Plant Disease • 2022 • 106:1031-1034 • https://doi.org/10.1094/PDIS-09-21-1959-A

RESOURCE ANNOUNCEMENT

Draft Genome Sequence Resource of '*Fragaria* × *ananassa*' Phyllody Phytoplasma Strain StrPh-CL from Chilean Strawberry

Weier Cui, Nicola Fiore, and Alan Zamorano†

Department of Plant Health, Faculty of Agricultural Sciences, University of Chile, Santiago, Chile

Funding
This research was supported by the National Fund for Scientific and Technological Development (FONDECYT) of Chile, Proyecto Postdoctoral 2017 no. 3170120 and Proyecto Iniciación 2020 no. 11200576.

Keywords
strawberry phyllody, phytoplasma, draft genome, small fruits, pathogen diversity

Genome Announcement

Strawberry phyllody has become a common disease on Chilean strawberry (*Fragaria* × *ananassa* Duch.) crops in recent years. The pathogen associated with this disease is a phytoplasma belonging to 16SrXIII ribosomal group (Mexican periwinkle virescence group). In this study, we obtained the draft genome sequence of '*Fragaria* × *ananassa*' phyllody phytoplasma strain StrPh-CL from infected periwinkle plants. The assembly consists of 33 contigs of 627,584 bp with an average sequencing coverage of 55.3 and a GC content of 25.4%. The genome is estimated to be 90% complete, with the possible presence of a plasmid of 4,173 bp. Among the 591 predicted protein-coding sequences (CDS), there are 26 putative secreted proteins, including the homologs of two better known pathogenic effectors, SAP54 (phyllogen) and TENGU. This genome resource serves the future research in pathogen-host interaction, evolution, epidemiology, and other aspects of this phytoplasma group.

Phytoplasmas are phloem-limited phytopathogenic bacteria naturally transmitted by insect vectors, causing plant diseases and significant agricultural losses. In Chile, phytoplasmas have been detected in economically important crops and ornamental plants including grapevine, cherry, pear, sugar beet, lettuce, Swiss chard, and peony (Arismendi et al. 2011; Facundo et al. 2017; Fiore et al. 2015a, b; González et al. 2011; Quiroga et al. 2017). Since 2015, strawberry crops showing severe phyllody symptoms have been reported in the central production regions, causing loss of entire plants and reducing fruit yields. Phytoplasmas belonging to the 16SrXIII ribosomal group (Mexican periwinkle virescence group) have been detected from the symptomatic strawberry plants (Cui et al. 2019).

Phytoplasmas of 16SrXIII group are exclusively found in the Americas, with the representative strain '*Candidatus* Phytoplasma (*Ca*. P.) hispanicum' (Davis et al. 2016). All the '*Fragaria* × *ananassa*' phyllody phytoplasma strains share >97.5% identity of the 16S ribosomal RNA sequence with '*Ca*. P. hispanicum,' suggesting that they are related to this reference strain (IRPCM 2004). Based on 16S rRNA sequence, 16SrXIII group is phylogenetically close to 16SrI and 16SrXII groups, both having been better studied with several genome sequences available (Davis et al. 2016). By contrast, studies of 16SrXIII group have stayed on detection and classification, and no genome sequence of '*Ca*. P. hispanicum'-related strain has been reported. In this study, we report the first draft genome sequence of '*Fragaria* × *ananassa*' phyllody phytoplasma strain StrPh-CL.

Genome sequencing and assembly. Strawberry (*Fragaria* × *ananassa* var. Monterrey) plants showing phyllody symptoms were collected from fields located in Litueche, O'Higgins Region of Chile. Periwinkle (*Catharanthus roseus*) plants were obtained by seeds. A strawberry fruit showing severe phyllody was grafted onto a healthy periwinkle approximately 2 months old to obtain infected periwinkles. Three months post grafting, newly emerged branches showing symptoms of virescence and phyllody were examined with nested PCR of

†Corresponding author: A. Zamorano; agezac@uchile.cl

The author(s) declare no conflict of interest.

