Peer Community In
Evolutionary Biology

The variety of bread-making practices promotes diversity conservation in food microbial communities

Tatiana Giraud *and* ***Jeanne Ropars*** *based on peer reviews by 2 anonymous reviewers*

Elisa Michel, Estelle Masson, Sandrine Bubbendorf, Leocadie Lapicque, Thibault Nidelet, Diego Segond, Stephane Guezenec, Therese Marlin, Hugo deVillers, Olivier Rue, Bernard Onno, Judith Legrand, Delphine Sicard (2022) Artisanal and farmers bread making practices differently shape fungal species community composition in French sourdoughs. bioRxiv, ver. 6, peer-reviewed and recommended by Peer Community in Evolutionary Biology. `https://doi.org/10.1101/679472`

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Domesticated organisms are excellent models for understanding ecology and evolution and they are important for our food production and safety. While less studied than plants and animals, micro-organisms have also been domesticated, in particular for food fermentation [1]. The most studied domesticated micro-organism is the yeast used to make wine, beer and bread, *Saccharomyces cerevisiae* [2, 3, 4].

Filamentous fungi used for cheese-making have recently gained interest, for example *Penicillium roqueforti* used to make blue cheeses and *P. camemberti* to make soft cheeses [5, 6, 7, 8]. As for plants and animals, domestication has led to beneficial traits for food production in fermenting fungi, but also to bottlenecks and degeneration [6, 7, 9]; *P. camemberti* for example does not produce enough spores any more for optimal culture and inoculation and *P. roqueforti* has lost sexual fertility [9]. The loss of genetic diversity and of species diversity in our food production system is concerning for multiple reasons : i) it jeopardizes future improvement in the face of global changes ; ii) it causes the loss of evolved diversity during centuries under human selection, and therefore of beneficial characteristics and specificities that we may never be able to recover ; iii) it leads to degeneration in the few cultivated strains; iv) it impoverishes the diversity of our food products and local adaptation of production practices.

The study of domesticated fungi used for food fermentation has focused so far on the evolution of lineages and on their metabolic specificities. Microbiological assemblages and species diversity have been much less studied, while they likely also have a strong impact on the quality and safety of final products. This study by Elisa Michel and colleagues [10] addresses this question, using an interdisciplinary participatory research approach including bakers, psycho-sociologists and microbiologists to analyse bread-making practices and their impact on microbial communities in sourdough. Elisa Michel and colleagues [10] identified two distinct groups of bread-making practices based on interviews and surveys, with farmer-like practices (low bread production, use of ancient wheat populations, manual kneading, working at ambient temperature, long fermentation periods and no use of commercial baker's yeast) versus more intensive, artisanal-like practices. Metabarcoding and microbial culture-based analyses showed that the well-known baker's yeast, *Saccharomyces cerevisiae*, was, surprisingly, not the most common species in French organic sourdoughs. *Kazachstania* was the most represented yeast genus over all sourdoughs, both in terms of read abundance and of species diversity. *Kazachstania* species were also often dominant in individual sourdoughs, but *Saccharomyces uvarum* or *Torulaspora delbrueckii* could also be the dominant yeast species.

Metabarcoding analyses further revealed that the composition of the fungal communities differed between the farmer-like and more intensive practices, representing the first evidence of the influence of artisanal practices on microbial communities. The fungal communities were impacted by a combination of bread-making variables including the type of wheat varieties, the length of fermentation, the quantity of bread made per week and the use of commercial yeast. Maintaining on farm less intensive bread-making practices, may allow the preservation of typical species and phenotypic diversity in microbial communities in sourdough. Farmer-like practices did not lead to higher diversity within sourdoughs but, overall, the diversity of bread-making practices allow maintaining a larger diversity in sourdoughs. For example, different *Kazachstania* species were most abundant in sourdoughs from artisanal-like and farmer-like practices. Interviews with the bakers suggested the role of dispersal of *Kazachstania* species in shaping sourdough microbial communities, dispersal occurring by seed exchanges, sourdough mixing or gifts, bread-making training in common or working in one another's bakery. Nikolai Vavilov [11] had already highlighted for crops the importance of isolated cultures and selection in different farms for generating and preserving crop diversity, but also the importance of seed exchange for fostering adaptation.

