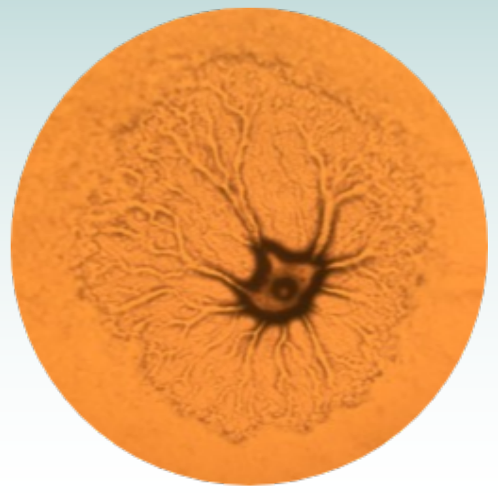

Image by Kara Ruff, 2019

Pathogens or symbionts? A study of the slime mold *Cavenderia aureostipes* var. Helvetia and its associated bacteria

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INTRODUCTION

- Studies on slime molds have found an intracellular bacterial symbiont able to persist through the "sterile" stalk and fruiting body stage[1]
- *Burkholderia* sp. confer the ability of *D. discoideum* to "farm" food bacteria[2,3] and secrete an effector that harms the fruiting body production of non-farmer amoeba[4]

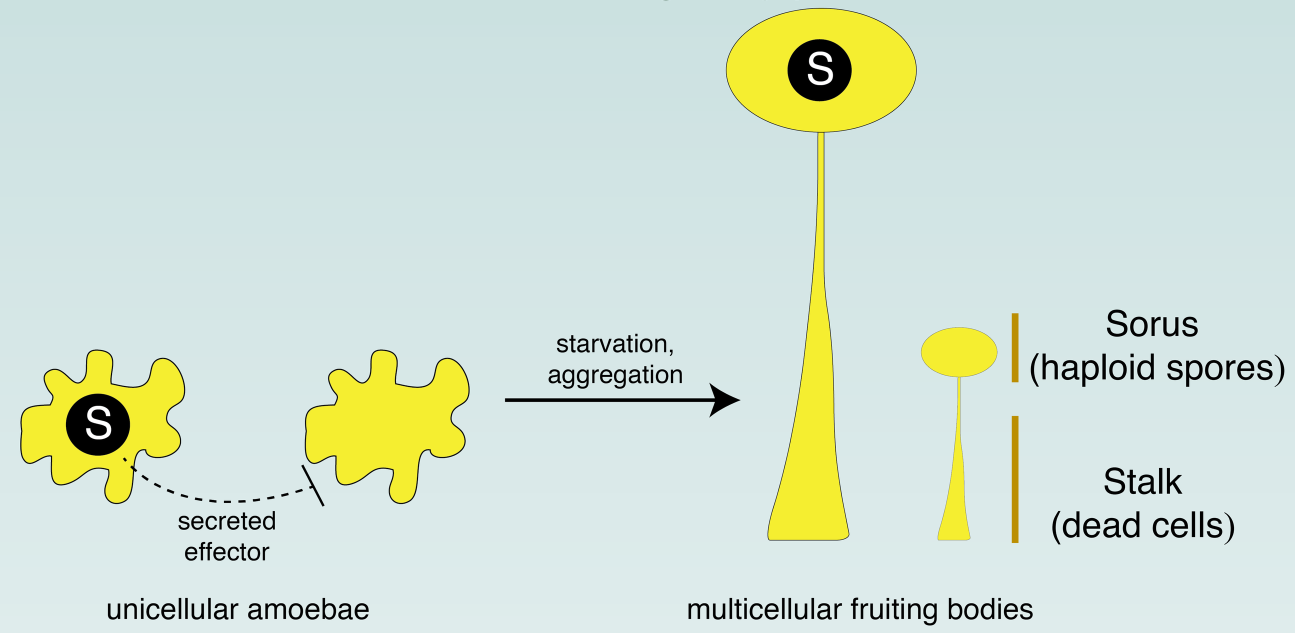


Figure 1. The cellular slime mole *Dictyostelium discoideum* has an intracellular symbiont (*Burkholderia* sp.) that secretes an effector molecule harming fruiting body production of "non-farming" amoeba. The symbiont can persist through multiple social cycles in the stalk and spores of *D. discoideum.*

- We surveyed wild slime mold isolates for possible symbionts
- We found 3 culturable bacterial species associated with ***C. aureostipes* var. Helvetia** that were somehow able to persist through multiple social cycles:
  - *Achromobacter* species 1
  - *Achromobacter* species 2
  - *Escherichia coli* strain 1

OBJECTIVES

To investigate the relationship between *C. aureostipes* var. Helvetia and associated bacteria by examining:

- location of the bacteria (**intracellular** or **extracellular**?)
- edibility of the bacteria (**edible** or **inedible**?)
- effect of the bacterial cell free supernatant (**promote** or **inhibit** growth?)

