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Biogeosciences
Discussions

Interactive comment on "Diversity and abundance of n-alkane degrading bacteria in the near surface soils of a Chinese onshore oil and gas field" *by* K. Xu et al.

K. Xu et al.

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Authors' Comments to Referee 1

The authors wish to thank referee 1 for his/her efforts in reviewing our manuscript and for the helpful and constructive comments provided. Below are our point by point responses to all issues raised by the referee. The manuscript has been revised accordingly.

Referee:In their study, the authors used the alkB gene as marker for alkane degrading microorganisms in the environment. They compared the communities in soils above an oil and gas field to the surrounding soils to evaluate the potential of alkB as indicator

for oil and gas prospecting. In general, it is a well conducted study and a well written manuscript which shows that the abundance of proteobacterial alkane degraders might be indicative for the presence of an oil or gas field. Nevertheless, I have some minor comments: Many alkane degraders have several different enzyme systems to catalyse the first oxidation step. Is there anything known if the Actinobacteria or the Proteobacteria have several AlkB-like monooxygenases? This should be considered when deducing abundance from gene copy numbers. Is there anything known on horizontal gene transfer of alkB, or how well does it reflect the 16S rRNA phylogeny? I think the manuscript could be improved by combining data and removing some of the figures such as Figure 3 or Table S1 (see below).

Response:We thank referee 1 for the encouraging assessment and the detailed and constructive criticism. In fact, as the kindly referee said, many alkane degraders have several different enzyme systems to catalyse the first oxidation step. Depending on the chain-length of the alkane substrate, different enzyme systems are required to introduce oxygen in the substrate and initiate biodegradation. For simplicity, there are three categories: C1–C4 (methane to butane, oxidized by methane monooxygenase-like enzymes), C5–C16 (pentane to hexadecane, oxidized by integral membrane non-heme iron (alkB) or cytochrome P450 enzymes), and C17+ (longer alkanes, oxidized by essentially unknown enzyme systems). While the mechanism of alkane mineralization has been not fully understood, it is known that alkane hydroxylase (alkB) is one of the key enzymes in the process. It has been detected in Proteobacteria as well as the Actinobacteria. Although several hydroxylases have been confirmed recently to be involved in alkane degradation, such as P450, almA, and ladA, alkB is most important and prevalent in aerobic oildegrading bacteria.

AlkB gene can be used as molecular marker to detect the ecological role of hydrocarbon-degrading bacteria in environments. All alkB proteins are conserved in six hydrophobic stretches that are likely to span the cytoplasmic membrane, and eight to nine histidines that are essential for the alkane-hydroxylizing activity. Based on these

conserved moieties, degenerate primers for polymerase chain reaction (PCR) detection of alkB genes have been designed. With these primers, the alkB gene has been detected in a variety of environments, including Mississippi shallow aquifers, California soil, Arctic and Antarctic soil, Brazilian soils, German barley fields and grassland soil, Mediterranean beaches, Alaskan and Antarctic marine sediments, and recently in the Timor Sea. Moreover, the alkB gene can be used to predict the potential of biodegradation of oil-related environments. A relationship between alkB gene abundance and n-alkane degradation has been confirmed. Quantification of alkB can be developed to monitor microbial community change during bioremediation of hydrocarbon-contaminated Antarctic soil. The knowledge about the diversity of alkB gene as well as their hosting bacteria in certain area helps to evaluate the impact of hydrocarbons. Therefore, alkB gene is the best biomarker which can reflect the 16S rRNA phylogeny to date.

The horizontal gene transfer of alkB might happen. However, recently, Shen et al. found that actinobacterial alkB gene sequences derived from the whole genome shotgun sequencing projects are phylogenetically characterized, and they exclude the possibility of horizontal gene transfer of the alkB gene in these bacterial groups. The possibility of the horizontal gene transfer of alkB between Actinobacteria and Proteobacteria will be tested in our further research.

References:

Beilen, J. B. v., and Funhoff, E. G.: Alkane hydroxylases involved in microbial alkane degradation, Appl Microbiol Biotechnol, 74, 13-21, 2007.

