

# Exposure to dense bacteria lawns does not cause the social amoeba *Dictyostelium discoideum* to carry bacteria through the social stage

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**Background.** Interactions between eukaryotic amoebae and bacteria are understudied and important. Bacteria inside of amoebae are protected from external forces including antibiotics. An excellent model for bacteria-amoeba interactions is the social amoeba *Dictyostelium discoideum* and its associated bacteria. A third of wild-collected clones of the soil-dwelling amoeba *Dictyostelium discoideum* exhibit a suite of characteristics that make them simple farmers of bacteria. They carry bacteria internally through the social spore-making stage. They then release these bacteria to grow and subsequently eat them, prudently stopping before they are entirely consumed so some bacteria can be carried to the next generation. *D. discoideum* defend their food bacteria with other inedible bacteria that produce compounds toxic to non-farmers. Both carried bacteria and social amoeba hosts have demonstrated co-evolved characteristics. Most farmer clones discovered to date carry inedible *Burkholderia* in addition to food bacteria, but it is not clear whether or not a preponderance of naïve bacteria might induce the farming state by overwhelming the phagocytic actions of the host amoebae. In this study we address this question with *D. discoideum* clones that naturally carry bacteria and those that do not. Will naïve bacteria in large numbers succeed in colonizing the amoebae?

**Methods.** We grew five non-farmer clones and five farmer clones of wild-collected *Dictyostelium discoideum* on three different concentrations of a highly palatable bacterial food source, *Klebsiella pneumoniae*. We then tested them to see if they carried bacteria through the social stage.

**Results.** We found that bacterial density did not have a significant effect on whether or not the clones carried bacteria through the social stage. Even those grown in very dense bacterial cultures were able to shed them successfully unless they were also carrying *Burkholderia*.

**Discussion.** Our results indicate that even a preponderance of food bacteria cannot overwhelm the ability of social amoebae to digest and not carry bacteria. Apparently, only the inedible *Burkholderia* have that effect. This points to the importance of understanding co-infection with multiple bacteria because those that cannot induce carriage can nevertheless become carried, foiling digestive processes, but only in the presence of another bacterium. Future studies of host bacteria interactions should consider using multiple bacteria simultaneously.

Running head:

Exposure to bacteria and bacteria carriage in the social amoeba

**Exposure to dense bacteria lawns does not cause the social amoeba  
*Dictyostelium discoideum* to carry bacteria through the social stage**

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# ABSTRACT (500 words)

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## Keywords

Bacteria; *Burkholderia*; *Dictyostelium discoideum*; symbiosis; co-infection; amoebae

Finding nutrients is essential for all organisms. However, certain environments contain fluctuating resources where preferred food is not always available. This is particularly likely to be true of highly heterogeneous soil environments. To maximize survival, many organisms must adapt to both high-resource and low-resource environments. Phenotypic plasticity is observed in many genera, is defined as the ability of an organism to adopt different phenotypes depending on its environment, and may impact the origination of novel phenotypes [1, 2]. For example, captive mallard ducks, *Anas platyrhynchos*, are able to alter their digestive organs based on diet and fiber availability [3]. Mallards eating a poor quality diet increase the size of their digestive organs to maximize digestion efficiency. Similar diet response changes have been documented in other organisms such as house sparrow nestlings [4], Mongolian gerbils [5], colocolo opossums [6], and African clawed frogs [7].

Alternatively, some organisms have evolved mechanisms to address this issue by using agriculture to grow their preferred food. Well-studied examples of agriculture are found in ants, termites, and ambrosia beetles, where these insect farmers became dependent on cultivated crops for food to minimize environmental variance [8-10]. These three types of insect agriculturists have obligate relationships with their cultivated food source. However, we might expect that a facultative farming phenotype, where both partners can grow independently, may exhibit plasticity and change based on the environment.

Some clones of the social amoeba *Dictyostelium discoideum* have a primitive type of bacterial farming similar to fungal farming in ants, termites, and ambrosia beetles [11]. *D. discoideum* is a eukaryotic amoeba found in the soil and leaf litter with a unique life cycle composed of a solitary, vegetative stage and a social, multicellular stage [12]. In the solitary stage, amoebae prey on bacteria and divide mitotically. If food bacteria become exhausted, *D. discoideum* amoebae starve and enter into the social stage. Here, *D. discoideum* amoebae aggregate in the thousands. Slugs form that are capable of migrating in search of new food sources. After migration, the slug forms a fruiting body containing about 80% reproductive spores and about 20% stalk cells that altruistically die to aid in spore dispersal [13].

