PHD SCHOOL OF SCIENCE
UNIVERSITY OF COPENHAGEN

Final assessment of the PhD thesis

1.General information

Name: Zhoufeng Yu

Danish CPR number: tdn691 Department: Biology

2. Supervisor(s)

Principal Supervisor
Title & Name: Søren Johannes Sørensen E-mail: sjs@bio.ku.dk

Co-supervisor
Title & Name: E-mail:

Place of employment:

Co-supervisor
Title & Name: E-mail:

Place of employment:

3. PhD thesis

Title of PhD thesis: The wastewater plasmidome and its derived resistome: insight into their dynamics in the urban water system

Date of defense: June 23 2022 Can the PhD degree be awarded? [x] **Yes** [ ] **No**

4. Assessment of the PhD thesis

Short overview of the structure of the thesis

The candidate indicates that the thesis is principally divided into two chapters with an introductory chapter showing the detailed background, aims and hypothesis of the research targets and a manuscript chapter describing the main subjects, results, and opinions. We prefer to assess that the thesis is structured in four parts the first one proposing a complete bibliography review on urban water systems, antibiotic resistance, mobile genetic elements, plasmidome and mobilome. We can also find in this 1st part a chapter on the aims and objectives of the work, another one on the results/discussion and the two last ones on conclusions and perspectives. The second part includes the three scientific manuscripts, the third part a list of the presentations in conferences and the last one is an appendix.

Objectives of the thesis' research

Considering that wastewater treatment plants (WWTPs) are unique reservoirs of antibiotic resistance genes (ARGs) and could be hotspots for the transfer of these genes between human and environmental bacteria the overall aim of the PhD work was to investigate the pool of plasmids in the urban water systems (UWSs). A specific focus on the associated antibiotic resistance encoded by genes localized on mobile genetic elements for a better understanding of whether these mobile ARGs can be transferred to opportunistic pathogens. This required to understand the interplay of ARGs and mobile genetic elements (MGEs) dynamics across the treatment compartments, justifying the extensive work on ARG associated MGEs, particularly plasmids.

Summary and quality of main findings and scientific achievements

In a first part of his thesis, the candidate presents an adequate and complete bibliography on his research field. It starts with the description of the urban water systems that is fundamental to understand why these environments are important to understand the spread of ARGs. The reader appreciates the paragraph entitled “Why urban water systems“, showing that the co-occurrence of antibiotics, biocides, metals, and microbes accelerates the horizontal gene transfer. The paragraph and subparagraphs “Antibiotic resistance and ARGs” are also important to clarify the definitions of antibiotic resistance on clinical and epidemiological points of view and to ascertain the true ARGs genes based on the mechanisms leading bacteria to resist these specific biocides in relation to their mode of actions. In the paragraph “Antibiotic resistance in the wastewater environment” the author assesses that “the current wastewater antibiotic resistance risk assessment and management are defective, and a comprehensive understanding of these contents is still missing”. This is partly true, but should be temperate by indicating that many studies all over the world investigates this problematic. The paragraph and subparagraphs on Mobile Genetic Elements are almost exhaustive and should be very useful for new students starting their research on the mobility of genes described in the paragraph and subparagraphs “canonical HGT mechanisms”. The fourth paragraph of this bibliography synthesis is fundamental because it deals with the plasmidome that is the key point of the thesis. The candidate must be congratulated for the exhaustivity of the analysis that describes the different wet and dry approaches and tools to specifically isolate and investigate the plasmid sequences from microbiota. In conclusion, this bibliography synthesis is an excellent introduction to the thesis and its objectives, which are clearly exposed as indicated above.

Transfer and retransfer potential of ESBL plasmids (Manuscript 1). By using innovative techniques, the author isolated a large IncN plasmid (pDK_DARWIN) that harboured and expressed multiple ARGs and a complete set of functional conjugative genes. The potential host range of this plasmid was revealed after it was tagged by gfp and tested for permissiveness for the wastewater bacterial communities. Important results were obtained including the demonstration that this plasmid could be efficiently transferred into native urban water community microbiomes. In addition, the analysis of several wastewater metagenomes from three different European countries led to the detection of this mainly in hospital sewers that demonstrates its prevalence in Europe. In addition, this plasmid exhibits the highest relative abundance in the hospital sewers in comparison to other compartments confirming the potential role of such a plasmid for transferring ARGs at a large geographical scale and at a high frequency. These results are excellent and should reach a large audience among the scientific community.

Insights into the circular: the cryptic plasmidome and its derived antibiotic resistome in the urban water systems (Manuscript 2)
The plasmidome was sampled at different stages of UWSs in three different countries (Spain, UK and Denmark). Using wet lab methods developed during this PhD project and analysed using state-of-the-art bioinformatical tools, the circular plasmidome from UWSs was analysed. More than 9500 novel putative circular plasmids are identified of which only 66 are identified as conjugative. ARGs of the plasmidome are also analyzed and described according to the group of encoding plasmid. Albeit the level of ARG-encoding plasmids decreased along the UWS flow, they would still persist in the effluent of wastewater treatment plants. The manuscript is missing some materials and methods to allow reproducibility, but is of overall excellent quality.

Plasmidome derived antibiotic resistome reveals the partitioning of different geographic regions and treatment compartments in the urban water systems (Manuscript 3)
The plasmidome was sampled at different stages of UWSs in the three different countries Spain, Denmark, and the United Kingdom, using the same samples as in Manuscript 2. However, in this manuscript, there is a clear focus on the resistome and linear putative plasmids are included. Resistance genes against the clinically important types of antibiotics, aminoglycoside, tetracycline, macrolide and phenicol drug, constituted more than half of the identified resistance genes. The Spanish samples contained a statistically higher amount of ARGs than the Danish & English samples, which likely relates to the higher usage of antibiotics in Spain. The wastewater treatment plants in Spain and Denmark where much more efficient in reducing the amount of ARGs than the UK WWTP plant, which is likely related to the UK plant using a different biological treatment proces. The manuscript is missing some materials and methods to allow reproducibility, but is otherwise of excellent quality.

Quality of all papers (unsubmitted, submitted as well as published)

Three papers with Zhuofeng Yu as 1st author are almost ready to be submitted. These papers are excellent and should be accepted for publication in specialized journals.

Other relevant factors

Excellent and efficient collaboration with the international partners of the DARWIN project.

Assessment of oral defence

Zhoufeng did a great presentation of his thesis work with excellent visualization. The candidate was able to entertain a stimulating discussion of the science and perspectives of his work.

Overall conclusion

The candidate performed an excellent wet and dry work that contributes to a better understanding of the spread of ARGs. Hence, the PhD thesis is therefore considered as fully sufficient and clearly represent the level of quality required for obtaining the PhD degree at the Faculty of Science, University of Copenhagen.

5. Members of the assessment committee

Member 1 (SCIENCE, chairman)

Title and Name:	Professor Anders Priemé
Department	Biology
Date: June 23 2022	Signature: 

Member 2 (External)

Title and Name:	Pascal Simonet
Place of employment:	Ecole Centrale de Lyon, Lyon, France
Date: June 23, 2022	Signature: 

Member 3 (External)

Title and Name:	Gisle Vestergaard
Place of employment:	Christian Hansen A/S, Hørsholm, Denmark
Date: June 23, 2022	Signature: