Identification of a New Haplotype of ‘*Candidatus Liberibacter solanacearum*’ in *Solanum tuberosum*

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Abstract

In 2017, potato tubers suspected of being infected with the bacterium ‘*Candidatus Liberibacter solanacearum*’ were received from the Animal and Plant Health Inspection Service in the United States. A total of 368 chipping tubers were observed for internal symptoms of zebra chip disease, which is associated with ‘*Ca. L. solanacearum*’ infection in the United States, Mexico, Central America, and New Zealand. A single tuber sliced at the stem end showed classic zebra chip symptoms of darkened medullary rays, with streaking and necrotic flecking. The symptomatic tuber was confirmed positive for the bacterium by polymerase chain reaction targeting three

different ‘*Ca. L. solanacearum*’ genes. Sequence analysis of these three genes, and subsequent BLAST analysis, identified the pathogen with 99, 98, and 97% identity to ‘*Ca. L. solanacearum*’ for the 16S ribosomal RNA gene, 50S ribosomal proteins L10/L12 genes, and the outer membrane protein gene, respectively. Sequence analysis did not identify the sample as one of the six known haplotypes of ‘*Ca. L. solanacearum*,’ indicating that a seventh haplotype of the pathogen was identified. This new haplotype, designated haplotype F, is now the third haplotype of the bacterium that infects *Solanum tuberosum* in the United States.

Since the early 2000s, the bacterium ‘*Candidatus Liberibacter solanacearum*’ has been economically damaging commercial crops of the Solanaceae family, including potato, tomato, and pepper, in the central and western United States, Mexico, Central America, and New Zealand (Liefing et al. 2008; Munyaneza et al. 2012). In potato, ‘*Ca. L. solanacearum*’ induces symptoms of terminal leaf purpling, leaf curling, aerial tuber formation, shortened internodes, and rapid plant death, leading to reduced yields (Munyaneza et al. 2012). In the potato tubers themselves, ‘*Ca. L. solanacearum*’ causes medullary rays to become darkened and show a distinctive striped pattern (a characteristic that led to the designation of ‘zebra chip’ disease), which renders the tubers unmarketable (Munyaneza et al. 2012).

In 2008, ‘*Ca. L. solanacearum*’ was found in Northern Europe for the first time in commercial carrot, and was subsequently identified in commercial crops of the Umbelliferae family in Spain, Greece, and on the African continent in Morocco (Alfaro-Fernández et al. 2012, 2017; Holeva et al. 2017; Munyaneza et al. 2010; Nelson et al. 2013; Tahzima et al. 2014; Teresani et al. 2014). In carrot, ‘*Ca. L. solanacearum*’ induces symptoms that include leaf purpling, leaf curling, shortened shoots and roots, and secondary root proliferation (Munyaneza et al. 2010). Symptoms in celery include stem curling and yellowing, and shoot proliferation (Teresani et al. 2014). These symptoms render the carrots and celery unmarketable, causing reduced economic returns for the growers.

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Although ‘*Ca. L. solanacearum*’ has been found in both solanaceous and umbelliferous crops, specific haplotypes of the pathogen have been shown to infect different host crops and are carried by different insect vectors. It has been hypothesized that the ‘*Ca. Liberibacter*’ species originated from a single original species, but have since endured long-standing geographical separation (Nelson et al. 2011). The differences in plant and vector hosts are likely a contributing factor in the global distribution of ‘*Ca. L. solanacearum*’ and regional specificities of the different haplotypes. ‘*Ca. L. solanacearum*’ haplotypes A and B have been found in solanaceous crops in the United States, Mexico, Central America, and New Zealand, and are vectored to these host plants by several haplotypes of the potato psyllid (also known as the tomato psyllid), *Bactericera cockerelli* Sulc. (Munyaneza et al. 2007; Swisher et al. 2014). ‘*Ca. L. solanacearum*’ haplotype C has been found in carrot crops in northern Europe, specifically in Sweden, Norway, Finland, and Germany, and is vectored by the carrot psyllid, *Trioza apicalis* Förster (Munyaneza et al. 2010). Haplotypes D and E of ‘*Ca. L. solanacearum*’ have been identified in the Mediterranean regions of Spain, the Canary Islands, Greece, and Morocco, and are associated with the carrot psyllid vector, *Bactericera trigonica* Hodkinson (Alfaro-Fernández et al. 2012; Holeva et al. 2017; Tahzima et al. 2014; Teresani et al. 2014). Very recently, ‘*Ca. L. solanacearum*’ haplotype U was also identified in northern Europe, found in the *Trioza urticae* psyllid and in stinging nettle (Haapalainen et al. 2018). The pierce-sucking nature of the psyllid results in acquisition of the various ‘*Ca. L. solanacearum*’ haplotypes and persistent transmission to their host crops, allowing for the rapid spread of the pathogen (Sengoda et al. 2014).

