Science in School
The European journal for science teachers

ISSUE 67 – April 2024 **Topics** Biology | Sustainability

Image courtesy of the author

Footprints in the agar: growing bacteria from ants' feet to combat plant diseases

Ida Cecilie Jensen

A walk on the wild side: invite some ants to take a walk on your petri dish and discover how bacteria from their feet could help us reduce pesticide use.

Introduction

Ants are superorganisms that are masters of cooperation. They use pheromones to communicate quickly and effectively with each other, which also makes them efficient hunters. Most ants eat a lot of different insects, which makes them a great alternative to chemical pesticides.

As ants live closely together, they are at high risk of catching and transmitting diseases. To protect themselves against diseases, they produce a wide variety of antibiotic chemicals,[1] and they even house bacteria and fungi on their bodies and feet, which, in some cases, also produce antibiotics.[1] Thus, when ants walk, they leave behind a trail of microorganisms. Some of these produce antibiotic chemicals that can be effective against a wide array of pathogens, including plant diseases. So, when ants walk across a plant, they can actually protect it against diseases. In this way, ants are guardians of the forest, as they can protect trees against harmful insects and diseases.

Ants live in dense, social societies, where they are in close contact with each other – they groom each other, and even feed their nestmates mouth to mouth. This increases the risk of catching and transmitting diseases.
Image courtesy of the author

Ants have been used in agriculture for centuries,[2] as their protective effect in forests can be moved into agricultural systems, like fruit orchards. Here, ants can be used to protect crops against both pest insects and crop diseases,[3,4] just like they naturally protect trees in a forest. Using ants instead of chemical pesticides is both sustainable and organic and serves as a promising tool for feeding the world in the future. The following experiments are suitable for students aged 11–19. The experiments give insights into the complex microbial community of ants, and showcase how ants and their associated microorganisms can be used to combat plant diseases, and maybe in the future even human diseases. The activities link to the following curriculum topics: microbiology, ecology, sustainability, biology, agrobiology, zoology, antibiotics, applied biology.

Activity 1: Discovering the diversity of bacteria and fungi on ants' feet

Through this experiment, students can investigate the diversity of bacteria and fungi that ants have on their feet. This can be done in smaller groups of two to three students. The experiment should be performed under as sterile conditions as possible.

The first part of the experiment (setting up the incubations) takes around an hour if the teacher knows where to find some ants nearby and the agar plates have been prepared beforehand, but can be done in 30 min if ants have already been collected. Time can vary depending on the students' age. The results will be observable after 2–3 days and a second session will be needed to analyse and discuss them.

Notes

- For these experiments, potato dextrose agar (PDA) is used. PDA is a very common medium used in microbiology, and all agar in the following activities is PDA. This can be bought pre-poured into Petri dishes, but if you want to prepare the culture plates yourself, see the teacher instructions in the supporting material.
- Microbiologists usually refer to agar-filled Petri dishes as 'agar plates' or 'culture plates', so when plate/plates are mentioned in the instructions, this refers to agar-filled Petri dishes.

Materials

- The Ant infosheet
- Agar plate with PDA
- Petri dish without agar (to capture or keep ants in)
- Inoculation loops
- Nitrile gloves
- Ziplock bags
- Timer