METHODS

E. coli B/r, *E. coli* Strain 1 *Achromobacter* sp. 1 and sp. 2 cultures grown at 28°C with shaking at 150rpm

Edibility of the bacteria

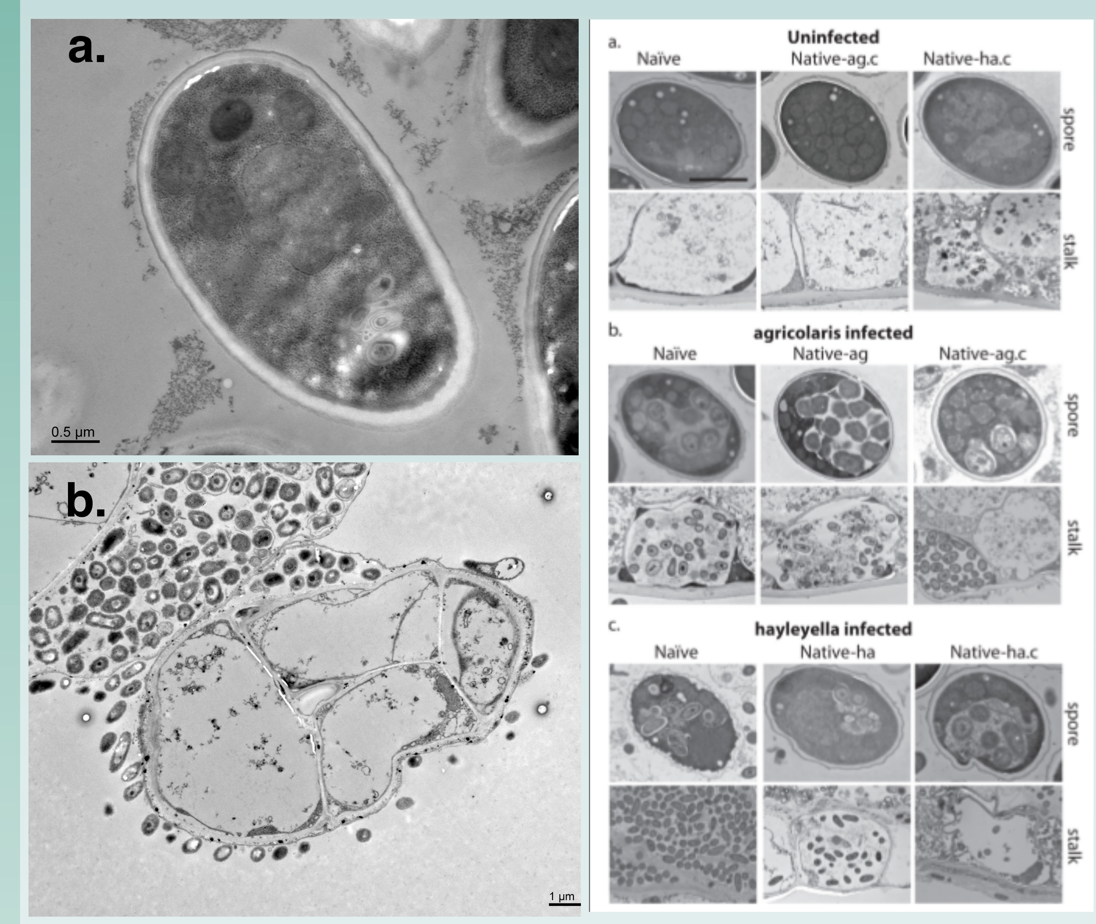
- Bacteria spread on SM/5 plates
- *C. aureostipes* var. Helvetia spores spotted in 4 quadrants
- Incubated at 22°C and scored after one week according to Brock et al.[5]
- Excellent / Good / Poor / Inedible

