Overview of four *Agaricus subrufescens* strains used in the last 15 years in Brazil and other countries and current potential materials for the future

Diego C. Zied 1 • Wagner G. Vieira Junior 2 • Douglas M. M. Soares 3 • Cassius V. Stevani 3 • Eustáquio S. Dias 4 • Matheus R. Iossi 2 • Arturo Pardo-Giménez 5

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Abstract
The mushroom *Agaricus subrufescens* has been synonymous with *Agaricus blazei* and *Agaricus brasiliensis* during the last decades, and there has been much discussion with regard to the origin, distribution, and nomenclature of this mushroom. Therefore, we conducted a genetic and morphological characterization of the mycelium and mushroom of four commercial strains currently cultivated in Brazil (ABL CS7, ABL 18/01, ABL 98/11, and ABL 16/01) together with an assessment of their agronomic behavior and compared these results with those published in works using other strains during the last 15 years. All the *A. subrufescens* strains characterized here are phylogenetically related to the Americas/Europe specimens, bearing an internal transcribed spacer region of type A (ABL 16/01) or both types A and B (ABL 18/01, ABL 98/11, and ABL CS7). We did not find any correlation between the morphological characteristics of the mycelial colonies and the agronomic behavior of the strains. Strains ABL 98/11 and ABL 16/01 produced the best yields and morphological characteristics for the mushrooms, indicating their high weight, which enhances the commercialization of the mushroom and justifies their longstanding commercial use over the last 15 years.

Keywords *Agaricus blazei* • *Agaricus brasiliensis* • Genetic characterization • Yield • Screening of mushroom quality

Introduction

*Agaricus subrufescens* Peck is synonymous with *Agaricus blazei* (Murrill) ss. Heinemann and *Agaricus brasiliensis* Wasser et al. (Dias et al. 2013). Over recent decades, there have been ongoing discussions as to the origin, distribution, and nomenclature of this mushroom (Kerrigan 2005, 2007; Wasser et al. 2002, 2005). *A. subrufescens* is alternatively known as the sun, almond, or medicinal mushroom, and the cultivation history can be divided into three periods. The first period was between approximately 1894 and 1918, as reported by Kerrigan (2005), when the species was cultivated in the USA (Falconer 1894; Anonymous 1904). The second period occurred between 1965 and 1997, based on reports by Iwade and Ito (1982), Iwade and Mizuno (1997), and Mizuno (1997) who described the cultivation in Brazil, Japan, China, and Korea and presented technological advances in the area of mushroom breeding. The final period began with the publication of the use of *A. subrufescens* in Brazil (Colauto et al. 2002; Eira et al. 2005a, b), which allowed an increase of research teams to study this mushroom in a greater range of countries, such as Argentina, Canada, France, Slovenia, Mexico, Taiwan, and Norway, and continues to the present day (Gregori et al. 2008; González Matute et al. 2010; Wasser et al. 2005, 2007; Wasser et al. 2002, 2005).
The strains used in experimental crops in the last 15 years came from Brazil, France, Spain, the USA, Mexico, Taiwan, Belgium, and Italy (Llarena-Hernández et al. 2011). In recent decades, studies have been conducted mainly with strains isolated from commercial crops (Brazil), with several strains collected from the wild (France, Spain, Thailand, and others). Cultivated mushrooms vary in the obtained yield, average weight of the mushrooms, and morphological characteristics (color and format) of the pileus and the stipe of mushrooms (Colauto et al. 2010a, b, c; Llarena-Hernández et al. 2013). To avoid these variations and combine the favorable characteristics of isolates and wild-collected specimens, several hybrids have been developed (Zied et al. 2011a; Jatuwong et al. 2014); however, it is unknown exactly why these hybrids have not yet been used commercially.

Studying the genetic variation of Japanese and Brazilian strains, Figueiredo et al. (2013) reported the low degree of difference between the isolates and suggested that the strains could have come from the same wild-type population of A. brasiliensis. Finally, the authors suggested that the development of studies of genetic divergence among strains should be integrated into several other aspects of growth such as physiology and productivity to enable a precise and consistent strain analysis and efficient development of superior strains for cultivation.

In this sense, the present manuscript studied in detail the commercial strains currently cultivated in Brazil and compared them with the strains used in the last 15 years in several publications. We carried out a genetic and morphological characterization of the mycelium and mushroom of four selected strains and also evaluated their agronomic behavior.

**Materials and methods**

The experiment was divided into four evaluations. The first referred to genetic characterization, the second to mycelial morphological characterization, the third to morphological characterization of the fruitbodies, and the fourth to the agronomic behavior of the mushroom strains. Four most cultivated commercial strains of *A. subrufescens* were used: ABL CS7 (acquired from the Federal University of Lavras, Brazil – MW200295.2 GenBank number); ABL 18/01 (isolated from a grower in the region of São Paulo, Brazil – MW200293.2 GenBank number); ABL 98/11 (isolated from growers in the region of Mogi das Cruzes, Brazil – MW200294.2 GenBank number); and ABL 16/01 (isolated from commercial spawn lab in Valinhos, Brazil – MW200292.1 GenBank number).

