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Isolation and characterization of a sporless mutant in the white button mushroom (*Agaricus bisporus*)

Rasouli Farzad*, Motallebiazar Alireza, Bolandnazar Sahebali, Zare Nahandi Fariborz and Panahande Jaber

Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Tabriz 51666-14766, Iran

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Abstract

Despite the long cultivation and importance of *Agaricus bisporus*, but efforts in breeding of this species are minimal. One of the major reasons for the little efforts is the life cycle. Because of the life cycle the cross cannot happen between single spores of different strains. So variation in *Agaricus bisporus* is very low and most of strains that are available in the markets are very similar. One of the ways to make variation is using mutagens like UV irradiation. We selected A15 strain that is one of the important strains in Iran. Fragments of mycelium were placed to irradiation by UV lamp 10-w for eight exposure periods (0, 4, 8, 16, 24, 48, 72 and 96 hours) at a distance of 10 cm. In order to pick out of isolate mycelia we examined irradiated fragments after spawning and fruit body production. We archived to pick out one mutated isolate in the exposure of 24 hour that it is different in gill morphology, the rate of spawn running and it cannot produce spores. It is the first report that one sporless isolate of *Agaricus bisporus* introduced. This study shows that mutagenesis by UV can be useful and a quick way method to make diversity and breeding of *Agaricus bisporus*.

***Corresponding Author:** Rasouli Farzad ✉ farrasoli@gmail.com

Introduction

Mushrooms have been valued not only for nutritional and medicinal values but also as helping to recycle of huge agriculture products waste and the other hand mushroom industry has made a good stand in Iran in the last decade. The total area for production of mushroom is increasing every day. The white button mushroom, *Agaricus bisporus*, is the most widely cultivated species of edible mushroom in Iran as the other countries. It is cultivated in more than 100 countries located on every continent (Chan and Miles, 2004). However, despite the long tradition cultivation, professionalization of the cultivation system and economic value, it is surprising that efforts in breeding of this species are minimal (Sonnenberg, *et. al.,* 2011). One of the major reasons for the little efforts of program for *A. bisporus* is the nature of its life cycle. All commercially produced strains and most wild strains have a predominantly secondary homothallic life cycle (Elliott and Langton, 1981). In secondary homothallic species, most basidia produce two spores. Meiosis takes place after fusion of nuclei in the basidial cell and the non-sister nuclei are usually paired in one nucleus. This leads to mycelia with opposing mating types and thus to fertile heterokaryons (Sonnenberg, *et. al.* 2005). Therefore, all the cells are binucleated or multinucleated, as opposed to most of the other basidiomycota where the cells are uninucleated. Mycelium in *Agaricus bisporus* is septate, and each of the cells contains all necessary information for independent growth. If a mycelium is fragmented, each fragment can regenerate to from a new colony (Goltape, *et al*. 2009).

Because of the life cycle as mentioned above, the cross cannot happen between single spores of different strains. Then recombination and diversity is very low in strains of *A. bisporus* and all commercial and wild types of strains are very similar to each other. Especially, commercial cultivars have been used for long periods of time and have limited genetic variability; future breeding for improved cultivars needs the genetic variation of wild collected strains (Chang and Miles, 2004). Also published observations confirm that there is relatively little genetic diversity in commercial isolates of *A. bisporus* (Royce and May, 1982a). In the other hand recombination and diversity are basis of each program breeding. For example, for any study of inheritance it is necessary to have two individuals that differ in some character that can be either visibly distinguished or detected by (1) growth or lack of growth, or (2) the production or lack of production of any product of metabolism, and the formation or lack of formation of any part of body, which may or may not be essential for its life activities. Those characters that commonly occur in nature are referred to as wild type (sometimes the term of normal is used), whereas a sudden, heritable change that can be distinguished from wild type is known as a mutation (Chang and Miles, 2004).

One way to introduce genetic variability is through mutation using chemical agents or ionizing radiation. Of course, mutations occur naturally, but at low frequencies. The mutation may introduce one or more change in characteristic of mutated organisms (Djajanegara and Harosyo, 2008). The characteristic change may occur at the gene level which will be passed to the next generation.

Mutants in which some aspect of the sexual development of the species has been altered by mutation are known in a number of fungi, and they are of particular interest to us in connection with the development of fruiting bodies of basidomycetes. One class of practical importance is composed of those that lead to a failure to produce spores or in which spore production is greatly restricted (Chang and Miles, 2004).

One of the goals in *A. bisporus* breeding compared with other mushroom is production of sporeless or low sporing individuals, because spores discharged from cultivated mushroom may give rise several problems: (a) reduction of commercial value because is spores deposited on mushrooms at markets, (b)

some growers of mushrooms have respiratory allergies that is caused by on the spore surface antigens, (c) population of mites and other microorganisms that feed on fungal spores will increased in cultivation room mushroom, (d) erosion of genic variation in natural population of the mushroom species. These problems may be solved by production of spore-deficient strain (Murakami, 1993). For *Agaricus bisporus* reduced commercial and genetic variation are most the problems.

So far, sporeless mutant have been isolated by physical and chemical mutation in some basidomycetes including *Pleurotus* spp. (Eger, *et. al.* 1976), *Schizophyllun commune* (Brombery and Schwalp, 1977) *lentinola edodes* (Hasebe *et al.* 1991) *pleurotus eryngii* (Obatake, *et. al.* 2003) *pl. Sajor-Caju* and *Pl. florida* (Ravishankar, *et al.* 2006). In *Agaricus bisporus* have not still been reported.

As it is mentioned above, mutation can be induced by irradiation mutation. In this technique ionizing radiation like X-rays, ultraviolet (UV) and gamma rays are used to increase the frequency of mutation. For most fungal investigators seeking to obtain mutant of fungi, it is simpler to use UV light than the other mutagenesis, because a simple, inexpensive, germicidal UV lamp is available (Chan and Miles, 2004). The germicidal effect is related to the absorption maximum of nucleic acid at 260 nm, which is included in the wavelengths delivered by the germicidal lamp (Chan and Miles, 2004). So in this study we use a UV lamp for mutation in *Agaricus bisporus*. This article will describe the first isolation of sporeless mutant in *Agaricus bisporus* with a considerable change in growth rate and we suggest this method for making variation more and more than before.

Materials and methods

Strain

In this research A15 strain of *Agaricus bisporus* was used for mutagenesis treatment. Spawn of A15 was brought from local producer of mushroom. Then, spawning carried out in compost.

Induction of mutation

Fruit bodies were harvested after production of at first flash. In order production of pure culture, the pileus were aseptically fragmented and all fragments inoculated on Potato Dextrose Agar (PDA) media in petri dishes under laminar flow hood and incubated at 25±1°c for 10 days. Since mycelia covered the PDA media, from the perimeter of the actively growing culture were aseptically excised to smaller length fragments (Approximately 2 × 2 mm^2) and for each petri dish 10 fragments were transferred to the fresh PDA media for irradiation treatment (Fig. 1) (Beejan and Nowbuth, 2009). The petri dishes were placed to irradiation by UV lamp Toshiba 10-w under a laminar air flow cabinet for eight exposure periods (0, 4, 8, 16, 24, 48, 72, 96 hours) at a distance of 10 cm. This procedure was carried out in darkness to inhibit photoreactivation (Chan and Miles, 2004). Because it has been demonstrated that exposure to the wavelength in the approximate range of 360 to 480 nm can repair the UV induced damage (Chan and Miles, 2004). The LD_{99} for mycelia of *Agaricus bisporus* was taken at 96 hours.

Spawn production and spawning

After irradiation, petri dishes (including irradiated fragments mycelia) were incubated at 25±1°C in the dark. After seven days among the fragments mycelia were selected based on the mycelia growth rate and sometimes accidentally, then transfer to another PDA media for the increasing growth of mycelia. After covering of PDA by mycelium, spawn was prepared by wheat seeds. Spawning as done by the method of thorough mixing in which the measured quantity of spawn has to thoroughly mixed with a measured quantity of compost and accommodated in bags to 1 kg compost/bag. For each spawn from each fragment we applied three replicate for evaluation.

Spore print

To examine sporing in fruit bodies, spore print was prepared on the petri dishes for each replicate four times in each flash of harvest. To make a spore print, the stem of fruit body cut off and the cap placed, with the gills facing down on a glass petri dish. The cap covered with a glass and leave for 24-72 hours, depending on the humidity and the freshness of the mushroom.

