Fermented foods offer up a versatile microbial model system

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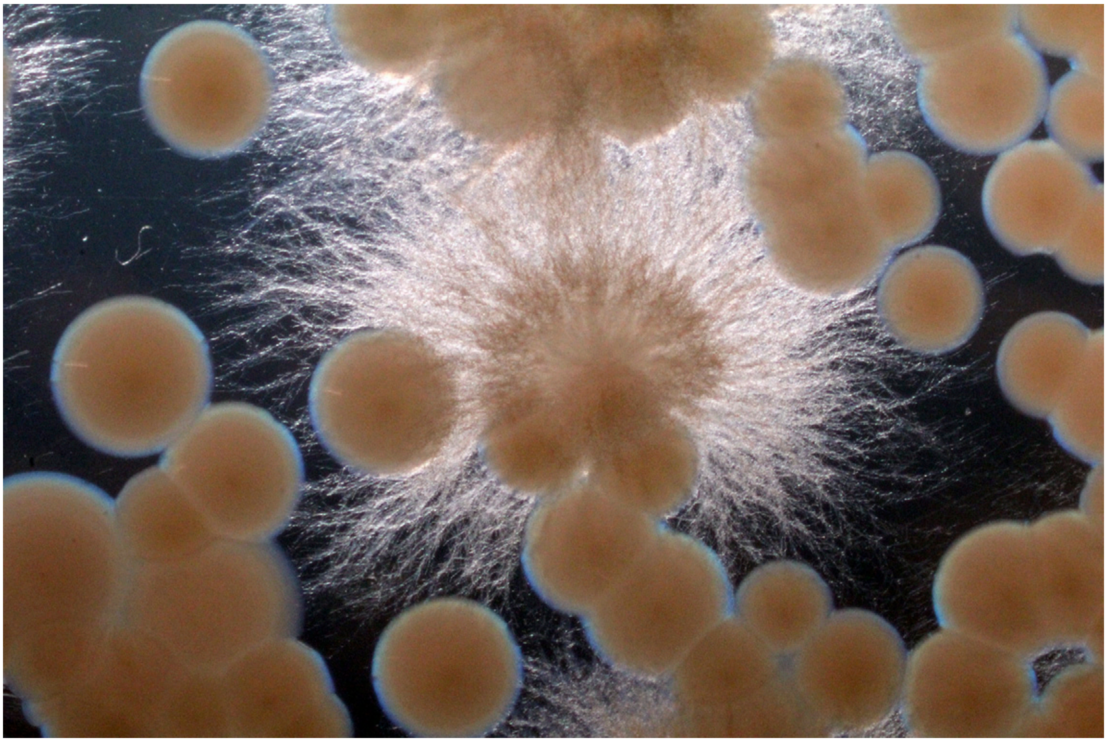
In a corner nook of Benjamin Wolfe's fourth floor laboratory at Tufts University sit two grow-lit shelves of baby Napa cabbages, each individual plant enclosed in a clear plastic box that keeps it sterile. When they get a little older, Wolfe's team will inoculate the leaves with different combinations of bacteria and watch the bugs grow. This spring, similar sterile Cruciferae will be transplanted to one of a handful of garden patches in Massachusetts, New York, and Vermont, from which they will later be harvested, then chopped up and fermented—essentially, turned into sauerkraut—all in the name of science.

Scientists have a lot to learn from sauerkraut, says Wolfe, and from other fermented foods, like kombucha and cheese. By studying how these microbial communities form and how their member species interact, he says, researchers can gain insight into the dynamics that shape much more complex communities, such as those found on the human body (1). Understanding how individuals in such communities interact and affect each other could point to efficient ways of manipulating the human microbiome, which, a growing body of research suggests, has a big influence on myriad aspects of health and well-being. "Nobody has ever tried to test these kinds of questions, yet they are very important for things, like fecal transplants, where [doctors] are trying to replace entire microbial communities."

"I just find it fascinating," says Jeffrey Dangl, a Howard Hughes Medical Institute investigator studying plant pathogen interactions at the University of North Carolina. "You can do repeated experiments perturbing the system, and then glean generalities" about more complex microbial interactions, he explains.

We now know that humans and other organisms, be they plant or animal, live symbiotically with collections of microorganisms that grow in, on, and around them, but the diversity of these communities is so vast and so complex that teasing out how the components of the system interact has so far been an overwhelming challenge. The microbial communities



As part of studies focused on microbial communities, colonies of filamentous fungi and bacteria are isolated from the rind of Robiola, an Italian cheese. Image courtesy of Benjamin Wolfe.

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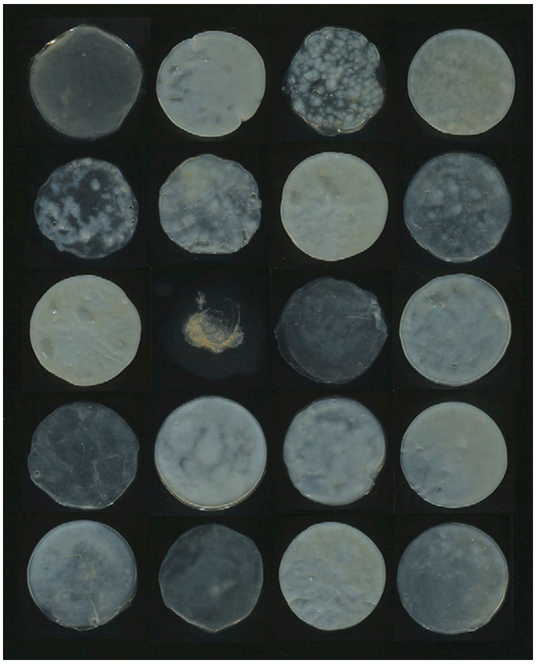

Researchers crafted a model system out of biofilms taken from 20 unique fermented tea (kombucha) microbial communities. Floating on top of the tea, each biofilm contains the yeast and acetic acid bacteria that ferment sucrose into alcohol and acetic acid. Image courtesy of Benjamin Wolfe.

responsible for food fermentation, however, hit a sort of diversity sweet spot: rather than hundreds or thousands of species, they might contain 30. These communities are relatively reproducible as well as experimentally manipulable; after all, they have been optimized by humans over thousands of years to produce specific flavors and textures, which makes them a powerful model system. "It's what we call tractable complexity," says Wolfe. "You can actually take all the members and understand how they're interacting."

