**\*bold = Rachel Vannette comments**

**\* red = Tobias comments**

**We could adjust/ shoot for a few journal options**

**Current microbiology**

* **Manuscript (6000 words, 50 ref, 5 figures)**
* **Short communication (3000 words, 30 ref, 5 figures)**

**BMC microbiology (unlimited length?)**

**Oecologia (35 pages double spaced total, ~4500 words, 50 ref, 5 fig) – not sure if this is a great fit**

**New phytologist – this seems like a great journal. Not sure why I feel this. (no short comm, manuscript has limit of 6500 )**

**\*seems like this reviewer thought it was not enough data; make short communication instead (alternatively think about other nectar data we could include/or I could ask Dino to run some more microbes or compounds?)**

This study assessed six compounds with one concentration level used for each compound except H2O2 with two concentration levels. The manuscript can benefit from some explanations why choosing these six compounds of antimicrobial effects. Are these six compounds of antimicrobial effects most common, most important, or most abundant (high concentrations) in nectar compared to other compounds? ~~Why did this study choose the particular concentration level for each compound given the wide range of their concentrations in nectar?~~

**\*okay, seems like we can do this**

Added line saying we chose the high end of documented concentrations. As for why we chose these 6, I added a line saying ----

The manuscript mentioned the 12 microbes commonly isolated from nectar, pollinators, and the environment (Table 1). It would be good to include how many host species these microbes have been detected to show how common they are and their frequency of isolation.

**\*sounds like some work but this seems possible to do; I have some data/papers compiled for the yeasts.**

~~The co-growth experiment did not include biological replicates for each of pairing categories (a facultative nectar yeast with a non-nectar yeast; a nectar specialist yeast with a nectar specialist bacteria; a non-nectar specialist yeast with a nectar specialist bacteria). That is, there was only one pair for each category.~~

**\*reduce the mention of these categories; simply mention that specific pairings were tested.**

I kept this but switch the order so instead of having the list of what we were testing and the microbes that filled that role in parentheses following it now lists the microbial pair tested and then in parentheses what each microbes “role” is. I think this down plays that that is what we are testing while still providing that interesting information.

This manuscript used scale value (treatment / control) as response variable in figures 1-4. It is better to use response ratio, that is, log of (treatment /control), to represent the effect size as response variable.

So response ratio is treatment/control but we could do log response ratio which is what their referring to. I’ll look into this. It shouldn’t change much is my understanding but can decrease slight bias when aggregating many response ratios(?)

When analyzing all the 12 microbes together (figures 1 and 4), it may need to consider the phylogenetic relatedness of these microbes as a random effect, because of data dependency.

**\*thoughts? Difficult to compare yeasts and bacteria so these would have to be done separately, reducing statistical power. Mention in the discussion if there are members of the same taxonomic group that respond similarly but mention that we did not have the replication necessary.**

My vote is to not do this but to just mention in the discussion that phylogenetic relatedness could impact results in the discussion. I agree with Rachel here

This study assessed both growth rate and abundance (OD) of individual microbes. Perhaps it is necessary to give the reason why including both measures especially the growth rate. Originally, I thought the growth rate measures of individual microbes would be used to inform the null expectations of the co-growth assay when two microbes growing together, but it was not the case. So some explanations of the measures and the connections between the two experiments in this study are needed.

**\*interesting idea: thoughts?**

~~In figure 5, it is unclear which treatment(s) the 'solo' came from. Some error bars are missing as well.~~

\***check**

This is fixed and the solo treatment now says control underneath. Not sure what error bars they’re referring to since there are none..? Not all boxes in the graph have whiskers but that’s not unusual

Reviewer #2: The manuscript describes an in vitro study of the effects of common nectar solutes on microbial communities. Congratulations to the authors on an interesting, relevant, and well written manuscript. This is the first substantial and systematic study of nectar microbe competition as influenced by metabolites found in nectar. The adoption of microbes ranging from low to high detection frequency in nectar is an elegant approach for investigating the role of environmental filtering. Their results confirm that metabolites influence microbe growth and competition. However, the variability in these effects is somewhat surprising, as are the limited impacts of some factors like sugar content, which have frequently been cited as reducing the growth of non-nectar specialists. The publication contributes to our understanding of nectar microbial ecology. It is therefore an excellent fit for the journal's theme, and I support its publication. Below, I offer some suggestions

and questions.

**\*nice to see; looks like most of these can be relatively easily addressed.**

~~Page 3 paragraph 3: I do not often see "in vitro" hyphenated, perhaps check this.~~

changed

~~Materials & Methods: Your base synthetic nectar is quite N rich with peptone, yeast extract and 50% 100x NEAA solution. Please explain this choice.~~

I added a line that said explained that many microbes didn’t grow in the original solution  
  
~~Page 5, paragraph 3: Instead of describing your freezer stock solution here once more, suggest keeping the description in "microbial strains" section only and mentioning the 15% sugar component of the solution there.~~

Eliminated redundancy and added sucrose to first mention  
  
~~Page 6, paragraph 1: "…however, the vials exploded during incubation due to extremely rapid fermentation" WOW! Interesting and nice detail to add.~~   
  
~~Page 7, paragraph 2: "If the OD increased and then returned to the starting value within 72 hours, we considered the well having no growth and set both parameters to zero." Is it possible that cells sedimented to the bottom of the well? Could cells have begun coagulating or clumping together, making variable readings? Why would OD increase and then decrease in this way?~~   
sedimentation, no, since they were constantly shaken.

Clusmping, yes, but it wouldn’t lead to a decrease back down to zero unless all cells move out of the reader path and cluster at the very edge.

Most often when a decrease in OD is seen however it is assumed to be due to apoptosis, autolysis, or death which breaks down cells and drops the OD.

I did look at many wells that showed a decrease under the microscope to look for extreme clumping and never saw evidence of it but I didn’t do this systematically so Im not sure if its worth adding. Perhaps something like “We examined many wells visually that showed a decrease in OD and never found evidence of clumping in the wells inspected”

Results:  
  
~~Page 8, paragraph 5: Without significance, I would not describe the effect of sucrose, LTP, and linalool as trends, although scientists differ on this. To my mind, if the models do not support a difference, there is insufficient evidence to claim one. Looking at figure 1, I also do not see strong evidence of a growth reduction trend. Also it seems odd to claim a trend for these three solutes but not ethanol, which has a p-value suggesting it is closer to significance.~~

I like this reasoning, I adjusted to get rid of “trend” phrasing and just said no significant effect

Discussion:   
  
Page 11, paragraph 3: "For microbes that do not experience growth suppression, it is possible that deltaline is a source of otherwise limiting nitrogen." It took a few reads to understand this sentence, suggest editing for clarity. Given what seems to be a rather generous dose of amino acids and N in your synthetic nectar, I wonder if microbes were very N limited in this experiment. I suspect if lower N levels were used, some of the co-growth results would have changed.

This is a very fair point. I adjusted this discussion point leaving it to say that it’s a potential nitrogen source but that our solution has much more nitrogen that nectar. (line 371)

Potentially relevant discussion point? Microbial growth can be stimulated by floral extracts, see the pathogen Colletotrichum fioriniae: <https://doi.org/10.1094/PHYTO-07-17-0263-R> and <https://doi.org/10.1094/PHYTO-01-20-0010-R>

This is very interesting from my skim through. I’ll read this and think about if/how to include/adjust.

~~The tolerance of Metschnikowia reukaufii to H2O2, and extension of this protection to Rosenbergiellla, is really interesting.~~

Fig 1: I cannot decipher which pinkish red dots correspond to which species here. Can new colors be used, or shapes added to make this clearer? I also recommend adding more space between treatments on the x-axis for this figure. Looking at Fig 2, I can see that the max ODs at 6 and above are associated with 1% EtOH, but I originally interpreted those points as belonging to deltaline.   
Yea these are good points. I’ll look into putting line breaks on the microbe names and stretching the graph part so its wider. Not sure what to do about colors – ill look into adjusting the pallet or decreasing alpha value here.

