Characteristics and suitability of various cereal grains in spawn production of button mushroom

Byung-Joo Lee*, Mi-Ae Lee, Yong-Gyun Kim, Kwang-Won Lee, Byung-Eui Lee[1] and Geon-Sik Seo[2]

Crop Research Division, Chungcheongnam-do Agricultural Research & Extension Services, Yesan 340-861, Korea
[1]Industry Academy Cooperation Foundation, Soonchunghyang University, Asan 336-745, Korea
[2]Korea National College of Agricultural and Fishery, Hwasung, Kyonggi-do 445-893, Korea

ABSTRACT: Spawn is the vegetative growth of the mycelium and serves as the inoculum or seed for cultivating mushrooms. Various cereal grains, such as wheat, millet, barley, sorghum, brown rice, rye, and oat were assessed to compare their characteristics and suitability for spawn production of button mushroom (*Agaricus bisporus*). The mycelial growth rates, density, and the number of completely colonized grains were measured from the twentieth day of inoculation. Wheat grains showed fastest mycelial growth with 8.4 cm followed by rye, oat, barley with 8.2, 7.5 and 7.3 cm, respectively. In the mycelial density, foxtail millet, barley, and sorghum were best compared with that of wheat grains. Especially, the number of grains which were completely colonized by mycelia were greatest in foxtail millet with 5,123 grains followed by proso millet, and wheat with 3,052 and 914, respectively. Based on the results obtained, barley, foxtail millet, and sorghum grains would be appropriate substituting for wheat grain in spawn production of button mushroom.

KEYWORDS: *Agaricus bisporus*, Button mushroom, Cereal grain, Mycelial growth, Spawn

Introduction

Edible mushrooms are cultivated for the production of food consumption due to its nutritive and medicinal values (Fan *et al*., 2006) including button mushroom (Beelman *et al*., 2003). The button mushroom is one of the most widely cultivated edible mushroom species placing first followed by *Pleurotus ostreatus* and *Lentinus edodes*. It is an edible basidiomycete mushroom native to grasslands in Europe and North America cultivated in more than 70 countries (Cappelli, 1984) increasing its popularity in Eastern Countries such as China and Korea.

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*Corresponding author
E-mail : byungjoo@korea.kr
Tel : +82-41-635-6061, Fax : +82-41-635-7920

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Spawn is pure culture of vegetative mycelium by which the compost is uniformly inoculated with the mushroom fungus. Spawn has an important role as the mushroom seed, comparable to the seed in crop plants. The success and productivity of mushroom cultivation depend to a large extent on the quality of the spawn used. Early mushroom spawns was produced on horse manure compose (Constantin and Matruchot, 1894). Sinden (1932) developed the procedure to produce grain spawn and later cereal grain spawns were substituted for manure compost (Stoller, 1962). Mostly grain spawn is used for mushroom cultivation. Grains favor rapid and vigorous development of the mycelium (Yang and Jong, 1987) because they are quick and easy to handle, distributed evenly over the compost providing more initial inoculum points (Vedder, 1978). A number of different cereal grains were used by different researches for making grain spawn such as wheat (Mansur *et al*., 1992; Elhami and Ansari, 2008; Chang, 2009; Stanley, 2010), rye (Chang, 2009; Stoller, 1962), rice (Oei, 1996), millet(Oei, 1996; Elhami and Ansari, 2008; Stanley, 2010), sorghum (Jiskani *et al*., 2007) etc.

The process of producing spawn on grains involves soaking and/or boiling to hydrate them. Colonization of mycelium depends on moisture content and soaking and/

or boiling of grains for different periods affect the absorption of water and rate of contamination (Frische, 1981; Kumar and Rana, 2000). Usually the calcium sulphate ($CaSO_4 \cdot 2H_2O$) and calcium carbonate ($CaCO_3$) are mixed when the grains hydrated and cooled. The calcium sulphate prevents the grains from sticking and calcium carbonate is used to adjust the pH (Stoller, 1962). The best results have been obtained by using 2% calcium sulphate and 0.5% calcium carbonate on dry weight basis of grains but different researchers used different ratios for mixing them (Sharma *et al*., 2013; Dehariya *et al*., 2011; Kumari and Achal, 2008; Ali *et al*., 2007)

The present study was to compare the characteristics and suitability of different cereal grains as single substrate for the production of spawns of button mushroom following their different hydration treatment to make approximately 50% moisture content. Calcium sulphate and calcium carbonate were not applied to understand the basic characteristics of grain materials.

