AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

AMPLICON SEQUENCE COLLECTIONS

16S rRNA Gene Amplicon Sequencing Data of Tailing and Nontailing Rhizosphere Soils of *Mimosa pudica* from a Heavy Metal-Contaminated Ex-Tin Mining Area

Saidu Abdullahi,[a,b] Hazzeman Haris,[a] Kamarul Z. Zarkasi,[a] Hamzah G. Amir[a]

[a]School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia
[b]Department of Botany, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT The 16S rRNA gene amplicon sequence data from tailing and nontailing rhizosphere soils of *Mimosa pudica* from a heavy metal-contaminated area are reported here. Diverse bacterial taxa were represented in the results, and the most dominant phyla were *Proteobacteria* (41.2%), *Acidobacteria* (17.1%), and *Actinobacteria* (14.4%).

The rhizosphere of plants is normally colonized by microbial communities which potentially benefit the host plants (1–3). Knowledge of the bacterial community of the metal-tolerant and leguminous *Mimosa pudica* (found abundantly in the studied heavy metal-contaminated soil) would explain their involvement in enhancing the growth and survival of the plant (4). Studies on microbial communities from different environments provide novel insights into their structure and function, leading to the discovery of novel functional genes (5, 6). The 16S rRNA gene amplicon sequence data presented here provide insight toward understanding how the bacterial diversity impacts plant growth promotion and heavy metal tolerance.

The rhizosphere soils of *M. pudica* were collected from six different sites (tailing sites, MRS1, MRS2, and MRS3; nontailing sites, MRS4, MRS5, and MRS6) of an ex-tin mining area (latitude 5°38′N, longitude 101°1′E) in Perak, Malaysia. Physicochemical analysis (7) indicated the soils to have acidic pH (4.90 to 5.75) and to be sandy loam in nature and salt free (electrical conductivity [EC] range, 0.12 to 0.79 mS/cm). Heavy metal analysis by inductively coupled plasma optical emission spectrometry (8) detected arsenic (14.3 to 1,242 mg/kg), cadmium (1.6 to 17.8 mg/kg), and lead (52.17 to 81.10 mg/kg). *M. pudica* roots were shaken gently to remove rhizosphere soil adhering to the surface of the roots. This was done in a laminar flow cabinet, and sterile forceps were used to remove soil tightly attached to the roots (9, 10). DNA was extracted from the rhizosphere soils using a NucleoSpin soil DNA extraction kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. PCR amplification of the V3/V4 hypervariable regions was performed using the recommended Illumina 16S rRNA gene amplicon library method (USA) (11) with the primers Bakt_341F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACHVGGGTATCTAATCC) (12). The libraries were sequenced using the MiSeq platform (Illumina).

Adapter sequences and low-quality reads were removed from the paired-end reads using BBDuk from the BBTools package (https://sourceforge.net/projects/bbmap/) (13), and forward and reverse reads were merged using USEARCH v11.0.667 (14, 15). Default parameters were used for all software unless otherwise specified. All sequences shorter than 150 bp or longer than 600 bp were removed from downstream processing. The reads were aligned with the SILVA 16S rRNA gene database (release 132) (16), inspected for chimeric errors using VSEARCH v2.6.2 (17), and then clustered *de novo* into operational taxonomic units (OTUs) at 97% similarity using

Citation Abdullahi S, Haris H, Zarkasi KZ, Amir HG. 2020. 16S rRNA gene amplicon sequencing data of tailing and nontailing rhizosphere soils of *Mimosa pudica* from a heavy metal-contaminated ex-tin mining area. Microbiol Resour Announc 9:e00761-20. https://doi.org/10.1128/MRA.00761-20.

Editor J. Cameron Thrash, University of Southern California

Address correspondence to Hamzah G. Amir, amirhg@usm.my.

Received 10 July 2020
Accepted 28 September 2020
Published 15 October 2020

TABLE 1 Summary description of sample data in this study

Site	Sample	SRA accession no.	No. of sequences	No. of OTUs	Amplicon read length (bp)
Tailing	MRS1	SRR10909566	9,995	957	608
	MRS2	SRR10909565	26,105	1,306	607
	MRS3	SRR10909564	9,199	942	603
Nontailing	MRS4	SRR10909563	28,389	1,213	604
	MRS5	SRR10909562	27,898	1,308	599
	MRS6	SRR10909561	23,572	1,285	568

UPARSE v11.0.667 (18). The taxonomic assignment of the OTUs was achieved using QIIME v1.9.1 (19–21) against the SILVA database (16). All statistical analyses were done in the R statistical package v3.6.1 (https://www.r-project.org/) (22).

The total number of reads obtained in this study was 125,158 (Table 1). The OTUs were assigned to 23 bacterial phyla, 72 classes, 165 orders, 248 families, and 357 genera. *Proteobacteria* (41.2%), *Acidobacteria* (17.1%), and *Actinobacteria* (14.4%) were the most represented phyla, and the relative abundance of the top bacterial phyla in each sample is shown in Fig. 1.

