Recent developments in vascular-restricted, walled bacteria of citrus: *Xylella fastidiosa* and the liberobacters, proteobacterial plant pathogens

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ABSTRACT

INTRODUCTION. Two major diseases of citrus are caused by walled bacteria restricted to the vascular tissues: citrus variegated chlorosis (CVC) and huanglungbin (ex greening). **CITRUS VARIEGATED CHLOROSIS AND *XYLELLA FASTIDIOSA*.** A bacterium, found in xylem vessels of CVC affected citrus in Brazil, was cultured and shown to be a strain of *Xylella fastidiosa*, a member of the gamma subclass of the *Proteobacteria*. The bacterium was subsequently demonstrated to be the causal agent of the disease. A sensitive serological detection method demonstrated that the *X fastidiosa* strain of CVC was related to *X fastidiosa* strains causing diseases in grapevines and other plants. **HUANGLUNGBIN AND THE LIBEROBACTERS.** In the case of huanglungbin, a bacterium restricted to the phloem tissues was also shown to be associated with the disease. It belongs to the alpha subclass of the *Proteobacteria* and was named *Candidatus* Liberobacter africanum for African strains, and *Candidatus* Liberobacter asiaticum for Asian strains. **DETECTION OF THE CVC AND HLB BACTERIA.** Specific and sensitive methods were developed for the detection of liberobacters in citrus and in the two psyllid vectors of the liberobacters, by DNA/DNA hybridization and PCR. **CONCLUSION.** *X fastidiosa* affects not only citrus where it is a major problem, but has recently been found in coffee. It is even possible that the coffee decline witnessed in Brazil, 20 years ago, could have been due to *X fastidiosa*. Today, 30 years after the HLB organism was first seen in citrus, it is well characterized owing to the development of molecular techniques.

KEYWORDS
Citrus, bacteria, chlorosis, phylogeny, liberobacter, proteobacteria, xylem, phloem.

Récents progrès chez les bactéries à paroi des agrumes localisées dans les faisceaux vasculaires : *Xylella fastidiosa* et les liberobacters, proteobactéries, pathogènes des plantes.

RÉSUMÉ

INTRODUCTION. La chlorose variégée des agrumes (CVA) et le huanglungbin (HLB, ex greening) sont deux graves maladies des agrumes, dues à des bactéries à paroi, localisées dans les tissus vasculaires. **LA CVA ET *XYLELLA FASTIDIOSA*.** Une bactérie, trouvée dans les vaisseaux du xylème des agrumes atteints de CVA au Brésil, a été cultivée et s'est révélée être une forme de *X fastidiosa* appartenant à la sous-classe gamma des *Proteobacteria*. La bactérie a ensuite été confirmée comme étant l'agent responsable de la maladie. Une méthode de détection sensible par sérologie a montré que la forme de *X fastidiosa* de la CVA était apparentée à d'autres formes de *X fastidiosa* responsables de maladies connues chez la vigne et d'autres plantes. **LE HUANGLUNGBIN ET LES LIBEROBACTERS.** Le HLB s'avère causé par une bactérie localisée dans le phloème, appartenant à la sous-classe alpha des *Proteobacteria*. Elle a été nommée *Candidatus* Liberobacter africanum pour les souches africaines et *Candidatus* Liberobacter asiaticum pour les souches asiatiques. **DÉTECTION DES BACTÉRIES RESPONSABLES DE CVA ET DE HLB.** Des méthodes spécifiques et sensibles d'hybridation d'ADN et de PCR ont été développées pour détecter les liberobacters dans les agrumes et dans les deux psylles vecteurs de ces organismes. **CONCLUSION.** *X fastidiosa* n'affecte pas seulement les agrumes, mais elle a été aussi trouvée récemment dans le caféier. Il n'est pas exclu que le déclin des caféiers observé au Brésil, il y a 20 ans, ait pu être causé par *X fastidiosa*. Trente ans après la première mise en évidence de l'agent responsable du HLB, cet organisme n'a toujours pas été cultivé, mais il a pu être caractérisé grâce au développement des techniques moléculaires.

MOTS CLÉS
Agrumes, bactérie, chlorose, phylogénie, liberobacter, proteobacteria, xylème, phloème.

