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CHARACTERISATION AND CULTIVATION OF WILD *AGARICUS* SPECIES FROM MEXICO*

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ABSTRACT

Germplasm preservation and genetic improvement of authentic wild species is fundamental for developing the mushroom industry of any country. In Mexico, strains of wild *Agaricus* species were isolated from diverse regions. Ten species were tentatively identified on the basis of fruit-body morphology: *A. abruptibulbus* Peck, *A. albolutescens* Zeller, *A. augustus* Fries, *A. bisporus* var. *bisporus* (Lange)Imbach, *A. bitorquis* (Quél.)Sacc., *A. campestris* Link : Fries, *A. hortensis* (Cooke)Pilàt, *A. osecanus* Pilát, *A. robustissimus* Panizzi, and *A. subrufescens* Peck; there was also a group of five strains classified as *A*. sp. These species were characterised considering several criteria (mycelial growth on different culture media and pH, fruiting tests on compost, macroscopic morphology and basidial spore number of fruit bodies), using strains of *A. bitorquis* as a standard reference. Colony morphology on culture media was variable, showing differences in density (high, low), aerial mycelia (abundant, scarce), and growth rates (fast, slow). The initial pH and the culture medium influenced colony growth rates, which ranged from 0.02-1.06 cm/day. In fruiting trials, wild *Agaricus* species also showed wide variations in the average time for compost (7-52 days) and casing soil (10-48 days) colonization, fruiting (2-17 days, after the casing soil is colonized), and fruit-body development (3-19 days, from primordia to mature sporophores), as well as in the number of flushes (1-5), mushroom yields (49.5-1,499.1

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g), and biological efficiency (1.8-55.5%). The period from spawning to harvesting the first flush, considering the average time for fruit-body development, ranged from 39-96 days. All species studied showed spore-bearing fruit bodies with normal morphology, having variable colour (white, off-white, cream, brown), scaliness, and size of caps and stipes. The average basidial spore number indicated that most species were of tetrasporic character showing a high proportion of normal four-spored basidia (87.2-99.5%). The exception was *A. bisporus* var. *bisporus* whose basidia were predominately bisporic (67.5%), with a lower proportion of three- (5.0%) or four-spored (27.5%) basidia.

Key words: ***Agaricus*, wild species, germplasm characterisation, mushroom cultivation, Mexico.**

INTRODUCTION

Most basic and applied research work in cultivated edible mushrooms has been concentrated on *Agaricus bisporus* (Lange)Imbach. At present, significant advances have been made in this species: the genetic diversity has broadened, there is a good knowledge of the life cycle, the genome has been characterised, and diverse genetic markers, as well as promising transformation systems, are available [2, 5, 13, 14, 16, 23, 28]. Systematic breeding of *A. bisporus* combining classical and molecular tools is actively being developed by several research groups worldwide. However, considerable less attention has been given to other wild *Agaricus* species [1, 3, 6, 21, 25, 26, 31], despite their scientific and commercial potential, as well as the fact that genetic techniques and production technologies developed for *A. bisporus* can also be applied to them.

Commercial production of *Agaricus* in Mexico started around 1933, and nowadays this country is the largest producer in Latin America [17, 22]. In the highlands, successful enterprises have evolved because there are suitable environmental conditions and increasing mushroom consumption. The authors established a programme since 1997 for recovery and preservation of wild germplasm from *Agaricus* species, some of which are normally collected and consumed in rural communities or popular markets during the rainy season [19, 20, 21]. We present the characterisation of more than ten wild *Agaricus* species from several regions of Mexico, whose cultivation potential has also been assessed.

MATERIALS AND METHODS

Strain isolation

Wild fruit bodies of *Agaricus*, tentatively identified on the basis of morphology [4, 10, 11, 27], were collected in several regions from Mexico. Strains were isolated by tissue culture, and maintained and stored at low temperatures. Standard complete yeast extract medium [CYM; composition (g/L distilled water: dextrose, 20; peptone, 2; yeast extract, 2; $MgSO_4.7H_2O$, 0.5; KH_2PO_4, 0.46; K_2HPO_4, 1; and agar, 20], malt extract agar medium (MEA; BBL), and potato dextrose agar medium (PDA; Bioxon), routinely autoclaved at 121°C for 15 min, were used for the isolation and subculturing of *Agaricus* strains. All

strains isolated are deposited at the CP's culture collection [19].

Mycelial growth on culture media and different pH

Strains were grown on petri dishes (90 mm) containing 20 ml of CYM, MEA, and PDA media, whose initial pH was adjusted to 6.5, 7.0, and 7.5, prior to autoclaving. Inoculated plates were incubated at 25°C in the dark. All experiments were carried out with three replicates, having the strains CP-43, CP-156, and CP-157 of the *A. bitorquis* (Quel.)Sacc. complex as a standard reference [18]. Mycelial growth rates were measured every 3-4 days.

