INSTYTUT IMMUNOLOGII I TERAPII DOŚWIADCZALNEJ
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Rada Wydziału Nauk Biologicznych
Uniwersytetu Wrocławskiego

Ocena pracy doktorskiej

Pani Katarzyny DANIS-WŁODARCZYK

Tytuł: *Characterization of lytic bacteriophages infecting Pseudomonas aeruginosa and their peptidoglycan and exopolysaccharide degrading enzymes*
Promotor pracy: Prof. dr hab. Zuzanna Drulis-Kawa

Recenzent: dr hab. Krystyna Dąbrowska,
Instytut Immunologii I Terapii Doświadczalnej, im. Ludwika Hirszfelda
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Oceniana praca została wykonana we współpracy z Katholieke Universiteit Leuven w Belgii, a rozprawa została przygotowana w języku angielskim i oceniona przez komitet o składzie międzynarodowym. Dlatego uwagi szczegółowe do ocenianej pracy formułuję w języku angielskim.

Szczegółowa ocena pracy doktorskiej

The evaluation of the content (originality and quality of the contribution, the analytical approach and the methodology, data analysis, the results and discussion and the conclusions).

Pseudomonas aeruginosa is a frequent cause of nosocomial infections. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week and Pseudomonas infections are complicated and can be life-threatening. This is mostly because of common antibiotic resistance in Pseudomonas strains, as well as because of its ability to form a biofilm. The biofilm as a structure protects the embedded bacteria from antimicrobial agents applied from without and it also contributes to evolution of antibiotic resistance. As an opportunistic pathogen,

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Pseudomonas often causes endogenous infections in immune deficient individuals, e.g. in cancer or cystic fibrosis patients.

Unarguably, new strategies for combating difficult, antibiotic resistant infections caused by Pseudomonas are awaited by the health care systems and by affected patients. Since there are many hurdles in the new antibiotic development, especially against resistant Gram-negative pathogens, bacteriophages and phage-derived enzymes have been proposed as a treatment alternative in Pseudomonas infections. The vast bacteriophage diversity, their high efficacy against the target bacteria, and their different to antibiotic mechanism of antibacterial action make phages and their enzymes prospective alternative or complementary to antibacterial therapies. Phage therapy in general have already been accepted and successfully used as experimental treatment and clinical trials are currently in progress.

Katarzyna Danis-Włodarczyk in her doctoral dissertation reports her research work with the main objective to characterize new bacteriophages lytic towards Pseudomonas aeruginosa and to characterize two classes of phage-borne enzymes: peptidoglycan degrading enzymes targeting bacterial cell walls and polysaccharide depolymerases as potential anti-biofilm agents. For this goal she has completed a very extensive and ambitious plan of studies that contained:

1. Characterization of basic biology of the new bacteriophages, including morphology, host surface receptor, reproduction cycle, viral particle stability and a host range.
2. Study of phage genome organization and proteome composition and their influence for phage structure and fitness.
3. Comparative genome analysis and construction of protein-sharing networks to reveal phage evolution.
4. Evaluation of phage antibacterial activity in vitro with the use of a novel Airway Surface Liquid model on non-CF and CF epithelial cells lines, to mimic in vivo conditions of the respiratory tract.
5. Bioinformatics search of a set of phage genes encoding peptidoglycan degrading enzymes and polysaccharide depolymerases, and identification of enzymatic activity and structure prediction, based on available homology.
6. Preparation of recombinant proteins, including gene amplification, protein expression optimization and purification to obtain pure and active phage enzymes.
7. Assessment of recombinant protein stability.

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8. Evaluation of bactericidal activity and biofilm eradication potential of the recombinant enzymes.

All the above elements are original and new contribution of Katarzyna Danis-Włodarczyk's dissertation to the current state of knowledge. Her work resulted in new genomic, structural and microbiological data related to new bacteriophages. It should be emphasized, that the collected data impress by their extensity and by the variety of applied methods. The applied methods have been rationally planned to increase the application potential of the study (e.g. a novel Airway Surface Liquid model on non-CF and CF epithelial cells lines). I would like to emphasize that a range of methods used by the PhD candidate covers a variety of bioinformatics tools (genome and protein analysis), molecular biology laboratory work (cloning, expression, purification), a wide variety of microbiological assays, microscopy, functional analysis of enzymes. Taking the main objective, the approach was good and effective, and obtained results correspond very well with the idea of delivery new antibacterial means able to be active against Pseudomonas. Discussion of the results is critical, with pointing out study limitations and further work that needs to be done for complete recognition of investigated mechanisms. Thus, presented conclusions are fully justified.

