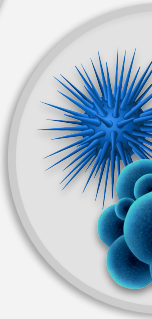

The Role of Cyanobacteria in Enhancing Radish Growth in Simulated Lunar Soils

Celestial Seedlings

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Introduction

Astrobiology with Plant the Moon

- Plant The Moon Challenge
  - Practiced in 44 states but not Colorado until RRCC and CCA
  - Finds most efficient way to grow plants in Lunar regolith
- Provide baseline for future RRCC Astrobiology groups

Hypothesis

Increasing cyanobacteria populations in lunar regolith will allow for more efficient lunar agriculture due to nitrogen fixation

- Growth in lunar regolith (soil)
  - Reduce transportation costs
    - Fertilizer
    - Soil
    - Food

Background

Lunar agriculture

Nitrogen is required for plant growth.

Nitrogen can be obtained from cyanobacteria or fertilizer.

Polymerase Chain Reaction (PCR): amplifies DNA.

Gel electrophoresis: lab procedure used to separate and visualize DNA sequences.

16S primers: amplifies bacterial DNA.

CYA primers: The specific DNA amplification of cyanobacterial gene.

Goals

1. Determine the effect of cyanobacteria on radish growth
    a. Growth
    b. Presence of cyanobacteria

1. Determine optimal substrate
    b. 100% soil
    c. 100% regolith
    d. 50/50 soil regolith mixture

Experimental Design

- Radishes were grown in three ratios of lunar regolith and soil (100:0, 50:50, 0:100)
- Each substrate composition was given autoclaved pond water and non-sterile pond water
- Plants were watered three times a week
  - 25-50ml on the surface
- Soil samples were taken each week from the surface of the pots
- Plants were grown for 60 days

Photo of all 6 plant samples (Black plate represents pond water and orange place sterilized water)

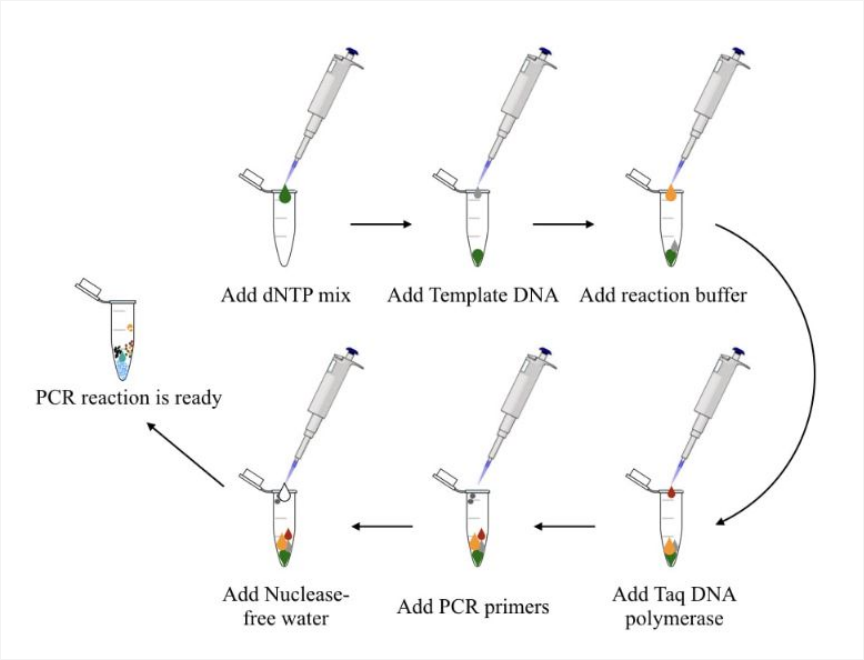
Experimental Design

- Autoclaved soil
  - Garden Kellogg Organics' All Natural Indoor Potting Mix
    - Sphagnum peat moss, recycled forest products, perlite, organic fertilizer, and dolomite lime (as pH adjuster)
      - Easily accessible
- Simulated Lunar Regolith
  - 74.4% Anorthosite, 24.7% Glass-rich Basalt, <1% Ilmenite, bronzite, olivine
- Cherry Belle Radishes
  - Rapid growth
  - Nutritional benefits - vitamin C, potassium, and calcium
- Cyanobacteria - nitrogen fixing bacteria that acts similarly to fertilizer
- Sterilized pond water vs. pond water
  - Allows for equal nutrient levels without the DNA from organisims

Materials and Methods – DNA extraction

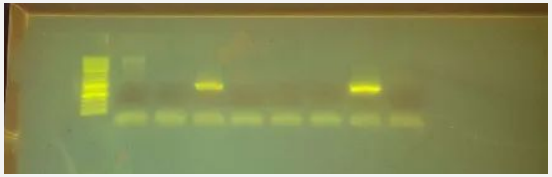
DNA extraction - Zymo Research Quick- DNA Fecal/Soil Microbe Miniprep Kit

Materials and Methods –PCR

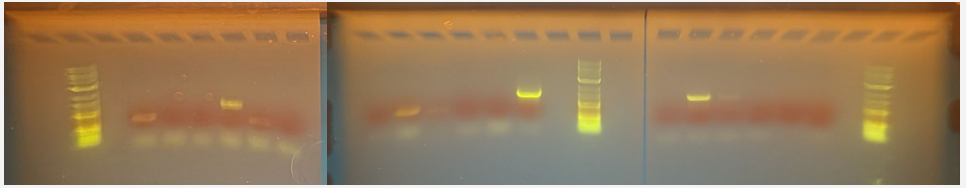


Polymerase chain reaction (PCR) on 16S and CYA gene - amplifies the DNA segment targeted by primers.

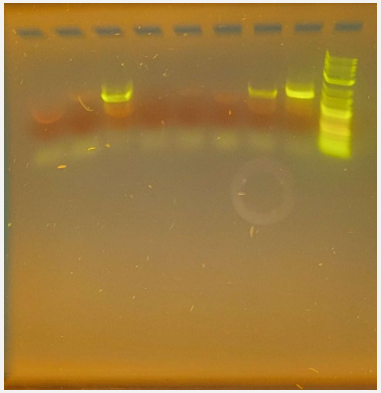
Materials and Methods – Gel Electrophoresis Results



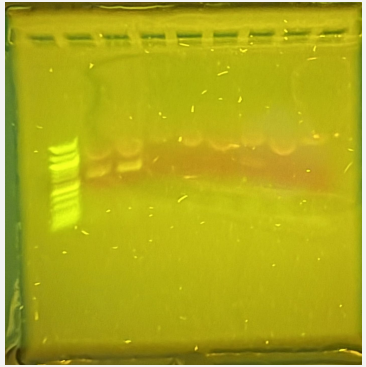
CYA PCR test for 10/4 sample. Positive control and sterile 50/50.



16S and CYA PCR test for 10/20 sample. Left to right: CYA soil non-sterile, positive control, 50/50 sterile, 50/50 non-sterile, 16S positive control, 50/50 non-sterile, and soil non-sterile.

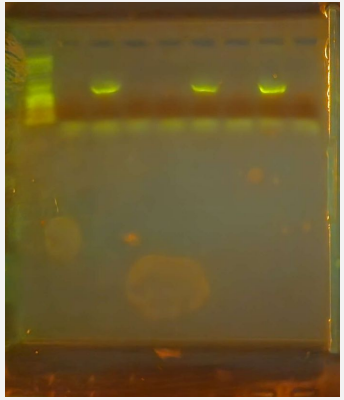


16S PCR test for 11/1 sample. Positive control, 50/50 sterile and non-sterile.



Left: CYA PCR test for 11/1 sample. 50/50 unsterile, 50/50 sterile, and positive control.

Right: 16S PCR test for 11/22 sample. Positive control, 50/50 sterile, and 50/50 non-sterile.



