

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

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Date of Thesis Defense: 30 September 2019

Name of the Reviewer: Prof. Claire Janoir (Université Paris-Sud)

<p>I confirm the absence of any conflict of interest</p> <p>(Alternatively, Reviewer can formulate a possible conflict)</p>	<p>Signature:</p>  <p>Date: August, 28, 2019</p>
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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

Review of Doctoral Thesis submitted by Ms Anna MAIKOVA entitled, "The CRISPR-Cas system of human pathogen *Clostridium difficile*: function and regulation"

Ms Anna Maikova presents a very interesting thesis with important breakthroughs on mechanistic studies of CRISPR-Cas systems in *Clostridium difficile* and their potential applications to inhibit this bacterium. This subject is perfectly on line with the research topics of her supervisors' labs. Given that Mme Maikova has presented her thesis in English, which I believe is not her first language, the overall standard of presentation is very high.

The manuscript is quite classically organized in five chapters.

The first chapter corresponds to a review of the literature, focusing on *C. difficile* and on CRISPR-Cas systems. To my point of view, it is the least completed part of the manuscript. Although classification and mechanism of CRISPR-Cas systems for bacterial immunity and immunization are very clearly depicted, with helpful figures and the review, as far as I can see, is up-to date, however impact of these systems in bacterial pathophysiology remains a little vague. The section on the pathogen she worked on during her PhD is very short and lacks detailed data on virulence factors and what is currently known on their regulation. To fully introduced her experimental work, Ms Maikova could have given information, as examples, on regulation of known or putative virulence factors by the second messenger c-di-GMP and on the suggested pleiotropic role of the RNA-chaperone Hfq protein in *C. difficile*. In addition, importance of *C. difficile* adaptation during the infectious cycle should have been emphasized and illustrated by some examples.

The following three parts correspond to her experimental work. Regrettably, the objectives, quickly mentioned in the abstract at the beginning of the manuscript, are not specified, but this can be easily corrected.

The first experimental work (described in the Chapter 2) corresponds to basic studies on the functionality of CRISPR-Cas systems in *C. difficile*, which are yet unpublished. Ms Maikova confirmed experimentally the sequence of efficient protospacer adjacent motifs (PAM), previously suggested by in silico analysis and proved the functionality of mostly of the CRISPR-Cas systems in two well-studied strains of *C. difficile*, at least for interference processus. In addition, deletion of the full cas-operon of the reference strain 630 showed a moderate impact on plasmid interference assay. Some methodologies issues will be discussed during thesis defense notably on the construction of plasmids to study immunization functionality of CRISPR-Cas systems in *C. difficile*.

The second experimental work (described in the Chapter 3) has been partially published in 2018 in the high-rank journal Nucleic Acids Research (IF = 11.147) and Ms Maikova signed as a co-first author. She investigated the functionality of type I toxin/anti-toxin systems and their association with CRISPR-Cas systems in *C. difficile*, with a fonctionnal analysis of two of these combinations. She suggested that their both increased expression in some stress conditions (i.e. biofilm conditions) could be related to sigma B factor-dependant co-regulation. Ms Maikova suggests this functional coupling between TA and CRISPR-Cas systems may be involved in the stabilization of *C. difficile* chromosomal regions carrying CRISPR-Cas systems and/or in induction of dormancy. In addition, interesting yet unpublished data strongly suggests a modulation of CRISPR-Cas functionality by the TA systems via c-di-GMP regulation.

The fourth chapter is dedicated to the development of a novel tool for genome editing in *C. difficile*, harnessing the endogenous type I-B CRISPR-Cas system of *C. difficile*. Ms Maikova applied this tool to the deletion of the Hfq protein for which previously existing deletion or inactivation tools were not successful. Until about 12 years ago, *C. difficile* could not be genetically engineered. Nowadays, some tools are available but they all have drawbacks and limits. The new deletion tool devised during this thesis is quicker and has the potentially to be used in all strains of *C. difficile* as far as they display

endogenous CRIPR-Cas systems. It is obviously a additionnal step towards the easy genetic manipulation of *C. difficile* and consequently the increase of our knowledge on the pathophysiology of this important human pathogen. This work (with Mme Maikova as the first author) has been recently accepted for publication in Applied Environmental Microbiology (IF = 4.077).

The last chapter corresponds to an short summary of her main findings and pave the way for future experiments to complete study of CRISPR-Cas systems in *C. difficile*.

During the viva examination, in addition to few methodologie questions, some overall issues will be addressed, e.g. the contribution of her results to decipher the pathophysiology of *C. difficile* which is allusive in the manuscript ; in particular, Ms Maikova suggested a link between CRISPR-Cas regulation and biofilm formation but the role of biofilm in *C. difficile* infection needs to be more clearly defined.

To conclude, her studies span complex basic biology, traditional and molecular microbiology, and bioinformatic in silico analyzes. Her results were fully in line with her research topic, thanks to use of relevant (although sometimes tricky) methods used during her PhD.

Given the difficulty to work with *C. difficile*, I consider this thesis to be excellent and to fully comply to international standard. Anna Maikova's work led already to two high-standard publications; the strategy to published the results described in the second chapter will be clarified during the thesis defense. In my view, Ms Maikova successfully satisfies two basic requirements before thesis defense. She has been is able to conduct work that stands up to an independent peer review process, and she has acquired high-level competences in microbiology and molecular biology rendering her capable of acting as an independent researcher.

Therefore, I strongly recommend that the candidate should defend the thesis.

Yours sincerely,

Claire Janoir

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