

Peer-Review Record:

Archaeal Lineages within the Human Microbiome: Absent, Rare or Elusive?

Hans-Peter Horz

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Reviewer 1: Anonymous

Reviewer 2: Anonymous

Editor: Hans-Peter Klenk, Michael W. W. Adams and Roger A. Garrett (Guest editor of special issue “Archaea: Evolution, Physiology, and Molecular Biology”)

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First Round of Evaluation

Round 1: Reviewer 1 Report and Author Response

With great interest I have read the article by H.-P. Horz about “Archaea in the human microbiome”. In general, it is a very interesting article, showing that there are currently major problems in the proper detection of human-associated archaea.

I have a few general comments and a few very specific ones, which are given below.

First of all, I’d suggest a proper proof-reading by a native speaker. The language is generally quite casual and could be adapted a little bit more towards a scientific language in some paragraphs of the text. In addition, there are severe problems with respect to comma placements. Some examples are given below (specific comments).

Response: The manuscript has now been proof-read by a native speaker.

Secondly, the author mentions, that this article is “not recapitulating exhaustively all studies on archaeal diversity in humans”. Well, this is what usually reviews do. The article itself is nicely written and highly interesting, but resembles strongly an opinion paper.

Response: I agree with that comment. However, in the case of human-associated Archaea there are already a number of recent and comprehensive review-articles, which are cited in the manuscript. Therefore, I would prefer to keep this article in its current way with the character of an “opinion”-paper.

Thirdly, I have some problems with the title. Archaea are not absent from the human microbiome. In the article, the author makes clear, that he is talking about specific archaeal lineages, which appear to be absent, but the title is misleading in this regard.

Response: In order to avoid confusion, the title has now been modified as follows: “Archaeal lineages within the human microbiome—absent, rare or elusive?”

Here are some specific comments:

- Page 1, Line 17: “However, they appear” - *Done*
- Page 1, Line 22: methodological concepts cannot describe them, but they can be used to describe them - *Done*
- Page 1, Line 24 (and elsewhere): please be clear in what you mean with “novel” archaea. Novel means undescribed (never seen so far). But you are also talking about archaea that have not been associated with humans so far, e.g. the Thaumarchaeota that are mentioned. The lineages found on human skin seem not to be novel lineages, but they have now been associated with humans.

Response: Throughout the manuscript this aspect has now been made clearer. “Novel archaea” in humans are now addressed as distinct archaeal lineages previously not associated with humans.

- Page 1, abstract: Be clear in tense usage, e.g., Line 27: demonstrated - *Done*
- Page 2, Line 9 and following: the definition of microbiome as the entire microbial world within a system is also well accepted. Nice discussion here: <http://schaechter.asmblog.org/schaechter/2013/10/stc-poll-1-the-definition-of-the-microbiome-.html>

Response: Thanks for this link. Indeed there appear to be different opinions about how the microbiome should be defined. I prefer to keep the definition in the manuscript according to the cited reference.

- Page 2, Line 15: The “emphasis” does not refer to the term “microbiology”. The studies are performed with a different emphasis.

Response: This has now been re-phrased.

- Page 2, Line 17 and elsewhere: correct citation style (avoid “e.g., [x]”)

Response: The citation style has been corrected.

- Page 2, Line 17: Rephrase sentence: “While by itself...”

Response: The sentence has been re-phrased.

- Page 2, Line 22: replace “custom” by “usual” - *Done*
- Page 2, Line 26: replace “Because” by “Since” and “neglected” by “ignored”; add comma after “microbiology” - *Done*
- Page 2, Line 33: rephrase “Maybe it would, however the crux is... is still so fuzzy” (typical example for a very casual wording) - *Done*
- Page 3, Line 1: add comma: “rare members, if” - *Done*
- Page 3, Line 9: change to “domains. However, the unique” - *Done*
- Page 3, Line 10: rephrase; it is not clear what is meant by “lend them the character”; use key species instead of keystone species

Response: The sentence has been re-phrased and the word “key species” has been used.

- Page 3, Line 24: add comma: “(methanogens), these” - *Done*
- Page 3, Line 25: what is meant by “were highly appreciated for their”

Response: The word “appreciated” has been changed by “recognized”, the sentence should be clear now.

- Page 3, Line 32 and following: More information about the effect of methane is provided here: <http://www.ncbi.nlm.nih.gov/pubmed/16293652>

Response: Thanks for this additional article however, I think that enough references are already cited about this issue.

- Page 3, Line 32: “ on these and later investigations” - *Done*
- Page 3, Line 39: “in vivo” should be written in italics; as also “par excellence” on Page 4, Line 12. - *Done*
- Page 4, Line 1: Remove “Besides methane as end-product” (does not fit together)

Response: This sentence has been re-phrased.

- Page 4, Line 2 and following: please be clear: what is the carbon and what is the energy source? Methanobrevibacter uses CO₂; Methanosphaera acetate (both are not mentioned)

Response: The sentence has been modified and the energy sources mentioned in order to avoid confusion.

- Page 4, line 12 and following: Unable to understand this sentence. Rephrase? In this paragraph, it might be good to add more information about syntrophy or hydrogen transfer (energy?). Interplay of sulfate-reducers and methanogens?

Response: This sentence has been re-phrased for better understanding. I think the aspect of interspecies hydrogen transfer is sufficiently addressed in this paragraph. For more detailed information the manuscript refers the reader to pertinent literature dealing with this issue.

- Page 4, Line 23 and following: Difficult discussion. Newest publication suggest to keep only two phyla: doi: 10.1093/gbe/evu274

Response: This additional reference has been included into the manuscript.

