AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

AMPLICON SEQUENCE COLLECTIONS

Microbial Community of Saline, Alkaline Lakes in the Nebraska Sandhills Based on 16S rRNA Gene Amplicon Sequence Data

Nicole A. Fiore,[a] David D. Dunigan,[b,c] Julie J. Shaffer,[d] Ryan Roberts,[e]* Sanjay Antony-Babu,[a]* Bradley A. Plantz,[a] Kenneth W. Nickerson,[a] Andrew K. Benson,[f] Karrie A. Weber[a,g,h]

[a]School of Biological Sciences, University of Nebraska—Lincoln, Lincoln, Nebraska, USA
[b]Department of Plant Pathology, University of Nebraska—Lincoln, Lincoln, Nebraska, USA
[c]Nebraska Center for Virology, University of Nebraska—Lincoln, Lincoln, Nebraska, USA
[d]Department of Biology, University of Nebraska at Kearney, Kearney, Nebraska, USA
[e]Department of Biochemistry, University of Nebraska—Lincoln, Lincoln, Nebraska, USA
[f]Department of Food Science and Technology, University of Nebraska—Lincoln, Lincoln, Nebraska, USA
[g]Department of Earth and Atmospheric Sciences, University of Nebraska—Lincoln, Lincoln, Nebraska, USA
[h]Daughtery Water for Food Institute, University of Nebraska—Lincoln, Lincoln, Nebraska, USA

ABSTRACT The Nebraska Sandhills region contains over 1,500 geochemically diverse interdunal lakes, some of which are potassium rich, alkaline, and hypersaline. Here, we report 16S rRNA amplicon pyrosequencing data on the water and sediment microbial communities of eight alkaline lakes in the Sandhills of western Nebraska.

The Nebraska Sandhills region is the largest sand dune region in the Western Hemisphere, covering 50,000 km^2 (1). Despite the semiarid climate, more than 1,500 lakes have formed in depressions between grass-stabilized dunes (2). Most of these lakes are shallow, with only 5% exceeding 2.5 m in depth (3). The lakes vary significantly in their geochemistry, with alkalinity from 0.0 mg/liter to >90,000 mg/liter (4), pH from neutral to 10.8 (1, 5), and salinity from 200 mg/liter to >100,000 mg/liter of total dissolved solids (TDS) (5). In addition to variance between the lakes, evaporation drives seasonal geochemical changes within the lakes (6). Though these alkaline systems are well described, their microbial communities remain undescribed.

Over a 2-year period (2007 to 2008), water and sediment samples were collected from the littoral zone of saline, alkaline lakes in the Nebraska Sandhills (Table 1). Water samples (1 liter) were collected from eight lakes in sterile, plastic bottles by immersion below the lake surface and then filtered through nitrocellulose membranes (Whatman 7182-002). Filters were lyophilized and stored with desiccant. DNA was extracted using the Qiagen BioSprint 96 One-For-All vet kit (7). Sediment samples were collected from five lakes (Border, Ellsworth, Kokjohn, Merritt, and Tree Claim) by collecting ca. 25 g of sediment directly into sterile polypropylene tubes. Sediment was pelleted by centrifugation (8) and stored at −20°C until DNA extraction with the Mo Bio PowerSoil DNA isolation kit (using the manufacturer's protocol). The V1-V2 region of the 16S rRNA gene was amplified with bacterium-specific primers and sequenced using the Roche-454 GS FLX system for all samples (7, 9). Sequence processing was completed using QIIME 1.8.0 (10). Chimeric sequences were identified with ChimeraSlayer (11), and reads of <150 bp or with a mean quality score (Q) of <25 were discarded. Fifteen samples yielded a total of 152,015 high-quality reads (230 bp mean length, 10,134 mean reads per sample). Taxonomy was assigned in reference to Greengenes v13_8 (12) with a 97% operational taxonomic unit (OTU) identity threshold.

The distribution of taxa varied among the lakes and seasons (Table 1). *Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the most frequently identified

Citation Fiore NA, Dunigan DD, Shaffer JJ, Roberts R, Antony-Babu S, Plantz BA, Nickerson KW, Benson AK, Weber KA. 2019. Microbial community of saline, alkaline lakes in the Nebraska Sandhills based on 16S rRNA gene amplicon sequence data. Microbiol Resour Announc 8:e00063-19. https://doi.org/10.1128/MRA.00063-19.

Editor Christina Cuomo, Broad Institute of MIT and Harvard University

Address correspondence to Karrie A. Weber, kweber@unl.edu.

* Present address: Ryan Roberts, United States Army, Fort Eustis, Newport News, Virginia, USA; Sanjay Antony-Babu, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, USA.

