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RESEARCH ARTICLE

A study of mycelium characterization of several wild genotypes of the button mushroom from Iran

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Abstract

Morphology characteristics of mushroom mycelia in solid media are important because these characteristics are means to predict further mycelial growth into the compost, casing layer, and total yield. It has been well-known that many species of edible mushrooms, including *Agaricus bisporus*, grow in wilderness of Iran. To the best of our knowledge, no study has been done to characterize the mycelial growth of Iranian wild isolates of *A. bisporus*. Mycelial characteristics of six samples of Iranian wild isolates, along with three samples of commercial isolates were studied in compost extract-based media. Characteristics of mycelial morphology in the solid media included texture, density, color, radial growth rate ($mm.day^{-1}$) and sectoring. In addition, mycelial morphology in the liquid media included texture, color, growth rate and duration of filling vials. Wild isolate As005 showed normal and strandy mycelia, while most of the other wild isolates showed a mixture of strandy-fluffy texture. The highest and lowest radial growth rates were recorded for As0011 and As001 with 2.87 and 1.97 mm per day, respectively. The results showed no symptom of severe sectoring, stromatal mycelia or color change during seven subsequent subcultures. Taken together, wild isolates As003 and As005 may be more likely to achieve higher productivity and best mycelial morphology, compared to the other wild isolates.

INTRODUCTION

Pure mycelium culture in a solid medium is the first step of cultivation of edible mushrooms. Mycelial growth into a solid substrate is affected by various factors, including temperature, pH, nutrient ingredients and environmental factors (Imtiaj et al., 2008). The time required for the growth of mycelium on a solid medium is relatively short, and thus an accurate and quick assessment can be facilitated. Therefore, the quality and quantity of a mushroom strain mycelium in a solid medium is one of the most important metrics to determine further nutritional requirements for the production of fruiting bodies of the mushroom. It also provides a tool to predict further mycelium growth into the compost, casing layer, and total yield (Khandakar et al., 2008). Different growth characteristics are used by researchers to investigate the quality and quantity of mushroom mycelia in a liquid or solid medium, including morphology, radial growth rate, colony mass, and color of the mycelium (Guadarrama-Mendoza et al., 2014). The effects of variables such as temperature, pH, and composition of the culture medium on mycelial characteristics have also been studied (Mai, 2014).

At the present, the button mushroom, *Agaricus bisporus*, accounts for 35 to 45 percent of total worldwide production of edible mushrooms (Hesami et al., 2014). The limited commercial strains of *A. bisporus* are constantly

replicated by spawn producers, which might lead to strain degeneration over a long period of time. Even though, the exact genetic mechanism of strain degeneration has not been well understood, it has been linked to DNA defect and chromosomal mutations during consecutive propagation of a strain (Health et al., 1995; Li et al., 1994). Strain degeneration may cause mycelial deformations and sectoring, which might change mycelial growth characteristics such as texture, growth rate, color and other properties of mycelia (Health et al., 1995; Li et al., 1994). Other than on solid medium, mycelial abnormalities can be observed on spawn, compost, and casing layer. Consequently, mycelial deformations may lead to a decrease in *mushroom quality or yield loss* (Begin and Spear, 1991). There are solutions suggested by researchers to eliminate the mycelia sectoring, including use of original strains (that have probably been deep-frozen), basidiospores cultures, and wild populations of *A. bisporus* (Li et al., 1994).

It has been well-known that many species of edible mushrooms, including *A. bisporus,* grow wild in Iran. The mycelial and tissue culture characterization is the first step is to determine the commercialization feasibility of these genotypes. To the best of our knowledge, no study has been undertaken to characterize the mycelial growth of Iranian wild isolates of *A. bisporus*. Therefore, the purpose of this study was to investigate the mycelium growth properties and possible developmental abnormalities in several well-authenticated indigenous strains of the button mushroom in comparison with several commercial strains. The findings of this study may be applicable to further mushroom breeding programs employing wild isolates of *A. bisporus*.

Materials and methods

Mushroom samples

The mushroom samples were comprised of six indigenous isolates, which have been authenticated through internal transcribed spacer (ITS) sequence analysis (data not yet published), as well as three commercial strains of *A. bisporus*. The wild isolates were designed as As001, As003, As004, As005, As007, and As0011. The commercially cultivated strains included H737, IM008 and A15. All the mushroom samples were prepared as pure mycelial cultures from the mushroom bio-bank of Industrial Fungi Biotechnology Research Department, ACECR-Mashhad Branch, Iran.

