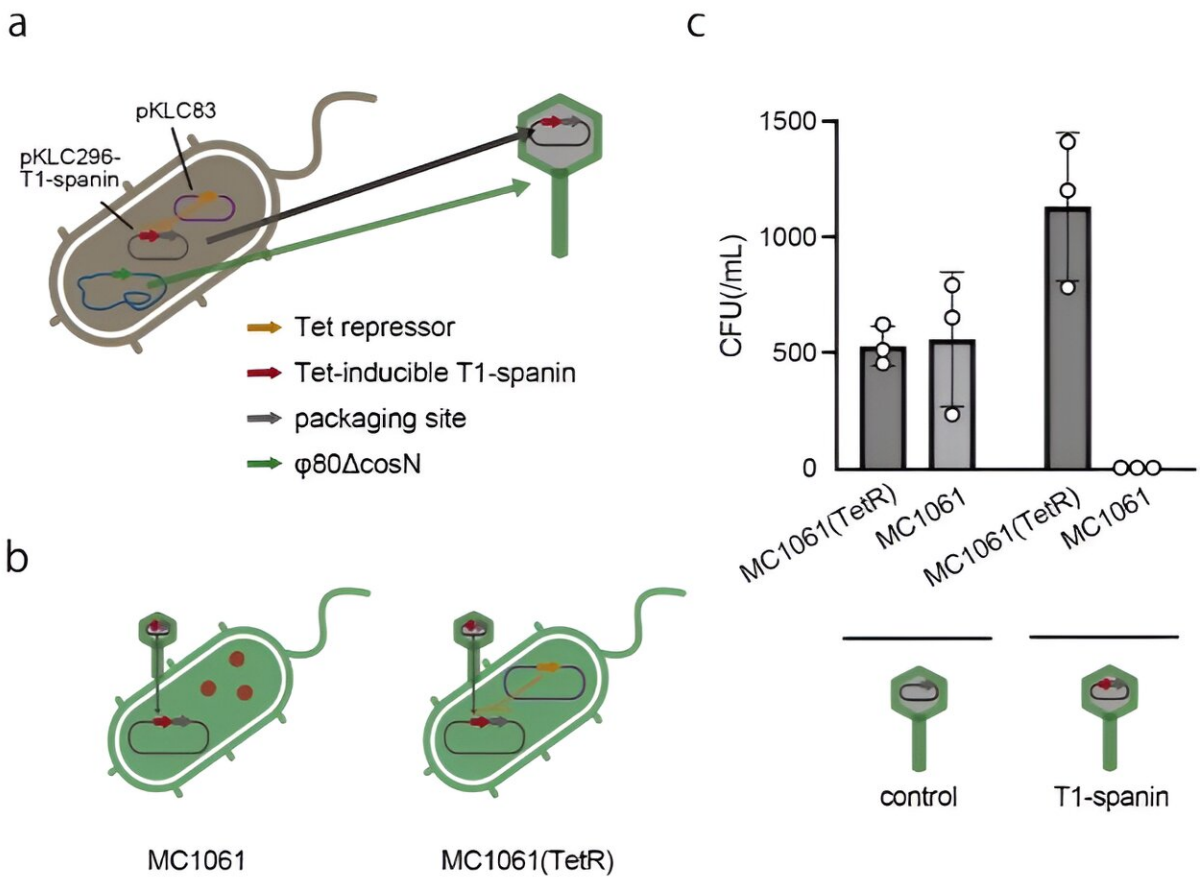
Exploring the bactericidal activity of T1-spanin against drug-resistant bacteria

March 12 2024



(a) Schematic diagram of the construction of a φ80 phage capsid containing T1-spanin gene. T1-spanin exhibits toxicity toward synthetic bacterium 594; therefore, its expression was suppressed using a Tet repressor-expressing plasmid (pKLC83). Due to the presence of a packaging sequence, the T1-spanin plasmid was encapsulated within a phage capsid. (b) The synthesized phage capsid (a) was used to infect Escherichia coli MC1061. Within bacterial cells lacking Tet

repressor expression, T1-spanin was expressed. (c) E. coliMC1061 were infected with phage capsids containing either the T1-spanin-encoding plasmid or a plasmid lacking T1-spanin (control). Credit: *BioDesign Research* (2024). DOI: 10.34133/bdr.0028

Given the worldwide prevalence of drug-resistant bacteria, the research community is on the lookout for alternative bactericidal treatment approaches. In a recent study, Japanese researchers have now compared bacteriophage-derived enzymes for combating drug-resistant bacteria.

