J Ind Microbiol Biotechnol (2008) 35:595–602
DOI 10.1007/s10295-008-0321-1

ORIGINAL PAPER

Utilization of new naturally occurring strains and supplementation to improve the biological efficiency of the edible mushroom *Agrocybe cylindracea*

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Received: 24 August 2007 / Accepted: 16 January 2008 / Published online: 14 February 2008

Abstract To evaluate the importance of searching new naturally occurring strains to raise yields in mushroom production, eight wild and four commercial strains of *Agrocybe cylindracea* were cultivated on wheat straw. The highest biological efficiencies (BE) (54.5–72.4%) were obtained with three wild and two commercial strains when cultured on non-supplemented wheat straw. Rolled oats or soybean flour supplementation were tested using three selected strains, increasing BEs up to 1.2, 0.5 and 0.7-fold, respectively. This effect of supplementation was stronger in the Asiatic wild strain, yielding up to 41.1 and 30% more than the two other strains with rolled oats and soybean flour, respectively. The Asiatic wild strain cultivated with soybean flour supplementation achieved an average biological efficiency of 179%, to our knowledge, the highest reported for this species. These results show the importance of searching for new naturally occurring strains in combination with supplemented wheat straw substrate for raising yields in *A. cylindracea* cultivation.

Keywords *Agrocybe cylindracea* · *Agrocybe aegerita* · Naturally occurring strains · Mushroom cultivation · Biological efficiency improvement

Introduction

Agrocybe cylindracea (Brig.) Singer (Basidiomycetes, Agaricales, Bolbitiaceae) [12] is an excellent edible mushroom appreciated for its culinary properties and pleasant odor. This mushroom is known worldwide as *A. aegerita*, nevertheless, we use the name *A. cylindracea* because this is the correct name [15]. It is currently being widely studied for its medicinal properties, especially for its content of antioxidant, antimutagenic and antitumor agents [11, 19, 22]. It is cultivated on a commercial scale in numerous Asiatic and European countries such as China, Thailand, Japan, Germany, Greece, Italy and Spain (see http://www.mushworld.com) [7]. The use of several low-cost substrates containing agricultural and forestry by-products such as barley, wheat straw (WS), orange peel, grape stalks, reed, rice husks, sunflower and poplar/willow tree sawdust has been reported for *A. cylindracea* cultivation [6, 8]. It can also be cultivated on stumps of cottonwoods, willows, poplars or box elders [13]. However, low yields obtained with commercial strains, and a short post harvest life compared with other cultivated mushrooms such as *Agaricus bisporus* and *Pleurotus ostreatus*, has limited its cultivation [16]. In this sense, a significant improvement in biological efficiency (BE), defined as the yield relative to the dry substrate weight, becomes essential to make this species economically attractive.

One of the problems in mushroom production is that commercial strains sometimes decline in their production performance after several consecutive subcultures and/or a long period of storage in culture medium, leading to a reduction in the yield. BE can be sometimes raised by optimization of cultural conditions, such as combining different substrates and/or adding nutritional supplements. Nevertheless, these practices are not always successful in recovering the production of commercial strain's performance. An alternative solution could be searching new natural occurring strains for cultivation, which could result in finding highly productive or quality strains.

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A. cylindracea is a widely distributed species which, although rare in northern latitudes, has been recorded in all continents [17]. In particular, it can be easily found in the central-east region of Argentina, as groups of basidiomata growing on living trees or dead trunks of several genera such as *Populus*, *Salix*, *Quercus*, *Ulmus*, *Acer*, *Robinia* and *Melia* [18].

In this paper we used the species *A. cylindracea* to evaluate the importance of searching for new wild strains for improving BE and quality in edible mushrooms production. Twelve wild and commercial strains were cultivated and all essential yield and quality parameters were recorded and analyzed. The effect of supplementation with soybean flour and rolled oats has been studied using selected strains to establish a mushroom production methodology capable of supporting relevant agro-economic activities. In addition, we evaluated the value of *A. cylindracea* as a different alternative mushroom for cultivation.

Methods

Strains

A. cylindracea strains used in this study are conserved in the IIB-INTECH collection of fungal cultures (ICFC). Geographic origins, collection dates, collectors, original substrates (if available) and collection numbers are listed in Table 1.

Culture media, spawn, and substrate preparation

Potato dextrose agar (PDA, Britania, Argentina, 39 g/l) culture medium was used for routine culture and storage purposes. Grain spawn was prepared in 1 l glass bottles filled with 300 g of boiled sorghum (*Sorghum bicolor*) grains supplemented with 1% w/w calcium carbonate ($CaCO_3$). The bottles were autoclaved at 120°C for 2 h and inoculated with mycelium grown on PDA. Bottles were incubated at 25°C for 1 month, in the dark, with periodical shaking.

