INSPIRE Pre-College Internship

Kennedy Space Center

Mutualism in a Reduced Gravity Environment (MuRGE)

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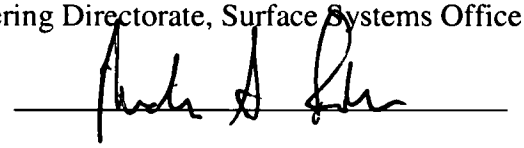

Abstract

MuRGE (Mutualism in a Reduced Gravity Environment) is a NASA flight-research experiment to investigate the microgravity effects associated with cell-cell communication and beneficial microbe-host interactions using a plant-fungal model system. This investigation will use a clinostat, an instrument that slowly rotates the plants to negate the effects of gravitational pull on plant growth (gravitropism) and development, to simulate microgravity. I will be using the endophytic fungus *Piriformospora indica* (Pi) and the model plant species *Arabidopsis thaliana* (At). *P. indica* has been shown to colonize roots of various plant species, including *A. thaliana*, and to increase plant growth and resistance to stress. The fungus has the ability to grow from spores or in axenic cultures without the presence of a host. *P. indica* spores and *P. indica* extract will be used to inoculate Arabidopsis seeds germinated on a clinostat in order to determine if simulated microgravity affects the interaction between the fungus and its plant host.

Introduction

Mutualism can be described as interactions between different organisms that are beneficial for both partners. One common effect of mutualism is increased growth of one or both organisms by increasing access to nutrients. Microgravity affects the physiology of organisms and the way they interact with one another and their environment. Plants and fungi go wherever humans go and are therefore a part of spacecraft environmental control and life support systems. It is necessary to study the effects of microgravity on microorganisms so we can be prepare for long duration missions in space. MuRGE will test the interaction between At and Pi in both normal gravity and microgravity environments. The null hypothesis is that *P. indica* will increase the growth of *A. thaliana* in both normal gravity and simulated microgravity.

Materials and Procedures

This experiment will germinate seeds from *A. thaliana* (L.) Heynh wild-type ecotype Columbia on a solid agar media consisting of 2.2 g of Murashige and Skoog salts (Murashige and Skoog, 1962), 0.5 g of MES buffer, 2.5 g of sucrose, and 1 mL of 1,000x Gamborg vitamins (Sigma-Aldrich, St. Louis) per liter at pH of 5.75. Phytagel (Sigma) is added to a concentration of 0.8% (w/v) and autoclaved. After autoclaving, 5 mL of the media is aliquoted into a sterile 60 mm Petri plate (Millipore Microbiological Dishes, 47mm). Each Petri plate will be planted

with18 sterilized Arabidopsis seeds per plate oriented in a single row across the plate on the solid surface. For *P. indica* spore treatments, *A. thaliana* seeds are coated with *P. indica* spores immediately prior to planting by immersing the seeds for 2 minutes in ~50 µL of a spore suspension containing ~1 x 10^4 spores mL^{-1} dI H_2O. For *P. indica* extract treatments, *A. thaliana* seeds are coated with *P. indica* extract immediately prior to planting by immersing the seeds for 2 minutes in ~50 µL of the extract.

Four slow-rotation clinostats rotating at 1 rpm, each holding 12 60-mm Petri plates arranged in 4 sets of 3, will be used during the experiment. Three Petri plates will contain only *Arabidopsis thaliana* (Treatment A), three plates will contain only *P. indica* (Treatment B), three will combine *A. thaliana* and *P. indica* spores (Treatment C), and a third set of plates will contain *A. thaliana* and an extract of *P. indica* (Treatment D). A third set of 12 60-mm Petri plates will be placed on the shelf as a no-centrifugal force control. Each Petri plate will be prepared in duplicate and one plate corresponding to each treatment will be covered in aluminum foil as an etiolated (dark) treatment. Two clinostats will be oriented vertically to rotate the Petri plates perpendicular to the Earth's surface (Simulated Microgravity) and the other clinostats will be oriented horizontally to rotate the Petri plates parallel to the Earth's surface (Normal Gravity Control). Petri plates loaded into the clinostats and the static controls will be placed in a Controlled Environment Chamber (CEC 15) under the following growth conditions: 18:6 Light:Dark, 300 µmol m^{-2} sec^{-1}, 400 ppm CO_2, 20°C, and 50% RH (relative humidity).

The growth of the plants will be monitored by measuring the length of roots and shoots of the *Arabidopsis* plants on the Petri plates and measuring the fresh-weight biomass. A single Petri plate will be harvested from each treatment at each of three four-day time intervals spanning twelve days. At each three-day interval, one plate from each treatment will be removed from the clinostat and the plants harvested for measurement under an Olympus SZX-12 zoom microscope.

