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Phenotypic and genomic characterization of two newly isolated salmophages and evaluation of their potential for food biopreservation

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Statement of the Problem: The human microbiome plays a significant role in the health state, and dysbiosis - the imbalance between beneficial and pathogenic microbes - affects the development of diseases in various organs, including the intestine. One of the main bacterial pathogens transmitted through food and water is Salmonella - the etiological agent of diseases such as typhoid, paratyphoid, or salmonellosis. Due to irrational antibiotic stewardship (AMS), an increase in the incidence of multi-drug resistance (MDR) of bacteria has been observed recently. Therefore, searching for new therapeutic methods for pathogen infection treatment seems necessary. One of the possibilities is the utilization of bacteriophages (phages) as natural enemies of bacteria. Thus, this study aimed at the genomic and functional characterization of two newly isolated phages targeting Salmonella and their biological potential for food biopreservation.

Methodology & Theoretical Orientation: The Salmonella phage KKP 3828 and Salmonella phage KKP 3953 were isolated against Salmonella enterica subsp. enterica serovar 6,8:1,v:- KKP 1008 host strain deposited in the Culture Collection of Industrial Microorganisms - Microbiological Resources Center (IAFB). The bacterial host range and the effect of phage addition at different multiplicity of infections (MOIs) on host growth kinetics were determined using the Bioscreen C Pro-growth analyzer. Bacteriophages genomic DNA was isolated from lysates using the PureLinkTM RNA/DNA Mini Kit, followed by next-generation sequencing technology using nanopore technology (GridION long-read sequencer). The resulting consensus sequences were analysed in bioinformatic software (i.a. Phanotate, Ublast, ViPTree, Prokka, RGI, PhageAI, VirSorter, Phigaro, and PhaGAA). Proteomic trees of the newly isolated phages were generated using ViPTree server.

Results: Based on the transmission electron microscopy (TEM) and whole-genome sequencing (WGS) analyses the phages were classified as members of tailed bacteriophages belonging to Caudoviricetes class. According to the WGS analysis, the genome of the Salmonella phage KKP 3828 consists of 43,099 bp linear dsDNA with total G+C content of 52.0%. Among the 71 coding sequences (CDS), 25 exhibited significant homology to reported functional genes, while the remaining 46 CDS were annotated as hypothetical proteins. The proteins encoded by Salmonella phage KKP 3828 were divided into several functional modules: phage structure/assembly (portal, capsid, collar, tail, and their fiber proteins); DNA replication/modification/regulation; phage lysis; phage packing; and some additional proteins. No tRNAs were found in the phage genome, suggesting that the Salmonella phage KKP 3828 did not take over the host transcription/translation system, but uses host tRNAs for the synthesis of phage proteins. In addition, in the Salmonella phage KKP 3828 genome neither antibiotic resistance genes (ARGs) nor genes associated with integration into the host genome were located (markers of temperate bacteriophages). BLASTn analysis revealed that the Salmonella phage KKP 3828 genome had high similarity to the tailed Koutsourovirus from the Autographiviridae family.

Analysis of the Salmonella phage KKP 3953 genome (115,522 bp linear dsDNA) showed 28 tRNAs. The overall G+C content of its genome is 39.4%. Among the 262 CDS, 96 exhibited significant homology to reported functional genes, while the remaining 166 CDS were annotated as hypothetical proteins. The proteins encoded by Salmonella phage KKP 3953 were divided into several functional modules: phage structure/assembly (portal, capsid, collar, baseplate, tail, and their fiber proteins); DNA

replication/modification/regulation; phage lysis (holin, endolysin); phage packing; and other proteins. Neither ARGs nor other genes associated with integration into the host genome were located. BLASTn analysis revealed that the Salmonella phage KKP 3953 genome had high similarity to the tailed Epseptimavirus from the Demerecviridae family.

Conclusion: Genome analysis showed that both phages do not encode virulence or toxin genes and can be classified as virulent bacteriophages. Virulent characteristics and no possible pathogen factors make them feasible to be potential candidates for food biocontrol.

Biography

Michał works in the Laboratory of Biotechnology and Molecular Engineering, which is part of the Department of Microbiology in the Institute of Agricultural and Food Biotechnology (IAFB). He does research in Food Science, Molecular Biology, and Microbiology. His main field of interest is the biocontrol of saprophytic bacteria and bacterial pathogens (i.e., Salmonella) in the food industry by lytic (virulent) phages. Furthermore, he is interested in the transfer of antimicrobial resistance among bacteria in food products

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