

Dear Editors and reviewers.

We thank you for the thoughtful comments and criticisms of our work. Here we indicate, point by point, how we have responded to your remarks. In addition, we have modified the manuscript format according to the PCI template and uploaded the revised version to bioRxiv.

Our responses to the reviewers' remarks are in **blue bold face** below each remark. Text highlighted in grey is part of the message received from the editors that did not require a response.

Thank you again for your work and the interesting debate and discussion.

For all of the authors,

Cindy Morris

=====

Dear authors,

As you can see, your manuscript has been thoroughly reviewed by three peers, two of which contributed to the one review. All reviewers like the paper and offered suggestion to improve the manuscript towards a recommendation in PCI.

The reviewers saw a need to improve the introduction including a revision and addition of relevant references. Further suggestions were given for improving the figures.

The reviewers brought up the topic of sampling method using a bucket. This is a rather -common method, I agree. However, please specify whether additional means were taken to prevent contamination of one sample by another.

The cts sequences generated in this study are provided in the supplementary material and also submitted to the NCBI. However, there is no mentioning of the accessio numbers and data availability in the main text. Please add this. (If I missed this, I apologize).

Additionally, please do not use the term 16S rDNA as this is incorrect - use instead 16S rRNA gene.

The sentence was modified in a way that this is now a moot point.

Looking forward to reading your revised version,

Mina

Reviews

Reviewed by Tiffany Lowe-Power, 23 Jan 2023 22:20

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Reviewed by António Machado, 08 Feb 2023 21:10

Reviewer – Manuscript ID 2022.09.06.506731v1.full

Congratulations to the authors for a beautiful study. The present version of the preprint could be improved for publication acceptance.

General comments

The abstract is too long and could be easily reduced without losing its message. Please revise it.

We have now reduced the abstract by a couple of sentences.

The references did not follow a chronological order, so I do not understand the logical citations in the main text of the manuscript and the introduction could be improved (see my minor comments).

The citation system we used was numbered, but in alphabetical order of the first authors. We have now changed to citation of authors by name in the text according to the PCI format recommendations.

A better organization of the Results and Discussion sections should be realized by the authors avoiding repetitions and reducing text but maintaining a narrative of the experimental design of this study (see my minor comments).

However, the preprint needs to be improved by the authors. The present version of the preprint needs to be improved for publication acceptance.

Minor comments

Abstract

As previously mentioned, the abstract is too long, please reduce it.

We have now reduced the abstract by a couple of sentences.

Introduction

The relevance of the *Pseudomonas syringae* (Psy) and the Soft Rot Pectobacteriaceae (SRP) species as plant pathogens should be explained to non-familiar Readers in the Introduction section. Although the authors explained the main virulence mechanisms present in both pathogens, it is not clear what kind of damage they realize in environmental plants or agriculture production, at least for this reviewer.

We have added information to explain that:

– they both have very large host ranges collectively. Furthermore, Psy and Pectobacteria are the most frequent causes of new disease emergences in crops among bacterial plant pathogens (Morris et al 2019).

Results

Page 5- Figure 1- Please add the scale bar in the illustrative map shown in the figure.

OK, we have added this

Page 5- Also, from what I understand the authors quantify both microorganisms through colony-forming units on the culture media plate, so the authors should state CFU instead of bacteria/L.

We think that the reader clearly understands that, whatever the technique used to quantify bacteria (as clearly described here), we are estimating the number of bacteria per liter of water.

Line 94- The 12 samples (7%) below the detection threshold belonged to the same location or time collection? Please detail information about these data.

The sites that have populations of Psy below the detection threshold are indicated on Fig 1 that is cited at the beginning of the paragraph. Particularities about these sites is revealed via the analyses that are described little by little in the subsequent results.

Lines 117-118- Please justify the reason why PO₄³⁻, NH₄⁺, NO₂⁻, and NO₃⁻ were only measured in 2017.

We did not have access to the appropriate equipment for these analyses in 2016.

Figure 2 –Figure 2 illustrated the average values of each chemical parameter and showed its correlation with total bacteria and each plant pathogen. However, nothing is told about the different sample locations. Please clarify to the Readers what was observed.