Received 14 August 1997
Accepted 6 October 1997

Fruits, 1997, vol 52, p 361-369

RESUMEN SPAÑOL, p 369

introduction

The vascular tissues of plants, both xylem and phloem, are known to be habitats of various phytopathogenic bacteria. The wall-less bacteria, ie, the mollicutes (spiroplasmas and phytoplasmas) are always restricted to the phloem sieve tubes, as indicated in the companion paper (BOVÉ and GARNIER, 1997). Walled bacteria also inhabit the sieve tubes, and the agent of citrus huanglungbin (ex greening) has become the best known of these walled, sieve-tube-restricted bacteria. A classic example of xylem-restricted bacteria is *Xylella fastidiosa*, the agent of several diseases, including Pierce's disease of grapevine (WELLS et al, 1987). We have shown that from 1989 to 1992 *X fastidiosa* was the causal agent of citrus variegated chlorosis in Brazil and in Argentina where the disease is known under the name 'Pecosita'.

The sap of sieve tubes, contrary to that of xylem vessels, is enriched by the products of photosynthesis, and the sieve-tube-restricted bacteria are probably more demanding than those of the xylem. This is probably the reason why culture media supporting their multiplication have not yet been developed, except for the spiroplasmas which were cultured as early as 1971 (SAGLIO et al, 1971). The xylem-limited *X fastidiosa* was first cultured in 1978 (DAVIS et al, 1978) from Pierce's disease affected grapevines. In this paper, we review the agents of huanglungbin and citrus variegated chlorosis. In the companion paper, the phytopathogenic mollicutes of stubborn and witchesbroom have been covered (BOVÉ and GARNIER, 1998).

citrus variegated chlorosis and *Xylella fastidiosa*

Citrus variegated chlorosis (CVC) was first observed in 1987 on sweet orange trees in the Southwestern part of Minas Gerais, Brazil and in the northern part of São Paulo State (Colina). The disease now (1997) affects a high percentage (25%) of trees. ROSSETTI et al (1990) were the first to show by electron microscopy that a xylem-limited bacterium, thought to be a strain of *X fastidiosa*, was present in all symptomatic leaves and fruits tested, but not in similar tissues from symptomless trees. CHAGAS et al (1992) confirmed these results.

It was our aim to culture the CVC bacterium. On several cell-free media known to support the growth of *X fastidiosa*, a bacterium could consistently be cultured from symptomatic twigs of sweet orange trees affected by CVC, but not from tissues of healthy trees (CHANG et al, 1993a). Bacterial colonies typical of *X fastidiosa* became visible in 5 days on PW agar medium, and in 7–10 days on CS20 and PD2 agar media. The cells of the CVC bacterium were rod-shaped, 1.4–3 µm in length, and 0.2–0.4 µm in diameter, with rippled walls. An antiserum against an isolate (8.1.b) of the bacterium gave strong positive reactions in double-antibody-sandwich (DAS), enzyme-linked immunosorbent assay (ELISA) with other cultured isolates from CVC-affected citrus, as well as with several type strains of *X fastidiosa*. DAS-ELISA was also highly positive with all leaves tested from CVC-affected shoots. Leaves from symptomless trees reacted negatively (CHANG et al, 1993a; GARNIER et al, 1993).

These results prove that the bacterium seen by electron microscopy in CVC-affected tissues and the bacterium cultured from such tissues is the same for the following reasons: i) both occur in CVC-affected citrus; ii) the bacteria as seen in situ in the xylem and in the cultures have the same size and morphology; and iii) the antiserum raised against the CVC bacterium gives highly positive DAS-ELISA reactions with CVC-affected leaves. Strong positive ELISA reactions were obtained with CVC-affected tissues from all the areas where CVC is present.

In addition, the following data indicate that the CVC bacterium is a strain of *X fastidiosa*: i) the CVC bacterium and *X fastidiosa* are both xylem-limited; ii) they have the same morphology in planta and in vitro; iii) they grow in media developed for *X fastidiosa*; iv) they have close serological relationships with other strains of *X fastidiosa*; and v) they are reported to have similar protein patterns

upon polyacrylamide gel electrophoresis (NETO et al, 1991). The serological reactions of the CVC-specific serum with other *X fastidiosa* strains indicate that the CVC bacterium is more closely related to group I than to group II strains, even though good reactions are observed with the Ragweed stunt strain. This is also evidenced by the ability of the CVC bacterium to grow in both PW and PD media.