Willow tree (*Salix* sp.) sawdust and wheat (*Triticum* sp.) straw substrates were prepared as follows: dried substrates were chopped up to a size particle of 1.5–3 mm (willow sawdust) and of length 30–50 mm (wheat straw). Chopped substrates were mixed with 1% w/w $CaCO_3$ for all experiments and with 20% w/w soybean flour or 20% w/w rolled oats for the supplementation experiments. Distilled water was added to all formulas and left overnight to moisturize completely in order to obtain 70% w/w of water content. 25 × 45 cm polypropylene bags (30 μm thick) were filled with 1 kg of wet substrate, and were sterilized twice at 120°C for 2.5 h. After cooling, the bags were inoculated with 5% w/w spawn and incubated in the dark at 25°C.

Experimental design and cultivation conditions for mushroom production

In a first experiment, two different non-supplemented substrates were evaluated: 100% willow tree sawdust and 100% wheat straw, using three *A. cylindracea* strains (ICFC 480/02, 492/03, and 106/99). In a second experiment, four commercial and eight naturally occurring *A. cylindracea* strains were cultivated on non-supplemented wheat straw. Four replicates per substrate and strain were used in these two experiments. In a third experiment, three selected strains were cultivated on non-supplemented

Table 1 Geographic origins, collection dates, collectors, original substrates and collection numbers of the *A. cylindracea* strains used in this study

Strains	Geographic origin[a]	Collection date (month/year)	Collector	Original substrate	Collection numbers (ICFC[b])
Naturally occuring	China, Taiwan	Unknown	Unknown	Unknown	621/04
	China, Guizhou Province	Unknown	Unknown	Unknown	622/04
	Guatemala, near Tecpan	1991	Ruth de Leon	Trunk of *Sambucus* sp. at 2,000 m altitude	558/03
	Arg., Bs. As., Llavallol, S. A.	1998	E. Albertó	Dead trunk	09/98
	Arg., Bs. As., Llavallol, S. A.	02/1999	B. Lechner	Unknown	106/99
	Arg., Bs. As., C. F.	04/1994	E. Blanchet	*Acer negundo* trunk	313/00
	Arg., Bs. As., Lanús	05/1989	J. R. Deschamps	*Acer negundo* trunk	440/01
	Arg., Bs. As., Chascomús, L. A.	03/2002	E. Albertó	Dead trunk	462/02
Commercially cultivated	Italy	12/2002	Unknown	Unknown	480/02
	Scotland	Unknown	Unknown	Unknown	587/03
	France	Unknown	Unknown	Unknown	492/03
	Belgium	Unknown	Unknown	Unknown	571/03

[a] *Arg* Argentina, *Bs. As.* Buenos Aires, *C. F.* Capital Federal, *S. A.* Santa Catalina forest, *L. A.* La Alameda forest

[b] IIB-INTECH collection of fungal cultures

wheat straw and on soybean flour or rolled oats-supplemented wheat straw. Eight replicates per formula and strain were performed. After complete colonization of the substrate (55 days in the first and second experiments and 40 days in the third experiment), the bags were removed and colonized substrates, referred hereafter as production blocks, were transferred to fruiting conditions (18−20°C, 70–80% humidity levels, with 9 h light/15 h dark photoperiod) to induce basidiome formation. A 20 W fluorescent light was used on a 2.5 × 4.5 m culture room.

