Project PN-III-P4-PCE-2021-1797

PCE 96/2022

Transcriptomic landscape of host-pathogen-antibiotic interactions in Drosophila melanogaster model infected with Gram-negative ESKAPE bacteria

Scientific and technical report - stage I

Planned objectives and activities

Ob1. Evaluation of the phenotypic effect of ESKAPE Gram-negative bacterial cells as well as of colistin and carbapenem antibiotics on *D. melanogaster* wild-type lines and simulation of different infection scenarios with different bacterial loads
1.1 Inoculation of *D. melanogaster* lines with colistin and carbapenems;
1.2 Infection of *D. melanogaster* lines with bacterial suspensions of different densities prepared from different ESKAPE strains;
1.3 Optimization of experimental infection parameters for each ESKAPE pathogen;
1.4 Identification of physiological responses of inoculated/infected *D. melanogaster* lines;
1.5 Evaluation of the composition and diversity of *D. melanogaster* microbiota using 16S rRNA sequencing;
1.6 Analysis and interpretation of data.

Summary of stage 1

During the first stage of the project, all the proposed activities for the fulfillment of the objective of the stage were fully accomplished. Thus, the establishment of the parameters of antibiotic inoculations and experimental infections for each ESKAPE pathogen, the optimization of the *D. melanogaster* lines that will be used in the next stages, the identification of the physiological responses of the considered *D. melanogaster* individuals, as well as the culture-independent analysis of the microbiota of the inoculated/infected *D. melanogaster* lines. The workflow optimized during this stage that will be used in the following stages of the project is summarized in Figure 1.

