"Science Stays True Here"
Biological and Chemical Research, Volume 2015, 297-305 | Science Signpost Publishing

The Research, Development and Application of the Fermentation Accelerator, a New Material for Edible Mushroom Cultivation on Unsterilized Substrate

Han Shenghua[1], Ye Caiyun[2], Han Hongyi[3]

1. Hangzhou Huadan Agricultural Products Company Limited, Add.: Zhongcun Village, Zhuantang Town, West Lake District, Hangzhou City 310024, Zhejiang Province, P.R.China.

2. Office for Foreign Investment in Agriculture, Ganzhou City Agriculture and Grain Bureau, Add.: Room 911 North Tower, Ganzhou Municipal Center, Ganzhou City 341000, Jiangxi Province, P.R.China.

3. Zhejiang Provincial Huadan Academy for the Application of Microbial Resources in Agriculture, Add.: Zhongcun Village, Zhuantang Town, West Lake District, Hangzhou City 310024, Zhejiang Province, P.R.China.

Received: May 28, 2015 / Accepted: July 03, 2015 / Published: October 25, 2015

Abstract: The fermentation accelerator is a product resulted from the research on the differences in biological characters among various fungi. The purpose of the research was to develop a substance that can accelerate fermentation of the substrate, thus realize sterilization at a lower temperature and in a shorter time period, as well as reduce consumption of energy, cost and labor. We found that by using it in the traditional sterilization method, the fermentation accelerator could help produce ammonia and beneficial micro-organisms to accelerate the death of harmful micro-organisms like weed moulds at a temperature between 70 and 80℃ in 5-7 hours, realizing the purpose of sterilization, The production of 10,000 bags of Lentinula edodes (Shiitake) or Auricularia auricular -judae (Black wood ear) could save 720 kgs of coal and 2 labor forces and increase the yield by over 10%. Our conclusion was that edible mushrooms could not only be cultivated on sterilized substrate, but also on unsterilized substrate with the help of the fermentation accelerator, which could bring about considerable economic and ecological benefits. This achievement passed the expert appraisal of scientific and technological administration sector and over 2 billion bags were spread and used in 26 provinces (regions, municipalities) across China.

Keywords: The fermentation accelerator, edible mushrooms, cultivation on unsterilized substrate, new material

Corresponding author:

Ye Caiyun, Office for Foreign Investment in Agriculture, Ganzhou City Agriculture and Grain Bureau, Ganzhou City 341000, Jiangxi Province, P.R. China. E-mail: 594835892@qq.com.

1. Concepts of Unsterilized Substrate and Its Cultivation

Edible mushroom cultivation on unsterilized substrate has many advantages and will greatly promote the reform of its cultivation procedures and the development of its industry.

Cultivation on unsterilized substrate is a concept comparative with cultivation on sterilized substrate. The former refers to the cultivation procedures in which mycelium of the mushroom inoculated on the unsterilized substrate can grow well and contaminants are inhibited or killed. The latter refers to the cultivation procedures of substitute substrate in which mycelium of the mushroom is inoculated on the steam sterilized substrate.

The ancestors of Chinese nationality began to cultivate edible mushrooms on unsterilized substrate a long time ago. Auricularia auricular - judae (Black wood ear) and Lentinula edodes (Shiitake) cultivation on wood logs were cultivation techniques on unsterilized substrate. The primitive cultivation on unsterilized substrate depends on the primitive materials, hardwood logs, and should be under an optimal natural environment, so it is limited by climatic conditions, especially humidity and temperature. Furthermore, cultivation on wood logs has such shortcomings as low biological efficiency, low output, unstable quality of mushroom products and the destruction of forest and tree resources, so it is banned in many places and is gradually replaced by the cultivation technique on substitute substrate (sterilized substrate) emerged in the late 1970s'.

Although cultivation on substitute substrate has the advantages of high yield, easy control by man and stable fruiting, it has the following deadly disadvantages: ① much waste of raw materials: equivalent amount of firewood or alternative energy source is needed to sterilize 1 kg of sawdust substrate; ② heavy nutrient loss of the raw materials at a high temperature or pressure; ③ varied quality of mushrooms from different flushes: loose and soft tissue, poor mouth feel; and ④ a long sterilization period and a big amount of manpower input. Therefore, cultivation technique on unsterilized substrate has long been dreamed by mushroom growers, but up to now it has not been successfully popularized and applied due to the severe contamination after inoculation. The few so-called successful cultivation examples had very high costs and much sequel. All the above set forth a research requirement for cultivation technique on unsterilized substrate.