Mycelial culture

A compost extract (CE)-based medium was prepared as follows. Three hundred grams of the phase II compost of the white button mushroom was boiled in one liter of distilled water for one hour. The boiled mixture was poured into 50-mL Falcon tubes and centrifuged (Hermle Labor Technik GmbH, Germany) at 5000 rpm for five minutes. The upper phase was collected, filtered and used as the compost extract. The distilled water was added to the resulting extract to reach a volume of one liter. The media was either used as a CE for mycelia liquid culture or mixed with 0.5% agar (Merck, Darmstadt, Germany), v/w(CEA) to serve as a solid media. The autoclaved media was distributed into gamma-sterilized 8-cm plastic petri dishes and kept in a dry and dark place until use. The solid media was inoculated with 1 cm^2 disks of the leading edges of mushroom mycelia. To prepare mycelia liquid culture, three-five pieces of the leading edges of mushroom mycelia with minimal agar were transferred into prepared CE in 50-mL glass vials. The plates were incubated in 25°C until they were seen to grow properly in the media.

Measurements of mycelial radial growth rate in solid medium

The radial growth rate of mycelium was examined daily within two weeks based on a method by Islam and Ohga (2013) with modifications. In brief, two vertical lines were drawn on the plates followed by marking four points of mycelia extensions at perpendicular angles. Recorded values per day were means of readings taken at the four points, using a caliper. At the end of the reading period, the overall average of the radial growth rate of mycelium was measured based on daily averages, expressed in mm per day.

Mycelia morphology

The main characteristics of mycelial morphology included texture (strandy, normal, stromatal, fluffy, and appressed types), density (high, regular or low), and color (off-white, white or pale pink). These characteristics were recorded by visual observations after complete colonization of the media, based on a modified method of Guadarrama-Mendoza et al. (2014). Stromatal, fluffy, and appressed textures were referred to as abnormal growth, according to Heath et al. (1995) and Li et al. (1994). In addition to the afore-mentioned mycelial morphology, sectors in growing mycelia of the tested mushrooms in the solid media were recorded over seven consecutive subcultures.

Statistical Analysis

All the treatments were applied in triplicate, and each experiment was independently repeated at least three times. The experiments were carried out based on a factorial experiment conducted in a completely randomized design

with three independent replications. Data analysis was performed with SAS software version 9.1 and was determined by ANOVA and Duncan test.

Results

Measurements of mycelial growth rate in the solid media

Over the period of two weeks, radial growth rate of mycelium in the solid media was recorded. The results showed that wild isolates As003, As004 As007, and As0011 along with all the commercial strains colonized the solid media within 14 days, while As001 and As005 filled 80% of the plate within the same period of time. Among the wild isolates, the highest and lowest radial growth rates were recorded for As0011 and As001 with 2.87 and 1.97 mm per day, respectively. According to statistical analysis, there was significant difference between wild isolate As0011 and commercial isolate A15 ($p < 0.01$). Among the commercial isolates, highest and lowest radial growth rates were recorded for H737 and A15 with 2.80 and 2.48 mm per day, respectively (Fig.1).

Based on the statistical analysis conducted for the radial growth rates of mycelia, three classes of mycelial growth rate were produced. Accordingly, wild isolates As0011, As003, and As007 and also commercial strains H737 and IM008 were placed in the high-growing class. While commercial strain A15 and wild isolate As004 showed a regular growth rate, wild isolates As001 and As005 were placed in the low growing class (Fig. 1).

Morphology characteristic of mycelia in the solid media

After two weeks of mycelial growth, main characteristics of mycelial morphology were evaluated. Wild isolate As005 and all the commercial strains exhibited a normal, strandy texture. Other wild isolates showed abnormal mycelia, including fluffy and stromatal (Table 1, Fig. 2). Wild isolates As003, As004, As007 and As0011 and also all the commercial strains had a high density of mycelia, while isolates As001 and As005 showed regular density in the solid media. No low density was observed among the tested mushrooms in the solid media. All tested mushrooms showed a white color of mycelia in the solid media (Table 1).

Sectoring of mycelia in solid media

There are various mycelial growth abnormalities in a solid media. However, sectoring is referred to as the most severe form of mycelial abnormality that might also be a symptom of strain degeneration. Since sectoring has been evidenced to be occurred during repeated sub-culturing mycelia, in this study the tested mushrooms were subjected to seven continuous subcultures. Mycelial abnormalities were recorded for all the subcultures. The results showed no symptom of severe sectoring during the repeated sub-culturing. In the last subculture, wild isolates As003, and As005 and all the commercial strains exhibited a strandy texture, while wild isolates As001, As004, As007 and As0011 had a strand-fluffy texture (Fig. 3).