- Page 5, Line 19: are the other cultured species mentioned in reference [40]?

Response: This reference focusses on one species, the text in the manuscript has been changed accordingly.

- Page 6, Line 1: not a novel lineage was discovered, but they were found to be human associated for the first time

Response: This aspect has been clarified throughout the manuscript now.

- Page 6, Line 9: “until recently archaea” - *Done*
- Page 6, Line 34: Add a question mark after microbiome. - *Done*

- Page 7, Line 5 and following: I guess, a large problem is the usage of certain databases. Sequences cannot be classified correctly, if the used database lacks archaea. Thus, the archaeal are removed from the dataset, representing “unclassified sequences”. I assume, the correct data analysis is a larger problem than DNA extraction and holds also true for metagenomics analyses.

Response: I would prefer to restrict the discussion about the methodological problem to the DNA extraction protocols and PCR-methods. The point is, that if someone intends to find archaeal lineages it should be obvious that appropriate databases need to be used. However, even with the intention to find archaeal lineages people are probably not so aware that some experimental steps in the laboratory are less appropriate for archaeal cells.

- Page 7, Line 42: “There are, however, some” - *Done*
- Page 8, Line 24: What does this mean: “methane..., which are involved in interspecies hydrogen transfer”? Please rephrase

Response: This sentence has been re-phrased for clarity.

Round 1: Reviewer 2 Report and Author Response

The aim of the manuscript of HP Horz is to question the low representation of one of the three domains of life, Archaea, within the human microbiome. Is it reflecting reality or a bias in our analyzing process? Belong they to our “rare biosphere” or is it a bias in samplings? And if being truly members of our “rare biosphere”, what can however be their clinical significance?

These questions are of importance as some archaea are suspected to be at least associated if not involved in various human diseases, and therefore they have to be considered in the present time of bloom of research projects undertaken in human microbiota analysis.

The manuscript is clearly written, hypotheses and arguments are well stated, with examples taken from recent research results, for various archaea and various microbiomes (gastrointestinal tract, oral cavity and the skin). It can be considered as an “opinion paper” with interesting comments, rather than a “standard review paper”. Moreover, there is an open and wise view of some current development.

I have only few comments, some being really trivial, listed below: about commas (often used in my native language, French), there are propositions but please ask a native English speaker to verify. Their presence should help the reader.

Page 2, Line 23: of course it is right to say the “prokaryote domain Archaea”: however, as archaea are still unfortunately not known as very different organisms compared to bacteria or eukaryotes for many people, I would prefer to not see “prokaryote”: it is a bit confusing for many people and maintains unconsciously a very close relationship between bacteria and archaea, which does not reflect the reality.

Response: I agree with this comment, the term “prokaryote” has been removed from the text.

Page 4, Line 16–17: Please add a sentence like “... three so-called enterotype networks” [30] even if likely more complex than initially thought (reference of Jeffery IB, Claesson MJ, O'Toole PW, Shanahan F. Categorization of the gut microbiota: enterotypes or gradients? *Nat Rev Microbiol.* 2012 Sep; 10(9): 591–592. PubMed PMID: 23066529.)

Response: This reference has now been incorporated into the manuscript and the text modified accordingly.

There is no ref 36 in the text, and in the reference list, ref 35 and 36 correspond to the same paper.

Response: This error has been corrected.

Page 5, Lines 21–22: to avoid any confusion, perhaps could be changed into “the seventh order of methanogenic archaea, belonging to the class Thermoplasma and neighboring the order Thermoplasmatales”. Ref considering this part are likely [41–44]

Response: The sentence has been modified as suggested, and an additional reference included.

Page 5, Line 24: to be more precise, could be changed into “... are like many other methanogens dependent on H₂ and use methanol for methanogenesis” (in fact methanol use is quite uncommon among methanogens, but seems common for human GIT-associated archaea).

Response: The sentence has been modified accordingly.

Page 5, Lines 25: likely change ref 44 by a more appropriate (precise) one: ref from pubmed = Borrel G, Gaci N, Peyret P, O'Toole PW, Gribaldo S, Brugère JF. Unique characteristics of the pyrrolysine system in the 7th order of methanogens: implications for the evolution of a genetic code expansion cassette. Archaea. 2014 Jan 27;2014:374146. doi: 10.1155/2014/374146. eCollection 2014.

Response: The references have been exchanged.

Page 7, Line 1: in order to moderate, proposition: “... while ignoring the frequent and unique rigid ... and the unique chemical composition of their membranes among life beings.”

Response: Thanks for the suggestion, but I would prefer to keep the sentence as it is.

Page 8, Line 9: “Black” or “Native Africans”? If “Black” (I don’t know if it is correct to use this term), should be written “Blacks”.

Response: Thanks for this comment. The sentence has been modified. The cited study has analyzed individuals working in a Canadian hospital. Accordingly the text has now been modified as follows: “... staff members of two Canadian hospitals from different geographic origins (i.e., Caucasians, Africans, Orientals and Indians) ...”

References: please check the journal rules, more specifically if words in the title should or not contain italics (for example, *Methanobrevibacter smithii*, in vitro, ...”

Response: Names of organisms are in italics now in the reference.

Second Round of Evaluation

Round 2: Reviewer 1 Report and Author Response

This paper has improved a lot. One comment:

- The statement on Page 4, Lines 4/5 is still not correct. It should read: “of distinct substrates as energy and carbon source, such as ...”.

- CO₂ should be listed.

Response: I thank this reviewer for evaluating the revised manuscript and for his comment. The requested correction has now been made as suggested, the term carbon source and CO₂ are now included in the text.

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