SUBSTRATE AND METHOD FOR CULTURE OF FUNGI, INCLUDING SHIITAKE (LENTINUS EDODES)

An improved substrate and method for culturing fungi, including Shiitake. The substrate is essentially cellulose-free and comprises a major portion of grain and minor portions of nutritional supplements. The grain is partially sterilized by boiling in order to kill bacteria, cooled in order to induce germination of the heat resistant spores, and steam sterilized before the germinated spores have matured sufficiently to create new spores. The substrate is inoculated with fungi, which are then cultured.
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DESCRIPTION

SUBSTRATE AND METHOD FOR CULTURE OF FUNGI,
INCLUDING SHIITAKE (LENTINUS EDODES)

Technical Field.

This invention relates to the cultivation of mushrooms and other fungi, especially shiitake (Lentinus edodes).

Background Art.

Inventors have long sought a method for efficiently and quickly cultivating fungi, especially shiitake, because of its great demand and relatively limited supply.

Shiitake and other mushrooms are usually cultivated on logs or in cellulose based substrates. Among the methods using a cellulose based substrate are those described in U.S. Patent No. 4,127,965 to Mee and U.S. Patent No. 4,637,163 to Pellinen. Mee also teaches the use of a cellulose based substrate in a microorganism impermeable flexible container which is then sealed and sterilized. However, as taught by U.S. Patent No. 4,674,228 issued to Murata, removal of the mycelium from such containers often causes damage that reduces productivity. Other methods also have been tried. For example, U.S. Patent No. 4,735,014 to Weber teaches the use of hemp stalks and U.S. Patent No. 4,741,122 to Becsy teaches the use of agricultural wastes.

There are many drawbacks to the various methods for growing shiitake currently in use. Growing shiitake on logs in the traditional manner is slow and inefficient. Cultivation of shiitake in microorganism impermeable flexible containers (commonly known as "space bags") offers advantages over traditional methods, but still does not provide a satisfactory production rate.

Thus, it is an object of this invention to provide an improved method of cultivating fungi, especially shiitake.
It is a further object of this invention to provide an improved culture medium for the culture of fungi, including shiitake.

It is a further object of this invention to provide a more efficient and faster method of raising fungi, including shiitake.

Disclosure of Invention.

The invention is a new substrate for the growth of fungi, especially shiitake, created using a new method of sterilizing the substrate to allow cultivation of the desired fungi without contamination by competing organisms.

The new substrate is grain that is essentially cellulose free and that has been sterilized in accordance with the process described herein. As indicated above, the prior art in the growth of mushrooms and other fungi requires growth on logs, sawdust or other substrates containing a major portion of cellulose. However, cellulose is not necessary for the cultivation of shiitake. Shiitake mushrooms have the ability to break down cellulose for essential nutrients, but can be more efficiently grown in a substrate containing these materials in an already usable form. Similarly, shiitake can break down lignin, which is a constituent of wood, but again shiitake can be cultivated more efficiently by providing the breakdown products instead of the lignin.

Prior art references have taught the use of grain as a nutritional supplement in a cellulose based substrate. See for example, Han, et. al, Physiology and Ecology of Lentinus Edodes (Berk) sing., Mushroom Science XI, Proceedings of the Eleventh International Scientific Congress on the Cultivation of Edible Fungi (1981). However, the substrate of this invention is essentially free of cellulose and the grain itself is the substrate.
The grain substrate must be sterilized for the cultivation of fungi, including shiitake. Unsterilized grain contains various bacteria and microorganisms that compete with mushrooms and other fungi and therefore reduce production efficiency. Further, conventional heat sterilization techniques, such as steam sterilization, are insufficient to sterilize the grain against all competing microorganisms. Accordingly, conventionally sterilized grain is unsuitable as a substrate. In fact, one prior art reference states that, in view of the well-established use of tree logs and the amount of energy necessary to sterilize a substrate, "widespread large scale use of any sterilized substrate to produce shiitake mushroom appears unlikely." San Antonio, "Cultivation of the Shiitake Mushroom", Hortscience, Vol. 16(2), April 1981.

The main problem with conventional heat sterilization of grain substrates is that certain bacteria, primarily of the genus *Bacillus*, form heat resistant spores that will survive such sterilization even though the bacteria themselves are killed. Accordingly, even though a grain substrate may be conventionally heat sterilized, it will still contain spores of *Bacillus* bacteria which will contaminate the substrate and render it unsuitable for production of fungi, including shiitake. This invention solves the problem of bacterial contamination in the grain so that an appropriately sterile substrate is provided.

In the invention, the substrate is boiled to kill the bacteria that are present. The substrate is then cooled to induce any heat resistant spores to germinate. The substrate then is steam sterilized after such germination, but before the bacteria have matured sufficiently to form heat-resistant spores.
Of course, non-heating methods of sterilizing the grain substrate also can be used, such as irradiation. However, irradiation of the substrate would require greater governmental regulation and may affect marketability of the resulting mushrooms. Other non-heating methods of sterilization could include, for example, chemical sterilization (in which chemical agents in solid, liquid or gaseous form are used for sterilization) or pressure sterilization (in which the substrate is subjected to extremes of high or low pressure (including vacuum), or both).

