



The diversity of citrus endophytic bacteria and their interactions with *Xylella fastidiosa* and host plants

João Lúcio Azevedo¹, Welington Luiz Araújo² and Paulo Teixeira Lacava³

¹*Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brazil.*

²*Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil.*

³*Departamento de Morfologia e Patologia, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, São Carlos, SP, Brazil.*

Abstract

The bacterium *Xylella fastidiosa* is the causal agent of citrus variegated chlorosis (CVC) and has been associated with important losses in commercial orchards of all sweet orange [*Citrus sinensis* (L.)] cultivars. The development of this disease depends on the environmental conditions, including the endophytic microbial community associated with the host plant. Previous studies have shown that *X. fastidiosa* interacts with the endophytic community in xylem vessels as well as in the insect vector, resulting in a lower bacterial population and reduced CVC symptoms. The citrus endophytic bacterium *Methylobacterium mesophilicum* can trigger *X. fastidiosa* response *in vitro*, which results in reduced growth and induction of genes associated with energy production, stress, transport, and motility, indicating that *X. fastidiosa* has an adaptive response to *M. mesophilicum*. Although this response may result in reduced CVC symptoms, the colonization rate of the endophytic bacteria should be considered in studies that intend to use this endophyte to suppress CVC disease. Symbiotic control is a new strategy that uses symbiotic endophytes as biological control agents to antagonize or displace pathogens. Candidate endophytes for symbiotic control of CVC must occupy the xylem of host plants and attach to the precibarium of sharpshooter insects to access the pathogen. In the present review, we focus on interactions between endophytic bacteria from sweet orange plants and *X. fastidiosa*, especially those that may be candidates for control of CVC.

Keywords: endophytes, *Citrus sinensis*, *Curtobacterium flaccumfaciens*, *Methylobacterium mesophilicum*, symbiotic control.

Received: March 17, 2016; Accepted: June 09, 2016.

Endophytic microorganisms and biological control

Endophytes can be isolated from surface-disinfected plant parts or the inner plant tissues and are defined as microorganisms, mainly bacteria and fungi, that live within a plant for at least a part of their life cycle without causing apparent harm to the host (Petrini *et al.*, 1989; Hallmann *et al.*, 1997; Azevedo *et al.*, 2000). A more comprehensive definition was proposed by Azevedo and Araújo (2007), who described endophytes as all microorganisms that may or may not be successfully cultured, internally colonize the host plant and do not cause apparent damage and/or visible external structures. This definition was amended by Mendes and Azevedo (2008) to divide endophytes in two types: type I, which does not produce external structures, and type

II, which produces external structures, such as nodules from nitrogen-fixing bacteria and fungi-plant mycorrhizal associations. Recently, Hardoin *et al.* (2015) proposed that the term “endophyte” should be used as a habitat only, not a function, including all microorganisms able to colonize the inner plant tissues.

Endophytes have been reported to contribute to host plant protection and, ultimately, survival (Sturz and Matheson, 1996; Hallmann *et al.*, 1998; Azevedo *et al.*, 2000; Newman and Reynolds, 2005; Rosenblueth and Martinez-Romero, 2006; Reinhold-Hurek and Hurek, 2011; Suryanarayanan, 2013; Nair and Padmavathy, 2014; Podolick *et al.*, 2015). Because endophytes colonize an ecological niche similar to that of phytopathogens, they are possible bio-control agents (Hallmann *et al.*, 1997; Hardoin *et al.*, 2015). The potential for practical applications of endophytes has led to studies investigating the ability of bacteria to control both disease and insect infestations, as well as promote plant growth (Azevedo *et al.*, 2000; Kozdrój *et al.*; 2004;

Kavino *et al.*, 2007; Podolick *et al.*, 2015). Indeed, previous work has suggested that endophytic microorganisms have the potential to control pathogens (Sturz and Matheson, 1996; Duijff *et al.*, 1997; Sharma and Nowak, 1998; Sturz *et al.*, 1998; Lacava *et al.*, 2004; 2007a), insects (Petrini *et al.*, 1989; Azevedo *et al.*, 2000), and nematodes (Hallmann *et al.*, 1997). In grass, infection by endophytic fungi and alkaloid production reduced aphid feeding but had no effect on viral titers in the host plant (Ruá *et al.*, 2013). However, the authors observed that the virulence of the viral infection was reduced in endophyte-infected plants, suggesting that although the endophyte had no effect on viral infection, the presence of the endophytes could trigger a host response that reduced virulence. The balance of this interaction among the host plant, endophytic fungi, aphids and viruses was influenced by the host and endophyte genotypes and was also driven by environmental conditions (Ruá *et al.*, 2013; 2014).

In addition, endophytes can also accelerate seed emergence, assist in establishing the plant under adverse conditions (Chanway, 1997), and increase plant growth and development (Bent and Chanway, 1998; Lazarovits and Nowak, 1997; Pillay and Nowak, 1997; Nassar *et al.*, 2005; Bao and Roossinck, 2011; Bezerra *et al.*, 2013; Verma *et al.*, 2015; Chebotar *et al.*, 2015).

Endophytic bacteria may play a significant role in protection against plant pathogens and in the overall productivity of an agricultural ecosystem (Hallmann *et al.*, 1997; Sturz *et al.*, 2000). These microorganisms produce molecules that function as growth-promoting metabolites, insect-pest repellents, antimicrobials against plant pathogens, and protectants against stress (Rai *et al.*, 2014). They can also produce unique secondary metabolites that can be exploited in pharmaceutical, agricultural and other industries (Golinska *et al.*, 2015).

The utilization of endophytic bacteria for biotechnological purposes has recently increased, especially with regard to insect and disease control and plant growth promotion. Endophytic bacteria promote plant growth in three major ways, by synthesizing compounds that are useful to the plants, by facilitating the uptake of certain nutrients from the soil, and by controlling or preventing disease (biological control). Growth promotion mediated by endophytic bacteria occurs via several mechanisms: the synthesis of enzymes; the production of hormones such as auxin [indoleacetic acid (IAA)]; symbiotic nitrogen fixation; antagonism against phytopathogens by siderophores, chitinases or antibiotics; and the solubilization and mineralization of nutrients, particularly insoluble mineral phosphates (Lacava and Azevedo, 2013, 2014). Indeed, interactions between endophytes and plants can promote plant health and play a significant role in low-input sustainable agriculture for both food and nonfood crops. Nonetheless, an understanding of the mechanisms that enable endophytes to interact with plants is essential for realizing the biotechno-

logical potential of these microorganisms (Quecine *et al.*, 2014).

In citrus trees, the endophytic environment becomes more stable and uniform over time. This may result from selection of particular genotypes within each local microbial population (Araújo *et al.*, 2002). Consequently, bacteria living in an endophytic environment may adapt to this more stable environment, resulting in intense interactions among the populations (Lacava *et al.*, 2004).

Several reports highlighted the relationships among bacterial populations and suggest that CVC symptoms in citrus plants could be influenced by the population balance among *Methylobacterium* spp., *Curtobacterium flaccumfaciens* and *X. fastidiosa* (Araújo *et al.*, 2002; Lacava *et al.*, 2004, 2006b, 2007a; Ferreira Filho *et al.*, 2012). Understanding the relationship among endophytic bacteria within sweet orange trees and *X. fastidiosa* may lead to strategies to control CVC using endophytic bacteria (Ferreira Filho *et al.*, 2012) or inducing physiological shifts in both the host plant and microbial community that result in more balanced and stable interactions.

The plant pathogen *Xylella fastidiosa*

The first report of symptoms caused by what we now call *Xylella fastidiosa* was in 1884 in the grape-growing region of southern California (US). A disease syndrome, known today as Pierce's disease (PD), was later described in detail (Pierce, 1892). Subsequently, similar diseases were reported in many fruit tree and ornamental species, especially in North and South America (Hopkins, 1989). *X. fastidiosa* is a fastidious, Gram-negative, xylem-limited, rod-shaped bacterium with distinctive rippled cell walls. It is non-flagellated, does not form spores and measures 0.1-0.5 1-5 μm (Nyland *et al.*, 1973; Bradbury, 1991). This Gram-negative bacterium was formally named only in 1987 (Wells *et al.*, 1987). It is extremely slow-growing in culture. These traits have made the pathogen difficult to study and contributed to its previous obscurity. The taxonomic position of *X. fastidiosa* (Wells *et al.*, 1987) is: Bacteria, *Gracilicutes*, aerobic rods, Category I, Group 4, Subgroup 4 A (Holt, 1994). Natural transmission occurs via insects feeding suctorially on xylem sap. Transmission efficiency varies widely among vector species. The bacterium overwinters in the xylem of the host plants as well as in weeds (Lopes *et al.*, 2003; Wistrom and Purcell, 2005).

X. fastidiosa (Wells *et al.*, 1987) resides in the xylem vessels of a broad range of perennial plants in the New World and has been shown to cause important diseases in a variety of fruit trees and vines. These include PD in grapevines (Davis *et al.*, 1981; Hopkins and Purcell, 2002), leaf scorch in pecan (Sanderlin and Heyderich-Alger, 2000; Sanderlin and Melanson, 2006), pear (Leu and Su, 1993), plum (Raju *et al.*, 1983), almond (Mircetich *et al.*, 1976), mulberry (Kostka *et al.*, 1986), elm, sycamore, oak (Hearon

et al., 1980), maple (Sherald *et al.*, 1987), coffee (de Lima *et al.*, 1998), oleander and olives (Saponari *et al.*, 2013), as well as alfalfa dwarf (Goheen *et al.*, 1979), phony peach disease (Wells *et al.*, 1981), periwinkle wilt (McCoy *et al.*, 1978), and citrus variegated chlorosis (Chang *et al.*, 1993; Hartung *et al.*, 1994). Strains of *X. fastidiosa* have a wide host range in the native flora, where they exist without inducing symptoms of disease, and they are transmitted by common sharpshooter insects (Freitag, 1951; Freitag and Frazier, 1954).

Following the publication of the *X. fastidiosa* genome (Simpson *et al.*, 2000), there was an increased number of articles describing new features of *X. fastidiosa*, such as the existence of plasmids and phages and the discovery of conjugation and recombination in the species as well similarities among genomes of distinct *X. fastidiosa* strains. Bhattacharyya *et al.* (2002) described a whole-genome comparative analysis of three phytopathogenic strains from almond, citrus and oleander. This study demonstrated that these genomes are closely related. Van Sluys *et al.* (2003) described limited genomic structural variability within *X. fastidiosa*, which suggested that phylogenetic groups colonizing different host plants have similar pathogenicity mechanisms. Varani *et al.* (2012) showed that comparative genomic databases were an important information resource to explore the annotation, genomic features and biology of different *X. fastidiosa* strains. Other results published after the *X. fastidiosa* genome release should also be mentioned. Marques *et al.* (2001) described the sequence of the plasmid pXF51 from the plant pathogen *X. fastidiosa*, showing that this plasmid contained genes for conjugation, replication and mobilization but apparently had no role in pathogenesis, only in conjugative transfer. Nunes *et al.* (2003) constructed a microarray and evaluated the occurrence of prophages, plasmids and genomic islands (18% of the genome). The authors showed that most of these elements are transcriptionally active and could explain the ability of *X. fastidiosa* strains to infect a wide range of plant species. Plasmids were also found and sequenced by other authors, such as Rogers and Stenger (2012). Additionally, Kung and Almeida (2011) demonstrated that recombination can occur at relatively high rates and may play a large role in shaping the genetic diversity of *X. fastidiosa*. Chen and Civerali (2008) demonstrated that phages particles are released by *X. fastidiosa* cultures for the first time, and Summer *et al.* (2010) carried out genomic and bacterial analyses of the phage Xfas53 and related prophages of *X. fastidiosa*. Guilhabert and Kirkpatrick (2003) successfully transformed *X. fastidiosa* using a broad host range plasmid.

