Bacterial biofilm: the new superorganism

Over 99% of bacteria are not free-living but exist in thin-layered colonies known as 'biofilms'. In the human body, biofilms can cause serious, chronic and intractable infections. Dr Céline Lévesque, of the University of Toronto, is exploring how the bacteria within our dental plaque biofilm communicate. She has discovered a molecular mechanism which triggers some cells to lie dormant, evading treatment, and others to die for the benefit of the colony. Disrupting these signals could lead to promising new treatments for disease.

Every time you brush your teeth, you clear away a sticky film, dental plaque, containing millions of microbes from hundreds of different species. In healthy people, this ubiquitous oral 'biofilm' of bacteria, like those in many other parts of our bodies, is usually harmless or even beneficial. However, many biofilms can opportunistically become disease-causing pathogens, for instance when one microbial species becomes too prevalent, when the biofilm becomes established in an organ it does not usually colonise, or when host immunity is compromised, even by something as simple and inevitable as old age.

Biofilms are now emerging as a key concern for human health, and they are extremely difficult to eradicate. They are highly tolerant to antibiotics: treatment may reduce the number of bacteria present but usually fails to remove the whole colony, leading to chronic, recurrent infections.

Among the opportunistically pathogenic biofilm bacteria are several species of *Streptococcus*. Streptococci make up some 80% of the bacteria in dental plaque and are implicated in infections ranging from tooth decay and sore throat to life-threatening diseases such as pneumonia, meningitis and an inflammation of the heart lining called infective endocarditis. Dr Lévesque's work focuses upon the 'model organism' *Streptococcus mutans*, one of the main causes of tooth decay, the most widespread infectious disease in the human population. Her work seeks to find new, more successful methods to treat biofilm infections, by understanding the underlying biology of oral *Streptococcus mutans* colonies.

COMMUNICATION IS KEY

Many scientists now consider bacteria to be 'social organisms,' with biofilms comprising structured communities, built up in a particular sequence, and protected within a matrix of defensive materials allowing them to survive in hostile environments such as the human body. Dr Lévesque believes that the key to understanding how biofilms behave, and thus their role in disease, lies in how the individual bacteria within the colony communicate with one another. To do this, they use a system known as 'quorum sensing.'

In quorum sensing, small pheromone molecules, which act as signals, are released by each bacterial cell and received by others. These molecules build up in the environment as a function of the number of ▸

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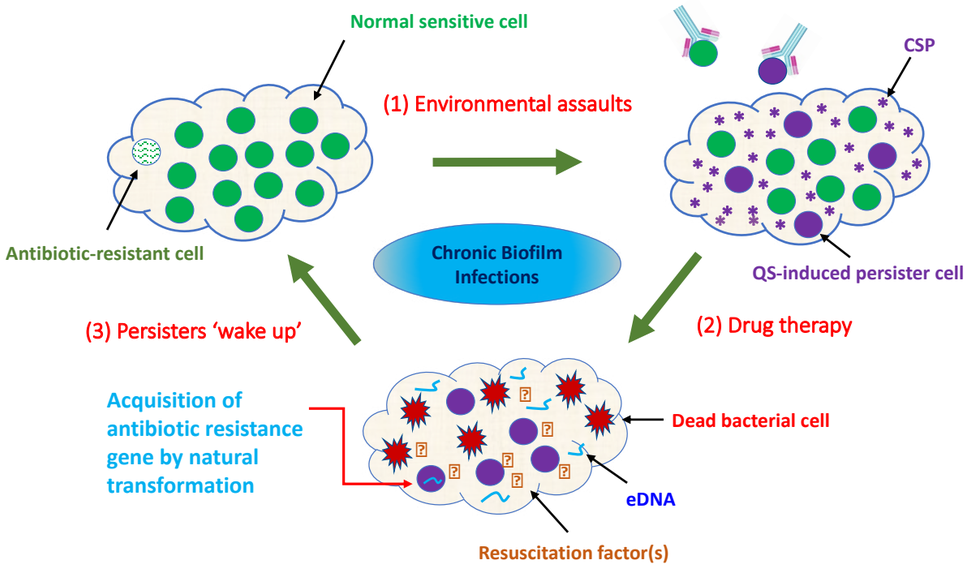
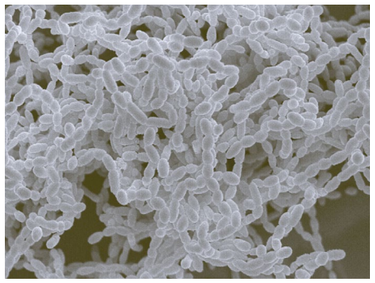
Chronic biofilm infections and QS-induced multidrug-tolerant persister cells





Scanning electron micrograph of *Streptococcus mutans*.

bacteria present. Once a certain threshold concentration of pheromones is reached, indicative of the colony attaining a certain size, this triggers a change in bacterial behaviour, usually by switching on or switching off key genes in the bacterial genome. This mechanism enables the population as a whole to elicit a coordinated response to its environment. Quorum sensing is essential for biofilm formation and many other behaviours including, it now appears, pathogenicity.