Of course, freezing can be used with the invention as well.

The invention can be practiced with the listed sterilization methods and all other sterilization methods that kill bacteria or other spore-forming microorganisms, but that normally leave surviving spores. As long as the spores are allowed to germinate after an initial sterilization, a second sterilization that kills the bacteria or other microorganisms will completely sterilize the substrate, if the second sterilization takes place before the bacteria or other microorganisms have matured sufficiently to form new spores. Thus, the particular methods of initial and secondary sterilization are not critical, as long as the spores are allowed to germinate after initial sterilization and the substrate is secondarily sterilized before the spores mature sufficiently to form new spores.

The substrate of the invention thus provides a more efficient medium for cultivation of mushrooms, including shiitake, because the nutrients required by the mushrooms are furnished directly, rather than being furnished in the form of cellulose and lignin that must be enzymatically broken down by the mushrooms. The invention also provides a more efficient method of cultivating mushrooms because
competing microorganisms, including bacteria, are eliminated from the substrate.

An advantage of the invention is the shortening of incubation times for the shiitake. The invention shortens the incubation time for forming mycelium to 21 days, as opposed to log cultivation, which requires 8 months to 1 year for incubation, and sawdust based substrates, which require approximately 80 days for incubation.

A further advantage of the invention is the increase in yield per pound of substrate. One hundred pounds of the substrate of the invention yields approximately 300 pounds of shiitake within 5 months. By comparison, 100 pounds of logs yields approximately 10 to 15 pounds of shiitake over more than 3 years, and 100 pounds of sawdust based substrate yields approximately 80 pounds of shiitake over 8 months.

A further advantage of the invention is that no special spawn material is necessary. The same material used for fruiting can be used as a spawn material to start new production units, so that production can be increased immediately instead of waiting for new spawn to be grown. Similarly, no spawn is wasted if production is decreased.

A still further advantage of the invention is that production units may be kept in incubation beyond the 21 day period for up to 6 months if, for example, market conditions are unfavorable. This also allows stockpiling of colonized units for large seasonal production outputs.

In the practice of the invention, various nutritional supplements (including proteins, sugars, starches and vitamins) are boiled in water until they are dispersed throughout the mixture. The grain for the substrate is then added and boiled for approximately one hour in order to kill the bacteria present and cause the absorption of the dispersed
nutritional supplements into the grain. The grain is then allowed to cool to induce germination of any heat-resistant spores. While the grain is cooling, it is mixed with permeability enhancing powders to prevent caking and packed into microorganism impermeable sterilizable containers, such as polypropylene bags. The bags are then steam sterilized in accordance with conventional practice before the germinated bacteria have matured sufficiently to form spores.

After sterilization of the bags, colonization of the bags is accomplished by introducing either pure spawn of the desired fungi or by introducing previously colonized grain. The bags are then shaken to mix the spawn or previously colonized grain with the grain in order to decrease the incubation time. The bags are then incubated for approximately three weeks at approximately 80 degrees Fahrenheit. During this time, the spawn will digest most, if not all, of the substrate to form a mycelium.

The mycelium can then be induced to fruit by subjecting the bags to a cold shock of 40 to 65 degrees Fahrenheit for 5 to 15 days under cool white fluorescent lighting. After the cold shock, fruiting to maturation is accomplished by removing the mycelium from the containers and exposing them to an intermittent chilled water mist, or otherwise placing the mycelium in a high humidity environment.

Alternatively, fruiting can be induced using only a cold water spray under lighted conditions.

**Brief Description of Drawing.**

Figure 1 is a flow chart of a preferred method of preparing the substrate of the invention.

