Profile of a pathogen

DR PING XU

Employing a holistic approach to amalgamate findings about gene functions in isolation and in interaction, **Professor Ping Xu** is compiling a comprehensive dossier on a bacterium that establishes tightly-knit communities in the human mouth. Here, he outlines his progress to date

By way of introduction, what motivates your studies into *Streptococcus sanguinis*?

Streptococcal *genera* are a large group of Gram-positive bacteria, many of which are important human pathogens. For example, the leading cause of uncomplicated bacterial pharyngitis and tonsillitis – strep throat – is *Streptococcus pyogenes*. The leading cause of meningitis and sepsis in newborns is *S. agalactiae*. *S. pneumoniae*, which has over 90 different antigenic types and causes pneumonia, meningitis and bacteremia, is the most common cause of sinusitis, acute bacterial otitis media and conjunctivitis beyond early childhood.

S. sanguinis is an opportunistic virulence pathogen in endocarditis and a pioneer coloniser in oral biofilm. It is an excellent model organism to study Gram-positive bacteria, because of its importance in human health and its straightforward genetic manipulations.

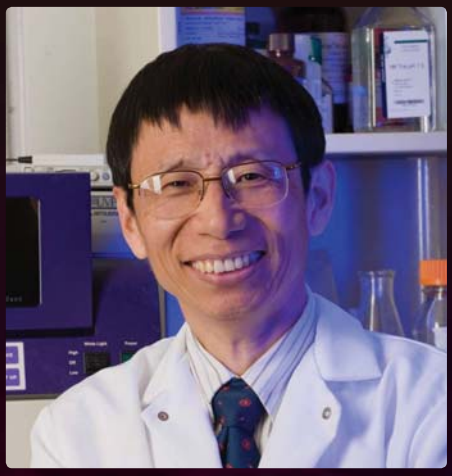
What prompted you to undertake genome-wide gene-by-gene deletions?

We are currently focusing on *S. sanguinis* genes that are essential for biofilm formation and for bacterial viability.

Many different biofilm genes have been identified from different bacterial species in previous studies. However, biofilm is a very complicated microbial phenotype. For example, biofilm genes are associated with many different biological processes including surface protein adhesion, cell wall and envelope membrane structures, signal transduction, two-component system, quorum-sensing, carbohydrate metabolism and exopolysaccharide biosynthesis. It would be difficult to explain biofilm formation from only a single or a small group of genes. We consider that a comprehensive gene mutant library will help us to understand biofilm formation in *S. sanguinis* and thence construct the genome-wide gene-by-gene deletion mutants.

Could you explain how studying biofilm applies to the development of antibacterial drugs?

Biofilms are very important for microorganisms: in biofilms, they are well-protected by the surface polymer matrix against environmental stresses such as antibiotics, extreme pH shifts, oxidants, high osmolarity or the host immune system. Nutrients and substrates are well shared among species inside biofilms. The tightly-arranged microorganisms in biofilms also help cell communications and genetic material exchanges. And up to 80 per cent of infective bacteria relate to biofilm. Scientists are exploring drugs against specific targets to control infectious diseases by reducing biofilm formation.



How might integrative studies of streptococcal essential genes using systems biology methods improve upon traditional methods?

In the post-genome era, the large datasets obtained from genomics, proteomics,

transcriptomics and metabolomics allow an integrative study of biological processes via genome-wide gene analysis.

On one hand, we will build a comprehensive gene interaction network for *S. sanguinis* via bioinformatic analyses using -omics data. On the other hand, we will collect all genes for a specific phenotype, such as essential genes or biofilm genes. We will then combine their intracellular interactions in the network with their extracellular biological phenotypes.

How would you summarise your findings on the critical roles of the essential genes in *S. sanguinis*?

An essential gene is one whose loss is lethal under certain conditions. All essential genes in *S. sanguinis* were identified and carefully confirmed in multiple experiments. When we linked the essential genes and studied them as a whole, we found that they were clustered together in a network associated with three basic biological functions: cell envelope maintenance, energy production and genetic information processing, which can explain many apparent inconsistencies in previous essential gene identifications in different bacteria. The identification of essential genes in bacteria is important because it will help (1) to identify crucial genes and pathways as new drug targets for controlling emerging bacterial pathogens; (2) to define necessary components required for creating designer bacteria in synthetic biology; (3) to study critical genes during evolution; and (4) to find fundamental elements for living organisms and to shed light on the origin of life.