The only method currently established for preventing the spread of zebra chip disease of potato that is associated with infection by either haplotype of ‘*Ca. L. solanacearum*’ (A and B), is targeting the psyllid vector by chemical control (Munyaneza 2015). The economic impact of insecticides and their application can be significant for those in the potato industry. In a 3-year period in the mid-2000s, it was estimated that the cost of chemical management of the potato psyllid in the state of Texas was 25.86 million U.S. dollars (CNAS 2006). Additionally, in the Pacific Northwest, where over 50% of the U.S. potatoes are grown, it was estimated to cost 11 million U.S. dollars to control the potato psyllid in a year (Greenway and Rondon 2018). The economic impact of zebra chip disease on the U.S. potato industry cannot be taken lightly.

In the winter of 2017, potato tubers were received from the Animal and Plant Health Inspection Service (APHIS) in the United States for

the detection of '*Ca. L. solanacearum*.' The goal was to identify if the '*Ca. L. solanacearum*' pathogen was present in the tubers, and if so, what haplotype of '*Ca. L. solanacearum*.' Analyses were done using standard conventional polymerase chain reaction (PCR) techniques, followed by routine cloning and sequencing of targeted genes.

Materials and Methods

Tuber samples. In December 2017, a total of 368 chipping potato tubers from a shipment rejected because of PCR detection of '*Ca. L. solanacearum*' were sent to the USDA-ARS Temperate Tree Fruit and Vegetable Research Unit in Prosser, WA, by APHIS. Tubers originated from the Klamath Basin in Southern Oregon. Tubers were stored at 10°C until processing. All tubers were labeled with a single number to identify each tuber and each processed sample. At sampling, a stem end slice was made, and each tuber was visually scored for zebra chip symptoms of medullary ray darkening and internal patterns of brown striping in and near the vascular system (Crosslin et al. 2012; Rashed et al. 2013).

Nucleic acid extractions and PCR analysis. A stem end slice (~1 g) of each tuber was placed into a grinding bag, and nucleic acids were extracted using the standard Dellaporta protocol (Dellaporta et al. 1983) with modifications made in Crosslin et al. (2006). All samples were resuspended in 400 µl purified H₂O for subsequent PCR analysis.

