Peer Community In Microbiology

Burkholderia strains go it alone

Romain Barnard *based on peer reviews by* ***Vittorio Venturi*** *and 1 anonymous reviewer*

Adrian Wallner, Agnieszka Klonowska, Ludivine Guigard, Isabelle Rimbault, Eddy LM Ngonkeu, Phuong V Nguyen, Gilles Bena, Lionel Moulin (2023) Comparative genomics and transcriptomic response to root exudates of six rice root-associated Burkholderia sensu lato species. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Microbiology. https://doi.org/10.1101/2022.10.04.510755

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The *Burkholderia* sensu lato group is predominant in the rhizosphere of rice. It includes both plant growth promoting rhizobacteria (typically members of the *Paraburkholderia* genus) and phytopathogens (typically members of the *Burkholderia* genus). Better understanding the interaction between *Burkholderia* sensu lato and their host plant is therefore crucial to advance our knowledge of the ecology of rice, a plant that feeds more than half of the humans on the planet.

The perception of root exudates from their host is key for rhizobacteria. Is the response to root exudates more related to the phylogeny of the bacteria, i.e. genus-dependent, or is it strain-specific? This question is not trivial for the *Burkholderia* sensu lato group, which has experienced shifting outlines over the last twenty years. During the early stages of rice root colonization, Wallner *et al.* [1] investigated the transcriptomic regulation of three strains of each *Burkholderia* and *Paraburkholderia* genera, in addition to a genomic comparison, in order to better understand their early colonization strategies.

While these six strains possess a large proportion of gene homologues, their experiment shows their response to root exudates to be strain-specific. In the study, rice root exudates affected several metabolic pathways of interest in most strains, noticeably including i) the Entner-Doudoroff pathway, which had never been reported to be activated in relation to root colonization and ii) the putrescine pathway, which may reflect signaling controlling root colonization.

The work by Wallner *et al.* provides new insights on the strain-level response of the transcriptomic regulation of *Burkholderia* sensu lato in response to root exudates in the early stages of root colonization. Beyond this, the next steps will hopefully shed light on what happens in more complex environments, within a complex bacterial community and during later colonization stages.

References:

Wallner A, Klonowska A, Guigard L, King E, Rimbault I, Ngonkeu E, Nguyen P, Béna G, Moulin L (2022) Comparative genomics and transcriptomic response to root exudates of six rice root-associated *Burkholderia* sensu lato species. BioRxiv, 2022.10.04.510755, version 2 peer-reviewed and recommended by PCI Microbiol. https://doi.org/10.1101/2022.10.04.510755

Reviews

Evaluation round #2

Reviewed by Vittorio Venturi, 26 February 2023

The authors have incorporated some of the more general comments provided by one reviewer and most of the comments of the other reveiwer. The manuscript is now clearer and more complete.

This is a major comparative study (genomics and transcriptomics) of a set of Burkholderia and Paraburkholderia strains; members of these genera are important players in the rhizopshere however thier clinical associations limits thier poteintial use in agriculutre.

This study is an important contribution in relation to plant colonization and plant gene expression opening many doors to future studies in relation to plant-bacteria signaling.

Reviewed by anonymous reviewer 1, 25 January 2023

The authors addressed all comments and did change most of the manuscript accordingly or otherwise explained why they disagree. From my side I do not see any further need of revision.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2022.10.04.510755
Version of the preprint: 2

Authors' reply, 20 December 2022

We thank the reviewers for their helpful insights and modified the manuscript with respect to their remarks as detailed below. The final formatted version was uploaded on BioRxiv with doi: https://doi.org/10.1101/2022.10.04.510755

One reviewer suggested the fusion of result and discussion sections. This had been attempted in a former version of the manuscript but was not deemed to improve the overall readability. If the recommender does not have any objections, we would prefer to keep the results and discussion sections separate.

Comments from reviewer 1

1. As root exudates are likely to vary considerably throughout the growth phase of rice, in the future of similar studies, a metabolomic analysis of the root exudate will add important data to this study. Ultimately RNAseq data can be associated with metabolomic profiles.

This would certainly be of interest to further refine our analysis. In the present study, we tried to focus on the comparative analysis of the transcriptomic responses induced in the 6 bacterial strains of interest to mitigate the lacking identification of exudate composition. We also relied on the description of rice exudate composition at similar growth stages and similar closed-environment and sterile growth conditions as well as identic rice cultivar available in the study by Suzuki et al (2010) cited in the manuscript.

2. This study could have benefited from some genetic validation studies especially in relation to the common loci regulated in all or most strains.

For genetic validation, we took advantage of one of our recent studies (Wallner et al., 2022) which analyzed gene essentiality for rice interaction by Tn-seq in one Burkholderia and one Paraburkholderia strain which are also included in the present study. Several conserved loci presented here have been identified in the past Tn-seq study as positively involved in plant colonization. This supports the present observation and we make mention of it in the manuscript at the appropriate places.

