

Peer-Review Record:

Regulation of Three Nitrogenase Gene Clusters in the Cyanobacterium *Anabaena variabilis* ATCC 29413

Teresa Thiel and Brenda S. Pratte

Life 2014, 4, 944-967, doi:10.3390/life4040944

Reviewer 1: Yuichi Fujita

Reviewer 2: Anonymous

Editors: John C. Meeks and Robert Haselkorn (Guest Editor of Special Issue “Cyanobacteria: Ecology, Physiology and Genetics”)

Received: 17 October 2014

First Revision Received: 21 November 2014

Accepted: 4 December 2014

Published: 11 December 2014

First Round of Evaluation

Round 1: Reviewer 1 Report and Author Response

Anabaena variabilis is a heterocystous cyanobacterium that is unique because of the presence of three sets of nitrogenases; *nif1*, *nif2* and *vnf*. Nif1 and Vnf encode sets of proteins for Mo-nitrogenase and V-nitrogenase, respectively, and both nitrogenases are localized only in heterocysts. *nif2* encodes proteins for another Mo-nitrogenase, which is mainly localized in vegetative cells only under anoxic conditions. This review by Thiel and Pratte summarize how these three gene sets for nitrogenase are regulated in *A. variabilis*; the paper discusses a series of experiments. The authors describe what is revealed and what has remained unclear in the regulation of three of the nitrogenase systems. I felt that this review was written very well. I have some comments for this review as follows:

- (1) I understand intuitively the uniqueness of the three nitrogenase systems in *A. variabilis*. However, I wonder how such a system is unique in cyanobacteria. I suggest that the authors mention briefly how many cyanobacterial strains have similar three sets of nitrogenases, or whether *A. variabilis* ATCC 29413 is the only known strain possessing three nitrogenase systems.
- (2) Introduction part: I suggest that more of the latest papers should be cited in the introduction of function of Nif gene products.

- Page 2, Line 62: The chemical formula for FeMo-co is now [7Fe-9S-Mo-C-homocitrate]; Spatzal *et al.* (2011) Science 334, 940; Lancaster *et al.* (2011) Science 334, 974–977.
 - Page 2, Line 66: The crystal structure of NifEN has been reported. Kaiser *et al.* (2011) Science 331, 91–94.
 - Page 2, Line 66: Detailed NifB function has been reported. Wiig *et al.* (2012) Science 337, 1672–1675.
 - Page 2, Line 68: For a latest review of FeMo-co biosynthesis, I suggest a JBC paper; Hu and Ribbe (2013) JBC 288, 13173–13177.
 - Page 2, Line 70: As to the function of NifZ, I suggest a PNAS paper; Hu *et al.* (2007) PNAS 104, 10424–10429.
 - Page 2, Line 72: The *nifM* gene encodes the peptidyl-prolyl *cis/trans* isomerase that is essential for the correct folding of NifH. (Gavini *et al.* (2006) J Bacteriol 188, 6020–6025.)
- (3) As to the comparison of organization of *nif/vnf* genes in cyanobacteria (Pages 4–5), I suggest citing a PNAS paper; Welsh *et al.* (2008) PNAS 105, 15094–15099.
- (4) As to proteins involved in the regulation of nitrogenase genes, I suggest mentioning a transcriptional activator CnfR, a PatB homolog, in a nonheterocystous cyanobacterium; Tsujimoto *et al.* (2014) PNAS 111, 6762–6767.
- (5) Page 7, Line 217; Page 9, Line 246 and Page 9, Line 276; I think that 30 nM, 3 nM, and 10 μ M, respectively, are better than just M indications.
- (6) Page 18, Line 497; The genome paper of “*Nostoc azollae*” 0708 should be cited; Ran *et al.* (2010) PLoS One 5, e11486.
- (7) Followings are just typos:
- Page 1, Line 22: micro-oxic rather than microxic
 - Page 3, Line 107: “*nifB-fdxN-nifS-nifU, nifHDK*” looks different font (Helvetica?).
 - Page 5, Line 154: “as well as” should not be italicized.
 - Page 5, Line 158: encodes the beta-subunit (beta is missing)
 - Page 5, Line 172: “(Figure 3, panels A, B and C)” rather than “(Figure 3A, 3B, 3C)”.
 - Page 6, Line 189: Insert a space between “was” and “photographed”.
 - Page 6, Line 191: Insert a space between “microscopy” and “Panels”.
 - Page 7, Line 201: “(Figures 5 and 12)” rather than “(Figure 5 and Figure 12)”.
 - Page 7, Line 217: Km should be italicized (m is a subscript).
 - Page 7, Line 218: Mb rather than MB
 - Page 8, Line 236: “A” should not italicized.
 - Page 9, Lines 252–253: “*Fischerella muscicola* PCC7414 have orthologs of the vanadate transport genes.” looks different font (Helvetica?). “*muscicola*” should be italicized.
 - Page 9, Lines 259–260: “Tungstate is transported by....a nitrogenase [78]. T” looks different font (Helvetica?).
 - Page 10, Line 304: mRNA rather than m-RNA (also Page 11, Line 312)
 - Page 13, Line 364: KEN should be italicized with *vnfDG*.
 - Page 13, Line 369: dinitrogenase rather than diinitrogenase
 - Page 17, Line 466: “as well as” rather than “a well as”

Response: The recent references suggested by the reviewer were very helpful and all were included in the appropriate places in the revised manuscript. All minor changes were also made.

Round 1: Reviewer 2 Report and Author Response

This review by Thiel and Pratte on the regulation of nitrogen fixation genes in *Anabaena variabilis* is very well written and provides a clear and comprehensive review and analysis of the topic covered. I have no major or even minor criticisms of the review. I found only a few small issues and typos that need to be corrected.

- Line 61: It is a little confusing to start with two genes and then switch to referring to proteins for the rest of this paragraph and the next.
- Line 153: Although I now prefer no space between PCC and the strain number, a space is needed here to conform to the style used in this paper.
- Line 158: The Greek letter is missing in my pdf file.
- Line 167: Italics missing.
- Line 182: Delete comma.
- Line 184: Insert space. Spaces are missing in other places, which I will leave for the copy editor to fix.
- Line 195: First “B” should be “D”.
- Line 236: Remove italics from “A”.
- Line 301 and elsewhere: Replace the smart quote with a prime mark.

Response: Corrections requested by the reviewer have been made in the revised manuscript.

© 2014 by the reviewers; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).