We recently discovered that some clones carry an inedible bacterium, *Burkholderia* sp., which allows them to also carry edible bacteria, essentially turning them into farmers [14]. Bacteria are carried inside the fruiting body and can seed new food populations when dispersed into food-scarce conditions. In addition to carrying bacteria, farmer clones have reduced slug migration distances, harvest food bacteria prudently (form fruiting bodies before all bacteria are eaten), and are resistant to toxic secretions of *Burkholderia* [11, 15]. For the prudent harvesting trait, farmers forego eating all available food bacteria to save some for transport while non-farmers instead maximize growth by eating all available food bacteria [11]. Therefore, when preferred food bacteria are absent or few, farmers are able to capitalize on their transported bacteria and produce many more spores than non-farmers. However, when preferred food bacteria are plentiful, non-farmers can produce more spores and out-compete farmers. One indication that bacteria density matters is that we have previously found Allee effects in *D. discoideum* interactions with bacteria [16]. In this case whether or not bacteria were toxic to *D. discoideum* depended on their density. In this study we ask another question related to density. Can dense populations of bacteria overwhelm *D. discoideum*'s digestive processes and become

carried? Or is bacteria carriage entirely controlled by *Burkholderia* already in *D. discoideum* spores?

To test this, we grew *D. discoideum* clones at three different bacterial densities, attained by varying the nutrient content of the agar plates and then adding a standard number of bacteria to proliferate. We did not find support for our hypothesis. Only prior carriage of *Burkholderia* influenced the ability of *D. discoideum* to carry food bacteria.

## MATERIALS AND METHODS

We grew amoebae from 10 different clones at 3 different bacterial densities to see if high bacterial density could induce carriage through the *D. discoideum* social cycle. Five clones were farmers, already carrying *Burkholderia*, and five were bacteria-free. We tested them by placing fruiting bodies on nutrient plates without bacteria and scoring bacterial growth. Figure 1 shows our experimental procedure.

### Wild *D. discoideum* isolates and culture conditions

We used five farmer clones (QS11, QS21, QS22, QS23, and QS155) and five non-farmer clones (QS1, QS6, QS9, QS17, and QS18) collected at Mountain Lake Biological Station in Virginia (GPS coordinates: N 37° 21', W 80° 31') for our test population. The five farmers used in this study each carry *Burkholderia* Clade 2 [11, 14] as well as the lab food *Klebsiella pneumoniae*. One farmer (QS155) also carries *Stenotrophomonas maltophilia* K279a (closest identity by 16S rRNA). We plated wild clones from spores on nutrient agar petri plates (2 g glucose, 2 g Oxoid bactopectone, 2 g Oxoid yeast extract, 0.2 g MgSO<sub>4</sub>, 1.9 g KH<sub>2</sub>PO<sub>4</sub>, 1 g K<sub>2</sub>HPO<sub>4</sub>, and 15.5 g agar per liter DDH<sub>2</sub>O) in association with *K. pneumoniae* at room temperature (22°C) to prepare stock plates for experiments. We prepared our bacterial food stock by growing shaking overnight cultures of *K. pneumoniae* in liquid Luria broth (LB) (10 g tryptone, 5 g Oxoid yeast extract, and 10 g NaCl per liter DDH<sub>2</sub>O) at 25°C.

