AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

AMPLICON SEQUENCE COLLECTIONS

16S rRNA Gene Amplicon Profiling of the New Zealand Parasitic Blowfly *Calliphora vicina*

Nikola Palevich,[a] Paul H. Maclean,[a] Luis Carvalho,[a] Ruy Jauregui[a]

[a]AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand

ABSTRACT Here, we present a 16S rRNA gene amplicon sequence data set and profiles demonstrating the bacterial diversity of larval and adult *Calliphora vicina*, collected from Ashhurst, New Zealand (May 2020). The three dominant genera among the adult male and female *C. vicina* blowflies were *Serratia* and *Morganella* (phylum *Proteobacteria*) and *Carnobacterium* (phylum *Firmicutes*), while the larvae were also dominated by the genera *Lactobacillus* (phylum *Firmicutes*).

Ectoparasitic flies (blowflies) are a significant animal welfare and production issue for farmers worldwide (1). Control of blowflies is problematic because the flies are unpredictable and highly mobile, and strike (or myiasis) is difficult to see initially but has an immediate impact on animal production and welfare. Currently, control relies heavily on the prophylactic application of long-acting chemicals to all sheep, but this approach is increasingly under threat due to the development of resistance to current treatments (2, 3). *Calliphora vicina* NZ_CalVic_NP (4, 5) was selected for microbiome assessment as a representative of a New Zealand field strain of *C. vicina*. In this study, we investigated the larval and adult male and female bacterial microbial profiles of *C. vicina* for the future development of new interventions such as probiotics, bioactive compounds, vaccines, or insecticides.

The *C. vicina* specimen larvae were collected from a farm site in the Ashhurst area of New Zealand (40°18′S, 175°45′E). Lab-reared blowflies were maintained on beef liver as the protein source and a 10% sugar solution, with the procedures for blowfly propagation and sample preparation based on those of Dear (6). To remove surface adherent bacteria from lab-reared *C. vicina*, pools of larvae and entire adult males and females were separated and washed twice in sterile phosphate-buffered saline (PBS; pH 7.4), snap-frozen in liquid nitrogen, and transferred to −80°C storage prior to DNA extraction. High-molecular-weight genomic DNA was isolated from *C. vicina* pooled samples of 100 larvae as well as 10 entire adult males and females per replicate ($n = 5$ for each), using a modified phenol-chloroform protocol recently applied to difficult samples (7–13). A DNA library was prepared using the Illumina (San Diego, CA) 16S V3 and V4 rRNA library preparation method according to the manufacturer's instructions (14) and sequenced on the Illumina MiSeq platform with the 2 × 250-bp paired-end (PE) reagent kit v2, producing a total of 3,017,007 PE raw reads.

The processing of the amplicon reads followed a modified version of the pipeline described in reference 15. Default parameters were used for all software unless otherwise specified. The reads produced by the sequencing instrument were paired using the program FLASH2 v2.2.00 (16). The paired reads were then quality trimmed using Trimmomatic v0.38 (17). The trimmed reads were reformatted as fasta files, and the read headers were modified to include the sample name. Mothur v1.45.2 (18) was used to remove reads with homopolymers longer than 10 nucleotides (nt) and to collapse the reads into unique representatives. The collapsed reads were clustered using Swarm v2 (19). The clustered reads were filtered based on their abundance, keeping representatives that were (i) present in

Citation Palevich N, Maclean PH, Carvalho L, Jauregui R. 2021. 16S rRNA gene amplicon profiling of the New Zealand parasitic blowfly *Calliphora vicina*. Microbiol Resour Announc 10: e00289-21. https://doi.org/10.1128/MRA.00289-21.

Editor Irene L. G. Newton, Indiana University, Bloomington

Address correspondence to Nikola Palevich, nik.palevich@agresearch.co.nz.

