Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 7

Cultivation Modes

SHELF CULTIVATION OF OYSTER MUSHROOM

With Emphasis on Substrate Fermentation

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Shelf cultivation of oyster mushrooms is a unique Korean production method. This method was adapted by Korean scientists in the early 1980's from button mushroom (*Agaricus bisporous*) growing on shelves. Button mushroom cultivation can be summarized as follows: Phase I (outdoor fermentation), Phase II (pasteurization and conditioning), Phase III (spawning and incubation), fruiting, harvesting and emptying. The unique characteristics of shelf cultivation of oyster mushroom in Korea include the fact that the substrate is fermented in three steps (pre-fermentation, pasteurization, and post-fermentation) rather than on the shelf itself. Unlike the pasteurization process that lasts for 2-6 hours in most countries, shelf cultivation requires the substrate materials to be pre-fermented outdoors for 2-3 days, then pasteurized at 60-65 °C for 8-10 hours, and finally post-fermented at 45-55 °C for 3-4 days. This process requires considerable time and generates significant expenses.

In Korea, the main methods of oyster mushroom growing are shelf cultivation, bag cultivation and bottle cultivation. Korean oyster mushroom growers ferment the substrate materials in shelf cultivation while they sterilize substrate materials in the cases of bag and bottle cultivation. Button mushroom (*A. bisporus*) is a secondary decomposer requiring the previous degradation of substrates by bacteria or other fungi in order to be able to absorb nutrients from the substrate. On the other hand, oyster mushrooms (*Pleurotus* spp.) are primary decomposers, so they have ability to break down and absorb the components of substrate materials that have not been composted or degraded. Korean growers have normally fermented substrate materials in spite of the high fuel costs generated during the fermentation process because fermentation is definitely helpful in producing high yields and high quality oyster mushrooms. This article will discuss the process of shelf cultivation and the events relative to the substrate materials during each fermentation step.

Shelf Cultivation at a Glance

The process of shelf cultivation is summarized as follows, with images

Pre-fermentation



Figure 1. Pre-fermentation and turning of cotton waste

As the first step of fermentation, pre-fermentation is a part of Phase I of button mushroom (*A. bisporus*) cultivation. Most Korean farmers utilize rice straw or cottonseed hull as substrate materials for shelf cultivation. Substrate material is piled up outdoors and then watered. The temperature of the heap gradually increases as microorganisms activated by the water begin to propagate themselves, eventually resolving the high molecular carbon sources into simpler molecules and absorbing them (Shim, 2001). The pile is turned (Fig. 1) to provide fresh air and prevent overheating. The temperature drops initially after turning but it increases again as the activities of the microorganisms continue (Shim, 2001). This step usually takes 2-3 days and the duration differs depending on the substrate

materials. When this method was first adapted from button mushroom cultivation methods by Korean scientists, farmers had always gone through this step. As time went by however, farmers came to understand that outdoor fermentation could be simplified in order to save labor costs. Nowadays some growers just water the substrate materials outdoors and keep them overnight, and then ferment them at $45-50^{\circ}$ C for about two days before pasteurization. Though this is a simplified version, many growers, especially the most successful growers, still go through this or a somewhat modified pre-fermentation process.

Pasteurization and post-fermentation

The substrate is pasteurized usually at 60 $^{\circ}$ C for 6-10 hours and then goes through the post-fermentation process at 50-55 $^{\circ}$ C for 3-4 days. The temperatures and times of pasteurization and post-fermentation vary slightly according to growers' experience.

Some growers in other countries pasteurize substrate materials. However, pasteurization and post-fermentation for shelf cultivation are very technology-intensive activities, and unlike bag cultivation, they require many years' experience to effect high productivity. Pasteurization and post-fermentation are the key factors for producing high yields in shelf cultivation. Through this process, the substrate becomes more of an appropriate food source for mushrooms, and microorganisms that can be possible competitors for nutrients are eliminated from the substrate (Shim, 2001).