### Experimental stock plate preparation and culture conditions

To test whether available food bacteria density changes the ability of a clone to carry bacteria through the social stage, we used a standard bacteria media and decreased nutrient amounts for food bacteria by five-fold and ten-fold. The three different medias are SM (10g peptone, 1g yeast extract, 10g glucose, 1.9g KH<sub>2</sub>PO<sub>4</sub>, 1.3g K<sub>2</sub>HPO<sub>4</sub>, 0.49g MgSO<sub>4</sub> anhydrous and 15.5g of agar per liter DDH<sub>2</sub>O), SM/5 (2g peptone, 0.2g yeast extract, 2g glucose, 1.9g KH<sub>2</sub>PO<sub>4</sub>, 1.3 g K<sub>2</sub>HPO<sub>4</sub>, 0.2 g MgSO<sub>4</sub> anhydrous and 15.5g of agar per liter DDH<sub>2</sub>O), and SM/10 (1g peptone, 0.1g yeast extract, 1g glucose, 1.9g KH<sub>2</sub>PO<sub>4</sub>, 1.3g K<sub>2</sub>HPO<sub>4</sub>, 0.1g MgSO<sub>4</sub> anhydrous and 15.5g of agar per 1L of DDH<sub>2</sub>O). We collected spores from five farmers and five non-farmers in starvation buffer (2.25g KH<sub>2</sub>PO<sub>4</sub> and 0.67g K<sub>2</sub>HPO<sub>4</sub> per liter DDH<sub>2</sub>O) and counted with dilution using a hemocytometer and light microscope to determine spore density. Next we plated 2x10<sup>5</sup> spores with 200μL of *K. pneumoniae* on each of the three media concentrations.

### Determining Bacterial density

To establish the link between plate composition and bacterial density, we determined bacteria colony forming units for *K. pneumoniae* grown on agar plates containing varying nutrient densities. We made an overnight liquid culture of *K. pneumoniae* and incubated it at 21°C. We set the concentration of bacteria at 1.5 O.D. A600. Next, we plated 200μL of *K. pneumoniae* on

SM, SM/5, and SM/10 nutrient agar plates (see stock plate preparation above). After two days of bacterial growth, we collected the bacteria in a measured volume of starvation buffer for each plate and determined the number of bacteria on the plate through serial dilutions to get colony forming units per microliter. We performed three replicates.

### **Bacteria presence assay to determine farmer or non-farmer status**

In order to determine if farmer or non-farmer status changes during growth on different bacterial densities, we set up bacteria presence assays (spotting tests) seven days after plating and growing clones on treatment plates. For each clone and each treatment, we collected 20 individual sori by touching the top of a sorus with a pipet tip with a filter using a dissecting scope. Then we transferred the contents of each sorus by touching the pipet tip to a fresh SM/5 agar plate with no added *K. pneumoniae*, making a grid of twenty individual spots on the plate. After three days, we checked the plates for bacterial growth. See Figure 2 for representative experimental spot test plates. We tabulated total number of positive spot tests for each clone in each nutrient density. We used these data to determine if farmer and non-farmer status changed for a particular clone based on whether or not we observed bacteria growth from individual spotted sorus contents on the nutrient agar plate. Positive bacterial growth for a majority of the twenty spots indicates farmer status based on bacteria carriage; negative bacterial growth for a majority of the spots indicates non-farmer status (Figure 4). For each clone and each nutrient level, we performed two replicates.

### **Statistical analyses**

We analyzed our data using a mixed model ANOVA with fixed effects (farmer and non-farmer) and a random affect (clone). Analysis specifics are found in the results section. Standard error and F-statistics were Kenward Rogers (KR) corrected so that degrees of freedom are approximated using variances and correlations in the observed data (Kenward and Rogers 1997). We used SAS software (Version 9-2 of the SAS System for Windows, Copyright 2002-2003, SAS Institute Inc.) to analyze our data.

## **RESULTS**

First we show that our way of manipulating bacterial density is effective. Then we show that food density does not change whether *D. discoideum* carry bacteria through the social stage.

### **Bacterial densities increase with plate nutrient density.**

For our experiments with *D. discoideum*, we added a fixed number of bacteria to each treatment, expecting them to proliferate more on plates with richer media. To show that this was the case, we grew *K. pneumoniae* on three different media with decreasing nutrient densities: SM, SM/5, and SM/10. SM contains five times more nutrients for bacteria than SM/5 and ten times more than SM/10. We plated a fixed amount of our food bacteria, *K. pneumoniae*, on each of the three densities in triplicate. After growth, we collected the bacteria and determined the number of colony forming units (CFUs) for each nutrient density under this regime. We found that the number of bacteria present on each media type was proportional to the nutrient density (Figure 3). SM produced  $55.63 \times 10^{10}$  CFUs  $\pm 5.33$ , SM/5 produced about one fifth the CFUs of SM at  $11.06 \times 10^{10}$  CFUs  $\pm 0.36$ , and SM/10 produced about one tenth the CFUs of SM at  $5.26 \times 10^{10}$  CFUs  $\pm 0.18$ . Based on these data, we used plate nutrient density as a proxy for bacterial density.