Extrapolating your methods to other bacteria, how viable is it that essential genes can now be predicted on the basis of their genome annotations? Also, how will identifying essential genes help to fight bacterial infections?

We have established a model to predict essential genes for different bacterial species. Besides *S. sanguinis*, we have already predicted and confirmed essential genes, using our model, for *S. pneumoniae*, *Streptococcus mutans*, *Bacillus subtilis* and *Staphylococcus aureus*.

A clear perception of gene essentiality in bacterial pathogens is important for identifying drug targets to combat emergence of new pathogens and antibiotic-resistant bacteria. The longer-term goals of our research focus on preventing streptococcal-related infectious diseases by developing vaccines or chemotherapeutic agents against those gene targets.

Integrative investigations of a model pathogen

Studies of *Streptococcus sanguinis* at the **Philips Institute, Virginia Commonwealth University** seek essential gene targets for combating bacteria in biofilm that are responsible for a range of infectious diseases

THE GENUS STREPTOCOCCUS includes a number of species of bacteria that cause inflammation and infection, such as meningitis, rheumatic fever and pneumonia. One species, a normal resident of the human mouth, *Streptococcus sanguinis*, is a primary initiator and coloniser of plaque, the biofilm containing many variants of bacteria and some saliva constituents that forms on teeth, gums, the tongue and the throat. *S. sanguinis* is an opportunistic pathogen that, on entering the bloodstream, can occasion bacterial endocarditis, a serious heart infection.

SEQUENCING *S. SANGUINIS*

In 2007, a team at the Virginia Commonwealth University (VCU) sequenced the genome of *S. sanguinis* and found that it was circular and larger than other Streptococcus variant species genomes, containing 2.4 million base pairs of DNA and about 2,270 protein-coding genes. Ping Xu, Associate Professor of Oral & Craniofacial Molecular Biology, Microbiology and Immunology at the Philips Institute at the University, was one of the lead investigators of this project and co-authored the manuscript that described the sequence. Since then, he has undertaken further explorations of the genetic makeup of *S. sanguinis* as part of his work on oral microbial diseases, initially in exploring targeted treatment to obviate bacterial endocarditis and now using the bacterium as a model system for wider studies because of its accessibility and utility; it has simple and reliable genetic manipulations and has a genome-wide mutants available.

LATEST INVESTIGATIONS

One aspect of Xu's latest research into *S. sanguinis* centres on the gene interactions that enable *S. sanguinis* and other microbes to form biofilm in the mouth: "Up to 90 per cent of the population worldwide may be infected by periodontal diseases and most of those diseases relate to oral biofilm," he states. "*S. sanguinis* is not only an opportunistic virulence pathogen in endocarditis, but also a pioneer coloniser in oral biofilm. The pioneer streptococci provide signals, metabolites and space for subsequent bacterial species to colonise and form matured biofilm, which eventually develops periodontal diseases." A second facet of Xu's research explores the essential genes that render the bacterium viable. He points out that in addition, this tranche of work analyses the associations of essential genes with infective diseases, with a view to developing directed antimicrobial treatments for infections: "The identification of essential genes in bacteria is important, because it will help to identify crucial genes and pathways as new drug targets for controlling emerging bacterial pathogens, and define necessary components required for creating designer bacteria in synthetic biology".

Excitingly, the research conducted by Xu and his collaborators also aims to further knowledge about the evolution of essential genes in bacteria and their interactions and processes in a much wider context: "We are looking to uncover fundamental elements for living organisms and shed light on the origin of life".

STUDIES IN STREPTOCOCCUS SANGUINIS

OBJECTIVES

- To understand streptococcal virulence and pathogen-host interactions
- To develop integrative analysis of streptococcal biofilm genes through systems biology
- To develop antibacterial drugs and investigate antibiotic resistance
- To compare microbial genomics and oral microbiome

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DR PING XU graduated as a star student from the Nanjing University in 1986 before attaining a scholarship of Sino-British Joint Education Programme at Oxford University in 1987. He then went on to attain a Rockefeller Foundation Career Fellowship before working as the honor professor at the Biotechnology and, later, the Medical colleges of Zhejiang University.