The strains were deposited in the collection of the Centro de Estudos em Cogumelos, from the Universidade Estadual Paulista, Câmpus de Dracena, with open access to other researchers.

**Genetic characterization of the A. subrufescens strains**

To genetically characterize *A. subrufescens* ABL 16/01, ABL 18/01, ABL 98/11, and ABL CS7 strains, we performed amplification, cloning, and DNA sequencing of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (rDNA), which is widely used in fungal taxonomy (Schoch et al. 2012). Initially, 100 mg of *A. subrufescens* mycelium was added to 2.0-mL imnuSPEED lysis tubes containing steel beads (5 × 4.7 mm) and 400 μL of buffer AP1 (DNeasy Plant Mini Kit, QIAGEN) for tissue disruption. Samples were homogenized for two cycles of 1 min each in the SpeedMill PLUS equipment (Analytik Jena). Then, 4 μL of RNase A was added to each tube, followed by incubation at 65 °C for 10 min. All following steps on genomic DNA extraction were performed as recommended by the manufacturer (DNeasy Plant Mini Kit, QiaGen).

Genomic DNA samples were eluted in sterile distilled water and quantitated using a NanoPhotometer® (Implen). PCR reactions were carried out in a final volume of 25 μL with Platinum Taq DNA Polymerase (Invitrogen) using 50 ng of genomic DNA and conserved primers that flank a rDNA region containing the ITS sequences: ITS5F (5′-GGAA GTAAAGTCGTAACAAGG-3′) and ITS4R (5′-TCCT CCGCTTAATGATATGC-3′) (White 1990). Reactions were incubated in a SimpliAmp thermal cycler (Applied Biosystems) at 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C for 30 s, and DNA extension at 72 °C for 1 min. PCR products were separated by electrophoresis on a 1.5% agarose gel for 35 min at 130 V. DNA bands (~700 bp) were excised, purified using the GenElute™ gel extraction kit (Sigma Aldrich) in a volume of 40 μL, and quantitated with the NanoPhotometer®. Purified PCR amplicons were cloned into pGEM-T Easy Vectors (Promega) at a molar ratio of 5 (insert):1 (vector), according to the manufacturer’s instructions.

Chemocompetent *Escherichia coli* stellar cells were transformed with ligation reactions. Reactions were plated in selective LB media containing ampicillin and incubated at 37 °C for 16 h. Positive clones were confirmed by plasmid DNA extraction, followed by EcoRI digestion and electrophoresis in 1.5% agarose gel. DNA sequencing reactions were prepared with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) using 5 μL of plasmid DNA (100 ng/μL) and 2.5 μL of 5 μM sequencing primers M13F (5′-CGCCAGGGTTTTCGATCGAC-3′) or M13R (5′-CAGGAAACAGCTATGAC-3′). Four clones of each sample were sequenced in duplicate using the Sanger method with an ABI 3730 DNA Analyzer (Applied Biosystems), at the Centro
de Pesquisa sobre o Genoma Humano e Células-Tronco da Universidade de São Paulo (CEGH-USP, Brazil).

Consensus sequences for each *A. subrufescens* strain were obtained from the analysis of all DNA sequencing replicates using the software Geneious Prime® 2020.2.4 (Biomatters). MUSCLE multiple alignments were performed using these consensus sequences and 23 *A. subrufescens* ITS sequences from different geographic regions (Table S2). A consensus tree was generated from the Geneious tree builder using the neighbor-joining method and Jukes-Cantor genetic distance model, with 10,000 replicates at the bootstrap resampling method.

**Mycelial morphological characterization**

For mycelial characterization, a petri dish compost agar was used, following the methodology reported by Jones et al. (2017). Fresh compost used for *A. subrufescens* production was dried for 48 hours at a temperature of 60 °C. A 60 g of dried compost was added with 1 l of distilled water and the mixture boiled for 30 min. The boiled mixture was filtered, and 15 g of agar was added and sterilized at 121 °C for 30 min. Culture medium was transferred to petri dishes (90 mm in diameter) and, after cooling, was inoculated with a mycelium disc (10 mm in diameter) from the respective strains. After 12 days at 28 °C, colonies were classified into different types (Jones et al. 2017), which were determined prior to analysis:

- **Type 1**: Cottony mycelium, with uniform radial distribution, presence of homogeneous aerial mycelia (throughout the colony), with rapid mycelial growth
- **Type 2**: Rhizomorphic mycelium, thick, with uniform radial distribution, presence of partial aerial mycelia (only in the center of the colony), and rapid mycelial growth
- **Type 3**: Cottony mycelium, with uneven radial distribution, presence of homogeneous aerial mycelia, and intermediate mycelial growth
- **Type 4**: Rhizomorphic mycelium, thick, with uneven radial distribution, presence of partial aerial mycelia (only in the center of the colony), with intermediate mycelial growth

**Mushroom morphological characterization**

For morphological evaluation, 30 mushrooms from each strain were randomly selected before the rupture of the ring (recommended point of harvest), and the following parameters were considered: total length of the mushroom, pileus and stem, width of the stem, stem base, pileus, and ring size. The measurements were performed with the aid of a 150-mm digital caliper with 0.1-mm precision, as shown in Fig. 1.