Harvesting, yield, BE and dry weight and size

Mushrooms were harvested with unbroken veil. Mushrooms were harvested, counted and weighed daily. At the end of third flush yield and number of fruit bodies were calculated. Yield was expressed as g/1kg bag. Average mushroom size was calculated as fresh mushroom weight harvested divided by the number of mushrooms per bag. Biological efficiency (BE) was determined as the ratio of (g) of fresh mushrooms harvested per dry weight substrate weight (g) and expressed as a percentage. Mushrooms were sliced into quarter or eighths depending on mushroom size then samples (100g) were placed in a paper bag and oven dried at 70°c for 72h. Five replicates per treatment were used and solid contents were recorded as percent dry mushroom weight. The number days to spawn running and first harvest also were measured.

Protein assay

Protein content of pericarp extracts were determined by the Bradford method (Bradford, 1976). A bovin serum albumin (2mg/ml) was prepared and then standards (0 to10 mg/ml) were provided by dilution. For reaction solution 0.1 ml of Bradford Reagent (50mg comassi blue g 250 was solved at 25 ml ethanol 95% and then 50 orthophosphoric acid 85% was added and the volume was reached to 100 ml by distilled water) was mixed with 0.1 ml of extract and allowed to develop for 15 minute at room temperature. Absorbance measurements were carried out at 595 nm.

Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA). This treatment was performed by using SPSS 17.0 program. Statistical differences with $p<0.05$ were considered significant.

Result

The genetic variability in a population can occurred naturally by multiple factors. Also it can be induced artificially through the use of some physical, biological, or chemical mutagens, which has attracted the interest of breeders for many decades (Fahad and Salim, 2009; liu eta, 2011). This mutation was done by applying UV radiation on mycelia grown on PDA. In spite of other studies that have declared the mutation of mycelium could not be achieved (Ravishankar *et al*, 2006), but we could success in this study.

Sporless isolate

In total 800 mycelia fragments were irradiated and 120 of them were examined to find sporeless and morphological mutated; only one isolate was found that didn't be able to produce spores. In the mycelia fragments exposed for 4, 8, 16, 24, 48 and 72 hours all of them survived, whereas in the mycelia exposed for 96 hours almost half of them couldn't continue the growth. After examination among irradiated fragmants, one mutated isolate was picked out in the exposure period of 24h and we named AG-M. Also the morphology of gills in AG-M was markedly different from the wild-type parental. Gills in AG-M were not parallel and interweaved (Fig. 1 and 2).

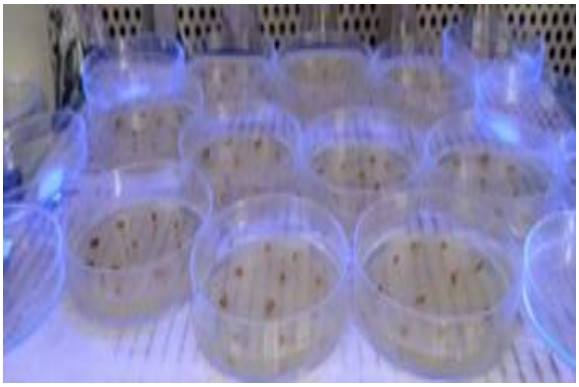

Fig. 1. The fragments of mycelium under the UV irradiation. The fragments that irradiated are indicated by arrows.

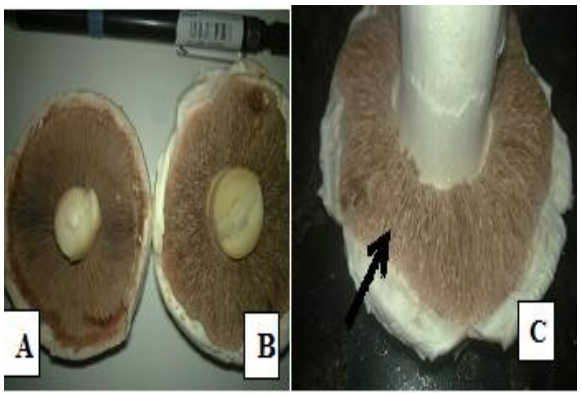

Fig. 2. Morphologies of parental wild-type and sporeless mutant. A – wild-type strain with normal gills. B and C- mutated strain (AG-M) that gill morphology have undergone a change.