Materials and Methods

Media and culture

Mycelium of button mushroom strain 'Dahyang' was cultured vegetatively at 25°C on CDA medium. For CDA 20 g compost was added to 1L distilled water, boiled for 15 min and 10 g dextrose and 15 g agar were added to liquid extract. The volume for 1L was made with distilled water. The medium was sterilized at 121°C for 30 minutes, and then preserved at 4°C. Agar blocks taken from actively growing colonies on CDA plates were inoculated into different cereal grains for mycelial growth.

Spawn substrate preparation

The cereal grains were washed with tap water and selected healthy and clean grains after removing dust and other particles. They were then hydrated by boiling or in boiled water, drained on sieve, air-dried in shade for 4 hrs, and autoclaved for 40 mins at 121°C of 120 kPa. The grains of 50±3% moisture contents were stuffed into 3 cm diameter and 20 cm long glass tube and plugged with sili stoppers after inoculation. They were incubated at 24°C in dark and the mycelial growth rates, density, and the number of completely colonized grains were measured after 20 days of incubation.

Grain types and moisture content

The cereal grains used were wheat (*Triticum aestivum* L.), proso millet (*Panicum miliaceum* L.), buck wheat (*Fagopyrum esculentum* Moench), foxtail millet (*Setaria italica* Beauvois), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* (L.) Moench), brown rice (*Oryza sativa* L.), rye (*Secale cereale* L.), oat (*Avena sativa* L.). The moisture contents of the grains were calculated using the formula as follows: Moisture content (%)= [(Initial weight - dry weight)/ Initial weight] × 100. Dry weight of the grains after hydration treatment and autoclave was determined by drying at 105°C for 4 hrs in a hot oven.

Determination of chemical properties

Total carbon and nitrogen contents were determined using an elemental analyzer (Vario Max CNS, Elementar, Germany), and the C/N ratio was calculated. The pH values were obtained in a 5:1 ratio with distilled water using a pH-Meter (Fisher model-50). The determination of inorganic elements were determined by ICP (ICP-OES, Varian, Nederland).

Results and Discussion

Different cereal grains viz. wheat, proso millet, buck wheat, foxtail millet, barley, sorghum, brown rice, rye, and oat were used to study the basic characteristics and suitability for spawn preparation of button mushroom (Fig. 1). Wheat grain was used as a control since it has been the most common grain by the choice of spawn makers in Korea. The preparation of wheat grain for commercial spawn production does not vary significantly. Boiling or steaming with water was followed by drying and coating the grain with calcium sulphate and calcium carbonate to prevent the grain from sticking and to correct the pH before sterilization.

The physical properties of different cereal grains such as 1000-kernel weight, number of kernels per milliliter, grain shape were very different, whereas the weight per liter and moisture content were similar (Table 1). The 1000-kernel weight varied ranging from 39.2 g of barley to 2.7 g of foxtail millet. Number of kernels per milliliter were greatest in foxtail millet with 281.5 followed by proso millet with 128.0. The least number of kernels per milliliter was barley with 19.0 followed by oat with 20.3. The very important advantage of grain spawn is the increased number of inoculum points

Fig 1. Different grains used in spawn preparation; A: wheat, B: proso millet, C: buck wheat, D: foxtail millet, E: barley, F: sorghum, G: brown rice, H: rye, I: oat.

compared to sawdust spawn because each individual kernel becomes such a point that mycelium can be distributed over the compost. A milliliter of colonized grain spawn can provide approximately 280 and 130 inoculum points in foxtail millet and proso millet compared with 20 points in barley and oat grains. Therefore, smaller grains are better because they cover larger surface area and give more inoculation points to the compost. However, larger grains such as wheat, rye, barley, oat can preserve the mycelium for longer periods of time because they provide greater nutrient reservoir. Grain moisture content affects the quality as well as storability. High moisture content also provides ideal conditon for mould and insect growth. Moisture contents of grains tested ranged from 12.5 to 15.2%. Some variation should be expected depending on a lot of factors such as the cultivar, harvest time, fertilization, soil, cultivation method etc. Different grains had different size and also different shapes such as oval, round, triangular. Smaller grains in a container fills the interstitial space and changes the gap or pore between kernels. Pore size is probably one of very important physical features affecting aeration needed for mycelial growth. Larger grains have larger pores compared to smaller grains of small pores. Similarly different shapes may have different aeration which may also affect mycelial growth during spawn production.