Accepted for publication 22 November 2021.

Table 1. General features of the draft genome of '*Fragaria* × *ananassa*' phyllody phytoplasma strain StrPh-CL

Features	StrPh-CL
16Sr group assignment	16SrXIII-F
GenBank accession	JAGVRH000000000
No. of contigs	33
Genome size (bp)	627,584
N50 (bp)	82,058
G+C content (%)	25.4
Number of predicted genes	635
Complete protein-coding sequences (CDS)	559
Partial CDS	32
rRNA operons	2
tRNA genes	32

16S rRNA gene, and the amplicon was sequenced for phytoplasma identification. The periwinkle plant confirmed infected by '*Fragaria* × *ananassa*' phyllody phytoplasma was used for DNA extraction. Total DNA was extracted from midribs, petioles, virescent flowers, and young shoots showing phyllody, according to Cui et al. (2019).

The total DNA was sent to Macrogen (Maryland, U.S.A.) for library preparation and sequencing. The library was prepared using TruSeq Nano DNA Kit, and the sequencing was performed with the Illumina platform, generating 83,467,822 paired-end reads with an average length of 151 bp. Quality trimming of the raw sequences was performed with Trimmomatic v0.39 (Bolger et al. 2014) to remove the adaptors, the 3′ and 5′ nucleotides with a Phred quality score below 20, and the reads shorter than 30 nt. The trimmed reads were aligned to the periwinkle genome (GenBank accession no. GCA_000949345) with Bowtie2 v2.4.2 (Langmead and Salzberg 2012). The unmapped reads were assembled into contigs with SPAdes v3.14.1 (Nurk et al. 2013). The assembled contigs were queried against the NCBI nucleotide database using BLAST+ v2.11.0 (Camacho et al. 2009), and only the contigs with hits to phytoplasma sequences were retained. The retained contigs were manually curated and ordered according to their lengths.

The draft genome of StrPh-CL consists of 33 contigs with a total size of 627,584 bp and an average coverage of 55.3, with an N50 value of 82,058 (Table 1). Based on a preliminary PFGE electrophoresis experiment, the completeness of this draft genome is approximately 90% (unpublished data). The GC content of the draft genome is 25.4%. RNAmmer web server v1.2 (Lagesen et al. 2007) identified two complete rRNA operons, and tRNAscan-SE web server v2.0 (Lowe and Chan 2016) identified 32 tRNA genes (Table 1). The two copies of 16S rRNA genes (5,286–6,529 on Contig_2 and 34,106–35,349 on Contig_3) share 98.7% and 98.8% identity with '*Ca.* P. hispanicum' (sequence ID: AF248960.1), respectively, supporting that StrPh-CL is related to this reference strain.

Gene annotation and pathogenic effector candidates. Protein-coding gene prediction was performed with Prodigal v2.6.3 (Hyatt et al. 2010). Gene annotation was performed with KAAS (Moriya et al. 2007), eggNOG-mapper (Huerta-Cepas et al. 2019), and WebMGA (Altschul et al. 1990), and the results were integrated. A total of 591 CDS were predicted (Table 1), including 559 complete CDS and 32 partial ones. Among the 591 genes, 440 were annotated with specific COG (cluster of orthologous genes) numbers. Six genes were predicted to encode replication initiator protein (Rep) similar to that of geminiviruses. Geminivirus-like Rep-coding genes have been observed on extrachromosomal DNA molecules of several phytoplasmas (Saccardo et al. 2011), and the presence of these genes suggests the existence of plasmids. All six genes in StrPh-CL are located on short contigs of 1,488 to 9,333 bp, and on one of these contigs, Contig_14, gene arrangement suggests that this contig could be joined into a plasmid, which would be 4,173 bp in size and contain five CDS.