Samples were first analyzed by PCR using the Go Taq DNA polymerase (Promega, Madison, WI), and results were confirmed using the Advantage Taq high-fidelity enzyme (Takara Bio USA, Inc., Mountain View, CA). For Go Taq, a 25-µl PCR reaction was prepared using 5 µl of the 5× Go Taq Green Buffer, 0.25 µl of a 10 mM dNTP (each) mix, 0.5 µl forward and reverse primers, 0.1 µl Go Taq DNA polymerase, 17.65 µl H₂O, and 1 µl nucleic acid extraction. For Advantage Taq, a 20-µl PCR reaction was prepared using 2 µl of the 10× Advantage 2 PCR Buffer, 0.5 µl of a 10 mM dNTP (each) mix, 0.5 µl forward and reverse primers, 2 µl of 10× Rediload (Invitrogen, Grand Island, NY), 0.5 µl 50× Advantage Taq polymerase mix, and 1 µl nucleic acid extract. Three different '*Ca. L. solanacearum*' genes were targeted, including the 16S rRNA, 50S ribosomal proteins L10 and L12, and the outer membrane protein, and primer sequences used for amplification are listed in Table 1. For the 16S rRNA gene, two different primer pairs were used, including OA2/OI2c and ClipoF/OI2c. For the 50S ribosomal protein gene, primers CL514F/CL514R were used, and for the outer membrane protein, primers OMB1482F/OMB2086R were used. Thermal cycling conditions for both primer pairs targeting the 16S rRNA gene were as follows: 94°C for 2 min, 40 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 5 min. Conditions for the 50S ribosomal protein gene were as follows: 94°C for 3 min, 40 cycles of 94°C for 45 s, 54°C for 45 s, and 72°C for 1 min, followed by an extension of 72°C for 10 min. Thermal cycling conditions for the outer membrane protein gene were as follows: 94°C for 2 min, 40 cycles of 94°C for 15 s, 55°C for 90 s, 72°C for 60 s, followed by a final extension of 72°C for 5 min. All PCR products were visualized on a 1.5% agarose gel with ethidium bromide staining.

Cloning and sequencing analysis. PCR products for the 16S rRNA, 50S ribosomal protein, and outer membrane protein genes, amplified with either Go Taq DNA polymerase (Promega) or Advantage Taq high-fidelity enzyme (Takara Bio USA), were cut from the agarose gels and purified using GenElute minus Ethidium Bromide Spin Columns (Sigma Aldrich, Burlington, MA). Purified products

were cloned using the TOPO TA cloning kit into pCR 2.1-TOPO vectors using TOP 10 chemically competent cells (Invitrogen, Grand Island, NY). Plasmid DNA was isolated from the TOP 10 cells using the QIAprep spin miniprep kit (Qiagen, Valencia, CA). Plasmids were sent to Molecular Cloning Laboratories (MCLAB; South San Francisco, CA) for DNA sequencing.

Sequences of cloned PCR products were loaded into Geneious R11 v11.1.2 (<https://www.geneious.com>; Kearse et al. 2012), and the vector sequences were removed. The trimmed sequences were assembled in Geneious using the pair-wise alignment tool to obtain the full-length sequence of each clone and to check for any ambiguous nucleotides. To generate the consensus sequences, full-length sequences from a minimum of four clones for each primer pair were aligned using the multiple sequence alignment tool in Geneious. Consensus sequences were deposited in GenBank for the 16S rRNA gene, 50S ribosomal protein L10/L12 genes, and the outer membrane protein gene as accession numbers MH259699, MH259700, and MH259701, respectively. The consensus sequences for each amplicon were checked for identity using the nucleotide BLAST function (megablast for highly similar sequences and blastn for somewhat similar sequences) on the National Center for Biotechnology Information (NCBI) website. (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Deduced amino acid sequences for the 50S ribosomal protein L12 (rpL12) and outer

Fig. 1. Fresh-cut symptomatic potato tuber received from APHIS showing classic zebra chip symptoms of darkened medullary rays and stripes in and near the vascular system.

Table 1. Primers used in this study to detect '*Ca. L. solanacearum*'

Target	Primer name	Primer sequence	Reference
16S rRNA	OA2	GCGCTTATTTTAATAGGAGCGGCA	Liefting et al. 2009
16S rRNA	OI2c	GCCTCGCGACTTCGCAACCCAT	Jagoueix et al. 1996
16S rRNA	ClipoF	TACGCCCTGAGAAGGGGAAAGATT	Secor et al. 2009
50S ribosomal protein	CL514F	CTCTAAGATTTTCGGTTGGTT	Munyanza et al. 2009
50S ribosomal protein	CL514R	TATATCTATCGTTGCACCAG	Munyanza et al. 2009
Outer membrane protein	OMB1482F	GGCGTGGTTATAAGCAGAGT	Crosslin et al. 2011
Outer membrane protein	OMB2086R	ATCTACACGCGGACCTATAC	Crosslin et al. 2011