Received 18 March 2021
Accepted 16 April 2021
Published 6 May 2021

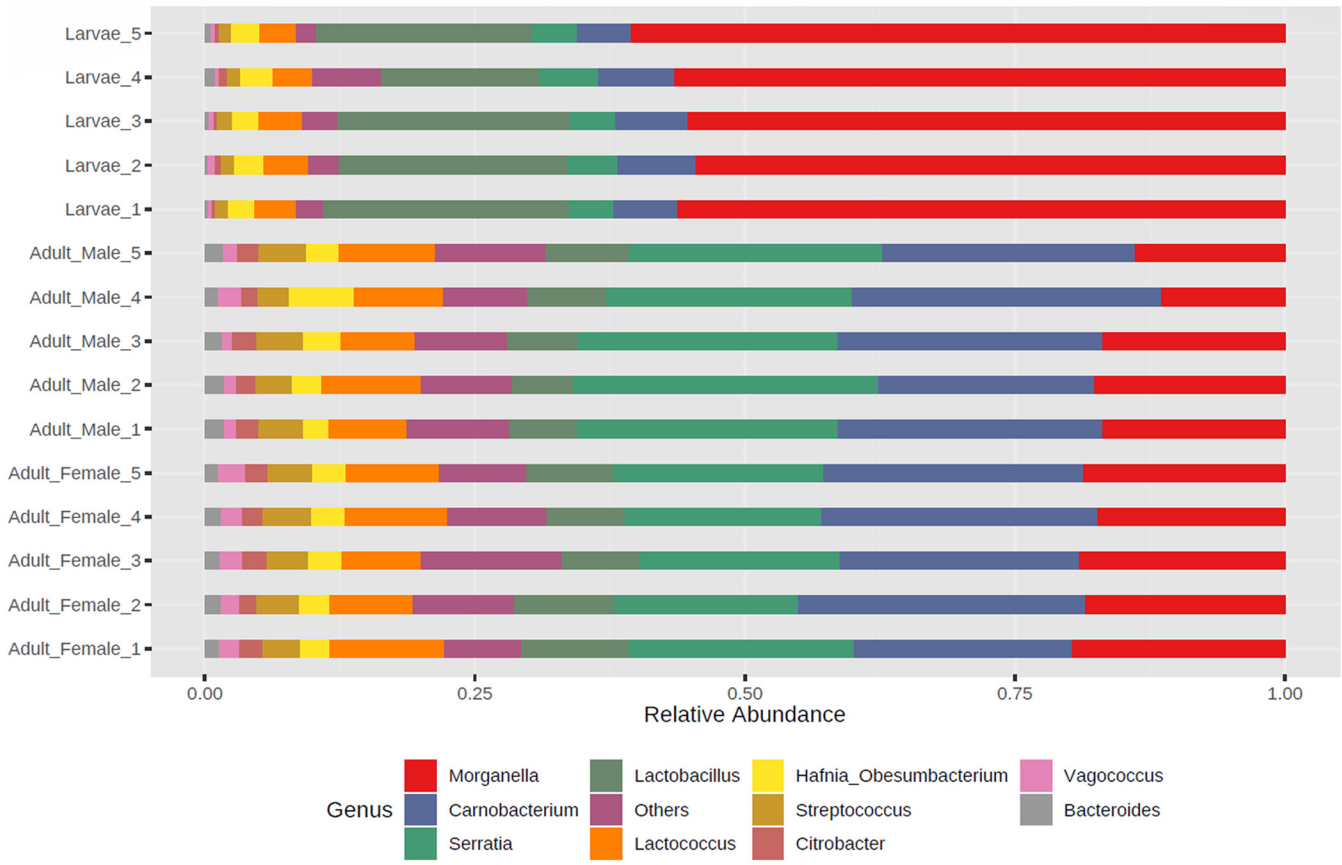

FIG 1 Taxonomic composition of the dominant bacteria of New Zealand *C. vicina*. The relative abundance of the dominant bacterial genera was obtained from 16S rRNA sequencing of *C. vicina* field strain NZ_CalVic_NP larvae and adult male and female samples. Genera with a relative abundance of less than 1% and unassigned amplicon sequence variants were grouped together as "others."

one sample with a relative abundance of >0.1%, (ii) present in >2% of the samples with a relative abundance of >0.01%, or (iii) present in 5% of the samples at any abundance level (Fig. 1). The selected representatives were annotated using Qiime 2 v2017.4 (20) with the Silva database v138 (21). The metagenomic 16S rRNA gene amplicon sequencing of *C. vicina* field strain NZ_CalVic_NP reported here is a valuable resource for future studies investigating the bacterial genetic mechanisms associated with flystrike.

Data availability. The 16S rRNA gene amplicon sequence data have been deposited in the GenBank Sequence Read Archive (SRA) under the BioProject accession number PRJNA667961.

ACKNOWLEDGMENTS

We thank Xiaoxiao Lin for assistance with the DNA sequencing and Paul Candy for sample collection.

This research was supported by the Agricultural and Marketing Research and Development Trust (AGMARDT) Postdoctoral Fellowships Programme (number P17001) and the AgResearch Ltd. Strategic Science Investment Fund (SSIF) (number PRJ0098715) of New Zealand.

