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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Julia Piskunova

PhD Program: Life Sciences

Title of Thesis: Structural and Functional Analysis of Ribosomally Synthesized and Post-Translationally Modified Microcins from *Escherichia coli.*

Supervisor: Professor Konstantin Severinov

Chair of PhD defense Jury: Professor Yuri Kotelevtsev ***Email:*** ***y.kotelevtsev@skoltech.ru***

Date of Thesis Defense: October 27, 2017

Name of Reviewer: Konstantinos Beis

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:**  **Date: 02-10-2017**

The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to forward a completed copy of this report to the Chair of the Jury at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevancy of the topic of dissertation work to its actual content
- The relevancy of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)

- The quality of publications
- The summary of issues to be addressed before/during the thesis defense

The work presented in the thesis investigated two different aspects of microcin action. The first was the role of microcin C on its role in bacterial cell persistence. The second was on a methodical mutagenesis study to identify new variants of microcin B to inhibit bacterial cell growth. The thesis is very well presented (some recommendations for improvement are made at the end of this report) with an extensive introduction on microcin biosynthesis, mode of action and mechanisms of persistence. The results sections (Chapter 1 and 2) contain the correct level of information and details.

The first chapter investigated the role of microcin C (McC) to induce persistence bacteria cell formation. The role of microcins as antibacterial agents is well studied but in this work a novel aspect of McC is studied, persistence. The antibacterial activity of McC is conferred by inhibiting the activity of aspartyl-tRNA-synthase. Previous studies have shown that increased levels of the second messenger (p)ppGpp resulted in bacterial cell persistence. In this study, it has been shown that inhibition of the aspartyl-tRNA-synthase by McC resulted in increased levels of persistence cells due to increased (p)ppGpp levels. It was further validated by deleting the relA gene that encodes the (p)ppGpp synthase. The data showed that McC requires (p)ppGpp to induce persistence. Similarly, cells that produce McC have increased levels of persistence.

The second chapter investigated the role of the heterocycles on the antibacterial activity of the microcin B (McB). McB has two sites that contain thiazole and oxazole rings. Its antibacterial activity is due to its interaction with DNA gyrase. In this elegant study, the two sites were mutated by a different set of amino acids that could preserve or abolish heterocycles and their antibacterial activity was measured in vitro and in vivo. The study identified that loss of heterocycles in site A significantly reduced antibacterial activity of McB compared to site B mutants in vivo. Interestingly, most of the mutants retained antibacterial activity against DNA gyrase in vitro but not in vivo suggesting that internalisation may have been affected. The findings may lead to the identification of novel antibacterials to combat antibiotic resistant bacteria.

The work presented here makes a significant and important advance on our understanding on the mode of action of microcins. The work has generated a significant publication.

The work that is described within Chapter 1 has been published in Molecular Microbiology proving the significance of this study. The results of Chapter 2 are not yet published but the quality and novelty of the findings will result in a future publication. Julia is also a mid author in a paper submitted in JACS.

Specific comments/recommendations to be addressed before the viva:
Both chapters lack some in depth discussion and conclusion (or future perspective) after the results section. A summary is included for both chapters but only lists the key findings. The thesis will benefit with a more extended discussion section for each section. The Conclusion section in p70-71 is not sufficient.

The Project Objectives (p47) should be moved before the Materials and Methods section since it will introduce the key questions of the thesis.

Since the McB variants produced in the thesis have not been characterised before, it might be good to show HPLC traces and mass spec data for key variants as an appendix.

Provisional Recommendation

☐ *I recommend that the candidate should defend the thesis by means of a formal thesis defense*

☒ *I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report*

☐ *The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense*