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EDIBLE MUSHROOM CULTIVATION AT THE INSTITUTE OF ECOLOGY IN MEXICO

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ABSTRACT

Research work on mushroom cultivation at the Institute of Ecology (INECOL) began in 1989 cultivating *Pleurotus* spp. At present, these studies have been extended to other genera including *Lentinula* and *Volvariella*. Different agricultural and forest by-products have been evaluated as substrates for commercial cultivation. More than one hundred strains of *Pleurotus* have been studied in order to select germplasm suited for commercial production. Hybrids obtained by crossing selected monokaryons give yields greater than the parents in reduced culture cycles. Some strains of *Lentinula edodes* have been cultivated on pasteurized non-conventional substrates, such as coffee pulp, sugar cane bagasse, and wheat straw, showing good adaptation to this process. The strain collection (INECOL) has over 300 strains of edible mushrooms, and nearly 100 strains of phytopathogenic fungi. More than 60% of them come from wild Mexican specimens, mainly isolated from tropical and subtropical regions. Training courses and workshops have been carried out in order to share practical experience, and to offer technical support and basic services to growers from Mexico and other countries of Latin America. Recently, some aspects of enzyme production and physiological adaptation have been investigated in *Pleurotus* and *Lentinula*. The antagonistic relations between mushrooms and other fungi (mainly *Trichoderma* spp.) are also important topics. Strain selection for greater yields, physiological adaptation to different substrates, disease resistance, spawn improvement, and germplasm conservation are basic lines for future research.

Key words: **Edible mushroom cultivation, *Pleurotus*, *Lentinula*, *Volvariella*, Latin America, agricultural and forest by-products, interbreeding, strain collection.**

INTRODUCTION

In Mexico, many species of fungi have been reported as edible and some of them are consumed since prehispanic times. Even today, they still maintain their original indigenous names [6]. The names of some towns show the importance of mushrooms in Mexico. For example, the Aztec name for mushrooms *nanacatl*, a word meaning "meat", appears in communities as *Nanacatepec* (mushroom hill) and *Nanacamilpa* (the place where mushrooms grow). Despite the enormous traditional knowledge about mushrooms in Mexico, there is no evidence that edible species were being cultivated in prehispanic times. Modern research on mushroom production began only a few decades ago. However, in the 1980s these investigations received an important impulse and different research groups were formed [17].

In the mid-1980s, a small facility for producing edible mushrooms was built at the *Instituto Nacional de Investigaciones sobre Recursos Bióticos* (INIREB). This experimental pilot plant was originally supported by the National Council of Science and Technology (CONACYT), under the supervision of G. Guzmán and D. Martínez-Carrera [8]. A laboratory of edible mushroom cultivation was also created. Both units served as a model for creating new production facilities, both for research and commercial production purposes [16, 18]. Since 1989, these facilities have been administrated by the Department of Mycology at the Institute of Ecology (INECOL). Recently, the research work carried out in these facilities has been focused on: 1) Strain selection and adaptation to different substrates; 2) The establishment of a collection to maintain strain viability over long periods of time, evaluating different conservation methods; 3) The support to conventional taxonomic research; and 4) The promotion of cultivation techniques across Latin America by training courses. In addition, cooperation with commercial growers has also been a very important subject.

ADAPTATION TO DIFFERENT CULTIVATION SUBSTRATES AND STRAIN SELECTION

More than 100 strains of *Pleurotus* have been cultivated, mainly strains of *P. ostreatus* (Jacq. : Fr.)Kumm., *P. columbinus* Bres., *P. pulmonarius* (Fr.)Quél., and *P. djamor* (Fr.)Boedijn (pink and white ecological varieties). Some strains have been obtained by crossing selected monokaryons [3, 4, 32, 33]. Notably, we have obtained significant reductions in culture cycle times and biological efficiencies greater than 100% in several strains of *Pleurotus* (**Table 1**).