Data availability. The 16S rRNA gene amplicon sequencing data from this study were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the BioProject accession number PRJNA601794 (Table 1).

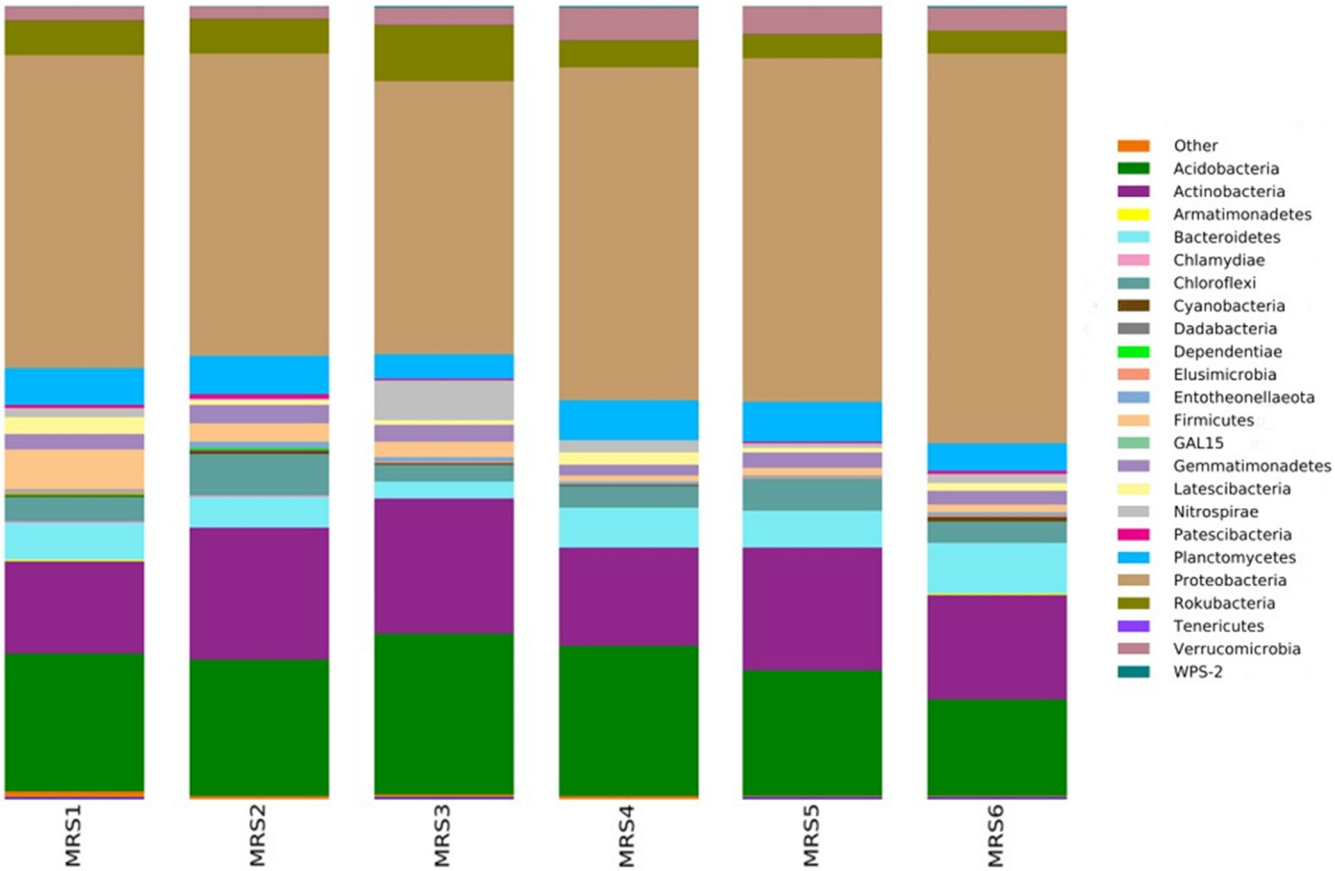

FIG 1 Relative abundance of the top bacterial phyla in each sample obtained from the sequencing data. Each color represents a different phylum.

ACKNOWLEDGMENTS

This work was supported by a Ministry of Higher Education, Malaysia (MOHE), grant (number 203/PBIOLOGI/6711774) and a Universiti Sains Malaysia Bridging Grant (grant number 304/PBIOLOGI/6316511). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

We also thank Mardani Abdul Halim of the School of Biological Sciences, Universiti Sains Malaysia, for constructive comments and suggestions.

All authors have read and approved the manuscript and declare no conflicts of interest in the submission.