Ecologically mushrooms are classified into three categories: saprophytes, parasites, and mycorrhiza and most common mushrooms belongs to saprophytes which are decomposers in an ecosystem growing on organic matters such as wood, leaves, straw and other organic matter (Chang 2008). For example, oyster mushroom (*Pleurotus ostreatus*) and shiitake mushroom (*Lentinula edodes*) have lignocellulosic enzymes and they can use raw materials as substrate. On the other hand, secondary decomposers like button mushroom require substrate that has already undergone a significant amount of degradation or composting process by bacteria or other fungi before the mushroom can grow. Such composted substrates provide carbon, nitrogen and inorganic compounds as its nutritional sources. They have a much higher proportion of nitrogen than the substrates preferred by the primary decomposers. Not only carbon and nitrogen but their balance are very important nutrition because a well balanced carbon to nitrogen ratio enhanced the growth of mushrooms while imbalance of them impeded their growth (Stamets and Chilton, 1983). Many researchers reported different values for optimal mycelial growth of button mushroom but the carbon to nitrogen ratio variation was in the range of 15 to 30 (Maccnna, 1984; Ma *et al*., 2014;

Table 1. Physical properties and moisture contents of different grains

Grains	1000-kernel weight (g)	Weight/L (g)	Kernels/mL	% moisture	Grain shape
wheat	32.0	785.1	24.5	14.1	oval
proso millet	6.1	774.2	128.0	13.9	round
buck wheat	23.8	750.9	31.5	13.8	triangular
foxtail millet	2.7	751.5	281.5	13.4	round
barley	39.2	743.5	19.0	13.7	oval
sorghum	19.5	770.4	39.6	14.6	round
brown rice	27.7	798.3	28.8	15.2	oval
rye	29.8	706.4	23.7	12.8	oval
oat	36.9	748.5	20.3	12.5	oval

Wuest, 1977; Chang and Miles, 2004). Wheat grain which is most widely used world wide (Singh *et al*., 2011) showed 41.0% of total carbon level and 2.49% of total nitrogen level resulting 16.6 C/N ratio (Table 2). Compared to wheat other grains had similar carbon content in the range of 39.8 and 41.5 except oat. Oat had highest carbon content while lowest in brown rice. Nitrogen contents of other grains were lower compared to that of wheat in the range of 1.67 and 2.23. The C/N ratio of other grains ranged from 18.4 to 35.5 which may be enough to support the mycelial growth of button mushroom.

The calcium sulphate and calcium carbonate supplementation are common practice for quality spawn production. They were added to prevent the grains from sticking and adjust the pH (Stoller, 1962) and the optimum pH for mycelial growth of button mushroom lied between 6 and 7.5 (Treschov, 1944; Volz, 1966; Imtiaj *et al*., 2009). For this purpose usually 2% calcium sulphate and 0.5% calcium carbonate on dry weight basis of grains were tried. However, different researchers used different ratios for mixing them (Dehariya *et al*., 2011; Kumari and Achal, 2008; Ali *et al*., 2007) and they seem to be not essential for the mycelial growth (Sharma *et al*., 2013). The pH level of wheat grain was 6.5 without supplementation of calcium sulphate and calcium carbonate. Compared to wheat other grains had similar pH in the range of 5.7 and 6.8.

Table 2. Carbon, nitrogen contents and pH of different grains

Grains	T-C (%)	T-N (%)	C/N	pH
wheat	41.0	2.49	16.5	6.5
proso millet	40.6	1.96	20.7	6.5
buck wheat	41.1	2.23	18.4	6.4
foxtail millet	41.5	1.76	23.6	6.7
barley	40.6	1.67	24.3	5.7
sorghum	40.5	1.39	29.1	6.7
brown rice	39.8	1.12	35.5	6.6
rye	40.8	1.66	24.6	6.8
oat	45.7	2.01	22.7	6.2

The inorganic elements identified in different grains are presented Table 3. Phosphorus and potassium were the major elements of grains. A wide range of minerals are required for mushroom nutrition and minerals such as phosphorus and potassium were the major elements in most mushrooms including button mushroom (Demirbas, 2001). Phosphorus, one of the essential minerals is involved in many metabolic processes such as adenosine triphosphate (ATP), nucleic acids, and in the phospholipids of membranes (Chang and Miles, 2004). Potassium is also one of the most abundant metabolic element found in many fungi, which is a important factor in some enzyme systems, carbohydrate metabolism, and maintenance of ionic balance (Chang and Miles, 2004). Although it may be required in lower concentration, other elements such as sulphur, magnesium, calcium and iron play an essential role and should be included in the media (Wood and Fermor, 1985). In other researches, however, mineral elements such as zinc, copper, iron, manganese were required in very minute quantities and did not show direct relationship with mycelial growth in button mushroom (Hayes, 1972; Sharma *et al*., 2013).

