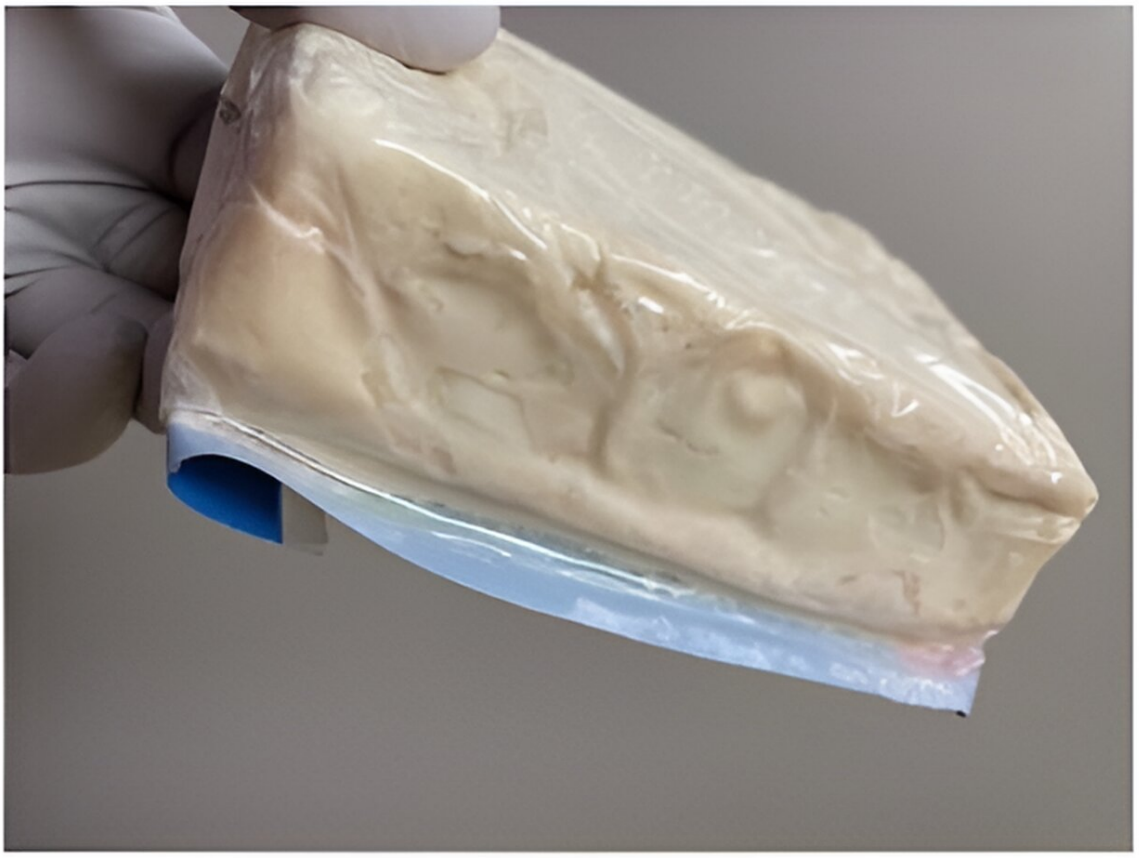
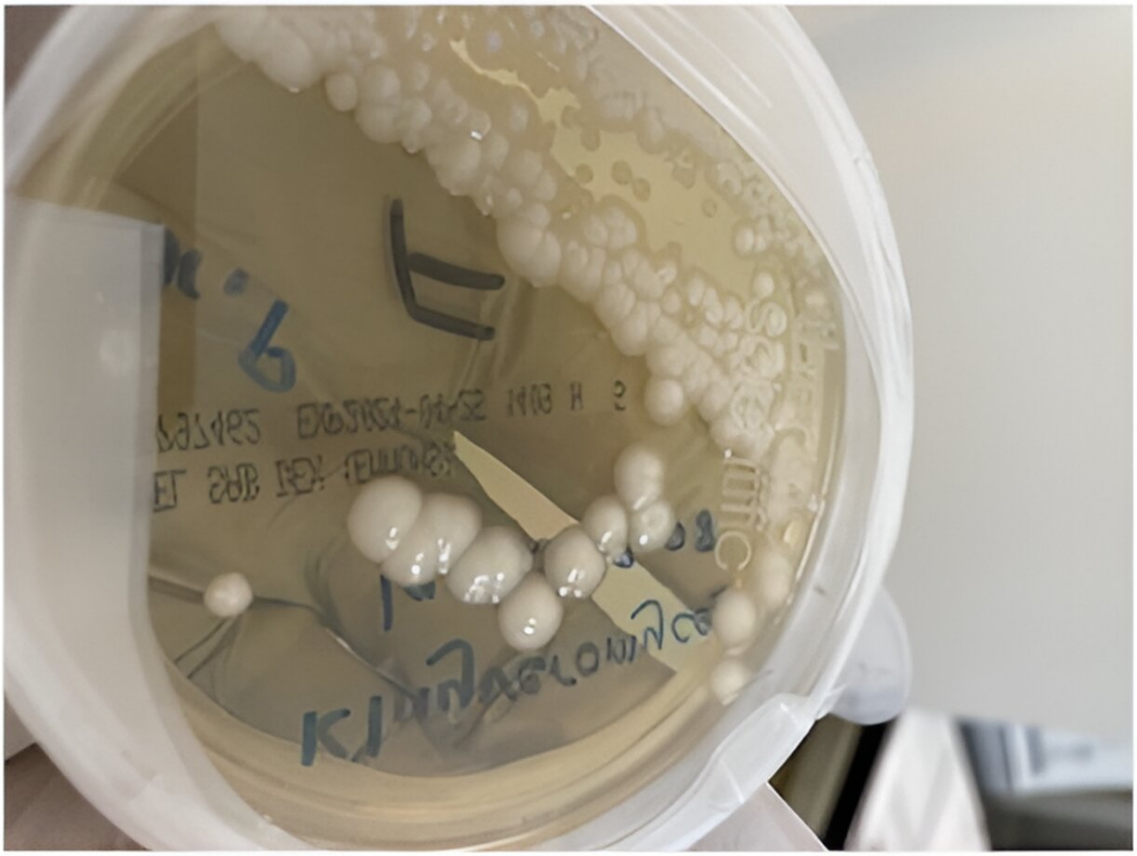
The microbiology of food spoilage in your refrigerator

