Mutualism in a Reduced Gravity Environment (MuRGE)

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ABSTRACT

MuRGE (Mutualism in a Reduced Gravity Environment) is a ground research study to determine the feasibility of assessing fungi-plant (*Piriformospora indica-Arabidopsis thaliana*) interactions in microgravity. Seeds from the plant species Arabidopsis thaliana (At) will be grown in the presence or absence of Piriformospora indica (Pi) an endophytic Sebacinaceae family fungus.

Pi is capable of colonizing the roots of a wide variety of plant species, including non-mycorrhizal hosts like At, and promoting plant growth similarly to AMF (arbuscular mychorrizal fungi). Unlike most AMF, Pi is not an obligate plant symbiont and can be grown in the absence of a host. In the presence of a suitable plant host, Pi can attach to and colonize root tips. Interaction visualization is accomplished with strong auto-fluorescence in the roots, followed by root colonization via fungal hyphae, and chlamydospore production. Increased root growth can be observed even before root colonization is detectable.

In addition, Pi chlamydospores generated from axenic culture in microgravity will be used to inoculate roots of At grown in 1g to determine the effect of microgravity upon the inherent virulence or beneficial effects. Based on recent reports of increased virulence of S. typhimurium, P. aeruginosa, and S. pneumoniae in reduced gravity, differences in microbial pathogenic responses and host plant systemic acquired resistance are expected.

The focus of this project within MuRGE involved the development of *P. indica* culture media evaluation and microscopy protocol development. High, clean spore harvest yields for the detection of fungi-plant interactions microscopically was the immediate goal of this experiment.

BACKGROUND

•Demonstrated sustainability for high yield spore extractions from Pi in short turnovers.

•Sporulation rates shown to be related towards stress exerted upon organisms. Little or no stress yielded light spore production, while higher stress and less resource availabilty yielded directs resouce usage towards increased spore prroduction

•The methodology revolves around the organisms survival mechanisms. When under increased stress conditions fungi direct more available energy towards reproducing. In a more harsh environment fewer individual organisms of a community are expected to reach maturity, and must increase reproduction to maintain existence.

•Spectrophotometer based concentration readings were compared against haemocytometer microscopically

• Both large (6mm) and small (2mm) glass beads were used to alleviate spores into solution during the extraction process

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DESIGN

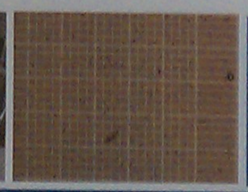
Objective- To provide PI culture protocol in which pure and high yields of basidiospore may be readily harvested.
Hypothesis- Fungal stress levels often regulate community growth rates, and is a reliable way to create a reproductive response via sporulation. The physiological state of PI in higher nutrient media will not be stressed to induce substantial sporulation. Significant stress should be achieved with a more limited media and environmental pressure.
Experimental Design- PI is subject to a range of morphological characteristics due to environmental stress or nutrient availability. Multiple trials were conducted on a considerably enriched media, and a more basic media.

METHODS

Organism Growth Conditions-
-Frozen stock is used initially to begin growth. Stock is allowed to warm to room temperature, vortexed, and then 300µl aliquots transferred to each of three plates. The stock is spread evenly until the plates are no longer moist.
-Eight milliliters of sterilized DI water is dispensed to a grown plate, and the surface is scrubbed heavily with a spreader to alleviate the fungi. From the liquid suspension over the plate 100µl aliquots are transferred to new plates, and the remainder harvested as described below.
-Cultures are transferred weekly in triplicate for each media type.
Incubation conditions are 30°C.

