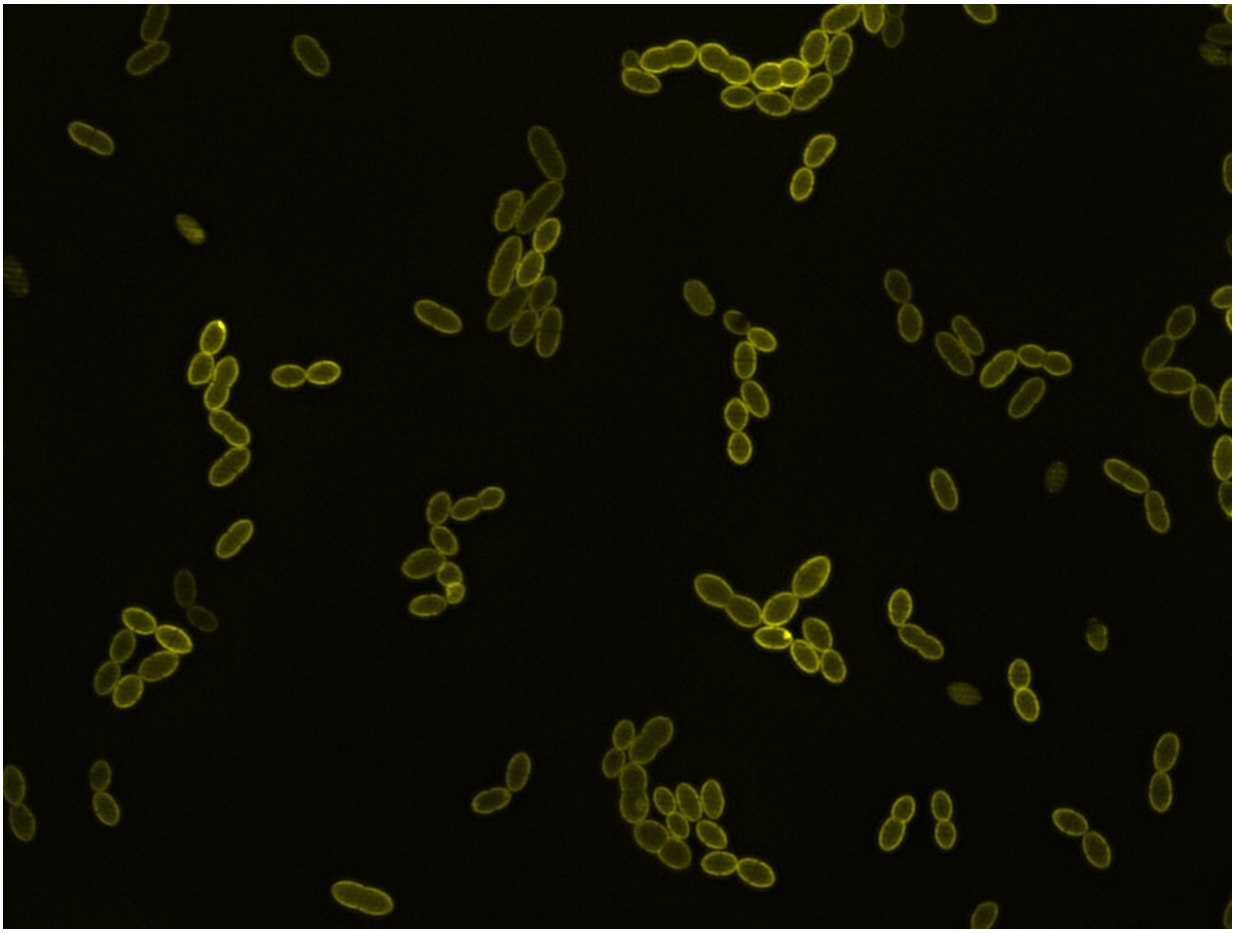
How pathogenic bacteria weather the slings and arrows of infection

April 2 2021



Streptococcus pneumoniae cells expressing fluorescent MurM and MurN. Credit: Sergia Filipe

Infectious diseases are a leading cause of global mortality. During an infection, bacteria experience many different stresses—some from the host itself, some from co-colonizing microbes and others from therapies employed to treat the infection. In this arms race to outwit their competition, bacteria have evolved mechanisms to stay alive in the face of adversities. One such mechanism is the stringent response pathway. Understanding how the activation of the stringent response pathway is controlled can provide clues to treat infection.

