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SHORT RESEARCH ARTICLE

UPDATE F_1F_0 ATP synthase a-subunit of *Stenotrophomonas* sp.**DL18 from Indian Soda Lake, Lonar: a brief report [version 2; peer review: 2 not approved]**Devendra Lingojar^{1,2}, Ravikant Jadhav², Kachru Gawai¹¹Department of Chemistry, University of Pune, Ganeshkhind, Pune, 411007, India²ATG LAB, Ganesh Nagar, Pimple Nilakh, Pune, 411027, India

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Abstract

Lonar Lake, an Indian soda lake with high alkaline conditions of pH 10.5, is well known for its biodiversity of extremophiles including alkaliphiles. Most of the molecular studies on Lonar Lake alkaliphiles are based on identification by 16S ribosomal RNA (16S rRNA). Various studies have reported alkaliphiles from different alkaline habitats other than Lonar Lake with alkaliphile specific amino acid residues in the F_1F_0 ATP synthase a-subunit. As the data on the alkaliphilic nature of bacteria from Lonar Lake is incompletely understood, the present report comprised of isolation and identification of alkaliphiles from Lonar Lake. Further, we studied the F_1F_0 ATP synthase a-subunit, with reference to alkaliphile specific domains, of one of the facultative alkaliphiles, *Stenotrophomonas* sp. DL18.

Keywords

ATP synthase a-subunit, alkaliphile, Lonar Lake India, 16S rRNA, *Stenotrophomonas* sp. DL18

Open Peer Review**Approval Status**

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1. Thomas Meier , Max-Planck Institute of Biophysics, Frankfurt, Germany 2. Gregory Cook , University of Otago, North Dunedin, New Zealand		
Any reports and responses or comments on the article can be found at the end of the article.		

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UPDATED Changes from Version 1

In response to the referee comments, changes were made including modifications in the title, abstract and the discussion along with the deletion of supplementary figures showing homology modeling of a subunit, growth studies in alkaline conditions i.e. pH 7.0 to pH 12.0 and the scanning electron microscopy study. The aim of this updated article is to report on alkaliphile specific motifs in the α -subunit of ATP synthase from the isolated alkaliphile, *Stenotrophomonas* sp. DL18.

Scope of the updated brief report

Lonar Lake in India is a well known source of alkaliphiles. This is the first report from Lake Lonar that is based on alkaliphile specific motifs in the α -subunit of ATP synthase from the isolated alkaliphile, *Stenotrophomonas* sp. DL18. Although the present study does not have X ray crystallography data of purified ATP synthase and mutational studies, this brief report may initiate investigations of other facultative alkaliphiles research from Lonar Lake.

See referee reports**Introduction**

ATP is molecular currency for a living cell, which is not only growing and dividing but also continuously responding to external environmental stimuli. To survive in extreme conditions, microorganisms devise specific adaptive mechanisms. Along with other transporter proteins, ATP synthase is widely considered one of the key molecules for adaptation in alkaline conditions. Hydrolysis of nucleoside triphosphates, specifically ATP, provides the chemical energy to drive a wide variety of cellular reactions. ATP synthesis is central to ATP production during oxidative phosphorylation. These are energy-coupling *factors* and hence called F_1F_0 -ATP synthases. The F_0 integral membrane protein complex provides a transmembrane pore for protons, whereas the peripheral protein F_1 is involved in catalysis¹. F_1 consists of five subunits, $\alpha_3\beta_3\gamma_1\delta_1\epsilon_1$, with a ring of α - and β -subunits alternating around a single γ -subunit². F_0 is a membrane embedded domain with subunits ab_2c_{10-15} . Out of these subunits, α -subunit is a stator and c -ring is a rotor ring through which ions (H^+ or Na^+) are translocated³⁻⁶. Each c -chain from the ring consists of two α -helices traversing the membrane and the polar loop extends out of the membrane to interact with the γ - and ϵ -subunits. A cytoplasmic F_1 catalytic domain is connected by a membrane-embedded F_0 domain by a central ($\gamma\epsilon$) and peripheral ($b_2\delta$) stalk²⁻⁶. In general, downhill ion translocation across the membrane through F_0 causes rotation of the c -ring, which induces conformational changes in the catalytic β -subunit and results in ATP synthesis. However, in alkaline conditions, the external pH is high i.e. above pH 8.0, which poses a major thermodynamic problem for ATP synthesis. Hence, the cytoplasmic pH needs to be maintained 1.5 to 2.3 pH units below the external environment, which generates an optimal condition for ATP synthesis.