Furthermore, one of the yeast frequently found in artisanal sourdoughs, *Kazachstania humilis*, displayed phenotypic differences between sourdough and non-sourdough strains, suggesting domestication. The sourdough strains exhibited significantly higher CO2 production rate and a lower fermentation latency-phase time.

The study by Elisa Michel and colleagues [10] is thus novel and inspiring in showing the importance of interdisciplinary studies, combining metabarcoding, microbiology and interviews for assessing the composition and diversity of microbial communities in human-made food, and in revealing the impact of artisanal-like bread-making practices in preserving microbial community diversity.

Interdisciplinary studies are still rare but have already shown the importance of combining ethno-ecology, biology and evolution to decipher the role of human practices on genetic diversity in crops, animals and food microorganisms and to help preserving genetic resources [12]. For example, in the case of the bread wheat Triticum aestivum, such interdisciplinary studies have shown that genetic diversity has been shaped by farmers' seed diffusion and farming practices [13]. We need more of such interdisciplinary studies on the impact of farmer versus industrial agricultural and food-making practices as we urgently need to preserve the diversity of micro-organisms used in food production that we are losing at a rapid pace [6, 7, 14].

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Reviews

Evaluation round #3

DOI or URL of the preprint: https://doi.org/10.1101/679472
Version of the preprint: https://doi.org/10.1101/679472

Authors’ reply, 14 October 2022

Download author’s reply

Decision by Tatiana Giraud, posted 28 September 2022

Very minor revisions

The revised manuscript has been greatly improved, but we still have some (very) minor suggestions, outlined below

-L40: of larger species and genetic diversities

-L47: the syntax issue remains: it should be ”other yeast species, belonging to the Kazachstania genus, were dominant in 54% of sourdoughs” : with commas, otherwise it means that Saccharomyces belongs to Kazachstania

-L82: delete the second occurrence of ”of S. cerevisiae” in the sentence.

-L83: who have or which has?

-L88-89: still unclear and awkward (you define them as ascomycete or basidiomycete and then say they’re mostly fungi, and then only later give their definition). I would define yeast first, e.g. ” Yeasts are organisms growing mainly as single cells and with sexual states not enclosed in fruiting bodies, typically belonging to ascomycete or basidiomycete fungi.”

-L94: ” The factors determining the presence of these species in sourdough”

-L161: define OTU at first occurrence

-L193: ”Different roots were tested and the root placement did not affect the tree topology.”

-L210: To investigate the relationship between beta-diversity and bakery practices, we…

-L267 and elswhere: bread-making

-L269 : breaK-making ?

-L270 : is presented

-L281 and elsewhere: practice group (no plural as it qualifies another term afterwards)

-L299 : fungal genus (no plural as it qualifies another term afterwards)

-L307 : replace « both » by « the » : as you compare the methods you cannot use « both » ; if you keep « both », delete « the same » and list them

-L309 : the Cladosporium genus

-L313 : as its ITS sequence is identical with that of Candida..

-L315 : has been further analysed using..

-L320 communities or the fungal community

-L328 : the beta diversity was analysed

-L344 : remove the comma or add one after cerevisiae

-L365 : comma after bulderi

-L375 : (K. australis) and sauerkraut

-L585-6 : analysis of the filamentous cheese fungi P. roqueforti and P. camemberti also revealed genetically differentiated cheese populations

-L588 : Roquefort cheeses

-L590 : of the soft-cheese making

-L591 : different kinds ; capitals for Brie and Camembert

-L596 : in organisms used for fermented product making

-L598 : standardize products OR to meet

-L604-605 : dynamicS..remainS

-L606 : « can evolve ».. but really the conclusion of this study is wrong, as shown in Ropars et al 2020 : the so-called « wild » strain belongs to the domesticated cluster, it is not at all a wild strain. The observations reported are therefore most likely due to phenotypic plasticity. I would delete this sentence.