Cultural Complexity

In a pilot study Wolfe's team conducted last summer, they found that their sterile and identical Napa cabbages replanted at different growing sites went on to form completely different microbial cultures. "We can now take these microbial cultures and do very controlled experiments to look at what determines their composition," Wolfe says. "Is it something to do with which microbe gets there first? Is it about microbes interacting with each other? Or is the host controlling which microbes are present?"

It was Rachel J. Dutton, a microbiologist at the University of California, San Diego, who came up with the idea of using fermented food to study the molecular mechanisms of microbial communities. As a doctoral student investigating bacterial genetics at Harvard, Dutton attended an intensive summer course on microbial diversity at the Marine Biological Laboratory in Woods Hole, Massachusetts. She emerged excited to explore microbial communities and the molecular mechanisms that underlie their formation. But few laboratories were doing such work, and there wasn't an appropriate model system.

That's when the self-described foodie was struck by an epiphany. Dutton, who had started reading about food science, realized that cheese rinds consisted of highly reproducible, well-formed biofilms made up of bacteria and fungi, exactly the set-up she was looking for. "They have just enough complexity to make them biologically interesting, but they're still simple enough to study," she says.

In 2010, Harvard awarded Dutton a 5-year independent fellowship to develop cheese as a simple, experimentally tractable system in which to study the basic processes occurring within microbial communities. Wolfe, her first hire, signed on as a postdoc. Along with a second postdoc, Julie Button, the researchers began by sequencing the bacterial and fungal components of 160 types of cheese. After characterizing the players and confirming that they could easily be grown in the laboratory, Dutton and her colleagues set out to reconstruct the pattern of community formation, remixing the microbial components in 96-well culture dishes to essentially reverse-engineer the cheeses' formation. What they saw resembled a forest going through stages of growth: reproducible ecological changes that occurred over time as communities formed (2). The researchers have now begun to probe the molecular and genetic basis of this characteristic succession.

"Each system has its own special properties that make it unique in asking different types of questions," says Wolfe, who has continued working on cheese and other fermented foods after establishing his own laboratory at Tufts. For example, the sauerkraut studies examine how and why different microbial populations might pop up in an identical host environment, providing a way to explore the interaction between microbes and their hosts.

Microbial Mix

Meanwhile, cheese rind contains both bacteria and fungi, providing a window for understanding how these different microbes interact, a topic that's generally overlooked in microbiology. In a study published last October, the group, working with Dutton's laboratory, surveyed the relative abundance of four *Staphylococcus* species in 25 cheese rinds. Curiously, the *Staphylococcus* species most prevalent in the rinds, *Staphylococcus equorum*, grew the slowest of the four when assayed alongside only bacteria. They found that the bacterium's colonizing oomph got a boost from the presence of *Scopulariopsis* fungi (3). "The fungi completely determine the distribution of these *Staph* species: they reshape [their] abundance," Wolfe says. "We have no idea for most bacteria why some are abundant and some are not." If a desirable bacterium relies on a fungal neighbor, you may need

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>
> **—Benjamin Wolfe**

to make sure to encourage the fungus to grow, whether in the course of making cheese or coaxing the proliferation of beneficial bacterial in the human gut, in plants, or in soil.

The focus of Wolfe's newest project is kombucha, a fizzy tea fermented by a culture of bacteria and yeast; the yeast species in the biofilm produce alcohol, and the bacteria, which are fewer in number, metabolize the alcohol into acetic acid. Enthusiasts of the drink keep these blobby biofilms alive for years in jars that serve as in-home mini beverage factories, and each version has a distinct taste and smell. Last summer, Wolfe scoured the e-commerce site Etsy and ordered some 25 kombucha cultures from around the country. Now his team is growing them in the laboratory to investigate the design principles that drive microbial communities and the role evolution plays in the process: questions such as whether new members can come in from the external environment to influence the community and how interchangeable the individual species within it might be.

Wolfe points out that the approach of using fermented foods does have limitations and may not translate perfectly to more complex microbial systems. For one thing, model set-ups like cheese and kombucha don't get input from a host immune system the way the human microbiome does. Also, these communities are assembling in a manner that's dependent upon humans.

Even so, the potential is powerful, says Dangl. "In biology there are dissectors and there are observers," he explains. "The dissectors can take systems apart and see how they tick and apply that to real problems." Molecular biology has flourished in the last 60 years by finding tools to do just that, he suggests, and with approaches such as this one in hand, microbial ecology can begin to do the same.

1 Wolfe BE, Dutton RJ (2015) Fermented foods as experimentally tractable microbial ecosystems. *Cell* 161(1):49–55.
2 Wolfe BE, et al. (2014) Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. *Cell* 158(2):422–433.
3 Kastman EK, et al. (2016) Biotic interactions shape the ecological distributions of *Staphylococcus* species. *MBio* 7(5):e01157-16.