Most studies have focused on the proton translocation channel in the α -subunit of ATP synthase⁷⁻⁹. However, similar experimental

evidence from other geographic locations such as highly alkaline soda lakes needs further exploration to understand pH homeostasis in facultative alkaliphiles. This study explores the ATP synthase α -subunit of a facultative alkaliphilic aerobe isolated from Lonar Lake.

Methods**Isolation and identification of bacteria from the soda lake**

In the present study, underwater sediment soil samples were collected 350 meters away from the Kamalaja Devi Temple end of Lonar Lake, Buldhana, Maharashtra, India. The initial screening was performed at pH 9.5. Then isolates were further studied in the range of pH 7 to pH 12. Across the pH gradient, morphologically different pink, orange and white bacterial colonies were observed. The bacterial genomic DNA of optimally grown bacteria at pH 9.5 with orange pigmentation was isolated by the DNAzol method¹⁰.

Further polymerase chain reaction (PCR) and sequencing of 16S rRNA was carried out for identification of bacterium¹¹.

PCR and DNA sequencing of ATP synthase α -subunit of *Stenotrophomonas* species DL18

The following primers (Integrated DNA Technologies, USA) were used for the amplification of the 1.7 Kbp PCR product of the ATP synthase F_0 subunit (consisting of *atpB*, *atpC* and *atpA* genes), based on a reference strain (i.e. *S. maltophilia* K279a): forward primer Steno atp1F (5' CCTGGCGGATCCTTATGATCTCCG 3') and reverse primer Steno atp1R (5' CAGTGAGGATCCTTAGATCTCCGAGGCCAGCT 3'). Briefly, a PCR reaction mixture of 100 μ l was prepared: 10x PCR buffer, 50 mM $MgCl_2$, 10 mM dNTP mix, 100 picomoles each of forward and reverse primers, 5 units of *Taq* DNA polymerase (Chromous Biotech, India) with *Pfu* and 200 ng of bacterial genomic DNA template in nuclease free water. The thermal cycling conditions were: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30s, primer annealing at 55°C for 30s and primer extension at 72°C for 3 min. A final extension was carried out at 72°C for 5 min. The amplified PCR product of 1.7 kbp was visualized on 1% agarose gel and the results were documented by *Bio-Rad* gel documentation system with Quantity One software (Bio-Rad, USA). Further, the DNA sequencing of the ATP F_0 subunit of the selected isolate was performed via primer walking. An ATP synthase comparative study was performed by multiple sequence alignment with other strains from different categories i.e. acidophiles, neutrophiles and alkaliphiles, as shown in Figure 1.

Results and discussion**Bacterial identification and ATP synthase F_0 subunit amplification**

The orange pigmented bacterium was identified as *Stenotrophomonas* sp. based on a NCBI BLAST analysis of the 16S rRNA

