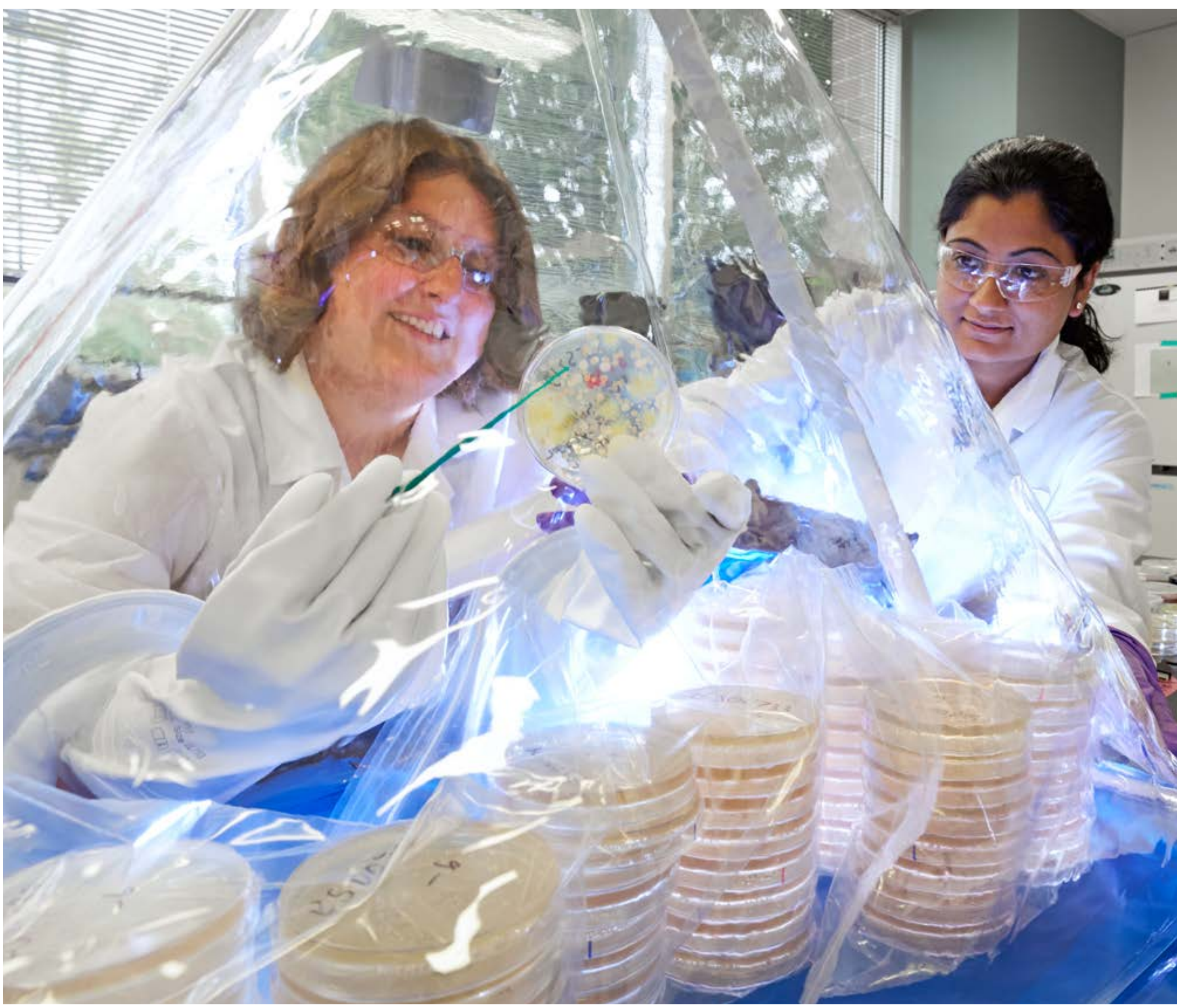
Frozen treasure

Genes from bacteria are helping Bayer scientists improve the characteristics of crop varieties for farmers. Through the use of biotechnology, today's seeds are tolerant to herbicides, resistant to damaging insects and nematodes and even have higher yield potential. Any of these benefits can be realized by transferring some of the tremendous genetic diversity of the bacterial kingdom to important agricultural crops. Recently, Bayer's ability to discover valuable new genes from bacteria has been greatly increased by technological improvements around automation and large scale bioinformatics. These advances give scientists a new ability to physically handle a large number of bacterial strains and learn about their functions through their genetic codes.



Preparing for new tests: Janelle Ciafardoni and Rakhi Singh (left to right) use an anaerobic tent to isolate microbes that can only survive in an oxygen-free environment.

Photos: Peter Ginter/Bayer AG (5)

Dr. Jon Giebel and his team from Trait Research at Bayer's Crop Science Division spend their days attending to the welfare of a remarkable collection of bacteria. Each one of the 116,000 bacterial strains which are locked away at minus 80° Celsius in the freezers of the Bayer Innovation Center in Morrisville, North Carolina, could hold the key to helping farmers meet the rapidly growing global demand for food. These microbes harbor valuable traits which will help the researchers develop crop varieties with improved characteristics. "Many of the bacteria contain genes which could make crops pest-resistant or herbicide-tolerant. Other strains can be used to produce new biological or chemical crop protection products," says Giebel, describing his living treasure trove. With an average of 5,000 genes per organism and 116,000 organisms, Bayer scientists have a vast library of over half a billion genes to choose from; each one could hold the key to unlocking greater yields for farmers.

New microbes for the collection: Sebastian Doerfert takes soil samples at a Bayer-owned site in North Carolina. The bacteria living in this soil will then be isolated in the laboratory.

Microbial diversity can provide answers to the challenges faced by farmers

The Bayer collection of 116,000 strains of bacteria continues to grow, and adding new biological diversity is a key focus for Giebel and his team. Bayer takes into account any applicable laws as to collection of environmental samples, including laws enacted by signatories to the Nagoya Protocol. Rather than searching the globe for new diversity, Bayer scientists are focusing on what can be found in the company's own back yard. "One tablespoon of soil from our parking lot contains more diverse organisms than we would find in all of the zoos in North America," explains Giebel. "It is hard for humans to picture the scale of the microbial community that is all around us. When we think of an earth worm and a lion we think of two very different organisms, but when we use the evolutionary scale of the microbial community, worms and lions are actually close cousins." The key is to capture that genetic diversity in the lab. In addition to diversity, Bayer scientists are trying to select for microbes that are more likely to have beneficial properties for plants.

New samples arriving in Morrisville are cultured in the lab. From these cultures, new unique microbes are isolated and safely stored. Then the hard work begins: screening for promising traits. Microbes are tested for their potential to combat insects and nematodes, inhibit the growth of pathogenic fungi, degrade chemical herbicides or increase overall plant health. Once a bacterial strain has demonstrated one of these valuable traits, researchers across Bayer's Crop Science Division try to understand the source of that trait and look for ways to deliver that to the farmer. If a microbial gene can be associated with one of these traits, that gene is then transferred into a crop such as soybeans to create a genetically modified plant with a new characteristic. Two recent innovations have accelerated the process. Eighteen months ago, Bayer researchers began sequencing the genomes of the entire collection, and at the beginning of 2017 a robot called the Automated Storage and Handling System (ASH) will come online to fully automate the storage, removal and preparation of microbe samples for testing – tasks that have always been done by hand in the past. "Instead of preparing 1,500 strains a week, we will soon be doing 1,700 strains a day," says Giebel. The large effort in time and resources invested in building this transformative pipeline is beginning to pay off in the speed with which new innovations can be made.

Scientists test whether selected bacteria are effective against key agricultural pests

Now that the genomes of bacteria in the collection have been sequenced, scientists can identify new genes which are active against pests faster and with greater precision than before. Thus, the quest for new genes is now less empirical and more driven by scientific knowledge.