To assess coloration, a Discovery V20 Binocular Stereoscope and an Axiocam 503 color camera (Zeiss®) were used. Readings were made with two X-shaped lines, each of which had approximately 1000 points. The average of all measured points was obtained, and thus each mushroom constituted a repetition. The color data were obtained in RGB format (red, green, and blue) and analyzed separately according to the color spectrum.

**Agronomic behavior**

The inoculum was prepared based on sorghum grains following the production steps for selection of mushroom and production of subculture, parent spawn, and grain spawn as described by Zied et al. (2011a, b). The compost formulation consisted of a mixture of wheat straw, chicken manure, and gypsum. The compost was produced using the traditional composting method described by Zied et al. (2014) with phase I (a total of 26 days, with 7 days of prewetting and 19 days of fermentation) and phase II (total of 9 days, with 8 h of pasteurization at 59 °C and 8 days of conditioning at 47 °C).

After the composting process, the substrate was distributed in plastic boxes, in equal amounts of 3.5 kg, with the inoculum added in the measure of 1% of the wet weight of the compost. Subsequently, the compost was incubated in a semi-controlled mushroom chamber (temperature and humidity control) for 20 days at a temperature of 28 ± 2 °C and relative humidity of 80 ± 5%. A casing layer based on peat moss was used to provide high water holding capacity and porosity and less compaction, thus allowing gas exchange between the compost and the environment. The casing layer was added to a thickness of 4 cm over the colonized compost.

After the colonization of the casing layer, ruffling was performed on the 27th day. The primordia induction was carried out by temperature oscillation and control of the relative
humidity. On the 28th day, the temperature was reduced from 28 ± 2 °C to 20 ± 2 °C with a humidity of 90 ± 5%, following the methodology proposed by Pardo-Giménez et al. (2020a) for rapid primordia induction. Then, the temperature was again increased to 28 ± 2 °C, with the same process being performed in each harvest flush. The total growth cycle was 85 days (20 days of mycelial run and 65 days of production phase).

The mushrooms were harvested twice a day manually and weighed after scraping the base of the stipe to remove residues from the casing layer. The yield (fresh weight of mushrooms divided by fresh weight of the compost, multiplied by 100, expressed as a percentage), number of mushrooms (count of mushrooms harvested), weight of mushrooms (fresh weight of mushroom divided by mushroom number), precocity (yield of half the production time, started after harvesting the first mushroom, divided by the total production time multiplied by 100 and expressed as a percentage), and earliness (number of days between adding the casing layer and the time required for the first harvest) were evaluated as described by Zied et al. (2010) and Navarro et al. (2020).

The experiment was carried out in a completely randomized design, with four treatments (strains of A. subrufescens: CS7, ABL 18/01, ABL 98/11, and ABL 16/01) each with eight replicates, which were represented by a 3.5-kg box of compost.

**Results**

**Genetic characterization of the A. subrufescens strains**

DNA sequencing analysis of the rDNA region from the four strains of A. subrufescens (ABL 16/01, ABL 18/01, ABL 98/11, and ABL CS7) revealed the successful amplification of a 773-bp genomic region, including a partial sequence of the small subunit ribosomal RNA gene and complete sequences for the ITS 1 5.8S ribosomal RNA gene and for ITS 2, a partial sequence of the large subunit ribosomal RNA gene. Consensus sequences for each A. subrufescens strain were generated from the analysis of nonredundant DNA sequences (Table S1) and deposited in the NCBI nucleotide database under the accession numbers MW200292 (ABL 16/01), MW200293 (ABL 18/01), MW200294 (ABL 98/11), and MW20029 (ABL CS7).

Analysis of the polymorphic regions from a multiple alignment performed between our sequences and A. subrufescens ITS types A, B, A/B, and C, previously characterized by Chen et al. (2016), revealed the occurrence of ITS type A in the ABL 16/01 strain and both ITS types A and B in the ABL 18/01, ABL 98/11, and ABL CS7 strains (Table 1).

In addition to polymorphisms at 13 positions (90, 168, 176, 184, 199, 200, 254, 323, 389, 521, 525, 614, and 704), previously reported by Chen et al. (2016), we found two other new polymorphisms at positions 208 and 644 for ITS type B and for ABL 18/01, ABL 98/11, and ABL CS7 strains. DNA sequencing of more clones is needed to confirm if the polymorphisms at positions 224, 240, and 673 represent true minor variants or PCR/sequencing artifacts.

A consensus tree built from the multiple alignments between the ITS sequences described here and another 23 ITS sequences from 21 A. subrufescens strains native of the Americas, Asia, Europe, and Oceania revealed that all the strains analyzed in this work are phylogenetically closer to the Americas and Europe specimens (ITS types A or B) than to the Asia and Oceania sequences, characterized by specimens bearing the ITS type C (Fig. 2).

**Mycelial morphological characterization**

Mycelial morphology was classified by the mycelial structure (rhizomorphic or cottony), radial growth distribution (uniform

| Polymorphic positions in ITS sequences | 90 | 168 | 176 | 184 | 199 | 200 | 208 | 224 | 240 | 254 | 323 | 389 | 521 | 525 | 614 | 644 | 673 | 704 |
|---------------------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| ITS A                                 | T  | A  | A  | A  | G  | A  | C  | T  | T  | T  | G  | A  | A  | T  | G  | A  | T  | T  |
| ABL 16/01                             | T  | A  | A  | A  | G  | A  | C  | T  | T  | T  | G  | A  | A  | T  | G  | A  | T  | T  |
| ITS B                                 | -  | G  | G  | G  | A  | T  | Y  | T  | T  | G  | A  | A  | T  | G  | A  | T  | T  | T  |
| ITS A/B                               | T/-| A  | R  | R  | R  | W  | Y  | T  | T  | Y  | G  | A  | A  | T  | G  | R  | T  | T  |
| ABL 18/01                             | T/-| A  | R  | R  | R  | W  | Y  | Y  | T  | Y  | G  | A  | A  | T  | G  | R  | Y  | T  |
| ABL 98/11                             | T/-| A  | R  | R  | R  | W  | Y  | T  | T  | Y  | G  | A  | A  | T  | G  | R  | T  | T  |
| ABL CS7                               | T/-| A  | R  | R  | R  | W  | Y  | T  | T  | Y  | G  | A  | A  | T  | G  | R  | T  | T  |
| ITS C                                 | -  | A  | G  | G  | A  | T  | C  | T  | T  | C  | A  | A  | C  | -  | G  | A  | T  | T  |

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and uneven), presence of aerial hyphae (homogeneous and partial), and time for colonization of the culture medium (10 and 15 days).

The ABL CS7 strain produced rhizomorphic and aerial mycelia at the beginning of growth (partial) and took longer for colonization (15 days) with uneven radial growth; therefore, this strain was classified as type 4. The ABL 98/11 strain had the same morphological characteristics as type 4 but differed with respect to having cottony mycelium and was therefore classified as type 3 (Fig. 3).

However, the ABL 18/01 strain produced rhizomorphic and aerial mycelia at the beginning of growth (partial), had only a short colonization time (10 days) with radial growth, and was classified as type 2. The ABL 16/01 strain showed the same morphological characteristics but differed with respect to having cottony mycelium and homogeneous aerial hyphae and was therefore classified as type 1.

**Mushroom morphological characterization**

Evaluation of the morphology of mushrooms revealed a significant difference between the strains, with mushroom length being the only nonsignificant factor. When analyzing the length and width of the pileus, the ABL 16/01 and 98/11 strains had the highest values, and ABL CS7 and ABL 18/01 strains had the lowest. Regarding the size of the ring, the ABL 16/01 strain was the largest, whereas the ABL CS7 and 18/01 strains were smaller. In terms of the length of the stipe, the ABL CS7 strain had the highest value. The widths of the stipe and of the base were largest in ABL 16/01 followed by those of the ABL 98/11 strain, which are considered to have more robust fruit bodies than those of the ABL CS7 and 16/01 strains (Table 2).

Another morphological characteristic evaluated was the color of the pileus. Significant differences were found between all parameters evaluated; the ABL CS7 strain had the lowest values for color and was classified as a strain with a darker pileus. The strains ABL 16/01 and ABL 18/01 had statistically equal values and the intermediate color of the pileus. Finally, the ABL 98/11 strain showed the highest color value, with a clear pileus color. The combination of the three colors can be transformed into a hexadecimal code, which can then be used as a universal color standard (Table 3).

**Agronomic behavior**

All agronomic variables exhibited significant differences in relation to the strains used, except for precociousness. ABL CS7, 98/11, and 16/01 strains had the highest yields, practically twice that of the ABL 18/01 strain (Table 4). Characterizing these strains, ABL 98/11 had the highest yield.
in the first flush while CS7 had the highest yield in the second flush. The precocity values were 94.98%, 93.44%, and 80.10% for ABL 98/11, ABL 16/01, and ABL CS7, respectively; ABL CS7 had a low value owing to reduced yield in the 1st flush of harvest (Fig. 4).

Regarding the number and weight of mushrooms, two opposite situations were found: in the first, the ABL CS7 strain presented a greater number of mushrooms harvested with reduced weight, whereas, in the second with the ABL 98/11 strain, fewer mushrooms were harvested but with a greater weight. Earliness and precocity complete the range of positive agronomic parameters that a strain must have and help position the ABL 98/11 strain as the best genetic material used in this research (Fig. 4).