<i>Bacillus pseudofirmus</i> OF4	157	FKIIEEFANTLT <u>LG</u> MRLFGNVYA KE ILMILLVTAGTS--	193
<i>Bacillus clausii</i> KSM	157	FKVIEEGSNALT <u>LG</u> MRLFGNIYA KE ILMLLLVSLGTT--	193
<i>Bacillus megaterium</i> DSM	154	LKIIEEFANTLT <u>LG</u> RLRYGNIYA GE ILLSLLAGLATT--	190
<i>Enterococcus hirae</i> ATCC9790	156	MKLIEEFTNLLT <u>LAL</u> RLYGN IA GEVLLTLIANMMNN--	192
<i>Escherichia coli</i> K12	195	LEGVSLLSKPVS <u>LG</u> RLFGN MYA GELIFILI-----	225
<i>Thioalkalivibrio</i> sp. K90mix	207	LEFINLLAKPAS <u>LAL</u> RLFGN LYA AEMIFILIGVMYS---	242
<i>Stenotrophomonas</i> sp. DL18	185	LNIVEWLSKPIS <u>LAM</u> RLFGN MFG GEIVFLLIWVLGG---	220
<i>Stenotrophomonas maltophilia</i>	185	LNIVEWLSKPIS <u>LAM</u> RLFGN MFG GEIVFLLIWVLGG---	220
<i>Acidithiobacillus ferrooxidans</i> ATCC53993	173	MSLIEEITKPLS <u>LG</u> RLFGN MFAG ELVFLLLAMLFP----	207
<i>Thioalkalimicrobium cyclicum</i> ALM1	183	MTFIEEIAPKIS <u>LAL</u> RLFGN IFAG ELVFMLIALIGGTMA	221
<i>Acidiphilium cryptum</i>	162	IEIVSFLSRPIS <u>LSV</u> RLFAN ITAG HVMWEVFAGFMLMLV	200
<i>B. pseudofirmus</i> OF4	194	GLIGMFGAFLPL-IVWQAF <u>GL</u> FIGAIQAYIFAMLAMVYMAHKVES	237
<i>B. clausii</i> KSM	194	AVYWGIFAFVPL-IVWQAF <u>S</u> IFIGSLQAYIFAMLAMVYMSHKVEQ	237
<i>B. megate</i> DSM	191	GFLGTIGAAIPM-LLWQGF <u>S</u> IFVGAIQAFIFTMLTMVYLSHKVSS	234
<i>E. hirae</i> ATCC9790	193	LGWFSPLAIPLEMVWIAF <u>S</u> FIGSIQAFVFTLSMVYMSHKIEV	237
<i>E. coli</i> K12	226	AGLLPWSQWILNVPWAF <u>H</u> ILIIITLQAFIFMVLTIIVYLSMA-SE	269
<i>Thioalkalivibrio</i> sp.K90mix	243	AGLILGLAGGVLVHVGWAF <u>H</u> ILVVPLQAFIFMVLTVVYLDMAYSE	287
<i>Stenotrophomonas</i> sp. DL18	221	AGFFGAIAGGAFGLGWMLF <u>H</u> LLVIPLQAFIFMMLSIVYLSLS-ED	264
<i>Stenotrophomonas maltophilia</i>	221	AGFFGAIAGGVFGLGWMLF <u>H</u> LLVIPLQAFIFMMLSIVYLSLS-ED	264
<i>A. ferrooxidans</i> ATCC53993	208	---WWG--GLVMGEVWSLF <u>E</u> SLIIILQAFIFTVLTVVYLGMANMQ	247
<i>T. cyclicum</i> ALM1	225	AALFWAPLQAILDLGWAF <u>H</u> ILIIITLQAFIFMVLTIIVYLSMAHDE	269
<i>Acidiphilium cryptum</i>	206	VGVVAIIIPGLNIALTAL <u>E</u> FLVAFLOAYVFALTCLYLHDAIHM	250

Figure 1 Alignment of α -subunit of ATP synthase from neutrophiles and alkaliphiles. Conserved sequences are underlined and significant variant residues for channel formation are shown in bold. These residues are mainly from TMH-4 and TMH-5. *Bacillus megaterium* DSM 319 (GenBank Accession number: [YP_003600289](#)) grows strictly in neutral pH environments. However, *Bacillus clausii* KSM K-16 (GenBank Accession number: [YP_177349](#)) is a reported alkaliphile.

gene sequence and titled *Stenotrophomonas* sp. DL18 (GenBank Accession number: [JN995612](#)). Microbiological studies we performed showed that *Stenotrophomonas* sp. DL18, which optimally grows at pH 9.5, is an aerobic, facultative alkaliphilic with curved rod morphology.

A BLAST analysis of the ATP synthase α -subunit of the *Stenotrophomonas* species DL18 suggests maximum identity with *Stenotrophomonas* species SKA14 (GenBank Accession number: [ZP_05136035](#)) at the amino acid level (259 amino acid residues were identical from a total of 266 amino acid residues in the SKA14 species).

