GROWING KNOWLEDGE

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The genetic revolution comes home

OSU Plant Clinic seeks new applications for whole genome sequencing

BY MELODIE PUTNAM

CHANGE IS INEVITABLE. Although the world may look more or less the same, you are not living in the one that existed when you were born.

We are in the midst of an ongoing genetic revolution that has radically changed our understanding of nearly every biological process. You have probably heard of the use of genetic engineering to create crop plants resistant to certain types of herbicides. You may also know that marker genes have been used to help plant breeders identify useful traits to speed up development of new lines of material.

However, our knowledge of genetics has deepened and expanded substantially beyond these two concepts in the last 20 years. For example, it is now possible to insert a novel gene with no functional purpose into a proprietary plant line to curtail illegal propagation. If the characters of a plant look too similar to one developed by a breeding company, that company can test their rival's material for the presence of the inserted gene. If present, they have evidence their competitor unlawfully reproduced their genetics.

This is one simple, but powerful, example of how knowledge of genetic information within an organism can be used for specific purposes.

The power of modern genetic tools

My colleagues and I in the Department of Botany and Plant Pathology at Oregon State University have been investigating

how to best use the power of modern genetic tools to help growers, specifically in the realm of disease detection, pathogen transmission, and disease management. Bacteria contain relatively little genetic information, compared with fungi and other more complex organisms, so we have been using bacteria as a model system for method development and to mine the genomes for useful information.

Let me back up here a minute. A genome is all the genetic material that exists within an organism. Bacteria usually have one chromosome (humans have 23 pairs of chromosomes), consisting of a long string of chemical structures called bases. There are four bases that are key portions of the nucleotides that comprise the DNA molecule (Figure 1). The genetic information contained in DNA allows the organism to grow, reproduce, and carry out all functions needed for life.

The method called whole genome sequencing allows researchers to "read" the building blocks of DNA, its bases, in the order in which they occur. It is somewhat like fingerprinting an organism, only much more precise and informative. Analysis of whole genome sequences of the Covid-19 virus allowed scientists to track the different variants as they moved through a population in real time, not retroactively.

Tracking newly formed lineages is possible because, as DNA replicates, small errors or changes in base order will occur, resulting in genetically distinct variants within a species over time. Bacteria, in addition to the chromosome, often have genes in structures called plasmids. These plasmids may be transferred to other bacteria of the same genus, or less frequently, to bacteria of a closely related genus. Plasmids often contain genes that govern the ability to cause disease, and hence are important drivers in the evolution of bacteria (Figure 2).

Plasmid DNA, aside from being shared among bacteria, may also show genetic drift through errors in replication.

It is by comparing these small changes in DNA composition, either in the chromosome or a plasmid, that one can determine how long ago variants arose. The greater the number of changes, the more distantly related two organisms are predicted to be.

These minor changes allow tracking of unique strains within a species from one place to another. And since bacteria have regeneration times of hours, distinct genetic changes can appear relatively quickly. They can happen in years instead of hundreds of years.

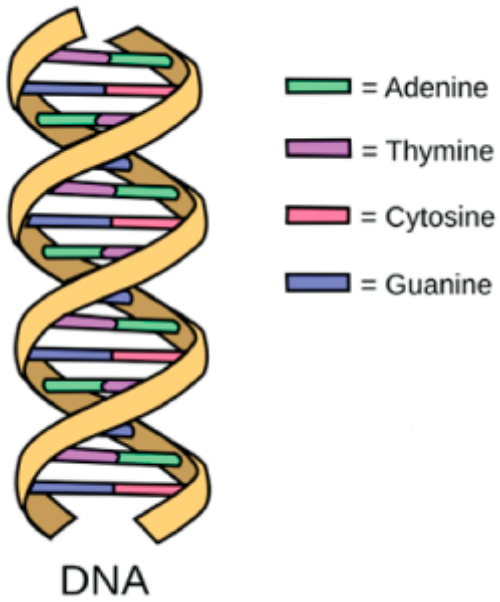

Fig. 1. A simplified diagram of a portion of a DNA molecule comprised of four nucleotides, containing, along with a sugar and phosphate molecule (not shown), the nucleobases adenine, thymine, cytosine, and guanine. The nucleotides are the "rungs" of the ladder.