**Best Mode for Carrying Out Invention.**

Figure 1 of the drawings sets forth generally a preferred method of preparing the substrate of the invention.
The ingredients in the substrate are preferably chosen to provide optimum nutrition for the fungi to be grown without requiring additional artificial supplements. This use of all-natural materials therefore makes sale and marketing of the cultivated fungi easier because fewer regulatory requirements are imposed. The preferred ingredients, their ranges and the optimum amounts are set forth below for preparing batches of the substrate.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Range</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Sorghum grain</td>
<td>150-300 lbs</td>
<td>200</td>
</tr>
<tr>
<td>Whole Oat grain</td>
<td>0-50 lbs</td>
<td>35</td>
</tr>
<tr>
<td>Russet Potatoes</td>
<td>5-20 lbs</td>
<td>10</td>
</tr>
<tr>
<td>Rolled Barley grain</td>
<td>0.5-15 lbs</td>
<td>5</td>
</tr>
<tr>
<td>Maple pea sprouts</td>
<td>0-15 lbs</td>
<td>5</td>
</tr>
<tr>
<td>Brewer’s yeast powder</td>
<td>2-35 lbs</td>
<td>6</td>
</tr>
<tr>
<td>Hulled sunflower seed</td>
<td>0-10 lbs</td>
<td>2</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>0-2.5 lbs</td>
<td>.15</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>0-2.5 lbs</td>
<td>1.5</td>
</tr>
<tr>
<td>Whole Garlic</td>
<td>0.5-4 lbs</td>
<td>1.5</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>0-20 tablespoons</td>
<td>10</td>
</tr>
<tr>
<td>Wheat germ oil</td>
<td>0-20 tablespoons</td>
<td>10</td>
</tr>
<tr>
<td>Molasses</td>
<td>0-20 tablespoons</td>
<td>6</td>
</tr>
<tr>
<td>Water</td>
<td>20-35 gallons</td>
<td>25</td>
</tr>
<tr>
<td>Milk</td>
<td>0-1 gallon</td>
<td>.25</td>
</tr>
</tbody>
</table>

The preferred coating ingredients, the ranges and the optimum for every two batches of the above substrate are set forth below:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Range</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limestone powder</td>
<td>25-75 lbs</td>
<td>50</td>
</tr>
<tr>
<td>Gypsum powder</td>
<td>100-200 lbs</td>
<td>160</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>0-60 lbs</td>
<td>40</td>
</tr>
</tbody>
</table>

The maple pea sprouts are preferably grown for 6 to 12 days under a mist system. Commercial bean sprouts may also be used, but more roots and larger cotyledons are available with maple pea sprouts.
1  Sorghum provides vitamins, carbohydrates, starches, protein and minerals such as Copper, Iron, Manganese, Zinc and Selenium. Oats provide vitamins, minerals, carbohydrates, starches, proteins and salicylic acid. Salicylic acid promotes shiitake fruiting. Rolled barley grain provides vitamins and carbohydrates and absorbs excess water. Soybean meal provides a source of minerals, proteins and vitamins. Brewer’s yeast powder provides high amounts of vitamins, especially B vitamins that promote mycelial growth. Sunflower seed and sunflower oil provide vitamins, minerals, proteins and saturated and unsaturated oils. The sunflower seed and oil also promote heavier secondary mycelial growth.

5  The pea sprouts promote a heavier amount of fruitings to occur. This allows some control over the size of the mushrooms. More sprouts allow for more mushrooms to form but the mushrooms are smaller in size. Fewer sprouts allow for fewer mushrooms to form but the mushrooms are larger in size. With no sprouts added, mushrooms with individual weights of from 3/4 lb to 1-1/2 lbs may form on the substrate.

10 Garlic provides natural antibacterial action in order to resist bacterial growth after boiling and sterilization of the substrate. Molasses provides sugars and wheat germ oil provides saturated and unsaturated oils as well as vitamin D. Corn gluten meal provides vitamins, minerals, protein and selenium. Potatoes provide starch. Milk provides cassein and cheese can be substituted instead of milk.

15 The coating ingredients serve additional functions besides increasing permeability of the substrate. Limestone powder adjusts the pH of the substrate to neutral (approximately 7 to 8). The gypsum powder also provides long term pH maintenance and makes the grain substrate loose and powdery. The cottonseed meal provides protein and oil.
It should be noted that the prior art teaches that, under certain conditions, calcium inhibits fruiting of mycelium. However, the substrate of this invention contains substantial amounts of calcium from the limestone and gypsum powder.

The size and number of mushrooms can be controlled prior to colonization by the amount of substrate that is packed in the bags, with larger bags that contain more substrate producing larger and more mushrooms. For example, eight pound bags will produce 3/4 pound mushrooms for approximately 6 months.

Mushroom size and number also can be controlled after colonization by allowing individual colonized units to come into contact with each other. The individual units will form one large continuous unit forming larger and more numerous mushrooms than an individual unit.

Fully colonized units can be placed on shelving or strung on rods to maximize production per unit area.

The following example illustrates the use of this invention using the optimum amounts set forth above.

EXAMPLE

The water is boiled in a 60 gallon capacity steam kettle with a bottom spigot. The potatoes are sliced and then added to the boiling water together with the milk, garlic, corn gluten meal, wheat germ oil, sunflower oil, molasses, hulled sunflower seed, brewer's yeast powder and soybean meal. The mixture is then boiled until all components break into small pieces. The mixture is preferably mixed with a portable paint mixer to help break clumps into small pieces. Maple pea sprouts are then added to the boiling mixture, which is stirred with a large paddle until the sprouts are soft. The oat grain, barley grain and sorghum grain are then added, together with sufficient water only to cover the grain. The
mixture is then boiled and stirred until the water level falls below the grain level by 3 to 4 inches and the heat source is then turned off. After approximately one hour, any remaining liquid is drawn off from the bottom of the pot. At this point, the grain should be half-cooked and semi-hard. The grain is then allowed to cool for approximately 24 hours, at which time it is removed from the pot.

