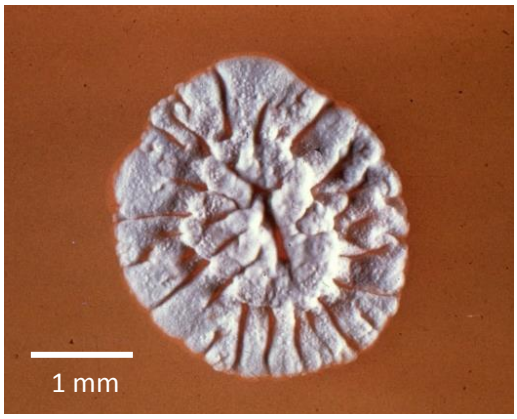
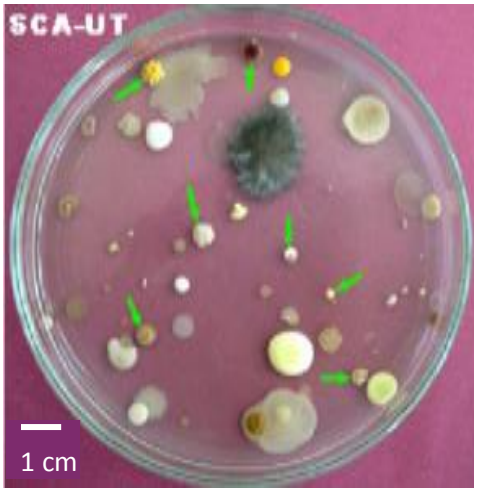
Measuring 'the smell of dirt'
Soil health test

GrassWorks, Inc.

What are you measuring? The smell of a freshly plowed field can be credited to compounds released by *Streptomycetes* bacteria as they die. These bacteria are very important in soil: they break down a wide range of organic molecules into humus. Humus is part of soil organic matter, which is the soil's storehouse for plant nutrients. *Streptomycetes* are slow-growing so they produce antibiotics to compete for food. Over 70% of the antibiotics in use for human and veterinary health (i.e. ivermectin, neomycin, tetracycline) are produced by Streptomyces. Antibiotic production may also reduce loss of soil organic matter by slowing soil microbial growth. The number of Streptomyces colonies may indicate the soil's ability to build organic matter.



Streptomyces colony by J.J. Goodman



Streptomyces colonies marked with green arrows on an agar plate by M.

What will you see? At a microscopic scale, Streptomyces grow branched structures called hyphae, which are like fungal mats. The hyphae form spores to reproduce. To the naked eye, the spores give the colonies a powdery or chalky look and the hyphae make the colonies appear tough and leathery, instead of soft and moist like most bacterial colonies. One way to identify colonies of Streptomyces is to expose them to ultraviolet (UV) light or "blacklight." Streptomyces produce pigmented compounds called phenazines that glow under UV. Other bacteria like *Psudomonas* produce antibiotics that glow under UV but the colonies appear soft and moist.

Precautions: All cultured organisms should be treated as able to cause disease. Wash hands and sterilize surfaces before and after. Avoid opening plates. Discard plates in a sealed trashbag.

Who developed the test? Robert Bauer adapted the test from a classroom exercise by Drs. Charles Hagedorn and Brian Badgley at Virginia Tech. NCR SARE, GrassWorks, and Wisconsin RC&D's support the Sharing Soil Health Knowledge and Practice through Grazing Networks project.

Instructions: The test involves: 2 hours of prep time, 1 week of incubation, and $20 of materials per soil.

- The grazing network project created a test kit that fits into a salad spinner: four alcohol wipes, one purple sharpie, four 14 mL dilution tubes, six starch casein agar (SCA or "nutrient agar") plates, ten 1 mL graduated plastic transfer pipettes, one roll of clear tape, and one salad spinner turntable. Store the agar plates in the refrigerator until you are ready to do the test.
- You provide: 20 g fresh soil, gram scale, container to weigh samples, spoon, oven, 50 mL distilled water, rack for dilution tubes, clock/timer, glass spreading rod/angled serving tongs/six disposable plastic razors, gas flame/butane lighter, and UV light/modified flashlight (see instructions below).

- How moist is your soil? Circle: dry (+15%), moist (+25%), wet (+35%). What is the soil texture? Circle: sandy (-10%), loamy (+0%), clayey (+10%). Add the two numbers and divide by 100: (*a*) ☐
- Weigh out 5 grams of fresh soil; break apart clumps. Place soil in an oven at 140°F for 1 hour to reduce competition from fast-growing bacteria. Streptomyces spores will survive heating.
- Pipette 9 mL of distilled water into each dilution tube. Label the tubes: A, B, C, D. Place 1 gram of oven-treated soil in tube A and shake for 1 min. Let the sample sit at room temp for 10 min. Then shake the tube again and transfer 1 mL from tube A to tube B with a pipette. Shake tube B for 30 sec. Use a new pipette to transfer 1 mL from tube B to tube C. Shake tube C for 30 sec. Use a new pipette to transfer 1 mL from tube C to tube D. Shake tube D for 30 sec.
- Label the bottom of sequential SCA plates: B, C, D. Label a duplicate set of plates. Then use separate pipettes for each tube: transfer 0.1 mL from the tubes onto the labeled plates.