Mycelial morphology in liquid media

After 21 days of mycelial growth in the liquid media, mycelial characterizations were evaluated. The results showed wild isolate As0011 and commercial strain IM008 had strandy texture, wild isolates As005 and As007 and commercial strains H737 and A15 showed strandy fluffy mycelia. Wild isolates As001, As003, and As004 had fluffy-stromatal texture (Table 2, Fig. 4). The color of mycelia was white in all the wild and commercial strains tested in the liquid media. Wild isolates As004, As005, As007 and As0011 and commercial isolates IM008 and H737 colonized the media within 17 days. Wild isolates As003 and As001 filled the vial in 20 days, while commercial strain A15 completed its growth in the liquid media within 21 days (Fig. 5). Based on the statistical analysis results ($p < 0.05$), four classes of mycelial growth rate in the liquid media were produced: high, regular, low, and very low. The results showed that wild isolates As004, As005, As007 and As0011, and also commercial strains IM008 and H737 had same class (high). Wild isolates As003 and As001 ranked in the classes regular and low, respectively. Commercial isolate A15 ranked in the class very low.

Figures

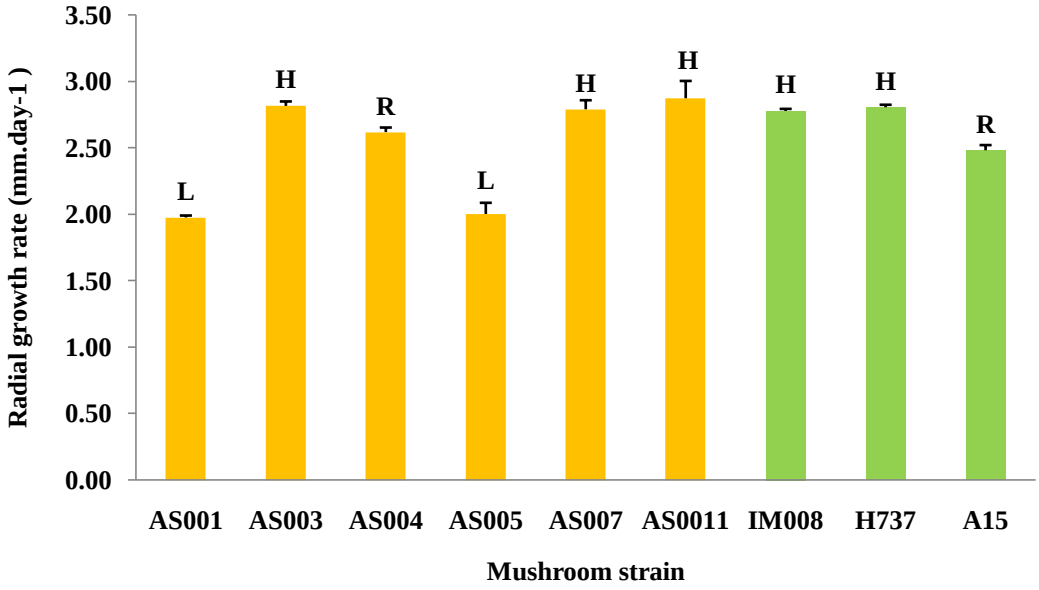
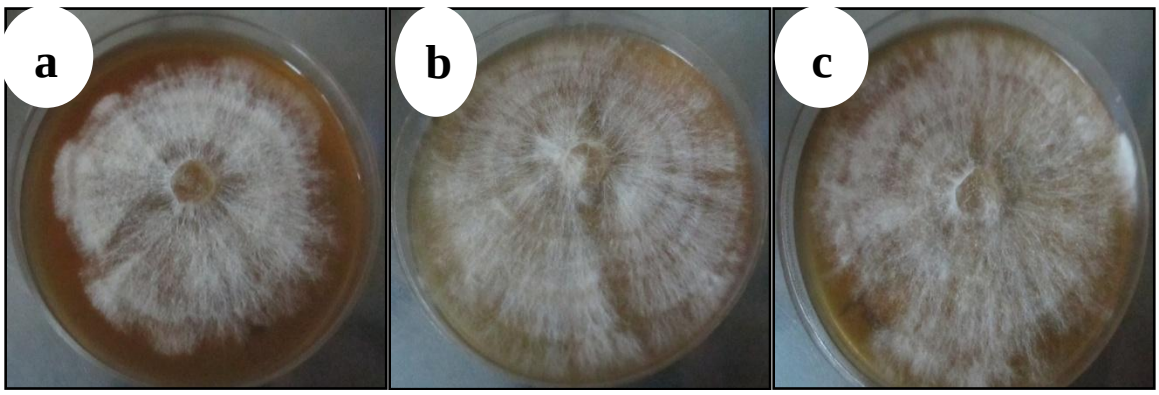


Figure 1. Mycelial growth rates of wild and commercial strains of *A. bisporus* in CEA. Values represent daily mycelial growth rates based on the average of three independent replications through which standard deviations were generated. In each independent experiment, mycelial growth rates were measured based on the mean of the recorded daily growth rates over a period of 14 days. The letters show the classes of growth rate based on statistical differences; H: High, R: Regular, and L: Low.



