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 (Phycomyces nicotiana-Arabidopsis thaliana) interactions in microgravity. Seeds from the plant species Arabidopsis thaliana (AT) will be grown in the presence or absence of Phycomyces byciclus (Pi) an endophytic Sebacicaceae 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 mycorrhizal fungus). 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 compatible plant host, Pi can attach to and colonize root tips. Interaction visualization is accomplished with strong autofluorescence in the root axes, 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 need to be evaluated.

The focus of this project within MuRGE involved the development of Pi 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 turnovs.
- Sporeulation rates shown to be related towards stress exerted upon organisms. Little or no stress yielded tight spore production, while higher stress and less resource availability yielded loose resource usage towards increased spore production.
- The methodology revolves around the organisms survival mechanisms. When under increased stress conditions fungi direct their available energy towards reproduction. 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 elevate spores into solution during the extraction process.

DESIGN

Objective: To provide Pi culture protocol in which pure and high yields of basidiospores may be readily harvested.

Hypothesis: Fungal stress levels often regulate community growth rates, and is a reliable way to determine a reproducible 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 medium and environmental pressure.

Experimental Design: Pi is subjected 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.

Sporulation Preparations:
- From the media plates growth was scraped on each of three plates (MS or PDA) with spreader and ~2ml sterile DI water. 5ml detached Pi mixture was collected from each (3) with 1ml pipette, and aliquoted into 5x105 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 2mm 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 25ml centrifuge tube, filtered through 41 micron sterile filter, spun down particulate from solution (0,000rpm for 5min), and decanted liquid and resuspended in 3ml for concentration determination via haemocytometer.
- Concentration of cells in original mixture = (concentration of 3ml)/5

RESULTS

Effects of Bead Size (2mm Left & 5mm Right)

Effects of Media Type

Effects of Analysis Method

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