Molecular and physiological adaptations to low temperature in *Thioalkalivibrio* strains isolated from soda lakes with different temperature regimes

Anne-Catherine Ahn, Evelien Jongepier, Jasper Schuurmans, W. Irene C. Rijpstra, Jaap Sinninghe Damsté, Erwin Galinski, Pawel Roman, Dmitry Sorokin, and Gerard Muyzer

Corresponding Author(s): Gerard Muyzer, University of Amsterdam

Review Timeline:  
Submission Date: November 16, 2020  
Editorial Decision: January 18, 2021  
Revision Received: March 8, 2021  
Accepted: March 30, 2021

Editor: Rachel Mackelprang

Reviewer(s): The reviewers have opted to remain anonymous.

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1128/mSystems.01202-20
January 18, 2021

Prof. Gerard Muyzer
University of Amsterdam
IBED
Science Park 904
Amsterdam NL-1098 XH
Netherlands

Re: mSystems01202-20 (Molecular and physiological adaptations to low temperature in Thioalkalivibrio strains isolated from soda lakes with different temperature regimes)

Dear Prof. Gerard Muyzer:

We are pleased to inform you that your manuscript has been accepted for publication contingent upon satisfactorily completing minor modifications based upon reviewer suggestions.

Below you will find the comments of the reviewers.

To submit your modified manuscript, log onto the eJP submission site at https://msystems.msubmit.net/cgi-bin/main.plex. If you cannot remember your password, click the "Can't remember your password?" link and follow the instructions on the screen. Go to Author Tasks and click the appropriate manuscript title to begin the resubmission process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only."

Due to the SARS-CoV-2 pandemic, our typical 60 day deadline for revisions will not be applied. I hope that you will be able to submit a revised manuscript soon, but want to reassure you that the journal will be flexible in terms of timing, particularly if experimental revisions are needed. When you are ready to resubmit, please know that our staff and Editors are working remotely and handling submissions without delay. If you do not wish to modify the manuscript and prefer to submit it to another journal, please notify me of your decision immediately so that the manuscript may be formally withdrawn from consideration by mSystems.

If your manuscript is accepted for publication, you will be contacted separately about payment when the proofs are issued; please follow the instructions in that e-mail. Arrangements for payment must be made before your article is published. For a complete list of Publication Fees, including supplemental material costs, please visit our website.

Corresponding authors may join or renew ASM membership to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.
Thank you for submitting your paper to mSystems.

Sincerely,

Rachel Mackelprang

Editor, mSystems

Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: peerreview@asmusa.org
Phone: 1-202-942-9338

Reviewer comments:

Reviewer #1 (Comments for the Author):

The manuscript presents a transcriptomic analysis of two Thioalkalivibrio strains grown at 10 and 30 degrees with the aim of identifying cold-response processes. The results are somewhat predictable, and very detailed and thorough. The major flaw in the manuscript is the presentation, differences between the two strains are lost in the middle of the text. Figure 1 is a very good illustration of the differences between the two strains, but is not sufficiently described or explained in the text.

Some minor comments:
• Line 106: revise the dilution rate, is it ml/h?
• Line 306: do the authors have any comment on why a BCCT transporter is downregulated at low temperature, and why the other transporters are not differentially expressed? De novo synthesis is not an energy efficient method.
• Lines 415 to 419, what is the significance of these data?

Reviewer #2 (Comments for the Author):

The manuscript entitled "Molecular and physiological adaptations to low temperature in Thioalkalivibrio strains isolated from soda lakes with different temperature regimes" by Ahn et al. is a well-focused exploration of low-temperature adaption in two haloalkaliphilic, sulphur-oxidizing species pertaining to the Thioalkalivibrio genus that is spread in soda lakes worldwide. The temperature-dependent responses in membrane lipid composition, compatible solute accumulation and RNA-Seq assisted gene expression were jointly interpreted to draw a picture on low-temperature acclimation in the tested bacterial strains.

Main Concerns:
1) The experiments were performed partly in batch and continuous culture but I missed clear explanation on why was done so. Clearer description and reasoning for experimental set-up is needed.
2) After reading the manuscript, I started wondering whether the observed increase in intracellular
gycine betaine (that act primarily as osmotic regulators) is linked to the decrease in temperature 
as concluded in Ins 468-469) or to the increase in osmotic pressure (or water activity) as main
physical drivers of adaption response. Previous investigations on the relationships between
compatible solutes and temperature (see references below) pointed that the vitality at low-
temperature is supported by the accumulation of `chaotropic` compounds (Chin et al., 2010) while
glycine betaine is categorized as `kosmotropic` agent (Cray et al., 2015). To strengthen inferences
that the observed changes in membrane lipid composition and the enhanced synthesis of glycine
betaine are unequivocally linked to low temperature and not to temperature-dependent changes
of external osmotic pressure, I suggest performing comparative measurements of the osmotic
pressure (or water activity) at 10 and 30°C, respectively. The discussion should be then adjusted
considering the suggested experimental results.
Chin, J. P., Megaw, J. et al. (2010). Solute determine the temperature windows for microbial survival
and growth. Proc Natl Acad Sci USA, 107(17), 7835-7840.
Cray, J. A., Stevenson, A. et al. (2015). Chaotropicity: a key factor in product tolerance of biofuel-
Abstract.
Ln. 25 (and same at lna. 50, 383) The genus name of Thioalkalivibrio should be followed either by
'sp.' or 'genus' (e.g. The sulphur-oxidizing bacteria of Thioalkalivibrio genus are...)
Ln. 36 -the glycine betaine is osmoprotectant or compatible solute in first place (see also In 56 -
'osmolyte' word is appropriately used there). I suggest rewording here.
I suggest that a take-home concluding remark to be added. What relevance have the findings to
the overall low temperature adaptations in the two investigated strains?
Importance
Ln. 45 In the statement `...extreme parameters, to which bacteria need to adapt.` the word
`bacteria` is not appropriately reflecting the ecosystem biodiversity that, indeed, needs to adapt to
the given environmental conditions. It is too restrictive and I doubt that only bacteria are living there
(see In 59 - `high microbial diversity`, Ins 67-68).
Introduction
I overall noticed the lack of current knowledge on low-temperature adaption in halophiles and/ or
haloalkaliphiles. Most if not entire introduction is dedicated to a very general description of life in
soda lakes without any indication of the scientific background that ingintes the main scientific
question of the manuscript.
Ln. 61 -what is the unit for '4.5'? Be accurate. If it's M (molar), then nature of salts should be
indicated either as total salts or as total sodium.
Ln. 63 The waters in Wadi An-Natrun (Egypt) are rather saline alkaline (with high NaCl contents)
than true soda (carbonate-based) lakes. Instead I suggest indicating only the East African Rift
Valley lakes here.
Lns 66-67 -English proofread is needed here or please, re-word.
Same for In 69, the awkward statement 'involved in the cycling of the biogeochemical elements'
needs correction.
Lns 89-93 -the paragraph here is a brief description of the employed methodology. Although
necessary, I do not think it is needed here.
As stated above, my feeling is that the Introduction lacks focus on the actual knowledge in the low
temperature adaption in salt-stressed microorganisms while large part of it is dedicated to (way
too) general statements.
Materials and methods
Parts of Methodology section are confusing. For example, it is not clear whether the growth rate
and other parameters are measured in batch (see statement at Ins 96-98) or continuous (steady-
state phase) cultures.
Ln 98 - In this paragraph, a batch mode growth is indicated although the perception of continuous
cultivation is induced in the mind of the reader by referring to chemostat/reactor set-up (Ins 105, 108, 118). Was the batch used for starting up the culture? On the other hand, from this section it seems that growth parameters were measured in batch while the adaption mechanisms were assayed in continuous cultivation (see from ln 104 onward) which is odd.