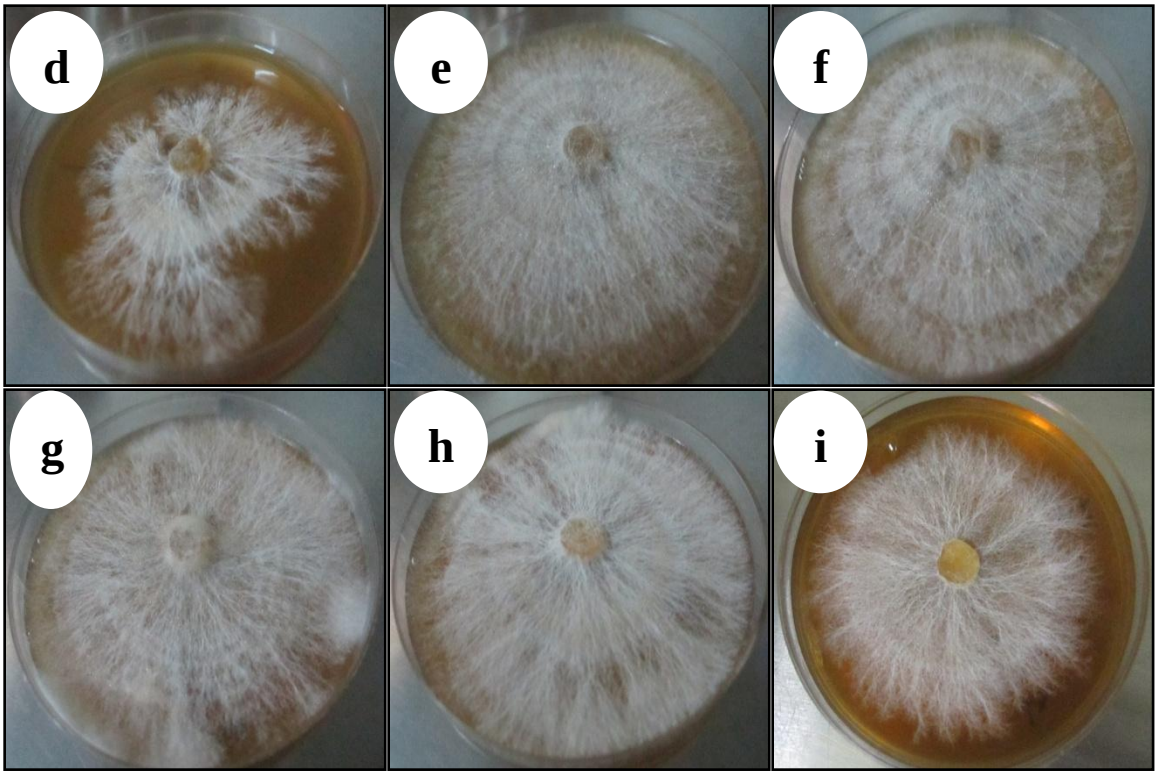
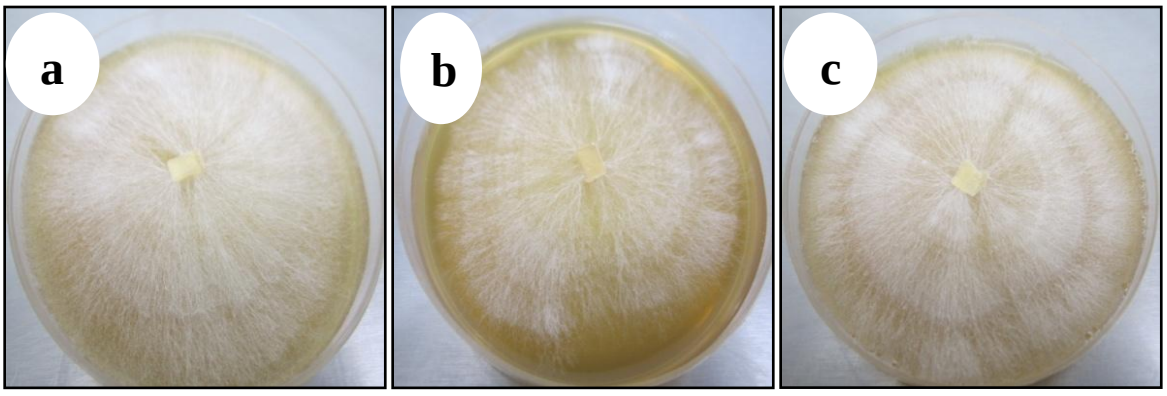


Figure 2. Illustrations of different mycelial textures of wild and commercial isolates of *A. bisporus* in the solid media (CEA). Photographs were taken with a digital camera on the fourteenth day of mycelial growth in the first subculture. Each strain was subjected to three independent replications. All the commercial strains and wild isolate As005 had strandy texture. Wild isolates As001, As003 and As007 had strandy fluffy texture. Only two wild isolates As004 and As0011 had sectoring growth fluffy stromatal and fluffy texture, respectively. Letters indicate the following strains: **a:** As001, **b:** As003, **c:** As004, **d:** As005, **e:** As007, **f:** As0011, **g:**H737, **h:** IM008, **i:** A15.



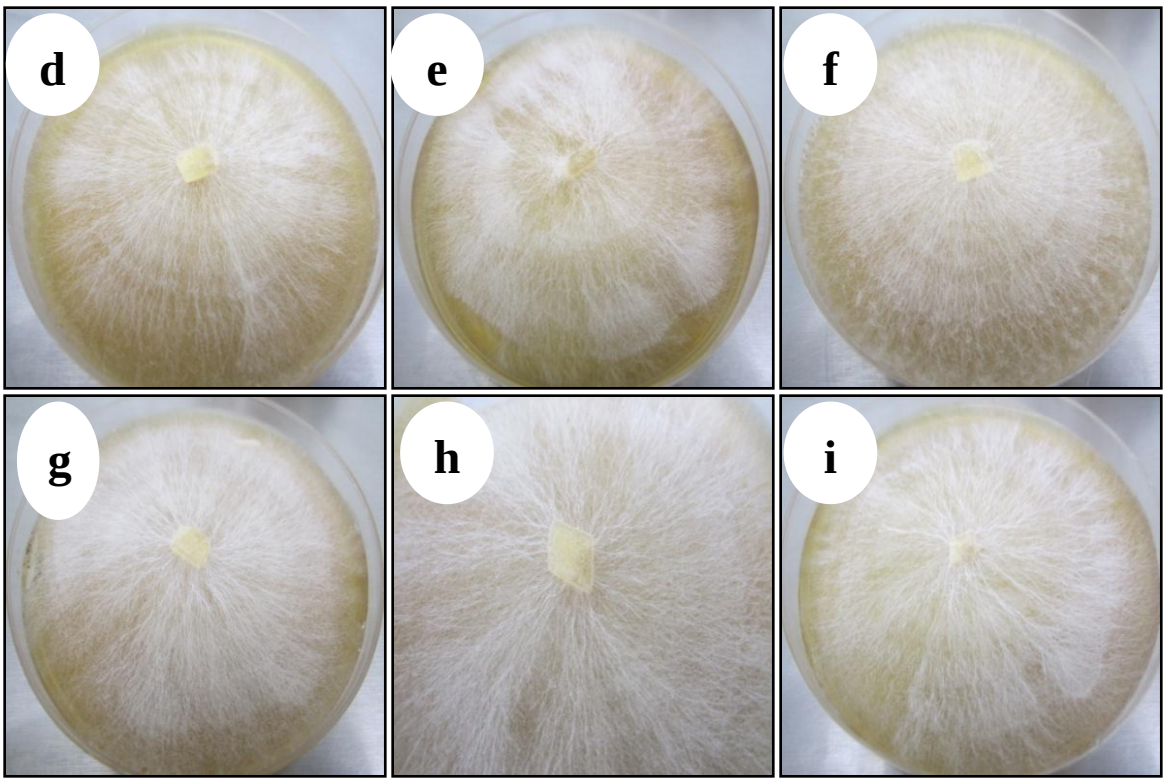
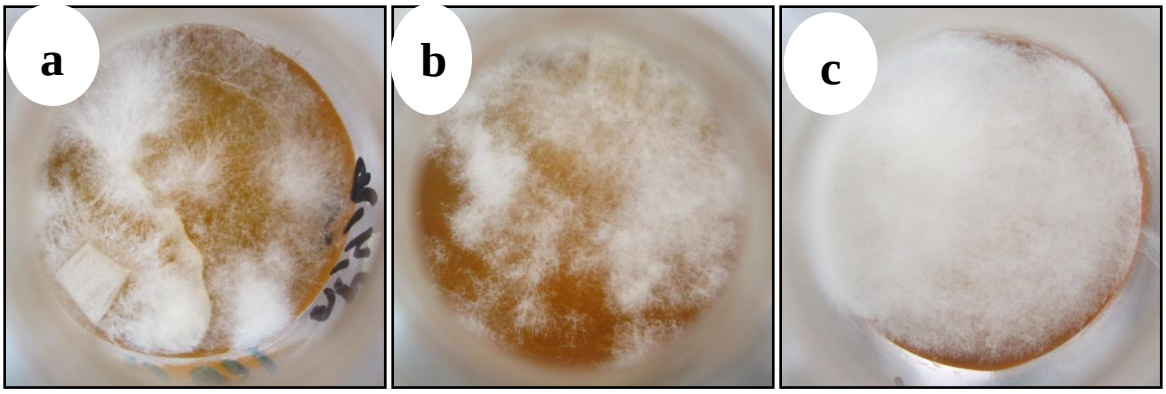


Figure 3. Illustrations of mycelial textures of the wild and commercial strains of *A. bisporus* after seven consecutive subcultures. Photographs were taken with a digital camera on the sixteenth day of the seventh subculture to evaluate mycelial morphology grown in CEA media. Each strain had three independent replications. No severe stromatal or fluffy textures was observed in the wild or commercial strains. Letters indicate the following strains**: a:** As001, **b:** As003, **c:** As004, **d:** As005, **e:** As007, **f:** As0011, **g:** H737, **h:** IM008, **i:** A15.



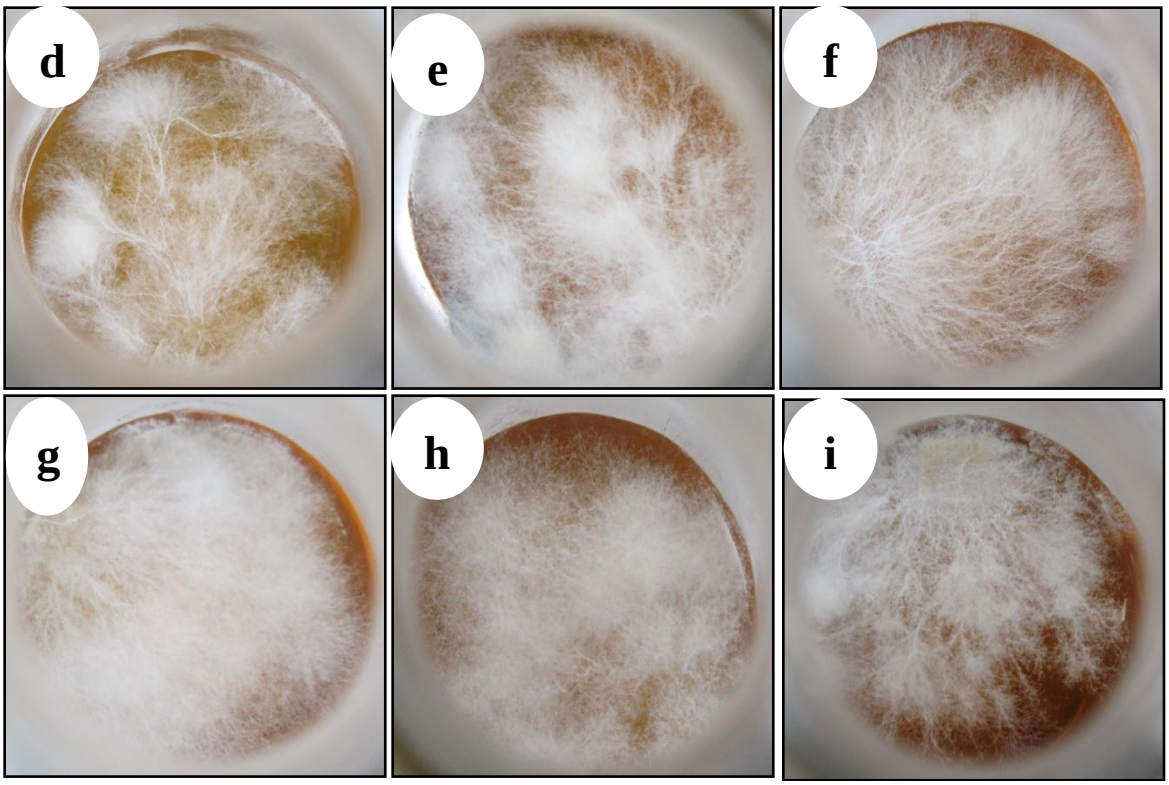


Figure 4. Illustrations of mycelial textures of wild and commercial isolates of *A. bisporus* in the liquid media (CE). Photographs were taken with a digital camera on twenty-first day of mycelium growth in liquid media. Wild isolate As0011 and commercial strain IM008 had strandy texture in the liquid media. Wild isolates As005 and As007 and commercial strains H737 and A15 had strandy fluffy texture. Wild isolates As001 and As003 had fluffy stromatal texture, while As004 showed a stromatal texture. Letters indicate the following strains: **a:** As001, **b:** As003, **c:** As004, **d:** As005, **e:** As007, **f:** As0011, **g:** H737, **h:** IM008, **i:** A15.