REFERENCES

1. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA. 2000. Climate change and vector-borne diseases: a regional analysis. Bull World Health Organ 78:1136–1147.
2. Hall M, Wall R. 1995. Myiasis of humans and domestic animals. Adv Parasitol 35:257–334. https://doi.org/10.1016/s0065-308x(08)60073-1.
3. Fischer OA, Matlova L, Dvorska L, Svastova P, Bartl J, Weston RT, Pavlik I. 2004. Blowflies *Calliphora vicina* and *Lucilia sericata* as passive vectors of *Mycobacterium avium* subsp. *avium*, *M. a. paratuberculosis* and *M. a. hominissuis*. Med Vet Entomol 18:116–122. https://doi.org/10.1111/j.0269-283X.2004.00477.x.
4. Palevich N, Carvalho L, Maclean P. 2021. The complete mitochondrial genome of the New Zealand parasitic blowfly *Lucilia sericata* (Insecta:

Diptera: Calliphoridae). Mitochondrial DNA B Resour 6:1267–1269. https://doi.org/10.1080/23802359.2021.1906774.
5. Palevich N, Carvalho L, Maclean P. 2021. Characterization of the complete mitochondrial genome of the New Zealand parasitic blowfly *Calliphora vicina* (Insecta: Diptera: Calliphoridae). Mitochondrial DNA B Resour 6:1270–1272. https://doi.org/10.1080/23802359.2021.1906775.
6. Dear JP. 1986. Calliphoridae (Insecta: Diptera). Fauna N Z 8:88.
7. Palevich N, Maclean PH, Baten A, Scott RW, Leathwick DM. 2019. The genome sequence of the anthelmintic-susceptible New Zealand *Haemonchus contortus*. Genome Biol Evol 11:1965–1970. https://doi.org/10.1093/gbe/evz141.
8. Palevich N, Maclean PH, Choi Y-J, Mitreva M. 2020. Characterization of the complete mitochondrial genomes of two sibling species of parasitic roundworms, *Haemonchus contortus* and *Teladorsagia circumcincta*. Front Genet 11:573395. https://doi.org/10.3389/fgene.2020.573395.
9. Palevich N, Kelly WJ, Ganesh S, Rakonjac J, Attwood GT. 2019. *Butyrivibrio hungatei* MB2003 competes effectively for soluble sugars released by *Butyrivibrio proteoclasticus* B316^T during growth on xylan or pectin. Appl Environ Microbiol 85:e02056-18. https://doi.org/10.1128/AEM.02056-18.
10. Palevich N, Kelly WJ, Leahy SC, Denman S, Altermann E, Rakonjac J, Attwood GT. 2019. Comparative genomics of rumen *Butyrivibrio* spp. uncovers a continuum of polysaccharide-degrading capabilities. Appl Environ Microbiol 86:e01993-19. https://doi.org/10.1128/AEM.01993-19.
11. Palevich N, Maclean PH, Kelly WJ, Leahy SC, Rakonjac J, Attwood GT. 2020. Complete genome sequence of the polysaccharide-degrading rumen bacterium *Pseudobutyrivibrio xylanivorans* MA3014 reveals an incomplete glycolytic pathway. Genome Biol Evol 12:1566–1572. https://doi.org/10.1093/gbe/evaa165.
12. Palevich N, Palevich FP, Maclean PH, Altermann E, Gardner A, Burgess S, Mills J, Brightwell G. 2021. Comparative genomics of Clostridium species associated with vacuum-packed meat spoilage. Food Microbiol 95:103687. https://doi.org/10.1016/j.fm.2020.103687.
13. Palevich N, Palevich FP, Maclean PH, Jauregui R, Altermann E, Mills J, Brightwell G. 2019. Draft genome sequence of *Clostridium estertheticum* subsp. *laramiense* DSM 14864^T, isolated from spoiled uncooked beef. Microbiol Resour Announc 8:e01275-19. https://doi.org/10.1128/MRA.01275-19.
14. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79:5112–5120. https://doi.org/10.1128/AEM.01043-13.
15. Camarinha-Silva A, Jáuregui R, Pieper DH, Wos-Oxley ML. 2012. The temporal dynamics of bacterial communities across human anterior nares. Environ Microbiol Rep 4:126–132. https://doi.org/10.1111/j.1758-2229.2011.00313.x.
16. Magoč T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963. https://doi.org/10.1093/bioinformatics/btr507.
17. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
18. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541. https://doi.org/10.1128/AEM.01541-09.
19. Mahé F, Rognes T, Quince C, de Vargas C, Dunthorn M. 2014. Swarm: robust and fast clustering method for amplicon-based studies. PeerJ 2:e593. https://doi.org/10.7717/peerj.593.
20. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena 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.
21. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and Web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219.