In new research published this week online in the journal *Proceedings of the National Academy of Sciences,* former Carnegie Mellon University graduate student Surya D. Aggarwal and his advisor, Associate Professor of Biological Sciences Luisa Hiller, observed that the deletion of a gene involved in surface remodeling caused a stress-dependent growth defect in a human pathogen that could not easily be explained. Deciphering the biological mechanism underlying this defect led to an international collaboration between Carnegie Mellon, the Universidade NOVA de Lisboa (Portugal) and the University of Warwick (UK). The joint effort combined the Carnegie Mellon team's expertise in pathogenesis with that of Assistant Professor Sergio Filipe of the Universidade NOVA de Lisboa and the University of Warwick Associate Professor Adrian Lloyd's work in the composition and biosynthesis of bacterial cell walls and associated biochemical processes.

"This has been one of the most fun and exciting projects in my career," said Hiller.

The joint project established that transfer RNAs (tRNAs) serve as a crucial component in the control of the activation of the stringent response pathway. tRNAs play a critical role in translation: they help to decode the genetic information into amino acids, the building blocks of proteins.

However, sometimes they can make a mistake, where the tRNA carrier and the amino acid building block are mismatched, rendering the combination toxic. In stressful conditions, tRNAs make more errors and accumulation of these errors is a trigger for the stringent response. This biological process is akin to the malfunction of a machine in an assembly line that results in flaws in the final manufactured product.

Many bacteria display a thick cell wall on their surface. Amino acids are a key component of this structure, and this research revealed that a protein involved in the addition of amino acids to this cell wall, the MurM enzyme, displays a strong preference for the tRNA loaded with mismatched building blocks. By diverting these toxic blocks towards cell wall synthesis and away from translation, MurM serves as a quality control manager who ensures that the flow line remains error-free and the manufacturing process can continue unabated.

In the absence of MurM, cells under stress activate the stringent response more easily than the parental strain. These findings suggested that MurM serves as a gatekeeper of this stress response pathway.

"It is highly rewarding when suddenly intriguing observations are explained by a simple and clear model," Filipe said. "The proposal that the cell wall can be used to divert the accumulation of toxic compounds is quite exciting. I wonder what other surprises will come from the study of the bacterial cell surface".

"To explore this further, we drew parallels between the bacteria we study and other species that do not encode MurM", said Aggarwal, who is now a postdoctoral fellow at NYU Langone Medical Center. In most domains of life, including human cells, the pathological consequences of these toxic tRNAs are mitigated by AlaXp, an enzyme that also corrects the defect by decoupling the tRNA from the incorrectly coupled building block.

However, Streptococcus pneumoniae, the bacteria in this study, as well as multiple other bacteria with thick cell walls, do not encode AlaXp. Aggarwal adds, "We wanted to test whether artificially introducing an additional gatekeeper in the form of AlaXp to pneumococcal cellular machinery would allow the flow line to remain functional even in the absence of MurM. This line of investigation set us on a road to test whether the stress-dependent growth defects we observed were attributable to the protein's role in preventing accumulation of toxic tRNAs."

The validation was a joint effort. The research at CMU employed genetic tools to decouple the role of the MurM in the architecture of the cell wall from its role in correcting toxic carrier-building block pairs. The work at Warwick made use of biochemical tools to reveal the underlying processes that render MurM optimal to correct the toxic molecules, while studies in Lisbon captured how the correction activity of the MurM enzyme impacts cell wall architecture. To quote Lloyd: "This international consortium was able to focus disparate yet connected areas of expertise to determine how previously considered disparate areas of microbial biochemistry collaborate to enable a crucial pathogen to navigate the stresses it endures during infection. This work provides a step change in our understanding of the resilience of bacteria as they cause infection."

The study suggests that MurM is an alternative evolutionary solution to the challenge of these toxic tRNAs. These findings implicate cell wall synthesis in the survival of bacteria as they encounter unpredictable and hostile conditions in the host. The association between cell wall synthesis and translational fidelity is likely to be active in many other pathogens, implicating these findings in the biology of many other pathogens.

This collaborative work sets the framework for future work exploring the molecular connection between two fundamental cell processes,

translation and cell wall synthesis, and stress responses. Moreover, the pivotal position of the stringent response in survival to stresses and to antibiotics, suggests these findings will also shed light on pathways associated with bacterial drug resistance, a major challenge for this century.

More information: Surya D. Aggarwal et al, A molecular link between cell wall biosynthesis, translation fidelity, and stringent response in Streptococcus pneumoniae, *Proceedings of the National Academy of Sciences* (2021). DOI: 10.1073/pnas.2018089118

Provided by Carnegie Mellon University