Examination of T1-spanin revealed that it shows superior bactericidal activity against various strains, including E.coli. Furthermore, a novel phage-based technology effectively delivers T1-spanin genes into target bacteria. This breakthrough holds promise for the development of innovative antimicrobial agents in the future.

In the recent past, global efforts have focused on tackling emergent and unprecedented health risks, such as those posed by the COVID-19 pandemic. Nonetheless, the continued prevalence of drug-resistant bacteria presents an even greater threat to global public health. For instance, in 2019 alone, drug-resistant bacteria were responsible for about 1.27 million annual deaths worldwide.

Antibiotic-resistant strains now claim more lives annually than HIV and malaria combined. Now, while the menace of antibiotic-resistant bacteria continues to plague the health care system, alternative treatment approaches, such as bacteriophage therapy, have emerged as potential treatment options.

Bacteriophages, also referred to as "phages," are viruses that specifically target and destroy bacteria, including those that have acquired resistance

to antibiotics. What makes some phages particularly effective is the presence of specialized enzymes, which they use in different ways to target and kill bacteria.

The phage-derived lytic enzyme is one such type of enzyme, which can break down and kill bacteria from the inside out. Scientists have now learned to harness the power of these enzymes through bacteriophage therapy, offering a promising approach for targeting drug-resistant pathogens with high precision and efficacy.

To further explore and improve this approach, a group of researchers from Japan, led by Satoshi Tsuneda and Kotaro Kiga conducted a study investigating T1 phage's unique properties, focusing on trying to understand the phage's mechanism of action. The findings of this study were published in *BioDesign Research* on 8 January 2024, and shed light on T1 phage's potential role in refining bacteriophage therapy for combating drug-resistant pathogens.

The researchers first compared enzymes derived from the T1 phage with those from the T7 phage. They analyzed endolysins, holins, and spanins to assess their ability to kill bacteria. Endolysins are known to degrade the bacterial cell wall from the inside, causing the cell to burst open. They work alongside holins to regulate their activity.

"Holins work by puncturing the inner membrane of bacteria through oligomerization, with the help of a transmembrane domain. Spanins, on the other hand, act by mediating the fusion between the outer and inner membranes," explained Prof. Tsuneda.

Spanins also help break down the bacterial cell membrane. The researchers found that among the enzymes studied, those derived from the T1 phage were the most efficient at killing bacteria.

In particular, T1-spanin stood out. When attacking bacteria, viruses hijack the internal DNA machinery of bacteria, using it to make copies of themselves, similar to the mechanism observed in humans. Once enough copies have been made inside the bacterium, the cell bursts open, releasing the newly formed virus particles into the environment. Spanins, like T1-spanin, play an important role in this process, as they help break down the bacterial cell membrane, facilitating the release of the virus particles.

The T1-spanin enzyme displayed an exceptional capability to penetrate the outer defenses of nearly 120 different bacterial strains. Employing an innovative strategy, the researchers developed a novel approach to directly introduce the T1-spanin gene into target bacteria. This involved integrating the T1-spanin gene into a template virus shell.

Prof. Tsuneda says, "Unlike natural bacteriophages, this synthetic virus is unable to reproduce itself, and by using the synthetic virus instead of the bacteriophage directly, we were able to reduce the risk of environmental contamination or any adverse effects."

Due to T1-spanin's broad applicability, the method developed by the researchers in this study can be used to effectively target a wide range of bacteria, diverging from traditional approaches. Although it may be difficult to imagine how something as small as a virus-derived enzyme can make a difference to the menace of antibiotic-resistant bacteria, this study shows us a ray of hope. It suggests how molecular-level innovative strategies can help address the most pressing challenges in global public health, illustrating the potential of novel strategies to combat drug-resistant pathogens and advance the development of therapeutic interventions.

More information: Wakana Yamashita et al, Harnessing a T1 phage-derived spanin for developing phage-based antimicrobial development, *BioDesign Research* (2024). DOI: 10.34133/bdr.0028

Provided by NanJing Agricultural University