Comparative studies of ATP synthase α -subunit of *Stenotrophomonas* species DL18

Table 1 lists the bacterial species used in the comparative studies. The amino acid residue arginine, which was found to be conserved in almost all bacterial species in the α -subunit, was observed at position 200 (Arg²⁰⁰) in DL18. Moreover, other amino acid residues that were conserved in most of the bacteria include Leu²⁰⁷, Arg²¹⁰, Leu²¹¹, Gly²¹³, Asn²¹⁴, Gly²¹⁸, Gln²⁵², Ala²⁵³ and Phe²⁵⁵ (*E. coli* numbering system for ATP synthase α -subunit) in the trans-membrane helix-4 (α TMH-4), α TMH-5 and the corresponding amino acids were also found in the DL18 alignment (Figures 1 and 2).

Table 1 List of bacterial species used in the comparative studies of the ATP synthase α -subunit of *Stenotrophomonas* sp. DL18 (first row).

Species	Accession number
<i>Stenotrophomonas</i> sp. DL18	AET99165
<i>Stenotrophomonas</i> sp. SKA14	ZP_05136035
<i>Bacillus pseudofirmus</i> OF4	YP_003426326
<i>Bacillus clausii</i> KSM-K16	YP_177349
<i>Enterococcus hirae</i> ATCC 9790	YP_006487510
<i>Bacillus megaterium</i> DSM 319	YP_003600289
<i>Stenotrophomonas</i> sp. K279a	YP_001973793
<i>Theoalkalivibrio</i> sp. K90mix	YP_003461818
<i>Escherichia coli</i> K12 DH10B	YP_001732559
<i>Acidithiobacillus ferrooxidans</i> ATCC 53993	YP_002221206
<i>Acidiphilium cryptum</i> JF-5	YP_001233541
<i>Thioalkalimicrobium cyclicum</i> ALM1	YP_004537849

It was observed that the most conserved arginine residue of the α -subunit (Arg²⁰⁰ of *Stenotrophomonas* sp. DL18) was aligned with the expected position of the facultative alkaliphile *Bacillus pseudofirmus* OF4 (i.e. Arg¹⁷²). This positively charged Arg²⁰⁰ plays an elemental role in the function of the F₀ rotor³. Lys¹⁸⁰ in

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1  MAGEALTPTSIIQHHLQNLTPVGNLGMFWHIIHVDFTLTAVLMGLVIVVAFWLGTRKAT 60
61 AGVPGKWQAFVEICLFEVDRQAKDTHGKSKLVTPIAITIFFWILMMNLIKMIPADFIK 120
121 PLELAGIHYWKPVPVTADVNTLGMASVFFLMLFFALKSKGLGGFIKEFLTAPFGKWMMP 180
181 FNLILNIVEWLSKPISLAMRLFGNMFGGEIVFLLIWVLGGAGFFGAIAGGAFGLGWMLFH 240
241 LLVIPLQAFIFMMLLSIVYLSLSEDAH 266

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Figure 2 Complete amino acid sequence of *Stenotrophomonas* species DL18 ATP synthase α -subunit. The most conserved amino acid residues are shown as bold and underlined.

B. pseudofirmus OF4 was replaced by Gly²⁰⁸ in the DL18 strain. In addition, glycine and alanine residues were observed at the same position in other alkaliphiles including Gly²⁰⁶ in *Thioalkalimicrobium cyclicum* ALM1, and Ala²³⁰ in *Theoalkalivibrio* sp. K90mix, as shown in Figure 1. On the other hand, glycine was also at the same corresponding position in alignment for the acidophiles, *Acidithiobacillus ferrooxidans* ATCC 53993 and *Acidiphilium cryptum* JF-5 (Figure 1). As reported by Ivey DM *et al.*, Lys¹⁸⁰ and corresponding amino acids were located in α TMH-4 in *Bacillus pseudofirmus* OF4 and other alkaliphiles^{12,13}. In the DL18 strain, a histidine residue, which is conserved in other reference species of the same genus (e.g. *Stenotrophomonas* species K279a, *Stenotrophomonas* sp. SKA14 and other alkaliphiles including *T. cyclicum* ALM1 (His²⁴⁴), and *Theoalkalivibrio* sp. K90mix (His²⁶²)), was present at position 240 (His²⁴⁰) (Figure 1).