Heat treatment of substrate such as boiling and steaming significantly reduced substrate contamination

Table 3. Inorganic elements contents of different grains

Grains	Concentration (mg%)								
	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
wheat	54.5	0.50	12.7	268.7	158.0	3.76	8.64	322.2	6.09
proso millet	39.2	0.49	11.3	127.7	92.8	0.56	9.46	170.7	3.99
buck wheat	42.5	0.55	10.9	338.8	220.9	1.29	6.86	366.3	2.62
foxtail millet	43.9	0.60	11.2	191.2	109.4	1.21	7.44	196.4	3.89
barley	49.5	0.44	11.7	212.3	88.0	1.43	8.95	217.2	3.58
sorghum	38.0	0.27	10.2	194.8	94.7	1.00	7.66	153.2	2.82
brown rice	35.9	0.37	10.5	124.7	74.4	2.26	8.64	184.4	7.62
rye	52.8	0.43	11.1	316.1	117.5	2.61	6.73	289.5	4.25
oat	69.5	0.37	12.7	234.0	131.5	3.98	12.76	313.2	6.75

Table 4. Hydration methods of different grains and resulting moisture content

Grains	Hydration treatment	Treatment time (min)	Moisture content (%)
wheat	boiling water	30	46.8
proso millet	boiling water	5	47.5
buck wheat	boiled water	10	46.7
foxtail millet	boiling water	5	46.8
barley	boiled water	10	48.2
sorghum	boiled water	30	46.6
brown rice	boiling water	30	51.7
rye	boiling water	30	47.9
oat	boiling water	30	49.5

Table 5. Mycelial growth characteristics in different grains

Grains	Mycelial growth (cm)	Mycelial density	Completed spawn (kernels)
wheat	8.4	+++[a]	914
proso millet	6.0	+++	3052
buck wheat	2.7	+	213
foxtail millet	6.2	++++	5123
barley	7.3	++++	752
sorghum	6.4	++++	594
brown rice	6.9	+	657
rye	8.2	++	901
oat	7.5	++	825

[a]+, very weak; ++, weak; +++, high; ++++, very high

and improved mushroom yield compared to unheated control (Jongman *et al*., 2013). Such heat treatment does not only decrease contamination but improve hydration needed for mycelial growth. Different grains were treated with different hydration methods and time resulting about 50% moisture content (Table 4). Proso millet and foxtail millet were needed least time of 5 minutes to reach that moisture content followed by buck wheat and barley with 10 minute treatment. The rest grains such as sorghum, brown rice, rye, oat including wheat were needed 30 minute treatment. Determination of different varieties of each grain for spawn production are needed to be tested in the future because different varieties of grain affected on spawn growth (Jiskani *et al*., 2007).

The mycelial growth characteristics of each individual grains that supports fast and dense growth were assessed in glass tubes (Table 5). Wheat grain was fastest in mycelial growth among all grains tested with 8.4 cm after 20 days of innoculation followed by 8.2, 7.5, 7.3, and 6.9 cm to colonize rye, oat, barley and brown rice, respectively. On the contrary, buck wheat was slowest in mycelial growth with 2.7 cm followed by 6.0, 6.2, and 6.4 cm to colonize proso millet, foxtail millet and sorghum, respectively. Mycelial density varied from weak to high. Foxtail millet, barley, sorghum was highest in mycelial density, while rye, oat, buck wheat, and brown rice were weak or very weak compared with that of wheat. When it comes to completely colonized kernels, foxtail millet, proso millet were greatest with 5,123 and 3052 compared with 914 of wheat.

Fig. 2. Mycelial growth on wheat (A), barley (B), foxtail millet (C), and sorghum compared with wheat grain (D).

In general, larger grains such as wheat, rye, oat and barley supported faster mycelial growth. The large grains can also preserve the mycelium for longer periods of time because they have a greater food reserve (Fritsche, 1988). Therefore, the spawn prepared with larger grains can withstand adverse conditions like poor composting. The colonized grain spawn is mixed with various composted substrate providing many points of inoculation. The smaller grains such as proso millet and foxtail millet resulted a greater number of fully colonized kernels providing a grater number of inoculation points than large grains such as wheat, barley, rye, oat. Given the smaller grains with more points of inoculum per gram of spawn, the spawn prepared with smaller grains may cover the compost sooner resulting faster spawn run and saving a few days for starting production. Similarly different spawn grains may affect fruiting body production as shown in

Lentinus squarrosulus (Nwanze *et al*., 2005).

Based on the results obtained, barley, foxtail millet, and sorghum grains would be most appropriate for grain spawn production substituting for wheat grain in button mushroom (Fig. 2). The advantage of barley was dense and fast mycelial growth with comparable to wheat grain. Sorghum and foxtail millet have smaller spherical kernels with dense and relatively fast mycelial growth. The distinct advantage of both grains is the increased number of inoculation points per gram or per liter compared to wheat grain.

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