

# Genome Sequences of *Lactobacillus* sp. Strains wkB8 and wkB10, Members of the Firm-5 Clade, from Honey Bee Guts

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**We sequenced two strains from the *Lactobacillus* Firm-5 clade, a dominant group of symbionts in the guts of honey bees and other social bees. The genome of strain wkB8, comprising a 1.93-Mb chromosome and a 6.4-kb plasmid, was fully closed, while strain wkB10 was assembled into 32 contigs. These genomes will provide insights into how gut symbionts evolve and interact with their host species.**

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Honey bees (*Apis* spp.) possess a highly specialized gut microbial community comprising about 8 bee-specific phylotypes (1). We recently sequenced the genomes of the major Gram-negative constituents, *Snodgrassella alvi* and *Gilliamella apicola* (2). Here, we present the genomes of two *Lactobacillus* strains of the Firm-5 clade, commonly identified as the most numerically abundant phylotype in honey bee guts (3, 4). This clade has also been reported in bumble bees (*Bombus* spp.) and stingless bees (*Meliponini*), which are close relatives of honey bees (5–7). Interestingly, host-associated *Lactobacillus* have not been found in other bee species, suggesting a uniquely coevolved symbiosis exists between Firm-5 and the social corbiculate bees (*Apis*, *Bombus*, and *Meliponini*) (8).

We isolated Firm-5 strains from the guts of the honey bee *Apis mellifera* as previously described (9), using Columbia agar with 5% sheep's blood as a growth medium. DNA was extracted using phenol-chloroform and purified on DNeasy spin columns (Qiagen). Total genomic DNA was sequenced on the Illumina MiSeq platform from 2 × 250-bp paired-end libraries, returning 3,046,168 (strain wkB8) and 2,613,160 (strain wkB10) reads. Overlapping reads were combined using FLASH (10). All read types were then assembled with Velvet version 1.2.10 (11), producing a total of 18 and 32 contigs for wkB8 and wkB10, respectively. We successfully closed the wkB8 genome by *in silico* assembly inspection and combinatorial gap-spanning PCRs, although 45 ambiguous bases remain due to polymorphisms in the multi-copy rRNA-encoding regions. Genomes were annotated with the RAST server (12).

The genome of wkB8 comprises a 1,926,135-bp chromosome and a 6,396-bp plasmid. It carries 1,772 predicted CDSs, 57 tRNAs, and 4 rRNA operons, and has a GC content of 36.7%. The wkB10 genome assembly was broken into 32 contigs with an  $N_{50}$  of 165,078, but appeared to represent a single chromosome. Examination of read coverage depth uncovered several contigs representing genomic regions likely present in multiple copies: contig018 (19 copies), contig019 (13 copies), and contig028 (5

copies). Thus, we estimate the wkB10 genome to be between 2.08 and 2.30 Mb in size, with 35.4% GC content. We detected 1,957 CDSs, 55 tRNAs, and at least 4 rRNA operons in wkB10. Both strains possess pathways to ferment various carbohydrates (e.g., fructose, lactose, mannose, *N*-acetylglucosamine, sorbose, sucrose, trehalose, xylulose) to lactic acid, and both encode a large number of predicted extracellular proteins that may allow adhesion to and degradation of environmental substrates such as chitin (13).

Strains wkB8 and wkB10 are 98.9% identical at their 16S rRNA locus but have only 86.0% average nucleotide identity across orthologous genomic regions. The bee-associated *Lactobacillus* Firm-5 cluster clearly comprises a diverse, deeply branching group of symbionts. Based on recent efforts to classify this group (14, 15), wkB8 is likely most related to *Lactobacillus helsingborgensis*, while wkB10 is part of *L. kullabergensis* or *L. kimbladii*; however, more genomes are required to unravel the murky relationships within the Firm-5 clade.

**Nucleotide sequence accession numbers.** The complete sequence of strain wkB8 has been deposited in GenBank under accession numbers CP009531 and CP009532, and the wkB10 whole-genome shotgun project has been deposited under accession number JQ00000000.

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## REFERENCES

- Martinson VG, Danforth BN, Minckley RL, Rueppell O, Tingek S, Moran NA. 2011. A simple and distinctive microbiota associated with honey bees and bumble bees. *Mol. Ecol.* 20:619–628. <http://dx.doi.org/10.1111/j.1365-294X.2010.04959.x>.