Previously, pre-fermented substrate was filled into the shelves of the growing room and then went through pasteurization and postfermentation in the growing room. However, the substrate wasn't thoroughly fermented and the extent of fermentation differed

Figure 2, 3. Room for pasteurization and post-fermentation (Farm A and B)

according to the specific layer of the shelf because temperature differences in the growing room were too large to evenly ferment substrate materials. In addition, this practice consumed a large amount of fuel. As a result, many growers nowadays have built a special room that is heated by steam for pasteurization and post-fermentation (Fig. 2). Baskets filled with pre-fermented substrate materials are stacked (Fig. 3) and pasteurized and post-fermented. Thanks to this room, growers can ferment substrate materials evenly in a relatively small space and save money by using this system.





Figures 2, 3. Room for pasteurization and post-fermentation (Farm A and B)

If the substrate is pre-fermented sufficiently outdoors, its temperature can be quite high when moved into the room. The temperature inside the room is increased by steaming and kept at 60° C for 8-10 hours in order to accomplish the pasteurization. Though the room temperature is kept as 60° C, the internal temperature of the substrate rises up to 65° C. After 8-10 hours the temperature is lowered and maintained at 48-53 °C for 4-5 days. Some growers say five-day is too long for post-fermentation and the substrate is like to be too wet after five days of post-fermentation.

If the substrate is just watered outdoors without pre-fermenting it, it can be fermented in the room before pasteurization. The room temperature should be slowly increased by steaming up to 45° C and then gradually raised up to 46° C and then 48° C and finally up to 53° C for two days to accomplish pre-fermentation.



Figure 4. Cross-sectional view of basket

fermentation.

Although the principles are same, each expert grower has his own know-how in this process. Some growers make an additional effort to keep each part of the substrate at a similar temperature for thorough fermentation. Figure 4 shows a cross-sectional view of a basket filled with substrate materials. The central part of the substrate is removed, and by doing this, the temperature difference among each part of the substrate in the same basket can be reduced down to 4° C. Otherwise, the temperature difference between the hottest part and the coolest part in the same basket reaches as far as $8-10^{\circ}$ C, which hinders thorough

When pasteurization and post-fermentation is completed, a white color is visible on the substrate. These are actinomycetes, one of the thermophiles produced in the last stage of post-fermentation. If actinomycetes exist in sufficient quantities on the substrate, the substrate can be said to be well fermented and suitable for mushroom growing. Actinomycete will be discussed further later in this article.

Filling and spawning

After pasteurization and post-fermentation is completed, the substrate is filled into shelves in the growing room. Filling and spawning is one of the most labor-intensive processes in shelf cultivation if it is done manually. The post-fermented substrate is poured from the box onto the shelf and this is repeated until each shelf has the allotted amount of substrate (Fig. 5). Though differences exist, growers usually fill 15kg of dry substrate per square meter of shelf, as is the usual case with cotton waste. As the moisture content of the substrate is about 70%, filling the proper amount of the fermented substrate can be calculated as 50kg per square meter. However, the filling weight varies depending on growers, substrate materials, and seasons. If rice straw is selected as the substrate, greater amounts are filled than if the substrate is cotton waste. More substrate is used in winter than in summer because the substrate is more likely to be overheated in summer.

After filling, the substrate is covered with a plastic sheet (Fig. 6) to keep in humidity, and stays overnight (Fig. 7) for cooling. The next day, when the substrate has cooled down to 20-25 °C, about 60-70% of the spawn is inoculated (Fig. 8) and thoroughly mixed with the substrate on the shelves. The substrate is then spread evenly on the shelves and the remaining 30-40% of the spawn is sprinkled onto the substrate surface (Fig. 9). The spawning rate is generally higher in shelf cultivation and can be as much as 14% of the wet weight of the substrate, namely 7kg of spawn is inoculated to 50kg of substrate per square meter. The shaped substrate is mulched with a plastic sheet that has very small holes for ventilation (Fig. 10).