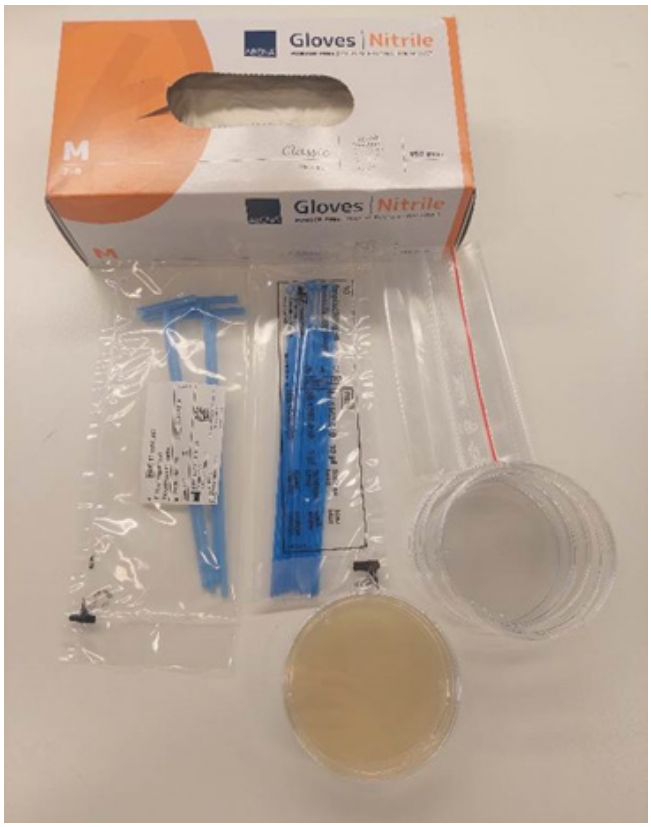

Figure1: Materials for Activity 1
Image courtesy of the author

Procedure

Before starting with the practical steps, the Ant infosheet can be used to provide students with some background information to understand the context. Alternatively, this can be discussed in the second lesson when the results are discussed.

Collecting ants

Ants can be found in many different habitats, including forests, parks, and even on pavements. To collect ants, bring a few empty Petri dishes (keep them in a ziplock bag, so they remain sterile), a pack of inoculation loops, and some nitrile gloves. Do not touch the Petri dishes or inoculation loops without gloves; they need to be kept sterile.

1. Wearing gloves, one team member holds the Petri dish, while another carefully grabs an ant using the inoculation loop (this can be done by putting the loop next to the ant; they will usually crawl onto the loop or bite it).
2. Gently put the ant into the Petri dish. You can use the lid to gently slide the ant off. You need one ant per agar plate, so collect ants accordingly, including a few ants as backup.

If you plan on doing Activity 2 following Activity 1, you can save time by collecting ants for this experiment as well.

Putting ants on the agar plates

Once the ants have been collected, they are brought back to the laboratory.

1. Wearing gloves, a team member takes a clean inoculation loop, and picks up an ant from the Petri dish.
2. Gently put the ant on the agar plate (keep the lid closed after adding the ant). Be careful not to touch the agar with the inoculation loop, to avoid potential contamination.
3. Start a timer. The ant should roam around on the agar for at least 10 min.
4. After 10 min, remove the ant and put it back into the Petri dish, so it can be released outside. The agar plates are then incubated at room temperature for 2–3 days. Plates should be stored in plastic bags with the lid of the Petri dish facing down.

Expected results

After a few days at room temperature, microbial growth is visible (figure 2). If you are lucky, the exact steps of the ant are visible as tiny microbial footprints.

Figure 2: An agar plate inoculated with grey mould upon which an ant has been roaming around. This is 24 h after the ant was removed, so no grey mould growth is visible yet. However, the microbial trail from the ant is visible.
Image courtesy of the author

Students can count the number of colonies, or describe their morphology (colour, form, structure, texture) and compare it to the other students' results. Older students can examine the different microorganisms under a microscope, to assess different cell structures (bacteria versus fungi). If different ant species are tested by different groups, they can compare their results to see if there are differences between the species in terms of microbial communities.

Activity 2: Antimicrobial effect of ant footprints

Some of the bacteria and fungi that ants have on their legs and feet are antimicrobial. Through this activity, students will investigate whether the ants transfer any antimicrobial microorganisms that are able to control a plant pathogen.

