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Review Report on PhD Thesis of Łukasz Mazurek

entitled:

Studies of interaction of pentapeptide repeat proteins with *Escherichia coli* DNA gyrase

Bacteria deal with various challenges during even most favorable life conditions, such as direct and indirect factors affecting the topology of their genetic material during the regular processes (DNA replication, gene expression) and the action of external agents (e.g. antibiotics). As a prokaryotes, bacteria have their chromosome not secluded in the separate space, which makes the necessity of the proper packing and unpacking of DNA even more important to ensure the access of cellular proteins to DNA. Thus, the number of proteins and processes are involved in maintaining adequate DNA topology, including the timely changes dependent on the status of the cell. Therefore the knowledge about the mechanisms responsible for adequate DNA winding and unwinding is crucial for understanding the main cellular processes in bacteria and also extrapolating them to higher organisms. Also, not to be underestimated, the information about functioning of bacterial indispensable homeostasis mechanisms could be used to design and employ novel antimicrobial strategies. Enzymes restoring proper DNA topology play very important role in both transcription and replication. Among them, gyrase is a popular target for antibiotic action. However, the resistance to the most common anti-gyrase antibiotics, fluorochinolones, become increasingly wide-spread, which makes them useless. Therefore, understanding of the mechanisms of quinolone resistance is a basis to both comprehend the gyrase function on molecular level and propose novel

antibacterial agents overcoming this resistance. Thus, the PhD thesis of Łukasz Mazurek covers timely the subject concerning the interactions of gyrase with proteins conferring resistance to quinolones. The work on this subject was conducted under the supervision of prof. dr. hab. Jonathan Heddle in the Bionanoscience and Biochemistry Laboratory, Małopolska Centre of Biotechnology, Jagiellonian University in Kraków.

The doctoral thesis of Mr. Mazurek is organized typically for the publications based on the experimental work. The structure is correct with all important parts present. The thesis contains 6 main chapters, preceded by the abstracts in both Polish and English, and followed by conclusions, bibliography and supplementary materials. Together with the list of abbreviations and properly presented table of contents it makes the thesis a complete and easy to follow publication. The Introduction covers the background of topoisomerases characteristics, presenting in details types of the enzymes and reactions performed by them. The schemes and models presented in figures help to understand the topic. As the main model of the thesis is gyrase, the information on this enzyme is given in more detail. The second part of the Introduction is dedicated to the group of gyrase interacting agents, poisons and proteins that could potentially neutralize the toxin action. This part is quite detailed however it smoothly transfers the reader to the well-formulated aim of the studies, complemented with two main objectives. The following chapter, Materials and methods, presents in a clear and concise way the broad techniques and methodology collection that was employed in this work. The presentation is clear (however I would recommend writing the bacterial species name in the strain list), especially the methods description which could be used as a manual for e.g. students. It is worth noting that the range of biochemical methods, protein purification and analysis is particularly impressive and shows the high level of skills of the doctoral Candidate. The main part of the thesis constitutes the Results section (74 pages). It contains 3 main parts, with the second one divided into numerous sub-chapters which facilities following the work flow. The variety of methods chosen to achieve the thesis aims resulted in the interesting systematic studies on the interaction between gyrase and specific type of proteins. The chapters usually end with a summary helping to pinpoint the findings and explain next steps of research. The results are then discussed in the next section, which is a strong point of this dissertation. This part is very clearly and interestingly written and presents the data in the light of the current literature and knowledge in the field. The possible shortcomings and limitations of the data are also mentioned which makes the discussion even more valuable. The concluding remarks clearly summarize the findings of the thesis. The bibliography presents comprehensive

reference list containing the current papers, and indicating the broad knowledge of the Candidate. The dissertation is written clearly, using in general good quality scientific language. However, as a reviewer I have to mention some mistakes that I spotted through the text. There are numerous typo, punctuation and spacing errors and persisting lack of italics in the names of bacterial species (also, using capital letter in both binominal parts of species names). In addition, Gram-negative should be written in capital letter "G" as it comes from the name of the scientist who invented this type of staining. The names of the genes also sometimes are not written in italics. There jargon phases are rare which is notable for so voluminous dissertation. In general, all these shortcomings are minor and they do not substantially affect the quality of this work.