Cropping period, crop yield, and quality traits assessment

One to three flushes were collected during the cropping period (time lapsed between the induction day until the last harvest day), which lasted from 17 to 60 days, depending on the strain and formula. All experimental units were discarded after 60 days under fruiting conditions. Mature fruiting bodies were collected and the following production and quality traits were registered: Production—(1) first harvest day: time (in days) from the start of incubation under fruiting conditions until harvest of the first fruiting bodies; (2) BE: fresh fruiting bodies weight (total yield)/dry substrate weight (expressed as a percentage); (3) percentage of yield distribution (first, second and third flush yield); (4) number of fruiting bodies collected on the first flush. Quality traits: (a) *Pileus* weight (expressed as a percentage of the total weight); (b) dry weight (expressed as a percentage of the fresh weight); (c) diameter of *pileus* (cm); (d) fruiting body fleshiness (g/cm^2): *pileus* thickness estimated by determining the ratio of *pileus* weight to surface. It was assumed that each *pileus* was circular; the surface was calculated as πr^2 (the ratio r was measured using a 0.5 cm rule), and that the density was the same for all genotypes [5]; (e) gracility index: (stem length)^2/pileus diameter × stem diameter [2]; (f) nutritional properties (protein, carbohydrate, fat and fiber content) were analyzed by the *Centro de Investigación y Desarrollo en Criotecnología de Alimentos* (CIDCA, La Plata, Argentina). For nutritional properties analysis, strains ICFC 621/04, 9/98 and 571/03 cultivated on soybean flour supplemented wheat straw, and strain ICFC 9/98 cultivated on non-supplemented wheat straw were used. All quality traits were recorded using, exclusively, basidiomata from the first flush. For all size measurements, fruiting bodies were grouped by similar size ± 1 mm. The number of groups depended on the size variation on each crop. One basidiocarp was chosen randomly from each group and measured using a 0.5 mm rule. *Pileus* diameter, fruiting body fleshiness, and gracility index were calculated as the weighted average of all basidioamata from the first flush.

In the first and second experiments, only BE was recorded, whereas in the third experiment BE and all other traits were measured.

Statistical analyses

Prior to analysis, the Kolmogorov–Smirnov test (with Lilliefors' correction) and Levene Median test were applied to test data for normality and equal variation, respectively. Data sets that did not conform the assumptions of normality and homoscedasticity were sine, log or ln-transformed and re-evaluated. Differences between mean values of treatments (substrates, genotypes, and nutritional supplements) were analyzed with two way ANOVA, followed by all pairwise multiple comparison procedures (post-hoc testing) using the Holm–Sidak method. A value of $P < 0.05$ was considered to be significant. Statistic analysis was carried out with the SigmaStat® program for windows, version 3.1 (Systat software inc.).

Results

Substrate selection

In order to choose a suitable substrate for *A. cylindracea* cultivation, three strains (ICFC 480/02, 492/03, and 106/99) were cultivated on 100% willow tree sawdust or 100% wheat straw. The BE average values for the three strains on wheat straw comprised between 25.5 and 31.0% (Fig. 1). These values were largely higher (7.5–8.2-fold) to those obtained using sawdust, where the BE average values obtained were from 0 to 4.1% (Fig. 1). Moreover, mycelial growth could not colonize the sawdust completely during the 55 days incubation period, whereas it completely colonized wheat straw at the end of the spawning run period. These results justified the use of the wheat straw as substrate for all other experiments in this work.

Yield comparison between wild and commercial strains

Figure 2 compares the BE of four commercial and eight wild *A. cylindracea* strains cultivated on wheat straw. It is possible to observe that five strains yielded significantly more (0.8–2.4-fold, $P < 0.05$) than all others, with BE average values comprised between 54.5 and 72.4%. Interestingly, three of these highest-yield strains were wild strains, two from Asia (ICFC 621/04 and 622/04) and one from South America (ICFC 9/98). The other two were commercial strains from Europe (ICFC 571/03 and 587/03).

Effect of the genotype and supplement in total and first flush yield

We used three highest-yielding strains (see Fig. 2) in order to analyze the effect of supplementation with soybean flour or rolled oats. These three selected strains were chosen

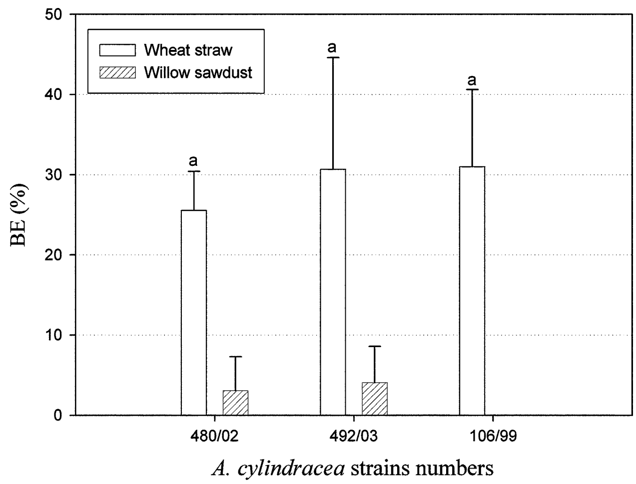

Fig. 1 BE (%) of three *A. cylindracea* strains cultivated on non-supplemented wheat straw or willow sawdust. Four replicates per substrate and strain were used. Values (means of four replicates) not sharing common letters are significantly different at $P = 0.05$

