

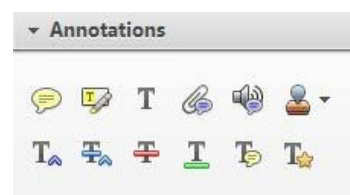
## Smart Proof System Instructions

It is recommended that you read all instructions below; even if you are familiar with online review practices.

Using the Smart Proof system, proof reviewers can easily review the PDF proof, annotate corrections, respond to queries directly from the locally saved PDF proof, all of which are automatically submitted directly to **our database** without having to upload the annotated PDF.

- ✓ **Login into Smart Proof** anywhere you are connected to the internet.
- ✓ **Review the proof** on the following pages and mark corrections, changes, and query responses using the **Annotation Tools**.

**Note:** Editing done by replacing the text on this PDF is not permitted with this application.



- ✓ **Save your proof corrections** by clicking the "Publish Comments" button.

Corrections don't have to be marked in one sitting. You can publish comments and log back in at a later time to add and publish more comments before you click the "Complete Proof Review" button below.

Publish Comments

- ✓ **Complete your review** after all corrections have been published to the server by clicking the "Complete Proof Review" button below.

### Before completing your review.....

Did you reply to all author queries found in your proof?

Did you click the "Publish Comments" button to save all your corrections?  
Any unpublished comments will be lost.

**Note:** Once you click "Complete Proof Review" you will not be able to add or publish additional corrections.

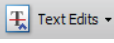
Complete Proof Review

## Adding Comments and Notes to Your PDF

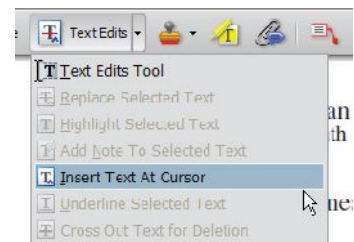
**IMPORTANT:** Composition cannot be completed until the author review is complete and all changes and author query answers have been added to this proof. Author queries are found at the end of this pdf.

To facilitate electronic transmittal of corrections, we encourage authors to utilize the comments and notes features in Adobe Acrobat. The PDF provided has been “comment-enabled,” which allows you to utilize the comments and notes features, even if using only the free Adobe Acrobat reader (see note below regarding acceptable versions). Adobe Acrobat’s Help menu provides additional details on the tool. When you open your PDF, the comments/notes/edit tools are clearly shown on the tool bar (though icons may differ slightly among versions from what is shown below).

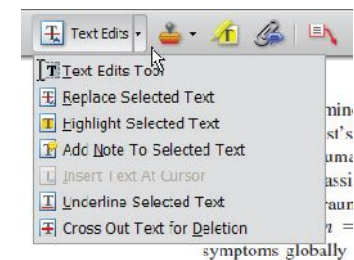
For purposes of correcting the PDF proof of your journal article, the important features to know are the following:

- Use the **Text Edits tool**, , to insert, replace, or delete text.

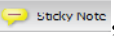
- To **insert text**, place your cursor at a point in text and select “Insert Text at Cursor” from the text edits menu. Type your additional text in the pop-up box.



- To **replace text** (do this instead of deleting and then re-inserting), highlight the text to be changed, select “Replace Selected Text” from the text edits menu, and type the new text in the pop-up box.



- To **delete text**, highlight the text to be deleted and select “Cross Out Text for Deletion” from the text edits menu (see graphic above).

- Use the **Sticky Note tool**, , to describe changes that need to be made (e.g., changes in bold, italics, or capitalization use; altering or replacing a figure) or to answer a question or approve a change that was posed by the editor. To use this feature, click on the sticky note tool and then click on a point in the PDF where you would like to make a comment, then type your comment in the pop-up box.



- Use the **Callout tool**, , to point directly to changes that need to be made. Try to put the callout box in an area of white space so that you do not obscure the text, as in the example below.

Table 5

*Experiment 4: Comparative Optimism as a Function of Self-Presentation and Event Valence*

Self-presentation	Event					
	Positive		Negative		Total	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Public/student	3.46	0.13	3.60	0.10	3.53	0.12
Public/expert	2.66	0.12	2.78	0.13	2.73	0.13
Control	2.39	0.11	2.46	0.09	2.43	0.11
Total	2.84	0.47	2.95	0.50		

The first column's entries should be flush left (except for "Total", which should be indented one em-space), as in Tables 1 and 2 previously.

- Use the **Highlight tool**, , to indicate font problems, bad breaks, and other textual inconsistencies. Describe the inconsistencies with the callout tool (shown) or a sticky note. One callout (or sticky note) can describe many changes.

Close up minus sign to lambda (3 times, highlighted)

$$du/dt = -\lambda v^\alpha = -\lambda u$$

$$du/u = -\lambda dt$$

$$u_t = ue^{-\lambda t}$$

An alternate method is to select the appropriate text with your cursor, select **“Add Note to Selected Text”** from the text edits menu, and then type your note in the pop-up box (the selected text is highlighted automatically).

As with hand-annotated proof corrections, the important points are to communicate changes clearly and thoroughly; to answer all queries and questions; and to provide complete information for us to make the necessary changes to your article so it is ready for publication.

To utilize the comments/notes features on this PDF you will need Adobe Reader version 7 or higher. This program is freely available and can be downloaded from <http://get.adobe.com/reader/>



## Draft Genome Sequence of *Nitrosomonas* sp. Strain APG5, a Betaproteobacterial Ammonia-Oxidizing Bacterium Isolated from Beach Sand

AQ: au **Hidetoshi Urakawa,<sup>a</sup> Jorie Skutas,<sup>b</sup> Jose V. Lopez<sup>b</sup>**

AQ: info <sup>a</sup>Department of Marine and Ecological Sciences, Florida Gulf Coast University, Fort Myers, Florida, USA

<sup>b</sup>Department of Biological Sciences, Halmos College of Natural Sciences and Oceanography, Nova Southeastern University, Dania Beach, Florida, USA

**ABSTRACT** *Nitrosomonas* sp. strain APG5 (=NCIMB 14870 = ATCC TSA-116) was isolated from dry beach sand collected from a supralittoral zone of the northwest coast of the United States. The draft genome sequence revealed that it represents a new species of the cluster 6 *Nitrosomonas* spp. that is closely related to *Nitrosomonas ureae* and *Nitrosomonas oligotropha*.

AQ:A

*Nitrosomonas* is a genus of nitrifying bacteria belonging to the class *Betaproteobacteria*. Together with ammonia-oxidizing archaea, ammonia-oxidizing bacteria (AOB) play important roles in both natural and artificial environments. However, AOB are sensitive to environmental stressors, such as hydrocarbon contamination (1), and serve as valuable ecological indicators (2).

In 2008, *Nitrosomonas* sp. strain APG5 was isolated from a dry sand sample collected from Edmonds Ferry Dock Beach, located in the Puget Sound, which is historically and routinely contaminated by small oil spills due to heavy ship transportation, including a state-run ferry system (3). Five grams of sand collected in a sterilized 50-ml centrifuge tube were incubated at 20°C with 10 ml of autoclaved ultrapure water supplemented with ammonium chloride to 100 μM as a final concentration. After the first indication of nitrite production (4), 1 ml of sample was transferred into 9 ml of APG medium, and the pure culture was obtained by a serial dilution technique (5). Initial phylogenetic analysis based on the 16S rRNA gene revealed that APG5 belongs to the cluster 6a *Nitrosomonas* spp. (6). To determine the genome sequence of APG5, cells were cultured accordingly (5), and genomic DNA was prepared as previously described (7). A draft genome sequence was obtained using the Illumina MiSeq platform and Nextera XT sample preparation kit v2 with 2 × 250-bp paired-end reads (3,338,462 reads). Genome assembly quality was evaluated using Quast (version 4.6.3) (8), and reads were assembled using Unicycler (version 0.4.6.0) (9) on Galaxy (10) using default settings. The draft genome was initially annotated with the Rapid Annotations using Subsystems Technology (RAST) server (version 2.0) (11) and SEED Viewer (12) to confirm some house-keeping genes and key functional genes. The NCBI Prokaryotic Genome Annotation Pipeline (Gene Mark S+ version 4.4) (13) was used in the last stage of genome annotation.

AQ: B

AQ: C

The assembled draft genome sequence comprised 3.75 Mbp at 222-fold coverage and consisted of 239 contigs with an average size of 12,702 bp and  $N_{50}$  length of 56,724 bp. The G+C content was 43.5%, and one plasmid was found (pAPG501, 14,708 bp). The draft genome contains 3,172 protein-coding DNA sequences, 40 tRNA genes, and a single 16S-23S-5S rRNA operon.

The two-way average nucleotide identity values (14) of strain APG5 with *Nitrosomonas ureae* Nm10 (GenBank accession number CP013341) (15), *Nitrosomonas* sp. AL212 (accession number CP002552) (16), and *Nitrosomonas* sp. Is79 (accession number

**Citation** Urakawa H, Skutas J, Lopez JV. 2019. Draft genome sequence of *Nitrosomonas* sp. strain APG5, a betaproteobacterial ammonia-oxidizing bacterium isolated from beach sand. *Microbiol Resour Announc* 8:e01573-18. <https://doi.org/10.1128/MRA.01573-18>.

**Editor** J. Cameron Thrash, University of Southern California

**Copyright** © 2019 Urakawa et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Hidetoshi Urakawa, [hurakawa@fgcu.edu](mailto:hurakawa@fgcu.edu).

**Received** 17 November 2018

**Accepted** 1 May 2019

**Published**

CP002876) (17) were 82.2%, 81.9%, and 77.9%, respectively, which were lower than the average nucleotide identity (ANI) value of 95%, which corresponds to a 70% species level cutoff determined by DNA-DNA hybridization (18). The results evidently indicate that these genomes do not belong to the same species.

Genes involved in ammonia oxidation, including those encoding ammonia-mono-oxygenase, hydroxylamine dehydrogenase, and c-type cytochromes, were identified, as well as that encoding nitrosocyanin. As in other AOB, genes encoding nitrite reductase (*nirK*) and urease were present. The APG5 genome encodes 95 chemotaxis and flagellum-associated proteins, based on the subsystem information in the SEED Viewer. Concomitantly, genes encoding catalase and superoxide dismutase (Fe) were found for protection from reactive oxygen species. The plasmid contained a gene used to stabilize plasmid function and some ecologically significant genes. For example, a gene encoding the universal stress protein UspA, which modulates the expression of a variety of genes that help to cope with stress (19), was found in the plasmid. In addition, a sodium-proton exchanger (which maintains the homeostasis of pH and sodium), ATP-dependent exonuclease SbcCD, antitoxin Phd family protein, and addiction module toxin RelE were found in the same plasmid. These genes may increase the fitness of APG5 in supralittoral beach sand, in which microorganisms need to cope with long periods of exposure to air, heat, cold, low nutrients, and freshwater exposure through precipitation (20). Further genome annotation and genome comparisons with other *Nitrosomonas* species will provide additional insights for ecological adaptation of this bacterium.

**Data availability.** The *Nitrosomonas* sp. APG5 whole-genome shotgun (WGS) project has the project accession number PXXU00000000. This version of the project (01) has the accession number PXXU01000000 and consists of the sequences PXXU01000001 to PXXU01000239. The sequencing reads (under Sequence Read Archive number SRP170980) can be accessed through BioProject number PRJNA438189 and BioSample number SAMN08707727. Data and additional information are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at <https://data.gulfresearchinitiative.org/data/R4.x262.000:0045> (doi:10.7266/n7-fnva-0v52).