However, despite the increasing knowledge of *X. fastidiosa* characteristics, the molecular mechanisms determining host plant specificity have not been elucidated (Almeida and Nunney, 2015). The interactions between *X. fastidiosa* and attacked plants were discussed in more detail in a review by Hopkins and Purcell (2002). More recently,

Almeida and Nunney (2015) reviewed the main processes that led to the emergence of the diseases caused by *X. fastidiosa*. Since 2002, it has become clear that the frequency and density of the pathogens and endophytic bacteria in citrus plants may be a result of a tripartite interaction associated with environmental conditions (Araújo *et al.*, 2001, 2002). Therefore, in the present review, the CVC status and the molecular and ecological aspects of this tripartite interaction will be discussed. In addition, a possible strategy based on symbiotic control will be proposed.

Citrus variegated chlorosis (CVC)

Citrus variegated chlorosis (CVC) is a disease of the sweet orange [*Citrus sinensis* (L.)], which is caused by *X. fastidiosa* (Chang *et al.*, 1993; Hartung *et al.*, 1994; Schaad *et al.*, 2004), a phytopathogenic bacterium that has been shown to infect all sweet orange cultivars (Li *et al.*, 1997a). CVC was first reported in Brazil in 1987 and has rapidly become one of the economically most important diseases affecting sweet orange production in Brazil (Rossetti *et al.*, 1990; Lee *et al.*, 1991). CVC rapidly spread to most major citrus-growing areas through unregulated movement of infected nursery stock due to a previous lack of certification programs and high CVC infection rates in Brazil.

Brazil is the largest producer of citrus fruits in the world (25% of the total world production), supplying most of the international market with concentrated orange juice. More than 80% of these products are produced in the state of São Paulo. Approximately 15 years ago, CVC was found in at least 90% of the orchards in Brazil (Lambais *et al.*, 2000). The incidence and severity of CVC are higher in the northern region than in the southern region of São Paulo (Ayres *et al.*, 2001; Gonçalves *et al.*, 2014). In 2011, more than 40% of the sampled plants in Brazil had CVC symptoms, approximately 5% more than in 2010, showing that the disease is still increasing (www.agriculture.gov.br/arq_editor/file/camaras_exterior).

CVC is considered one of the most important diseases affecting the Brazilian citrus industry, and economic losses due to CVC can reach \$120 million per year (Bové and Ayres, 2007). In an effort to reduce losses, additional regulations have been placed on the production and commercialization of citrus seedlings. In 2003, it became mandatory in São Paulo to propagate citrus in protected, screened houses, increasing the cost of production (Carvalho, 2003). CVC affects mostly oranges (*C. sinensis*); it has mainly been observed on the cultivars 'Pera', 'Hamlin', 'Natal' and 'Valencia'. It occurs in trees propagated on all commonly used rootstocks in Brazil: *C. limonia*, *C. reshni* and *C. volkameriana* (Li *et al.*, 1997c). The disease has not been observed in limes (*C. latifolia*) or mandarins (*C. reticulata*), even when the trees were planted in severely affected orange groves (Li *et al.*, 1997b). Some weed species are also hosts and act as reservoirs of infection (Smith *et al.*, 1997). This disease continues to show an increase in sever-

ity, as mentioned, with 35%–40% of the sweet orange trees in São Paulo, Brazil currently showing yield losses (www.fundecitrus.com.br). Citrus plants with CVC show a notable leaf chlorosis, similar to zinc deficiency, as the initial symptom (Laranjeira *et al.*, 1998; Machado *et al.*, 2006). Later symptoms include wilting, canopy dieback, necrotic leaf lesions, and undersized, hard fruit (Derrick and Timmer, 2000; Hopkins and Purcell, 2002). The causal agent of CVC has been found to be transmitted by sharpshooter leafhoppers (Cicadellidae) in Brazil (Lopes *et al.*, 1996; Almeida and Purcell, 2003). CVC has been experimentally transmitted by 11 different sharpshooter species tested in Brazil (www.fundecitrus.com.br). Additionally, the pathogen can be transmitted through seeds (Li *et al.*, 2003). Although *X. fastidiosa* was the first plant pathogen to have its genome sequenced (Simpson *et al.*, 2000), there is still no effective control for CVC. The pathogen is known to have an extraordinary host range among higher plants in New World ecosystems (Freitag, 1951). Interestingly, within the majority of native host plants, *X. fastidiosa* does not damage the host plant and behaves as an endophyte (Purcell and Saunders, 1999). In contrast, the horticultural crops that suffer from diseases caused by *X. fastidiosa* are those that have been introduced into New World ecosystems (Chen *et al.*, 2000). The observation that a few asymptomatic trees persist in some infected orchards may lead to new approaches to the control of CVC. These asymptomatic plants have the same genotype as diseased plants and are located in the same grove under similar climatic and edaphic conditions, suggesting that some other factor is responsible for resistance to CVC. One factor that may influence the resistance to CVC is the nature of the endophytic microbial community colonizing individual *C. sinensis* plants (Araújo *et al.*, 2002).

Endophytic bacteria from citrus plants and interactions with *Xylella fastidiosa*

We have focused on the interaction between members of the endophytic bacterial community, such as *Methylobacterium* spp. and *C. flaccumfaciens*, which occupy the same ecological niche as *X. fastidiosa* in the xylem vessels of citrus plants (Araújo *et al.*, 2002). The genus *Methylobacterium* is classified in the $\alpha 2$ subgroup of the Proteobacteria and includes a group of strictly aerobic, Gram-negative, pink-pigmented, facultatively methylotrophic (PPFM) bacteria characterized by their ability to utilize single-carbon compounds, such as methanol and formaldehyde, via the serine pathway, as well as a wide range of multi-carbon growth substrates (Green, 1992; Urakami *et al.*, 1993; Wood *et al.*, 1998; Doronina *et al.*, 2000, 2002; McDonald *et al.*, 2001; Van Aken *et al.*, 2004; Anesti *et al.*, 2004; Van Jourand *et al.*, 2004; Gallego *et al.*, 2005a,b,c, 2006). In the host plant, during plant interactions, biofilm formation on surface of the root and hypocotyl of *C. roseus*

occurred prior to endophytic colonization (Andreote *et al.*, 2006). In addition, the authors observed that *M. mesophilicum* SR1.6/6 induced shifts in the indigenous endophytic α - and β -Proteobacteria populations, using DGGE techniques. In soybean, Araújo *et al.* (2015) observed that during the initial step of plant colonization (including plant exudate recognition and adaptation), based on transcriptomic analysis, several genes involved in membrane transport were expressed, suggesting metabolic activation in the presence of root exudate. In addition, the results showed that genes encoding proteins related to suppression of oxidative stress, such as glutathione peroxidase and glutathione synthetase, were induced, suggesting that these genes are probably related to cellular detoxification during plant root colonization. Additionally, Dourado *et al.* (2013) showed that bacterial density is an important characteristic during plant colonization because some genes related to metabolism, stress and pathogenesis were induced by quorum-sensing systems, indicating that plant colonization depends on bacterial coordination of events related to host recognition and stress suppression.

Araújo *et al.* (2001) isolated several endophytic bacteria from different citrus rootstocks and showed that *Alcaligenes* sp., *Bacillus* spp. (including *B. cereus*, *B. lentus*, *B. megaterium*, *B. pumilus*, and *B. subtilis*), *Burkholderia cepacia*, *Curtobacterium flaccumfaciens*, *Enterobacter cloacae*, *Methylobacterium extorquens*, and *Pantoea agglomerans* were the dominant species. Furthermore, the frequency of endophytic bacteria in healthy, escape, and CVC-affected *Citrus sinensis* plants was studied using cultivation as well as cultivation-independent methods (Araújo *et al.*, 2002). Bacteria from the genus *Methylobacterium* were the most frequently isolated endophytes from CVC-symptomatic citrus plants (*C. sinensis*) (Araújo *et al.*, 2002; Lacava *et al.*, 2004, 2006a,b); however, Araújo *et al.* (2002) observed that *M. extorquens* was only isolated from CVC-affected plants while *M. mesophilicum* was isolated from healthy plants, indicating that specific *Methylobacterium* species may be related to the citrus plant physiological condition. Furthermore, in *in vitro* studies, Lacava *et al.* (2004) showed that *M. mesophilicum* could reduce the growth of *X. fastidiosa*, while *M. extorquens* had no effect on the *X. fastidiosa* growth. In addition, in co-inoculation experiments using *Catharanthus roseus* as a model plant, Lacava *et al.* (2006a) showed that the population of *M. mesophilicum* was lower in the presence of *X. fastidiosa* compared to inoculation of this endophytic bacterium alone and that the population of *X. fastidiosa* was in turn reduced by the inoculation of *M. mesophilicum*. The results suggest that these bacteria could compete for nutrients and colonization sites inside the host plant. In fact, using microarray analysis, Dourado *et al.* (2015) showed that the *M. mesophilicum* strain SR1.6/6 directly down-regulated genes related to bacterial growth, such as DNA replication and protein syn-

thesis genes (50S ribosome protein and topoisomerase enzyme genes) in *X. fastidiosa*. In contrast, *C. flaccumfaciens* strain ER1/6, another citrus endophyte, up-regulated genes related to protein synthesis. Additionally, despite the *X. fastidiosa* growth reduction, genes related to energy generation (fumarate hydratase and dihydrolipoamide dehydrogenase from the Krebs cycle) were up-regulated in *X. fastidiosa* in response to *M. mesophilicum*, suggesting that although the CVC causal agent is not growing, energy is necessary to maintain the interaction profile, including genes related to stress response and membrane transporters.

Therefore, the development of CVC symptoms in citrus plants could be influenced by the population balance among the endophytic bacteria *Methylobacterium* spp. and *X. fastidiosa* (Lacava *et al.*, 2004; Dourado *et al.*, 2015) and environmental conditions, which affect the host physiology and response to the presence of the microbial community.

The genus *Curtobacterium* has been defined by Yamada and Komagata (1972) as Gram-positive aerobic bacteria, with some so-called motile brevibacteria. *Curtobacterium* strains have been isolated from rice and other plants, and *C. flaccumfaciens*, in particular, is a well-established plant pathogen (Collins and Jones, 1983). However, *Curtobacterium* has been isolated as an endophyte from many crops, including red clover (Sturz *et al.*, 1998), potato (Sturz and Matheson, 1996), yam (Tor *et al.*, 1992), prairie plants (Zinnier *et al.*, 2002), and citrus (Araújo *et al.*, 2001). Several reports have indicated that *C. flaccumfaciens* can function as a biological control agent against many pathogens and may function either by triggering induced systemic resistance (Raupach and Kloepper, 1998) or by antibiosis (Sturz and Matheson, 1996).