2. The Basic Objectives of Mushroom Cultivation on Unsterilized Substrate

Compared with cultivation on sterilized substrate, cultivation on unsterilized substrate has the following advantages: simplify the procedures, save labor and cost, the inoculation operator is exempt from the injury by disinfectants in the inoculation room, reduce the use of energy and maintain the eco-balance.

Hangzhou Huadan Agricultural Products Company Limited developed a fermentation accelerator after over 20 years of constant research. It can basically meet the above-mentioned requirements in edible mushroom cultivation.

3. The Functions and Principles of the Fermentation Accelerator

(1) The sterilization principle and function.

The fermentation accelerator is a product resulted from the research on the differences in biological characters among various fungi. On the substrate not thoroughly sterilized, the major contaminants in the growing bags are aspergillum and bacteria. But on those thoroughly sterilized, they are mainly Trichoderma spp. and green moulds. The spawned and cultivated edible mushrooms are macro-fungi, the fermentation accelerator will primarily kill aspergillum and bacteria, and will also kill such contaminants as Trichoderma spp., green moulds and Neurospora spp. (Agent Orange).

(2) The fermentation acceleration principle and function.

At a temperature between 70 and 80℃, the fermentation accelerator will volatilize onto the substrate to speed up fermentation and realize the effectiveness of composting for over 1 week. The ammonia and beneficial microorganisms produced in a short time period accelerate the death of contaminants like weed moulds, thus realize the effectiveness of a thorough sterilization. The nutrient loss of substrate is very small in this process.

(3) The catalytic fermentation principle and function.

At a substrate temperature between 70 and 80℃, the fermentation accelerator can decompose the substrate into carbon dioxide, water, urease and special－effect catalytic enzyme, which will volatilize and penetrate on the substrate consisting mainly of sawdust, straw and cottonseed husk etc. After mixing with these ingredients, the fermentation accelerator can be decomposed into NH4+and OH- with the help of enzyme. OH- can break the ester bond and the mosaic structure between the molecules of lignin and cellulose in the substrate, thus dissolves them into semi-cellulose and cellulose. The NH4+ decomposed from the fermentation accelerator can be adsorbed on the surface and fracture of the substrate and form a compound of NH4+—N with the free nitrogen. During the mycelia growth, this kind of compound will be synthesized into the microorganism protein, which will be freely adsorbed on the surface of the substrate and is more optimal for the growth of mushroom mycelium. The research shows that the fermentation accelerator can increase the biological efficiency of the substrate by about 10%～20% and the mushroom yield by more than 10%.

4. Effects of the Fermentation Accelerator Applied to Mushroom Production

The fermentation accelerator is suitable for all wood-rotting mushrooms cultivated with the technique of normal pressure sterilization (steam boiler, earth stove). It can also be used to compost for Agaricus

bisporous to make the compost temperature rise and ferment in a short time period. After a long period of experimentation and extension, it showed the effects as follows:

(1) A low sterilization temperature at 70～80℃ and a short sterilization period in 7 or 5 hours. Fuel, time and labor were saved by about one half, but effect was improved by one fold.

(2) A wide-range application. It can be applied to almost all edible mushrooms cultivated at present (with very few exceptions). The percentage of mushrooms fruited and harvested in large-scale cultivation exceeded 95%.

(3) The amount of bran in the regular formula could be reduced to 10%～15%, thus decreased the cost. 20% of wastes could be used to increase the resource utilization.

(4) The robust growth and fast colonization of the mycelium could make the spawn run the entire bag advanced by 7 days.

(5) Sterilization at a low temperature would cause less nutrient loss of the substrate and less deformed mushrooms as well as high quality and yield.

(6) The improved resistance to high temperatures would reduce the number of rotten bags, slow down the shrinking and loosening of growing bags, strengthen the after– effect and prolong the fruiting period.

(7) It could resist the infestation of Neurospora spp. (Agent Orange) and Fastigiated bacteria, which are massive and severely harmful.

(8) The reduced occurrence of pests and diseases made the pesticides unnecessary.

(9) The application to straw rotting mushroom cultivation could make the temperature of fermented substrate rise in a short time period, thus promoted the substrate fermentation.

5. The Main Points of Operating the Fermentation Accelerator in Edible Mushroom Cultivation

The fermentation accelerator is simple to use. What you need to do is to add it and other ingredients successively from a small amount to a large amount based on the percentages in the formula, then mix them evenly, and finally stir the mixture with water.

(1) Add 0.35%～0.5% of the fermentation accelerator to the total dry weight of the substrate. Take the Black wood ear as an example: 1 bag of the fermentation accelerator weighing 1,000 grams can be formulated in about 600 17×33 cm growing bags.

(2) Mix the raw materials. Add the fermentation accelerator and other ingredients in the sequence from

a small amount to a large amount based on the proportions in the formula: the fermentation accelerator → gypsum powder → bran (corn meal, soybean meal etc.) →sawdust, straw (cottonseed husk, corncob etc, which can be pre-wetted). Mix these ingredients evenly in sequences, and then stir the mixture with water.

