Fighting Microbes with Microbes

Doctors turn to good microbes to fight disease. Will the same strategy work with crops?

By Amy Coombs | January 1, 2013

ike humans, with their complement of microbes that aid in everything from immune responses to nutrition, plants rely on a vast array of bacteria and fungi for health and defense. Over the last decade, research has revealed many new functional aspects of the crosstalk between human-associated microbes and human cells, but plant biologists are only beginning to scratch the surface of the often surprising ways that soil microbiota impact plants, from underground fungus-wired alarm systems to soil bacteria that can trigger defensive plant behavior or even act as a sort of vaccine. But despite these benefits, microbes are still primarily thought of as harbingers of disease.

"Since the discovery of antibiotics, medical research has been dominated by a 'bazooka mentality,'" and so has agricultural research, says Alexandre Jousset, a plant scientist at the Georg-August University in Göttingen, Germany. "Traditionally, microbes have been viewed negatively, and focus has been placed on eradication." Today, scientists and some medical doctors are becoming increasingly aware of their utility, and botanical researchers have also begun to debate whether the same may be true of plants.

While the Human Microbiome Project has discovered that some 10,000 species of microorganisms live in and on the human body, outnumbering our own cells by ten to one, plant scientists have found that any given soil sample contains more than 30,000 taxonomic varieties of microbes. Soil microflora not only provide nutrients for plants, but also suppress disease. In exchange, roots secrete fixed carbon into the soil and feed their bacterial symbionts.

Although the medical community now warns that overprescribing antibiotics kills beneficial organisms and encourages the formation of resistant strains, a similar change in opinion has not occurred in agriculture, where a kill-all approach to plant pathogens has given rise to biocides that indiscriminately wipe out the beneficial along with the pathogenic. "Biocides can nuke the soil, but they never kill everything," says Mike Cohen, a biologist at Sonoma State University in California. "This creates a biological vacuum that becomes filled by opportunistic survivors and organisms from the surrounding soil." Biocides create a strong selective pressure: the few pathogens that survive face little competition and proliferate, giving rise to pathogenic communities that can evade standard treatments.

Beneficial soil organisms, however, can protect plants more selectively than biocides do. They displace pathogens and produce toxins that kill pathogenic microbes, and they also trigger plants' own defense mechanisms. "Native bacteria are the first and most powerful barrier to prevent the establishment of pathogens," says Jousset. "A diverse community is especially important to keeping pathogens away—this is true in the human gut and in the soil."

"The idea is that we can reduce pesticide and fungicide use by utilizing the microbiome," says Harsh Bais, a plant biologist at the University of Delaware in Newark. "But we need to know more about the mechanisms of action; relationships between microbes and plants are very complex."

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Life Underground

According to a recent study published in PLOS One, underground networks of fungi help tomato plants "eavesdrop" on the alarm signals produced by their neighbors.[1] Even when plants are not able to communicate with chemical cues released through their leaves, they can link up and share vital information under the soil.

Researchers at South China Agricultural University in Guangzhou inoculated tomato plant leaves with the early blight fungus *Alternaria solani*, which creates brown and dead patches on leaves and can rot the tomato fruit. They then covered all research plants with airtight plastic bags, which prevented the transmission of airborne signals. Despite being covered, the tomato plants were able to communicate. Uninoculated plants growing several feet away activated defense-related genes and started making disease-fighting enzymes.

Researchers traced communication back to the fungus *Glomus mosseae*, which forms a symbiotic relationship with plant root hair known as a mycorrhizal network by inserting itself into the root cell's membrane. Bagged tomato plants grown in soil that lacked this underground network were unable to receive the "activate-defenses!" signal from infected neighbors and did not produce disease-fighting compounds. In contrast, in soils containing Glomus mosseae, uninfected plants detected the warning signs of disease and produced higher levels of six defense-related enzymes, including peroxidase (POD), polyphenol oxidase (PPO), chitinase, β-1,3-glucanase, phenylalanine ammonia-lyase (PAL), and lipoxygenase (LOX). (See diagram below.)

Because the mycorrhizal network can extend from one set of plant roots to another, it's possible that the network of fungal mycelia acts like telephone wires, allowing the plants to communicate underground. If this hypothesis is proven by identifying compounds that relay the chemical signal through the fungi, it might be possible to prevent plant disease by cultivating an appropriate mix of microbes in the soil. "The problem is that we don't know how plants and microbes select one another," says Bais.

Plant scientists have found that any given soil sample contains more than 30,000 taxonomic varieties of microbes.

To try to answer that question, Bais and his colleagues turned to *Arabidopsis thaliana* plants and *Bacillus subtilis*, a bacterium known to improve plant health. Despite the plant's antimicrobial defenses, *B. subtilis* somehow becomes established in the soil. The team found that *B. subtilis* secretes an antimicrobial peptide that temporarily suppresses toxins secreted by the root, allowing the beneficial bacterium to colonize the soil around the roots.[2] The peptide secreted by *B. subtilis* may also help ward off soil-borne pathogens while the plant's defenses are compromised, says Bais.

In a prior study, Bais and his colleagues found that plants can pick and choose the beneficial bacteria species recruited during pathogen attacks.[3] The team infected *Arabidopsis* seedlings with the bacterium *Pseudomonas syringae* pv. *tomato*, which causes bacterial speck—a major disease of tomato crops. Plant roots soon began secreting L-malic acid, a food source for *B. subtilis*. As a result, *B. subtilis* colonized the roots, which in turn triggered production of the plant's defense chemical salicylic acid, helping it fight the bacterial infection. "This isn't a typical symbiotic relationship," Bais says, "but there is an interesting reciprocity here." (See diagram below.)

Even after a bacterial community wanes, the biochemical pathways developed by the plants in response to bacterial colonization remain intact.

Plants may even be able to recruit different bacterial species as their need for food and water changes. Researchers from Ain Shams University in Cairo, Egypt, recently dissected the root systems of drought-sensitive pepper plants (*Capsicum annuum*) grown with varying amounts of water.[4] After comparing the structure and diversity of bacterial communities in the rhizosphere, the team found that plants grown in the desert with little water have larger populations of plant growth–promoting (PGB) bacteria which can enhance photosynthesis and biomass synthesis by as much as 40 percent under drought stress. Although PGB's mechanism of action has not been worked out, the bacteria are known to alleviate salt stress by reducing the production of ethylene in tomato seedlings.

Surprisingly, there is some evidence that the effects of beneficial bacteria can endure across generations. Even after a bacterial community wanes, the biochemical pathways developed by the plants in response to bacterial colonization remain intact. "This suggests the bacteria function as a vaccine of sorts," says Bais. This heightened disease response can then be passed to the next generation of plants. For example, even when progeny are not exposed to *B. subtilis*, they are better able to fight disease if parent plants fostered a relationship with the bacterium. "The bacteria help prime the plant to respond more quickly to disease, and they pass this memory to the next generation," says Bais. The effects appear to last the duration of the offspring plant's life, but are not passed on to a third generation.

Although most microorganisms that are beneficial to plants reside in the soil, their effects are not always localized to the roots. In a third study, published in *The Plant Journal*,[5] Bais and his colleagues showed that beneficial soil microbes encourage the closure of stomatal pores in the leaves of *Arabidopsis* plants. Stomata allow carbon dioxide to diffuse into the leaf and release expired oxygen and water into the air. Hot and dry conditions are known to trigger stomatal closure to preserve a plant's water, but Bais was the first to show that soil bacteria can trigger the response—an important finding, as some pathogenic bacteria, such as *P. syringae* pv. *tomato*, enter the plant through the stomata.

