Peer Community In Microbiology

Genetically controlled biomineralization in *Cyanobacteria*: diel fluctuations of ccyA transcript abundances and identification of neighboring genes putatively involved in the precipitation of intracellular amorphous calcium carbonates in *Microcystis aeruginosa* PCC7806

Rutger De Wit *based on peer reviews by* **Rutger De Wit** *and 1 anonymous reviewer*

Apolline Bruley, Juliette Gaëtan, Muriel Gugger, Claire Pancrace, Maxime Millet, Geoffroy Gaschignard, Manuela Dezi, Jean-François Humbert, Julie Leloup, Fériel Skouri-Panet, Isabelle Callebaut, Karim Benzerara, Elodie Duprat (2024) Diel changes in the expression of a marker gene and candidate genes for intracellular amorphous CaCO3 biomineralization in *Microcystis*. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Microbiology. https://doi.org/10.1101/2024.07.07.602159

Submitted: 12 July 2024, Recommended: 14 January 2025

Cite this recommendation as:
De Wit, R. (2025) Genetically controlled biomineralization in *Cyanobacteria*: diel fluctuations of ccyA transcript abundances and identification of neighboring genes putatively involved in the precipitation of intracellular amorphous calcium carbonates in *Microcystis aeruginosa* PCC7806. *Peer Community in Microbiology*, 100153. 10.24072/pci.microbiol.100153

Published: 14 January 2025

In this interesting study by Bruley et al. (2024), the cyanobacterium *Microcystis aeruginosa* PCC7806 is taken as a model organism for intracellular CaCO3 precipitation in *Cyanobacteria*, i.e. in the form of intracellular amorphous calcium carbonates (iACC). This phenomenon, which was first described in 2012, is an example of genetically controlled biomineralization in bacteria. Hence, a gene coding for the protein calcyanin (*ccyA*) has been documented in iACC biomineralizing cyanobacteria. Nevertheless, so far, the functioning of the calcyanin protein remains unknown. As a first step to elucidate its role in iACC biomineralization the authors study the diel variations of ccyA expression. An approximately 2.5-fold variation in abundance of *ccyA* transcripts has

been observed with the highest values of *ccyA* expression observed during the second half of the dark period. In addition, the authors made a thorough investigation of transcriptomics data, to detect gene-expressions with temporal patterns that positively or negatively correlate with *ccyA*. A particular focus was made on neighboring genes (both upstream and downstream) to detect a possible operon gathering *ccyA* with other genes. Very interestingly, the authors discovered that some neighboring genes coding for Ca2+/H+ antiporter systems, showed transcripts with abundances that correlate with that of *ccyA*.

This study raises many interesting questions on genetically controlled biomineralization in bacteria and more particularly the function of iACC biomineralization in *Cyanobacteria*. As the authors write, iACC biomineralization could be involved in carbon-concentrating mechanisms (CCM), intracellular pH buffering, and create "ballast" for buoyancy and floatability regulation. Nevertheless, these roles would require mechanisms for the dissolution of iAAC in concert with its precipitation ; fine-tuning of both resulting in homeostasis or cyclic temporal patterns of iAAC increase/decrease. As a perspective, the response of *Microcystis* to fluctuations in calcium and/or pCO2 levels could provide valuable insights into the molecular mechanisms underlying the biomineralization of iACC, as well as comparisons with non-iACC biomineralizing strains or with a mutant of PCC 7806 with a deactivated/deleted *ccyA* gene.

References:

Bruley A, Gaëtan J, Gugger M, Pancrace C, Millet M, Gaschignard G, Dezi M, Humbert J-F, Leloup J, Skouri-Panet F, Callebaut I, Benzerara K and Duprat E (2024) Diel changes in the expression of a marker gene and candidate genes for intracellular amorphous CaCO3 biomineralization in *Microcystis*. bioRxiv, ver.3 peer-reviewed and recommended by PCI Microbiol.
https://doi.org/10.1101/2024.07.07.602159

Reviews

Evaluation round #2

DOI or URL of the preprint: https://www.biorxiv.org/content/10.1101/2024.07.07.602159v2
Version of the preprint: 2

Authors' reply, 23 December 2024

Download author's reply
Download tracked changes file

Decision by Rutger De Wit, posted 19 December 2024, validated 20 December 2024

Dear Dr. Benzerara and co-authors,

Thank you very much for the submission of the revision this paper and for carefully considering the reviewers comments and suggestions. I have checked these points and consider that they have been carefully addressed and where possible correctly accommodated in the revision. Nevertheless, I have spotted a certain number of mainly editorial points that need correction. Therefore, I kindly ask you to post a new revised version with these corrections. This is the last step before I can agree on a recommendation. I am looking forward to receive the revision in due course and subsequently will write the recommendation as is the custom for PCI.