Different agricultural by-products have been used as substrates for *Pleurotus* spp. cultivation [20, 29, 37]. Among them, coffee pulp is perhaps the most important agricultural residue in Mexico, particularly in the State of Veracruz. Mexico is the fifth largest producer of coffee in the world, and the state of Veracruz accounts for about 30% of the national production of coffee. Although the pulp represents more than 40% by volume of the coffee fruit, its commercial utilization has been limited [30]. At present, we have diversified our studies, including other genera such as *Lentinula*,

Table 1. Strains of *Pleurotus* spp. obtained by intercrossing in the laboratory, which showed the highest yields on barley straw.

Strain	BE (%)	Reference
P. djamor		
IE-202	123	[4]
IE-220	101	
P. ostreatus		
IE-240	119	
IE-241	100	
P. pulmonarius		
IE-226	105	
IE-227	105	

BE= Biological efficiency.

Neolentinus and *Volvariella* in our investigations [1, 2, 23, 34, 35].

Several strains of *Lentinula edodes* (Berk.)Pegler (the Japanese shiitake) have adapted very well to different substrates, including coffee pulp, sugar cane bagasse, and wheat straw, reaching biological efficiencies greater than 100% (**Table 2**) [19, 34]. In addition, wild strains of *L. boryana* (Berk. & Mont.)Pegler (the "American shiitake"; common from the southeastern United States to South America) are under evaluation for commercial production [22, 23].

Recent efforts have been directed towards obtaining mushroom strains that grow rapidly on non-conventional substrates, mainly agricultural by-products. Selected strains show high yields of fruiting bodies on non-sterilized substrates. Additional studies have been undertaken on strains of *Pleurotus* and *Lentinula* that show good cultivation possibilities on pine wood residues [2, 29]. A few strains of *P. pulmonarius* and *L. edodes* have shown biological efficiencies for commercial production purposes over relatively short periods of time [38].

STRAIN COLLECTION

The strain collection at the INECOL has over 300 edible mushroom strains, and nearly 100 strains of phytopathogenic fungi. More than 60% of these strains originate from wild Mexican specimens that were isolated from tropical and subtropical regions. The collection is registered in the World Data Center for Microorganisms with the number 782 [36].

The section of edible mushrooms currently maintains more than 30 strains of mushrooms that were selected through genetic improvement. The following taxa are included: *Agaricus* [*A. bisporus* (Lange)Imbach, *A. bitorquis* (Quél.)Sacc.], *Auricularia*, *Flammulina velutipes* (Curt.:Fr.)Sing., *Ganoderma applanatum* (Pers.:Wallr)Pat., *Grifola frondosa* (Dicks:Fr.)S.F.Gray, *Hypsizygus tessulatus* (Bull.:Fr.)Sing., *Lentinula* (*L. boryana, L. edodes*), *Lepista*, *Laetiporus sulphureus* (Fr.)Murr., *Morchella*, *Neolentinus suffrutescens* (Brot.:Fr.)May &

Table 2. Biological efficiencies reached by shiitake strains grown on agroindustrial by-products available in some regions of Mexico.

Substrate	BE (%)	Reference
Sugar cane bagasse	130-133	[34]
Sugar cane leaves	83-98	[34]
Coffee pulp	63	[19]
Bracts of pineapple crown	36-37	[34]
Wheat straw	25-55	

BE= Biological efficiency.

Wood, *Pleurotus* (*P. cystidiosus* Miller, *P. eryngii* (DC.:Fr.)Quél., *P. djamor*, *P. columbinus*, *P. ostreatus*, *P. pulmonarius*, *P. smithii* Guzmán), and *Volvariella volvacea* (Bull.:Fr.)Sing (**Fig. 1**). Some of these species are cultivated commercially in many places around the world. All strains are stored in liquid nitrogen, using spawn stocks as substrate for freezing. Several strains of *Pleurotus* and *Lentinula* have been evaluated for viability and productivity after freezing in liquid nitrogen [14, 15, 25, 26, 28]. Results have confirmed the high efficiency of freezing spawn prepared from gramineous seeds. This technique permits a rapid recovery of mycelia from just a few grains of frozen spawn. Furthermore, no morphological variations or reductions in productivity have been recorded in the strains recovered, even after 1, 3 and 5 years of frozen storage.

