Isolation, characterization, and application of a novel *Salmonella* phage

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ABSTRACT

Enteric bacteria *Salmonella* is the causative agent for gastroenteropathy and enteric (typhoid) fever. The population of multi-drug-resistant (MDR) bacteria has considerably expanded over the past several years due to the overuse and misuse of antibiotics; consequently, developing an alternative antibacterial therapy to treat MDR bacterial strains is of utmost importance. Thus, there has been a recent, remarkable surge in phage research involving isolation, characterization, and application.

In this study, an enteric *Salmonella* bacteriophage STWB21 was isolated from a lake water sample, Kolkata, and found to be a novel lytic phage with promising potential against the host bacteria *Salmonella Typhi*. In addition, the phage STWB21 was also able to infect *S. Paratyphi*, *S. Typhimurium*, *S. Enteritidis*, and a few other bacterial species such as *Sh. flexneri 2a*, *Sh. flexneri 3a*, and *ETEC*. The phage morphology study using a transmission electron microscope revealed that the phage STWB21 belongs to the *Siphoviridae* family with an icosahedral head and a long flexible non-contractile tail. Phage stability was analyzed under various environmental conditions. Phage STWB21 was found relatively stable under a wide range of pH (4-11) and temperatures (4°C-40°C) for both typhoidal and non-typhoidal *Salmonella strains*. A one-step growth curve of bacteriophage was performed to assess the population kinetics of this isolated lytic phage. The latent period and burst size of phage STWB21 against *S. Typhi* were 25 min and 161 plaque-forming units per cell. The whole genome sequencing study revealed that phage STWB21 contained a dsDNA of 112,834 bp in length with a GC content of 40.37%. The existence of lytic genes and the absence of any lysogeny or toxin genes were also confirmed by genomic analysis. Furthermore, phylogenetic analysis revealed that the phage STWB21 cluster together with T5-like *Salmonella* phages. A detailed proteomic characterization identified 19 proteins in phage STWB21 by high-resolution Nano LC-MS/MS, which provided insight into the structural architecture of the phage. The structural models of the morphogenesis proteins were predicted using deep learning and homology-based methods. An *in-vitro* assay was performed to evaluate the capability of this phage for therapeutic purposes. Since *Salmonella* is a foodborne pathogen, it was found that bacteriophage STWB21 treatment significantly reduced biofilm not only on a 96-well microplate but also on food samples. The antibiofilm activity of phage STWB21 was also evaluated against *S.* Typhi, and *S.* Enteritidis alone and in comparison, with antibiotic cephalosporin. In both cases, a significant reduction was observed in the bacterial population of *S.* Typhi biofilm. The prophylactic and therapeutic efficacy of phage STWB21 was studied in a preclinical mouse model of *S.* Typhi infection. After introducing phage treatment to the infected mice, the phage showed reduced colonization in the liver and spleen in both the treatment and prevention groups. Overall, this thesis work showed phage STWB21 has a promising ability as a biocontrol agent of *Salmonella spp*. and proposes its application in food industries and for therapeutic purposes.

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