We disagree with the reviewer's observation. Firstly, Figure 2 indicates the full range of values of each environmental parameter grouped by each of the 3 basins of the water shed – not just the means. This indicates site-to-site variability and the homogeneity (or not) of the values between the three basins. In terms of the effect of the parameters at the different sample locations, this was the point of the Principal Component Analysis and the ANOVA presented in the results section. The results of these analyses reveal that there were significant correlations of population densities and physical-chemical factors for only a limited number of the physical-chemical factors, with temperature being the most influential.

Lines 91, 120, and 123-There is no need to abbreviate Tables 1, 2, and 3. However, the Table 3 abbreviation is missing the final dot.

We added the dot. Thank you.

Table 2 does not deliver any useful information to the Readers, nor a correlation with any of the plant pathogens, and it is well-known that several chemical parameters are related to each other. I recommend explaining the purpose of the data in Table 2 or to delete it.

We present this table because, indeed, physical-chemical traits can be correlated with each other. By revealing the correlations, we can better understand what might be underlying the seeming effect of any given physical-chemical trait on bacterial population density. Although this information may be obvious and well known for ecologist it is not so clear for plant pathologists and we think this information is useful. For the 21 correlations we assessed, only 6 were significant. This suggests that if we observed a significant effect of any one of most of the physical-chemical factors, the effect would most likely not be due to its correlation with another factor. Take the case of temperature. This factor is significantly correlated only with DOC and NO₂ (both are positive correlations). This suggests that the significant correlation of Psy population densities with NO₂ in the middle basin (Fig 2) might just be due to a residual effect of temperature. However, DOC and temperature are strongly opposed in their effect on Psy population density even though they have a positive correlation with each other. This suggests that the influence of each of these factors is independent

from the other factor and probably involves different mechanisms. Without understanding the correlations between physical-chemical factors, we could not discern these differences.

Page 9- The comparison with the published data of reference 3 (Moussa et al., 2022) should be realized in the Discussion section. Please amend it.

We are not sure what the reviewer means here. In the part on page 9 where we describe results for SRP, we refer to the work of Ben Moussa et al three times. These remarks are about the results they describe in that reference. Furthermore, as our work is based on the comparison of the SRP and the *P. syringae* species complex, we thought it was important to recall the analysis performed by Ben Moussa et al in the result section before describing the result for the *P. syringae* species complex.

Lines 199-200- Please rectify the term “Unlike SRP species...”.

This means “In contrast to SRP species”. We have changed to clarify

Line 204- Please specify the location of the detection of 128 haplotypes.

The original sentence was “Nearly half (128) of these haplotypes appeared endemic and were found at only one of the 21 sampling sites (Fig. 4)”. We understand how this is confusing. We have changed the wording to reflect that by endemic we mean that a haplotype is not widely distributed, being found at only 1 sampling site. There were 128 haplotypes that had this limited distribution. The sentence now reads: “Nearly half of the haplotypes were endemic in that each was found only at a single sampling site. There were 128 haplotypes with this limited, endemic distribution (Fig. 4).”

Line 212-Please avoid discussing previous studies in the Results section.

This information about the nature of the DD.1 haplotype is a result from this work. The realization of this similarity resulted from the data set of sequences from >900 strains of *P. syringae* that we established for this research. Although the remarks about the DD.1 haplotype could have been introduced in the discussion section, however, we wanted to focus the discussion on the comparison between the two species complexes. We think that introducing this information in the discussion section would have diluted the focus on the comparison between the two groups and on the generic perspectives of our findings.

In general, Page 10 is already a discussion and comparison with previous reports. I recommend the authors revise in detail the Results section or merely merge the Results and Discussion sections.

As mentioned in the response to the previous comment, these observations are the result of constructing the data set for this work.

Discussion

The Discussion section has more than six pages, being too long, and also adding that the last page of the Results section is a discussion. Due to the extension of the present study, I recommended the authors merge both Results and Discussion sections and reduce repetitions.

This reviewer requests that we reduce the Discussion section. We feel that it would be difficult to highlight the key findings if they were mixed with Results. Furthermore, the other reviewer requests us to add information to the Discussion (which we have done).

Lines 262-263- Please rectify the sentence, in particular “... as well as human pathogenic potential...”.