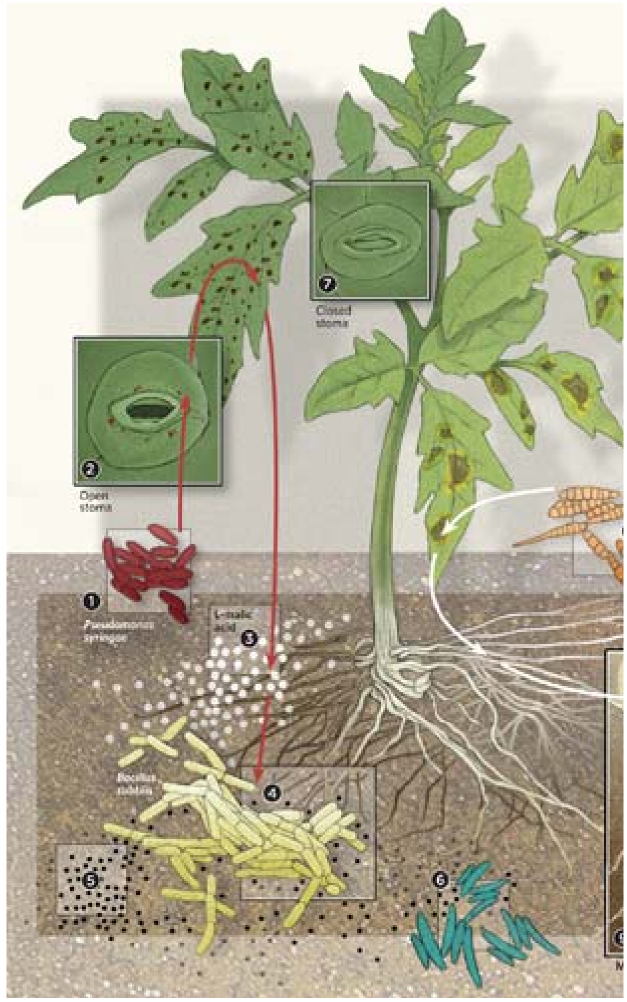
To see if root microbes could help counteract already established plant infections, Bais and his colleagues grew plants infested with *P. syringae* and then inoculated the soil with the beneficial *B. subtilis*. As the roots recruited new colonies of *B. subtilis*, the plants began producing abscisic acid—a chemical known to regulate stomatal closure. After three hours, only 43 percent of stomata were open in *B. subtilis*-treated plants. In control groups, 56 percent of stomata remained open. "This difference was significant and helped reduce disease," says Bais.

Unearthing the mechanism of action

It's much harder for pathogens to take over the human gut when beneficial microflora coat its surface. A similar mechanism is at play in the soil. When it comes to preventing plant disease, some microbes kill pathogens directly; others consume resources, taking up the niches that invading bacteria might otherwise inhabit.

"When a community is composed of species that use distinct resources, there is less free room for invading species," says Jousset. He and his colleagues set out to determine whether a broader genetic diversity of beneficial bacterial strains was more important than simply cultivating a large variety of bacteria, regardless of their genetic makeup.

The researchers grew 95 microbial communities, each containing between one and eight strains of *Pseudomonas fluorescens* bacteria—another species known to improve plant health. Each group had varying degrees of genetic similarity. The team then exposed the colonies to the invading bacterial species *Serratia liquefaciens*, which colonizes soil, water, and even the human gut and urinary tract, where pathogenic strains cause infection. After 36 hours, *S. liquefaciens* was able to invade communities that contained genetically similar species, but it was not able to gain a foothold in more genetically diverse communities. Indeed, as genotypic dissimilarity increased threefold, researchers saw a linear decrease in the colonization by *S. liquefaciens*.[6]



A look at the soil microbiome View full size JPG | PDF

However, the number of beneficial species in the soil was nearly as important as the degree of genetic dissimilarity between them. Communities with four to six species were better able to ward off invasion. Interestingly, communities were more susceptible to invasion by *S. liquefaciens* when a lower or higher number of bacterial species was present. Most likely, says Jousset, this is due to the variety of toxins produced. The colonies containing too many species produced a large amount of toxins, some of which also harmed beneficial strains of bacteria, whereas communities with too few species had low levels of toxin production, thus making invasion more likely.