The first part of the experiment (setting up the incubations) takes around an hour if the teacher knows where to find ants or if the ants were already collected during Activity 1, and if the agar plates were inoculated with the pathogen by the teacher beforehand. However, it can be extended to 2 h for older students, who can inoculate the plates themselves (15–19 years is recommended for this). Results will be observable after 2–3 days and a second session will be needed to analyse and discuss them.

See the teacher instructions for details on how to autoclave the water and grow initial plant pathogen cultures (*Botrytis cinerea* or *Fusarium graminearum* are recommended; the

former is used as an example here). This experiment should be performed under as sterile conditions as possible.

Part 1: Inoculating the agar plates

This can be done by students on the day of the experiment (the agar plates need to dry for 5 minutes before ants are added) or by the teacher beforehand. If the teacher chooses to inoculate the agar plates beforehand, this should not be done more than 12 h in advance.

Materials

- Drigalski spatula
- 6 ml syringe
- Sterile water (autoclaved Milli-Q water or demineralized water)
- Agar plate with *B. cinerea* grown until the pathogen is visible
- Agar plate with PDA
- Pipette and pipette tips

Figure 3: Materials for Activity 2
Image courtesy of the author

Procedure

1. Wearing gloves, take the syringe, and fill it with sterile water (5 ml).
2. Add the sterile water (5 ml) to the agar plate containing *B. cinerea*.
3. Use an inoculation loop to gently scrape the entire surface of the agar plate, to loosen hyphae and spores. Once the solution is murky, it is ready to use (figure 4).

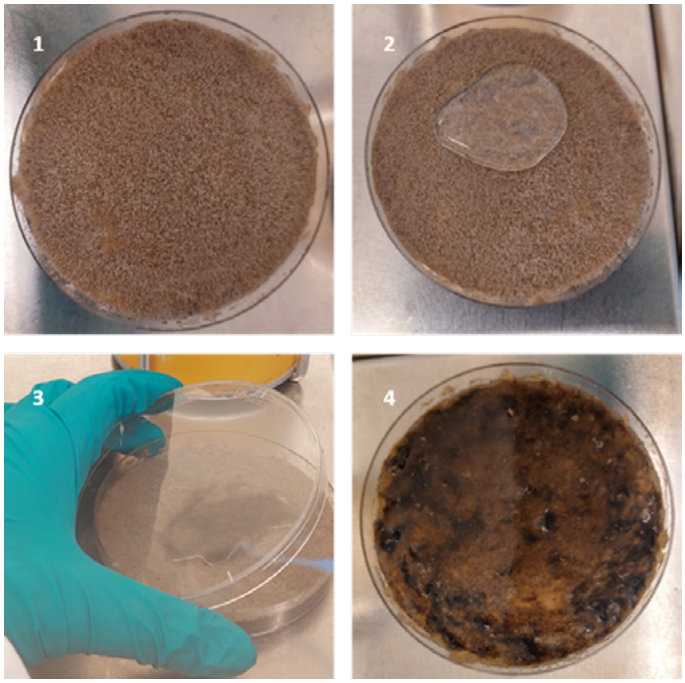

Figure 4: How to make the plant pathogen inoculum: 1) take a fully overgrown plate with pathogen (in this case *B. cinerea*); 2) add sterile water (5 ml) using a syringe; 3) gently scrape the surface of the agar plate to loosen the hyphae and spores and suspend them in the water; 4) once the whole plate has been scraped and the solution is murky, it is ready to be used.
Image courtesy of the author

4. Pipette the *B. cinerea* solution (50 µL) onto a clean agar plate.
5. Use a Drigalski spatula to evenly distribute the solution on the agar plate (figure 5).

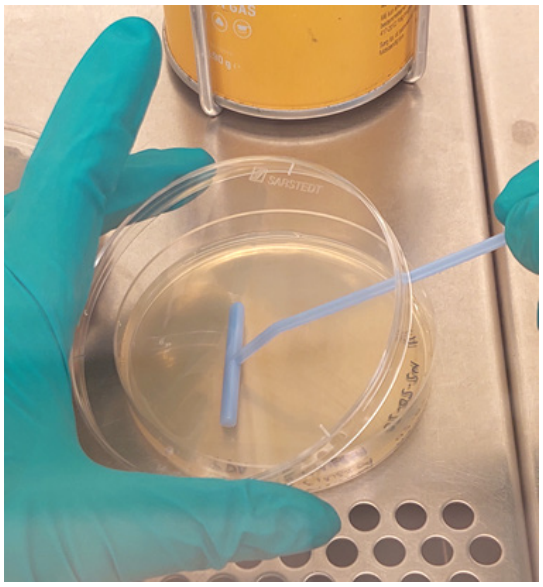

Figure 5: How to use the Drigalski spatula. Open the lid of the Petri dish, add the inoculum, and then place the Drigalski spatula on the surface and let it slide gently over the agar without applying pressure. Make sure to evenly distribute the inoculum over the entire surface of the plate, and keep the lid as closed as possible to avoid contamination.
Image courtesy of the author

6. Once the agar plate has been inoculated, leave it to dry for 5 min, before proceeding to the next step (testing the antimicrobial effect of ants).

Part 2: Testing the antimicrobial effect of ants

Materials

- Agar plate inoculated with *B. cinerea*
- Petri dish without agar (to collect or keep ants in)
- Inoculation loops
- Nitrile gloves
- Ziplock bags
- Timer

Procedure

1. Wearing gloves, take an inoculation loop and use it to grab an ant from the Petri dish.
2. Gently put the ant onto the inoculated agar plate.
3. Let the ant roam around for 5–15 min.
4. Remove the ant and incubate the agar plates at room temperature (20–25°C) for 2–3 days. Agar plates should be stored in plastic bags with the lid of the Petri dish facing down.

Expected results

After incubation, the growth of *B. cinerea* should be visible on the agar plates, as well as the microorganisms from the ant footprints (figure 6).

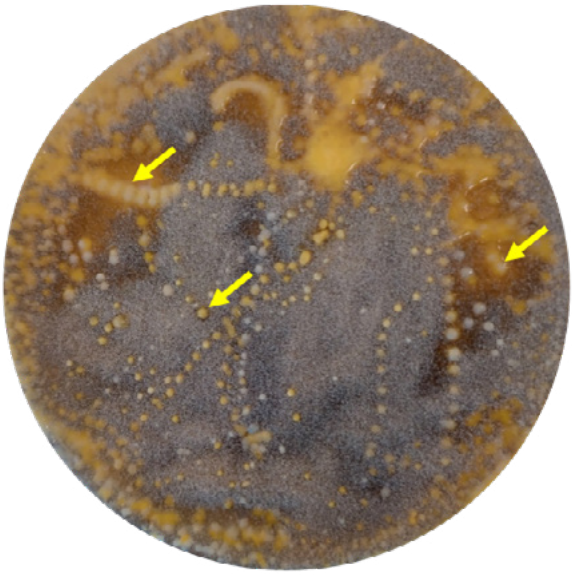

Figure 6: Same agar plate as that shown in Figure 2 after 3 days of incubation at room temperature. Grey mould growth is visible. Yellow arrows indicate antimicrobial microorganisms.
Image courtesy of the author

If any of the ant-associated microorganisms are able to control the pathogenic fungus, it can be seen as an inhibition zone or clearing zone, which is a clear zone around the microorganisms (figure 7). This means that the microorganism is producing antibiotic substances that are killing the fungus or keeping it from growing.

Figure 7: Agar plate inoculated with *Fusarium graminearum*, where a single ant has been roaming on the agar. Yellow arrows indicate the antimicrobial microorganisms transferred by the ant, shown by a visible clearing zone.
Image courtesy of the author

Students can assess the number of antimicrobial microorganisms their ants have transferred and further describe their morphology. Older students can, as described in Activity 1, assess the antimicrobial microorganisms under a microscope to further assess cell structure (bacteria versus fungi).