We have changed to: “In comparison, for *Listeria monocytogenes* that has potential to be a saprophyte as well as a human pathogen...”

Lines 297-302- Please add references to support your assumptions.

We have added additional precision and also citation of Portier et al to this section.

Line 310- Please when citing for the first time a species name in the Discussion section, it should be written with full names. Please check it in the remaining section.

This species was mentioned for the first time on L188-189 where it is clear that it corresponds to *Dickeya oryzae*.

In general, the Discussion section is well-written and the narrative is very informative. However, I still consider that the authors should merge the Results and Discussion sections to avoid some repetitions and reduce the narrative in a useful manner for the Readers.

We feel that having a separate discussion section really helps to focus attention on the marked findings of this work.

The shortcomings of the present study must also be added at the end of the Discussion section.

In our responses to the reviewer’s criticisms we have addressed the remarks about possible shortcomings.

Methods

Line 396- Please replace “Experimental Procedure” with “Methods”. It is more common terminology.

OK we modified this.

Line 397- Please put the subsection titles in a different line from the text, as done in the “Statistical analyses.” subsection, maintaining the same structure during the text.

OK we modified this.

Line 400- The abbreviation “FR” should be followed first by the full names, I guess it is France. Please add it.

We have now spelled out “France” instead of using its official, internationally recognized two-letter country code. We note that the reviewer did not ask us to spell out the abbreviation USA. Should we also spell this out?

Lines 419-420- Please briefly explained the total culturable bacteria quantification procedure, it is based on the Murray et al. (2010) study, right?

Does the reviewer mean Morris et al (2010)? We have now added more explanation to the sentence: “The concentrated suspension was dilution-plated on 10% tryptic soy agar as previously described and using the same number of replicates and volumes for quantification of *Psy* described below.”

Line 442-Please clarify if the *cts* gene (citrate synthase) is specific to *P. syringae* after the growth of putative colonies in the selective KBC medium. If you are not sure, it should be considered a limitation and written in the shortcomings of the study.

Citrate synthase is an essential enzyme that is part of the Krebs cycle, common to all aerobic organisms. The sequence of the gene can be used to identify organisms. The work from our team by Berge et al (2014) established how to use the sequence of this gene to identify and classify *P. syringae* and to distinguish it from other closely related organisms. Because of the overall diversity of the gene sequence throughout the biological world, the primers used to sequence the gene have some degree of specificity. The design of the primers has been established by our previous work as well as that of other teams. This method of characterization is well accepted. Furthermore, it helps to overcome the former biases of identifying *P. syringae* based on traits of colonies.

Line 511- Please replace “Statistical analyses.” with “Statistical analysis” eliminating the final remark/dot.

We removed the dot. However, in this section we describe the multiple types of statistical analyses that we conducted. For our methods section we would not entitle it “Method”. Indeed, the reviewer recommended us to entitle it “Methods”.

Line 514- Please properly cite R software.

The reference was listed in the list of cited literature. However, we had not inserted it correctly in the text as a citation. This has been corrected.

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.

Thank you for this summary.

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.

“Paucity” does not mean absence. It means rareness or scarceness – suggesting relative abundance. In the introduction, this description is clearly used to illustrate a relative abundance of information compared to that available for human pathogens. This is not an exaggerated (hyperbolic) statement. If you line up the number of studies on human pathogens in water vs plant pathogens – and even if expressed as a fraction of the studies on each type of organism – those for plant pathogens are rare.

Nevertheless, in light of the reviewer's additional remarks below, we have modified the text (see further below)

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:

Thank you for these references. Comments about them are listed after each reference. These references will lead us to modify the statement (as described further below).

♣ <https://journals.asm.org/doi/full/10.1128/AEM.00960-07> This is a laboratory study where *Ralstonia* was introduced into microcosms. It does not give information about natural occurrence.

♣ <https://doi.org/10.1099/mic.0.2008/019448-0> Another microcosm study in the laboratory.

♣ <https://journals.asm.org/doi/full/10.1128/AEM.71.1.140-148.2005> Isolation and quantification of *Ralstonia* from river water. This provides a useful comparison for our objectives.