2. Kwong WK, Engel P, Koch H, Moran NA. 2014. Genomics and host specialization of honey bee and bumble bee gut symbionts. *Proc. Natl. Acad. Sci. U. S. A.* 111:11509–11514. <http://dx.doi.org/10.1073/pnas.1405838111>.
3. Moran NA, Hansen AK, Powell JE, Sabree ZL. 2012. Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. *PLoS One* 7:e36393. <http://dx.doi.org/10.1371/journal.pone.0036393>.
4. Corby-Harris V, Maes P, Anderson KE. 2014. The bacterial communities associated with honey bee (*Apis mellifera*) foragers. *PLoS One* 9:e95056. <http://dx.doi.org/10.1371/journal.pone.0095056>.
5. Koch H, Schmid-Hempel P. 2011. Bacterial communities in central European bumblebees: low diversity and high specificity. *Microb. Ecol.* 62: 121–133. <http://dx.doi.org/10.1007/s00248-011-9854-3>.
6. Vásquez A, Forsgren E, Fries I, Paxton RJ, Flaberg E, Szekeley L, Olofsson TC. 2012. Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. *PLoS One* 7:e33188. <http://dx.doi.org/10.1371/journal.pone.0033188>.
7. Leonhardt SD, Kaltenpoth M. 2014. Microbial communities of three sympatric Australian stingless bee species. *PLoS One* 9:e105718. <http://dx.doi.org/10.1371/journal.pone.0105718>.
8. McFrederick QS, Cannone JJ, Gutell RR, Kellner K, Plowes RM, Mueller UG. 2013. Specificity between lactobacilli and hymenopteran hosts is the exception rather than the rule. *Appl. Environ. Microbiol.* 79: 1803–1812. <http://dx.doi.org/10.1128/AEM.03681-12>.
9. Kwong WK, Moran NA. 2013. Cultivation and characterization of the gut symbionts of honey bees and bumble bees: description of *Snodgrassella alvi* gen. nov., sp. nov., a member of the family *Neisseriaceae* of the *Beta-proteobacteria*, and *Gilliamella apicola* gen. nov., sp. nov., a member of *Orbaceae* fam. nov., *Orbales* ord. nov., a sister taxon to the order '*Enterobacteriales*' of the *Gammaproteobacteria*. *Int. J. Syst. Evol. Microbiol.* 63: 2008–2018. <http://dx.doi.org/10.1099/ijs.0.044875-0>.
10. Magoč T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27:2957–2963. <http://dx.doi.org/10.1093/bioinformatics/btr507>.
11. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
12. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res.* 42: D206–D214. <http://dx.doi.org/10.1093/nar/gkt1226>.
13. Sánchez B, González-Tejedo C, Ruas-Madiedo P, Urdaci MC, Margolles A. 2011. *Lactobacillus plantarum* extracellular chitin-binding protein and its role in the interaction between chitin, Caco-2 cells, and mucin. *Appl. Environ. Microbiol.* 77:1123–1126. <http://dx.doi.org/10.1128/AEM.02080-10>.
14. Killer J, Dubná S, Sedláček I, Švec P. 2014. *Lactobacillus apis* sp. nov., from the stomach of honeybees (*Apis mellifera*), having an *in vitro* inhibitory effect on the causative agents of American and European foulbrood. *Int. J. Syst. Evol. Microbiol.* 64:152–157. <http://dx.doi.org/10.1099/ijs.0.053033-0>.
15. Olofsson TC, Alsterfjord M, Nilson B, Butler E, Vásquez A. 2014. *Lactobacillus apinorum* sp. nov., *Lactobacillus mellifer* sp. nov., *Lactobacillus mellis* sp. nov., *Lactobacillus melliventris* sp. nov., *Lactobacillus kimbladii* sp. nov., *Lactobacillus helsingborgensis* sp. nov. and *Lactobacillus kullabergensis* sp. nov., isolated from the honey stomach of the honeybee *Apis mellifera*. *Int. J. Syst. Evol. Microbiol.* 64:3109–3119. <http://dx.doi.org/10.1099/ijs.0.059600-0>.