The bacterium *C. flaccumfaciens* has been more frequently isolated from CVC-asymptomatic than from CVC-symptomatic orange (*C. sinensis*) and tangerine (*Citrus reticulata*) plants (Araújo *et al.*, 2002; Lacava *et al.*, 2004), and it was also suggested, based on *in vitro* interaction experiments, that the growth of *X. fastidiosa* could be inhibited by endophytic *C. flaccumfaciens*. Symptoms of *X. fastidiosa* infection in *C. roseus*, such as shortened internodes, reduced flowering and stunting and chlorosis of leaves with occasional scorch symptoms, were reduced or prevented entirely by co-inoculation with *C. flaccumfaciens* (Lacava *et al.*, 2007a). Madagascar periwinkle, *C. roseus* (L.) G. Don, has been identified as an excellent experimental host for *X. fastidiosa* (Monteiro *et al.*, 2001). Symptoms of *X. fastidiosa* infection in periwinkle include shortened internodes, reduced flowering, stunting, and leaf chlorosis with occasional scorch symptoms and wilting (Monteiro *et al.*, 2001). In comparison with the sweet orange, the Madagascar periwinkle is significantly easier to maintain in a greenhouse, and symptom induction following inoculation with *X. fastidiosa* is both more rapid and more reliable. The Madagascar periwinkle has also been utilized to study the interactions between *X. fastidiosa*

and other endophytic bacteria (Lacava *et al.*, 2006a, 2007a).

Biological control of CVC

Lacava *et al.* (2004) reported that the growth of *X. fastidiosa* was inhibited by endophytic *C. flaccumfaciens* *in vitro*, and Lacava *et al.* (2007a) demonstrated that *C. flaccumfaciens* reduced the severity of disease symptoms when co-inoculated with *X. fastidiosa* in periwinkle (*C. roseus*) plants.

Isolation and denaturing gradient gel electrophoresis (DGGE) techniques revealed several genera of bacteria as colonizers of glassy-winged sharpshooter (GWSS) heads. These bacteria were identified by 16S sequencing and included *M. extorquens* and *C. flaccumfaciens*. The GWSS *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae) [formerly *H. coagulata* (Takiya *et al.*, 2006)] is the most widespread sharpshooter insect vector of *X. fastidiosa* in the United States. In addition, Kirkpatrick and Wilhelm (2007) have also isolated strains of *C. flaccumfaciens* as part of the endophytic bacterial community of grapevines in California. In Brazil, *C. flaccumfaciens* is consistently isolated as an endophytic bacterium from citrus plants (Araújo *et al.*, 2002; Lacava *et al.*, 2004).

It is likely that endophytic bacteria are introduced into sweet orange trees by sharpshooter insects in the same manner as *X. fastidiosa*. Gai *et al.* (2011) showed that *Curtobacterium* sp. were the most important bacteria colonizing the heads of the insect vectors of *X. fastidiosa* in Brazil.

Curtobacterium flaccumfaciens was shown to play an important role in the prevention of CVC symptoms in citrus trees (Araújo *et al.*, 2002; Lacava *et al.*, 2004, 2007a). The presence of the citrus endophyte *Curtobacterium* sp. in insect heads could explain why the transmission efficiency of *X. fastidiosa* by vectors is low (5 to 10%) compared to the transmission of *X. fastidiosa* subsp. *fastidiosa* by GWSS, which transmit PD (45%) (Krügner *et al.*, 2000; Redak *et al.*, 2004).

Endophytic bacteria could influence disease development by reducing the efficiency of transmission by insects due to competition with pathogens in host plants and also in insect foreguts (Gai *et al.*, 2011). In addition, the bacterial communities in the foregut of insect vectors of *X. fastidiosa* change with time and environmental conditions and in different insect species. However, because members of the genus *Curtobacterium* were consistently detected in the insect vectors of *X. fastidiosa* (Gai *et al.*, 2011), they may be candidates for biological control of *X. fastidiosa*, which requires endophytic bacteria (Lacava *et al.*, 2007a) that can colonize both the insect vectors of CVC and citrus plants.

Symbiotic control (SC)

The technique of paratransgenesis was developed as a novel method to create conditions that render insect vectors incompetent. The symbiotic control (SC) strategy employs both paratransgenic (defined below) and non-recombinant methods to control disease or health problems. In some cases, these solutions may result in competitive displacement of the pathogen with a more benign microbe.

Paratransgenesis was developed to prevent the transmission of pathogens from insect vectors to humans (Beard *et al.*, 1998, 2001, 2002; Rio *et al.*, 2004; Hurwitz *et al.*, 2011). The key concept in paratransgenesis is the genetic alteration of symbiotic microbes that are carried by insects (therefore, they are paratransgenic insects). The genetic alterations of the symbiotic microbes are designed to increase their competitiveness in the insect vector at the expense of the pathogen. This overall strategy of disease prevention is an example of SC and is a variation on the theme of symbiotic therapy (Ahmed, 2003; Hurwitz *et al.*, 2011). Genetic manipulation has fitness costs that must be factored in to the application (Durvasula *et al.*, 1997; Miller, 2007, 2011).

The key to SC, and therefore paratransgenesis, is to find a local candidate microbe with an existing association with the pathosystem that is being investigated. The local candidate microbe should occupy the same niche as, or have access to, the target pathogen or condition (Durvasula *et al.*, 1997; Hurwitz *et al.*, 2011). The local origin of the biocontrol microbe in SC differs from classical biological control, where microbes, herbivores, parasites or predators are sought from outside the local ecosystem for establishment in the local ecosystem to control a pest, such as a plant or invertebrate (Miller, 2011; Hurwitz *et al.*, 2011). In SC, all elements originate at the local site and have already co-evolved with and been established in the pathosystem; foreign exploration is not only unnecessary but also most likely counter-productive. Because of these strict requirements, a suitable symbiotic candidate may not always be found or may not be amenable to practical manipulation (Miller, 2011; Hurwitz *et al.*, 2011).

Microbes chosen for symbiotic control must be able to pass subsequent regulatory scrutiny (Miller, 2011; Bourtzis *et al.*, 2012). Once a candidate symbiont is identified as a control agent for paratransgenesis, all genetic or other manipulations can be local. Indeed, a symbiotic control or paratransgenic solution developed for a specific location may not be suitable for another site or condition elsewhere (Durvasula *et al.*, 1999, 2003; Miller, 2011).

Once a microbe is identified as having potential for symbiotic or paratransgenic control, it is studied to define the requirements for culture and reintroduction into the pathosystem and the suitability for genetic alteration, if necessary. The methods selected must be adaptable to ordinary practices in the target area. In the case of paratransgenic control, a gene or genes to be introduced into the endosymbiont to influence its interaction with the pathogen

must be identified. Beard *et al.* (2001) isolated and characterized symbiotic bacteria from various triatomine species, which are vectors of Chagas disease, and developed a method for genetically transforming them. These authors reintroduced them into triatomine species, thereby producing stable paratransgenic insects that express heterologous gene products.

Pierce's disease (PD) was first detected in Southern California in 1884, where it destroyed approximately 40,000 acres of grapes in Anaheim, CA during a 5-year outbreak (Pierce, 1892; Goodwin and Purcell, 1992). After this devastating experience, PD became only an occasional concern to West Coast viticulture for decades until the mid-1990s, when the GWSS became established in California. The GWSS is a major concern for horticultural industries beyond viticulture due to its ability to transmit *X. fastidiosa* strains causing scorch diseases in a number of host plants, including *X. fastidiosa* subsp. *fastidiosa* that causes PD in grapevines (Wistron and Purcell, 2005). As with other sharpshooter insects, *H. vitripennis* is a xylophagous insect that feeds on hundreds of plant species (Purcell and Hopkins 1996; Purcell and Saunders 1999); citrus is one of its preferred hosts (Blua *et al.*, 2001). Perring *et al.* (2001) demonstrated a relationship between PD incidence in grapes and the proximity of vineyards to citrus orchards. This leafhopper, which can serve as a vector of *X. fastidiosa*, has the capacity to feed on more than 70 different plant species and can survive winter temperatures as low as -6 °C (Park *et al.*, 2006). Moreover, compared with other *X. fastidiosa*-carrying insects associated with PD that are native to California, GWSS has a longer flight range (up to a quarter mile). These traits make the GWSS a very serious threat to the wine industry of southern and central California (Castle *et al.*, 2005). Indeed, since the first identification of GWSS in California vineyards, programs aimed at controlling the dissemination of this insect to prevent PD outbreaks have involved more than US\$ 160 million of direct investments (<http://www.cdfa.ca.gov/phpps/pdcp/>). Control of any of the GWSS-transmitted diseases of horticultural crops in California by an SC or paratransgenic approach would be of immediate interest to other industries as well. The objective or rationale for developing a method of SC for PD is to disrupt vector transmission with minimal effects on other crops. SC would be available to local vineyards for local control instead of the area-wide treatments of alternative host plants that are currently used. Treatment of citrus with systemic insecticides for GWSS to reduce the chance of acquiring and spreading pathogens in adjacent vineyards cannot be considered a long-term solution. SC would be more selective and have fewer side effects than other biological control practices. The SC organisms inhabit the xylem fluid of the target plants yet do not contaminate the berries of the grapevines. It remains to be determined if one treatment would be effective for an entire season (Miller, 2011).

Three potential bacterial candidates, *Alcaligenes* sp., *Chryseomonas* sp., and *Ralstonia* sp., for SC of PD were collected from GWSS in southern California (Bextine *et al.*, 2004). All were endophytes transmitted to different host plants by GWSS in a manner analogous to the pathogen; thus, the candidates had access to the pathogen in host plants or in the insect vector, providing the needed access. *Alcaligenes denitrificans* var. *xylosoxidans* (Axd) was selected for further development because the endophytic bacterium should have most of the requirements for a successful paratransgenesis strategy such as: a) a population of microbes that is amenable to culture and genetic manipulation *in vitro* must exist within a disease-transmitting vector; b) facile methods for isolating and transforming the endophytic bacteria must be present; c) transformation of the symbiotic/endophytic bacteria must result in stable mutants; d) genetic manipulation of the bacteria should not affect their symbiotic functions in the host vector; e) genetic manipulation of symbiotic bacteria should not render them virulent, either to the target vector or other organisms in the environment. Furthermore, bacteria chosen as gene-delivery vehicles must not be pathogens themselves.

Successful delivery to and colonization of Axd in the foregut regions of GWSS suggest that a paratransgenic approach to manage, prevent, and/or control Pierce's disease is possible (Bextine *et al.*, 2004).

Lacava *et al.* (2007b) used isolation and denaturing gradient gel electrophoresis (DGGE) techniques to identify several genera of bacteria as colonizers of the heads of GWSS collected in orange groves. As identified by 16S rRNA sequencing, these included *Bacillus*, *Cryocolla*, *Microbacterium*, *Micrococcus* and *Pedobacter*. In addition, *Methylobacterium extorquens*, *Curtobacterium flaccumfaciens*, *Baumannia cicadellincola* and various *Pseudomonas* and *Wolbachia* species were found. Of these genera, *Bacillus*, *Pseudomonas*, *Methylobacterium* and *Curtobacterium* were previously described as endophytes that are able to colonize citrus plants. The work of Araújo *et al.* (2002) strongly suggested that there are interactions among *Methylobacterium* spp., *C. flaccumfaciens* and *X. fastidiosa*. These results reinforced the idea that all of these bacteria could interact in the insect vector as well as in the host plant.

Furthermore, Lacava *et al.* (2004) suggested that CVC symptoms in citrus plants could be influenced by the interactions among these three species. In a study of the diversity of bacterial communities associated with GWSS foreguts, they used culture-dependent methods as well as procedures based on sequence polymorphisms (DGGE) of the 16S rRNA gene in total DNA extracted from GWSS foreguts. Lacava *et al.* (2007b) suggested that the diversity profiles obtained with culture-dependent (isolation in culture) techniques indicated a low bacterial diversity. However, the same authors described higher bacterial diversity when using PCR-DGGE, a culture-independent method.

