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RESOURCE ANNOUNCEMENT

Complete Genome Sequence Resource of a Strain of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, the Causal Agent of Bacterial Wilt of Common Bean, from Turkey

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Abstract

Curtobacterium flaccumfaciens pv. *flaccumfaciens* is the causal agent of bacterial wilt of common bean (*Phaseolus vulgaris*), a disease that can reduce yields of this economically important crop worldwide. Current genomics resources for this bacterial pathogen are limited. Therefore, long-read sequencing was used to determine the complete genome sequence of a pathogenic *C. flaccumfaciens* pv. *flaccumfaciens* strain isolated from common bean leaves showing irregular necrotic lesions with yellow borders collected in a commercial field in Turkey in 2015.

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Keywords

bacterial diseases, bacteriology, *Curtobacterium flaccumfaciens*, *Phaseolus vulgaris*, plasmid

Genome Announcement

Curtobacterium flaccumfaciens pv. *flaccumfaciens* is a Gram-positive coryneform bacterium that causes bacterial wilt disease of common bean (*Phaseolus vulgaris*). Some *C. flaccumfaciens* pv. *flaccumfaciens* strains also cause bacterial wilt of other legume crops, including cowpea (*Vigna unguiculata*), soybean (*Glycine max*), and pea (*Pisum sativum*) (EPPO 2011; Osdaghi et al. 2015; Soares et al. 2013). Symptoms of bacterial wilt disease include wilting, leaf scorch and, less typically, necrotic lesions with yellow borders that closely resemble those of common bacterial blight, caused by *Xanthomonas phaseoli* and *X. citri* pv. *fuscans* (Zaumeyer and Thomas 1957). *C. flaccumfaciens* pv. *flaccumfaciens* is seed-transmissible, and infected seed may be shriveled or discolored.

Bacterial wilt was first described in South Dakota in the 1920s (Hedges 1922), and caused economic losses to common bean production in the Midwestern United States. By the 1970s, the disease was effectively managed through practices such as planting pathogen-free seeds, crop rotation, and sanitation (Harveson et al. 2015). However, in the early 2000s, bacterial wilt reemerged as an economically important disease in the United States, e.g., in the states of Colorado, Nebraska, and Wyoming (Harveson et al. 2006). The disease has also been reported from other countries including Australia, Brazil, Canada, Germany, Iran, Mexico, South Africa, Spain, and Turkey (EPPO 2011; Harveson et al. 2015; Osdaghi et al. 2015).

In 2015, a survey of bacterial diseases of common bean was conducted in Turkey. The predominant disease observed was common bacterial blight based on (i) observation of blighted

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leaves having irregular lesions with water-soaked spots and lemon-colored borders, and (ii) consistent isolation of *X. phaseoli* on 523 medium, a general medium for isolation of Gram-negative and -positive plant pathogenic bacteria (Kado and Heskett 1970), and MXP, a semiselective medium for xanthomonads (Claflin et al. 1987). However, isolations from 5 of 18 such leaf samples, collected from three fields in the Burdur province, consistently yielded yellow mucoid colonies on 523 but not on MXP. A representative strain from a single colony of these yellow bacteria from each of these five samples was subcultured, and all were Gram-positive based on the KOH reaction (Suslow et al. 1982). Sequence analysis of the PCR-amplified ribosomal 16S-23S internal transcribed spacer region (Jensen et al. 1993) of the five strains revealed >90% identity with sequences of strains of *C. flaccumfaciens*, including pathovars *betae*, *flaccumfaciens*, *poinsettiae*, and *oortii*. Furthermore, the expected-size ~300-bp fragment was amplified from total genomic DNA of these five strains by PCR with the *C. flaccumfaciens* pv. *flaccumfaciens*-specific primer pair CffFOR2 and CffREV4 (Tegli et al. 2002). Pathogenicity of these strains was assessed by inoculating the common bean cultivars Topcrop, Elinda, and Volare by stem-stabbing with 20 μl of bacterial suspensions of approximately 10^8 cfu/ml (EPPO 2011). Plants were maintained in a temperature-controlled greenhouse. By 21 days postinoculation, all five strains induced wilting and interveinal necrotic lesions with yellow borders on trifoliolate leaves. Together, these results indicated that these are strains belonging to *C. flaccumfaciens* pv. *flaccumfaciens* and are designated Cff1035 to Cff1039. Furthermore, these results demonstrated that some of the necrotic lesions with yellow borders observed on common bean leaves in the three fields in Burdur were symptoms of bacterial wilt caused by *C. flaccumfaciens* pv. *flaccumfaciens* rather than common bacterial blight caused by *X. phaseoli* or *X. citri* pv. *fuscans*.