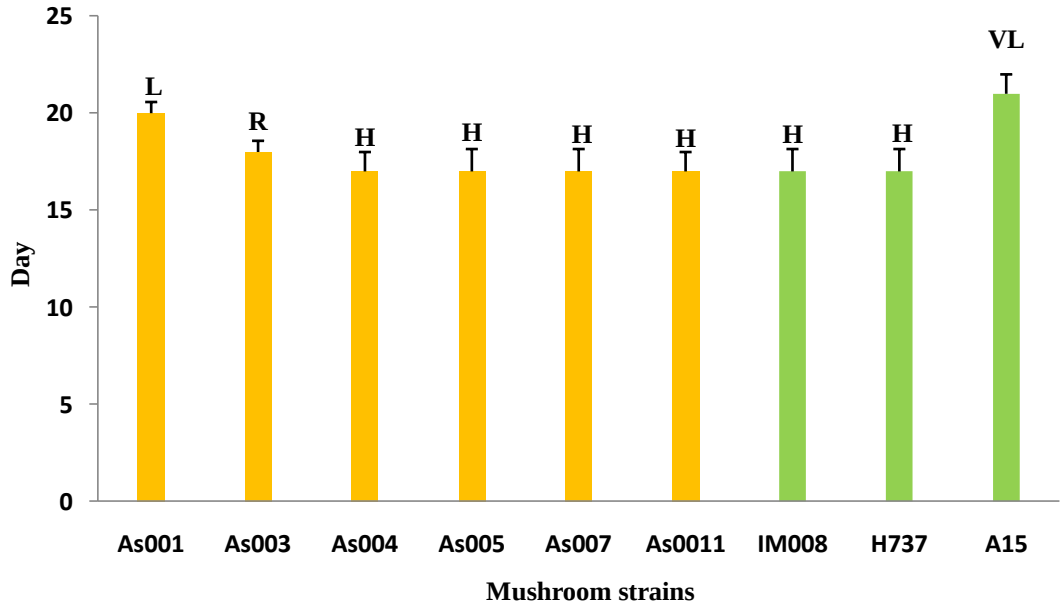


Figure 5. Mycelial growth rates of wild and commercial isolates of *A. bisporus* in the liquid media. All the wild and commercial isolates were recorded every day in CE culture media. Wild isolates As004, As005, As007 and As0011 and commercial strains IM008 and H737 could fill the whole vial within 17 days. This duration was 20 days for wild isolates As003 and As001, and 21 days for commercial strain A15. The letters show the class of growth rate, based on the results of statistical analysis; H: High, R: Regular, L: Low, and VL: Very Low.

Isolates	Texture	Color	Growth rate
As001	Fluffy stromatal	White	Low
As003	Fluffy stromatal	White	Regular
As004	Stromatal	White	High
As005	Strandy fluffy	White	High
As007	Strandy fluffy	White	High
As0011	Strandy	White	High
IM008	Strandy	White	High
H737	Strandy fluffy	White	High

Table 1. Morphology characteristic of mycelia of *A. bisporus* in the solid media (CEA)

Table 2. Mycelial characterization in the liquid media (CE)

Isolate	Texture	Density	Color	Growth rate
As001	Strandy fluffy	Regular	White	Low
As003	Strandy fluffy	High	White	High
As004	Fluffy stromatal	High	White	Regular
As005	Strandy	Regular	White	Low
As007	Strandy fluffy	High	White	High
As0011	Fluffy	High	White	High
IM008	Strandy	High	White	High
H737	Strandy	High	White	High
A15	Strandy	High	White	Regular

A15	Strandy fluffy	White	Very Low

1. A, France, pp. 35-42.

Discussion

In this study, various mycelial morphologies were investigated among different ITS-authenticated wild isolates of *A. bisporus*, compared against several commercial strains. It was depicted that mycelia of wild isolates As0011, As007, and As003 were able to grow as fast as those of commercial strains H737 and IM008 in the solid media. However, amounts of mycelial abnormalities were observed in the mycelia of some of the wild isolates. Wild isolates As005 showed normal and strandy mycelia, while most of the other wild isolates showed a mix of strandy-fluffy texture. As004 was the only wild isolate to show stromatal-fluffy mycelia. In order to accurately evaluate whether the mycelial morphology of the tested mushrooms will change during repeated subcultures, seven consecutive subcultures were performed during the time which mycelial characteristics were carefully recorded. The results showed no symptom of severe sectoring, stromatal mycelia or color change during the subcultures. In the last subculture, wild isolates As003, and As005 and all the commercial strains exhibited a strandy texture, while wild isolates As001, As004, As007 and As0011 had a strandy-fluffy texture.

