



## Microorganisms as biocontrol agents against bacterial citrus diseases

Jorge Poveda<sup>a,b,f,1,\*</sup>, Roxana Andrea Roeschlin<sup>c,d</sup>, María Rosa Marano<sup>e</sup>,  
María Alejandra Favaro<sup>f,1</sup>

<sup>a</sup> Institute for Multidisciplinary Research in Applied Biology, Universidad Pública de Navarra, Campus Arrosadia, Pamplona 31006, Spain

<sup>b</sup> Institute of Environment, Natural Resources and Biodiversity, University of León, Av/ de Portugal, 42, León 24071, Spain

<sup>c</sup> Instituto Nacional de Tecnología Agropecuaria (INTA), Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Estación Experimental Agropecuaria, Ruta 11 km 773, Reconquista S3560WAA, Santa Fe, Argentina

<sup>d</sup> Facultad de Ciencias Agropecuarias, Universidad Católica de Santa Fe, Ludueña 612, Reconquista S3560DYR, Santa Fe, Argentina

<sup>e</sup> Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET. Área Virología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Ocampo y Esmeralda S/N, Rosario S2000FHN, Santa Fe, Argentina

<sup>f</sup> Instituto de Ciencias Agropecuarias del Litoral (ICI Agro Litoral), Universidad Nacional del Litoral, CONICET, Facultad de Ciencias Agrarias, Kreder 2805, Esperanza 3080 HOF, Santa Fe, Argentina

### HIGHLIGHTS

- Citrus crop is seriously threatened by various bacterial diseases.
- Cultural practices and chemical pesticides do not control these diseases.
- Microbiological control is a very promising strategy.

### ARTICLE INFO

#### Keywords:

Citrus  
Asiatic citrus canker  
Citrus variegated chlorosis  
Huanglongbing  
*Xanthomonas citri*  
*Xylella fastidiosa*  
*Candidatus Liberibacter*

### ABSTRACT

Citrus represents one of the most widely grown crops on the planet, extensively cultivated for both the fresh fruit and juice markets. The productivity of citrus orchards can be seriously affected by highly aggressive pathogenic bacteria, such as *Xanthomonas citri* subsp. *citri*, *Xylella fastidiosa* subsp. *pauca* and currently *Candidatus Liberibacter asiaticus*. Different microbiological biocontrol agents have been described against these pathogens, such as antagonistic bacteria (mainly species from *Pseudomonas* and *Bacillus* genus) and bacteriophages. This review summarizes all the microbiological control strategies reported so far against bacterial diseases that affect citrus, highlighting those fields of study where there is great potential yet to be discovered.

### 1. Introduction

Citrus is one of the most widespread cultivated fruits worldwide, grown for both the fresh fruit and fresh and processed juice markets. Most citrus cultivars and rootstock varieties belong to the *Fortunella*, *Poncirus* or *Citrus* genera, in the Rutaceae family (order Geraniales, suborder Geraniineae) (Cuenca et al., 2018; Jaouad et al., 2020; Zhong and Nicolosi, 2020). The main growing areas include countries such as China (with a total production of more than 32.7 million tons), Brazil (16.55 million tons), India (9.7 million tons), United States (7.8 million tons), or Spain (6.8 million tons), the latter leading the world ranking of

citrus exports for consumption as fresh fruit, being a key sector within the agricultural system of various growing areas (González-González et al., 2020). Citrus fruits have become an important dietary source of nutrients for many countries (Zou et al., 2016).

As many tropical and subtropical crops, citrus are hosts of numerous fungal, viral and bacterial diseases. Some of the non-bacterial pathogens that affect citrus cultivation are *Citrus tristeza virus* (CTV disease), the fungi *Mycosphaerella citri* (greasy spot disease), *Alternaria* spp. (brown spot and black rot disease), *Phyllosticta citricarpa* (black spot disease), *Colletotrichum acutatum* and *C. gloeosporioides* (anthracnose disease), and the oomycete *Phytophthora* spp. (root and collar rot and brown rot on

\* Corresponding author at: Institute for Multidisciplinary Research in Applied Biology, Universidad Pública de Navarra, Campus Arrosadia, Pamplona 31006, Spain.

E-mail address: [jorge.poveda@unavarra.es](mailto:jorge.poveda@unavarra.es) (J. Poveda).

<sup>1</sup> These authors contributed equally to this work.

fruit) (Guarnaccia et al., 2019; Sun et al., 2019; Jaouad et al., 2020).

## 2. Bacterial citrus diseases

Bacterial diseases pose a constant threat to citrus cultivation and cause substantial reductions in production in all growing areas around the world. Among them, huanglongbing (HLB), Asiatic citrus canker (ACC) and citrus variegated chlorosis (CVC) cause significant economic impacts, due to the death of millions of trees every year and the imposition of quarantine restrictions (Mendonça et al., 2017; Gabriel et al., 2020).

HLB and ACC are currently endemic diseases in most commercial citrus producing regions of the world, whereas CVC is restricted to the Americas. In the European continent, all of them are not known to occur (Mendonça et al., 2017). The risk of introduction of these bacteria into citrus growing regions represents a constant preoccupation for citrus growers and trade. For ACC, the most significant impact derives from the restrictions to interstate and international fruit trade coming from endemic areas. For HLB and CVC, strict quarantine measures for the introduction of propagative citrus material are implemented in several producing countries worldwide (Gabriel et al., 2020).

Although these diseases infect a common host, they adopt different strategies for virulence and dispersion, showing different tissue specificity and causing different symptoms. Recent reviews are focalized on secretion systems and effectors, cell-to-cell signaling pathways, and pathogenicity mechanisms that are related to the specific lifestyle, dispersion or symptoms produced by these bacteria (Vojnov et al., 2010; Mendonça et al., 2017; Gabriel et al., 2020; Timilsina et al., 2020).

ACC, caused by the hemibiotrophic bacteria *Xanthomonas citri* subsp. *citri* (*X. citri*), is a widely distributed disease that affects most commercial citrus cultivars. Typical symptoms of the disease are erumpent, corky and raised pustules on the surface of leaves, fruits and twigs which serve as sources of bacterial inoculum. Infected fruit have decreased commercial quality and are rejected by most important markets (FERENCE et al., 2018). Defoliation, twig dieback and premature fruit drop are also observed as a plant response to the infection (Graham et al., 2004). The presence of a water film over the lesions promotes *X. citri* exudation to the surface, and rain splash combined with wind dispersed bacteria to new susceptible tissue (Graham et al., 2004; Bock et al., 2005).

Bacterial attachment, microcolony formation and biofilm development of *X. citri* on citrus surfaces are essential steps in the pathogenesis of the disease (Rigano et al., 2007; Vojnov and Marano, 2015). Quorum sensing or cell-to-cell signaling, mediated by a diffusible signal molecule called DSF, plays an important role in this process (Siciliano et al., 2006). Biofilm formation is conditioned by bacterial strain and the presence of exopolysaccharides (EPS), weather conditions, cultivar resistance and phenological stage (Favaro et al., 2014, 2020; Roeschlin et al., 2017; Chiesa et al., 2019). After this phase of epiphytic growth, mesophyll colonization is accomplished through natural openings in young susceptible tissues. The presence of wounds caused by insects such as citrus leafminer, pruning tools and storms facilitates bacterial ingress in older tissues (Martins et al., 2020).

Citrus blast and black pit caused by *Pseudomonas syringae* pv. *syringae* (*P. syringae*) is a minor bacterial disease of citrus worldwide, although was reported causing severe outbreaks in some Mediterranean countries. In Montenegro, the current incidence of blast disease in citrus nurseries is around 10–30% (Ivanović et al., 2017). Recent research aims to characterize the genetic structure of the pathogen involved and to develop disease management strategies (Ivanović et al., 2017; Mougou and Boughalleb-M'hamdi, 2018; Islam et al., 2020). Colonization and dispersion of *P. syringae* are favoured by rain and wind in spring when the shoots and fruit development begins, resembling the *X. citri* pathogenicity cycle. Characteristic disease symptoms begin as water-soaked lesions in leaves, reaching the mid-vein and the twigs surrounding the base of the petiole. Diseased leaves eventually fall and the necrotic twigs and shoots dieback after a few weeks. (Ivanović et al., 2017; Islam et al.,

2020).

CVC is caused by *Xylella fastidiosa* subsp. *pauca* (*X. fastidiosa*), which also produces coffee leaf scorch and olive quick decline syndrome, even though it is a different sequence type. The disease affects sweet orange to a greater extent and is endemic to some countries of the American continent (Gabriel et al., 2020). *X. fastidiosa* colonizes two environments: the plant host xylem and the mouthparts of its vector (Rapicavoli et al., 2018). CVC is transmitted by sharpshooters belonging to the family Cicadellidae, and by infected budwood. The CVC pathogenesis cycle starts when sharpshooters carrying the bacteria feed on the xylem sap of a healthy tree (Gabriel et al., 2020). After that, the disease is characterized by the obstruction of xylem caused by bacterial biofilm and EPS, leading to insufficient translocation of water in the vessels (Vojnov et al., 2010; Mauricio et al., 2019). Symptoms include foliar wilt and interveinal chlorosis on the adaxial surfaces of the leaves, similar to zinc deficiency. These symptoms are accompanied by necrotic, gum-like regions on the abaxial side of the leaves. Fruit from infected branches are smaller, harder and exhibit changes in organoleptic quality. Young and water stressed trees are more susceptible to disease (Gabriel et al., 2020). Over many years, CVC was an epidemic disease in Brazil causing annual economic losses of \$120 million. However, the prevalence of HLB during recent years significantly diminished its importance (Mendonça et al., 2017; Rapicavoli et al., 2018).