According to the current consensus, pathogenic effectors of phytoplasmas are secreted into the host cytoplasm via the Sec-dependent protein translocation system (Sugio et al. 2011). To identify effector candidates, the predicted proteins were first screened with SignalP web server v5.0 (Almagro Armenteros et al. 2019) to select those with a signal peptide. The selected proteins were then loaded onto TMHMM web server v2.0 (Krogh et al. 2001) to remove those with more than one transmembrane domain, which would be integrated into the plasma membrane. Finally, the remaining candidates were examined with BLASTp and the

Table 2. Pathogenic effector candidates of StrPh-CL

CDS ID[a]	Contig no.	Length (aa)	BLASTp result and known homologs	Sequence ID
FRU_045	Contig_1	102	SVM family proteins	WP_212330693.1
FRU_059	Contig_1	271	Hypothetical proteins	WP_212330729.1
FRU_078	Contig_1	194	SAP61	WP_212330782.1
FRU_080	Contig_1	105	TENGU	WP_212330783.1
FRU_107	Contig_1	284	No homologs found	WP_212330856.1
FRU_180	Contig_2	160	SAP67	WP_212331043.1
FRU_181	Contig_2	102	SAP42	WP_212331045.1
FRU_183	Contig_2	85	No homologs found	WP_212331047.1
FRU_199	Contig_2	169	SAP42	WP_212331080.1
FRU_284	Contig_4	109	SVM family proteins	WP_212331392.1
FRU_287	Contig_4	268	Hypothetical proteins	WP_212331397.1
FRU_288	Contig_4	81	SVM family proteins	WP_212331400.1
FRU_383	Contig_6	162	SAP67	WP_212331757.1
FRU_384	Contig_6	109	SVM family proteins	WP_212331760.1
FRU_442	Contig_9	372	SAP49	WP_212331922.1
FRU_462	Contig_10	131	SVM familly proteins	WP_212331973.1
FRU_467	Contig_10	191	SAP40	WP_212331981.1
FRU_506	Contig_15	261	SVM family proteins	WP_212332077.1
FRU_507	Contig_15	361	SAP49	WP_212332080.1
FRU_525	Contig_17	117	SAP54 / phyllogen	WP_212332119.1
FRU_526	Contig_17	199	Hypothetical protein	WP_225885840.1
FRU_536	Contig_18	166	SAP42	WP_212332161.1
FRU_551	Contig_20	117	SVM family proteins	WP_212332188.1
FRU_564	Contig_22	114	Hypothetical proteins	WP_212332212.1
FRU_568	Contig_23	151	SAP08	MBS2126629.1

[a] CDS = protein-coding sequences.

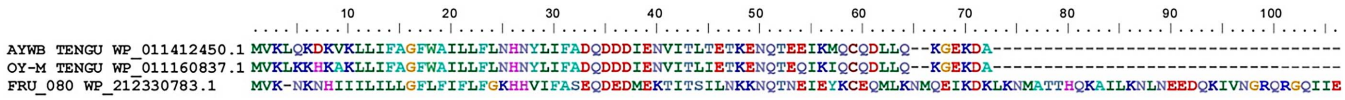

Fig. 1. Protein sequence alignment of three TENGU homologs.

MOTIF Search web tool (Kanehisa et al. 2010) to exclude those with well annotated functions other than secreted effectors. These analyses resulted in a total of 26 pathogenic effector candidates (Table 2), homologs of 24 of them having been found in other phytoplasma genomes.

Among these candidates, homologs of two better studied effectors, SAP54 (phyllogen) and TENGU, were identified, whereas homologs of another two effectors, SAP11 and SAP05, were not found (Table 2). Members of the phyllogen family induce phyllody in host plants and promote insect colonization (MacLean et al. 2014). Interestingly, the phyllogen homolog of StrPh-CL, FRU_525, shares 95.73% identity with the homolog of *Bellis* virescence phytoplasma from 16SrIII group but only 50.45% identity with that of aster yellows witches'-broom phytoplasma from 16SrI group, which is phylogenetically closer to 16SrXIII group. Phytoplasmas from group 16SrIII have been detected in various host plants in Chile, and two insect vectors have been identified (Fiore et al. 2015a, b; González et al. 2011; Quiroga et al. 2019). These data suggest that this effector might have undergone horizontal transfer among phylogenetically distant but geographically overlapping strains.