TREATMENTS (18 seeds per Petri plate; 3 Petri plates per treatment)

A. *A. thaliana*	CTL ; HC ; VC	3 plates/treatment = 9 plates
B. *P. indica*	CTL ; HC ; VC	3 plates/treatment = 9 plates
C. *A. thaliana* and *P. indica* spores	CTL ; HC ; VC	3 plates/treatment = 9 plates
D. *A. thaliana* and *P. indica* extract	CTL ; HC ; VC	3 plates/treatment = 9 plates
		36 plates incubated in light
		36 plates incubated in dark

	DOE4	DOE8	DOE12
Control	CTL: A1/B1/C1/D1	CTL: A2/B2/C2/D2	CTL: A3/B3/C3/D3
Horizontal Clinostat	HC: A1/B1/C1/D1	HC: A2/B2/C2/D2	HC: A3/B3/C3/D3
Vertical Clinostat	VC: A1/B1/C1/D1	VC: A2/B2/C2/D2	VC: A3/B3/C3/D3

Results

1st Experiment-Day 4

Plate			Orientation					
	# of seeds	Germinated	Up	Down	Left	Right	Unknown	Notes
A1 HC	18	14	6	4	2	2		
A1 N	18	17	15	2				
A1 VC	18	13	2	10	1			
B1 HC	18							16 spots of Pi (fungal contaminant?)
B1 N	18							Cannot distinguish individual spots, fungal growth along center
B1 VC	18							Cannot distinguish individual spots, fungal growth along entire line, one large growth on right
C1 HC	18	17	7	2	4	4		Spores and fungal growth not visible
C1 N	18	15	10	3	2			14 seem to have spores, 2 of those overtaken by Pi
C1 VC	18	16	5	5	3	3		11 visibly affected by Pi, others not visible
D1 HC	18	15	5	3	4	3	3	Plate had bubbles, unable to determine if affected by Pi
D1 N	18	13	11			2		Longest roots, all affected by Pi, 1 overtaken by Pi
D1 VC	18	16	3	10	1	2		Fungal growth visible across entire line, 1 overtaken

The data above was collected from the first set of plates, which contained eighteen seeds each. In most cases, more than three-fourths of the seeds germinated. In the neutral plates, a majority of the plants sprouted upwards. However, a few of the seeds that had recently germinated grew in other directions. The plates that were placed on the horizontal clinostat had similar results to one another. A majority of plants in each plate grew upwards. The plates on the vertical clinostat had a majority of seeds that grew downwards. The Pi growth was undistinguishable from the fungal contamination.

1st Experiment-Day 8

Plate			Orientation					
	# of seeds	Germinated	Up	Down	Left	Right	Unknown	Notes
A2 HC	18	17	14	3				
A2 N	18	17	17					
A2 VC	18	18	4	9	8	2		
B2 HC								10 visible colonies of contaminant (?)
B2 N								2 types of fungal contaminant
B2 VC								2 types of fungal contaminant
C2 HC	18	17	5	2	4		6	Pi/ contaminant has overtaken At- unable to observe roots/orientation
C2 N	18	16	6	5			5	Pi/ contaminant has overtaken At- unable to observe roots/orientation
C2 VC	16?	14	4	5	2	3		Plate contaminated, 5 overtaken
D2 HC	18	17						Plate dropped? All plants are loose and floating
D2 N	18	17	17					Bubbles around left side of plate, contaminate on right side
D2 VC	18	18	8	5	1		4	Many are shriveled, germinated at dif. times, newly germinated have been overtaken by contaminant

The data above was collected from the second set of plates from the first experiment. All of the plates,except one, showed to have eighteen seeds. A majority of those seeds germinated. In the *Aribidopsis* only and *Aribidopsis* + *P. indica* extract, all of the germinated seeds grew upwards.

However, the *Arabidopsis+ P. indica* spores had a randomly distributed amount of plants in all directions. The plates that were placed on the horizontal clinostat had a majority of plants that grew upwards. One plate had floating seeds, which could have resulted from the plate being dropped. The plates on the vertical clinostat had a randomly distributed amount of seeds growing in all directions. The direction and rate of Pi growth was unable to be quantified because of the presence of fungal contaminant(s).

2nd Experiment-Day 4

Plate			Orientation					
	# of seeds	Germinated	Up	Down	Left	Right	Unknown	Notes
A1 HC	18	18	9	4	4	1		
A1 N	18	15	15					
A1 VC	18	15	5	10				
B1 HC								1 distinguishable spot
B1 N								Pi not visible
B1 VC								Pi not visible
C1 HC	18	15	7	6		2		Pi not visible
C1 N	18	16	16					Root hairs visible
C1 VC	18	15	5	8	2			
D1 HC	18	14	7	4			3	A couple of roots are interwoven-unable to determine orientation
D1 N	18	18	18					Longest roots, root hairs somewhat visible
D1 VC	18	15	6	6		3		Pi not visible

The data above was collected from the first set of plates from the second experiment. In most cases, more than three-fourths of the seeds germinated. In the neutral plates, all of the seeds that germinated grew upwards. The plates that were placed on the horizontal clinostat had a majority of the plants grew upwards. The plates on the vertical clinostat had a majority of seeds that grew downwards. The Pi did not show any visible growth on these plates.

Discussion

MuRGE investigated the microgravity effects associated with cell-cell communication and beneficial microbe-host interactions using a plant-fungal model system. This investigation used a clinostat to simulate the effects of microgravity. The plant *Aribidopsis thaliana* and the fungus *Piriformaspora indica* were used to investigate these microbe-host interactions.

Two experiments were completed using the clinostats. While collecting data from the day four plates, a contaminant was spotted on the plates. The spores were the likely source of this. The inconclusive experiment resulted in starting a second experiment. Unfortunately, there was no visible spore growth in the first eight days of the experiment. Due to time restraints of my internship, I was unable to collect data for the day twelve plates of my second experiment. As a result of two inconclusive experiments, I was unable to determine the affects of Pi on At in both normal gravity and microgravity.

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