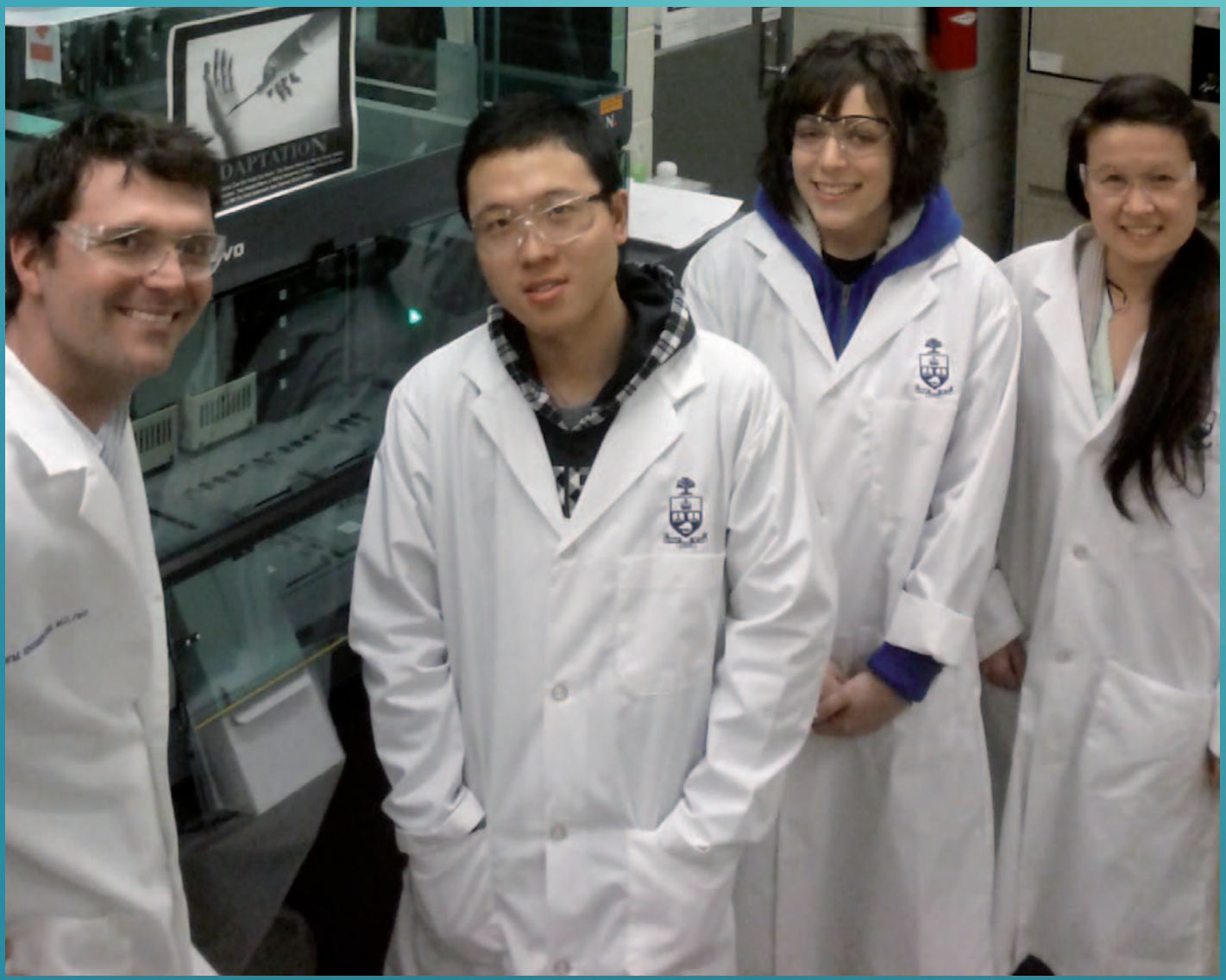
Too gracious a host

Legionnaires' disease, a potentially fatal infection caused by *Legionella pneumophila* has recently been in the headlines due to a number of serious outbreaks around the world. Scientists at the University of Toronto are using Tecan's Infinite® M200 PRO and Gas Control Module to study host-pathogen interactions for *Legionella* bacteria.