Several studies have investigated mycelial growth rates of commercial strains of *A. bisporus* in solid media. Since this trait depends on various factors (particularly strain, composition of nutrient media, and method of measurement), different radial growth rates have been reported by researchers, including 3, 5, and 8 $mm.day^{-1}$ (Straatsma et al. 1991). However, little research has been undertaken to compare mycelial growth rates between wild isolates and commercial strains of the button mushroom, unlike other mushrooms such as wild *Pleurotus eryngii* from Korea (Lee et al., 2014) and wild *Pleurotus* spp from Mexico (Guadarrama-Mendoza et al., 2014).

In addition to growth rate, different mycelial characteristics could also be evaluated for growing mushroom mycelia in solid media, including texture, color, mass and other characteristics. In addition to normal growing mycelia, abnormalities might also appear in a mushroom mycelial culture, including fluffy, aerial mycelium, color change, and sectoring, some of which might associate to strain degeneration. Observation of strain degeneration in *A. bisporus* dates back to several decades ago (Fritsche, 1966, 1970; Elliott, 1985). Although genetic determinants (including chromosomal instabilities) have been suggested to cause degeneration in commercials strains of *A. bisporus* (Horgen et al., 1996), this phenomenon is frequently seen during the repeated sub-cultures of the same commercial strains (Health et al. 1995). Use of wild populations of *A. bisporus* has been suggested as an option to overcome sectoring and degeneration caused by repeated subcultures of the limited commercial strains (Li et al. 1994). It was shown that mycelium of wild strains of *A. bisporus* did not produce mycelial sectoring, whereas commercial strain of U1 produced irreversible sectors or abnormalities.

The commercial strains utilized in this study have been prepared from a non-degenerate and low sub-cultured stock, and thus they were not supposed to show mycelial degeneration or severe abnormalities. This study was, thus, aimed to examine mycelial characteristics of the wild isolates in order to select good candidates for further mushroom breeding programs. Mycelial growth rate could be used as a criterion to select for fast-growing isolates, as it is assumed that mushroom fast growing strains colonize compost or casing layer much faster and achieve high production yields compared to the slower strains (Guadarrama-Mendoza et al., 2014). Yet, growth rate is not the sole criterion and there are other important mycelial characteristics that should be taken into account. Based on the findings of this study, wild isolate As003 showed a fast-growing mycelia and a strandy texture in the solid media even after seven repeated sub-cultures. Although being placed in the low-growing class, wild isolate As005 showed a strandy texture after seven repeated sub-cultures in both the solid and liquid media. Other wild isolates showed amounts of fluffy textures, while were placed in high or regular growth rate class. However, wild isolate As004 showed more obvious stromatal texture during sub-culturing in both the solid and liquid media. Taken together, wild isolates As003 and As005 may be more likely to achieve higher productivity and best mycelial morphology, compared to the other wild isolates. However, wild isolates As007 and As0011 could still be considered good candidates for further studies,

In addition to *A. bisporus*, a number of studies have reported mycelial morphology and abnormalities in other edible mushroom, and the role of strain degeneration in decrease or complete loss of mushroom yield. Recent examples include *P.eryngii* (Lee et al., 2014), indigenous *Lentinus squarrosulus* from Ghana (Mensah and Obodai, 2014), *P. eryngii* from Korea (Lee et al., 2014) and *Pleurotus* spp. from Mexico (Guadarrama-Mendoza et al., 2014), and 29 wild mushroom species from Tibet (China) (Gayane et.al.,2011).

Conclusions

Thus far, no study has been reported to evaluate the potential of the Iranian wild populations of *A. bisporus* in mushroom breeding. Mycelial morphology of a mushroom isolate or strain would be an important step towards selecting best candidates. Also, it is very important to recognize and avoid propagation of degenerate mushroom mycelia. The findings of this study revealed that two wild isolates As003 and As005 could be considered good candidates as compared with high quality commercial strains of *A. bisporus*. Following As003 and As005, two isolates As007 and As0011 could also be considered good candidates according to their mycelial growth rate and morphology.

Acknowledgements

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Declaration of interest

There is no conflict of interest.

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