Mixed-culture Cultivation of *Tremella fuciformis* on Synthetic Logs

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INTRODUCTION

The white jelly mushroom, *Tremella fuciformis*, was discovered in China. It is an important and popular medicinal and gourmet mushroom in Southeast Asia where it is best known by its Chinese name, 金银耳 (Yin Er) or silver ear. Cultivation on natural logs began in China in 1894 during the Ching Dynasty. Successful cultivation on synthetic logs was first achieved in Gutein County, Fujian Province, China, in 1978. Cultivation in substrate bags has been used almost exclusively for the production of *T. fuciformis* in China since the 1980s. However, natural logs are still used in Taiwan. The discussion below is primarily based upon Chinese methodology.

Due to some unusual characteristics of *T. Fuciformis*, some background information is essential. Prior mushroom cultivation experience will also be helpful.

BACKGROUND INFORMATION

1. Carbohydrate metabolism : *T. Fuciformis* can not degrade and use either cellulose nor lignin efficiently, if at all.

2. Mixed culture: for growth on complex substrate containing cellulose and lignin it is necessary to use a mixed culture of *Tremella fuciformis*, a basidiomycete, and *Hypoxylon acheri*, an ascomycete, to insure that nutritional needs will be met.

3. Specificity of pairing *T. fuciformis* and *H. acheri*: The two fungi must be isolated, in the wild, from the same log in the same ecological niche. Strains of these two fungi of different origins should not be mixed.

4. Reproduction: The life cycle of *T. fuciformis* involves both basidia in sexual reproduction and yeast-like conidia in asexual reproduction.

5. Differential mycelial growth rate: mycelial growth of *T. fuciformis* is much slower than the mycelial growth of *H. acheri*.

6. Different growth parameters: *T. fuciformis* growth parameters are controlled to produce the desired phases (sexual vs asexual reproductive phases) during its life cycle .

7. Unstable mixed cultures or spawn: go back to mother culture as necessary.

SUBSTRATE FORMULATION

Supplemented sawdust-bran substrates are generally used. Other agricultural wastes may also be used (Table 1). Cotton-seed hull has been reported to give higher yields.

Table 1. Substrate Formulation

Supplemented Sawdust-bran Substrate A:	
sawdust, hardwood	70 kg
rice or wheat bran	19 kg
gypsum or lime	1 kg
sucrose	1 kg
water, approximately	140 kg
Supplemented Sawdust-bran Substrate B:	
sawdust, hardwood	77 kg
bran	18 kg
sucrose	1 kg
gypsum	1.5 kg
calcium superphosphate	1 kg
soybean powder	1.5 kg
water	140 kg
Supplemented Cotton-seed Hull and Bran Substrate:	
cotton-seed hull	100 kg
bran	25 kg
soybean powder	3 kg
sucrose	1.5 kg
calcium superphosphate	1.5 kg
water	120-140 kg

PRODUCTION OF MIXED MOTHER CULTURE (OR MOTHER CULTURE SPAWN):

Production of the mixed mother culture requires that *T. fuciformis* be started first, then *H. acheri* is added into the same test tube. *T. fuciformis* is not fastidious and can be cultured on most laboratory media. First, subculture *T. fuciformis* into the desired number of agar slants. Incubate at 25^{o}C. When the colony of *T. fuciformis* reaches 1 cm in diameter, inoculate a minute amount (a few hypha) of *H. acheri* into each of the tubes to create a mixed culture (*T. fuciformis*: *H. acher*i = 1000:1 approximately). When these two fungi have grown together, the mixed cultures are ready to be sold as mother-culture spawn or used to produce the primary spawn.

Alternatively, *H. acheri* can be started in the test tube first. After the *Hypoxylon* mycelia has become established, yeast-like conidia of *T. fuciformis*, in massive amounts, can then be smeared onto the substrate surface. A larger *Tremella* to *Hypoxylon* ratio is required in this approach. It can be tricky to use the yeast-like conidia of *T. fuciformis* for the production of mixed culture, since not all the conidia germinate.

Recently Paul Stamets (2000a, b) of Fungi Perfecti produced the mixed mother culture spawn of Yin Er (*T. fuciformis*) by using liquid cultures. 24 drops of *H. acheri* were added to 2 liters of *T. fuciformis* (liquid or submerged fermentation). The ratio of mixing is larger than 1:1,000,000,000.

PRODUCTION OF MIXED PRIMARY SPAWN

Use generous amount of mother-culture spawn to inoculate the substrate for primary-spawn, usually supplemented sawdust-bran substrate with 65% moisture content in bottles. Drier substrate favors the growth of *Tremella*. Use the entire mixed culture from a mother-culture agar slant in a tube for 1-4 bottles of primary spawn. Incubate at 25^{o}C until the formation of primordia. Primordia resemble 1/2 a grain of rice. Formation of primordia ensures that the primary spawn contains both *T. fuciformis* and *H. acheri* and is capable of producing *Tremella* fruiting bodies.