Ins 100-102 - what is the electron donor/energy source? This is however indicated for the continuous cultures (Ins 115).

Ins 105 - why was a growth rate of 0.001 h-1 chosen? Is this value supported by the previous batch experiments? It is close to the maximum growth rates of both strains or it is a 'conventional' value? For all these, an explanatory statement is needed. At Results, Ins 2013-2015, the maximum growth rates for both strains are much higher.

Ins 116-117 - a confusing explanation is given here, please clarify as follows: at ln 116 the authors claimed that `run in two biological replicates`, then, at ln 117 `providing four replicates for each condition`. Please be consistent.

In the same paragraph, it worth clarifying that the second run was initiated starting from a steady-state inoculum.

Ins 120, the verb `to ensure` is inappropriately used here. I suggest `to assess` or `estimate`.

While reading the cell count by flow-cytometry I again missed the point at ln 140: were the cells harvested from the continuous (steady state) culture counted or those collected from the batches? If one wishes (ln 120) `To ensure steady-state, cell count measurements by flow cytometry`, why also counting the batch cultures? See also my points raised above about the misleading on `batch` versus `continuous`.

Ins 140 - the statement `Growth of the batch cultures was modelled` sounds odd. Why modelling the growth in batch?

Please indicate a reference for the analytical procedure described at Ins 146-153. The thiosulfate and sulphate ions determination in high salt solutions using ion chromatography is challenging and a reference to detailed standard methodology would be helpful.

Ins 155 - please specify the origin of bacterial biomass either batch or steady-state continuous culture.

Ins 178 - please explain what `triplicate biological samples` means here. The explanations given above suggested that two biological replicates are analysed from each steady-state cultures (Ins 116 - `run in two biological replicates`). It is not clear whether each replicate culture was GC-analysed in triplicate or one biological sample collected from a steady-state culture was assayed in triplicate. Please clarify throughout the manuscript.

Ins 182 - same as above.

Ins 184-185 - I am confused why RNA-seq is done in continuous (or batch?) and glycine-betaine was measured in batch. Please clarify this. In my opinion, all measurements should have been done in the similarly grown biomass. Explanation on why some determinations were done in batch and others in steady-state continuous cultures is needed.

Ins 199-200 - Define the aim of total protein analysis.

Ins 203 - please be consistent in writing `RNA-Seq` or `RNA-seq` (uppercase or lowercase). Both are accepted but its writing must be the same throughout the manuscript.

Results and Discussion

Ins 207-212 - there are repetitive information that is already provided in the introduction. This part should be omitted.

As stated above (se main concerns and other related little concerns), the reasons for not measuring the growth parameters in continuous (substrate-limiting) conditions is not clear. Instead maximum growth rate is measured in batch (substrate excess).

Ins 232-233 - The statement `on the functioning of essential cellular metabolisms inside the membrane, such as the electron transfer chain` is oddly formulated. I suggest `on the functioning of membrane-associated metabolic pathways such as the respiratory electron transfer`
I think `low concentrations` (or `abundances` as in Table 2 caption) is more appropriate than `low quantities`.

As I suggested above, to rule out the hypothesis that lipid composition is modulated in response to temperature-dependent changes in osmotic pressure, the measurement of this parameter is advisable.

The strains were grown aerobically while the Fab pathway is suggested as an `anerobic` process. How do the author explains activation of Fab conversion at low temperature during the aerobic growth?

Thioalkalivibrio is a genus of Gram-negative bacteria thus lacking cell wall. Peptidoglycans are typical to cell-walled (Gram-positive) bacteria. Could this finding be corroborated by any previous evidences of PG in these Gram-negative strains?

As stated above, the role of glycine betaine as cryoprotective molecule is debatable. The indicated references (44-46, 47, 49) are quite old by comparing with the recent ones I suggested at the beginning of my review. A careful discussion on this issue should be considered in the light of up-to-date literature.

The statement `Intracellular damage of nucleotides and proteins is repaired by an RNA helicase and chaperones` is weird as RNA helicases are not acting in repair of `nucleotides` and the proteins are never `repaired`. Please read this part very carefully and be back with a scientifically sound sentence. In fact, the entire statement at Lns 472-474 needs thorough revision.

In the statement 'Moreover, the essential cryoprotector glycine betaine is also one of the key factors in protecting against osmotic stress.' I found two mistyped terms (cryoprotector/ cryoprotectant, osmic/ osmotic) but more important, the emphasizing of glycine betaine as cryoprotectant and not as omoprotectant (as it was earlier demonstrated). This conclusion might be overall much better supported if osmotic pressure will be measured in the growth medium at both tested temperature.

Most of this paragraph is a rehearsal of the results. In my opinion, meaningful statements denoting the most important lessons authors have learned from their investigations are missing here.

The statement `Intracellular damage of nucleotides and proteins is repaired by an RNA helicase and chaperones` is weird as RNA helicases are not acting in repair of `nucleotides` and the proteins are never `repaired`. Please read this part very carefully and be back with a scientifically sound sentence. In fact, the entire statement at Lns 472-474 needs thorough revision.

In the statement 'Moreover, the essential cryoprotector glycine betaine is also one of the key factors in protecting against osmotic stress.' I found two mistyped terms (cryoprotector/ cryoprotectant, osmic/ osmotic) but more important, the emphasizing of glycine betaine as cryoprotectant and not as omoprotectant (as it was earlier demonstrated). This conclusion might be overall much better supported if osmotic pressure will be measured in the growth medium at both tested temperature.

