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MycoMaterials: Metal-Fungi Hybrids

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Abstract

Eight different species of fungi were grown such as Blue Oyster (*Pleurotus columbinus*), Reishi Ling Chi (*Ganoderma lucidum*), and Shaggy Mane (*Coprinus comatus*). Species of fungi were chosen based on their potential growth rate, potential strength and non-toxicity to humans. Metal-mycelium hybrids were developed by using both the cell and mycelium life stages. In experiments involving the mycelium stage, varying amounts of sterilized sawdust (0.5 to 1 g), zinc sulfide (0.5 to 1 g), and small pieces of aluminum foil (0.1 to 1 g) were applied on top of the mycelium growing on petri dishes. For experiments using the cell stage, 8 to 9 drops of the fungal sample were applied to zinc sulfide (0.5 to 1 g) and aluminum foil (0.5 to 1 g). Mycelium (Blue Oyster) were grown on to differing quantities of aluminum foil. Cells (Shaggy Mane) have been grown onto aluminum foil with and without nutrients from the petri dish. Shaggy Mane cells also grew onto 0.5 g of zinc sulfide.

Introduction

There is an interest in creating aerospace structures that regenerate, communicate and that are capable of using carbon dioxide as a fuel source. Before such a vision is achieved several fundamental questions must first be answered. For example, what are the optimal conditions and laboratory settings for mycelium growth and is it possible for mycelium to digest different metals to form metal-mycelium hybrid structures? Recent research has shown that fungi and photosynthetic organisms can absorb harmful metals.

Fungi are essential decomposers; they use the mycelium to digest and absorb numerous substances such as compost, sawdust, and nematodes, and concentrations of nickel (Ref. 1). Mycelium are threadlike structures of the fungi which bind onto polymers to absorb and degrade nutrients and produce fruiting bodies known as mushrooms. This process can be used in two ways. First, mycelium can grow and regenerate if there is a steady food source present within a range of conducive temperatures. Second, if the mycelium and the potential food source (such as sawdust) are heated under compression above 93 °C while the mycelium is digesting the food source, the material forms a rigid structure bound by the enzymes secreted by the mycelium during digestion. Different species of mycelium are currently being used in producing building material (myco-bricks) because of their strong binding properties. In addition, myco-materials have been found to exhibit multifunctionality (e.g., fire resistance, radiation absorption, acoustic absorption). Systems using myco-materials may thus have reduced weight and improved efficiency (or reduced energy to produce) that are critical to aerospace applications.

A review of growing techniques for fungal organisms is found in Stamets (Ref. 2). This includes ideal incubation times and temperatures (21 to 27 °C for 5 to 7+ days), relative humidity (80 to 100 percent),

inoculation techniques (Mason jars and petri dishes), mycelial characteristics, best substrates for each species, and their natural habitats. For example, hen of the woods (*Grifola frondosa*) prefer temperatures between 21 to 24 °C, a relative humidity of 95 to 100 percent, and digest deciduous hardwoods such as oak. Hen of the woods is not used here but may be a useful species to explore. McCoy (Ref. 3) provides information on the general characteristics of mycelium, known sexual reproduction/life cycles, knowledge of harmful and beneficial species, methods and emphasis on pasteurizing substrates (such as straw), using clean distilled water, and overall handling of cultures.

Bellettini et al. (Ref. 4) stated the effects of chemicals, pH, and luminosity for *Pleurotus* species. Mycelium can grow in a pH range of 4.0 to 7.0, but for optimal growth, a pH of 6.5 to 7.0 is preferred. Many species can initially grow in partial light or darkness; it is most likely that all species (especially *Pleurotus* and Shiitake) need light to form the basidioma (fleshy structure of mushroom which produces spores) and the primordia (developing cells). In addition, fungi should receive between 200 to 640 lux for 8 to 12 hr a day at a warm temperature. Fungi which receive more than the required amount can be deformed and pale in color.