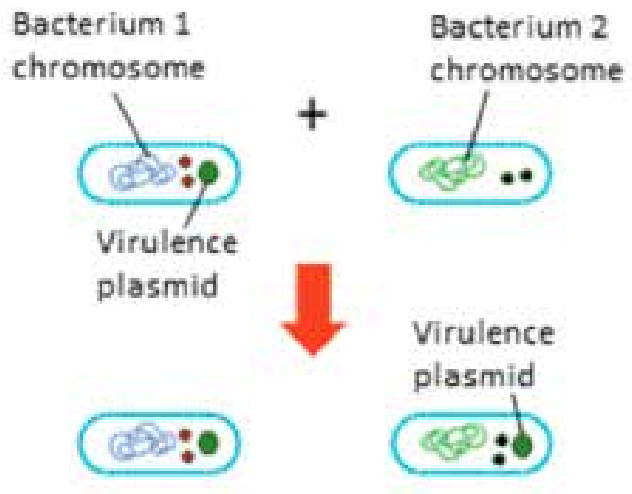

Fig. 2. A donor cell, Bacterium 1, contains a virulence plasmid and two smaller accessory plasmids. The virulence plasmid contains genes that allow the bacterium to infect plants. Bacterium 2 lacks the virulence plasmid and does not cause disease; it also contains two smaller plasmids. When the two bacteria are in physical contact, they fuse and Bacterium 1 donates a copy of its virulence plasmid to Bacterium 2, which can now infect plants and cause disease. This is one way new lineage of bacteria can rapidly develop.

Fig. 3. Pathogenic isolates of *Rhodococcus fascians* cause growth abnormalities called leafy galls, shown here on a *Lavatera*. The multiple buds produce stunted leaves that do not grow to full size.

The next steps with genome sequencing

Significant and rapid improvements in sequencing technology since the first genomes were "read" has placed whole genome sequencing within reach of individual researchers.

To take advantage of this, the OSU Plant Clinic — with funding from a Nursery Research grant from the Oregon Department of Agriculture and the Oregon Association of Nurseries — purchased a DNA sequencer capable of "reading" the order of bases in DNA from a bacterial cell, which could then be organized into a whole genome.

My colleagues — Drs. Jeff Chang, Nik Grünwald, Alexandra Weisberg, and their students and post-doctoral scholars — have been helping me to delve into the genomes of multiple bacterial species to learn about their evolution and movement over time. One of our projects involved the bacterial pathogen *Rhodococcus fascians*, which causes leafy gall disease of plants (Figure 3).

Not all *Rhodococcus fascians* isolates can cause disease. In *R. fascians*, the virulence genes that enable the bacterium to infect a plant are carried on a plasmid, which may not be present in some members of the species. However, the plasmid can be transferred to harmless *R. fascians* cells, which then have the ability to cause disease.

Leafy gall disease is of particular concern in ornamentals nurseries, because there is no control or cure available. I was interested in the problem that some nurseries were having with recurring disease. Did these nurseries just need a better sanitation program? Or were they reintroducing the bacteria with plants they were bringing on site?

We tackled this question by analyzing the whole genome sequences of nearly 100 isolates of *R. fascians*, most of which were collected from nursery samples. We gained some valuable insights into the biology of the bacteria, and discovered an interesting story.

We had in our collection many isolates of *R. fascians* recovered from different species of plants from multiple nurseries over 15 years. When we analyzed the genetics of these bacteria, we found that one nursery had eight different genetic lineages of *R. fascians*. A second nursery had six different genetically distinct variants, suggesting that both nurseries had obtained the pathogen multiple times, probably from different sources.

We found other instances where multiple nurseries, located in different states, had bacterial isolates that were essentially genetically identical, suggesting that infected plants had been shipped to the nurseries. This can happen with *R. fascians* because symptoms may not be recognized or even visible at time of shipping.