<Manual Filling and Spawning>



Figure 5. Filled substrate on shelf



Figure 6. Covering filled substrate with plastic sheet



Figure 7. Cooling substrate overnight



Figure 8. The first spawning



Figure 9. The second spawning after flattening the surface



Figure 10. Covering with a plastic sheet after spawning

These processes are very labor intensive, so some growers utilize equipment such as filling machine and spawn mixing machine. Farm A saves 50% of their labor costs of the spawning operation by using a spawn mixing machine. Filling is done manually one day before spawning, and 60% of the spawn is inoculated by sprinkling (Fig. 11). Then the substrate and spawn are mixed (Fig. 12) by the wires of the machine (Fig. 14), which rides on the rails along the both sides of shelf (Fig. 13). The rail for this machine is attached to both edges of the shelves (Fig. 13). The substrate is then flattened (Fig. 15) and the rest of spawn is sprinkled on the surface (Fig. 16).

<Farm A: Spawning with spawn mixing machine>



Figure 11. The first spawning by sprinkling



Figure 12. Mixing substrate and spawn



Figure 13. Rail for the machine at the edge of shelf



Figure 14. The wires for mixing in the machine



Figure 15. Flattening substrate after mixing



Figure 16. The second spawning on the surface

Farm B saves much more labor by using a filling machine (Fig. 17). In this case, filling and spawning are done simultaneously. The post-fermented substrate stays overnight for cooling down to the 20-25 °C temperature

appropriate for spawning, and then the substrate in baskets is poured into the filling machine (Fig. 18) from where it moves onto the shelves via the conveyer of the machine. As soon as the substrate falls onto the woven textile mat on the shelf, two workers sprinkle spawn, mix it with the substrate, and flatten the substrate by hand on both sides of shelf (Fig. 19). This procedure takes some time, so another worker controls the speed of the filling machine (Fig. 20). When the substrate is flattened, the woven textile under the substrate is winched toward the other end of the shelf (Fig. 21, 22). The rest of the spawn is then sprinkled on the surface of the substrate.

<Farm B: Filling with filling machine>



Figure 7. Filling machine



Figure 18. Filling substrate



Figure 19. Substrate on shelf is Spawned and mixed.



Figure 20. Controller of filling machine



Figure 21. Substrate winched toward the end of the shelf



Figure 2. Winch machine

Incubation

Mushroom mycelia are incubated for 17-23 days while covered with a plastic sheet. The temperature should be maintained at 20-22 °C during the first stage, and then increased gradually up to 25 °C. As the incubation progresses, the substrate emits heat by itself due to mycelial growth. Therefore, the temperature should be set at 22-23 °C though the optimal temperature for growing of the oyster mushroom mycelia is 25 °C (Cha *et al.*, 1997). The mycelia don't require much ventilation during vegetative growth, but it should be kept in mind that they do require sufficient oxygen during this stage.

Pinning and fruiting

When the spawn has fully colonized the whole substrate (Fig. 23), the environment in the growing room is adjusted and made appropriate for reproductive growth and fruiting. To convert to the reproductive growth stage, the factors such as adding light, performing a cold shock, maintaining a high relative humidity, and providing enough oxygen are implemented. Light levels are raised to 80-120 lux, sufficient for newspaper to be readable, for 3-4 days before the plastic sheet is removed for ventilation. The temperature of the growing room is lowered to

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15-18 °C, but the optimal temperature for pinning varies from 10-24 °C, depending on species and strains (Cha *et al.*, 1997). Growers should be aware of the characteristics of the species and strains they are growing. Relative humility inside the growing room should be kept as high as 85-95% by watering once or twice a day. Pinheads (Fig. 24) should be observed within several days and grow to full size soon thereafter.









Figure 25. Fruitbodies growing from perforated parts

Some growers cover the whole shelves with black perforated plastic sheets with 5-10cm holes every 10-15cm. At spawning, 60-70% of the spawn is mixed with the whole shelf and then the shelf is covered with the perforated plastic sheet and the rest of the spawn is inoculated into the holes. Then, the shelf has 80% of mulched area and 20% of perforated area. The fruitbodies grow only from the perforated parts (Fig. 25) in clusters. The mulching with a plastic sheet provides many benefits. It reduces the labor input during harvest and the abortion of small pins, and produces quality mushrooms with favorable color and long stipes, which are more marketable in Korea, and most importantly, it produces greater yields (Oh *et al.*, 2003b). In addition, mulching with a plastic sheet is effective for in preventing diseases such as bacterial brown blotch and various fungal diseases by preventing invasion of the pathogens and reducing waterlogged areas on the shelves (Oh *et al.*, 2003a).

