

Review Form 1.7

Journal Name:	Biotechnology Journal International
Manuscript Number:	Ms_BJI_118870
Title of the Manuscript:	MECHANOSENSITIVE CHANNELS, UTILISING THE BACTERIAL CELL'S DEFENCE MECHANISM AGAINST IT
Type of the Article	Review Article

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<p>Compulsory REVISION comments</p> <ol style="list-style-type: none"> 1. Is the manuscript important for scientific community? (Please write few sentences on this manuscript) 2. Is the title of the article suitable? (If not please suggest an alternative title) 3. Is the abstract of the article comprehensive? 4. Are subsections and structure of the manuscript appropriate? 5. Do you think the manuscript is scientifically correct? 6. Are the references sufficient and recent? If you have suggestion of additional references, please mention in the review form. <p><u>(Apart from above mentioned 6 points, reviewers are free to provide additional suggestions/comments)</u></p>	<ol style="list-style-type: none"> 1. The title is suitable 2. The abstract of the article is not comprehensive. It does not establish any background regarding the significance of antibiotic resistance to global health outcomes. At the same time, the authors include details that are not critical to the explanation of the text, such as the subgroups of MscS. Overall, there is no clear thesis outlining the contents of the manuscript, which reflects the lack of focus in the manuscript itself. 3. The subsections of the manuscript are appropriate, and the structure does show a clear flow from an overview of mechanosensitive channels to their role in infectious disease and antibiotic susceptibility. However, the structure is not anchored to a conclusion that would encompass the work as a whole. 4. The manuscript largely appears to be scientifically correct, but the misapplication of references throughout leaves major concerns as to the accuracy of the report overall. I detail specific concerns about referencing in response 6. Other concerns regarding accuracy are below. <ol style="list-style-type: none"> a. On page 5 line 19 the authors propose three points that they claim the data supports. However, the authors fail to clearly support these claims in the remaining text. b. Page 11 Line 10 states: "Research has indicated that the unique ability of aminoglycosides to eliminate bacterial persisters is enhanced when hypoionic shock is applied" This statement is demonstrably false. Aminoglycosides do not have a unique ability to 'eliminate' persisters. Persistence is well studied to include tolerance to aminoglycosides. See Harms et al. 2016 (DOI: 10.1126/science.aaf4268) and Shan et al. 2015 (DOI: 10.1128/mbio.00078-15) 5. The references are recent, but many places in the manuscript are missing sufficient references and references are frequently misapplied and falsely represented. When using bullet pointed lists each bullet point should have one or more references. If subsequent bullet points share the same reference, I recommend adding the reference at the end of each sentence to avoid reader confusion. Below are notes for points at which referencing is needed or misapplied. <ol style="list-style-type: none"> a. Pg 3. Lines 13-44: Ensure each bullet point has one or more reference. b. Figure 2 legend: Reference 16 (Rasmussen <i>et al.</i> 2019) focuses on MscS structure, not osmotic regulation. c. Pg. 4 Line 17: Reference 16 (Reuter et al. 2014), does not describe different species and thus cannot be used to support the statement "The exact mechanisms of mechanosensitive channel activation vary depending on the specific channel type and the bacterial species" d. Page 5 Line. 24: Reference 2 (Rasmussen et al. 2019) does not discuss ion flow as the preceding text (MscS family may ... signal in ways that can be distinguished from ion flow) would suggest. e. Page 5 Line 28: Reference 13 (Kawasaki et al. 2020) solely examines <i>Corynebacterium</i> and thus cannot be broadly applied to bacteria as the preceding statement would suggest. f. Page 5 Line 32: The statement "Gd³⁺ ions have been demonstrated to block MscL activity in <i>E. coli</i> spheroplasts, preventing lactose and ATP from leaving shocked cells." does not have an appropriate reference. The following sentence again cites reference 13, which solely studied <i>Corynebacterium</i>, and specifically examined CsMscL – a MscL like protein – and thus the results are not directly applicable to <i>E. coli</i> as the authors writing would suggest in this manuscript. g. Page 5. Line 37: Reference 5 (Li et al. 2002) does not test bacteria devoid of both 	

