**Title: Nectar compounds impact bacterial and fungal growth and shift community dynamics in a nectar analog**

**Authors**: Tobias G. Mueller[1][2]\*, Jacob S. Francis[1], Rachel L. Vannette[1]

Dear Editor,

I am writing to **resubmit** our original research manuscript titled “Nectar compounds impact bacterial and fungal growth and shift community dynamics in a nectar analog” (**Previously submission** **ID: EMI-2022-0802**) for consideration for the **journal Microbial Ecology Reports**.

We believe that this paper is of general interest to your readership. We examine the hypothesis that filtering due to inhospitable nectar chemistry is a main driver of the low diversity of microbes found in floral nectar. This study is the first to broadly compare if microbes, isolated from nectar and other habitats, vary in resistance, and therefore colonizing ability, to a range of nectar compounds and if these compounds impact microbe-microbe interactions. Our results suggest that compounds that plants produce in nectar, often assumed to evolve primarily in response to plant interactions with macro-organisms including pollinators or herbivores, can also affect microbial growth and assembly on plants.

No part of this paper or the findings within are in any separate work, published or unpublished, or are currently submitted to another journal. This paper has been made available as a preprint at biorxiv (<https://doi.org/10.1101/2022.03.29.485809>). All authors have read and approved the submitted version and give consent for publication in EMI/EMIR if accepted. All local, national, and international regulations and conventions, and normal scientific ethical practices, have been respected and followed.

We thank the previous reviewer for their valuable feedback and have incorporated all suggestions and improvements. Please see below for individual responses to the reviewers’ comments and line number changes.

Thank you for your time and consideration,

Tobias G. Mueller, for the authors

**Improvements in response to reviewer feedback**

- How many isolates of each species were tested? Was intraspecies variability taken into account in any way?

Thank you for this concern. Only 1 isolate of each species was tested, and we admit this is a potential limitation, but not invalidation, of the interpretation of the results. We added a mention on lines 228-230 about the possibility of intraspecies variation and this intriguing future line of research.  
  
- Lines 154-155: normally, the floral nectars of most plant specied is nitrogen-poor. I guess that authors used a high-nitrogen nectar analog to secure microbial growth, but by doing so they are neglecting the (potential) combined effect of nitrogen limitation and other nectar hurdles.

We appreciate the reviewer’s comment. There are likely to be combined effects of nitrogen limitation, however, to be able to test solely how secondary compounds interacted with microbial growth and colonization we chose to remove nitrogen as a limiting factor and provide a hospitable environment for all microbes as the control. This allowed us to compare different microbes’ response solely to the tested compounds. We have added a mention of this deviation from “field realism” and the reason for this choice on line 22-26 of the supplemental methods.

- Lines 167-168: it seems that the 96-well plates used in the in vitro assays were triple parafilmed before incubation. Was this really neccesary? Why? Do the authors expect it to have any effect on the growth of strictly (or preferentially) aerobic species?  
The plates were triple parafilmed to decrease evaporation of wells that we found to occur occasionally when using a single parafilm wrap. The parafilm should not influence microbial growth of aerobic species (although no strict aerobes were tested) as it allows for gas diffusion. The triple parafilming mainly served to ensure any seams in parafilming were adequately covered and sealed as occasionally the overlap of a single wrap of parafilm would peel up during the duration of the plate reader assay.

- Were biological replicates included in the co-growth experiments? Besides, the authors should justify why a nectar bacterium/non-nectar bacterium pair was not included in the such experiments.

Biological replicates were not used for the co-growth experiment. The same single species isolates were used as from the other experiment in the manuscript. The isolated used as well as their source are described in table 1. We added a mention on lines 228-230 about the possibility of intraspecies variation and admit how this is a potential limitation, but not invalidation, of the interpretation of the results.

We did not include a nectar bacterium/non-nectar bacterium in the co-growth assay as preliminary experiments using the combination showed that we were not able to differentiate the colonies on the plate. A mention of this reasoning and the previous attempt has been added on line 190-192.

- Lines 191-192: the fact that 30% sucrose did not affect the growth of individual microbial species doesn't exclude the possibility that it might have some impact on species-species interactions.

This is true and a good point from the reviewer. We do not intend to suggest that our exclusion of sucrose from the cogrowth experiment suggests in any way that it might not influence growth in coculture. We have reworded this section to ensure this does not come across so.

- Lines 265-269: apart from comparing growth differences between yeasts and bacteria, it seems that the authors did not take into account the phylogenetic (un)relatedness of tested species in their data analyses.

Thank you for this suggestion. We have added a new analysis of phylogenetic signal run on both maximum growth and growth rate. We found no significant effect of phylogenetic signal via both Pagel’s lambda and Blomberg’s K. This has been added to the results (lines 177-181) and is described in the supplemental methods (lines 104-112).

Other minor comments:  
  
- Publication year for Zemenick et al. is missing in lines 77 and 82.  
Thank you, this has been corrected.

- Line 183: What do the authors mean by "exploded"? Complete destruction of PCR tubes or just that their lids opened because of the increased pressure? (Document not available)

Thank you, this has been clarified on line 190 to more accurately describe what occurred.