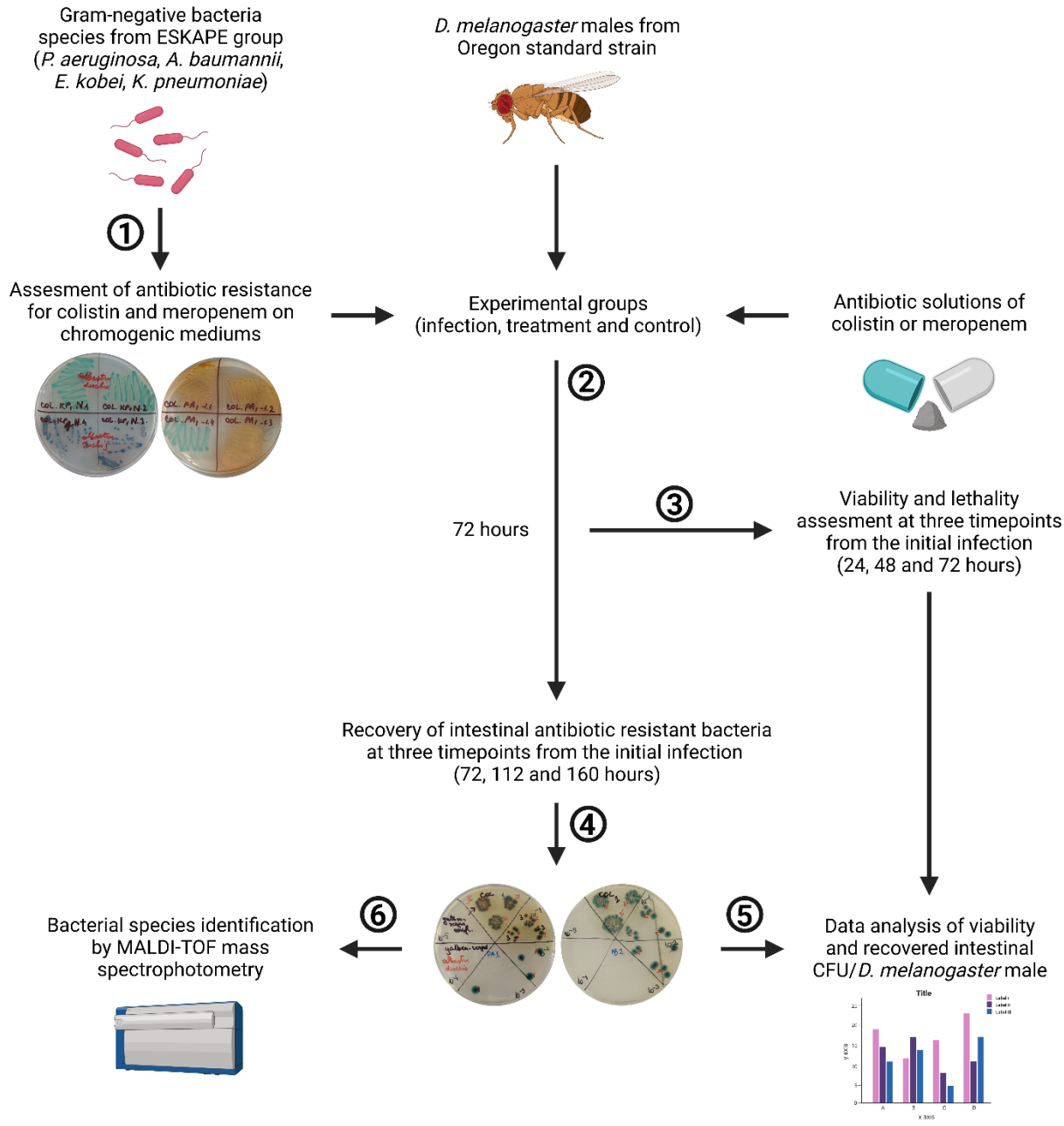

Fig. 1 Graphical representation of the optimized workflow of the project

For antibiotic inoculations and experimental infections, adult males from the Oregon wild-type (standard) line will be used. In particular cases, to confirm certain working hypotheses (e.g., *D. melanogaster* lines harboring mutations in the genes involved in the immune response will respond differently to infection/antibiotic treatment compared to normal, wild type line), Oregon females and other lines optimized in the first stage of the project can also be used. For the experimental

infections, four multi-drug resistant strains of Gram-negative bacilli from the ESKAPE group, previously characterized at genetic level showing complex genes profiles of resistance, virulence and mobile genetic elements will be used.

The density of the bacterial inoculum used for the experimental infection by ingestion will be 4.5 × 10^9 CFU (colony forming units), and the duration of the ingestion interval 72 hours. Physiological parameters such as viability, motility and negative geotropism will be monitored at 24, 48 and 72 hours. Depending on the minimum inhibitory concentration (MIC) values for colistin and meropenem against the selected ESKAPE strains, the antibiotic concentration to be administered by ingestion was standardized, namely 256 μg/mL for meropenem and 32 μg/mL for colistin. The antibiotic administration and the experimental infection with the ESKAPE bacterial strains did not induce significant lethality and behavioral changes in *D. melanogaster* males. These results will allow modulation of the infection period so that we can optimally capture the interactions among the infecting strains, gut microbiota and the host organism.

To evaluate the diversity of *D. melanogaster* microbiota by 16S rRNA gene sequencing, the optimization of the protocols for *D. melanogaster* DNA isolation and purification, obtaining and sequencing of amplicon libraries using the Ion Torrent PGM system and the Ion Sequencing 400 kit (Life Technologies) were carried out. At the same time, optimizations were also implemented for the analysis of the obtained data, to determine the composition and diversity of the microbiota and its dependence on the genetic background, sex and antibiotic treatment (colistin, meropenem).

The preliminary results reveal high levels of Enterobacteriaceae and Pseudomonadaceae in all analyzed lines, which show intrinsic resistance to the antibiotics selected for the study, but at the same time, could acquire resistance genes from the ESKAPE strains used for experimental infections, by horizontal transfer. The abundance of bacteria from the families Enterobacteriaceae and Pseudomonadaceae has decreased following the treatment with the two antibiotics. This dynamic is favorable for our experimental approach, as it will allow us to highlight the role of the commensal microbiota in the transmission and dissemination of resistance in the absence and presence of antibiotic treatments, respectively.

Dissemination of results

The results were disseminated through participation with oral presentation in an international conference and publication of two articles in Q1 journals with impact factor>6. The project web page was created and updated with general data about the project and the results obtained in the first stage.

Project website

- https://unibuc.ro/cercetare/promovarea-rezultatelor-cercetarii/proiecte-de-cercetare/proiecte-cu-finantare-nationala/transcend/?lang=en

WOS papers

1. Chifiriuc MC, Filip R, Constantin M, Pircalabioru GG, Bleotu C, Burlibasa L, Ionica E, Corcionivoschi N andMihaescu G (2022). Common themes in antimicrobial and anticancer drug resistance. Front. Microbiol. 13:960693. doi: 10.3389/fmicb.2022.960693, **IF. 6.**
2. Ecovoiu, A.A.; Bologa, A.M.; Chifiriuc, D.I.M.; Ciuca, A.M.; Constantin, N.D.; Ghionoiu, I.C.; Ghita, I.C.; Ratiu, A.C. Genome ARTIST_v2—An Autonomous Bioinformatics Tool for Annotation of Natural Transposons in Sequenced Genomes. Int. J. Mol. Sci. 2022, 23,12686. https://doi.org/10.3390/ ijms232012686, **IF 6.2.**

International conference

1. Chifiriuc M.C. - *Drosophila melanogaster* - a Versatile Model for Human Cancer Research, 2nd OncoHub Conference, Romania, 21-23 sept. 2022 (https://www.oncohub.ro/agenda).