TENGU, SAP05, and SAP11 enhance the proliferation of axillary meristems, inducing dwarfism and witches' broom in host plants (Chang et al. 2018; Hoshi et al. 2009; Huang et al. 2021). To date, TENGU homologs have been found exclusively in phytoplasmas from 16SrI group (Sugawara et al. 2013). The TENGU homolog of StrPh-CL shares <42% identity with the other homologs and includes an additional 35 aa at the C terminus (Fig. 1), which could affect its function as a pathogenic effector. The possible altered function of TENGU, in addition to missing SAP11 and SAP05 homologs, might explain the lack of symptoms of dwarfism and witches' broom on the strawberry plants infected by StrPh-CL.

The draft genome of '*Fragaria* × *ananassa*' phyllody phytoplasma strain StrPh-CL is the first sequence of a strain related to '*Candidatus* Phytoplasma hispanicum.' It provides a

valuable resource for future research of phytoplasmas from 16SrXIII group and will serve as a reference for sequencing approaches and comparative study. The identification of the homologs of the two pathogenic effectors, phyllogen and TENGU, provides intriguing topics on pathogenicity, epidemiology, and the evolutionary history of phytoplasmas in the Americas.

Data Availability

The whole-genome shotgun project of '*Fragaria* × *ananassa*' phyllody phytoplasma strain StrPh-CL has been deposited in GenBank under the accession number JAGVRH000000000.

Literature Cited

Almagro Armenteros, J. J., Tsirigos, K. D., Sønderby, C. K., Petersen, T. N., Winther, O., Brunak, S., von Heijne, G., and Nielsen, H. 2019. SignalP 5.0 improves signal peptide predictions using deep neural networks. Nat. Biotechnol. 37:420-423.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Arismendi, N., González, F., Zamorano, A., Andrade, N., Pino, A. M., and Fiore, N. 2011. Molecular identification of '*Candidatus* phytoplasma fraxini' in murta and peony in Chile. Bull. Insectol. 64:95-96.

Bolger, A. M., Lohse, M., and Usadel, B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T. L. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421.

Chang, S. H., Tan, C. M., Wu, C.-T., Lin, T.-H., Jiang, S.-Y., Liu, R.-C., Tsai, M.-C., Su, L.-W., and Yang, J.-Y. 2018. Alterations of plant architecture and phase transition by the phytoplasma virulence factor SAP11. J. Exp. Bot. 69: 5389-5401.

Cui, W., Quiroga, N., Curkovic, S. T., Zamorano, A., and Fiore, N. 2019. Detection and identification of 16SrXIII-F and a novel 16SrXIII phytoplasma subgroups associated with strawberry phyllody in Chile. Eur. J. Plant Pathol. 155:1039-1046.

Davis, R. E., Harrison, N. A., Zhao, Y., Wei, W., and Dally, E. L. 2016. '*Candidatus* Phytoplasma hispanicum', a novel taxon associated with Mexican periwinkle virescence disease of *Catharanthus roseus.* Int. J. Syst. Evol. Microbiol. 66:3463-3467.

Facundo, R., Quiroga, N., Méndez, P., Zamorano, A., and Fiore, N. 2017. First report of '*Candidatus* Phytoplasma pyri' on pear in Chile. Plant Dis. 101:830.

Fiore, N., González, X., Zamorano, A., Quiroga, N., Paillalef, R., and Pino, A. M. 2015a. Phytoplasmas associated with yellow wilt disease of sugar beet in Chile. Phytopathogenic Mollicutes 5:S63-S64.

Fiore, N., Zamorano, A., and Pino, A. M. 2015b. Identification of phytoplasmas belonging to the ribosomal groups 16SrIII and 16SrV in Chilean grapevines. Phytopathogenic Mollicutes 5:32.

González, F., Zamorano, A., Pino, A. M., Paltrinieri, S., Bertaccini, A., and Fiore, N. 2011. Identification of phytoplasma belonging to X-disease group in cherry in Chile. Bull. Insectol. 64:235-236.