HLB is one of the oldest diseases in citrus and has been known in East Asia for over a century. Currently, it is the most destructive citrus disease worldwide, present in Asia, the Middle East, Africa, and more recently in the Americas. HLB is associated with a phloem-limited fastidious  $\alpha$ -proteobacterium provisionally named as *Candidatus Liberibacter* spp. (*Ca. L.*). Three species of *Ca. L.* are recognized in trees with HLB disease according to the origin: *Ca. L. africanus*, *Ca. L. americanus*, and the most widespread *Ca. L. asiaticus* (Wang and Trivedi, 2013; da Graça et al., 2016). The bacteria are transmitted in a persistent manner by the psyllid vectors *Diaphorina citri* and *Trioza erytreae*, and also by grafting. After vector inoculation in *Citrus* phloem, a long incubation period arises before symptom expression. Sieve plug formation and accumulation impedes nutrient transport and causes the characteristic HLB symptoms such as asymmetric blotchy mottles on the leaves, parti-colored fruit, loss of productivity, and eventual decline and death of infected trees after several years (Munir et al., 2018; Gabriel et al., 2020). HLB greatly damages the citrus industry by shortening the lifespan of infected trees and reducing fruit yield and quality characteristics. HLB can debilitate the productive capacity of citrus trees, with reported losses of 30 to 100%, and all commercial citrus species and cultivars are susceptible to HLB infection, though to varying degrees (Wang and Trivedi, 2013; Blaustein et al., 2018).

## 3. Current management of citrus bacterial diseases

Taking into account the worldwide importance of citrus production, industry and global trade, the development and application of strategies to reduce the damage occasioned by bacterial diseases are essential. Although the management of these diseases has some common aspects, different lifestyles and methods of transmission determine specific strategies for each disease.

Exclusion and eradication efforts are carried out in canker-free countries or regions to avoid ACC disease (Vojnov et al., 2010). On the other hand, an integrated approach successfully manages ACC in areas where the disease is endemic, including planting pathogen free certified trees, disinfestation of tools, citrus leafminer control with insecticides, orchard protection through the installation of windbreaks, copper-based spray programs and selection of resistant or tolerant cultivars (Behlau et al., 2010; Mendonça et al., 2017; Gabriel et al., 2020). A faster phenological development of the leaf, a smaller stomatal density, size and aperture, and also higher epicuticular wax content were associated with partial resistance to ACC (Wang et al., 2011; Favaro et al., 2014, 2020; Gonçalves-Zuliani et al., 2016). It has been

demonstrated that copper compounds could disrupt or avoid *X. citri* biofilm formation (Redondo et al., 2015; Favaro et al., 2017). Numerous copper sprays are required during the growing season when susceptible cultivars are exposed to favorable weather conditions for disease. Efforts to reduce and replace copper sprays with more sustainable strategies are essential to avoid the negative ecological impact of the accumulation of copper in soil, the risk of phytotoxicity to roots and fruits, and the emergence of copper-resistance in *X. citri* populations (Behlau et al., 2013, 2020).

CVC management efforts include eradication of infected trees, pruning of symptomatic branches and intensive control of the vector (Gabriel et al., 2020). Recent research has demonstrated that the substitution of diseased buds with healthy ones in 'Rangpur' lime or 'Cleopatra' rootstocks, is an effective and economic strategy to manage the disease (Lopes 2020). Moreover, it has been demonstrated that high concentrations of copper inhibit *X. fastidiosa* biofilm formation *in vitro*, although copper supplementation in the watering solution in tobacco model plants is ineffective to inhibit *X. fastidiosa* growth (Cobine et al., 2013; Ge et al., 2020).

Management of HLB continues to be a challenge due to the lack of effective curative treatments and the long incubation period of the disease. Nowadays, HLB management relies on production of budwood in insect-proof nurseries, frequent inspection, detection and eradication of diseased trees, added to the control of the vector in order to diminish the spread of the disease (Gabriel et al., 2020). Other approaches such as the use of broad spectrum antimicrobials, small molecule compounds, thermotherapy, and compounds that stimulate plant growth or trigger host defenses, have been assayed for HLB treatment with promising results, although more efforts and studies should be performed before their implementation in the field (Munir et al., 2018; Blaustein et al., 2018).

Strategies for developing genetic resistance to the phytopathogen have been undertaken (Blaustein et al., 2018). Research into the microbiome of HLB-diseased plants has produced new insights to find promising microbial communities for disease management (Ginnan et al., 2018, 2020; Blaustein et al., 2017; Blacutt et al., 2020).

In spite of the significant progress achieved in the management of citrus bacterial diseases, some key aspects remain unsolved, such as the lack of genotypes presenting full resistance to the diseases, the inexistence of effective chemical compounds for CVC and HLB treatment, the risk of emergence of copper-resistant *X. citri* populations, and the accumulation of this compound in soil. In this context, biocontrol is a promising alternative and it has many advantages in terms of sustainability, mode of action and toxicity compared to chemical pesticides (Marin et al., 2019). The incorporation of biocontrol in the integrated management of ACC could reduce the rate and frequency of copper sprays and the risk of copper resistance development. Moreover, biocontrol agents could constitute useful tools for the management of HLB and CVC, overcoming the present limitations in their control.

#### 4. Microbiological biocontrol in citrus diseases

The current awareness of the environmental and health damage caused by the use of copper bactericides and antibiotics, added to the lack of efficient control measures for citrus bacterial diseases, support the development of alternative strategies using biocontrol agents (BCA).

Biocontrol includes strategies to suppress diseases by the application of a biological agent, such as fungus, bacterium, or bacteriophage to the plant or the soil. Biocontrol mechanisms include the production of antimicrobial substances or lytic enzymes, nutrient and space competition, parasitism, signal interference and induced systemic resistance (ISR) in plants. Previous studies have shown that one antagonist will often use more than one mechanism. Generally, BCA are highly specific for a pathogen and hence are considered harmless to non-target species, although it is necessary to know how they act to implement an effective biocontrol program (Weller, 2007; Höfte and Altier, 2010; Nunes, 2012;

O'Brien, 2017; Chen et al., 2020).

As far as citrus non-bacterial pathogens are concerned, there are several studies showing promising results with BCA. The ability of different bacteria to promote plant growth and act as biocontrol agents in *Citrus* has been extensively studied (Giassi et al., 2016).

A good source of microbiological control agents against citrus pathogens is the plant's own microbiota, with fully developed methodologies for their identification and analysis against pathogens, such as *Ca. L.* (Blacutt et al., 2020). Different microbiological biocontrol agents have been described against citrus bacterial diseases (Table 1), which will be analyzed in detail in the following sections and are summarized in an infographic (Fig. 1).

#### 5. Bacterial biocontrol against bacterial citrus diseases

Endophytic bacterial diversity in citrus trees has been described as a potential resource for effective antimicrobial compounds, such as antibiotics. In a study carried out in *C. aurantifolia*, it has been possible to verify how the bacterial species isolated from leaves, such as *Bacillus cereus*, *B. subtilis*, *B. pumilus* and *Pantoea agglomerans*, produce antibiotics capable of inhibiting the growth of pathogenic bacteria such as *Streptococcus mutans*, *Vibrio cholerae*, *Salmonella thypii*, *S. thyposa* and *Enterobacter faecalis* (Jannah et al., 2018).

Numerous rhizosphere and phyllosphere species from the genera *Pseudomonas* and *Bacillus* are effective biocontrol agents against citrus bacterial diseases. Moreover, *Xanthomonas* isolates reduce ACC severity. On the other hand, bacterial communities present in the microbiome of citrus plants are promising sources of BCA. In the following sections we will focus on biological control reported for these agents so far.

##### 5.1. *Pseudomonas* genus as BCA

The *Pseudomonas* genus includes a wide variety of species which have been studied and used as biocontrol agents against various plant diseases. Plant-associated *Pseudomonas* spp. include pathogenic, saprophytic and plant-growth-promoting species (Höfte and Altier, 2010). The agricultural and economic importance of this genus in plant health is due to the ability of *Pseudomonas* spp. to grow rapidly *in vitro* and to be mass produced and adapt to environmental stresses. Diverse *Pseudomonas* species rapidly utilize seed and root exudates, colonize and multiply in the rhizosphere, phyllosphere, internal plant tissues or even in structures produced by phytopathogens (Weller, 2007; Mercado-Blanco and Bakker, 2007).

Mechanisms involved in biocontrol by *Pseudomonas* spp. include production of bioactive metabolites (i.e., antibiotics, siderophores, volatiles), competition for niches and resources with other microorganisms, induction of systemic resistance and plant-growth promotion (Mercado-Blanco and Bakker, 2007; Weller, 2007; Höfte and Altier, 2010; Biessy and Filion, 2018).

Numerous *Pseudomonas* spp. isolates obtained from the citrus phyllosphere were effective against *X. citri*, both *in vitro* and in greenhouse conditions. Caicedo et al. (2016) identified two *Pseudomonas* spp. (SJ01 and SJ02) able to reduce ACC when sprayed on *C. sinensis* leaves at a concentration of  $10^7$  CFU ml<sup>-1</sup>, in mixtures with the pathogenic bacteria *X. citri*. These biocontrol agents act by disruption of *X. citri* quorum sensing, resulting in a reduced attachment to the surface of leaves, compromising biofilm formation and canker development. Likewise, de Oliveira et al. (2011) and de Oliveira et al. (2016) demonstrated the antibiotic activity of secondary metabolites from *Pseudomonas* spp. and *P. aeruginosa* (LV strain) isolated from an old canker symptom in *C. sinensis*. Preventive and curative sprays, applied a day after or before *X. citri* ( $10^7$  CFU ml<sup>-1</sup>), reduced the number of cankers between 65 and 97%. The secondary metabolites from the soluble fraction in ethyl acetate altered EPS, inducing cell lysis and interrupting biofilm formation in orange leaves. An organocopper antibiotic compound (OAC) was identified to be the pure active compound (de Oliveira et al., 2016).

**Table 1**

Microorganisms used in the biocontrol of bacterial citrus diseases. *X. citri*: *Xanthomonas citri* subsp. *citri*; *X. fastidiosa*: *Xylella fastidiosa* subsp. *pauca*; *Ca. L.*: *Candidatus Liberibacter asiaticus*; *P. syringae*: *Pseudomonas syringae* pv. *syringae*.