AQ: D

## ACKNOWLEDGMENTS

We thank David A. Stahl at the University of Washington and Willm Martens-Habbena at the University of Florida for their early contributions to this study. We also thank Megan E. Feeney (Florida Gulf Coast University) for her laboratory assistance.

A part of this research was supported by NSF Division of Environmental Biology grant DEB-1664052 to H.U. A part of this work was also supported via funding provided by the Office of Research and Graduate Studies of Florida Gulf Coast University (H. U.) and funding from the Gulf of Mexico Research Initiative through the Alabama Center for Ecological Resilience, administered by the Dauphin Island Sea Lab (H. U.).

J.V.L. is part of the DEEPEND consortium ([DEEPENDconsortium.org](http://DEEPENDconsortium.org)).

## REFERENCES

- Urakawa H, Rajan S, Feeney ME, Sobecky PA, Mortazavi B. 2019. Ecological response of nitrification to oil spills and its impact on the nitrogen cycle. *Environ Microbiol* 21:18–33. <https://doi.org/10.1111/1462-2920.14391>.
- Urakawa H, Bernhard AE. 2017. Wetland management using microbial indicators. *Ecol Eng* 108:456–476. <https://doi.org/10.1016/j.ecoleng.2017.07.022>.
- Washington State Department of Ecology. 2018. 30 years of spill prevention, preparedness, and response. Publication 18-08-012. Spill Prevention, Preparedness, and Response Program, Olympia, WA.
- Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* 461:976–979. <https://doi.org/10.1038/nature08465>.
- Urakawa H, Garcia JC, Nielsen JL, Le VQ, Kozłowski JA, Stein LY, Lim CK, Pommerening-Roser A, Martens-Habbena W, Stahl DA, Klotz MG. 2015. *Nitrosospora lacus* sp. nov., a psychrotolerant, ammonia-oxidizing bacterium from sandy lake sediment. *Int J Syst Evol Microbiol* 65:242–250. <https://doi.org/10.1099/ijs.0.070789-0>.
- Purkhold U, Pommerening-Roser A, Juretschko S, Schmid MC, Koops HP, Wagner M. 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys. *Appl Environ Microbiol* 66:5368–5382. <https://doi.org/10.1128/AEM.66.12.5368-5382.2000>.
- Urakawa H, Martens-Habbena W, Stahl DA. 2010. High abundance of ammonia-oxidizing Archaea in coastal waters, determined using a modified DNA extraction method. *Appl Environ Microbiol* 76:2129–2135. <https://doi.org/10.1128/AEM.02692-09>.

8. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
9. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
10. Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Čech M, Chilton J, Clements D, Coraor N, Grüning BA, Guerler A, Hillman-Jackson J, Hiltmann S, Jalili V, Rasche H, Soranzo N, Goecks J, Taylor J, Nekrutenko A, Blankenberg D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res* 46:W537–W544. <https://doi.org/10.1093/nar/gky379>.
11. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
12. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang H-Y, Cohoon M, de Crécy-Lagard V, Diaz N, Disz T, Edwards R, Fonstein M, Frank ED, Gerdes S, Glass EM, Goessmann A, Hanson A, Iwata-Reuyl D, Jensen R, Jamshidi N, Krause L, Kubal M, Larsen N, Linke B, McHardy AC, Meyer F, Neuweger H, Olsen G, Olson R, Osterman A, Portnoy V, Pusch GD, Rodionov DA, Rückert C, Steiner J, Stevens R, Thiele I, Vassieva O, Ye Y, Zagnitko O, Vonstein V. 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. *Nucleic Acids Res* 33:5691–5702. <https://doi.org/10.1093/nar/gki866>.
13. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
14. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57:81–91. <https://doi.org/10.1099/ijs.0.64483-0>.
15. Kozłowski JA, Kits KD, Stein LY. 2016. Complete genome sequence of *Nitrosomonas ureae* strain Nm10, an oligotrophic group 6a nitrosomonad. *Genome Announc* 4:e00094-16. <https://doi.org/10.1128/genomeA.00094-16>.
16. Suwa Y, Norton JM, Bollmann A, Klotz MG, Stein LY, Laanbroek HJ, Arp DJ, Goodwin LA, Chertkov O, Held B. 2011. Genome sequence of *Nitrosomonas* sp. strain AL212, an ammonia-oxidizing bacterium sensitive to high levels of ammonia. *J Bacteriol* 193:5047–5048. <https://doi.org/10.1128/JB.05521-11>.
17. Bollmann A, Sedlacek CJ, Norton J, Laanbroek HJ, Suwa Y, Stein LY, Klotz MG, Arp D, Sayavedra-Soto L, Lu M. 2013. Complete genome sequence of *Nitrosomonas* sp. Is79, an ammonia oxidizing bacterium adapted to low ammonium concentrations. *Stand Genomic Sci* 7:469–482. <https://doi.org/10.4056/signs.3517166>.
18. Konstantinidis KT, Tiedje JM. 2005. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci U S A* 102:2567–2572. <https://doi.org/10.1073/pnas.0409727102>.
19. Kvint K, Nachin L, Diez A, Nyström T. 2003. The bacterial universal stress protein: function and regulation. *Curr Opin Microbiol* 6:140–145. [https://doi.org/10.1016/S1369-5274\(03\)00025-0](https://doi.org/10.1016/S1369-5274(03)00025-0).
20. McLachlan A. 1983. Sandy beach ecology—a review, p 321–380. *In* McLachlan A, Erasmus T (ed), *Sandy beaches as ecosystems*. Springer, Dordrecht, the Netherlands.

AQ: E



## AUTHOR QUERIES

### AUTHOR PLEASE ANSWER ALL QUERIES.

1

**Composition will not be completed until a response is received for each query listed below.**

---

AQau—Please confirm the given-names and surnames are identified properly by the colors.

■ = Given-Name, ■ = Surname

AQinfo—Please review all affiliations and contact information for accuracy, including the corresponding author emails in the citation box on page 1 of the proof.

AQ: Links have been added to the proof for readers to access information concerning accession numbers cited in this paper. Please review the links to verify that they are opening to the correct information site online.

AQfund—The table below includes funding information that you provided on the submission form when you submitted the manuscript. This funding information will not appear in the article, but it will be provided to CrossRef and made publicly available. Please check it carefully for accuracy and mark any necessary corrections. If you would like statements acknowledging financial support to be published in the article itself, please make sure that they appear in the Acknowledgments section. Statements in Acknowledgments will have no bearing on funding data deposited with CrossRef and vice versa.

Funder	Grant(s)	Author(s)	Funder ID
National Science Foundation (NSF)	DEB-1664052	Hidetoshi Urakawa	<a href="https://doi.org/10.13039/100000001">https://doi.org/10.13039/100000001</a>
Gulf of Mexico Research Initiative (GoMRI)	ACER	Hidetoshi Urakawa	<a href="https://doi.org/10.13039/100007240">https://doi.org/10.13039/100007240</a>

AQA—Please verify edits to the sentences beginning "The draft genome sequence revealed..." and "Genes involved in ammonia oxidation..." or edit further if necessary.

AQB—If APG is an abbreviation, please provide an expansion upon first use in "APG medium."

AQC—Note that "Galaxy v" was changed to "Galaxy"; if a version number was missing, please edit to add it if desired.

AQD—Please verify edits per ASM style to the "Data availability" paragraph. Note that the data website information was moved here from the Acknowledgments.

AQE—Ed: The web link/DOI ([\[https://doi.org/10.4056/sigs.3517166\]](https://doi.org/10.4056/sigs.3517166)) is broken/nonfunctioning or does not seem to be targeting the proper webpage. Please provide an updated link.

---