Isolation and characterization of a novel *Shigella* bacteriophage by electron microscopy

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Bani Mallick, M.Sc.

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Registration No: SLSBT1211719

Division of Electron Microscopy

ICMR-National Institute of Cholera and Enteric Diseases

P-33 C.I.T. Road, Scheme-XM,

Beliaghata, Kolkata-700010

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ABSTRACT

Enteric disease such as Shigellosis is one of the leading causes of morbidity and mortality worldwide. The increased prevalence of multidrug-resistant *Shigella* spp. has revived the importance of bacteriophages as an alternative therapy to antibiotics. Based on the bactericidal properties of lytic bacteriophages, they are considered potential biocontrol and therapeutic agents. Therefore, phage research involving isolation, characterization, and applications has recently significantly increased.

In this study, a *Shigella* bacteriophage Sfk20 was isolated from the lake water of the diarrheal outbreak area of Kolkata and found to be a novel lytic phage with promising potential against the host bacteria *Shigella flexneri 2a*. The morphological study (transmission electron microscopy and scanning electron microscopy) revealed that the bacteriophage had a prolate head and a long contractile tail indicating a member of the *Myoviridae* family. Phage Sfk20 showed infectivity against *Shigella flexneri*, *Shigella sonnei*, *Shigella dysenteriae 1*, and two non-typhoidal *Salmonella* strains. The one-step growth curve study of Sfk20 revealed a latent period (20 mins) and large burst size (123 pfu per infected cell). Phage Sfk20 showed high stability at a 4-37°C temperature range and a pH range of 7-9. A study on phage stability conducted at different salinity revealed phage Sfk20 remains active within 0-5% salt concentration. A study on understanding the nature of the phage Sfk20 host receptor suggested that the outer membrane LPS of the *Shigella flexneri 2a* acts as a receptor for the phage Sfk20. The bacteriolytic activity of phage Sfk20 study at various MOI showed that the growth of the host bacteria became restricted at high MOI. The whole-genome sequencing study revealed that the bacteriophage Sfk20 contains a linear double-stranded genome consisting of 164878 bp, 35.62% G+C content 241 ORF, and ten tRNA. The genomic analysis also confirmed the presence of lytic genes and the absence of lysogeny, virulent genes, and toxic genes. The comparative genomic study and phylogenetic analysis suggested that phage Sfk20 belongs to the family of *Myoviridae*. Using the LC-MS/MS technique in proteomic analysis, around 40 Sfk20 phage proteins were detected and identified, which helped to understand the structural landscape of phage Sfk20. The structural characterization of the phage Sfk20 was carried out using single-particle cryo-electron microscopy and image processing and the structure of the phage proteins was predicted using deep learning and homology-based approaches. The structural characterization of phage and its proteins further expands our knowledge of phage biology. The attachment of the phage particle to its host and subsequent intracellular development of phage and host cell lysis were visualized in a time-dependent experiment using thin-section transmission electron microscopy (FEI Tecnai 12 BioTwin) and scanning electron microscopy (FEI Quanta 200). This study further confirmed the lytic cycle of phage Sfk20. Bacteriophage Sfk20 showed antibiofilm activity against *Shigella* bacteria alone and in combination with ampicillin. The synergistic effect of Sfk20 and ampicillin on the removal of biofilm was observed by scanning electron microscopy. The efficiency of the phage cocktail was confirmed based on the ability to remove the biofilm. The result of this study implies that Sfk20 has the potential to work as a biocontrol agent and phage therapy candidate.

Bani Mallick 18.08.2023
Signature of Candidate

Moumita Dutta 18-08-2023
Signature of Supervisor

डॉ॰ मोमिता दत्ता / Dr. Moumita Dutta
वैज्ञानिक-सी / Scientist-C
आईसीएमआर-राष्ट्रीय कोलरा और आत्रं रोग संस्थान
ICMR-National Institute of Cholera and Enteric Diseases
कोलकाता-700 010 / Kolkata-700 010