Finally, in order to fulfil Koch's postulates, sweet orange seedlings were mechanically inoculated with the CVC bacterium. The CVC bacterium could be detected by DAS-ELISA in these plants 3 months after inoculation, had become systemic 4 months after inoculation, and could be reisolated from the inoculated seedlings. Symptoms of chlorotic variegation characteristic of the disease started to develop 6 months after inoculation and were conspicuous 3 additional months later. These results indicate that the CVC strain of *X fastidiosa* is the causal agent of CVC (CHANG et al, 1993a, b).

Hence, for the first time, we detected a bacterium in the xylem of CVC affected citrus (ROSSETTI et al, 1990), we cultured the bacterium (CHANG et al, 1993a, b), raised an immunoserum against the bacterium and showed the CVC bacterium to be a strain of *X fastidiosa* (CHANG et al, 1993a; GARNIER et al, 1993), and reproduced the disease by mechanically inoculating the CVC strain of *X fastidiosa* to citrus, thus fulfilling Koch's postulates (CHANG et al, 1993a, b). These results have been confirmed by others (LEE et al, 1993; HARTUNG et al, 1994).

Recently, new plants have been found to be invaded by *X fastidiosa*. In Brazil, *X fastidiosa* not only affects citrus, but also coffee, causing coffee leaf scorch or scalding (Requeima do cafeeiro) (BERETTA et al, 1996; DE LIMA et al, 1996). The *X fastidiosa* isolates from coffee appear to be very similar to strains of CVC. In California, a lethal new disease of oleander (leaf scorch) is caused by a strain of *X fastidiosa*, apparently new to California, and representing a new pathotype. The sharpshooter *Homalodisca coagulata*, only recently detected in California, but native to the southeastern states is closely associated with the spread of the disease. It is an efficient vector of *X fastidiosa* to peach and grape in Florida and Georgia. In California, the sharpshooter likes not only oleander, peach and grape but also citrus. Whether it will be capable of introducing *X fastidiosa* into citrus and produce in California a disease similar to CVC in Brazil is a matter of speculation. In Brazil, the sharpshooters *Acrogonia terminalis*, *Dilobopterus costalimai* and *Oncometopia fascialis* occur regularly on citrus and have been shown to be vectors of the CVC bacterium (LOPES et al, 1996; ROBERTO et al, 1996).

huanglungbin and the liberobacters

The micro-organism associated with citrus greening disease, now renamed huanglungbin (HLB), was first observed in 1970 by LAFLÈCHE and BOVÉ (1970a, b) in the phloem of affected sweet orange leaves. It was initially thought that the HLB organism was a 'mycoplasma-like organism' (MLO), but the organism was soon found to be enclosed by a 25-nm-thick envelope, which was much thicker than the unit membrane envelopes characteristic of MLOs (thickness, 7–10 nm) (SAGLIO et al, 1971). These properties suggested that the HLB organism is a walled bacterium and does not resemble mycoplasmas. Organisms similar to the HLB agent occur in plants other than citrus and are associated with more than 20 different diseases. As far as is known, these organisms are always restricted to the sieve tubes within the phloem tissue. None of them have been obtained in pure culture. By analogy with MLOs, the HLB-organism has been designated 'bacterium-like organism' (BLO) (MOLL and MARTIN, 1974), and was shown by GARNIER et al (1984) to be a Gram-negative bacterium.

HLB is one of the most severe diseases of citrus. It has a large geographic distribution, because it is transmitted by two psyllid insect vectors, *Diaphorina citri* in Asia and *Trioza erytreae* in Africa (MCCLEAN and OBERHOLZER, 1965; CAPOOR et al, 1967). Symptoms of HLB in Asia occur even when temperatures are well above 30 °C, while in Africa the disease

is present only in cool regions. These temperature effects have been reproduced under phytotron conditions (BOVÉ et al, 1974). In addition, when the HLB-BLO was experimentally transmitted from citrus to periwinkle plants, the HLB reaction was the same as that observed in citrus (GARNIER and BOVÉ, 1983). Therefore, the African BLO is heat sensitive and the Asian BLO is heat tolerant. This is the only known biological difference between the African and Asian HLB diseases.

