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Discovering and Domesticating Wild Tropical Cultivatable Mushrooms

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ABSTRACT

Tropical regions have the potential to be one of the richest sources of cultivatable fungal species. Even though there has been considerable research on the taxonomy and phylogeny of these mushrooms, there has been far less research on their domestication. The purpose of this paper is to review and detail the methods we have used for the discovery and domestication of wild tropical mushrooms. As it is difficult to cultivate mycorrhizal species we have mainly concentrated on saprobic species. Methods include collection, isolation, spawn production and fruiting body production testing in sawdust and compost media. We also discuss a semi-industrial approach of inoculating wild edible mushroom spawn into the natural environment to produce seasonal mushrooms. We have collected and isolated numerous strains of species of wild mushrooms and present initial results on domestication attempts. It is hoped to be able to introduce these to the mushroom growing industry in the future.

Keywords: cultivatable mushrooms, domestication, edible mushrooms, fungal species, mushroom spawn, mushroom compost

1. INTRODUCTION

For thousands of years, wild edible mushrooms have been collected and consumed, and provide a major source of income and significant additions to the diets of poor people in developing countries [1]. The use by humans of edible mushrooms 13,000 years ago in Andes has been confirmed through archeological records [2]. The consumption of wild mushrooms in China was first reliably

noted, more than 2,000 years ago [3]. Edible mushrooms were gathered from forests in ancient Greek and Roman times and were highly valued, though more by high-ranking people than by peasants [4]. The British colonial records in Africa contain little information about the local use of wild edible mushrooms, despite the fact that people throughout southern Africa have eaten them for centuries [5, 6].

While commercial harvesting of wild mushrooms continues today, most of the world's supply comes from commercial mushroom growers [1, 7, 8]. The Chinese first cultivated Shiitake (*Lentinula edodes*) mushrooms around 1,100 AD, with domestication efforts beginning century's earlier [7, 8]. The white button mushroom (*Agaricus bisporus*), which are most familiar to Americans and Europeans, was first domesticated in France in 1650 [8, 9]. The first truffle plantation took place in Italy and France in 1970s [10]. For approximately 160 years, *A. bisporus* was grown in open fields. At some point, it was realized that mycelium, or what is referred to as the spawn of the mushroom, was what gave rise to the fruiting bodies and could be utilized much like the seed of plants to grow mushrooms [11-13]. The first cultivation in a cave (i.e. production year round) began in Paris around 1800 and during the 19th century production of spawn by industrial spawn producers began [14]. The first experimental culture of mycelium from spores was carried out by Costantin and Matruchot [15] in Paris at the Pasteur Institute [14, 16], whereas the first strain isolation from cultures from mushroom tissue was by Boyer [17]. The first white cultivar derived from a white capped button mushroom was from a culture in the USA in 1926 [18]; and the first commercial hybrid strains were developed by Fritsche [19]. Commercial production of mushrooms began in the United States in the 1880s [8].

Agaricus is the leading mushroom crop worldwide and accounted for 99% of the United States' mushroom production in 1997 [7, 20]. The oyster mushroom (*Pleurotus* spp.) has been domesticated more recently and now ranks second in world production [21]. The Shiitake mushroom, which is very popular in Asian cultures, ranks third in world production [22]. Many other edible mushrooms, such as the straw mushroom (*Volvariella volvacea*) and wood ear mushrooms (*Auricularia auricula*), are gaining in popularity [7]. It is estimated between 650-700 mushroom species are edible [23], but only approximately 130 have been domesticated (Table 1). The button, oyster, and shiitake mushrooms make up about 70% of the world's production [24]. During the past 30 years, mushroom production worldwide increased twenty-fold, with much of that increase occurring in the 1980s and 1990s [1, 8]. Increased demand for specialty mushrooms has been particularly strong. Asian countries continue to dominate world production and consumption; however, consumption in the United States has increased sharply in recent years, providing potential opportunities for mushroom growers [1, 7, 8].

During the period between 1990-1994, world mushroom production increased by 30.5%, reaching about 4,909 thousand tons in 1994 [25-27]. The global economic value, although difficult to evaluate, has been estimated to be more than 9.8 billion dollars per annum [27]. In Thailand mushroom growing has also increased steadily [28, 29]. The saprobic mushrooms species that to our knowledge are cultivated

in Thailand are listed in Table 2. The Thai government is trying to enhance people's life by encouraging mushroom growing, and an increase of mushroom production at the community level is expected [29].

Even though mushrooms have been domesticated for cultivation since early times, the most commonly grown strains are temperate species [30-32]. Tropical mushrooms are, however, numerous, and recent studies on various genera have shown them to be specious. Numerous new species have been introduced to science with some being edible and other having medicinal use [33-48]. Therefore domesticating tropical mushrooms provides an enormous opportunity for tropical and subtropical countries [1, 49-52]. Most tropical mushrooms grow rapidly and produce fruiting bodies at 25°C or higher and thus can be produced more quickly than temperate species [51]. The tropical mushrooms can also be produced on readily available and cheap waste products such as saw dust, corn cobs, rice straw, sugarcane bagasse, and other forest and agricultural wastes [53]. Therefore growing new tropical mushrooms will help recycle agricultural and forest waste products, provide income to various entrepreneurs and local industries, provide nutritional and medicinal foods and prevent pollution through less dumping and burning of agricultural waste [41, 51, 54].

Several new wild edible mushrooms have been successfully domesticated over the last few years, especially in tropical areas [42, 48, 51, 52, 54-58]. Klomklung *et al*. [51], has shown that it is possible to domesticate local strains of *Pleurotus giganteus* that can grow at temperatures consistent with Thailand farm production. More recently, medicinal mushrooms such as reishi (*Ganoderma lucidum*) and lion's mane (*Hericium erinaceus*) have been introduced to Thailand [29, 51]. A new hybrid strain from Thai and French strains of *Agaricus subrufescens* were developed successfully between INRA, France and Mae Fah Luang University, which fructifies in tropical climates [52].