September 4 2024





Slimy, discolored feta cheese. Credit: Matthew Pettengill, Ph.D.

Anyone who has ever lost track of some bread, produce or leftovers, and later returned to observe that nature has run its course, has experience using the five senses (eyes, nose, [hopefully less often] taste buds and even ears—container lid "popping"), to evaluate food spoilage. But have you ever wondered what microorganisms you might be able to grow in the lab from the decomposing dishes in your refrigerator?

To satisfy his curiosity, Matthew Pettengill, Ph.D., D(ABMM), Scientific Director of Clinical Microbiology at Thomas Jefferson University Hospital in Philadelphia, Pa., decided to sample a variety of common food items from his own home (or local grocery store) that either showed visible sign(s) of decay or were past the printed expiration date.

The goal was to identify what organisms were involved using microscopy with Gram stains and lactophenol cotton blue, and a basic bacterial workup (basic media, such as chocolate and MacConkey agar, and MALDI-TOF). Here he describes his observations and shares his findings. Follow along, take a good look at the provided images and see if you can identify the organisms before he does.

Case 1: Unopened feta cheese before its expiration date

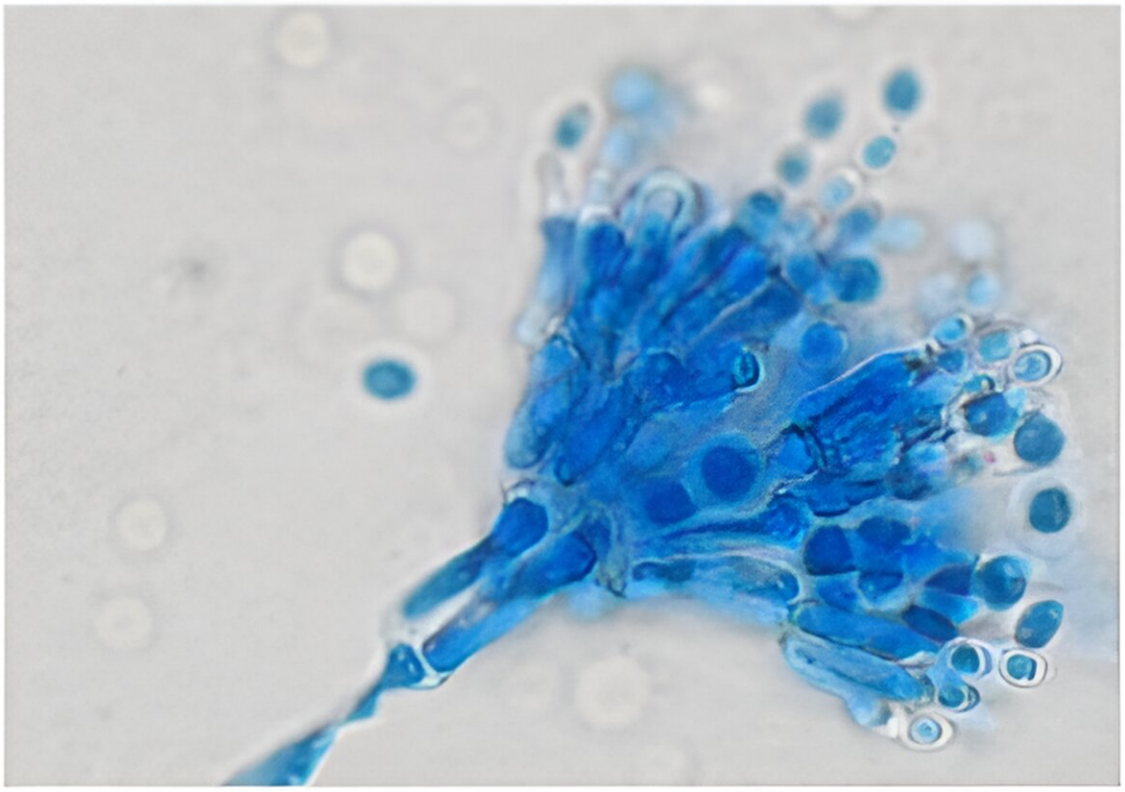
Observations: I really wanted to make a Greek salad, but when I went to grab some feta cheese from my refrigerator, it didn't look right, with a

