General comments

The authors studied the denitrifying bacterium Marinobacter hydrocarbonoclasticus strain 617, (1) by describing the gene clusters encoding the 4 denitrification reductases (deduced from the strain ST2 genome) and some of the regulatory denitrification genes, (2) by performing in silico analysis of DNA binding sequence of FNR and NarL, (3) by measuring the transcript levels of the 4 denitrification genes under microoxic culture conditions, (4) by determining that the nosRZDFYL gene cluster is in fact a polycystronic operon, (5) by comparing (i) the growth and (ii) the dynamic of nitrate and nitrite (measuring nitrate and nitrite) of strain 617 cultured under microoxic and anoxic conditions, and (iii) the dynamic of the activities of the NO and N2O reductase in whole-cell (measuring enzymes activity) in these cultures. The manuscript is in general well written but suffers of some inconsistencies.

We can start with the letter to the reviewer and editor (which raised some expectations of what I was expected to see in the text)

1- "The denitrification pathway of a marine bacterium Marinobacter hydrocarbonoclasticus is characterized for the first time." Not necessary since the same research group did publish data on strain 617 for the enzymatic activities of denitrification enzymes. Besides the genome of Marinobacter hydrocarbonoclasticus ST2 is in NCBI since 2012 and with annotations related to the denitrification pathway.

2- "The approaches used go from transcriptomics to the measure of metabolites." Transcriptomics is usually referred to RNA seq. Here qPCR assays were used. One cannot measure metabolites from qPCR. This provide indication of the level of gene expression.

3- "The results support the hypothesis that this organism mitigates the release of nitrous oxide to the atmosphere." This is a big jump here from artificial culture conditions (with sufficient nitrate and carbon source) to open ocean. N2O was not measured in the cultures. The N2O reductase is active yes, but it does not mean that N2O is not released.

Format:

The authors did not respect the format for the "Abstract" as defined in the "Author guides", as well as the interlined spaces (should have been 2 line space)

Introduction

Throughout all the manuscript, "aerobic culture conditions" has to be changed to "oxic culture conditions"; "microaerobic" to "microoxic" and "anaerobic" to "anoxic".

The section is too detailed for some aspects, and not enough detail in other aspects.

For instances, lines 61-62 can be deleted and also lines 63-75 (the whole paragraph), as the concept described in these lines were not a subject of study in this work.

In line 77, not all denitrifying bacteria have their denitrification genes clustered in close vicinity, as it seems to be with M. hydrocarbonoclasticus. For instance, Hyphomicrobium denitrificans.

Line 89: Reference to Zumft 1997. Although Zumft 1997 did a very comprehensive review on Denitrification at that time, it is getting a bit old reference and some more recent reviews can be added.

Line 90: delete "other families of bacteria, especially" It is true that not many marine bacteria with denitrifying activities are subject of studies. However, some works should be mentioned. For instance the marine bacterium Methylophaga nitratireducenticrescens JAM1 with the work of Mauffrey et al 2015 (In Frontiers in Microbiol) and Mauffrey et al. 2017 (in PeerJ). Another marine denitrifying bacterium is Shewanella baltica that some works were reported.

Line 98. Replace "of urge" by "a need". Still in this line, I would delete "as is the case of Marinobacter hydrocarbonoclasticus." However, this could be better developed down to why Marinobacter hydrocarbonoclasticus should be used as a model to the characterization of the denitrification pathway in marine bacteria.

Line 100. Replace "species" by "spp." Also, replace "are one of" by "among". For the abundance, I looked to the two references. Yoon 2003 described only Marinobacter litoralis, and provided no indication on the abundance of Marinobacter. Kaye JZ, and Baross JA. 2000 provided some evidence of abundance of halotolerant bacteria in pelagic water and it seems that Marinobacter spp. are found in several samples. I would refer as Marinobacter spp. been ubiquitous in marine environments.