It has been proposed that Gly¹²⁰ and Lys¹⁸⁰ forms a channel for the proton uptake pathway of the α -subunit through which protons pass onto the neighboring c -subunit in *B. pseudofirmus* OF4⁷. However, one study has reported that His²⁴⁵ along with the Gly²¹⁸ and Glu²¹⁹ positioning plays a critical role in F_0 ion translocations in *E. coli*⁸, and in the present study on *Stenotrophomonas* sp. DL18, His²⁴⁰, Gly²⁰⁸ and Glu²⁰⁹ were observed at the same corresponding positions as shown in Figure 1. McMillan¹⁴ *et al* demonstrated the importance of residues with a basic side chain along with its pKa value in ATP synthesis in alkaline environments. However, that mutation study was carried out in the *Bacillus* sp. TA2.A1, specifically involving the 180th residue in the α -subunit. In that amino acid substitution study, amino acid residue Lys¹⁸⁰ in the α -subunit was mutated to Gly¹⁸⁰, His¹⁸⁰ and Arg¹⁸⁰. These mutations indicated the ATP synthesis at neutral, neutral to alkaline and only in alkaline conditions (>pH 8.5), respectively, i.e. residue with a strong base, such as lysine or arginine, was ideally appropriate to function at alkaline pH. This was particularly marked for histidine, which has a pKa in a neutral range, showing significant ATP synthesis activity at pH 9.0.

E. coli K12 DH10B considered a neutrophile, can adapt to slightly alkaline conditions up to pH 8.0 and this may be due to the presence of His²⁴⁵. In addition, exchange mutations at α ^{E219-H} and α ^{H245-E} showed similar ATP synthase activity in *E. coli*. Moreover, the α G218-K substitution effect was suppressed by

α ^{H245-G} mutation in *E. coli*. Hence, these studies in *E. coli* signified that the positions Gly²¹⁸ and His²⁴⁵ along with Glu²¹⁹ had a critical interaction with the F_0 subunit function. In addition, equivalent amino acid residue studies were also reported from the facultative alkaliphile *B. pseudofirmus* OF4 α -subunit⁷⁻⁹.

From alignment, Glu²⁰⁹ of the DL18 strain was conserved in alkaliphiles, neutrophiles and some acidophiles except His¹⁸⁶ in *Acidiphilium cryptum* (Figure 1). In addition, the position of Gly²¹² in *B. pseudofirmus* OF4 corresponds to Ser²¹² in *Enterococcus hirae* ATCC 9790 (neutrophile), His²⁴⁵ in *E. coli* and His²⁴⁰ in the *Stenotrophomonas* sp. DL18 while glutamate in acidophiles is positioned at Glu²²² in *A. ferrooxidans* and Glu²²⁵ in *A. cryptum* as shown in Figure 1. This showed that channel formation may involve a glycine residue along with other residues, specifically those with acidic, basic and neutral side chains, which play vital roles in ATP synthesis in acidophile, alkaliphiles and neutrophiles. Hence, these residues are found to be critical in channel formation⁷⁻⁹. Similarly, in *Stenotrophomonas* sp. DL18, amino acid residues. Gly²⁰⁸ and His²⁴⁰ may form the proton translocation channel. Thus, the basic side chain residue His²⁴⁰ and other amino acid residues may responsible for growth of the *Stenotrophomonas* sp. DL18 at high alkaline pH.

Author contributions

Devendra Lingojar contributed to the conception and design of the project, acquired, analyzed and interpreted the data, review of literature, and prepared the manuscript. Ravikant Jadhav helped prepare the manuscript, contributed to data collection and conducted Bioinformatics analysis. Kachru Gawai provided overall guidance for the project, helped prepare the manuscript and revised it critically for intellectual content. All authors approved the final version of the article.

Competing interests

No competing interests were disclosed.

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Thomas Meier

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The authors have slightly improved the manuscript by removing some questionable supplementary figures and some modifications in the text.

The science presented is still quite poor and it does not really answer any biological question (at least I could not find such a question in the manuscript). This is a report about the sequence of the a-subunit. The presented alignments with other species are incomplete and do not explain the criteria used to state what is considered as conserved.