Many solutes studied here are not unique to nectar, which the authors point out, but I think is worth emphasizing further. For example, H2O2 is also present in other environmental contexts like sunlit waters. Linalool is produced by leaves and fruit. Alkaloids and other defensive chemicals and enzymes are produced throughout plant tissues and exuded into the soil. This more widespread occurrence could explain more generalized tolerance of these "filtering" compounds. 

This is an interesting point.

~~Perhaps worth mentioning that hydrogen peroxide has a short half life in solutions rich with solutes as found in your nectar. The concentration of hydrogen peroxide almost certainly rapidly declined in the first 24 hours of the experiments. Other solutes tested were probably persistent, but this one was likely no longer present by 72 hours.~~   
I put a sentence on this in the discussion (~line 322)  
  
  
~~Reviewer #4: This manuscript describes a nice study on the tolerance of different yeast and bacterial species (including nectar specialists and non-specialists), either alone or in co-growth experiments, to a set of chemical inhibitors present in floral nectar. While this is a very interesting and relevant topic, I have several major concerns about the experimental methods. In particular:~~  
  
1) The authors should clarify how many isolates per tested species were included in the experiments (and provide isolate names, e.g. in Table 1). It seems to me that they just used one isolate per species, which would neglect possible intra-species variation in tolerance to the different nectar compounds.

This expectation of testing lots of subspecies seems a little onerous to me but maybe this in common in microbiology world? I’ve added a line in the discussion that says that there may be intra-species variation to these results (~line 315)

Part of me thinks though this is too early in the discussion and limits the impacts of results? Thoughts?   
  
2) In order to minimize the impact of edge effects (see, e.g., <https://doi.org/10.1016/j.bbrep.2021.100987>) or diffusion of volatiles produced in one well to neighboring wells, the order of treatments and strains should have been randomized in each plate (or, at least, on each batch of plates inoculated on the same day).

Like yes this probably would have been ideal. But a little late now. I can add to the discussion that this could impact things when comparing between microbes, but within microbe treatment effects (the majority of the papers analysis) these edge effects should be consistent and should not impact those comparisons.

3) Master microbial cell suspensions were stored as glycerol stocks at -80 ºC. Did the length of the freezing storage have any effect on microbial viability?

**\*check lag time variation in controls with time since freezing?**

4) 96-well plates were covered with a lid and triple parafilmed before incubation in an optical reader. Besides, it is no clear if the PCR tubes used in the co-growth experiments remained closed during the incubation. Did the authors consider using in both cases (96-well plates and PCR tubes) breathable membranes to allow gas interchange during incubation? Note that some of the microbial species included in the experiments are strictly aerobic (e.g., Acinetobacter), whereas other are facultative anaerobes. Limited oxygen diffusion might result in lower growth performance of obligate aerobes vs. facultative anaerobes.

Parafilm is a permeable membrane and does allow oxygen diffusion. However the pcr tubes were closed the whole time …. hmm

5) Co-growth trials focus on yeast-yeast and yeast-bacterium interactions, but do not include any bacterium-bacterium pair. Is there any reason for that?

**\*able to distinguish on plates...**

Im not going to change anything on this unless it comes up in the next review round?

~~6) The authors state that "Rosenbergiella did not form single colonies", which seems very weird to me. Did they try using more diluted suspensions?~~  
  
Other minor comments:  
  
~~- Abstract, 2nd paragraph: it should be indicated that Metschnikowia reukaufii is a yeast species. Besides, it would be nice if the authors provided some examples of the "many non-nectar specialist microbes" they mention.~~  
  
~~- Methods, 2nd paragraph: If the experiments started from axenic (i.e., pure) cultures of tested strains, I do not see the need of supplementing YMA with chloramphenicol or TSA with cycloheximide. Besides, determining bacterial cell densities with an hemocytometer is challenging due to the small cell size. Therefore, I would recommend the authors to provide further details on the protocol they used.~~

**~~\_\_\*hmmm, well I can count them! (need photos?)~~**