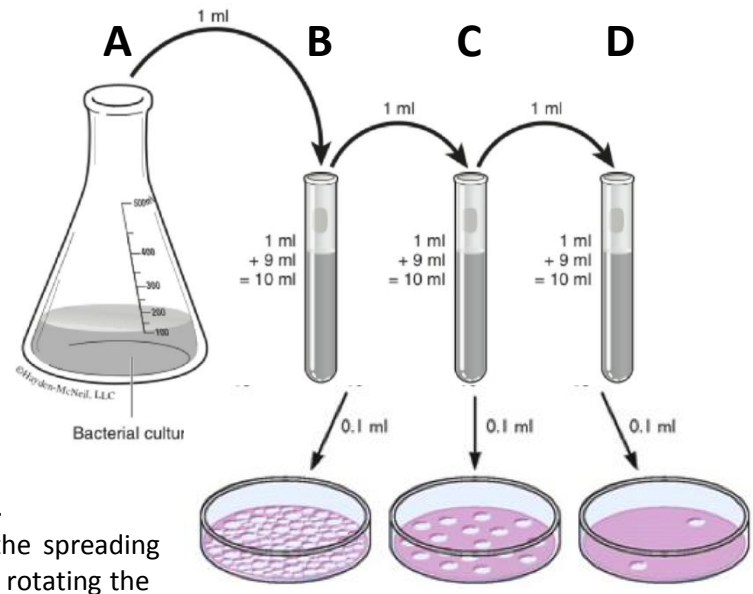
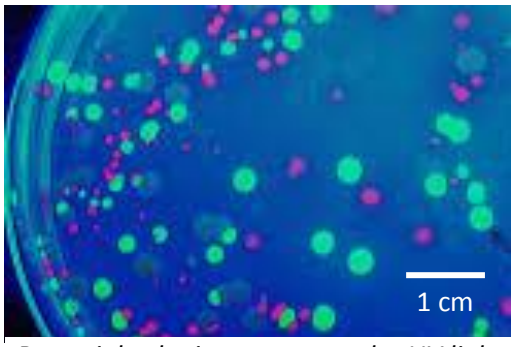


Diagram of dilution and transfer to plates

- Spread the liquid on the plates by moving the spreading rod in an arc on the surface of the agar while rotating the plate on a turntable. Sterilize the spreading rod before each spread: pass it back and forth through a flame for 5 sec then cool it for 10 sec.
- Tape down the lids of the plates at two spots. Turn the plates over so the lids are facing down so condensation will collect on the agar, not the lid. Put the plates in a spot that will stay at room temp (77°F) for one week to incubate. Avoid knocking the plates during the incubation period.
- After incubation, turn the plates over and examine growth. Plates B and C may be overgrown but plate D should have distinct colonies. Count the total number of colonies (total bacterial count) on the D plate duplicates. Do not open the lid; you can mark a dot on the lid above each colony with a sharpie to help you count. Write down the average of total bacterial colony counts: (*b*) ☐
- Next count the *Streptomycete* colonies on the D plate duplicates. They are usually small, hard, powdery, and surrounded by a zone of no other growth. The colonies will not be gooey or shiny. They can be various colors above and below the surface of the agar (white, grey, yellow, or red). Write down the average of the counts of *Streptomycete* colonies: (*c*) ☐



Bacterial colonies on agar under UV light by T. Le and others

- Expose the plates to UV light in the dark. You can make a UV light by covering a flashlight with pieces of clear tape colored with blue and purple sharpie. How many colonies glow? Write down the average of the counts of glowing colonies from plate D duplicates: (*d*) ☐
- How many of the glowing colonies are shiny (*Pseudomonas*)? Write down the average of the counts of shiny colonies from plate D duplicates: (*e*) ☐
- Use your numbers (*a*) and (*c*) to calculate the colony-forming units of Streptomyces in your soil: (*f*) ☐

$$Colonies\ per\ gram\ of\ dry\ soil\ = \frac{(\ c\ \times 11{,}000\ )}{(\ 1-a\ )}$$

- Send a completed copy of this sheet and a photo of your plate to Kirsten Jurcek, Glacierland RC&D, N2437 Brattset Lane, Jefferson, WI 53549, email kjurcek1@centurytel.net, or call (920) 342-9504.