# ***D. discoideum* grown with more bacteria are not more likely to carry them through the social stage.**

To determine if bacterial carriage is a plastic trait dependent on the density of bacteria available, we used five farmers and five non-farmers grown independently on three different types of nutrient agar plates (SM, SM/5, and SM/10) as a proxy for bacterial density. We tested twenty sori for bacteria presence from each clone in each nutrient density. We found bacteria carriage is not dependent on food abundance (Figure 4). Carriage for both farmers and non-farmers is statistically unchanged in the three nutrient levels (Farmer\*Nutrient Level  $F_{2,46} F = 0.17 p = 0.8462$ ); individual farmers and non-farmers do not differ from each other in each nutrient level (Nutrient Level  $F_{2,46} = 3.18 p = 0.0506$ ); and farmer status also remains unchanged in the three nutrient densities (Farmer  $F_{1,8} = 368.86 p < 0.0001$ ). We used a post hoc Tukey HSD test to correct for multiple comparisons and significant differences are indicated by different letters. Error bars equal s.e.m; N equals 10 with two biological replicates. These data show that the bacterial density found in the environment of a *D. discoideum* clone does not play a role in whether or not bacteria are carried through the clearing stage during development into a fruiting body.

## **DISCUSSION**

Our results suggest that farmers carry bacteria with the same frequency regardless of bacterial density. Non-farmers tested were unable to farm, irrespective of food density. Thus, the amount of bacteria available in the environment during the vegetative stage had no effect on the ability of clones to carry bacteria through the social stage. Clearly *Burkholderia* are necessary for clones to also carry food bacteria.

We still have many questions about this system. We might have expected that the relationship between bacterial density and carriage went the opposite direction if *D. discoideum* were in control, since they might have more need of bacteria in a new place if edible ones are generally scarce. Our results did not support this opposite hypothesis.

We can also ask why have *Burkholderia* not swept through the population of *D. discoideum*, since they confer the advantage of allowing bacterial carriage through the social stage? There may be costs to carrying *Burkholderia* in the vegetative stage where clones of *D. discoideum* simply eat bacteria and then divide through mitosis. Perhaps digestion slows this form of proliferation. However, we have prior evidence against this where we showed that farmer clones did not proliferate less well in the vegetative stage than non-farmer clones [11].

It is unusual that farming is not universal in *D. discoideum*, present in some but not other clones. Indeed, the insect examples we mentioned in the beginning all have fixed farming across the species. That it is variable in *D. discoideum* means that there are great opportunities for future research on the costs and benefits of the interaction, and on what changes occur in all the partners over evolutionary time. Completely novel symbiosis interactions will also inform this endeavor [17].