REFERENCES

1. Lopez S, Goux X, Echevarria G, Calusinska M, Morel JL, Benizri E. 2019. Community diversity and potential functions of rhizosphere-associated bacteria of nickel hyperaccumulators found in Albania. Sci Total Environ 654:237–249. https://doi.org/10.1016/j.scitotenv.2018.11.056.
2. Ju W, Liu L, Fang L, Cui Y, Duan C, Wu H. 2019. Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper contaminated soil. Ecotoxicol Environ Saf 167:218–226. https://doi.org/10.1016/j.ecoenv.2018.10.016.
3. Khanna K, Jamwal VL, Gandhi SG, Ohri P, Bhardwaj R. 2019. Metal resistant PGPR lowered Cd uptake and expression of metal transporter genes with improved growth and photosynthetic pigments in Lycopersicon esculentum under metal toxicity. Sci Rep 9:5855. https://doi.org/10.1038/s41598-019-41899-3.
4. Xavier JC, Costa PES, Hissa DC, Melo VMM, Falcão RM, Balbino VQ, Mendonça LAR, Lima MGS, Coutinho HDM, Verde LCL. 2019. Evaluation of the microbial diversity and heavy metal resistance genes of a microbial community on contaminated environment. Appl Geochem 105:1–6. https://doi.org/10.1016/j.apgeochem.2019.04.012.
5. Klonowska A, Moulin L, Ardley JK, Braun F, Gollagher MM, Zandberg JD, Marinova DV, Huntemann M, Reddy TBK, Varghese NJ, Woyke T, Ivanova N, Seshadri R, Kyrpides N, Reeve WG. 2020. Novel heavy metal resistance gene clusters are present in the genome of Cupriavidus neocaledonicus STM 6070, a new species of Mimosa pudica microsymbiont isolated from heavy-metal-rich mining site soil. BMC Genomics 21:214. https://doi.org/10.1186/s12864-020-6623-z.
6. Gu Y, Van Nostrand JD, Wu L, He Z, Qin Y, Zhao F-J, Zhou J. 2017. Bacterial community and arsenic functional genes diversity in arsenic contaminated soils from different geographic locations. PLoS One 12:e0176696. https://doi.org/10.1371/journal.pone.0176696.
7. Motsara MR, Roy RN. 2008. Guide to laboratory establishment for plant nutrient analysis. FAO Fertilizer Plant Nutr Bull 19.
8. Bettinelli M, Beone GM, Spezia S, Baffi C. 2000. Determination of heavy metals in soils and sediments by microwave-assisted digestion and inductively coupled plasma optical emission spectrometry analysis. Anal Chim Acta 424:289–296. https://doi.org/10.1016/S0003-2670(00)01123-5.
9. McPherson MR, Wang P, Marsh EL, Mitchell RB, Schachtman DP. 2018. Isolation and analysis of microbial communities in soil, rhizosphere, and roots in perennial grass experiments. J Vis Exp 2018:57932. https://doi.org/10.3791/57932.
10. Qiao Q, Wang F, Zhang J, Chen Y, Zhang C, Liu G, Zhang H, Ma C, Zhang J. 2017. The variation in the rhizosphere microbiome of cotton with soil type, genotype and developmental stage. Sci Rep 7:3940. https://doi.org/10.1038/s41598-017-04213-7.
11. Illumina. 2013. 16S metagenomic sequencing library preparation: preparing 16S ribosomal RNA gene amplicons for the Illumina MiSeq system. Illumina, San Diego, CA.
12. Herlemann DPR, Labrenz M, Jurgens K, Bertilsson S, Waniek JJ, Andersson AF. 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J 5:1571–1579. https://doi.org/10.1038/ismej.2011.41.
13. Bushnell B, Rood J, Singer E. 2017. BBMerge—accurate paired shotgun read merging via overlap. PLoS One 12:e0185056. https://doi.org/10.1371/journal.pone.0185056.
14. Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. https://doi.org/10.1093/bioinformatics/btq461.
15. Liu T, Chen C-Y, Chen-Deng A, Chen Y-L, Wang J-Y, Hou Y-I, Lin M-C. 2020. Joining Illumina paired-end reads for classifying phylogenetic marker sequences. BMC Bioinformatics 21:105. https://doi.org/10.1186/s12859-020-3445-6.
16. Glöckner FO. 2019. The SILVA database project: an ELIXIR core data resource for high-quality ribosomal RNA sequences. Biodivers Inf Sci Stand 3:e36125. https://doi.org/10.3897/biss.3.36125.
17. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584. https://doi.org/10.7717/peerj.2584.
18. Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998. https://doi.org/10.1038/nmeth.2604.
19. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, 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.
20. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9.
21. Wu H, Irizarry RA, Bravo HC. 2010. Intensity normalization improves color calling in SOLiD sequencing. Nat Methods 7:336–337. https://doi.org/10.1038/nmeth0510-336.
22. R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.