Peer Community In
Microbiology

Unraveling disease ecology: insights from soft rot *Pectobacteriaceae* co-infections

Clara Torres-Barceló based on peer reviews by 2 anonymous reviewers

Marie-Anne Barny, Sylvia Thieffry, Christelle Gomes de Faria, Elisa Thebault, Jacques Pedron (2024) Bacterial pathogens dynamic during multi-species infections. bioRxiv, ver. 2, peer-reviewed and recommended by Peer Community in Microbiology.
https://doi.org/10.1101/2023.12.06.570389

Submitted: 30 January 2024, Recommended: 12 April 2024

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Torres-Barceló, C. (2024) Unraveling disease ecology: insights from soft rot *Pectobacteriaceae* co-infections. *Peer Community in Microbiology*, 100082. 10.24072/pci.microbiol.100082

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Few studies deal with the understanding of disease ecology, especially in the agricultural domain. Soft rot *Pectobacteriaceae* are major plant pathogens that frequently co-infect potato tubers. Exploring their ecological relationships can provide valuable insights for effective monitoring and preventing disease. The study of Barny et al (2024) explores the dynamics of synthetic communities of soft rot *Pectobacterium* species (SRP) following *in vitro* and *in vivo* inoculations, focusing on the implications for disease development. To delve into co-infection dynamics, the authors constructed mixed populations comprising six strains, with three strains from each of two species. Through inoculations of both liquid cultures and potatoes, they observed outcomes using amplicon sequencing targeting the gapA gene, along with monitoring bacterial population sizes and symptoms on potato tubers. Results reveal intriguing patterns: competition among strains of the same species, cooperation through trophic interactions, and interference due to toxicity. Thanks to a modelling approach, they suggest that the presence of a cheater strain may be favoured when it is associated with an aggressive strain. This finding is crucial for field sampling strategies, as there is a risk that during an outbreak, only the cheater strain may be detected, potentially overlooking the problematic aggressive strain.

While the study conducted by Barny et al. (2024) provides valuable insights into strain interactions, it also highlights areas for further exploration to enhance understanding. First, the extent to which different species occupy similar niches in real agricultural scenarios remains unclear. Additionally, comparative genomics analysis on strains and investigating specific gene candidates could offer valuable mechanistic insights into strain dynamics. These areas for future research offer chances to build up our knowledge base in this field and improve how we understand the interactions between bacteria in nature. The implications of the study extend beyond plant pathogens like SRP. Similar scenarios of complex diseases involving closely related species or strains competing within the same niche are observed in human pathogens as well.

References:

Barny, M.-A., Thieffry, S., Gomes de Faria, C., Thebault, E., Pedron, J. (2024). Bacterial pathogens dynamic during multi-species infections. https://doi.org/10.1101/2023.12.06.570389

Reviews

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.12.06.570389
Version of the preprint: 2

Authors' reply, 02 April 2024

Dear recommender,

Please find attached a revised version of our manuscript (https://doi.org/10.1101/2023.12.06.570389),

The main changes are
- Supplements, which are now provided online as requested (zenodo file https://zenodo.org/doi/10.5281/zenodo.10886378)
- A minor error was found in Table 2, a revised Table 2 is provided.

Other changes made to improve the manuscript in response to reviewer comments are listed below in the response to reviewers.

We hope that this revised version is now suitable for PCI recommendation.

Response to reviewers

Dear reviewers,

First of all, we would like to thank both of you for your helpful comments on how to improve the manuscript.

The specific responses to your comments are given below in italics.

Reviewer 1

Species of the soft rot Pectobacteriaceae are an important group of plant pathogens and are known to co-infect potato tubers. The authors tried to get insight into the dynamics of coinfection by creating synthetic communities made of 6 strains (3 strains from two species each). They inoculated liquid cultures and potatoes and observed the outcome of the co-infections using amplicon sequencing (of the gapA gene), bacterial population sizes, and symptoms on potato tubers. The obtained results suggest that there is competition among strains of the same species, there is cooperation through trophic interaction, and there is toxicity interference.