Mushrooms are not only used in traditional medicines but are known to contain various bioactive components which can be used in cosmetics [29, 59], and medicine [54, 60, 61, 62]. People in most parts of the world enjoy eating mushrooms and therefore there is enormous potential for introducing new tropical mushrooms to the global market. Even though there has been considerable research on the taxonomy and phylogeny of these mushrooms, there has been far less research on their domestication. The purpose of this paper is to review and detail the methods we have used for the discovery and domestication of wild tropical cultivatable mushrooms.

1.1 Conservation of Unknown Genetic Diversity During the Domestication Process

The success of a newly cultivated strain depends on both economical and biological factors. There are two groups of biological factors: the first group relates to mushroom production and the second to mushroom consumption (edibility/toxicity, the nutritional and medicinal aspects, and appetency). Biological factors affecting the success of production depend on the environment and any methods to control it, and equally on genetic factors. These genetic factors depend on the intraspecific variability, of the genetic structure of the population, of epigenetic factors and genomic stability [52]. At each step of the domestication process we have to make a

choice and thus we make a genetic or even epigenetic selection: which field specimens to choose; from which part of the specimen to isolate; which isolate to choose, and later which subculture. There is some experimental knowledge guiding these decisions, however these are also species dependent.

To search for new species that can be cultivated we make some general recommendations that the senior author P. Callac has realized from decades of research in mushroom growing:

1) If several sporocarps are found from the same site make isolates from at least two.

2) If you have a good specimen make at least two isolates, one from the cap and one from the stipe; all isolates must have different identification code numbers even if they come from the same piece of cap of the same sporocarp.

3) Any isolate growing very slowly or developing sectors with different aspects or growth rates can be discarded immediately.

4) Although the optimal conditions for each species is unknown, where possible strain isolation must be made on a culture medium similar the one that will be used to maintain the strain over many years.

5) Avoid taking too much precaution against potential contamination; for example in most cases it is not necessary to use antibiotics in the culture medium to isolate a strain and it is not necessary to use very thin slices of mushroom tissue. Thus the strain may be less fastidious in it sterile requirements when producing fruiting bodies.

6) Obtaining a good isolate is more important than obtaining a spore print, since the isolate will allow you to get a spore print from a future fruit body, while the spore print will never allow you to get the parental strain; however a quickly made spore print from the wild specimen (= the parent) can save time when performing hybridization.

7) For any isolate that successfully passes the fruiting test and which is maintained on non-synthetic medium (such as compost extract medium for *Agaricus*), make in parallel a mycelium growth rate test on a synthetic or semi synthetic reproducible medium (for example 14 days-test on malt agar medium). The growth rate is characteristic of the strain that will be further improved.

8) Each retained strain should be conserved through at least two independent clonal lineages.

Although the above recommendations are general, one should be careful not to generalize results obtained from a single field collection, as these may not be representative of the entire species. For example, isolates from some wild specimens of *A. subrufescens* never produce fruiting bodies when domesticated; in *A. bisporus* isolates from field specimens generally fructify, but some with a very low yield and some have yields as great as the best cultivars (but not their level of quality).

2. RECOMMENDATIONS FOR DISCOVERING CULTIVABLE MUSHROOMS

2.1 Edible Cultivatable Mushrooms

To discover new industrial mushrooms, one must 1) make certain if it is edible and 2) establish if it can readily be cultivated. Information on edibility can be gained from local traditional or scientific knowledge. Local traditional knowledge is the best way to find out whether a species is edible or medicinal. Leaning from indigenous people including local names, seasonality, harvest techniques and habitat management is a short-cut for mushroom identification.

Key informants include local mushroom collectors, housewives, and herbal doctors. A scientific name can also be used to find out whether a species is edible or poisonous, or if it has medicinal or other useful properties [1, 63, 64]. Local traditional knowledge, taxonomic and hybrid knowledge (scientific and local) can also establish whether species are edible. In the genus *Agaricus* all the species are edible except in the section *Xanthodermatei* [42, 65], and excellent edible frequently eaten species belong to sections *Bivelares*, *Arvenses, Sanguinolentes*, and *Agaricus* [40, 41]. Species of *Minores* are also probably good, but generally they are too small [66]. Species of section *Chitonioides* are also sometimes eaten, although they can have a bad smell before cooking. Edibility of other sections is not well known. In *Lentinus*, almost all the species are edible unless they are too hard to eat [40-42]. *Volvariella* species are also edible. Local people did not realize that a giant *Volvariella* growing at the Mushroom Research Centre in northern Thailand was edible (they said no with horror). It was identified using morphology and named as a *Volvariella* species and we were able to safely consume the delicious collection (MRC unpub. data). *Pleurotus giganteus* is considered as a non-edible mushroom by locals in Thailand, but is known as a delicious edible mushroom in Sri Lanka [51, 67].

Once it is established that the collection is edible we must then establish if it can be cultivated as not all mushrooms are cultivatable. Hints to their cultivatability can be gleaned from existing knowledge. If other species in the genus or section of the genus are already cultivated, it is more likely to have success with novel cultivatable species (INRA unpub. data). For an example *Agaricus subrufescens* is a well known edible, medicinal, and cultivated mushroom in the section *Arvenses*. We have collected and described the new species, *Agaricus flocculosipes*, from northern Thailand, in the same section, and obtained successful cultivation results [42, INRA unpub. data]. On the other hand the new species *Agaricus flavicentrum*, which is a delicious member of section *Agaricus* is unlikely to be cultivatable as others species in this section cannot be produced commercially (MFU unpub. data).

Table 1. Presently cultivated mushrooms.