Characterization of the HLB agent has been slow and difficult because the BLOs have resisted in vitro cultivation. Electron microscopy combined with cytochemistry revealed that the HLB-BLO was surrounded by a peptidoglycan-containing membranous cell wall of the Gram-negative type (GARNIER et al, 1984). Later, monoclonal antibodies (mAbs) were obtained against two Asian BLO strains, strain Poona from India and strain Fujian from China, and one African strain, strain Nelspruit from South Africa (GARNIER et al, 1987, 1991). These mAbs are highly strain specific, and seven different BLO serogroups have been identified so far in this way (GAO et al, 1993). Recently, a 2.6 kbp DNA fragment of the Poona BLO genome (fragment In–2.6) has been cloned and sequenced (VILLECHANOUX et al, 1992, 1993). This fragment corresponds to the rather well-conserved *rplKAJL-rpoBC* bacterial operon and, in particular, codes for four ribosomal proteins (proteins L1, L10, L11 and L12). When, for taxonomic purposes, the sequence of the BLO operon was compared with the sequences from other bacteria obtained from the GenBank data base, the HLB-BLO was unambiguously identified as a member of the eubacteria. However, a comparison of the protein sequences deduced from the genes with their counterparts in other bacterial species failed to reveal a specific relationship between the BLO and any previously described bacterial species.

Southern hybridizations of fragment In–2.6 with DNAs extracted from HLB affected citrus plants obtained from various geographic regions (VILLECHANOUX et al, 1992) revealed that In–2.6 was able to hybridize under high-stringency conditions with all of the Asian strains, but not with the African strain tested. However, at lower stringencies, hybridization was also obtained with the African strain, but Southern hybridization profiles revealed DNA polymorphism (VILLECHANOUX et al, 1993).

In order to determine the phylogenetic position of the HLB-BLO and the evolutionary distance between African and Asian BLOs, we PCR-amplified, cloned, and sequenced the 16S ribosomal DNAs (rDNAs) of Asian strain Poona and African strain Nelspruit of the HLB-BLO. Sequence comparisons revealed that the two BLOs are members of the alpha subclass of the class Proteobacteria (JAGOUEIX et al, 1994).

The Proteobacteria (former 'purple bacteria') comprise most of the Gram-negative bacteria (WOESE, 1987; STACKEBRANDT et al, 1988). The alpha subclass of the *Proteobacteria* is a diverse group of microbes that includes, both plant pathogens or symbionts with some distinctive properties (*Agrobacterium tumefaciens, Bradyrhizobium* spp) and human pathogens (*Rochalimea* spp, *Bartonella baciliformis, Brucella abortus, Afipia* spp, etc). The organisms in this group live in intimate association with eucaryotic cells and, in many cases, have acquired the ability to survive and grow within an arthropod vector. The HLB organism fits this description nicely: it grows in a specialized niche of its eukaryotic plant host, the phloem sieve tubes, and it is transmitted by two arthropod vectors, the psyllids *T erytreae* and *D citri*, in which it multiplies both in the hemolymph and within the cells of the salivary glands.

Previously, we have shown that HLB-BLO strains from Africa could be distinguished between HLB-BLO strains from Asia on the basis of temperature sensitivity (BOVÉ et al, 1974; GARNIER and BOVÉ, 1983), serology (GAO et al, 1993) and genomic properties (VILLECHANOUX et al, 1992, 1993). A comparison of the 16S rDNAs of the Asian BLO strain Poona and the African BLO strain Nelspruit showed that they are 97.7% homologous. The close phylogenetic relationship between Indian and African HLB-BLOs is not surprising as these organisms cannot be distinguished morphologically, and they induce similar disease symptoms in citrus and periwinkle plants. While, in nature, the Asian BLO is transmitted by *D citri* and the African BLO by *T erytreae*, thus reflecting

the geographical distribution of these psyllids, experimentally each psyllid vector can transmit the two BLOs (MASSONIÉ et al, 1976; LALLEMAND et al, 1986). However, we recently cloned and sequenced the rather well-conserved *rplKAJL-rpoBC* operon of BLO strain Nelspruit strain and observed only 70% homology between this organism and the Indian BLO strain (PLANET et al, 1995). This finding and the 16S rDNA results suggest that these two strains might be members of two different species of the same genus. On the basis of our results, it is clear that the HLB-BLO is a member of the alpha subclass of the Proteobacteria and that its closest relatives are members of the alpha-2 subgroup. However, the presence of only one oligonucleotide signature of the alpha-2 subgroup and the presence of signatures characteristic of the alpha-1 and alpha-3 subgroups indicate that the HLB-BLOs do not belong to the alpha-2 subgroup, a finding concordant with the phylogenetic tree. Consequently, the HLB-BLOs might represent descendants of an early offshoot and the first members of a new subgroup in the alpha subclass.