We might be able to encourage disease-fighting bacterial communities by selecting for the right number and combination of species.

—Alexandre Jousset, Georg-August University, Göttingen, Germany

"This suggests we might be able to encourage disease-fighting bacterial communities by selecting for the right number and combination of species," says Jousset. Like the gut, the soil is an open system that allows bacteria to come and go, and competition for food and nutrients determines community structure. By manipulating food sources and growing conditions in the soil, it may be possible to select for genetically diverse communities. In a recent field study, Jousset sampled the disease-fighting genes found in the soil and discovered that a diverse mixture of planted herbs and grasses gives rise to the best ratio of disease-fighting genes and helps suppress soil-born pathogens. (See "Down and Dirty," *The Scientist*, September 2012.)

Creating healthy soil

Just as antibiotics indiscriminately kill both good and bad bacteria in the gut, fungicides and biocides impede the soil's innate defenses. Studies have shown that gentler practices such as crop rotation, tillage, and fertilization can influence ecological processes in the soil, and may encourage the establishment of microbial communities capable of suppressing disease.

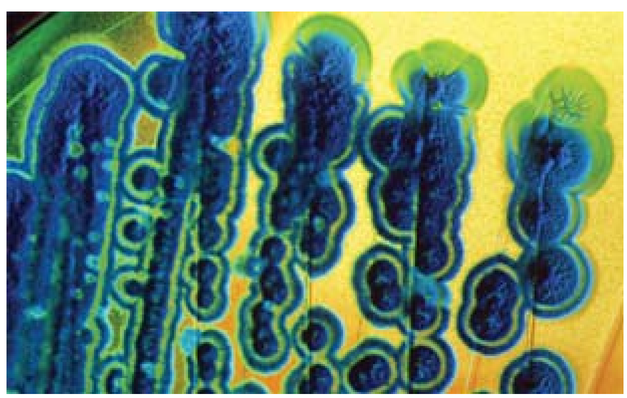
RAPSEED FLOWER

In search of a way to supplement the soil that encourages the growth of beneficial bacteria, Mike Cohen of Sonoma State University joined colleagues at the US Department of Agriculture to test rapeseed (*Brassica napus*) meal—a waste product from processing rapeseed into cooking oil or biodiesel.

The researchers split the roots of an apple tree seedling so that the plant had roots potted in two different containers. They then introduced the pathogen *Rhizoctonia solani*, which causes root rot, into one container. Rapeseed meal was incorporated into the soil of the other container at about 0.5 percent of the total volume, whereas the soil inoculated with the pathogen was left untreated. "This allowed us to test the indirect impacts of seed meal on the plant," says Cohen.[7]

The rapeseed meal reduced root rot by about 50 percent relative to control groups grown without the treatment. In fact, the researchers observed that the entire plant benefited from the rapeseed meal even though only half of the roots were exposed. Cohen and colleagues think that rapeseed meal fosters colonization by species of beneficial Streptomyces, known to trigger systemic defenses in plants. There were 10 times as many *Streptomyces* bacteria in soils amended by rapeseed meal, a finding that was later corroborated by field trials.

Indeed, when the researchers directly inoculated the split-root soil with Streptomyces instead of rapeseed meal, they found that *Streptomyces* encouraged plant defenses much as the seed meal did. "We can't say for sure how *Streptomyces* benefit the plant," says Cohen, "but some evidence indicates it's related to induction of the jasmonic acid signaling pathway," a hormonal signaling system that triggers plant defenses. (See "How Plants Feel," *The Scientist*, December 2012.)



