Biocontrol treatments for bacterial citrus illnesses using microorganisms

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ABSTRACT

Citrus is one of the most frequently farmed crops on the globe, with a large market for both fresh fruit and juice. Highly aggressive pathogenic bacteria, such as, *Xylella fastidiosa subsp.* pauca, *Xanthomonas citri* and now Candidatus *Liberibacter asiaticus,* can have a significant impact on orange orchard productivity. Antibiotic bacteria (mostly from the Pseudomonas and Bacillus genera) and bacteriophages have been described as microbiological biocontrol agents against these diseases. This review outlines all of the microbiological control measures for citrus bacterial infections that have been documented so far, stressing those areas of research where there is still a lot of potential to be discovered.

Key Words: *Xanthomonas citri; Biocontrol; Bacterial citrus illness*

INTRODUCTION

Citrus is one of the most widely farmed fruits in the world, with markets for fresh fruit as well as fresh and processed juice. The Fortunella, Poncirus, and Citrus genera in the Rutaceae family are home to the majority of citrus cultivars and rootstock variants (order Geraniales, suborder Geraniineae). 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), the United States (7.8 million tons), or Spain (6.8 million tons) lead the world ranking of citrus exports for consumption as fresh fruit, with 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, Citrus fruits have become a major source of nutrition in many countries' diets. Citrus, like many other tropical and subtropical crops, is susceptible to a variety of fungal, viral, and bacterial diseases. Citrus tristeza virus (CTV disease), 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.* (brown spot and black rot disease) (root and collar rot and brown rot on fruit).

Bacterial infections are a continual hazard to citrus farming, resulting in significant output decreases in all growing regions across the world. Huanglongbing (HLB), Asiatic Citrus Canker (ACC), and Citrus Variegated Chlorosis (CVC), for example, have major economic consequences owing to the annual mortality of millions of plants and quarantine limitations. HLB and ACC are endemic diseases in most commercial citrus-growing locations across the world, but CVC is only found in the Americas. All of them are not known to exist on the European continent. The threat of these bacteria being introduced into citrus-growing countries is a perennial source of concern for citrus producers and traders. The limits on interstate and international fruit commerce emanating from endemic areas have the greatest impact on ACC. In various producing nations across the world, severe quarantine restrictions for the entry of propagative citrus material are in place for HLB and CVC. Despite the fact that these diseases all infect the same host, they use various virulence and dispersal mechanisms, have varying tissue specificity, and cause diverse symptoms. Recent reviews have focused on secretion systems and effectors, cell-to-cell communication pathways, and pathogenicity processes associated to the bacteria's specialized lifestyle, dispersion, and symptoms. ACC is a widespread disease caused by the hemibiotrophic bacterium Xanthomonas citri subsp. citri (X. citri), which infects nearly all commercial citrus varieties. Erumpent, corky, and elevated pustules on the surface of leaves, fruits, and twigs, which serve as sources of bacterial inoculum, are typical signs of the illness. Infected fruit has a lower commercial grade and is rejected by the majority of major markets. Plant responses to the infection include defoliation, twig dieback, and premature fruit drop. The presence of a water film over the lesions encourages X. citri exudation to the surface and the spread of germs to new susceptible tissue via rain splash and wind dispersion.

Bacterial adhesion, micro colony growth, and biofilm formation by X. citri on citrus surfaces are all important processes in the disease's pathophysiology. In this mechanism, quorum sensing or cell-to-cell communication, which is mediated by a diffusible signal molecule called DSF, is critical. The presence of Exopolysaccharides (EPS), climatic conditions, cultivar resistance, and phenological stage all influence biofilm development. Mesophyll colonization occurs after this period of epiphytic development, through natural apertures in immature sensitive tissues. Insect-induced wounds, such as those made by citrus leaf miner, pruning tools, and storms, make it easier for germs to enter older tissues. Pseudomonas syringae *pv. syringae* (*P. syringae)* causes citrus blast and black pit, a minor bacterial disease that has been observed to create major outbreaks in several Mediterranean nations. The present incidence of blast disease in citrus nurseries in Montenegro is roughly 10%–30%. Recent study attempts to describe the pathogen's genomic structure and create illness control techniques. Rain and wind favor colonization and dispersal of *P. syringae* in the spring, when shoot and fruit development begins, similar to the *X. citri* pathogenicity cycle. The disease manifests itself in leaves as water-soaked lesions that spread to the mid-vein and the twigs around the petiole's base. After a few weeks, the diseased leaves fall off and the necrotic twigs and shoots die back.