(3) Sterilization. ① 2～3 cm of gap should be maintained between rows when stacking the bags in the container so as to facilitate efficient steam circulation and reduce the dead spaces. ② during sterilization, the temperature displayed on the thermometer should be the actual substrate temperature inside the bag, so the thermometer should be inserted inside the bag at the bottom of the container on the stove. ③ when the substrate temperature inside the bag reaches 80℃, maintain it for 5 hours, and then let it cool down naturally to a proper temperature and take out the bags from the container. During the cooling period, the container is allowed to be tightly closed and the artificial logs should be prevented from dust contamination. ④ the temperature of 80℃ inside the sterilization container is not constant, so the fire can be stopped only when the reading of the thermometer exceeds 80℃ and should be continued when it is reduced to 80℃. The effective temperatures of the fermentation accelerator range between 50 and 150℃, so it will not lose its efficacy at a temperature of 100℃.

(4) Inoculation. It should be carried out in accordance with the regular sterile operation methods. Excellent spawn at a proper fungus age should be used for inoculation.

(5) Sealing the inoculation bags. The cotton plugs and rubber bands (plastic rings) are not proper for sealing the inoculation bags, because in the same container as the substrate, they will be contaminated instead of sterilized at a temperature of 80℃. For those edible mushrooms fruiting on bags, it is suggested that the bags be sealed by tying or folding them. The sticky tape or white wax (20% of leather belt wax can be mixed) is well fit for sticky sealing the inoculation holes in the bags for Shiitake and Tremella Fuciformis (Silver ear) cultivation. The surface mycelium on the inoculated squares shall be kept in a good, wet and aseptic microclimate to speed up their germination and colonization. If the inoculation holes are sealed with the spawn itself, more spawn shall be used and no gap is allowed on the surface, which shall be kept dry. The incubation room shall be frequently disinfected to kill contaminants in the air and shall have a good ventilation and aeration to prevent the inoculation surface from infection.

(6) Spawn running. During the first 7-day period after inoculation, the spawn with the fermentation accelerator used in the substrate colonizes slower than that of the regular substrate, but after that period, it becomes faster. The mycelium can colonize the entire bag 7 days ahead of the regular

substrate. The temperature in the artificial log pile ought to be monitored all the time to prevent it from burning.

(7) Compost. It shall be done according to the varied requirements of the regular production for different edible mushrooms. The quantity of the fermentation accelerator to be added is 0.5%.

(8) Storage. The fermentation accelerator is very easy to be moist and stuck together, so it should be stored in a closed, shady, cool and dry place.

(9) Bans. The fermentation accelerator can never be used by directly adding water to, as it is easy to become moist and compact even form sticky mass after moistened, making it unable to use.

6. Benefit Analysis on the Fermentation Accelerator Application

Take Shiitake, a typical wood rotting mushroom, as an instance, to produce 1,000 bags adding the fermentation accelerator, a direct input of 111 Yuan would be reduced. A cost comparison of the sterilized substrate and that of adding the fermentation accelerator in Shiitake cultivation are listed in table 1.

7. Advantages to Use the Fermentation Accelerator

(1) Simplify procedures. High pressure sterilization is unnecessary, so the operation is not limited by sites.

(2) Save labor and costs. With the help of the fermentation accelerator, sterilization can be realized at a lower temperature in a shorter time period, so it can reduce the consumption of energy and costs. A strictly disinfected inoculation room (cabinet) is also unnecessary. The unsterilized substrate bag cultivation can save a cost of 12%～35% than the sterilized substrate bag cultivation. In mushroom cultivation on substitute sterilized substrate, it consumes a large amount of firewood or other fuel to sterilize at the normal pressure. Calculated at 1,000 bags on 1 sterilization unit, 300 kgs of firewood are to be burnt. Cultivation on unsterilized substrate can make use of the firewood saved to produce more Shiitake so that the contradiction between mushroom cultivation and forests can be alleviated and the eco-balance is maintained, which is advantageous for the sustainable development of mushroom industry.

(3) Improve the inoculation conditions. The sterile room (cabinet) for the inoculation of sterilized substrate is replaced by a clean room, which can prevent the irritant odor of the disinfectants from injuring the human body.

(4) Save time. Unsterilized substrate bag cultivation saves time for sterilization and multiple handling

procedures.

(5) The change from bag cultivation to tray cultivation facilitates and simplifies spawn running, coloring, fruiting and management.

