Methods

**DNA Extraction, Sequencing, and read processing**

Strain Xap\_Cur was cultured overnight in nutrient broth. DNA was extracted using the Qiagen Blood and Tissue kit (Qiagen, Germantown, MD, United States) following the manufacturer’s instructions and sent to SeqCenter (previously known as MiGS, Pittsburg, PA, United States) for whole genome sequencing. DNA libraries were prepared with Illumina tagmentation kit and paired-end short read sequencing (2 x 150 bp) was conducted on a NextSeq 2000 system. Short read quality was assessed with fastQC v0.11.9 and reads were trimmed with BBduk in the BBmap suite v38.73. Long read libraries were generated with the Genomic DNA by Ligation kit (Oxford Nanopore Technologies, New York, NY, United States) and sequenced on an R9 flow cell using Guppy v5.0.16 in high accuracy mode for base calling (CITE).

**Genome Assembly and Polishing**

Genome assembly was conducted with a standard pipeline using Trycycler (CITE Trycycler). First, four draft genomes were generated with default parameters using both Flye and Raven, totaling eight draft assemblies, respectively (CITE FLYE, CITE RAVEN). Contigs from all draft assemblies were clustered via Trycycler Cluster and a modified draft assembly was reconciled with clusters remaining after manual inspection using Trycycler reconcile using a --min\_1kbp\_identity 23. The remainder of the Trycycler pipeline was run under default parameters, resulting in a consensus assembly composed of one circular chromosome and two circular plasmids. Genome polishing was conducted with both long reads and short reads. Long read polishing was completed with Medaka, using the medaka\_consensus command with the –bacteria flag (CITE medaka). Finally, the long read polished genome was further polished using Illumina short reads filtered and trimmed with bbmap v38.73 w k of 31and PolyPolish ran with default parameters (CITE, bbmap, polypolish). The final assembly is composed of one circular 4996128 bp chromosome and two plasmids of sizes 247284 base pairs and 41102 base pairs, respectively. Genome quality metrics were assessed with BUSCO and Merqury.

BLASTN of the small plasmid revealed a 99.96% sequence similarity at 41017 bases to the previously characterized pXap41 plasmid (Genbank: CP076702.1).

**Assembly results**

Quality assessment with BUSCO resulted in a BUSCO score of 99.9 % in Xanthomonadales (CITE). Completeness and overall quality values were assessed with Merqury v1.3 which yielded a 98.94% completeness score and quality values of the chromosome, mega plasmid, and pXap41 of 52.29, 57.52, and +inf, respectively (CITE).

Genome annotation was conducted on the Bakta webserver (CITE). This produced The completed genome is composed of 4458 CDS, 6 rRNAs, 54 tRNAs, and 1 tmRNA.

**Gene content**

Present in Xap CuR are a myriad of genes involved in the metabolism of heavy metals. These include a duplicated copLABGCD cluster, duplicated cusAB, one chromosomal and two plasmid copies of actP, two plasmid copies of czcAD, and one chromosomal copy of the cutAC and cohLAB operons, respectively. Moreover, Xap CuR shares a 98 % nucleotide identity and a pairwise amino acid identity of 97.3 % to the colRS regulated membrane protein (XAC1347) involved in copper homeostasis and virulence in Xanthomonas citri pv. citri (Fan et al, 2018).

This Xap CuR strain carries genes being reported for the very first time in Xap including many copper and heavy metal

copLABGCD X2 plasmid

cusAB X2 plasmid

actP X3 – 1 chromosome, two on plasmid

czcAD X2 plasmid

cutAC – chromosomal

cohLAB – chromosomal

BUSCO Xanthomonadales

99.9% BUSCO

1151 BUSCOS, single copy

1 fragmented busco