 Figure 2 and Table 1 - Specify the number of measurements (or biological replicates) from which
standard deviation is calculated.

Figure S1 - Please specify whether the reactor shown in images are in batch or steady-state continuous cultivation modes.

(B) - The cell count was performed both in batch and steady-state modes. As far as I understood, the steady-state is basically corroborated by more or less constant cell number. However, in this figure, the cell count of ALJ2 strain grown at 10°C increases during the steady-state growth in the second reactor or it fluctuates significantly at 30°C. Are there any explanations?

(C) - `principal` (instead of `Principle`). Add clear explanation on how were data sorted for this PCoA.

Table S1 - Define `nd` (`not detected` as in Table 2 OR `not determined`?)
The manuscript entitled "Molecular and physiological adaptations to low temperature in Thioalkalivibrio strains isolated from soda lakes with different temperature regimes" by Ahn et al. is a well-focused exploration of low-temperature adaption in two haloalkaliphilic, sulphur-oxidizing species pertaining to the Thioalkalivibrio genus that is spread in soda lakes worldwide. The temperature-dependent responses in membrane lipid composition, compatible solute accumulation and RNA-Seq assisted gene expression were jointly interpreted to draw a picture on low-temperature acclimation in the tested bacterial strains.

**Main Concerns:**

1) The experiments were performed partly in batch and continuous culture but I missed clear explanation on why was done so. Clearer description and reasoning for experimental set-up is needed.

2) After reading the manuscript, I started wondering whether the observed increase in intracellular glycine betaine (that act primarily as osmotic regulators) is linked to the decrease in temperature (as concluded in Lns 468-469) or to the increase in osmotic pressure (or water activity) as main physical drivers of adaption response. Previous investigations on the relationships between compatible solutes and temperature (see references below) pointed that the vitality at low-temperature is supported by the accumulation of ‘chaotropic’ compounds (Chin et al., 2010) while glycine betaine is categorized as ‘kosmotropic’ agent (Cray et al., 2015). To strengthen inferences that the observed changes in membrane lipid composition and the enhanced synthesis of glycine betaine are unequivocally linked to low temperature and not to temperature-dependent changes of external osmotic pressure, I suggest performing comparative measurements of the osmotic pressure (or water activity) at 10 and 30oC, respectively. The discussion should be then adjusted considering the suggested experimental results.


**Abstract.**

Ln. 25 (and same at ln. 50, 383) The genus name of Thioalkalivibrio should be followed either by 'sp.' or 'genus' (e.g. The sulphur-oxidizing bacteria of Thioalkalivibrio genus are...)

Ln. 36—the glycine betaine is osmoprotectant or compatible solute in first place (see also In 56 – ‘osmolyte’ word is appropriately used there). I suggest rewording here.

I suggest that a take-home concluding remark to be added. What relevance have the findings to the overall low temperature adaptations in the two investigated strains?

**Importance**

Ln. 45 In the statement ‘...extreme parameters, to which bacteria need to adapt.’, the word ‘bacteria’ is not appropriately reflecting the ecosystem biodiversity that, indeed, needs to adapt to the given environmental conditions. It is too restrictive and I doubt that only bacteria are living there (see ln 59 - 'high microbial diversity', Ins 67-68).

Introduction
I overall noticed the lack of current knowledge on low-temperature adaption in halophiles and/or haloalkaliphiles. Most if not entire introduction is dedicated to a very general description of life in soda lakes without any indication of the scientific background that ingintes the main scientific question of the manuscript.

Ln. 61 – what is the unit for ‘4.5’? Be accurate. If it’s M (molar), then nature of salts should be indicated either as total salts or as total sodium.

Ln. 63 The waters in Wadi An-Natrun (Egypt) are rather saline alkaline (with high NaCl contents) than true soda (carbonate-based) lakes. Instead I suggest indicating only the East African Rift Valley lakes here.

Lns 66-67 – English proofread is needed here or please, re-word.

Same for ln 69, the awkward statement ‘involved in the cycling of the biogeochemical elements’ needs correction.

Lns 89-93 – the paragraph here is a brief description of the employed methodology. Although necessary, I do not think it is needed here.

As stated above, my feeling is that the Introduction lacks focus on the actual knowledge in the low temperature adaption in salt-stressed microorganisms while large part of it is dedicated to (way too) general statements.

Materials and methods

Parts of Methodology section are confusing. For example, it is not clear whether the growth rate and other parameters are measured in batch (see statement at Lns 96-98) or continuous (steady-state phase) cultures.

Ln 98 – In this paragraph, a batch mode growth is indicated although the perception of continuous cultivation is induced in the mind of the reader by referring to chemostat/reactor set-up (Lns 105, 108, 118). Was the batch used for starting up the culture? On the other hand, from this section it seems that growth parameters were measured in batch while the adaption mechanisms were assayed in continuous cultivation (see from ln 104 onward) which is odd.

Lns 100-102 – what is the electron donor/ energy source? This is however indicated for the continuous cultures (ln 115).

Ln 105 – why was a growth rate of 0.001 h⁻¹ chosen? Is this value supported by the previous batch experiments? It is close to the maximum growth rates of both strains or it is a ‘conventional’ value? For all these, an explanatory statement is needed. At Results, Lns 2013-2015, the maximum growth rates for both strains are much higher.

Lns 116-117. A confusing explanation is given here, please clarify as follows: at ln 116 the authors claimed that ‘run in two biological replicates’, then, at ln 117 ‘providing four replicates for each condition’. Please be consistent.

In the same paragraph, it worth clarifying that the second run was initiated starting from a steady-state inoculum.

Ln 120, the verb ‘to ensure’ is inappropriately used here. I suggest ‘to assess’ or ‘estimate’.
While reading the cell count by flow-cytometry I again missed the point at ln 140: were the cells harvested from the continuous (steady state) culture counted or those collected from the batches? If one wishes (ln 120) `To ensure steady-state, cell count measurements by flow cytometry`, why also counting the batch cultures? See also my points raised above about the misleading on `batch` versus `continuous`.

Ln 140 – the statement `Growth of the batch cultures was modelled` sounds odd. Why modelling the growth in batch?

Please indicate a reference for the analytical procedure described at lns 146-153. The thiosulfate and sulphate ions determination in high salt solutions using ion chromatography is challenging and a reference to detailed standard methodology would be helpful.

