Draft Genome Sequences of Two Phytoplasma Strains Associated with Sugarcane Grassy Shoot (SCGS) and Bermuda Grass White Leaf (BGWL) Diseases

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Abstract

We performed whole-genome sequencing of two phytoplasmas associated with sugarcane grassy shoot (SCGS) and Bermuda grass white leaf diseases. These are the first draft genomes of SCGS phytoplasma (strain SCGS) and '*Candidatus* Phytoplasma cynodontis' (strain LW01) and may help to delineate these phytoplasmas at a finer taxonomic level.

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Keyword

phytoplasmas

Genome Announcement

'*Candidatus* Phytoplasma' is a cell wall-less, not-yet-cultivated, obligate bacterial pathogen associated with diseases of hundreds of plant and crop species (Rao et al. 2017). sugarcane grassy shoot (SCGS) phytoplasma causes severe proliferation of tillers leading to the typical grassy appearance of sugarcane (*Saccharum officinarum* L.), hence the name 'grassy shoot' (Nasare et al. 2007; Yadav et al. 2017). The SCGS disease contributes to losses of 5 to 20% in the plant crop of sugarcane and these losses are higher in the ratoon crop (Yadav et al. 2013). The Bermuda grass white leaf (BGWL) phytoplasma causes 'white leaf' disease in *Cynodon dactylon* (L.) Pers. and has been described as '*Candidatus* Phytoplasma cynodontis' (Marcone et al. 2004). A sugarcane plant sample (strain SCGS) exhibiting grassy shoot symptoms and a Bermudagrass sample (strain LW01) showing white leaf symptoms were collected from Pune, Maharashtra, India. The presence of phytoplasmas was confirmed by detection and sequencing of the 16S ribosomal RNA (rRNA) gene amplicons from SCGS (MN889545) and LW01 (LT558776) samples. These phytoplasma strains belonged to the 16SrXI and 16SrXIV groups, respectively, according to the restriction fragment length polymorphism patterns of their 16S rRNA gene sequences, which were digested *in silico* with selected restriction endonuclease enzymes, using online tool *PhyClassifier* (Zhao et al. 2009).

Total nucleic acids were extracted from the SCGS and LW01 samples and were enriched for prokaryotic DNA selection using the NEBNext microbiome enrichment kit (New England BioLabs). The enriched DNA of both samples was amplified using illustra Ready-To-Go GenomiPhi V3 DNA amplification kits and was sequenced on the Illumina HiSeq platform. Simultaneously, the enriched DNA of SCGS and LW01 were sequenced on the Oxford Nanopore Technology (ONT) platform.

Sequencing of the SCGS and LW01 samples resulted in 22,765,620 reads (150 × 2 chemistry) and 22,630,326 reads (150 × 2 chemistry), respectively, using the Illumina HiSeq

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platform. All Illumina reads were quality checked with FastQC v0.11.8 (Brown et al. 2017). The ONT sequencing data were base-called with quality filtering (>Q7) using GUPPY, which generated 50,541 reads for SCGS and 41,237 for LW01. All QC-passed Illumina reads (>Q30) were subjected to metagenomic assembly using MEGAHIT v1.1.3 (Li et al. 2016). This assembly was subjected to binning, using MetaBAT2 v2.12.1 (Kang et al. 2015). Raw reads were mapped on bins corresponding to phytoplasma. Additionally, Illumina reads were mapped on all available phytoplasma genome sequences, using Bowtie2 v2.2.6 (Langmead and Salzberg 2012). Finally, all mapped reads and QC-passed ONT reads were used to generate the hybrid assembly in SPAdes v3.11.1 (Bankevich et al. 2012) and Unicycler v0.4.8 (Wick et al. 2017). Bin quality check, genome completeness, and taxonomic assignments were performed using CheckM v1.0.14 (Parks et al. 2015). Assembly quality was checked using QUAST v5.0.2 (Gurevich et al. 2013). The genome coverage was calculated using BBMap (Bushnell, 2014). All obtained scaffolds were used as the queries to run a BLASTX search against the National Center for Biotechnology Information (NCBI) nonredundant protein database, using Blast2GO to identify contaminating scaffolds. Both genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Zhao et al. 2012).

The final SCGS assembly contained 29 scaffolds corresponding to 505,173 bp, including a putative plasmid of 2,976 bp. This genome was found to be 95.43% complete with 333.98x coverage and the GC content was 19.86%. The SCGS genome was predicted to have 404 protein-coding genes, 12 transfer RNA (tRNA), and two rRNA genes. Similarly, the final LW01 assembly contained 21 scaffolds corresponding to 483,935 bp. This genome was found to be 91.32% complete with 373.6x coverage and the GC content was 20.46%. The LW01 genome was predicted to have 425 protein-coding genes, 13 tRNA, and three rRNA genes. The 16S rRNA gene sequences of SCGS and LW01, obtained from their respective assemblies, showed 98.07 and 99.59% similarity, respectively, with the reference sequence of '*Candidatus* Phytoplasma cynodontis' strain BGWL-C1, when analyzed on EzBioCloud Database to find out their closest taxonomic relative (Yoon et al. 2017).

Data Availability

The Whole Genome Shotgun sequence for strains SCGS and LW01 have been deposited in the DDBJ/ENA/GenBank database under accession numbers VVXMM000000000 and VWOH000000000, respectively. The version described in this paper is VVXMM010000000 and VWOH010000000. The sequence of the SCGS plasmid (pSCGS) is available under accession number NZ_CM018785.1

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