Peer-Review comments and authors responses
Editorial team

Dear Dr. Fregni,
Thank you for the opportunity to revise and resubmit our manuscript “The use of probiotic for pain reduction in patients with fibromyalgia: a systematic review” for consideration in the PPCR Journal. We are grateful to you and the reviewers for the time and effort dedicated to providing such insightful and constructive feedback. The reviewer’s comments have significantly helped us to improve clarity and strengthen the overall quality of our manuscript. We have carefully addressed every comment and have made revisions accordingly.
Below is a point-by-point response to the reviewer’s comments. All changes in the manuscript have been implemented and reflected in the clean copy. In addition, we are submitting a track change copy.

Reviewer 1:

Dear Reviewer, 1, we appreciate and highly value your feedback as we find it important for the improvement of the overall clarity and consistency of our paper. Please fin

> **Comment:** Major points for review:Flow of the text. Some information, especially in the Methods and the Results sections, are either repetitive or not where they should be. As a result, it is difficult for the reader to follow the text. Please address the following:

Response: *We appreciate the time you took to make this review and recommendations. We conducted an overall internal editorial team review to enhance clarity and readability.*

> **Comment:** Under Methods - Selection of Studies and Data Extraction: "a total of 439 records were removed before the screening phase" this belongs to the Results section.

Response: *Thank you for your observation. We agree that the current description is included in the result section, and this was removed from the methods to avoid repetition.*

> **Comment:** Under Methods - Data synthesis: "All studies, except Calandre et al (2021), reported the use of the VAS to evaluate how probiotics interventions affected the degree of pain experienced by fibromyalgia patients, from baseline to a maximum of 8 weeks": this belongs to the Results section

Response: *Thank you for your observation. We agree that the current description is included in the result section, and this was removed from the methods to avoid repetition.*

> **Comment:** Under Results - Outcomes: "One study presented their data as means and standard error of the mean (Roman et al, 2018), while another study presented the data as means and standard error, reflected in a bar chart, as well as the mean difference (ES d or Cohen’s d) in change and standard deviation". I believe this level of detailing isn't very relevant to be written at this section, especially as the measures will be mentioned when you describe the findings on the endpoints. Moreover, Cohen's d could be calculated by the authors in papers where it was not reported.

Response: *Thank you for your feedback. We agree that the level of detail in this section was not adding value, and we removed it from the section. The results have the referenced values, and no additional data was available. Authors were contacted unsuccessfully to obtain missing data from the studies.*

> **Comment:** Under Results - Outcomes: "This score consists of 36 items grouped into eight domains: physical, social, emotional, mental health, vitality, overall health, and changes in health over time". I understand why it was written here, but I believe this information is more pertinent to the Methods section.

Response: *Thank you for your feedback, this was considered to be added to the methods section.*

> **Comment:** Under Results - Main results: This entire section seems to echo the information previously outlined in the results and could be deleted entirely. If the idea is to summarize what was written above, this would belong to the Discussion section, where you usually begin with a summary of your findings.

Response: *Thank you for your feedback. This section had been removed to avoid repetition of what is included in the discussion section.*

> **Comment:** Under Results - Individual Study Results: a lot of this section echoes the previous ones. I do not think it is necessary to echo information from the tables when referencing the studies (e.g., Roman et al. (2018) conducted an 8-week pilot randomized controlled trial to assess the effects of a multispecies probiotic on cognitive, emotional, and functional outcomes in 31 FMS patients. The intervention group (n = 16) received a daily probiotic containing various Lactobacillus and Bifidobacterium strains). I also think you do not need to mention the findings on the pain scales that were already outlined in a previous section. Instead, you can use this section to report secondary endpoints that were not mentioned before.

Response: *Thank you for your feedback. We agree with your suggestion, and this section has incorporated language describing secondary endpoints across studies in general.*

> **Comment:** Under Discussion: "In the Aslan et al. study, although data were not available to draw or confirm conclusions for the VAS outcome, despite multiple requests to the corresponding author". This sentence could be seen as provocative and I would not mention communication attempts in the manuscript.

Response: *This sentence has been reworded to reflect the lack of data without provocative, unintended interpretations.*

> **Comment:** Under Discussion: The paragraph that starts with "While the three studies..." could be broken down into two paragraphs since it is quite long.

Response: *Thank you for your review. This has been addressed to improve clarity*

> **Comment:** I believe you could at least briefly mention safety in the Discussion. I know probiotics are considered a very safe intervention, but a brief sentence with a reference, saying that probiotics are safe for most people, would probably suffice.