Ln 155 – please specify the origin of bacterial biomass either batch or steady-state continuous culture.

Ln 178 – please explain what `triplicate biological samples` means here. The explanations given above suggested that two biological replicates are analysed from each steady-state cultures (ln 116 - `run in two biological replicates`). It is not clear whether each replicate culture was GC-analysed in triplicate or one biological sample collected from a steady-state culture was assayed in triplicate. Please clarify throughout the manuscript.

Ln 182 –same as above.

Lns 184-185 –I am confused why RNA-seq is done in continuous (or batch?) and glycine-betaine was measured in batch. Please clarify this. In my opinion, all measurements should have been done in the similarly grown biomass. Explanation on why some determinations were done in batch and others in steady-state continuous cultures is needed.

Lns 199-200 –Define the aim of total protein analysis.

Ln 203 –please be consistent in writing `RNA-Seq` or `RNA-seq` (uppercase or lowercase). Both are accepted but its writing must be the same throughout the manuscript.

**Results and discussion**

Lns 207-212 –there are repetitive information that is already provided in the introduction. This part should be omitted.

As stated above (se main concerns and other related little concerns), the reasons for not measuring the growth parameters in continuous (substrate-limiting) conditions is not clear. Instead maximum growth rate is measured in batch (substrate excess).

Lns 232-233 –The statement `on the functioning of essential cellular metabolisms inside the membrane, such as the electron transfer chain` is oddly formulated. I suggest `on the functioning of membrane-associated metabolic pathways such as the respiratory electron transfer`.

Ln 239, 241 – I think `low concentrations` (or `abundances` as in Table 2 caption) is more appropriate than `low quantities`.

Lns 242-243 – English proofread is needed ‘

Lns 245-246 – (`were detected as a result of the difference in cultivation temperature ....`) - As I suggested above, to rule out the hypothesis that lipid composition is modulated in response to
temperature-dependent changes in osmotic pressure, the measurement of this parameter is advisable.

Lns 266-273—the strains were grown aerobically while the Fab pathway is suggested as an `anaerobic` process. How do the author explains activation of Fab conversion at low temperature during the aerobic growth?

Ln 286—Thioalkalivibrio is a genus of Gram-negative bacteria thus lacking cell wall. Peptidoglycans are typical to cell-walled (Gram-positive) bacteria. Could this finding be corroborated by any previous evidences of PG in these Gram-negative strains?

Ln 294—as stated above, the role of glycine betaine as cryoprotective molecule is debatable. The indicated references (44-46, 47, 49) are quite old by comparing with the recent ones I suggested at the beginning of my review. A careful discussion on this issue should be considered in the light of up-to-date literature.

Ln 337, correct the wording `‘(Table -> S9)’ to (Table S9)

Ln 380—‘genes coding for the vitamin B12 biosynthesis have been shown...’

Ln 381—‘linked to oxidative stress protection’

Ln 383—what is the meaning of ‘normally’ here?

Ln 413—English proofread is needed here ‘For genes coding for proteins in the electron transport chain’

Same for sentence at lns 417-419—a rephrasing is needed as it sounds weird in English.

Lns 421-423—rewording and correction are needed as ‘Organisms can sense fluctuations in environmental temperatures by means of changes in the membrane fluidity, as well as by structural changes in...’ is not really well formulated. Organisms can sense by employing means like sensory molecules interlinked in signalling pathways. Instead of ‘means’ (in the meaning of ‘receptors/sensors’), authors describe the ‘response’ (‘changes in membrane fluidity’/structural changes). I am not sure whether I am clear with this. However, a careful reading followed by wording can help. I suggest ‘either Organisms can respond to temperature fluctuations by...’ (if RESPONSE is meant) or ‘Organisms can sense temperature fluctuations by... (followed by listing of sensory/signalling systems)’

Lns 446-449—English proofread for better rephrasing is needed here.

Same at lns 460-461, 469-471, 483-484

Lns 462-463—the sentence is too general and little informative.

Conclusions

Most of this paragraph is a rehearsal of the results. In my opinion, meaningful statements denoting the most important lessons authors have learned from their investigations are missing here.

Lns 472-473—the statement `Intracellular damage of nucleotides and proteins is repaired by an RNA helicase and chaperones` is weird as RNA helicas are not acting in repair of `nucleotides` and the proteins are never ‘repaired’. Please read this part very carefully and be back with a scientifically sound sentence. In fact, the entire statement at lns 472-474 needs thorough revision.
Lns 485-486 – In the statement ‘Moreover, the essential cryoprotector glycine betaine is also one of the key factors in protecting against osmic stress.’ I found two mistyped terms (cryoprotector/cryoprotectant, osmic/osmotic) but more important, the emphasizing of glycine betaine as cryoprotectant and not as omoprotectant (as it was earlier demonstrated). This conclusion might be overall much better supported if osmotic pressure will be measured in the growth medium at both tested temperature.

Figure 2 and Table 1 – Specify the number of measurements (or biological replicates) from which standard deviation is calculated.

Figure S1 – Please specify whether the reactor shown in images are in batch or steady-state continuous cultivation modes.

(B) – The cell count was performed both in batch and steady-state modes. As far as I understood, the steady-state is basically corroborated by more or less constant cell number. However, in this figure, the cell count of ALJ2 strain grown at 10oC increases during the steady-state growth in the second reactor or it fluctuates significantly at 30oC. Are there any explanations?

(C) `principal` (instead of `Principle`). Add clear explanation on how were data sorted for this PCoA.

Table S1 – Define `nd` (`not detected` as in Table 2 OR `not determined`?)
Reviewer #1 (Comments for the Author):

The manuscript presents a transcriptomic analysis of two *Thioalkalivibrio* strains grown at 10 and 30 degrees with the aim of identifying cold-response processes. The results are somewhat predictable, and very detailed and thorough. The major flaw in the manuscript is the presentation, differences between the two strains are lost in the middle of the text. Figure 1 is a very good illustration of the differences between the two strains, but is not sufficiently described or explained in the text.

➔ We thank the reviewer for her/his comments, which improved the manuscript. We increased the number of citations referring to Figure 1 throughout the text. The differences in responses between the strains are actually minimal for most points. Statistical differences are mentioned with the interaction effects, which we now explained as this might not be evident.

Some minor comments:
1. Line 106: revise the dilution rate, is it ml / h?

➔ The dilution rate (D) is expressed in h\(^{-1}\). What you mean is the flow rate (F). Both are interconnected: \( F = D \times V \) (\( V \)=volume of the medium in the reactor, which is in our case 0.5 L). Therefore, in our reactors, the flow rate was approximately 5 mL/h.

