Skoltech
Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Anna Maikova

PhD Program: Life Sciences

Title of Thesis: The CRISPR-Cas system of human pathogen *Clostridium difficile*: function and regulation

Supervisor: Prof. Konstantin Severinov

Co-Supervisor: Prof. Olga Soutourina

Chair of PhD defense Jury: Prof. Mikhail Gelfand ***Email**: m.gelfand@skoltech.ru*

Co-Chair of PhD defense Jury: Prof. Harald Putzer

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Date of Thesis Defense: 30 September 2019

Name of the Reviewer:

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:**  **Date: 29-08-2019**

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

The doctoral thesis presented by Anna MAIKOVA describes the functional characterization of the CRISPR-Cas system and its link to type I toxin-antitoxin systems in the pathogenic bacteria *Clostridium difficile.* It provides novel insights into the function and capacity of this defense system in *Clostridia* and highlights unique features related to the its potential co-regulation under adverse conditions with closely linked toxin-antitoxin modules. Moreover, it describes the feasibility of using the endogenous CRISPR-Cas system as a novel tool for gene editing in *C. difficile.*

This study is highly relevant and important to a better understanding of the defense strategies of the pathogen *C. difficile* and its capacities to adapt to changing environments inside the host.

The thesis is very well written and presented. The introduction is concise, well-illustrated, informative and up to date.

Major experimental results include the following:

Experimental prove for *in silico* defined PAM sequences.

Demonstration using interference assays that most of the CRISPR arrays are functional. Importantly, this was also shown to be valid for the hypervirulent strain R20291. Efficiency of CRISPR interference generally correlated with array expression even though this was not always clear to me from the figures presented.

Albeit not very efficient, some arrays were shown to be capable of new spacer acquisition.

The role of the multiple CAS operons remains unclear as the deletion in one case showed no clear-cut effect.

Chapter 3 presents the demonstration of a unique co-localization of type I Toxin-antitoxin (TA) moduls with various CRISPR arrays, a feature conserved in the majority of sequenced *Clostridia* strains. The functionality of these TA modules was demonstrated (toxicity and effect on cell length)

A potential co-regulation of the TA moduls with CRISPR-Cas system via the stress response sigmaB and biofilm related factors (c-di-GMP levels) was shown.

As discussed in the thesis, the co-localization of the CRISPR arrays and TA moduls could contribute to the stabilization of the chromosomal regions carrying the CRISPR arrays in order to maintain the increased defensive capacities.

The usability of the endogenous CRISPR-Cas system for genome editing was demonstrated and extends the range of biotechnological techniques in *Clostridia.* Active killing of *C. difficile* cells by CRISPR self-targeting also opens new perspectives for developing alternative strategies for *C. difficile* infection treatment.

Anna MAIKOVA has applied a remarkable spectrum of *in vitro* and *in vivo* genetics and molecular biology approaches to study these questions and the data presented throughout this thesis are of excellent quality.

The results obtained in this thesis led to the publication of three articles in well-known peer-reviewed journals, in all of which Anna is first or co-first author: 1 in Nucleic Acids Research, 1 in Front. Microbiol. Frontiers and 1 in Applied and Environmental Microbiology (under revision). Two further manuscripts are in preparation.

In summary, I fully recommend the acceptance of this excellent thesis by Anna MAIKOVA for defense by the Graduate Research Schools of Skoltech and Université de Paris.

Provisional Recommendation
X *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*