**Discussion**

*Agaricus subrufescens* is only grown commercially in a few countries, such as Brazil, Japan, China, Taiwan, South Korea, and the USA, and this confined cultivation range has limited technological advancement to increase yield. Japan was the first country to adapt *A. bisporus* cultivation technologies to *A. subrufescens* (1997). Recently, Spanish and French researchers have also applied this type of study (Pardo-Giménez et al. 2020a, b; Llarena-Hernández et al. 2014). A greater diversity of strains was used in the third period (2005 to 2020) of cultivation compared with those used in the second period (1965 to 1997); these more recent strains were

| Strain    | Mushroom length | Pileus length | Pileus width | Ring size | Stipe length | Stipe width | Base width |
|-----------|-----------------|---------------|--------------|-----------|--------------|-------------|-----------|
| ABL CS7   | 102.35 a        | 37.34 c       | 34.92 c      | 14.22 c   | 94.35 a      | 19.12 c     | 30.24 c   |
| ABL 18/01 | 95.25 a         | 37.35 c       | 28.30 d      | 10.50 c   | 83.25 b      | 18.45 c     | 27.75 c   |
| ABL 98/11 | 98.53 a         | 46.94 b       | 38.10 b      | 14.94 b   | 85.78 b      | 21.84 b     | 33.83 b   |
| ABL 16/01 | 97.29 a         | 65.15 a       | 41.10 a      | 16.61 a   | 82.24 b      | 26.68 a     | 42.46 a   |
| CV (%)    | 10.28           | 15.98         | 12.78        | 18.75     | 11.92        | 19.48       | 15.66     |
significantly superior than earlier ones as they included wild, isolated, and hybrid varieties.

In Brazil, the number of strains used commercially has also increased, although only three strains are still cultivated (ABL CS7, ABL 98/11, and ABL 16/01) when comparing the strains used in this study with those used in the last 15 years (Table 5). This selection is justified by the favorable agronomic behavior and morphological characteristics of these mushrooms (Tables 2 and 4). In Argentina, the strains studied came from the commercial laboratory Brasmicel at the beginning of 2010 and were sent by Edson de Souza (González Matute et al. 2010, 2011, Matute et al. 2012).

In Europe, after 2010, two countries led the experimental studies carried out with A. subrufescens. In Spain, studies were carried out using Brazilian strains (Zied et al. 2011a, b; Pardo-Giménez et al., 2016), and, in France, wild and hybrid strains were used (Thongklang et al. 2014a, b; Foulongne-Oriol et al. 2016). Furthermore, French researchers had a great influence on the identification and dissemination of A. subrufescens in Thailand (Wisitrassameewong et al. 2012; Thongklang et al. 2016), whereas North American research influenced the experimental cultivation in Norway (Stoknes et al. 2013).

A. subrufescens mushrooms exhibit a high level of polymorphism in the ITS of the nuclear rDNA region (Kerrigan 2005). Comparing A. subrufescens ITS sequences and samples from different geographic regions, Chen et al. (2016) observed a distribution of ITS type A, B, or both A/B in American and European A. subrufescens specimens, whereas specimens from Asia or Oceania exhibited ITS type C, with the exception of the wild French isolate CA487 that had ITS of all three types.

Our results revealed that all the A. subrufescens strains characterized here were phylogenetically related to the Americas/Europe specimens, bearing an ITS of type A (ABL 16/01) or both types A and B (ABL 18/01, ABL 98/11, and ABL CS7). A previous study carried out with other strains and molecular markers showed that the strains used in Brazil had a substantial genetic similarity, suggesting a common origin (Tomizawa et al. 2007). Similar findings were reported from genetic analysis of both Brazilian and European A. subrufescens cultivars using simple sequence repeat (SSR) markers, a molecular tool widely used to study genetic diversity and reproductive biology (Foulongne-Oriol et al. 2012, 2014). A genetic distance tree from this analysis revealed that the Brazilian cultivars belong to the same cluster, different from those European cultivars where a significant polymorphism was detected (Llarena-Hernández et al. 2013). Thus, any differences in agronomic traits, mainly among Brazilian strains, should be related to other DNA

### Table 3 Pileus color and hexadecimal code of A. subrufescens strains

| RGB code (0-255) | Hexadecimal code |
|-----------------|-----------------|
| Strain          | Red  | Green | Blue  | |
| ABL CS7         | 132.41 c | 108.82 c | 77.13 c | #846c4d |
| ABL 18/01       | 198.55 b | 169.30 b | 145.15 b | #c6a991 |
| ABL 98/11       | 221.45 a | 211.20 a | 186.15 a | #ddd3ba |
| ABL 16/01       | 188.20 b | 161.55 b | 123.80 b | #bca17b |
| CV (%)          | 16.28 | 17.13 | 21.68 |

### Table 4 Agronomic behavior of A. subrufescens strains after 95 days of cultivation

| Strain   | Yield, % | Number of mushroom, u | Weight of mushroom, g | Precocity, % | Earliness, days |
|----------|----------|------------------------|-----------------------|--------------|-----------------|
| ABL CS7  | 11.02 a  | 20.0 a                 | 18.13 c               | 80.10 a      | 34.2 b          |
| ABL 18/01| 6.64 b   | 12.8 b                 | 16.53 c               | 91.91 a      | 24.8 a          |
| ABL 98/11| 14.94 a  | 11.6 b                 | 43.05 a               | 94.98 a      | 20.0 a          |
| ABL 16/01| 13.21 a  | 13.0 b                 | 34.29 b               | 93.44 a      | 23.4 a          |
| CV (%)   | 29.09    | 27.98                  | 11.87                 | 15.74        | 14.71           |

