

## Jury Member Report – Doctor of Philosophy thesis.

**Name of Candidate:** Tatyana Zyubko


**PhD Program:** Life Sciences

**Title of Thesis:** Efficient in vivo Synthesis of Lasso Peptide Pseudomycolidin Proceeds in the Absence of Leader and Leader Peptidase

**Supervisor:** Prof. Konstantin Severinov

**Date of Thesis Defense:** 19 December 2019

**Name of the Reviewer:** Konstantin Lukyanov

I confirm the absence of any conflict of interest	<b>Signature:</b>  <b>Date: 18-11-2019</b>
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*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 dissertation studies a novel lasso peptide biosynthetic gene cluster from *Bacillus pseudomycooides*. The author first identified it bioinformatically, and then studied in detail experimentally. Gene deletion analysis of this cluster revealed its unique properties, which differ it from all other known lasso peptide biosynthetic pathways. Most importantly, the novel lasso peptide called pseudomycoidin can be efficiently produced by a single enzyme – specific lasso-cyclase – in the absence of other processing enzymes and leader sequence. This unexpected feature was clearly demonstrated and studied in a series of elegant experiments. Overall, this excellent work provides significant contribution to our understanding of basics of lasso peptide biosynthesis. Importantly, it also opens new perspectives for biotechnology and medicine by finding the greatly simplified cassette for production on pseudomycoidin, its variants, and possibly other lasso peptides.

Author applied a broad range of modern techniques – bioinformatics sequence analysis, DNA cloning, gene deletion analysis, site-directed mutagenesis, heterologous protein expression and purification, mass-spectrometry, chromatography, and NMR (the latter in collaboration). The used methods are adequate to the processes under investigation; all experiments are well designed and contain all appropriate controls.

Results have been published in 2019 in two peer-reviewed papers in high-ranked journals: in Chemical Science journal (impact factor 9.6) with first authorship of Tatyana Zyubko, and in MBio journal (impact factor 6.7).

I do not have any significant concerns about this work; some minor points are listed below:

Literature citation in the text could be simplified, e.g., “[1], [2], [3], [3][4], [5], [6], [7], [8], [9]” -> [1-9]; “[2], [4], [6], [11]” -> [2, 4, 6, 9]; etc.

Page 15, missed symbol: “attached to the -carboxyl group”.

Page 51, misprint “The psmCA genes were cloned on the sane...” (-> same).

Figure 20B is of too low resolution (not all letters are clear).

Page 78: “With the help of collaborators, we recorded NMR spectra...” I think it is worth mentioning their names and institution.

#### **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*