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# LITERATURE CITED

1. Pigliucci, M., *Phenotypic plasticity: beyond nature and nurture*. 2001: JHU Press.
2. West-Eberhard, M.J., *Phenotypic plasticity and the origins of diversity*. Annual review of Ecology and Systematics, 1989: p. 249-278.
3. Kehoe, F.P., C.D. Ankney, and R.T. Alisauskas, *Effects of dietary fiber and diet diversity on digestive organs of captive Mallards (Anas platyrhynchos)*. Canadian Journal of Zoology, 1988. **66**(7): p. 1597-1602.
4. Brzęk, P., K. Kohl, E. Caviedes-Vidal, and W.H. Karasov, *Developmental adjustments of house sparrow (Passer domesticus) nestlings to diet composition*. Journal of Experimental Biology, 2009. **212**(9): p. 1284-1293.
5. Liu, Q.-S. and D.-H. Wang, *Effects of diet quality on phenotypic flexibility of organ size and digestive function in Mongolian gerbils (Meriones unguiculatus)*. Journal of Comparative Physiology B, 2007. **177**(5): p. 509-518.
6. Cortés, P.A., M. Franco, P. Sabat. S.A. Quijano, and R.F Nespolo, *Bioenergetics and intestinal phenotypic flexibility in the microbiotherid marsupial (Dromiciops gliroides) from the temperate forest in South America*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2011. **160**(2): p. 117-124.
7. Sabat, P., J.M. Riveros, and C. López-Pinto, *Phenotypic flexibility in the intestinal enzymes of the African clawed frog Xenopus laevis*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2005. **140**(1): p. 135-139.
8. Mueller, U.G., N.M. Gerardo, D.K. Aanen, D.L. Six, and T.R. Schultz, *The evolution of agriculture in insects*. Annual Review of Ecology, Evolution, and Systematics, 2005: p. 563-595.
9. Aanen, D.K., P. Eggleton, C. Rouland-Lefèvre, and T. Guldberg-Frøslev, *The evolution of fungus-growing termites and their mutualistic fungal symbionts*. Proceedings of the National Academy of Sciences, 2002. **99**(23): p. 14887-14892.
10. Farrell, B.D., A.S. Sequeira, B.C. O'Meara, B.B. Normark, J.H. Chung, and B.H. Jordal, *The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae)*. Evolution, 2001. **55**(10): p. 2011-2027.
11. Brock, D.A., T.E. Douglas, D.C. Queller, and J.E. Strassmann, *Primitive agriculture in a social amoeba*. Nature, 2011. **469**(7330): p. 393-396.
12. Kessin, R.H., *Dictyostelium - Evolution, cell biology, and the development of multicellularity*. 2001, Cambridge, UK: Cambridge Univ. Press. xiv + 294.
13. smith, j., D.C. Queller, and J.E. Strassmann, *Fruiting bodies of the social amoeba Dictyostelium discoideum increase spore transport by Drosophila*. BMC evolutionary biology, 2014. **14**(1): p. 105.
14. DiSalvo, S., T.S. Haselkorn, U. Bashir, D. Jimenez, D.A. Brock, D.C. Queller, and J.E. Strassmann, *Burkholderia bacteria infectiousy induce the proto-farming symbiosis of Dictyostelium amoebae and food bacteria*. Proceedings of the National Academy of Sciences, 2015. **112**(36): p. E5029-E5037.
15. Brock, D.A., S. Read, A. Bozhchenko, D.C. Queller, and J.E. Strassmann, *Social amoeba farmers carry defensive symbionts to protect and privatize their crops*. Nature Communications, 2013. doi: 10.1038/ncomms3385.



16. DiSalvo, S., D.A. Brock, j. smith, D.C. Queller, and J.E. Strassmann, *In the social amoeba, Dictyostelium discoideum, density, not farming status, determines predatory success on unpalatable Escherichia coli*. BMC Microbiology, 2014. **14**(1): p. 328.
17. Momeni, B., C.-C. Chen, K.L. Hillesland, A. Waite, and W. Shou, *Using artificial systems to explore the ecology and evolution of symbioses*. Cellular and molecular life sciences, 2011. **68**(8): p. 1353-1368.

## FIGURE LEGENDS

**Figure 1. Diagrammatic representation of the spot test (bacteria presence or absence) experiment.** Tan rectangular bars are nutrient agar plates in three densities: high (SM), medium (SM/5), and low (SM/10).

**Figure 2. Sample spot test plates.** A. Random spore masses spotted from a representative *D. discoideum* farmer clone are positive for bacteria presence. Spots show bacteria and *D. discoideum* fruiting bodies. B. Random spore masses spotted from a representative *D. discoideum* non-farmer clone are negative for bacteria presence. Spots are blank. (Photo credit Debra Brock)