Two batches of grain are then placed in a large flat bin and the limestone powder, gypsum powder and cottonseed meal are mixed with the grain until all the grain is coated with powder. The grain should appear coated and should not stick in clumps. Two batches will yield approximately 1,200 pounds of prepared substrate.

The prepared substrate is then packed into double polypropylene plastic bags (1.5 mil.). Each of these double bag units has a polypropylene collar, a cotton plug and an aluminium foil cover over the plug. The bags from 4 batches of the grain (approximately 2,400 pounds) are then loaded in a steam retort (5 foot diameter, 13 feet long) and steam-sterilized at 250°F, 15 pounds per square inches steam pressure for 7 hours. Each load is then cooled for 24 hours before seeding.

After the bags of substrate have been sterilized, they are preferably seeded under sterile conditions in laminar airflow hoods. Seeding is accomplished by introducing pure spawn or, preferably, colonized grain from previous production runs. Approximately 5 to 10 tablespoons of colonized grain is added into each 2-pound bag. Each of the bags is then shaken to mix the colonized grain throughout the new unit. This thorough mixing of the previously colonized grain with the substrate reduces the normal incubation time considerably. Thus, a 2-pound bag will usually be fully colonized after approximately 3 weeks of incubation at 80°F. Usually 15 new 2-pound
units may be started from each colonized 2-pound unit. The preferred size of bag is 8 pounds because of the disproportionately greater number of buds per 8 pound bag when compared with 2 pound and 4 pound bags.

After approximately 3 weeks, the grain substrate will be mostly or completely digested, leaving only the mycelium in the bag. The bag can be retained in the mycelial stage for approximately 3 to 4 months for shipment or storage. When mushroom production is desired, the bags containing the mycelium are subjected to a cold shock by chilling them at 40 to 65° F for 5 to 15 days under cool white fluorescent lighting of 25 to 100 lux. The preferred cold shock is at a temperature of 45° F for 7 to 9 days, although a cold water bath for 24 to 48 hours also may be used.

The bags can be shipped in a refrigerated container during this cold shock stage.

As an alternative to the cold shock method of inducing fruiting, the mycelium may be removed from the bags and exposed to an intermittent cold water mist. It is preferred that the misting take place during daylight hours and also during a 2 hour period during the night. The water used for misting is chilled to 50 to 75° F and misting occurs for 2 to 120 seconds at 2 to 10 minute intervals for 6 to 15 hours during the daylight period. Approximately 10 to 20 days after the mycelium is exposed to mist, shiitake mushrooms may be harvested. Subsequent crops from the bags may occur 20 to 30 days apart. The relative humidity in the misting environment must be at least 80%.

As an alternative to the intermittent chilled water mist, the mycelium may be removed from the bags and allowed to fruit using previously known methods.

After the substrate has been spent, it may be
used for other purposes, such as compost, animal feed, mushroom compost for other mushrooms or insect feed.

After formation of the mycelium, but before fruiting, the mycelium also may be used as animal feed or for human food. Useful biochemicals also may be extracted from the mycelium.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the invention, as described in the claims. For example, and not by way of limitation, the substrate described herein is suitable for growing many species of mushrooms, including those listed in Mushroom List 1, which is a part of this description and incorporated herein by reference, and many genera of fungi, including those listed in Fungal List 2, which is a part of this description and incorporated herein by reference. Many of these fungi are useful for their biochemical or other properties. Thus, the substrate can be used for growing penicillin mold, weed molds, yeasts and medicinal mushrooms.

Accordingly, no limitation is to be inferred except as set forth in the claims.

MUSHROOM LIST 1

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus arvensis</td>
<td>Horse Mushroom</td>
</tr>
<tr>
<td>Agaricus augustus</td>
<td>The Prince</td>
</tr>
<tr>
<td>Agaricus bernardii</td>
<td></td>
</tr>
<tr>
<td>Agaricus bisporus</td>
<td></td>
</tr>
<tr>
<td>Agaricus bitorquis</td>
<td></td>
</tr>
<tr>
<td>Agaricus campestris</td>
<td></td>
</tr>
<tr>
<td>Agaricus excellans</td>
<td>Common Field Mushroom</td>
</tr>
<tr>
<td>Agaricus langei</td>
<td></td>
</tr>
</tbody>
</table>
1  Agaricus macrosporus
    Agaricus silvaticus
    Agaricus silvicola
    Agaricus vaporarius

5  Agrocybe aegerita
   Armillaria Caligata
   Armillaria ponderosa
   Armillariella mellea
   Armillariella tabescens

10  Auricularia polytricha
    Auricularia auricula
    Calvatia craniiformis
    Calvatia gigantea
    Clitocybe geotropa

