競合により133CsのTFが顕著に減少したが、原木中の窒素濃度はTFに有意な影響を与えなかった。加えて、シイタケを実験室で育成する方法の開発も行った。

研究成果の学術的意義や社会的意義
There is now good evidence that high potassium concentrations in logs could be used as a potential mitigating strategy to reduce the uptake of radiocesium in log-cultivated shiitake. We also established a method to grow shiitake in the lab, creating a new avenue for advancing research in this area.

研究成果の概要（英文）：To better understand the transfer of radiocesium (134Cs and 137Cs) between contaminated logs and shiitake, we utilised stable cesium (133Cs) as a proxy for radiocesium. We first developed a rapid method to collect wood and shiitake samples from logs for 133Cs quantification, and then we collected logs from throughout Japan, inoculated these logs with shiitake spawn and measured the transfer factor (TF) of 133Cs from wood to shiitake at harvest time; the TF of 133Cs was approximately 4.0 on a fresh weight basis. The TF of 133Cs significantly decreased with higher concentrations of K in logs, likely due to competition between 133Cs and K. The nitrogen concentration in logs had no significant effect on the TF. Lastly, we developed a method to cultivate shiitake in the laboratory.

研究分野：Mycology

キーワード：Shiitake　133Cs　Transfer factor　oak logs　stable cesium　Potassium　K　Lentinula edodes

様式 C-19,　F-19-1,　Z-19（共通）

1. 研究開始当初の背景

There is a strict limit of radiocesium (^{134}Cs + ^{137}Cs, <50 Bq/kg) permitted in logs by the Japanese Forestry Agency (Forestry Agency, 2012) to ensure the concentration will be lower than the maximum tolerable level of radioactivity in food of 100 Bq/kg set by the Japanese government in 2012 (Ministry of Health, Labour, and Welfare, 2012). Mushrooms, like shiitake, are wood-decaying fungi (= saprotrophic) that can obtain all their nutrients directly from dead trees. Mushrooms can also take up various trace elements (e.g., cesium) and accumulate them in their fruit bodies (= the reproductive structure of a mushroom) (Falandysz & Borovicka, 2013). The ratio of an element in a fruit body to its concentration in a substrate such as wood, known as the transfer factor (TF), is a measure of the ability of a mushroom to accumulate an element such as cesium. A higher TF between log and mushroom increases the probability that radiocesium concentration in shiitake will exceed the 100 Bq/kg level. Mushrooms do not discriminate between stable (e.g., ^{133}Cs) and decaying isotopes (e.g., ^{137}Cs) (Falandysz & Borovicka, 2013). Because ^{133}Cs originates from the mineral component of soil, it is taken up and homogenously distributed within oak trees (Mahara et al., 2014), and thus by determining the TF of ^{133}Cs, we can estimate the log to shiitake TF of ^{137}Cs.

2. 研究の目的

Part 1: The purpose of Part 1 was to determine the TF of ^{133}Cs between logs and shiitake to provide clarity to all stakeholders whether limiting ^{137}Cs in logs to <50 Bq/kg is scientifically sound or not. If the current limit is too low, there is a greater risk to consumers of exposure to high levels of ^{137}Cs from consuming log-cultivated shiitake, however, if the limit is too high the risk to consumers reduces significantly but the recovery of the forestry industry in the less contaminated regions of Fukushima prefecture is unnecessary delayed. We also wanted to know the relationship between K and N in logs and the TF of ^{133}Cs.

Part 2: The purpose of Part 2 was to develop a protocol to cultivate shiitake in the laboratory to produce fruiting bodies. Shiitake is known to be extremely fastidious when it comes to producing fruiting bodies, with only a few records in the literature where researchers succeeded in producing fruiting bodies in a laboratory setting. This protocol will be useful to other researchers who want to investigate how shiitake takes up and moves radiocesium internally through its body.

3. 研究の方法

Part 1A: Develop an efficient method to sample wood and shiitake on logs.