PRODUCTION OF SPAWN

Screen primary spawn carefully for the production of spawn, on the basis of :

-formation of vigorous primordia
-deep mycelial penetration of *T. fuciformis* into the substrate
-presence of robust feather-like mycelia of *H. acheri*

These are also the criteria to determine the number of subcultures of spawn bottles to be made from each chosen primary spawn bottle.

Remove and discard the primordia from the primary spawn, mix the mycelia thoroughly under aseptic conditions. Keep the moisture content of the spawn substrate at 65-70%. Wetter substrate facilitates the formation of yeast-like conidia which can be easily distributed in liquid droplets into the deeper layer of substrate. This offers better substrate utilization and can compensate for lack of depth of mycelial penetration by *Tremella*.

Incubate the spawn at 26^{O}C for 18-22 days. Readiness of the spawn is indicated by the appearance of white mycelial globules (balls). Two to three days after the formation of such globules, the spawn is ready for use. Use only the upper portion of the spawn since the lower part contains less mycelia. Mix mycelia thoroughly before spawning. Use 1 bottle of spawn for approximately 20 synthetic logs in bags, unless the spawn is of exceptional quality and well mixed. The spawn may be further incubated for 3-5 days after mixing to enhance success.

SPAWNING (INOCULATION)

Use only the top 3 cm of the mixed mycelial spawn. Before spawning, crumble and mix the culture thoroughly to insure a proper combination of *Tremella* and *Hypoxylon*. Narrow and long synthetic bags, 12 cm x 60 cm, are used in China. Four inoculating holes, 1.5 cm in diameter and 1.2-1.5 cm deep are made with a mechanical tool. Commonly available wide plastic adhesive tapes are used to seal the inoculation holes. With the availability of polypropylene and polyethylene bags with microfilter windows, these can be adopted for the cultivation of silver ears.

SPAWN RUN

Incubate at 25-28^{O}C for 3 to 5 days to facilitate the growth of *Hypoxylon* around inoculation sites and to prevent contamination. The Chinese continue incubation at 23-25^{O}C and increase aeration to bags after 3 to 5 days to favor the growth of *Tremella* mycelia. After 10-15 days of spawn run, the bags are moved to the fruiting room. Refer to Table 2 for the control of growth parameters.

MUSHROOM GROWTH (fruiting-body formation)

Formation of white mycelial globules and yellowish exudate at the inoculating sites signals the onset of primordia formation, in order words, the readiness of synthetic logs to fruit. Drain if excessive amount of exudate is produced. For the regulation of growth parameters following the formation of the white mycelial globules, see Table 2.

Table 2. Growth Parameters

	Spawn Run	Primordia Induction	Fruiting-Body Development
Temperature	25-28^{O}C, 3-5 days (to favor Hypoxylon) 23-25oC, 7-10 days (to favor *Tremella*)	18-23^{O}C	23-24^{O}C 20-27^{O}C Oei, 1996 (woodlogs)
Relative Humidity	tolerance of drought	85-95% R. H.	85-95% R. H. 95-100% R. H. (for medium to large fruitings)
Light	-	-	50-600 lux No light required according to Oei, 1996
Ventilation	-	increase	increase to 3-5 times daily (3-10 min each)
Duration	10-15 days	15-20 days (16 days) following the appearance of white mycelial globules	35-40 days after spawning

INDUCTION OF PRIMORDIA

(critical period during fruiting)

Mycelial metabolism reaches the peak during the period from the formation of the whitish mycelial globules to the development of primordia. A tremendous amount of heat is generated. Management of growth parameters should be closely monitored, particularly to maintain the proper temperature and gradual and careful increase of ventilation to prevent abortion of primordia.

Primordia are developed approximately 16 days (15-20 days) after the appearance of the white mycelial globules. Mature fruiting bodies ready for harvest are produced in 35-40 days after spawning. A second flush is possible.

CONCLUSION

Tremella fuciformis is one of the major medicinal mushrooms and a treasured mushroom delicacy in China. Cultivation of this mushroom is a challenge for the most enthusiastic and the advanced

growers. Adoption of the basic methodology, as developed in detail in China, should be a very worthy step forward. The practical knowledge of how to eat the mushroom will be a topic for future discussion.

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Figure 1: Stock cultures on agar slants. *Tremella fuciformis* (right) is whitish and *Hypoxylon archeri* (left) is greenish. Mixed culture cultivation of the two species is used for the production of *Tremella* fruiting bodies.

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Figure 2: Prolific production of whitish *Tremella fuciformis* fruiting bodies by mixed culture cultivation on synthetic logs (substrate blocks in bags)

Figure 3: Delicate fruiting bodies of *Tremella fuciformis*, whitish and gelatinous