Some specific points:

- Abstract: The authors write that (more than 1) alkaliphile was reported in this work but the work actually reports just about 1 species, i.e. *Stenotrophomonas* sp. DL18 (?)
- Intro: Reference 1 is from 1992 and outdated as a review
- Figure 1: The alignment does not show the complete a-subunit and the discussion includes residues, which are not shown here, e.g. Gly120
- In general the language could be improved. There are examples throughout the text. It should be read and edited by a native speaker.
- The authors use the expression "F₀ subunit". The F₀ is a subunit complex, consisting of several proteins. The expression is misleading to say the least.
- Fig. 1, legend: It is not clear how TMH-4 and TMH-5 is defined. This should also be referenced in the main text (page 3). What is the criterium to say that some residues are conserved, while others are not? This should be defined in the text too.
- Fig. 2, legend: "The most conserved amino acid reside...": improve language and give criterium

for conservation.

- The text on page 4 jumps back and forth in the topic: from Lys180 to His262, then back to Lys180 (and now also Gly120) for three lines, then five lines about Gly218 and Glu219, then back again to Lys180 (with reference to McMillan *et al.*).... This is very confusing and cannot be understood.

- Finally what is the reason for the authors stating that DL18 is an alkaliphile despite the fact that this a-subunit does not have Lys180 (?)? I just could not follow the arguments any more and got lost.

- A conclusion sentence at the end perhaps helps.

Being fully aware of how tight the funding situation of the authors might be, I have to stay with my conclusion that the scientific content of this work is not enough to be published in *F1000Research*. As long as no new experimental data is supplementing this work and no biological question is addressed, I do not see this work as suitable for this journal. In the current form, the authors should consider to deposit the sequence in the sequence database with a remark that it contains some (but not all) alkaliphilic motifs in the a-subunit sequence of the ATP synthase.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Version 1

Reviewer Report 18 January 2013

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Gregory Cook

Department of Microbiology, University of Otago, North Dunedin, New Zealand

I don't see this study has much relevance to the field as it adds nothing to the literature in this area and is not great science e.g. the homology model is not based on a structure but just a model. The authors should have also included a c subunit analysis to check for the presence/absence of Na⁺-binding motifs. Overall the work is not of a standard in which others in the field can benefit.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 09 January 2013

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Thomas Meier

Department of Structural Biology, Max-Planck Institute of Biophysics, Frankfurt, Germany

- The overall content of this work is very poor. It contains: growth of bacteria in medium, PCR, sequencing, sequence alignment and a homology model. It is what is done today in a student practicum.
- The homology model (structure of subunit α , Supplementary Figure 3) is a homology model based on a model (this was NO real structure), which basically also means, one could just take 4 random alpha helices and put them together in a bundle.
- The authors should mention in the methods section which genes from F_0 were amplified by PCR.
- Supplementary material figure 1: The window ledge and the arm of the photographer is visible on the picture.
- Supplementary material figure 2: The quality of the left EM micrograph is poor, the picture is not in focus. The picture on the right side is distorted, I cannot see anything there.
- Page 5 line 1: The Cook lab did not use *Bacillus pseudofirmus* OF4 but rather *Bacillus* TA2.A1
- Figure 2 and Figure 3: The alignment should contain *Bacillus* TA2 because the authors refer to it in the text on Reference 25 (McMillan *et al.*).
- Figures 2 and 3 appear to be very similar. It may have made more sense to include all the information in one figure to avoid redundancies and save space.

The scientific value of this work is questionable. It is a good start to investigate the α -subunit from *Stenotrophomonas* but it is too early to publish as such.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Comments on this article

Version 2

Author Response 16 May 2013

Devendra Lingojar, ATG LAB, India

This article is all about first time reporting alkaliphile without alkaliphile specific sequences in the ATP synthase a subunit. Most of the earlier reported studies were all about a few bacterial strain ATP synthase studies, including that of *Bacillus pseudofirmus* OF4. Most of the articles, including a [Nature review in 2011 by Dr. Krulwich TA](#), stressed greater importance of these amino acid residues in bacterial pH homeostasis with reference to alkaliphiles.

Both the referees have queries and objections as to why crystallographic studies were not reported and why mutation studies were not reported. I ask a question, how many alkaliphiles were reported from alkaline sources without alkaliphile specific sequences? What is mentioned in this updated version of this article is all about reporting an alkaliphile from the source known for decade. But at the molecular level, studies on Lonar Lake alkaliphiles are incompletely understood or rather not reported earlier.

Competing Interests: No

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