Citrus tree endophytic bacterial diversity has been reported as a possible source of antimicrobial chemicals like antibiotics. It was possible to verify how bacterial species isolated from leaves, such as *Bacillus cereus, Bacillus subtilis, Bacillus pumilus, and Pantoea agglomerans,* produce antibiotics capable of inhibiting the growth of pathogenic bacteria like *Streptococcus mutans, Vibrio cholera, Salmonella thypii, Salmonella thyposa, and Enterobacter faecalis*, in a study carried out in *C. aurantifolia*. Several Pseudomonas and Bacillus rhizosphere and phyllo sphere species are efficient biocontrol agents against citrus bacterial infections. Furthermore, Xanthomonas isolates lessen the severity of ACC. Bacterial communities found in the microbiome of citrus trees, on the other hand, are prospective sources of BCA.

In vitro and in the greenhouse, the use of bacteriophages against bacterial infections in citrus has yielded some promising results against *X. citri*. The capacity of bacteriophages Cp1 and Cp2 to lyse bacterial cells in

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vitro has been described. In addition, the filamentous phage XacF1, which belongs to the Inoviridae family, causes a number of physiological changes in the bacterial host cells in vitro, including lower levels of extracellular polysaccharide production, reduced motility, slower growth rate, and a significant decrease in virulence. Furthermore, treatment of leaves with a combination of bacteriophages Cp2, Xac2005-1, cc7, and cc13 24 hours before inoculation with *X. citri* gave considerable disease reduction on *C. paradisi* under greenhouse conditions, and was as effective as copper-bactericide. Furthermore, twice-weekly field treatment of a bacteriophage combination obtained from wild type X. citri strains decreased ACC disease in C. aurantifolia through bacterial cell lysis. In vitro investigations recently revealed that the commercially available phage 6 inhibited P. syringae development in vitro by causing bacterial cell lysis. If the delivery technique can be adjusted, phage-based treatment offers the potential to manage citrus bacterial illnesses like HLB. Prophages are highly dynamic components of the Ca. L. genome that play a key role in intra-species variation, bacterial population dynamics in plant and insect hosts, and their relationship to disease transmission and development.

In recent years, the potential of various mycorrhizal and endophytic filamentous fungi to manage various diseases has been extensively researched, both through direct mechanisms and through the activation of plant defensive responses. Trichoderma species, for example, release proteins that aid in the development of plant resistance to the fungal disease *P. citricarpa* (teleomorph: Guignardia citricarpa) in citrus. In the case of bacterial infections in citrus, it would be interesting to see if various Trichoderma strains may produce resistance, albeit no research has yet been conducted in this area. It is used HLB to sequence the endophytic fungus populations found in the roots of several Citrus species in 2018. Liberibacter crescens, a culturable surrogate for the unculturable HLB-associated bacterium, was shown to be significantly inhibited by the fungus *Cladosporium cladosporioides* and *Epicoccum nigrum*. Cladosporols A, C, and D, pure bioactive natural compounds with anti-Ca. L action, were discovered in the fungus *C. cladosporioides.*

CONCLUSION

Citrus crops are important for agriculture and economics across the world, generating more than 125 billion tons per year, highlighting the necessity to produce with as little plant pathogen impact as feasible. Copper treatment is commonly used to treat ACC, although this promotes the development of bacterial resistance and has negative environmental consequences. Effective techniques to manage CVC and HLB, on the other hand, are currently restricted. As a result, it's critical to create efficient biocontrol agents to combat these diseases, which impact a wide range of commercial citrus types. HLB, ACC, and CVC are bacterial citrus diseases that have a substantial impact on agricultural yield and kill millions of trees each year. Numerous research has been conducted in this regard, demonstrating the efficiency of antagonistic bacteria in combating various illnesses under in vitro, greenhouse, and field settings. Antibiosis, competition for space and resources, growth encouragement, and activation of plant defensive responses are all strategies that species like *P. fluorescens, P. aeruginosa, B. subtilis, B. amyloliquefaciens*, or *C. flaccumfaciens* use to suppress bacterial citrus diseases. In this regard, it is critical to emphasize their ease of use in the field, since they are species that are extensively disseminated in a variety of goods on the market today, and whose field use has been established for years. As a result, we're talking about goods that are easy to use, lucrative, and efficient.

The microbiome of damaged and healthy citrus trees is now being studied to find effective microbial communities for HLB and CVC control. However, like with many other bacterial illnesses, the use of bacteriophages in biocontrol of these diseases requires further investigation, since the number of lines of research in this subject is now beginning to expand dramatically. As a result, they offer biocontrol agents with a lot of promise, promising outcomes, and the potential for significant economic and agronomic advantages for the citrus industry. Finally, the use of fungus against this category of citrus illnesses has been proposed, indicating some prospective future research directions in the field of very hazardous bacteria biocontrol.