Phylogenetic analyses. Phylogenetic analyses of *Liberibacter* 16S rRNA and 50S ribosomal protein sequences were performed in Geneious R11 v11.1.2. For 16S rRNA analysis, sequences used were ‘*Ca. L. solanacearum*’ Haplotype A (FJ498802), ‘*Ca. L. solanacearum*’ Haplotype B (FJ939136), ‘*Ca. L. solanacearum*’ Haplotype C (GU373048), ‘*Ca. L. solanacearum*’ Haplotype D (HQ454302), ‘*Ca. L. solanacearum*’ Haplotype E (KF737348), ‘*Ca. L. africanus*’ (KY000560), ‘*Ca. L. americanus*’ (EU921623), ‘*Ca. L. asiaticus*’ (KY990822), ‘*Ca. L. brunswickensis*’ (KY077741), ‘*Ca. L. europaeus*’ (JX244259). For 50S ribosomal protein analysis, sequences used were ‘*Ca. L. solanacearum*’ Haplotype A (EU834131), ‘*Ca. L. solanacearum*’ Haplotype B (FJ498807), ‘*Ca. L. solanacearum*’ Haplotype C (GU373051), ‘*Ca. L. solanacearum*’ Haplotype D (HQ454317), ‘*Ca. L. solanacearum*’ Haplotype E (KY777461), ‘*Ca. L. africanus*’ (U09675), ‘*Ca. L. americanus*’ (EF122254), ‘*Ca. L. asiaticus*’ (DQ471904). For outer membrane protein analysis, sequences used were ‘*Ca. L. solanacearum*’ Haplotype A (JN848754), ‘*Ca. L. solanacearum*’ Haplotype B (KC768327), ‘*Ca. L. solanacearum*’ Haplotype C (KP760078), and ‘*Ca. L. solanacearum*’ Haplotype D (KY595979). Alignments were performed with the ClustalW (version 2.1; Larkin et al. 2007) function contained within the Geneious program using the default settings (Gap open cost = 15, Gap extend cost = 6.66). Phylogenetic trees were generated using the RAxML (Randomized Axelerated Maximum Likelihood plugin version 8.2.11; Stamatakis 2014) within Geneious using the GTR Gamma nucleotide model and rapid hill climbing algorithm with 1,000 bootstraps.

Upon visual inspection of all 368 stem-end sliced tubers, a single tuber was identified with characteristic zebra chip symptoms of striped, darkened medullary rays (Fig. 1). Subsequent nucleic acid extraction and PCR analysis of the 368 tubers identified the same tuber as positive for the presence of '*Ca. L. solanacearum*': 16S rRNA

gene using the OA2/OI2c primer pair (Fig. 2). No other tuber displayed visual symptoms characteristic of zebra chip disease, and none were PCR-positive for the presence of the '*Ca. L. solanacearum*' pathogen when targeting the 16S rRNA gene.

To confirm the presence of the unculturable '*Ca. L. solanacearum*' pathogen in the single positive tuber, additional standard genes were targeted (Crosslin et al. 2012; Nelson et al. 2013), including the 50S ribosomal subunit protein L10 (rplJ) and L12 (rplL) genes using primer pair CL514F/CL514R, and the outer membrane protein gene using primer pair OMB1482F/OMB2086R, and an alternate 16S rRNA amplicon using primer pair ClipoF/OI2c. Positive PCR bands were identified for all three of these genes from the single tuber identified with visual zebra chip symptoms and '*Ca. L. solanacearum*' 16S rRNA-positive (Fig. 2).

