Abstracts of papers published in other scientific journals during 2007-2015

Papers with TMI contribution numbers

No. 391

Intersterility between populations of *Lentinula edodes* from Papua New Guinea

Norihiro Shimomura, Kazuhisa Terashima, and Kozaburo Hasebe

Mycosciense **50**: 240-243, 2009.

Mating tests among strains of *Lentinula edodes* distributed in Asia-Australasia were conducted. As a result, 26 strains were classified into three groups: 2 strains from Mt. Wilhelm in Papua New Guinea（PN1 group）showed intersterility with 7 strains from Mt. Albert Edward and Mt. Kaisenik in Papua New Guinea（PN2 group）and semicompatibility（clamp formation restricted to contact zone between paired monokaryons）with 17 strains from Asia-Australasia（AA group）, whereas the strains of the PN2 group showed compatibility with the AA group. These results suggest that the shiitake populations distributed in Asia-Australasia including Papua New Guinea are in the process of speciation.

Key words: geographic lineage, intersterility, *Lentinula edodes*, mating compatibility.

Contribution No.391 of the Tottori Mycological Institute. 菌蕈研究所研究業績 第 391 号.

No. 392

Possibility of three loci for *B* incompatibility factor in *Lentinula edodes*

Norihiro Shimomura, Yoshitaka Shimohiro, Tadanori Aimi and Kozaburo Hasebe

Mushroom Science and Biotechnology **19**: 22-24, 2011.

The genetic constitution of *B* incompatibility factors in *Lentinula edodes* was estimated by a recombination test. We isolated *B* factor recombinants in monosporous isolates from a cultivated strain from Japan and a wild strain from Papua New Guinea. By an intrastrain mating test between *B* factor recombinants, these recombinants were classified into two groups in a cultivated strain from Japan and at least four groups in a wild strain from Papua New Guinea. These results suggest that the third mating type locus, *Bγ*, is linked to *Bα* or *Bβ* in a wild strain from Papua New Guinea and imply that a putative *Bγ* locus is the same in allelic specificity or absent in the two component homokaryons in a cultivated strain from Japan.

Key words: *B* incompatibility factor, *Lentinula edodes*, mating test, recombination.

シイタケの *B* 不和合性因子における 3 遺伝子座の可能性

霜村典宏・下廣慶宜・会見忠則・長谷部公三郎

日本きのこ学会誌 **19**: 22-24, 2011.

シイタケの *B* 不和合性因子組換え型菌株間の交配試験により, 本因子の構成について解析した. 日本産の栽培品種 1 菌株およびパプアニューギニア産野生菌株 1 菌株より *B* 不和合性因子組換え型菌株を分離した. *B* 不和合性因子組換え型菌株間の系統内交配をした結果, 今回用いた日本産栽培品種では 2 群に, そしてパプアニューギニア産野生菌株では 4 群に分類できた. これらの結果から, パプアニューギニア産野生菌株の *B* 不和合性因子においては, *Bα* または *Bβ* に第 3 の座位 *Bγ* が連鎖している可能性が考えられた. また, 日本産栽培品種では *Bγ* がホモであるか, あるいは存在しない可能性があると思われた.

Contribution No. 392 of the Tottori Mycological Institute. 菌蕈研究所研究業績第 392 号.

No. 393

PCR-based gene marker with a high efficiency genome scanning（HEGS）system: application for distinguishing among cultivars in *Lentinula edodes*

Kazuhisa Terashima, Chisato Funato, Teruyuki Matsumoto, Asa Maeda, and Kozaburo Hasebe

World J Microbiol Biotechnol **28**: 1315-1319, 2012.

To establish a high throughput and cost-efficiency procedure for distinguishing among cultivars in *Lentinula edodes*, the polymorphism in the genes reported previously in this fungus was examined using PCR or PCR–RFLP techniques with a high-efficiency genome scanning（HEGS）system. As a result, PCR-based markers derived from eight genes（*tyr, cap, ppa*, *IGS*-RFLP, *pri B*-RFLP, *mfb* C-RFLP, *gla*-RFLP, *xy*-RFLP）showed polymorphisms among cultivars in this fungus and consequently, enabled to distinguish seventy-nine cultivars used in this study.

Key words: the shiitake mushroom, HEGS gene marker, breeder's right, premium production.

Contribution No.393 of the Tottori Mycological Institute. 菌蕈研究所研究業績 第 393 号.