2. Line 306: do the authors have any comment on why a BCCT transporter is downregulated at low temperature, and why the other transporters are not differentially expressed? De novo synthesis is not an energy efficient method.

➔ We thank the reviewer for this interesting and challenging question. First of all, we would like to emphasise that the uptake and release systems of glycine betaine are quite complex and that there is only limited knowledge available so far. A possible reason why the BCCT transporters are not differently expressed is that they are not strongly used as we did not supplement the culture medium with glycine betaine. The only external source of glycine betaine might be from lysed cells or cells excreting their surplus of glycine betaine. Most of the glycine betaine, *Thioalkalivibrio* has to produce *de novo*. It is likely that the BCCT transporters work bi-directional. In this case, a downregulation of the BCCT would avoid the release of glycine betaine above a certain concentration. As our hypothesis is speculative, we prefer to leave the reason why a BCCT transporter is downregulated open.

3. Lines 415 to 419, what is the significance of these data?

➔ These lines of the text discuss the up- and downregulation of the electron transport chain (NADH-quinone oxidoreductase) and the ATP synthase. The increase in expression at 10°C compared to 30°C for genes coding for these proteins mean that the cell has an increased need for ATP, and therefore, it increases its production. This can be explained by the extra energy costs the bacteria face to perform the adaptations to the cold, especially for the glycine betaine production. We included this explanation to the text: “The upregulation of the genes involved in energy generation reflects an increased need for ATP to perform adaptations towards low temperature as for example the *de novo* production of glycine betaine (86)”
Reviewer #2 (Comments for the Author):

The manuscript entitled "Molecular and physiological adaptations to low temperature in Thioalkalivibrio strains isolated from soda lakes with different temperature regimes" by Ahn et al. is a well-focused exploration of low-temperature adaption in two haloalkaliphilic, sulphur-oxidizing species pertaining to the Thioalkalivibrio genus that is spread in soda lakes worldwide. The temperature-dependent responses in membrane lipid composition, compatible solute accumulation and RNA-Seq assisted gene expression were jointly interpreted to draw a picture on low-temperature acclimation in the tested bacterial strains.

Main Concerns:
1) The experiments were performed partly in batch and continuous culture but I missed clear explanation on why was done so. Clearer description and reasoning for experimental set-up is needed.

2) After reading the manuscript, I started wondering whether the observed increase in intracellular glycine betaine (that act primarily as osmotic regulators) is linked to the decrease in temperature (as concluded in lns 468-469) or to the increase in osmotic pressure (or water activity) as main physical drivers of adaption response. Previous investigations on the relationships between compatible solutes and temperature (see references below) pointed that the vitality at low-temperature is supported by the accumulation of ´chaotropic´ compounds (Chin et al., 2010) while glycine betaine is categorized as ´kosmotropic´ agent (Cray et al., 2015). To strengthen inferences that the observed changes in membrane lipid composition and the enhanced synthesis of glycine betaine are unequivocally linked to low temperature and not to temperature-dependent changes of external osmotic pressure, I suggest performing comparative measurements of the osmotic pressure (or water activity) at 10 and 30oC, respectively. The discussion should be then adjusted considering the suggested experimental results.


➔ We thank the reviewer for her/his kind words and the English corrections, which improved the manuscript.
➔ Regarding your comment on the batch and continues culture (chemostat) experiments, we hope that we could clarify now our text to easy the understanding of when and why batch or chemostats (continuous cultures) were used. Please see our answers to your questions number 12 and 13 for your better understanding.
➔ Thank you also for your very interesting question whether the increase in intracellular glycine betaine content is triggered by temperature or by osmotic pressure. However, it is known that osmotic pressure decreases with a drop in temperature. Furthermore, we know that at the salinities we used (0.6 M Na+), sodium carbonates are fully dissociated even at 10°C. In previous work, it was seen that the activity coefficient of the sodium carbonate solution decreased above its half saturation point (2 M total Na+), which was determined by direct measurement of osmotic pressure. As sodium carbonates are weak electrolytes, the temperature effect only starts to show at high concentrations, which would be for sodium carbonates above 1.5-2 M Na+ and especially at close to saturation. So, in conclusion, at the concentration of carbonates we used, the temperature would...
not influence the osmotic pressure, so the increase in glycine betaine as was found in our study is an effect of decrease in temperature. Furthermore, even if we would work at a critical salinity (>2 M Na$^+$), the expected effect of decreasing temperature (from the physico-chemical prospect) would been opposite: decrease in solubility/dissociation leading to a decrease in intracellular osmolytes. Please find some references supporting our argumentation:


Abstract.

1. Ln. 25 (and same at Ina. 50, 383) The genus name of *Thioalkalivibrio* should be followed either by 'sp.' or 'genus' (e.g. The sulphur-oxidizing bacteria of *Thioalkalivibrio* genus are...)

➔ We corrected this in the manuscript.

2. Ln. 36 -the glycine betaine is osmoprotectant or compatible solute in first place (see also ln 56 -'osmolyte' word is appropriately used there). I suggest rewording here.

➔ We corrected this in the manuscript.

3. I suggest that a take-home concluding remark to be added. What relevance have the findings to the overall low temperature adaptations in the two investigated strains?

➔ The take-home message is already included in the importance section. Including a take-home message in the abstract was not possible due to the limitation of 250 words.

Importance

4. Ln. 45 In the statement ´...extreme parameters, to which bacteria need to adapt., the word ´bacteria´ is not appropriately reflecting the ecosystem biodiversity that, indeed, needs to adapt to the given environmental conditions. It is too restrictive and I doubt that only bacteria are living there (see ln 59 - ´high microbial diversity´, Ins 67-68).

➔ We changed ´bacteria´ into ´microbial communities´.
5. I overall noticed the lack of current knowledge on low-temperature adaption in halophiles and/or haloalkaliphiles. Most if not entire introduction is dedicated to a very general description of life in soda lakes without any indication of the scientific background that ignites the main scientific question of the manuscript.

➔ We added a paragraph to the introduction discussing the low-temperature effects.

6. Ln. 61 -what is the unit for '4.5'? Be accurate. If it's M (molar), then nature of salts should be indicated either as total salts or as total sodium.

➔ (4,5) are the numbers of the citations.

7. Ln. 63 The waters in Wadi An-Natrun (Egypt) are rather saline alkaline (with high NaCl contents) than true soda (carbonate-based) lakes. Instead, I suggest indicating only the East African Rift Valley lakes here.