Lines 102-103. Replace "species" by "strains". Also this sentence has to be rewritten. Grimaud 2012 described the genome of M. hydrocarbonoclasticus SP17 (Type strain), Ling 2017 the genome of M. hydrocarbonoclasticus STW2, and Singer 2011 the genome of Marinobacter aquaeolei, not M. hydrocarbonoclasticus. Delete "pointing out its biological importance" in line 103 (too trivial).

Line 109, at the end of the sentence (sources.), please provide at least one reference. This would be a good place to explain why M. hydrocarbonoclasticus is a good candidate to study the denitrification pathway in marine environments. Related to M. hydrocarbonoclasticus 617, what makes strain 617 different from the two other M. hydrocarbonoclasticus strains?

Lines 109-110. Delete "Therefore, a detailed analysis of the denitrification pathway in this organism is timely important,"

Lines 111-112. The authors seem to have done a good amount of works with the enzymatic systems of this denitrifying bacterium. Briefly summarized the results of these studies. The Figure 1 provides no substantial information that it is not been described from other systems, and can be moved in supplemental data.

Line 117. add "strain 617" after hydrocarbonoclasticus

M&M

Move (lines 128-132) "Identification of putative gene functions was performed by BLAST search using the web platforms of blastp and blastn suites (NCBI) (Altschul et al. 1997) (statistic data are provided in Supplementary Information Table S1), gene and protein alignments using ClustalOmega (McWilliam et al. 2013), and comparison of gene organization with other denitrifying microorganisms." with the Table S1

Line 143. Starkey 1938. This reference is very old. Some reader may have problem getting this reference. Provide the recipe.

Line 149. Confusing: Described the dimension of the reactor. 2-L: is the work volume of the reactor? Also, what do you mean as 10% volume? Is the reactor is 20 L work volume with 2L medium? Or did you inoculated 200 mL of the final inoculum with 1.8L medium in the reactor?

Line 157: "Cultures were visualized under an optical microscope to confirm its purity." I have strong reservation that observation of the cultures by optical microscope can provide indication that the cultures are contaminated or not.

Line 159. "small aliquots" How the aliquots were sampled and what was the volume? Provide a schematic of the reactor with location(s) of where you took the samples.

Line 164: Cultures in the 100 ml flasks. Are these flasks flushed with N2 gas?

Line 173. Again, how the aliquots were sampled and what was the volume?

Line 176: What was the volume taken from the reactor for nucleic acid extraction?

Line 194. What is this reference "2004"? How can I find this in the list of reference? This is probably taken from instructions provided by Applied Biosystem. This is not a proper reference. Normalization of the transcript levels of the targeted genes was done with the transcript levels of the 16S ribosomal RNA gene determined also by qPCR. In line 202 (and Figure 3), the authors claimed that the expression of the 16S genes remained stable. If it is so, provide all the data in raw data files (not only for one culture but also for the three cultures with the 16S results and the calculation). I have strong reservation of using 16S gene as normalization control. First, there are 3 copies of this gene in the genome, which makes complicate whether these copies are expressed equivalently. Second, 16S rRNA genes are not a housekeeping gene and their expression are strongly influenced by the growth phases, meaning that per cell there are more 16S transcripts during the exponential growth than during the stationary phase, which is not desirable for a housekeeping gene. A real housekeeping gene keeps the same number of transcripts per cell whatever the growth phase. Finally, more than one housekeeping gene have to be used to get reliable results.

Lines 210-212. Please provide data for the two other replicates in the raw data. Another way to illustrate the results is to have Standard deviation to the time points (i.e. samples taken at 3, 6, 8 h, could be 5.7 h ±SD)

Line 231: defined "RT"

Line 237: "NO-saturated water to a final concentration of 9.6 μM" . Provide the source of NO (company). How NO-saturated water was made? How the concentration of NO was measured?

Line 249. N2O-saturated water. How this solution was made? How the concentration of N2O was measured?