Scientific names	Common names	References
Agaricus arvensis	Horse mushroom	[1, 68]
Agaricus bisporus	Button mushroom	[29, 69-73, 86]
Agaricus bitorquis	Rodman's agaricus	[71, 74, 75]
Agaricus subrufescens	Almond mushroom	[69, 76-78]
Agrocybe chaxingu	-	[79]
Agrocybe cylindracea (=*Agrocybe aegerita*)	Yanagi-mutsutake	[1, 29, 30, 69, 73, 80, 81]
Agrocybe molesta (=*Agrocybe dura*)	Cracked-cap *Agrocybe*	[1, 82]
Agrocybe praecox	-	[1, 30]
Albatrellus spp.	-	[1, 30]

Table 1. Continued

Scientific names	Common names	References
Antrodia cinnamomea	Stout camphor fungus, Zhan ku, Niú zhāng zhī, Kusunoki shiba, Niu-chang-ku, Cinnamomum kanehir, Grib kamphorniy	[83]
Antrodia camphorata	Stout camphor fungus, Zhan ku, Niú zhāng zhī, Kusunoki shiba, Niu-chang-ku, Cinnamomum kanehir, Grib kamphorniy	[83]
Armillaria mellea	Chiodini, honey	[80, 84-86]
Armillaria tabescens		[86]
Auricularia auricula	Wood ear	[25, 73, 80, 86, 87]
Auricularia fuscosuccinea	Ear fungus	[1, 73, 86]
Auricularia nigricans (=Auricularia polytricha)	Black chinese mushroom, Wood ear fungus, Wood fungus, Ear fungus, or Tree ear fungus	[30, 80, 86, 88]
Auricularia cornea	Hairy jew's ear, Wood ear, Cloud ear.	[86]
Auricularia peltata	-	[86]
Auricularia mesenterica	-	[86]
Calvatia gigantea	Giant puffball	[1, 89]
Coprinus comatus	Lawyer's wig, shaggy-mane mushroom	[30, 71, 73, 86, 89]
Coprinus atramentarius	Inky cap	[29]
Cordyceps sinensis	*Cordyceps* Mushroom, Caterpillar fungus	[83]
Cordyceps militaris	Caterpillar fungus, Orange club	[83]
Cordyceps sobolifera	The vegetable fly, Cordyceps cicadae, Insect flower, Crown cicada	[83]
Collybia reinakeana	-	[89]
Creolophus pergamenus	Bear's head	[30, 78]
Daedalea quercina	Oak mazegill, Maze-gill fungus	[1, 90]
Dictyophora duplicata	Netted stinkhorn, Wood witch	[1, 86, 91]
Dictyophora indusiata	Stinkhorn	[80, 91]
Dictyophora rubrovolvata	Stinkhorn	[86]

Table 1. Continued

Scientific names	Common names	References
Flammulina velutipes	Enokitake, Winter mushroom, Velvet stem	[25, 29, 30, 70, 71, 73, 86]
Fomes fomentarius	Tinder fungus, Hoof fungus, Tinder conk, Tinder polypore, Ice man fungus	[1, 91]
Ganoderma applanatum	Artist's bracket, Artist's conk, or Flacher lackporling	[92-95]
Ganoderma australe	Southern bracket, Reishi australe	[83, 92, 94]
Ganoderma curtisii	Southern bracket, Reishi australe	[83, 94]
Ganoderma lucidum	Reishi	[29, 30, 70, 73, 86, 89, 92-94, 96]
Ganoderma oregonense	Western varnish shelf, Oregon polypore, American reishi	[83, 93]
Ganoderma resinaceum	Lacquered bracket, Língzhī resinaceum de, Reishi resinaceum, Ganoderme résineux	[83, 92, 93]
Ganoderma sinense	Black reishi	[1, 83, 86, 93]
Ganoderma tenus		[1, 83, 93]
Ganoderma tsugae	Hemlock varnish shelf	[83, 93]
Grifola frondosa	Maitake	[25, 30, 70, 97]
Grifola albicans	Choreimaitake	[86, 98]
Hericium coralloides	Icicle tooth fungi, Comb tooth, Conifer coral, Coral spine, Coral tooth	[1, 99]
Hericium erinaceus	Lion's mane mushroom	[29, 30, 70, 73, 86, 99, 100]
Hypholoma capnoides	-	[1, 30, 101]
Hypholoma sublateritium	Brick cap	[1, 30, 102]
Hypsizygus marmoreus	Bunashimeji	[25, 86, 100, 103]
Hypsizygus tessulatus	Shimeji	[30, 70, 100, 104]
Hypsizygus ulmarius	Shirotamogitake	[30]
Inonotus obliquus	Chaga, Huà shù gū, Naname inonotus, Polypore cendré, Polypore oblique	[83, 105]
Inonotus levis	NA	[106]
Kuehneromyces mutabilis	Sheathed woodtuft	[1, 107]
Laetiporus sulphureus	Chicken-of-the-woods	[1, 108]
Laricifomes officinalis (*Fomitopsis officinalis*)	Agaric, Agarikon, Quinine conk	[109]

Table 1. Continued

Scientific names	Common names	References
Lentinula edodes	Shii-take	[29, 30, 70, 71, 73, 110]
Lentinus connatus	-	[111, 112]
Lentinus cladopus	-	[111, 112]
Lentinus polychrous	-	[111]
Lentinus sabnudus	-	[111]
Lentinus squarrulosus	Tropical white rot fungus	[89, 111, 112]
Lentinus strigosus (=Panus rudis)	Ruddy panus	[1, 113]
Lentinus tigrinus	-	[1, 89]
Lentinus tuber-regium	King tuber mushroom	[1, 86]
Lepista nuda	Blewit	[71, 73, 114]
Lepista sordid	Flesh-brown blewit	[115]
Lignosus rhinoceros	Tiger milk mushroom, Tiger's milk ling-zhi	[116]
Lyophyllum decastes	Fried chicken, Hatakeshimaji	[80]
Lyophyllum fumosum	-	[1, 117]
Lyophyllum ulmarium (=Hypsizygus ulmarium)	Ulmenräsling, Elm leech	[1]
Macrocybe gigantea (=Tricholoma giganteum)	Giant mushroom	[1, 118]
Macrolepiota gracilenta	Parasol mushroom	[29, 119]
Macrolepiota dolichaula	Parasol mushroom	MFLU unpub. data
Macrolepiota procera	Parasol mushroom	[1, 120, 121]
Marasmius oreades	The scotch bonnet	[1]
**Morchella angusticeps*	Black morel	[1, 122]
**Morchella esculenta*	Common morel, Morel, Yellow morel, True morel, Morel mushroom, Sponge morel	[1, 123]
Naematoloma sublateritivum	Bricktop, Chestnut, Kuritake	[80]
Neolentinus lepideus (=Lentinus lepidus)	Scaly *Lentinus*, Train wrecker	[1, 124]
Oligoporus spp.	-	[1]
Oudemansiella radicata	Rooted *Collybia*, Rooted agaric	[1, 86]
Oudemansiella submucida	Porcelain fungus	[1, 125]
Oxyporus nobilissimus	Giant polypore fungus	[1]
Panaeolus cyanescens	Pan cyan	[30, 71]
Panaeolus subbalteatus	The belted cap *Panaeolus*	[71]

