Pseudomonas

Orphan Gene Clusters Offer Potential Protection Against Plant Diseases

JOYCE LOPER (D1088-1)

The tobacco hornworm larva on the left was inoculated with *P. fluorescens* Pf-5 bacteria and has lost body rigidity as a result. On the right is a healthy larva not inoculated with bacteria. Each larva is about 2 inches long.

The term "pseudomonad" refers to a diverse group of bacteria found in a variety of natural environments. Some species can be pathogenic for plants and animals, but others—such as the biological control agent *Pseudomonas fluorescens* Pf-5—can actually promote plant health.

What determines whether a pseudomonad is helpful or harmful? The answer may lie in its genes. New genomic research has revealed novel, potentially beneficial compounds and toxins that are giving scientists insight into pseudomonad-plant interactions.

Every year, new genomes are sequenced and made publicly available. With this information, scientists have been able to isolate many chunks of DNA and identify the products they code for. But there are many DNA segments whose functions are still unknown.

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Technician Brenda Shaffer (left) and plant pathologist Joyce Loper load samples for quantitative PCR analysis, a procedure to evaluate gene expression by *P. fluorescens* Pf-5.

Some of these isolated segments are similar to other genes that code for secondary metabolites—organic compounds that can contribute to beneficial biological processes. Because the specific metabolite produced is unknown, these segments are known as "orphan gene clusters."

The ABCs of Gene Clusters

It's likely that some orphan gene clusters produce enzymes that could make new antibiotics. Identifying these gene clusters and their products would give scientists new tools to fight harmful microorganisms.

"Orphan gene clusters represent the potential to discover new antibiotics, drugs, and chemicals," says Joyce Loper, a plant pathologist at ARS's Horticultural Crops Research Laboratory (HCRL) in Corvallis, Oregon. "What are these clusters producing? Could those products have positive applications? That's what we want to know."

Working with scientists at Scripps Institution of Oceanography at the University of California-San Diego; Oregon State University; and Northland College, in Ashland, Wisconsin, Loper investigated a new method for determining the products of orphan gene clusters.

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The scientists used what they call a "genomisotopic" approach. It's a two-step procedure that combines genomic sequence analysis and isotope-guided fractionation—the process of isolating compounds.

The first step is to make an educated guess, based on existing knowledge about DNA sequences, about the type of compound produced by the orphan genes. But DNA sequences provide limited information about the compound.

JOYCE LOPER (D1089-1)

Entomologist Denny Bruck injects a larva of the tobacco hornworm, *Manduca sexta,* with *P. fluorescens* Pf-5.

"We can gather enough information through observation of an organism's genome to make a reasonable prediction about the nature of a compound's structure," says Scripps professor William Gerwick, who collaborated on the project. For example, information in the genome sequence can sometimes be used to predict the precursors—or chemical building blocks—of the final product.

The next step is to verify whether that prediction proves true.

To do this, the scientists label the chemical building blocks with isotopes and then place the labeled molecules in a culture of the *Pseudomonas* bacterium that has the orphan gene cluster. The isotopes enable the scientists to track the molecules to see whether they form the predicted compound. Since the compound will also be labeled, it can easily be isolated from other chemical compounds that are present in the bacterial culture. Once the product is isolated, chemists can determine its structure.

Analyzing genomic sequences is a common practice, as is using isotopes to isolate and identify compounds. But the experiment above was the first that used them together to discover a new compound, Gerwick says.

Potentially Protective Properties

With Gerwick, Loper and her colleagues applied the genomisotopic method to the genome of Pf-5, a pseudomonad that produces antibiotics that suppress plant diseases caused by soilborne pathogens such as *Pythium ultimum* and *Rhizoctonia solani*.

Scientists have long known that Pf-5 makes antibiotics, but they've now identified two previously unknown ones. The first, discovered in the above study, is a metabolic byproduct called "orfamide A."

A joint project conducted by Loper and Harry Gross, at the University of Bonn, Germany, demonstrated that a second *Pseudomonas* orphan gene cluster codes for biosynthesis of compounds related to rhizoxin—an antibiotic and potential antitumor substance. Both orfamide A and the rhizoxin-related compounds are toxic to some plant pathogens.

"The genomic sequence data has definitely expanded our understanding of the biological control capacity of this organism," Loper says. "It's likely that both compounds could contribute to biological control of plant diseases."

Scientists have also long known that Pf-5 suppresses plant diseases. Recent research—in collaboration with HCRL entomologist Denny Bruck and European colleagues—showed that the pseudomonad, when injected into larvae, can also kill some insects. The team identified a gene that codes for production of a toxin related to *mcf,* a powerful toxin that "makes caterpillars floppy"—hence the acronym.

Tests show that the toxin kills tobacco hornworm and waxworm larvae, but it isn't Pf-5's only defense against insects. Mutants of Pf-5, in which the toxin gene has been removed, also kill larvae, albeit less effectively. From this, the researchers infer that the genome codes for additional insect-killing toxins, which they are currently working to identify.

These studies were funded in part by ARS, the National Institutes of Health, the German Research Foundation, and the Microbial Genomic Sequencing Program, part of USDA's Cooperative State Research, Education, and Extension Service (CSREES). In 2007, CSREES funded the sequencing of seven other *Pseudomonas* species, four of which are under investigation in ARS laboratories.

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Further research could identify new natural products with valuable properties for use as antibiotics, drugs, or pesticides.—By **Laura McGinnis,** ARS.

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