No. 394

Intraspecific comparison of three complete mitochondrial genome sequences from the oyster mushroom, *Pleurotus ostreatus*

Mai Morinaga, Yasuhito Okuda, Rika Takagi, Teruyuki Matsumoto and Yukitaka Fukumasa-Nakai

Mushroom Science **18**: 293-299, 2012. Proceedings of the 18th Congress of the International Society for Mushroom Science.

Mitochondria, ATP-producing organelles in cells, have their own independent genomes called the mitochondrial（mt）genome. The effects of the mt genome structure on cellular phenotype during the fruiting body formation of basidiomycetes are presumably important; however, these effects have not been eluciadated. Intraspecific variation of mt genome structures, which is indispensable information for assessing the effects of mt genome structure, has been assessed mainly by using restriction fragment length polymorphism（RFLP）patterns. Here, we used complete mt genome sequences of *Pleurotus ostreatus* isolates to examine intraspecific variation among mt genome structure among three genome types, I（accession number: AB573642）, II（accession number: AB573692）, and III（NC_009905）, and compared this variation with that determined using RFLP patterns.

The mt genome of each *P. ostreatus* type was a circular DNA molecule ranging in size from 71 947 bp（type I）to 73 242 bp（type III）. Each of the genomes contained 14 common mt genes, one ribosomal small subunit protein 3 gene, one RNA polymerase gene（only in type III）, two DNA polymerase genes, and two rRNA genes. Twenty-four tRNA genes were determined to form a common set among the three types of mt genomes. The type I mt genome had two additional tRNA genes. The gene orders in each mt genome were almost identical, but each had different open reading frames（ORFs）and introns. Comparison of nucleotide sequences showed that there were many substitutions and indels in the intergenic regions. Futhermore, nucleotide sequence differences were observed in gene-coding regions, the majority of which were related to polymorphisms in amino-acid coding sequences. These results indicated both conservation of mt genome primary structure in *P. ostreatus* species and sequence polymorphisms that corresponded to the mtDNA RFLPs.

Key words: comparative genomics, genome organization, mitochondrial genome（mtDNA）, *Pleurotus ostreatus*.

Contribution No. 394 of the Tottori Mycological Institute. 菌蕈研究所研究業績第 394 号.

No. 395

Characterization of vitamin B_{12} compounds in the fruiting bodies of shiitake mushroom (*Lentinula edodes*) and bed logs after fruiting of the mushroom

Tomohiro Bito, Fei Teng, Noriharu Ohishi, Shigeo Takenaka, Emi Miyamoto, Emi Sakuno, Kazuhisa Terashima, Yukinori Yabuta, Fumio Watanabe

Mycoscience **55**: 462-468, 2014. DOI: 10.1016/j.myc.2014.01.008

This study determined the vitamin B_{12} content in commercially available dried fruiting bodies of shiitake mushroom, *Lentinula edodes*. The vitamin B_{12} contents in dried donko-type fruiting bodies with closed caps (5.61 ± 3.90 μg/100 g dry weight), did not significantly differ from those of dried koushin-type fruiting bodies with open caps (4.23 ± 2.42 μg/100 g dry weight). The bed logs after fruiting of the mushroom also contained the vitamin B_{12} levels similar to that in the dried shiitake fruiting bodies. To determine whether the dried shiitake fruiting bodies and their bed logs contained vitamin B_{12} or other corrinoid compounds that are inactive in humans, we purified corrinoid compounds using an immunoaffinity column and identified vitamin B_{12} using vitamin B_{12}-dependent *Escherichia coli* 215 bioautograms and liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) chromatograms. Dried shiitake fruiting bodies rarely contained an unnatural corrinoid vitamin B_{12}[*c*-lactone] that is inactive in humans. Given that shiitake mushroom lacks the ability to synthesize vitamin B_{12} *de novo*, the vitamin B_{12} found in dried shiitake fruiting bodies must have been derived from the bed logs.

Key words: chloramine-T, cobalamin, pseudovitamin B_{12}, vegetarians, vitamin B_{12}[*c*-lactone].

Contribution No.395 of the Tottori Mycological Institute. 菌蕈研究所研究業績 第 395 号.

No. 396

Development of the specific rDNA-ITS and SSR primers to detect the ectomycorrhizal fungus *Rhizopogon roseolus* (In Japanese)

Yasuhito Okuda, Chisato Funato, Norihiro Shimomura and Teruyuki Matsumoto

Japanese Journal of Mycology **55**: 29-34, 2014.