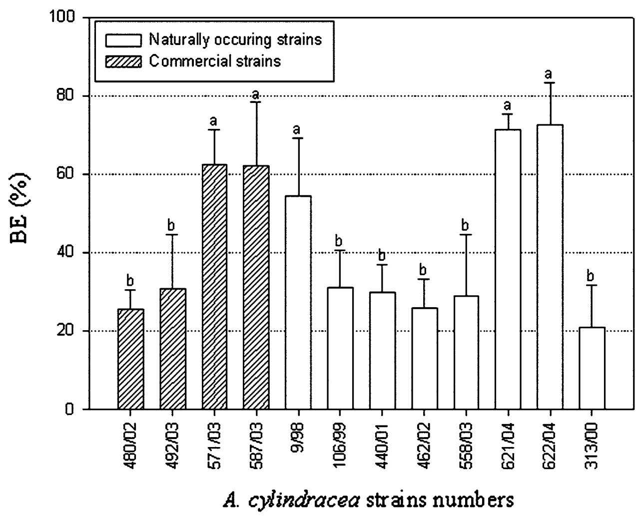

Fig. 2 BE (%) of four commercial and eight naturally occurring *A. cylindracea* strains cultivated on non-supplemented wheat straw. Four replicates per substrate and strain were used. Values (means of four replicates) not sharing common letters are significantly different at $P = 0.05$

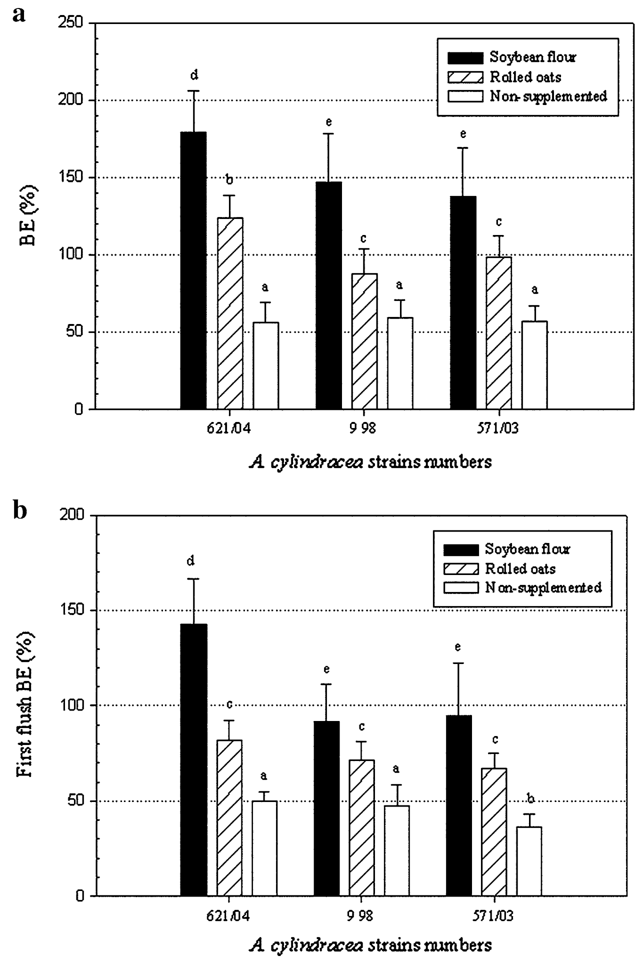

Fig. 3 BE (%) of three *A. cylindracea* highest yield strains cultivated on soybean flour or rolled oats supplemented and non-supplemented wheat straw. Eight replicates per formula and strain were performed. Comparisons were made between treatments on each strain and between strains on each treatment. Values (means of eight replicates) not sharing common letters are significantly different at $P = 0.05$

within the five highest-yield strains according to their type: one commercial and two wild strains were chosen; and their geographical origin: one from Europe, one from Asia and one from America. One of the two European commercial strains (ICFC 571/03) and one of the two wild Asiatic strains (ICFC 621/04) were chosen randomly. The American wild strain ICFC 9/98 was also selected for the supplementation experiment.

In this third experiment, the non-supplemented BEs of the three selected strains ranged from 56.5 to 59.4%, without significant differences (Fig. 3a). Regardless of the strain used, the BE was highly improved by rolled oats supplementation and even more enhanced by soybean flour supplementation: comparing with the non-supplemented condition, the BE was increased 1.2, 0.5, and 0.7-fold with rolled oats ($P < 0.05$) and 2.2, 1.5, and 1.4-fold with soybean flour ($P < 0.05$) in the two wild (ICFC 621/04 and 9/98) and in the commercial (ICFC 571/03) strains, respectively (Fig. 3a). No significant differences were found between the mean BE values of the American wild strain ICFC 9/98 and the commercial strain ICFC 571/03 in any condition.