Ten oak logs with ready-to-harvest shiitake fruiting bodies were cut into nine 10-cm discs and each disc was separated into bark, sapwood, and heartwood. All samples were dried, milled and digested with 60% nitric acid and the concentration of ^{133}Cs was measured in the bark, sapwood, heartwood, sawdust (generated from cutting each disc) and fruiting bodies (collected separately from each individual disc), and the wood-to-shiitake transfer factor (TF) was calculated.

Part 1B: Pilot study to determine the TF of ^{133}Cs between logs and shiitake, and factors that may affect the TF

Logs inoculated with shiitake were sourced from 13 locations throughout Japan (n = 2 logs/location). Fruiting was induced in a humidity- and temperature-controlled environment. Using the sampling method developed in Part 1A, representative sawdust and fruit body samples were collected from each log. All log and fruiting body samples were dried, milled, and digested with 60% nitric acid, and then analysed for ^{133}Cs and K concentrations using ICP-MS and ICP-OES, respectively. Nitrogen concentration in logs was measured with a NC analyser. Exchangeable ^{133}Cs and K was also measured in logs by placing the dried and milled samples into 1 M ammonium acetate for 18 hours, and then analysed for ^{133}Cs and K concentrations using ICP-MS and ICP-OES, respectively.

Part 2: Cultivation of shiitake in the laboratory to produce fruiting bodies

The method of **Tan & Moore (1992)** was used to produce shiitake fruiting bodies in the laboratory. However, modifications to the Tan and Moore protocol were necessary to induce fruiting. The shiitake strain, Hokken 600, was incubated under each of the following four main conditions: (1) constant temperature (23°C) + no cold shock, (2) a constant temperature (23°C) + cold shock, (3) fluctuating temperature (12 h @ 10°C & 12 h @ 23°C) + no cold shock, and (4) a fluctuating temperature (12 h @ 10°C & 12 h @ 23°C) + cold shock. We also investigated if growing shiitake in narrow-mouthed conical flasks vs wide-mouthed conical flasks vs plant pots and using cotton vs silicone rubbers plugs could affect fruiting.

4. 研究成果

Part 1A: Develop an efficient method to sample wood and shiitake on logs (full report in O'Brien et al., 2019).

We developed an alternative method to sample logs for stable cesium (^{133}Cs). The previous method involved mechanically breaking the wood into smaller pieces, whereas the new method involved cutting the log multiple times along its length, and the resultant sawdust samples were collected and mixed to produce one representative sample per log. We found that the sawdust-to-shiitake TF of ^{133}Cs did not differ (P = 0.223) compared to either the sapwood-to-shiitake TF or heartwood-to-shiitake TF. ^{133}Cs concentration in sawdust and fruiting bodies collected along the length of the logs also did not differ (P > 0.05). This data shows that sawdust samples can be used instead of wood samples to estimate the log-to-shiitake TF of ^{133}Cs and collecting 12 – 16 fruiting bodies randomly along the length of a log is representative of the shiitake crop growing on the log.

Part 1B: Pilot study to determine the TF of ^{133}Cs between logs and shiitake, and factors that may affect the TF

The average log-to-shiitake TF on a dry weight basis was 22 (range 5 to 35) for total ^{133}Cs and 27 (range 11 – 39) for exchangeable ^{133}Cs; the TF was approximately 4.0 on a wet weight basis. With an increasing concentration of K in logs, the log-to-shiitake TF of both **total ^{133}Cs** (r = -0.570, P<0.01) and **exchangeable ^{133}Cs** (r = -0.576, P<0.01) significantly decreased (Fig. 1A – B). To determine the absorption competition between K and ^{133}Cs, we calculated the ^{133}Cs /K ratio, and found that there was a positive correlation between this ratio and the log-to-shiitake TF of **total ^{133}Cs** (r = 0.538, P<0.01) and **exchangeable ^{133}Cs** (r = 0.328, P>0.05) (Fig. 1C – D). This means that shiitake growing on logs with higher concentration of K will take up less ^{137}Cs. Nitrogen concentration in logs did not correlate significantly with the log-to-shiitake TF of either **total ^{133}Cs** (r = 0.146, P>0.05) or **exchangeable ^{133}Cs** (r = - 0.063, P>0.05).

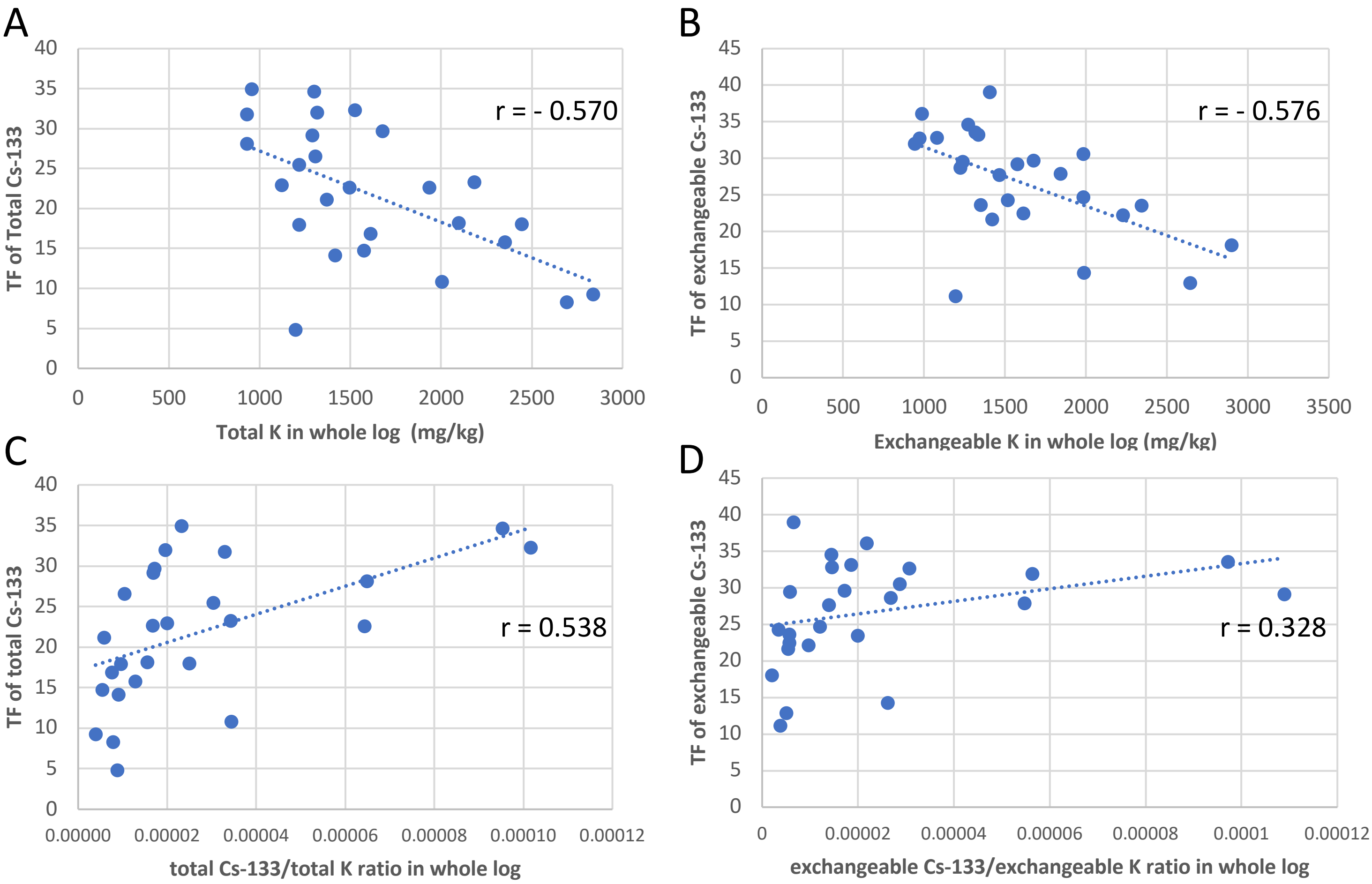

Figure 1. Relationships between the log-to-shiitake TF of total ^{133}Cs and total K in logs (A), log-to-shiitake TF of exchangeable ^{133}Cs and exchangeable K in logs (B), log-to-shiitake TF of total ^{133}Cs and total ^{133}Cs/total K ratio (C), and log-to-shiitake TF of exchangeable ^{133}Cs and exchangeable ^{133}Cs/exchangeable K ratio (D). All values are on a dry weight basis (n = 26 logs).

<u>**Part 2:** Cultivation of shiitake in the laboratory to produce fruiting bodies</u>

Table 1 shows the culturing conditions that allowed us to successfully produce shiitake fruiting bodies. From inoculation to fruiting took an average of 59 days (range: 47 – 71 days). Figure 2 shows examples of the shiitake fruiting bodies produced. The protocol to produce shiitake fruiting bodies in the laboratory will require further optimisation to produce larger fruiting bodies necessary for experimental work.

Table 1. Culturing conditions used to produce shiitake fruiting bodies in the laboratory.

1. **Shiitake stain:** Hokken 600
2. **Cultivation medium:** Leatham medium (after Leatham, 1983)
3. **Amount of medium in flask:** 25 ml
4. **pH of medium:** 4.0
5. **Quantity of Hokken 600 inoculum:** 1 ml (i.e., 1 ml of inoculum was added to 25 ml of medium)
6. **Mycelial biomass in 1 ml of inoculum:** 0.750 mg dry weight
7. **Type of flasks:** Both narrow- or wide-mouth conical flasks (250 ml) were used
8. **Plug type for flasks:** Cotton
9. **Mycelial growth (day 1-30):** temp, 23 C; humidity, 50%; light/dark cycle, 9 h light & 15 h dark
10. **Cold shock to induce fruiting (day 31-33):** temp, 5 C; humidity, n/a; light/dark cycle, 24 h dark