By the comparison of sequences of rDNA-ITS (internal transcribed spacer) region and SSR (simple sequence repeat) loci from 45 isolates of *Rhizopogon roseolus*, the five specific primer sets, one rDNA-ITS primer and four SSR primers, were designed for detection of *R. roseolus*. All these primer sets amplified the specific PCR products for the total DNAs from mycelia of 45 *R. roseolus* isolates, respectively, but no amplified product was detected for the DNAs from host plant (*Pinus thunbergii*) and 16 ectomycorrhizal isolates consist of 13 species including two *Rhizopogon* species, *R. luteolus* and *R. superiorensis*. Furthermore, target regions of these primer sets were also specifically amplified using by DNA templates from ectomycorrhizas of this fungus. Thus, the five sets of primer pair represent a potent tool for the monitoring of introduced isolates of *R. roseolus* and for molecular ecology applications.

Key words: ectomycorrhiza, molecular marker, multiplex PCR, Shoro.

外生菌根菌ショウロの検出用特異的 rDNA-ITS および SSR プライマーの開発

奥田康仁・舩戸知聖・霜村典宏・松本晃幸

日本菌学会会報 **55**: 29-34, 2014.

日本産ショウロ 45 株の rDNA-ITS および SSR 座位領域の配列比較を行い，ショウロに特異的な rDNA-ITS および SSR 座位増幅用プライマーセットを作製した．各プライマーにより，ショウロ 45 株の菌糸から抽出した全 DNA すべてから特異的な PCR 増幅が認められたが，宿主クロマツや本菌と同属の 2 種を含む 13 種 16 株の外生菌根菌の全 DNA では認められなかった．ショウロの菌

根から抽出したDNAからも特異的な増幅が認められた．このことはこれらのプライマーが菌根からのショウロの検出および分子生態学的調査に利用できることを示している．

Contribution No.396 of the Tottori Mycological Institute. 菌蕈研究所研究業績 第396号.

No. 397

Development of a method for rapid strain-typing of a shiitake cultivar, Kinko 115, by high-resolution melting（HRM）analysis（In Japanese）

Asa Maeda, Kazuhisa Terashima and Kozaburo Hasebe

Mushroom Science and Biotechnology **23**: 114-119, 2015.

High-resolution melting（HRM）analysis is a closed-tube method for detecting nucleotide polymorphisms, enabling rapid genotyping. In this study, we have developed a rapid strain-typing method for Kinko 115, a premium shiitake cultivar, by HRM analysis. When HRM analyses of *tyrosinase*（*tyr*）genes of 64 shiitake cultivars were carried out, 3 cultivars including Kinko 115 could be distinguished from another 61 cultivars. After that, HRM analyses of the ribosomal intergenic spacer（IGS）region in these 3 cultivars revealed that Kinko 115 was distinguished from the other 2 cultivars. Consequently, Kinko 115 could be distinguished from other cultivars by HRM analyses of the *tyr* gene and the IGS region. Furthermore, Kinko 115 could be distinguished from the others by HRM analyses using not only purified DNA, but also crude DNA extracts from mycelia or gills of dried fruiting bodies. Thus, the HRM analyses developed in this study would be useful for rapidly and easily identifying Kinko 115.

Key words: high-resolution melting（HRM）analysis, *Lentinula edodes*, premium shiitake cultivar, strain-typing.

High-resolution melting（HRM）解析を用いたシイタケ品種「菌興115号」の迅速DNA品種識別法の開発（和文）

前田亜紗・寺島和寿・長谷部公三郎

日本きのこ学会誌 **23**: 114-119, 2015.

本研究では，High-resolution melting（HRM）解析を用い，各地でブランド化が進行中である，極厚肉で食味に優れた原木シイタケ用品種「菌興115号」の，迅速･簡便な識別法を開発した．まず，チロシナーゼ遺伝子座の3' 末端領域を標的として，64品種のシイタケを解析した結果，菌興115号を他61品種と識別することができた．さらに，IGS領域を標的として残る3品種の解析を行った結果，菌興115号を他の2品種と識別することができた．これらの結果から，上記2箇所の遺伝子領域のHRM解析を行うことにより，菌興115号を他の63品種と区別できることが明らかになった．また，培養菌糸体および乾シイタケのヒダから粗抽出したDNAを用いても菌興ll5号を識別できることを明らかにした．以上の結果から，HRM解析法は，菌興115号の迅速，簡便な検出に有効であることが示された．

Contribution No.397 of the Tottori Mycological Institute. 菌蕈研究所研究業績 第397号.