Table 1. Continued

Scientific names	Common names	References
Panaeolus tropicalis	Magic mushroom	[1]
Panellus serotinus	Green oyster, Last fall oyster, Hiratake	[80]
Phallus impudicus	Common stinkhorn	[1]
Phellinus linteus	Black hoof fungus, Fire sponge	[83]
Pholiota nameko	Nameko, Viscid mushroom	[25, 30, 86, 100]
Pholiota adipose	Fat *Pholiota*	[80]
Piptoporus betulinus	Birch polypore,Birch bracket, Razor strop	[1, 126]
Piptoporus indigenous	-	[1]
Pleurocybella porrigens	Angel's wings	[1]
Pleurotus abalones	Abalone	[80, 86]
Pleurotus citrinopileatus	Golden oyster mushrooms	[1, 29, 30, 86, 73]
Pleurotus cornucopiae	Golden oyster	[80, 127, 128]
Pleurotus cystidiosus	Oyster mushrooms, Ohritake	[29, 30, 80, 127, 129]
Pleurotus djamor	Rose, Pink oyster	[30, 80]
Pleurotus eryngii	King oyster mushrooms	[29, 30, 73, 86,127, 128, 130]
Pleurotus euosmus	Oyster mushrooms	[1, 30]
Pleurotus florida	Oyster mushrooms	[127]
Pleurotus ostreatus	Oyster, white mushrooms	[29, 30, 70, 71, 131]
Pleurotus pulmonarius	Phoenix-tail	[30, 80]
Pleurotus rhodophyllus	-	[86]
Pleurotus sajor-caju	Oyster mushrooms, Himarayahiratake, Angel mushroom	[86, 89, 100, 112, 127, 132, 133]
Pleurotus sapidus	-	[69, 73]
Pleurotus giganteus	Seri pagi in Malaysia, Urupaha in Sri Lanka	[73]
Pluteus cervinus	Deer, Fawn mushroom	[1]
Polyporus indigenous	-	[1]
Polyporus grammocephalus	-	[89]
Polyporus saporema	-	[1]
Polyporus umbellatus (= Dendropolyporus umbellatus)	Lumpy bracket, Umbrella polypore	[1, 30]
Poria cocos (Wolfporia extensa)	Hoelen, poria, Tuckahoe, China root, Fu ling, and Matsuhodo.	[69, 134]
Psilocybe cubensis	San Lsidro, Cubensis	[71]
Psilocybe cyanescens	Cyan, Grandote	[71]

Table 1. Continued

Scientific names	Common names	References
Psilocybe mexicana	Mushroom of the gods, Pajaritos	[71]
Psilocybe tampanensis	The tempa *Psilocybe*	[71]
Schizophyllum commune	Split gill	1, 89, 135]
Sparassis crispa	Cauliflower mushroom	[1, 136]
Stropharia rugoso-annulata	The wine red *Stropharia*, The giant *Stropharia*	[30, 69, 71, 73]
Trametes cinnabarina	-	[1]
Trametes versicolor	Turkey tail	[1, 69]
Tremella fuciformis	Snow-fungus, Jelly fungus	[25, 29, 69, 70, 73]
Tremella aurantia	-	[69]
Tremella cinnabarina	-	[73]
**Tricholoma giganteum*	Nioushimeji	[69, 73, 100, 137]
**Tricholoma crassum*	Matsutake crassum	[29]
**Tuber aestivum*	Summer truffle	[80, 138-140]
**Tuber indicum*	Chinese truffle	[138-141]
**Tuber magnatum*	Piedmont white truffle	[80, 140]
**Tuber melanosporum*	Perigord black truffle	[80, 138-140]
Volvariella bombycina	Silky sheath, Silky rosegill, Silver-silk straw mushroom, Tree mushroom	[1, 30]
Volvariella diplasia	Banana, Straw	[80]
Volvariella volvacea	Padi straw mushroom, The Chinese mushroom	[29, 30, 69-71, 73, 89]
V. volvacea var. *gloiocephala*	Padi straw mushroom, The Chinese mushroom	[1, 30]

* Semi-industrial mushrooms

3. METHODOLOGY TO DISCOVER CULTIVABLE MUSHROOMS

3.1 Collection, Description, Identification and Isolation

Collection is important as it will not only result in new species and collections of known species, but will provide a diverse collection of strains. Collection should be carried out at optimal times, which in Thailand is the warm wet season [142]. However, if rains occur in the cool season mushrooms should also be collected as these strains may have differing requirements for fruiting; for an example *Tricholoma crassum* has been collected in the cool season in Thailand [143].

Wild collections of mushrooms are described in detail with macrocharacters being recorded at collection and microcharacters being recorded later from dried material [144, 145]. It is recommended that for every fleshy mushroom to be found, a specimen photograph and an individual Field Data Sheet should be completed [146]. The collected samples are stored separately in aluminium foil. Small

samples are placed in a plastic container with small compartments and returned to the laboratory. Published references are used for correct identification, i.e. Heinemann's taxonomic system [147] for *Agaricus* species which utilizes morphology [146] are recommend publications to use, as is Kerrigan's work on *Agaricus* and molecular approaches [65, 148-154] and for other mushroom genera [155-159]. The method for obtaining cultures is well-established and routinely used by the INRA laboratory and our group. These methods of obtaining cultures from *Agaricus blazei, A. bisporus, Coriolus versicolor, Lentinula edodes*, *Pleurotus ostreatus, P. tuber-regium,* and *Tremella fuciformis* and their cultivation are detailed in Stamets [30, 71]. In addition single spore isolates of the strains can be obtained following the protocols of Raper [160], prior to sub-culture for breeding.