11. **Fruiting period (d 34 – 70):** temp, 9 h @ 10 C & 15 h @ 23 C; humidity, > 80%; light/dark cycle, 9 h light & 15 h dark

Figure 2. Examples of shiitake fruiting bodies produced in our laboratory.

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O'Brien, M., Hiraide, M., Ohmae, Y., Nihei, N., Miura, S., Tanoi, K. (2019). Efficient sampling of shiitake-inoculated oak logs to determine the log-to-mushroom transfer factor of stable cesium. *PeerJ 7*:e7825.

Tan, Y.H. & Moore, D. (1992). Convenient and effective methods for in vitro cultivation of mycelium and fruiting bodies of *Lentinus edodes*. *Mycological Research*, *96*(12), 1077-1084.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 O`Brien, M., Masakazu, H., Ohmae, Y., Nihei, N, Miura, M., Tanoi, K.	４．巻 7
２．論文標題 Efficient sampling of shiitake-inoculated oak logs to determine the log-to- mushroom transfer factor of stable cesium	５．発行年 2019年
３．雑誌名 PeerJ	６．最初と最後の頁 e7825
掲載論文のDOI（デジタルオブジェクト識別子） 10.7717/peerj.7825	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 O`Brien, M., Masakazu, H., Ohmae, Y., Nihei, N, Miura, M., Tanoi, K.
２．発表標題 Sampling oak logs to determine the log-to-shiitake transfer factor of 133Cs
３．学会等名 The 56th Annual Meeting on Radioisotopes and Radiation Research
４．発表年 2019年

１．発表者名 O`Brien, M.
２．発表標題 サンプリング方法の提案：安定セシウムを利用したシイタケ原木におけるセシウム移行に関する研究
３．学会等名 第15回放射能の農畜水産物等への影響についての研究報告会
４．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

Lab. of Radio-Plant Physiology http://park.itc.u-tokyo.ac.jp/radio-plantphys/

6．研究組織

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7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関