-L617 : revealed instead of evidenced

-L619 : insightS

-Figure 4 : change fonts, species names are not readable ; I still don't understand this figure : what is represented on the Y axis of bars ? It's really difficult to see the difference between the bars from farmer or artisanal practices.. Maybe two separated maps would help, as two panels of the figure ? Or at least a symbol (e.g. an asterisk) below the bars of one of the practices ? As it stands, it is not useful to illustrate the difference between practices.

-L857 : LAB : define all abbreviations in all legends, a figure should be understandable by itself

-L891 : based on the Unifrac distance (define briefly)

-The legend of the figure 5 is unclear. Make complete sentences (the term « A) » cannot be a subject or remove the bracket and say « the panel A shows »). Panel A : what are the colors ? (everything should be described in a figure).

-L894 : the panels B to E show (not shows)

-L894, L896 : remove the signs « : »

-L896-7 : number of groupS ; what groups ? I don't understand what these bars represent, neither the X or the Y axes

-L902 : define all abbreviations in all legends, including genus names

-L906 : coma after axes

-L908 : a symbol or symbolS, but symbols should be specified with their meaning as well as colors, in the text and not only on the figure.

-L912 : Cellt27 is not defined and it would be easier to define abbreviations in the same order as on the plot from left to right ; explain the color meaning in the legend text too. A legend should describe everything on the figure, as often the authors often don't realize how hard it is to understand a figure we have not drawn.

Evaluation round #2

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Version of the preprint: `https://doi.org/10.1101/679472`

Authors' reply, 26 September 2022

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Decision by Tatiana Giraud, posted 16 August 2022

Minir revision

The revised manuscript has been greatly improved, but I still have some suggestions before the manuscript can be recommended, in particular about the clarity of the text and exactitude of the terms.

-L40: unclear: do you mean diversity within species or species diversity? Both are important and threatened and could be highlighted

-L45: why surprisingly? explain or delete

-L47: put ”of the closely related Kazachstania genus » between brackets, otherwise it means that Saccharomyces is included in the Kazachstania genus

-L48 : unclear « the distribution of yeast species » : distribution across what ? among what, or do you mean composition ?

-L49 : differences between what and what ? Do you mean « the most striking bread-making practice effect « ?

-L51 : syntax issue in « Phenotyping of these two species in laboratory sourdough mimicking media revealed » and sounds partly redundant with the rest of the sentence

-L53, L521 : no plural at practices as it qualifies diversity

-L54 : this is not a problem of taxonomy (classification) but of biological diversity ‘eg species diversity)

-L66 : « again » does not seem the right term here, better to just delete (or explain when was the forst episode of conservation this implies ?)

-L67 : distribution across what ? among what ? or just « on species composition « ? (or diversitu ? this is very different and it’s unclear here what you mean)

-L70 : « The origin … originated » : redundant

-L71 : the reader miss here a date for plant domestication and what was the first domesticated plant

-L72 : not sure about the term « combined » here… « linked » ?« associated »?

-L76 : is « process » the right term here ? you mean a here a particular event not the way of doing it

-L82 « and have improved » sounds too much active and anthromopomorphic, replace by « which has improve » (domestication has improved fermentation, it’s not the yeasts that have decided to improve their fermentation)

-L83 : why « however » ? delete ?

-L90 : Pichia in italics

-L91 : distribution across what ? among what ? or just «presence in surdough » ?

-L94 : « or » instead of a coma before house

-L96 : professional bakers plural

-L99-100 : fermers-bakers of farmer-bakers ? choose one and keep it consistent

-L139 : with instead of according to

-L143, L191, L202, L415 : no capital within sentences even for defining acronyms

-L147 : no hyphen in 33-yeast

-L159, L182 : no s at OTU as it qalifies another, following word

-L162 : was assessed

-L181 : fungal communities, or just delete « To analyse fungal community » that is redundant with the following

-L183 : trees

-L185 : why did you test different roots and how did you assess which one to chosse ?

-L186 : « It did » : who ?

-L187, L193 : a tree does not « classify » taxa, it represents evolutionary relationships ; classifying is a human decision.. « misassigned to» ?

