**Methods & Materials**

**Fungal Strains**

A total of 10 species were collected for this study. Seven species were collected from wild specimens in and around Pullman, WA; and three species were ordered from the commercial provider Mushroom Mountain in SC.

All fungal strains were grown in laboratory conditions on PDA media. Before beginning the experiment, grain spawn was used to propagate each species.

|  |  |  |
| --- | --- | --- |
| **Table 1: Fungi Species Used** | |  |
| **Species:** | **Source:** | **Cultivation Technique:** |
| *Agaricus augustus* | Wild | Tray |
| *Agaricus avrensis* | Wild | Tray |
| *Hericium americanum* | Wild | Bag |
| *Pleurotus* | Wild | Bag |
| *Pisolithus* | Wild | TBD |
| *Coprinus comatus* | Wild | Tray |
| *Ganoderma applanatum* | Wild | Bag |
| SRUG1 *- Stropharia rugoso-annulata* | Mushroom Mountain. | TBD |
| LEDO2 *- Lentinula edodes - Cold Outdoor* | Mushroom Mountain. | Bag |
| AAUR1 *- Auricularia auricula - Wood Ear - Wild type, SC* | Mushroom Mountain. | Bag |

**Selection of Substrates:**

Four types of raw materials were used as substrates: Wheat straw, sawdust, spent Brewer’s grain, and coffee grounds. These were combined in different concentrations to create X different substrates.

Optimal substrate mixtures for most mushroom species consists of approximately 80% lignin/carbon source, 20% nitrogen-rich source, and other micronutrients**. (Sokól et al., 2015),(Stamets, 2000) (Sánchez, 2004), (Jang, Lee, Liu, & Ju. 2009).**

**Create a table showing concentrations of substrates x mixtures.**

**Treatment/Preparation of Substrate**

For each replicate, 3kg of substate was added to an autoclavable mushroom cultivation bag and autoclaved at 121°C for 15 minutes. The bags were left to fully cool to room temperature (25°C). Once cooled, X g of spawn was added to each bag. Each replicate bag was then sealed and mixed evenly. The replicates were then left to spawn for 1-2 weeks.

Bag or tray cultures were used depending on the species.

**Inspired by the procedure in (Jasińska et al. 2014)**

**Bags:**

Seven L capacty, 50 micron polypropylene bags with linear ventilation filters were used.

**Trays:**

**Inoculation and Growing procedures**

Once full colonization was achieved, bags were opened to allow for fruiting.

* Conditions in which growth occurred

Growth conditions were held within recommendations for each species.

Describe the growing conditions: temperature, air moisture, light, CO2, etc.

**Measurement:**

* Sporocarp mass.
* Nutrient analysis (?)

At the end of growth, the sporocarp mass per initial substrate mass was measured. Sporocarps were collected, dried, and weighed.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 2: Substrate Mixtures** | | | | |
| Mixture | **Material Concentrations (%)** | | | |
|  | **Sawdust** | **Straw** | **Brewer's Grain** | **Coffee Grounds** |
| **M1** | 80 |  | 20 | 0 |
| **M2** | 75 |  | 20 | 5 |
| **M3** | 70 |  | 20 | 10 |
| **M4** | 65 |  | 20 | 15 |
| **M5** | 60 |  | 20 | 20 |
| **M6** |  | 80 | 20 | 0 |
| **M7** |  | 75 | 20 | 5 |
| **M8** |  | 70 | 20 | 10 |
| **M9** |  | 65 | 20 | 15 |
| **M10** |  | 60 | 20 | 20 |
| **M11** | 65 |  | 25 | 10 |
| **M12** | 60 |  | 30 | 10 |
| **M13** | 55 |  | 35 | 10 |
| **M14** | 50 |  | 40 | 10 |
| **M15** |  | 65 | 25 | 10 |
| **M16** |  | 60 | 30 | 10 |
| **M17** |  | 55 | 35 | 10 |
| **M18** |  | 50 | 40 | 10 |

**Sources:**

**Jang, M. J., Lee, Y. H., Liu, J. J., & Ju, Y. C. (2009). Optimal conditions for the mycelial growth of Coprinus comatus strains. *Mycobiology*, *37*(2), 103-108.**

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**Lemke, G. (1971). Mycelen zucht und Fruchtkö rper produktion des Kulturchampignons Agaricus bisporus (Lange). Sing. Gartenbauwissenschaft 36, 19–27**

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**Jo WS, Cho YJ, Cho DH, Park SD, Yoo YB, Seok SJ. Culture Conditions for the Mycelial Growth of Ganoderma applanatum. Mycobiology. 2009 Jun;37(2):94-102. doi: 10.4489/MYCO.2009.37.2.094. Epub 2009 Jun 30. PMID: 23983516; PMCID: PMC3749412.** [**https://pubmed.ncbi.nlm.nih.gov/23983516/**](https://pubmed.ncbi.nlm.nih.gov/23983516/)

**Vetayasuporn, S. (2006). Oyster mushroom cultivation on different cellulosic substrates. *Res J Agric Biol Sci*, *2*(6), 548-551. From: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1056.3983&rep=rep1&type=pdf**

**Mushroom Strains (Included in lit review)**

* ***Hericium / lion’s mane***

Traditional cultivation of *Hericium erinacium* uses the log method, where fresh logs are inoculated and then left to fruit under natural conditions. While this technique is not resource-optimal, it is easy to replicate. (Given the climate of Pullman, not recommended). Intensive cultivation uses the bottle or bag method, with sawdust and wheat bran. Sokol et al. article suggests several combinations based on approximately 75% organic carbon, 20% wheat bran, 3% corn meal, and 1% each of gypsum and sugar; which yield high volume and quality fruiting bodies. Many other substrates from agricultural and food waste were beneficial to growth. Sawdust from hardwoods is preferred, although pine colonized by other species first may also be used. **(Sokol et al., 2015)**

* ***Pleurotus / oyster***

Numerous sources emphasize that this is not a picky mushroom. Stamets, Cotter…

* ***Auricularia* /wood ear**
* ***Gandoerma / reishi***

Grows well on PDA, YMA, and MCM.

Woo-Sik et al. found that the optimal conditions for growing *Ganoderma applanatum* consisted of 25~30 ºC and a C/N ratio (using glucose and NaNO3) between 10:1 and 5:1 (consistent with other species). Beneficial supplements included, “thiamine-HCl and biotin as vitamins, succinic acid and lactic acid as organic acids, and MgSO4·7H2O, KH2PO4 and NaCl as mineral salts” **(Woo- sik et al., 2009).**

* ***Stropharia rugosoannulata / Wine cap***
* ***Lentinula / shiitake***
* ***Coprinus comatus / Shaggy Mane***

(Stamets, 2000) – Grows on most lab agars. Prefers growing on straw and manure mixtures. Can also work with paper. Peat-moss casing soil. Grow from trays.

0.5% N content had 80% BE

* ***Agaricus Augustus***

Complicated preparation of substrates. (Sánchez, 2004).

Due to this mushroom’s role as a secondary decomposer and its requirement for composted substrates cultivation (Beyer 2017, Stamets, 2000), it may not lend itself to small-scale cultivation.

* ***Agaricus Avrensis***

This species does not seem to be cultivated much possible because *A. augustus* is much more popular and has a similar cultivation technique.