➔ We adapted our sentence from “soda lakes” to “These hypersaline alkaline lakes with a large fraction of sodium carbonates” to be more precise.

8. Lns 66-67 -English proofread is needed here or please, re-word.

➔ We corrected the sentence. The original sentence was: “Apart from pH and salinity as stress factors, soda lakes can, depending on their geographical location, also be subjected to different environmental temperature regimes.” and reads now: “Soda lakes can exert multiple types of stress factors on their microbial community in addition to their haloalkaline conditions. For instance, certain lakes are exposed to fluctuating temperatures throughout the year, whereas others are located in regions with a stable temperature profile”

9. Same for ln 69, the awkward statement ‘involved in the cycling of the biogeochemical elements’ needs correction.

➔ We corrected the sentence to: “These communities are actively involved in the biogeochemical cycling of carbon, nitrogen and sulfur”

10. Lns 89-93 -the paragraph here is a brief description of the employed methodology. Although necessary, I do not think it is needed here.

➔ The paragraph briefly describes the methodology used in the manuscript, which we think is important to make the understanding easier for the rest of the manuscript.

11. As stated above, my feeling is that the Introduction lacks focus on the actual knowledge in the low temperature adaption in salt-stressed microorganisms while large part of it is dedicated to (way too) general statements.

➔ Please see our response to comment nº 5.
Materials and methods

12. Parts of Methodology section are confusing. For example, it is not clear whether the growth rate and other parameters are measured in batch (see statement at Ins 96-98) or continuous (steady-state phase) cultures.

- Growth parameters such as maximum growth rate, lag phase, and stationary phase can only be calculated in batch experiments. The batch experiment was performed to determine these growth parameters that were subsequently used in the continuous cultivation experiment. The continuous culture experiment, in which the bacteria are after five-volume changes in the steady state, is essential to study the influence of temperature on the gene expression and the membrane lipid composition as all changes in the RNA-Seq and membrane lipids originate from the difference in temperature and strain between the reactors, and are not influenced by the growth state of the culture as it is the case in batch cultivation. To emphasise that the reactors are in batch mode, we changed the sentence to: “grown in duplicate 500 mL batch-mode reactors at 10°C and 30°C by cell count measurements using flow cytometry. The batch reactors were magnetically stirred”.

13. Ln 98 - In this paragraph, a batch mode growth is indicated although the perception of continuous cultivation is induced in the mind of the reader by referring to chemostat/reactor set-up (Ins 105, 108, 118). Was the batch used for starting up the culture? On the other hand, from this section it seems that growth parameters were measured in batch while the adaption mechanisms were assayed in continuous cultivation (see from ln 104 onward) which is odd.

- As mentioned under comment n°12, the batch experiment was done to determine the different growth parameters. To start the chemostats used for the continues cultivation of the bacteria, we needed to know the maximum dilution rate. In addition to the determination of the growth parameters, we also used in a second time the batch cultivation to obtained enough starting material for the continues cultivation. Once we obtained enough biomass, we switched to the continuous cultivation. After 20 days (i.e., after five volume changes of the culture medium), the cultures reached their steady state and we could take samples for RNA-seq and lipid analysis.

14. Lns 100-102 - what is the electron donor/ energy source? This is however indicated for the continuous cultures (ln 115).

- We used thiosulfate as an electron donor and energy source in all our experiments.

15. Ln 105 -why was a growth rate of 0.001 h-1 chosen? Is this value supported by the previous batch experiments? It is close to the maximum growth rates of both strains or it is a 'conventional' value? For all these, an explanatory statement is needed. At Results, Lns 2013-2015, the maximum growth rates for both strains are much higher.

- The dilution rate of 0.001 h⁻¹ was chosen as it should corresponds to 75% of the lowest maximum growth rate found in the batch cultivation for the different conditions (temperature and strains), which was in our case the one for ALJ2 at 10°C. In the begin,
we had estimated the maximum growth rate just by manual calculations and without the model. This value was lower than the one obtained by the model. Therefore, it is true, we could have used a dilution rate way higher, but which we did not know at that time.

16. Lns 116-117. A confusing explanation is given here, please clarify as follows: at ln 116 the authors claimed that ´run in two biological replicates´, then, at ln 117 ´providing four replicates for each condition´. Please be consistent.
In the same paragraph, it worth clarifying that the second run was initiated starting from a steady-state inoculum.

We cannot say 4 biological replicates as this would mean 4 independent reactors, whereas here, the cultures for the second run were started with biomasses from the first run and with this, they have a common history. We simplified our text to make the understanding easier and we added that the second run was initiated with a “steady state inoculum”.

17. Ln 120, the verb ´to ensure´ is inappropriately used here. I suggest ´to assess´ or ´estimate´.
While reading the cell count by flow-cytometry I again missed the point at ln 140: were the cells harvested from the continuous (steady state) culture counted or those collected from the batches? If one wishes (ln 120) ´To ensure steady-state, cell count measurements by flow cytometry´, why also counting the batch cultures? See also my points raised above about the misleading on ´batch´ versus ´continuous´.

We replaced “to ensure” by “to assess”.

Flow cytometry was used to follow the growth of the bacteria. We first measured the cell numbers over time in the batch-cultivation to obtain the growth curve of the different conditions. With these results, we were able to calculate the growth parameters. Subsequently, we used flow cytometry to followed growth in our chemostat reactors to ensure that our culture was in steady-state at the sampling time. We added an explanatory sentence in the material methods section of the flow cytometry measurement.

18. Ln 140 - the statement ´Growth of the batch cultures was modelled´ sounds odd. Why modelling the growth in batch?

We changed the sentence in “was fit in a model”. The growth data we obtained were introduced into the best-fitting growth curve model, which was in our case the logistic fit model in order to precisely calculate the growth parameters.

19. Please indicate a reference for the analytical procedure described at Lns 146-153. The thiosulfate and sulphate ions determination in high salt solutions using ion chromatography is challenging and a reference to detailed standard methodology would be helpful.
The group of Pawel Roman routinely measures these components in high salt medium. Many papers describe such a methodology, we added two to our Material & Methods in the manuscript. Please find a list of articles, in which a similar method is used:


20. Ln 155 - please specify the origin of bacterial biomass either batch or steady-state continuous culture.

We changed the sentence to “Bacterial cells were harvested from the steady-state cultures”.

21. Ln 178 - please explain what ‘triplicate biological samples’ means here. The explanations given above suggested that two biological replicates are analysed from each steady-state cultures (Ln 116 - ‘run in two biological replicates’). It is not clear whether each replicate culture was GC-analysed in triplicate or one biological sample collected from a steady-state culture was assayed in triplicate. Please clarify throughout the manuscript.