Response: *Thank you for your valuable feedback. We agree that the commercial products used in the studies are generally considered safe. We have revised the discussion to add their favorable safety profile.*

> **Comment:** Abstract - Results: Where it reads "3 double-blind, placebo-controlled RCTs (2018–2024; n=213; 198 women; ages ~46–55; Spain/Turkey) met inclusion" should read "3 double-blind, placebo-controlled RCTs (2018–2024; n=213; 198 women; ages ~46–55; Spain/Turkey) met eligibility criteria and were included"

Response: *Thank you for your feedback. We consider that your revised version improves the clarity of the statement, and it has been changed as suggested.*

> **Comment:** Abstract - Results: "Interventions were multistrain probiotics for 8–12 weeks; outcomes included VAS pain, FIQ/FIQR, and SF-36" this should be moved to Methods.

Response: *This sentence was removed and added in the methods section. "The multistrain probiotics for 8-12 weeks" was added in the third paragraph of the "Description of studies and population" in the methods section. The rest of this sentence was not added to the methods section, as discussed in "outcomes" in the methods section.*

> **Comment:** Abstract - Conclusion: ", despite the limited number of studies for this topic, the evidence for pain improvement using VAS, FIQ (FIQR), or SF-36 remains weak and inconclusive". The word "despite" infers contrast, but I don't see contrast here. Suggest rewriting as "Partly due to the limited number of studies..."

Response: *Thank you for your suggestion. We agree the change will improve clarity. The sentence was reworded.*

Comment: Methods - Search strategy: "Four independent reviewers searched six databases". This step of the review does not require independent reviewers, so I don't believe it is necessary to report it this way. Suggest rewriting as "Six databases were searched"

Response: *Thank you for your feedback, we agree that concise reporting improves clarity. The reviewers part was removed from the text.*

Comment: Methods - Selection of Studies and Data Extraction: Where it reads "and any unclear or incomplete information, the corresponding authors were contacted to obtain additional details." I suggest rewriting as "study authors were contacted if additional information was necessary."

Response: *We agree that a concise statement improves clarity. The sentence was reworded.*

Comment: Throughout the paragraphs, make sure you are being consistent with spacing before the first word.

Response: *Thank you for the recommendation. We addressed this with a thorough review to ensure consistency.*

Comment: Table 2 is never formally referenced in the text. I believe it should be referenced here "The table describes in detail the interventions of the three RCTs, double-blind, placebo-controlled trials, including the type of intervention, dosage, duration, and route of administration.".

Response: *Thank you for your observation, we agree that this was missing and the number 2 was added to the initial sentence of the second paragraph for the "Description of studies and populations" in the methods section.*

Comment: Results: Before "The table describes in detail the interventions of the three RCTs, double-blind, placebo-controlled trials, including the type of intervention, dosage, duration, and route of administration.", you should consider including a subheader "Interventions".

Response: *Thank you for your suggestion. A subheader has been added before this paragraph to address clarity*

Comment: Results - results according to the pain scale: Where it reads "however the study's conclusions were limited due to the lack of methodologically rigorous presentation and analysis of the VAS data.", I would consider a more neutral language.

Response: *Thank you for your feedback. The sentence has been reworded to ensure neutral language.*

Comment: Discussion: "The Clinical Trials Registry (Clinicaltrials.gov) was used to assess grey literature, limiting the number of available studies.". Including grey literature does not limit the number of available studies. It actually increases, as you are looking also for unpublished material.

Response: *Thank you for your observation. We reworded the sentence to reflect the accurate statement.*

Reviewer 2:

Dear Reviewer 2,
We greatly appreciate your feedback and revision to our manuscript. Please find the list of the items addressed below.

> **Comment:** The article addresses a clinically relevant topic, exploring the potential role of probiotics in managing fibromyalgia. The authors followed the PRISMA guidelines, providing clear descriptions of the methodology, information sources, eligibility criteria, search strategy, data collection, and risk of bias assessment. Limitations of the included studies were acknowledged transparently by the authors.

Response: *Thank you for your feedback. It helps to outline our work and reassure our collective research process*

Comment: Search Strategy - Only English-language studies were included, which limited the generalizability.

Response: *We appreciate your thoughtful comment regarding the inclusion of only English language publications and the potential impact on generalizability. While our eligibility criteria required studies to be published in English, it is important to note that two of the three studies included in our final analysis were conducted in Spain and the third in Turkey. All three studies translated their work into English for publication, indicating that English language dissemination is not restricted to countries where English is the primary language. Rather, English serves as a common scientific medium that facilitates broader accessibility and international knowledge exchange. Therefore, although we acknowledge the inherent risk of language bias in systematic reviews, in this case, limiting the search to English did not confine the sample to English-speaking regions and still allowed for representation of geographically and culturally diverse populations. We added this clarification to the manuscript in the discussion section. The following statement was added to the discussion in the first paragraph: "Although our search included only English publications, the included studies originated from Spain and Turkey, demonstrating that English dissemination did not restrict geographic diversity and the risk of language bias was considered minimal."*

> **Comment:** Grey literature was assessed only through ClinicalTrials.gov, which restricted the scope of available studies.

