Summary: This is a valuable study that highlights the freshwater ecology of two groups of phytopathogenic bacteria: the *Pseudomonas syringae* species complex (called "Psy" hereafter) and the soft rot Pectobacteriaceae species complex (called "Pectos" in our review and "SRP" in the paper). The detailed ecological analysis yields multiple lines of evidence that Psy are generally better adapted to freshwater than Pectos. First: Psy are more abundant than Pectos, there are more Psy haplotypes/clades identified than Pectos, and Psy were recovered from more locations that spanned a larger temperature range than Pectos. By taking a culture-based approach, this study builds a strain collection that can bolster future studies. The study took a low-resolution phylogenetic analysis of thousands of isolates, including an Illumina-based metabarcoding approach of a Psy marker gene (*cts*: citrate synthase, a central metabolism gene) that the authors previously validated. Most of the Psy isolates are from clades that can be plant pathogenic whereas the majority of the aquatic Pecto species are not known to be plant pathogens.

Radically candid critique of the study:

- The introduction is overly strong when framing the gaps in knowledge on freshwater ecology of plant pathogens. I agree that there is significantly more known about aquatic phases of human pathogens, but the introduction's current framing veers on hyperbolic.
    - For example the repetition of "paucity of information of plant pathogens in surface waters" in L33 and L36.
    - The statement in L35 of a "20-year hiatus in studies of aquatic phases of plant pathogen ecology" overlooks the strong aquatic Ralstonia papers that have been published since 2002/2003:
        - **https://journals.asm.org/doi/full/10.1128/AEM.00960-07**
        - **https://doi.org/10.1099/mic.0.2008/019448-0**
        - **https://journals.asm.org/doi/full/10.1128/AEM.71.1.140-148.2005**
        - **https://link.springer.com/article/10.1007/s10658-009-9508-1**
        - **https://journals.asm.org/doi/full/10.1128/AEM.01219-13**
        - **https://pubmed.ncbi.nlm.nih.gov/30764299/**
        -
    - **As well as some great papers by the authors:**
        - **https://www.nature.com/articles/ismej2007113**
        - https://doi.org/10.1016/j.meegid.2006.05.002
- We appreciated the rigor of the statistical analysis, e.g. the Spearman correlation analysis on Psy vs. Pecto community abundance for all samples and the independent analysis after separating the sites to the upper, middle, and lower river basins. The consistent correlation of the bacterial communities with water temperature is persuasive.
- **Figure 1** is a very nice figure that conveys complex information in an almost clear format, but it would be stronger with a few revisions. (1) label the y-axes. (2) Add a more obvious legend for the meaning of the bars' colors (seasons). This could be put at the top of the figure. (3) It is hard to read the names of the rivers (blue text) over the blue squiggles of the water ways.
- **Figure 2**. It would be useful to use different colors to differentiate the positive and negative correlations. I am fond of the color-blind friendly diverging color schemes https://colorbrewer2.org/

- Fig 3. It would be easier to understand this data if it was presented on two panels. The black circles of Psy communities obscures the open circles showing the Pecto communities.
- **We have some concern about the method for sampling the bacteria from the water.** L407-408 state that "the bucket was rinsed twice with water from the sample site before each sample was collected." On first read, we thought this sentence referred to a method for rinsing previous microbial communities from the bucket. To alleviate confusion, please mention how the bucket was sterilized between samples, especially since the first author has co-authored a review on biofilms.
- **L419-420**. Minor quibble: Incubating plates for 2 days at 18C before counting colonies seems like it would only allow the counting of fast-growing strains.
- **Minor confusion on the cts sequencing methods:** Did you purify the strain's DNA prior to the PCR amplification of the cts gene, or did you perform a colony PCR? It would be useful to know if the method requires DNA extraction or is feasible directly from bacterial cell suspension.
- **Suggestion:** There would be value in discussing the ecology of Pectos and Psy to other bacterial phytopathogens. For example the aquatic stages of Ralstonia wilt pathogens (in addition to the pre-2002 studies above, there are earlier studies from Elphinstone and colleagues).
- **Suggestion:** Because it seems like Pectos could form biofilms on plant detritus, it would be worth discussing additional unmeasured variables that could correlate with Pecto community size. (e.g. TOC, which would capture organic polymers). This may inspire someone to test additional factors and bolster knowledge on aquatic biology of these bugs.
- **Suggestion:** The discussion would be bolstered by discussing the value of epidemiological approaches that can identify ways to mitigate pathogen sources (e.g. Microbial Source Tracking). This approach is not common for plant pathogens, and I wonder if it is because plant health is managed more reactively whereas there is more funding for human health and sometimes more proactive management of human infectious agents.
- **L321-L323:** We hope that the authors investigate this question in a future study using a Microbial Source Tracking approach.

Wordsmithing:

- We think that it would be more accurate to say "Psy Communities/SRP Communities" than populations (single-species). This especially applies to the SRP Pectos which have a taxonomic breadth of the family-level. Moreover, saying "total cultural bacterial community" would communicate the methods and results more clearly than "total bacterial population".
- L59 has a sentence fragment.
- L160-161, L167, and L176-177 – Did you mean to cite Fig 3 here? It seems like Fig 2 or a supplemental might have been the correct choice in one or more of these citations.
- L282 and L289 – 16s rDNA sequencing is not metagenomics (sequencing of large genomic fragments within a community). Please replace with "16S community profiling" or "metabarcoding approaches that target 16S rDNA"
- L304 – It would be stronger to add a few words to highlight how P. peruviense was detected (MLST, WGS, gapA sequencing?)
- L356-346. The growth of Psy and Pectos in river water is cited as unpublished data from the authors. When this data is published, it would be nice if the findings are contrasted with

Ralstonia, who seem to survive in water more than divide. https://journals.asm.org/doi/full/10.1128/AEM.00960-07

- L508-509. “could not have led to the wrong identification” seems too confident/strong. Is it absolutely impossible that another bacteria could horizontally acquire the cts gene from a Psy strain? I suggest a slightly more nuanced “is highly unlikely to…”

This review is signed by Matthew Cope-Arguello and Tiffany Lowe-Power.