Harvest



Figure 26. Oyster mushroom on conventional shelf



Figure 27. Oyster mushroom on vinyl mulching

Oyster mushrooms are harvested when they grow to full size. Several flushes are harvested because the abundant quantity of substrate on the shelves still has plenty of nutrition for oyster mushrooms even after several harvests. Most growers harvest 3-4 flushes from the shelf, and

about 50% of the yield is from the first flush. Accumulated biological efficiency reaches 100%, but it fluctuates according to seasons.

After 3-4 flushes, the shelf can still produce more mushrooms due to the existence of sufficient nutrients on the shelves. However, harvesting more than 3-4 flushes is not economically reasonable in Korea where land and labor costs are very high and high fuel expenses for growing room control are required in summer for cooling and in winter for heating. More flushes could be harvested in countries with low land costs and labor costs and tropical or sub-tropical climates, and in those situations the biological efficiency could reach much higher rates.

Oyster mushrooms from shelf cultivation are considered to be of a higher quality than those from bag or bottle cultivation thanks to the rich nutrients available for mushrooms grown on shelves. This high quality produce also earns more money. In the Korean mushroom market, high quality oyster mushrooms are usually three times more

expensive than low quality ones.

Emptying

When the substrate has produced an economically reasonable amount of mushrooms, the shelves are emptied. Emptying is also very labor-intensive, but many growers have discovered more convenient methods that save labor. In principle, the substrate on the shelves is steamed and then removed. However, many farms have a steaming facility only in the room used for pasteurization and post-fermentation, so growing rooms are disinfected by fungicides or insecticides such as diluted formalin solution, Benlate, or Panmashi. The spent substrate is moved away from the farm to prevent the infection of new crops. Sometimes the spent substrates are utilized as pig's fodder.

Fermentation, the Art of Microorganism

The following section is excerpted and translated from 'The essence of mushroom cultivation - Fermentation of Substrate' (in Korean), written by Dr. Moon-soo Shim and excerpted here with the permission of the author.

Fermentation can be defined as the process of converting or decomposing organic matters into unique final products by the function of microorganisms' enzymes. However, fermentation in mushroom cultivation can be defined as the converting by microorganisms of the nutrients of substrates into proteins.

Selecting substrate material

Button mushrooms naturally grow from materials with a relatively high nitrogen content such as horse manual (1.8% nitrogen) and wheat straw (0.65% nitrogen). The optimal C : N ratio for cultivating button mushrooms is 17 : 1. On the other hand, oyster mushrooms and shiitake grow from wood with a relatively low nitrogen source, of which the C : N ratio is 350 to 500 : 1. The optimal C : N ratio differs according to mushroom species. Therefore, the C : N ratio of substrate material should be first considered in substrate preparation. Table 1 shows composition contents of each substrate material.

Material	pН	Cellulose	Lignin	Total Carbon	Total Nitrogen	C:N ratio
Cotton Waste	6.2	73	6	24	0.41	59:1
Rice Straw	6.7	42	13	46	0.63	72:1
Wheat Straw	6.9	48	20	47	0.48	97:1
Corncob	7.2	47	25	47	0.48	97:1
Sawdust	5.5	54	29	49	0.1	491:1

As shown in Table 1, the main substrate material alone sometimes cannot provide enough nitrogen required for optimal growth of mushrooms, so additives such as rice and wheat bran are supplemented as a nitrogen source. The amounts supplemented vary depending on which substrate is chosen. If cotton waste is selected, a smaller amount of nitrogen source is added than when wheat straw is selected.

