Regulation of fitness and virulence in oral streptococci

Professor Ping Xu outlines his innovative research into the virulence of oral streptococci, and explains how this could benefit those at risk of infective endocarditis and biofilm formation

Can you provide an overview of your research goal?

Our research goal is to understand the fundamental biological roles of oral streptococci in infectious diseases. We have used *Streptococcus sanguinis* as a model organism to study its role in oral biofilm formation for periodontal disease and in infective endocarditis for heart disease. We currently focus on three research areas of *S. Sanguinis*:

- Its virulence in infective endocarditis
- Its role in dental biofilm formation and relation with periodontal diseases
- Identification of targets for therapeutic intervention to control *S. sanguinis* and other oral streptococci related infectious diseases

The long-term goal of the research is to develop chemotherapy and immunotherapy strategies to control oral streptococcal related infectious diseases.

Could you offer an insight into the *S. sanguinis*; what knowledge has been gained in terms of its occurrence and mechanisms, and how does its impact differ in human teeth and heart?

S. sanguinis is a common gram-positive bacteria inhabitant in human indigenous oral microbial flora. It has long been recognised as a pioneer coloniser of human teeth. It forms biofilm on teeth and provides a base for other periodontal pathogen attachment and colonisation. In human teeth, other oral pathogens interact with *S. sanguinis* in oral biofilm to develop periodontal diseases. *S. sanguinis* is an opportunistic pathogen. In human heart, *S. sanguinis* has long been recognised as one of principal causative agents of a serious heart disease -infective endocarditis.

Conversely, what gaps in knowledge are there in relation to the bacterium and in what ways is your research seeking to address these?

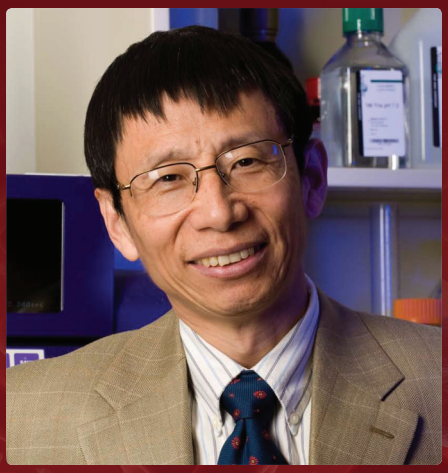
Researchers are seeking answers to many questions regarding *S. sanguinis*. We are particularly focused on three questions:

- How does *S. sanguinis* cause infective endocarditis?
- How does it interact with other pathogens and involve in oral biofilm development?
- How can we control this opportunistic pathogen?

To solve these questions, we first identify all possible genes that contribute to one biological function, for example essential genes that are required for bacterial viability. Then all the essential genes will be linked in a network based on their relations in genome location, transcription regulation, protein-protein interaction or pathways. We then apply bioinformatics and systems biology to study the relationships of these essential genes and to understand their functions for bacterial viability altogether. An analysis of their associations in the network should provide us with a comprehensive understanding of the *S. sanguinis* genome.

The genome of *S. sanguinis* has successfully been mapped. What significance does this bear and in what ways has it helped to shape your research?

The completion of *S. sanguinis* genome provides opportunities to study *S. sanguinis* in the post-genomic era. Because the genome provides detailed genetic information, *S. sanguinis* can be used as a model microbe to understand oral streptococci biological roles. In our research, we collect a large amount of data on *S. sanguinis* using genomics, transcriptomics, proteomics and metabolomics. The data is then integrated and studied as a whole using a systems biology approach. Our hypothesis is that a global analysis of all associated genes in one organism will provide a comprehensive understanding of gene biological functions for bacterial phenotypes that cannot be revealed by any single or small group genes. All these complicated studies are based on the availability of *S. sanguinis* genome.