slimy discolored surface. So, I brought it to the lab for testing.

Experimentation: As far as I could tell, the container was still well sealed, so I opened it and performed a Gram stain, which revealed budding yeast when viewed under a compound microscope. The organism was plated on Sabouraud dextrose agar and allowed to grow at 30ºC. One of my colleagues ran the sample on MALDI-TOF a couple of days later.

Identification: The isolated organism was identified as Kluyveromyces lactis. K. lactis is a yeast used in cheese production as a replacement for animal rennet, which functions to coagulate milk. Perhaps the growth on this particular package of feta cheese was due to a pasteurization or manufacturing process failure of some sort—K. lactis has been detected in spoiled feta from a study in Sardinia.

Case 2: Anti-streptococcal applesauce



Top: Greenish-grey mold colonies on applesauce surface. Bottom: Lactophenol cotton blue stain of organism from applesauce. Credit: Matthew Pettengill, Ph.D.

Observations: In the Fall, my family likes to make our own applesauce and jar some of it to eat the rest of the year. One day I noticed some common mold overgrowth in an applesauce jar that had been left in the fridge for a few months.

Experimentation: To determine what was growing on my applesauce, I first made a mold tape print directly off of the food item—carefully lifting out 1 of the heaped, green, fluffy colonies using a lab tape product similar to packing tape (sometimes called a Scotch tape print, but we don't actually use Scotch brand tape). I then performed a lactophenol cotton blue stain and examined it under the microscope.

Identification: The conidiophore and phialide structure and greenish-gray colony color were strongly suggestive of Penicillium species, a common environmental mold that has done the world a lot of good by giving us penicillin. I don't recommend making penicillin at home though—you should only use it if prescribed by a physician and acquired from a pharmacy (not a mold).

