

European and Mediterranean Plant Protection Organization
 Organisation Européenne et Méditerranéenne pour la Protection des Plantes

EPPO Data Sheets on pests recommended for regulation
Fiches informatives sur les organismes recommandés pour réglementation

‘*Candidatus Liberibacter solanacearum*’

Identity

Name: ‘*Candidatus Liberibacter solanacearum*’ (Liefiting *et al.*, 2009c)

Synonym: ‘*Candidatus Liberibacter psyllauros*’ (Hansen *et al.*, 2008)

Taxonomic position: Bacteria: Proteobacteria: Alphaproteobacteria: Rhizobiales: Rhizobiaceae

Common names: zebra chip (English), zebra complex (English), psyllid yellows (English), punta morada (Spanish), papa manchada (Spanish), papa rayada (Spanish)

EPPO code: LIBEPS

Phytosanitary categorization: EPPO A1 list no 365 (for Solanaceae haplotypes).

Hosts

‘*Candidatus Liberibacter solanacearum*’ is known to primarily infect solanaceous species, including potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), tomatillo (*Physalis peruviana*), tamarillo (*Solanum betaceum*), tobacco (*Nicotiana tabacum*), and several weeds in the family Solanaceae (Hansen *et al.*, 2008; Liefiting *et al.*, 2008a,b, 2009a,c; Abad *et al.*, 2009; Crosslin & Munyaneza, 2009; Lin *et al.*, 2009; Munyaneza *et al.*, 2009a,b,c; Secor *et al.*, 2009; Wen *et al.*, 2009; Brown *et al.*, 2010; Crosslin *et al.*, 2010; Munyaneza, 2010, 2012; Rehman *et al.*, 2010; Sengoda *et al.*, 2010). This *Liberibacter* species is transmitted to solanaceous species by the potato/tomato psyllid, *Bactericera cockerelli* (Šulc). Recently, ‘*Ca. L. solanacearum*’ has been detected in carrot plants (*Daucus carota*) in Finland and is vectored by the carrot psyllid, *Trioza apicalis* Förster (Munyaneza *et al.*, 2010a,b, 2011b). Subsequently, this bacterium was also detected in carrots and *T. apicalis* in Sweden and Norway (Munyaneza *et al.*, 2012b,c). Most recently, ‘*Ca. L. solanacearum*’ has been reported in carrot, celery (*Apium graveolens*) and the psyllid *Bactericera trigonica* in the Canary Islands and mainland Spain (Alfaro-Fernández *et al.*, 2012a,b). These recent findings on the association of ‘*Ca. L. solanacearum*’ with non-solanaceous species and other psyllids suggest that it is likely to have more insect vectors and host plants than currently known.

Geographical distribution

‘*Ca. L. solanacearum*’ has been detected in several European countries in carrot crops (and to a lesser extent in celery) in association with other psyllid species (*Bactericera trigonica* and *Trioza apicalis*). However, ‘*Ca. L. solanacearum*’ has not been detected in potato or tomato crops in the EPPO region.

EPPO region: Finland, France (transient; only in 2 carrot fields), Norway, Spain (mainland and Canary Islands), Sweden.

North America: Mexico, USA (Arizona, California, Colorado, Idaho, Kansas, Montana, Nebraska, Nevada, New Mexico, Oregon, Texas, Washington Wyoming).

Central America: Guatemala, Honduras, Nicaragua.

Oceania: New Zealand (recently introduced).

Biology

‘*Ca. L. solanacearum*’ is a phloem-limited, Gram-negative, unculturable bacterium that is spread from infected to healthy plants by psyllid insect vectors (Hansen *et al.*, 2008; Munyaneza *et al.*, 2008, 2010b; Secor *et al.*, 2009; Munyaneza, 2012). It may be spread experimentally by grafting (Crosslin & Munyaneza, 2009; Secor *et al.*, 2009). This *Liberibacter* species has also been shown to be transmitted both vertically (transovarially) and horizontally (from feeding on infected plant hosts) in *B. cockerelli* (Hansen *et al.*, 2008; Munyaneza, unpublished data). No information is currently available on vertical transmission for *T. apicalis* and *B. trigonica*. Although only limited experiments have been conducted on *Liberibacter* transmission, it appears that ‘*Ca. L. solanacearum*’ is not transmitted through true seed from infected plants (Munyaneza, 2012).