These results from Lacava *et al.* (2007b) showed that PCR-DGGE is suitable for the analysis of bacterial diversity in GWSS heads. In the future, species such as *C. flaccumfaciens* and *Methylobacterium* spp., found as part of the bacterial community in GWSS, could be investigated as potential candidates for use in an SC or SC paratransgenic-based strategy to control the spread of *X. fastidiosa*.

Using methods perfected in previous studies (Lampe *et al.*, 1999, 2000), Axd was genetically altered to contain a DsRed fluorescent marker gene in the chromosome (Bextine *et al.*, 2004) to demonstrate the ability of DsRed Axd to colonize the cibarial region of the GWSS foregut for up to 5 weeks post-exposure. Axd was shown to occupy the same region in the foregut as the pathogen *X. fastidiosa* (Bextine *et al.*, 2004). DsRed Axd was transmitted by GWSS and colonized various plants (Bextine *et al.*, 2004, 2005). DsRed Axd could be introduced into grapevines by misting the leaves, by soil drenching or by direct injection into the stem of the grapevine. Interestingly, Axd appeared to be better adapted to citrus than to grapevine (Bextine *et al.*, 2005). Indeed, the original samples of GWSS from southern California were obtained from citrus groves in the Agricultural Operations plots at the University of California, Riverside; therefore, it is likely that the endophytes in the GWSS originally came from citrus. Bextine *et al.* (2004) describe the successful delivery of Axd to, and the colonization of, the foregut of GWSS. These results suggest that a paratransgenic approach to manage, prevent, and/or control PD by SC may be possible.

A number of candidate antimicrobial peptides were screened against *X. fastidiosa* (Kuzina *et al.*, 2006). In this study, the authors showed that antibiotics and antimicrobial peptides have some activity against *X. fastidiosa* and may have applications in protecting plants from developing PD. The potential use of these antimicrobial peptides in the protection of grapevines will depend on the development of a delivery system, such as SC (Kuzina *et al.*, 2006). Additionally, Lampe *et al.* (1999, 2000) further screened single-chain antibodies from a phage antibody library for the ability to bind the coat protein of *X. fastidiosa*. These authors selected an antibody fragment, designated S1, that was specific to the strain of *X. fastidiosa* causing PD and did not recognize closely related *X. fastidiosa* strains.

Azizi *et al.* (2012) presented a simple robust approach for the generation of panels of recombinant single-chain antibodies against the surface-exposed elements of *X. fastidiosa* (PD) that may have potential use in diagnosis and/or disease transmission blocking studies. *In vitro* combinatorial antibody ribosome display libraries were assembled from immunoglobulin transcripts rescued from spleens of mice immunized with heat-killed *X. fastidiosa*. The libraries were used in a single round of selection against an outer membrane protein, MopB, resulting in the isolation of a panel of recombinant antibodies. The potential use of selected anti-MopB antibodies was demonstrated

by the successful application of the 4XfMopB3 antibody in an enzyme-linked immunosorbent assay (ELISA), a western blot assay, and an immunofluorescence assay (IFA). These immortalized *in vitro* recombinant single-chain antibody libraries generated against heat-killed *X. fastidiosa* are a resource for the PD research community that may be readily accessed for the isolation of antibodies against a plethora of *X. fastidiosa* surface-exposed antigenic molecules.

Recently, Arora *et al.* (2015) reported a novel strategy for the delivery of genetically engineered bacteria in a paratransgenic system that targets the glassy-winged sharpshooter (*Homalodisca vitripennis*), an insect vector of grapes and citrus that transmits the phytopathogen *X. fastidiosa* (Dandekar *et al.*, 2012; Rathe *et al.*, 2014). Using simple and inexpensive materials for bioencapsulation (Weinbreck *et al.*, 2010; Burgain *et al.*, 2011) of the engineered symbiotic bacterium, *Pantoea agglomerans*, they demonstrated targeting of the sharpshooter *H. vitripennis* under controlled conditions with an alginate hydrogel that is tuned to release its bacterial payload during xylem flow into the foregut of the insect. By deploying a microencapsulation system that permits gated delivery of the bacterial payload to the arthropod, while greatly minimizing release in the environment, these authors concluded that robust field-applicable technologies for paratransgenic control of arthropod-borne diseases will be possible. According to these authors, this is the first example of the use of microencapsulation to deliver recombinant bacteria to an insect gut. They demonstrated that transgenic symbiotic bacteria can be delivered to the appropriate physiological niche within a disease-transmitting arthropod. Additionally, this platform may be expanded to deliver recombinant bacteria to other disease-transmitting arthropod vectors, thus facilitating field use of paratransgenic control.

Strategy of symbiotic control for CVC

The key to symbiotic control is finding a candidate microbe with an existing association to the ecosystem that includes the problem or condition under investigation and that occupies the same niche as or has access to the target pathogen (Miller, 2011). Bacteria of the genus *Methylobacterium* are known to occupy the same niche as *X. fastidiosa* inside citrus plants (Araújo *et al.*, 2002; Lacava, *et al.*, 2004). During feeding, insects could acquire not only the pathogen but also endophytes from host plants. Gai *et al.* (2009) and Gai *et al.* (2011) reported the localization of the endophytic bacterium *M. mesophilicum* in the *C. roseus* model plant system and the transmission of this endophyte by *Bucephalagonia xanthophis*, a sharpshooter insect vector of *X. fastidiosa*.

Methylobacterium mesophilicum, originally isolated as an endophytic bacterium from citrus plants (Araújo *et al.*, 2002), was genetically transformed to express GFP (Gai *et al.*, 2009). The GFP-labeled strain of *M.*

mesophilicum was inoculated into *C. roseus* (model plant) seedlings and was observed colonizing its xylem vessels. The transmission of *M. mesophilicum* by *B. xanthophis* was verified with insects feeding on fluids containing the GFP-labeled bacterium. Forty-five days after inoculation, the plants exhibited endophytic colonization by *M. mesophilicum*, confirming this bacterium as a nonpathogenic, xylem-associated endophyte (Gai *et al.*, 2009). These data demonstrate that *M. mesophilicum* not only occupies the same niche as *X. fastidiosa* inside plants but also may be transmitted by *B. xanthophis*. The transmission, colonization and genetic manipulation of *M. mesophilicum* are prerequisites for it to potentially be used for paratransgenic SC to interrupt transmission of *X. fastidiosa* by insect vectors. We propose *M. mesophilicum* as a candidate for a paratransgenic SC strategy to reduce the spread of *X. fastidiosa*. It is known that *X. fastidiosa* produces a fastidial gum (da Silva *et al.*, 2001), which may be responsible for the obstruction of xylem in affected plants (Lambais *et al.*, 2000); therefore, the production of endoglucanase by genetically modified endophytic bacteria may transform the endophytes into symbiotic control agents for CVC (Ferreira Filho *et al.*, 2012). Azevedo and Araújo (2003) have used the replicative vector pEGLA160 to produce genetically modified *Methylobacterium* expressing antibiotic resistance and endoglucanase genes. Furthermore, other strategies can be evaluated, such as the production of genetically modified *Methylobacterium* to secrete soluble anti-*Xylella* proteins in citrus. Lampe *et al.* (2006) suggested a similar strategy with the *Escherichia coli* α -hemolysin system for use in Axd to secrete soluble anti-*Xylella* protein effectors in grapevines and GWSS. Additionally, Lampe *et al.* (2007) suggested the evaluation of proteins secreted from the grapevine bacterial symbiont *P. agglomerans* for use as secretion partners of anti-*Xylella* protein effectors. One strategy that can be adopted as the next step for SC control of CVC is producing a genetically modified endophytic bacterium, such as *Methylobacterium*, to secrete anti-*Xylella* protein effectors.

Another strategy to control *X. fastidiosa* is to degrade the EPS (exopolysaccharides) formed by this plant pathogen that are directly related to biofilm formation. In *X. fastidiosa*, the fastidial gum may be directly linked to pathogenicity (da Silva *et al.*, 2001) because it may be involved in the biofilm formation required for the attachment and survival of this bacterium in xylem vessels and the sucking pumps of the insect vectors. A lack of EPS would therefore prevent the plant symptoms caused by vessel occlusion (and/or embolism) and the spread of the disease as well (da Silva *et al.*, 2001). Based on the premise of symbiotic control, Ferreira Filho *et al.* (2012) genetically modified the citrus endophytic bacterium *Methylobacterium extorquens*, strain AR1.6/2, and evaluated its capacity to colonize a model plant and its interaction with *X. fastidiosa*. The strain AR1.6/2 was genetically transformed to express

heterologous GFP and endoglucanase A (EglA), generating the strains ARGFP and AREglA, respectively. Using fluorescence microscopy, it was shown that ARGFP was able to colonize the xylem vessels of the *C. roseus* seedlings. Using scanning electron microscopy, it was observed that AREglA and *X. fastidiosa* may co-inhabit the *C. roseus* vessels. *M. extorquens* was observed in the xylem with the phytopathogen *X. fastidiosa* and appeared to cause a decrease in biofilm formation. AREglA stimulated the production of the resistance protein catalase in the inoculated plants. These results demonstrate the successful transformation of AR1.6/2 to generate two different strains with a different gene and also indicate that AREglA and *X. fastidiosa* could interact inside the host plant, suggesting a possible strategy for the symbiotic control of CVC disease.

According to Ferreira Filho *et al.* (2012), the endophytic AR1.6/2 expressing the *EglA* or *gfp* genes showed most of the prerequisites listed by Durvasula *et al.* (2003) and Miller (2011) for a successful strategy of symbiotic control. For example, the AR1.6/2 strain that colonized citrus plants is amenable to isolation, culturing and transformation with foreign genes, and the heterologous expression of GFP and EglA by AR1.6/2 did not affect its growth and survival inside the host.

Conclusions and Future Perspectives

Our strategy is similar to that developed by Bextine *et al.* (2004) for a paratransgenic strategy for SC of PD in grapevines. Bextine *et al.* (2004) suggested that the genus *Alcaligenes*, an endophytic bacterium that can colonize the GWSS vector of *X. fastidiosa*, would be a candidate for paratransgenic SC of PD in the USA. We believe that the endophytic bacterium *M. mesophilicum* from citrus plants is likewise a candidate for paratransgenic SC of CVC. Our results indicate that this endophyte colonizes the same niche as *X. fastidiosa* in citrus plants (Araújo *et al.*, 2002; Lacava *et al.*, 2004, 2006a; Andreote *et al.*, 2006). *M. mesophilicum* is also transmitted by an insect vector of *X. fastidiosa* (Gai *et al.*, 2009).

Bacteria chosen as gene delivery vehicles for paratransgenesis SC must not be pathogens themselves. *M. mesophilicum* is not a pathogen, and several requirements for a successful paratransgenesis SC strategy as described by Durvasula *et al.* (2003) and Miller (2011) have been demonstrated: a) *M. mesophilicum* is amenable to culture and genetic manipulation *in vitro*; b) facile methods for isolating and transforming the endophytic bacteria have been developed; c) transformation of the symbiotic/endophytic bacteria has resulted in mutants that were stable *in planta*. Future genetic manipulation of *M. mesophilicum* to achieve paratransgenic SC should not affect its symbiotic functions in the plant host and insect vector, and genetic manipulation of symbiotic bacteria should not render them virulent, either to the host plant or target.

