

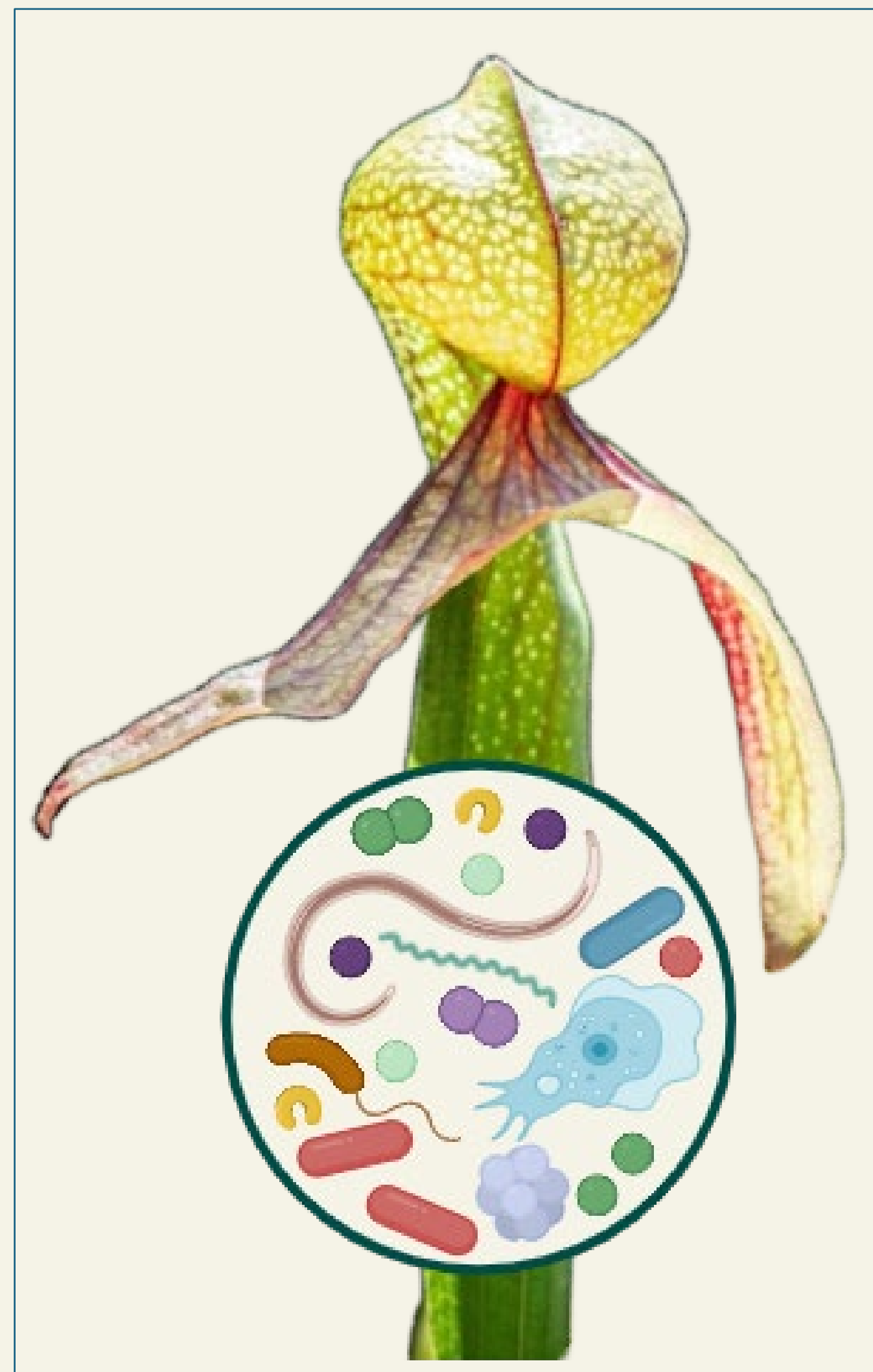
Developing Culture-Based Methods to Assess the Function of *Burkholderia sensu lato* in Pitcher Plants