Current research on metal-fungi and chemical-fungi interactions is focused on removing hazardous chemicals and metals in eco-friendly ways. Xia Tang et al. (Ref. 5) conducted several experiments to observe if *Pleurotus eryngii* could digest and grow in different potting soils (sandy, sandy loam, and loamy clay) contaminated by nickel and fluoranthene. They decided to use fluoranthene because it is a toxic organic chemical and nickel is one of the largest metal pollutants in China. They analyzed the concentration of nickel by using a flame atomic absorption spectrometer. To determine fluoranthene, gas chromatograph mass spectroscopy was used. *Pleurotus eryngii* was able to successfully absorb fluoranthene and nickel in all soils; fluoranthene positively affected its growth, in contrast with nickel. In all of the experiments, Xia Tang et al. discovered that *Pleurotus eryngii* was best suited for absorbing fluoranthene in sandy soil. More fungal species (Ref. 4) are currently being tested for bioremediation purposes.

Methodology and Results

Fungi Growth

For proper growth and development, fungi must have a non-contaminated surface, the appropriate humidity, temperature and low light. In the lab, the relative humidity varied between 51.9 to 65.5 percent and the temperature ranged from 21.4 to 24.4 °C. Complete darkness was preferred during the initial growth stages. Three methods were used: the open air method, the glove box method, and the laminar flow hood method. The open air method and the glove box (cardboard box) method are not recommended because the samples are easily contaminated with airborne contaminants such as *Trichoderma*, which can easily grow onto any nutrient rich substance. A laminar flow hood (12 in. model 200 air cleaner, Sentry Air Systems) was thus used. Positive pressure blowing and filtration keeps out mold spores and other unwanted organisms. Personal protective equipment (coverall or a lab coat, eye protection, face mask, and nitrile gloves) was used for all experiments. The lab area and laminar flow hood were sanitized, ideally with 99 percent isopropyl alcohol. For growing mycelium in Mason jars, instructions from Midwest Grow Kits (Ref. 6) were followed. An alcohol wipe was used to clean the top of the syringe to reduce contamination underneath a laminar flow hood. Then, a sterile needle was connected to the syringe and nominally 2 mL (± 1 mL) of the sample was injected into a Mason jar. The Mason jar was sealed shut by taping aluminum foil to the jar. The jars were then labelled with the name of the species, amount of sample used, date of inoculation (transferring the sample into the Mason jar), and any other distinguishing information. Lastly, the jar was placed into the incubator (model 10-180 Quincy Lab, Inc.) at 25 to 30 °C

for three to four weeks or until the Mason jar is full of mycelium. If a different color other than white (such as purple or green) was observed in the jar, the jar was discarded due to likely contamination.

For petri dishes, 8 to 9 drops of mycelium solution were transferred onto a clean/unused petri dish while ensuring that the solution covered the entire dish. The petri dish was sealed and properly labeled with the inoculation date, species name, batch number and date of the syringe, and other distinguishing information. The petri dish was then placed into the incubator for at least 5 to 7 days.

Metal-Fungi Hybrid Experiments

The lab manual for experiments conducted in this research are available by contacting the corresponding author. Initially, sodium cobaltinitrite and silver nitrate were used to observe the possible effects of growth in Golden Oyster. In the first part of the experiment, three 250 mL Erlenmeyer flasks were cleaned and labeled “Golden Oyster control,” “Golden Oyster + 1.0007 g silver nitrate,” and “Golden Oyster + 1.0002 g sodium cobaltinitrite” (the chemicals were measured to be approximately 1 g). This is shown in Figure 1 Left. Figure 1 Right shows growth for the control sample. For the control, 16 g of rice flour was added into the flask (ideally, the control would be given 17 g). The top of the syringe was loosened and an alcohol wipe was placed over it to reduce contamination. Next, 1 to 2 mL of the sample was injected onto the rice flour. The rice flour was covered with a piece of wax paper. The flask was placed into an area with low light (dark room). For the experimental groups, the only difference in methodology was to add 16 g of rice flour plus 1 g of a chemical, such as zinc sulfide or silver nitrate, and then transfer 1 to 2 mL of the sample. For example, the flask labeled with silver nitrate received 15.9986 g of rice flour and the flask labeled with sodium cobaltinitrite was given approximately 15.9901 g of rice flour. In theory, the Golden Oyster would obtain nitrogen from either the sodium cobaltinitrite or the silver nitrate and thus grow. However, only the controlled Erlenmeyer flask exhibited growth and became contaminated by *Trichoderma* within 1 to 2 days after inoculation shown in Figure 2 Left. The other flasks did not show any visible growth (Figure 2 Middle and Right), which corresponds with current information provided by Bellettini et al. (Ref. 4). Nitrates are known to discourage growth in some species of basidiomycetes because it is difficult for the nitrate ions to move through the fungal membrane where growth can be encouraged. Both nitrates and nitrites could possibly inhibit growth in basidiomycetes and ascomycetes because *Trichoderma* and other molds did not grow as well.

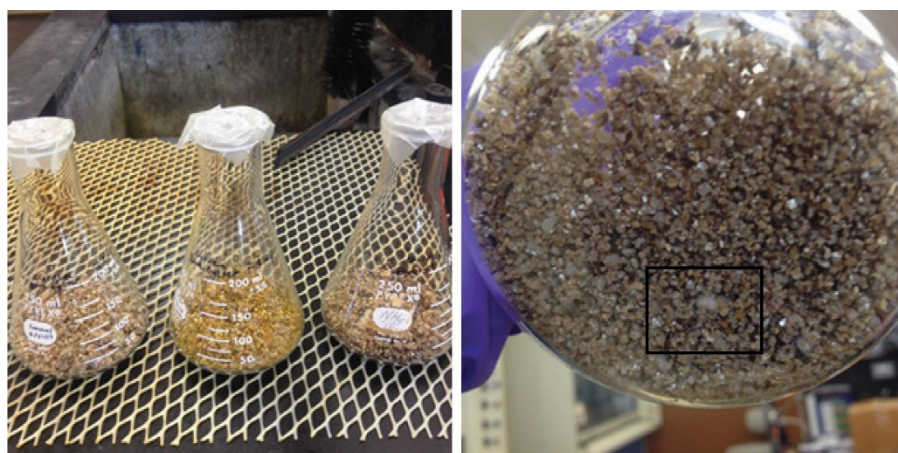


Figure 1.—Left photo: from left to right, Golden Oyster control, Golden Oyster with sodium cobaltinitrite and Golden Oyster with silver nitrate. 6/30/17. Right photo: noticeable growth on control. 6/30/17.



Figure 2.—Left photo: contamination of control. 7/3/17. Middle photo: Golden Oyster with silver nitrate. Right photo: Golden Oyster with sodium cobaltinitrite. 6/30/17.

The primary goal is to find a combination of elements or a non-toxic metal (alloy) which will encourage and facilitate basidiomycete growth. In this experiment, two life stages (cell and mycelium) were used. For growing metal-fungi hybrids in the mycelium stage it would be best to gather a large number of petri dishes with relatively the same amount of mycelium, same species, batch number, and inoculation/incubation dates. In reality, only 3 to 5 petri dishes were used because some petri dishes became contaminated. Ideally, the first petri dish would be the control, the second would be given a desired amount of zinc sulfide (0.5 to 1 g), the third would receive pieces of aluminum foil (0.5 to 1 g), the fourth would obtain sterilized sawdust (0.5 to 1 g), the fifth would be given sterilized sawdust (0.5 g) plus zinc sulfide (0.5 g), and the sixth would receive sterilized sawdust (0.5 g) plus pieces of aluminum foil (0.5 g). The sawdust was sterilized by using the model 50× All American Sterilizer. For example in the Blue Oyster group, one petri dish was designated as the control, one received 0.5 g of aluminum foil (Figure 3 Left and Figure 4), and the other received 0.5 g of zinc sulfide. Growth of fungi was then observed and recorded on a daily basis. Figure 3 Right shows Reishi Ling Chi growing on sawdust. For growing metal-fungi hybrids in the cell stage, a laminar flow hood was used and broken aluminum foil pieces were placed onto a clean/new petri dish (the petri dishes labeled Shaggy Mane were reused because there was no visible growth). The amount of Reynold's aluminum foil and zinc sulfide used was approximately 0.5 to 1.0 g each. In the Blue Oyster (mycelium) experiment, the mycelium attached itself to the foil, but not the zinc sulfide. In the Shaggy Mane (cell) experiment, there were similarities and differences. The samples applied to aluminum foil grew even without the nutrients from the petri dish (Figure 5 Left). The sample applied to 0.5 g of zinc sulfide showed growth (Figure 5 Right). Figure 6 Left shows the growth of Shaggy Mane on aluminum with nutrients and Figure 6 Right shows growth of Reishi onto aluminum. One of the petri dishes that contained approximately 1 g of zinc sulfide was contaminated by mold, which might suggest that sulfates have the potential to encourage growth in basidiomycetes and ascomycetes. Further research is necessary to understand why aluminum and zinc sulfide are non-toxic to certain species of fungi. Preliminary studies were also conducted using Nostoc (cyanobacteria) and Oedogonium (green algae) in an attempt to create lichen-metal hybrids but were unsuccessful due to contamination of samples.

