Complete Genome Sequences of Six Copper-Resistant *Xanthomonas* Strains Causing Bacterial Spot of Solanaceous Plants, Belonging to *X. gardneri*, *X. euvesicatoria*, and *X. vesicatoria*, Using Long-Read Technology

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**ABSTRACT** *Xanthomonas vesicatoria*, *Xanthomonas euvesicatoria*, and *Xanthomonas gardneri* cause bacterial spot disease. Copper has been applied since the 1920s as part of integrated management programs. The first copper-resistant strains were reported some decades later. Here, we fully sequenced six *Xanthomonas* strains pathogenic to tomato and/or pepper and having a copper-resistant phenotype.

Bacterial spot disease causes yield loss and impairs fruit quality in most tomato- and pepper-producing areas. The disease is caused by several distinct *Xanthomonas* species: *X. vesicatoria*, *X. euvesicatoria*, *X. gardneri*, and *X. perforans*, with *X. perforans* being recently reclassified as *X. euvesicatoria* (1). These phylogenetically distant species display similar symptoms and host range (2). Tomato (*Lycopersicon esculentum*), sweet pepper (*Capsicum annuum*), and chili pepper (*Capsicum frutescens*) are the main natural host species. The large-scale spread of the pathogens occurs through contaminated seeds, requiring strict disease management strategies to be imposed. A research effort is being made on biocontrol strategies, such as bacteriophage use, because resistant cultivars are scarce and rapidly overcome, and bacterial resistance to antimicrobial compounds is often reported. For example, widely used on pepper since the 1920s in the United States (3), a failure of copper sprays to control bacterial spot disease was first observed in 1968 (4), and the first associated genetic determinant (i.e., a transmissible plasmid) was reported more than 20 years later (2, 5).

In order to obtain a better understanding of copper resistance determinants and their spread among xanthomonads, we sequenced six tomato and pepper pathogen strains displaying a copper-resistant phenotype: *X. euvesicatoria* LMG930 and LH3, *X. vesicatoria* LMG911 and LM159, and *X. gardneri* JS749-3 and ICMP7383.

The long-read PacBio RSII technology was used to fully sequence the six strains, using one single-molecule real-time (SMRT) cell for each strain. Assembly of the raw reads was then performed using a SMRT Analysis HGAP version 2.3 protocol, and circularization of the contigs was done using a combination of the Minimus assembler (6) and the SMRT Analysis resequencing version 1 protocol. Assembly of transcription activator-like genes was improved using a custom version of the method previously described (7).

We obtained six closed chromosomes with sizes ranging from 4,969,893 bp to 5,313,102 bp, along with two to four closed plasmids per strain (31,328 bp to 31,328 bp).
TABLE 1 Characteristics of the six sequenced _Xanthomonas_ strains

| Strain      | Other no. | Species         | Copper resistance location | Country of isolation | Yr of isolation | Host              | Chromosome size (bp) | G+C content (%) | ANI score (%) | Accession no.       |
|-------------|-----------|-----------------|----------------------------|----------------------|-----------------|-------------------|---------------------|-------------------|----------------|---------------------|
| LMG930      |           | _X. euvesicatoria_ | Plasmid                    | United States        | 1969            | Pepper            | 5,079,107           | 64.7              | 99.98          | CP018463 to CP018467 |
| LMG911      | CFBP2537  | _X. euvesicatoria_ | Plasmid                    | New Zealand          | 1955            | Tomato            | 5,110,163           | 63.4              | 99.95          | CP018725 to CP018727 |
| LM159       |           | _X. euvesicatoria_ | Plasmid                    | Argentina            | 1987            | Pepper            | 5,086,726           | 64.2              | 99.88          | CP018468 to CP018471 |
| LH3         | CFBP7993  | _X. euvesicatoria_ | Plasmid                    | Mauritius            | 2010            | Tomato            | 4,969,893           | 64.9              | 98.68          | (99.98)            |
| JS749-3     |           | _X. gardneri     | Plasmid                    | Réunion              | 1997            | Tomato            | 5,158,913           | 63.7              | 99.91          | CP018728 to CP018730 |
| ICMP7383    | CFBP7999  | _X. gardneri     | Plasmid                    | New Zealand          | 1980            | Tomato            | 5,313,102           | 63.5              | 98.29          | CP018731 to CP018734 |

222,061 bp) and un circularized contigs. Average nucleotide identity (ANI) scores with type strains were used to confirm species assignation (Table 1). The six strains possessed the previously described copper resistance gene system _copLAB_ (8) carried on a conjugative plasmid. Interestingly, the plasmid was very well conserved among five strains (dating from 1955 to 2010), while the other (LMG930) had no similarities apart from the copper resistance gene cluster. On five strains, this plasmid also comprised the _cuzAB-smmD_ from the copper resistance gene cluster. LH3 solely lacked _arsBHCR_.

Evaluation of the minimum inhibitory concentrations was achieved on casitone yeast extract glycerol (CYE) medium, as previously reported (9, 10): these were zinc chloride, 16 to 32 mg/liter; copper sulfate, 128 to 256 mg/liter; sodium arsenite, 16 mg/liter (LH3), 128 to 512 mg/liter (other strains); cadmium sulfate, 6.4 to 12.8 mg/liter; and cobalt chloride, 16 to 32 mg/liter.

The very high similarity of the plasmids hosting the copper resistance adaptive trait among four species pathogenic to solaneous plants suggests pervasive events of horizontal gene transfer at the niche level.

**Accession number(s).** The six closed genome sequences have been deposited at GenBank. The genome accession numbers are shown in Table 1.

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