15  Coorinus comatus
    Dictyophora duplicata
    Flammulina velutipes
    Galerina mutabilis
    Ganoderma lucidum

20  Grifola frondosa
    Grifola umbellata
    Hericium coralloides
    Hericium erinaceus
    Laetiporus sulphureus

25  Lentinus edodes
    Lepiota naucina
    Lepiota procera
    Lepiota rachodes
    Leoiota nuda

30  Leucopaxillus giganteus
    Lycoperdon gemmatus
    Lycoperdon pyriforme
    Lyophyllum cecastes
    Lyophyllum ulmarium

35  Macrolepiota procera
    Marasmius oreades
    Morchella angusticeos
    Morchella deliciosa

   Wood Mushroom
   Brown Swordbelt
   Wood Ear
   Wood Ear
   Skull-shaped Puffball
   Giant Puffball
   Shaggy Inky Cap
   Netted Stinkhorn
   Enoki
   Reishi
   Hen of the Woods
   Zhu Ling
   Pom Pom
   Sulfur Polypore
   Shiitake
   Smooth Lepiota
   Parasol Mushroom
   Scaly Lepiota
   Wood Blewit
   Gem-Studded Puffball
   Pear-Shaped Puffball
   Honshimeji
   Parasol
   Fairy Ring
   Black Morel
| 1  | Morchella esculenta | 14  | White Morel          |
|    | Morchella conica    |     | Conical Morel        |
|    | Morchella crassipes |     | Thick-Footed Morel   |
|    | Morchella elata     |     |                      |
| 5  | Morchella semilibera|     | Common Morel         |
|    | Morchella vulgaris  |     |                      |
|    | Panellus serotinus  |     |                      |
|    | Panus sp.           |     |                      |
|    | Pholiota adiposa    |     |                      |
| 10 | Pholiota nameko     | 15  | Fat Pholiota         |
|    | Pleurotus columbinus|     | Nameko               |
|    | Pleurotus cornucopiae|   | Blue Oyster          |
|    | Pleurotus cystidiosus| | Canary               |
|    | Pleurotus eryngii   |     | Abalone              |
|    | Pleurotus flabellatus| 20 | Pink Oyster          |
|    | Pleurotus florida   |     | Florida Oyster       |
|    | Pleurotus ostreatus |     | Oyster               |
|    | Pleurotus pulmonarius| |                     |
|    | Pleurotus sajor-caju|     | Phoenix              |
|    | Pleurotus salmoned stramineus| |                     |
|    | Sparassis crispa    | 25  | Cauliflower          |
|    | Stropharia rugosoannulata | | Wine Red Stropharia  |
|    | Tremella fusciformis|     | White Jelly          |
|    | Tricholomopsis rutilans |     |                      |
|    | Volvariella bakeril |     |                      |
|    | Volvariella bombycina|    |                      |
|    | Volvariella volvacea |     |                      |

**Fungal List 2**

List of Fungal Genera That May be Grown on the Substrate

| 35 | Abortiporus   |    | Amylostereum        |
|    | Absidia      | 35  | Anomoporia          |
|    | Achlyya      |     | Antrodia            |
|    | Acremonium   |     | Apiotrichum         |
|    | Acrophialophora | | Arachnomyces        |
|    | Acrospeira   |     | Armillariella       |
1 Actinomucor
Agaricus
Agrocybe
Aleurodiscus

