ノート（Note）

# *Lyophyllum shimeji* confers morphological changes on the roots of *Populus nigra* and promotes its aboveground growth *in vitro*

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*Lyophyllum shimeji* (Kawam.) Hongo naturally associates with Pinaceae and Fagaceae plants as an ectomycorrhizal symbiont and produces the prized mushrooms “hon-shimeji” (Kawai 1997, Ohta 1994). In addition to the availability of a mycorrhizal nursery plantation system that enables fruiting in the wild (Kawai et al. 1997), *L. shimeji* can be cultivated as spawns for fruiting in the absence of host plants in protected facilities (Ohta 1994), which may allow the fungus to be regarded as a model for edible ectomycorrhizal mushrooms. *Populus nigra* L. (Salicaceae) is a model tree species that naturally harbors both ectomycorrhizal and arbuscular mycorrhizal fungi (Lukac et al. 2003, Biswas et al. 2012, Joner 2013), but it is not regarded as a natural host of *L. shimeji*.

We previously reported that *Prunus speciosa* (Koidz.) Nakai (Rosaceae), which naturally harbors arbuscular-mycorrhizal fungi, allows *Tricholoma matsutake* (S.Ito & Imai) Sing. and *Suillus luteus* (L) Roussel, both of which are ectomycorrhizal symbionts of *Pinus* plants, to form root endophytic but not ectomycorrhizal symbiosis in axenic in vitro dual cultivation (Murata et al. 2014, 2015). Subsequently, we found that *L. shimeji* associates with *Pr. speciosa*, but not ectomycorrhizal ones, as a root endophyte in vitro (unpublished). This turns the lateral roots of *Pr. speciosa* brown and causes its root tips to swell into a beer bottle-like shape; while the hyphae penetrated the lateral root tissues, they did not penetrate the swollen root tips (Fig. 1). We questioned whether *L. shimeji* associates with *Po. nigra* in vitro in the same manner as it does with *Pr. speciosa*. Thus, our aim was to analyze the in vitro association of an unusual plant–microbe combination, which could ultimately prove to be a model plant system.

*Lyophyllum shimeji* YG6L (ATCC 201196, NBRC 100038; Ohta 1994) and somatic *Po. nigra* plants, which were generated through shoot cultures that were derived from the peeled twigs of a mature tree (Mohri et al. 1996, Biswas et al. 2012); were axenically dual cultivated on a granite-based soil substrate in vitro (Murata et al. 2014). Plants and fungal mycelia were individually cultured in the substrate as negative

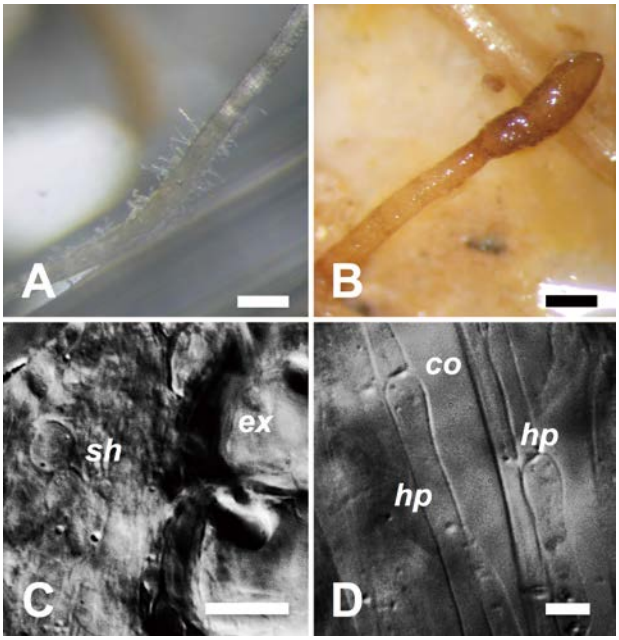


**Fig. 1 *Prunus speciosa* roots colonized by *Lyophyllum shimeji* mycelia *in vitro*.**
A–B, Dissecting micrographs: (A) Roots of *Pr. speciosa* without *L. shimeji*. (B) Roots of *Pr. speciosa* associated with *L. shimeji*. *Scale bars* 1 mm. C–D, Differential interference contrast Nomarski micrographs: (C) Cross-section at the exodermis. (D) Longitudinal section of the root endophyte association between *Pr. speciosa* and *L. shimeji*. Abbreviations: *co* cortical cell, *ex* exodermis, *hp* hyphae, *sh* mycelial sheath. *Scale bars* 10 μM.

ホンシメジ *Lyophyllum shimeji* は閉鎖実験系で、外生菌根共生を伴わずにセイヨウハコヤナギ *Populus nigra* の根の形態を変化させ、植物の地上部の成長を促進する

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controls. Five replicates were conducted for each experimental set. One hundred and sixty days after the incubation period, the root systems were thoroughly washed with water and examined under a microscope. Plant vigor was analyzed by measuring the aboveground and belowground dry weights of each plant.

The lateral roots of *Po. nigra* that were cultivated with *L. shimeji* were brown, while those that were cultivated in the absence of the fungus were pale green (Fig. 2A, B). The lateral roots of *Po. nigra* that associated with *L. shimeji* became swollen (Fig. 2B). The control fungal mycelia that were cultured in the absence of plants did not grow over the substrate, and the inocula remained at the inoculation site. Sections of live, lateral roots of *Po. nigra* that associated with *L. shimeji* were examined under a microscope, and the results showed that the fungus only colonized the lateral root surfaces, forming a mycelial sheath, but it did not penetrate the root tissues (Fig. 2C, D). The plant growth-promoting effect of *L. shimeji* was detected in both the aboveground and the belowground portions (Table 1).