Summary

These experiments reveal the antimicrobial effects of ants and their associated microorganisms, and how they can be used to combat plant diseases in agriculture. You will get first-hand experience with microbiology, when you discover the diversity of microorganisms that can be found in ant footprints and test the antimicrobial effect of these feet bacteria and fungi. The power of antibiotics can be viewed with the naked eye when antibiotic bacteria and fungi excrete antibiotic chemicals that kill or inhibit plant diseases, resulting in a clearing zone. These are the chemicals that are important in agriculture, but especially medicine, where resistance to antibiotics is a big problem. Therefore, we need to continuously find new antibiotics – maybe you just found something that could be used as medicine in the future! «

Cutting-edge science: related EIROforum research

European XFEL

Antibiotic resistance poses a significant challenge in hospitals globally. At the European XFEL, researchers are using very bright x-ray lasers to shed light on the structure of structure of enzymes linked to antibiotic resistance and to analyze the key reaction steps from a beta-lactamase enzyme responsible for antibiotic resistance in tuberculosis bacteria.

www.xfel.eu

References

[1] Offenberg J, Jensen IC, Hansen RR (2021) Combatting plant diseases with ant chemicals: a Review. *Journal of Applied Ecology* **59**: 25–38. doi: 10.1111/1365-2664.14017

[2] Huang HT, Yang P (1987) The ancient cultured citrus ant. *BioScience* **37**: 665–671. doi: 10.2307/1310713

[3] Jensen IC et al. (2023) Implementing wood ants in biocontrol: suppression of apple scab and reduced aphid tending. *Pest Management Science* **79**: 2415–2422. doi: 10.1002/ps.7422

[4] Schifani E et al. (2020) New tools for conservation biological control: testing ant-attracting artificial nectaries to employ ants as plant defenders. *Insects* **11**: 129. doi: 10.3390/insects11020129

Resources

- Read a short article on how ants can be a source of new antibiotics, and why this is important.
- Watch a video on fungus-farming ants and how they grow antibiotic bacteria on their bodies, which they use to protect their fungus gardens.
- Explore antibiotic resistance and drug development with these fun microbiology activities: Deumal Fernandez M, Lladonosa Soler M, Godinho T (2021) Microbiology: discovering antibacterial agents. *Science in School* **55**.
- Try this role-playing activity to understand how research projects are funded and the importance of basic research: McHugh M (2022) What is it good for? Basic versus applied research. *Science in School* **55**.
- Gain insights into toxicology and the physiological effects of drugs through the use of Daphnia as a model organism: Faria HM, Fonseca AP (2022) From drugs to climate change: hands-on experiments with Daphnia as a model organism. *Science in School* **59**.
- Learn about the biochemical aspects of honey through a series of simple experiments: Scheuber T (2023). To bee or not to bee: the biology of bees and the biochemistry of honey. *Science in School* **62**.
- Explore chemotaxis and the scientific method with these slimy experiments: Buchta A, Dunthorn M (2023) Moving slime: exploring chemotaxis with slime mould. *Science in School* **62**.
- Learn about how plants defend themselves against pathogens: Harant A, Pai H, Cerfonteyn M (2023) Plant pathology: plants can get sick too! *Science in School* 62.
- Read about the development of lab-grown meat substitutes: Noble M (2023) From Petri dish to plate: the journey of cultivated meat. *Science in School* **63**.
- Discover how mealworms could offer a sustainable alternative source of animal protein: Bonin L, Jeran M (2024) Towards sustainable nutrition: could mealworms provide a solution? *Science in School* **64.**

AUTHOR BIOGRAPHY

Ida Cecilie Jensen is a biologist and PhD student at Aarhus University, Denmark. She studies ants and how their associated microorganisms can be used as sustainable alternatives to chemical pesticides.

Image courtesy of Nicolai Hildebrand/Aarhus University