Results

Genomic organization of denitrification genes in M. hydrocarbonoclasticus

Lines 257-352. First of all, this subsection described the gene annotations of strain SP17, not strain 617. The authors assumed that both genomes are the same, for the denitrifications genes, which seems to be true, as they have no difficulty to get PCR amplification from primers derived from the strain SP17 genome. However, the authors should acknowledge that differences could occur between their strain and strain SP17. The part of this subsection is a summary of the annotations of strain SP17 that Grimaud reported in 2012 and the annotations are already available in NCBI since then. For these lines, no new information is provided. The authors should only point out the new information not on the NCBI site.

Line 379. The NarL5 and NaL6 sites sit in the narK gene. This should be mentioned and goes with the probability that this is not a proper binding site. For instance, narK gene and narGHJI are part of a polycistronic operon in Methylophaga nitratireducenticrescens. See Mauffrey et al 2015 (Front in Microb).

Line 391 (and this subsection). Are these the results of the 2L-reactor cultures?

Lines 398-403. Without proper statistical analysis (results from replicate cultures), you cannot draw any conclusion on whether one gene is more expressed than the other genes. Here the only information is relative to the time of higher expression for specific gene.

Figure 3. Use linear scale to show the OD as this is the most usual manner to illustrate growth. Also, because it is difficult to clearly see distinctly the first 10 hours, the growth should be shown (or expand) for the first 20 hours only.

Line 420. State why M. hydrocarbonoclasticus cultures were performed under microoxic conditions (this is only explained at the end of the manuscript).

Figure 5. Use linear scale to show the OD. Here, the way the two panels is presented, we have to look carefully to notice that under anoxic conditions the OD did not grow more than 0.1 log OD (or 1.2 OD) and under microoxic conditions it reached upto 0.4 log OD or 3.5 OD. This is 3 times the biomass yield. This is a substantial difference that could be explained by the presence of oxygen. Although oxygen was recorded at 0% in the microoxic conditions, that means that aerobic respiration occurred and generated more biomass than under anoxic conditions.

Line 425. What is a dead phase?

Line 425. The diauxic growth observed by Hahnke 2014 is observed during the transition of nitrite accumulation after nitrate reduction, to the beginning of nitrite reduction. During this transition, the growth slowed before resuming when nitrite started to be reduced. In figure 5, the log scale for growth is misleading. With the raw data provided, when I was able to draw at linear scale, and it was obvious that for the first 10 hour of culture, the grow rate was faster than the 10 to 50 hours period with a linear growth at slower growth rate. The authors mentioned that the other replicates behaved the same, but this should be presented in the raw data. As mentioned for the comment above for Figure 5, the first period probably illustrates both respirations (oxygen and nitrate) that occurred in the culture and the second period is the growth with oxygen as the only electron acceptor. A proper control should have be done with strain 617 cultured under oxic conditions (full aeration) with and without nitrate.

Line 435. No aeration provided so no substantial growth afterward as nitrate is exhausted.

Lines 465-471. The authors measured the NO and N2O reductase activities, but never measured the NO and N2O concentration generated in the cultures. The recording of activities in whole-cell (which I found elegant) only measured the reductase abundance in the cell (as mg protein extract). Therefore, in line 466 referring to "maximum NO reduction rate" should be "maximum NO reductase production by the cells". See also line 540. The same goes with the N2O

Figure 6.
Except for putative motifs found upstream of denitrification gene clusters, none of the regulatory pathway was proven from the results. This remains speculative and replicates what was proven in other denitrifying bacteria. This can be moved in the supplemental data

Table 1. Delete. This is information available in many reviews or books or moved this in supplemental data

Table 2.
Added the expected length of the PCR products, and indicate which primers were used for qPCR

Discussion
First paragraph is a repetition of the Results

Line 491. Delete Remarkably.

Lines 497-498. As mentioned before, although oxygen was recorded at 0, it does not mean it was absent, but because it was consumed by cells as electron acceptor (respiration).

Line 504-508. Too speculative. As mentioned before, low aeration provided O2 in the medium. A control is missing with qPCR assays done with cultures under anoxic conditions flushed with N2 so no oxygen is provided.