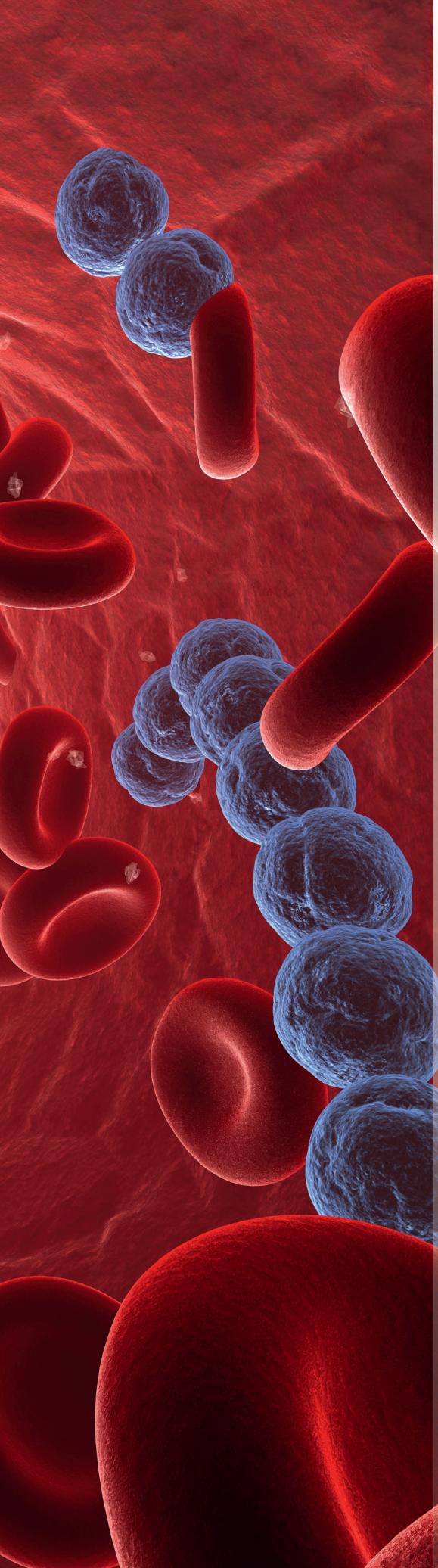


Has any headway been made in developing a model to identify essential genes in other bacteria?

Based on our essential genes analysis in *S. sanguinis*, we have developed a model to predict essential genes for other bacteria. The model works well to identify essential genes in testing bacteria by comparing published data. This model for identification of essential genes will help to recognise new antibacterial targets in other bacteria. It will become more important as new infectious pathogens and antibiotic resistant bacteria emerge.

What would you delineate as your most significant discoveries or achievements to date?

To date there are two significant achievements in our research. The first achievement is the current research on genome-wide functional study of *S. sanguinis* using systems microbiology. We have constructed a comprehensive set of *S. sanguinis* mutants and identified a set of essential genes. The second achievement is the completion of several microbial genomes and the identification of several novel properties in their metabolic pathways. These discoveries on genomics result in several publications in peer-reviewed journals, such as *Nature*, *Science* and the *Journal of Bacteriology*.



The genetics of oral health

New research conducted by a team at **Virginia Commonwealth University** into *Streptococcus sanguinis* could offer encouraging new treatments to control the condition's many related diseases

THE GRAM-POSITIVE bacterium *Streptococcus sanguinis* is a member of human indigenous oral microbial flora. It has long been recognised as a key player in the bacterial colonisation of the mouth. The growth and metabolism of this species has a considerable impact on the oral environment, altering surrounding conditions and enabling more fastidious and harmful organisms to gain a foothold. Furthermore, recent studies have highlighted the prominent role that *S. sanguinis* plays in the inflammatory heart disease infective endocarditis.

Infective endocarditis occurs when bacteria enter the bloodstream through exposure to foreign objects, including interaction with medical instruments, dental surgical procedures, or even daily activities such as flossing or brushing one's teeth. When bacteria are able to infiltrate previously injured heart valves, they colonise the valve surface and form vegetation made of platelets, fibrin and microorganisms, which cause infective endocarditis. Acute endocarditis may become life threatening within days. It usually begins suddenly with a high fever, fast heart rate, fatigue, and rapid and extensive heart valve damage. However, sub-acute bacterial endocarditis is harder to recognise, due to vaguer symptoms such as fatigue, mild prolonged fever, a moderately fast heart rate, weight loss, night sweating, and a low red blood cell count. Infective endocarditis affects older people than other ages and twice as many men as women. Echocardiography may be used to detect the damaged heart valves.

THE PROJECT

Professor Ping Xu, of Virginia Commonwealth University, is investigating the biological functions of oral streptococci in infectious diseases, seeking to identify virulence factor-related genes so that better diagnosis and treatments may be developed. The severe nature of the disease and the urgent requirement for more effective treatments is clear: "Infective endocarditis is the fourth leading cause of life-threatening infectious disease syndromes. Its complications include congestive heart failure, aneurysm and stroke," outlines Xu. "It is uniformly fatal if left untreated. Even in the present era of antibiotic availability, it causes substantial morbidity and mortality. Recent retrospective studies have reported endocarditis mortality rates ranging from 12–46 per cent". Xu has amassed a multidisciplinary team to aid in his investigations into identifying the virulence factors and to research protein functions using bioinformatics and systems biology approach. Utilising researchers with a wide range of skills, Xu hopes that his approach will advance the rate of research and provide a greater level of understanding of the biological gene functions for bacterial phenotypes. These methods have already paid great dividends, enabling the group to identify a comprehensive set of essential genes that affect the viability of *S. sanguinis*.

> Current evidence suggests that biofilm formation is an essential character of oral infectious diseases

BIOFILM

Xu, and his primary partner, Dr Todd Kitten, are now investigating the role of biofilm in the development of infective endocarditis. In most oral ecosystems, microbes such as bacteria, fungi and viruses exist as a biofilm on moist, saliva-coated surfaces. Xu is quick to highlight the persistent nature of the streptococci and how they influence the strength of the biofilm: "*S. sanguinis* has been isolated on freshly cleaned tooth surfaces within 4 hours. After the initial streptococci colonisation, other microbes interact with them to form a 'mature' biofilm and some pathogenic bacterial develop periodontal diseases," he explains. "In this process, the pioneer streptococci provide a new surface and appropriate metabolites and other signals for the attachment of succeeding microbes. Current evidence suggests that biofilm formation is an essential character of oral infectious diseases."