3.2 Media and Long Term Storage

Isolates of mushrooms are grown in a wide range of culture media and used for inoculation spawn and maintaining strains. Most mycologists develop preferences for certain types of media based on experience and peculiarities of the type of fungi/mushrooms that are routinely grown [161]. Media normally affects colony morphology and colour, whether particular structures are formed or not, and may affect whether the fungus/mushroom will even grow in culture [162]. The widely used media for mushroom culture is Potato Dextrose Agar (PDA) [163]. The mushroom mycelia grow well on PDA. Other media: Potato Sucrose agar (PSA), Potato Dextrose Yeast Agar (PDYA), Malt Extract Agar (MEA), Yeast Malt Agar (YMA) and, Mulasaki and Skoog (MS) are also used to culture mushrooms [164, 165].

Maintenance of strains and genetic characteristics of axenic cultures are important for mushroom cultivation. There are various methods to maintain short term and long term viability of strains such as storage in liquid nitrogen, mineral oil, distilled water, or sawdust-freezing method [166-170].

3.3 Testing Cultivability

Approximately 30,000 mushroom species have been described [70] however, less than 150 species are cultivated (Table 1) [1, 30, 71] and in Thailand only about 22

Figure 2. Mushroom cultures on different media.
A. *Agaricus subrufescens* on compost media after 7 days
B. *Agaricus* sp. on PDA media after 25 days
C. *Laetiporus sulphureus* on PDA after 10 days

species are produced industrially (Table 2). The number being cultivated is steadily increasing and information concerning their production is readily available [30].

Over the previous five years, we have isolated several wild mushrooms strains collected from Northern Thailand (e.g. species of *Agaricus*, *Lentinus*, *Lepista*, *Lepiota*,

Table 2. Mushrooms cultivated in Thailand.

Common name	Latin name	Thai name	Market price (THB*/ kg)
Button mushroom	*Agaricus bisporus*	Hed kradum	80-120
Black poplar mushroom	*Agrocybe cylindracea*	Hed yanagi	250-300
Wood ear	*Auricularia auricula*	Hed hu-noo	30-50
Wood ear	*A. polytricha*	Hed hu-noo	20-80
Inky cap	*Coprinus atramentarius*	Hed muerk	120-160
Enokitake	*Flammulina velutipes*	Hed khemthong	150-200
Reishi	*Ganoderma lucidum*	Hed lin juer	1,000-1,500
Lion's mane	*Hericium erinaceus*	Hed hua ling	1,000 (dry)
No common name recorded	*Macrolepiota gracilenta*	Hed nok yoong	400-500
Seri pagi in Malaysia, Urupaha in Sri Lanka	*Pleurotus giganteus*	Hed thon fon	60-100
Oyster mushroom	*P. ostreatus*	Hed nanglom	30-40
Grey albalone oyster	*P. sajor-caju*	Hed nang fah	60-70
King oyster mushroom	*P. eryngii*	Hed nanglom Luang	200-250
No common name recorded	*Lentinus squarrulosus*	Hed khon	50-90
Tropical white rot fungus	*L. polychrous*	Hed lom	120-150
Shii-take	*Lentinula edodes*	Hed mom	160-180
Split gill	*Schizophyllum commune*	Hed klang	80-150 400-500 (dry)
Matsutake crassum	*Tricholoma crassum*	Hed teen rad	100-200
Turkey's tail mushroom	*Coriolus versicolor*	Hed win chu	NA
Song-gen in China, Sang-hwang in Korea, and Mesimakobu in Japan	*Phellinus linteus*	Hed piman	300-3000
Snow fungus, Silver ear fungus, White jelly mushroom	*Tremella fuciformis*	Hed hu noo kao	300-350
Straw mushroom	*Volvariella volvacea*	Hed fang	90-120

*THB (Thai Baht, THB1= USD 0.031 in March 2014
(Kwon and Thatithatgoon [29], [171-174])

Macrolepiota, Oudemansiella, Pleurotus, Gasteromycetes, *Pholiota, Flammulina, Hericium, Coprinopsis*, *Schizophyllum, Agrocybe, Lentinula* and *Volvariella*). Research is being carried out towards the cultivation of these wild strains and possible introduction to the local market. Testing cultivability includes establishing best growth media, best spawn constituents [30, 71] and testing their ability to produce fruiting bodies in compost and sawdust bags. In order to develop mushroom species for cultivation we have adopted and developed certain protocols which we detail herein.

3.4 Cultivation Parameters for the Mushroom Growing

3.4.1 Media

The composition of media is very important when optimizing mushroom mycelia prior inoculate to the spawn. The media can also be used to establish optimum pH, temperature and carbon and nitrogen sources best for the strain of mushroom [161]. It is very important to test the media to find suitable media for copious mycelium growth [175] (Figure 2). The temperature and light should be controlled and the growth diameter measured daily to establish growth rates [164, 176].

3.4.2 pH

The optimal pH for each mushroom is different, for example *Agaricus bisporus* grows well at pH 7.2-8.2 [177], *Pleurotus ostreatus* at pH 7.5-8.5 [178] and *Lentinus edodes* in pH 4-6 [167]. To establish optimal pH conditions for mushroom growth, we should first find out the optimal pH of each mushroom. The pH is adjusted with 1 N HCl for acidity condition and 1 N KOH for alkalinity condition [176, 179]. All mushroom cultures are incubated in the same condition, and the diameter measured daily to establish growth rates.

3.4.3 Temperature

Temperature is important for mycelium growth in both agar media in spawn running and also for fruiting body development. Optimal temperatures can be different for each species and probably strains of the same species [161]. For example, the optimal temperature for fruit body production of *P. eryngii* is 13-18°C, while *P. sajor-caju* produces fruiting bodies at 15-25°C. *Pleurotus cornucopiae* and *P. cystidiosus* produce fruiting bodies well at 30°C. Temperature also affects the colour of the pileus of some mushrooms [127]. To cultivate mushrooms it is therefore essential to establish optimum temperatures for growing mycelium and those at which fruiting bodies are best produced. Normally in topical areas temperatures between 21-26°C or higher are suitable for mushroom growing. It is very important to vary the temperature, media, pH, and light and find out the suitable conditions for the mushrooms, as different mushrooms have different preferences [161, 176].

3.4.4 Carbon and Nitrogen Sources

Carbon (C) and nitrogen (N) are significantly affect mycelial growth and enzyme production in mushrooms [180]. The composition of C and N sources are important for growing mushrooms because they are the major components in growing media [181]. There are several methods for testing suitable C and N ratio in solid or liquid media [174, 176, 182] and glucose, fructose, galactose, starch, lactose, maltose, dextrose, mannitol, xylose, sucrose, citric acid, cellobiose, and yeast extract can be used as C sources. Malt extract, yeast

extract, and peptone, ammonium hydrogen phosphate ($(NH_4)_2 HPO_4$), ammonium nitrate (NH_4NO_3) and calcium nitrate ($Ca(NO_3)_2$) can be used as N sources [176, 180]. Media should be supplemented with each C and N source and the volume depends on the media and mushroom. Subculture of the mushroom mycelia in different media and incubation in the same conditions can be used to establish optimum growth conditions by measuring the diameter of the mushroom colony daily [183].

3.4.5 Spawn Production

Spawn is the media for transfer of the mushroom mycelium to the growing substrate by the cultivator [175]. Depending on the substrate to be inoculated, the vehicle can be grain, sawdust, wood chips, dowels, or rope. There are many types of spawn, including virgin spawn (spawn collect from the pastures & meadows), flake spawn (breaking of beds through which mushroom mycelium has run), mill track spawn (bricks dried and made from mixture of horse dung, cow dung and lawn soil) and manure spawn (on sterilized horse manure or manure compost) [16]. Technological developments have meant that there is good equipment available to produce high quality spawn. For example in INRA we use rye grain spawn made by Euromycel, France [184, 185]. In Thailand we mostly use sorghum as the spawn medium, because it is readily available and inexpensive. The grains of sorghum are cleaned manually to remove inert matter, stubble and debris. The cleaned grains are thoroughly washed and soaked in tap water for 12 hours or overnight. Thereafter, the soaked grains are drained until the excess water is removed. The cleaned dry grains (50 g) are filled in to narrow neck bottles and autoclaved twice at 121°C, 15 psi for 15 minutes. After cooling, the mycelia from the pure mushroom cultures with a portion of PDA are inoculated to sorghum grain medium [183]. The grains invaded by mycelia are used as spawn after the mycelium has invaded all grain medium inside the bottles [183].

3.4.6 Preparing Sawdust Bags

Wood inhabiting mushrooms can be cultivated in any type of lignocellulosic material such as straw, sawdust, and rice hull [186]. It has been shown that oyster mushroom cultivation (*P. ostreatus*) on various sawdust types, gives different fruiting yields [187]. Presently sawdust is commonly used and is the preferred medium at the commercial scale [188]. Furthermore, it has been shown that *P. ostreatus* can give maximum biological efficiency on rubber tree sawdust [187]. Softwood sawdust such as coconut, cashew, mango, and rubber are known to be more suitable than hardwood sawdust [189]. The availability of raw materials (sawdust, rice straw, sugarcane wastes) are key factors for choosing agricultural wastes for growing mushrooms in any region [187]. The most commonly and easily cultivated mushrooms in Thailand and in South East Asian countries are oyster mushrooms, ear mushrooms, and straw mushrooms. *Lentinula edodes, Lentinus* species (e.g. *Lentinus squarrosulus*), *Ganoderma* species (e.g. *Ganoderma lucidum*), *Macrolepiota* sp. (*Macrolepiota gracilenta), Agrocybe* sp. (e.g. *Agrocybe cylindracea,*) can also be cultivated successfully [29]. It is recommended that a newcomer to mushroom cultivation starts with easy to grow and commercially viable mushrooms such as shitake, straw and oyster mushrooms.

In tropical areas different types of

sawdust are used depending on the area and the trees available, with *Hevea brasiliensis* being most popular [29, 51, 190], followed by *Acacia auriculiformis* [191], *Mangifera indica* [180] and *Tamarindus indica*. For 1 kg saw dust bag, 10 g of calcium carbonate, 50 g of rice bran, 10 g of pumice, 10 ml of molasses, 10 g of flour, 10 g of brewers waste are added. The components are mixed with water to make moisture around 65-70% and then 800 g of the substrate are tightly packed in 25 × 8 cm poly propylene bags and capped with plastic ring or bottle neck leaving a space to later inoculate the mycelium [29-30, 51, 100]. The sawdust bags are sealed with cotton wool plugs and covered with newspaper, and tied with a rubber band. The sawdust bags are sterilized at 121°C for 15 minutes or at 90–100°C for 3 h. After the temperature drops down to 25°C, spawn of 10% of weight of the sawdust bag is inoculated to the sawdust bags. Sawdust bags are kept at room temperature (25°C) with humidity around 70-80% in order to produce fruiting bodies [51].

3.4.7 Preparing Compost

The compost process for mushroom cultivation in Thailand is prepared from rice straw as the main substrate. The chopped rice straw is supplemented with rice bran, urea and ammonium phosphate to increase the N source in compost. Gypsum and calcium carbonate are added to compost for buffering the pH to 7.5–8 (Royal Project method, unpub. data). The compost stack is homogeneously mixed and moistened to 70% [water added]. The outdoor process of compost lasts 20 to 25 days with eight turn cycles. The compost is heated to 55–60°C for 3–6 hrs with hot air and steam for pasteurization, which neutralizes all harmful microbes and insects in the compost. It is then cooled for 24 hrs to 25°C, when the compost is generally used and inoculated with spawn.

3.4.8 Trying Out Strains

Fruiting tests of wild strains are important to introduce new mushrooms to market. Depending on the type of the mushroom we use compost or saw dust media. As a rule of thumb, for wood-inhabiting mushrooms we use sawdust media in bags and for soil-inhabiting mushrooms (e.g. *Agaricus, Macrolepiota*) we used rice straw compost.

For wood-inhabiting mushrooms protocols adapted from Klomklung *et al*. [51] and Namphung Klomklung (pers. comm.) are followed. Spawn (2% weight of bags) is inoculated onto the surface of sawdust growing bags. The bags are kept in a dark incubation room at 25 ± 1°C with 70%-80% relative humidity and opened when the mycelium had completely colonized the substrate. The upper portions of the bags are then opened and surface of the substrate is scraped slightly with a sterile teaspoon to remove the thin whitish mycelia. The substrate bags are then placed on the shelf and covered with black cloth to give appropriate ventilation. To maintain 80-85% relative humidity in the growing house, water is sprinkled inside the growing house. Water spraying is carried out daily until pin heads and eventually fruiting bodies fully develop. The fruiting bodies are manually harvested, counted and weighed [51].

For soil-inhabiting mushrooms protocols adapted from Klomklung *et al*. [51] and Naritsada Thongklang (pers. comm.) are followed. Spawn (2% weight of compost) is inoculated into compost media and incubated at 25°C. After the mycelia fully covers and grows throughout

the compost a casing layer is applied [192]. A casing layer can be made from soil and sand (1:1) and mixed with 2% calcium carbonate at a pH 7.5–8 and a thickness of 1–1.5 inch is added and incubated at 25°C for *Agaricus, Lepista,* and *Macrolepiota* [184], Royal Project method, unpub. data. An ideal environment, e.g. 90–95% of humidity at 25–27°C and low CO_2 concentration is maintained. After casing, watering begins usually once a day. The fruiting test is carried out with four replicates trays. The fruiting bodies, including those with open and closed caps, are manually harvested, and counted and weighed [193].

4. SEMI-INDUSTRIAL APPROACH OF INOCLATING WILD EDIBLE MUSHROOM SPAWN INTO THE NATURAL ENVIRONMENT TO PRODUCE SEASONAL MUSHROOMS

4.1 Introducing Edible Mushrooms to Grasslands, Orchards or Forests

Uncertainty of harvests of wild mushrooms from one year to the next makes commercial exploitation difficult and some attempts have been made to overcome this by cultivating high demand mycorrhizal species such as *Tricholoma matsutake,* as then these mushrooms become available year round [23, 194, 195]. Trees have successfully been infected with truffles [194, 196-198] and managed under controlled conditions in Italy and elsewhere to naturally produce these expensive mushrooms [1, 138, 139, 141, 196, 197].

Although industrial cultivation is the only way to provide a year round supply of edible mushrooms, this can be supplemented by semi-industrial methods where the mushrooms are inoculated directly into natural environments and sporulate during ideal weather. This section deals with the semi-industrial methodologies that are useful for saprobic as well as mycorrhizal mushrooms. There have however, been a few studies on inoculating edible mushrooms in natural environments so that they can be naturally harvested [1, 194, 199]. Research on planting mycelia in soils, could lead to the establishment of successful and sustainable production technologies [200]. In this paper we term this semi-industrial mushroom cultivation.

4.2 Choosing Mushrooms and Location for Semi-Industrial Mushroom Cultivation

Saprobic mushrooms colonize and derive their nutrition from rotting wood and organic matter found in soils [1, 201, 202] and there are numerous species that could be utilized in semi-industrial mushroom production. Mycorrhizal mushrooms on the other hand form symbiotic relationships with their host trees and plants and have fundamental roles in determining plant health and in the functioning of forest ecosystems [1, 23, 203]. Tree species can form mycorrhizal associations with more than one fungus, and a fungus may be associated with more than one tree [1, 204-211]. Both types of fungus can be used in semi-industrial mushroom production, but research is need for the best methodologies for each potential species. To semi-industrialize mycorrhizal mushrooms, their spawn is inoculated directly in the soils of specific hosts in forests. As the trees grow, besides timber, a secondary crop of mushrooms is produced. Normally inoculation has to be done twice (spring and autumn) in the first year and it will fruit in the following 20 years [138, 139, 141, 195-197]. Tree seedlings in pots can also be inoculated with mycorrhiza, so that they already have mycorrhizal associations with edible mushrooms when they are

planted out [212, 213].

Inoculating saprobic mushrooms in the soil or specific media and obtaining a harvest may be much easier than inoculating the mycorrhizal mushroom mycelia with the natural host trees and obtaining a harvest [214]. Careful research and consideration is required to select species and location for the application of semi-industrial production of saprobic and mycorrhizal tropical mushrooms [1, 194]. Each edible species is ecology distinct. For example, *Agaricus subrufescens* [54, 60, 184, 215], *Lepiota* and *Macrolepiota* species [46] grow under trees, at the edge of the forest or in grasslands, but mushrooms such as *Tricholoma matsutake* (Matsutake), and *Tuber* spp. (truffles) grow in forests forming symbiotic associations with trees [1, 196, 197]. It is therefore recommended that mushrooms should be inoculated in locations similar to their natural habitats. Trees have been inoculated with mushroom mycelia of a small number of edible tropical species (e.g. *Astraeus* species, *Phlebopus portentosus*) with some success in mushroom production [138, 139, 141, 195-197]. For example *P. portentosus* [176, 216, 217] grows under fruit trees and thus could be planted in mango and other fruit orchards. *Astraeus* species [23] grow in Dipterocarp forests and could be inoculated under the tree species of Dipterocarpaceae [218]. In Northern Thailand spawn of *P. portentosus* is now sold in local markets for inoculating under fruit and other trees, so that locals can obtain a seasonal harvest of these valued mushrooms [176, 219].

4.3 Potential with *Macrolepiota dolichaula*

In Northern Thailand we are attempting to domesticate *Macrolepiota dolichaula* (Hed Nok Yoong). This wild edible species grows abundantly during the rainy season in grasslands. Due to issues surrounding the cultivation of *M. dolichaula* on rice straw substrates, alternative options were investigated, such as planting of mycelia in natural soils. This proved successful, and after a period of 90 days, fruiting bodies were found around the mycelia inoculated area (L.M. Rizal, pers. observation).