1,700

bacterial strains

can now be processed each day thanks to the automated system.

Source: Bayer

Dr. James Doroghazi and Dr. Jon Giebel (photo above left, left to right) analyze the genetic profiles of bacteria at the computer. To test new strategies against pests in the laboratory, the scientists (photo above) run feed tests on stink bug larvae that they breed in an incubator (photo left).

A promising microbe that is required to test hypotheses from the data analysis is sent to the ASH system. The bulky machine – the size of a small studio apartment – then retrieves sets of the desired strains from the depths of the freezer and transports them to the surface. Within minutes the samples are thawed and ready to be cultured in the lab in preparation for feed trials with insects and nematodes.

In the next step, the selected bacterial strains are tested for activity to confirm the scientists' in silico prediction of activity. The bacteria are mixed with artificial diet and fed to the target pest. Lab technician Ellis Driver demonstrates the results of a test on the southern green stink bug, which attacks soybeans and other legumes: six out of eight insect larvae which were fed the bacteria-laced diet lie dead at the bottom of one of the flasks in her hand. In a second flask containing the same bacteria-laced diet, all the bugs are dead. "That's a pretty good result," says Driver. "The larvae in the untreated control group are all fine but the treated group is nearly all dead."

The next step is to confirm which genes are responsible for the activity. One powerful way to do this is a process known as 'comparative genomics'. As Doroghazi explains, "Let's say we have five bacterial strains which kill the pest and five which don't, even though they show a lot of similarity in their genome sequences. We then look for genes that are present in the former, but not in the latter, and evaluate those genes for potential activity."

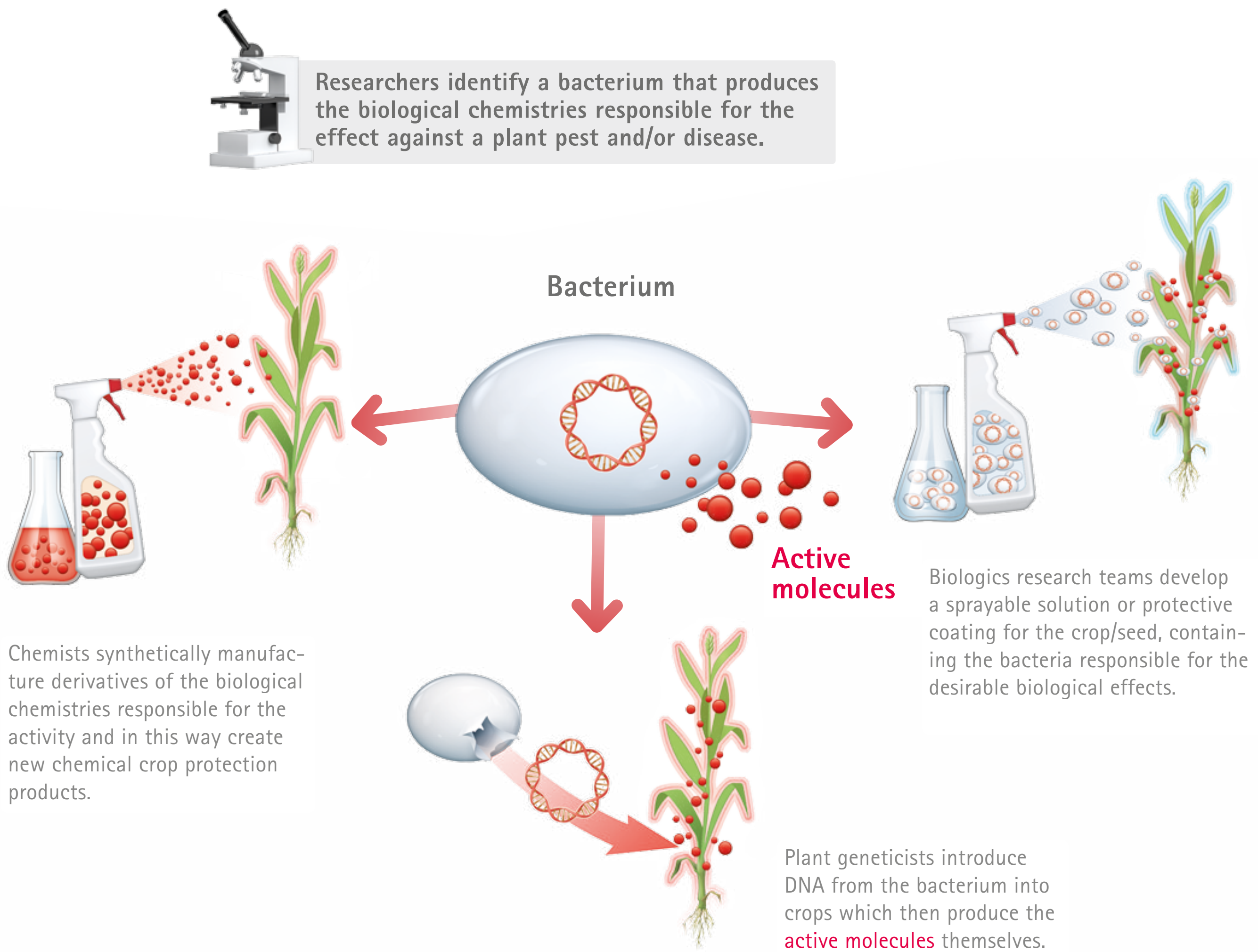
In addition to comparative genomics, Bayer researchers have other methods of determining what makes a strain lethal to insects. Biochemists can extract and separate proteins from pesticidal bacteria and test them with insect feeding experiments to see which group displays the pesticidal activity. The goal of this work is to isolate a single protein or a small number of proteins that are responsible for the pesticidal activity; the genes that encode for these active proteins can then be determined by matching the protein sequence to the genome DNA sequence.