Papers without TMI contribution numbers

Luminescent *Mycena*: new and noteworthy species

Dennis E. Desjardin, Brian A. Perry, D. Jean Lodge, Cassius V. Stevani and Eiji Nagasawa

Mycologia **102**: 459-477, 2010. DOI: 10.3852/09-197.

Seven species of *Mycena* are reported as luminescent, representing specimens collected in Belize, Brazil, Dominican Republic, Jamaica, Japan（Bonin Islands）, Malaysia（Borneo）and Puerto Rico. Four of them represent new species（*Mycena luxaeterna*, *M. luxarboricola*, *M. luxperpetua*, *M. silvaelucens*）and

three represent new reports of luminescence in previously described species (*M.* aff. *abieticola*, *M. aspratilis*, *M. margarita*). *Mycena subepipterygia* is synonymized with *M. margarita*, and *M. chlorinosma* is proposed as a possible synonym. Comprehensive descriptions, illustrations, photographs and comparisons with phenetically similar species are provided. A redescription of *M. chlorophos*, based on analyses of type specimens and recently collected topotypical material, is provided. The addition of these seven new or newly reported luminescent species of *Mycena* brings the total to 71 known bioluminescent species of fungi.

Key words: Agaricales, bioluminescence, Mycenaceae, mycenoid fungi, taxonomy.

Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*)

Bryn T.M. Dentinger, Joseph F. Ammirati, Ernst E. Both, Dennis E. Desjardin, Roy E. Halling, Terry W. Henkel, Pierre-Arthur Moreau, Eiji Nagasawa, Kasem Soytong, Andy F. Taylor, Roy Watling, Jean-Marc Moncalvo and David J. McLaughlin

Molecular Phylogenetics and Evolution **57**: 1276–1292, 2010.

Porcini (*Boletus* section *Boletus*: Boletaceae: Boletineae: Boletales) are a conspicuous group of wild, edible mushrooms characterized by fleshy fruiting bodies with a poroid hymenophore that is "stuffed" with white hyphae when young. Their reported distribution is with ectomycorrhizal plants throughout the Northern Hemisphere. Little progress has been made on the systematics of this group using modern molecular phylogenetic tools because sampling has been limited primarily to European species and the genes employed were insufficient to resolve the phylogeny. We examined the evolutionary history of porcini by using a global geographic sampling of most known species, new discoveries from little explored areas, and multiple genes. We used 78 sequences from the fast-evolving nuclear internal transcribed spacers and are able to recognize 18 reciprocally monophyletic species. To address whether or not porcini form a monophyletic group, we compiled a broadly sampled dataset of 41 taxa, including other members of the Boletineae, and used separate and combined phylogenetic analysis of sequences from the nuclear large subunit ribosomal DNA, the largest subunit of RNA polymerase II, and the mitochondrial ATPase subunit six gene. Contrary to previous studies, our separate and combined phylogenetic analyses support the monophyly of porcini. We also report the discovery of two taxa that expand the known distribution of porcini to Australia and Thailand and have ancient phylogenetic connections to the rest of the group. A relaxed molecular clock analysis with these new taxa dates the origin of porcini to between 42 and 54 million years ago, coinciding with the initial diversification of angiosperms, during the Eocene epoch when the climate was warm and humid. These results reveal an unexpected diversity, distribution, and ancient origin of a group of commercially valuable mushrooms that may provide an economic incentive for conservation and support the hypothesis of a tropical origin of the ectomycorrhizal symbiosis.

Key words: molecular systematics, molecular clock, biogeography, Boletales, evolution, synapomorphy, partial veil, ITS, RPB1, ATP6, stuffed pores, sustainable non-timber forest product, conservation.

Taxonomic reevaluation of a fungus described as "*Mucidula mucida*" in Japan

Shuji Ushijima, Norihiro Shimomura, Eiji Nagasawa and Nitaro Maekawa

Mushroom Science and Biotechnology **20**: 22-30, 2012.