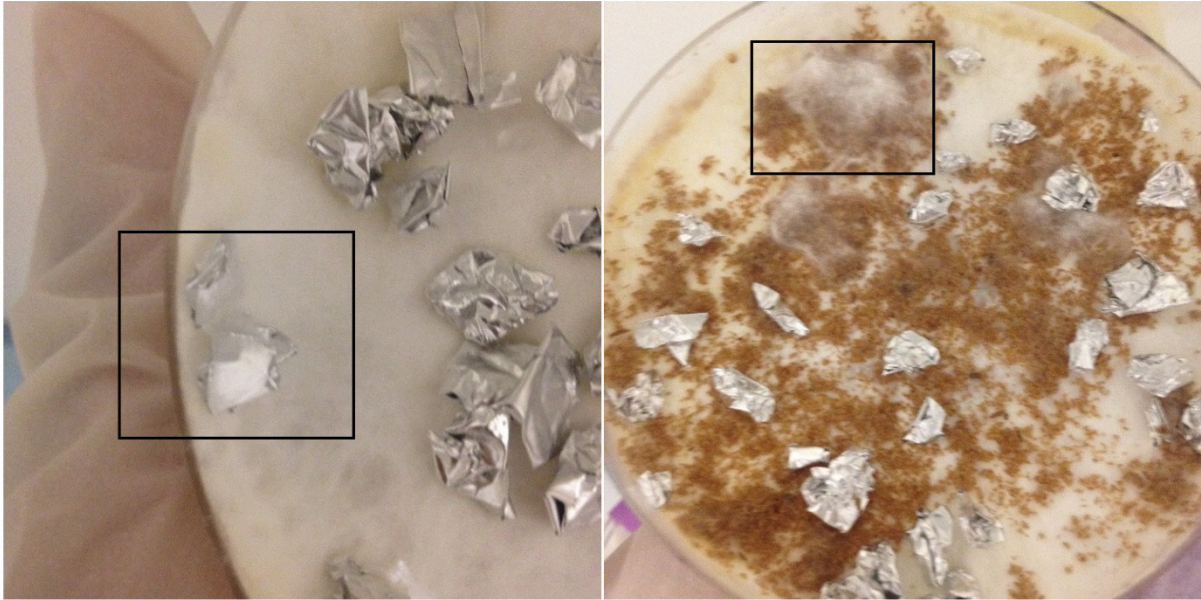


Figure 3.—Left photo: Blue Oyster (mycelium) growth onto aluminum foil. 7/25/17. Right photo: Reishi Ling Chi (mycelium) growing onto sawdust. 7/25/17.