Consensus DNA and deduced amino acid sequences from cloned PCR products for each gene were used in BLAST searches of the NCBI database to confirm identification of the newly detected Liberibacter as a '*Ca. Liberibacter solanacearum*.' For each gene consensus sequence, the results returned from BLAST searches with highest homology were from '*Ca. Liberibacter solanacearum*' DNA sequences deposited in GenBank. For the 16S rRNA gene, the sequences most homologous were from '*Ca. L. solanacearum*,' with nucleotide identities of 99%. Individual BLAST searches were performed against other Liberibacter species, and results showed homology to the 16S rRNA genes of '*Ca. L. africanus*' (98% identity), '*Ca. L. asiaticus*' (97% identity), '*Ca. L. brunswickensis*' (97% identity), '*Ca. L. americanus*' (95% identity), and '*Ca. L. europaeus*' (94% identity). For the 50S ribosomal protein genes L10 and L12 (rplJ and rplL), BLAST results showed the most similarity to '*Ca. L. solanacearum*,' with nucleotide identities of 97–98%. Individual

BLAST searches to other Liberibacter species showed reduced homology toward '*Ca. L. asiaticus*' (78% identity), '*Ca. L. africanus*' (77% identity), and '*Ca. L. americanus*' (68% identity). For the deduced amino acid sequence of the 50S ribosomal protein L12 (rplL), BLAST results were highest to sequences from '*Ca. Liberibacter solanacearum*' with 96% identity. For other Liberibacter species, results were obtained for '*Ca. L. africanus*' (75% amino acid identity), '*Ca. L. asiaticus*' (70% amino acid identity), and '*Ca. L. americanus*' (59% amino acid identity). BLAST searches for the outer membrane protein results showed highest similarity to '*Ca. Liberibacter solanacearum*' with nucleotide identities of 97%. BLAST results for other Liberibacter species included '*Ca. L. americanus*' (71% identity), '*Ca. L. asiaticus*' (70% identity), and '*Ca. L. africanus*' (68% identity). At the amino acid level, top BLAST results were to '*Ca. Liberibacter solanacearum*' having 93–95% identity. BLAST results for other Liberibacter species included '*Ca. L. africanus*' (54% amino acid identity), '*Ca. L. asiaticus*' (51% amino acid identity), and '*Ca. L. americanus*' (44% amino acid identity).

To validate the Liberibacter identified in the diseased potato is a '*Ca. Liberibacter solanacearum*,' a ClustalW alignment of '*Ca. Liberibacter*' species 16S rRNA and 50S ribosomal protein gene sequences deposited in GenBank was performed. For the 16S rRNA sequences, the phylogenetic tree generated with RAxML clusters the newly identified Liberibacter in a clade with the known '*Ca. Liberibacter solanacearum*' haplotypes (Fig. 3). Similar results were observed in the phylogenetic tree generated using the 50S ribosomal protein gene sequences (Fig. 4). To determine if the new Liberibacter is a novel haplotype, ClustalW alignment of the consensus sequences for 16S rRNA, 50S ribosomal proteins, and outer membrane proteins with those of previously identified as unique '*Ca. Liberibacter*

Fig. 4. Unrooted phylogenetic tree constructed using 50S ribosomal proteins L10 and L12 gene segments from '*Ca. Liberibacter*' species. Abbreviations used were Ca. Lso ('*Ca. L. solanacearum*'), Ca. Laf ('*Ca. L. africanus*'), Ca. Lam ('*Ca. L. americanus*'), and Ca. Las ('*Ca. L. asiaticus*'). GenBank accession numbers are indicated in parentheses. Bar length represents substitutions per site.

solanacearum' haplotypes were performed. The ClustalW alignments revealed distinct nucleotide differences for each of the genes compared (Tables 2 and 3), indicating that the new *Liberibacter* is a unique haplotype.

Discussion

Sequencing and phylogenetic analyses were conducted to identify the haplotype of '*Ca. Liberibacter solanacearum*' found in a rejected shipment of potatoes in the United States. Phylogenetic analyses identified the pathogen as most closely related to '*Ca. L. solanacearum*,' rather than to other *Liberibacter* species, including '*Ca. L. asiaticus*,' '*Ca. L. brunswickensis*,' '*Ca. L. africanus*,' '*Ca. L. americanus*,' and '*Ca. L. europaeus*.' Although the sample was closely related to the '*Ca. L. solanacearum*' species, it did not belong to one of the six known haplotypes (A–E, U). Thorough sequencing and phylogenetic analyses therefore identified the pathogen as a novel haplotype of '*Ca. L. solanacearum*,' designated here as "Haplotype F".

The identification of this novel '*Ca. L. solanacearum*' haplotype found in a potato tuber, documents a third '*Ca. L. solanacearum*' haplotype capable of infecting potato in the United States. '*Ca. L. solanacearum*' haplotypes A and B are known to cause zebra chip disease symptoms of vascular striping and darkened medullary rays in the tuber. This new genetically distinct '*Ca. L. solanacearum*,' haplotype F, induces similar symptoms in the potato tuber. With comparable tuber infection between haplotypes A, B, and F, it is possible that, like haplotypes A and B, haplotype F could infect all members of the Solanaceae family. Analyses to compare the pathogenicity of '*Ca. L. solanacearum*' haplotype F will be important to conduct to grasp the full impact this new haplotype could have on the potato, and possibly tomato and pepper industries in the United States.

Following the identification of this new '*Ca. L. solanacearum*' haplotype, research must be conducted to determine its host range, as well as the insect that vectors this pathogen. Because the positive

Table 2. Comparison of single nucleotide polymorphisms in the 16S rRNA and 50S ribosomal L10/L12 protein genes between haplotypes A through E, U, and the new haplotype, '*Ca. L. solanacearum*' haplotype F

Gene	Nucleotide position	' <i>Ca. L. solanacearum</i> ' haplotype						
		A	B	C	D	E	U	New
16S rRNA	g.115A>G	A	A	A	A	G	A	A
	g.116C>T	C	C	C	T	C	C	C
	g.151A>G	A	A	A	A	G	A	G
	g.212T>G	T	G	T	T	T	T	T
	g.359A>C	A	A	A	A	A	A	C
	g.524G>A	G	G	G	G	G	G	A
	g.581T>C	T	C	T	T	T	T	C
	g.959C>T	C	C	C	C	T	C	C
	g.1039A>G	A	A	G	G	A	G	G
	g.1073G>A	G	G	G	A	G	G	G
50S ribosomal L10/L12 proteins	g.558T>G	T	T	T	T	T	T	G
	g.583G>C	G	G	C	G	G	G	G
	g.622A>G	A	A	A	G	A	A	A
	g.640C>T	C	C	T	C	C	C	C
	g.669G>C	G	C	G	G	G	G	G
	g.689C>T	C	C	C	T	T	T	C
	g.691G>T	G	T	T	G	G	G	T
	g.695G>A	G	G	G	G	G	G	A
	g.697A>G	A	A	A	A	A	A	G
	g.700A>G	A	A	A	G	A	A	A
	g.712G>T	G	T	G	G	G	G	T
	g.722G>A	G	G	G	G	A	G	G
	g.749C>A	C	C	C	A	C	C	C
	g.779_780delA	A	A	A	A	A	A	-
	g.780_781insA	-	-	A	A	-	A	-
	g.785G>A	G	A	G	G	G	G	G
	g.849T>C	T	T	T	C	C	T	T
	g.909T>C	T	C	C	C	C	C	C
	g.919_920ins[C/T]TG	-	-	CTG	-	-	-	TTG
	g.920T>C	T	C	T	T	T	T	C
	g.938C>T	C	C	C	C	C	T	C
	g.955G>T	G	G	T	G	G	G	G
	g.961G>T	G	G	G	G	G	T	G
	g.987T>G	T	G	G	G	G	G	G
	g.993A>G	A	A	G	A	A	A	A
	g.1005T>C	T	T	T	T	T	T	C
	g.1041G>A	G	A	A	G	G	A	A
	g.1042A>G	A	A	A	A	A	A	G
	g.1049A>G	A	G	A	A	A	A	A
	g.1068C>T	C	C	C	T	C	C	C
	g.1107G>A	G	A	G	G	G	G	A
	g.1110C>T	C	C	C	C	C	T	C
	g.1111_1112insC	-	-	C	-	-	-	-
	g.1122G>A	G	A	A	A	A	A	A
	g.1137A>G	A	A	A	A	A	A	G
	g.1143G>A	G	A	G	G	G	G	A

