Newly-discovered bacterial regulatory mechanism has implications for antibacterial control measures

June 27 2024

Agrobacterium tumefaciens. Credit: Wikimedia Commons Jerzy Opioła Creative Commons Attribution-Share Alike 4.0 International license.

Research spearheaded by four biologists within the College of Arts and Sciences at Indiana University Bloomington has uncovered a new regulatory mechanism shared by many bacteria, which may have profound implications for anti-bacterial control measures in medical and agricultural settings.

In addition, this novel regulatory mechanism also has significant commercial potential in the production of bioadhesives—nontoxic, biological alternatives to petroleum-based, synthetic adhesives which have multiple uses in medicine and other sensitive applications.

The study, titled "Control of biofilm formation by an Agrobacterium tumefaciens pterin-binding periplasmic protein conserved among diverse Proteobacteria," published in the *Proceedings of the National Academies of Sciences*, examines biofilms, which are bacterial communities prevalent in nature that assemble on organic and inorganic surfaces. Notably, biofilms are a common cause of persistent infections in humans, animals, and plants.

Jennifer Greenwich, a post-doctoral scientist in the Department of Biology within the College of Arts and Sciences is lead author on the study, and the research was performed in the laboratory of study co-author Clay Fuqua, the Clyde Culbertson Professor of Biology. Other co-authors include IU alumni Nathan Feirer, a 2017 Microbiology Ph.D. and a research scientist in the private sector, and Justin Eagan (Biology B.S. 2016), now at the University of Wisconsin, Madison.

"The study is rooted in an interest in bacterial biofilms, which are more than the sum of their parts and have emergent properties such as increased resistance to antibiotics," said Professor Fuqua.

"Bacterial biofilms also have higher rates of what is termed 'horizontal gene transfer'—this leads to spreading of genes among bacteria, including those for antibiotic resistance, which drives the evolution of disease-causing pathogens."

Biofilms are particularly prevalent in hospital and surgical environments. For example, many patients who undergo artificial joint replacement or have long-term catheters frequently develop antibiotic-resistant bacterial biofilm infections.

Greenwich, Fuqua, and co-authors studied a model bacterium named Agrobacterium tumefaciens, which functions as a plant pathogen. Previous research, conducted over multiple years, has elucidated the mechanisms of how biofilm formation and surface attachment occurs within this system.

It works as follows, said Fuqua, "We know that there is a 'glue' called UPP, or unipolar polysaccharide, that is produced on one end of the rod-shaped cell and functions to stick bacteria to uncolonized surfaces such as non-living materials or host tissues, to form a biofilm.

"The bacterial production of UPP glue is highly regulated by a self-produced signal molecule inside of cells known as c-di-GMP, common to many different bacteria that stimulates biofilm formation. Simply put, high levels of the internal signal c-di-GMP molecules fuel biofilm formation and attachment to surfaces, while lower levels curb these processes."

Importantly, this internal signal is common in a vast number of different bacteria, and almost always regulates biofilm formation and how bacteria attach to their hosts to form biofilms.

In their study, Greenwich, Fuqua, and their fellow researchers discovered that the production of this internal signal c-di-GMP in Agrobacterium is regulated by a second external signal outside of the cells. This external signal is a biological molecule called a pterin, a class of compounds that fulfill a variety of biological roles, including human and animal metabolism, and are produced across all domains of life.

"Pterins are synthesized by animals, plants, fungi, and on and on—virtually all branches of the tree of life—but importantly, pterins are also synthesized by bacteria," said Fuqua. "Thus, 'pterin-dependent signaling' may be a new form of chemical communication between hosts and bacteria, and/or between bacteria and other bacteria. Mechanistic insights into this regulatory circuit could lead to new advances in antibiofilm treatments."

Greenwich, Fuqua, and their co-authors describe how the excreted pterins are recognized by interaction with cells via a receptor on the bacterial cell periphery. The pterin-receptor interaction regulates a second protein that spans the bacterial membrane. In turn, the portion of this membrane protein on the inside of the bacterial cell can drive the production and degradation of the internal signal, thereby controlling formation of a biofilm.

"An exciting finding," said Fuqua, "is that we have discovered a new regulatory mechanism shared by many bacteria. It turns out that this pterin response and control system is not unique to the Agrobacterium, but is found across a large group called the Proteobacteria that includes human pathogens such as Klebsiella, Vibrio and Pseudomonas and many other disease agents that widely impact human, animal and plant health."

Further, since bacteria and other organisms excrete pterins, this may provide the scientific community with insights as to how bacteria collect information about the other organisms in their environments, as well the environmental conditions that stabilize or destabilize the pterin molecules.

"In an applied sense, understanding these mechanisms, and all of these properties, have major consequences not only for how we treat disease, but also how we may harness microbes to do positive things," said Fuqua.

"We may be on the cusp of creating strategies to target biofilm formation, and control biofilm formation and attachment among bacteria. Another exciting aspect of the discovery's commercial potential is how scientists can use the control pathway to regulate glue production, and some day, in the not-too-distant future, we may be able to produce this glue for use as a biologically compatible, non-toxic adhesive."

More information: Jennifer L. Greenwich et al, Control of biofilm formation by an Agrobacterium tumefaciens pterin-binding periplasmic protein conserved among diverse Proteobacteria, *Proceedings of the National Academy of Sciences* (2024). DOI: 10.1073/pnas.2319903121

Provided by Indiana University