In Japan, a fungus described as *Mucidula mucida* ("Japanese *M. mucida*", Japanese name: "Numeritsuba-take") was identified as *M. mucida* var. *asiatica* based

on morphological features and phylogenetic analysis of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. This variety differs macromorphologically from *M. mucida* var. *venosolamellata* (Japanese name: "Numeritsubatakemodoki") mainly in that the latter possesses reticulate, anastomosed veins on the lamellae. However, mating compatibility tests between *M. mucida* var. *asiatica* (= "Japanese *M. mucida*") and var. *venosolamellata* appeared compatible in all pairings. Furthermore, the basidiomata derived from the mating-compatible mycelia possessed intermediate macro- and micromorphological features between *M. mucida* var. *asiatica* and var. *venosolamellata*. In our molecular phylogenetic tree, the two fungi formed a clade of "Japanese *M. mucida*"/*M. mucida* var. *asiatica*/*M. mucida* var. *venosolamellata*, and specimens of the two fungi were randomly scattered within this clade. These results strongly suggest that fungi belonging to the clade should not be divided into intraspecific taxa. *Mucidula mucida* var. *venosolamellata* (= var. *asiatica*) differed from *M. mucida* var. *mucida* in their pileipellis structure, basidiospore size and ITS-based phylogenetic analysis. These differences indicate that *M. mucida* var. *venosolamellata* and var. *mucida* are different at the specific level.

Key words: mating compatibility, molecular phylogenetic analysis, morphology, *Mucidula venosolamellata*, taxonomy.

日本産ヌメリツバタケ（*Mucidula mucida*）の分類学的再検討

牛島秀爾・霜村典宏・長澤栄史・前川二太郎

日本きのこ学会誌 **20**: 22-30, 2012.

日本産ヌメリツバタケ（Japanese *M. mucida*）として知られている菌は，形態的特徴および核rDNAのITS領域を用いた分子系統解析の結果, *M. mucida* var. *asiatica* と同定された．本変種は，ひだに皺を持つ日本固有種のヌメリツバタケモドキ（*M. mucida* var. *venosolamellata*）とは，肉眼的特徴に基づいて区別されてきた．しかし，両菌は交配可能であり，両菌の中間的な形態的特徴を示す捻性な子実体を形成した．さらに，ITS領域に基づく分子系統樹において，両菌は1つのクレード内に混在した．これらの結果は，両菌を *M. mucida* の種内分類群として分割すべきでないことを強く示唆する．また，日本産ヌメリツバタケ（ヌメリツバタケモドキを含む）は，担子胞子の大きさ，傘表皮組織の構造およびITS領域を用いた分子系統解析結果において，*M. mucida* var. *mucida* とは明らかに種レベルで異なった．

The genus *Ponticulomyces* (Physalacriaceae, Agaricales) from Japan

Shuji Ushijima, Eiji Nagasawa, Hiroto Suhara, Nitaro Maekawa

Mycoscience **53**: 156-160, 2012. DOI:10.1007/S10267-011-0147-Y

Two species of the genus *Ponticulomyces* collected from Japan for the first time are described and illustrated. *Ponticulomyces kedrovayae* is characterized by its lamellae staining yellow when bruised and in age, stipe lacking an annulus and a pseudorhiza, scattered pileal hairs, and large amygdaliform basidiospores. It mainly occurs on dead wood of *Fagus crenata*. *Ponticulomyces orientalis* is characterized by its scattered pileal hairs and broadly ellipsoid to ellipsoid basidiospores. It was collected mostly on dead wood of *Cameria japonica*.

Key words: Agaricomycetidae, new geographic distribution, *Oudemansiella*, taxonomy, *Xerula*.

Productivity of the toxic substance, fasciculol E, in fruiting bodies of *Hypholoma fasciculare* and some other members of Strophariaceae (In Japanese)

Kumiko Oka, Marina Nishida, Eiji Nagasawa, Shuji Ushijima, Atsushi Ishihara and Nitaro Maekawa

Mushroom Science and Biotechnology **22**: 147-152, 2015.

