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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Viktor Mamontov

PhD Program: Life Sciences

Title of Thesis: Escape mechanisms of mobile genetic elements against CRISPR-Cas system and diversity in microbial communities

Supervisor: Professor Konstantin Severinov

Name of the Reviewer:

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Edze Westra** **Date: 14-12-2023**

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 is an interesting thesis that consists of 4 chapters ; an introduction, first author experimental chapter about CRISPR-plasmid co-existence, a chapter about benchmarking of DNA Isolation Methods for Marine Metagenomics Studies, and finally a chapter that combines in silico, in vitro and invivo analysis of Bacteriocin-Producing Escherichia coli Q5 and C41 and examines potential probiotic properties of these strains.

The work is of high quality, and all work is published or in review. I particularly enjoyed the Mamontov et al PNAS study (close to my area of expertise), which shows that bacterial CRISPR-Cas immune systems can co-exist in the same cells with the plasmids they target. The CRISPR-Cas immune system is imperfect, and modelling and data suggest that once a plasmid reaches sufficiently high copy number, the CRISPR immune system is not able to catch up and remove the plasmid from the infected cell. The authors use a combination of plating experiments, single cell microscopy, flow cytometry and modelling to test their hypotheses. This work is of very high quality, and challenges the view that CRISPR-Cas immune systems are perfect barriers for plasmid uptake. Instead, the data and the model suggest that CRISPR immune systems can enable formation of stable equilibria, where a stable proportion of a CRISPR immune population carry the target plasmid. The authors suggest that this imperfection of CRISPR immunity may even be adaptive - it increases genetic variation in the population on which selection can act if the environment fluctuates (e.g. if antibiotics are added).

The main point I would like to discuss during the defence is whether the data demonstrate the existence of stable equilibria. Are the experiments in the paper of sufficient long duration to conclude this? Is it appropriate to include pulses of positive selection (plating cells on media that contain antibiotics at each transfer)? Has the candidate considered comparing the decline in plasmid-bearing cells in CRISPR immune and non-immune populations over time, and if so, can they estimate whether or not rates of plasmid loss are higher in cells with CRISPR immunity? Alternatively, did they use their microfluidics setup to track cells over longer timescales to measure rates of plasmid loss from CRISPR immune and non-immune cells ? I envisage the discussion to revolve around the issue whether the experiments adequately test the model prediction that plasmid-free and plasmid-containing cells stably coexist or whether it is possible that the detection of plasmid-containing cells is part of a transient dynamic.

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*