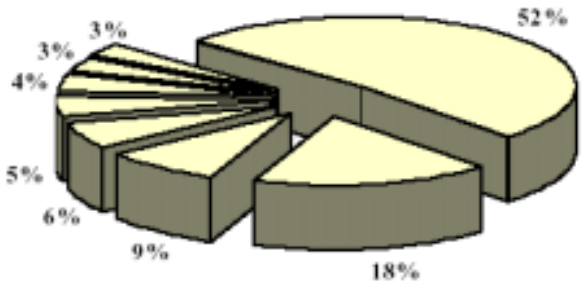

Fig. 1. Genera of fungi represented in the strain collection (section of edible mushrooms) at the Institute of Ecology: *Pleurotus* (52%), *Lentinula* (18%), *Volvariella* (9%), *Agaricus* (5%), *Laetiporus* (4%), *Auricularia* (3%), *Neolentinus* (3%), and others (3%).

SUPPORT TO CONVENTIONAL TAXONOMIC RESEARCH

Several species of *Pleurotus* have been misidentified, despite of their economical importance. Main problems in identification are attributed to the great variation and wide distribution of the genus. More than 1000 *Pleurotus* species have been described worldwide in more than 25 related and/or confusing genera, but approximately only 50 valid species are recognized [7]. In order to define taxonomically and genetically wild and commercial strains of *Pleurotus* in Mexico, interbreeding tests were carried out amongst Mexican, European and Asiatic strains. It was found that several commercial strains of *Pleurotus* reported as *P. ostreatus* have been confused with other species, such as *P. columbinus*, *P. pulmonarius*, "*P. sajor-caju*", "*P. ostreatus* var. *florida*", "*P. floridanus*", "*P. flabellatus*", and *P. djamor* [11]. In the case of white and pink forms of *P. djamor*, they can be considered as ecological varieties of this species: *P. djamor* var. *djamor*, *P. djamor* var. *roseus*, and *P. djamor* var. *salmoneostramineus* [10, 12].

Interbreeding tests between *Lentinula* species were carried out to define relationships between the Japanese shiitake, *L. edodes*, and the American shiitake, *L. boryana*. In fact, both species are different but ecologically equivalent [13, 21].

TRAINING COURSES AND SERVICES

Since 1989, the INECOL has organized short courses, in Mexico, for sharing practical experiences, provided technical support, and offered basic services, such as spawn preparation. Colleagues from other Latin American countries (Argentina, Chile, Colombia, Cuba, El Salvador, Guatemala and Panama) have been technically supported. The book "El Cultivo de los Hongos Comestibles" (The Cultivation of Edible Mushrooms) [9], one of the first written in Spanish in Latin America, is now a classic book that has been useful for people

starting a commercial production, mainly for *Pleurotus* cultivation. The INECOL has also become a member of the CYTED network (Ibero American Programme of Science and Technology for Development), and a project on the cultivation of edible mushrooms is currently funded by the subprogramme on biomass production. As a result of this cooperation, a handbook about edible mushroom cultivation has been published and sponsored by four Latin American institutions [5].