A statistically significant interaction was found between the genotype and supplement used ($P < 0.05$). The yield of the Asiatic wild strain ICFC 621/04 responded more to the use of supplemented substrate: when we added rolled oats to the substrate, this strain yielded 41.4 and 25.2% more than the other two strains (ICFC 9/98 and 571/03, $P < 0.05$); similarly, when we used soybean flour as supplement, this strain yielded 22 and 30% more ($P < 0.05$) than the other two strains, achieving a BE average value up to 179% (Fig. 3a). The addition of supplement to the substrate had a similar effect on the first flush BE (Fig. 3b) and on the total BE (Fig. 3a). First flush BE was improved by rolled oats supplementation (0.7, 0.5, and 0.8-fold, $P < 0.05$) and much more enhanced by soybean flour supplementation (1.9, 0.9, and 1.6-fold, $P < 0.05$) in the two wild (ICFC 621/04 and 9/98) and in the commercial (ICFC 571/03) strains, respectively (Fig. 3b). Using soybean flour supplementation, the Asiatic wild strain (ICFC 621/04) BE on the first flush was 142.4% in average, a value that was significantly higher ($P < 0.05$) than those of the other two strains (91.4 and 94.5% BEs), whereas no differences were found between the three strains using rolled oats supplementation (67–82.1% BEs). A difference between first flush BE and total BE was observed in the non-supplemented condition, in which the wild strains (ICFC 621/04 and 9/98) first flush BEs were significantly higher (35.8 and 29.8%, $P < 0.05$) than the commercial strain (ICFC 571/03) first flush BE (Fig. 3b), whereas total BEs in this condition were similar for the three strains (Fig. 3a).

Effect of the genotype and supplement in other production traits

First harvest day, cropping period, number of flushes, yield, yield distribution and number of basidiomes are represented in Table 2 for the three strains and treatments. Concerning the first harvest day, the three strains were harvested during the same interval time, from day 12 to day 20, using the non-supplemented condition (Table 2). As regards the three strains supplemented with rolled oats and soybean flour, strains ICFC 621/04 and 571/03 were harvested for the first time from 1 to 7 days before, whereas the first harvest day of strain ICFC 9/98 was the same as in the non-supplemented condition (Table 2).

Total yield was distributed in two flushes in non-supplemented experiments using strains ICFC 9/98 and 571/03, and in one or two flushes using strain ICFC 621/04 (Table 2). Flushes were separated by seven or more days in which no basidiomata were present. Rolled oats supplementation caused a third flush in 13 and 29% of the experimental units using strains ICFC 621/04 and 571/03, but no third flush was registered for the strain ICFC 9/98. Soybean flour supplementation had a stronger effect, raising the number of flushes to three in 43–75% of the three strains' production blocks. The first flush yielded the most important fraction (50–100%) of the total crop; the second and third flush represented a much lower fraction, ranging from 0 to 50%, and from 0 to 35% of the total yield, respectively (Table 2).

The average number of fruiting bodies on the first flush ranged between 31.2 and 113.4, without significant differences between strains and supplements (Table 2).

Effect of the genotype and supplement in quality traits

Pileus weight, *pileus* diameter, fruiting body dry matter and gracility index were evaluated for the three selected strains (ICFC 9/98, 621/04, and 571/03) cultivated on supplemented or non-supplemented wheat straw. Fruiting body fleshiness was recorded for both, a commercial and a wild strain (ICFC 571/03 and 9/98) (Table 3).

The percentage of weight corresponding to *pileus* varied between 64.6 and 80.2% in average (Table 3). Supplementation had no significant effect on this trait. Concerning the genotype, the commercial strain ICFC 571/03 had a higher (0.1–0.2-fold, $P < 0.05$) percentage of *pileus* weight than the Asiatic wild strain ICFC 621/04 (Table 3). The mean values of the *pileus* diameter varied between 2.6 and 4.2 cm. Supplementation had no effect on the *pileus* size;

Table 2 First harvest day, cropping period, number of flushes, yield, yield distribution and basidiome number of *A. cylindracea* selected strains cultivated on non-supplemented and soybean flour or rolled oats supplemented with wheat straw