BIOCONTROL AGENT		BACTERIAL-CITRUS PATHOGEN	EXPERIMENT CONDITIONS	CITRUS SPECIES	MECHANISMS	REFERENCES				
GROUP	SPECIES									
Bacteria	<i>Acinetobacter baumannii</i>	<i>X. citri</i>	<i>In vitro</i> Greenhouse	<i>C. limon</i>	Antibiosis	Tan et al. 2006				
	<i>P. fluorescens</i>		<i>In vitro</i> Greenhouse	<i>C. limon</i>	Not indicated	Khodakaramian et al. 2008				
	<i>Pseudomonas</i> spp.		<i>In vitro</i> Greenhouse	<i>C. sinensis</i>	Antibiosis, Cell lysis, Biofilm disruption	de Oliveira et al. 2011				
	<i>P. fluorescens</i>		<i>In vitro</i>	–	Antibiosis	Montakhabi et al. 2011				
	<i>P. viridiflava</i>		Greenhouse	<i>C. limon</i>	Not indicated	Dong et al. 2012				
	<i>P. syringae</i>									
	<i>Bacillus</i> spp.									
	<i>Streptomyces</i> spp. <i>Pseudomonas</i> spp.									
	<i>Burkholderia</i> spp.									
	<i>B. subtilis</i>						<i>In vitro</i> Greenhouse	<i>C. aurantifolia</i>	Antibiosis, Biofilm disruption	Huang et al. 2012
	<i>B. subtilis</i>						<i>In vitro</i>	–	Antibiosis	Long and Guanhua, 2012
	<i>P. fluorescens</i>						<i>In vitro</i> Greenhouse	<i>C. aurantifolia</i>	Not indicated	Al-Saleh, 2014
	<i>B. subtilis</i>	Field Greenhouse					<i>C. latifolia</i>	Space competition	Das et al. 2014	
	<i>P. aeruginosa</i>	<i>In vitro</i> Greenhouse					<i>C. sinensis</i>	Antibiosis, Cell lysis	Spago et al. 2014	
	<i>P. aureginosa</i>	<i>In vitro</i> Greenhouse	<i>C. aurantifolia</i>	Defense response induction Plant growth promotion	Sudyoung et al., 2020					
	<i>P. entomophila</i>	Greenhouse	<i>C. limon</i>	Antibiosis	Villamizar et al. 2020					
	<i>B. subtilis</i>									
	<i>Pseudomonas</i> spp.									
	<i>Pseudomonas</i> spp.									
	<i>Bacillus</i> spp.									
	<i>P. aeruginosa</i>					<i>In vitro</i> Greenhouse	<i>C. sinensis</i>	Antibiosis, Cell lysis, Biofilm disruption	de Oliveira et al. 2016	
	<i>B. subtilis</i>					Greenhouse	<i>C. aurantifolia</i>	Antibiosis	Ibrahim et al. 2016	
	<i>P. protegens</i>					<i>In vitro</i> Greenhouse	<i>C. limon</i>	Defense response induction	Michavila et al. 2017	
	<i>P. geniculata</i>					Greenhouse	<i>C. paradisi</i>	Defense response induction	Riera et al. 2018	
	<i>B. amyloliquefaciens</i>					Greenhouse	<i>C. aurantifolia</i>	Antibiosis	Daungfu et al. 2019	
	<i>B. tequilensis</i>	<i>X. fastidiosa</i>	Field	<i>C. sinensis</i>	Not indicated					
	<i>B. subtilis</i>									
	<i>Bacillus thuringiensis</i>					<i>In vitro</i>	–	Antibiosis, Cell lysis	Islam et al. 2019	
	<i>Bacillus velezensis</i>					<i>In vitro</i>	–	Antibiosis, Cell lysis	Rabbee et al. 2019	
	<i>X. citri</i> A <sup>T</sup>					Greenhouse	<i>C. limon</i>	Defense response induction	Roeschlin et al. 2017	
	<i>Xcc</i>					Greenhouse	<i>C. limon</i>	Defense response induction	Chiesa et al. 2019	
	<i>Xfa</i>					Field	<i>C. sinensis</i>	Not indicated	Araújo et al. 2002	
<i>Methylobacterium</i> sp.	<i>In vitro</i>					–	Antibiosis	Lacava et al. 2004		
<i>Curtobacterium flaccumfaciens</i>	Field					<i>C. sinensis</i>	Antibiosis	Azevedo et al. 2016		
<i>Methylobacterium mesophilicum</i>	<i>Ca. L.</i>					Field	<i>C. sinensis</i>	Not indicated		
<i>C. flaccumfaciens</i>										
<i>Methylobacterium</i> sp.										
<i>Sphingobacterium</i> sp.										
<i>Methylobacterium</i> sp. <i>Sphingomonas</i> sp.		Field	<i>C. sinensis</i> , <i>C. paradise</i> , <i>C. unshiu</i> , <i>C.x tangelo</i>	Competition	Blaustein et al. 2017					
<i>Methylocystaceae</i> sp.										
<i>Bacillus</i> sp., <i>Lactobacillus</i> sp., <i>Streptomyces</i> sp., <i>Methylobacterium</i> sp., <i>Hymenobacter</i> sp., <i>Pantoea</i> sp., <i>Curtobacterium</i> sp., <i>Spirosoma</i> sp.		Field	–	–	Ginnan et al., 2018, 2020					
<i>P. aureginosa</i>		Greenhouse	<i>C. sinensis</i>	Antibiosis	Pistori et al. 2018					
<i>Burkholderia metallica</i>		<i>In vitro</i>	–	Antibiosis	Riera et al. 2017					
<i>B. territorii</i>		Greenhouse	<i>C. sinensis</i>	Defense response induction	Tang et al. 2018a					
<i>P. granadensis</i>										
<i>P. geniculata</i>										
<i>Rhodococcus jialingiae</i>										
<i>B. pumilus</i>										
<i>B. amyloliquefaciens</i>										
<i>B. amyloliquefaciens</i>	Greenhouse					<i>C. sinensis</i>	Defense response induction	Tang et al. 2018b		
<i>B. amyloliquefaciens</i>	Greenhouse					<i>C. tangerine</i> <i>C. madurensis</i>	Defense response induction	Tang et al. 2018b		

(continued on next page)

Table 1 (continued)

BIOCONTROL AGENT		BACTERIAL-CITRUS PATHOGEN	EXPERIMENT CONDITIONS	CITRUS SPECIES	MECHANISMS	REFERENCES
GROUP	SPECIES					
	<i>B. subtilis</i> <i>Bacillus</i> spp.	<i>P. syringae</i>	Field <i>In vitro</i> Greenhouse	Several <i>Citrus</i> spp. <i>C. limon</i>	Defense response induction – Antibiosis	Munir et al. 2020 Mougou and Boughalleb-M'hamdi, 2018 Islam et al. 2020
	<i>Bacillus</i> spp.		<i>In vitro</i> Greenhouse	<i>C. sinensis</i> <i>C. limon</i>	Antibiosis	
Bacteriophages	Cp2, ΦXac2005-1, ccΦ7 and ccΦ13 Cp1 and Cp2 XacF1 Unidentified Φ6	<i>X. citri</i>  <i>P. syringae</i> <i>Ca. L.</i>	Greenhouse <i>In vitro</i> <i>In vitro</i> Greenhouse Field <i>In vitro</i>	<i>C. paradisi</i> – – <i>C. aurantifolia</i> –	Not indicated Cell lysis Virulence reduction Cell lysis Cell lysis	Balogh et al. 2008 Ahmad et al. 2014a Ahmad et al. 2014b Ibrahim et al. 2017 Pinheiro, et al. 2019 Ginnan et al. 2018
Fungi	<i>Cladosporium</i> spp. <i>Sporobolomyces</i> spp. <i>Symmetospora</i> spp. <i>Camptophora</i> spp. <i>Hannaella</i> spp. <i>Exophiala</i> spp. <i>Fusarium</i> spp. <i>Glomus</i> spp. <i>Rhizophagus</i> spp. <i>Acrocalymma</i> spp. phylum <i>Glomeromycota</i>			Field  <i>In vitro</i>	Several <i>Citrus</i> spp.  Several <i>Citrus</i> spp.	Possible Growth promotion Growth inhibition by bioactive natural products

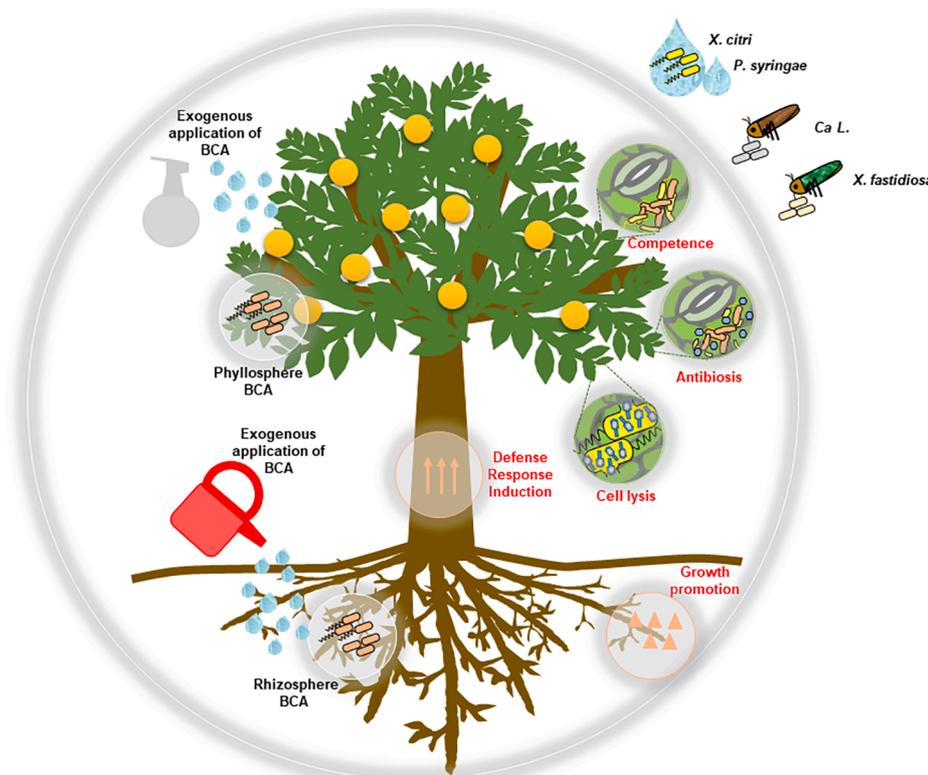


Fig. 1. Schematic representation of biocontrol agents (BCA) mechanisms against citrus bacterial diseases: *Xanthomonas citri* subsp. *citri* (*X. citri*), *Pseudomonas syringae* pv. *syringae* (*P. syringae*), *Candidatus Liberibacter* spp. (*Ca. L.*) and *Xylella fastidiosa* subsp. *pauca* (*X. fastidiosa*). BCA present in the phyllosphere and rhizosphere or applied as a soil drench or spray avoid bacterial pathogen infection and spread by direct antagonistic effect on the pathogen (antibiosis, competition, cell lysis) or indirect response by induction of plant defense or growth promotion.