Case 3: Unopened bread after its expiration date

Top: Moldy store bought bread. Bottom: Lactophenol cotton blue stain of

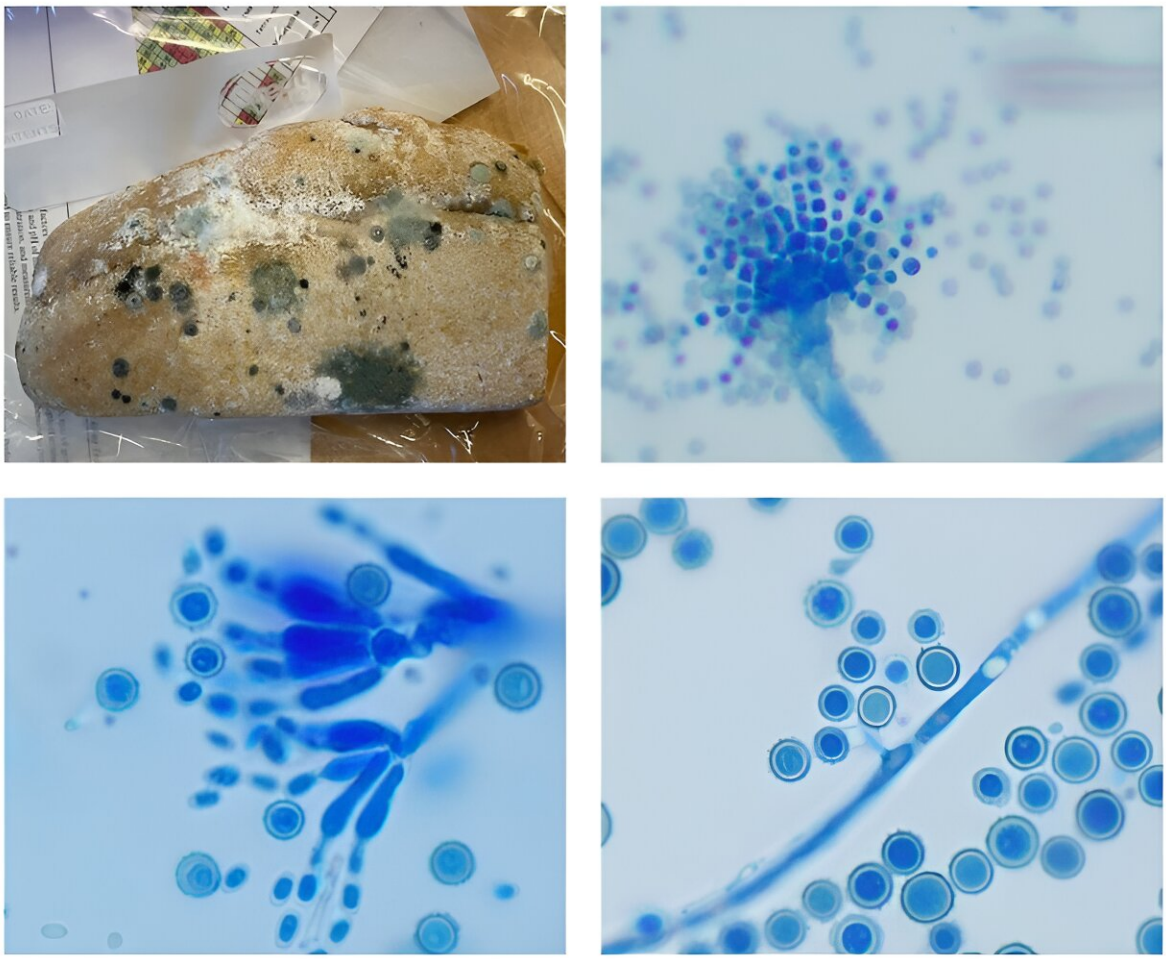
organism from bread sample. Credit: Matthew Pettengill, Ph.D.

Observation: Next, I decided to examine an unopened, store-bought loaf of bread that had gotten lost in the pantry and had a nice-sized green mold colony in the unopened packaging.

Experimentation: I again made a mold tape print directly off of the food item and made a lactophenol cotton blue stain and observed the slide on a microscope.

Identification: This is another example of Penicllium. You can see it reaching out its phialide fingered hands to grab my baguette.

Case 4: Homemade bread way after its expiration date



Top left: Moldy store bought bread. Top right and bottom: Lactophenol cotton blue stains of organisms from bread sample. Credit: Matthew Pettengill, Ph.D.

Observation: Next, I became curious to see how the microbial growth on an expired loaf of store-bought bread compared to microbial growth on a homemade loaf of bread, which lacked preservatives and had also been exposed to outside air (beside an open window). When I noticed the first mold colony on my homemade loaf, I put it in a plastic baggie to bring to work for testing. At that point, I forgot about it and left it in my backpack for a few weeks. When I remembered the loaf was there, I

pulled it out for testing.

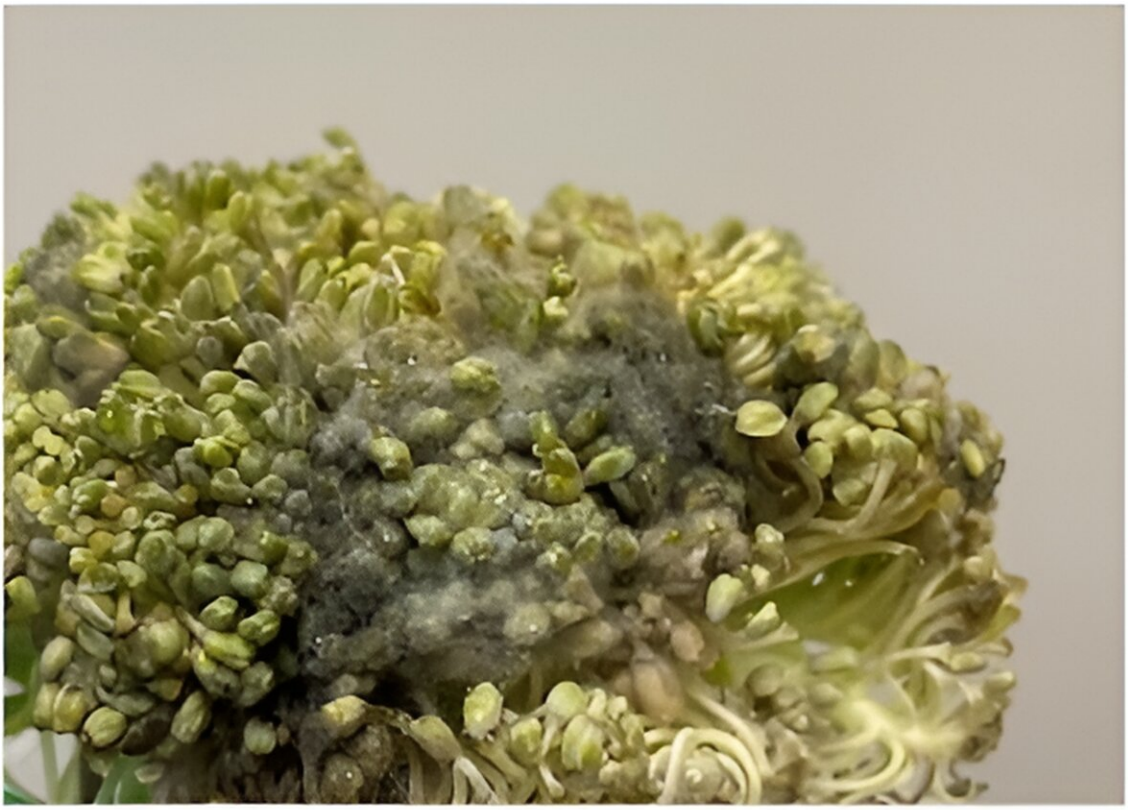
Experimentation: I again made a mold tape print directly off of the food item, made a lactophenol cotton blue stain and observed the slide on a microscope.