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*Note: CV (%) represents the coefficient of variation.*
sequences that may be assessed by various molecular methods, including the single-nucleotide polymorphisms (SNPs). Likewise SSR markers, SNPs have become the best choice for population genetic studies (Chattopadhyay et al. 2017), being potentially useful to correlate slight modifications of the genes with phenotypic variations, helping us to understand the genomic and functional diversity of fungi (Kim et al. 2015; Tsykun et al. 2017; Guo et al. 2020).

Studies have shown that the mycelia of *A. subrufescens* have intermediate growth, taking an average of 10 to 20 days to colonize a 90 mm-diameter petri dish, depending on the culture medium and strains used (Neves et al. 2005). This differs from *Pleurotus ostreatus*, which has a more rapid growth, taking only 6 days to colonize a petri dish of the same size (Donini 2006). In the present study, strains ABL 18/01 and ABL 16/01 took 10 days to colonize the entire culture medium, which in a commercial cultivation provides advantages as faster mycelium develops decreases the possibility of contamination of the compost by other fungi and bacteria. We did not find any correlation between the morphological characteristics of the colonies and the agronomic behavior of the strains.

Morphological characteristics of mushrooms are extremely important when selecting strains for commercial production. In Brazil, the commercialization of mushrooms is based on standards such as length (height) of mushroom, width of base and stipe, and level of ring opening (more closed mushrooms have better market value), and mushrooms are classified as extra, grade A and B, and opened, respectively (Zied et al. 2017). Therefore, mushrooms from the ABL 16/01 strain would be classified as extra mushrooms, meeting all quality standards, when the mushroom length reaches above 80 mm and base width is between 35 and 50 mm with a closed pileus. With regard to this last parameter, this strain presented an excellent ring size (16.61 mm) before veil rupture. The ABL 98/11 strain also has favorable morphological characteristics. Given all these results, the weights of harvested mushrooms underlined the excellent commercial quality for strains ABL 16/01 (34.29 g) and ABL 98/11 (43.05 g). However, it should be noted that on some occasions, the weight of the mushroom has a negative correlation with the number of mushrooms harvested (Straatsma et al. 2013).

The color of the mushroom pileus is related to the level of maturation of the mushroom (near the rupture of the mushroom-veil, this becomes clearer) and the number of small scales over the pileus. Pardo-Gimenez et al. (2020a) found that the primordial induction method influences the color of the pileus so that slow induction keeps the pileus darker when using the ABL 99/30 strain.

We found a negative correlation between mushroom weight and mushroom number (r = −0.723 and P = 0.003). This has also been observed in other studies (Chu et al. 2012; Dias et al. 2013) but differs from those obtained in research with Chinese and Japanese cultivars (Wang et al. 2010, 2013; Win and Ohga 2018).

Finally, yield is an important parameter to be considered as an agronomic behavior, being directly related to the cultivation earliness, precocity, and crop time (growth cycle). Wild and hybrid strains from Spain, France, and Thailand have low earliness, with values from 15.1 to 30 days, whereas cultivars from Brazil, China, and Japan are tardy, with earliness values between 26.8 and 48.8 days after the addition of casing (Llarena-Hernández et al. 2014; Horm and Ohga 2008; Wang et al. 2013; Jatuwong et al. 2014).

Nevertheless, the most recent published yields reached 28.16%, with ABL 16/01 strain during a 108-day growth cycle (Zied et al. 2018), and 20.91%, with ABL 99/30 strain during an 83-day growth cycle (Pardo-Giménez et al. 2020b). These yields were similar to the excellent yield obtained by the wild strains CA 487 (24.4%) and 438-A (26.2%), during their 85-day growth cycle, and the M7700 cultivar (21.1%), which had a 130-day growth cycle (Llarena-Hernández et al. 2014). Our current highest yield was 14.94%, with ABL 98/11 strain during an 85-day growth cycle, with commercial quality mushroom grade A, in a semi-controlled mushroom chamber, which allows lower energy expenditure for Brazilian growers. This strain provides more than 90% of the total harvest in the first two flushes (Fig. 3), which opens the possibility of reducing the duration of the growing cycle.