Secondary metabolites purified from the strain LN of *P. aeruginosa*, obtained from a young canker in *C. sinensis*, were also able to damage EPS and bacterial cells, reducing citrus canker when applied as preventive sprays (Spago et al., 2014). Moreover, Murate et al. (2015) found that secondary metabolites obtained from a *Pseudomonas* spp. (LN strain) protected *C. sinensis* leaves from *X. citri*, diminishing ACC.

An antagonistic *P. aeruginosa* strain (isolate SWUC02) obtained from

canker symptomatic trees reduced *X. citri* growth in evaluations performed *in vitro* (Sudyoung et al., 2020). Likewise, in *C. aurantifolia* seedlings and adult trees, the pre-inoculation of leaves with this bacteria ( $10^8$  CFU ml<sup>-1</sup>) 24 h before *X. citri* infection ( $10^6$  CFU ml<sup>-1</sup>), significantly reduced ACC severity, leading to a control efficacy of 84%. The improvement of tree growth was associated with the induction of indoleacetic acid and siderophore synthesis, phosphate solubilization

and nitrogen fixation, promoted by *P. aeruginosa* extracellular compounds.

Different mechanisms of biocontrol have been found for other *Pseudomonas* species. *P. protegens* CS1 isolated from healthy lemon phyllosphere was capable of inhibiting the growth of *X. citri* both *in vitro* and in greenhouse conditions in *C. limon*. Spray application of the BCA one day after inoculation with *X. citri* significantly reduced canker symptoms through the active compound enantio-pyochelin and the generation of reactive oxygen species (Michavila et al., 2017). On the other hand, the antibacterial effect of the strain JS2 of *P. entomophila* isolated from soil against *X. citri* was attributed to multiple factors, including diketopiperazine production. Strikingly, this *Pseudomonas* species is known for its capacity to naturally infect insects, but this is the first time it has shown antibacterial activity. Highly susceptible *Citrus* × *limonia* species sprayed with a mixture of *P. entomophila* and *X. citri* ( $10^7$  CFU ml<sup>-1</sup>) showed a significant reduction in ACC severity (Villamizar et al., 2020).

The induction of systemic resistance is another mechanism proposed for biocontrol in the genus *Pseudomonas*. Considering the devastating symptoms caused by HLB, healthy looking citrus trees identified in severely HLB-diseased citrus groves, are considered as promising rhizosphere microbial communities enriched in beneficial traits. A strain of *P. geniculata*, isolated from this habitat, was found to activate a defense response against ACC by the induction of salicylic acid signaling pathways. The application of the beneficial bacteria as a soil drench ( $10^8$  CFU ml<sup>-1</sup>) to Duncan *C. paradisi* rhizosphere seven days before *X. citri*-spray inoculation to leaves ( $10^8$  CFU ml<sup>-1</sup>), significantly reduced the canker disease severity (Riera et al., 2018). On the other hand, a reduction in *Ca. L. asiaticus* population was observed for preventive and curative treatments with secondary metabolites produced by a *P. aeruginosa* (LV strain), sprayed to orange leaves 7 days before or 30 days after bud-graft inoculation of HLB, respectively. This reduction in pathogen infection was attributed to the antibacterial activity of the active compound phenazine, capable of eliciting a systemic resistance response (Pistori et al., 2018).

Other species of the genus *Pseudomonas* also inhibit the growth of *X. citri* *in vitro*, such as *P. viridiflava*, *P. syringae* and *P. fluorescens* (Montakhabi et al., 2011). In particular, *P. fluorescens* strains obtained from the phylloplane of healthy citrus trees act as BCA against *X. citri* in greenhouse conditions, reducing citrus canker in *C. limon* and *C. aurantifolia* when sprayed 3 days before the pathogenic bacteria. The mechanisms involved in the antagonistic effect have not been elucidated in these reports (Khodakaramian et al., 2008; Al-Saleh, 2014).

## 5.2. *Bacillus* genus as BCA

*Bacillus* species represent a large group of Gram-positive bacteria that are natural inhabitants of both, the phyllosphere and rhizosphere of plants. The ability to form endospores and synthesize various antimicrobial metabolites (antibiotics, enzymes and secondary metabolites) identifies the *Bacillus* genus as the most common endophytic bacteria to exhibit remarkable biocontrol against plant diseases. It has been reported that approximately 5–8% of the whole genomes of *Bacillus* spp. are dedicated to biosynthesis of structurally diverse antimicrobial compounds (Fira et al., 2018; Kaspar et al., 2019; Chen et al., 2020).

Different *Bacillus* spp. have shown biocontrol effects to *X. citri* as *in vitro* inhibition of growth (Long and Guanhua, 2012; Liu et al., 2015) or by lysis of cell bacteria (Islam et al., 2019; Rabbee et al., 2019). In particular, it has been shown that *B. subtilis* presents antagonistic activity with both protective and curative effect against ACC. Huang et al. (2012) demonstrated that the application of two soil isolates of *B. subtilis* (WG6-14 and TKS-1) 1 day before *X. citri* inoculation was able to reduce ACC development in *C. aurantifolia*, by interference of colonization and biofilm formation of the pathogen. Likewise, similar mechanisms were observed when leaves of *C. sinensis* were co-inoculated with *Bacillus* spp. ( $10^7$  CFU ml<sup>-1</sup>) by spraying (Caicedo et al., 2016). Moreover,

satisfactory reduction of ACC under field and greenhouse conditions was obtained by single spray pre-inoculation of *B. subtilis* in *C. aurantifolia* (Das et al., 2014; Ibrahim et al., 2016). On the other hand, Daungfu et al. (2019) showed curative control of ACC by *Bacillus* endophytes isolated from healthy *Citrus* spp. phyllosphere and rhizosphere. In this case, the inoculation with *B. subtilis* LE24, *B. tequilensis* PO80 or *B. amyloliquefaciens* LE109 ( $10^8$  CFU ml<sup>-1</sup>) 24 h after *X. citri* infection significantly reduced canker development (0–10% disease incidence). The same results were obtained in leaves treated with crude bioactive compounds from each strain, demonstrating that the bioactive compounds were within the group of lipopeptides (Daungfu et al., 2019). On the other hand, the inoculation with *Bacillus* spp. ( $10^8$  CFU ml<sup>-1</sup>) 72 h after *P. syringae* infection reduced the extent of stem necrosis in *C. sinensis* and *C. limon* after 10 weeks after inoculation (Mougou and Boughalleb-M'hamdi, 2018; Islam et al., 2020).

On the other hand, some *Bacillus* spp. have been reported as promising BCA for HLB. Munir et al. (2020) compared the core endophyte communities on leaves from distinct citrus varieties with different degrees of HLB disease. A higher frequency of *B. subtilis* was found in the healthy/asymptomatic plants compared to the symptomatic plants, suggesting a role for *B. subtilis* in HLB resistance. Root-associated *B. amyloliquefaciens* is known for producing sub-lethal concentrations of cyclic lipopeptides and volatiles that trigger induced systemic resistance (ISR) in plants (Chowdhury et al., 2015). It has been shown that *B. amyloliquefaciens* GJ1 isolated from healthy leaves of *C. sinensis* controls HLB development by reducing 50% of *Ca. L. asiaticus* in infected *C. sinensis* plants. The curative effect performed by root irrigation of the biocontrol bacterial (OD600nm ~ 1) once every 7 days for 45 days triggered several defense responses and promoted starch degradation (Tang et al., 2018a, 2018b). Furthermore, there are *Bacillus* species that can act as indirect BCAs against *Ca. L.* This is the case of endophytic strains of *B. thuringiensis* which translocate from citrus seedling roots to shoots and control *Diaphorina citri* nymphs in the tree canopy (Dorta et al., 2020).

## 5.3. *Xanthomonas* genus as BCA

The induction of resistance pathways by host and non-host *Xanthomonas* spp. is a mechanism proposed for ACC biocontrol. Roeschlin et al. (2017) demonstrated that the pre-inoculation of leaves with a natural variant of *X. citri* (*X. citri* A<sup>T</sup>,  $10^9$  CFU ml<sup>-1</sup>) significantly reduced canker symptoms in *C. limon*. The hypersensitive response triggered by this isolate involves autophagy-associated vacuolar processes and induction of salicylic acid defense responses that mediates the protecting effect. Similar protective results were observed with *Xanthomonas fuscans* ssp. *aurantifolii* strain C (*Xfa*) that induced gene-for-gene host defense response in *C. limon*. The pre-inoculation of leaves with *Xfa* ( $10^9$  CFU ml<sup>-1</sup>), 2 and 7 days before *X. citri* infection ( $10^9$  CFU ml<sup>-1</sup>), significantly reduced ACC (Chiesa et al., 2019). Remarkably, when the same experiment was carried out with the non-host *X. campestris* pv. *campestris* (*Xcc*), the protection was more effective, suggesting that *Xcc* acts as an endophyte in *C. limon* plants. The mechanisms employed by these BCA were associated with oxidative stress and the expression of secondary metabolites (Chiesa et al., 2019).