(6) There is no limit to production scales. The unsterilized substrate cultivation can be in bags or on ridged fields according to labor availability, busy or slack seasons and sizes of the incubation rooms. The production scale is unlimited, which can be small or large. Cultivation can be flexibly arranged according to local altitudes and climatic conditions in any season so long as the temperature is in the range optimal for the mycelium growth of the spawned mushroom strain.

(7) 95% of mushrooms can be fruited and harvested from the unsterilized substrate cultivation technique, which considerably increases the economic benefit.

Under the authorization of Zhejiang Provincial Department of Science and Technology, Hangzhou City Science and Technology Bureau hosted the appraisal meeting for the project of Research on the Application Technique of the Fermentation Accelerator on August 6, 2006. The project appraisal committee consisted of 8 experts from the Microbiology Research Institute of the Chinese Academy of Sciences, Jilin Agricultural University, Edible Mushroom Research Institute of Shanghai Academy of Agricultural Sciences, Zhejiang University and Zhejiang Provincial Academy of Agricultural Sciences etc. Wei Jiangchun, an academician, was appointed to host the appraisal as chairman of the committee.

The experts of the committee gave the following evaluations on the technique: compared with traditional sterilization method, the application technique of the fermentation accelerator had such characteristics as a low sterilization temperature, a short time period, low costs and high efficiency. Ideal results were obtained in applying it to sterilize the substrate for mushroom cultivation at a substrate temperate of 70～80℃ for 5～7 hours.

The formulation of Enterprise Standards of the Fermentation Accelerator provided a technical support for safe and standardized production of the new product. This research result was applied in Zhejiang, Anhui, Gansu and Fujian Provinces etc, with a total of 290 million bags cultivated and an accumulative of 116 million Yuan obtained from saving labor and costs and increasing efficiency. All these showed that it had prominent social and economic benefits. The experts approved of the product's passing the appraisal and suggested that the result be transformed and spread as soon as possible to create larger social and economic benefits.

Mushrooms can be cultivated on unsterilized substrate using the fermentation accelerator. Producing 10,000 bags of Shiitake or Black wood ear can save 720 kgs of coal and 2 labor forces and increase the output by over 10%, realizing substantial economic and ecological benefits. During the more than 1 year

period since the appraisal meeting, over 2 billion bags of the fermentation accelerator were spread to 26 provinces (municipalities, regions) across China, getting obvious benefits.

Table 1 A Comparison of Cost Calculation for the Recommended Formulas of Per Ton Substrate

Ingredients of the substrate		Sterilized substrate（CK）（100℃ for 12～16 hours）			The fermentation accelerator（70℃ for 7 hours or 80 ℃ for 5 hours）		
		Quantity (kg)	Unit price (Yuan/kg)	Amount (Yuan)	Quantity (kg)	Unit price (Yuan/kg)	Amount (Yuan)
Costs of raw materials							
	Hardwood sawdust	780	0.44	343	850	0.44	374
	Wheat bran	200	1.1	220	140	1.1	154
	Gypsum powder	10	0.8	8	10	0.4	8
	Brown sugar	10	3.6	36	…	…	…
	Fermentation accelerator	…	…	…	3.5～5	20	70～100
	Firewood for sterilization	300	0.4	120	100	0.4	40
	Laborer for sterilization	2	30 Yuan/ laborer	60	1	30 Yuan/ laborer	30
Total		…	…	787	…	…	676～706
Variable costs							
	Survival rate	80%	…	984	Survival rate	90%	751
		85%	…	926		95%	711
		90%	…	874		98%	690

Notes: (1) Formula of sterilized substrate: sawdust 78%, wheat bran 20%, gypsum powder 1%, brown sugar 1%; (2) Formula using the fermentation accelerator: sawdust 85%, wheat bran 14%, gypsum powder 1%, the fermentation accelerator 0.4%; (3) The expenditures for plastic bags, production and miscellaneous laborers are the same; (4) 1 ton of the above substrate can be filled in 1,000 15×55×0.0045～0.005 cm polyethylene bags for Shiitake production; (5) The figures in this table are for reference only, the specific figures are subject to the cultivation varieties and local prices.

References

[1]. Yang Xinmei, The Cultivation Science of Edible Mushrooms in China, published by China Agricultural Press, Beijing in 1988.

[2]. Han Shenghua, The Cultivation and Utilization of Edible Mushrooms, published by China Forestry Press, Beijing in 2006.

[3]. Han Shenghua etc., The Research and Development of the Fermentation Accelerator and the Research on its Application Techniques, in Edible Mushrooms in China, the 6th edition, 2002.

[4]. Wu Xiaoping etc., the Separation, Appraisal and Preliminary Exploration on White Neurospora spp. Contaminating Growing Bags of Edible Mushrooms, in Edible Mushrooms in China, the 3rd edition, 2008.