Four geographic haplotypes of ‘*Ca. L. solanacearum*’ have recently been described (Nelson *et al.*, 2011, 2012). Two haplotypes (LsoA and LsoB) are associated with diseases caused by this bacterium in potatoes and other solanaceous plants, whereas the other two (LsoC and LsoD) are associated with diseased carrots. Haplotype LsoA has been found primarily from Honduras and Guatemala through Western Mexico to Arizona, California, Oregon, Washington, Idaho and in New Zealand. Haplotype LsoB is currently found from Eastern Mexico and northwards through Texas. These two haplotypes show some range

overlap in Texas, Kansas and Nebraska. Haplotype LsoC has been found in Finland, Sweden and Norway (Nelson *et al.*, 2011, 2012) and is associated with *T. apicalis*. LsoD was very recently described from infected carrots and the psyllid *B. trignonica* in Spain and the Canary Islands (Nelson *et al.*, 2012). The four haplotypes are not yet known to elicit biological differences in the plant or insect hosts. These apparently stable haplotypes suggest separate long-lasting populations of the bacterium. Interestingly, LsoA and LsoC were found to be genetically very close, despite their large geographic separation and the differences in both plant and insect hosts (Nelson *et al.*, 2012). Furthermore, a study on the genetic diversity of Lso strains using simple sequence repeat (SSR) markers identified two major lineages of this bacterium in the United States and only one lineage in Mexico (Lin *et al.*, 2012).

Effects of environmental conditions on ‘*Ca. L. solanacearum*’ are not well known. However, temperature has a significant effect on development of this bacterium. Compared to citrus greening *Liberibacter* species, ‘*Ca. L. solanacearum*’ appears heat sensitive as it seems not to tolerate temperatures above 32°C (Munyanzeza *et al.*, 2012a). *B. cockerelli*, the insect vector of this bacterium in the Americas and New Zealand, appears to have similar sensitivity to heat.

Detection and identification

Symptoms

The characteristic above-ground plant symptoms of ‘*Ca. L. solanacearum*’ infection in potato, tomato and other solanaceous species resemble those caused by phytoplasmas (see Fig. 1) and include stunting, erectness of new foliage, chlorosis and purpling of foliage with basal cupping of leaves, upward rolling of leaves throughout the plant, shortened and thickened terminal internodes resulting in plant rosetting, enlarged nodes, axillary branches or aerial tubers, leaf scorching, disruption of fruit set, and production of numer-



Fig. 1 Tomato plant infected by ‘*Candidatus Liberibacter solanacearum*’ (photo courtesy of JE Munyanzeza).

ous, small, misshapen, and poor quality fruits (Munyanzeza *et al.*, 2007a,b; Liefiting *et al.*, 2009a; Secor *et al.*, 2009; Crosslin *et al.*, 2010; Munyanzeza, 2010, 2012). In potato, the below-ground symptoms include collapsed stolons, browning of vascular tissue concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues, all of which can affect the entire tuber (see Fig. 2). Upon frying, these symptoms become more pronounced and crisps or chips processed from affected tubers show very dark blotches, stripes, or streaks, rendering them commercially unacceptable (Munyanzeza *et al.*, 2007a,b, 2008; Secor *et al.*, 2009; Crosslin *et al.*, 2010; Miles *et al.*, 2010; Munyanzeza, 2012; Munyanzeza & Henne, 2012). The symptoms in potato tubers have led to the disease being named ‘zebra chip’ (Munyanzeza *et al.*, 2007a,b; Munyanzeza, 2012).

Symptoms in carrots infected with ‘*Ca. L. solanacearum*’ include leaf curling, yellowish, bronze and purplish discoloration of leaves, stunting of the carrot shoots and roots, and proliferation of secondary roots (Munyanzeza *et al.*, 2010a,b, 2012b,c; Alfaro-Fernández *et al.*, 2012a,b). Collectively, these symptoms resemble those caused by leafhopper-transmitted phytoplasmas and *S. citri* in carrots (Font *et al.*, 1999; Lee *et al.*, 2006; Cebrián *et al.*, 2010; Munyanzeza *et al.*, 2011b).