The C : N ratio is also important because it affects the fermentation process. Through the fermentation process, nitrogen is converted into ammonia nitrogen, restraining the growth of mushroom mycelia as well as making nitrogen available for the mycelia. If available nitrogen increases, ammonia nitrogen also increases, and ammonia

nitrogen decreases when available nitrogen decreases. Therefore, the amount of ammonia produced by fermentation should be considered in substrate selection. Table 2 shows the result of comparison of oyster mushroom yields after supplementing different amounts of rice bran. As supplemented rice bran increases, total nitrogen and ammonia nitrogen increase and the oyster mushroom yield is affected as a result. When 0.98% of total nitrogen added, the amount of ammonia nitrogen (28 ppm) is too little to influence oyster mushroom yields. When 1.48% of total nitrogen is added however, the yield decreases to 45.2kg because the ammonia nitrogen levels are enough to restrain the oyster mushroom from growing. In conclusion, oyster mushroom yield decreases when the ammonia concentration is higher than 68 ppm as well as when total nitrogen is smaller than the optimal amount.

Total Nitrogen (%)	Ammonia nitrogen (ppm)	Yield (kg/m ²)
0.98	28	173.9
1.08	68	193.4
1.48	84	149.2

Table 2. Oyster mushroom	vield according	g to total nitrogen and	l ammonia nitrogen

Therefore, C : N ratio and the amount of ammonia nitrogen should be both considered. If cotton waste is chosen as the main substrate material for oyster mushroom, a nitrogen source such as rice bran should be supplemented considering the optimal C : N ratio. The amount of nitrogen source supplemented should be up to the total amount of nitrogen from which the amount of ammonia nitrogen created during fermentation doesn't restrain the mycelial growth of the mushroom.

Cotton waste and sawdust, the major substrate materials, have naturally occurring microorganisms that participate in fermentation of the substrate. Figures 28 and 29 show incubated microorganisms from sawdust and cotton waste. Both substrate materials were soaked in water and then the water was inoculated on nutrient agar and incubated. The first two Petri dishes were incubated at 30 °C and several kinds of mesophiles were propagated on them (Fig. 28). The other two Petri dishes were incubated at 50 °C and some thermophiles were cultivated on them (Fig. 29).



A. from sawdust B. from cotton waste Figure 28. Mesophiles incubated at 30℃



A. from sawdust B. from cotton waste Figure 28. Thermophiles incubated at 50° C

Table 3 calculated the number of microorganisms in cotton waste, hardwood sawdust, and rice bran. Cotton waste has the most microorganisms while sawdust has less and rice bran has the lowest amount. Though the results vary a little bit according to how and how long the materials are stored, the result will be same as below if they are stored under similar conditions. Sawdust has as many mesophiles as cotton waste, but cotton waste has 390 times more thermophiles than sawdust. This is because cotton waste is more easily exposed to microorganisms in nature than sawdust. Therefore, materials with more microorganisms are desirable as

mushroom substrate if the substrate is to be fermented.

Substrate materials	Cotton waste	Hardwood sawdust	rice bran	note
Mesophiles	75×10^{4}	54×10^{4}	8×10^{2}	Cotton waste has 1.3 times more
(incubated at 30° C)				mesophiles than sawdust.
Thermiphiles	47×10^{4}	12×10^{2}	12	Cotton waster has 390 times more
(incubated at 50°C)				themophiles than sawdust.

Table 3. Numbers	of microo	rganisms	according to	o materials

Pre-fermentation

Fermentation aims at repressing microorganisms that might possibly compete with oyster mushrooms and converting substrate materials into a superior nutritional source for mushroom through the actions of a succession of microorganisms. Outdoor fermentation is the first step. Substrate materials in nature have microorganisms attached on their surfaces and these microorganisms are suppressed on dry material. Once water is applied, microorganisms on substrate material can propagate themselves. If the initial temperature of the substrate is $20 \,^{\circ}\text{C}$, microorganisms suitable for this temperature range will increase and begin consuming the water-soluble carbon source that is relatively easy to absorb. Generally, the organism utilizes 35% of the nutrients for energy but the other 65% cannot be utilized and is emitted as heat. As the microorganisms increase by geometric progression, the heat emitted by the microorganisms is accumulated and the temperature of substrate increases to $30 \,^{\circ}\text{C}$. Then, growth of the microorganisms that prefer the temperature of substrate increases up to $50 \,^{\circ}\text{C}$.