Usually, the pheromones are produced arbitrarily by each bacteria cell, and thus the concentration of these signalling molecules in the colony environment depends solely upon how many bacteria are present. However, Dr Lévesque's team made the ground-breaking discovery that the production of one pheromone, 'competence-stimulating peptide' (CSP), by *Streptococcus mutans*, is dramatically increased when the bacteria are stressed by adverse environmental conditions, such as heat, acid or the action of the host immune system. This, she says, means that CSP is not just a pheromone but an 'alarmone,' produced in response to unfavourable conditions to illicit responses key to the bacteria's survival.

SLEEPING BEAUTIES

Dr Lévesque's second major discovery was to elucidate a novel response promoted by the CSP alarmone. Under stressful conditions – such as the presence of antibiotics – quorum sensing in oral *Streptococcus mutans* leads to a dramatic increase in what are known as 'persister' cells – members of the bacterial colony that lie in a dormant state. Because they are not actively growing, these cells are not susceptible to antibiotics. Even if the rest of the colony is wiped out, once the course of antibiotics is finished the persister cells can awaken to re-establish the biofilm. Persister cells are now thought likely to be the main cause of recurrent, chronic infections throughout the human body, even though they do not display standard antibiotic resistance mechanisms. Dr Lévesque's hope is that, by understanding the molecular basis of quorum sensing, we may find ways to manipulate or disrupt it so as to prevent the formation of persisters, making conventional antibiotic treatment more effective.

FOR THE SAKE OF THE SUPERORGANISM

Even more remarkably, Dr Lévesque's recent studies have suggested that the same mechanism of quorum sensing, using the CSP pheromone, not only leads to the formation of persister cells but may also lead other bacteria in the colony to commit suicide through a mechanism known as 'programmed cell death.' This is thought to be beneficial to the remainder of the biofilm for several possible reasons: for instance, it might release nutrients to the living population, or release chemicals damaging to the host. In this way, bacterial biofilms are thought to act analogously to multicellular organisms, with different cells performing specialised functions – even as extreme as suicide – of benefit to the whole.

> **The key to understanding how biofilms behave lies in how the individual bacteria within the colony communicate**

The fact that CSP stimulates *Streptococcus mutans* individuals to take up two very different roles (dormancy and suicide) even when cells are in very close proximity, is a remarkable feat and something that Dr Lévesque would very much like to explain in more detail at the molecular level. "We need to completely understand the mechanism of action of the *Streptococcus* CSP pheromone," she says.

The study of quorum sensing is a relatively new and very fast-growing field which, Dr Lévesque believes, has great potential to impact upon the treatment of infectious disease. The implications of her research extend beyond the mouth to all parts of the human body and many kinds of infections. As she states, "Oral health is a 'window' to overall health," and overall health depends upon maintaining stable populations of bacterial biofilms whilst controlling their pathogenic effects.

Q&A

What first led you to the study of dental plaque?
During my microbiology bachelor's degree at Laval University in Québec City, I was particularly fascinated by a group of researchers studying the microbes living in the mouth. The group called 'Oral Ecology Research Group' ('Groupe de Recherche en Écologie Buccale' in French), comprised a dynamic and vibrant community of researchers and teachers. I decided to join their group for my PhD. I was thrilled! What I got fascinated by was how all these microbes found in our mouth can exist in harmony with one another and at some other times, the same inoffensive microbes can become pathogenic.

Can you briefly describe the makeup of a typical bacterial biofilm?
The development of a microbial biofilm is a dynamic process involving successive steps. The first step is the attachment of the bacteria to the selected abiotic or biotic surface. The second step corresponds to the development of micro-colonies promoted by the growth and division of the first attached cells. The micro-colonies progressively enlarge and coalesce to form the first layer of bacteria covering the surface. When multiple layers of bacteria pile up on the surface, the third step of the formation is obtained, indicated by the presence of a mature biofilm characterised by the presence of macro-colonies surrounded by water channels that help distribute nutrients and signalling molecules.

Do you think your findings from Streptococcus mutans can be extended to other species of bacteria?
Because *Streptococcus mutans* is a Gram-positive pathogen which has evolved in close association with the human host, it constitutes a very useful model organism for providing a better understanding of the biology of other important Gram-positive human pathogens, such as the superbugs MRSA (methicillin-resistant *Staphylococcus aureus*) and VRE (vancomycin-resistant enterococci).

How do you envisage your findings being translated into successful treatments for biofilm infections?
Biofilm infections compromise quality of life and may be associated with mortality. Chronic biofilm infections are fundamentally different than acute infections, and different interventional approaches are necessary to treat these biofilm infections more efficiently. Besides, inappropriate use of antibiotics has created a global crisis of drug resistance, putting a severe strain on the nation's healthcare and economy. Programmed population control through the quorum system of the bacteria may be envisioned as one requisite for biofilm organisms to survive and persist within the human body. The opportunity to interfere with the bacterial cell-to-cell communication system, using anti-quorum sensing molecules, could provide a sophisticated means for manipulating the composition of pathogenic biofilms, and possibly eradicating the infection by bypassing the need for conventional antibiotics.

Detail

RESEARCH OBJECTIVES

Dr Lévesque's work focuses on biofilms – 'sticky' colonies of microbes. She has identified dormant microbes in our mouth termed 'persister cells' which may play a key role in chronic oral infections.

FUNDING

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BIO

Dr Lévesque received her PhD from Laval University in Québec and did her postdoctoral training at the University of Toronto. She is Associate Professor at the University of Toronto, Faculty of Dentistry and holds a Canadian Research Chair in Oral Microbial Genetics, the only Research Chair in Oral Microbiology in Canada.

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