## CHARACTERIZATION OF THE BACTERIOPHAGE ACTIVE AGAINST *PROTEUS* GENUS BACTERIA

**Hamova D[1], Kostiuk I[1], Zelena P[1], Rokush K[1], Faidiuk Yu[1,2].**
[1] Taras Shevchenko National University of Kyiv, Institute of Biology and Medicine
[2] D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine,
Department of bacteriophage molecular genetics
*e-mail: gamovadd@gmail.com*

*Proteus* genus harbors the causative agents of serious diseases such as urinary tract infections (UTI) in urinary catheter patients, gastrointestinal, respiratory system, skin and eye infections. Because of a wide variety of adaptational mechanisms exhibited by the bacteria, including biofilm formation, transition to swarmer state, etc. antibiotic treatment hasn't been proven to be largely successful. This situation calls for development of novel therapeutical methods with the use of phages (or phage-derived products) being among the most promising. Some of the benefits of phage therapy include specific host targeting, unique activity mechanisms, cost efficiency and a comparative easiness of development. Isolation and characterization of broad-host-range phages are of specific interest.

Recently a *Proteus mirabilis* strain (assigned according to the biochemical identification) was isolated from tomato plants (Kharina, 2015). A phage, able to cause productive infection, was isolated after application of the sewage water on the bacterial lawn. The aim of the present work was to characterize the bacteriophage and perform the molecular-genetic identification of the isolated bacterial strain to prove its species assignment.

The morphology of the phage and its interaction with the host were studied by TEM of the sample obtained directly from the plaque. Detected phage particles featured B1 morphology (*Siphoviridae*), with icosahedral capsid (Dmax of nearly 50 nm) and a long non-contractile tail. Along with it, highly flagellated bacterial cells at different stages of lysis were observed. The intact phage tails were difficult to detect, the ones detected on the microphotographs reached a length of 119 to 278 nm. It has been noted that the tails have a transverse arrangement of subunits. We detected the presence of bacteriocins of poly-sheath type with a length from 422 to 529 nm as well. It was also established that some phage particles or individual tails were attached to the flagellum, which may indicate the probable flagellotropic nature of a phage. In order to characterize the host range of the phage, *P. vulgaris* UCM B-905T, as well as *E. coli* C600 and *Erwinia* sp. 60 were used along with the host. The bacterial virus was able to develop plaques on the lawn of *P. mirabilis* and *P. vulgaris* strains, while the bacteria of other genera were not infected.

With respect to current guidelines on species assignment, partial 16S rRNA gene sequencing was used to prove the identification of *P. mirabilis*. Using a specific set of primers (a forward 27F-5'-GAGTTTGATCMTGGCTCAG-3' and a reverse 803R-5'-CTACCRGGGTATCTAATCC-3') we obtained amplicons of approximately 800 bp that contained variable regions V1, V2, V3 and V4. Sanger sequencing of the amplicon resulted in obtaining 708 bp fragment. Comparative analysis has shown that our sequence shares significant similarities with *P. mirabilis* strain MPE4069 – with 0 mismatch count, but as well with *P. vulgaris* JCM 20013 – with 0 mismatches, *P. vulgaris* strain P190036 – with 1 mismatch.

Thus, the ability of the phage to overcome the species barriers, while proved up to biochemical and morphological studies of the hosts, remains unproved on molecular genetic level, as the 16S rRNA comparison appears to be poorly informative for within-genus discrimination for *Proteus*. Usage of other phylogenetic markers or approaches is planned.