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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: Konstantin Lukyanov

I confirm the absence of any conflict of interest	**Date:** 12-08-2022

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

Ribosomally synthesized Posttranslationally-modified Peptides (RiPPs) are a diverse group of heavily modified peptides naturally produces by various organisms. Molecular mechanisms of their synthesis, modifications, transport and biological function are very diverse and in most cases poorly studied. From the point of view of practical importance, RiPPs often possess antibacterial activity and thus represent a promising source of novel antibiotics for medicine and agro technology. Since RiPPs are genetically encoded, they can be artificially mutated to create libraries of compounds for further screening.

The present dissertation by Dmitrii Travin is a comprehensive study of a novel RiPP from a symbiotic nitrogen-fixing bacterium *Rhizobium*. The work includes several consecutive stages. First, Dmitrii identified a novel biosynthetic gene cluster for a putative RiPP by genome mining. Then, chemical structure of this RiPP named phazolicin (PHZ) was deciphered. Moreover, the exact mechanism of PHZ action as a ribosome exit channel-blocking antibiotic of a narrow spectrum was revealed. Also, mechanism of import into target bacteria via two distinct transporters was demonstrated. Next, biological importance of PHZ production to ensure competitive advantages of PHZ-producing strains in different environment was studied. Finally, Dmitrii performed extensive bioinformatic search of PHZ homologs and biosynthetic gene clusters, which could be further studied as a source of new RiPPs.

The main results of the work are published in the Nature Communication (IF 17.7) paper with the first authorship of Dmitrii Travin. Dmitrii also a coauthor of the paper in mBio (IF 6.8) and the first author of two reviews, in Frontiers in Genetics (IF 4.4) and RSC Chemical Biology (preliminary IF 2.7).

Overall, this is a fantastic work, perhaps the best dissertation in my practice, considering all together – scientific content, style, logic and clarity of the narrative.

Minor comments:

1. Page 77: “The proteolytic degradation of PHZ by the aminopeptidases is apparently not possible, as the carbonyl of Ala1 (Ala29 of the precursor) is involved in the formation of the first thiazole cycle, which protects the peptide bond from the cleavage”. In fact, Figure 5.2.2D shows that peptide bond between Ala1 and Thr2 is intact and thus can potentially be cleaved by a peptidase.
2. Page 92: “A notable feature of the PHZ molecule in its ribosome-bound state is the formation of complex intramolecular interactions that include both face-to-face and edge-to-face π–π stacking of Thz12, Oxz21, Thz6, and Oxz18, along with the nucleobase U2609 from the 23S rRNA (Fig. 5.6.3F)”. It is a textbook knowledge that exact “face-to-face” π–π stacking is energetically unstable, since partial negative charges of π electrons from the opposite molecules repulse from each other. Instead, “displaced face-to-face” π–π stacking is commonly observed; probably, this is also the case for PHZ. You might want to specify this in the text.
3. Page 128: DSRed -> DsRed.
4. Page 132: “This unexpected result may be explained by the relatively large size of the PHZ molecule and the presence of azole cycles, which can mediate interactions with soil-forming organic polymers”. I think that inorganic particles of clay and silica can be efficient absorbers of PHZ molecules.

5. Page 134: “The majority of nodules on legume plants are initiated by a single rhizobium bacterium and are therefore colonized by clonal populations”. Is it reasonable to expect competition between bacterial strains if each nodule contains only one strain (derived from a single bacterium)? Would not it be better to count CFU for strains isolated from mixed-infected nodules?

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*