The evaluation of the presentation (structure, style, layout) of the draft.

The whole presentation includes up to 312 pages which is an outstanding size of dissertation. The general structure of the dissertation includes all required elements such as: introduction, objectives, materials and methods, results, discussion, conclusions and references. Introduction gives a good insight into the current state of knowledge and into the problem undertaken by the PhD candidate. Objectives are clear and they correspond well with the problem. Results are very detailed, with interesting and helpful supplementary material related. Materials and methods section is comprehensive. Extensive list of references contains more than 500 positions. PhD candidate has also proposed a critical discussion: intermediate dedicated to each chapter, as well as a general one, that summarises and puts into context all findings of this doctoral work. There are also supporting parts, including an abbreviation list, supplementary materials, and summaries. The clearly made table of contents allow to easily navigate through the text. All elements were presented in details written in easy understandable grammatical style and in a very logical

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sequence. Beautiful "old-fashioned" pictures illustrating each chapter make this dissertation outstanding in terms of presentation aesthetic.

Summary and overall assessment

To summarize the Individual Assessment of Katarzyna Danis-Włodarczyk PhD Thesis entitled: Characterization of lytic bacteriophages infecting Pseudomonas aeruginosa and their peptidoglycan and exopolysaccharide degrading enzymes

I would like to emphasize the outstanding amount of research work that was completed by the PhD candidate, high quality of presentation and originality of scientific contribution. In my opinion this doctoral thesis work brings highly applicable data on new types of phage-derived enzymes and their characteristics, together with solid basic knowledge related to new phage strains active against Pseudomonas. Methods used by the PhD candidate range from advanced bioinformatics to solid laboratory techniques of molecular biology. I conclude that the trial meets the conditions imposed dissertations prepared for the doctoral degree. I submit the Katarzyna Danis-Włodarczyk PhD dissertation evaluation to Chairmann of KU Leuven Examination Committee, asking for his admission to further stages of doctoral procedure and permission for public defence.

Minor specific comments

My comments relate mostly to the part of the dissertation dedicated to protein expression, purification, and testing.

1. Figure 7.14 panel B needs to be improved by its quality (sharpness, intensity) to make protein bands more visible
2. Page 192 lines 6-7: "The size exclusion chromatography was not applied due to low protein concentration". It is not clear for me why any standard protein concentration method was not applied (e.g. vivaspin, amicon, etc). Possibly the protein was unstable in high concentration or other reason existed? It would be helpful if any explanation was given.
3. Page 192, description of gp49 domain 1, sentence: "After FPLC purification 6 fractions (0.5 ml each) were collected and their concentrations were as follows: A6: 0.26, A7:

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0.23, A7: 0.26, A9: 0.19, A10: 0.19, A11: 0.11 mg/ml.". When I compare these data to a related figure 7.15, it is unclear how contribution of target protein was divided from numerous impurities that were also present in the samples. According to materials and methods protein concentration was measured in a way that reveals total protein content (M&M: "The protein concentration was determined by spectrophotometric measurement of absorption at 280 nm").

4. Page 194: similarly to domain 1, some comments regarding protein yield/concentration would be helpful, particularly with regard to protein oligomerisation.
5. Chapter 7. Final preparations of investigated enzymes should be presented (final step of purification, as used for activity testing; SDS-page)
6. Page 225: "To verify whether PG hydrolases also demonstrate antibacterial activity towards E. coli APEC and S. Enteritidis strains, an antibacterial assay was performed with freshly purified proteins" – how many and what strains were tested?
7. Chapter 8. Final preparations of investigated peptidoglycan degrading enzymes should be presented (final step of purification, as used for activity testing; SDS-page)
8. Materials and methods: it would be useful to include full genetic background information for the E. coli strain used for cloning
9. Additionally, Summary in Polish should be corrected in regard to language accuracy since it contains minor linguistic errors, probably resulting from direct translation.

PODSUMOWANIE

Po wnikliwym zapoznaniu, się z pracą doktorską Pani mgr Katarzyny-Danis-Włodarczyk uważam, że przedstawiona do recenzji rozprawa spełnia warunki określone w ustawie z dn.14.03.2003 roku o stopniach naukowych i tytule naukowym (Dz.U. nr 65 poz.595) z późniejszymi zmianami.

Wnoszę do Rady Wydziału Nauk Biologicznych Uniwersytetu Wrocławskiego o dopuszczenie mgr Katarzyny-Danis-Włodarczyk do dalszych etapów przewodu doktorskiego. Jednocześnie wnoszę do Wysokiej Rady o wyróżnienie niniejszej rozprawy doktorskiej.

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