Overall, the manuscript describes a clear hypothesis and a well-designed experiment to answer it and results are described well and discussed objectively. I have no major concerns about the study but I list comments that I hope can improve the manuscript. Also, there are minor English language issues throughout the manuscript that need to be fixed since they distract from the science.

L107

The authors should explain here or at the beginning of the results section the rationale for creating synthetic communities of 6 strains with 3 strains per species.

- *We added the following explanation at the beginning of the results section (lane 265-270):*

"In brief, each synthetic SRP community was a mixture of 6 strains belonging to two different species. The mixture of two species was preferred because the mixture of two different species is often observed in the field (Ge et al. 2021; Motyka-Pomagruk et al. 2021; de Werra et al. 2021; Degefu 2021; Smoktunowicz et al. 2022). The addition of three strains per species allows to disentangle the "species" effect from the "strain" effect."

Moreover, the authors need to explain how strains were chosen and what is known about the strains. Some strains could be distinguished from each other using gapA sequencing but others could not. This suggests that some strains are closely related (possibly identical) to each other while others trains in other species are more divergent.

- *Yes, some species are more divergent than others. For example, it is well known that D. solani is a highly homogeneous species. We added the following information in the Materials and Methods section (lane 118-122)*

"For each species, where possible, the bacterial strains were selected to allow strain discrimination using the gapA barcode (see below). For some species, such as D. dianthicola, D. solani and P. polaris/P. parvum, we did not find discriminating strains in our collection (Table S2). This is probably due to the fact that these latter species are quite homogeneous (Hugouvieux-cotte-pattat 2023, Pasanen et al 2020). "

Do the authors have a phylogenetic tree of the strains that could be shown as a Supplementary Figure? How these strains are related to each other could be an important factor contributing to the outcome of the entire experiment so the authors definitely need to provide more information about the chosen strains.

- *We do not have draft genomes for all the strains; therefore, it is not possible to make a robust phylogenetic tree with all the strains. The added references above show example of the species homogeneity.*

L327 That is too bad that a CFU to OD analysis was not made ahead of time in order to have a ratio closer to 50%. Also, did the authors make sure that the ration of species based on cfu corresponded to the ratio based on gapA sequence counts? It is unlikely but it is possible that there would be some amplification/sequence bias.

- *The time zero inoculum showed that in most of the cases, there is not amplification bias with the gapA barcoding and the strains are correctly equilibrated in the mix. We just noticed a bias with the mucoid strain. To make it clear we added the following sentence (lane 340-341)*

"For all the others synthetic communities tested, no obvious bias was detected and the strains are correctly equilibrated in the initial mix."

L334 In the extreme case where P. aquaticum represented 79% to start with, going to 90% would be really minimal.

- *It is important to remember that the initial population size is only about 1% of the final population size and that in many cases a single strain will be eliminated by competition. Therefore, reasoning from the initial strain ratio at time zero is not correct to predict the outcome.*

Would it be helpful to represent the data as a change in ration instead or besides a percentage. In other words: show how species changed in ratio in the different synthetic communities so you could conclude independently of the absolute percentages which species outcompeted the other species in which communities.

- *We tried as you suggested and the conclusions remained unchanged. The disadvantage of the ratio presentation is that you do not see the initial inoculum at time zero. We fell that it will be easier for the reader if we keep the data presentation unchanged.*

L373 Consider changing "coexistence between the two species twice was more frequent" to "the frequency of co-existence was twice as high in TSB medium compared to withing potato tubers:

- *Changed (lane 386-387)*

"Overall, out of the 16 tested combinations, the two species coexisted twice as much in TSB medium (12/16 SRP communities tested) compared to within potato tuber (6/16 SRP communities tested)."

L385-386 Is it known which cell wall degrading enzymes are encoded in the genome of P. aquaticum compared to the other species? In other words, is the genetic basis of this phenotype known?

