Complete Genome Sequence Resource for *Xanthomonas fragariae* Causing Crown Infection Pockets in Strawberry

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Abstract

Xanthomonas fragariae is a global quarantine pathogen, which typically inflicts angular leaf spots. In the present study, we report a new 4.11-Mb high-quality genome sequence of *X. fragariae* YL19. YL19 can cause the typical angular leaf spot symptoms on strawberry plants in China as well as crown infection pocket symptoms. This new symptom has not been reported in other *X. fragariae*. Compared with typical *X. fragariae* strains, including PD885, NBC2815, PD5205, Fap21, and Fap29, the genome and plasmid in YL19 were smaller in size, lacking 109 coding genes, and have more carbohydrate-active enzyme and secondary metabolism genes. The YL19 genome ought to clarify the molecular mechanisms of genome evolution, host adaptation, and pathological process of *X. fragariae* and help improve strawberry management strategies.

Genome Announcement

Strawberry is a perennial herb of the genus *Fragaria* in the family Rosaceae and is known as the “queen of berries,” one of the most widely consumed fruits in the world (Amil-Ruiz et al. 2011). *X. fragariae* is a bacterial quarantine pathogen of strawberry and the cause of angular leaf spot, which can significantly affect the production of strawberries. It was initially described within the United States in 1962 and has since unfolded globally (Kennedy and King 1962). Under favorable conditions, the pathogen might cause vital harm to nurseries and production of strawberries (Kim et al. 2016; Roberts et al. 1997).

Recently, it was reported that the isolate of *X. fragariae* YL19 can result not only in the typical symptoms of angular leaf spot but also crown infection pocket symptoms in the infected strawberry plants (Li et al. 2021; Song et al. 2021). Since the 2017 growing season, the strawberry crown infection pocket disease caused by *X. fragariae* YL19 emerged in multiple provinces or regions in China, including Liaoning, Yunnan, Zhejiang, and Beijing (F. F. Sui, *personal communication*). The disease incidence is estimated to be 5 to 10% in general but could reach 30 to 40% in some strongly affected plastic tunnels (F. F. Sui, *personal communication*). Until now, a total of 62 genome sequences of *X. fragariae* were made publicly available (Gétaz et al. 2017; Henry and Leveau 2016; Vandroemme et al. 2013). However, no strain was reported to cause crown infection pockets affecting strawberry plants (Fig. 1). Therefore, revealing the *X. fragariae* YL19 genome sequence could increase our understanding of crown infection pocket symptoms and provides a reference for facilitating each pathogenicity mechanism study.

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Keywords

crown infection pockets, genome, strawberry, *Xanthomonas fragariae*

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Fig. 1. The crown infection pocket symptoms and a whole-genome map of *Xanthomonas fragariae* YL19. **A**, The crown infection pocket symptoms in strawberry. **B**, Whole-genome map of *X. fragariae* YL19. The outer circle is the genomic sequence position coordinates. Rings from the outside to inside represent gene function annotation (Cluster of Orthologous Groups, Gene Ontology, and Kyoto Encyclopedia of Genes and Genomes), noncoding RNA, and GC content. The GC content is counted by window (chromosome length/1,000) bases, step (chromosome length/1,000) bases.

In the present study, we report the genome sequence of the *X. fragariae* YL19 strain, which was isolated from infected strawberry stems (*Fragaria × ananassa* Duch.) within the Liaoning province of China in 2019 (Li et al. 2021; Song et al. 2021). It was confirmed by the internal transcribed spacer region (accession number MT513132.1, MW233896, MW233897, and MW233895) and was plated on sucrose peptone agar medium at 25°C in constant darkness (Li et al. 2021). Genomic DNA was extracted with the sodium dodecyl sulfate method (Moslem et al. 2010). The harvested DNA was detected by the agarose gel electrophoresis and was quantified with a Qubit 2.0 fluorometer (Thermo Scientific). The whole genome of YL19 was sequenced using the Nanopore PromethION platform and Illumina NovaSeq PE150 at the Beijing Novogene Bioinformatics Technology Co., Ltd (Beijing). Libraries for

Table 1. Comparison of YL19 with FaP21, FaP29, PD5205, PD885, and NBC2815 genomes