Effect of the bacterial cell free supernatant:

- *E. coli* B/r collected → *C. aureostipes* var. Helvetia spores added → Spread on 36 SM/5 plates[6]
- Cultures pelleted → Supernatant collected through 2μm filter tip → Diluted in LB to 1/10 and 1/100
- Undilute, 1/10 dilution, and 1/100 dilution of cell free supernatants of each bacterial species spread in triplicate
- Plates incubated at 22°C for one week and average number of sori per square centimeter determined
- Statistical analysis

RESULTS

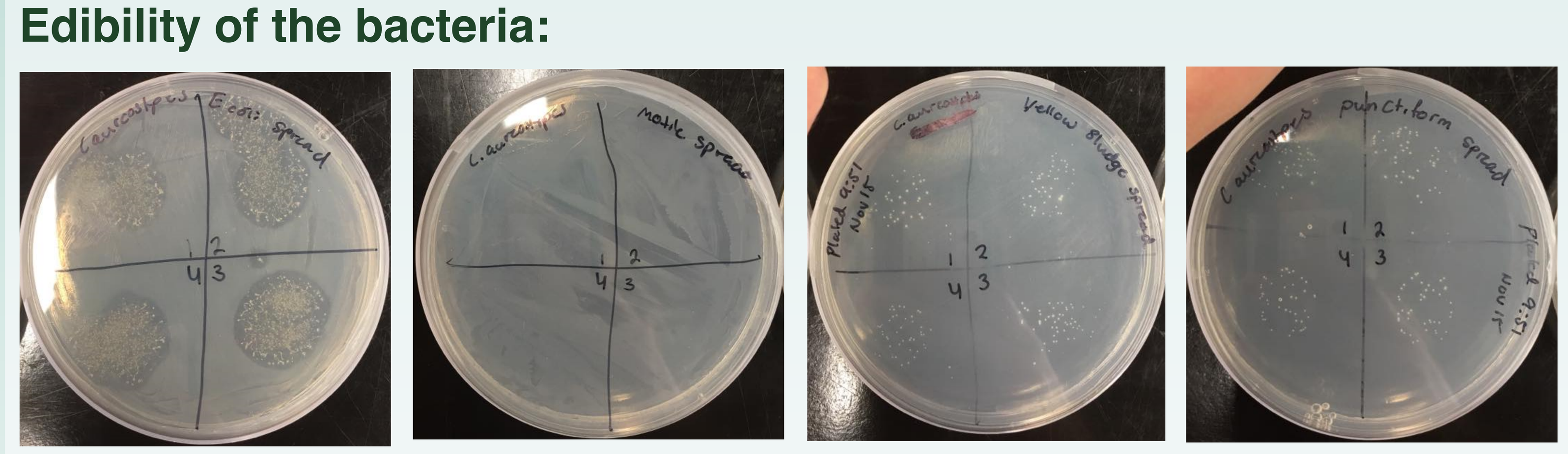
Location of the bacteria:



- No intracellular infection of spores or stalk
- Appearance of bacterial aggregates in a possible biofilm suggests bacteria may be maintained through multiple social cycles simply by "hanging on" to sori

Figure 2. Comparison of TEM images of a) spores and b) stalk cells to Figure 6 by Shu et al.[1]

Edibility of the bacteria:



E. coli B/r	*E. coli* strain 1	*Achromobacter* sp.1	*Achromobacter* sp.2
Control	**Inedible**	**Poor**	**Inedible**

Figure 3. Co-cultured bacteria are poorly edible in comparison to food bacteria (*E. coli* B/r). *Achromobacter* inedibility may be due to biofilm formation[7]. *E. coli* str. 1 may be pathogenic toward *C. aureostipes* based on observations and previous reports[8,9].

