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Opinion

Assessment of the M.Sc. Fairoz Ali Al-Wrafy's doctoral dissertation "Immunochemical and biochemical characterization of phage receptors in two clinical strains of *Pseudomonas aeruginosa*"

executed under the supervision of: Professor Andrzej Gamian

The reviewed work concerns the evaluation of the effect of bacteriophages, and in particular, proteins isolated from selected clinical strains of *Pseudomonas aeruginosa*. Bacteriophages are bacterial viruses; they are characterized by the ability to infect, they can multiply in bacterial cells and destroy their specific bacterial strains. In view of the increasing resistance of medicines to microorganisms, the occurrence of multi-drug resistant strains (MDR), and in recent years - resistant to all available drugs (PDR), it is very important to intensively search for new effective compounds, including biological preparations that would allow the eradication of clinically relevant, dangerous bacteria. It is currently believed that phage therapy can be a viable alternative to the treatment of chronic infections. However, the work on the application of phagotherapy in medicine is conducted in only a few centers in the world. One of them is the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences in Wrocław where Professor Stefan Ślopek was the initiator of this research in the 1980s. Very important achievements in this area enabled Professor Andrzej Górski to establish in 2005

the Phage Therapy Center at the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences in Wroclaw. In the last decade, a new research direction has been developed in connection with phagotherapy, involving treatment of infections with the use of lytic enzymes produced by bacteriophages. In the context of the above data, the subject of the work is therefore completely justified, as it follows current and original issues.

The work is presented in English; has a typical layout for a doctoral dissertation. It counts 125 pages of computer printouts, is divided into an introduction, with separate assumptions and purpose of the work, methodical part, presentation of results and discussion with conclusions. The author quotes 173 references, of which about 70% are from the last decade. The work is illustrated with 35 prints and 12 tables.

The introduction presents the current taxonomy of bacteria of the *Pseudomonas* genus, the pathogenicity of *Pseudomonas aeruginosa* and its mechanisms of drug resistance, virulence factors with particular emphasis on the description of exopolysaccharide (EPS) polymers produced. At the same time, the author has very broadly characterized bacteriophages and their clinical applications. The introductory part is a broad knowledge study on the opportunistic pathogen - *Pseudomonas aeruginosa* and the possibility of using bacteriophages to treat infections.

The purpose of the work and research tasks are presented and then consistently implemented. Two strains of *Pseudomonas aeruginosa* (PAR21 and PAR50) from diabetic foot ulcers were used for the study. In the first stage of the work, two bacteriophages were obtained from wastewater, demonstrating beekeeping activity only against the PAR50 strain and two proteins were isolated from them (PA-PP1 and PA-PP2), which were purified using chromatographic technique and identified by comparative analysis of peptide masses (NCBL, UniProt database). However, it is a pity that the obtained bacteriophages were not classified. The basic data in this area are necessary not

only to supplement the description of bacteriophages used in the work, but also to include the presented results in comparative studies on the activity of their enzymes against Gram-negative bacilli. The second stage of the work involved searching for the receptor for isolated PA-PP1 and PA-PP2 proteins as well as assessing the antibiotic susceptibility of the PAR50 strain in the presence of isolated proteins. The enzymatic activity of PA-PP1 and PA-PP2 was analyzed against the exopolysaccharide produced by the tested *Pseudomonas aeruginosa* strains - EPS and its purified fractions and isolated by polyacrylamide gel electrophoresis of the outer membrane protein (OMP) of strain PAR50. The research was carried out using classic immunochemical analyzes, immunoenzymatic test - ELISA for EPS determinations (developed in this work), produced by strains PAR21 and PAR50, as well as spectrophotometric method, spot assay, zymography and modern instrumental analysis methods: mass spectrometry and nuclear magnetic resonance spectroscopy as well as scanning electron microscopy. In the evaluation of PAR50 strain drug susceptibility, a disk-diffusion test was used. The research techniques applied in the work meet international standards, which was undoubtedly the merit of the promoter - Professor Andrzej Gamian. However, doubts may arise from the methods of obtaining exopolysaccharide - EPS directly from the *Pseudomonas aeruginosa* bacteria biomass, as well as from the supernatants of their culture. It is already well known that the optimal *in vitro* production of EPS polymers by various bacterial species, including *Pseudomonas aeruginosa*, occurs as a result of their adhesion to synthetic surfaces during the accumulation phase of the forming microcolonies. The material from the liquid culture medium obtained in this work could not be adequate for assessing the anti-EPS activity of isolated PA-PP1 and PA-PP2 enzymes.

The obtained results have been documented in detail in figures and tables. The author determined the molecular mass of the obtained phage proteins - PA-PP1 and PA-PP2, in the range of 45-66 kDa and showed that they are part

of the family of serine protease. At the same time, it was found that the receptor for these enzymes is one of the outer membrane proteins (OMP_s) with a molecular mass in the range of 31-45 kDa. Enzymes with serine protease activity are also produced by many bacterial species of Gram-negative bacilli, including those of *Pseudomonas* genus. Earlier, it has already been shown that some porin proteins of OMPs of Gram-negative rods can act as a receptor for bacteriophages. However, in the study, it is indicated that the substrate for porins may also be phage proteins - serine proteases. This new data can be important for medical practice. However, they appear to be in contrast to existing knowledge about the participation of serine proteases in the development of bacterial infections and destruction of host tissues. In turn, when analyzing the drug susceptibility of the tested strains, a synergy of the interaction of phage proteins PA-PP1 and PA-PP2 with the antibiotic β-lactam - piperacillin, leading to the reversion of previously found resistance of the strain PAR50 to this drug was demonstrated. Similar results of the PAR50 growth inhibition zone were obtained in the presence of piperacillin combined with the β-lactamase inhibitor - tazobactam (p.64), which is an interesting observation.

The discussion covered the own results obtained and the results about the current literature. The discussion is detailed, well carried out, and addresses many aspects of the issue. This testifies to a good theoretical preparation of a doctoral student, her scientific maturity. The conclusions of the study were clearly derived from the results of the research.

In summary, I would like to emphasize that the above critical remarks do not significantly reduce the substantive value of the work, and may be useful to the author in further research and preparation of the publication.

The assessed doctoral dissertation meets the conditions set out in art. 13 paras. 1 of the Act of 14 March 2003 on academic degrees and academic title, and on degrees and title in the field of art (Journal of Laws No. 65, item 595, as

amended). Therefore, I have the honour to ask the High Scientific Council of the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy of Polish Academy of Sciences in Wrocław for the acceptance of this doctoral dissertation and its admission to further stages of the doctoral thesis.

Poznań, 14.08.2018r.

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