REGULATION OF FITNESS AND VIRULENCE IN ORAL STREPTOCOCCI

OBJECTIVES

- To understand streptococcal virulence and pathogen-host interactions
- To develop integrative analysis of streptococcal biological gene functions through systems biology
- To compare microbial genomics and oral microbiome

KEY COLLABORATORS

Dr Gregory Buck
Dr Todd Kitten
Dr Cindy Munro
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DR PING XU graduated as a star master student from the Nanjing University in 1986 before obtaining a scholarship of Sino-British Joint Education Programme at the University of Oxford in 1987. He then went on to attain a Rockefeller Foundation Visit Scholarship before receiving the honor professor at the Agriculture and Biotechnology and, later, the Medical colleges of Zhejiang University.

His particular fields of interest are molecular biology, microbial genomics, streptococcal virulence and host interaction and systems microbiology. He currently holds a number of positions, including being a Member of the Centre for the Study of Biological Complexity, VCU, Associate Professor with the Philips Institute, VCU, and Affiliate Associate Professor in the Microbiology and Immunology Department VCU.

Treatment of biofilms is extremely difficult, due to the multitude of properties inherent in the bacteria that comprise the film itself. This is further complicated by the dispersal of the large number of cells that detach from the biofilm, which can instigate further infections in other locations. These breeding grounds can soon become a stronghold for bacteria, which can lead to further complications in their treatment, as Xu points out: "Medical surveillance indicates that up to 80 per cent of infectious diseases are related to bacterial biofilms," he asserts. "The bacteria in these films are much better protected against environmental stresses such as antibiotics, extreme pH shifts, oxidants, high osmolarity, or host immune system – making it more difficult to eradicate bacteria in biofilms".

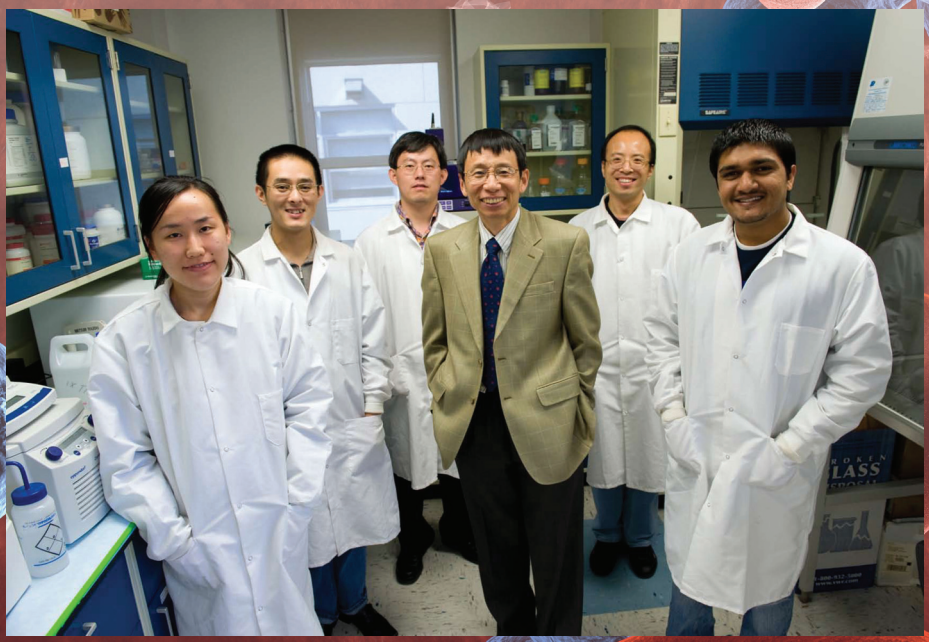
The key to treating biofilm infections lies in the speed of diagnosis and subsequent action. This reduces the number of additional infections and stems the amount of material exchange between cells, which can increase drug resistance and form new strains of infection that could increase in persistence and severity.

THE GENOME BREAKTHROUGH

Recent research into the genome has made this study possible and the impact of this new information has already heralded many significant advances. "We have identified a large set of the essential genes of *S. sanguinis*," remarks Xu. "Many of them are new essential genes that have not been reported before. As the essential genes affect the viability of *S. sanguinis*, they are potential targets for antibacterial drug development."

Encouragingly, research into understanding the genetic functioning of these genes and the diversity of the microbial populations from clinical patients could hold great potential in the development of viable new treatments and sequencing technology, with direct application for use in patient care. Xu and his team are utilising novel DNA sequencing technologies to gain new knowledge about uncultured oral microorganisms, which has led to team to validate antibacterial targets which could be used in the direct treatment of oral streptococci. The study will be supplemented by studying the medical microbial diversities in different biological niches to find candidates for more effective drugs which do not have any existing resistance against them.

This represents a positive step in the fight against infective endocarditis and a better understanding of oral infections and their impact on the rest of the body. The work of Xu and his team at Virginia Commonwealth University looks set to yield considerable advances in more effective treatments of and could have a substantial impact on treatments for this deadly infection, thereby improving the lives of those at greatest risk of inflammatory heart disease.



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