

Haplotypes of “*Candidatus Liberibacter europaeus*” also separate by geography and plant/insect host species

Warrick R. Nelson

Abstract

“*Candidatus Liberibacter europaeus*” (Leu) is one of six currently known *Liberibacter* species. It is known primarily from pear and related species across Europe, and from Scotch broom and its associated psyllids in New Zealand (introduced from Britain). The psyllids were introduced to New Zealand as a biocontrol agent for broom and it is thought the bacterium may have been introduced as an endosymbiont of the psyllids. No symptoms in apple or pear trees have been reported, but mild symptoms can occur in broom.

16S and 16S–23S intergenic spacer region DNA sequences of this species are available from GenBank. Analysis of these sequences and associated ecological descriptions indicates the occurrence of two haplotypes, LeuA and LeuB, defined not only genetically, but also by geographic range as well as by plant/psyllid host species composition. *Liberibacter* species are new to science and haplotype identifications enable historical accounts of relationships and spread to be generated.

Introduction

Liberibacter species have been shown to be members of the α -proteobacteria (Jagoueix et al., 1994). They are a major cause of damage to various Rutaceae, Solanaceae and Apiaceae crop plants (Haapalainen, 2014). “*Candidatus Liberibacter europaeus*” (Leu) was first described in Italy from pear trees and associated *Cacopsylla pyri* psyllids (Raddadi et al., 2010). It has subsequently been noted to be more widely spread across Europe, including in apple and other closely related plant species as well as their associated *Cacopsylla* psyllid vectors (Camerota et al., 2012). No symptoms ascribed to Leu have yet been reported in these plants.

The same species has been found in Scotch broom, *Cytisus scoparius*, showing symptoms such as stunting and leaf chlorosis (Thompson et al., 2013). It is suggested that Leu likely arrived in New Zealand within the broom psyllid, *Arytainilla spartiophila*, imported to New Zealand from England as a biocontrol agent against broom (Fowler, 2013).

The New Zealand partial 16S rDNA sequences were noted as being 99.7% similar to the Italian sequences (Thompson et al., 2013). This, combined with differences in geographic range, plant and insect hosts, suggests the potential to determine the presence of genetically, geographically and host species defined Leu haplotypes.

Materials & Methods

GenBank was searched for sequences identified as “*Candidatus Liberibacter europaeus*” (<http://www.ncbi.nlm.nih.gov/nuccore/?term=liberibacter+europaeus>). Nine sequences were discovered, covering either the 16S or the 16S-23S intergenic spacer region (ISR) or both. BLAST searches failed to identify any further relevant sequences. These sequences were aligned using ClustalX (Larkin et al., 2007). The sequences used were JX629241, JX629242, JX629243, JX629244, JX244258, JX244259, JX244260, FN678792, and FN678796.

In addition, the 16S through to the 5' end of the 23S gene of “*Ca. L. americanus*” (Lam), the closest species phylogenetically to Leu (Nelson et al., 2013), was also aligned (GenBank reference CP006604, nucleotides 585 655-587 709) from the Lam genome (Wulff et al., 2014).

Results & Discussion

Two single nucleotide polymorphisms (SNPs) on the 16S gene and 16 on the 16S-23S intergenic spacer region (ISR) were noted between the Italian and New Zealand sequences (Table 1). Of the 18 SNPs in total, 10 are shared between Leu (New Zealand) and Lam while none are shared between Leu (Italy) and Lam.

With relatively few sequences available and derived from investigations at only two laboratories, identification of haplotypes in Leu must be taken with caution. However, there are 8 sequences of the 16S gene and 5 of the ISR, including single sequences covering both gene segments, and both

The New Zealand Institute for Plant & Food Research Ltd., Private Bag 4704, Christchurch 8140, New Zealand
✉ warrick.nelson@plantandfood.co.nz

haplotypes have sequences derived from the respective plant and psyllid hosts. Further, these sequences lack random sequencing errors or ambiguities thus increasing the degree of confidence of identification that these are indeed biologically relevant SNPs.

Phylogenetically, Lam is the closest *Liberibacter* species to Leu (Nelson et al., 2013). The similarity of nucleotides at equivalent positions between the New Zealand sequences and Lam suggests that the New Zealand haplotype is closer to the common ancestor than is the Italian haplotype. This fits the biogeographic inference derived from molecular dating and plate tectonics, indicating a historical geographic link some millions of years old separating Lam in the Americas and Leu in central Europe (Nelson et al., 2013).

Two other *Liberibacter* species have been studied for haplotype presence. “*Ca. L. asiaticus*” (Las) lacks haplotypes on the 16S gene despite a very wide geographic range in commercial citrus orchards (Nelson, 2012). “*Ca. L. solanacearum*” (Lso) is a *Liberibacter* species vectored by a number of species of Triozid psyllids and causes considerable damage in both Solanaceae and Apiaceae crops in North America and western Europe respectively (Munyanza, 2013). Five haplotypes have now been described within this bacterial species through SNPs on the 16S, 16S-23S intergenic spacer region and 50S genes (Nelson, Fisher & Munyanza, 2011; Teresani et al., 2014), as well as describing differences in geographic and

plant/insect host ranges. Haplotyping has a number of important disease management aspects, for example as a means of aiding in determining the source of incursions (Nelson et al., 2014; Thomas et al., 2011), or apparent differences in host responses (Rashed et al., 2014).

Interestingly, Leu infection is symptomatic in Scotch broom but reported to be asymptomatic in pear trees. The symptoms in broom cover essentially the same main foliar symptoms commonly ascribed to other *Liberibacter* species. The lack of symptoms in the pear trees is somewhat puzzling, although a delay between detection via DNA tests and visual symptoms as long as 8 months post-inoculation is known for Las (Coletta-Filho et al., 2010). However, somewhat similar symptoms in apple and pear trees in Europe are usually ascribed to Pear Decline associated with “*Ca. Phytoplasma*” species (Seemüller & Schneider, 2004). Parry’s Disease of pears in England was concluded as being the same as Pear Decline (Davies et al., 1992), but the range of symptoms described suggests that Parry’s Disease might potentially be associated with Leu rather than a “*Ca. Phytoplasma*” species. Similarly, one of the haplotypes of Lso is recorded to produce symptoms on celery very similar to those associated with “*Ca. Phytoplasma*” (Teresani et al., 2014).

Conclusions

SNPs in 16S and 16s-23S ISR genomic DNA indicate the presence of genetically defined haplotypes of Leu. These haplotypes are also described by the geographic range as well as plant/psyllid host combinations. They are designated LeuA (Italian material FN678792) described from pear and *Cacopsylla pyri*, and LeuB (New Zealand material JX244259) described from Scotch broom *Cytisus scoparius* and the broom psyllid *Arytainilla spartioiphila*.

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Table 1 Nucleotide differences between Leu sequences from two geographic sources on the 16S and 16S-23S intergenic spacer region (ISR). Nucleotide numbers on the reference sequence FN678792, and indicating the nucleotide at the equivalent position on Lam.

Gene	Nucleotide number	New Zealand	Italy	Lam
16S	20	G	A	G
16S	515	T	C	T
ISR	1450	A	G	A
ISR	1359	G	A	T
ISR	1510	C	A	T
ISR	1593	G	T	G
ISR	1605	T	A	T
ISR	1610	T	A	T
ISR	1621	-	G	-
ISR	1702	-	A	T
ISR	1789	G	A	T
ISR	1879	C	T	C
ISR	1886	T	A	T
ISR	1895	G	A	T
ISR	1938	A	G	A
ISR	1968	T	A	G
ISR	1976	G	A	G
ISR	1977	A	G	T

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