Best wishes, Rutger de Wit

Recommender PCI-Microbiology

Comments

1- a) After reading again I consider that the statement (lines 30-31, "The ccyA gene shows a clear day/night expression pattern with a maximum transcript abundance at the end of the night." Could be misleading as the maximum of expression concerns the whole of the second half of the night (see Fig. 3) and not just a single time point prior to "sunrise". I suggest to change as follows:

"The ccyA gene shows a clear day/night expression pattern with maximum transcript abundances during the second half of the night."

b) Same point lines 249-251 replace as follows: "A clear day/night pattern can be observed in the expression of ccyA, with maximum transcript abundances during the second half of the night (t7_N and t8_N, Table 1) and a minimum transcript abundance at 12 am during daytime (t3_D, Table 1)".

c) Same point lines 256-258 replace as follows: "When compared with the mean expression of the 4440 genes of the whole transcriptome, the mean expression of the ccyA gene was significantly higher (p-value < 0.05) during the second half of the night, i.e., at the two-time points : 2 am (t7_N) and 7 am (t8_N) (Table 1).

2- To make things a bit more clear I suggest that you replace (lines 73-75) "About the later hypothesis, we note that buoyancy is not just controlled by cell density but also by additional parameters such as cell aggregation. For example, Gu et al. (2020) showed that Ca induces EPS production by M. aeruginosa, which can increase buoyancy" by :

"About the later hypothesis, we note that buoyancy is not just controlled by cell density but also by additional parameters such as EPS adhering to the cells and cell aggregation. For example, Gu et al. (2020) showed that Ca induces EPS production by M. aeruginosa, which particularly when incorporated in the cell aggregates, can increase buoyancy".

3- Note the ref Gu et al (2020) is missing in the list.

4- You may wish to change (line 111) "Overall, one way of gaining a better understanding" into "A first step of gaining a better understanding", which makes the justification for your approach a bit stronger.

5- Legend Fig. 7, replace "Figure 7 - Abundance profiles of three genomic neighbors of" by "Figure 7 – Time course of the Abundances of three genomic neighbors of"

6- a) Line 456, I think the "alternation" is a more appropriate term than "compartmentalization" in this context, as compartmentalization implies occurring in different spaces separated by some sort of boundaries.

b) Same point, lines 475-476, I suggest: "is compartmentalized" should be replaced by "alternates"

7- There is an error in a name of a co-author in the Straub et al. publication

First name: Nicole

Family name: Tandeau de Marsac

Correct Reference:

Straub, C., Quillardet, P., Vergalli, J., Tandeau de Marsac, N., Humbert, J.-F., 2011. A day in the life of 830 Microcystis aeruginosa strain PCC 7806 as revealed by a transcriptomic analysis. PLOS ONE 831 6, e16208. https://doi.org/10.1371/journal.pone.0016208

Evaluation round #1

DOI or URL of the preprint: https://biorxiv.org/cgi/content/short/2024.07.07.602159v1
Version of the preprint: 1

Authors' reply, 14 December 2024

Download author's reply
Download tracked changes file

Decision by Rutger De Wit, posted 25 October 2024, validated 25 October 2024

Montpellier, 25 October 2024

Re: M/S submitted to PCI Microbiology
Diel changes in the expression of a marker gene and candidate genes for intracellular amorphous CaCO3 biomineralization in Microcystis
by Bruley & Gaëtan et al.

Dear Drs Bruley, Gaëtan, Benzerara, Duprat and other co-authors.

Thank you very much for submitting the aforementioned M/S for evaluation to PCI-Microbiology. In the first place, I want to apologize for the delay of my response. I have had enormous problems in finding reviewers, among the more than 20 invited I only succeeded in obtaining one report, which to me appears very valuable and helpful. To advance the process I decided to do the second review myself, despite the fact that I only master this area only partially and I am not a specialist in transcriptomics. Nevertheless, I hope that you will find my report useful.

For the time-being, albeit promising, I cannot yet recommend this paper and thus advice formally "This preprint merits a revision" (considering the 3 possibilities for PCI recommendors). Hence, I ask you to prepare a carefully revised version. Please carefully consider the points raised by the reviewers and take them into account for the preparation of the revised version. I highly appreciate if you can prepare a reply where you pointwise address the issues raised by the reviewers and indicate how you have accommodated them in the revised version. I am looking forward to receiving the revised version with the reply in due course. Upon receipt, I will contact the other reviewer for additional assessment and also prepare my own assessment.

Yours sincerely,

Rutger De Wit
Recommender for PCI-Microbiology

Reviewed by anonymous reviewer 1, 30 August 2024

Download the review

Reviewed by Rutger De Wit, 25 October 2024

Download the review