Draft Genome Sequence of *Chryseobacterium* Strain CBo1 Isolated from *Bactrocera oleae*

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Running Head: Draft genome sequences of bacteria associated with the agricultural pest *Bactrocera oleae [limit: 54 characters and spaces]*

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**Abstract**

Bacteria of the genus *Chryseobacterium* have previously been identified as mutualists of plants and insects. *Chryseobacterium* CBo1 was cultured from the gut of the agricultural pest *Bactrocera oleae* and whole genome sequenced. This genomic resource will aid investigation in to the transition of microbes between plant and invertebrate hosts.

**Genome Announcement**

Bacteriaof the genus *Chryseobacterium* (family *Flavobacteriaceae*) exploit a diverse range of habitats including soil, water, and eukaryotic organisms (1). *Chryseobacteria* can promote growth in plants (2,3), and have been isolated from *Oleae europaea* olive groves (4), which are also occupied by the agricultural pest *Bactrocera oleae*. *Chryseobacteria* constitute an element of the gut microbiota in a broad range of invertebrates including mosquitoes (5,6), moths (7), cockroaches (8) and termites (unpublished- accession KF257250.1), and have now been discovered in the gut of *B. oleae*.

*Chryseobacterium* CBo1 was cultured from the homogenate of 10 dissected guts from surface-sterilized adult *B. oleae*. Guts were homogenized in Schneider’s Insect Medium supplemented with 10% Foetal Bovine Serum and spread on to Brain Heart Infusion (BHI) agar plates. Plates were incubated at 25oC for 72h and individual colonies subsequently streaked on to BHI plates and incubated at 25oC for 72h. DNA was isolated from single colonies by boiling at 95oC for 5 minutes and used as template for PCR of the 16S rRNA gene with the primers A16SF (5’-AGAGTTTGATCMTGGCTCAG-3’) and B16SR (5’-CCCCTACGGTTACCTTGTTACGAC -3’). Sanger sequencing was performed on the resulting fragment to identify the genus of bacterium as *Chryseobacterium*. Single colonies were inoculated in to BHI broth and incubated at 25oC for 72h and genomic DNA was extracted using the Zymo Quick DNA Universal Kit (Zymo) following the manufacturers’ instructions for biological fluids and cells. The following amendments to the protocol were employed: samples were incubated with proteinase K at 55oC for 30 minutes rather than 10 minutes, and were eluted twice in a volume of 40μl to give a total of 80μl per sample. Library preparation was performed with the NEBNext Ultra DNA library preparation kit (New England Biolabs) following the manufacturers’ instructions, and sequencing was performed on an Illumina MiSeq sequencer at the Centre for Genomic Research, University of Liverpool, with paired-end 250bp reads.

The resulting 2,122,794 reads were assembled with SPAdes version 3.7.1 (9). SPAdes generated a 4.5Mb assembly comprising 71 contigs with an N50 of 143,840 and an average GC content of 35.7%. Genes were annotated using PROKKA version 1.5.2 (10), which produced a total of 4144 protein coding and 76 RNA genes.

In combination with draft genome sequences from other members of the *B. oleae* gut microbiota (11-13), this draft genome sequence of *Chryseobacterium* CBo1 will allow further investigation in to the interactions between insects and their microbial communities. These genomic resources will also allow us to examine the transition that microbes undergo when shifting between plant and animal hosts on a range of evolutionary timescales.

**Nucleotide sequence accession numbers**. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession MAUH00000000. The version described in this paper is version MAUH01000000.

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**References**

1. **Bernardet JF, Hugo C, Bruun B**. 2005. The genera Chryseobacterium and Elizabethkingia. In *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*; Springer-Verlag: New York.
2. **Dardanelli MS, Manyani H, González-Barroso S, Rodríguez-Carvajal MA, Gil-Serrano AM, Espuny MR, López-Baena FJ, Bellogín RA, Megías M, Ollero FJ**. 2010. Effect of the presence of the plant growth promoting rhizobacterium (PGPR) Chryseobacterium balustinum Aur9 and salt stress in the pattern of flavonoids exuded by soybean roots. Plant Soil 328: 483 - 93.
3. **Gutiérrez Mañero FJ, Probanza A, Ramos B, Colón Flores JJ, Lucas García JA**. 2003. Effects of culture filtrates of rhizobacteria isolated from wild lupine on germination, growth, and biological nitrogen fixation of lupine seedlings. J Plant Nut 26: 1101 - 15.

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| 1. **del Carmen Montero-Calasanz M, Göker M, Rohde M, Spröer C, Schumann P, Busse HJ, Schmid M, Klenk HP, Tindall BJ, Camacho M**. 2014. Chryseobacterium oleae sp. nov., an efficient plant growth promoting bacterium in the rooting induction of olive tree (Olea europaea L.) cuttings and emended descriptions of the genus Chryseobacterium, C. daecheongense, C. gambrini, C. gleum, C. joostei, C. jejuense, C. luteum, C. shigense, C. taiwanense, C. ureilyticum and C. vrystaatense. Syst Appl Microbiol 37: 342 - 50.
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1. **Campbell CL, Mummey DL, Schmidtmann ET, Wilson WC**. 2004. Culture-independent analysis of midgut microbiota in the arbovirus vector Culicoides sonorensis (Diptera: Ceratopogonidae). J Med Entomol 41: 340 - 8.
2. **Kämpfer P, Chandel K, Prasad GB, Shouche YS, Veer V**. 2010. Chryseobacterium culicis sp. nov., isolated from the midgut of the mosquito Culex quinquefasciatus. Int J Syst Evol Microbiol 60: 2387 - 91.
3. **Walenciak O, Zwisler W, Gross EM**. 2002. Influence of Myriophyllum spicatum-derived tannins on gut microbiota of its herbivore Acentria ephemerella. J Chem Ecol 28: 2045 - 56.
4. **Dugas JE, Zurek L, Paster BJ, Keddie BA, Leadbetter ER**. 2001. Isolation and characterization of a Chryseobacterium strain from the gut of the American cockroach, Periplaneta americana. Arch Microbiol 175: 259 - 62.
5. **Nurk S, Bankevich A, Antipov D, Gurevich A, Korobeynikov A, Lapidus A, Prjibelsky A, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R**. 2013. Assembling genomes and mini-metagenomes from highly chimeric reads. Res Comput Mol Biol 158-170.
6. **Seemann T**. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 30: 2068-2069.
7. **Blow F, Gioti A, Starns D, Ben-Yosef M, Pasternak Z, Jurkevitch E, Vontas J, Darby AC**. 2016. Draft Genome Sequence of the Bactrocera oleae Symbiont “Candidatus Erwinia dacicola”. Genome Announc 4:e00896-16.
8. **Blow F, Vontas J, Darby AC**. 2016. Draft genome sequence of Stenotrophomonas maltophilia SBo1 isolated from Bactrocera oleae. Genome Announc 4:e00905-16.
9. **Blow F, Koukidou M, Vontas J, Darby AC**. 2016. Draft genome sequence of Tatumella Strain TA1 isolated from Bactrocera oleae. Genome Announc (Submitted).