Identification: I included images of three distinctive mold type structures, but there were probably more. The upper right mold type displayed conidiophore, phialide and conidia structures suggestive of Aspergillus, a common environmental mold that can cause serious infections in humans, particularly those who are immunocompromised. However, this did not appear to be one of the common clinical varieties, and I hypothesized, based on comparison to known phialide and conidia distribution, that this may be A. glaucus.

The conidia shape of the lower left mold type appeared to favor Paecilomyces, even if the phialides are more suggestive of its close cousin Penicillium. Paecilomyces is another genus of fungi that is commonly found in the environment. A number of Paecilomyces species are known plant pathogens. However, while infection in humans is possible (especially in immunocompromised hosts), it is less common.

The lower right structure consisted of abundant, large, knobby conidia that are characteristic of Sepedonium an environmental mold not typically associated with human disease (few cases of infection have been reported).

Case 5: Fuzzy, shrink-wrapped broccoli



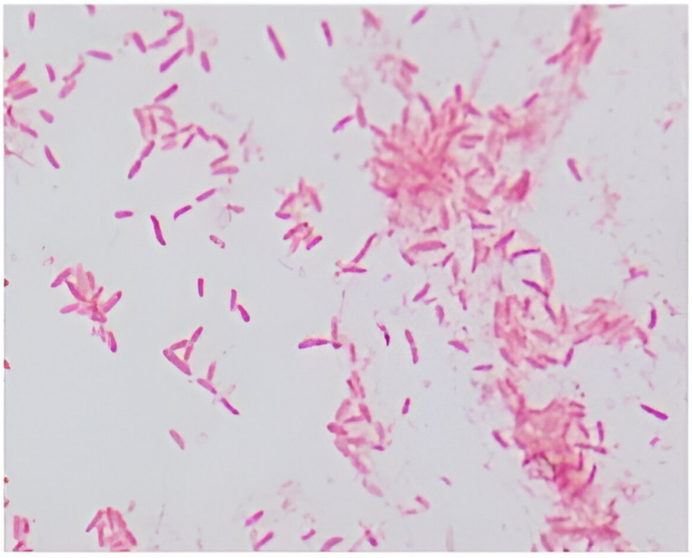
Top: Photo of moldy broccoli floret. Bottom: Lactophenol cotton blue stain of organism from broccoli sample. Credit: Matthew Pettengill, Ph.D.

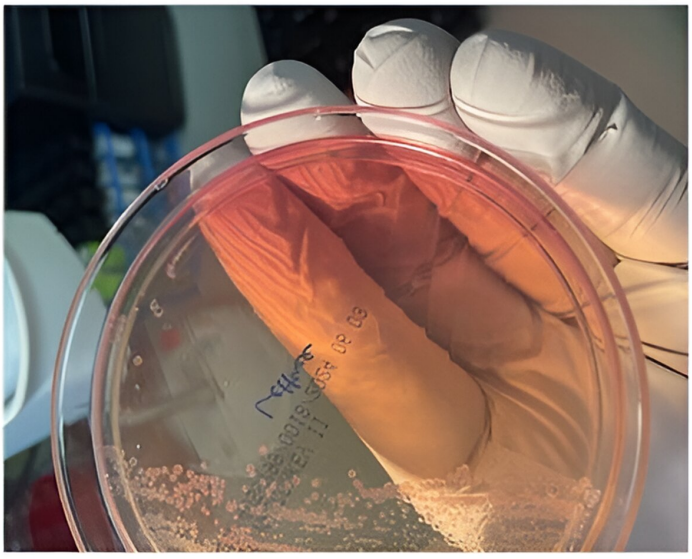
Observations: This head of broccoli went unnoticed in the back of the refrigerator and was fuzzy and gray on the florets—still in its shrink-wrapped packaging—when I found it. It did not appear to have an expiration date listed.

