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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

Supervisors: Prof. Konstantin Severinov;

Prof. Olga Soutourina, University of Paris-Saclay, France

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

Professor Harald Putzer, Paris Diderot University, France ***Email:*** putzer@ibpc.fr

Date of Thesis Defense: 30 September 2019

Name of the Reviewer: Ekaterina Semenova

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:** E. Semenova **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 PhD thesis prepared by Anna Maikova describes the work on CRISPR-Cas system of human pathogen *Clostridium difficil*e. The candidate investigated different aspects of functioning and regulation of this defense system in native clostridial cells. Assuming the importance of *C. difficile* studies for human health, there is no doubt that this work is of a general significance. The thesis is well organized and comprises 5 chapters. Chapter 1 presents a review of current knowledge on CRISPR-Cas systems as well as provides a background for better understanding of the presented results. Chapters 2, 3, and 4 report the experimental results. Chapter 2 focuses on functional analysis of *C. difficile* CRISPR-Cas system aimed to determine PAM (protospacer adjacent motif) requirements for CRISPR interference and CRISPR adaptation in this human pathogen. Significant progress has been achieved in studying the spacer acquisition process, referred to as CRISPR adaptation, in *C. difficile*. The cells with elevated expression level of cas genes essential for adaptation were used and acquisition events were detected for two CRISPR arrays. The results revealed PAM requirements for new spacer selection and shed light on spacer choice preference. So far, only one mode of spacer acquisition referred to as naïve adaptation was analyzed. However, the experimental set up developed in this work paves a way for further research of the adaptation process in *C. difficil*e. It helps to study primed adaptation and also determine a function of ancillary adaptation protein Cas4 in *C. difficile* CRISPR-Cas system. Chapter 3 describes the study of type I Toxin-Antitoxin system, riboswitches and adjacent CRISPR-Cas loci. The results of this work shed light on the interaction and coordinated regulation of the different defense mechanisms and led to the candidate's first author publication in *Nucleic Acids Researches*. Chapter 4 describes a developing of genome editing tool for *C. difficile* using its endogenous CRISPR-Cas system. Genomic manipulations are challenging for *C. difficile*. CRISPR-based editing of *C. difficile* genome can be extremely useful for biomedical applications. As a proof-of-principle several genes in *C. difficile* were deleted using the developed genome-editing tool and the mutants' genotype and phenotype were characterized. The results have been published in *Applied and Environmental Microbiology*. Chapter 5 presents the summarized results and discussion of research perspectives. The topic of the dissertation work matches the presented results. Combination of microbiological, genetic, molecular biological approaches has been employed in this work, among them are cell cultivation, plasmid conjugation, and sporulation assay at anaerobic conditions, different methods for genomic manipulations in *C. difficile*, deep-sequencing and data analysis. The methods are described clearly and in detail. Overall, the candidate's work significantly contributed to understanding of CRISPR-Cas functions in *C. difficile* and interaction of this defense system with toxin-antitoxin systems and riboswitches during sporulation and biofilm formation. The work led to the publication of two first-authored research papers in *NAR* and *Applied and Environmental Microbiology*, and one mini review in *Frontiers in Microbiology*. Two more manuscripts are in preparation. The results were presented at several national and international meetings including the oral presentations at 6th International *C. difficile* Symposium in Slovenia and at National Congress of the French Society for Microbiology in France. Presented work meets the requirements for granting a PhD degree. I have a few minor suggestions for correction. Some of them may be addressed during the

thesis defense:

- Page 3, “in interference” should be replaced with “CRISPR interference”.
- Page 17, a reference on *C. difficile* database should be added. Is it correct that genomes of 3,000 *C. difficile* strains have been sequenced?
- “CrRNA” should be replaced with “crRNA” throughout the text.
- Page 33, Figure 1.9, it might be better to present more recent model for spacer integration indicating the first nucleophilic attack at the leader-repeat junction (Nunez et al., *Molecular Cell*, 2016). This model explains a polarity of spacer integration at the leader proximal end.
- Pages 10, 43, use “complementary” instead of “complimentary”.
- Page 40, replace “bought to the limelight” with “brought to the limelight”.
- Consider indicating PAM consensus as YCN rather than TCN/CCN.
- Figures 2.13 and 2.14, please correct a description of a full cas operon mutant as “full cas operon deleted” instead of “full cas operon”.
- Page 66, replace “catalytic centers of the Cas2 protein are not required…” with a more precise “nuclease activity of the Cas2 protein is not required…”
- Page 50 and Figures 2.5, 2.6, it was suggested that the efficiency of CRISPR interference provided by various arrays depends on the crRNA expression level. However, only RNA-seq profiles were presented. The candidate should be ready to discuss the correlation between interference efficiency and expression level in more quantitative terms.

Provisional Recommendation

V *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*