THE GOOD STREP: *Streptomyces* sp. growing on agar for antibiotic research

Unfortunately, seed meal can also nourish pathogenic organisms. In some studies, disease-causing microbes proliferated in soils treated with seed meal. However, combining seed meals from mustard, rapeseed, and other plants can help minimize the growth of pathogenic microbes, says Cohen. This is because seed meals contain glucosinolates—chemicals that release pathogen-killing fumigants as they break down in water. As the chemicals released by rapeseed may be slightly different than those of mustard seed,"seed meals are more promising when used in combination," says Cohen. "One seed meal might target a pathogen, while another will help build beneficial communities of *Streptomyces*."

As gastroenterologists are now reporting the efficacy of transplanting gut bacteria from healthy individuals into human patients suffering from intestinal inflammation and infection, plant researchers may also find that multiple treatments with different concoctions of beneficial microorganisms will have a great impact on soil ecology. Even if one species doesn't curtail a pathogen, a full remake of the microbial community might help kick the problem. The goal is to gradually build the soil over time to establish a favorable microbial ecosystem. In rich, healthy soil, the microbial community may be more resistant to disease.

Researchers are now turning to field experiments to test the best combinations of species, and treatments like seed meal are already being used on organic farms in Northern California. If greater microbial diversity improves plant health in large-scale field trials, it could eventually help reduce chemical loads on industrial farms. "It might not work exactly the same way in the gut, but the mechanisms in the soil are very similar," says Jousset. "If we can protect and cultivate the soil microbiome rather than kill important species, we might need fewer chemicals in the field."

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soil microbiome, plant defenses, plant biology, pesticide resistance, pesticide, pathogen, microbiota, microbiology, gut bacteria, ecology, bacterial toxin, antibiotic resistance and agriculture

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Comments

Iwona Grad
Posts: 7

January 7, 2013

The experiments of using beneficial microbes to improve soil are not new, especially in organic farming. Look up Effective Microorganisms technology of prof. Teruo Higa.

after wiki: "He reported in the 1980s that a combination of approximately 80 different microorganisms is capable of positively influencing decomposing organic matter such that it reverts into a "life promoting" process. Higa invokes a "dominance principle" to explain the effects of his "Effective Microorganisms". He claims that three groups of microorganisms exist: "positive microorganisms" (regeneration), "negative microorganisms" (decomposition, degeneration), "opportunist microorganisms". In every medium (soil, water, air, the human intestine), the ratio of "positive" and "negative" microorganisms is critical, since the opportunist microorganisms follow the trend to regeneration or degeneration. Therefore, Higa believes that it is possible to positively influence the given media by supplementing with "positive" microorganisms."

Though the soil-microorganisms-plants interactions are no doubt very complex, there is multiple field evidence that the proposed microbial mixture restores soil capacity to support growth of healthy plants. I think a lot of further research needs to be done, especially in the context of geographical diversity, but the field is very promising.

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jeenious
Posts: 37

January 8, 2013

The war against animal and plant pathogens shares some homogeneous challenges comparable to those of any other kind of war. The greatest challenge is that the more sophisticated our solutions, the more sophistication is demanded of us on two fronts:

The first is that the organisms we seek to defeat, or neutralize, continue to become more sophisticated in response to the challenges to which we subject them;

The second is that as we create designer organisms to use against the enemy, the greater become the chances one or more of those designer organisms will evolve into something unwanted, or that the pathogens will succeed in utilizing them for the pathogens' own benefit in unexpected ways.

The history of agriculture has been characterized by an escalating sophistication by both us humans and our pathogen enemies.

Those of us most advanced in understanding "the enemy" are aware that our sciences have not -- as less science literate might suppose -- conquered our pathogen enemies at all. We have won battles only by staying ahead in the two-way escalation of resources of the one side against resources of the other side.