Genetic typing studies (e.g., repetitive element sequence-based PCR, amplified fragment length polymorphism) of *C. flaccumfaciens* pv. *flaccumfaciens* strains have shown a large degree of heterogeneity (Agarkova et al. 2012; Gonçalves et al. 2019), but genomic resources necessary for further investigation of the genetic diversity among *C. flaccumfaciens* pv. *flaccumfaciens* strains are limited. Genome assemblies for two *C. flaccumfaciens* pv. *flaccumfaciens* strains known to be pathogenic on common bean are publicly available: a draft genome of the type strain (CFBP3418), which was isolated from common bean in Hungary in 1957, and a recently released complete genome sequence of a strain (P990) isolated from bell pepper (*Capsicum* sp.) in Iran in 2015 (Table 1). Four additional draft genomes of *C. flaccumfaciens* strains are also available, but most of these came from environmental samples, and it is not known if these strains are pathogenic on plants.

Therefore, the complete genome sequence of one of the pathogenic *C. flaccumfaciens* pv. *flaccumfaciens* strains from Burdur, Turkey, Cff1037, was determined. A Cff1037 culture was grown in 50 ml of liquid 2× yeast tryptone medium in a 250-ml Erlenmeyer flask overnight at 28°C on a rotary shaker at 150 rpm. Cells were recovered by centrifugation and total genomic DNA was extracted with a CTAB method (Koike et al. 1999). This DNA was used to prepare a 15-kb BluePippin size-selected sequencing library by the UC Davis Genome Center. Sequencing was done on the Pacific Biosciences RSII platform with P6-C4 chemistry and generated 185,909 reads, with an N50 length of 11,598 and containing 1,503,939,033 total bases, after filtering via SMRTpipe (v1.87). Filtered reads were corrected, trimmed, and assembled de novo with Canu (v1.7.1), using options *–genomeSize=4.0*, *–correctedErrorRate=0.120*, *–corMaxEvidenceErate=0.15*, and *–corOutCoverage=100*, yielding five contigs (Koren et al. 2017). Three of these contigs were derived from a small number of reads (11 or fewer) with very low coverage relative to the largest assembled contig (less than 6×), suggesting they are most likely spuriously assembled contigs and, thus, were removed from the assembly. For the remaining two contigs, self-complementary contig ends were identified and removed with Circlator (v1.5.5), and the assembly was polished via SMRTpipe (Hunt et al. 2015). The genomic sequence was annotated with NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016).

The complete genome of Cff1037 is composed of a 3.7 Mb circular chromosome and a putative 113-kb linear plasmid, with mean assembly coverage of 396.0×. Cff1037 is predicted to encode 3,510 CDS, 47 tRNAs, three complete rRNA operons, and has a GC content of 71%. These properties are similar to those of the publicly available *C. flaccumfaciens* genomes (Table 1). Analysis with fastANI revealed that Cff1037 has an average nucleotide identity (ANI) of 96.25% with the type strain, CFBP3418, consistent with Cff1037 being a strain of *C. flaccumfaciens* (Jain et al. 2018). Annotation with dbCAN2 predicted Cff1037 encodes 44 cazymes, seven of which contain a signal peptide (Zhang et al. 2018). Sec and Tat secretion

Table 1. Characteristics of the genome of a strain of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* from Turkey (Cff1037) and comparison with publicly available *Curtobacterium* genome sequences

Strain	Genomic feature	Length (bp)	GC %	CDS	tRNAs	rRNA operons	Sequencing depth	Pathovar	Isolation source	Strain ANI to CFBP3418[a]	Source	GenBank accession number
CFBP3418[b]	WGS contigs[c]	3,827,492	71.0	3,571	47	2	200×	*flaccumfaciens*	*Phaseolus vulgaris*	100.00	GenBank	PUEZ01000000
P990								*flaccumfaciens*	*Capsicum* sp.	97.41		
	Chromosome	3,736,959	71.0	3,491	48	3	Unknown[d]			NA[e]	GenBank	CP045287
	pCff1	148,580	66.2	147	0	0	Unknown			NA	GenBank	CP045288
	pCff2	25,571	33.6	22	0	0	Unknown			NA	GenBank	CP045289
	pCff3	22,642	35.7	12	0	0	Unknown			NA	GenBank	CP045290
Cff1037								*flaccumfaciens*	*Phaseolus vulgaris*	96.25		
	Chromosome	3,667,996	71.1	3,393	47	3	329.9×			NA	This study	CP041259
	pCFF113	113,440	66.1	117	0	0	428.1×			NA	This study	CP041260
MEB126	WGS contigs	3,684,420	70.9	3,444	46	2	87×	ND[f]	*Arabidopsis thaliana*	94.32	GenBank	JXQU01000000
S5.26	WGS contigs	3,812,089	70.7	3,588	49	3	77×	ND	Ice-wedge polygon	94.31	GenBank	RCZL00000000
UCD-AKU	WGS contigs	3,692,614	70.9	3,386	47	3	261×	ND	Residential carpet	94.21	GenBank	APJN00000000
JUb65	WGS contigs	3,799,493	71.5	3,549	47	2	395×	ND	*Caenorhabditis elegans*	87.13	GenBank	SNVW00000000