Received 17 January 2019
Accepted 4 February 2019
Published 21 March 2019

TABLE 1 Summary of geochemical and sequence data by sample site[a]

Lake	Coordinates	pH[b]	Alkalinity[b] (mg/liter $CaCO_3$)	Conductivity[b,e] (μS/cm)	Sample date (mo/yr)	Source material	No. of reads	Taxonomic identification (no. of reads [%])[c]	Total no. of reads identified as chloroplasts (%)[d]
Border	41.79386°N, 102.53521°W	9.8–10.3	9,300–71,400	24,500–65,945	6/2007	Water	11,587	*Proteobacteria* (6,500 [56.1]), *Cyanobacteria* (2,344 [20.2]), *Bacteroidetes* (793 [6.8])	1,725 (14.9)
					6/2008	Water	11,862	*Cyanobacteria* (6,332 [53.4]), *Proteobacteria* (2,039 [25.6]), unassigned (1,297 [10.9])	3,027 (25.5)
					10/2008	Water	12,771	*Proteobacteria* (33,916 [30.7]), *Cyanobacteria* (3,769 [29.5]), *Bacteroidetes* (2,233 [17.5])	181 (1.4)
Ellsworth	42.06078°N, 102.28409°W	9.7	2,290	13,210	10/2008	Water	10,946	*Actinobacteria* (7,174 [65.6]), *Proteobacteria* (2,048 [18.7]), unassigned (650 [5.9])	169 (1.5)
Kokjohn	41.78245°N, 102.52274°W	9.5–9.9	2,672–27,200	6,070–70,000	6/2008	Water	10,651	*Actinobacteria* (4,647 [43.6]), *Proteobacteria* (3,277 [30.8]), unassigned (1,027 [9.6])	31 (0.3)
					10/2008	Sediment	7,334	*Proteobacteria* (2,811 [38.3]), unassigned (1,729 [23.6]), *Firmicutes* (795 [10.8])	30 (0.4)
Merritt	42.06846°N, 102.29020°W	9.4	390–3,220	8,330	10/2008	Water	9,803	*Cyanobacteria* (6,630 [67.6]), *Proteobacteria* (1,721 [17.6]), *Actinobacteria* (859 [8.8])	13 (0.1)
					10/2008	Sediment	4,198	*Proteobacteria* (1,138 [27.1]), unassigned (752 [17.9]), *Bacteroidetes* (652 [15.5])	324 (7.7)
Perrin	41.76924°N, 102.51555°W	8.6–9.0	450–522	800–1,040	6/2007	Water	12,099	*Proteobacteria* (5,854 [48.4]), *Bacteroidetes* (3,391 [28.0]), *Actinobacteria* (1,725 [14.3])	184 (1.5)
Smith	41.78609°N, 102.52386°W	8.3–8.9	470–502	148–890	6/2007	Water	11,732	*Cyanobacteria* (3,659 [31.2]), *Proteobacteria* (3,500 [29.8]), *Actinobacteria* (2,090 [17.8])	115 (1.0)
					10/2008	Water	10,566	*Cyanobacteria* (4,617 [43.7]), *Proteobacteria* (2,501 [23.7]), unassigned (1,875 [17.7])	4,119 (39.0)
Tree Claim	41.78248°N, 102.49649°W	7.5–9.9	501–9,800	1,700–17,866	6/2008	Water	9,404	*Actinobacteria* (3,748 [39.9]), *Proteobacteria* (2,155 [22.9]), *Bacteroidetes* (1,078 [11.5])	79 (0.8)
					10/2008	Water	9,749	*Cyanobacteria* (6,553 [67.2]), *Proteobacteria* (2,045 [21.0]), unassigned (563 [5.8])	80 (0.8)
					10/2008	Sediment	7,016	*Cyanobacteria* (3,573 [50.9]), *Proteobacteria* (1,506 [21.5]), unassigned (815 [11.6])	2,066 (29.4)
Louden	42.07929°N, 102.20402°W	9.3	2,810	9,450	6/2008	Water	12,297	*Cyanobacteria* (10,525 [85.6]), *Proteobacteria* (784 [6.4]), unassigned (318 [2.6])	104 (0.8)

[a] Geochemical values are reported as a range of observed values, when possible, to account for seasonal variation.
[b] Values from Shaffer et al. (6), Roberts (7), Zlotnik et al. (14), and Shinneman et al. (15).
[c] Read numbers were calculated by multiplying the total number of reads by the percentage of reads assigned to the taxon. *Cyanobacteria* include chloroplast-identified sequences.
[d] Total number of reads and percentage of total reads identified as chloroplasts.
[e] μS, microSiemens.

phyla in the water samples. In some cases, the majority of cyanobacterial reads were classified as chloroplasts (Table 1). Several sandhill lakes have abundant algal populations (13). Chloroplast sequences were therefore not removed, as they are a marker of potential eukaryotic primary productivity.

Cyanobacteria, *Proteobacteria*, and *Actinobacteria* were also commonly identified in sediment samples (Table 1). Sediment samples from Border and Ellsworth were excluded from downstream analysis due to low read counts (<2,000). Sequences associated with taxa capable of anoxygenic photosynthesis (*Chromatiaceae*) were identified in Kokjohn sediment, consistent with purple pigments observed during sample processing. A lack of archaeal identification in the samples is expected as a consequence of bacterium-specific primers.

These samples indicate that microbial populations vary among the alkaline lakes. More detailed analyses of aqueous and sedimentary geochemistry and hydrology across diurnal and seasonal timescales are required to discern meaningful differences in community structures.

Data availability. DNA sequences from this project were deposited in the NCBI Sequence Read Archive under the accession no. SRP156869.

ACKNOWLEDGMENTS

This project was supported by the Nebraska Tobacco Settlement Fund to K.A.W.; the Nebraska Center for Energy Sciences to K.A.W., D.D.D., K.W.N., J.J.S., and B.A.P.; the Nebraska Research Initiative to K.W.N., B.A.P., D.D.D., and J.J.S.; an NU Research Strategic Cluster Grant to D.D.D., K.W.N., B.A.P., and J.J.S.; the National Science Foundation under grant 1736030, and the UNL Agricultural Research Division and Office of Research and Economic Development to D.D.D. National Institute for General Medical Science (NIGMS) (5P20GM103427), a component of the National Institutes of Health (NIH) to J.J.S.

The contents of this publication are the sole responsibility of the authors and do not necessarily represent the official views of NIGMS or NIH.

REFERENCES

1. Loope D, Swinehart B, Mason J. 1995. Dune-dammed paleovalleys of the Nebraska Sand Hills: intrinsic versus climatic controls on the accumulation of lake and marsh sediments. Geol Soc Am Bull 107:396–406. https://doi.org/10.1130/0016-7606(1995)107<0396:DDPOTN>2.3.CO;2.
2. Schneider R, Humpert M, Stoner K, Steinauer G. 2005. The Nebraska Natural Legacy Project, Nebraska Game and Parks Commission Publications 25. Nebraska Game and Parks Commission, Lincoln, NE. https://digitalcommons.unl.edu/nebgamepubs/25.
3. Zhang L, Fang J, Joeckel RM. 2013. Microbial biomass and community structure in alkaline lakes of the Nebraska Sand Hills, USA. Chem Geol 356:171–180. https://doi.org/10.1016/j.chemgeo.2013.08.017.
4. Schnagl JA. 1980. Seasonal variations in water chemistry and primary productivity in four alkaline lakes in the Sandhills of western Nebraska. MS thesis. University of Nebraska—Lincoln, Lincoln, NE. http://digitalcommons.unl.edu/opentheses/65/. Accessed 18 September 2018.
5. Gosselin D. 1997. Major-ion chemistry of compositionally diverse lakes, Western Nebraska, U.S.A.: implications for paleoclimatic interpretations. J Paleolimnol 17:33–49. https://doi.org/10.1023/A:1007908909148.
6. Shaffer JJ, Peterson BC, Koupal KD. 2017. Assessment of seasonal changes in abiotic and zooplankton communities in highly and moderately alkaline Sandhills lakes. Great Plains Res 27:109–116. https://doi.org/10.1353/gpr.2017.0019.
7. Roberts R. 2010. The in situ function of a microbial community profiled by FT-IR: a snapshot in time. MS thesis. University of Nebraska—Lincoln, Lincoln, NE. http://digitalcommons.unl.edu/biochemdiss/5. Accessed 18 September 2018.
8. Weber KA, Urrutia MM, Churchill PF, Kukkadapu RK, Roden EE. 2006. Anaerobic redox cycling of iron by freshwater sediment microorganisms. Environ Microbiol 8:100–113. https://doi.org/10.1111/j.1462-2920.2005.00873.x.
9. Benson AK, Kelly SA, Legge R, Ma F, Low SJ, Kim J, Zhang M, Oh PL, Nehrenberg D, Hua K, Kachman SD, Moriyama EN, Walter J, Peterson DA, Pomp D. 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc Natl Acad Sci U S A 107:18933–18938. https://doi.org/10.1073/pnas.1007028107.
10. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Gonzalez Pena A, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303.
11. Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabbaa D, Highlander SK, Sodergren E, Methé B, DeSantis TZ, Petrosino JF, Knight R, Birren BW. 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res 21:494–504. https://doi.org/10.1101/gr.112730.110.
12. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072. https://doi.org/10.1128/AEM.03006-05.
13. McCarraher DB. 1960. The Nebraska Sandhill lakes: their characteristics and fisheries management problems, Nebraska Game and Parks Commission paper 7. Nebraska Game and Parks Commission, Lincoln, NE. http://digitalcommons.unl.edu/nebgamewhitepap.
14. Zlotnik VA, Burbach M, Swinehart J, Bennett D, Fritz SC, Loope DB, Olaguera F. 2007. A case study of direct push methods for aquifer characterization in dune-lake environments. Environ Eng Geosci 13:205–216.
15. Shinneman ALC, Bennett DM, Fritz SC, Schmieder J, Engstrom DR, Efting A, Holz J. 2010. Inferring lake depth using diatom assemblages in the shallow, seasonally variable lakes of the Nebraska Sand Hills (USA): calibration, validation, and application of a 69-lake training set. J Paleolimnol 44:443–464. https://doi.org/10.1007/s10933-010-9427-3.