- *Ben Moussa et al. (2023) conducted a genomic analysis of P. aquaticum. The analysis revealed that this species has slightly fewer PCWDEs. Additionally, they also showed that in vitro the production of pectinases is affected.*

To make it clear we changed the sentence to (lane 397-399)

"We noticed here that the P. aquaticum strains have a poor capacity to rot potato tubers and multiply within symptoms (Fig. 2). This is a previously described phenotypic trait that could be linked to a reduced production of PCWDEs (Ben Moussa et al 2023)."

L524 either "bacterial community" or "microbial community"

- *Changed to "microbial community"*

L459 instead of saying "more numerous" maybe say "had a higher percentage" since it is the percentage that is reported and not the absolute CFUs.

- *Changed (lane 471-472):*

"The strains had different outcomes when grown on potato tubers and TSB. Overall, the outcompeted strains made up a higher percentage within the potato tuber (66%) than after growth on TSB (32%)"

L468 Since in L467, you point out a single strain, I expected here to see the specific strain of P. parmentieri listed instead of just the species.

- *Changed (lane 481-482)*

"only the P. parmentieri strain CFBP8575 dominates in potato tubers when associated with P. aquaticum and, in TSB medium, the P. brasiliense strain CFBP6617 dominates when associated with P. versatile"

L493 should it be BRASI 3230 since it says CFBP3230?

- *Throughout the figures and tables BRASI 3230 is the abbreviation for "The P. brasiliense strain CFBP3230" as written in this sentence.*

Reviewer 2

This is an interesting paper containing novel information on the dynamics of soft rot Pectobacterium species (SRP) after inoculation of TSB and potato tubers with mixed populations of SRP species. The research is sound and in general the paper is well written.

Nevertheless, I have some critical comments:

The paper is focused on population studies of SRP in TSB and in potato tubers. It does not describe the dynamics during the development of blackleg. The fact that authors focus on tuber soft rot should be more clearly outlined in the introduction

· *To make it clear we added (lane 103-104) "during soft rot within potato tubers"*

The sentence is now "The aim of the present work was to experimentally assess the fate of mix infection ***during soft rot within potato tubers"***

The two different species (existing of three strains) are inoculated together in potato tubers. A limitation of the study is also that the effect of space is not taken into account. In naturally infected tubers, species often separated (different lenticel infections, different parts of the vascular system). To what extend different species occupy the same niche in practice is unknown. This should also be discussed in the paper.

· *We add the following paragraph between (lane 579-585) to discuss this.*

"As the outcome of the community is highly dependent on the environment, it is likely that each of the communities studied will evolve as the infection progresses in the field under natural conditions. In particular, each of the analysed communities is likely to evolve differently within the stem during blackleg infection in the field, as the environmental conditions within the potato tuber and within the potato stem are drastically different. Contrasting SRP fitness in stem and tuber has been reported previously (Blin et al 2021) and further work is required to analyse how our synthetic consortia will evolve in potato stem."

I am not convinced by the modelling work. For example, the interactions related to the production of antimicrobial compounds (toxicity interference) are not included. Another factor in the interactions is temperature and humidity. There will also be an interaction with (the defense mechanism) of the potato cultivar. I suggest to remove this part. The model has not been properly validated.

· *The conceptual and mechanistic modelling approach developed in this paper is not designed to catch up with reality (that is the aim of a different type of modelling approach) but to reflect on a specific question and assess the relative importance of different and general mechanisms involved in the competition between cheater and non-cheater species. This is a mechanistic and theoretical model meaning that its purpose is here to investigate the relative importance of general mechanisms involved in the competition between cheater and non-cheater species, and not to quantitatively predict epidemic dynamics of specific strains in potatoes. As indicated in the text we wish " To investigate the potential mechanisms by which P. aquaticum might persist when co-inoculated with other species and become even dominant". The addition of toxins would make the model too complex and would be a clouding of the issue. Temperature and humidity are important to validate other type of model design to assess the risk of pathogen blooming for farmers but our model is not focused on epidemiological surveillance. One of the key results of our modelling study is to show that the cheater could be favored when associated with an aggressive strain. This has important implications for sampling design in the field, as there is a risk of not detecting the problematic strain but only the cheater in an outbreak if sampling does not take this risk into account.*

I regret that P. aquaticum has been chosen as a model, as it has never been isolated from potato and seems not endemic in potato.