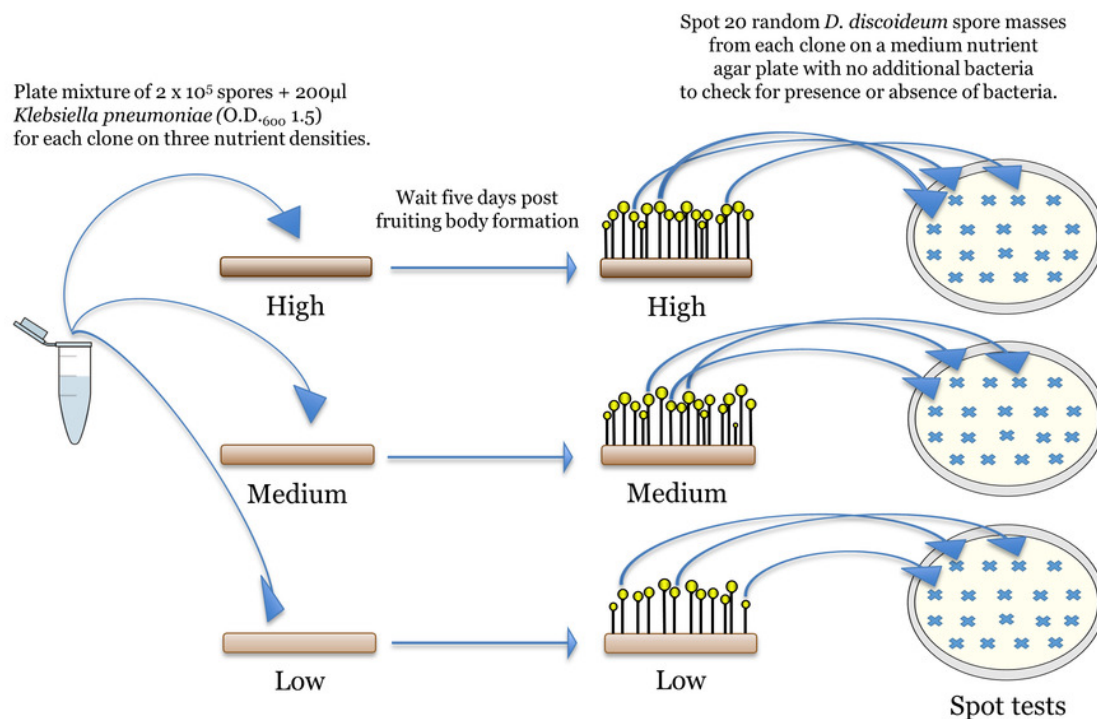
**Figure 3. Bacteria increase on plates made with more nutrients.** We began our plates with equal numbers of *K. pneumoniae* bacteria and relied on their proliferation under different nutrient conditions to generate the variation in prey for the social amoebae *D. discoideum*. This figure shows that greater nutrient density results in greater numbers of prey bacteria. CFUs equal colony forming units of culturable bacteria.

**Figure 4. Bacteria carriage by *D. discoideum* farmers is not dependent on food abundance.** We tested twenty random fruiting body sori for bacteria presence from each of five different farmer and five different non-farmer clones grown on three different types of nutrient agar plates as a proxy for bacteria density. SM plates are nutrient rich and so support the highest bacteria density if bacteria are present in the fruiting bodies while SM/10 plates have the least. We scored for positive or negative bacteria growth after 5 days. We found bacteria present in nearly all tested farmer fruiting bodies regardless of the nutrient density of the medium they were plated on while bacteria were absent in nearly all the non-farmer fruiting bodies under the same conditions. We used a post hoc Tukey HSD test to correct for multiple comparisons and significant differences are indicated by different letters. Error bars equal s.e.m; N equals 10 with two biological replicates.

# Figure 1

Diagrammatic representation of the spot test (bacteria presence or absence) experiment.