-L188 : « in » insetad of « according » and delete « were »

-L196 : define Chao1

-L197 : index as it qualifies another, following word

-L203 : I doubt you really performed the analysis on the bakers themselves ?

-L204, L206, L208 : practice as it qualifies another, following word ; define FDR and add a reference

-Overall, the passive for mis much too used, it renders the text cumbersome to read. The active form should be preferred, it's lighter and more dynamic.

-L208 : I guess you do not expect a single link between all these variables so plural would be better

-L220, L303, L361, L450 : delete « finally », it's not likel you only expected to arrive there

-L222 : proxies

-L226 : fermentation latency phase (no hyphen there) ; define tlg

-L227 : cut the sentence

-L235, L399 and elsewhere : all numbers below ten should be written in letters, except in equations

-L249 : R package (no hyphen there)

-L250 : move the bracket at the end of the sentence, i twill be easier to understand

-L261 : unneeded repeat of « bread making process »

-L266, L275 and elsewhere : keep the past tense

-L268 : what does « ancient wheat populations » mean ? harvested centuries ago ? Otherwise it would rather be « ancient varieties » ? or what ?

-L270 : why using « baker's yeast » ? starter ? S. cerevisiae ? other yeasts ?

-L282 : delete « see M&M section » but explain when this is needed

-L288 : coma instead of space at 194 557

-L289 : « were identified as non-yeast » : really ? by molecular markers ? « yeast » is not a taxonomic status, it is a growth form opposed to « filamentous », there are also basidiomycetous yeasts ; same issues at other places in the manuscript, e.g. L292, L297 (and I don't know what yeast-like means)

-L306 : unclear what you mean exactly by « distribution of fungal species diversity » ? what does « distribution of » add ? across what ?

-L308, L309 and eslwhere : explicit the genus when you did not mention the species yet, it is nclear what you're referring to

-L310 : what do you mean by « distribution pattern » ? what is the question ? I have a hard time with this term, I don't understand what you mean when using it ? It seems like just a vague term lacking clear questions.

-L313 : lower relative abundanceS : I guess they don't all have the same abondance value ?

-L316 : differences in terms of what ? species presence/absence ? species diversity ? abundances ?

-L320 : keep the same difit numbers

-L320 : shown

-Figure 1 : whay all this white space ?

-Figure 2B : the text is still too small to be readable

-The figure 4 could probably be improved

-Figure 5 :labels are not readable on trees and PCAs ; give whole species names without abreviation (also Figure 7) ; « Practices clustering » : practice clustering or just practices ? « unknown » instead of « missing » or explicit what is missing

-L323 : again unclear what you mean by « distribution » ; yeastS (or just fungi ? « yeast » is not a very precise term)

-L325 : again unclear what is a « yeast species » ? do you have a taxonomic meaning ? or just growing as unicellular ? or able of fermentation ? you should define at the very beginning of the manuscript hat you mean by yeasts ?

-L341 : delete « indeed »

-L363 : replace « evidenced » by « reported » or « observed »

-L365 : be consistent all acros the ms in « bread-making » with or without a hyphen

-L368 : fewer instead of less

-L370 : keep the past tense

-L381 Group 1 encompassed

-L384 : harbored surgoughs with

-L389, 390 and elsewhere : for each test, do not just give the P value, but also the degree of freedom, the statistic value and all information needed for repeatibility and check

-L429 : farmers-liker ?

-P18-19 : all these results are a bit cumbersome to read and it is difficult to extract the main message.. would it be possible to summarize also on a figure the main features of the different groups ?

-L446 : avoid this abbreviation

-L447 : fungal and bacterial would be more consistent

-L479 : comparisonS or A comparison

-L491 : unclear what « these findings » refers to ? and « the diversity found in France » : do you mean in the present study ? This sentence is highly unclear

-L493 : delete the comas before Wickerhanomyces and after castellii

-L495 : replace the coma after australis by « and »

-L496 : do no compare a species and a genus

-L498 : if you list them all, « include » is not the right word

-L502 : avoid the unclear « it » by fusing the two sentences

-L504-5 : unclear why this is an issue to study genetic diversity

-L506 : delete « the » unless you mean a secific surdough ? but which one ?