The water-soluble carbon sources in substrate are all consumed by microorganisms as the fermentation proceeds. At that point microorganisms begin to consume high molecular carbon sources that are relatively hard to absorb, such as cellulose, hemi-cellulose and lignin. In theory, the increase of substrate temperature up to 50° C means that microorganisms have decomposed the high molecular carbon sources that are relatively hard to utilize as well as the water-soluble carbon sources that are easy to utilize, and that the nutritive substances of the substrate are accumulated in the microorganisms in the form of protein. However, the substrate temperature is not uniform within a pile in actual fermentation, so the substrate pile is turned several times to encourage thorough fermentation by repeating the process described above several times. In addition, turning helps aerobic fermentation by providing more air to the pile.

Some growers may wonder what would happen if the substrate materials were fermented at 50 °C. Would this make the fermentation process faster and easier? The answer is negative. The initial microorganisms that exist on cotton waste or cereal straw come mainly from the soil and many of them are mesophilic microorganisms best cultivated at about 30 °C. Because 50 °C is not a usual temperature in natural environments, there are not many or many varieties of thermophiles that are best incubated at 50 °C. There are not many microorganisms available to ferment the substrate if the fermentation starts from 50 °C. In addition, various kinds of microorganisms are more able to ferment the whole substrate depending on the diverse nutritional components. However, oyster mushrooms can be still cultivated successfully even though the substrate has been fermented only from 50 °C if the mushrooms are well managed in each step of cultivation. This is because oyster mushrooms grow very well on various substrates without fermentation.

Pasteurization

The substrate is pasteurized at 65 °C for 6-8 hours. Pasteurization is sometimes said to aim at killing insects and

mold spores, but this is not a sufficient explanation of the process of pasteurization. The spores of molds are generally killed at temperatures over 80 °C, and therefore the pasteurizing temperature of 65 °C is not enough for killing most mold spores. Moreover, the spores are more durable in a 65 °C substrate with relative humidity of 60-70% than in 65 °C water. This can be easily understood if you think that people can stay in an 80 °C sauna but not in 80 °C water. Some growers pasteurize substrate at 80 °C, but this temperature can kill useful microorganisms as well as mold spores. The substrate is pasteurized in order to soften the substrate materials and kill mesophilic microorganisms, not mold spores.

Post-fermentation

After pasteurization is completed, the substrate is post-fermented at 50-55 °C for 3-4 days. Though prefermentation is completed before pasteurization, the whole substrate is not fermented. Post-fermentation aims at a thorough and even fermentation of the whole substrate. During pre-fermentation, mesophiles are converted to thermophiles, but mesophiles are still abundant because considerable parts of the substrate are not fully fermented. These mesophilic microorganisms later compete with mushroom mycelia because both grow well at similar temperatures. Once these mesophiles are converted into thermophiles, however, these thermophilic microorganisms cannot grow at the incubation temperature of mushroom, and so cannot compete with mushroom



Figure 30, 31. Actinomycetes on cotton waste(above) and rice straw (below)

mycelia.

White actinomycete grows at the last stage of the fermentation (Fig. 30, 31). The presence of actinomycete indicates that the substrate is well fermented aerobically and has become appropriate for the mycelial growth of mushrooms. The presence of actinomycete on the substrate indicates that the pH of the substrate is more than pH 7, a level that suppresses the growth of green molds.

After post fermentation, the substrate has become a superior nutritional material for mushroom mycelia. Useful nutrients are possessed by microorganisms as proteins and these proteins are not spoiled because they are inside the living organisms. Mushroom mycelia vegetative growth cells are able to secrete a greater variety of digestive enzymes than any other microorganisms. Once mushroom mycelia are inoculated into the substrate in the form of spawn, by using various digestive enzymes the mycelia can digest materials that other microorganisms could not process. Moreover, the mycelia have enzyme-digesting microorganisms, so they can dissolve and absorb the proteins, lipids, minerals, and vitamins of the microorganisms.