Experimentation: Once again, I made a mold tape print directly off of the food item, made a lactophenol cotton blue stain and observed the slide on a microscope.

Identification: The print yielded some nice septate hyphae and large conidia with longitudinal and transverse septation, typical of the mold Alternaria. The dark gray color of the fuzz on the broccoli was suggestive of it being a dematiaceous mold, which produces melanin pigment. Alternaria is also common in the environment but can cause serious cutaneous and ocular infections in (primarily immunocompromised) humans.

Case 6: Spring Greens With a Fluorescent Dressing





Top: Decaying spring mix. Middle: Gram stain of organism from spring mix sample. Bottom: Organism on MacConkey agar. Credit: Matthew Pettengill, Ph.D.

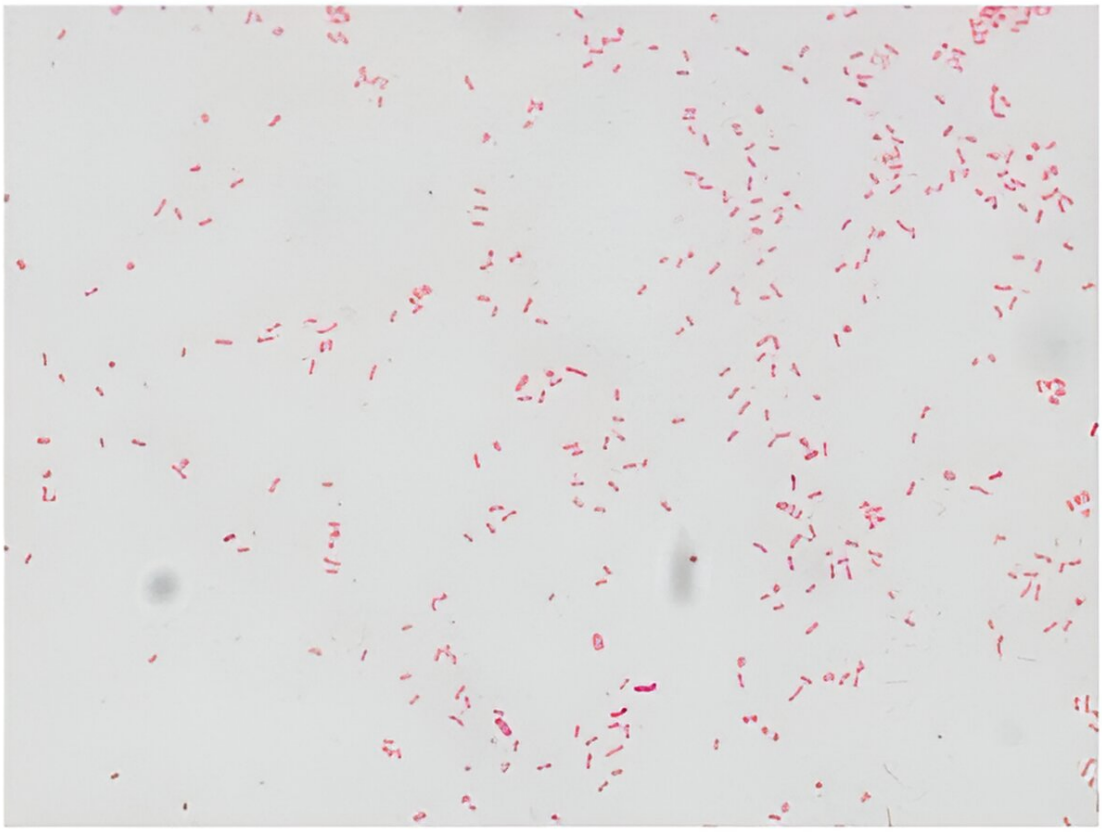
Observations: Spring mix usually contains tender baby lettuce, spinach and other edible leaves. This item was pulled from the fridge in its original packaging and sampled before its expiration date. I observed black, liquefied growth on some of the leaves.

Experimentation: The sample for this food item was made by sticking an inoculation loop directly into a black piece of red romaine or red oak leaf. Next, I performed a Gram stain, which revealed abundant, gram-negative bacilli. I plated the organism on MacConkey agar.