His particular fields of interest are systems microbiology, microbial genomics, streptococcal virulence and host interaction and oral biofilm. He currently holds a number of high profile 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.

ANALYSIS IN THE ROUND

Recent developments in genomics, proteomics and bioinformatics have transformed biological investigations. It is now possible to obtain genomic information about emerging pathogens and so identify targets for drug development or other types of therapy comparatively quickly. However, the profile of bacterial and viral infections is constantly changing in the context of environmental shifts brought about by climate change, global travel and trade and the wholesale use of antibiotics to counteract bacterial infections in the 20th Century. To Xu, the advances in knowledge and technology are offset by this contextual complexity: "It is still a challenge to combat emerging pathogens," he muses, "and their incidence is increasing. Many resistant strains and 'superbugs' have emerged, posing a real threat to the global economy".

To identify genes involved in biofilm formation and maintenance, with the aim of identifying novel associations and any emerging biological properties, Xu's technique is to construct a logical network of gene functions, pathways and their interactions via bioinformatics analysis of genome-wide gene, protein and metabolite data. The driver of this approach is that exhaustive analysis will exclude false-positive and false-negative identifications that are apparent in prior single gene studies; the VCU group's analysis of those investigations pointed to conflicting or inconsistent results describing essential genes and pathways, making it difficult to predict gene essentiality and identify treatments for emerging pathogens. "A living cell is a complex network that relies on associations among genes, proteins and metabolic subsystems," outlines Xu. "We have constructed a gene network for *S. sanguinis* using gene interactions from comparative genomics, microarray analysis, protein-protein interactions and metabolic pathways and are studying gene associations in the network."

TESTING FOR ESSENTIALITY

In testing for essentiality, Xu's approach is to delete every gene and to obtain the non-essential gene mutants so that the essential genes may be more readily identified. He then tests the effects of modifying each candidate gene one by one and examining the effects on the whole. Xu's laboratory has now developed a simplified predictive model of essential pathways from this analysis, which has so far been extended to largely successful prediction of gene essentiality for different species of microorganisms: "The difference between studying the whole by systems biology and studying a part by traditional methods can be effectively explained by the tale where six blind men are asked to describe an elephant by touching and feeling it," reflects Xu. "Each of them gives a conflicting or incomplete description. The story demonstrates the range of truths and fallacies under limited information – a global analysis of all associated genes in one organism provides a comprehensive understanding of gene biological functions that cannot be revealed by analysis of a single gene or a small group of gene mutations."

IDENTIFICATION OF GENES

For *S. sanguinis*, Xu's lab has identified 218 essential genes, which fall into two main types: genes that resist mutation, of which there are 60, and 158 double-band essential genes. 96 essential genes were found to be responsible for biosynthesising amino acids. Some genes that were predicted to be essential were found to not be, and these findings were reconfirmed. Analysis of the pathways with which each gene is associated has been performed. The VCU researchers have also compared 48 other streptococcal species genomes and found that 202 of the 218 essential genes for *S. sanguinis* are present in all of them, as are 787 of the non-essential genes: "We have completed sequencing the *S. sanguinis* genome and have established simple genetic manipulation systems," he affirms. "All of our efforts establish *S. sanguinis* as a model Gram-positive organism for oral biology studies."

> A global analysis of all associated genes in one organism provides a comprehensive understanding of gene biological functions

The results of all the tests and analyses in Xu's lab are being entered into a growing comprehensive database of essential genes and non-essential gene mutants. Included in the library are full datasets about the design of the mutant genes, their properties, characteristics and effects. The collaborators are currently using a number of these mutant genes for further investigations into gene functions.

Xu believes that his model and method will pave the way to more rapid identification of targets for antimicrobial treatment against a broad range of present and emerging pathogens, where there is an annotated genome sequence available: "Our findings of essential and biofilm genes in *S. sanguinis* will be applicable not only for oral microbiology but for other streptococcal-related infectious diseases," he concludes.