



Draft Genome Sequences of the Type Strains of Three *Clavibacter* Subspecies and Atypical Peach-Colored Strains Isolated from Tomato

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ABSTRACT Here, we present the draft genome sequences of 10 *Clavibacter* sp. strains, including the type strains of different subspecies of *Clavibacter michiganensis* and a potentially novel species within the genus. Genome lengths of the strains varied between 2,982,864 and 3,288,331 bp, with G+C contents of 72.23 to 73.50%.

The genus *Clavibacter* (*Microbacteriaceae*) comprises Gram-positive actinobacteria, most of which are pathogenic on agriculturally important crops (1). Traditionally, the genus *Clavibacter* has been considered to include only one species, *Clavibacter michiganensis*, comprising five plant-pathogenic subspecies, i.e., *C. michiganensis* subsp. *insidiosus*, *C. michiganensis* subsp. *michiganensis*, *C. michiganensis* subsp. *nebraskensis*, *C. michiganensis* subsp. *sepedonicus*, and *C. michiganensis* subsp. *tessellarius* (2). Recently, plant-associated but nonpathogenic members of *C. michiganensis* were assigned to new subspecies, including *C. michiganensis* subsp. *californiensis* and *C. michiganensis* subsp. *chilensis* (3). Additionally, *C. michiganensis* subsp. *phaseoli* and *C. michiganensis* subsp. *capsici* were identified as the causal agents of bacterial bean leaf yellowing and bacterial canker of pepper, respectively (4, 5). On the other hand, peach color-pigmented nonpathogenic *Clavibacter* sp. strains were isolated from tomato phyllosphere and remain to be taxonomically evaluated (6, 7).

Recently, a reclassification of *C. michiganensis* members into two new species and three new combinations was proposed (8). However, due to the lack of genomic information from every newly identified subspecies, further investigations are warranted to clarify the taxonomy of *Clavibacter* spp. Here, we present the draft genome sequences of 10 *Clavibacter* species strains (Table 1), including the type strains of *C. michiganensis* subsp. *californiensis* (CFBP 8216), *C. michiganensis* subsp. *chilensis* (CFBP 8217), and *C. michiganensis* subsp. *phaseoli* (CFBP 8627), as well as the atypical peach-colored strains (CFBP 8615 and CFBP 8616) isolated from tomato in Iran (6, 7). The strains CFBP 8615 and CFBP 8616 resulted from the screening of microbial communities associated with tomato plants at Shiraz University in Iran (7, 9) and were isolated from asymptomatic tomato leaves on yeast extract-peptone-glucose agar (YPGA) medium as previously described (6), whereas the remaining eight strains were provided by CIRM-CFBP in France (2).

The *Clavibacter* sp. strains (Table 1) were grown on YPGA medium as previously described (2), and DNAs were extracted using the Wizard genomic DNA purification kit (Promega, Madison WI). The DNAs were sequenced using the Illumina HiSeq X platform at BGI Tech Solutions (Hong Kong), and the shotgun sequencing yielded 150-bp paired-end reads. A combination of Velvet (v 1.2.10), SOAPdenovo (v 2.04), and SOAPGapCloser (v 1.12) platforms (10, 11) was used for genome assembly. The genomes were assembled using the same strategy and the default settings of the

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TABLE 1 Source, place, and date of isolation, as well as genome information, for each *Clavibacter* species strain used in this study

