Skoltech
Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Dmitrii Travin

PhD Program: Life Sciences

Title of Thesis: Phazolicin — a novel azole-modified peptide antibiotic: structure, mechanisms of action, transport, and biosynthesis

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Douglas A Mitchell

I confirm the absence of any conflict of interest	**Signature:** **Date: 31-07-2022**

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 submit signed copy of the report 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 relevance of the topic of dissertation work to its actual content
- The relevance 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

This is a high-quality thesis that is well-organized, thoughtfully constructed, and highlights several significant scientific achievements of Mr. Travin's research. I have no concerns about the methods applied nor the relevance of the document to the thesis research completed by Mr. Travin. As indicated by his publications in peer-reviewed, international journals that are renowned, the scientific advancements described are high caliber. I fully endorse an oral defense should be pursued. I have reviewed the entire document and I only have minor points, some of which contain substance while others are simply cosmetic.

1. Consider more broadly the biological role of RiPPs. Signaling molecules? Not always a growth-suppressing factor. Concentration gradient near the produced (i.e., like an early warning system). We can discuss this at the thesis defense.
2. Phazolicin is a LAP. You should define that in the abstract and in the abbreviations list.
3. Prokaryote means bacteria and archaea. Does phazolicin inhibit archaeal protein translation?
4. Page 18: the local genes rarely hint towards MOA, in my experience.
5. Please reconsider using green/red in the same figures owing to color blindness.
6. Page 24: RiPPs are harder to genomically locate, no? Has RiPPER solved this problem?
7. Page 27, ref 49: are these "RiPPs"?
8. Page 28: for multi-precursor peptide BGCs, the precursors do not always resemble one another
9. Fig 2.1.3: typically Z-alkenes are formed; E-alkenes (as drawn) are much rarer. Also, when stereochemistry is known (R vs. S) isomers should be drawn. Oxime spelling issue.
10. Fig 2.1.5: indicate that telomestatin is a non-RiPP. Also, the molecular drawing settings need to be consistent (bond length, width, atom label size, etc).
11. Page 36: the role of Ec YcaO in uL16 thioamidation is hypothetical, not yet demonstrated experimentally. Further, TfuA is not always required.
12. Page 35: Have the RRE domains fused to the cyanobactin dehydrogenases been shown to be functional?
13. Page 38: the target of plantazolicin is proposed to be the membrane (not proven). References missing
14. Page 40: the molecular target of streptolysin S is not necessarily band3. It could be a direct or indirect interaction.
15. Page 42: we have described the confusion around the TfuA nomenclature in our 2021 Nat Chem Biol publication.
16. Do not use "Alfa", it is alpha, best to use the Greek symbol instead.
17. Section 2.2: How did Nif11 get "co-opted" for RiPP biosynthesis? We can discuss at the defense.
18. Methods sections: avoid starting statements with quantities. Also, always use x g instead of rpm since the latter depends on rotor diameter.
19. Page 69: I am a little confused how you determine a priori what the false-positives are.
20. Page 71: the YcaO protein is not "class-defining" for thiopeptides.
21. Page 77: I am unfamiliar with the term "brutto". We typically call this the empirical formula.
22. Page 85: are there PHZ non-susceptible bacteria that encode a PhzE ortholog but do not produce PHZ?
23. Fig 5.5.1: confusing to say "inhibition of luciferase mRNA" when it fact you are inhibiting translation. The mRNA is the substrate and it is the ribosome that is inhibited.
24. Once PHZ binds, how quickly does it dissociate? Is that ribosome dead forever?
25. Page 141/1422: Is PZN production a metabolic burden? How much is made? And is there an optimal number of nodules per plant (i.e., are too many growth-inhibitory?)

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*