NEW EXPERIMENTAL MYCOLOGY UNIT (EMU)

In 1999, the INECOL began the construction of a new production plant at the same place as the former and smaller facility built in 1985. This EMU was inaugurated on March 23, 2000, during the First Latin American Symposium on the Cultivation of Edible Mushrooms. The unit covers an area of approximately 450 m^2, and was designed to maintain strict control over the environmental factors influencing mushroom development. The EMU has the following sections: 1) Laboratory for the production of spawn; 2) Sterile area for seeding of inocula; 3) Inoculation chamber; 4) Substrate treatment; 5) Pasteurization tunnel using steam; 6) Seeding area with filtered air; 7) Two incubation areas with controlled temperature; 8) Two production areas with controlled ventilation, temperature, and humidity; 9) Cold room; 10) Area for drying mushrooms; and 11) Office for the staff (**Fig. 2**). It is hoped that this unit will become an important site for research, capacity building, technical training, and the validation of cultivation techniques at the pilot plant scale, which may have a high potential impact in Mexico and Latin America.

RESEARCH IN PROGRESS

Aspects of enzyme production and physiological adaptation have recently been under investigation using strains of *Pleurotus* and *Lentinula* [31, 37]. This research is oriented towards the production of enzymes which are important in the degradation of lignocellulosic materials. We plan to study strains capable of degrading phenolic compounds through the production of laccase, manganese-dependent peroxidase, and other oxidases [37]. We will also assess mushroom strains for their capacity to substantially reduce quantities of lignin, hemicellulose, and cellulose in the substrate. Feasibility studies will be undertaken on the utilization of residues from mushroom cultivation in other biotechnological processes, such as vermiculture.

Other important research will be the study of *Pleurotus* and *Lentinula* species that form antagonistic relationships with certain fungi, especially *Trichoderma*. Studies are being undertaken to select strains that show natural resistance to mold attacks [27], as well as high fruiting-body yields and a strong ability for adapting to diverse substrates.

A research programme has been initiated to study the inductive capacity of different strains for producing enzymes during the preparation of spawn. In this case, the base grain is supplemented in order to obtain spawn more adapted to the substrate of cultivation [24]. We will continue to focus on strain viability for germplasm conservation, with a special emphasis on the recovery of viability without the use of cryo-protecting substances.

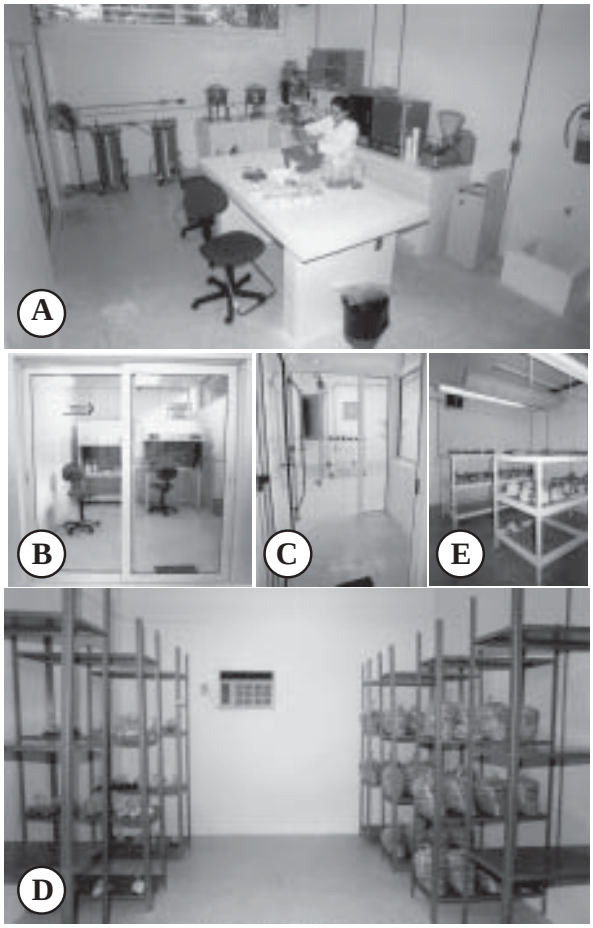

Fig. 2. Experimental Mycology Unit (EMU) at the Institute of Ecology. A: Laboratory, B: Inoculation chamber, C: Seeding room, D: Incubation area, E: Production zone.

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