We changed the sentence into: “The measurement was done on three samples per condition.”

22. Ln 182 -same as above.

We changed the sentence into: “The measurement was done on two samples per condition.”

23. Lns 184-185 -I am confused why RNA-seq is done in continuous (or batch?) and glycine-betaine was measured in batch. Please clarify this. In my opinion, all measurements should have been done in the similarly grown biomass. Explanation on why some determinations were done in batch and others in steady-state continuous cultures is needed.

RNA-seq analysis was done on biomass from the steady-state chemostat cultures, while the glycine betaine quantification was done on biomass from batch cultures. This was due to the fact that our steady state cultures did not generate enough biomass to perform all three analyses (i.e., RNA-Seq, lipid analysis, and glycine betaine measurement) with it. We have added this to the text.
24. Lns 199-200 -Define the aim of total protein analysis.

➔ The amount of glycine betaine was given in relation to the amount of protein. We added this explanation to our text.

25. Ln 203 -please be consistent in writing `RNA-Seq` or `RNA-seq` (uppercase or lowercase). Both are accepted but its writing must be the same throughout the manuscript.

➔ We corrected this throughout the text and used “RNA-Seq”.

Results and discussion

26. Lns 207-212 -there are repetitive information that is already provided in the introduction. This part should be omitted.

As stated above (se main concerns and other related little concerns), the reasons for not measuring the growth parameters in continuous (substrate-limiting) conditions is not clear. Instead maximum growth rate is measured in batch (substrate excess).

➔ We prefer to give a short introduction at the beginning of the results and discussion section as a brief reminder. Regarding the confusion on the batch and continuous cultivation, please see our answer to n°12 and n°13.

27. Lns 232-233 -The statement `on the functioning of essential cellular metabolisms inside the membrane, such as the electron transfer chain` is oddly formulated. I suggest `on the functioning of membrane-associated metabolic pathways such as the respiratory electron transfer`.

➔ We changed the text according to your suggestion.

28. Ln 239, 241 - I think `low concentrations` (or `abundances` as in Table 2 caption) is more appropriate than `low quantities`.

➔ We replaced “quantity” by “abundance”.

29. Lns 242-243 -English proofread is needed`

➔ We adapted the text.

30. Lns 245-246 - (`were detected as a result of the difference in cultivation temperature ....`) - As I suggested above, to rule out the hypothesis that lipid composition is modulated in response to temperature-dependent changes in osmotic pressure, the measurement of this parameter is advisable.

➔ Please see our answer to your introduction. In addition, it is well known that the lipid composition of the bacterial cell wall is modulated depending on the temperature (and of course to other environmental fluctuations). As is published in the papers mentioned below:

31. Lns 266-273 -the strains were grown aerobically while the Fab pathway is suggested as an ‘anaerobic’ process. How does the author explain activation of Fab conversion at low temperature during the aerobic growth?

⇒ The Fab pathway is called “anaerobic” as it does not require oxygen in its catalytic reaction and the pathway is not inhibited under aerobic conditions. We changed this in the text to avoid confusion.

32. Ln 286 - *Thioalkalivibrio* is a genus of Gram-negative bacteria thus lacking cell wall. Peptidoglycans are typical to cell-walled (Gram-positive) bacteria. Could this finding be corroborated by any previous evidences of PG in these Gram-negative strains?

⇒ Both, Gram-positive and Gram-negative bacteria possess peptidoglycan-containing cell walls. However, Gram-negative bacteria have a thin peptidoglycan layer between their cytoplasmic and outer membrane.

33. Ln 294 - As stated above, the role of glycine betaine as cryoprotective molecule is debatable. The indicated references (44-46, 47, 49) are quite old by comparing with the recent ones I suggested at the beginning of my review. A careful discussion on this issue should be considered in the light of up-to-date literature.

⇒ Please see our answer to your introduction. Moreover, we are well aware of the kosmotropic and chaotropic concept. Nevertheless, there is ample evidence from a whole range of bacterial groups demonstrating beyond doubt the involvement of glycine betaine in a cold-induced protection mechanism. Although it has not yet been clarified as what makes glycine betaine such a good cold-protectant among the kosmotropes, we present here another piece of evidence for its role in cold acclimation. Please see our references in the text, to which we have added also a more recent literature reference.

34. Ln 337, correct the wording `'(Table -> S9)' to (Table S9)

⇒ We corrected the mistake.
35. Ln 380 - `genes coding for the vitamin B12 biosynthesis have been shown...`
   ➔ We corrected this in the text.

36. Ln 381 - `linked to oxidative stress protection'
   ➔ We changed the text according to your suggestion.

37. Ln 383 - `what is the meaning of `normally’ here?`
   ➔ Normally, under standard conditions (cultivation at 30°C), *Thioalkalivibrio* directly completely oxidizes thiosulfate to sulfate as the electron donor, thiosulfate, is the limiting factor in our chemostat. However, we found out that at 10°C the second part of sulfur oxidation (the zero-valent sulfur oxidation to sulfate) is downregulated, and therefore elemental sulfur accumulated in the reactors. We adapted the text to improve the understanding.

38. Ln 413 - English proofread is needed here `For genes coding for proteins in the electron transport chain`  
   ➔ We changed the text accordingly.

39. Same for sentence at Lns 417-419 - a rephrasing is needed as it sounds weird in English.
   ➔ We changed the sentence.

40. Lns 421-423 - rewording and correction are needed as `Organisms can sense fluctuations in environmental temperatures by means of changes in the membrane fluidity, as well as by structural changes in...` is not really well formulated. Organisms can sense by employing means like sensory molecules interlinked in signalling pathways. Instead of `means` (in the meaning of `receptors/sensors`), authors describe the `response` (`changes in membrane fluidity`/structural changes). I am not sure whether I am clear with this. However, a careful reading followed by wording can help. I suggest `either Organisms can respond to temperature fluctuations by...` (if RESPONSE is meant) or `Organisms can sense temperature fluctuations by...(followed by listing of sensory/signalling systems)`
   ➔ We see what you mean, but we believe that this depends on the angle we are looking. They are all the inducers of the signalling cascade. All of them are classified as thermosensors. The structural changes in DNA and RNA have a direct response to transcription and translation respectively. The structural changes in protein thermometers triggers the induction of the signal transmission cascade. For the changes in membrane fluidity, yes, sensing proteins sense the change in the membrane and then transmit the information. However, they react to the changes in fluidity of the membrane and not to temperature. So, in the first line, it is the changes in the membrane fluidity, and the structures of the DNA, RNA and proteins, which react to changing temperatures. For this reason, we prefer to do not adapt the phrasing of the sentence.