C. flaccumfaciens is also a candidate for biological control of CVC. Interaction and antagonism between *C. flaccumfaciens* and *X. fastidiosa* was strongly indicated on the basis of the frequency of *C. flaccumfaciens* isolation from sweet orange (Araújo *et al.*, 2002). In addition, *in vitro* interactions between *X. fastidiosa* and *C. flaccumfaciens* have been described, including the inhibition of *X. fastidiosa* growth by cell-free supernatants of nutrient medium in which *C. flaccumfaciens* had been grown (Lacava *et al.*, 2004). Additionally, Lacava *et al.* (2007a) demonstrated that *C. flaccumfaciens* interacted with *X. fastidiosa* in *C. roseus* and reduced the severity of the disease symptoms induced by *X. fastidiosa* in this model plant to study the interaction of this plant pathogen and endophytic bacteria (Monteiro *et al.*, 2001; Lacava *et al.*, 2006a). The ability of *C. flaccumfaciens* to colonize plant tissues in the presence of *X. fastidiosa* and the reduction of disease symptoms caused by *X. fastidiosa* (Lacava *et al.*, 2007a) are prerequisites for the use of this endophytic bacterium as a biocontrol agent. Because members of the genus *Curtobacterium* were consistently detected in the insect vectors of *X. fastidiosa* (Gai *et al.*, 2011), they fulfill another requirement of candidates for biological control of *X. fastidiosa* (Lacava *et al.*, 2007a), i.e., they can colonize both the insect vectors of *X. fastidiosa* and citrus plants. In the case of biocontrol of *X. fastidiosa* and CVC disease, it would be desirable if *C. flaccumfaciens* could be transmitted by budwood, but this has yet to be demonstrated. The reduction of disease symptoms caused by *X. fastidiosa* in the presence of *C. flaccumfaciens* may be attributable to direct killing of *X. fastidiosa* by *C. flaccumfaciens*. Consistent with this hypothesis, three bacteriocins showing activity against *X. fastidiosa* have been described in *C. flaccumfaciens* (Cursino, 2005, PhD thesis, University of São Paulo, Piracicaba, São Paulo, Brazil).

We propose two complementary strategies for control of CVC using endophytic bacteria from citrus plants. We suggest the endophytic bacterium *C. flaccumfaciens* as a classical biological control agent and the endophytic bacterium *M. mesophilicum* as a qualified candidate for a paratransgenic SC strategy. The details of these strategies are summarized in Figure 1.

In addition to paratransgenic processes, the balance among endophytic microorganisms and *X. fastidiosa* is very important in the control of CVC. Approximately 15 years ago, a study conducted by the Microbial Genetics group in the Department of Genetics at Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ/USP), Brazil (Araujo *et al.*, 2001, 2002) showed that in the same citrus plantations, *X. fastidiosa* is found in both symptomatic (showing disease symptoms) and asymptomatic plants (healthy plants). Although no genetic differences were found in these plants, distinctions were found in the composition of their endophytic bacteria. Bacteria of the genera *Curtobacterium* and *Methylobacterium*

Strategies of control of CVC using endophytes

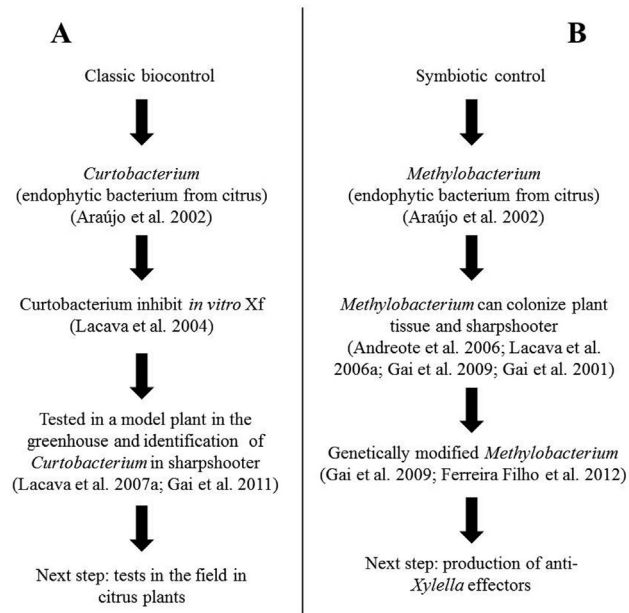


Figure 1 - Hypotheses and strategies to control citrus variegated chlorosis (CVC) using endophytic bacteria from citrus plants. (A) We suggest the endophytic bacterium *Curtobacterium flaccumfaciens* as a classical biological control agent. *C. flaccumfaciens* has the ability to colonize plant tissues in the presence or absence of *Xylella fastidiosa* (Xf). This is a prerequisite for the use of this bacterium as a biocontrol agent. The data indicate that *C. flaccumfaciens* interacted with *X. fastidiosa* in *Catharanthus roseus* and reduced the severity of the disease symptoms induced by *X. fastidiosa* (Araújo *et al.*, 2002; Lacava *et al.*, 2004; ; Lacava *et al.*, 2007a; Gai *et al.*, 2011). (B) Additionally, the endophytic bacterium *Methylobacterium* has been suggested as a qualified candidate for a paratransgenic symbiotic control (SC) strategy because there have been reports on the transmission, colonization and genetic manipulation of *Methylobacterium*, which are prerequisites for the potential use of this bacteria to interrupt transmission of *X. fastidiosa*, the bacterial pathogen causing CVC, by insect vectors (Araújo *et al.*, 2002; Andreote *et al.*, 2006; Lacava *et al.*, 2006a; Gai *et al.* 2009, 2011; Ferreira Filho *et al.*, 2012).

distinguished the endophytic communities of healthy and diseased plants, respectively. There may be many causes for this endophytic imbalance, including the use of agricultural chemical products, intensive cultures, distinct agricultural management and other abiotic and biotic factors (Laranjeira *et al.*, 2005). It appears that the balance among endophytes, which has been maintained for thousands of years by co-evolution, can be altered, and some endophytic bacteria, such as *X. fastidiosa*, may become pathogenic due to this endophytic imbalance (Figure 2). Similar cases have been found; *Vitis vinifera* plants also display differences in disease severity and symptoms, and this was shown to be due to the presence of endophytic fungi, such as *Cochliobolus* sp., which inhibited *X. fastidiosa* by producing the antimicrobial radicinin. The absence of these fungi may result in the emergence of *X. fastidiosa* as a pathogen (Aldrich *et al.*, 2015). Additionally, studies (Dourado *et al.*, 2013, 2015; Lacava *et al.*, 2004, 2006a) have shown that the development of CVC symptoms in citrus plants could be a result of an unbalanced endophytic population, including *Methylobacterium* spp. and *X. fastidiosa*. Therefore, the understanding of this interaction will allow the development of potential strategies to prevent CVC and other diseases caused not only by *X. fastidiosa* but also by other phytopathogens and physiological shifts, which may be due to the disequilibrium in the microbial community that is induced by agricultural management during crop production.

Acknowledgments

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support (grants: 98/16262-2; 02/08786-9; 02/14143-3; 06/55494-4; 12/24217-6) and fellowships (process No.

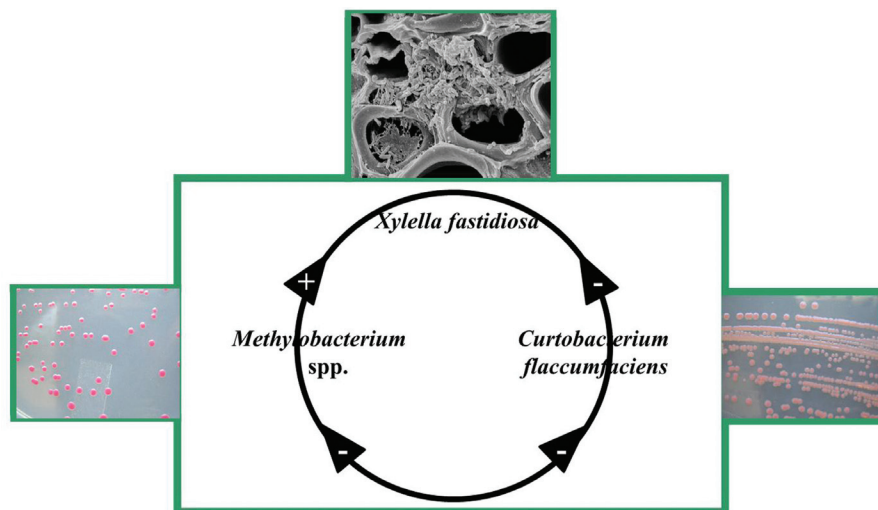


Figure 2 - Balanced interactions among endophytic bacteria from *Citrus sinensis* and *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis. Photos of endophytic strains of *Methylobacterium* and *Curtobacterium* grown in Petri dishes by the authors. Photo of scanning electron micrograph of the bacterium *X. fastidiosa* by E. W. Kitajima, ESALQ/USP/Brazil (<http://aeg.lbi.ic.unicamp.br/xf/>).

00/08498-8; process No. 00/10699-1; process No 00/10721-7; process No. 00/14987-1).