| Nomenclature | CIRM-CFBP ^a code | Host of isolation | Yr of isolation | Country of isolation | G+C content (%) | Genome length (bp) | No. of contigs | Sequencing coverage (x) | No. of protein-coding genes | No. of RNA genes | No. of pseudogenes | Accession no. | | Reference or source | |
|---|-----------------------------|-----------------------------|-----------------|------------------------|-----------------|--------------------|----------------|-------------------------|-----------------------------|------------------|--------------------|-----------------|-----------------------|---------------------|------------|
| | | | | | | | | | | | | DBJ/ENA/GenBank | Sequence Read Archive | | |
| <i>Clavibacter michiganensis</i> subsp. <i>insidiosus</i> | CFBP 1195 | <i>Medicago sativa</i> | 1964 | United Kingdom | 72.84 | 3,203,470 | 805 | 450 | 3,333 | 52 | 199 | QWZD01000000 | SRR7977544 | SRX4810326 | 16 |
| <i>Clavibacter michiganensis</i> subsp. <i>insidiosus</i> | CFBP 6488 | <i>Medicago sativa</i> | 1998 | Czech Republic | 72.23 | 3,225,729 | 1,892 | 435 | 3,890 | 52 | 329 | QWEA01000000 | SRR7977605 | SRX4810381 | 2 |
| <i>Clavibacter michiganensis</i> | CFBP 7491 | <i>Solanum lycopersicum</i> | ND ^b | ND | 73.02 | 3,288,331 | 921 | 475 | 3,560 | 51 | 122 | QWEB01000000 | SRR7977581 | SRX4810357 | This study |
| <i>Clavibacter michiganensis</i> | CFBP 7493 | <i>Solanum lycopersicum</i> | ND | ND | 72.91 | 3,275,884 | 782 | 570 | 3,487 | 51 | 91 | QWEC01000000 | SRR7977687 | SRX4810463 | This study |
| <i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> | CFBP 7577 | <i>Zea mays</i> | ND | ND | 72.75 | 2,982,864 | 1,273 | 510 | 3,291 | 53 | 373 | QWED01000000 | SRR7977688 | SRX4810464 | 2 |
| <i>Clavibacter michiganensis</i> subsp. <i>californiensis</i> | CFBP 8216 ^T | <i>Solanum lycopersicum</i> | 2000 | United States (Hawaii) | 72.71 | 3,193,415 | 811 | 515 | 3,367 | 51 | 105 | QWEE01000000 | SRR7983517 | SRX4814786 | 3 |
| <i>Clavibacter michiganensis</i> subsp. <i>chilensis</i> | CFBP 8217 ^T | <i>Solanum lycopersicum</i> | 2007 | Netherlands | 73.50 | 3,044,807 | 1,002 | 450 | 3,356 | 54 | 114 | QWGS01000000 | SRR7983516 | SRX4814785 | 3 |
| <i>Clavibacter</i> spp. | CFBP 8615 | <i>Solanum lycopersicum</i> | 2015 | Iran | 73.23 | 3,129,097 | 620 | 580 | 3,236 | 50 | 78 | QWGT01000000 | SRR7983538 | SRX4814807 | 6 |
| <i>Clavibacter michiganensis</i> subsp. <i>phaseoli</i> | CFBP 8616 | <i>Solanum lycopersicum</i> | 2015 | Iran | 73.15 | 3,094,686 | 961 | 555 | 3,342 | 50 | 151 | QWGU01000000 | SRR7983541 | SRX4814809 | 6 |
| <i>Clavibacter michiganensis</i> subsp. <i>phaseoli</i> | CFBP 8627 ^T | <i>Phaseolus vulgaris</i> | 2009 | Spain | 73.46 | 3,052,098 | 1,009 | 460 | 3,289 | 53 | 205 | QWGV01000000 | SRR7983540 | SRX4814808 | 4 |

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^bND, not determined.

platforms. In brief, different values of kmers were tested to obtain a preassembly with SOAPdenovo. Then, the resulting preassemblies were treated as long reads and introduced in Velvet to obtain final assemblies. Libraries were prepared using 170 to 800 bp. For each strain, genome length (bp) and G+C content (%) are summarized in Table 1. Additionally, genome annotation was performed using the GeneMarkS+ (v 4.6) suite implemented in the NCBI Prokaryotic Genome Annotation Pipeline with default settings (12). Total numbers of protein-coding genes, RNA genes, and pseudogenes were determined for all the genomes, as shown in Table 1.

Average nucleotide identity (ANI) analysis using the JSpeciesWS Web server (13) showed that the atypical peach-colored strains CFBP 8615 and CFBP 8616 have only 89.00 to 93.00% sequence identity with the type strains of previously identified and/or newly introduced *Clavibacter* species/subspecies. These ANI values are far below the accepted threshold (95 to 96%) for the definition of prokaryotic species (14), suggesting that the strains CFBP 8615 and CFBP 8616 could be defined as a new species. A comprehensive multiphasic taxonomic study using the genome sequences provided in this study to reevaluate the taxonomy of *Clavibacter* spp. and clarify the position of the strains CFBP 8615 and CFBP 8616 within the genus is ongoing.

Data availability. These whole-genome shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers shown in Table 1. For all sequences, the first versions of the accession numbers are described in this paper. The raw reads of the sequences of all strains were submitted to the Sequence Read Archive (SRA) database (15), and the corresponding accession numbers are shown in Table 1. All of the strains listed in Table 1 are available at CIRM-CFBP, the French Collection of Plant-Associated Bacteria (http://www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria).

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REFERENCES

- Davis MJ, Gillaspie AG, Vidaver AK, Harris RW. 1984. *Clavibacter*: a new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. and *Clavibacter xyli* subsp. *cynodontis* subsp. nov., pathogens that cause ratoon stunting disease of sugarcane and bermudagrass stunting disease. *Int J Syst Bacteriol* 34: 107–117. <https://doi.org/10.1099/00207713-34-2-107>.
- Jacques MA, Durand K, Orgeur G, Balidas S, Fricot C, Bonneau S, Quillévéré A, Audusseau C, Olivier V, Grimault V, Mathis R. 2012. Phylogenetic analysis and polyphasic characterization of *Clavibacter michiganensis* strains isolated from tomato seeds reveal that non-pathogenic strains are distinct from *C. michiganensis* subsp. *michiganensis*. *Appl Environ Microbiol* 78:8388–8402. <https://doi.org/10.1128/AEM.02158-12>.
- Yasuhara-Bell J, Alvarez AM. 2015. Seed-associated subspecies of the genus *Clavibacter* are clearly distinguishable from *Clavibacter michiganensis* subsp. *michiganensis*. *Int J Syst Evol Microbiol* 65:811–826. <https://doi.org/10.1099/ijsem.0.000022>.
- Gonzalez AJ, Trapiell E. 2014. *Clavibacter michiganensis* subsp. *phaseoli* subsp. nov., pathogenic in bean. *Int J Syst Evol Microbiol* 64:1752–1755. <https://doi.org/10.1099/ijsem.0.058099-0>.
- Oh E-J, Bae C, Lee H-B, Hwang IS, Lee H-I, Yea MC, Yim K-O, Lee S, Heu S, Cha J-S, Oh C-S. 2016. *Clavibacter michiganensis* subsp. *capsici* subsp. nov., causing bacterial canker disease in pepper. *Int J Syst Evol Microbiol* 66:4065–4070. <https://doi.org/10.1099/ijsem.0.001311>.
- Osdaghi E, Ansari M, Taghavi SM, Zarei S, Koebnik R, Lamichhane JR. 2018. Pathogenicity and phylogenetic analysis of *Clavibacter michiganensis* strains associated with tomato plants in Iran. *Plant Pathol* 67:957–970. <https://doi.org/10.1111/ppa.12801>.
- Osdaghi E, Taghavi SM, Hamzehzarghani H, Fazliarab A, Harveson RM, Tegli S, Lamichhane JR. 2018. Epiphytic *Curtobacterium flaccumfaciens* strains isolated from symptomless solanaceous vegetables are pathogenic on leguminous but not on solanaceous plants. *Plant Pathol* 67: 388–398. <https://doi.org/10.1111/ppa.12730>.
- Li X, Tambong J, Yuan KX, Chen W, Xu H, Lévesque CA, De Boer SH. 2018. Re-classification of *Clavibacter michiganensis* subspecies on the basis of whole-genome and multi-locus sequence analyses. *Int J Syst Evol Microbiol* 68:234–240. <https://doi.org/10.1099/ijsem.0.002492>.
- Osdaghi E, Taghavi SM, Hamzehzarghani H, Fazliarab A, Lamichhane JR. 2017. Monitoring the occurrence of tomato bacterial spot and range of the causal agent *Xanthomonas perforans* in Iran. *Plant Pathol* 66: 990–1002. <https://doi.org/10.1111/ppa.12642>.
- Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW, Yiu SM, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam TW, Wang J. 2012. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. *Gigascience* 1:18. <https://doi.org/10.1186/2047-217X-1-18>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
- Borodovsky M, Lomsadze A. 2014. Gene identification in prokaryotic genomes, phages, metagenomes, and EST sequences with GeneMarkS suite. *Curr Protoc Microbiol* 32. <https://doi.org/10.1002/9780471729259.mc01e07s32>.
- Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. 2016. JSpeciesWS: a Web server for prokaryotic species circumscription based

- on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>.
14. Kim M, Oh HS, Park SC, Chun J. 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 64:346–351. <https://doi.org/10.1099/ijs.0.059774-0>.
 15. Kodama Y, Shumway M, Leinonen R. 2012. The Sequence Read Archive: explosive growth of sequencing data. *Nucleic Acids Res* 40:D54–D56. <https://doi.org/10.1093/nar/gkr854>.
 16. Dye DW, Kemp WJ. 1977. A taxonomic study of plant pathogenic *Corynebacterium* species. *N Z J Agric Res* 20:563–582. <https://doi.org/10.1080/00288233.1977.10427375>.