4.4 Inoculating Spawn into Soils

Once the spawn is prepared it is inoculated into the soil. Here we describe a basic protocol for inoculating spawn in soils which is adapted from Paul [220]. Mature mycelia from the spawn bottle are directly inoculated in the soil in a 15×45 cm deep pit. The mycelium is covered with soil and litter. The area is marked and watered regularly during the dry season. Each mushroom species has different requirements [200]. Very little data is available concerning the best time to carry out inoculation in natural environments. Accurate information on the ecological niches in which the mushrooms grow is likely to improve the success of inoculation. The main recommendation we could provide at present is to understand the ecology of the fungus and utilize this knowledge in the inoculation protocols.

5. RESULT OF OUR WORK

Testing on media including agar media and spawn production

We have isolated more than 300 collections of edible mushrooms and these are maintained in MFLUCC culture collection. We have also attempted to cultivate more than ten species of tropical mushrooms. The optimal condition for growing mushrooms depends on the species and differs during the various stages of the cultivation process. For example, the suitable temperature for spawn run of *P.*

ostreatus (Figure 3) is 25°C, while primordia formation and fruiting body production were 10–1°C and 10–17°C, respectively [127]. In the case of *P. cystidiosus* the suitable temperature for spawn running is 25-35°C, and primordia formation and fruiting body production is 20–25°C and 25–30°C respectively [127]. Both mushrooms are from the same genus; illustrating how knowledge of optimum conditions for media and spawn running and fructification are important for growing mushrooms.

Figure 3. Effect of temperature on fruit body color of *P. ostreatus* [127].

The genus *Agaricus* is particularly interesting for study as most species are edible and others (e.g. *A. bisporus*, *A. subrufescens*) have medicinal properties [221, 222]. In Thailand we have potentially discovered more than 70 new *Agaricus* species and have isolated several strains (e.g. *A. subrufescens*, *A. flocculosipes*) for fruiting testing. The mycelium was isolated and subcultured on compost agar media and inoculated onto spawn grain media. The spawns were inoculated into standard compost media based on wheat straw mixed with horse manure and various additives like rice bran, gypsum and calcium carbonate. Comparative studies of the cultivation of the Thai (*A. subrufescens* and *A. flocculosipes*) and French strains of *A. subrufescens*, as a control, were carried out. The mycelium fully covered the compost within 15 days and a casing layer was applied. The first primordia of the French *A. subrufescens*, and Thai *A. subrufescens* strains appeared after 12 days, and 24 days after casing, respectively. Significant yields were obtained. The yield of Thai *A. subrufescens* strain was lower than the French control strain, but had a better yield than the Brazilian commercial strain (INRA unpub. data), while *A. flocculosipes* strains produced mushrooms after 32 days. This is the first report of cultivation of a wild strain of the *A. subrufescens* and *A. flocculosipes* collected in Asia (Thailand), and fruiting test as a tool and concluded that it is possible to grow the wild strains [185, 193].

Our effort to grow *Pleurotus giganteus* has been partially successful [51]. *P. giganteus* has been shown to have medicinal properties [223], and the dry weight protein content of *P. giganteus* is 37.8%, which is high in comparison to most other cultivated mushrooms [51, 67]. Our study has also shown that it is possible to domesticate local strains of *P. giganteus* that can grow at temperatures consistent with Thailand farm production [51]. However, the yields were relatively low and methodology or strain selection needs improving to make

industrial *P. giganteus* production commercially viable. Experiments with producing *Clitocybe* sp. using compost medium and *Lentinus connatus* using compost medium and sawdust bags have show that cultivation of these species is feasible (MFLU unpub. data). In the latter case it is interesting that the yield on compost is quite high.

6. CONCLUSIONS

Mushrooms can be found in forests around the world. Given the proper environment, mushrooms will grow and can offer a good source of natural vitamins and minerals, [51, 143, 196, 197, 224-226] generate income and livelihood activities [10, 23]. However, mushrooms can also result in illness or even death to people who are unable to accurately recognize wild mushrooms [56, 58, 143, 227, 228]. Cultivated mushrooms are therefore the preferred and most reliable source of supply of nutrition for the rural and urban poor in developing countries. There are nearly 130 species of saprobic mushrooms that can be cultivated (Table 1). The technique for cultivation is often simple and duplicable particularly for women and housewives. Commercial markets are dominated by *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus* spp. and these accounts for nearly three quarters of the cultivated mushrooms grown worldwide [100]. The majority of cultivated species are grown on a variety of organic substrates, including saw dust, cotton, coffee, rice, maize, and pineapple waste.

Mushroom cultivation offers economic opportunities as well as nutritional and health benefits [27, 229, 230]. Small-scale cultivation takes place throughout China and could provide a suitable model for technology transfer into other developing countries. In the Philippines, Thailand and Vietnam the cultivation of the paddy straw fungus is integrated with rice-based agricultural system [1, 50, 85]. The number of saprobic species being cultivated is steadily increasing and information and practical advice are readily available [30].

The most commonly and easily cultivated mushrooms in South East Asian countries are oyster mushrooms, ear mushrooms, and straw mushrooms. Other types of mushrooms such as *Lentinula edodes, Lentinus* spp., *Ganoderma* spp., *Macrocybe* spp., and *Agrocybe* spp. can also be cultivated successfully, but require more research and refinement [143]. With the latest technologies there are possibilities for expanding the cultivation of edible mushrooms. In most countries, there is a well-established consumer acceptance for cultivated mushrooms such as *Agaricus bisporus*, *A. subrufescens*, *Auricularia* spp., *Pleurotus* spp., *Lentinula edodes,* and *Volvariella volvacea* [217]. Larger-scale methods are unsuitable for local communities that lack the funds to establish large industries. Smaller-scale approaches ("backyard cultivation") are described in Stamets [30] and widely used throughout China. These have a greater potential for rural folk who, for example, cultivate paddy-straw mushrooms as part of integrated farming systems in Vietnam, and Philippines [1, 50]. As a conclusion mushroom cultivation can create a valuable contribution to sustainable livelihoods for both rural and urban poor, because they are highly compatible with other livelihood activities, requiring minimal physical and financial inputs and resources [1, 50]. Future research should also concentrate on the nutritional value [231-234], biochemical and medicinal properties [60-62, 225-227, 235, 236] for those mushrooms we can newly cultivate.

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