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	<p>MscS and MscL and thus cannot support the statement “bacteria devoid of both MscS and MscL activities are nearly incapable of withstanding an osmotic downshock of 0.5 M”</p> <ul style="list-style-type: none">h. Page 7. Line 11 – The statement “Transgenic expression of E. coli MscL can protect certain marine organisms from extreme hypoosmotic stress” is not supported by reference 8. The reference does not have transgenic expression of MscL in marine organisms, and while the results may leave one to conclude that such expression may protect against osmotic stress the paper itself makes no such assertions.i. Page 8 Lines 15-27: Each bullet point needs one or more references.j. Page 9. Line 20: Reference 4 (Boer et al. 2011) does not study S. typhimurium and thus cannot support the statement, “Research has indicated that the mechanosensitive channel YnaI is necessary for colonizing the host's intestinal tract, and that S. typhimurium internalization increases when the ynaI gene is deleted”k. Page 11. Line 11. Reference needed for, “Surprisingly, mechanosensitive-channel activators as parabens or indole significantly amplified this impact.”l. Page 11. Line 12. Reference 36 (Wray et al. 2022) does not contain any experiments regarding persistence to glycosides or flash-freezing.m. Page 11. Line 21. Reference 42 (Cama et al. 2019) does not reference tetracycline.n. Page 12. Line 20. Reference 44: Shen et al. 2023. studies the effects of OaBac5mini and does not have any results examining gramicidin A, gramicidin S, nisin, and aureinso. Page 12. Line 23. Reference 45 Huan et al. 2020 makes no mention of mechanosensitive channels.p. Page 12. Line 26. Reference 44 does not discuss glutamate excretion.q. Page 13. Line 2. Reference 46 (Hirasawa et al. 2013) studies gene editing in yeast and does not examine penicillin stress.r. Page 13. Lines 4-7. These statements require references. “Similarly, it has been demonstrated that ampicillin causes E. coli to produce mscL and mscS transcription. Other antibiotics including gentamicin, ofloxacin, and norfloxacin did not cause this induction, which is consistent with conditions that compromise the cell envelope.”s. Page 13. Lines 33-36. Reference needed for bullet point.t. Page 15. Line 6. Ramizol is in the antibiotic class styrylbenzene - these are not two separate drugs as the authors suggest in the text.u. Page 15. Line 36. Reference needed for “It has been discovered that the more potent derivative, IRS-16, and SCH-79797, bind to the MscL channel directly and cause membrane permeabilization when they activate it.”v. Page 15. Line 39. SCH-79797 is not discussed in reference 59. The effects of SCH-79797 are reported in reference 66 (Wray et al. 2022). <p>6. I have several comments on figures.</p> <ul style="list-style-type: none">a. Figure 2: The figure legend needs revision. What is the purpose of parts I – V? The figure itself gives a good idea, but further explanation in the legend is warranted. Additionally, the source, [2] Rasmussen et al. 2019, is not appropriate as a reference for the figure since the cited manuscript focuses on the structure of MscS.b. Figure 3: This legend is insufficient to describe the figure. Note the original figure (which is used here with no changes) has a lengthy figure legend describing the contents of the figure. One sentence fails to describe the figure with enough detail for the reader to understand the diagram.c. Figure 4 is taken directly from Wray et al. 2022, which is not referenced here. Furthermore, the figure legend does not sufficiently describe the figure.	
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Minor REVISION comments	There are typos throughout the work that overall do not affect the readability of the article.	
1. Is language/English quality of the article suitable for scholarly communications?		
Optional/General comments		

PART 2:

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Are there ethical issues in this manuscript?	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	

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