Effect of the bacterial cell free supernatant (between species):

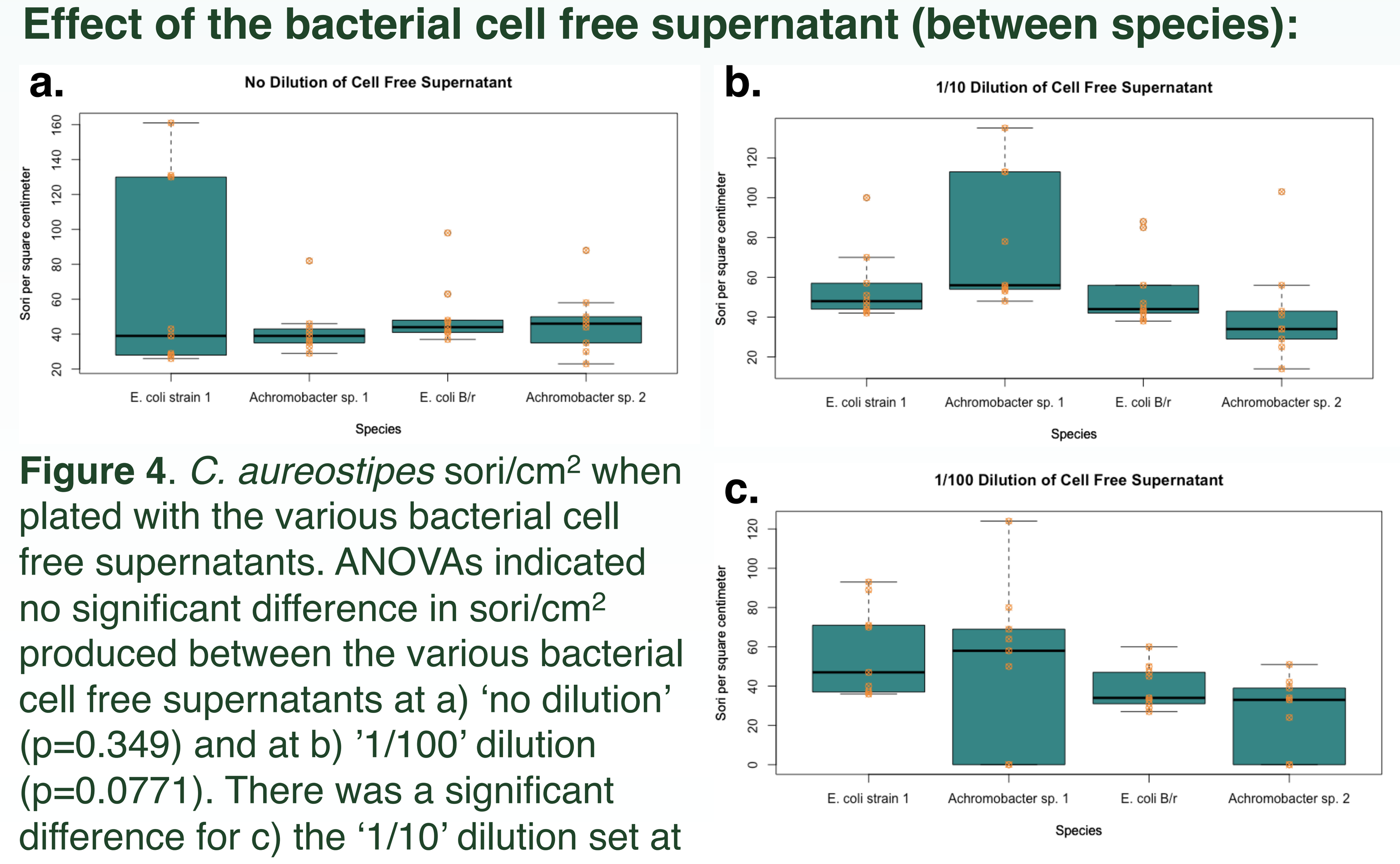


Figure 4. *C. aureostipes* sori/cm^2 when plated with the various bacterial cell free supernatants. ANOVAs indicated no significant difference in sori/cm^2 produced between the various bacterial cell free supernatants at a) 'no dilution' ($p=0.349$) and at b) '1/100' dilution ($p=0.0771$). There was a significant difference for c) the '1/10' dilution set at $p=0.032$. F-test indicated that difference was due to *Achromobacter* sp. 1 ($p=0.046$).