[a] ANI includes all contigs and plasmids if applicable.
[b] *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*-type strain.
[c] WGS = whole genome sequence.
[d] Sequencing depth indicated on the associated BioSample (SAMN13022323) is unclear.
[e] NA = not applicable.
[f] ND = not determined.

systems were detected with KofamKOALA annotation (Aramaki et al. 2019). Two genes encoding proteins associated with the type VII secretion system (FK523_11590 and FK523_11600) were identified by BLAST search against the UniProtKB database (Consortium 2018) and are located in a putative operon along with six other genes of unknown functions (FK523_11575-11610). In *Mycobacterium tuberculosis*, type VII secretion systems are required for virulence; additionally, some contribute to maintaining iron and zinc homeostasis (Chang et al. 2014). Comparatively little is known about the role of this secretion system in plant pathogenic bacteria, although deletion of a locus containing type VII secretion system homologs in *Streptomyces scabies* did not affect virulence (Chang et al. 2014; Fyans et al. 2013).

The putative linear plasmid, pCFF113, has high nucleotide identity (99.7 to 100%) to two contigs from the draft assembly of the *C. flaccumfaciens* pv. *flaccumfaciens*-type strain CFBP 3418 (GenBank accession numbers PUEZ01000006.1 and PUEZ01000008.1), and to pCff1 from *C. flaccumfaciens* pv. *flaccumfaciens* strain P990. Plasmids pCFF113 and pCff1 are colinear, though pCff1 contains additional 5.5 and 29.6 kb sequences at the 5′ and 3′ ends, respectively. Read mapping analysis with minimap2 (v2.17) does not support a circular structure for pCFF113 (Li 2018), nor does pCFF113 contain self-complementary ends, consistent with the failure of Circlator to circularize this plasmid. These results suggest that this plasmid is not unique to Cff1037, but that genetic diversity in the sequence and even structure of this plasmid may exist among *C. flaccumfaciens* pv. *flaccumfaciens* strains. pCFF113 is not present in the four other *C. flaccumfaciens* draft genomes, consistent with a role in plant pathogenicity, although an ~5.6 kb region of pCFF113 that includes genes involved in carbohydrate metabolism (FK523_17505-17525) was detected in all *C. flaccumfaciens* genomes, except JUb65. A linear plasmid, pCSL1, occurs in *Clavibacter sepedonicus* strain ATCC33113 (GenBank accession number AM849036.1), a Gram-positive coryneform bacterial plant pathogen related to *Curtobacterium* (Bentley et al. 2008). Comparison of the complete sequences of pCFF113 and pCSL1 with PROmer (v3.07) revealed eight conserved regions ranging from 1.1 to 4.3 kb (totaling 18.4 kb) with 55 to 70% translated amino acid identity (Kurtz et al. 2004). This suggests that pCFF113 is distantly related to pCSL1.

Clavibacter michiganensis strain NCPPB382 (GenBank accession number AM711866) encodes many serine proteases, including *pat-1*, which is required for virulence (Bentley et al. 2008; Burger et al. 2005; Thapa et al. 2017). *Clavibacter sepedonicus* strain ATCC33113 also encodes numerous serine proteases, including one on pCSL1, and these genes are often associated with mobile elements (Bentley et al. 2008). Cff1037 is predicted to encode at least

five serine proteases, four of which are located on pCFF113. Three of the pCFF113-encoded serine proteases (FK523_17305, FK523_17310, and FK523_17320) are clustered together and are associated with an IS481 family-transposase, whereas the fourth (FK523_17500) is located ~35 kb away and is associated with an IS3 family-transposase and a gene encoding a tra-like protein. BLASTp comparisons indicate that these *C. flaccumfaciens* pv. *flaccumfaciens* serine proteases have between 30 to 40% amino acid identity with the *Clavibacter michiganensis* serine protease *pat-1*, indicating that they are related but may not be orthologous. Together, these results further support a role for pCFF113 in pathogenicity and an evolutionary history of horizontal gene transfer.

Data availability. The complete genome sequence for Cff1037 has been deposited into GenBank under the accession numbers CP041259 and CP041260 (BioProject PRJNA551505; BioSample SAMN12153678). Raw reads have been deposited into Sequencing Read Archive in association with BioProject PRJNA551505.

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