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Drosophila melanogaster as bioindicator of microbiome dysfunction

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The gastrointestinal (GI) microbiota is tightly linked to intestinal homeostasis and crucial for the overall host health. Several factors such as bird mode, genetics, diet, stress, and pollution have been reported to highly shape microbial communities. When the microbiota is impaired or altered, common inflammatory, metabolic disorders, as well as oncologic and neurologic diseases are more likely to be developed. In general, GI disorders in the human population have exponentially increased during the last decade and are linked to anthropogenic pollution and contamination. Therefore, there is an urgent need to develop new approaches methodologies to rapidly study the impact of emergent contaminants on the microbial GI. Because of their unique properties (e.g., time and cost-effective handling, large offspring, and genetic manipulation is fast and inexpensive) we propose the use of the fruit fly, Drosophila melanogaster, to study microbial dysbiosis. In this study, we aimed to develop and optimize an innovative protocol describing (1) the best bacterial isolation method from the host, (2) the finest bacterial DNA isolation kit, and (3) the optimal concentration of bacterial DNA needed for sequencing, as well as the impact of these factors to the microbial gamma and alpha diversity. This will be assessed by using a MinION sequencer (Oxford Nanopore Technologies). The results indicate that separating the gut from the larvae body ameliorates the bacterial DNA (~ 2.5 ng/µL) discrimination from the host DNA (~ 40 ng/µL). Using the larvae phase, instead of adult flies, makes possible the gut separation from the rest of the body. On the other hand, we have seen that a wide number of bacteria is lost when filtering the lysates with a 10 µm-mesh filter to isolate bacteria from undigested tissues. The kit that yielded more bacterial gDNA per sample was the QIAamp PowerFecal Pro DNA Kit. We finally found that in 50 larvae guts there were an average of 2 ng/µL of bacterial DNA. At least 40 ng are needed to proceed with the 16s rRNA gene amplification PCR > barcoding PCR > End-prep > and MINIon sequencing. Future experiments will conclude whether the above-mentioned conditions alter the alpha and beta diversity. Further experiments are planned to work with (i) 150 or 300 larvae guts and with (ii) adult flies to study the overall adult microbiome.

Funding:
This work was partially supported by the EU Horizon 2020 programme (965196, PlasticHeal), the Spanish Ministry of Science and Innovation (PID2020-116789, RB-C43), the Generalitat de Catalunya (2021-SGR-00731), and the ICREA-Academia programme to AH.

Keywords:
Microbiota; gastrointestinal; Drosophila; experimental models; contaminants.