The point of this work was not to place blame on particular nurseries for distributing infected material, but to get a better handle on sources of the bacteria to allow improved management strategies. The nursery with the chronic bacterial reservoir has adopted much more stringent sanitation, scouting, and testing measures to try to exclude the pathogen. The managers are much more aware of the extent of the problem and are acting accordingly.

Tracking how diseases spread

In the examples given above, we used the genetic similarity and difference between isolates to make inferences on pathogen movement. We assumed it was unlikely that the same genetic background would show up in different nurseries unless the nurseries had purchased plants from the same source.

But is that a valid assumption? Or is it wishful thinking? We decided to use a recent outbreak of *Xanthomonas hortorum* pv. *pelargonii*, cause of bacterial blight of geranium, to test our assumptions.

In the spring of 2022, *X. hortorum* pv. *pelargonii* was distributed on cuttings from a facility in Central America, and clients were duly notified of this event. An outbreak such as this, with a known source, was an excellent opportunity to demonstrate the ability to track a genetic trail.

We obtained 35 bacterial isolates from plants sent to diagnostic laboratories in Maryland, Indiana, and New York, including some isolates from previous outbreaks, and analyzed their whole genome sequences. We found that all of the geranium isolates from the 2022 outbreak were essentially clonal, which is what you would expect from material that originated from a single source. The bacterial isolates from previous outbreaks had accumulated multiple changes in their genetic code, and were only distantly related to the bacteria in the 2022 incident.

We also found evidence, in one nursery, of disease spread from the plants originating from Central America to plants obtained from a different breeder. Both sets of plants were growing in the same greenhouse.

This test case, using a known

outbreak from a single source, verified our assumptions regarding the use of whole genome sequencing for tracking the movement of bacterial isolates over time and space.

Currently, I am collecting isolates of the crown gall bacterium, *Agrobacterium*, from blueberry, raspberry, and grape grown in western states to determine whether there are similarities between isolates from diverse sources (Figure 4).

This work is being conducted with Dr. Alexandra Weisberg and is funded by the Western Integrated Pest Management Center. The goal is to help growers identify how the pathogen is getting into their vineyards and fields.

Since the bacteria may be present on nursery plants without causing symptoms, might *Agrobacterium* be getting around this way? We also wish to learn if any new traits are developing in the bacteria over time. Another goal is to exploit the information present in the bacterial genes for insights into more effective disease management.

There are two similar biocontrol products for crown gall bacteria that, when used preventively, are very effective in shutting down the disease. However, these products only work against certain strains of *Agrobacterium* – those that have the ability to produce certain types of opines.

Opines are metabolites produced by the bacteria and are used as nutrients and for other cell functions. There are over 20 different kinds of opines that may be produced by a given *Agrobacterium* cell, and which opine is produced depends on what type of plasmid is present in the bacterium.

Tracking how diseases spread

If the biocontrol products are only effective against those agrobacteria that contain certain opines, what about the other isolates of agrobacteria? Simple: they are not affected in the slightest, and growers using these products who have insensitive agrobacteria in their fields and vineyards are wasting money. If we have Agrobacterium cultures from a grower's field, orchard, or vineyard, we should be able to determine what type of opine genes are present and whether the biocontrol products will be effective.

Ferreting out this, and other types of information, is only possible when there is a large collection of genomic resources available for analysis. To understand trends in pathogen development, we need to analyze the genes of populations of bacteria to determine the breadth of variation of types present. It isn't much help to deeply know a few individual types when there are hundreds or thousands present.

Using *Rhodococcus*, *Xanthomonas*, and *Agrobacterium* model systems, we are developing the sequencing skills and analytical pipelines that will allow us to eventually and routinely analyze all types of bacteria and, eventually, fungi. Uncovering the genetic information within the tiny pathogens will allow us to exploit their vulnerabilities to allow better informed management solutions and understanding of how growing practices can influence pathogen evolution in real time.

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Melodie Putnam was the director of the OSU Plant Clinic for 30 years. She has recently retired, but the work described in the article continues. She may be contacted at putnamm@oregonstate.edu.

Fig. 4. *Agrobacterium* is a genus of bacteria that can cause the disease known as crown gall, shown here as the swollen tissue at the graft union of these grape plants.

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