Legionnaires' disease is an uncommon form of pneumonia which is fatal in 10-15 % of the general population. Although the bacteria are widely distributed in the environment where they can live in all types of water, they only become a risk to health in conditions that allow *Legionella* to grow rapidly, such as in poorly designed or maintained water systems.

Researchers at the Ensminger Lab, part of the Department of Molecular Genetics at the University of Toronto, Canada, are investigating host-pathogen interactions, specifically for *Legionella pneumophila*, using cutting-edge genomics, experimental evolution and high throughput robotic solutions. Assistant Professor Alex Ensminger discussed the laboratory's approach to studying this potentially deadly pathogen: "*Legionella pneumophila* normally grows inside host cells; it is not thought to grow in the water as such, but rather in amoebae and protozoa present in the water. The bacteria are basically professional pathogens of amoebae and amateur pathogens of human



The Ensminger Lab team (left to right): Alex Ensminger, Chitong Rao, Carly Weiss and Amy Chung

cells; the human lung is an evolutionary dead end where the bacteria cause disease. We need to understand how these pathogens interact with both their natural hosts, and accidental hosts – more specifically, patients' lungs – to learn how growth in those two situations is similar but, equally importantly, different. This knowledge is critical to understanding how these bacteria persist in different environments, growing to high levels in cooling towers, for example, and how this offers them the opportunity to cause human disease."

The Group's starting point was to experimentally evolve the bacteria, passaging them repeatedly in specific types of amoebae or macrophages, and observing any changes to see if they became more specialized for different hosts. A key part of this procedure is to monitor bacterial growth under certain conditions, which is a very labor intensive process, involving plating out, counting colony forming units, and comparing different strains with each other. Alex soon identified an alternative: "I had seen recent publications describing the use of the Infinite M200 PRO to measure CO_2-independent growth of *Legionella* using a green fluorescent protein (GFP) marker. As soon as I read that the instrument was also available with a Gas Control Module (GCM™), I was sold on buying one. I realized that I could put the bacteria in host cells, place them in the plate reader and leave them there for three or four days, watching their growth. By measuring every 20 minutes or so, we have seen growth curve shapes that disappear if readings are taken less frequently, even hourly. These shapes match what we might predict from cell biology, and allow more subtle distinctions between phenotypes than was previously possible. Growth is observed for 14 hours, followed by a pause of about four hours before it begins again, which reflects what we know, that an initial round of infection takes 14 hours, and then the bacteria lyze out of that first set of host cells and cause further infection."

"We use both fluorescence and luminescence measurement in our experiments and, with the high throughput that the Infinite M200 PRO offers, we are able to look at the same strain under a variety of conditions simultaneously, minimizing day-to-day variations and giving us extra confidence in our results. We soon realized that we needed another reader, and it made sense to integrate this with a Freedom EVO® 100 platform to give us even more flexibility and the capacity to increase throughput going forward. An incubator stack maintains 96-well plates at a set temperature, and every 20 minutes the Robotic Manipulator (RoMa) Arm transfers a plate to the reader. After dark adapting to prevent autoluminescence background signals, a luminescence measurement is taken. Fluorescence measurement is even simpler."

Alex and his team have widened their studies as they have become more familiar with their Tecan instruments: "We have some evidence that nutritional requirements influence the host range of the bacteria and how they are recognized by the host cell. To investigate this, we are using the Freedom EVO to prepare batches of 96-well plates where each well is lacking a different nutrient, and can seal and freeze them for future use. Along with our colleagues at Public Health Ontario, we are also using this technology to look at clinical and environmental strains that have been collected over the last 30 years, trying to identify phenotypic and genetic diversity and determine whether strains from specific outbreaks are more specially adapted to different hosts. We are moving towards a situation where we will be able to establish the specific isolate or type of bacterium causing an outbreak and make predictions regarding its host range and virulence. The aim is to see if there are conditions which select for more virulent bacteria that are more likely to cause human disease. The idea is that there may be specific natural hosts which select for more virulence, or perhaps if a specific type of protozoan or amoeba is present in the water system, that could act as a biomarker for the system being at increased risk for selecting more virulent bacteria. Increased throughput and the capability to do phenotypic screening in a huge number of host cells simultaneously is the thing that's making this possible."

To find out more about the Infinite M200 PRO and GCM, visit **www.tecan.com/infinite200pro**

To find out more about the Ensminger Lab, visit **individual.utoronto.ca/ensminger_lab**