Fruiting tests

The general methodology for assessing the fruiting competence of strains isolated has previously been described [21]. Wheat grain spawn was prepared in jars according to standard methods [7, 29]. After inoculation, the jars were incubated at 27°C, and shaken at weekly intervals to promote rapid and even growth. Complete colonization varied from 2-4 weeks.

Strains were cultivated and produced with virtually the same compost, methods, and facilities used for the commercial cultivation of *A. bisporus*. Fruiting trials were carried out using a standard commercial compost formulation based on wheat straw, horse manure, and gypsum, which was provided by the Mushroom Company "Hongos Leben, S. A.", Guadalupe Victoria, Mexico. Strains were cultivated in plastic bags (50 x 70 cm) containing 4-5 kg of compost. Spawning was done by hand, at a rate of *ca*. 30 g/kg fresh compost weight. Five to twelve replicate bags of each strain were incubated in an experimental chamber, having also the strains CP-43, CP-156, and CP-157 of the *A. bitorquis* as a standard reference [18]. A mixture of local black soil (humic andosol) and chalk to a depth of 2.5-3 cm was used for casing all bags, 8-53 days after spawning. When the compost and casing material was colonized by the mushroom mycelium, ventilation and watering were increased to promote fruiting. The yield was recorded for three weeks, and fruit bodies were harvested at different stages for studying ontogenic development, as well as main morphological characters. Biological efficiency (BE) was determined by expressing the yield of fruit bodies (fresh weight) as a percentage of the dry weight of compost at spawning [30].

Estimation of basidial spore number

Fruit bodies at stages 2-3 of development [9] derived from fruiting trials were checked microscopically to determine the spore number. Gill fragments were rested on small agar blocks and placed onto glass slides [24]. Counts of spore numbers per basidium of each strain were made in different lamellae and sectors of the hymenium.

RESULTS AND DISCUSSION

A total of 46 strains were isolated by tissue culture from various localities of the States of Chiapas (1), Mexico (2), Puebla (39), and Tlaxcala (4) [**Table 1**]. Several strains were difficult to handle in the laboratory due to their poor mycelial development (CP-81, CP-82, CP-89, CP-101, CP-108, CP-109, CP-110, CP-111, CP-115, CP-117, CP-119, CP-135, CP-136, CP-146, CP-147, CP-151, CP-152). Strains studied were grouped within ten *Agaricus* species, and a group of five

Table 1. Origin and code of *Agaricus* strains studied.

State	Location	Code	Number of isolates
Chiapas	Unión Juárez	CP-89	1
Mexico	Texcoco (surroundings)	CP-80, CP-82	2
Puebla	Atexcac	CP-144	1
	Ignacio Zaragoza	CP-115, CP-117, CP-119	3
	Puebla city (surroundings)	CP-81, CP-101, CP-109, CP-110, CP-111, CP-121, CP-123, CP-125, CP-132, CP-135, CP-147, CP-148, CP-150, CP-151, CP-152	15
	San Miguel Canoa	CP-136	1
	Tecalli	CP-54, CP-74, CP-130, CP-131	4
	Tlatlauquitepec	CP-146	1
	Tlaxcalancingo	CP-108	1
	Valsequillo	CP-55, CP-73, CP-83, CP-84, CP-85, CP-87, CP-126, CP-127, CP-128, CP-129	10
	Xoxtla	CP-138, CP-139, CP-140	3
Tlaxcala	La Malinche	CP-124, CP-134, CP-149	3
	Tlaxcala city (surroundings)	CP-142	1

strains whose specific epithet could not be determined at this stage: *A. abruptibulbus* Peck, *A. albolutescens* Zeller, *A. augustus* Fries, *A. bisporus* var. *bisporus* (Lange)Imbach, *A. bitorquis* (Quél.)Sacc., *A. campestris* Link : Fries, *A. hortensis* (Cooke)Pilàt, *A. osecanus* Pilát, *A. robustissimus* Panizzi, *A. subrufescens* Peck, and *A*. sp. (**Table 2**).

Colony morphology on culture media was variable in 28 strains belonging to the species studied, showing differences in density (high, low), aerial mycelia (abundant, scarce), and growth rates. The initial pH and the culture medium influenced colony growth rates, which ranged from 0.02-1.06 cm/day (**Table 3**). Most strains showed higher growth rates on MEA. Two categories can be identified considering the highest growth rate of every strain: Group 1 included slow growing strains (0.02-0.40 cm/day) from all species studied, whereas fast growing strains (0.41-1.06 cm/day) of *A. abruptibulbus* (CP-139), *A. bitorquis* (CP-85, CP-129, CP-130), *A. osecanus* (CP-125), and *A.* sp. (CP-126) were in Group 2.