♣ <https://link.springer.com/article/10.1007/s10658-009-9508-1> Isolation and quantification of *Ralstonia* from river water. This provides a useful comparison for our objectives.

♣ <https://journals.asm.org/doi/full/10.1128/AEM.01219-13> For this study the authors developed a typing method to trace the origin of *Ralstonia* in epidemics of crops. The origin was traced to a plant growing in river water and thus the movement of the bacterium with the river is suspected. But it should be noted that the habitat identified here for *Ralstonia* was a plant.

♣ <https://pubmed.ncbi.nlm.nih.gov/30764299/> Isolation and quantification of *Ralstonia* from pond water and aquatic weeds. This provides a useful comparison for our objectives.

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>

Yes indeed we published on this subject, but we did not want to consider our work in this analysis as it is “more of the same” info on *P. syringae* from our perspective.

In light of the information above, we have modified the first two paragraphs of the introduction.

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.

Thank you

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.

1) label y axes: OK we have modified this

2) We have added an additional copy of the bar labels on top of the figure as well as on the bottom. We will add a comment in the figure legend to explain the colors.

3) The names of the rivers on an integral part of the map that we used to make the figure. We can't change it here. But we will indicate the names of these main rivers in the figure legend.

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/>

The colors in the figure are used to indicate the degree of significance and not whether the correlations are positive or negative. The symbols "+" and "-" are used for this latter purpose. If we used colors to indicate positive and negative correlations, this would require the use of 4 colors: two for positive correlations (significant at 5% and significant at 10%) and likewise for the negative correlations.

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 are disappointed by this remark. We feel that this figure really illustrates the striking contrast between the two groups of bacteria. The regression lines highlight the trends and the sets of points have a very different spread from each other. In fact, all of the open circles are visible. The full circles do not cover completely any of the open circles.

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.

Indeed, this sentence is meant to indicate how the bucket was cleaned to prevent influence from previous sites. The bucket was dedicated to sampling river water, never in contact with high level of bacterial populations. It was rinsed twice with water from the sampled site before collecting the sample. In this way, water from previous sampling sites was washed away. The efficiency in the method is illustrated by the fact that for SRP populations that were irregularly found in water, there is no site-to-site correlation of presence and abundance of the bacteria. The sentences were modified to add precision to how we cleaned the bucket

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.

We state that we incubate for 2-4 days "when plate counts were recorded". We mean that the plate counts were recorded at all of these times. We start checking at 2 days and continuing noting for up to 4 days. The new sentences now reads: "Plates were incubated at ambient temperature (18 to 25°C) for 2 to 4 days. Colonies were counted regularly during the incubation period up to 4 days."

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.

As stated in the Methods section, growth from colonies of putative *P. syringae* were put into suspension in wells of microtiter plates. PCR was conducted directly on the suspensions in the wells. We did not describe any DNA extraction step because there was not one.

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).

We have now added modifications to the discussion to highlight comparison with the *Ralstonia solanacearum* species complex.

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.

In running water, biofilms would form on a fixed surface. In the case of *P. syringae*, they were part of biofilms on rocks in rivers [Morris C.E., et al. 2007. Surprising niche for the plant pathogen *Pseudomonas syringae*. *Infection, Genetics and Evolution* 7:84-92]. In such cases, fixed biofilms are probably not collected in bulk water and hence the polysaccharides of the biofilm matrix would not contribute to the TOC of the sample. If bacteria were growing on detritus, as sediment or in suspension, it is difficult to assume that they would be growing as biofilms. It could be argued that detritus would be a rich nutrient source and the bacteria could grow “normally” in the tissue matrix without the expense of producing bulky exopolysaccharides. On the other hand, it could also be speculated that growth in such a rich environment could foster production of EPS.

Rather than suggesting to readers to assess TOC as another possible indicator of the ecology of plant pathogenic bacteria in water, we have added a sentence to suggest looking directly for biofilms (see modifications for the previous critique). In our early work with *P. syringae* on epilithic biofilms, we found *Brennaria*-like strains in biofilms that had a morphology distinct from those in which *P. syringae* was found. We have not reported this finding, so there is still room for someone to make a novel discovery.

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.