Notable, *A. subrufescens* differs from *A. bisporus* because of the wide variation in yield and other agronomic behaviors obtained with the same strains in different publications. Another important point when producing commercial cultivars from other countries is the period of domestication in...
Table 5 Strains used in the last 15 years in different countries and regions

| Strains         | Origin                  | References                                                                 |
|-----------------|-------------------------|-----------------------------------------------------------------------------|
| ABL-97/11 = CS4 | São Paulo, SP, Brazil   | Eira et al. (2005a, b)                                                      |
| ABL-97/12       | Piedade, SP, Brazil     | Braga (1999); Braga et al. (2006)                                           |
| ABL-98/11 = CA 571 | Mogi-da-cruzes, SP, Brazil | Llarena-Hernández et al. (2011)                                          |
| ABL-99/25 = CS5 | Araçatuba, SP, Brazil   | Eira et al. (2005a, b); Sousa et al. (2016)                                 |
| ABL-99/26       | São José dos Campos, SP, Brazil | Colauto et al. (2010a, b, c, 2011)                                      |
| ABL-99/28 = CA 560 | Botucatu, SP, Brazil | Eira et al. (2005a, b); Llarena-Hernández et al. (2011); Farnet et al. (2013); Zied et al. (2012a, 2014) |
| ABL-99/29 = CS7 | Porto Alegre, RS, Brazil | Eira et al. (2005a, b); Kopytowski Filho and Minhoni (2004); Colauto et al. (2010c, 2011); Sousa et al. (2016) |
| ABL-99/30 = CA 561 | Piedade, SP, Brazil | Eira et al. (2005a, b); Kopytowski Filho (2006); Kopytowski Filho and Minhoni (2007); Llarena-Hernández et al. (2011, 2013, 2014); Favara et al. (2014); Zied et al. (2012a, 2012b, 2014); Pardo-Giménez et al. (2014, 2020a, b) |
| ABL-01/29 = CA 570 | Rio de Janeiro, RJ, Brazil | Llarena-Hernández et al. (2011, 2013, 2014)                             |
| ABL-01/44       | São José do Rio Preto, SP, Brazil | Kopytowski Filho et al. (2008)                                           |
| ABL-03/44 = CA 562 | Lençóis Paulista, SP, Brazil | Kopytowski Filho (2006); Llarena-Hernández et al. (2011); Zied et al. (2012a, 2014); Pardo-Giménez et al. (2014); |
| ABL-03/49 = CA 565 | Boituva, SP, Brazil | Llarena-Hernández et al. (2011, 2013, 2014)                             |
| ABL-04/49 = CA 563 | São José do Rio Preto, SP, Brazil | Kopytowski Filho (2006); Andrade et al. (2007); Llarena-Hernández et al. (2011); Favara et al. (2014); Zied et al. (2010, 2011b, 2012a, 2014); Pardo-Giménez et al. (2014); Martos et al. (2017) |
| ABL-05/51 = CA 564 | Bariri, SP, Brazil | Llarena-Hernández et al. (2011)                                           |
| ABL-06/53 = CA 566 | Brazilia, DF, Brazil | Llarena-Hernández et al. (2011); Zied et al. (2012a)                         |
| ABL-06/59       | Brazilia, DF, Brazil     | Zied et al. (2014)                                                          |
| ABL-07/58 = CA 572 | Suzano, SP, Brazil | Llarena-Hernández et al. (2011); Farnet et al. (2013)                       |
| ABL-07/59 = CA 574 | Atibaia, SP, Brazil | Llarena-Hernández et al. (2011)                                           |
| ABL-16/01       | Valinhos, SP, Brazil     | Zied et al. (2018)                                                          |
| ABL-16/02 = ABL 18/01 | Valinhos, SP, Brazil | Zied et al. (2018)                                                          |
| ABL-16/03       | Minas Gerais, MG, Brazil | Zied et al. (2018)                                                          |
| A. blazei      | Fazenda Guirra, SP, Brazil | Gern et al. (2010)                                                        |
| BZ-04          | Brasímicel, Suzano, SP, Brazil | Cavalcante and Gornes (2005); Cavalcante et al. (2008)                    |
| BZ-ae          | Brasímicel, Suzano, SP, Brazil | González Matute (2009)                                                      |
| BZ-7           | Brasímicel, Suzano, SP, Brazil | González Matute (2009)                                                      |
| BZ-PL          | Brasímicel, Suzano, SP, Brazil | González Matute (2009); González Matute et al. (2011)                     |
| CS1            | Vitória, ES, Brazil      | Silva et al. (2009, 2011); Dias et al. (2013, 2014); Sousa et al. (2016)  |
| CS2            | Belo Horizonte, MG, Brazil | Dias et al. (2013, 2014); Martos et al. (2017); Sousa et al. (2016)       |
| CS9            | Elói Mendes, MG, Brazil  | Sousa et al. (2016)                                                        |
| CS10           | UFLA, Brazil             | Figueirêdo et al. (2013); Sousa et al. (2016)                               |
| WC837 = CA454 = ATTC 76739 | Brazil (Penn State Mushroom Spawn Lab) | Bechara et al. (2008); Llarena-Hernández et al. (2011, 2013, 2014); Farnet et al. (2013) |
| WC838 = CA 455 | Brazil (Penn State Mushroom Spawn Lab) | Llarena-Hernández et al. (2011)                                           |
| CA567          | Bois de Berquit, Dion de Val, Belgium | Llarena-Hernández et al. (2011)                                           |
| M7700 = CA 646 | Mycelia BVBA, Belgium    | Gregori et al. (2008); Llarena-Hernández et al. (2011); Stoknes et al. (2013); Farnet et al. (2013) |
| M7703 = CA 647 | Mycelia BVBA, Belgium    | Llarena-Hernández et al. (2011); Farnet et al. (2013)                       |
| PA93 = CA 487  | Saint-Léon, Gironde, France | Llarena-Hernández et al. (2011, 2013, 2014); Farnet et al. (2013)          |
| CA 516         | Saint-Léon, Gironde, France | Llarena-Hernández et al. (2011)                                           |
| CA 643         | Le Pian Médoc, Gironde, France | Llarena-Hernández et al. (2011, 2013, 2014)                               |
the place where the cultivation has migrated to. We verified
this with the 99/30 strain that was first cultivated in Spain,
with a yield of 4.7% after 80 days of cultivation (Zied et al.
2011a). After re-isolation in several subsequent crops, we ob-
tained a productivity of 20.2% after 85 days of cultivation
(Pardo-Giménez et al. 2020a).