3. The putrescine hypothesis is potentially interesting; again verification of the presence of putrescine in the root exudate will be of vital importance.

We are confident that putrescine is present in the recovered root exudates as it has been detected before by Suzuki et al (2010) using GC-MS in every root exudate sample, produced in very similar conditions to those of the present study and with the same rice variety (Nipponbare).

4. As a general point, it is always advisable to remind the reader that the experimental set-up used here is an oversimplification of the conditions which occur in the wild. The strains used here, in the wild they will be members of highly complex rhizosphere microbial communities and are present at much lower concentrations. Hence the expression profiles in the wild are likely to vary considerably from the results presented here.

The following reminder was added to the discussion, line 300 : “As the reported observations were made in a gnotobiotic setting, they are unlikely to reflect the full spectrum of bacterial adaptations in a complex natural environment. Still, species of Burkholderia s.l. were repeatedly described to be dominant in the rice rhizosphere which suggests that they can exist in communities where they exert the most influence beside their host (Ikeda et al., 2014; Yu et al., 2018).” The citation list was updated accordingly.

Comments from reviewer 2

1. Definition of Rhizosphere is broadly known and there is no need to explain it in an Abstract.

The definition of rhizosphere (L19-21) was removed from the abstract.

2. L25: it is not true that they are mostly studied for human opportunistic traits. Change to often. The Abstract in general should be more concise, focusing more on the why and what was done in the study without being lost in basic rhizosphere definitions.

Suggested changes were incorporated.

3. L43-46 To this list references should be added

The statement of L43-46 is further deepened in the subsequent sentences L46-64 and references are provided at this moment.

4. L88ff: please try to use the genus name and be more precise – only stating bacteria is not enough for making clear what bacterial strains/genus you isolated and compared

Genera names were added.

5. L103 ff Please explain how many strains you isolated and how many of these were new. Please also explain if ABIP444 is a new strain in this study

Table 1, which is referred to L102, was updated to clearly state which strains are new to this study and provide references to the original studies for every other strain used. As stated L108-110, ABIP444 has been the subject of a past publication. Here, we only present it under its updated taxonomy, Burkholderia orbicola.

6. L122 “and” is missing after (Table 1); please also add how you validated their colonization in a few words

The following description of colonization-efficiency assessment was added L121: “Plantlets growing in a sterile hydroponic environment were inoculated with each strain individually and the attached strains were

enumerated by plate counting after 3, 7 and 14 days."

Results

7. 102ff: to increase readability of this first section I would recommend to refer to a table with the coverage information and only mention the critical values (e.g. ABIP659)

We agree that this first section is a rough start, but we feel that replacing some of the mentioned information by a table would not substantially improve its readability. We would still have to comment on ABIP444 and ABIP659 as well as ABIP441 which has no hit to any validated species so far. Still, we attempted to simplify the section by removing some redundant formulations without altering the message.

8. Table 1: Please also add Chromosomes and Plasmids

Abbreviation were removed on the table.

9. Figure1: I would recommend to not add the accession number in the figure's label but rather main factors for the tree construction

Accession numbers were removed and the following sentences explaining tree construction were added in Figure 1 legend: "Distances between whole genomes was computed using Mash (Ondov et al., 2016) and plotted using the rapid Neighbor-Joining method (Simonsen et al., 2008). The displayed distances are correlated to average nucleotide identity (ANI) such as D ≈ 1-ANI."

10. L150: please mention for the transcriptome how many replicates were sequenced

The section was amended as suggested L153-154.

11. L153: rich minimal medium: please call it the same way in result and method section

Rich minimal medium was changed to VSG medium.

12. L170: please add how many fold change down/upregulation you received

Fold change values are available for every mentioned gene in supplementary table 2 and on figure 7 for those involved in the putrescine and Entner-Doudoroff pathways. We feel that adding the counts to the manuscript would not improve readability (especially for the DE genes conserved across all six strains).

Discussion

13. L303 "one would have" is very colloquial

Sentence was modified to: "To avoid such biases induced by temporal shifts, a transcriptomic kinetic analysis would have been required."

14. L367 The conclusion of why analysing 6 instead of 2 strains doesn't make much sense here – did you plan to do only two or do you mean that other studies rely only on 2 strains?

We understand that L367 could be easily misinterpreted in its past form (it is 6 strains instead of 2 genera that are analyzed) and it was thus clarified as such: "Thus, instead of analyzing one Burkholderia and one Paraburkholderia group composed of three species each, we rather have six distinct strains that independently underline the importance of certain pathways in plant-bacteria interactions."

Materials and Methods

15. Please also reference and describe the Loess method? Please explain what dpi is?

Additional information for the non-parametric regression model LOESS was added to its site of mention in the legend of figure 2 and the original reference of the method was added. The meaning of dpi (days post inoculation) has been added at its first occurrence L.125

16. L377 Please describe how you collected the hydroponic media

The method section was amended with additional details on the medium collection procedure.