Hoshi, A., Oshima, K., Kakizawa, S., Ishii, Y., Ozeki, J., Hashimoto, M., Komatsu, K., Kagiwada, S., Yamaji, Y., and Namba, S. 2009. A unique virulence factor for proliferation and dwarfism in plants identified from a phytopathogenic bacterium. Proc. Natl. Acad. Sci. 106:6416-6421.

Huang, W., MacLean, A. M., Sugio, A., Maqbool, A., Busscher, M., Cho, S.-T., Kamoun, S., Kuo, C.-H., Immink, R. G. H., and Hogenhout, S. A. 2021. Parasitic modulation of host development by ubiquitin-independent protein degradation. Cell 184:5201-5214.

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., et al. 2019. EggNOG 5.0: A hierarchical, functionally, and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res. 47:D309-D314.

Hyatt, D., Chen, G. L., LoCascio, P. F., Land, M. L., Larimer, F. W., and Hauser, L. J. 2010. Prodigal: Prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119.

IRPCM. 2004. '*Candidatus* Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. Int. J. Syst. Evol. Microbiol. 54:1243-1255.

Kanehisa, M., Goto, S., Furumichi, M., Tanabe, M., and Hirakawa, M. 2010. KEGG for representation and analysis of molecular networks involving diseases and drugs. Nucleic Acids Res. 38:D355-D360.

Krogh, A., Larsson, B., Von Heijne, G., and Sonnhammer, E. L. L. 2001. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. J. Mol. Biol. 305:567-580.

Lagesen, K., Hallin, P., Rødland, E. A., Stærfeldt, H. H., Rognes, T., and Ussery, D. W. 2007. RNAmmer: Consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100-3108.

Langmead, B., and Salzberg, S. L. 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9:357-359.

Lowe, T. M., and Chan, P. P. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. 44:W54-W57.

MacLean, A. M., Orlovskis, Z., Kowitwanich, K., Zdziarska, A. M., Angenent, G. C., Immink, R. G. H., and Hogenhout, S. A. 2014. Phytoplasma effector SAP54 hijacks plant reproduction by degrading MADS-box proteins and promotes insect colonization in a RAD23-dependent manner. PLoS Biol. 12: e1001835.

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., and Kanehisa, M. 2007. KAAS: An automatic genome annotation and pathway reconstruction server. Nucleic Acids Res. 35:W182-W185.

Nurk, S., Bankevich, A., Antipov, D., Gurevich, A. A., Korobeynikov, A., Lapidus, A., et al. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J. Comput. Biol. 20:714-737.

Quiroga, N., Bustamante, M., Gamboa, C., Molina, J., Zamorano, A., and Fiore, N. 2017. 16SrIII- J phytoplasmas infecting lettuce and Swiss chard crops in Chile. Phytopathogenic Mollicutes 7:91-94.

Quiroga, N., Longone, V., González, X., Zamorano, A., Pino, A. M., Picciau, L., Alma, A., Paltrinieri, S., Contaldo, N., Bertaccini, A., and Fiore, N. 2019. Transmission of 16SrIII-J phytoplasmas by the leafhoppers *Paratanus exitiousus* and *Bergallia valdiviana.* Phytopathol. Mediterr. 58:231-237.

Saccardo, F., Cettul, E., Palmano, S., Noris, E., and Firrao, G. 2011. On the alleged origin of geminiviruses from extrachromosomal DNAs of phytoplasmas. BMC Evol. Biol. 11:185.

Sugawara, K., Honma, Y., Komatsu, K., Himeno, M., Oshima, K., and Namba, S. 2013. The alteration of plant morphology by small peptides released from the proteolytic processing of the bacterial peptide TENGU. Plant Physiol. 162: 2005-2014.

Sugio, A., MacLean, A. M., Kingdom, H. N., Grieve, V. M., Manimekalai, R., and Hogenhout, S. A. 2011. Diverse targets of phytoplasma effectors: From plant development to defense against insects. Annu. Rev. Phytopathol. 49:175-195.