Better colony morphology and growth rate were observed at an initial pH of 6.5 for *A. campestris*, *A. hortensis*, and *A. subrufescens*; 6.5-7.0 for *A. bitorquis*, *A. abruptibulbus*, and *A. osecanus*; 7.0 for *A. albolutescens*, and *A. robustissimus*; 7.5 for *A. augustus*, and *A. bisporus* var.

bisporus; and 6.5-7.5 for the group of strains belonging to *A. sp*. Several strains of different species did not show a clear pH preference during experiments (CP-43, CP-156, CP-124, CP-129).

In fruiting trials, wild *Agaricus* strains also showed wide variations in the average time for compost and casing soil colonization, fruiting, and fruit-body development, as well as in the number of flushes, yields, and biological efficiency (**Table 4**). The compost was colonized in a period ranging from 7-52 days, in comparison with a range of 13-15 days in standard strains of *A. bitorquis*. Strains of *A. abruptibulbus* colonized the compost in 7-26 days; *A. albolutescens* in 11 days; *A. augustus* in 23 days; *A. bisporus* var. *bisporus* in 52 days; *A. bitorquis* in 13-18 days; *A. campestris* in 14 days; *A. hortensis* in 22 days; *A. osecanus* in 15-16 days; *A. robustissimus* in 34 days; *A. subrufescens* in 13 days; and the group of strains classified as *A*. sp. in 10-39 days.

The casing soil took 10-45 days to be colonized, compared to 15-48 days in standard strains of *A. bitorquis* (Table 4). Strains of *A. abruptibulbus* colonized the casing in 17-28 days; *A. albolutescens* in 42 days; *A. augustus* in 20 days; *A. bisporus* var. *bisporus* in 10 days; *A. bitorquis* in 13-23 days; *A. campestris* and *A. robustissimus* in 19 days; *A. hortensis* in 28 days; *A. osecanus* in 16-18 days; *A. subrufescens* in 26 days; and the group of strains classified as *A*. sp. in 20-45 days.

Table 2. Species of *Agaricus* studied in this work, which were tentatively identified on the basis of fruit-body morphology.

Species	Code	N	Edibility
A. abruptibulbus Peck	CP-87, CP-138, CP-139, CP-140	4	Confirmed Nd
A. albolutescens Zeller	CP-149	1	Nd
A. augustus Fries	CP-80	1	Nd
A. bisporus var. *bisporus* (Lange)Imbach	CP-124	1	Confirmed
A. bitorquis (Quél.)Sacc.	CP-84, CP-85, CP-127, CP-128, CP-129, CP-130, CP-131	7	Confirmed
A. campestris Link : Fries	CP-54	1	Confirmed
A. hortensis (Cooke)Pilàt	CP-74	1	Confirmed
A. osecanus Pilát	CP-83, CP-125	2	Confirmed
A. robustissimus Panizzi	CP-73	1	Confirmed
A. sp.	CP-55, CP-126	5	Confirmed
	CP-132, CP-134, CP-148		Nd
A. subrufescens Peck	CP-123	1	Nd

N= Number of isolates. Nd= Not determined in this study.

Table 3. Mycelial growth rate of wild *Agaricus* species on different culture media and pH, in comparison with standard strains of *A. bitorquis* (CP-43, CP-156, CP-157).

Species	Strain	pH	AGR (cm/day)		
			PDA	MEA	CYM
A. bitorquis	CP-43	6.5	0.13	0.25	0.15
		7.0	0.10	0.26	0.10
		7.5	0.15	0.25	0.18
	CP-156	6.5	0.14	0.16	0.22
		7.0	0.16	0.25	0.16
		7.5	0.20	0.24	0.14
	CP-157	6.5	0.14	0.29	0.10
		7.0	0.12	0.32	0.12
		7.5	0.13	0.29	0.10
A. abruptibulbus	CP-87	6.5	0.14	0.27	0.20
		7.0	0.10	0.18	0.10
		7.5	0.11	0.15	0.06
	CP-138	6.5	0.20	0.35	0.15
		7.0	0.11	0.30	0.26
		7.5	0.15	0.35	0.21
	CP-139	6.5	0.05	0.11	0.12
		7.0	0.05	0.46	0.18
		7.5	0.26	0.14	0.10
	CP-140	6.5	0.14	0.13	0.08
		7.0	0.10	0.06	0.07
		7.5	0.10	0.08	0.06
A. albolutescens	CP-149	6.5	0.10	0.08	0.13
		7.0	0.10	0.18	0.10
		7.5	0.05	0.10	0.10
A. augustus	CP-80	6.5	0.02	0.13	0.08
		7.0	0.02	0.14	0.05
		7.5	0.02	0.20	0.08
A. bisporus var. *bisporus*	CP-124	6.5	0.10	0.16	0.03
		7.0	0.07	0.12	0.05
		7.5	0.08	0.20	0.04

Table 3 (continued)

