Skoltech
Skolkovo Institute of Science and Technology

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 Pseudomycoidin Proceeds in the Absence of Leader and Leader Peptidase

Supervisor: Prof. Konstantin Severinov

Date of Thesis Defense: 19 December 2019

Name of the Reviewer: Mikhail Gelfand

I confirm the absence of any conflict of interest	**Signature:**  **Date: 11-11-2019**

Reviewer's Report
The thesis is a nice combination of bioinformatics used to obtain an initial guess about a promising candidate lass peptide and in-depth experimental analysis of this candidate. Tatyana has identified a new lasso peptide gene cluster; developed an in vivo system for its production; determined its structure and characterized variant produced by subsets of biosynthetic genes, hence providing an initial characterization of these genes' function; isolated the encoded enzymes and identified their mode of action; reproduced these results in vitro; characterized biochemical properties of the system; studied mutant forms of the precursor to characterize their synthesis and functionality. The manuscript is well written, logically organized, and almost completely devoid of minor or editorial errors (a rare example being "It is suggest that the lasso topology in itself is not sufficient for antibacterial activity" on page 80). The review section of the dissertation is well-organized, detailed yet not boring, lays a base for the research project and contains a lot of food for thought for further development – an obvious direction might be evolution of lasso-peptide loci. However, the author is interested in biochemistry rather than evolution, and it is a reasonable choice. The applied methods are relevant and diverse. Overall, Tatyana has demonstrated perfect command of a wide variety of biochemical techniques and an ability to identify, select, and use relevant bioinformatic

approaches. This is how the modern molecular biology should work in general. The conclusions follow from the obtained results, are clearly formulated and, to repeat, relevant and important.

Given the discovery void in antibiotic development and the coming era of resistant and multiresistant pathogenic bacteria, all studies of antibiotics, especially new ones, are of major potential interest for applications.

The results of the research are published in two good papers in reputable journals. In one of the papers Tatyana is the first author. They have been reported at two recognized international conferences; one of the reports have been awarded the second prize. Hence, all formal SkolTech requirements are fulfilled.

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*