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# Whole-genome sequence of *Enterobacter hormaechei*, isolate *jjbc* recovered from the gut of *Plutella xylostella* feeding on cabbage

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**AUTHOR AFFILIATIONS** See affiliation list on p. 3.

**ABSTRACT** We present the whole-genome sequence of *Enterobacter hormaechei* (previously *Enterobacter cloacae*) obtained from long and short reads. It is a dominant gut symbiont of the notorious crop pest *Plutella xylostella*, highly prevalent in lepidopteran midguts and a useful model for the evolution of resistance to antimicrobials.

**KEYWORDS** gut microbiota, lepidoptera, host-microbe interactions, antimicrobial resistance

*E. hormaechei* symbionts isolated from lepidopterans encode genes with functions such as digestion of plant cell wall carbohydrates, detoxification of plant allelochemicals, and the breakdown of insecticides (1–4), capabilities that are consistent with a niche as symbionts

of herbivores. The ease of use of *Enterobacter hormaechei* in gnotobiotic insect rearing systems and the possession of an AmpC beta-lactamase also mean that this bacterium is a valuable model for antimicrobial resistance in the *Enterobacteriaceae* (5).

*E. hormaechei* isolate *jjbc* was isolated by homogenizing *Plutella xylostella* larvae feeding on Chinese cabbage, *Brassica pekinensis*, at the Department of Zoology, University of Oxford. Homogenates were plated on Luria-Bertani (LB) agar and incubated at 30°C (6). After streaking clones, bacteria were grown in LB broth overnight at 30°C. DNA extraction from these cultures and sequencing were done at MicrobesNG (Birmingham, UK). Briefly, 40 µL of bacterial cell suspension was lysed in 120 µL of TE buffer-containing lysozyme, metapolyzyme, and RNase A. Proteinase K (final concentration 0.1 mg/mL) and SDS [final concentration 0.5% (vol/vol)] were added and incubated for 5 min at 65°C. Genomic DNA was purified using an equal volume of SPRI beads and resuspended in Elution buffer (10 mM Tris-HCl, pH 8.0). Genomic DNA libraries were prepared using the Nextera XT Library Prep Kit (Illumina, San Diego, USA) with these modifications: input DNA was increased twofold, and PCR elongation time was increased to 45 seconds. For the short reads, libraries were sequenced on a Illumina NovaSeq 6000 (Illumina, San Diego, USA) using a 250 bp paired-end protocol. For the long reads, DNA libraries were prepared using the Oxford Nanopore SQK-LSK109 and Native Barcoding EXP-NBD104/114 kits (ONT, UK) with 400–500 ng High Molecular Weight DNA, fragmented without size selection. Barcoded samples were pooled and loaded in a Library prepFLO-MIN111 (R10.3) flow cell, in a GridION (ONT, UK).

We obtained 1,172,223 Illumina reads and 13,666 ONT reads. The read quality was verified using FastQC (v. 0.12.1) (7) and NanoPlot (v. 1.42.0) (8), respectively. Genome assembly was performed using the fully automated hybrid assembly pipeline Unicycler (v. 0.5.0) (9). Default settings were used for all software (10). Manual intervention was required to resolve the following issues in the automatic assembly: (i) erroneous removal of genuine sequences during graph cleaning of the short-read assembly and (ii) collapse of inter-plasmidic repeats (11). The correction of these assembly errors resulted in a fully resolved genome consisting of one circular chromosome

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The authors declare no conflict of interest.