## 5.4. Other bacterial genera as BCA

Plant-associated microbiota can evade or reduce pathogen infection through direct competition or by stimulating plant immunity (Ginnan et al., 2020). Bacterial citrus diseases, such as HLB, are capable of completely modifying the existing microbial communities in the different organs of the plant. The presence of bacterial genera common to all healthy individuals could identify potential biocontrol agents (Trivedi et al., 2012). In recent years several studies on the citrus microbiome have provided new insights about bacterial communities in citrus trees affected by HLB (Trivedi et al., 2010, 2012; Blaustein et al.,

2017; Ginnan et al., 2018, 2020). It has been demonstrated that the composition and diversity of the leaf and root microbiota were strongly associated with HLB symptom severity (Blaustein et al., 2017). These authors found a negative relationship between *Ca. L.* and three bacterial families (Burkholderiaceae, Xanthomonadaceae, and Micromonosporaceae) reported to have plant-beneficial properties. They also found that other Alphaproteobacteria (*Methylobacterium*, *Sphingomonas*, and *Methylocystaceae*) were present in greater proportions in asymptomatic trees, suggesting a resource-related competition between the pathogen and other members of its bacterial class. On the other hand, Riera et al. (2017) obtained six rhizosphere bacteria from healthy looking citrus trees in severely HLB-diseased citrus groves, that showed *in vitro* antibacterial activity for two bacteria closely related to *Ca. L. asiaticus*. Among them, four belong to gram-negative strains (*Burkholderia metallica*, *B. territori*, *P. granadensis* and *P. geniculata*) and two to gram-positive strains (*Rhodococcus jialingiae* and *B. pumilis*). In the same way, bacteria from the genera *Bacillus*, *Lactobacillus*, *Streptomyces*, *Methylobacterium*, *Hymenobacter*, *Pantoea*, *Curtobacterium* and *Spirosoma*, obtained from infected citrus budwood, leaves, and roots were reported as possible biocontrol agents against *Ca. L.* (Ginnan et al., 2018, 2020).

Other reports highlighted the relationships among xylem endophytic bacterial populations of *Methylobacterium* spp. and *Curtobacterium flaccumfaciens* with the balance of *X. fastidiosa* in citrus plants (Azevedo et al., 2016). The secondary metabolites produced by these bacteria promote down-regulation of gene expression related to energy production, stress, transport and motility in *X. fastidiosa* inhibiting CVC development (Lacava et al., 2004; Azevedo et al., 2016). Moreover, different isolates of *Methylobacterium* spp. have been described as effective biocontrol agents against *X. fastidiosa* (Araújo et al., 2002) and *Ca. L.* (Trivedi et al., 2010) in *C. sinensis*.

On the other hand, the human and animal pathogen *Acinetobacter baumannii* isolated from citrus orchard soil was capable of reducing populations of *X. citri* both *in vitro* and in *C. limon* plants more than 50% (Tan et al., 2006). However, the mechanisms involved in this interaction remain uncertain.

## 6. Bacteriophage biocontrol against bacterial citrus diseases

The use of bacteriophages has received increased research interest in recent years as a realistic environmentally friendly means of controlling bacterial diseases in agriculture. Their use presents a viable control measure for a number of destructive bacterial crop diseases, with some phage-based products already becoming available on the market. Phage biocontrol possesses advantages over chemical controls in that tailor-made phage cocktails can be adapted to target specific disease-causing bacteria, and can be easily adapted for bacterial resistance which may develop over time (Buttimer et al., 2017; Kering et al., 2019). After invading a living bacterial cell, bacteriophages can multiply through two different mechanisms, the lytic cycle as phage and the lysogenic cycle as prophage. The identification of phages to use as part of a biological control strategy starts with different criteria for selection but clearly the pre-screening of phages for their potential value as biocontrol agents is preferred rather than arbitrarily selecting them based on lytic activity alone (Jones et al., 2012).

The use of bacteriophages against bacterial diseases in citrus has reported some success cases both *in vitro* and in greenhouse against *X. citri*. *In vitro*, the ability to lyse bacterial cells by bacteriophages Cp1 and Cp2 has been described (Ahmad et al., 2014a). Additionally, the filamentous phage XacF1, member of the family Inoviridae, causes several physiological changes to the bacterial host cells *in vitro*, including lower levels of extracellular polysaccharide production, reduced motility, slower growth rate and a dramatic reduction in virulence (Ahmad et al., 2014b). Moreover, under greenhouse conditions the treatment of leaves with a mixture of bacteriophages Cp2, ΦXac2005-1, ccΦ7 and ccΦ13, 24 h before the inoculation with *X. citri* provided significant disease reduction on *C. paradisi*, being equally effective as

copper-bactericide (Balogh et al., 2008). In addition, field application of a mixture of bacteriophages isolated from wild type *X. citri* strains twice a week significantly reduced ACC disease in *C. aurantifolia* through bacterial cell lysis (Ibrahim et al., 2017). Recently, *in vitro* studies have demonstrated that the commercially available phage Φ6 controlled *P. syringae* development through bacterial cell lysis *in vitro* (Pinheiro et al., 2019).

Phage-based therapy has potential for controlling citrus bacterial diseases, such as HLB, if the delivery method can be optimized (Blaustein et al., 2018). Prophages are highly dynamic components in the *Ca. L.* genome and play an important role in intra-species variations, mediating dynamics of bacterial populations in plant and insect hosts, and their correlation with insect transmission and disease development (Zhou et al., 2013).

## 7. Fungal biocontrol against bacterial citrus diseases

The ability of different mycorrhizal and endophytic filamentous fungi to control different types of diseases has been widely studied in recent years, both through direct mechanisms and thanks to the activation of plant defense responses (Poveda et al., 2020). For example, *Trichoderma* species secrete proteins related to the induction of plant resistance in citrus against the fungal pathogen *P. citricarpa* (teleomorph: *Guignardia citricarpa*) (de Lima et al., 2017). Regarding bacterial diseases in citrus, it would be interesting to assay if different *Trichoderma* strains could be able to induce resistance, although there are no studies yet developed in this regard. Despite this, in 2018, Ginnan et al. performed a massive sequencing of the endophytic fungal communities present in the roots of various Citrus species with HLB. The study revealed how all the analyzed individuals had in common several genera of endophytic fungi (*Cladosporium* sp., *Sporobolomyces* sp., *Symmetterspora* sp., *Campophora* sp., *Hannaella* sp., *Exophiala* sp., *Fusarium* sp. and *Acrocalymma* sp.) and mycorrhizal fungi (*Glomus* sp. and *Rhizophagus* sp.), hypothesizing their possible role as suppressors of the disease (Ginnan et al., 2018). Further studies demonstrated that the decrease in potentially beneficial mycorrhizal fungi of the phylum Glomeromycota coupled with the increase in *Fusarium* or *Gibberella* fungal parasites were major contributors to the root decline observed in HLB affected trees (Ginnan et al., 2020).

Blacutt et al. (2020) studied the microbiome from citrus roots, stems and leaves to identify potential anti-*Ca. L.* BCA. The fungi *Cladosporium cladosporioides* and *Epicoccum nigrum* showed significant inhibitory activity against *Liberibacter crescens*, a culturable surrogate for the unculturable HLB-associated bacterium. The purified bioactive natural products cladosporols A, C, and D with anti *Ca. L.* activity were identified from the fungus *C. cladosporioides* (Blacutt et al., 2020).

## 8. Conclusions

The agricultural and economic importance of citrus crops worldwide, producing more than 125 billion tons per year, highlights the need to achieve production with the least possible affection by plant pathogens. Generally, ACC is managed with copper application, but this favors the development of bacterial resistance and harmful effects on the environment. On the other hand, effective strategies to control CVC and HLB are limited to date. Therefore, it is essential to develop effective biocontrol agents against these diseases, which affect numerous commercial varieties of citrus.

HLB, ACC and CVC represent bacterial citrus diseases that significantly reduce crop productivity and cause the death of millions of trees every year. In this sense, numerous studies have been carried out that demonstrate the effectiveness under *in vitro*, greenhouse and field conditions to combat these diseases using antagonistic bacteria. Species such as *P. fluorescens*, *P. aeruginosa*, *B. subtilis*, *B. amyloliquefaciens* or *C. flaccumfaciens* represent good alternatives in the control of bacterial citrus diseases thanks to mechanisms such as antibiosis, competition for

space and nutrients, growth promotion or activation of plant defense responses. In this sense, it is important to highlight the easy accessibility for their use in field, since they are species widely distributed in different products on the market today, whose field application has been developed for years. Therefore, we are talking about accessible, profitable, established use and efficient products.

Currently, the study of the microbiome of citrus diseased and healthy plants is providing information about promising microbial communities for HLB and CVC management. On the other hand, the use of bacteriophages in the biocontrol of these diseases still needs further study, as is the case with many other bacterial diseases, as the number of lines of research in this field is now starting to increase significantly. Therefore, they represent biocontrol agents with great potential, with very promising results and whose research may have important economic and agronomic benefits for the citrus sector.

Finally, the use of fungi against this group of citrus diseases has been hypothesized, which begins some potential future lines of research for effective biocontrol in the field of highly harmful bacteria.

### Author contributions

J.P. conceived and designed the manuscript. J.P. performed the bibliographic search and analyzed the information. J.P. wrote the first manuscript. R.A.R., M.R.M. and M.A.F. contributed to the manuscript writing and the correction and critical reading, as well as to the knowledge on the bacteria and citrus crops fields.