17. L384ff: please include if the strains were deposited to a strain collection

The strains were not deposited in any collection which is why we omitted the mention of it.

18. L404: Where the roots washed before the pulverisation? If not, I would suggest mentioning in the discussion that you can't divide between colonizers of the outside or inside of root

Indeed, roots were not washed thoroughly enough to separate between rhizoplane and endosphere colonizers. It is rather the total root colonization capacity that we report. We added the mention of root-association instead of surface attachment in the result section L122.

19. L410: Please mention the number of plants treated

The section was amended with the number of 20 treated plants.

20. L415: Please add references to R and the different packages; Please also add the. access date to homepages

The required references to the R packages were added L416. We do not feel the access date for any homepage is applicable here.

21. L420: as described above? Please write abbreviations in brackets when used the first time and not the other way around.

Abbreviation was corrected

22. L430: did you measure the quantity of the RNA also with other system e.g. Fluorescence? Did you check the successful DNA depletion?

We did not check RNA levels with additional methods as we find the Bioanalyzer approach to be precise and reliable. However, RNA quantity was independently verified by the sequencing platform which validated both quantity and quality to be sufficient for a reliable analysis. No validation of DNA removal was carried out as this is usually an unambiguous step in RNA preparation.

23. L447: Which sequencing kit was used?

The kit used by the platform was the NextSeq 500/550 High Output Kit v2 and mention of it was added in the manuscript L449.

24. L450: Please reference to the results table to the sequencing results

Comment unclear

25. What was your threshold p value for RNA Sequencing analysis / gene expression analysis. Please also make sure to mention how many replicates you sequenced, if technical or biological

The mention of replicate amount and nature was added on L448-449: "...on ribodepleted RNA samples originating from three biological replicates per strain and per growth condition". The mention of threshold p-value and fold change was added L457-458: "Genes that had a positive or negative differential expression superior to 1.5-fold at pval < 0.05 were taken into consideration."

26. DNA Extraction: Although referenced, please mention which kits/extraction method you used

No kit was used, the mentioned and referenced JGI procedure follows a classic CTAB/phenol-chloroform DNA extraction procedure.

27. Please add a reference to all tools you used– e.g. Cutadapt

The required references were added L450

28. Please give more details concerning your data analysis.

We thank the reviewer for the previous comments which substantially improved the clarity of the manuscript. However, without further guiding we do not feel that any specific methodological part requires additional describing. Data analysis strategies are presented in the results section.

29. Figures: Please don't use any abbreviation in the figure labels.

Care was taken to remove abbreviations from figure legends.

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Decision by Romain Barnard, posted 18 November 2022, validated 24 November 2022

Request for revision

Dear Authors,

Both reviewers and I agree this is a well-conducted study which yields interesting results. It nevertheless requires moderate revision, following some constructive comments by the reviewers, mostly to clarify a few points and better explicit the limits of the study.

I am looking forward to the improved version of the paper.

Best regards,
Romain Barnard

Reviewed by Vittorio Venturi, 09 November 2022

Mnaucript by Wallner et ., 'Comparative genomics and transcriptomics resone...'

This study investigates the gene expression in response to root exudates via an RNAseq approach of 6 rice rhizosphere bacterial strains, 3 belonging to the Burkholderia genus and 3 to Paraburkholderia. Results are rather surprising since regardless the close origin and phylogenetic relationships of the strains, the response is very much species specific displaying considerable differences. There are some commonaliites in the expression profiles and the authors have focused on these, as for example amino acid and putrescine metabolism as well as c-di-GMO signaling.

General comment:

The study addresses the important question of bacterial gene expression in the rhizosphere as influenced by root exudates. The experiments are well described, performed and appropriately discussed. The focus on commonalities is understandable and interesting results in relation to signaling are presented. Novel data is presented in this study with respect to Burkholderia and Paraburkholderia gene expression in the rhizosphere and sets the scene for future experiments aimed at deciphering bacteria-pant interactions. The study also presents new genomic sequences of rhizosphere strains and the genomes of the 6 strains have also been comparatively analyzed.

Specific comments:

1. As root exudates are likely to vary considerably throughout the growth phase of rice, in the future of similar studies, a metabolomic analysis of the root exudate will add important data to this study. Ultimately RNAseq data can be associated with metabolomic profiles.
2. This study could have benefited from some genetic validation studies especially in relation to the common loci regulated in all or most strains.
3. The putrescine hypothesis is potentially interesting; again verification of the presence of putrescine in the root exudate will be of vital importance.
4. As a general point, it is always advisable to remind the reader that the experimental set-up used here is an oversimplification of the conditions which occur in the wild. The strains used here, in the wild they will be members of highly complex rhizosphere microbial communities and are present at much lower concentrations. Hence the expression profiles in the wild are likely to vary considerably from the results presented here.

Reviewed by anonymous reviewer 1, 06 November 2022

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