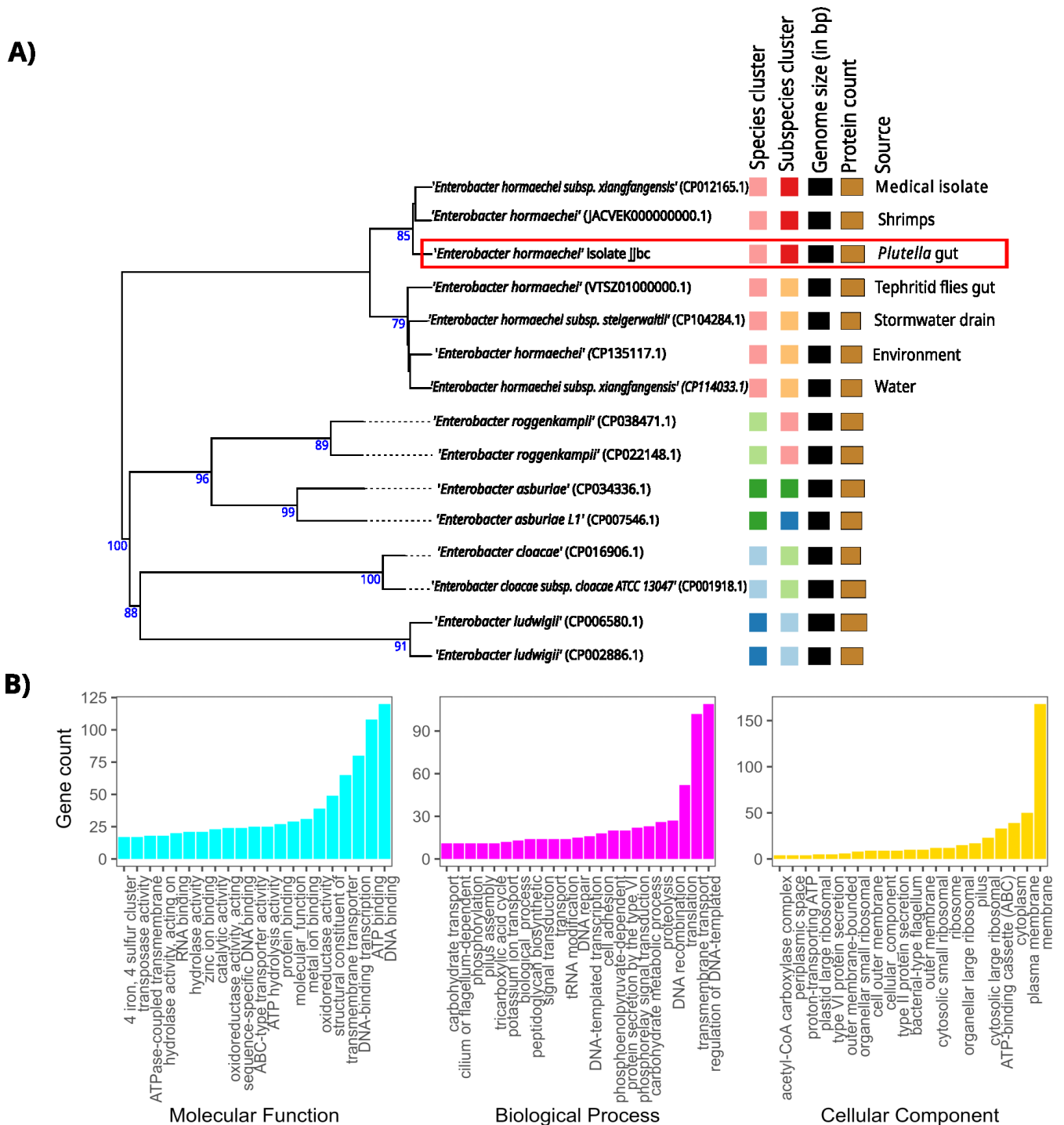
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**FIG 1** (A) Whole-genome phylogeny generated using the TYGS server confirming isolate jbc belongs to the species *E. hormaechei* and clusters with isolates from environmental samples, shrimps, and insect guts. Colored squares show five species clusters and seven sub-species clusters and the relative genome size and protein count of different genomes. Numbers above the branches represent branch support values inferred from 100 pseudo-bootstrap replicates based on Genome BLAST Distance Phylogeny under the algorithm distance formula (18). (B) Top 20 Gene Ontology terms showing the number of genes associated with each function for the categories of molecular function, biological process, and cellular component.

and four circular plasmids (Table 1), with a BUSCO completeness score of C:98.8% (S:98.6%,D:0.2%),F:0.2%,M:1.0%,n:440 (12) and a mean read depth of 110x. The closest matching species confirmed using the Type (Strain) Genome Server (TYGS) (13) is *E.*

TABLE 1 Summary of the genome assembly statistics for *E. hormaechei* isolate jjbc<sup>a</sup>

Replicon	Length (bp)	GC content (%)	Topology	GenBank accession
Chromosome	4,854,432	55.3	Circular	CP136935
Plasmid 1	87,769	50.5	Circular	CP136936
Plasmid 2	82,397	53.2	Circular	CP136937
Plasmid 3	64,969	52.4	Circular	CP136938
Plasmid 4	53,743	50.8	Circular	CP136939

<sup>a</sup>Circularity of contigs was confirmed through Unicycler's final assembly graph (gfa1) in Bandage (v 0.8.1) (17).

*hormaechei* (Fig. 1A). This genome was annotated using PGAP v.6.6 (14) and contains 4,951 predicted genes. Functional annotation shows multiple genes associated with carbohydrate metabolism (Fig. 1B). Using ResFinder 4.4.2 server (15), we identified two antimicrobial resistance genes: *fosA* for Fosfomycin resistance (96.71% alignment and 100% coverage) and *blaACT-16*, an AmpC beta-lactamase (99.74% alignment and 100% coverage) common in *Enterobacter* spp. (16).

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## DATA AVAILABILITY

This Whole-Genome Shotgun project and the annotate genome have been deposited in GenBank under the Bioproject number [PRJNA1026073](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1026073) and BioSample accession number [SAMN39460432](https://www.ncbi.nlm.nih.gov/biosample/SAMN39460432). The raw long and short reads are available from NCBI Sequence Read Archive (SRA) under the accession numbers [SRR28014489](https://www.ncbi.nlm.nih.gov/sra/SRR28014489) and [SRR28014490](https://www.ncbi.nlm.nih.gov/sra/SRR28014490).

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