Response: *We appreciate your insightful feedback regarding our approach to grey literature assessment. We acknowledge that relying solely on a single registry may limit the breadth of grey literature capture. However, ClinicalTrials.gov was selected as the primary source because it is the largest international registry for interventional clinical studies, routinely including submissions from multiple countries, not just the US. Given our specific focus on interventional trials, we considered it a highly relevant and efficient resource for identifying unpublished or ongoing studies that could meet our eligibility criteria. Alternative grey literature sources such as conference abstracts, dissertations, or institutional reports, would have been less likely to contribute eligible.*

> **Comment:** Methodological Concerns: High risk of bias due to missing outcomes, selective reporting, and lack of pre-specified statistical analysis plans. High attrition rates were observed in two studies, and a lack of transparency in reporting outcomes was noted in one study. Substantial heterogeneity across studies in design, diagnostic criteria, outcome measures, and probiotic strains used.

Response: *Thank you for your feedback. We agree with your observations and acknowledge them in the discussion section to demonstrate the transparency of our results.*

> **Comment:** Results and Generalizability. Only one study reported statistically significant pain reduction, limiting the strength of the conclusions. Small sample sizes resulted in underpowered analyses, unable to detect clinically meaningful differences. Studies were primarily conducted in outpatient settings, with limited diversity in the population. Female predominance may affect generalizability, particularly as some evidence suggests men are underdiagnosed.

Response: *Thank you for your revision, and we agree that the results and discussion sections address these concerns.*

> **Comment:** Complementary materials: The tables and figures are clearly organized and well-presented, effectively supporting the manuscript's narrative.
>
> **Recommendations:** Although the manuscript offers valuable insights, it contains minor grammatical issues, and some sections could be rephrased for greater clarity. Those sections are highlighted in the uploaded document.

Response: *Thank you for the review of our study. This helps us to keep our efforts to the highest standards and encourages our future research.*

Reviewer 3:

Dear reviewer 3, we highly value your review and recommendations made to our manuscript. Please find below our responses to the items addressing your feedback.

> **Recommendation:** Revisions Required. This systematic review addresses an important and clinically relevant question regarding the potential role of probiotics in the management of fibromyalgia-related pain. The paper is well-structured, using PRISMA 2020 guidelines and appropriate quality assessment tools. However, I would suggest the following:
>
> **Comment:** Search and Scope
> Restriction to English-language studies may have introduced language bias and limited the search. Including non-English studies or broader grey literature sources could reduce this risk.

Response: *We appreciate your thoughtful comment regarding the inclusion of only English language publications and the potential impact on generalizability. While our eligibility criteria required studies to be published in English, it is important to note that two of the three studies included in our final analysis were conducted in Spain and the third in Turkey. All three research teams translated their work into English for publication, indicating that English language dissemination is not restricted to countries where English is the primary language. Rather, English serves as a common scientific medium that facilitates broader accessibility and international knowledge exchange. Therefore, although we acknowledge the inherent risk of language bias in systematic reviews, in this case, limiting the search to English did not confine the sample to English-speaking regions and still allowed for representation of geographically and culturally diverse populations. We added this clarification to the manuscript in the discussion section. The following statement was added to the discussion in the first paragraph: "Although our search included only English publications, the included studies originated from Spain and Turkey, demonstrating that English dissemination did not restrict geographic diversity and the risk of language bias was considered minimal."*

> **Comment:** Synthesis of Results:
> No meta-analysis was conducted due to heterogeneity, which is reasonable. However, limited subgroup analyses might still have been feasible and would have added a little more detail to the results, such as doing so by probiotic strain.

Response: *Thank you for your insightful review. We agree that, ideally, subgroup analysis could provide valuable information, and our group considered this option. However, as addressed in the results and discussion, heterogeneity was found substantially in the methods, including different types of combinations of probiotic strains, making the comparability complex and probably not clinically meaningful. In addition, outcome results were reported with variations. As we describe in the paper, VAS was applied in two studies, but the results may not be comparable due to one of the studies modifying the tool, adding specific gastrointestinal symptoms other than pain. The FIQ was also variable, as studies used the FIQ and FIQR (revised version). Reports on the FIQ and FIQR were different, as one study reported a global score, and the other reported by domain of interest, only adding complexity to the comparability. Following your feedback, we further clarified this in the discussion, ensuring these limitations were discussed in the manuscript.*

Comment: The results section sometimes repeats details from individual trials extensively. A more concise synthesis emphasizing cross-study trends would improve readability.

