GROWERTALKS

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Unseen “Cities”: Bacterial Biofilms

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A biofilm isn’t the same as a biopic. It’s more similar to the film that accumulates in your greenhouse gutters or, perhaps, more personally, on your teeth. Dental plaque is a biofilm that builds up naturally on your teeth and which must be removed daily by brushing to prevent development of cavities or gum disease.

So what is a biofilm then? Biofilms are aggregates of bacteria or fungi attached to surfaces in a matrix that provides protection from the elements. Biofilms of bacteria on the interior of catheters inserted into cardiovascular or gastrointestinal tissues are responsible for serious infection and disease in hospitals. This article will address bacterial biofilms that can contribute to disease in plant production.

Bacteria not within a biofilm are said to be free-living, often as single cells or in small clumps (Figure 1). Bacteria within a biofilm matrix (Figure 2) are surrounded by long chains of gummy sugar molecules (extracellular polysaccharides), lipopolysaccharides, DNA and cellular debris, all produced by the resident bacteria. The polysaccharides provide the physical structure of the film and, like a house, protect occupants from exterior assaults from predation, antibiotics and disinfectants, desiccation, changes in pH and the harmful ultraviolet spectrum of the sun.

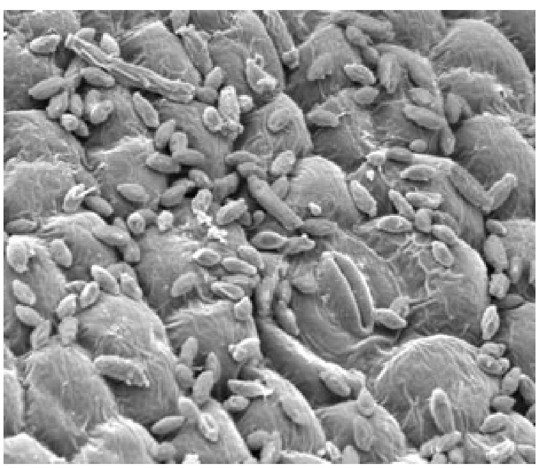

Figure 1. Football-shaped bacteria lie on the surface of a plant leaf, prior to biofilm formation. The image was taken using a scanning electron microscope by Teresa Sawyer, Oregon State University Electron Microscopy Facility.

The film is not just a slime, but is a three-dimensional structure with its own characteristic architecture—tunnels, bridges and pores may be present. Water and nutrients diffuse into the biofilm to nourish the bacteria in the interior and wastes are removed. In nature, biofilms aren’t composed of a single type of bacteria, but of multiple different species, including non-bacterial residents, such as yeasts. Each component member contributes something of value to the whole, which adds to the resiliency of the biofilm. The organization has been likened to a city and a term has been coined for the study of these structures and their inhabitants: sociomicrobiology.

Attachment to a plant or surface is the first step in establishment of a biofilm. Once adhered, the bacteria reproduce and increase in numbers. As the community grows, members communicate in a process called quorum sensing. This communication is in the form of chemical signals produced and perceived by the members of the biofilm, both between bacteria of the same, and of different, species. This communication can include signals that modify the mode of bacterial life from living harmlessly in association with a plant on the surface to being able to overcome plant defenses and initiate disease.

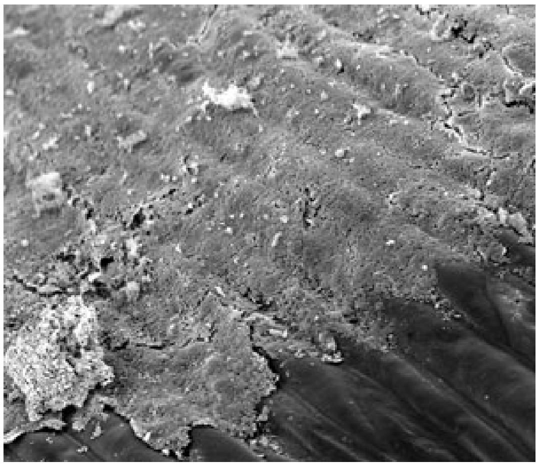

Figure 2. A bacterial biofilm has formed on the surface of these root epidermal cells. At lower right are plant cells not yet covered by the biofilm. This image was taken using less magnification than Figure 1, so the bacteria appear much smaller. Photo by Teresa Sawyer.