The discovery of a gene which codes for anti-pest activity is only half the battle. The next challenge is to insert the microbial gene into the plant genome. Hopefully, the plant will then express that gene, enabling it to produce the same proteins as the microbe to help the plant defend itself against the pests. It's a complicated step: if the microbial gene does not work properly in a plant or causes damage to the plant, the experiment will have failed, and Giebel, Doroghazi and the rest of the team will have to go back to the drawing board to do some further improvement.

If all goes to plan, the researchers have taken the first step to developing a new genetically engineered plant with built-in resistance. In the past years, several genes with activities against key pests of corn, cotton and soybean have been identified and validated in plants using this approach. Experimental varieties of

How genes from bacteria can protect plants

Some microbes release biological chemistries that fight plant pathogens and/or pests. Once scientists have identified a bacterium with a desired biological activity, they can employ at least three different strategies to use this discovery to protect crops against a pest.



these crops are tested in greenhouses a few miles from the labs at Bayer's U.S. Crop Science headquarters in Research Triangle Park. With the new capabilities developed in the trait discovery groups, the hope is to now intensify the rate of novel gene discovery to provide farmers with gene-based solutions to pests that reduce their yields.

Bacteria strengthen the resilience of plants in a number of different ways

Using the same collection, researchers searching for new biologicals take a different approach; they use whole, living bacteria to manufacture crop protection and crop enhancement products for foliar sprays and seed coatings. The microbes in these products provide many benefits to the plant including protection from fungal diseases or insect damage.

The Poncho™/VOTiVO™ seed treatment is a prime example. Designed for soybean, corn and cotton seeds, it provides dual protection: the seeds are coated with a mixture of living bacterial spores creating a living barrier that prevents nematodes from causing damage and a systemic chemical that is absorbed into the roots, providing control of many critical early-season insect pests. Moreover, in the United States this seed treatment is often used on seeds that have built-in herbicide tolerance and pest resistance due to GM traits similar to those described above.

Microbiologist Giebel has no doubt that many more Bayer products have yet to emerge from his bacterial collection. So he and his team will continue adding to and nurturing their collection of frozen treasures.