References

- Ahmed FE (2003) Genetically modified probiotics in foods. *Trends Biotechnol* 21:491-497.
- Aldrich TJ, Rolshausen PE and Roper MC (2015) Radicinin from *Cochliobolus* sp. inhibits *Xylella fastidiosa* the causal agent of Pierce's disease in grapevine. *Phytochemistry* 116:130-137.
- Almeida RPP and Purcell AH (2003) Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). *J Econ Entomol* 96:264-271.
- Almeida RPP and Nunney L (2015) How do plant diseases caused by *Xylella fastidiosa* emerge. *Plant Dis* 99:1457-1467.
- Andreote FD, Lacava PT, Gai CS, Araújo WL, Maccheroni Jr W, Van Overbeek LS, Van Elsas JD and Azevedo JL (2006) Model plants for studying the interaction between *Methylobacterium mesophilicum* and *Xylella fastidiosa*. *Can J Microbiol* 52:419-426.
- Anesti V, Vohra J, Goonetilleka S, McDonald IR, Sträubler B, Stackebrandt E, Kelly DP and Wood AP (2004) Molecular detection and isolation of facultatively methylotrophic bacteria, including *Methylobacterium podarium* sp. nov., from the human foot microflora. *Environ. Microbiol* 6:820-830.
- Araújo WL, Maccheroni Jr W, Aguilar-Vildoso CI, Barroso PAV, Saridakis HO and Azevedo JL (2001) Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. *Can J Microbiol* 47:229-236.
- Araújo WL, Marcon J, Maccheroni Jr W, Van Elsas JD and Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. *Appl Environ Microbiol* 68:4906-4914.
- Araújo WL, Santos DS, Dini-Andreote F, Salgueiro-Londoño JK, Camargo-Neves AA, Andreote FD and Dourado MN (2015) Genes related to antioxidant metabolism are involved in *Methylobacterium mesophilicum*-soybean interaction. *Antonie van Leeuwenhoek*. 108:951-963.
- Arora AK, Forshaw A, Miller TA and Durvasula R (2015) A delivery system for field application of paratransgenic control. *BMC Biotechnol* 15:e59.
- Ayres AJ, Gimenes-Fernandes N and Barbosa JC (2001) Intensity of the citrus variegated chlorosis in the state of São Paulo and south of Triângulo Mineiro. *Summa Phytopathol* 27:189-197.
- Azevedo JL and Araújo WL (2003) Genetically modified crops: Environmental and human health concerns. *Mutat Res* 544:223-233.
- Azevedo JL and Araújo WL (2007) Diversity and applications of endophytic fungi isolated from tropical plants. In: Ganguli BN and Deshmukh SK (eds) *Fungi: Multifaceted Microbes*. Anamaya Publishers, New Delhi, pp 189-207.
- Azevedo JL, Maccheroni Jr W, Pereira JO and Araújo WL (2000) Endophytic microorganisms: A review on insect control and recent advances on tropical plants. *Electron J Biotechnol* 3:40-65.
- Azizi A, Arora A, Markiv A, Lampe DJ, Miller TA and Kanga AS (2012) Ribosome display of combinatorial antibody libraries derived from mice immunized with heat-killed *Xylella fastidiosa* and the selection of MopB-specific single-chain antibodies. *Appl Environ Microbiol* 78:2638-2647.
- Bao XD and Roossinck MC (2011) Multiplexed interesting virus of endophytic fungi. *Adv Virus Res* 80:32-58.
- Beard CB, Durvasula RV and Richards FF (1998) Bacterial symbiosis in arthropods and the control of disease transmission. *Emerg Infect Dis* 4:581-591.
- Beard CB, Dotson EM, Pennington PM, Eichler S, Cordon-Rosales C and Durvasula RV (2001) Bacterial symbiosis and paratransgenic control of vector borne Chagas disease. *Int J Parasitol* 31:621-627.
- Beard C, Cordon-Rosales C and Durvasula R (2002) Bacterial symbionts of the triatominae and their potential use in control of Chagas Disease transmission. *Annu Rev Entomol* 47:123-141.
- Bent E and Chanway CP (1998) The growth-promoting effects of a bacterial endophyte on lodgepole pine are partially inhibited by the presence of other rhizobacteria. *Can J Microbiol* 44:980-988.
- Bextine B, Lauzon C, Potter S, Lampe D and Miller TA (2004) Delivery of a genetically marked *Alcaligenes* sp. to the glassy-winged sharpshooter for use in a paratransgenic control strategy. *Curr Microbiol* 48:327-331.
- Bextine B, Lampe D, Lauzon C, Jackson B and Miller TA (2005) Establishment of a genetically marked insect-derived symbiont in multiple host plants. *Curr Microbiol* 50:1-7.
- Bezerra JDP, Santos MGS, Barbosa RN and Svedese VM (2013) Fungal endophytes from cactus *Cereus jamacaru* in Brazilian tropical dry forest: A first study. *Symbiosis* 60:53-63.
- Bhattacharyya A, Stilwagen S, Ivanova N, D'Souza M, Bernal A, Lykidis A, Kapatral V, Anderson I, Larsen N, Los T, *et al.* (2002) Whole-genome comparative analysis of three phytopathogenic *Xylella fastidiosa* strains. *Proc Natl Acad Sci U S A* 99:12403-12408.
- Blua MJ, Redak RA, Morgan DJW and Costa HS (2001) Seasonal flight activity of two *Homalodisca* species (Homoptera: Cicadellidae) that spread *Xylella fastidiosa* in southern California. *J Econ Entomol* 94:1506-1510.
- Bourtzis K, Crook S, Daffonchio D, Durvasula R, Hanboonsong Y, Infante F, Lacava PT, Miller TA and Vega FE (2012) *International entomology*. *Am Entomol* 58:234-246.
- Bové JM and Ayres AJ (2007) Etiology of three recent diseases of citrus in São Paulo State: Sudden death, variegated chlorosis and huanglongbing. *IUBMB Life* 59:346-354.
- Bradbury JF (1991) *CMI Descriptions of Pathogenic Fungi and Bacteria*. No. 105. CAB International, Wallingford, 1049 p.
- Burgain J, Gaiani C, Linder M and Scher J (2011) Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *J Food Eng* 104:467-483.
- Carvalho SA (2003) *Regulamentação atual da Agência de Defesa Agropecuária para a produção, estocagem, comércio, transporte e plantio de mudas cítricas no Estado de São Paulo*. *Laranja* 24:199-239.
- Castle SJ, Byrne FJ, Bi JL and Toscano NC (2005) Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* Wells populations. *Pest Manag Sci* 61:75-84.
- Chang CJ, Garnier M, Zreik L, Rossetti V and Bove JM (1993) Culture and serological detection of the xylem-limited bacterium causing citrus variegated chlorosis and its identifica-

- tion as a strain of *Xylella fastidiosa*. *Curr Microbiol* 27:137-142.
- Chanway CP (1997) Inoculation of tree roots with plant growth promoting soil bacteria: An emerging technology for reforestation. *Forest Sci* 43:99-112.
- Chebotar NK, Malfanova MV, Shcherbakov AV, Ahtemakova GK, Luemberg B and Tikhenovich IA (2015) Endophytic bacteria in microbial preparations that improve plant development. *Appl Biochem Micro* 51:271-277.
- Chen J and Civerali EL (2008) Morphological evidence for phages in *Xylella fastidiosa*. *Virology* 375:75-81.
- Chen J, Jarret RL, Qin X, Hartung JS, Chang CJ and Hopkins DL (2000) 16SrDNA analysis of *Xylella fastidiosa*. *Syst Appl Microbiol* 23:349-354.
- Collins MD and Jones D (1983) Reclassification of *Corynebacterium flaccumfaciens*, *Corynebacterium betae*, *Corynebacterium oortii* and *Corynebacterium poinsettiae* in the genus *Curtobacterium*, as *Curtobacterium flaccumfaciens* comb. nov. *J Gen Microbiol* 129:3545-3548.
- da Silva FR, Vettore AL, Kemper EL, Leite A and Arruda P (2001) Fastidiosis gum: The *Xylella fastidiosa* exopolysaccharide possibly involved in bacterial pathogenicity. *FEMS Microbiol Lett* 203:165-171.
- Dandekar AM, Gouran H, Ibáñez AM, Uratsu SL, Agüero CB, McFarland S, Borhani Y, Feldstein PA, Bruening G, Nascimento R, *et al.* (2012) An engineered innate immune defense protects grapevines from Pierce's disease. *Proc Natl Acad Sci U S A* 109:3721-3725.
- Davis MJ, French WJ and Schaad NW (1981) Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. *Curr Microbiol* 5:311-331.
- de Lima JEO, Miranda VS, Hartung JS, Brlansky RH, Coutinho A, Roberto SR and Carlos EF (1998) Coffee leaf scorch bacterium: Axenic culture, pathogenicity, and comparison with *Xylella fastidiosa* of citrus. *Plant Dis* 82:94-97.
- Derrick KS and Timmer LW (2000) Citrus blight and other diseases of recalcitrant etiology. *Annu Rev Phytopathol* 38:181-205.
- Doronina NV, Trotsenko YA, Tourova TP, Kuznetsov BB and Leisinger T (2000) *Methylopila helvetica* sp. nov. and *Methylobacterium dichloromethanicum* sp. nov. - novel aerobic facultatively methylotrophic bacteria utilizing dichloromethane. *Syst Appl Microbiol* 23:210-218.
- Doronina NV, Trotsenko YA, Kuznetsov BB, Tourova TP and Salkinoja-Salonen MS (2002) *Methylobacterium suomiense* sp. nov. and *Methylobacterium lusitanum* sp. nov., aerobic, pink pigmented, facultatively methylotrophic bacteria. *Int J Syst Evol Microbiol* 52:773-776.
- Dourado MN, Bogas AC, Pomini AM, Andreote FD, Quecine MC, Marsaioli AJ and Araújo WL (2013) *Methylobacterium*-plant interaction genes regulated by plant exudate and quorum sensing molecules. *Braz J Microbiol* 44:1331-1339.
- Dourado MN, Santos DS, Nunes RL, Costa de Oliveira RLB, Oliveira MV and Araújo WL (2015) Differential gene expression in *Xylella fastidiosa* 9a5c during co-cultivation with the endophytic bacterium *Methylobacterium mesophilicum* SR1.6/6. *J Basic Microbiol* 55:1357-1366.
- Duijff BJ, Gianinazzi-Pearson V and Lemanceau P (1997) Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. *New Phytol* 135:325-344.
- Durvasula RV, Gumbs A, Panackal A, Kruglov O, Aksoy S, Merrifield RB, Richards FF and Beard CB (1997) Prevention of insect-borne disease: An approach using transgenic symbiotic bacteria. *Proc Natl Acad Sci U S A* 94:3274-3278.
- Durvasula RV, Panackal A, Taneja J, Gumbs A, Kruglov O, Richards F and Beard CB (1999) A strategy for spreading anti-trypanosomal genes in populations of the Chagas disease vector, *Rhodnius prolixus*. *Ann Entomol Soc Am* 92:937-943.
- Durvasula RV, Sundaram RK, Cordon-Rosales C, Pennington P and Beard BC (2003) *Rhodnius prolixus* and its symbiont, *Rhodococcus rhodnii*: A model for paratransgenic control of disease transmission. In: Bourtzis K and Miller TA (eds) *Insect Symbiosis*. CRC, Boca Raton, pp 83-95.
- Ferreira Filho AS, Quecine MC, Bogas AC, Rossetto PB, Lima AOS, Lacava PT, Azevedo JL and Araújo WL (2012) Endophytic *Methylobacterium extorquens* expresses a heterologous β -1,4-endoglucanase A (EglA) in *Catharanthus roseus* seedlings, a model host plant for *Xylella fastidiosa*. *World J Microbiol Biotechnol* 28:1475-1481.
- Freitag JH (1951) Host range of the Pierce's disease virus of grapes as determined by insect transmission. *Phytopathology* 41:920-934.
- Freitag JH and Frazier NW (1954) Natural infectivity of leafhopper vectors of Pierce's disease virus of grape in California. *Phytopathology* 44:7-11.
- Gai CS, Lacava PT, Quecine MC, Auriac MC, Lopes JRS, Araújo WL, Miller TA and Azevedo JL (2009) Transmission of *Methylobacterium mesophilicum* by *Bucephalagonia xanthophis* for paratransgenic control strategy of citrus variegated chlorosis. *J Microbiol* 47:448-454.
- Gai CS, Dini-Andreote F, Andreote FD, Lopes JRS, Araújo WL, Miller TA, Azevedo JL and Lacava PT (2011) Endophytic bacteria associated to sharpshooters (Hemiptera: Cicadellidae), insect vectors of *Xylella fastidiosa* subsp. *pauca*. *J Plant Pathol Microbiol* 2:102-109.
- Gallego V, Garcia MT and Ventosa A (2005a) *Methylobacterium hispanicum* sp. nov. and *Methylobacterium aquaticum* sp. nov., isolated from drinking water. *Int J Syst Evol Microbiol* 55:281-287.
- Gallego V, Garcia MT and Ventosa A (2005b) *Methylobacterium variabile* sp. nov., a methylotrophic bacterium isolated from an aquatic environment. *Int J Syst Evol Microbiol* 55:1429-1433.
- Gallego V, Garcia MT and Ventosa A (2005c) *Methylobacterium isbiliense* sp. nov., isolated from the drinking water system of Sevilla, Spain. *Int J Syst Evol Microbiol* 55:2333-2337.
- Gallego V, Garcia MT and Ventosa A (2006) *Methylobacterium adhaesivum* sp. nov., a methylotrophic bacterium isolated from drinking water. *Int J Syst Evol Microbiol* 56:339-342.
- Goheen AC, Raju BC, Lowe SK and Nyland G (1979) Pierce's disease of grapevines in Central America. *Plant Dis Rep* 63:788-792.
- Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H and Rai M (2015) Endophytic actinobacteria of medicinal plants: Diversity and bioactivity. *Antonie van Leeuwenhoek* 108:267-289.
- Gonçalves FP, Stuchi ES, Lourenço SA, Kriss AB, Gottwald TR and Amorim L (2014) The effect of irrigation on develop-