The cases in which we have eradicated an enemy have been rare to none. We have enjoyed the upper hand over staphylococcus, tuberculosis, syphilis, some strains of gonorrhea, some strains of influenza... only to discover that newer, more formidable successors of them tend to take their places.

Hopefully a time will come when some kinds of prevention will be permanent or when some organisms can be eradicated entirely. Certainly our science in 2013 is nowhere near such a level. And maybe it will never be.

But as long as we stay ahead in the game, the alternative of just quitting the war is unthinkable.

Here's to keeping ahead on our side of the scoreboard.

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Mounthell
Posts: 23

January 9, 2013

@jeenious: Speaking of organisms as "enemy" suggests a deterministic or Newtonian view of nature; the connotation suggests we're conducting a shooting-gallery operation and knocking off the "bad" ones as they pop up.

However, we will always have some variation of most of those that plague us because we can't stop nature from optimizing, aka adapting: nature, via its ecosystem dynamics always finds a path forward because organisms' malleability and persistence are intrinsic.

That view suggests we will increasingly find cases of once benign organism types turning pathogenic or parasitic as we continue to modify our environments across all length scales and thereby decrease our insulating and protective margins which healthy and vibrantly rich ecosystems once were able to provide.

As you know, humans can only continue to exist if we are upstanding and paid-up members of the complex ecosystems we infect, no matter our tricky technologies. And yes, "a time will come when some kinds of prevention will be permanent or when some organisms can be eradicated entirely."

Guess which familiar, charismatic organism type, along with its icons, deities and hard-won but insufficiently practiced wisdom, will be found to be in the latter group were some future visitor to bother to investigate.

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January 9, 2013

JToeppen
Posts: 28

Acorns need to planted in soils with certain mushroom mycelia to thrive. The spores may reside on the acorn, but some decaying leaf mass helps prevent mold and encourage white and brown "rot". Deliberate inoculation with Pisolithus arrhizus spores is also practiced.

Terra preta soils was used to produce food for millions of people where tens of thousands currently live in Bolivia;

http://en.wikipedia.org/wiki/Terra_preta

This soil must be a host to many biologically diverse organisms. Unlike a simple yogurt culture, such soils are probably a collection of complementary synergistic organisms existing in a complex ecosystem.

The era of bulldozer man will not end soon. However, there is a good chance that we will learn how to use organisms to transform our biological waste materials into long lasting self sustaining soils. The principle of using charcoal and ceramics to provide homes and reservoirs for spores and life seems self evident in hindsight. If we apply this understanding to waste management, biological management, land use, and our energy future many of todays problems become tomorrows resources. Composting has been around for a long time but applying modern understandings to practical matters is oddly uncommon in the modern world. The peoples of South and Central America developed potatoes, tomatoes, peppers, corn, squash, using methods that are not currently understood or practiced. We have quite a bit to learn about things that were known and yet forgotten.

Scientists often have gardens and experiment with things on their personal time. We are connected by topical networks now and share information in less formal and more artistic ways. Scientists are also becoming more involved in social and environmental issues, and more people embrace science as part of a larger Epicurean perspective. Our concepts of nature and culture need to be updated and applied to embrace "new" opportunities.

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eisenia
Posts: 2

February 1, 2013

Don't forget the other beneficial decomposers that trigger the mass production of the beneficial soil microbes naturally.

The plough is one of the most ancient and most valuable of man's inventions; but long before he existed the land was in fact regularly ploughed, and still continues to be thus ploughed by earth-worms. It may be doubted whether there are many other animals which have played so important a part in the history of the world...

Charles R. Darwin (1881)

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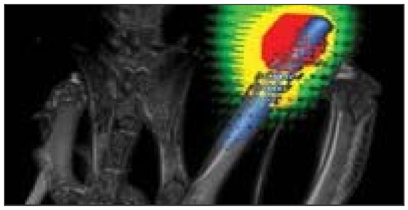

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