Spore Preparation-
-From the media plates growth was scraped on each of three plates (MS or PDA) with spreader and ~8ml sterile DI water. 5ml detached Pi mixture was collected from each (3) with 1ml pipette, and aliquoted into a15ml centrifuge tube
-The Pi was spun out of media @ 5,000rpm for 3min, supernatant discarded, and resuspended in 8ml – sterile DI water. The solution was then heavily vortexed on top speed for ~60s, and pulse vortexed for ~15s; then these steps were repeated two more times
-5x sterilized 6mm glass beads were added , heavily vortexed on top speed for ~60s, pulse vortexed for ~15s, and tubes shaken for ~60s
-The volume was transferred to a 50ml centrifuge tube, filtered through 41 micron steriflip filter, spun down particulate from solution (5,000rpm for 5min), and decanted liquid and resuspend in 3ml for concentration determination via haemocytometer
-concentration of cells in original mixture = $\left(\frac{\text{number of cells counted}}{(\text{proportion of chamber counted})(\text{volume of chamber})}\right)\left(\frac{\text{volume of sample dilution}}{\text{volume of original mixture in sample}}\right)$





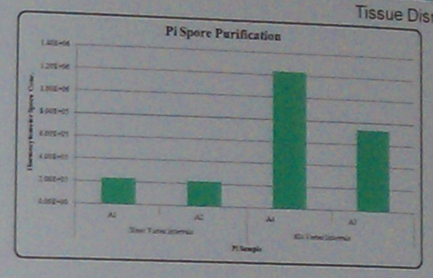

CONCLUSIONS

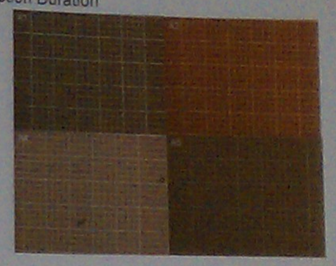
•Extended vortex duration with beads (60s vs 30s) yielded a 300%-600% increase in spore concentration during from Pi cultures
•6mm glass beads vital in recovering utile spore mass
  •2mm beads crushed most spores
•The highest recovery of basidiospores from PI culture occurred under PDA media
  •~130% increase from MS
•Spectrophotometer readings were not reliable to determine spore concentration
  •Haemocytometer must be used to count manually under microscope for any degree of accuracy

•Average spore concentrations of upper 1E5 to mid 1E6 are achieved with reliability and purity in a weeks incubation period. The spores have been used with more MuRGE projects to determine mutualistic growth interactions as well as determining staining techniques for visualization of interactions.

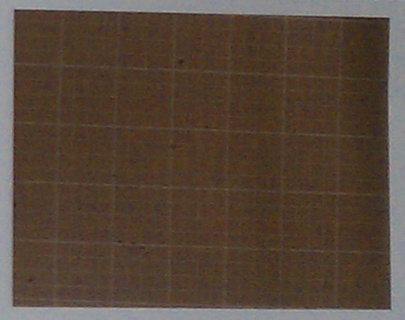
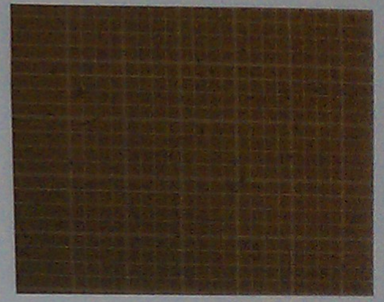
RESULTS

Tissue Disruption Duration

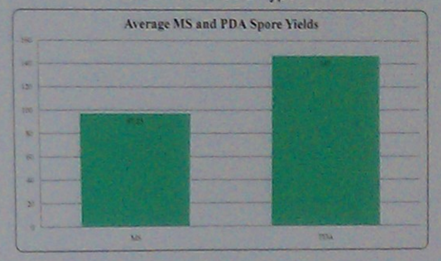




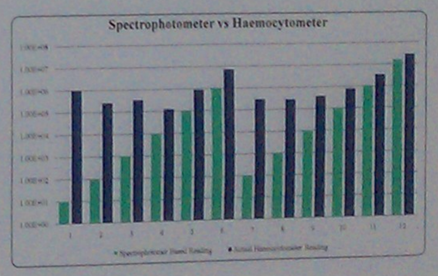
Effects of Bead Size (2mm Left & 6mm Right)

Effects of Media Type



Effects of Data Analysis Method



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