INSTYTUT IMMUNOLOGII I TERAPII DOŚWIADCZALNEJ
im. Ludwika Hirszfelda
Polska Akademia Nauk

Rudolfa Weigla 12, 53-114 Wrocław
tel. (4871) 337 11 72, fax: (4871) 373 2587
http://www.iitd.pan.wroc.pl/
NIP: 896 000 56 96

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Rada Wydziału Nauk Biologicznych
Uniwersytetu Wrocławskiego

Ocena pracy doktorskiej

Pani Agnieszki ŁĄTKI

Tytuł: *Characterization and in vitro engineering of exopolysaccharide (EPS) depolymerases originated from Klebsiella phages*
Promotor pracy: Prof. dr hab. Zuzanna Drulis-Kawa

Recenzent: dr hab. Krystyna Dąbrowska, Instytut Immunologii I Terapii Doświadczalnej, im. Ludwika Hirszfelda Polska Akademia Nauk, Rudolfa Weigla 12, 53-114 Wrocław

Oceniana praca została wykonana we współpracy z Uniwersytetem w Gandawie (Ghent University) 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).

Due to the increasing frequency of antibiotic resistant bacterial infections, new therapeutic strategies are awaited by the health care systems and by 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

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Klebsiella infections. Phages and phage-derived enzymes are a promising alternative in antibacterial therapies. The PhD candidate: Agnieszka Łątka has presented her work focusing on new phage-derived antibacterial enzymes: depolymerases. Depolymerases are enzymes able to degrade compounds that can be found in the bacterial cell surface, such as polysaccharides, or they are produced by bacteria when forming biofilms. By this way, depolymerases either decrease bacterial fitness or make bacteria more accessible for all-type antibacterial agents or for immune system. For these reasons depolymerases have a high potential for combating difficult bacterial infections.

My overall assessment of this PhD thesis is very high and 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 work results with highly applicable data on new types of phage-derived enzymes and their characteristics, and it brings new knowledge related to relationships between bacteriophage host range, specificity of phage-derived enzymes, and capsular serotype of sensitive bacteria. Further, this dissertation presents novel findings regarding depolymerase domains shuffling and its potential for engineering enzyme and phages, including phage specificity. Impressive selection of methods (including a novel, original technology: VersaTile by prof. Yves Briers) parallels very good presentation and discussion of results. Only minor comments regarding possible extension for discussion can be given.

Minor comments:

1. Statements related to the perspective of practical use of engineered bacteriophages (page 5 lines 29-33, page 25 lines 13-14, page 167 lines12-17) need to be toned down, since engineered bacteriophages become GMO thus resulting in many formal, ethical and public acceptance problems in case of attempts of therapeutic use. Please add more critical comments.
2. Figure 1.3, although adapted from a published review by Li et al 2013 (thus not being produced by the PhD candidate), is inappropriate in the style of cell presentation, particularly neutrophils, epithelial cells and dendritic cells).
3. Table 1.1 has also been adapted from another author, but it would be good to extend "resistance development" part with CRISPR/cas system

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4. In the same table (1.1) and in some other sites (tab 1.2) a reader may find information that phage penetration and circulation is regulated by RES (reticuloendothelial system)- the up to date name for this system is MPS (the mononuclear phagocyte system)

5. Table 1.2. contains information that phage-derived enzymes are effective on growing and non-growing cells, while phages "on growing" only. This statement goes too far, since there are phages that successfully propagate on stationary phase bacteria. This has been even proposed as a reason for differences (between phage strains) in phage-bacteria dynamics in mammalian guts, including phage propagation and selection for phage-resistant bacteria.

6. Unfortunately, one cannot agree with the statement that isolation of new phages is "fast and easy" (e.g. table 1.2). Please have in mind that there are still many bacterial species for which effective and applicable therapeutic phages have not been found yet.

7. In the section 3.3.4. "% of activity" is given many times. However, this looks like not calculated (from enzymatic kinetics) but estimated by the researcher who subjectively assessed plaques on plates (+, -, +/-). If so, more critical comments for this estimation and comparisons between proteins and conditions described in the section would be appropriate. Importantly: what was N for this assessment (how many plaques were assessed each time, how many times repeated)?

8. This assessment generates unclear conclusion for KP36gp50, that has been once described as having "preference to a more acidic environment" while next as "more stable in alkaline conditions".

9. Introduction to Chapter 4 contains large repetitions of information already given in Chapter 1, I guess this was decided due to the style of individual publication intended for this chapter?

10. Chapter 4 contains very interesting presentation of a few RBP systems in Klebsiella viruses. I understand that all figures 4.2-4.7 are original models developed by the PhD candidate, based on the in silico analyses described in this chapter. If so, this needs to be explicitly stated. First, because this will emphasize original contribution of this thesis into the field, second, because in silico analysis is not fully equivalent to structure analysis as identified by experimental work. As a special example serves Fig 4.5: a very complex structure with suggested protein-protein ratio needs to be critically presented. Alternatively, any source for Figs 4.2-4.7 should be cited.

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11. In the very interesting and well written Chapter 5, a little more developed introduction to horizontal gene transfer principles would be appropriate.

I do not find any major information missing. Minor points:

1. In the section 3.2.3 (p. 56), method for DNA isolation before its sequencing has been omitted
2. In the section 3.2.5. (p. 59) description of chromatography should be completed with volumes (or times+volumes per time unit) for all mentioned steps like "long" wash, "short" wash, elution.
3. Section 3.2.6.p. 59 what is "reducing sample buffer"?
4. Section 3.2.8. page 61: please give information on the concentration of EPS/CPS in zymography (in gels)
5. Figure 3.1. panel D: length identification for the bar is missing
4. Section 5.2.4 (bottom line 4) "supernatant" must be a mistake, "pellet" would be appropriate

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

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, very nicely presented with schematic graphics, and they correspond well with the problem. Results are appropriately detailed, with extensive listing in tables when necessary. Materials and methods sections are comprehensive with only minor elements missing (listed above). List of references is well completed and it contains all major positions related to this work. Discussion is in most cases well balanced, it addresses all major points in this work, both intermediate discussion sections in each chapter, as well as the general one, that summarizes and puts into context all findings of this doctoral work. The dissertation also contains all required technical elements, including an abbreviation list, list of content, or summaries. All sections are clearly written in easy understandable grammatical style and in a very logical sequence.

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This dissertation is very carefully edited, rare corrections that need to be done:

1. Tables 4.3-4.6 (caption) should contain "given" instead of "give"
2. tab. 4.4. "not apllicable" could be moved to correct position in a column
3. Page 125: "section 5.1.2" instead of "5.1.2" and "section 5.1.3" instead of "5.1.3"

PODSUMOWANIE FORMALNE

Po wnikliwym zapoznaniu się z pracą doktorską Pani mgr Agnieszki Łątki 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 Agnieszki Łątki do dalszych etapów przewodu doktorskiego. Jednocześnie wnoszę do Wysokiej Rady o wyróżnienie niniejszej rozprawy doktorskiej.

Dr hab. Krystyna Dąbrowska, Prof. PAN

Samodzielne Laboratorium Bakteriofagowe
Instytut Immunologii i Terapii Doświadczalnej
Polskiej Akademii Nauk
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