A. bitorquis	CP-84	6.5	0.12	0.10	0.05
		7.0	0.10	0.09	0.05
		7.5	0.06	0.06	0.10
	CP-85	6.5	0.23	0.38	0.30
		7.0	0.21	0.41	0.28
		7.5	0.30	0.33	0.35
	CP-127	6.5	0.24	0.21	0.15
		7.0	0.20	0.23	0.12
		7.5	0.09	0.18	0.12
	CP-128	6.5	0.24	0.28	0.16
		7.0	0.18	0.24	0.17
		7.5	0.18	0.30	0.30
	CP-129	6.5	0.35	0.64	0.35
		7.0	0.46	1.00	0.30
		7.5	0.42	1.06	0.20
	CP-130	6.5	0.32	0.50	0.13
		7.0	0.26	0.20	0.30
		7.5	0.17	0.17	0.28
	CP-131	6.5	0.21	0.15	0.12
		7.0	0.14	0.20	0.12
		7.5	0.24	0.20	0.15
A. campestris	CP-54	6.5	0.37	0.34	0.18
		7.0	0.33	0.35	0.27
		7.5	0.34	0.25	0.20
A. hortensis	CP-74	6.5	0.25	0.18	0.22
		7.0	0.18	0.22	0.20
		7.5	0.08	0.18	0.14
A. osecanus	CP-83	6.5	0.18	0.18	0.16
		7.0	0.14	0.21	0.07
		7.5	0.16	0.08	0.08
	CP-125	6.5	0.48	0.60	1.02
		7.0	0.42	0.55	0.95
		7.5	0.60	0.84	0.73
A. robustissimus	CP-73	6.5	0.26	0.07	0.22
		7.0	0.26	0.29	0.29
		7.5	0.20	0.18	0.18

Table 3 (continued)

A. sp.	CP-55	6.5	0.16	0.23	0.16
		7.0	0.15	0.25	0.17
		7.5	0.14	0.20	0.14
	CP-126	6.5	0.45	0.33	0.48
		7.0	0.46	0.40	0.38
		7.5	0.36	0.36	0.30
	CP-132	6.5	0.06	0.09	0.06
		7.0	0.04	0.04	0.09
		7.5	0.08	0.12	0.10
	CP-134	6.5	0.11	0.06	0.08
		7.0	0.13	0.06	0.08
		7.5	0.05	0.10	0.05
	CP-148	6.5	0.08	0.12	0.09
		7.0	0.08	0.12	0.12
		7.5	0.08	0.12	0.13
A. subrufescens	CP-123	6.5	0.08	0.12	0.05
		7.0	0.06	0.10	0.09
		7.5	0.06	0.09	0.09

AGR= Average growth rate.
PDA= Potato dextrose agar.
MEA= Malt extract agar.
CYM= Complete yeast extract medium.

Fruit bodies began to appear between 2-17 days after casing soil colonization, whereas in standard strains of *A. bitorquis* ranged from 5-8 (Table 4). Strains of *A. abruptibulbus* began to fruit in 4-11 days; *A. albolutescens* in 8 days; *A. augustus* in 12 days; *A. bisporus* var. *bisporus* in 13 days; *A. bitorquis* in 2-7 days; *A. campestris* and *A. robustissimus* in 4 days; *A. hortensis* in 3 days; *A. osecanus* in 3-13 days; *A. subrufescens* in 17 days; and the group of strains classified as *A.* sp. in 3-16 days.

Complete development of fruit bodies (from primordia to mature sporophores) took 3-19 days, in comparison with 6-8 days in standard strains of *A. bitorquis* (Table 4). Fruit bodies of *A. abruptibulbus* developed in 4-17 days; *A. albolutescens* and *A. augustus* in 9 days; *A. bisporus* var. *bisporus* in 7 days; *A. bitorquis* in 4-7 days; *A. campestris* in 12 days; *A. hortensis* in 3 days; *A. osecanus* and *A. robustissimus* in 4 days; *A. subrufescens* in 16 days; and the group of strains classified as *A.* sp. in 4-19 days.

The period from spawning to harvesting the first flush, considering the average time for fruit-body development, ranged from 39-96 days (Table 4). Only several strains of *A. bitorquis*, *A. osecanus*, and *A.* sp. showed a shorter period for harvesting than standard strains of *A. bitorquis*, which had a range of 45-72 days. Some strains of these species were also within the Group 2 of fast growing strains on culture media (CP-85, CP-129, CP-126).

The number of flushes was variable (1-5) in comparison with standard strains of *A. bitorquis* (3). Strains of *A. abruptibulbus* and the group of strains classified as *A.* sp. showed 1-4 flushes; *A. albolutescens* and *A. subrufescens* had 1 flush; *A. augustus* and *A. bisporus* var. *bisporus* had 3 flushes; *A. bitorquis* and *A. hortensis* had 4 flushes; *A. campestris* had 2 flushes; *A. osecanus* had 3-4 flushes; and *A. robustissimus* had 5 flushes. Strains showing 1-2 flushes had also a longer period of fruit-body development (9-19 days), in comparison with standard strains studied.

