Cracking the communication codes of bacteria

Despite the deceptively simple appearance of bacteria, they possess the ability to both understand and respond to their environment through chemical signalling mechanisms. By using such chemical codes, bacteria can signal to each other when is the perfect time to launch an attack on a weakened host to cause a devastating infection or to change their behaviour to survive under harsh conditions. Interpreting, understanding and potentially being able to disrupt these signalling mechanisms is one of the main aims of **Dr Emily Weinert's** research at Emory University, with the hope of finding a way to alter or prevent bacterial growth.

Bacteria are everywhere. Recent studies estimate as many as 40 trillion bacteria are hosted in the average human body. Every surface in our homes is covered in biofilms, slimy structures that are host to bacterial communities. When the biofilms become sufficiently large, we can feel the slippery nature of the films on places like our teeth or unclean surfaces.

Biofilms act as microscopic bacterial cities. The infrastructure of the city, known as the extracellular matrix, helps anchor the bacteria to surfaces and brings together the different microbes to form their local communities. The biofilm is a constantly evolving environment that can respond to external factors such as temperature or attacks by antibiotic drugs.

The question is, how do seemingly simple microorganisms like bacteria communicate and coordinate all of this work? Bacteria speak the language of chemistry – they produce chemicals to tell other bacteria that now is the ideal opportunity to attack and infect an unsuspecting host or detect the presence of other chemicals as a warning of an incoming antibiotic assault.

To decipher these communication techniques, Dr Emily Weinert at Emory University is employing a cross-disciplinary approach, spanning microbiology and biochemistry. This involves characterisation of the proteins in bacteria thought to be the 'language centres' that bind and release such chemicals and allow them to respond to changes in their external environment.

ROTTEN OXYGEN

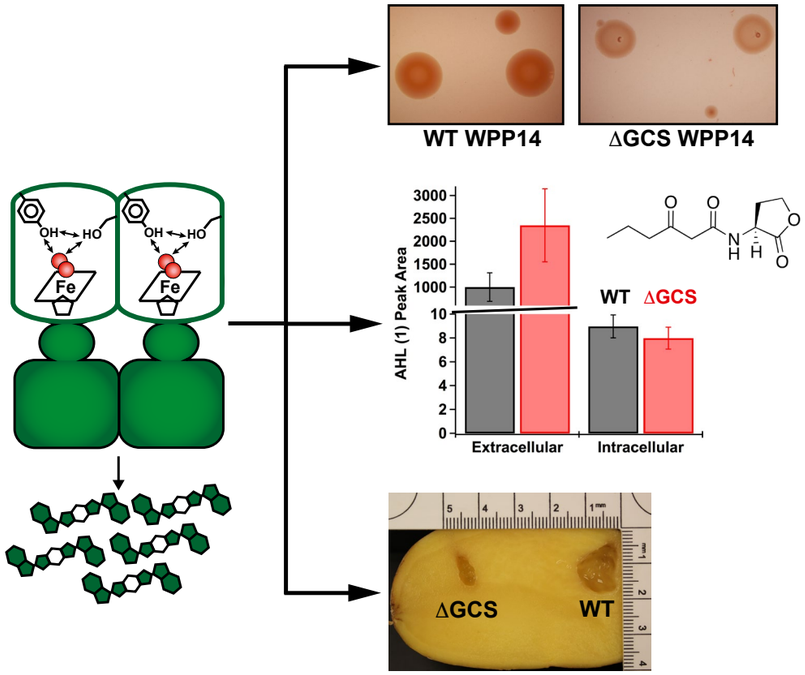
While there are millions of kinds of bacteria in the world, Dr Weinert is particularly interested in the bacteria responsible for whooping cough, rotting of agricultural plants, cholera and sepsis. These might seem like very different diseases, but all

By using a convergence of chemical and biological approaches…my research generates a comprehensive molecular-level understanding of complex biological systems

these bacteria have a common feature – they all contain a haem protein.

Haem proteins contain a central iron surrounded by ligands – chemical groups bound to the central metal species. Other examples of haem proteins are some enzymes, including those responsible for converting food into a useable source of energy for us. Probably the most famous of these proteins is haemoglobin – the protein in our blood that is responsible for the uptake and transport of oxygen in our body.

The bacteria that Dr Weinert has been studying (*Bordetella pertussis*, *Pectobacterium carotovorum* and *Vibrio brasiliensis*) use haem proteins termed globin-coupled sensors (GCS). Much like haemoglobin, all these bacteria can bind and release oxygen and, as a result, are sensitive to changes in the oxygen concentrations of their surrounding environments.



Globin coupled sensor signalling within *P. carotovorum* controls biofilm formation (top), AHL production and export (middle), and virulence on a potato host (bottom).

CHEMICAL SIGNALLING

The next question is, as the bacteria can respond to changes through these globin-coupled sensors (GCS), what do they do with this information? Dr Weinert's group have successfully been able to identify the changes in chemical structure the GCS protein sites undergo on the binding or release of oxygen.