Strain no. (ICFC)	Supplement	First harvest day	Cropping period (days)	No. of flushes	Yield[a] (g)	Yield distribution (%)			Basidiome number
						Flush 1	Flush 2	Flush 3	
621/04	Soybean flour	8–17	30–53	2–3	[d]537.4 ± 80.7	65–88	0–25	0–35	[a]74 ± 22.9
	Rolled oats	8–13	28–57	2–3	[b]370.1 ± 44.6	63–74	0–37	0–33	[a]52.9 ± 18.5
	Non-supplemented	12–20	17–60	1–2	[a]169.3 ± 39.3	69–100	0–31	0	[a]31.2 ± 17.1
9/98	Soybean flour	12–20	53–60	2–3	[e]440.9 ± 93.1	53–74	1–36	5–27	[a]83 ± 35.6
	Rolled oats	17–19	39–46	2	[c]262.0 ± 48.5	69–83	17–31	0	[a]102.2 ± 56.7
	Non-supplemented	15–18	49–51	2	[a]178.2 ± 34.8	76–85	19–24	0	[a]113.4 ± 71.8
571/03	Soybean flour	12–17	26–64	2–3	[e]413.4 ± 93.4	57–88	0–39	0–18	[a]97.3 ± 38.7
	Rolled oats	11–14	28–49	2–3	[c]295.8 ± 41.3	37–81	19–46	0–20	[a]93.3 ± 52.1
	Non-supplemented	13–20	45–54	2	[a]170.5 ± 31.0	50–85	18–50	0	[a]62.3 ± 37.2

[a] Values (means of eight replicates) not sharing common letters are significantly different at $P = 0.05$. Eight replicates per formula and strain were performed. Comparisons were made between treatments on each strain and between strains on each treatment

Table 3 *Pileus* weight, *pileus* diameter, fruiting body fleshiness, fruiting body dry matter and gracility index of *A. cylindracea* selected strains cultivated on non-supplemented and soybean flour or rolled oats supplemented with wheat straw

Strain no. (ICFC)	Supplement	*Pileus* weight (%)	*Pileus* diameter (cm)	Fruiting body fleshiness (g/cm^2)	Fruiting body dry matter (%)	Gracility index
621/04	Soybean flour	^{a}64.6 ± 3.9	^{a}3.9 ± 0.5	Not measured	^{a,e}6.3 ± 1.1	^{a}7.9 ± 2.6
	Rolled oats	^{a}64.8 ± 5.6	^{a}3.6 ± 0.7	Not measured	^{a}7.2 ± 0.9	^{a}6.1 ± 1.4
	Non-supplemented	^{a}68.9 ± 7.4	^{a}4.2 ± 0.8	Not measured	^{a,d}6.2 ± 0.9	^{a}8.2 ± 3.3
9/98	Soybean flour	^{a,b,d,e}70.3 ± 3.3	^{a,b}3.2 ± 0.6	^{a}0.21 ± 0.03	^{b,e}5.6 ± 1.1	^{b}3.9 ± 1.3
	Rolled oats	^{a,d}66.8 ± 5.2	^{b}2.7 ± 0.4	^{a}0.20 ± 0.05	^{b}5.0 ± 0.5	^{b}4.3 ± 1.4
	Non-supplemented	^{b,c,e}77.0 ± 3.7	^{b}2.6 ± 0.5	^{a}0.21 ± 0.03	^{b,d}5.0 ± 0.2	^{b}2.6 ± 0.7
571/03	Soybean flour	^{b}76.9 ± 3.7	^{a,c}3.3 ± 0.7	^{a,b,c}0.27 ± 0.02	^{c,e}5.7 ± 0.9	^{c}2.1 ± 0.6
	Rolled oats	^{b}77.0 ± 8.5	^{a,b,c}3.0 ± 0.7	^{c}0.30 ± 0.12	^{a,b,c}6.4 ± 1.9	^{c,d}2.0 ± 1.0
	Non-supplemented	^{b}80.2 ± 9.3	^{b,c}3.1 ± 0.9	^{a,b}0.20 ± 0.06	^{c,d}6.2 ± 0.5	^{d}1.4 ± 1.0

Values (means of eight replicates) in the same trait not sharing common letters are significantly different at $P = 0.05$. Eight replicates per formula and strain were performed. Comparisons were made between treatments on each strain and between strains on each treatment

whereas the genotype had a significant effect on this trait: under the non-supplemented condition, the Asiatic wild strain ICFC 621/04 *pileus* was significantly bigger (0.4 and 0.6-fold, $P < 0.05$) than the other two strains (Table 3). The mean values of the fruiting body fleshiness were between 0.2 and 0.3 g/cm^2 (Table 3). Compared to the non-supplemented condition, rolled oats supplementation raised ($P < 0.05$) this trait by 50% in the commercial strain ICFC 571/03 but not in the wild strain ICFC 9/98. No differences in the fruiting body fleshiness were found between the two strains in soybean flour supplemented and non-supplemented conditions. Fruiting bodies dry matter mean values ranged between 5 and 7.2% (Table 3). No significant effect of genotype or supplementation was observed on this trait. Gracility index was strongly influenced by the genotype but was not affected by the supplementation treatment (Table 3). The Asiatic wild strain ICFC 621/04 had the higher ($P < 0.05$) gracility index: the mean values ranged between 6.1 and 8.2. The American wild strain ICFC 9/98 had intermediate ($P < 0.05$) gracility index mean values (between 2.6 and 4.3) and the European commercial strain ICFC 571/03 had smaller ($P < 0.05$) gracility index mean values (between 1.4 and 2.1).

The protein, carbohydrate, fat and fiber content of *A. cylindracea* analyzed in this work by using the strains ICFC 571/03, 9/98, and 621/04 cultivated on 20% soybean flour supplemented wheat straw are shown in Table 4 in comparison with those previously reported for *A. bisporus*, *Lentinus edodes* and *P. ostreatus*.

Table 4 Crude protein, carbohydrate, fat and fibre content of *A. cylindracea*, *A. bisporus*, *L. edodes*, and *P. ostreatus*

	A. cylindracea	*A. bisporus*[a]	*L. edodes*[a]	*P. ostreatus*[a]
Protein	21.8–29.8	23.9–34.8	13.4–17.5	10.5–30.4
Carbohydrate	34.3–45.5	51.3–62.5	67.5–78.0	57.6–81.8
Fat	1.4–3.8	1.7–8	4.9–8	1.6–2.2
Fibre	14.9–17.8	8.0–10.4	7.3–8.0	7.5–8.7

All values were expressed as percentage of dry weight

[a] Values taken from [1]

Discussion

In the first experiment, three *A. cylindracea* strains were cultivated on wheat straw or willow sawdust in order to choose a suitable substrate for cultivating this mushroom. Surprisingly, mycelium growth could not colonize the sawdust during the 55 days incubation period, which could be probably due to the weak lignolytic system of this mushroom [14, 16] or to the compactness of the wet sawdust, whose particle size is smaller than the wheat straw, thus causing deficient gaseous exchange. Although the natural substrate of *A. cylindracea* is wood, and it can be found growing naturally on willow trunks, these are likely colonized over a relatively long period of time; i.e., logs inoculated with *A. cylindracea* spawn fructified one year later [13]. In contrast, wheat straw was completely colonized after the incubation period and the three strains yielded significantly higher on this substrate, with BE mean values ranging between 25.5 and 31% (Fig. 1). Wheat straw is the most commonly employed substrate for *Pleurotus* cultivation [8], and our results showed that it is more suitable than willow sawdust as a substrate for *A. cylindracea* cultivation. Furthermore, the use of wheat straw in mushroom cultivation is of particular interest for the agricultural economy of temperate and subtropical countries, since it is produced in large quantities (25 million tons/year, see http://www.fao.org) and its post-harvest treatment is mainly accomplished through burning or incorporation into the soil [8].

In a second experiment, 12 commercial and naturally occurring *A. cylindracea* strains were cultivated on wheat straw in order to compare their BEs (Fig. 2). Five strains, including two commercial and three wild strains, yielded significantly more than the others, with BEs ranging between 54.5 and 72.4%. These values were higher than those previously obtained (47.5–49.8% BE) on wheat straw supplemented with wheat bran, and on other substrates such as cotton wastes and peanut shells [8].

In a third experiment, three of the highest-yielding strains were cultivated on supplemented (soybean flour or rolled oats) and non-supplemented wheat straw. Supplementation strongly affected production traits such us first harvest day, BE and number of flushes, but had few or no effect on quality traits, meaning that soybean flour or rolled oats supplementation can be used for increasing these production traits on high-quality strains without changing their desirable characteristics. In particular, BE was the most positively affected production trait, which was highly raised by the addition of supplements, especially by soybean flour. Supplementation with soybean flour leads to the highest BE average values ever reported for *A. cylindracea*: 137.8–179.1%. Furthermore, these BE values were high even compared with those previously obtained with more widely cultivated edible fungi, such as *P. ostreatus*, *P. pulmonarius*, *P. djamour* and *P. eryingii* [8, 10, 20], *P. sajor-caju* and *P. cornucopiae* [3, 21] and *L. edodes* [9]. The effect of supplementation on BE depended on the cultivated genotype: interestingly, the Asiatic wild strain ICFC 621/04 yielded 22 and 30% more on soybean flour supplemented wheat straw and 41.4 and 25.2% more on rolled oats supplemented wheat straw than the other two strains (ICFC 9/98 and 571/03), respectively (Fig. 2). These results are in accordance with those of Ko et al. [4], who pointed out the importance of testing several *Hericium erinaceum* strains for their ability to utilize supplements.