Mushroom yields and biological efficiencies varied considerably, from 49.5-1,499.1 g (BE: 1.8-55.5%), in comparison with standard strains of *A. bitorquis* [1,055.5-4,058.3 g (BE: 42-150.3%)]. *A. abruptibulbus* had a yield ranging from 257-976.6 g (BE: 9.5-

Table 4. Average time for mycelial colonization of compost and casing soil, the period for fruiting, and mushroom yields from wild *Agaricus* species, in comparison with standard strains of *A. bitorquis* (CP-43, CP-156, CP-157).

Species	Strain	MC (days)		T_1 (days)	T_2 (days)	T_3 (days)	Yield (g)	F	BE (%)
		C	CS						
A. bitorquis	CP-43	15	15	8	7	45	4,058.3	3	150.3
	CP-156	13	43	8	8	72	1,055.5	3	42.0
	CP-157	13	48	5	6	72	1,266.5	3	50.4
A. abruptibulbus	CP-87	15	22	4	4	45	976.6	4	36.2
	CP-138	11	25	8	13	57	738.5	3	27.3
	CP-139	26	17	11	17	71	257.0	1	9.5
	CP-140	7	28	11	8	54	541.0	3	20.0
A. albolutescens	CP-149	11	42	8	9	70	437.7	1	17.4
A. augustus	CP-80	23	20	12	9	64	660.0	3	24.4
A. bisporus var. *bisporus*	CP-124	52	10	13	7	82	526.0	3	20.9
A. bitorquis	CP-84	15	18	4	5	42	755.0	4	27.9
	CP-85	16	22	2	4	44	1,108.5	4	41.0
	CP-127	18	17	7	4	46	711.8	4	26.4
	CP-128	14	18	3	4	39	575.3	4	21.3
	CP-129	13	13	6	7	39	616.5	4	22.8
	CP-130	15	23	5	7	50	568.3	4	21.0
	CP-131	16	20	4	4	44	603.7	4	22.3
A. campestris	CP-54	14	19	4	12	49	927.6	2	34.3
A. hortensis	CP-74	22	28	3	3	56	681.7	4	25.2
A. osecanus	CP-83	15	18	3	4	40	1,499.1	4	55.5
	CP-125	16	16	13	4	49	494.6	3	18.3
A. robustissimus	CP-73	34	19	4	4	61	1,240.8	5	45.9
A. sp.	CP-55	18	20	3	5	46	490.0	4	18.1
	CP-126	10	25	3	4	42	889.7	4	32.9

Table 4 (continued)

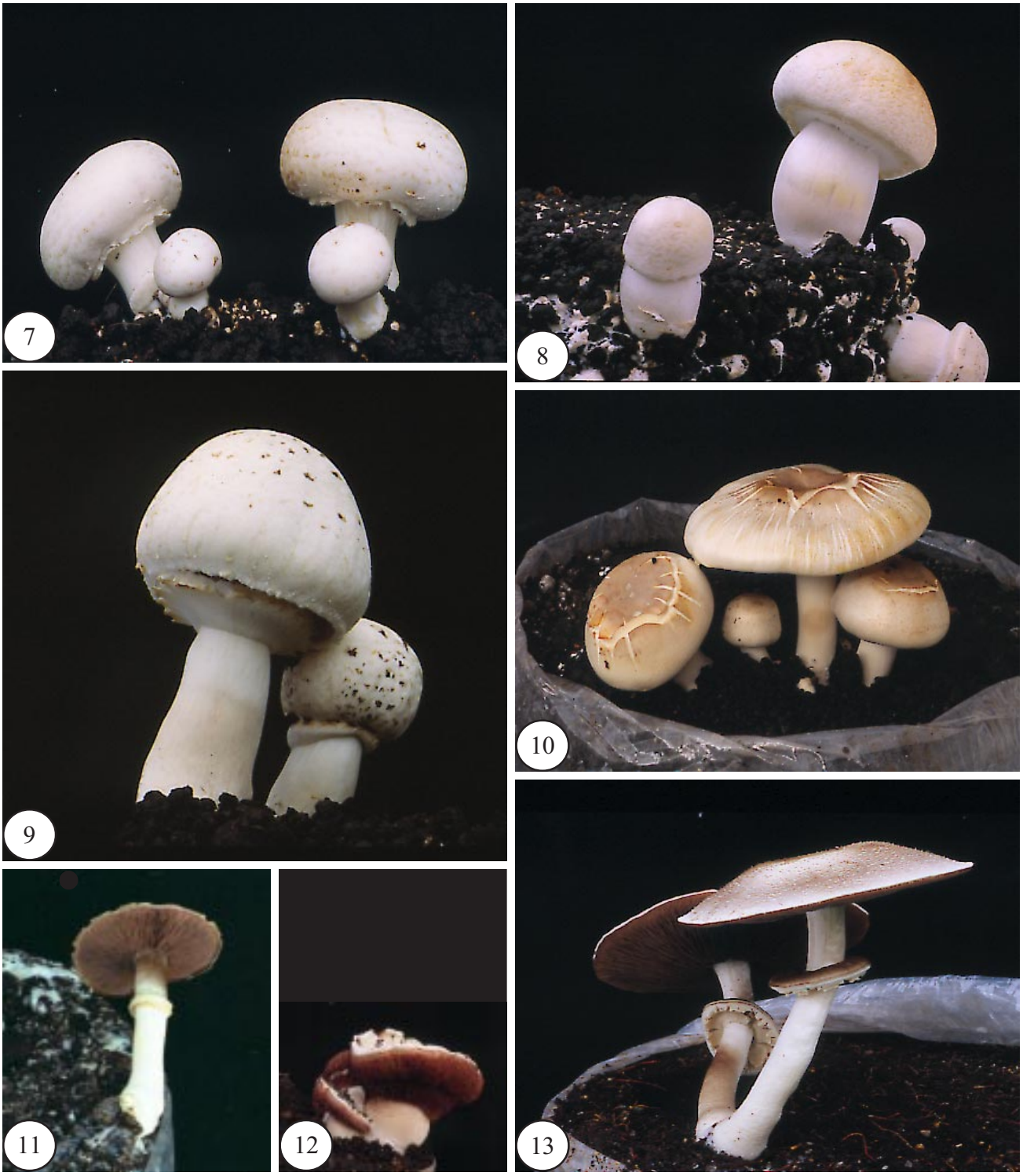
	CP-132	25	36	16	19	96	52.0	1	1.9
	CP-134	39	32	12	13	96	477.0	3	19.0
	CP-148	24	45	11	10	90	247.0	2	9.8
A. subrufescens	CP-123	13	26	17	16	72	49.5	1	1.8