Perhaps the reviewer does not mean “mitigate” pathogen sources but rather they mean “find” pathogen sources. In the case of Psy, the take home message from this and our previous work is that this group of bacteria are almost everywhere in temperate environments. There are currently far too many sources of this group of bacteria in the environment to identify a specific source. If the reviewer indeed means “mitigate” pathogen sources, if Psy also has a beneficial role in the environment via its leveraging of rainfall due to its ice nucleation activity, then mitigation might have negative consequences elsewhere. This is why in our conclusion we focused on the environmental factors that are critical to disease emergence (changes in land use, crop types, inputs, climate conditions, water traits) because they can lead to much earlier warning than does direct detection of the pathogen. Furthermore, we are clearly living with Psy and there is little disease relative to this bacterium’s ubiquity and abundance. This is a testament to the role of those other factors in maintaining a balance that mostly favors plant health – for the time being.

For SRP, Microbial Source Tracking as currently practiced would involve far more work than is necessary. Because of the much rarer occurrence of this group and the greater host specificity (or seeming need for a host plant to assure survival) than compared to *P. syringae*, scouting of wild

plants and weeds near sites where these bacteria are found in water might be the most rapid approach to finding reservoirs. This is currently under way by some of the authors.

L321-L323: We hope that the authors investigate this question in a future study using a Microbial Source Tracking approach.

Firstly, please note that the current Microbial Source Tracking is often based on 16S sequences. This is very imprecise and is not the means to find sister organisms. It can only orient towards communities with like profiles. In epidemiological studies where we have a good idea of the genetic perimeter of the pathogen, the imprecision of 16S information is not useful. Besides that, SRP in water are below the threshold of detection with culture independent microbial source tracking method (see Pédrón et al publication)

We have developed a tool that we suspect is more powerful than the current microbial source tracking tool. We will use data from this paper and elsewhere to put the different analytical approaches to a comparative test. Furthermore, we are also developing a model describing the accumulation of bacteria in run-off water as it slides across land cover into the river. Coming soon to journals near you!

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”.

We do not agree. Firstly, we defined Psy and SRP as species complexes. Hence, that is already a recognition of the diversity underlying “Psy populations” and “SRP populations”. We could talk about metapopulations, but this could give the impression that we are referring to the whole of the metapopulations for this bacterial groups.

Community is a charged word. It suggests interaction. In flowing water, where these organisms are at such low concentrations that they are essentially galaxies apart in their physical world, it is difficult to attribute the idea/supposition that there is anything other than a collection gathered onto a filter in a lab for the purposes of counting. Likewise for “total culturable” bacteria. We are simply counting numbers; we do not want to imply anything else.

L59 has a sentence fragment.

If one supposes that the verb “dominate” needs an object, then we added one to the end of the sentence: “dominate the landscape.”

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.

Yes indeed we meant to cite Fig 2. This has been corrected.

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”

We have replaced with “16S community profiling”.

L304 – It would be stronger to add a few words to highlight how *P. peruvienne* was detected (MLST, WGS, gapA sequencing?)

We added the phrase “– with the same techniques described in this work –”.

For information, *P. peruvienne* was first detected through its specific species sequence signature of the gapA barcode and this was confirmed for the two strains by a draft genome of the two isolates. Of note, both strains were isolated at two different time points and at two different sites and the draft genome confirmed their difference (Faye et al, <https://doi.org/10.1186/s40793-018-0332-0>)

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>

Thank you for the advice :)

In the *Ralstonia* paper mentioned by the reviewer, it is indicated that for “*R. solanacearum* populations, sterile river water was co-inoculated with the strain IVIA-1602.1 at about 10^6 CFU/ml”. We note that this initial concentration is too high to allow bacterial multiplication in such oligotrophic water. For SRP we do find multiplication, but multiplication stops when the population density reaches 10^6 CFU/ml (Ben Moussa et al 2022).

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...”

We are very confident about this method concerning false positives. We have modified this part to read: “Hence, the MiSeq isolate identification approach described here might have led to an under-estimation of the number of *P. syringae* colonies in water samples. However, based on our experience in validating identification of thousands of isolates over the past decades, we have not encountered a case of attribution of identity as *P. syringae* to strains that are clearly outside this species complex. Therefore we are confident that the likelihood of false positives is rare enough to not be of concern for this work.”