Table 3. Comparison of single nucleotide polymorphisms in the outer membrane protein gene between haplotypes A through D, and the new haplotype, ‘*Ca. L. solanacearum*’ haplotype F

Gene	Nucleotide position	‘ <i>Ca. L. solanacearum</i> ’ haplotype				
		A	B	C	D	New
Outer membrane protein	g.37T>C	T	C	T	T	T
	g.74T>G	T	G	T	T	G
	g.80T>C	T	T	T	T	C
	g.90G>A	G	G	G	G	A
	g.92T>A	T	T	T	T	A
	g.106G>C	G	G	G	C	G
	g.113G>A	G	A	G	G	G
	g.114G>A	G	A	A	A	A
	g.116T>G	T	G	T	T	T
	g.137C>A	C	C	C	C	A
	g.150C>T	C	C	C	C	T
	g.165G>A	G	G	A	G	G
	g.169C>T	C	C	T	C	C
	g.202C>A	C	C	A	C	C
	g.206C>T	C	C	T	T	C
	g.220C>T	C	C	T	T	C
	g.225C>A	C	C	C	C	A
	g.233A>G	A	G	G	G	G
	g.236C>T	C	C	C	T	C
	g.244G>A	G	G	A	A	A
	g.245A>C	A	A	C	A	A
	g.249A>G	A	G	A	A	A
	g.258A>G	A	A	A	A	G
	g.293C>T	C	C	C	C	T
	g.303T>G	T	G	T	T	T
	g.348A>G	A	A	A	A	G
	g.405G>T,C	G	G	T	C	G
	g.430G>T	G	T	G	G	G
	g.431T>G	T	T	G	T	T
	g.483G>T	G	T	T	T	T
	g.491T>G	T	G	T	T	T
	g.522T>A	T	T	T	T	A
	g.575T>C	T	T	C	T	T
	g.581C>T	C	T	C	C	T

tuber sample was identified following processing and shipment, the insect vector was not identified, nor was symptomatic foliage available to enable further greenhouse studies. Due to similarities in disease pattern of the tuber between ‘*Ca. L. solanacearum*’ haplotype F and haplotypes A and B, it is highly possible that the potato psyllid (*B. cockerelli*) will also vector this new haplotype. Potato psyllid populations found in potato fields appeared to be low in the Southwestern region of Oregon where the tuber with ‘*Ca. L. solanacearum*’ haplotype F originated in 2017 (Charlton 2018). At the same time, psyllids other than the potato psyllid were collected at slightly higher numbers (Charlton 2018), indicating that studies will need to be conducted to discern the true vector of ‘*Ca. L. solanacearum*’ haplotype F in the Klamath Basin.

In addition to studying host and vector biology of this novel ‘*Ca. L. solanacearum*’ haplotype, understanding the dynamics between haplotypes A, B, and F will be important. To our knowledge, this is the first time zebra chip disease has been identified in the Klamath Basin, so the potential for geographic overlap of ‘*Ca. L. solanacearum*’ haplotypes A, B, and F is unknown. In-depth surveys for ‘*Ca. L. solanacearum*’-infected plants and insects from this region of Oregon will be necessary to determine prevalence and the potential impact this novel haplotype will have on the potato industry of Southwestern Oregon.

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