· *P. aquaticum was deliberately chosen to show that cheating can occur. It would have been more difficult to show this with other SRP species because all other species are able to induce soft rot on tubers to varying degrees depending on the strain tested. Cheating between other SRP strains is likely to occur, but with a more subtle balance between rotting capacity and cheating behaviour.*

Minor comments

I suggest to find an alternative for the word 'cheater'. You could speak about commensal or secondary invader…

- *The term commensal or secondary invaders are usually associated with bacteria that do not belong to the SRP species's complex. Here we are looking at the relative ratio of SRP within the symptoms, and we are not looking at non-SRP commensals. We prefer to stay we "cheater" as introducing the term commensal or secondary invader seems ambiguous. Here we refer as cheating strategy within the SRP cohort of some weakly aggressive SRP strains that could be associated with more aggressive SRP strains and benefit from it.*

L36-49. This part of the introduction is lengthy and very general. It is also unclear if you speak about plant pathogens. Reduce the text and focus it on SRP

- *The beginning of the introduction is deliberately very general. In fact, diseases that could be induced by species complexes are not limited to SRP or even to plant pathogens. Several examples are also described for human pathogens, and the mechanism we have unraveled here with our plant SRP pathogens may have some connection with the one that occurs in other species complexes that share similar characteristics (closely related species/strains competing within the same niche). Most importantly, the questions we raised at the beginning of the introduction are common to all pathogens acting in a species complex and have been instrumental in our work.*

L55. Reference is missing for the 38 species. Recently Pectobacterium jejuense was described, isolated from cucumber stem tissue.

- *We have not accurately described all SRP species, as our article is not focused on taxonomy, but mainly on those relevant to potato (with the exception of P. aquaticum, as explained above). We also noted that P. jejuense is found on cucumber and described on the basis of a single isolated strain. Other new proposed species, such as P. colocasium isolated from Taro, also occurred during the submission of our paper. Adding these references is not really informative in our context.*

L99-101. Information unclear, please rephrase

- *The sentence has been shortened to "Furthermore, mix of SRP spp. are also frequently reported on asymptomatic potato tubers".*

L120 give formula of dipotassium hydrogen phosphate

- *done*

L128 DO must be OD

- *corrected*

L295. Except for P. parmentieri in competition with P. versatile, which showed a significant higher maceration rate than in P. aquaticum in competition with P. versatile

- *We added the following sentence (lane 305-307)*

"Similarly, the severity of rot symptoms was similar for all co-inoculations, except for P. parmentieri in competition with P. versatile, which showed a significantly higher maceration rate than P. aquaticum in competition with P. versatile."

Table 2 requires more explanation in the title or legend.

- *We changed "nb of tested combinations" for nb of tested synthetic communities" within Table 2. We hope it makes the table clearer than before*

L516 include a reference

- *Added reference: McGowan et al, 1997*

L609 But did you find a relatively high density of P. aquaticum in communities with this bacterium?

- *The proportion of P. aquaticum strains increase more than the other strains in 5 consortia out of 9 tested.*

Some typo’s
L84 stem not steam
L92 aggressiveness not aggressivity
L384 aquaticum fully in italics
L451 graph not grap

- *All Typo’s corrected*

Decision by Clara Torres-Barceló, posted 19 March 2024, validated 21 March 2024

Dear authors,

We thank you for your consideration on submitting your manuscript to PCI Microbiology. The reviewers find the work robust and interesting but they suggest ways to improve it before recommendation in PCI. You will find the two revisions attached. Please provide a detailed response to their comments. We look forward to the new version of your work.

Best wishes,

Clara Torres-Barceló

Reviewed by anonymous reviewer 2, 14 March 2024

Download the review

Reviewed by anonymous reviewer 1, 04 March 2024

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