Identification: The culture grew a non-lactose-fermenting gram-negative bacilli, with a good MALDI score for Pseudomonas fluorescens. Although P. fluorescens may occasionally cause disease in humans (primarily immunocompromised hosts), it has some beneficial properties in agricultural applications, such as controlling the growth of other, more harmful organisms, and it is possible that the bacteria were intentionally used on the farms where the spring green mix came from.

Case 7: Spoiled spinach


KEEP REFRIGERATED



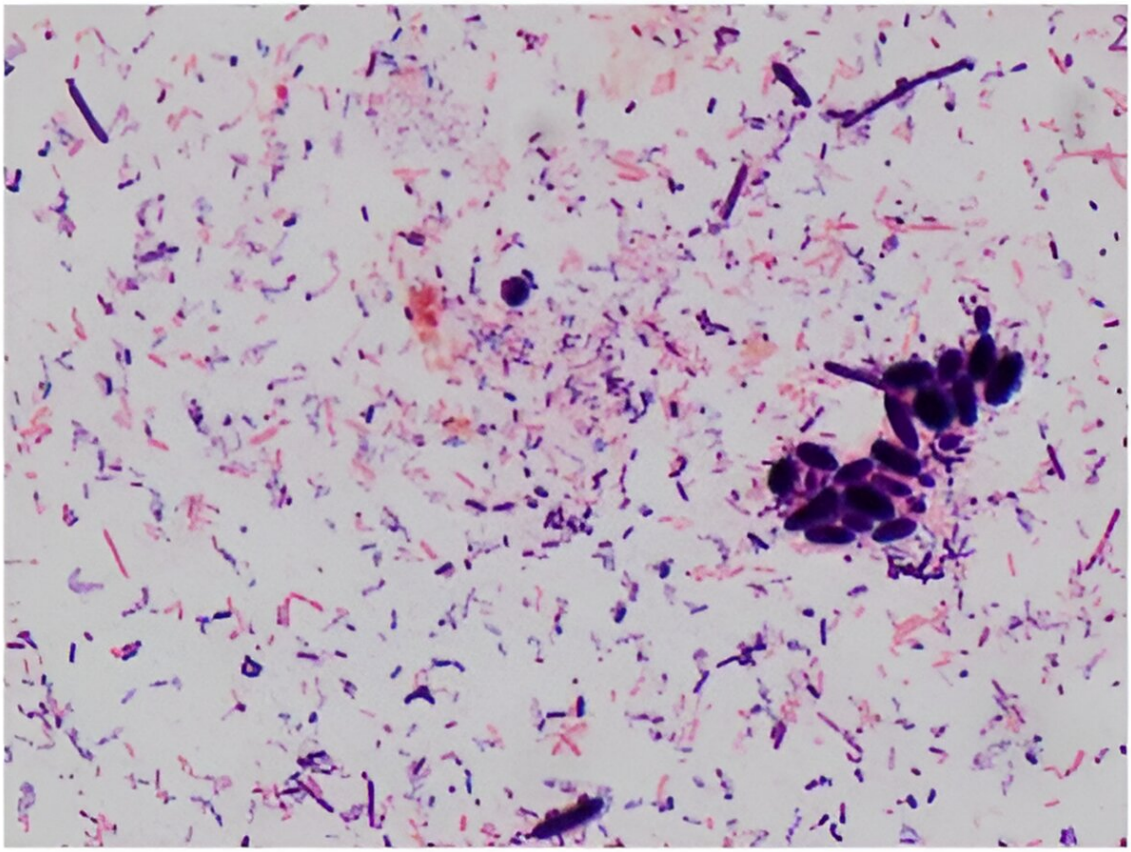
Top: Decaying spinach. Bottom: Gram stain of organism from spinach sample. Credit: Matthew Pettengill, Ph.D.

Observations: Shopping at our local grocery store, I noticed a bag of spinach that was going bad, with some darker liquifying leaves—I bought it to include in this project.

Experimentation: The morning after I made this purchase, I sampled the decaying leaves, performed a Gram stain and plated to MacConkey agar.

Identification: Abundant gram-negative bacilli grew once again. MALDI identified 2 different non-lactose-fermenters, Erwinia persicina and Serratia liquefaciens (the latter may have demonstrated slow lactose fermentation with some patience). Both of these bacteria are associated with crop damage in lettuce (and apparently spinach).

Case 8: Rotting onion from the back of the pantry



Top: Rotting onion. Bottom: Gram stain of organisms from onion sample. Credit: Matthew Pettengill, Ph.D.

