# Mycobacteria metabolism discovery may pave way for new tuberculosis drugs

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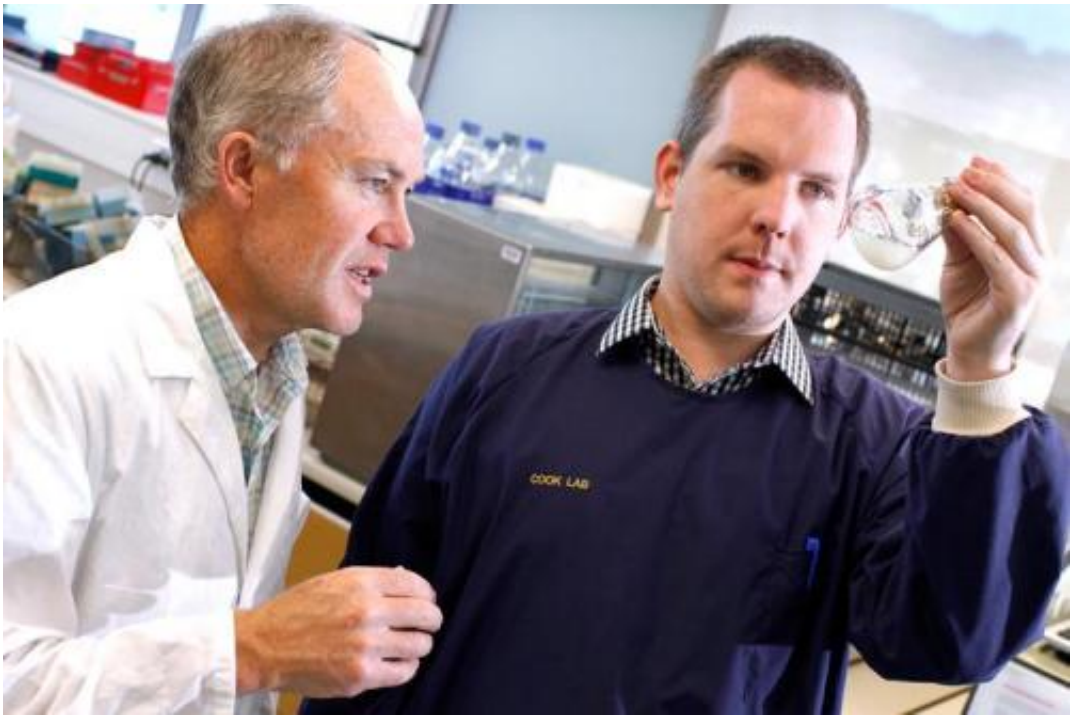

Professor Greg Cook and Dr Chris Greening (picturerd) are among an international team unravelling the mystery of why mycobacteria -- a family that includes the microbe that causes -- are extraordinarily hardy organisms. Credit: Sharron Bennett

The mystery of why mycobacteria—a family that includes the microbe that causes TB—are extraordinarily hardy organisms is being unravelled by University of Otago, New Zealand, research that offers new hope for developing a revolutionary class of antibiotics to tackle TB.

In collaboration with researchers in the US and Germany, Otago microbiologists have teased out the mechanisms by which the aerobic soil microbe Mycobacterium smegmatis is able to persist for extreme lengths of time in the absence, or near-absence, of oxygen.

Their findings, published this week in the prestigious US journal *PNAS*, show that hydrogen is a key factor that enables mycobacteria to survive oxygen-limitation over long periods.

The team, led by Professor Greg Cook, found that in such conditions the bacterium is able to quickly switch its cellular metabolism from a primarily oxygen-based one over to one that uses fermentation for energy production instead.

This metabolic mode depends on the production and recycling of molecular hydrogen, a high-energy fuel and diffusible gas. These cells produce hydrogen to ensure their survival until they once more have access to sufficient oxygen for growth.

Professor Cook says it had long been a puzzle how mycobacteria generate energy when in their oxygen-starved dormant states.

"Mycobacteria grow through combusting their preferred carbon-based fuel sources using oxygen. However, they can also somehow survive for months or years when their oxygen supply is exhausted.

"For example, in people with latent TB infections, *Mycobacterium tuberculosis* bacteria are walled in by clumps of immune and other body cells in what is thought to be an extremely low oxygen environment. However, such patients must be monitored for the rest of their lives in case the bacteria become active again," he says.

Professor Cook's team have established that *Mycobacterium smegmatis*

metabolises molecular hydrogen using three enzymes called hydrogenases. One hydrogenase produces hydrogen, whereas the other two consume it. These hydrogenases are activated under oxygen starvation by a master regulator called DosR.

The researchers found that strains of *Mycobacterium smegmatis* in which the genes for the hydrogenases or the regulator DosR had been 'knocked out' experienced a hundredfold reduction in the long-term survival compared to the normal bacterium, he says.

His team is currently testing whether these findings are extendable to *Mycobacterium tuberculosis*, which activates a further predicted hydrogenase under low oxygen conditions.

"If knocking out this other hydrogenase also drastically reduces long-term survival, the enzyme might end up being an excellent next-generation drug target in latent TB infections, which around one-third of the world's population suffer.

The Otago researchers' studies on hydrogen fermentation in mycobacteria have been performed in collaboration with Professor William Jacobs Jr., a world-leading bacterial geneticist at the Albert Einstein College of Medicine in New York, who is known as the 'TB terminator'.

One of the paper's lead authors is Dr Michael Berney, a former research fellow in Professor Cook's laboratory who is now an Assistant Professor associated with Professor Jacobs' laboratory. The other co-authors include Professor Cook, Dr Chris Greening, who also co-led the study and recently completed his PhD studies in the Cook Laboratory, and Professor Dr Ralf Conrad, director of the Max-Planck Institute for Terrestrial Microbiology in Marburg, Germany.

The research was funded by the Royal Society of New Zealand's Marsden Fund.

## Why hydrogen is important in allowing mycobacteria to survive oxygen starvation

As the most fundamental chemical compound, H2 has several impressive properties. It yields more energy per unit weight than any other molecule; hence combusting this molecule as a fuel can drive cellular processes in a surviving cell.

However, it's also a highly diffusible gas; hence, H2 production allows mycobacteria to produce energy by dissipating excess fuel as H2.

H2 production is a fermentation process, whereas H2 recycling is a respiration process. Both processes enable the cell to produce energy, but in different ways; respiration yields more energy overall, but fermentation can occur even when oxygen is completely absent. By maintaining a careful balance between fermentation and respiration, the mycobacterial cell can remain energised and unstressed. Hence, the cell can both cope with sudden downturns in environmental conditions and maximise energy-generation when conditions improve.

It is now clear that our previous models of how mycobacteria generate their energy are simplistic and that they are significantly more flexible than we previously thought. While it is clear that respiration is needed for growth, it is clear that fermentation supplements this process during long-term survival.

**More information:** An obligately aerobic soil bacterium activates fermentative hydrogen production to survive reductive stress during hypoxia, *PNAS*, www.pnas.org/cgi/doi/10.1073/pnas.1407034111

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