Yield, BE, size, dry weight and number days and proteins

Traits such as the number of fruit body, fresh weight, size, diameter of pileus, dry weight, biological efficiency (BE) and proteins were measured that presented in Table 1. There were no significantly differences in fresh weight, the number of fruit body, Size, Diameter of pileus, Days to pin production, Days to harvest and BE between the mutant and wild-type.

Also mycelium growth of AG-M was significantly faster than wild-type after spawning. Mycelium of AG-M completely filled the compost (spawn running) after 7 days, whereas it long 11 days in wild-type (figure 3) in other words spawn running of AG-M was faster than control. Although, the rest of growth and fruit body production phases were similar. Good spawn running is one the important characters in edible mushrooms. Protein in AG-M was significantly lower than wild-type. It may be because of the lower dry weight although difference in dry weight wasn't significant.

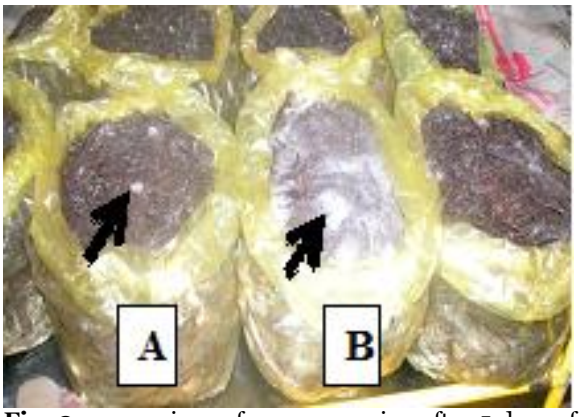

Fig. 3. comparison of spawn running after 5 days of spawning between wild –type (A) and AG-M (B).

Table 1. Growth traits of the of the white button mushroom mutant (AM-G) and compared to control.

	The number of fruit body/ bag (3flash)	Fresh weight (gr)/ 1kg bag	Size (g)	Diameter of pileus	Days for spawn running	Days to pin production	Days to harvest
wild-type	20 a	335.22 a	16.76 a	4.47 a	9.6 b	10.3 a	17.3 a
AD-M	19 a	324.91 a	17.10 a	4.32 a	6.6 a	11.6 a	18.3 a

-Means within a column followed by the same letter are not significantly different with P≤ 0.05

Table 2. Dry weight, BE ana protein of the white button mushroom mutant (AM-G) and compared to control.

	Dry weight (%)	BE	Proteins (g/100fg FW)
wild-type	8.40 a	64 a	3.3 a
AD-M	8.00 a	61 a	2.2 b

-Means within a column followed by the same letter are not significantly different with P≤ 0.05

Discussion

It is a goal of mushroom breeders to produce a sporeless mutant whose fruiting body has qualities equivalent to those of accepted commercial spore-forming stocks in yield, flavor, texture, fruiting time, and nutrient value (Chang and Miles. 2004). We could achieve an isolate that it can't produce spores. This is the first report of the production of a sporeless mutant in *Agaricus bisporus*. The process of spore production in *A. bisporus* is very important because

its life cycle limits process of breeding and it is major problem in the cross breeding. Therefore manipulating and changing of spore production can be useful in the future research. Also in this mutant lack of spore simultaneously happened to changing of gill morphology. It maybe shows that the traits are controlled by one gene.

The various irregular morphologies generated by the mutation will be very useful resources to understand the molecular mechanism (Shimomura *et al.*, 2007). We make a new diversity in *A. bisporus* that it can be useful in marketing also it can be useful to understand the molecular mechanism responsible for spore production. If we can identify the process of spore production and gene or genes in relation to sporing, we can manipulate the life cycle. In the future if we change the life cycle, it will be a great revolution in the breeding of *A. bisporus*.

As commercial cultivars have been used for long period of time and have limited genetic variability, future breeding for improved cultivars needs the genetic variation of wild collected strains (Chan and Miles, 2004). So mutation can be a method for making variation in of *A. bisporus* and it is demonstrated that UV irradiation can improve White Button mushroom by this study. Also we can isolate new cultivar by mycelia fragment irradiation as discussed by Raper *et al.,* (1972).

Resource

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