The effect of supplementation on other production traits also depended on the genotype; soybean flour and rolled oats supplementation had similar effects on advancing the first harvest day. Both advanced the first harvest day for 3–6 days using strains ICFC 621/04 or 571/03, but no effect was observed for the strain ICFC 9/98 cultivated on supplemented wheat straw (Table 2). Soybean flour supplementation had a stronger effect than rolled oats supplementation in the number of flushes: it raised the number of flushes to three in 43–75% of the production blocks of the three strains whereas rolled oats addition caused a third flush in 13 and 29% of the production blocks using strains ICFC 621/04 and 571/03, respectively, but no third flush appeared when using strain ICFC 9/98 (Table 2). Interestingly, despite the fact that the first harvest day of the strain ICFC 9/98 was not advanced by using supplements and no third flush appeared using rolled oats, this wild strain's total BE values were similar to those obtained with the commercial strain ICFC 471/03 in both supplemented and non-supplemented conditions. Concerning the effect of supplementation on quality traits, it only raised fleshiness of the commercial strain ICFC 571/03, but had no effect on the fleshiness of the wild strain ICFC 9/98. The fleshiness values obtained in this study for *A. cylindracea* strains were similar to those of *P. ostreatus* growing on straw [5]. Supplementation had no effect on the other quality traits studied (Table 3).

Comparing the BEs obtained on non-supplemented wheat straw with the three selected genotypes, the two wild strains (ICFC 621/04 and 9/98) first flush BEs were 29.8 and 35.8% higher than the first flush BEs of the commercial wild strain ICFC 571/03. This was not the case if total BE was considered, in this case the three strains' BEs were similar. Nevertheless, as in other edible mushrooms and as previously reported [8], yield distribution in *A. cylindracea* was not homogeneous, with the most important fraction of the yield (50–100%) occurring during the first flush. Moreover, this flush occurred during the first 22 days of the cropping period, where the maximum was fixed in this work on 60 days (all experimental units were discarded after this period under fruiting conditions), meaning that discarding the substrate blocks after harvesting the first flush could be an interesting option for solving space problems in a fruiting room. The Asiatic wild strain ICFC 621/04 has several advantages: it is the most productive genotype on supplemented wheat straw, it takes less days to be harvested for the first time, and it has a bigger *pileus*, which can be considered as a desired feature for edible mushrooms. The commercial strain ICFC 571/03 has a higher percentage of weight corresponding to *pileus*, which is also a desired feature. Breeding between these two strains should be attempted.

Table 4 compares some nutritional properties reported here for *A. cylindracea* with those previously reported for *A. bisporus* and *L. edodes*, the two most important cultivated mushrooms in the world, and *P. ostreatus*, the second most important cultivated mushroom in South America. *A. cylindracea* stands out for its highest fibre content, 0.4–1.4-fold higher than the other compared species. The protein content of *A. cylindracea* is comparable to that of *A. bisporus* and *P. ostreatus*, and higher than the protein content of *L. edodes*. The high fibre and protein content is a health contributing factor of edible mushrooms [1]; these results evidence the high nutritional value of *A. cylindracea*, making it an interesting mushroom for industrial cultivation.

In conclusion, utilization of new naturally occurring strains and using soybean flour supplemented wheat straw can highly raise BEs in *A. cylindracea* cultivation. Further investigations should be performed to test other supplements and their combinations. The high BE reached for the first time in this work will allow for re-evaluation of this species for commercial cultivation purposes worldwide. In addition, this article clearly evidenced the high nutritional value of *A. cylindracea*, meaning that this mushroom represents an excellent alternative to more traditionally cultivated species.

Acknowledgments This work was supported by the research project PIP 5516 from *Consejo Nacional de Investigaciones Científicas y Técnicas* (CONICET, Argentina). E. Albertó is a staff member and M. Uhart is a fellow from CONICET. We thank Dr. J. Labarère (Bordeaux, France) for providing strains and M. Sierra Marina for technical assistance.

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