Kloos, K., Munch, J. C., and Schloter, M.: A new method for the detection of alkane monooxygenase homologous genes (alkB) in soils based on PCR-hybridization, J Microbiol Methods, 66, 486-496, 2006.

Wasmund, K., Burns, K. A., Kurtböke, D. I., and Bourne, D. G.: Novel Alkane Hydroxylase Gene (alkB) Diversity in Sediments Associated with Hydrocarbon Seeps in the

Timor Sea, Australia, Appl Environ Microbiol, 75, 7391-7398, 2009.

Wang W, Wang L, Shao Z.:Diversity and Abundance of Oil-Degrading Bacteria and Alkane Hydroxylase (alkB) Genes in the Subtropical Seawater of Xiamen Island, Microb Ecol, 60, 429-39, 2010.

Shen F.T, Young L.S, Hsieh M.F, Lin S.Y, Young C.C. Molecular detection and phylogenetic analysis of the alkane 1-monooxygenase gene from Gordonia spp. Syst Appl Microbiol, 33, 53-59, 2010.

Referee:Page 14869 Line 5: full stop after bacteria

Response:Sorry for this confusion. The full stop have been added.

Referee:Page 14870 Line 2: van Beilen and Funhoff

Response:Thanks for the referee's careful review. This item has been changed.

Referee:Page 14870 Line 4: full stop after reference

Response:The full stop after reference have been added.

Referee: Page 14874 Line 26: remove the listed T-RF sizes, the link to Figure 2 is sufficient

Response:We have deleted the listed T-RF sizes

Referee:Page 14875 Line 16-20: The T-RFLP data only represents relative abundance. Comparing the T-RFLP results to the qPCR, the total abundance of alkane oxidizers seems to increase in the oil and gas field (if the total bacteria stay the same). It would be actually helpful to not only plot the ration of alkB to 16S, but also to give the absolute numbers. To me, it rather seems like the Actinobacteria do not change, whereas the Proteobacteria are becoming more frequent.

Response:This is a very valuable suggestion for improving the quality of our manuscript. We have given the absolute numbers in our revised manuscript. See

the updated Table S2 and Fig. S1 in “Electronic Supplementary Material”.

Referee:Page 14880 Line 12: please correct the reference: the authors are Matthias Noll, Diethart Matthies, Peter Frenzel, Manigee Derakshani, Werner Liesack

Response:We must apologize for being so careless. The authors of this reference have been corrected.

Referee:Figure 3: I do not understand this figure. Which are the sites used for this graph? Just a random subset of sites plotted in the ordination? Or the three sites from which the clone libraries were constructed? I think it would make more sense to include this information into the ordination and remove this figure. The ordination gives a better overview of the data structure, and also points out that T-RF74 is indicative for oil and gas field sites.

Response:Thanks for the referee’s good suggestion. As the kindly referee said, the three sites were from which the clone libraries were constructed. They are B3, B14 and B34 sampling site (see Fig 1), respectively. We will add the annotation in our revised manuscript. Figure 3 was used to directly indicate the differences in oil and gas field sites. Therefore, we would like to retained the figure 3.

Referee:Table S1:The OTU definition for representative OTUs is not explained. Is it based on identity or a similarity cutoff (e.g. 95%)? It is furthermore redundant as most information is shown in the phylogenetic tree in Figure 2. The number of clones for each representative OTU could be included within the tree and the table could be removed. Figure S1 is not well described and labeled. It consists of 3 sub-parts of which each includes four graphs. These graphs are all labeled Fig.1 to Fig.4. Please remove. Also, the labels for the x-axes are missing. Although the shapes of the curves have meaning in these figures, in my opinion, it would be enough to condense the information to one overview table. It is also not explained what definition is used for phylotype.

Response:This is a very valuable suggestion for improving the quality of our

manuscript. A similarity cutoff of 97% is used to define an OTU (operational Taxonomic Units). We will indicated in the text and Figure 2. The redundant Figure S1 has be removed. The numbers of clones for each representative OTU are included within Figure 2. Moreover, Figure S1 have also been removed. The information has been condensed to one overview table (Table S1). The definition of phylotype is same as the definition of OTU.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/9/C7956/2013/bgd-9-C7956-2013-supplement.pdf

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