Morphology

Similarly to other *Liberibacter*s, scanning electron microscopy images of ‘*Ca. L. solanacearum*’ in sieves of infected plants revealed that this bacterium has a rod-shaped morphology (Liefiting *et al.*, 2009a; Secor *et al.*, 2009). The bacterium is about 0.2 µm wide and 4 µm long (Liefiting *et al.*, 2009a).

Detection and inspection methods

The whole genome of ‘*Ca. L. solanacearum*’ isolated from zebra chip-infected potatoes has recently been sequenced



Fig. 2 Zebra chip infected potato tuber (photo courtesy of JE Munyanzeza).

(Lin *et al.*, 2011). Detection methods for 'Ca. L. solanacearum' have been developed and include conventional and quantitative real-time polymerase chain reaction (PCR) (Hansen *et al.*, 2008; Crosslin & Munyaneza, 2009; Li *et al.*, 2009; Liefting *et al.*, 2009a; Lin *et al.*, 2009; Wen *et al.*, 2009; Crosslin *et al.*, 2011; Munyaneza, 2012). Uneven distribution and variation in the Liberibacter titer in different parts of infected plants has been observed, making detection of this bacterium by PCR sometimes inconsistent (Crosslin & Munyaneza, 2009; Li *et al.*, 2009). Development of better and more accurate detection methods is currently underway. Visual symptom inspections in some infected plants such as potato tubers can be very reliable (see symptoms above). Mixed infections of 'Ca. L. solanacearum' and phytoplasmas have been reported in potato (Liefting *et al.*, 2009b; Munyaneza, unpublished data) and carrot (Munyaneza *et al.*, 2011b). Furthermore, mixed infections of Liberibacter, phytoplasmas, and *S. citri* have been detected in carrots in the Mediterranean region (Alfaro-Fernández *et al.*, 2012a).

Pathways for movement

'Ca. L. solanacearum' can be moved by its vectors or in host plants meant for propagation.

During international trade, infected planting material could carry the disease, or possibly also infective vectors (most likely as eggs). Seed potatoes infected with 'Ca. L. solanacearum' generally do not germinate but, in rare cases, may produce infected plants (Henne *et al.*, 2010; Pitman *et al.*, 2011). However, these seed-borne infected plants are often weak and short-lived and do not significantly contribute to the disease spread (Munyaneza, 2012). Most importantly, the psyllids have to be present to spread the bacterium.

Pest significance

Economic impact

'Ca. L. solanacearum' was first identified in 2008 (Hansen *et al.*, 2008; Liefting *et al.*, 2008a,b) and shown to be associated with zebra chip disease of potato, which has been observed since the 1990s with increasing impacts and linked to *B. cockerelli* for the first time in 2007 (Munyaneza *et al.*, 2007a,b). A comprehensive review of the history and association of this bacterium with zebra chip and other diseases of solanaceous crops was provided by Crosslin *et al.* (2010), Munyaneza (2010, 2012), and Munyaneza & Henne (2012). First reported in Mexico in the 1990s, zebra chip was documented causing serious economic damage in parts of Southern Texas in 2004-2005. The disease is now widespread in the South-Western, Central, and North-Western USA, Mexico, Central America, and New Zealand (Munyaneza *et al.*, 2007a,b, 2009a; Liefting *et al.*, 2009a; Secor *et al.*, 2009; Crosslin *et al.*, 2010, 2012a,b;

Munyaneza, 2010, 2012; Rehman *et al.*, 2010). This Liberibacter species also severely affects other important solanaceous crops, including tomato, pepper, eggplant, tamarillo (Liefting *et al.*, 2009a; Munyaneza *et al.*, 2009b,c; Brown *et al.*, 2010), and tobacco (Munyaneza, unpublished data). It has been established that 'Ca. L. solanacearum' is transmitted to solanaceous species by the psyllid *B. cockerelli*. Recently, Munyaneza *et al.* (2010a,b) detected 'Ca. L. solanacearum' in carrots affected by the psyllid *T. apicalis* in Finland, which constitutes the first report of Liberibacter in Europe and 'Ca. L. solanacearum' in a non-solanaceous species. The bacterium was subsequently detected in carrots and *T. apicalis* in Sweden and Norway (Munyaneza *et al.*, 2012a,b). Most recently, 'Ca. L. solanacearum' has been detected in carrot, celery and the psyllid *B. trigonica* in the Canary Islands and mainland Spain (Alfaro-Fernández *et al.*, 2012a,b; EPPO, 2012); symptoms in diseased plants had previously been attributed to phytoplasmas and spiroplasmas in this area.

