Review of: "Vertical transmission of tissue microbiota in Caenorhabditis elegans"

Mojibur Khan

Potential competing interests: The author(s) declared that no potential competing interests exist.

Reviewer's details: Mojibur R. Khan, Associated professor, Molecular Biology and Microbial Biotechnology Laboratory, Division of Life Sciences, Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam 781035, India

The current study by Zheng et al. on '**Vertical transmission of tissue microbiota in *Caenorhabditis elegans***' has provided some interesting observations related to the dogma on 'sterile womb hypothesis'. Previous studies by Samuel et al. (2016), Berg et al. (2016), and Dirksen et al. (2016) indicated that gut of *C. elegans* is colonized by a core microbiota. This study by Zheng et al. (2021) showed that symbiotic microbes could be vertically transmitted to the embryo and parallelly distributed into the worm tissue during the development of *C. elegans*. It also investigated the effect of antibiotic treatment on the vertical transmission of symbionts after several generations of *C. elegans*. However, manuscript preparation is very poor and very difficult to follow. English needs to be improved. Also, I feel that some of the information are misleading such as (Page 2, lines 41-43) where the cited reference does not mention 'the N2 wild type worm harbors some bacteria (genus Exiguobacterium, Mucilaginibacter, and Virgibacillus) that…….'. Moreover, the inferences from the PCoA plot based on very low number of worms are inconclusive which was also not supported by statistical comparisons between the groups. Also the variances (PC1 and 2) are very low.

There are some concerns as below.

1. The study compared the microbiota of isolated embryos and laboratory enriched N2 worms and compared it with other studies (Samuel et al. (2016); Berg et al. (2016); and Dirksen et al. (2016)). Culturing of the microbes would have provided information on their functionalities.

2. The study stated that correlative light and electron microscopy (CLEM) and FISH procedure were used to localize the gut bacteria in embryo and worm tissue. The study lacked to provide more evidence on how these embryo's microbiota is distributed during the development and adulthood of *C. elegans.*

3. The study suggested that tissue microbiota showed differences depending on the type of antibiotic treatments. However, there was no justification on it.

4. The study observed that vitellogenin-2 of yolk in the embryo allowed these gut microbes to vertically transmit to their

embryos and their effect on the lifespans of *C. elegans*. A previous study by Sornda et al. (2019) found that an increase in expression of yp170 promotes intestinal senescence in *C. elegans*. Therefore, it would be interesting to investigate how the vertical transmission of microbiota influences the expression of yp170 in F0 and F1 generation and affect their lifespans.

5. Next, the study reported that the diversity of tissue microbiota was restored after a few generations by different antibiotic treatments. Still, again, the study failed to report how diverse antibiotic treatment altered core microbiota and its restoration after several generations. And also the source of restoration of the eliminated microbes.

6. The study examined the effect of tissue microbiota on the worm's immunity against pathogens but did not address the involved immune mechanisms. Additionally, the role of this vertically transmitted microbiota should also be investigated on immune maturation in *C. elegans*. It would be interesting to study how tissue microbiota activates the immune mechanisms and increases their survival against pathogenic infections.

7. The study failed to address the importance of vertically transmitted microbiota on several other aspects of worm's biology, including fecundity.

References

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