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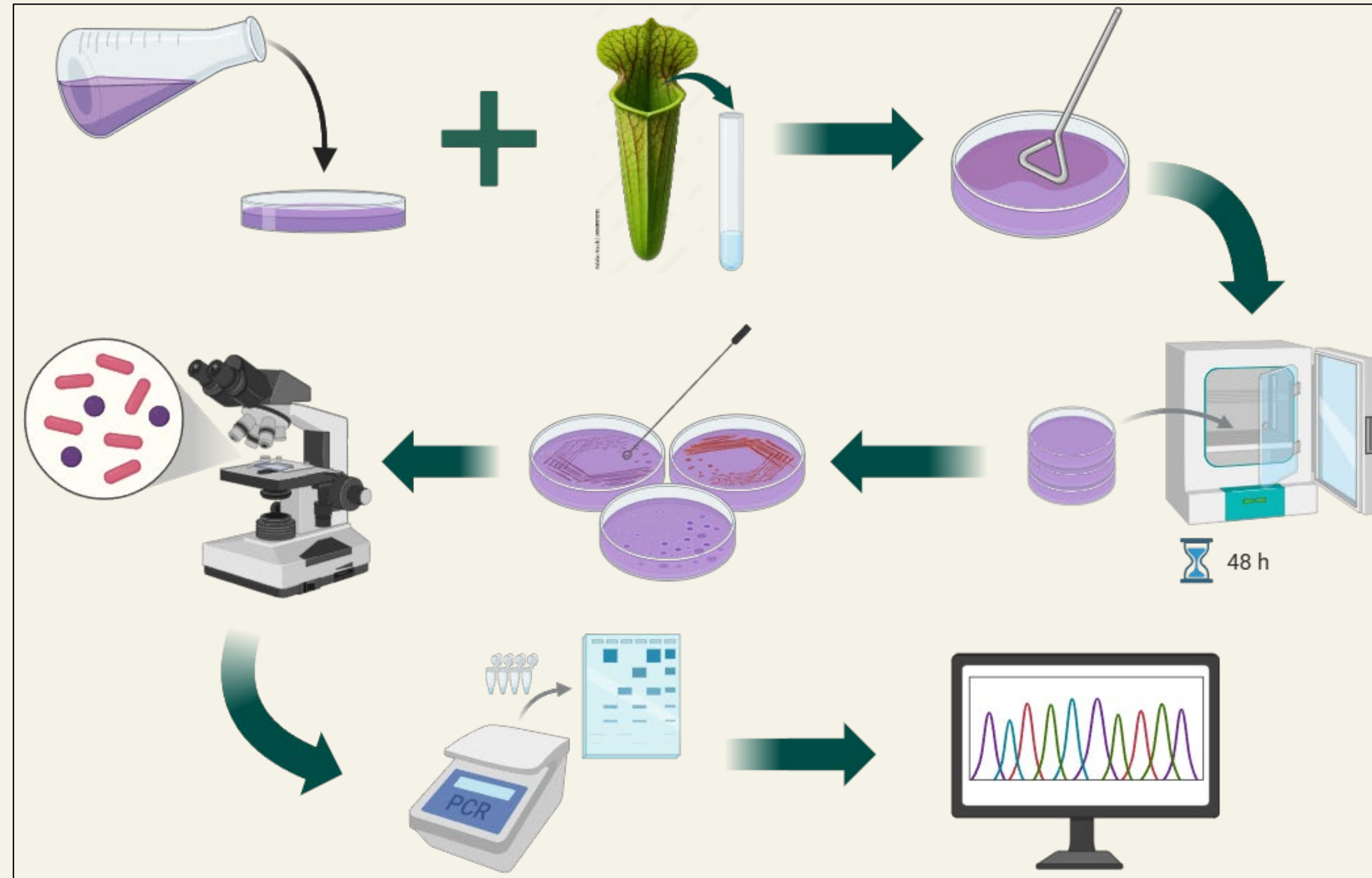
BACKGROUND



- Pitcher plants are ecological models that are host to a diverse array of microorganisms
- *Burkholderia* s.l. is a group of bacteria with diverse biochemical pathways that is poorly understood in pitcher plants despite a positive association with function in *Sarracenia purpurea*¹
- Assessing the biochemical characteristics of *Burkholderia* s.l. isolated from *Sarracenia* sp. will lend insight into its functional role in pitcher plants

METHODS

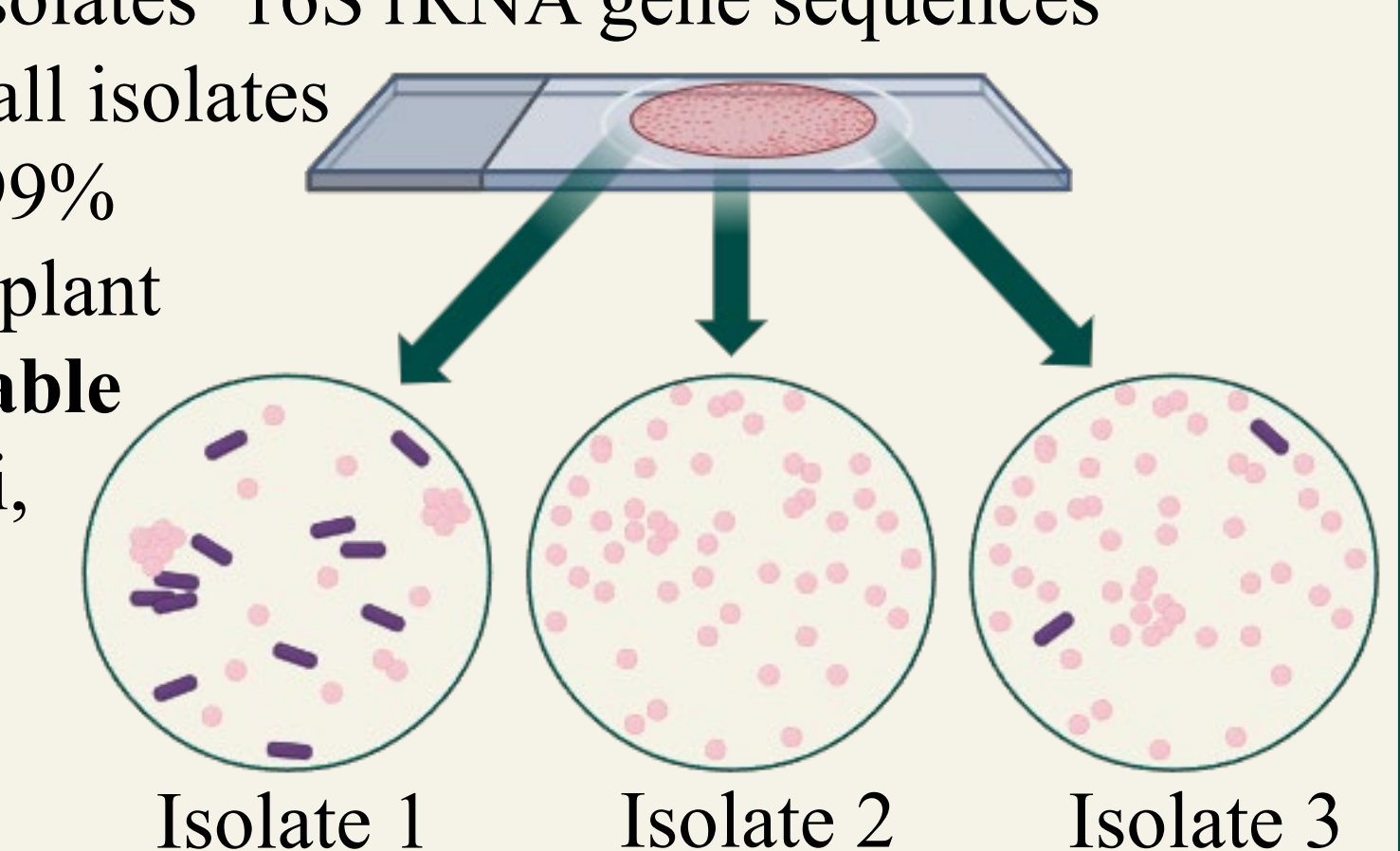
Selective and Differential Culturing of microbial communities from *Sarracenia* sp., Isolating *Burkholderia* s.l., and Sanger Sequencing of 16S rRNA gene



I conducted thioglycolate tests, motility agar stab tests, and nitrate reductase tests to assess the biochemical features of *Burkholderia* s.l.⁷