- ment of citrus variegated chlorosis symptoms. *Crop Prot* 57:8-14.
- Goodwin P and Purcell AH (1992) Pierce's Disease. In: *Grape Pest Management*. 2nd edition. University of California, Oakland, pp 76-84.
- Green PN (1992) The genus *Methylobacterium*. In: Balows A, Trüper HG, Dworkin M, Harder W and Schleifer KH (eds) *The Prokaryotes*. 2nd edition. Springer, New York, pp 2342-2349.
- Guilhabert MP and Kirkpatrick BC (2003) Transformation of *Xylella fastidiosa* with broad host range RSF 1010 derivative plasmid. *Mol Plant Pathol* 4:273-285.
- Hallmann J, Quadt-Hallmann A, Mahaffee WF and Kloepfer JW (1997) Bacterial endophytes in agricultural crops. *Can J Microbiol* 43:895-914.
- Hallmann J, Quadt-Hallmann A, Rodríguez-Kábana R and Kloepfer JW (1998) Interactions between *Meloidogyne incognita* and endophytic bacteria in cotton and cucumber. *Soil Biol Biochem* 30:925-937.
- Hardoin PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M and Sessitsch A (2015) The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev* 79:293-320.
- Hartung JS, Beretta J, Brlansky RH, Spisso J and Lee RF (1994) Citrus variegated chlorosis bacterium: Axenic culture, pathogenicity, and serological relationships with other strains of *Xylella fastidiosa*. *Phytopathology* 84:591-597.
- Hearon SS, Sherald JL and Kostka SJ (1980) Association of xylem-limited bacteria with elm, sycamore, and oak leaf scorch. *Can J Bot* 58:1986-1993.
- Holt JG (1994) *Xylella*. *Bergey's Manual of Determinative Bacteriology*. 9th edition. Williams and Wilkins, London, pp 100-115.
- Hopkins DL (1989) *Xylella fastidiosa*: Xylem-limited bacterial pathogen of plants. *Annu Rev Phytopathol* 27:271-290.
- Hopkins DL and Purcell AH (2002) *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. *Plant Dis* 86:1056-1066.
- Hurwitz I, Fieck A, Read A, Hillesland H, Klein N, Kang A and Durvasula R (2011) Paratransgenic control of vector borne diseases. *Int J Bot Sci* 7:1334-1344.
- Jourand P, Giraud E, Bena G, Sy A, Willems A, Gillis M, Dreyfus B and de Lajudie P (2004) *Methylobacterium nodulans* sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen fixing bacteria. *Int J Syst Evol Microbiol* 54:2269-2273.
- Kavino M, Harish S, Kumar N, Saravanakumar D, Damodaran T, Soorianathasundaram K and Samiyappan R (2007) Rhizosphere and endophytic bacteria for induction of systemic resistance of banana plantlets against bunchy top virus. *Soil Biol Biochem* 39:1087-1098.
- Kirkpatrick B and Wilhelm M (2007) Evaluation of grapevine endophytic bacteria for control of Pierce's disease. In: *Proceedings Pierce's Disease Research Symposium*, San Diego, pp 203-206.
- Kostka SJ, Tattar TA, Sherald JL and Hurtt SS (1986) Mulberry leaf scorch, new disease caused by a fastidious xylem-inhabiting bacterium. *Plant Dis* 70:690-693.
- Kozdrój J, Trevors JT and Van Elsland JD (2004) Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. *Soil Biol Biochem* 36:1775-1784.
- Krügner R, Lopes MTV, Santos JS, Beretta MJG and Lopes JRS (2000) Transmission efficiency of *Xylella fastidiosa* to citrus by sharpshooters and identification of two new vector species. In: *Proceedings 14th Conference of International Organization of Citrus Virologists*, Campinas, p 423.
- Kung SH and Almeida RPP (2011) Natural competence and recombination in plant pathogenic *Xylella fastidiosa*. *Appl Environ Microbiol* 77:5278-5284.
- Kuzina LV, Miller TA and Cooksey DA (2006) *In vitro* activities of antibiotics and antimicrobial peptides against the plant pathogenic bacterium *Xylella fastidiosa*. *Lett Appl Microbiol* 42:514-520.
- Lacava PT and Azevedo JL (2013) Endophytic bacteria: A biotechnological potential in agrobiological system. In: Maheshwari DK, Sarah M and Aeron A (eds) *Bacteria in Agrobiological: Crop Productivity*. Springer-Verlag, Berlin, pp 1-44.
- Lacava PT and Azevedo JL (2014) Biological control of insect-pest and diseases by endophytes. In: Verma VC and Gange AC (eds) *Advances in Endophytic Research*. Springer-Verlag, New Delhi, pp 231-243.
- Lacava PT, Araújo WL, Marcon J, Maccheroni Jr W and Azevedo JL (2004) Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacterium *Xylella fastidiosa*, causal agent of citrus variegated chlorosis. *Lett Appl Microbiol* 39:55-59.
- Lacava PT, Li WB, Araújo WL and Hartung JS (2006a) Rapid, specific and quantitative assays for the detection of the endophytic bacterium *Methylobacterium mesophilicum* in plants. *J Microbiol Methods* 65:535-541.
- Lacava PT, Andreote FD, Araújo WL and Azevedo JL (2006b) Caracterização da comunidade bacteriana endofítica de citros por isolamento, PCR específico e DGGE. *Pesq Agropec Bras* 41:637-642.
- Lacava PT, Li W, Araújo WL, Azevedo JL and Hartung JS (2007a) The endophyte *Curtobacterium flaccumfaciens* reduces symptoms caused by *Xylella fastidiosa* in *Catharanthus roseus*. *J Microbiol* 45:388-393.
- Lacava PT, Parker J, Andreote FD, Dini-Andreote F, Ramirez JL and Miller TA (2007b) Analysis of the bacterial community in glassy-winged sharpshooter heads. *Entomol Res* 37:261-266.
- Lambais MR, Goldman MHS, Camargo LEA and Goldman GH (2000) A genomic approach to the understanding of *Xylella fastidiosa* pathogenicity. *Curr Opin Microbiol* 3:459-462.
- Lampe DJ, Ackerley BJ, Rubin EJ, Mekalanos JJ and Robertson HM (1999) Hyperactive transposase mutants of the Himar1 mariner transposon. *Proc Natl Acad Sci U S A* 96:11428-11433.
- Lampe DJ, Walden KKO, Sherwood JM and Robertson HM (2000) Genetic engineering of insects with mariner transposons. In: Handler A and James T (eds) *Insect Transgenesis: Methods and Applications*. CRC Press, Boca Raton, pp 237-248.
- Lampe D, Lauzon C and Miller TA (2006) Use of the *E. coli* α -hemolysin secretion system in bacteria designed for symbiotic control of Pierce's disease in grapevines and sharpshooters. In: *Proceedings Pierce's Disease Research Symposium*, San Diego, pp 240-241.

- Lampe D, Kang A and Miller TA (2007) Native secretion systems for the grapevine endophyte *Pantoea agglomerans* useful for the delivery of anti-Xylella effector proteins. In: Proceedings Pierce's Disease Research Symposium, San Diego, pp 218-220.
- Laranjeira FF, Pompeu Junior J, Harakava R, Figueiredo JO, Carvalho AS and Coletta-Filho HD (1998) Cultivares e espécies cítricas hospedeiras de *Xylella fastidiosa* em condição de campo. Fitopatol Bras 23:147-154.
- Laranjeira FF, Amorim L, Bergamim-Filho A, Aguilar-Vildoso CI and Coletta-Filho HD (2005) Fungos, procariotos e doenças abióticas. In: Mattos Jr D, Negri JD, Pio RM and Pompeu Jr J (eds) Citrus. Instituto Agronômico, Campinas, pp 532-538.
- Lazarovits G and Nowak J (1997) Rhizobacteria for improvement of plant growth and establishment. HortScience 32:188-192.
- Lee RF, Kerrick KS, Beretta MJG, Chagas CM and Rossetti V (1991) Citrus variegated chlorosis: A new destructive disease of citrus in Brazil. Citrus Industry 72:12-13.
- Leu LS and Su CC (1993) Isolation, cultivation and pathogenicity of *Xylella fastidiosa*, the causal bacterium of pear leaf scorch disease in Taiwan. Plant Dis 77:642-646.
- Li WB, Donadio LC, Beretta MJG, Rosetti V and Sempionato OR (1997a) Practical methods of resistance evaluation of citrus varieties to citrus variegated chlorosis. Proc Int Soc Citricult 1:276-279.
- Li WB, Donadio LC, Beretta MJG, Rossetti V, Sempionato OR, Lemos EGM and Miranda V (1997b) Resistance or tolerance of citrus species and varieties to citrus variegated chlorosis. Proc Int Soc Citricult 1:283-285.
- Li WB, Donadio LC, Sempionato OR, Stuchi ES, Rossetti V and Beretta MJG (1997c) Effect of 20 rootstocks on the severity of citrus variegated chlorosis (CVC) of sweet orange 'Pera' (*C sinensis* L. Osbeck). Proc Int Soc Citricult 1:286-289.
- Li WB, Pria Jr WD, Lacava PM, Qin X and Hartung JS (2003) Presence of *Xylella fastidiosa* in sweet orange fruit and seeds and its transmission to seedlings. Phytopathology 93:953-958.
- Lopes JRS, Beretta MJG, Harakava R, Almeida RPP, Krüger R and Garcia Júnior A (1996) Confirmação da transmissão por cigarrinhas do agente causal da clorose variegada dos citros, *Xylella fastidiosa*. Fitopatol Bras 21:343.
- Lopes SA, Marcussi S, Torres SCZ, Souza V, Fagan C, França SC, Fernandes NG and Lopes JRS (2003) Weeds as alternative hosts of the citrus, coffee, and plum strains of *Xylella fastidiosa* in Brazil. Plant Dis 87:544-549.
- Machado EC, Oliveira RF, Ribeiro RV, Medina CL, Stuchi ES, Marin FR, Silva JAB and Silva SR (2006) Fluxo de seiva e fotossíntese em laranjeira 'Natal' com clorose variegada dos citros. Pesq Agropec Bras 41:911-918.
- Marques MV, Silva AM and Gomes SI (2001) Genetic organization of plasmid pXF51 from the plant pathogen *Xylella fastidiosa*. Plasmid 45:184-199.
- McCoy RE, Thomas DL, Tsai JH and French WJ (1978) Periwinkle wilt, a new disease associated with xylem delimited rickettsialike bacteria transmitted by a sharpshooter. Plant Dis Rep 62:1022-1026.
- McDonald IR, Doronina NV, Trotsenko YA, McAnulla C and Murrell JC (2001) *Hyphomicrobium chloromethanicum* sp. nov. and *Methylobacterium chloromethanicum* sp. nov., chloromethaneutilizing bacteria isolated from a polluted environment. Int J Syst Evol Micr 51:119-122.
- Mendes R and Azevedo JL (2008) Valor biotecnológico de fungos endofíticos isolados de plantas de interesse econômico. In: Maia LC, Malosso E and Yano-Melo AM (eds) Micologia: Avanços no Conhecimento. Sociedade Brasileira de Micologia, Recife, pp 129-140.
- Miller TA (2007) Symbiotic control in agriculture and medicine. Symbiosis 42:67-74.
- Miller TA (2011) Paratransgenesis as a potential tool for pest control: Review of applied arthropod symbiosis. J Appl Entomol 135:474-478.
- Mircetich SM, Lowe SK, Moller WJ and Nyland G (1976) Etiology of almond leaf scorch disease and transmission of the causal agent. Phytopathology 66:17-24.
- Monteiro PB, Renaudin J, Jagoueix-Eveillard S, Ayres AJ, Garnier M and Bové JM (2001) Madagascar periwinkle (*Catharanthus roseus*): An experimental host plant for the citrus strain of *Xylella fastidiosa*. Plant Dis 85:246-251.
- Nair DN and Padmavathy S (2014) Impact of endophytic microorganisms in plants, environments and humans. Sci World J 2014:e250693.
- Nassar AH, El-Tarabily KA and Sivasithamparam K (2005) Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.) roots. Biol Fert Soils 42:97-108.
- Newman LA and Reynolds CM (2005) Bacteria and phytoremediation: New uses for endophytic bacteria in plants. Trends Biotechnol 23:6-8.
- Nunes LR, Rosato YB, Muto NH, Yanay GM, da Silva VS, Leite DB, Gonçalves ER, de Souza AA, Colleta Filho HD, Machado MA, *et al.* (2003) Microarray analyses of *Xylella fastidiosa* provide evidence of coordinated transcription control of laterally transferred elements. Genome Res 13:570-578.
- Nyland GA, Goheen AC, Lowe SK and Kirkpatrick HC (1973) The ultrastructure of a rickettsia-like organism from a peach-tree affected with phony disease. Phytopathology 63:1275-1278.
- Park YL, Perring TM, Farrar CA and Gispert C (2006) Spatial and temporal distributions of two sympatric *Homalodisca* spp. (Hemiptera: Cicadellidae): Implications for area wide pest management. Agric Ecosyst Environ 113:168-174.
- Perring TM, Farrar CA and Blua MJ (2001) Proximity to citrus influences Pierce's disease in Temecula Valley vineyards. Calif Agric 55:13-18.
- Petrini LE, Petrini O and Laflamme G (1989) Recovery of endophytes of *Abies balsamea* from needles and galls of *Paradiplosis tumifex*. Phytoprotection 70:97-103.
- Pierce NB (1892) The California Vine Disease. USDA Division of Vegetable Pathology Bulletin No. 2. USDA, Washington, D.C., 222 p.
- Pillay VJ and Nowak J (1997) Inoculum density, temperature and genotype effects on *in vitro* growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. Can J Microbiol 43:354-361.
- Podolick O, Ardanov P, Zaets I, Pirtilla AM and Kozyrovska N (2015) Reviving of the endophytic bacterial community as a putative mechanism of plant resistance. Plant Soil 388:367-377.