Characteristics of microorganisms participating in fermentation

One of thermophiles and one of actinomycete were separated and their optimal pH and temperature were examined. According to Figure 32, the thermophilic microorganism showed optimal growth at pH 7-8 while the optimal pH for the actinomycete was 8-9. Therefore, both grew well on alkaline (over pH 7) substrate. Considering only microorganisms, pH 8 would be the optimal for substrate, but the optimal pH for mushroom growth is 6-8. Therefore, pH 7 is the best level for the growth of both mushrooms and thermophilic microorganisms. Figure 33 shows that the thermophiles and the actinomycete grow best at 50° C. The thermophiles propagate well enough below 50° C, but their growth is much repressed above 50° C. On the other hand, the

actinomycete prefer 45-55 $^{\circ}$ C. This result indicates that the thermophiles participate in fermentation at the early stage while actinomycete participate actively later, when the temperature is over 50 $^{\circ}$ C.



Figure 32. Optimal growth of thermophile and actinomycetes according to pH





Figure 33. Optimal growth of thermophile and actinomycetes according to temperature

Various microorganisms participate in the fermentation process as dominant species in each step in the succession. To monitor the changes of microorganisms during fermentation according to substrate materials, substrates were prepared by mixing different rates of cotton waste and sawdust and incubated at 30 °C for 2 days and then at 50 °C for 5 days, after which the propagation of mesophiles and thermophiles were examined (Fig. 34, 35, 36).

According to Figure 34, mesophilic microorganisms begin to propagate after the first day and multiplied in number over 1,000 times until the first day of incubation at 50 $^{\circ}$ C began. On the first day of incubation at 30°C mesophiles did not grow at all, because this was a preparatory period when appropriate microorganisms for temperature, pH, oxygen and nutritional condition of the substrate adapt themselves and microorganisms participating in fermentation are selected. When ready, the mesophiles increased explosively. However, they begin to decrease rapidly at the beginning of the first day of incubation at $50\,^\circ\!\mathrm{C},$ and their numbers dropped down to the level where fermentation started. On the other hand, thermophiles increased from the first day at 50 $^{\circ}$ C until the last day. Seeing that mesophiles did not stagnate at 50° C but decreased and disappeared, it can be inferred that they were utilized as nutrients for the thermophiles.

Figure 33, 34 and 35 shows how important the choice of substrate material in fermentation process is for two reasons. As the rate of sawdust increases among substrate (Fig. 35), thermophiles as well as mesophiles cannot increase because sawdust doesn't contain enough microorganisms that are

participating in fermentation. However, the number of microorganisms doesn't increase as much as when the rate of cotton waste is higher (Fig. 33, 34) even though the incubation days are extended. The second reason is that sawdust lacks nutrients easily utilized by microorganisms. Sawdust has more hemi-cellulose and lignin, which are relatively hard to utilize, while cotton waste has more cellulose, which is dissolved relatively easily by microorganisms. Therefore, the growth of microorganisms is restrained and fermentation is not increased as the amount of sawdust increases within a substrate. Another important finding is that if mesophiles don't increase, thermophiles also cannot increase. Mesophilic microorganisms affect the growth of thermophilic microorganisms. **Note : End of excerpt.**

Conclusion

Shelf cultivation of oyster mushroom adopts the composting technology of button mushroom cultivation. Though not essential, fermentation contributes to the high quality and high yield of oyster mushrooms. Nowadays, many Korean growers have converted from shelf cultivation to bag cultivation due to the high risk of shelf cultivation. The fermentation of substrate requires many years of experience and skill, so many inexperienced oyster mushroom growers fail to produce profitable amounts of mushrooms. Moreover, shelf cultivation entails high expenses due to the large amounts of substrate and spawn, the high fuel cost for fermentation, and so forth. On the other hand, bag cultivation is relatively easy and safe because it produces appropriate yields though not of as high of a quality. Nevertheless, it is expected that the principles of substrate fermentation could be applied to the respective situations of oyster mushroom growers. Fermentation might require far less costs in tropical or subtropical regions because less fuel is required.

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