### References

- Ahmad, A.A., Ogawa, M., Kawasaki, T., Fujie, M., Yamada, T., 2014a. Characterization of bacteriophages Cp1 and Cp2, the strain-typing agents for *Xanthomonas axonopodis* pv. *citri*. *App. Environ. Microbiol.* 80, 77–85. <https://doi.org/10.1128/AEM.02310-13>.
- Ahmad, A.A., Askora, A., Kawasaki, T., Fujie, M., Yamada, T., 2014b. The filamentous phage XacF1 causes loss of virulence in *Xanthomonas axonopodis* pv. *citri*, the causative agent of citrus canker disease. *Front. Microbiol.* 5, 321. <https://doi.org/10.3389/fmicb.2014.00321>.
- Al-Saleh, M.A., 2014. Evaluation of saudi fluorescent *Pseudomonads* isolates as a biocontrol agent against citrus canker disease caused by *Xanthomonas citri* subsp. *citri* a. *Egypt. Acad. J. Biol. Sci.* 6, 1–7.
- Araújo, W.L., Marcon, J., Maccheroni, W., van Elsland, J.D., van Vuurde, J.W., Azevedo, J. L., 2002. Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. *App. Environ. Microbiol.* 68, 4906–4914. <https://doi.org/10.1128/AEM.68.10.4906-4914.2002>.
- Azevedo, J.L., Araújo, W.L., Lacava, P.T., 2016. The diversity of citrus endophytic bacteria and their interactions with *Xylella fastidiosa* and host plants. *Genet. Mol. Biol.* 39, 476–491. <https://doi.org/10.1590/1678-4685-gmb-2016-0056>.
- Balogh, B., Canteros, B.L., Stall, R.E., Jones, J.B., 2008. Control of citrus canker and citrus bacterial spot with bacteriophages. *Plant Dis.* 92, 1048–1052. <https://doi.org/10.1094/PDIS-92-7-1048>.
- Behlau, F., Belasque Jr., J., Graham, J.H., Leite Jr., R.P., 2010. Effect of frequency of copper applications on control of citrus canker and the yield of young bearing sweet orange trees. *Crop Prot.* 29, 300–305. <https://doi.org/10.1016/j.cropro.2009.12.010>.
- Behlau, F., Hong, J.C., Jones, J.B., Graham, J.H., 2013. Evidence for acquisition of copper resistance genes from different sources in citrus associated xanthomonads. *Phytopathology* 103, 409–418. <https://doi.org/10.1094/PHYTO-06-12-0134-R>.
- Behlau, F., Gochez, A.M., Jones, J.F., 2020. Diversity and copper resistance of *Xanthomonas* affecting citrus. *Trop. Plant Pathol.* 45, 200–212. <https://doi.org/10.1007/s40858-020-00340-1>.
- Biessy, A., Filion, M., 2018. Phenazines in plant-beneficial *Pseudomonas* spp.: biosynthesis, regulation, function and genomics. *Environ. Microbiol.* 20, 3905–3917. <https://doi.org/10.1111/1462-2920.14395>.
- Blacutt, A., Ginnan, N., Dang, T., Bodaghi, S., Vidalakis, G., Ruegger, P., et al., 2020. An *in vitro* pipeline for screening and selection of citrus-associated microbiota with potential anti-*Candidatus* Liberibacter asiaticus” properties. *Appl. Environ. Microbiol.* 86, e02883–19. <https://doi.org/10.1128/AEM.02883-19>.
- Blaustein, R.A., Lorca, G.L., Meyer, J.L., Gonzalez, C.F., Teplitski, M., 2017. Defining the core citrus leaf- and root-associated microbiota: factors associated with community structure and implications for managing huanglongbing (citrus greening) disease. *Appl. Environ. Microbiol.* 83, e00210–17. <https://doi.org/10.1128/AEM.00210-17>.
- Blaustein, R.A., Lorca, G.L., Teplitski, M., 2018. Challenges for managing *Candidatus* Liberibacter spp. (huanglongbing disease pathogen): current control measures and future directions. *Phytopathol.* 108, 424–435. <https://doi.org/10.1094/PHYTO-07-17-0260-RVW>.
- Bock, C.H., Parker, P.E., Gottwald, T.R., 2005. Effect of simulated wind-driven rain on duration and distance of dispersal of *Xanthomonas axonopodis* pv. *citri* from canker-infected citrus trees. *Plant Dis.* 89, 71–80. <https://doi.org/10.1094/PD-89-0071>.
- Buttimer, C., McAuliffe, O., Ross, R.P., Hill, C., O'Mahony, J., Coffey, A., 2017. Bacteriophages and bacterial plant diseases. *Front. Microbiol.* 8, 34. <https://doi.org/10.3389/fmicb.2017.00034>.
- Caicedo, J.C., Villamizar, S., Ferro, M.I.T., Kupper, K.C., Ferro, J.A., 2016. Bacteria from the citrus phylloplane can disrupt cell–cell signalling in *Xanthomonas citri* and reduce citrus canker disease severity. *Plant Pathol.* 65, 782–791. <https://doi.org/10.1111/ppa.12466>.
- Chen, K., Tian, Z., He, H., Long, C., Jiang, F., 2020. *Bacillus* species as potential biocontrol agents against citrus diseases. *Biol. Control* 151, 104419. <https://doi.org/10.1016/j.biocontrol.2020.104419>.
- Chiesa, M.A., Roeschlin, R.A., Favaro, M.A., Uviedo, F., Campos Beneyto, L., D'Andrea, R., Gadea, J., Marano, M.R., 2019. Plant responses underlying nonhost resistance of *Citrus limon* against *Xanthomonas campestris* pv. *campestris*. *Mol. Plant Pathol.* 20, 254–269. <https://doi.org/10.1111/mpp.12752>.
- Chowdhury, S.P., Hartmann, A., Gao, X., Borriss, R., 2015. Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42—a review. *Front. Microbiol.* 6, 780. <https://doi.org/10.3389/fmicb.2015.00780>.
- Cobine, P.A., Cruz, L.F., Navarrete, F., Duncan, D., Tygart, M., De La Fuente, L., 2013. *Xylella fastidiosa* differentially accumulates mineral elements in biofilm and planktonic cells. *PLoS One* 8, e54936. <https://doi.org/10.1371/journal.pone.0054936>.
- Cuenca, J., Garcia-Lor, A., Navarro, L., Aleza, P., 2018. Citrus genetics and breeding. In: Al-Khayri, J.M., Jain, S.M., Johnson, D.V. (Eds.), *Advances in Plant Breeding Strategies: Fruits*. Springer, Cham, pp. 403–436.
- da Graça, J.V., Douhan, G.W., Halbert, S.E., Keremane, M.L., Lee, R.F., Vidalakis, G., Zhao, H., 2016. Huanglongbing: an overview of a complex pathosystem ravaging the world's citrus. *J. Integr. Plant Biol.* 58, 373–387. <https://doi.org/10.1111/jipb.12437>.
- Das, R., Mondal, B., Mondal, P., Khatua, D.C., Mukherjee, N., 2014. Biological management of citrus canker on acid lime through *Bacillus subtilis* (S-12) in West Bengal, India. *J. Biopest.* 7, 38–41.
- Daungfu, O., Youpensuk, S., Lumyong, S., 2019. Endophytic bacteria isolated from citrus plants for biological control of citrus canker in lime plants. *Trop. Life Sci. Res.* 30, 73. <https://doi.org/10.21315/tlsr2019.30.1.5>.
- de Lima, F.B., Félix, C., Osório, N., Alves, A., Vitorino, R., Domingues, P., et al., 2017. *Trichoderma harzianum* T1A constitutively secretes proteins involved in the biological control of *Guignardia citricarpa*. *Biol. Control* 106, 99–109. <https://doi.org/10.1016/j.biocontrol.2017.01.003>.
- de Oliveira, A.G., Murate, L.S., Spago, F.R., de Paula Lopes, L., de Oliveira Beranger, J.P., San Martin, J.A.B., et al., 2011. Evaluation of the antibiotic activity of extracellular compounds produced by the *Pseudomonas* strain against the *Xanthomonas citri* pv. *citri* 306 strain. *Biol. Control* 56, 125–131. <https://doi.org/10.1016/j.biocontrol.2010.10.008>.
- de Oliveira, A.G., Spago, F.R., Simionato, A.S., Navarro, M.O., da Silva, C.S., Barazzetti, A. R., et al., 2016. Bioactive organocopper compound from *Pseudomonas aeruginosa* inhibits the growth of *Xanthomonas citri* subsp. *citri*. *Front. Microbiol.* 7, 113. <https://doi.org/10.3389/fmicb.2016.00113>.
- Dong, Y.L., Tang, Q.J., Yi, T.Y., Xiao, Q.M., 2012. Screening and identification of antagonistic bacteria against citrus canker from soil and determination of its control efficacy. *Hunan Agric. Sci.* 9, 026.
- Dorta, S.D.O., Ballinotte, J., Monnerat, R., Lopes, J.R.S., da Cunha, T., Zanardi, O.Z., et al., 2020. Selection of *Bacillus thuringiensis* strains in citrus and their pathogenicity to *Diaphorina citri* (Hemiptera: Liviidae) nymphs. *Insect Sci.* 27, 519–530. <https://doi.org/10.1111/1744-7917.12654>.
- Favaro, M.A., Micheloud, N.G., Roeschlin, R.A., Chiesa, M.A., Castagnaro, A.P., Vojnov, A.A., Gmitter Jr., F.G., Gadea, J., Rista, L.M., Gariglio, N.F., Marano, M.R., 2014. Surface barriers of mandarin 'Okitsu' leaves make a major contribution to canker disease resistance. *Phytopathol.* 104, 970–976. <https://doi.org/10.1094/PHYTO-10-13-0277-R>.
- Favaro, M.A., Roeschlin, R.A., Ribero, G.G., Maumary, R.L., Fernández, L.N., Lutz, A., Sillon, M.R., Rista, L.M., Marano, M.R., Gariglio, N.F., 2017. Relationships between copper content in orange leaves, bacterial biofilm formation and citrus canker disease control after different copper treatments. *Crop Prot.* 92, 182–189.
- Favaro, M.A., Molina, M.C., Roeschlin, R.A., Gadea Vacas, J., Gariglio, N.F., Marano, M., 2020. Different responses in mandarin cultivars uncover a role of cuticular waxes in the resistance to citrus canker. *Phytopathol.* <https://doi.org/10.1094/PHYTO-02-20-0053-R>.
- Ference, C.M., Gochez, A.M., Behlau, F., Wang, N., Graham, J.H., Jones, J.B., 2018. Recent advances in the understanding of *Xanthomonas citri* ssp. *citri* pathogenesis and citrus canker disease management. *Mol. Plant Pathol.* 19, 1302–1318. <https://doi.org/10.1111/mpp.12638>.
- Fira, D., Dimkić, I., Berić, T., Lozo, J., Stanković, S., 2018. Biological control of plant pathogens by *Bacillus* species. *J. Biotechnol.* 285, 44–55. <https://doi.org/10.1016/j.jbiotec.2018.07.044>.
- Gabriel, D., Gottwald, T., Lopes, S.A., Wulff, N.A., 2020. Bacterial pathogens of citrus: Citrus canker, citrus variegated chlorosis and Huanglongbing. In: Talon, M., Caruso, M., Fred, G., Gmitter, F.G. (Eds.), *The Genus Citrus*. Woodhead Publishing, Cambridge, pp. 371–389.
- Ge, Q., Cobine, P.A., De La Fuente, L., 2020. Copper supplementation in watering solution reaches the xylem but does not protect tobacco plants against *Xylella fastidiosa* infection. *Plant Dis.* 104, 724–730. <https://doi.org/10.1094/PDIS-08-19-1748-RE>.