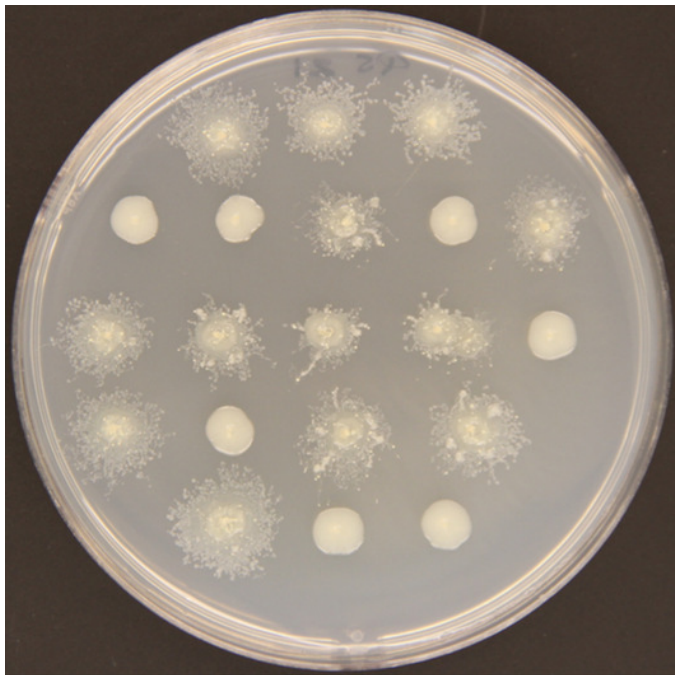
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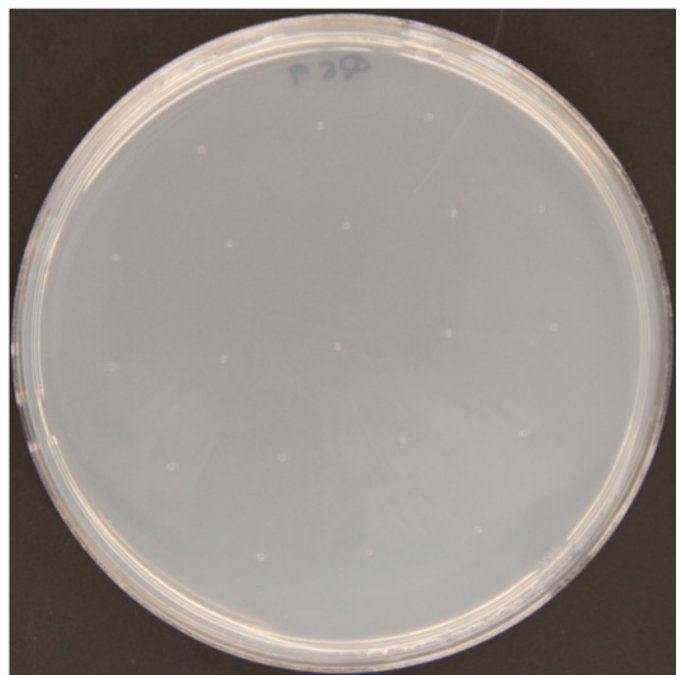
## Figure 2

Sample spot test plates.

A. Random spore masses spotted from a representative *D. discoideum* farmer clone are positive for bacteria presence. Spots show bacteria and *D. discoideum* fruiting bodies. B. Random spore masses spotted from a representative *D. discoideum* non-farmer clone are negative for bacteria presence. Spots are blank. (Photo credit Debra Brock)



Farmer

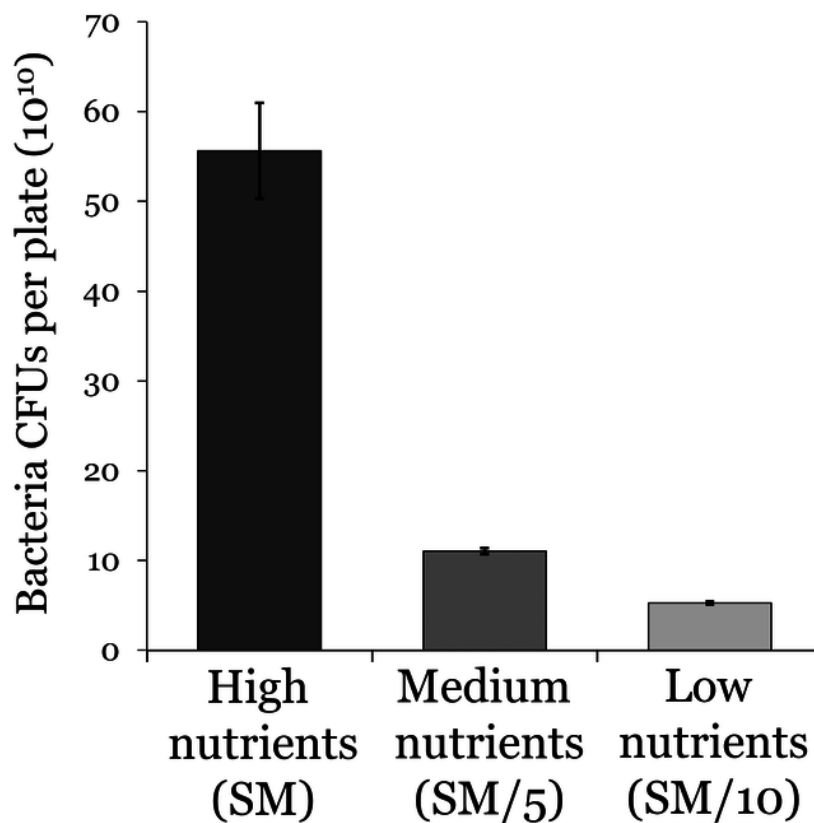


Non-farmer

## Figure 3

Bacteria increase on plates made with more nutrients.

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