41. Lns 446-449 - English proofread for better rephrasing is needed here. 
   Same at Lns 460-461, 469-471, 483-484  
   ➔ We changed these sentences accordingly.

42. Lns 462-463 - the sentence is too general and little informative.
Conclusions

43. Most of this paragraph is a rehearsal of the results. In my opinion, meaningful statements denoting the most important lessons authors have learned from their investigations are missing here.

We shortened the conclusions and focused on our concluding statement.

44. Lns 472-473 - The statement ‘Intracellular damage of nucleotides and proteins is repaired by an RNA helicase and chaperones’ is weird as RNA helicases are not acting in repair of ‘nucleotides’ and the proteins are never ‘repaired’. Please read this part very carefully and be back with a scientifically sound sentence. In fact, the entire statement at Lns 472-474 needs thorough revision.

The sentence was deleted.

45. Lns 485-486 - In the statement ‘Moreover, the essential cryoprotector glycine betaine is also one of the key factors in protecting against osmic stress.’ I found two mistyped terms (cryoprotector/ cryoprotectant, osmic/ osmotic) but more important, the emphasizing of glycine betaine as cryoprotectant and not as omoprotectant (as it was earlier demonstrated). This conclusion might be overall much better supported if osmotic pressure will be measured in the growth medium at both tested temperatures.

The sentence was deleted.

46. Figure 2 and Table 1 - Specify the number of measurements (or biological replicates) from which standard deviation is calculated.

Figure 2: Glycine betaine concentration was measured on three biological replicates and the standard deviation was calculated from these results.

Table 1: The batch growth to measure the growth parameters was performed in two reactors. Each sample (each time point of a reactor) was measured as well in triplicates on the flow cytometry. The averages of each reactor at each time point were calculated based on the three measurements, and those averages were used to feed the model, in which the different growth parameters were calculated with their standard deviations.

47. Figure S1 (A) Please specify whether the reactor shown in images are in batch or steady-state continuous cultivation modes. (B) - The cell count was performed both in batch and steady-state modes. As far as I understood, the steady-state is basically corroborated by more or less constant cell number. However, in this figure, the cell count of ALJ2 strain grown at 10oC increases during the steady-state growth in the second reactor or it fluctuates significantly at 30oC. Are there any explanations. C) ‘principal’ (instead of ‘Principle’). Add clear explanation on how were data sorted for this PCoA.
(A) The reactor shown in Figure S1A is in continues cultivation mode, seen by the in- and out-flow of the medium. We added the specification that it is in continues cultivation to the Figure’s caption. All three panels (A, B, C) concern the chemostat cultivation.

(B) A chemostat culture needs around five-volume changes of its culture medium to reach steady state. In our case the volume of the reactor was 0.5 L, and therefore we needed to have an exchange of 2.5 L. The time to reach steady state it is of course depending on the dilution rate. With a dilution rate of 0.01 h⁻¹, we reached our steady state in approximately 21 days. It is normal that fluctuations in the cell numbers occur during the run until the culture finds its steady state. As you can see in the figure S1B, the last 5 days in reactor 2 of ALJ2 at 10°C showed a stable cell number and the same counts for the reactors at 30°C.

(C) Plot S1C depicts the results of a Principal Component Analyses (PCA), not those of a Principal Coordinate Analyses (PCoA). For the later it would be appropriate to report the distance or dissimilarity matrix, on which the plot is based, but given that the plot depicts the Principal Component Analyses, this is irrelevant. To make clear what this plot depicts we expanded the caption which now reads: “Figure S1C. Principal component analysis (PCA) based on the expression data of the identified orthologs of *Tv. versatus AL2* and *Tv. nitratis* ALJ2. The symbols represent the projections of samples onto the principal components 1 and 2 based on sleuth-normalized est_counts using the plot_pca utility of the Sleuth R package (Pimentel & McGee 2020).”

- Table S1 - Define `nd` (‘not detected’ as in Table 2 OR `not determined`?)

- Thank you for pointing this out. “nd” stands for “not detected” as the value was below the detection limit. We added this information to the table’s caption.
March 30, 2021

Prof. Gerard Muyzer
University of Amsterdam
IBED
Science Park 904
Amsterdam NL-1098 XH
Netherlands

Re: mSystems01202-20R1 (Molecular and physiological adaptations to low temperature in *Thioalkalivibrio* strains isolated from soda lakes with different temperature regimes)

Dear Prof. Gerard Muyzer:

Your manuscript has been accepted, and I am forwarding it to the ASM Journals Department for publication. For your reference, ASM Journals' address is given below. Before it can be scheduled for publication, your manuscript will be checked by the mSystems senior production editor, Ellie Ghatineh, to make sure that all elements meet the technical requirements for publication. She will contact you if anything needs to be revised before copyediting and production can begin. Otherwise, you will be notified when your proofs are ready to be viewed.

As an open-access publication, mSystems receives no financial support from paid subscriptions and depends on authors' prompt payment of publication fees as soon as their articles are accepted. You will be contacted separately about payment when the proofs are issued; please follow the instructions in that e-mail. Arrangements for payment must be made before your article is published. For a complete list of **Publication Fees**, including supplemental material costs, please visit our [website](http://msystems.asm.org/).

Corresponding authors may [join or renew ASM membership](http://msystems.asm.org/) to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.

**For mSystems research articles**, you are welcome to submit a short author video for your recently accepted paper. Videos are normally 1 minute long and are a great opportunity for junior authors to get greater exposure. Importantly, this video will not hold up the publication of your paper, and you can submit it at any time.

Details of the video are:

- Minimum resolution of 1280 x 720
- .mov or .mp4 video format
- Provide video in the highest quality possible, but do not exceed 1080p
- Provide a still/profile picture that is 640 (w) x 720 (h) max

We recognize that the video files can become quite large, and so to avoid quality loss ASM
suggests sending the video file via https://www.wetransfer.com/. When you have a final version of the video and the still ready to share, please send it to Ellie Ghatineh at eghatineh@asmusa.org.

Thank you for submitting your paper to mSystems.

Sincerely,

Rachel Mackelprang
Editor, mSystems

Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: peerreview@asmusa.org
Phone: 1-202-942-9338

Table S8: Accept
Table S1: Accept
Table S4: Accept
Table S7: Accept
Table S6: Accept
Table S3: Accept
Table S5: Accept
Table S9: Accept
Figure S1: Accept
Table S2: Accept