- Giassi, V., Kiritani, C., Kupper, K.C., 2016. Bacteria as growth-promoting agents for citrus rootstocks. *Microbiol. Res.* 190, 46–54. <https://doi.org/10.1016/j.micres.2015.12.006>.
- Ginnan, N.A., Dang, T., Bodaghi, S., Ruegger, P.M., McCollum, G., England, G., et al., 2020. Disease-induced microbial shifts in citrus indicate microbiome-derived responses to Huanglongbing across the disease severity spectrum. *Phytobiomes J.* <https://doi.org/10.1094/PBIOMES-04-20-0027-R>.
- Ginnan, N.A., Dang, T., Bodaghi, S., Ruegger, P.M., Peacock, B.B., McCollum, G., et al., 2018. Bacterial and fungal next generation sequencing datasets and metadata from citrus infected with 'Candidatus Liberibacter asiaticus'. 2, 64–70. DOI:10.1094/PBIOMES-08-17-0032-A.
- Gonçalves-Zuliani, A.M.O., Cardoso, K.A.K., Belasque Jr., J., Zanutto, C.A., Hashiguti, H. T., Bock, C.H., Nakamura, C.V., Nunes, W.M.C., 2016. Reaction of detached leaves from different varieties of sweet orange to inoculation with *Xanthomonas citri* subsp. *citri*. *Summa Phytopathol.* 42, 125–133. <https://doi.org/10.1590/0100-5405/2143>.
- González-González, M.G., Gómez-Sanchis, J., Blasco, J., Soria-Olivas, E., Chueca, P., 2020. CitrusYield: a dashboard for mapping yield and fruit quality of citrus in precision agriculture. *Agronomy* 10, 128. <https://doi.org/10.3390/agronomy10010128>.
- Graham, J.H., Gottwald, T.R., Cubero, J., Achor, D.S., 2004. *Xanthomonas axonopodis* pv. *citri*: factors affecting successful eradication of citrus canker. *Mol. Plant Pathol.* 5, 1–15. <https://doi.org/10.1046/j.1364-3703.2004.00197.x>.
- Guarnaccia, V., Gehrmann, T., Silva-Junior, G.J., Fourie, P.H., Haridas, S., Vu, D., et al., 2019. *Phyllosticta citricarpa* and sister species of global importance to Citrus. *Mol. Plant Pathol.* 20, 1619–1635. <https://doi.org/10.1111/mpp.12861>.
- Höfte, M., Altier, N., 2010. Fluorescent pseudomonads as biocontrol agents for sustainable agricultural systems. *Res. Microbiol.* 161, 464–471. <https://doi.org/10.1016/j.resmic.2010.04.007>.
- Huang, T.P., Tzeng, D.D.S., Wong, A.C., Chen, C.H., Lu, K.M., Lee, Y.H., et al., 2012. DNA polymorphisms and biocontrol of *Bacillus* antagonistic to citrus bacterial canker with indication of the interference of phyllosphere biofilms. *PLoS One* 7, e42124. <https://doi.org/10.1371/journal.pone.0042124>.
- Ibrahim, Y.E., Saleh, A.A., El Komy, M.H., Al Saleh, M.A., 2016. *Bacillus subtilis* QST 713, copper hydroxide, and their tank mixes for control of bacterial citrus canker in Saudi Arabia. *J. Citrus Pathol.* 3.
- Ibrahim, Y.E., Saleh, A.A., Al-Saleh, M.A., 2017. Management of asiatic citrus canker under field conditions in Saudi Arabia using bacteriophages and acibenzolar-S-methyl. *Plant Dis.* 101, 761–765. <https://doi.org/10.1094/PDIS-08-16-1213-RE>.
- Islam, M.N., Ali, M.S., Choi, S.J., Hyun, J.W., Baek, K.H., 2019. Biocontrol of citrus canker disease caused by *Xanthomonas citri* subsp. *citri* using an endophytic *Bacillus thuringiensis*. *Plant Pathol. J.* 35, 486. <https://doi.org/10.5423/PPJ.OA.03.2019.0060>.
- Islam, M.S., Sultana, R., Hasan, M.A., Alam, M.S., Sikdar, B., Kamaruzzaman, M., Islam, M.A., 2020. Characterization and biocontrol measures of *Pseudomonas syringae* pv. *syringae* associated with citrus blast disease. *Vegetos* 33, 555–569. <https://doi.org/10.1007/s42535-020-00138-1>.
- Ivanović, Ž., Perović, T., Popović, T., Blagojević, J., Trkulja, N., Hrnčić, S., 2017. Characterization of *Pseudomonas syringae* pv. *syringae*, causal agent of citrus blast of mandarin in Montenegro. *Plant Pathol. J.* 33, 21–23. <https://doi.org/10.5423/PPJ.OA.08.2016.0161>.
- Jannah, M., Agustien, A., Zam, S.I., Lalfari, R.S., Aldi, Y., Dewi, A.P., Djamaan, A., 2018. Isolation and characterization of antibiotic-producing endophytic bacteria from *Citrus aurantifolia* swingle. *J. Pure and Appl. Microbiol.* 12, 1473–1481. <https://doi.org/10.22207/JPAM.12.3.51>.
- Jaoud, M., Moinina, A., Ezrari, S., Lahlali, R., 2020. Key pests and diseases of citrus trees with emphasis on root rot diseases: an overview. *Mor. J. Agri. Sci.* 1, 149–160.
- Jones, J.B., Vallad, G.E., Iriarte, F.B., Obradović, A., Wernsing, M.H., Jackson, L.E., Balogh, B., Hong, J.C., Momol, M.T., 2012. Considerations for using bacteriophages for plant disease control. *Bacteriophage* 2, 208–214. <https://doi.org/10.4161/bact.23857>.
- Kaspar, F., Neubauer, P., Gimpel, M., 2019. Bioactive secondary metabolites from *Bacillus subtilis*: a comprehensive review. *J. Nat. Prod.* 82, 2038–2053. <https://doi.org/10.1021/acs.jnatprod.9b00110>.
- Kering, K.K., Kibii, B.J., Wei, H., 2019. Biocontrol of phyto-bacteria with bacteriophage cocktails. *Pest Manag. Sci.* 75, 1775–1781. <https://doi.org/10.1002/ps.5324>.
- Khodakaramian, A., Heydari, A., Balestra, G.M., 2008. Evaluation of pseudomonads bacterial isolates in biological control of citrus bacterial canker disease. *Int. J. Agric. Res.* 3, 268–272.
- Lacava, P.T., Araújo, W.L., Marcon, J., Maccheroni Jr, W., Azevedo, J.L., 2004. Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa*, causal agent of citrus-variegated chlorosis. *Let. Appl. Microbiol.* 39, 55–59. <https://doi.org/10.1111/j.1472-765X.2004.01543.x>.
- Liu, B., Song, S., Liu, X.L., Yang, M.X., Gong, L.L., 2015. Screening, identification of bio-control endophytic bacterium against citrus canker and stability of its bioactive metabolites. *Acta Agric. Slov.* 27, 2152–2158.
- Long, Y., Guanhu, M., 2012. Screening of antagonistic spore bacteria for biocontrol against *Xanthomonas axonopodis* pv. *citri*. *South China Fruits* 4.
- Lopes, S.A., 2020. Scion substitution: a new strategy to control citrus variegated chlorosis disease. *Plant Dis.* 104, 239–245. <https://doi.org/10.1094/PDIS-02-19-0302-RE>.
- Marin, V.R., Ferrarezi, J.H., Vieira, G., Sass, D.C., 2019. Recent advances in the biocontrol of *Xanthomonas* spp. *World J. Microbiol. Biotechnol.* 35, 1–11.
- Martins, P.M.M., de Oliveira Andrade, M., Benedetti, C.E., de Souza, A.A., 2020. *Xanthomonas citri* subsp. *citri*: host interaction and control strategies. *Trop. Plant Pathol.* 45, 213–236.
- Maurício, F.N., Soratto, T.A.T., Diogo, J.A., Boscariol-Camargo, R.L., De Souza, A.A., Coletta-Filho, H.D., et al., 2019. Analysis of defense-related gene expression in citrus hybrids infected by *Xylella fastidiosa*. *Phytopathol.* 109, 301–306. <https://doi.org/10.1094/PHYTO-09-18-0366-FI>.
- Mendonça, L.B.P., Zambolim, L., Badel, J.L., 2017. Bacterial citrus diseases: major threats and recent progress. *J. Bacteriol. Mycol.* 5, 00143. <https://doi.org/10.15406/jbmoa.2017.05.00143>.
- Mercado-Blanco, J., Bakker, P., 2007. Interactions between plants and beneficial *Pseudomonas* spp.: exploiting bacterial traits for crop protection. *Antonie Van Leeuwen* 92, 367–389. <https://doi.org/10.1007/s10482-007-9167-1>.
- Michavila, G., Adler, C., De Gregorio, P.R., Lami, M.J., Caram Di Santo, M.C., Zenoff, A. M., et al., 2017. *Pseudomonas protegens* CS 1 from the lemon phyllosphere as a candidate for citrus canker biocontrol agent. *Plant Biol.* 19, 608–617. <https://doi.org/10.1111/plb.12556>.
- Montakhabi, M.K., Rahimian, H., Falahati, R.M., Jafarpour, B., 2011. *In vitro* investigation on biocontrol of *Xanthomonas axonopodis* pv. *citri* cause of citrus bacterial canker by citrus antagonistic bacteria. *J. Plant Prot.* 24, 368–376.
- Mougou, I., Boughalleb-M'hamdi, N., 2018. Biocontrol of *Pseudomonas syringae* pv. *syringae* affecting citrus orchards in Tunisia by using indigenous *Bacillus* spp. and garlic extract. *Egypt. J. Biol. Pest Control* 28, 1–11.
- Munir, S., He, P., Wu, Y., He, P., Khan, S., Huang, M., et al., 2018. Huanglongbing control: perhaps the end of the beginning. *Microbiol. Ecol.* 76, 192–204. <https://doi.org/10.1007/s00248-017-1123-7>.
- Munir, S., Li, Y., He, P., Huang, M., He, P., Cui, W., Wu, Y., He, Y., 2020. Core endophyte communities of different citrus varieties from citrus growing regions in China. *Sci. Rep.* 10, 3648. <https://doi.org/10.1038/s41598-020-60350-6>.
- Murate, L.S., de Oliveira, A.G., Higashi, A.Y., Barazetti, A.R., Simionato, A.S., da Silva, C. S., et al., 2015. Activity of secondary bacterial metabolites in the control of citrus canker. *Agri. Sci.* 6, 295–303.
- Nunes, C., 2012. Biological control of postharvest diseases of fruit. *Eur. J. Plant Pathol.* 133, 181–196. <https://doi.org/10.1007/s10658-011-9919-7>.
- O'Brien, P.A., 2017. Biological control of plant diseases. *Australas. Plant Pathol.* 46, 293–304. <https://doi.org/10.1007/s13313-017-0481-4>.
- Pinheiro, L.A., Pereira, C., Frazão, C., Balcão, V.M., Almeida, A., 2019. Efficiency of phage  $\phi 6$  for biocontrol of *Pseudomonas syringae* pv. *syringae*: an *in vitro* preliminary study. *Microorganisms* 7, 286. <https://doi.org/10.3390/microorganisms7090286>.
- Pistori, J.F., Simionato, A.S., Navarro, M.O., Andreatta, M.F., Santos, I.M., Meneguim, L., et al., 2018. Low-molecular-weight metabolites produced by *Pseudomonas aeruginosa* as an alternative to control Huanglongbing in *Citrus sinensis* cv. Valencia. *Trop. Plant Pathol.* 43, 289–296. <https://doi.org/10.1007/s40858-018-0231-3>.
- Poveda, J., Abril-Urias, P., Escobar, C., 2020. Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: *Trichoderma*, mycorrhizal and endophytic fungi. *Front. Microbiol.* 11, 992. <https://doi.org/10.3389/fmicb.2020.00992>.
- Rabbee, M.F., Ali, M., Baek, K.H., 2019. Endophyte *Bacillus velezensis* isolated from *Citrus* spp. controls streptomycin-resistant *Xanthomonas citri* subsp. *citri* that causes citrus bacterial canker. *Agronomy* 9, 470. <https://doi.org/10.3390/agronomy9080470>.
- Rapicavoli, J., Ingel, B., Blanco-Ulate, B., Cantu, D., Roper, C., 2018. *Xylella fastidiosa*: an examination of a re-emerging plant pathogen. *Mol. Plant Pathol.* 19, 786–800. <https://doi.org/10.1111/mpp.12585>.
- Redondo, C., Sena-Vélez, M., Gell, I., Ferragud, E., Sabuquillo, P., Graham, J.H., Cubero, J., 2015. Influence of selected bactericides on biofilm formation and viability of *Xanthomonas citri* subsp. *citri*. *Crop Prot.* 78, 204–213. <https://doi.org/10.1016/j.cropro.2015.09.010>.
- Riera, N., Handique, U., Zhang, Y., Dewdney, M.M., Wang, N., 2017. Characterization of antimicrobial-producing beneficial bacteria isolated from Huanglongbing Escape citrus trees. *Front. Microbiol.* 8, 2415. <https://doi.org/10.3389/fmicb.2017.02415>.
- Riera, N., Wang, H., Li, Y., Li, J., Pelz-Stelinski, K., Wang, N., 2018. Induced systemic resistance against citrus canker disease by rhizobacteria. *Phytopathol.* 108, 1038–1045. <https://doi.org/10.1094/PHYTO-07-17-0244-R>.
- Rigano, L.A., Siciliano, F., Enrique, R., Sendin, L., Filippone, P., Torres, P.S., Questa, J., Dow, J.M., Castagnaro, A.P., Vojnov, A.A., Marano, M.R., 2007. Biofilm formation, epiphytic fitness, and canker development in *Xanthomonas axonopodis* pv. *citri*. *Mol. Plant-Microbe Interact.* 20, 1222–1230. <https://doi.org/10.1094/MPMI-20-10-1222>.
- Roeschlin, R.A., Favaro, M.A., Chiesa, M.A., Alemanno, S., Vojnov, A.A., Castagnaro, A.P., Filippone, M.P., Gmitter Jr, F.G., Gadea, J., Marano, M.R., 2017. Resistance to citrus canker induced by a variant of *Xanthomonas citri* ssp. *citri* is associated with a hypersensitive cell death response involving autophagy-associated vacuolar processes. *Mol. Plant Pathol.* 18, 1267–1281. <https://doi.org/10.1111/mpp.12489>.
- Siciliano, F., Torres, P., Sendin, L., Bermejo, C., Filippone, P., Vellice, G., et al., 2006. Analysis of the molecular basis of *Xanthomonas axonopodis* pv. *citri* pathogenesis in *Citrus limon*. *Elect. J. Biotech.* 9, 200–204. <https://doi.org/10.2225/vol9-issue3-20>.
- Spago, F.R., Mauro, C.I., Oliveira, A.G., Beranger, J.P.O., Cely, M.V.T., Stanganelli, M.M., et al., 2014. *Pseudomonas aeruginosa* produces secondary metabolites that have biological activity against plant pathogenic *Xanthomonas* species. *Crop Prot.* 62, 46–54. <https://doi.org/10.1016/j.cropro.2014.04.011>.
- Sudyoung, N., Tokuyama, S., Krajangsang, S., Pringsulaka, O., Sarawaneeyaruk, S., 2020. Bacterial antagonists and their cell-free cultures efficiently suppress canker disease in citrus lime. *J. Plant Dis. Prot.* 127, 173–181. <https://doi.org/10.1007/s41348-019-00295-9>.
- Sun, L., Ke, F., Nie, Z., Wang, P., Xu, J., 2019. Citrus genetic engineering for disease resistance: past, present and future. *Int. J. Mol. Sci.* 20, 5256. <https://doi.org/10.3390/ijms20215256>.
- Tan, X.Y., Huang, S.L., Ren, J.G., Yan, W.H., Cen, Z.L., 2006. Study on a bacterial strain Bt8 for biocontrol against citrus bacterial canker. *Acta Microbiol. Sin.* 4, 292.
- Tang, J., Ding, Y., Nan, J., Yang, X., Sun, L., Zhao, X., Jiang, L., 2018a. Transcriptome sequencing and ITRAQ reveal the detoxification mechanism of *Bacillus* GJ1, a