What is even more remarkable is also what occurs downstream of the changes in chemical structure of the protein. In low oxygen, or anaerobic, conditions, Dr Weinert's group have been able to identify changes in the response of particular bacteria, resulting in the formation of 'stress-signalling' chemicals. To do this, they have used a combination of microbiology techniques for growing and expressing the proteins, enzymatic assays to determine the effects of oxygen, as well as spectroscopic analytical techniques that help characterise the haem based on the colours of light they absorb and that identify chemicals that have been formed based on mass. These stress-signalling chemicals are thought to go on to alter the transcription response of the bacteria, essentially changing the genes that will be encoded into proteins.

The ability of bacteria to alter their transcription response is part of what makes them so hardy and difficult to kill. By changing the operating conditions of proteins and gene expression, they can rapidly multiply or become more resistant to current temperature conditions, all as part of these chemical signalling pathways.

Bacteria – despite their apparent simplicity – exhibit a wide range of biological functions and are globally important for human and environmental health

COMBATTING ANTIBIOTIC RESISTANCE?

Understanding the mechanistic details of chemical signalling as Dr Weinert is doing is a challenging task as each protein will be made up of hundreds and thousands of individual atoms and bacteria have a huge range of proteins (known as sigma factors) that can adjust as part of the overall stress response mechanism.

However, there are immense potential benefits to a full understanding of these signalling mechanisms. *Pectobacterium carotovorum*, whose name literally translates as 'carrot-eater', is the bacteria responsible for soft rot in many crop and plant species. Using techniques such as excreted enzymes assays, which quantify the production of virulence factors, and rotting assays, Dr Weinert has found that decreasing the expression of the GCS protein responsible for oxygen sensing also results in a reduced amount of rotting in potato hosts.

By understanding this chemical signalling pathway, Dr Weinert has also uncovered a potential route of rescuing crops blighted with this bacterium. Disruption of this signalling route could help to protect crops by reducing the rotting caused by the pathogenic bacteria. There are also some more creative possibilities with the disruption of chemical signalling pathways. One possibility would be mimicking bacterial signals to hijack and reprogramme the bacteria for different purposes.

Oxygen-sensing bacteria are found in a wide variety of organisms and, as a result, Dr Weinert's work has far-reaching implications in our understanding of how we can fight infections in humans as much as crops and potentially provide valuable ammunition in the growing problem with antibiotic-resistant bacteria.

Q&A

What was the motivation for looking at haem protein containing bacteria?
Haem proteins (like haemoglobin) are well known to bind oxygen and bacteria must be able to sense oxygen levels due to the importance of oxygen in metabolism, suggesting that haem proteins could provide another mechanism by which bacteria could sense oxygen levels in their surrounding environment. Since altered oxygen levels have been linked to key bacterial phenotypes (biofilm formation, virulence, motility, etc.), we thought that globin-coupled sensors could be important signalling proteins.

What do you think are the most important implications of your work?
So far, I think the most important implications of my group's work are that bacterial signalling pathways, even those that are seemingly completely unrelated, can be interlinked and that environmental signals (like oxygen) can cause widespread changes to bacterial metabolism and gene expression. Identifying and characterising these pathways also has the potential to provide new targets for antibacterial compounds that can regulate bacterial growth phenotypes and/or virulence.

How difficult is it to work with the samples in the lab?
We must work with anaerobic samples inside a glove bag, which makes experiments a bit more difficult since you're working on samples inside the bag through thick gloves, and any samples that we want to keep anaerobic must be kept in air-tight containers (vials, cuvettes, etc.) for analysis. In addition, since the proteins have a non-covalently bound haem, we always have to worry about keeping the proteins stable, so the haem doesn't fall out. But, overall, the proteins and bacteria are amenable to our investigations.

How do you think your knowledge of oxygen-binding bacteria will transfer to receptors for other small chemicals?
I think that our work's aim to understand how globin coupled sensor proteins transmit the ligand binding event through multiple domains will add to the general understanding of how a small molecule can cause subtle rearrangements in a receptor protein that results in large conformational changes and altered downstream activity. Also, we've found that there are considerable differences in oxygen-dependent activation, even within this haem protein family. Furthermore, I think that our current studies will help delineate some of the factors that control the activation mechanism, which hopefully will allow us to more accurately predict the effects of chemical binding in the future.

What bacterial species is your next challenge?
We're currently investigating the roles of globin-coupled sensors in a species of *Shewanella*, which is an environmental bacterium that can use a wide variety of alternative electron acceptors in the absence of oxygen, but switch back to oxidative metabolism once oxygen concentrations increase. We're also starting to look at additional Gram-negative human pathogens to determine if the globin coupled sensors in those bacteria also affect virulence and quorum sensing, like it does in *Pectobacterium carotovorum*.

Detail

RESEARCH OBJECTIVES

Dr Emily Weinert's work at Emory University looks to determine the mechanism of signal transduction and the role of globin-coupled sensor signalling in diverse bacteria.

FUNDING

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BIO

Emily Weinert graduated from Duke University in 2002 with a BSc in Chemistry after working with Professor John Simon. She then joined Professor Steve Rokita's group at the University of Maryland for her PhD. In 2006, Emily began her postdoctoral work in Professor Michael Marletta's laboratory at the University of California, Berkeley. She joined the faculty of Emory University in 2011.

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