The poisonous mushroom *Hypholoma fasciculare* (Japanese name: Nigakuritake), belonging to the family Strophariaceae (Basidiomycota, Agaricales), produces fasciculol E as a major toxic agent. Fasciculol E has also been reported from a few related species, but the range of species producing it is not clear. In the present study, the content of fasciculol E in fruiting bodies of *H. fasciculare* and related species in the family Strophariaceae was analyzed by UPLC/MS/MS. The results revealed that all species of *Hypholoma* examined in this study stably produce fasciculol E. Moreover, a very small amount of the toxin was detected from several species belonging to the genera *Pholiota* and *Stropharia*. Of these, some species are known as edible mushrooms, but the amount contained in their fruiting bodies was much lower than the LD_{50} values of fasciculol E. Phylogenetic analysis using the internal transcribed spacer (ITS) region sequences suggests that the productivity of fasciculol E is correlated with phylogenetic distance from *H. fasciculare* within the family Strophariaceae.

Key words: fasciculol E, *Hypholoma fasciculare*, phylogenetic analysis, Strophariaceae, UPLC/MS/MS.

ニガクリタケおよびモエギタケ科属種子実体における毒成分ファシキュロール E の生産性（和文）

岡久美子・西田麻理奈・長澤栄史・牛島秀爾・石原　亨・前川二太郎

日本きのこ学会誌 **22**: 147-152, 2015

モエギタケ科に所属する *Hypholoma fasciculare*（ニガクリタケ）は毒きのこであり，主要な毒成分としてファシキュロールＥを生産する．これまでに本種以外のきのこ種子実体にもファシキュロールＥが含まれていることが報告されているが，詳細な調査は行われていなかった．そこで本研究では，*H. fasciculare* およびモエギタケ科属種子実体におけるファシキュロールＥの含有量を調査した．その結果，供試した *Hypholoma* 属種の子実体すべてよりファシキュロールＥが検出され，本属の近縁属である *Pholiota* 属や *Stropharia* 属種のいくつかの子実体中においても極めて少量であるが検出された．検出された子実体には食用きのこ種も含まれていた．さらに，分子系統解析の結果，ファシキュロールＥ生産性はモエギタケ科内の *Hypholoma* 属との系統的類縁性と密接に関係していることが示唆された．

A new species of *Dactylosporina* (Physalacriaceae, Agaricales) from Japan

Shuji Ushijima, Eiji Nagasawa, Shiro Kigawa, Nitaro Maekawa

Mycoscience **56**: 10-13, 2015. DOI: 10.1016/j.myc.2014.01.012

Dactylosporina brunneomarginata sp. nov., belonging to the family Physalacriaceae (Agaricales) is described and illustrated. This fungus differs from previously described species of *Dactylosporina* macroscopically by its viscid pileus and stipe surface when wet, the lamellae with dark brown edges, and microscopically by its gloeocystidioid pileo-, cheilo-, and caulocystidia with olive to yellow-brown oily content. This is the first report on *Dactylosporina* from Asia.

Key words: Agaricomycetidae, echinulate basidiospores, taxonomy, *Xerula/Oudemansiella* complex.

Relationship between *Panus lecomtei* and *P. strigellus* inferred from their morphological, molecular and biological characteristics

Ruby Vargas-Isla, Marina Capelari, Nelson Menolli Jr., Eiji Nagasawa, Keisuke Tokimoto, Noemia Kazue Ishikawa

Mycoscience **56**: 561-571, 2015. DOI: 10.1016/j.myc.2015.05.004

Panus strigellus was first recorded in Amazonas State of Brazil. This edible mushroom has macroscopic characteristics similar to those of *P. lecomtei*. In this

study, we used evidence obtained from a morphological comparison, molecular analyses and mating tests to clarify the taxonomic status of *P. strigellus* and show differences compared to the sympatric species of *Panus*. In addition, this paper discusses and reports some morphological characteristics of *P. strigellus*. The tetrapolar mating system of *P. strigellus* and the incompatibility between *P. lecomtei* and *P. strigellus* was confirmed. The geographical distributions of both species in the Americas are presented.

Key words: edible mushroom, *Lentinus strigellus*, *Lentinus strigosus*, *Panus rudis*.

Identification and symbiotic ability of Psathyrellaceae fungi isolated from a photosynthetic orchid, *Cremastra appendiculata* (Orchidaceae)

Takahiro Yagame, Eriko Funabiki, Eiji Nagasawa, Toshimitsu Fukiharu and Koji Iwase

Am. J. Bot. **100**(**9**): 1823-1830, 2013. DOI: 10.3732/ajb.1300099

• Premise of the study: Photosynthetic orchids found in highly shaded forests are often mixotrophic, receiving part of their carbon energy via ectomycorrhizal fungi that had originally received carbohydrate from trees. A photosynthetic orchid, *Cremastra appendiculata*, is also found under highly shaded forest, but our preliminary data suggested that its associated fungi were not ectomycorrhizal. We tested whether their relation is an unusual example of a mixotrophic orchid associating with saprotrophic fungi by direct detection of fungal DNAs in conjunction with isolation of the fungus in pure culture and experimental inoculation of orchid seeds with the fungus.