Response: *Thank you for your feedback. We appreciate these observations and revised the results section to avoid repeated information.*

Comment: Reporting and Interpretation - Greater use of confidence intervals, in addition to p-values, would help show the precision and clinical relevance of the results.

Response: *Thank you for your feedback. Only one of the three studies reported confidence intervals in their results and was described in the results section. Language addressing the lack and inconsistencies in the full disclosure of statistical results was added in the Methods and Discussion sections.*

Comment: The inconsistencies in outcome measures (VAS variants, FIQ vs. FIQR, partial vs. total SF-36 scores) are noted but could be expanded into a recommendation for standardized outcome sets in future trials.

Response: *Thank you for your feedback. We incorporated language in the conclusion section, making a recommendation on the use of standardized and validated tools to improve the quality of the results.*

Comment: Generalisability - The review includes predominantly female participants and studies conducted only in Spain and Turkey, which limits external validity. The discussion could place stronger emphasis on these limitations.

Response: *Thank you for your feedback. We agree that these observations are important limitations to validity and were added in the discussion*

Comment: Differences in diagnostic criteria (1990, 2010, 2016 ACR) likely introduced heterogeneity. This important methodological issue deserves clearer elaboration.

Response: *Thank you for your observation. We initiated this discussion; however, we agree that a clear statement will improve clarity. A sentence was added to state that the differences between the criteria used for diagnosis introduced heterogeneity, and later in the paragraph, we discuss how this limited the generalizability, along with the other inconsistencies.*

Comment: Conclusions and Future Directions - Maybe calirying/indicating what future RCTs should address: standardized pain outcomes, probiotic strain-specific analyses, longer follow-up periods, and better powered sample sizes.

Response: *Thank you for your observation. This was improved to the current statement in the conclusions to add clarity.*

Reviewer 4:

Dear reviewer 4, we appreciate your time reviewing our manuscript and took your valuable feedback into the highest level of consideration. Please find below the responses addressing each observation you kindly shared with us.

Recommendation: Resubmit for Review.

Comment: About the clarity of aim: The goal of the study must be clear enough, leaving no room for doubts and confusion. Your manuscript uses different expressions for the same. In one line uses the expression "…*aims to synthesize the available evidence*…", later uses another one: "…*aiming to assess their potential role as a complementary therapeutic option* …", and then a different and longer one: "…*aimed to describe the effects of probiotic supplementation on pain and associated fibromyalgia symptomatology in adult patients*…". These expressions are obvious and correct but together competing to explain the mission of the paper might be misleading for the reader and you authors lose the chance to be resounding. Right after these sentences, and instead of finishing the introduction section presenting the

aim of the study, you decide to retake the arguments of justification of the study which should have been exposed before you culminate the introduction when you show the aim of the study.

Response: *We acknowledge the inconsistency of the language and have addressed it to be aligned with our primary endpoint, aiming to assess the effects of probiotic supplementation on pain in patients with fibromyalgia. This was revised and updated accordingly.*

Comment: About the conclusion: As we know, the aim of a study should be strictly tied to the conclusion, but in this case your manuscript loses the chance of key concomitance. The two paragraphs you wrote as conclusion, by being long, might dilute interest in the reader. Both paragraphs could easily reduce to one without getting rid of all the ideas you included in the twelve lines.

Response: *We appreciate your feedback and agree that a concise message should improve clarity. The conclusion was reworded.*

Comment: About the search strategy: On the other hand, there should be only one method of search, but you said you used two different search protocols, one through six sources and then you said again you just did it with only of them (PubMed). Using both protocols is contradictory.

Response: *Thank you for your feedback. We agreed that the paragraph may use clarity to address that there was only one search strategy, but adaptations on the symbols for syntax were used as requirements are different on each database.*

Comment: About the risk of bias: Its assessment could be more detailed because you are working with the highest level of quality of research, RCTs. For instance, you said you had two reviewers testing for bias, but you don't explain what the mechanism you plan for the "consensus" if needed, as you name it. Sometimes we might be pragmatic, but when you work with pieces of research like RCTs you then need to be as transparent as possible.

Response: *Thank you for your feedback, the paragraph in the Methods section (Risk of Bias Assessment) was revised accordingly. Language was incorporated to address the missing mechanism to reach a consensus.*