The complex bacterium/vectors has caused serious damage to the potato and tomato industries in the Americas and New Zealand (Munyaneza *et al.*, 2007a,b, 2008; Liefting *et al.*, 2009a; Secor *et al.*, 2009; Crosslin *et al.*, 2010; Rehman *et al.*, 2010; Guenther *et al.*, 2012) and to the carrot industry in Europe (Munyaneza, 2010; Munyaneza *et al.*, 2010a,b, 2012b,c; Alfaro-Fernández *et al.*, 2012a,b). In the case of potato, plant growth is negatively affected; crisps or chips made from zebra chip-infected tubers show dark stripes that become markedly more visible upon frying, and hence are commercially unacceptable. Whole crops might be rejected because of high levels of the disease, occasionally leading to abandonment of entire potato fields. Potatoes for the fresh market are severely affected by zebra chip (Munyaneza *et al.*, 2011a). Infected tubers usually do not sprout and if they do, produce hair sprouts or weak plants (Henne *et al.*, 2010; Pitman *et al.*, 2011). The bacterium is also associated with economically damaging diseases of other important solanaceous crops, including tomato, pepper, eggplant, tobacco, and tamarillo. In Europe, damage to carrots by Liberibacter-infected carrot psyllids can cause up to 100% crop loss (Munyaneza *et al.*, 2010a,b, 2012b,c; Alfaro-Fernández *et al.*, 2012a,b).

Control

At present, applications of insecticides targeted against the potato and carrot psyllids are the only means to effectively manage diseases associated with 'Ca. L. solanacearum' (Munyaneza, 2012). See the datasheet for *B. cockerelli* for details. No plant resistance to the disease has yet been identified (Munyaneza *et al.*, 2011a).

Phytosanitary risk

'Ca. L. solanacearum' and its insect vector *B. cockerelli* have been found to be serious and economically important

pests of potatoes, tomatoes, and other solanaceous crops in Western and Central USA, Mexico, Central America and New Zealand. They would result in similar damage if introduced in the EPPO region. Quarantine considerations have already emerged in some regions where ‘*Ca. L. solanacearum*’ has been documented. Some countries are now requiring specific testing for ‘*Ca. L. solanacearum*’ prior to allowing import of potatoes (Crosslin *et al.*, 2010; Munyaneza, 2012). Furthermore, Australia put in place additional quarantine requirements for the importation of fresh tomato and pepper from New Zealand after 2006, where growers need to ensure that crops for export have been produced in areas free of *B. cockerelli* or the exported produce must be free of the psyllid.

‘*Ca. L. solanacearum*’ has already been documented in carrots in Finland, France, Sweden, and Norway and it is suspected that this bacterium is more likely to be widespread in parts of Northern and Central Europe where its insect vector *T. apicalis* occurs or at least in regions where damage by this psyllid has been observed. Very recently, this bacterium was reported in carrots and the psyllid *B. trigonica* in Spain and the Canary Islands. Nevertheless results of a preliminary study indicate that inoculation of ‘*Ca. L. solanacearum*’ to potato by *T. apicalis* is not possible (Munyaneza, unpublished data). These observations suggest that the main pathway of introducing ‘*Ca. L. solanacearum*’ into solanaceous species would be the introduction of infective *B. cockerelli* into the EPPO region.

Phytosanitary measures

EPPO recommends that vegetative material for propagation and produce (such as fruits) of Solanaceae should come from areas free of *B. cockerelli* and ‘*Ca. L. solanacearum*’. Seed and ware potatoes should come from areas free of zebra chip. Alternatively high grade seed potato may be imported under post-entry quarantine, and ware potatoes may be imported only for industrial processing purposes.

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