MC= Mycelial colonization. C= Compost. CS= Casing soil. T_1= Average time for fruiting after the casing soil is completely colonized. T_2= Average time for fruit-body development, from primordia to mature sporophores (at stages 5-6 of development [9]), in all flushes. T_3= Total number of days from spawning to harvesting the first flush, considering the average time for fruit-body development. F= Number of flushes. BE= Biological efficiency.

36.2%); *A. albolutescens* of 437.7 g (BE: 17.4%); *A. augustus* of 660 g (BE: 24.4%); *A. bisporus* var. *bisporus* of 526 g (20.9%); *A. bitorquis* from 568.3-1,108.5 g (BE: 21-41%); *A. campestris* of 927.6 g (BE: 34.3%); *A. hortensis* of 681.7 g (BE: 25.2%); *A. osecanus* from 494.6-1,499.1 g (BE: 18.3-55.5%); *A. robustissimus* of 1,240.8 g (BE: 45.9%); *A. subrufescens* of 49.5 g (BE: 1.8%); and the group of strains classified as *A*. sp. from 52-889.7 g (BE: 1.9-32.9%).

All strains studied showed spore-bearing fruit bodies with normal variable morphology, as well as free and dark brown gills at maturity (**Figs. 1-15**). Main characteristics of fruit bodies (at stage 5 of development [9]) from each species are shown in **Table 5**. *A. abruptibulbus* had white to off-white cap, 5.2-18.0 cm wide, smooth to regularly scaly, 1.0-2.5 cm cap thickness; stipe 6.3-8.5 cm long, white to off-white, smooth. *A. albolutescens* showed off-white cap, 13 cm wide, regularly scaly, 2.3 cm cap thickness; stipe 15.2 cm long, off-white, smooth. *A. augustus* had off-white cap, 16.0-17.5 cm wide, scaly, 1.3 cm cap thickness; stipe 13 cm long, off-white, regularly scaly. *A. bisporus* var. *bisporus* showed white cap, 8.0-9.5 cm wide, regularly scaly, 2.0 cm cap thickness; stipe 5.0 cm long, white, smooth. *A. bitorquis* had off-white to cream cap, 4.4-7.0 cm wide, slightly scaly to scaly, 0.4-1.0 cm cap thickness; stipe 3.0-7.2 cm long, cream to off-white, slightly to regularly scaly. *A. campestris* showed off-white cap, 2.4-3.5 cm wide, scaly, 0.3 cm cap thickness; stipe 2.5 cm long, off-white, regularly scaly. *A. hortensis* had off-white cap, 5 cm wide, regularly scaly, 0.9 cm cap thickness; stipe 5.5 cm long, cream, smooth. *A. osecanus* showed off-white cap, 6.2-18.0 cm wide, regularly scaly to scaly, 0.8-1.8 cm cap thickness; stipe 6.0-12.5 cm long, off-white, smooth. *A. robustissimus* had off-white cap, 4.8-5.0 cm wide, regularly scaly, 1.1 cm cap thickness; stipe 4.5 cm long, off-white, smooth. *A. subrufescens* showed cream cap, 8.5-9.0 cm wide, regularly scaly, 1.5 cm cap thickness; stipe 5.6 cm long, cream, smooth. The group of strains classified as *A*. sp. had off-white,

Figs. 1-6. Wild *Agaricus* species from Mexico cultivated in compost. 1: *A. abruptibulbus*. 2: *A. albolutescens*. 3: *A. augustus*. 4: *A. bisporus* var. *bisporus*. 5: *A. bitorquis*. 6: *A. campestris*.