RESULTS

- BLASTn confirmed the identity of isolates as *Burkholderia sensu lato*
- *Paraburkholderia* is the closest identity match to a single genus, though other genera also matched the isolates' 16S rRNA gene sequences
- Query Cover was 100% across all isolates
- Percent Identity Range: 93% - 99%
- *Burkholderia sensu lato* in pitcher plant fluid is **Gram variable**, with **variable cell morphology** (mixture of cocci, bacilli, and club-shaped cells)



- All isolates demonstrated **similar biochemical characteristics**

	Thioglycolate Test: growth at the top of the broth and bottom of the broth, indicating capacity to grow with and without oxygen present		Motility Agar Stab: motile; results were confirmed by observing bacterial movement phase contrast microscopy		Nitrate Reductase: negative; red coloration after addition of zinc powder; no gas produced in Durham tube
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HYPOTHESES & EXPECTED RESULTS

- ★ *Burkholderia* s.l. can be selectively and differentially cultivated from microbial communities found in pitcher plant fluid.
- 🔍 Colonies of *Burkholderia* s.l. will become yellow when grown on Media 1 and dark purple when grown on Media 2.
- 🔍 *Burkholderia* s.l. are gram-negative bacteria with bacillus cell morphology.
- 🔍 *Burkholderia* s.l. is an obligate aerobe, is motile, and can reduce nitrate.

METHODS

I developed two selective and differential media² to cultivate *Burkholderia* s.l. on 1.5% agar plates.

1

- Deionized water
- Ribose
- Lactose
- 1% Bromothymol blue
- Tryptophan³

2

- Deionized water
- 1% Neutral red⁴
- 0.1% Crystal violet⁴
- Yeast extract⁵
- Soluble starch
- Dipotassium phosphate
- Ferrous sulfate⁶

RESULTS



Media 1 Community Spread Plate:

- Visible growth occurred after 96 hrs of incubation at 30 °C
- Colonies were a light pink-purple coloration
- No bacterial colonies isolated from media



Media 2 Community Spread Plate:

- Visible growth occurred after 48 hrs of incubation at 30 °C
- Fungal growth grew ~1 cm above agar surface
- Bacterial colonies grew under fungi, often small, round, dark purple colonies
- 4 bacterial colonies isolated from media

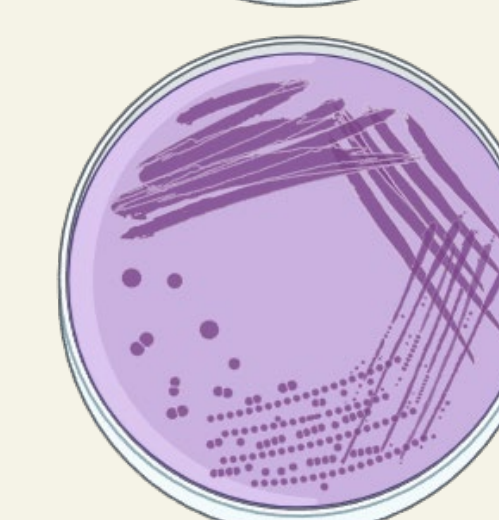
Media 1 Burkholderia Isolates Streak Plate:

ovular-to-round, smooth, off-white, mucosal colonies



Media 2 Burkholderia Isolates Streak Plate:

round, smooth, convex, dark purple colonies the size of a pin



KEY TAKEAWAYS

- ✓ Successful isolation of *Burkholderia sensu lato* from pitcher plant fluid using Media 2
- 🔍 *Burkholderia* is a gram variable bacterium with either cocci or bacilli cell morphology when inhabiting pitcher plant fluid
- 🔍 *Burkholderia sensu lato* occupies a role in pitcher plants which is potentially associated with oxygen-variable regions of the pitcher plant fluid, motility, and variability in cellular physiology

FUTURE QUESTIONS TO ADDRESS

- 🔍 What is the relationship between *Burkholderia* metabolism and pitcher plant nutrient acquisition?
- 🔍 What factors drive *Burkholderia* aggregation in pitcher plant fluid⁸?
- 🔍 What are the community dynamics between *Burkholderia* and the greater microbial community in pitcher plant fluid?

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