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Prof. dr hab. Dariusz Skarżyński
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Dear Professor Skarżyński:

I would like to thank you for honour of being appointed the external examiner on the Examination Committee to evaluate the PhD thesis of Ms Katarzyna Danis - Wlodarczyk. My assessment follows.

Yours sincerely

Andrew M. Kropinski Ph.D.
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Chair, Bacterial & Archaeal Virus Subcommittee, International Committee on Taxonomy of Viruses
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The candidate, Ms Katarzyna Danis-Włodarczyk , and her two supervisors, Professors Zuzanna Drulis-Kawa (Uniwersytet Wrocławski) and Rob Lavigne (KU Leuven), are to be congratulated for a comprehensive body of work that significantly adds to our knowledge of how to combat infections caused by biofilm-forming pathogens. The thesis was well-written, easy to read and the work it describes has resulted in five publications in high impact journals: Scientific Reports (Impact Factor: 5.228), PLoS One (3.234; 2 papers), Applied Microbiology and Biotechnology (3.376) and Archives of Virology (2.570).

The thesis follows what, in Canada, would be termed a "modified manuscript format," with chapters which resulted in manuscripts following an Introduction and background (Chapter 1), Study objectives (Chapter 2) and Materials & Methods (Chapter 3). Chapter 1 appropriately provides the reader with sufficient background information on *Pseudomonas*, bacteriophages, and biofilms, to put the Results chapters (4-7) into context. The word "appropriate" is not used in a negative manner since any one of these topics could be the subject of a book-length thesis. The Materials and Methods were thoroughly covered; indeed I think future students at Uniwersytet Wrocławski and KU Leuven will use her thesis as the reference for their methodologies.

I shall deal with Chapters 4 ("Bacteriophages KTN6 & KT28 represent the widespread and conserved *Pbunavirus* genus"), 5 ("Characterization of KTN4 phage, the novel φKZ isolate") and, 6 ("Characterization of PA5oct phage, the novel *Pseudomonas* jumbo phage") together since the basic methodologies are largely the same. The phages were isolated in Wrocław (Poland) on *Pseudomonas aeruginosa*, and characterized based upon morphology, host range, one-step growth analysis, and stability (temperature, pH, chloroform). What raises this work well above other studies is that (a) the cellular receptor involved in phage adsorption was investigated through the use of a panel of well-characterized host mutants, (b) the one-step growth curves were complemented by time-lapse microscopy, and (c) the genome sequence was analyzed fully i.e. termini, promoters and terminators were annotated. It was also impressive to see that Ms Danis-Włodarczyk used, amongst other tools, protein-sharing network and reticulate relationship analysis to position her phages relative to other fully-sequenced viruses.

Furthermore, the effect of the phages on bacterial biofilms was measured using LIVE/DEAD BacLight stain and confocal laser scanning microscopy. Pseudomonas phages KTN4 and PA5oct were also analyzed for their ability to mitigate bacterial infection of normal and Cystic Fibrosis-derived bronchial epithelial cell lines. These were the first incidences of assessments of phages by the Airway Surface Liquid infection model.

A special mention needs to be made on Chapter 6. Not only did the candidate employ all of the above-mentioned techniques; she also analyzed the proteomes and transcriptomes to fully characterize *Pseudomonas* phage PA5oct.

This is a phage that possesses an unusually large genome. It is impressive that Ms Danis-Włodarczyk actively employed classical phages techniques and modern ‘omic-era procedures to characterize her phages; then investigated their therapeutic applicability using a variety of state-of-the-art methodologies.

In the Chapters 7 and 8 (“Characterization of polysaccharide depolymerases domains associated with phage tail apparatus” and “Characterization of phage-encoded peptidoglycan degrading enzymes”) the candidate describes the cloning and comprehensive analysis of phage genes identified in previous research (i.e., phages LUZ7 and LKA1) and in her current studies which specified either EPS- or peptidoglycan-degrading actiivity. Ms Danis-Włodarczyk also employed protein structure modelling and motif analysis to predict biologically active regions of the proteins.

The experimental results were thoroughly discussed in each chapter, and the final General discussion (Chapter 9) nicely wrapped up the whole study.

In summary this is the most comprehensive thesis on bacteriophages that I have had the privilege of reviewing. In her research Ms Danis-Włodarczyk employed the full spectrum of the modern ‘omic sciences, together with expression of proteins and the biological and biochemical analysis of their properties.

Final assessment: A firt rate thesis.