Based on the results of the present study, the in vitro *L. shimeji–Po. nigra* system may not be suitable for elucidating ectomycorrhizal fungus–non-host plant interactions in vitro. However, the root swelling phenomenon, which was consistently observed in *Po. nigra*, as well as *Pr. speciosa*, may be an interesting subject for further analysis in light of the hormones that are produced by *L. shimeji*, which has never been reported in the family Lyophyllaceae, although there is precedence for hormone production by other families belonging to the Agaricomycetes.

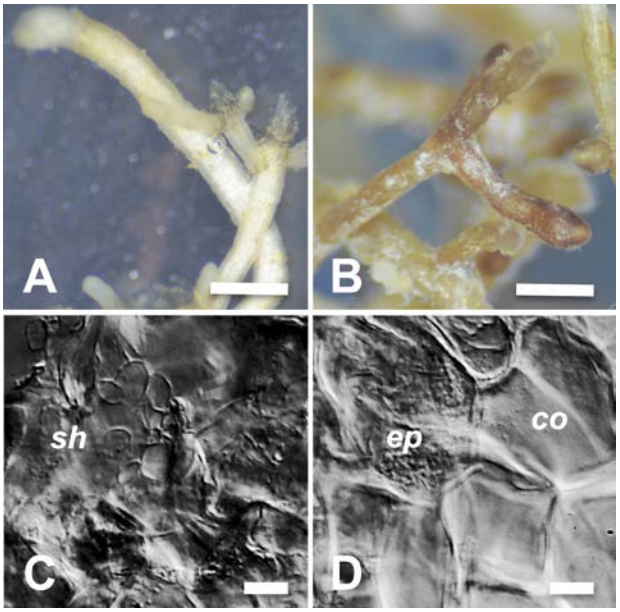


**Fig. 2 The *Po. nigra* root systems associated with *L. shimeji in vitro*.**
A–B, Dissecting micrographs: (A) Roots of *Po. nigra* cultivated in the absence of *L. shimeji*. (B) Roots of Po. nigra associated with *L. shimeji*. *Scale bars* 1 mm. C–D, Differential interference contrast Nomarski micrographs: (C) The mycelial sheath area. (D) The root tissues underneath the mycelial sheath; no fungal mycelia were observed. Abbreviations: *co*, cortical cell; *ep*, epidermis; *sh*, mycelial sheath. *Scale bars* 10 μM.

**Table 1. Parameters related to growth of *Po. nigra* in association with *L. shimeji*.**

| Replicate/ Mean, SE | Measured parameter (dry weight: mg)[a] | | | | | |
|---|---|---|---|---|---|---|
| | *Po. nigra* with *L. shimeji* | | | *Po. nigra* without *L. shimej* | | |
| | Above | Below | Total | Above | Below | Total |
| 1 | 177.5 | 24.7 | 202.2 | 65.0 | 28.1 | 93.1 |
| 2 | 339.0 | 85.4 | 424.4 | 75.9 | 20.6 | 96.5 |
| 3 | 597.8 | 106.5 | 704.3 | 130.6 | 11.0 | 141.6 |
| 4 | 358.3 | 265.3 | 623.6 | 107.5 | 35.7 | 143.2 |
| 5 | 231.2 | 92.6 | 323.8 | 83.4 | 26.0 | 109.4 |
| Mean | 340.8 | 114.9 | 455.7 | 92.5 | 24.3 | 116.8 |
| SE | 72.5 | 40.1 | 92.9 | 11.8 | 4.1 | 10.8 |

[a]Row data of 5 replicates along with their mean and SE are given. Above = aboveground, Below = belowground,Total = Above+Below.

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**References**

Biswas, K. K., Mohri, T., Kogawara, S., Hase, Y., Narumi I., and Oono, Y. (2012) An improved system for shoot regeneration from stem explants of Lombardy Poplar (*Populus nigra* L. var. *italica* Koehne). Am. J. Plant Sci. 3, 1181–1186.

Joner, E. J. (2013) Effects of biotic and abiotic amendments on phytoremediation efficiency applied to metal polluted soils, (eds) Eds Anjum, N. A., Pereira, M. E., Ahmad, I., Duarte, A. C., Umar, S., Khan N. A., Phytotechnologies: remediation of environmental comtaminants, CRC Press Boca Raton FL pp 283–291.

Kawai, M. (1997) Artificial ectomycorrhiza formation on roots of air-layered *Pinus densiflora* saplings by inoculation with *Lyophyllum shimeji*. Myocologia 89, 228–232.

Lukac, M., Calfapierta, C., and Godbold, D. L. (2003) Production, turnover and mycorrhizal colonization of root systems of three *Populus* species grown under elevated $CO_2$ (POPFACE). Global Change Biology doi, 10.1046/ j.1365-2486.2003.00582.x

Murata, H., Yamada, A., Yokota, S., Maruyama, T., Endo, N., Yamamoto, K., Ohira, T., and Neda, H. (2014). Root endophyte symbiosis *in vitro* between the ectomycorrhizal basidiomycete *Tricholoma matsutake* and the arbuscular mycorrhizal plant *Prunus speciosa*. Mycorrhiza 24, 315–321.

Murata, H., Yamada, A., Yokota, S., Maruyama, T., Shimokawa T., and Neda, H. (2015). Innate traits of Pinaceae-specific ectomycorrhizal symbiont *Suillus luteus* that differentially associates with arbuscular mycorrhizal broad-leaved trees *in vitro*. Mycoscience 56, 606–611.

Ohta, A. (1994) Production of fruit bodies of a mycorrhizal fungus, *Lyophyllum shimeji*, in pure culture. Mycoscience 35, 147–151.