Effect of the bacterial cell free supernatant (within species):

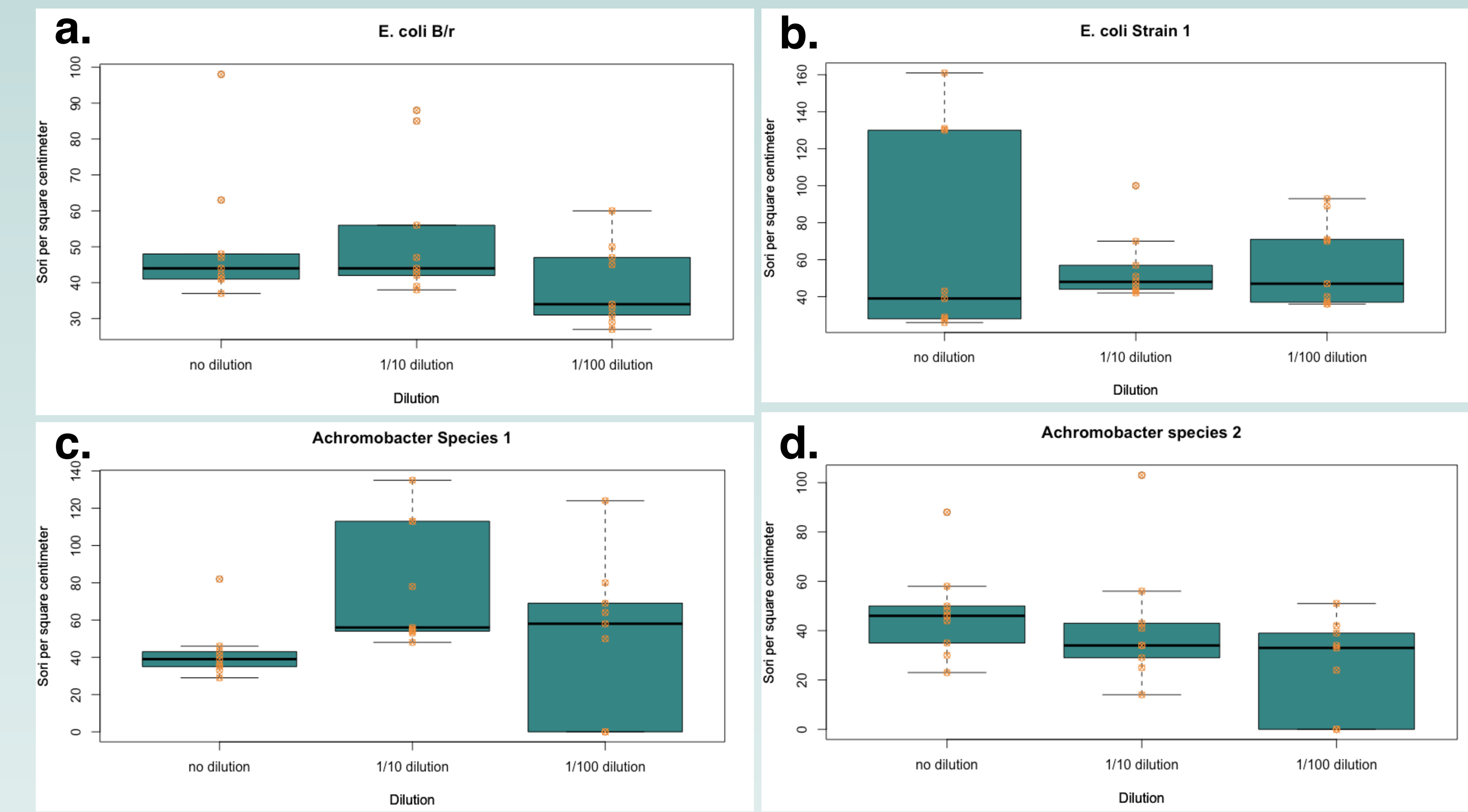


Figure 5. No dosage-dependent effect of co-cultured bacteria on *C. aureostipes* fruiting body density. Sori/cm^2 plated with cell free supernatant from a) *E. coli* B/r ($p=0.195$), b) *E. coli* Strain 1 ($p=0.731$), c) *Achromobacter* sp. 1 ($p=0.065$), and d) *Achromobacter* sp. 2 ($p=0.096$).

CONCLUSIONS

- The bacteria are **NOT** intracellular symbionts of *C. aureostipes*
- *Achromobacter* sp. 1 has poor edibility and *Achromobacter* sp. 2 is inedible, while *E coli* species 1 may be pathogenic
- Significant reduction in sori/cm^2 for the 1/10 dilution of *Achromobacter* sp. 2 likely due to human error: dose-dependent effect not observed
- Analysis into the genetic basis for inedibility/resistance may provide insight into the possibly pathogenic basis of these bacteria for both Dictyostelids

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