5 Allescheria
Alternaria
Alysidiium
Amanita
Amauroascus

10 Amylomyces
Backusella
Beauveria
Bispora
Bjerkandera

15 Blakeslea
Blastomyces
Boletopsis
Cadophora
Calbovista

20 Calcarisporium
Caldariomyces
Calocera
Calocybe
Calonectria

25 Calvatia
Camarops
Candida
Cantharellus
Celphalosporium

30 Cephalophora
Cephaloascus
Ceratocystis
Cercospora
Cerinomyces

35 Ceriosporopsis
Cerrena
Chaetomella
Chaetomium
Arthrinium
Arthrobothrys
Arthrographis
Ascotricha
Ashbya
Aspergillus
Athelia
Aureobasidium
Auricularia
Boletus
Bondarzewia
Botryodiplodia
Botryotrichum
Botrytis
Bovista
Byssochlamys
Coccospora
Cochliobolus
Colletotrichum
Collybia
Columnocystis
Conidiobolus
Coniella
Coniophora
Coniothyrium
Conoplea
Coprinus
Cordyceps
Coridus
Coriolus
Corticium
Cortinarius
Coryne
Corynesperma
Coryneum
Craterellus
Craterellus
1 Chalara
   Chalaropsis
   Choanephora
   Chondrostereum
  5 Chroogomphus
   Chrysosporium
   Circinella
   Cladosporium
   Clavariadelphus
  10 Claviceps
   Clavicorona
   Clavispora
   Clavulina
   Clitocybe
  15 Clitopilus
   Dacrymyces
   Dacryopinax
   Dactylium
   Daealea
  20 Debaryomyces
   Dekkera
   Dendryphion
   Dentinum
   Dermaloma
  25 Dichomitus
   Echinodontium
   Elsinoe
   Emericella
   Emericellopsis
  30 Entoloma
   Favolus
   Femsjonia
   Filobasidium
   Fistulina
  35 Flammula
   Ganoderma
   Geotrichum
   Gerlachia
   Crebrothecium
   Cryphonectria
   Cryptococcus
   Cryptopus
   Cryptosporiopsis
   Cunninghamella
   Curvularia
   Cushingophora
   Cyathus
   Cylindrocarpon
   Cylindrocephalum
   Cylindrocladium
   Cystostereum
   Cytospora
   Cytospora
   Dictyostelium
   Diheterospora
   Diplocarpon
   Diplodia
   Discina
   Discula
   Ditiola
   Doratomyces
   Dothistroma
   Drechslera
   Epicoccum
   Eupenicillium
   Eutypa
   Exophiala
   Flammulina
   Fomes
   Fomitopsis
   Fusarium
   Fuscoboletinus
   Gnomonia
   Gomphidius
   Gomphus
1 Gibberella
Gilmanniella
Gliocladium
Glomastrix
Gloeophyllum
Gloeoporus
Gloeosporium
Glomerella
Hanseniaaspera
10 Hansenula
Haploporous
Helicostylum
Helmintosporium
Helvella
Hendersonula
Hericium
Heterobasidion
Hirschioporus
Horomodendrum
20 Incrustoporia
Inocybe
Inonotus
Kloeckera
Laccaria
Lactarius
Laetisaria
Laurilia
Leccinum
Lentinellus
30 Lentinula
Lentinus
Lentodium
Macrophomina
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**Industrial Applicability.**

The invention can be used for the inexpensive and efficient cultivation of fungi, especially shiitake. Other fungi also may be cultivated, including fungi useful for food or medicinal purposes.
What is claimed is:

1. A method for killing *Bacillus* bacteria spores in a culture medium, comprising:
   heating said culture medium for approximately 1 hour to kill *Bacillus* bacteria cells;
   cooling said culture medium for approximately 8 to 24 hours to induce germination of spores; and
   killing said germinated spores by sterilizing said culture medium.

2. A method for preparing a substrate for culture of fungi, comprising:
   preparing a grain mixture by mixing water in approximately one to one-fourth parts by weight per part of a dry mixture containing a major portion of grain and minor portions of starch, protein and nutrient sources;
   boiling said grain mixture for a sufficient time to allow dispersal of said starch, protein and nutrient sources into said grain mixture;
   cooling said grain mixture for a sufficient time to allow spores of any heat resistant bacteria to germinate; and
   sterilizing said grain mixture before said germinated spores mature sufficiently to produce more spores.

3. A method as described in claim 2, further comprising:
   draining said grain and water after said boiling step.

4. A method as described in claim 3, further comprising:
   mixing a permeability improving additive into said grain mixture.

5. A method as described in claim 4, wherein said starch, protein and nutrient supplements are preselected to meet the nutritional requirements of said fungi.
6. A method for preparing a substrate for culturing of fungi, comprising:
  boiling approximately 25 gallons of water in a 60 gallon capacity steam kettle;
  adding approximately 10 pounds of sliced Russet potatoes, 6 pounds of brewer’s yeast powder, one quart of milk, 2 pounds of hulled sunflower seed, 1.5 pounds of soybean meal, 1.5 pounds of whole garlic, 1.5 pounds of corn gluten meal, 10 tablespoons of wheat germ oil, 10 tablespoons of sunflower oil, 6 tablespoons of molasses to form an intermediate mixture;
  boiling said intermediate mixture using a heat source;
  mixing said intermediate mixture to break clumps into small pieces;
  adding approximately 5 pounds of maple pea sprouts;
  stirring said intermediate mixture until said sprouts are soft;
  adding approximately 200 pounds of whole sorghum grain, 35 pounds of whole oat grain and 5 pounds of rolled barley grain to form a grain mixture;
  adding a sufficient amount of water to immerse said grain mixture;
  boiling and stirring said grain mixture only until the water level falls below said grain mixture level by approximately 3 to 4 inches;
  removing said heat source;
  approximately one hour after removal of said heat source, draining said grain mixture;
  approximately 8 to 24 hours after said draining step, mixing said grain mixture with approximately 25 pounds of limestone powder, 80 pounds of gypsum powder and 20 pounds of cottonseed meal until said grain mixture is completely coated;
introducing measured portions of said grain mixture into sterilizable microorganism impermeable containers; and
steam sterilizing said sterilizable microorganism impermeable containers.

