

Interactive comment on “Microbial communities responsible for fixation of CO₂ revealed by using *mcrA*, *cbbM*, *cbbL*, *fthfs*, *fefe-hydrogenase* genes as molecular biomarkers in petroleum reservoirs of different temperatures” by J.-F. Liu et al.

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The study by Liu et al. aimed to evaluate the potential for microbial CO₂ fixation and CH₄ production in subsurface oil reservoirs through analysis of functional genes. While the aim of the study is useful for understanding the long-term microbial impacts on CO₂ sequestration, the approach used by the authors fails to provide meaningful data to address the objective as only microbial diversity was assessed. The authors use a cloning and sequencing approach to assess the phylogenetic diversity of five functional genes involved in CO₂ fixation in petroleum reservoirs. While knowledge of the diversity of

C751

microbial groups involved in CO₂ fixation is useful it does not provide any information about the potential activity of these groups in petroleum reservoirs. Parallel analyses looking at the abundance of genes using quantitative PCR and/or analysis of gene transcripts and cultivation showing that CO₂ fixation can be carried out under reservoir conditions would provide the necessary data needed to understand potential microbial function in petroleum reservoirs.

Reply: Thanks for this valuable comment.

(1) In addition, the authors should perform additional statistical analysis to correlate geochemical conditions in the reservoirs with the different phylogenetic groups detected.

Reply: Thanks for this valuable suggestion. The CCA analysis with “Vegan” package in R has been conducted and added to the revised manuscript.

(2) The study does show that microbial communities harboring functional genes for microbial CO₂ fixation inhabit four oil reservoirs; however, analysis of only 4 oil reservoirs is not enough to support the statement that these functional groups “inhabit widely in oil reservoirs.”

Reply: Thanks for this valuable comment. The sentence in abstract of “. . .inhabit widely in oil reservoirs, which . . .” has been rewritten as “. . .inhabit in different oil reservoirs, which . . .”.

(3) The paper would benefit from having a combined results and discussion section as the results are currently too detailed and the discussion contains a lot of results.

Reply: The results and discussion sections have been rewritten.

Specific Comments:

L. 1-3: The title should be changed to reflect the actual study, which was a phylogenetic assessment of CO₂-fixation genes in petroleum reservoirs.

C752

Reply: Thanks for this valuable suggestion. The title of this manuscript has been changed to “The phylogenetic diversity of functional microorganisms for CO₂ fixation and bioconversion revealed by using *mcrA*, *cbbM*, *cbbL*, *fthfs*, *fefe*-hydrogenase genes as molecular biomarkers in petroleum reservoirs with different physiochemical properties”.

L. 16: this was not a comprehensive survey. A comprehensive survey would have included more analyses that provided a full picture of microbial diversity, e.g., quantitative PCR, total community analysis.

Reply: The word “comprehensive” in this sentence has been replaced with “specific” which seems to be more suitable.

L. 23: correct the spelling of Methanomicrobiales

Reply: “Methanomicrobiales” has been changed to be “Methanomicrobiales”

L. 59-61: please reference this statement

Reply: This statement is further instructed and elaborated by sentences followed.

L. 62: incorrect usage of “respectively”

Reply: This sentence has been revised.

L. 82-85: revise

Reply: this sentence has been revised to be “This study focus on the investigation of microorganisms involved in in situ microbial fixation and conversion of CO₂ into CH₄ in subsurface oil reservoirs through analysis of functional genes (*cbbM*, *cbbL*, *fthfs*, [FeFe]-hydrogenase encoding gene and *mcrA*) by characterization of the functional microbial communities which inhabited in the production waters.”

L. 96-97: revise for clarity and please give the volume filtered here

Reply: The water phase was separated from the oil/water mixture by heating the sam-

C753

ples to 50 °C and by phase separation with sterilized separatory funnels. The microbial biomass in the water fraction was finally concentrated onto membrane filter (0.22- μ m-pore-size).

L. 113: correct the reference

Reply: Revised as suggested.

L. 117-118: please clarify

Reply: The PCR primers and conditions for these five functional genes were all instructed just before this sentence.

L. 130: was bidirectional sequencing performed

Reply: Only the *fthfs* gene portion (1102 bp) was sequenced bidirectionally, single-read sequencing was used for the fragments of other genes in this study.

L. 135: BLAST is not a network service but an algorithm that searches the GenBank database. Please revise.

Reply: This sentence has been revised. “. . . one representative sequence was chosen from each OTU to compare with sequences in the GenBank database using the BLAST algorithm to identify nearest related ones.”

L.130-138: please add the methods for translating the nucleotide sequences into amino acid sequences.

Reply: Sequences of representative OTUs from clone libraries as well as reference from GenBank were translated into corresponding amino acid sequences using EMBOSS Transeq tool (http://www.ebi.ac.uk/Tools/st/emboss_transeq/) with default parameter (Standard Genetic Code). This has been incorporated into the proper part of the revised manuscript.

L. 144: please add a section discussing the geochemistry

C754

Reply: The concerning information on the sampling oil fields was added in section 2.1.

L. 145: the heading is incorrect. This is not a section on CO₂ fixation but a section on the diversity of CO₂ fixation functional genes.

Reply: The heading has been rewritten as "The diversity of CO₂ fixation functional genes"

L. 149-150 and elsewhere: please refer to figure 6 for the relative abundance data.

Reply: Revised as suggested.

L. 151 and elsewhere: This is the incorrect nomenclature for the Proteobacteria classes. It should be Betaproteobacteria or β -Proteobacteria.

Reply: Revised as suggested.

L. 151: clone cbbL-XJ6-32 is not in the tree. Please verify that clone names are consistent between the text and the tree.

Reply: The "cbbL-6-32" was written with a mistake in the phylogenetic tree (Fig. 1) and has been corrected to "cbbL-XJ6-32" in the revised manuscript.

L. 164 and elsewhere: it is not necessary to mention GenBank in the results if it is stated in the methods.

Reply: Revised as suggested.

L. 166: add the phylogenetic groups to the tree as brackets for clarity.

Reply: Revised as suggested.

L. 187: this is not acetogenesis but detection of acetogen functional genes. This applies to all headings.

Reply: Revised as suggested. All the headings have been checked /changed.

L. 226-227: this is too strong of a statement. You cannot make global oil
C755

reservoir statements from your limited dataset, tone down throughout the paper.

Reply: Thanks for this comment! This sentence has been rewritten to be "All these results show that the CO₂-reducing methanogens inhabit predominantly in these four oil reservoirs with different physiochemical properties."

L. 248 and elsewhere: use proper formatting for genus names (capitalize proper names).

Reply: Revised as suggested.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/12/C751/2015/bgd-12-C751-2015-supplement.zip>

Interactive comment on Biogeosciences Discuss., 12, 1875, 2015.