• Methods: For isolated mycobionts of *C. appendiculata* plants, two regions of nuclear ribosomal DNA, the internal transcribed spacer (ITS) and the large subunit (LSU), were sequenced, and fruiting bodies of the one isolate, SI1-1 were induced. In addition, two fungal isolates, SI1-1 and KI1-1, were grown in symbiotic cultures with *C. appendiculata* to verify their status as mycobionts.

• Key results: In phylogenetic analyses, all isolates clustered with fungi belonging to *Coprinellus* in Psathyrellaceae of Agaricales. Phylogenetic analyses of these DNA sequences showed that five fungal isolates from *C. appendiculata*, including SI1-1 and two mycobionts isolated from the mycoheterotrophic orchid *Epipogium roseum*, have very similar ITS sequences. Isolate SI1-1 was identified as *Coprinellus domesticus* based on the morphological characteristics of the fruiting body. Isolates SI1-1 and KI1-1 induced seed germination of *C. appendiculata* as mycobionts.

• Conclusions: This report is the first of a mycorrhizal symbiosis between a fungus in Psathyrellaceae and a photosynthetic orchid, revealing a new pathway to full mycoheterotrophy and contributing to our understanding of the evolution of mycoheterotrophy.

Key words: *Coprinellus domesticus*, mycorrhizal symbiosis, Orchidaceae, photosynthetic orchids, saprobic fungi.

A comparison of dried shiitake mushroom in log cultivation and mycelial cultivation from different geographical origins using stable carbon and nitrogen isotope analysis (In Japanese)

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We determined carbon and nitrogen isotopic compositions (δ^{13}C and δ^{15}N) of dried shiitake mushroom (Lentinula edodes) samples from Japan, China, South Korea and Brazil in order to discriminate their geographical origins. In log cultivation, the δ^{13}C values of Japanese dried shiitake samples were lower than those of Chinese samples, depending on the δ^{13}C values

of log and their growth conditions. In mycelial cultivation, the $\delta^{13}C$ and $\delta^{15}N$ values of Japanese dried shiitake samples were higher than those of Chinese samples. By using the $\delta^{13}C$ and $\delta^{15}N$ values, 87.4% of Japanese dried shiitake samples (n = 95) and 87.9% of Chinese dried shiitake samples (n = 66) in log cultivation, 90.0% of the Japanese dried shiitake samples (n = 50) and 93.9% of Chinese dried shiitake samples (n = 114) in mycelial cultivation, were correctly classified according to the production site. These results suggested that the $\delta^{13}C$ and $\delta^{15}N$ values will be potentially useful for tracing their geographical origin of dried shiitake samples.

Key words: geographical origin, stable isotope ratio, *Lentinula edodes*, Mushroom.

炭素・窒素安定同位体比分析による原木栽培及び菌床栽培乾シイタケの産地間比較（和文）

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原木栽培及び菌床栽培の乾シイタケについて，炭素・窒素安定同位体比（$\delta^{13}C$，$\delta^{15}N$）を用いて，栽培方法及び産地判別の可能性を検証した．原木栽培乾シイタケは，国産は中国産よりも $\delta^{13}C$ は低く，$\delta^{15}N$ は高い傾向が得られ，韓国産は国産と中国産の中間的な値を示した．菌床栽培乾シイタケは，国産は中国産よりも $\delta^{13}C$，$\delta^{15}N$ が高い傾向を示した．ブラジル産については，原木栽培品・菌床栽培品ともにほかの地域よりも $\delta^{15}N$ が高い特徴が得られた．国産・中国産の乾シイタケについて，炭素・窒素安定同位体比分析を行い，産地判別の可能性を検証した結果，原木栽培の正答率は，国産 87.4%，中国産 87.9%，菌床栽培の判別率は，国産 90.0%，中国産 93.9% となった．以上より，原木栽培及び菌床栽培の乾シイタケの $\delta^{13}C$，$\delta^{15}N$ は，栽培方法及び産地によって特徴的な分布を示し，中国産と国産については産地判別の可能性が示唆された．