7. A substrate for culturing of fungi prepared according to the method of any one of the preceding claims.

8. A method for culturing tree mushrooms, comprising:
preparing a grain mixture by mixing water in approximately one to one-fourth parts by weight per part of a dry mixture containing a major portion of grain and minor portions of starch, protein and nutrient sources;
boiling said grain mixture for approximately 1 hour;
cooling said grain mixture for approximately 8 to 24 hours;
introducing said grain mixture into a microorganism impermeable sterilizable container;
sterilizing said container and said grain mixture;
introducing tree mushroom spawn into said grain mixture;
shaking said container to mix said tree mushroom spawn throughout said grain mixture;
incubating said tree mushroom spawn in said container for approximately 21 days to allow said tree mushroom spawn to consume said grain mixture and to form mycelium;
chilling said container and said mycelium for 7 to 9 days at a temperature of approximately 45 degrees fahrenheit;
removing said mycelium from said containers; and
misting said mycelium with chilled water until tree mushrooms of the desired size are grown.
9. A substrate for culture of fungi, comprising:
   approximately 50% sorghum grain;
   approximately 26.6% water;
   approximately 1% yeast powder;
   approximately 1.67% potatoes;
   approximately 0.3% garlic;
   approximately 0.3% barley grain;
   approximately 4.2% limestone powder; and
   approximately 16% gypsum powder;
wherein said sorghum grain, water, yeast powder, potatoes, garlic and barley grain is prepared by:
   boiling for a sufficient time to kill any microorganisms to form an intermediate mixture;
   cooling said intermediate mixture for a sufficient time to allow any spores to germinate; and
   heat sterilizing said intermediate mixture before said germinated spores have matured sufficiently to form new spores.

10. A substrate according to claim 9, wherein said limestone powder and said gypsum powder is mixed into said intermediate mixture after said cooling step.

11. A method for sterilizing a culture medium containing sporulation capable microorganisms, comprising:
   first, sterilizing said culture medium to kill said microorganisms;
   second, inducing germination of any spores in said sterilized culture medium to form germinated spores; and
   third, sterilizing said culture medium to kill said germinated spores before said germinated spores have matured sufficiently to be capable of forming further spores.

12. A method according to claim 11, wherein said first sterilizing step is performed by boiling said culture medium in water for approximately one hour,
wherein said inducing step is performed by cooling said culture medium for a period from 8 to 24 hours, and wherein said third sterilizing step is performed by steam sterilizing said culture medium to approximately 250° Fahrenheit for approximately seven hours.

13. method for culturing tree mushrooms, comprising:

preparing a grain mixture by mixing water in approximately one to one-fourth parts by weight per part of a dry mixture containing a major portion of grain and minor portions of starch, protein and nutrient sources;

boiling said grain mixture for approximately 1 hour;

cooling said grain mixture for approximately 8 to 24 hours;

introducing said grain mixture into a microorganism impermeable sterilizable container;

sterilizing said container and said grain mixture;

introducing tree mushroom spawn into said grain mixture;

shaking said container to mix said tree mushroom spawn throughout said grain mixture;

incubating said tree mushroom spawn in said container for approximately 21 days to allow said tree mushroom spawn to consume said grain mixture and to form mycelium; and

inducing said mycelium to fruit.

14. A method for culturing tree mushrooms, according to claim 13, further comprising:

mixing a permeability improving additive into said grain mixture during said cooling step.

15. A method for culturing tree mushrooms, according to claim 13, wherein:

said grain comprises:

sorghum grain.
16. A method for culturing tree mushrooms, according to claim 13, wherein:
said protein comprises maple pea sprouts.

17. A method for culturing tree mushrooms, according to claim 13, wherein:
said sterilizing step is accomplished by steam sterilizing said container and said grain mixture at a temperature of approximately 250°F and a pressure of approximately 15 pounds per square inch, for approximately 7 hours.

18. A method for culturing tree mushrooms, according to claim 13, wherein said tree mushroom spawn comprises:
grain that has been previously colonized with tree mushroom spawn.

19. A method for culturing tree mushrooms, according to claim 13, wherein:
said inducing step is accomplished by removing said mycelium from said containers and exposing said mycelium to an intermittent cold water mist.

20. A method for culturing tree mushrooms, according to claim 13, wherein said intermittent cold water mist is accomplished by using water chilled to 50 to 75°F for 2 to 120 seconds at 2 to 10 minute intervals for 6 to 15 hours during daylight hours and for 2 hours at night.

21. A method for culturing tree mushrooms according to claim 13, wherein:
said starch, protein and nutrient sources are preselected to meet the nutritional requirements of said mushrooms.

22. A method for culturing tree mushrooms according to claim 13, further comprising adding whole garlic to said grain mixture before said boiling step.

23. A method for culturing tree mushrooms according to claim 13, wherein:
said tree mushroom spawn is shiitake mushroom spawn.

24. A method for culturing tree mushrooms according to claim 13, wherein:

said tree mushroom spawn is oyster mushroom spawn.