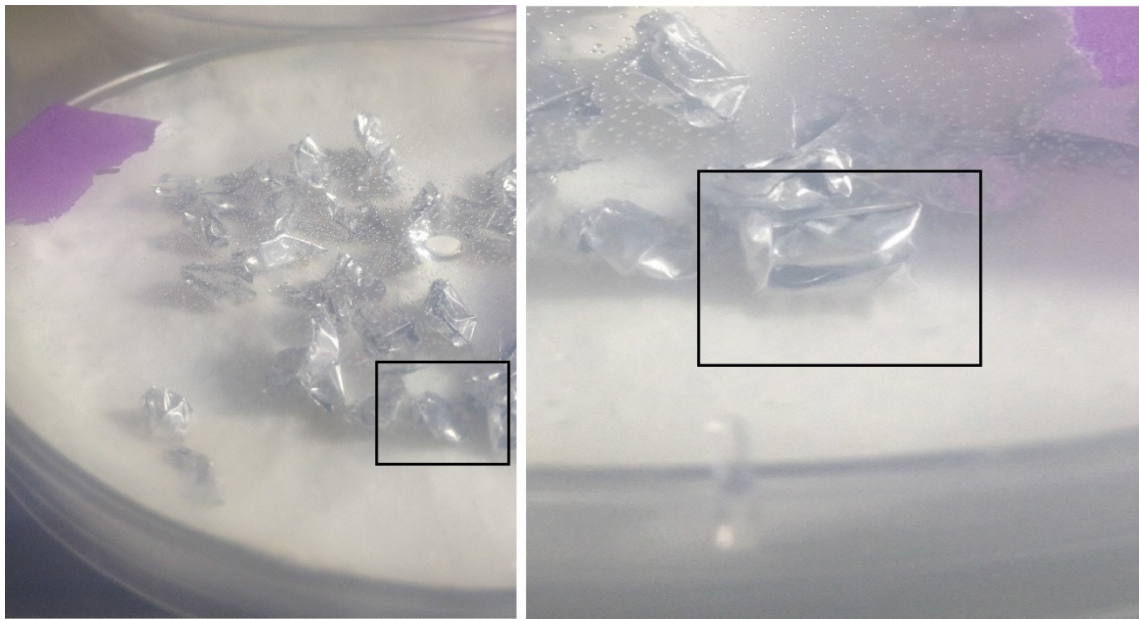


Figure 4.—Left photo: Blue Oyster (mycelium) growth onto aluminum foil. 7/27/17. Right photo: enhanced version of Blue Oyster growth onto aluminum foil. 7/27/17.

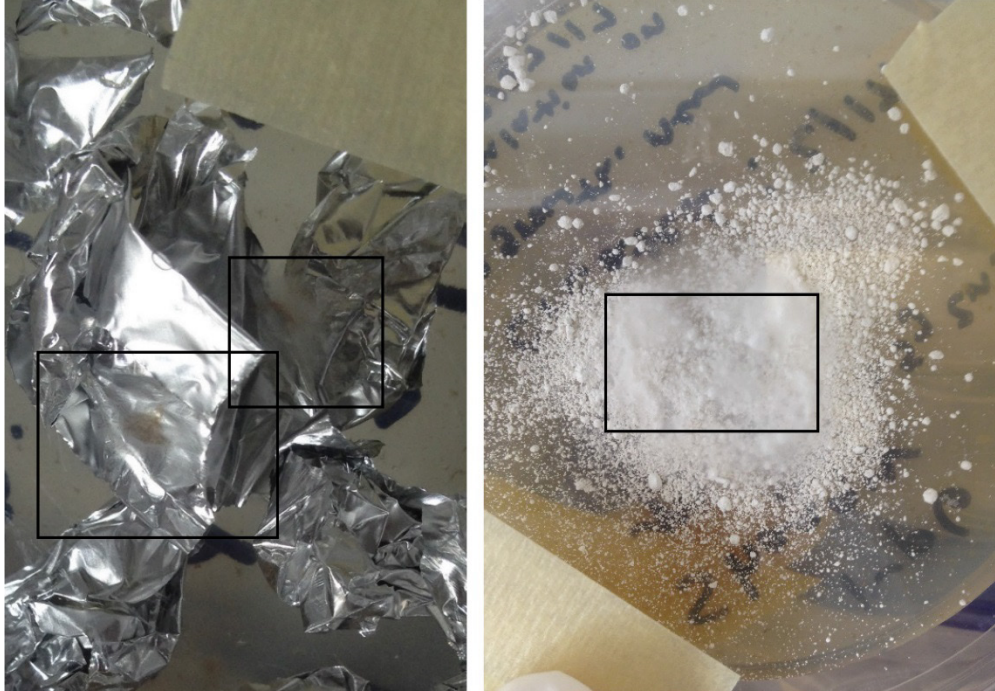


Figure 5.—Left photo: Shaggy Mane (hyphae) growing onto aluminum foil without nutrients from the petri dish. 8/4/17. Right photo: Shaggy Mane (hyphae) growing onto zinc sulfide. 8/10/17.