Many bacteria have a small piece of DNA called a plasmid that's carried outside of the chromosome. These plasmids carry additional genetic information that's often beneficial to the bacteria, but not essential. This is somewhat akin to carrying a wallet containing credit cards, cash and other documents useful in everyday life. Your wallet is small and portable, and if desired, can be shared. Here the analogy breaks down a bit, because your wallet is not self-reproducing, and plasmids are. However, I think you get the picture.

Plasmids can be transferred from one bacterium to another of the same species and sometimes to bacteria of another species. The information that's carried on plasmids can confer to the recipient traits, such as antibiotic resistance, the ability to withstand heavy metals or genes that allow the bacteria to cause disease. Bacteria must make physical contact for this exchange of genetic information and biofilms offer that intimacy, suggesting the possibility of harmless bacteria turning into pathogens or picking up antibiotic resistance by acquisition of a plasmid.

Why should you care about biofilms?

The bacteria in biofilms do not remain stationary. Under some conditions, the film can break away and the residents can be transported in water to establish a new city on another surface, including plants. *Agrobacterium tumefaciens*, the crown gall bacterium, has been shown to attach to and form biofilms on polystyrene and polypropylene, of which pots, pot carry trays, pot labels, groundcovers and bench tops are made. These materials, if not properly sanitized before reuse, can act as a source of infection for plants. Biofilms formed by *Agrobacterium* in irrigation lines are thought to contribute to crown gall disease of cucumbers and tomatoes in hydroponic systems.

Bacteria within biofilms are more difficult to eradicate because of the protection the film affords. The matrix in which the bacteria are immersed acts as a physical barrier to antimicrobial compounds, slowing down diffusion of the material into the matrix, delaying or preventing contact. Additionally, the matrix itself may react with the sanitizing agent, such that it's much less effective, or perhaps completely ineffective, by the time contact with the target bacteria occurs. The lethal action of nearly of all sanitizers and disinfectants is evaluated using free-living bacteria. This method of evaluation can underestimate the amount of product needed to kill bacteria in biofilms by 100 to 1,000 times.

This brings up an important issue: sanitizers and disinfectants may not be adequate to actually degrade and destroy biofilms on non-plant surfaces. This makes it essential that sanitizers be used correctly. Each type of product has its own characteristics that must be considered when using and storing the product, and failure to do so means your sanitizer may do little more than water.

Tools, pots, benches and other surfaces must first be scrubbed clean prior to using a sanitizing agent. Dumping a plant out of a pot and whacking the pot against the bench may remove large chunks of mix, but that's all. Putting such a pot into a sanitizing bath without further cleaning is like taking a shower with your clothes on: you can soap up, but little of it will reach the skin. For example, chlorine in a bleach solution will react equally with organic matter, inorganic matter and microorganisms. The more potting mix, fertilizer or plant debris that's present, the faster the bleach solution becomes useless. In one study, the addition of 1% dry milk powder completely neutralized a 10% bleach solution within 60 minutes. Each sanitizer has its own susceptibilities and product labels offer a wealth of useful information to get the best performance from the sanitizer you choose.

We at Oregon State University are currently investigating the effect disinfectants and sanitizers have on two plant pathogenic bacteria: *Agrobacterium* and *Rhodococcus fascians*. We're looking at the ability of currently registered and potential new products to control these bacteria in biofilms. Our goal is to be able to make sound recommendations to ensure growers don't have biofilms of these bacteria lurking in their production systems. Stay tuned. **GT**

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