Here, we emphasize the importance of obtaining a high
yield, with morphologically suitable mushrooms (extra
mushroom or mushroom grade A) and high dry matter. All
these characteristics are required in a promising strain for
commercial use with regard to studies published in the last
15 years.

Table 5 (continued)

| Strains         | Origin                                      | References                               |
|-----------------|---------------------------------------------|------------------------------------------|
| ATCC 76739-3 x CA487-100 = CA454-3 x CA487-100 | Hybrid, INRA, France                     | Llarena-Hernández et al. (2011, 2013, 2014) |
| CA 603          | Tlaxcala, Mexico                            | Llarena-Hernández et al. (2011)          |
| ER-1 = CA 462   | Hawái, USA                                  | Llarena-Hernández et al. (2011)          |
| ARAN559 = CA 438-A | Coll. LA Parra, Spain                      | Llarena-Hernández et al. (2011, 2013, 2014) |
| 24b-01 = CA 536 | Comacchio, Italy                            | Llarena-Hernández et al. (2011)          |
| 837             | Corvinus University of Budapest, Hungary    | Geössel (2011); Geössel and Györfi (2008) |
| 838             | Corvinus University of Budapest, Hungary    | Geössel (2011); Geössel and Györfi (2008) |
| 853             | Corvinus University of Budapest, Hungary    | Geössel (2011); Geössel and Györfi (2008) |
| 1105            | Corvinus University of Budapest, Hungary    | Geössel (2011); Geössel and Györfi (2008) |
| 2603            | Corvinus University of Budapest, Hungary    | Geössel (2011); Geössel and Györfi (2008) |
| Brazil          | Corvinus University of Budapest, Hungary    | Geössel (2011); Geössel and Györfi (2008) |
| Ma-He           | Corvinus University of Budapest, Hungary    | Geössel (2011); Geössel and Györfi (2008) |
| Si-2.2          | Corvinus University of Budapest, Hungary    | Geössel (2011); Geössel and Györfi (2008) |
| BCRC36814T      | Food Industry Research and Development Institute, Hsinchu, Taiwan | Chu et al. (2012)                          |
| CA 276          | Coll. W Chen, Taiwan                        | Llarena-Hernández et al. (2011)          |
| KS-72           | Kyushu University, Fukuoka, Japan           | Pokhrel and Ohga (2007); Horm and Ohga (2008) |
| KUMB1221        | Kyushu University, Fukuoka, Japan           | Win and Ohga (2018)                       |
| SH26            | Institute of Edible Fungi, Shanghai, China  | Wang et al. (2010, 2013)                 |
| MFLUCC 11-0653 = CA 918 | Chiang Rai, Thailand                 | Jatuwong et al. (2014); Thongklang et al. (2014a, b) |
| CA918-075 x CA454-4 | Hybrid, Mae Fah Luang University, Thailand | Jatuwong et al. (2014)                    |
| CA918-076 x CA454-4 | Hybrid, Mae Fah Luang University, Thailand | Jatuwong et al. (2014)                    |
| CA918-075 x CA487-35 | Hybrid, Mae Fah Luang University, Thailand | Jatuwong et al. (2014)                    |
| CA918-076 x CA487-35 | Hybrid, Mae Fah Luang University, Thailand | Jatuwong et al. (2014)                    |
| VAB             | România                                     | Rózsa et al. (2017)                      |

Conclusions

All the A. subrufescens strains characterized in this work are
phylogenetically related to the Americas/Europe specimens
and harbor an ITS of type A (ABL 16/01) or of both types
A and B (ABL 18/01, ABL 98/11, and ABL CS7). We did not
find any correlation between the morphological characteristics
of the mycelial colonies and the agronomic behavior of the
strains. Strains ABL 98/11 and ABL 16/01 obtained the best
yield results and morphological characteristics of the mush-
rooms, including their high weight. These characteristics en-
hance the commercialization of these strains and justify their
continued use over the last 15 years and can additionally be the subject of a breeding program aimed at improving yield and reducing agronomic variability.

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**Declarations**

**Ethics approval** Not applicable.

**Conflict of interest** The authors declare no competing interests.

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