- potential biocontrol agent for Huanglongbing. *PLoS One* 13, e0200427. <https://doi.org/10.1371/journal.pone.0200427>.
- Tang, J.Z., Ding, Y.X., Deng, L., Nan, J., Yang, X.Y., Zhao, X.Y., 2018b. Response of *Candidatus Liberibacter asiaticus*-infected citrus plants to *Bacillus amyloliquefaciens* GJ1. *Austin J. Plant Biol.* 4, 1018.
- Timilsina, S., Potnis, N., Newberry, E.E., Liyanapathirana, P., Iruegas-Bocardo, F., White, F.F., Goss, E.M., Jones, J.B., 2020. *Xanthomonas* diversity, virulence and plant-pathogen interactions. *Nat. Rev. Microbiol.* 18, 415–427. <https://doi.org/10.1038/s41579-020-0361-8>.
- Trivedi, P., Duan, Y., Wang, N., 2010. Huanglongbing, a systemic disease, restructures the bacterial community associated with citrus roots. *Appl. Environ. Microbiol.* 76, 3427–3436. <https://doi.org/10.1128/AEM.02901-09>.
- Trivedi, P., He, Z., Van Nostrand, J.D., Albrigo, G., Zhou, J., Wang, N., 2012. Huanglongbing alters the structure and functional diversity of microbial communities associated with citrus rhizosphere. *ISME J.* 6, 363–383. <https://doi.org/10.1038/ismej.2011.100>.
- Villamizar, S., Ferro, J.A., Caicedo, J.C., Alves, L.M.C., 2020. Bactericidal effect of entomopathogenic bacterium *Pseudomonas entomophila* against *Xanthomonas citri* reduces citrus canker disease severity. *Front. Microbiol.* 11, 1431. <https://doi.org/10.3389/fmicb.2020.01431>.
- Vojnov, A.A., Do Amaral, A.M., Dow, J.M., Castagnaro, A.P., Marano, M.R., 2010. Bacteria causing important diseases of citrus utilise distinct modes of pathogenesis to attack a common host. *Appl. Microbiol. Biotechnol.* 87, 467–477. <https://doi.org/10.1007/s00253-010-2631-2>.
- Vojnov, A.A., Marano, M.R., 2015. Biofilm formation and virulence in bacterial plant pathogens. In: Wang, N., Jones, J.B., Sundin, G.W., White, F.F., Hogenhout, S.A., Roper, C., de La Fuente, L., Ham, J.H. (Eds.), *Virulence Mechanisms of Plant-Pathogenic Bacteria*. APS Press, St. Paul, pp. 21–34.
- Wang, Y., Fua, X.Z., Liua, J.H., Hong, N., 2011. Differential structure and physiological response to canker challenge between 'Meiwa' kumquat and 'Newhall' navel orange with contrasting resistance. *Sci. Hortic.* 128, 115–123. <https://doi.org/10.1016/j.scienta.2011.01.010>.
- Wang, N., Trivedi, P., 2013. Citrus huanglongbing: a newly relevant disease presents unprecedented challenges. *Phytopathol.* 103, 652–665. <https://doi.org/10.1094/PHYTO-12-12-0331-RVW>.
- Weller, D.M., 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathol.* 97, 250–256. <https://doi.org/10.1094/PHYTO-97-2-0250>.
- Zhong, G., Nicolosi, E., 2020. Citrus origin, diffusion, and economic importance. In: Gentile, A., La Malfa, S., Deng, Z. (Eds.), *The Citrus Genome*. Springer, Cham, pp. 5–21.
- Zhou, L., Powell, C.A., Li, W., Irey, M., Duan, Y., 2013. Prophage-mediated dynamics of 'Candidatus Liberibacter asiaticus' populations, the destructive bacterial pathogens of citrus Huanglongbing. *PLoS One* 8, e82248. <https://doi.org/10.1371/journal.pone.0082248>.
- Zou, Z., Xi, W., Hu, Y., Nie, C., Zhou, Z., 2016. Antioxidant activity of citrus fruits. *Food Chem.* 196, 885–896. <https://doi.org/10.1016/j.foodchem.2015.09.072>.