Performance of crop-boosting bacteria may depend on delivery method

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Sorghum growing in a research field on the northeast edge of Lincoln. A Nebraska team recently tested whether the method of delivering soil- and root-dwelling bacteria to sorghum could influence the growth of the cereal grain. Credit: Craig Chandler | University Communication and Marketing

Soil bacteria may be the microscopic building blocks to greater crop growth and higher yields—while knocking down chemical fertilizer use—but University of Nebraska–Lincoln researchers recently found that more blocks do not always build taller towers.

For more than a million years, plants have co-evolved to closely associate with the billions of bacteria that live in the soil. In this micro-scale community, soil bacteria interact and compete for plant-given nutrients, producing a complex but balanced microbiome that can benefit plant growth. Microbiologists are now capitalizing on this prehistoric relationship by providing crops with ample growth-promoting bacteria.

Despite the decades-long progress toward using soil microbes to promote plant growth, no studies in the public literature have directly compared the multiple methods for introducing live bacteria to plants. A recent Nebraska study discovered that the most effective methods for promoting growth depends on the type of bacteria. Matching method with microbe could benefit agriculture and the environment by increasing crop yields and reducing chemical fertilizer use, respectively.

"We really believe that the nature of microorganisms can do something for agriculture," said Yen Ning Chai, a postdoctoral researcher at Nebraska and lead author of the study, published in the journal *Frontiers in Microbiology*. "We believe that, in the long run, we can slowly replace or supplement fertilizers with the microbes."

As plants stretch their roots deep into the microbe-rich soils, those roots recruit beneficial bacteria by secreting carbon deposits—an essential nutrient for bacteria, Chai said. Bacteria flock to these plant-provided carbon resources through the vast, carbon-limited soil. Many bacteria thrive and foster plant growth from the soil layer, called the rhizosphere, that clings to the root. Yet some bacteria can penetrate the inner root, or endosphere, where the space between cells holds even greater carbon reserves.

"It's not really known that well why some bacteria end up outside and some end up inside the root," said Daniel Schachtman, co-author and George Holmes University Professor of agronomy and horticulture at Nebraska. "(Bacteria) may be able to circumvent the plants' defense mechanisms or to fake the plants out to be able to get into the endosphere."

Bacteria have evolved specialized functions to survive challenging conditions. Adaptations of bacteria's protective cell walls have separated bacteria into two general groups: gram-positive and gram-negative, detectable by a staining technique developed by Hans Christian Gram in 1884. Bacterial cell walls contain a rigid layer, called peptidoglycan, that consists of a chain-link fence of sugars and amino acids around the cell, giving it shape and protection. Gram-positive bacteria have a thick layer of peptidoglycan, while gram-negative bacteria have a thin layer. The roles that bacteria play in the plant are likely related to the specialized functions they have adapted.

"Just like humans, some bacteria just colonize our skin, while some bacteria colonize the gut," Chai said. "They have different functions; the gut bacteria help to digest food.

"The (bacteria) that live inside the root have more genes related to carbon utilization than the ones in the rhizosphere."

The close association between microbes and plants was discovered around the turn of the 20th century, when specialized bacteria were spotted on the roots of legumes—a plant group including beans, peas and lentils. The first agricultural applications of bacteria hit the markets about 80 years later and are widely used in India and South America today. Directly coating seeds with bacteria is the most practical method currently in use. Alternative methods, which include applying bacteria to the soil or to recently sprouted seedlings, require a higher concentration of bacteria that can prove costly and time-consuming.

Microbiologists are now testing those different methods of loading crops with helpful bacteria. While the various approaches to applying bacteria have been implemented and tested separately, Chai, Schachtman and their colleagues compared the application methods using diverse types of bacteria. This allowed them to evaluate these practices more directly than when comparing studies that use a single method and microbe. In addition to monitoring plant growth, the researchers quantified the target bacteria on the plants over time, allowing them to correlate bacterial abundance with plant growth.

In comparing delivery methods, the team used a gram-positive bacterium and a gram-negative bacterium that occupy the rhizosphere, along with a gram-negative bacterium that can live in the endosphere. In some cases, the researchers applied these target bacteria to sorghum seeds or seedlings before planting in bacteria-free soil. In other cases, they applied the microbes directly to sterile soil.

"We originally isolated these bacteria from field-grown sorghum," said Chai. "We are interested in whether these bacteria that were originally associated with sorghum can help sorghum to grow."

Coating a sorghum seed with gram-positive bacteria was the most effective way to promote sorghum growth—better than coating the seedlings or drenching the soil, the team found. But the plants that received those gram-positive bacteria actually showed the smallest bacterial populations after eight weeks, an interesting result that suggests that more is not always better, Chai said.

Gram-negative bacteria, by contrast, were the better growth promoters when applied using the less-practical applications, especially the coating of sprouted seedlings. However, prolonged seed-coating boosted the numbers of gram-negative bacteria that could inhabit the endosphere.

When the seed-coating methods were taken to the field, leaving the applied bacteria to battle it out with the soil community for a coveted spot in the plant's microbiome, the results were inconclusive. The concentration of the bacteria may have been too low to give the bacteria a competitive advantage over the natural microbial community, Chai said.

"There are a bunch of other bacteria, and also fungi and other micro-organisms," she said. "When you put the seed that was inoculated in the soil, you don't just have the bacteria that you inoculate; you also have other microbes that can compete with the bacteria."

The best method for promoting crop growth, then, seems to follow a familiar adage of research: It depends. The effectiveness of the method varies by the type of bacteria and crop, the team said, and may even depend on the condition of the environment. So far, applying bacteria to crops has been most beneficial under unfavorable crop conditions, such as low water or nutrient availability, where the plant's microbiome can help provide what the soil lacks.

Though there's much left to learn, Schachtman said scientists are building toward an eco-friendly technique for promoting crop growth by getting to the root of the plant's native bacteria. The upside of those efforts, he said, should also extend beyond the environment.

"(Roots) are one of the most important organs on Earth, in that they take up nutrients and minerals from the soil, (transport) them to the edible parts, and we eat them," Schachtman said. "Soils are where we get most of our minerals."

More information: Yen Ning Chai et al, Assessment of Bacterial

Inoculant Delivery Methods for Cereal Crops, *Frontiers in Microbiology* (2022). DOI: 10.3389/fmicb.2022.791110

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