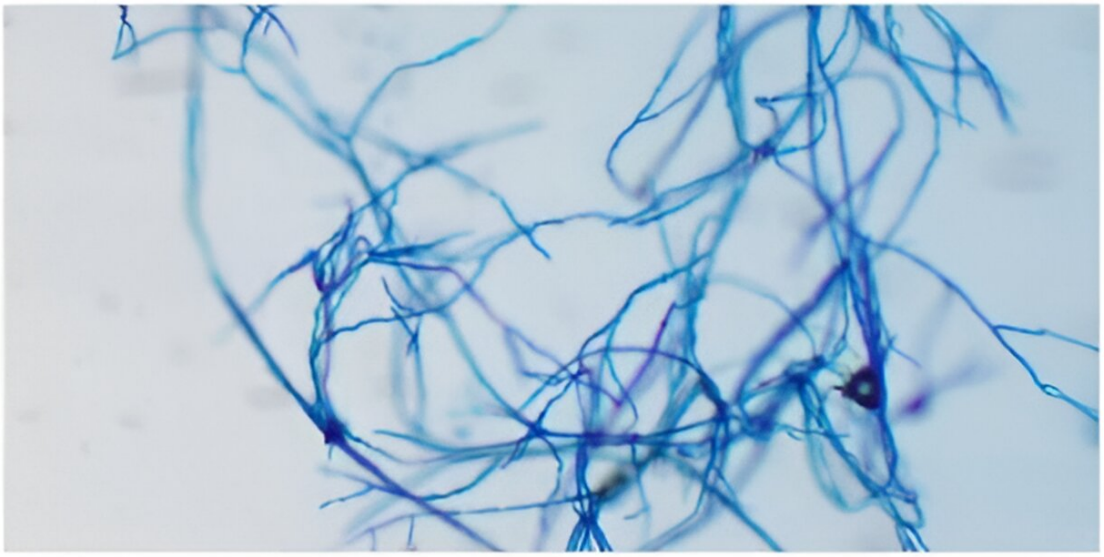
Observations: Nothing smells worse than spoiled potatoes or onions. Obviously, that is a subjective statement, but my family and I will occasionally be led by our noses to find a potato or onion that has gotten lost in the back of the pantry. This onion was long gone and putrid when found (soft/liquified in the outer skin), so I brought it to the lab in a plastic baggie for testing—my attempt to contain the considerable aroma.

Experimentation: I stuck a 1 uL loop into the putrefied fluid on the onion's surface to obtain a sample for Gram stain, and again I plated to agar media (based on the Gram stain I included MacConkey, Sheep's blood, Chocolate and Sabouraud dextrose agar).

Identification: The sample revealed both yeast and bacteria. The budding yeast didn't grow on subculture for some reason, but I observed that they were morphologically consistent with Candida (and a lot of other common yeast).

For bacteria we got a nice selection identified by MALDI: Lysinibacillus boronitolerans (presumably the gram-positive rod seen in the Gram stain), Enterobacter cloacae, Achromobacter xylosoxidans, Klebsiella oxytoca, Ewingella americana and Sphingobacterium multivorans. Some of these are commonly recognized pathogens, particularly the Enterobacter and Klebsiella.

Case 9: Fuzzy strawberries



Top: Moldy strawberries. Bottom: Lactophenol cotton blue stain of organisms from strawberry sample. Credit: Matthew Pettengill, Ph.D.

Observations: Strawberries have a pretty short shelf life, making it relatively common to find moldy fruit at home or even in the store. These berries had been in our refrigerator for a few days before I noticed they were turning white.

Experimentation: I again made a mold tape print directly off of the food item, made a lactophenol cotton blue stain and observed the slide on a microscope.

Identification: In this case, the mold prints yielded abundant septate hyphae and enough distinctive conidia to point to the Scedosporium species (although I didn't see good enough conidiophore examples to say it wasn't Lomentospora prolificans). Scedosporium is a common environmental mold, which can be a real clinical problem due to antifungal resistance. It is associated with serious infections following near-drownings and wounds acquired or exposed during wet, natural disasters (flooding, tornadoes, hurricanes).

Conclusion

Pettengill's refrigerator reconnaissance revealed a mixture of bacterial and fungal species feeding on his bread and produce. Interestingly, and perhaps not surprisingly, many of the species he identified are commonly found in the environment and either function as opportunistic pathogens or are not typically associated with human disease.

When asked if working in a microbiology lab has made him more or less concerned about microorganisms in and outside of the laboratory, Pettengill replied, "Growing up, at the first sign of spoilage the food item went straight in the trash. I'm still that way with growth that seems bacterial and smells bad, but a little green, fuzzy spot on bread doesn't scare me anymore."

He explained that many of the clinical microbiologists that he's worked with have accepted bacteria and fungi as part of life and seem less concerned about them than the average person.

"I'd be more concerned about bread that won't support mold growth, as it likely has a bunch of added preservatives I may not want to eat," he said, adding that he enjoyed learning more about the microbiology in his refrigerator and pantry and hopes others will as well. "I'll try to do a better job staying ahead of the (growth) curve in the future," he said.

Provided by American Society for Microbiology

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