25. A method for culturing tree mushrooms according to claim 13, wherein:

said tree mushroom spawn is morel mushroom spawn.

26. A method for culturing mushrooms, comprising:

boiling between 25 and 35 gallons of water;

adding between 5 and 20 pounds of Russet potatoes, between 2 and 35 pounds of brewer’s yeast powder and between 1/2 and 4 pounds of garlic to form an intermediate mixture;

boiling said intermediate mixture using a heat source;

mixing said intermediate mixture to break clumps into small pieces;

adding between 150 and 300 pounds of whole sorghum grain and between 1/2 and 15 pounds of rolled barley grain to form a grain mixture;

adding a sufficient amount of water to immerse said grain mixture;

boiling and stirring said grain mixture only until the water level falls below said grain mixture level;

removing said heat source;

approximately one hour after removal of said heat source, draining said grain mixture;

approximately 8 to 24 hours after said draining step, mixing said grain mixture with between 12 and 37 pounds of limestone powder and 50 to 100 pounds of gypsum powder until said grain mixture is completely coated;
introducing measured portions of said grain mixture into sterilizable microorganism impermeable containers;
sterilizing said sterilizable microorganism impermeable containers and said grain mixture;
introducing mushroom spawn into said grain mixture;
mixing said mushroom spawn throughout said grain mixture;
incubating said mushroom spawn in said container for approximately 21 days to allow said mushroom spawn to consume said grain mixture and to form mycelium; and
inducing said mycelium to fruit.

27. A method for culturing mushrooms, according to claim 26, wherein said mushroom spawn is shiitake mushroom spawn.

28. A method for culturing mushrooms, according to claim 26, wherein said mushroom spawn is oyster mushroom spawn.

29. A method for culturing mushrooms, according to claim 26, wherein said mushroom spawn is button mushroom spawn.

30. A method for culturing mushrooms, according to claim 26, wherein said mushroom spawn is paddy straw mushroom spawn.

31. A method for culturing mushrooms, according to claim 26, wherein said mushroom spawn is enoki mushroom spawn.

32. A mushroom cultured according to the method of any one of claims 8 or 13 to 31.

33. A method of sterilizing a material containing spore forming microorganisms, comprising:
initially sterilizing said material, whereby said microorganisms form surviving spores and said microorganisms are killed;
allowing said spores to germinate; and
secondarily sterilizing said material before said germinated spores have matured sufficiently to become capable of forming new spores, whereby said germinated spores are killed.

34. A method according to claim 33, wherein said microorganisms comprise bacteria.

35. A method according to claim 34, wherein said material comprises a grain substrate.

36. A method according to claim 33, wherein said initially sterilizing step is selected from the group consisting of heating, cooling, steam sterilizing, chemical sterilizing, pressure sterilizing and irradiating.

37. A method according to claim 33, wherein said secondarily sterilizing step is selected from the group consisting of heating, cooling, steam sterilizing, chemical sterilizing, pressure sterilizing and irradiating.
**INTERNATIONAL SEARCH REPORT**

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all) 

According to International Patent Classification (IPC) or to both National Classification and IPC  

**IPC(5):** A01G 1/04; A61L 2/06  
**U.S.CL.:** 47/1,1; 71/5; 422/1,21,26,28; 426/511,521; 435/254

**II. FIELDS SEARCHED**

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Documentation Search other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched.

**III. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>US, A, 4,127,965 (MEE) 05 December 1978, see the entire document.</td>
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<td>US, A, 2,520,318 (LESCARBOURA) 29 August 1950, see the entire document.</td>
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<td>Y</td>
<td>US, A, 4,542,608 (TAN) 24 September 1985, see the entire document.</td>
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<td>Y</td>
<td>US, A, 4,741,122 (BECSY, et al.) 03 May 1988, see the entire document.</td>
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* Special categories of cited documents:  
**"A"** document defining the general state of the art which is not considered to be of particular relevance  
**"E"** earlier document but published on or after the international filing date  
**"L"** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
**"O"** document referring to an oral disclosure, use, exhibition or other means  
**"P"** document published prior to the international filing date but later than the priority date claimed  
**"T"** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
**"X"** document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step  
**"Y"** document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art  
**"Z"** document member of the same patent family

**IV. CERTIFICATION**

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<td>Jill Johnston</td>
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

| Y.P | US, A, 4,874,419 (WI) 17 October 1989, see the entire document. | 2-7,9,10 |
| Y.P | US, A, 4,915,606 (SHIMOKAWA) 10 April 1990, see entire document. | 1,11,12, 33-37 |

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers , because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 8.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1, 11,12 & 33-37, drawn to sterilization methods; Class 422, subclass 11.

II. Claims 2-10 & 13-32, drawn to mushroom culturing; Class 47, subclass 21.1.

☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

☐ TELEPHONE PRACTICE, CHECK SUBMITTED.

☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest
☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.