Figs. 7-13. Wild *Agaricus* species from Mexico cultivated in compost. 7: *A. hortensis*. 8: *A. osecanus*. 9: *A. robustissimus*. 10: *A. subrufescens*. 11: *A.* sp. (strain CP-55). 12: *A.* sp. (strain CP-126). 13: *A*. sp. (strain CP-132).

Figs. 14-15. Wild *Agaricus* species from Mexico cultivated in compost. 13: *A.* sp. (strain CP-134). 14: *A.* sp. (strain CP-148).

cream, and brown caps, 2.5-9.5 cm wide, regularly scaly to scaly, 0.8-1.2 cm cap thickness; stipe 4.5-8.2 cm long, cream to off-white, smooth to regularly scaly.

The average basidial spore number in *Agaricus* species studied is shown in **Table 6**. Environmental conditions for mushroom cultivation in the experimental chamber involved a temperature varying from 26º-28ºC, and a relative humidity ranging from 60-70%. Most species were of tetrasporic character (87.2-99.5%) having a high proportion of normal four-spored basidia: *A. abruptibulbus*, 91.9-99.5%; *A. albolutescens*, 96.5%; *A. augustus*, 93.9%; *A. bitorquis*, 95.9-99.5%; *A. campestris*, 98.8%; *A. hortensis*, 96%; *A. osecanus*, 97.8-98.4%; *A. robustissimus*, 97.2%; *A. subrufescens*, 96%; and the group of strains classified as *A*. sp. from 87.2% to 98.5%. The exception was *A. bisporus* var. *bisporus* whose basidia were predominately bisporic (67.5%), with a lower proportion of three- (5.0%) or four-spored (27.5%) basidia. Although the basidial spore number is affected by environmental factors [8, 15], it is primarily determined by a single genetic locus (*BSN*), which is linked to the mating type (*MAT*) on chromosome *I* [12]. On the basis of this, we can infer that tetrasporic species appear to be heterothallic producing primarily homokaryotic basidiospores, whereas the predominately bisporic species seems to be secondarily homothallic producing mainly heterokaryotic basidiospores.

Research work is now in progress in order to study the pattern of sexuality from *Agaricus* species studied, their breeding relationships, and their genetic diversity at the molecular level.

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Table 5. Fruit-body morphology, at stage 5 of development [9], from wild *Agaricus* species cultivated in compost, in comparison with standard strains of *A. bitorquis* (CP-43, CP-156, CP-157).

Species	Strain	Cap				Gills at	Stipe		
		Size (cm)	Scales	T (cm)	Colour	maturity	L (cm)	Scales	Colour
A. bitorquis	CP-43	6.0x5.8	Scarce	1.0	White	Free, db	5.3	Scarce	White
	CP-156	7.0x9.5	Scarce	1.7	White	Free, db	7.2	Regular	White
	CP-157	16.3x16.3	Scarce	2.0	White	Free, db	10.1	Regular	White
A. abruptibulbus	CP-87	5.2x5.7	Absent	1.0	White	Free, db	6.3	Absent	White
	CP-138	11.5x12.7	Scarce	1.5	White	Free, db	8.5	Absent	White
	CP-139	17.5x18.0	Regular	1.8	Off-w	Free, db	8.5	Absent	Off-w
	CP-140	16.0x16.5	Scarce	2.5	Off-w	Free, db	7.9	Absent	Off-w
A. albolutescens	CP-149	13.2x13.7	Regular	2.3	Off-w	Free, db	15.2	Absent	Off-w
A. augustus	CP-80	16.0x 17.5	Abundant	1.3	Off-w	Free, db	13	Regular	Off-w
A. bisporus var. *bisporus*	CP-124	8.0x9.5	Regular	2.0	White	Free, db	5.0	Absent	White
A. bitorquis	CP-84	4.4x4.6	Scarce	0.4	Off-w	Free, db	6.2	Scarce	Off-w
	CP-85	4.9x5.0	Abundant	0.8	Off-w	Free, db	7.2	Regular	Off-w
	CP-127	5.5x6.7	Abundant	1.0	Off-w	Free, db	5.5	Regular	Off-w
	CP-128	5.0x6.5	Abundant	1.0	Off-w	Free, db	3.0	Scarce	Off-w
	CP-129	5.3x5.8	Regular	0.7	Cream	Free, db	6.8	Scarce	Cream
	CP-130	6.5x7.0	Abundant	0.9	Off-w	Free, db	4.6	Scarce	Off-w
	CP-131	5.9x6.8	Abundant	1.0	Off-w	Free, db	5.0	Scarce	Off-w
A. campestris	CP-54	2.4x3.5	Abundant	0.3	Off-w	Free, db	2.5	Regular	Off-w
A. hortensis	CP-74	5.5x5.7	Regular	0.9	Off-w	Free, db	5.5	Absent	Cream
A. osecanus	CP-83	6.2x6.3	Abundant	0.8	Off-w	Free, db	6.0	Absent	Off-w
	CP-125	17.0x18.0	Regular	1.8	Off-w	Free, db	12.5	Absent	Off-w
A. robustissimus	CP-73	4.8x5.0	Regular	1.1	Off-w	Free, db	4.5	Absent	Off-w
A. sp.	CP-55	6.2x6.3	Abundant	1.1	Off-w	Free, db	5.5	Absent	Off-w
	CP-126	5.4x6.2	Regular	0.8	Off-w	Free, db	6.0	Regular	Off-w
	CP-132	9.0x9.5	Abundant	1.2	Cream	Free, db	7.0	Absent	Cream
	CP-134	3.5x4.0	Abundant	0.8	Brown	Free, db	4.5	Absent	Off-w
	CP-148	2.5x5.3	Abundant	0.8	Brown	Free, db	8.2	Absent	Cream
A. subrufescens	CP-123	8.5x9.0	Regular	1.5	Cream	Free, db	5.6	Absent	Cream