The main aim of this study was to obtain the information on the molecular level about the interaction between gyrase and specific pentapeptide repeat proteins (PRP). These proteins are known to affect the gyrase-poison complexes protecting the gyrase activity in this manner. However the exact mechanism of this effect is mostly unknown with contradictive hypotheses proposed so far. Thus, PhD thesis of Łukasz Mazurek deals with this subject which is of great importance in both biochemical and potentially therapeutical fields. The model in this study was gyrase from *Escherichia coli* and three proteins that can rescue gyrase from inhibitor effects. These PRP proteins were derived from three different bacterial species: *Klebsiella pneumoniae* (QnrB1 protein), *Xanthomonas albilineans* (AlbG) and *E. coli* (McbG). The proteins protects bacteria from flurochinolones, albicidin and B17 microcin, respectively. **Here, I would like to ask what was a basis to choose these models to study?** *E. coli* seems quite obvious, but why others? Especially, while later in the discussion the problem of incompatibility between *E. coli* gyrase and PRP proteins from other species is mentioned. Entire work presented in the thesis was very well planned and performed. Notably, technical problems and difficulties were overcome by searching for alternative approaches, e.g. with protein purification. The Candidate established a wide set of molecular and biochemical tools to achieve the thesis goals, including crosslinking, Cryo-EM and mass spectrometry. The number of purified and defined proteins in the course of this work is impressive. The particular research objectives were pursued consequently which resulted in the completion of project aims and the comprehensive set of data.

As the most important results and achievements of the dissertation I would list the following:

- the purification of three pentapeptide repeat proteins from various bacterial species

- characterization of biochemical and structural features of two PRP, QrnB1 and AlbG
- determination of the structure of PRP-gyrase-antibiotic-DNA complex which helped also resolving the specific PRP residues taking part in the interaction
- finding that PRP stimulates ATP hydrolysis which is indispensable for abolishing of toxin effect towards gyrase
- resolving the question about interactions of PRP with specific DNA segment

Particularly important is presenting a new model of the interaction between PRP and gyrase, and consequently, the mode of action, which contradicts the models proposed so far by other teams. It opens new area of exploration of proteins interaction and in particular gyrase as a target for antibacterial agents. It is worth noting that important part of this dissertation has already been published in the renowned journal Nucleic Acids Research – which indicates the importance of this work and findings as well as making it available for general scientific world.

Here, I would like to ask some questions about the presented data and their interpretation:

1. What is a specificity of the PRP binding? Are there other than gyrase proteins that can be targets for PRP?
2. Do the antibiotics targeting gyrase induce the stringent response and its alarmone, (p)ppGpp accumulation? And, do (p)ppGpp affect the expression and activity of PRP?
3. I have a specific question about marbofloxacin which is a third-generation quinolone. Is this antibiotic used specifically in the veterinary medicine and how a resistance to it is widespread?
4. If the gyrase-targeting antibiotics induce the SOS response by double strand DNA break, they are likely to induce prophages (and by that, they will be unsuitable for treating phage-toxin dependent infections, as EHEC). Is this possible to introduce in such treatment the low-level PRP effect to diminish the SOS response, and still gives satisfactory growth inhibition by antibiotics?
5. I would like the Candidate to present a hypothesis what could be a selective pressure and advantages for the PRP to occur, especially that some of them are not evolutionary connected.

6. In the protein purification protocols many proteins were tagged (e.g. His), so why the removing of the tag was not undertaken? Could the presence of the tag affect protein activity and features?
7. As the gene encoding albicidin and *albG* gene are in one cluster, are they expressed together and regulate their own expression? By that, can they be classified as a typical toxin-antitoxin system?
8. As the structural studies on the complex of *E. coli* gyrase and QnrB1 protein from *K. pneumoniae* were performed, can they be directly translated into understanding of the interactions between same-species PRP and gyrase? What are the limitations of presented conclusions of heterologous interactions?

Final evaluation statement

To summarize, the Candidate for PhD degree, Łukasz Mazurek, presented **very interesting research and obtained results of high novelty and scientific value**. Thus, I express the opinion, that **this thesis definitely meets all the requirements for the degree of Ph.D**. by the statutes in the Journal of Laws of the Republic of Poland (Act on higher education and science, - art. 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce, tekst jednolity: Dz.U. z 2018 r. poz. 1668 z późn. zm). Therefore, I recommend admitting Mr. Łukasz Mazurek, MSc, to the public defense of his dissertation. Moreover, as a scientific level and novelty of this dissertation is high and the results have been already partially published, **I recommend the thesis for the relevant award**.

M. Szalewska-Pałasz