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 interactions in microbiality. Seeds from the plant species Arabidopsis thaliana (At) will be grown in the presence or absence of Piriformospora indica (Pi), an endophytic Sebacinaeae family fungus. Pi is capable of colonizing the roots of a wide variety of plant species, including non-mycoherizal hosts like At, and promoting plant growth similar to AMF (arbuscular mycorrhizal fungi). Unlike most AMF, Pi is 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 autofluorescence 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 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.
- Sporelation rates shown to be related towards stress exerted upon organisms. Little or no stress yielded high spore production, while higher stress and less resource availability yielded lower resource usage towards increased spore production.
- 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 elevate spore into solution during the extraction process.

CONCLUSIONS

- Extended vortex duration with beads (0s vs 30s) yielded a 300%-400% increase in spore concentration during from Pi culture.
- 6mm glass beads vital in recovering utilize spore mass
- 3mm beads crushed most spores
- The highest recovery of basidiospores from Pi culture occurred under PDA media
- 130% increase from MS
- Microscopy 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 1E5 are achieved with reliability and purity in a weeks incubation period. The spores have been used with more MuRGE projects to determine mutaMutualism in a Reduced Gravity Environment (MuRGE)Ristic growth interactions as well as determining staining techniques for visualization of interactions.

ACKNOWLEDGMENTS

Research support was provided by the NASA Research Opportunities for Flight Experiments in Space Life Sciences - NRA NNX10AG39G.