Parameters	Statistics					
	YL19 (2020) ^a	FaP21 (2011) ^b	FaP29 (2011) ^b	PD5205 (2005) ^c	PD885 (1960) ^c	NBC2815 (2011) ^c
No. bases (bp)	6,016,963,541	513,923,760	513,912,240	1,058,133,921	1,185,992,834	866,435,465
Genome coverage	1,471	120x	120x	247x	274x	203x
Genome size (bp)	4,107,640	4,282,698	4,282,602	4283943	4328441	4268155
No. plasmids	1	2	2	2	2	1
Plasmid size (bp)	pYL19, 20,156	pFap21-1, 29,232 pFap21-2, 20,831	pFap29-1, 29,138 pFap29-2, 20,831	pPD5205-30, 469 pPD5205.21, 20,830	pPD885-27, 27,106 pPD885-29, 29,233	pNBC2815-21, 21,045
No. scaffolds	2	3	3	3	3	2
GC (%)	62.75	62.27	62.27	62.27	62.25	62.3
Genes (total)	3,929	3,994	4,005	4,005	4,041	3,972
Genes (coding)	3,129	3,215	3,238	3,236	3,292	3,207
Transfer RNAs	54	52	52	52	52	52
Ribosomal RNAs	6	6	6	6	6	6
Other RNAs	245	86	86	86	86	87
CRISPR no.	2	1	1	1	1	1
Carbohydrate-active enzyme functional classification and corresponding genes						
Carbohydrate-related molecules	23	20	20	20	20	22
Carbohydrate esterases	4	4	4	4	4	4
Glycoside hydrolases	82	76	76	76	76	78
Glycosyl transferases	53	51	51	51	51	53
Polysaccharide lyases	3	4	4	4	5	4
Auxiliary activities	2	2	2	2	2	2
Total	167	157	157	157	158	163
Secondary metabolites related synthetic genes						
Nonribosomal peptide synthetases	83	0	0	0	41	0
Siderophores	9	0	0	0	0	0
Bacteriocins	19	0	0	0	0	0
Arylpolyenes	34	31	31	31	0	31
Type N secretion systems						
T2SS	10	10	10	10	10	10
T3SS	159	160	161	161	156	157
T4SS	7	5	5	5	9	6

^a This study.^b Henry and Leveau (2016).^c Gétaz et al. (2017).

Nanopore sequencing were constructed with an insert size of 10 kb. For the Illumina sequence, an Illumina library with an insertion size of 350 bp was built and was quantified using the Agilent 2100 Bioanalyzer (Agilent Technologies). Finally, the Nanopore assembly was polished using the Illumina reads to correct base errors.

After that, the genome assembly was performed using Unicycler software (Wick et al. 2017), using Illumina and Nanopore generation data. A total of 4.27 Gb of Nanopore short reads, representing 1,043x coverage of the YL19 genome, and 1.75 Gb of Illumina short reads (428x) were generated (Table 1). The final genome size of YL19 was 4.11 Mb, which contains a 0.02 Mb plasmid (Fig. 1). The strain PD 885, also known as LMG708, is the type *X. fragariae* strain, isolated in the United States by B. W. Kennedy in 1962 (Kennedy and King 1962). Strains NBC 2815 and PD 5202 were both isolated in the Netherlands from *Fragaria × ananassa* in 2011 and 2005, respectively (Gétaz et al. 2017). Strains FaP21 and FaP29 are originated from the United States in 2011 (Henry and Leveau 2016). The size of YL19 genome and plasmid were smaller than PD885, NBC2815, PD5205, FaP21, and FaP29, but the GC content of YL19 was similar to PD885, NBC2815, PD5205, FaP21, and FaP29 (Table 1). Coding gene prediction for YL19 was used by GeneMarkS (version 4.17) (Besemer et al. 2001). The YL19 genome has a total of 3,929 genes, of which 3,129 are coding genes. Compared with PD885, NBC2815, PD5205, FaP21, and FaP29, YL19 lacked 109 coding genes (Table 1). Using Gene Ontology (Ashburner et al. 2000), Kyoto Encyclopedia of Genes and Genomes (Kanehisa et al. 2004), Cluster of Orthologous Groups of proteins

(Galperin et al. 2015), and carbohydrate-active enzymes (CAZymes) (Cantarel et al. 2009) databases for gene function annotation. Compared with PD885, NBC2815, PD5205, Fap21, and Fap29, YL19 has more CAZyme genes, especially glycoside hydrolases (Table 1). antiSMASH (version 2.0.2) (Medema et al. 2011) was used for secondary metabolism gene clusters annotation. YL19 has more secondary metabolic genes than PD885, NBC2815, PD5205, Fap21, and Fap29, especially in the nonribosomal peptide synthetases, siderophore, and bacteriocins (Table 1). The protein sequence-function database and EffectiveT3 (version 1.0.1) (Eichinger et al. 2016) were used for the type N secretion system (TNSS) annotations and T3SS effector protein prediction, respectively. All the strains evaluated in the study have only three types of secretion systems, and there was no significant difference in the number of each secretion system between strains (Table 1).

The sequence of the *X. fragariae* YL19 genome can be employed in intraspecific and interspecies comparative genomic studies. In addition, this new genome ought to clarify the molecular mechanisms of genome evolution, host adaptation, and pathological process of *X. fragariae*.

Data Availability

The YL19 genome sequence data has been deposited at DDBJ/ENA/GenBank database under accession number CP071955. The sequenced strain is stored at Northwest A&F University in China.

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