-L508 : a bit strange to cite only cichlids here ? cite other cases, and at least a few of domesticated organisms, including fungi

-L511: replace the coma after bulderi by « and »

-L513 : this is a bit short and unclear

-L522 : you showed that it's higher but not that it increased, you don't know the ancestral state

-L531 : similarly, delete « of selection » : you would have only be able to detect different levels not whether this would have been due to selection

-L536 : twice « during the week » while neither seems important

-L544 : as already said, silage, not ensilage

-L561 : not sure that « diverge among themselves » is correct or mean something ? diverged one from each other ?

-L565 : and instead of et

-L576 : « important » has not the same meaning as in Frenc, it is not quantitative but qualitative, and bottleneckS

-L577 : italics at species name (also L580, 584) and add soft-cheese before P. camemberti to avoid confusion with the adjective blue cheese placed before

-L578 : diversity erosion in the doesticated organisms used for fermented product making ; and maybe you can be more specific by citing the sudies having revealed degeneration in domesticated fungi (eg loss of sex ability) ; this would help justifying your conclusion where you say that we need to preserve microbial diversity in food. Some additional examples of the benefit of having microbial diversity in food would be good.

-L580 : AOP is PDO in English and should be defined (protected designation of origin) ; maybe cite the P. camemberti study (Ropars 2020) for the issue associated with PDO but Dumas et al 2020 showed in contrast that PDO had protected diversity in the Roquefort P. roqueforti population.

-L584 : had ; different from

-L586 : a reference is missing, but if you mean the Ben Wolfe's study, this study is actually flawed in its conclusion as discussed in the Ropars 2020 paper : the strain used as collected in a cellar where cheese is cultivated and it belongs to a domesticated clade, so this was just not a wild-type strain

Evaluation round #1

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Authors' reply, 18 July 2022

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Decision by Tatiana Giraud, posted 16 March 2022

Revision

The manuscript has been evaluated by two referees, who agree that this interdisciplinary study on differences in terms of species composition between sourdoughs from artisanal and farmer bread is sound and interesting for the evolutionary biology community. Both referees nevertheless provide a list of excellent additional suggestions, such as improving the readability of figures, adding discussion on a broader context and be more cautious about the term domestication.

In addition, I recommend to add a genuine discussion and not just a brief conclusion lacking context. An extended discussion should replace the findings in a larger context, e.g. comparing with other systems where industry decreases micro-organism diversity (eg Penicillium camemberti DOI : 10.1016/j.cub.2020.08.082 and Penicillium roqueforti `https://doi.org/10.1111/mec.15359`) and highlighting better the importance of the results and the insights gained. Some points of uncertainty should also be discussed, eg whether the silage strains could be feral rather than wild representatives?

The figures can be much improved and this is very important for rendering a paper understandable and appealing. The figure 2 is much too small, colors would help clarifying; "artisanal" and "farmer" are hard to distinguished from point labels; placing the three panels as lines rather than columns would help make them larger and readable. The figure 3 should show the bread types. The figure 4 is also hard to read and understand, in particular the black and white rectangle do not make easy to distinguish bread types. I cannot read labels in the figure 5. Redefine all abbreviations in all figure legends.

I would encourage resubmission if you are able to revise the manuscript along all these lines.

In addition, please also address the comments below:

-In the title and in several places in the manuscript, there may be a confusion between species diversity and composition. If I understand correctly, the differences were tested in terms of species composition rather than diversity? Please clarify the terms and add some more direct analyses of diversity, such as Shannon or Simpson index.

-In the title and elsewhere: no plural when a word is used as an adjective before another word (e.g. no s for bread in the title, fermentation L159, sourdough L315)

-L41: delete "their", the reference is two sentences away.