T= Thickness. L= Length. db= Dark brown. Off-w= Off-white.

Table 6. Basidial spore number in fruit bodies from wild *Agaricus* species cultivated in compost, in comparison with standard strains of *A. bitorquis* (CP-43, CP-156, CP-157).

Species	Strain	T (°C)	RH (%)	FB	B	Number of spores (%)						
						1	2	3	4	5	6	7
A. bitorquis	CP-43	26-28	60-70	3	1,472	0	0	0.8	99.2	0	0	0
	CP-156	26-28	60-70	3	1,191	0	1.0	2.0	97.0	0	0	0
	CP-157	26-28	60-70	3	1,451	0	1.0	9.0	90.0	0	0	0
A. abruptibulbus	CP-87	26-28	60-70	3	1,138	0.4	0.3	6.1	91.9	0.5	0.8	0
	CP-138	26-28	60-70	3	1,184	0	0	1.4	98.6	0	0	0
	CP-139	26-28	60-70	3	230	0	0	0.9	99.1	0	0	0
	CP-140	26-28	60-70	2	1,511	0	0.1	0.4	99.5	0	0	0
A. albolutescens	CP-149	26-28	60-70	3	1,184	0	0	2.7	96.5	0.8	0	0
A. augustus	CP-80	26-28	60-70	2	1,168	0	0	6.1	93.9	0	0	0
A. bisporus var. *bisporus*	CP-124	26-28	60-70	3	1,231	0	67.5	5.0	27.5	0	0	0
A. bitorquis	CP-84	26-28	60-70	3	1,148	0	0.3	1.7	97.7	0.3	0	0
	CP-85	26-28	60-70	3	1,047	0	0	1.3	98.4	0.3	0	0
	CP-127	26-28	60-70	3	1,328	0	0.07	0.43	99.5	0	0	0
	CP-128	26-28	60-70	3	1,566	0	0	0.8	99.2	0	0	0
	CP-129	26-28	60-70	3	1,697	0	0.3	0.7	99.0	0	0	0
	CP-130	26-28	60-70	3	1,482	0	1.1	3.0	95.9	0	0	0
	CP-131	26-28	60-70	3	1,229	0	1.3	2.4	96.3	0	0	0
A. campestris	CP-54	26-28	60-70	3	976	0	0	1.0	98.8	0.2	0	0
A. hortensis	CP-74	26-28	60-70	2	1,118	0	0	3.6	96.0	0.4	0	0
A. osecanus	CP-83	26-28	60-70	3	1,212	0.2	0.2	0.7	97.8	1.0	0.1	0
	CP-125	26-28	60-70	3	2,363	0.04	0.6	0.7	98.4	0.3	0	0
A. robustissimus	CP-73	26-28	60-70	3	1,170	0	0	2.1	97.2	0.7	0	0
A. sp.	CP-55	26-28	60-70	3	1,030	0	0.5	0.9	97.1	1.0	0.3	0.2
	CP-126	26-28	60-70	3	1,031	0	0.2	1.2	87.2	11.4	0	0
	CP-132	26-28	60-70	2	1,102	0	0	1.5	98.5	0	0	0
	CP-134	26-28	60-70	2	939	0	0	9.5	90.5	0	0	0
	CP-148	26-28	60-70	3	883	0	0	6.0	94.0	0	0	0
A. subrufescens	CP-123	26-28	60-70	3	1,287	0	3.4	0.2	96.0	0.5	0	0

T= Temperature.
RH= Relative humidity.
FB= Number of fruit bodies studied.
B= Total number of basidia scored.

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