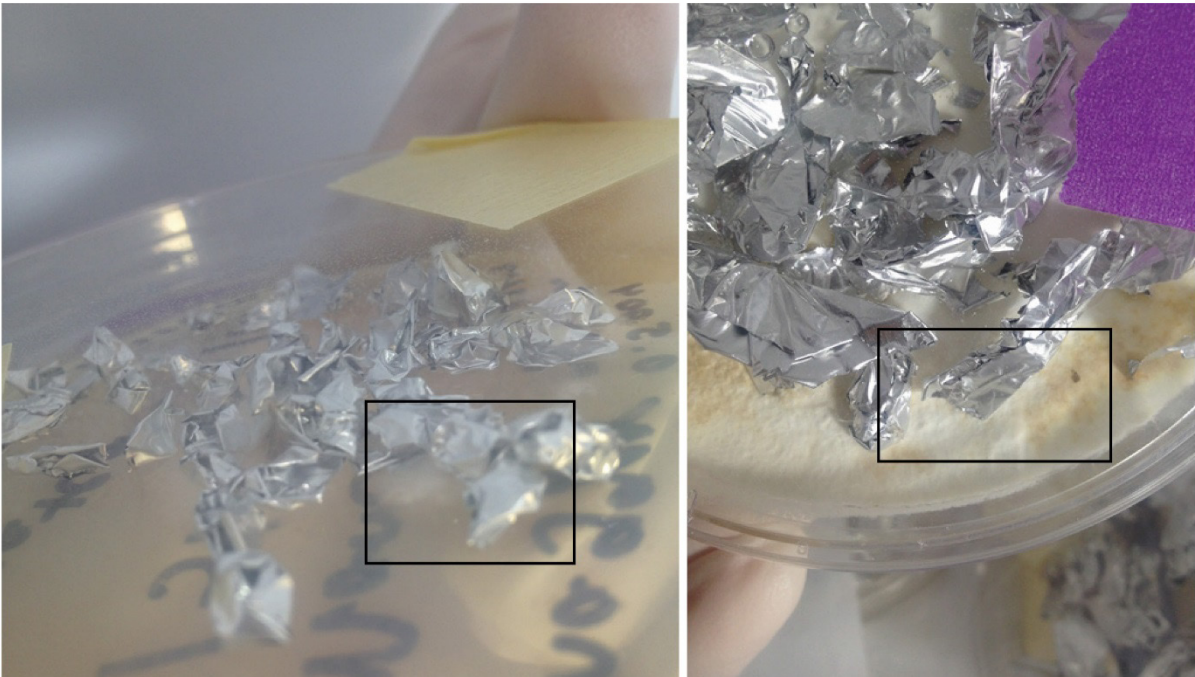


Figure 6.—Left photo: Shaggy Mane (hyphae) growing onto aluminum foil and the petri dish. 8/2/17. Right photo: Reishi Ling Chi (mycelium) growing onto aluminum foil. 8/2/17.

Conclusions

Blue Oyster was observed to grow onto aluminum foil while Shaggy Mane was observed to grow onto zinc sulfide. It is essential to work in a clean environment and to properly sterilize all working tools, equipment and samples to prevent contamination. In future experiments, pH, weight, and chemical concentration (such as zinc) will be recorded throughout the growth of the species to determine the mechanism of growth, the composition variation of the mycelium and the impact of environment on growth. In particular, a 0.5×0.5 cm sample may be measured every 10 to 15 days from the inoculation date to determine if the mycelium have digested the metal. In addition, the metal-fungi hybrids experiment will be altered by inoculating the samples directly onto substances such as aluminum powder and aluminum oxide, with and without nourishment. Further avenues of study include growth of mycelium by electrical pulses and chemical communication (e.g., farnesol). Mycelium have the potential to contribute to a new class of building materials. Future questions include: What is the effect of light on mycelium growth? What are the structural properties of the hybrids? Are the properties comparable to or better than current aircraft materials? Can biodegradable building material be 3D printed? Is it possible to replicate mycelium using synthetic materials? Applications of mycelium-metal hybrids may be found in regenerative structures for aircraft that are constructed out of bio-materials. These materials may store energy, communicate, and provide structural integrity. Symbiosis with cyanobacteria may also enable absorption of carbon dioxide from the atmosphere and conversion to hydrogen and oxygen for fuel cells.

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