-L61: roqueforti not roquefortii

-L68 "that" should not be omitted after suggest in written text

-L70, L74 : bread-making

-L75: delete the space before the point

-L89: delete may

-L96: farmers

-L160: no number at the beginning of a sentence, or only written in full letters

-L164, 165: silage not ensilage (French)
-L220 habitats
-L315: if you compare sourdoughs, it should be difference and not diversity, or a word is missing
-L331, L352, 355, 357, 470 and elsewhere: no abbreviation at the beginning of a sentence
-L346: I missed information about known abilities of Kazachstania species for fermentation?
-L370, 381, 528: diversity and/or composition?
-L319: associated with instead of related to
-References: check format, eg species name italics, no capital at words, ...

Reviewed by anonymous reviewer 1, 15 March 2022

I very much enjoyed reading this ms that reports on an interdisciplinary and participatory research concerning the investigation of the yeast communities associated with sourdough in France. This is a well-designed and carefully conduced investigation. Having no major concerns about thus ms, my comments should be understood as a contribution to improve this fine work.

Major
Fig 3 depicts very important results for the study but I think it is very difficult to locate a particular species given that small differences in colour tones. For example, I cannot distinguish S. cerevisiae from P. kudriavzevii on a printed image. My suggestion of that you color-code a restricted number, of the more relevant species. The other could be grouped in genera (or other groups) and given the same colour. For example, same colour for the genus Cryptococcus (a supplemental figure could provide the entire set of species or even a series of detailed images for those species that were grouped in the main fig. For example a supplemental fig where Cryptococcus species are coloured while the remaining are in shades of grey.

Fig. 4. Again some species - S. cerevisiae vs Pichia and species of Kazachstania are undistinguishable.

Fig 5 is very interesting but the lettering is so small that it cannot be read. For the first clustering indicate some species for each of the 3 groups so that they have a more relevant meaning.
Line 315 – 320. Could you please be more specific when analysing unifrac distancies. – which communities (samples) are similar, and which are not? Perhaps adding also a few more sentences on the approach and data interpretation would also help.

Finding that several species of Kazachstania can dominate sourdough fermentations is a very interesting result. Given that this genus has many species, it would be relevant to know what the sourdough species have in common? Are they phylogenetically related?

The authors have shown that the sourdough community can change. Similarly, the human microbiome project has shown that the gut microbiome can change among healthy individuals. Nevertheless, it appears that, at a general level, the main (gene) functions that the different communities perform are similar. Perhaps this point could be addressed with respect to the sourdough communities – different in composition but perhaps similar in function.

The section "phenotypic signatures of domestication" appears to require some caution in the interpretation of the results. First the number of sourdough isolates is disproportionately high when compared with non-sourdough strains. Moreover, you are comparing fresh isolates with collection strains. Secondly, given that these phenotypes were not investigated at the genetic level, assuming domestication appears to be premature. Domestication must have a genetic basis and studying phenotypes without studying genotypes appears is a problem if one wants to make statements about domestication (which, in fact are not that much relevant for the study). Moreover, you devote two figures to this unbalanced comparison and conclude that the strains have been domesticated without providing, as mentioned above, any genetic evidence.

"Therefore, our results suggest that populations of K. humilis have been selected by humans and can therefore be considered domesticated". Here and in the conclusion section, I think that these type of statements should be softened

Minor

Line 55. Perhaps rephrase since the Neolithic did not "emerge". It is just a time period designated by humans

Line 58. I think "selected strains" were not in use in the 19th century.

Line 58. "reduction in species". Please explain.

"reduction in genetic diversity" Indeed this is typically observed in plant crops but can you cite studies in microbes where this was observed?

Line 82. I know the cited work but cannot avoid two comments. First, you are claiming that a domestication process is contributing for a species diversity. However, a few lines earlier you claimed that domestication reduces diversity. Secondly, "indicating that sourdough bread making contributes to the conservation of S. cerevisiae diversity" entails a complex rationale. In the first place this reasoning assumes that diversity was already present (before sourdough) and this practice helps preserving it. The problem is that sourdough strains arose after domestication and therefore do not occur in nature. If one wants to measure the diversity of S. cerevisiae, considering the diversity of its truly wild populations is the relevant thing to do. Anything else would mean that the diversity of this organism is a human construction, due to domestication. I think this reasoning is true for any microbe. In brief I agree that sourdough practices are important to maintain the diversity of bread-related lineages (in a given species) but I have doubts that an artificial fermentation can maintain or expand the diversity of a species.