- Purcell AH and Hopkins DL (1996) Fastidious xylem-limited bacterial plant pathogens. *Annu Rev Phytopathol* 34:131-151.
- Purcell AH and Saunders SR (1999) Fate of Pierce's disease strains of *Xylella fastidiosa* in common riparian plants in California. *Plant Dis* 83:825-830.
- Quecine MC, Batista BD and Lacava PT (2014) Diversity and biotechnological potential of plant-associated endophytic bacteria. In: Ananda Kumar P and Govil JN (eds) *Biotechnology*, Vol. 2: Plant Biotechnology. Studium Press LLC, Houston, pp 377-423.
- Rai M, Rathod D, Agarkar G, Dar M, Brestic M and Marostica Junior MR (2014) Fungal growth promoter endophytes: A pragmatic approach towards sustainable food and agriculture. *Symbiosis* 62:63-79.
- Raju BC, Goheen AC and Frazier NW (1983) Occurrence of Pierce's disease bacteria in plants and vectors in California. *Phytopathology* 73:1309-1313.
- Rathe AA, Pilkington LJ, Hoddle MS, Spohr LJ, Daugherty MP and Gurr GM (2014) Feeding and development of the glassy-winged sharpshooter, *Homalodisca vitripennis*, on Australian native plant species and implication for Australian biosecurity. *PLoS One* 9:e90410.
- Raupach GS and Kloepper JW (1998) Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology* 88:1158-1164.
- Redak RA, Purcell AH, Lopes JRS, Blua MJ, Mizell III RF and Andersen PC (2004) The biology of xylem fluid feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. *Annu Rev Entomol* 49:243-270.
- Reinhold-Hurek B and Hurek T (2011) Living inside plants: Bacterial endophytes. *Curr Opin Plant Biol* 14:435-443.
- Rio RVM, Hu Y and Aksoy S (2004) Strategies of the home-team: Symbioses exploited for vector-borne disease control. *Trends Microbiol* 12:325-336.
- Rogers EE and Stenger DC (2012) A conjugative 38 kB plasmid is present in multiple subspecies of *Xylella fastidiosa*. *PLoS One* 7:e52131.
- Rosenblueth M and Martinez-Romero E (2006) Bacterial endophytes and their interactions with hosts. *Mol Plant Microbe Int* 19:827-837.
- Rossetti V, Garnier M, Bove JM, Beretta MJG, Teixeira ARR, Quaggio JA and deNegri JD (1990) Presence de bacteries dans le xyleme d'orangers atteints de chlorose variegée, unenouvelle maladie des agrumes au Brasil. *C R Acad Sci* 310:345-349.
- Rúa MA, McCulley RL and Mitchell CE (2013) Fungal endophyte infection and host genetic background jointly modulate host response to an aphid transmitted viral pathogen. *J Ecol* 101:1007-1018.
- Rúa MA, McCulley RL and Mitchell CE (2014) Climate drivers, host identity and fungal endophyte infection determine virus prevalence in a grassland ecosystem. *J Ecol* 102:690-699.
- Sanderlin RS and Heyderich-Alger KI (2000) Evidence that *Xylella fastidiosa* can cause leaf scorch disease of pecan. *Plant Dis* 84:1282-1286.
- Sanderlin RS and Melanson RA (2006) Transmission of *Xylella fastidiosa* through pecan rootstock. *HortScience* 41:1455-1456.
- Saponari M, Boscia D, Nigro F and Martelli GP (2013) Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (Southern Italy). *J Plant Pathol* 95:668.
- Schaad NW, Postnikova E, Lacy G, Fatmi M and Chang C-J (2004) *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex*, subsp. nov., *X. fastidiosa* subsp. *pauca*, subsp. nov. *Syst Appl Microbiol* 27:290-300.
- Sharma VK and Nowak J (1998) Enhancement of verticillium wilt resistance in tomato transplants by in vitro co-culture of seedlings with a plant growth promoting rhizobacteria (*Pseudomonas* sp. strain PsNJ). *Can J Microbiol* 44:528-536.
- Sherald JL, Wells JM, Hurtt SS and Kostka SJ (1987) Association of fastidious xylem-limited bacteria with leaf scorch in red maple. *Plant Dis* 71:930-933.
- Simpson AJG, Reinach FC, Arruda P, Abreu FA, Acencio M, Alvarenga R, Alves LMC, Araya JE, Baia GS, Baptista CS, et al. (2000) The genome sequence of the plant pathogen *Xylella fastidiosa*. *Nature* 406:151-159.
- Smith IM, McNamara DG, Scott PR, Holderness M and Burger B (1997) *Quarantine Pests for Europe*. 2nd edition. CAB International, Wallingford, 1425 p.
- Sturz AV and Matheson BG (1996) Populations of endophytic bacteria which influence host-resistance to *Erwinia*-induced bacterial soft rot in tubers. *Plant Soil* 184:265-271.
- Sturz AV, Christie BR and Matheson BG (1998) Association of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. *Can J Microbiol* 44:162-167.
- Sturz AV, Christie BR and Nowak J (2000) Bacterial endophytes: Potential role in developing sustainable systems of crop production. *Crit Rev Plant Sci* 19:1-30.
- Summer EJ, Enderle CJ, Ahern J, Gill JJ, Torres CP, Appel DN, Black MC, Young R and Gonzalez CF (2010) Genome and bacterial analyses of phage Xfas53 and related prophages of *Xylella fastidiosa*. *J Bacteriol* 192:179-190.
- Suryanarayanan TS (2013) Endophyte research: Going beyond isolation and metabolic documentation. *Fungal Ecol* 6:561-568.
- Takiya DM, McKamey SH and Cavichioli RR (2006) Validity of *Homalodisca* and of *H. vitripennis* as the name for glassy-winged sharpshooter (Hemiptera: Cicadellidae: Cicadellinae). *Ann Entomol Soc Am* 99:648-655.
- Tor M, Mantel SH and Ainsworth C (1992) Endophytic bacteria expressing beta-glucuronidase cause false positives in transformation of dioscorea species. *Plant Cell Rep* 11:452-456.
- Urakami T, Araki H, Suzuki K and Komagata K (1993) Further studies of the genus *Methylobacterium* and description of *Methylobacterium aminovorans* sp. nov. *Int J Syst Evol Microbiol* 43:504-513.
- Van Aken B, Peres CM, La Fferty-Doty S, Yoon JM and Schnoor JL (2004) *Methylobacterium populi* sp. nov. a novel aerobic, pink pigmented, facultatively methylotrophic, methane-utilizing bacterium isolated from poplar trees (*Populus deltoids* x *nigra* DN34). *Int J Syst Evol Microbiol* 54:1191-1196.
- Van Sluys MA, de Oliveira MC, Monteiro-Vitorello CB, Mitald CY, Furlan RL, Camargo LEA, da Silva ACR, Moon DH, Takita MA, Lemos EGM, et al. (2003) Comparative analy-

- ses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. *J Bacteriol* 185:1018-1026.
- Varani AM, Monteiro-Vitorello CB, de Almeida LGP, Souza RC, Cunha OL, Lima WC, Civerolo E, Van Sluys MA and Vasconcelos ATR (2012) *Xylella fastidiosa*: Comparative genomic databases is an information resource to explore the annotation, genomic features and biology of different strains. *Genet Mol Biol* 35:149-152.
- Verma P, Yadav AK, Khanna KS, Kumar S, Saxena AK and Suman A (2015) Alleviation of cold stress in wheat seedlings by *Bacillus amyloliquefaciens* iari-hhs2-30 an endophytic psychrotolerant K-solubilizing bacterium from NW Indian himalayas. *Nat J Life Sci* 12:105-110.
- Weinbreck F, Bodnár I and Marco ML (2010) Can encapsulation lengthen the shelf-life of probiotic bacteria in dry products? *Int J Food Microbiol* 136:364-367.
- Wells JM, Raju BC, Hung H-Y, Weisburg WG, Mandelco-Paul L and Brenner DJ (1987) *Xylella fastidiosa* gen. nov., sp. nov.: Gram-negative, xylem limited, fastidious plant bacteria related to *Xanthomonas* spp. *Int J Syst Evol Microbiol* 37:136-143.
- Wells JM, Raju BC, Thomson JM and Lowe SK (1981) Evidence of the common etiology of phony peach and plum leaf scald diseases. *Phytopathology* 71:1156-1161.
- Wistrom C and Purcell AH (2005) The fate of *Xylella fastidiosa* in vineyard weeds and other alternate hosts in California. *Plant Dis* 89:994-999.
- Wood AP, Kelly DP, McDonald IR, Jordan SL, Morgan TD, Khan S, Murrell JC and Borodina E (1998) A novel pink pigmented facultative methylotroph, *Methylobacterium thiocyanatum* sp. nov., capable of growth on thiocyanate or cyanate as sole nitrogen sources. *Arch Microbiol* 169:148-158.
- Yamada K and Komagata K (1972) Taxonomic studies on coryneform bacteria. IV. Morphology, cultural, biochemical, and physiological characteristics. *J Gen Appl Microbiol* 18:399-416.
- Zinnier DK, Lambrecht N, Harris B, Feng Z, Kuezmarski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG and Vidaver AK (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Appl Environ Microbiol* 68:2198-2208.

Associate Editor: Carlos F. M. Menck

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License (type CC-BY), which permits unrestricted use, distribution and reproduction in any medium, provided the original article is properly cited.