Pond Water Sample

Sterilized Pond Water Sample

September 30, 2024

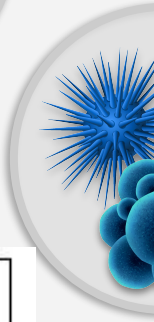
November 22, 2024

December 2, 2024

100% regolith 50/50 100% soil

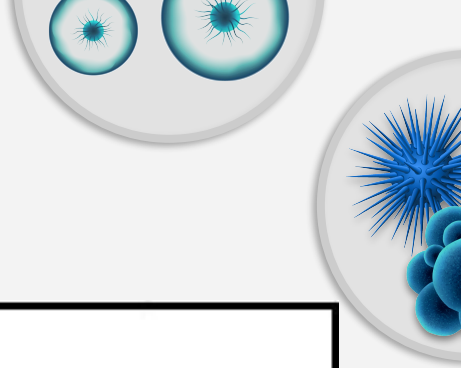
100% regolith 50/50 100% soil

Plant Viability



	Watered with non-sterile pond water			Watered with sterile pond water		
	Soil Pond	50/50 Pond	Reg Pond	Soil Sterile	50/50 Sterile	Reg Sterile
9/30	Alive	Alive	Alive	Alive	Alive	Alive
10/04	Alive	Alive	Alive	Alive	Alive	Alive
10/16	Alive	Alive	Alive	Alive	Alive	Dead
11/22	Alive	Alive	Dead	Alive	Alive	Dead
12/02	Dead	Dead	Dead	Alive	Dead	Dead

DNA Quantification Results



DNA Concentration (ng/μL)						
	water source	30-Sep	4-Oct	20-Oct	1-Nov	22-Nov
regolith	sterile pond	0.0		0.0	0.0	0.1
	non-sterile pond	0.0		0.0	0.0	0.0
soil & reg	sterile pond	0.1		3.4	17.0	28.0
	non-sterile pond	0.0		0.3	7.7	4.6
soil	sterile pond	0.5		0.8	14.0	23.0
	non-sterile pond	0.3		0.6	9.1	9.5

PCR Test Dates and Results

PCR targeting 16S						
	water source	30-Sep	4-Oct	20-Oct	1-Nov	22-Nov
regolith	sterile pond	-	-	-	-	-
	non-sterile pond	-	-	-	-	-
soil & regolith	sterile pond	-	-	-	+	+
	non-sterile pond	-	-	-	+	+
soil	sterile pond	-	-	-	-	-
	non-sterile pond	-	-	+	-	-

PCR targeting cyanobacteria						
	water source	30-Sep	4-Oct	20-Oct	1-Nov	22-Nov
regolith	sterile pond	-	-	-	-	-
	non-sterile pond	-	-	-	-	-
soil & regolith	sterile pond	-	+	+	+	+
	non-sterile pond	+	-	-	+	+
soil	sterile pond	-	-	+	-	-
	non-sterile pond	-	-	+	-	-

Conclusion

Key Findings:

✔ Microbial-enriched water significantly enhanced radish germination and growth in regolith.

✔ Confirmed ability to detect cyanobacteria with molecular techniques

✔ Supports potential for lunar agriculture using bio-enriched regolith.

✔ Substrates inoculated with non sterile pond water positively affect the growth of radishes

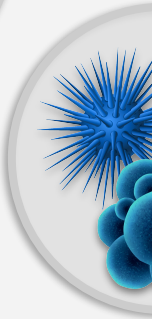
Why It Matters:

These results demonstrate a practical approach to cultivating crops in extraterrestrial substrates by harnessing microbial ecosystems.

Additionally:

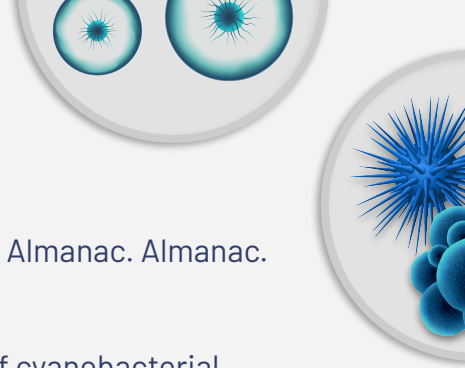
- ★ Created the one of the first COSGC Branch of Plant the Moon Challenge
- ★ Won Best in Show Award for Most Innovative Experiment

Future Recommendations



- Validate cyanobacteria's role with molecular tracking to confirm nitrogen fixation.
- Optimize DNA extraction for success in regolith.
- Refine DNA extraction methods for silica-rich substrates to improve microbial analysis.
- Test mutualism by analyzing root-adjacent microbes.
- Expand to different plants, particularly ones that work symbiotically with nitrogen-fixing bacteria.
- Develop irrigation methods (drip vs. wicking) to optimize moisture delivery for the differing water retention in soil vs regolith.

Citations



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Questions?