Line 280. You mention that you isolated 20 to 40 yeast strains per sourdough sample and cite previous works. I presume these were aleatory isolations and that this approach allowed you to calculate relative frequencies. I understand you cite two other studies but a brief description should perhaps be included as this part must be made clear as it is essential for understanding your work.

Line 289. "T. aestivum". Please do not abbreviate the genus name the first time you use it.

Line 289. "... assigned to plant pathogen fungi, such as Alternaria, Aspergillus or Fusarium, Gibberella". Is the labelling "plant pathogenic" the most appropriate, given that these genera also include many saprobes? Perhaps "... assigned to genera that include plant pathogen species together with saprobic ones"

Line 345. I have mixed fillings about the title of this section. Clearly finding several species of Kazachstania as dominant is a very interesting result. The problem is that the genus has lots of species, more that 30, and therefore the title is not very accurate.

Still on this topic in line 349: "In addition, eight species of the Kazachstania genus were found in sourdough, a much higher figure than for the Saccharomyces genus, represented by only two species (S. uvarum and S. cerevisiae)" If the genus Kazachstania is 4 times larger than the genus Saccharomyces (aprox 40 species vs aprox 10 species) then these numbers are not so special.

Line 456 “Previous studies on the bacteria content of the same sourdoughs showed that…” Perhaps changing the sentence to “same type of sourdough”.

The first reference in the reference list is incomplete. See also ref #25.

Reviewed by anonymous reviewer 2, 08 March 2022

Michel et al. integrate reported data from farmer-bakers and artisanal bakers in France coupled with sequencing and lab experiments to identify what practices influence yeast species diversity and community in French sourdough breads. They identify a number of differences that separate farmer and artisanal bakers. Using a culture based and culture-free sequencing approach, they identify species diversity in each of the sourdoughs. For the most part, the culture based method does a good job at identifying the dominant species in the sourdough, but the culture free method picks up more rare species and some unculturable species that are more prevalent in a couple of starters. Their most notable finding is that farmer-bakers sourdoughs do differ from artisanal bakers in species composition, which is mostly driven by whether the baker uses commercial yeast. I also found the results differentiating Kazachstania humilis and bulderi really interesting – the authors show that the two species are differentiated in a number of fermentation metrics, which follows their usage in either farmer-bakers or artisanal bakers. The K. humilis sourdough strains perform better than K. humilis strains isolated from other environments (although there are only a couple samples here). As the authors mention, follow up work with dough is needed, and the language about domestication could be tempered slightly, but I felt the work done was adequate for the scope of this manuscript.

I found the interdisciplinary approach of this study to be elegant. While other factors may be contributing to their findings differentiating species composition via farmer-bakers and artisanal-bakers (as they acknowledge), this study supports that preserving cultural practices can preserve/cultivate microbial diversity in our foods. It also catalogues a huge number of variables in breadmaking that have been hypothesized to contribute to a particular species community in sourdough. This is a very nice addition to the literature. A few minor comments below:

Line 279-280 isolated 20-40 yeast strains? Does this mean colonies? Throughout the manuscript there was reference to strains, but to me, a strain means a distinct genetic entity, which I don’t think is known in this context.

I had a couple of comments on the figures. First, I would suggest an additional figure or figure panel, which combines the Unifrac tree with the metabarcoding sequencing data to help visualize the species composition for each branch.

Figure 1 – I do not see the center of the groups denoted with a triangle (and not sure what this would represent). The labels next to the triangles in 1B are not always clear from comparison to Figure 1C.

Figure 5- please label each panel with letters for reference in the text. Also just visually, the scale of the font differs very dramatically between the legends and titles and is a bit hard to read the small font sizes.

Figure 6 – could you include the trait data for the non-sourdough strains on this figure as well? Also, I would be curious to know how K. humilis and K. bulderi differ in these fermentation metrics from S. cerevisiae sourdough and commercial strains.