Bacteriologists have hitherto had a conservative attitude and refrained so far from latin binomial names to non-cultured organisms. However, with the development of PCR and DNA-sequencing, it is now possible to characterize such organisms at the molecular and phylogenetical level. On the basis of such considerations, MURRAY and SCHLEIFER (1994) have proposed the designation '*Candidatus*' as an interim taxonomic status to provide a proper record of sequence-based potential new taxa at the genus and species level. This possibility was used in the case of the HLB-BLOs by naming the African HLB-BLO '*Candidatus* Liberobacter africanum' and the Asian HLB-BLO '*Candidatus* Liberobacter asiaticum' (JAGOUEIX et al, 1994).

detection of the CVC and HLB bacteria

X fastidiosa

Polyclonal and monoclonal antibodies have been produced against isolate 8.1.b of the CVC strain of *X fastidiosa*. Using polyclonal antibodies, detection of the CVC bacterium has been achieved by ELISA with a very good sensitivity. Homogenates of 1 g of CVC-affected leaf-midribs in 3 ml of buffer gave optical densities (OD) at 405 nm higher than 2.0 in many cases. Midribs from healthy sweet orange trees, from HLB-affected trees grown in the Bordeaux greenhouse, and from asymptomatic trees in CVC-affected orchards or orchards without CVC, gave OD readings close to zero (CHANG et al, 1993a; GARNIER et al, 1993). Leaves from Tabay (Argentina) with symptoms of Pecosita disease were also tested. They gave strongly positive ELISA reactions, indicating that CVC and Pecosita are similar, and that the disease is present not only in Brazil but also in Argentina. An ELISA kit is commercially available from Sanofi-Diagnostic Pasteur, France. Detection of the CVC-bacterium by direct tissue blot immunoassay (DTBIA) has also been achieved. Cross sections were cut with a scalpel through leaf midribs or petioles from healthy or CVC-affected citrus trees and the sectioned surfaces were immediately blotted onto nitrocellulose membranes. After incubation of the membrane with a 2 000-fold dilution of the IgG-alcaline phosphatase conjugate, followed by a solution of 5-bromo-4-chloro-3-indolyl phosphate in nitroblue tetrazolium, a purple-blue coloration was clearly seen in the xylem on prints from infected midribs, but not in that from healthy midribs, indicating that this technique can be used for quick detection of the bacterium (GARNIER et al, 1993). It must however be further evaluated with field material. A dot-immunobinding assay (DIBA) has been described (BERETTA et al, 1993). PCR detection assays have also been developed (MINSAVAGE et al, 1994; POOLER and HARTUNG, 1995). A comparison between ELISA, DTBIA, DIBA and PCR is required to assess the sensitivity of these techniques.

liberobacters

Two DNA probes, In-2.6 and AS-1.7, containing genes for ribosomal proteins (as part of the well known, so-called ß operon), have been produced respectively for L asiaticum and L africanum (VILLECHANOUX et al, 1992; PLANET et al, 1995). Used in dot-blot hybridization radioactively or digoxigenin-labelled, in 2.6 and AS 1.7 probes allow detec-

tion of the respective greening liberobacter species in citrus leaf samples collected in infected orchards (BOVÉ et al, 1993; HOCQUELLET et al, 1997). The sensitivity of the hybridization assay is similar to that of electron microscopy, the only detection method that was previously available. The necessity of using two different probes for the detection of the two liberobacter species (which occur concomitantly in areas such as the Arabic Peninsula, Reunion and Mauritius), and the fact that DNA extraction for dot-blot hybridization is time consuming, prompted us to look for additional procedures. PCR, known to be simple, quick and sensitive, has been envisaged. Indeed, for characterization purposes we have previously cloned and sequenced the 16S rDNA of both an Asian and an African liberobacter strain. From sequence comparisons, primers suitable for amplification of liberobacter ribosomal DNA were defined. Using three different primer combinations, we have been able to specifically amplify 16S rDNA from the two '*Candidatus*' Liberobacter species known today: L asiaticum and L africanum (JAGOUEIX et al, 1996). The primer pair OI1/O12c is able to amplify DNA of the two species, while the pair O12c/OA1 amplifies preferentially L africanum DNA. Thus, in countries where the two species are known or suspected to be present, we recommend the use of the three primers OI2c/OI1/OA1, in the same PCR mixture. With both liberobacter species, the amplified DNA has a size of 1 160 bp, and the two species cannot be distinguished from the size of their amplicons. However, the L asiaticum amplicon has one *Xba*I restriction site and yields two fragments (650 bp and 520 bp) when restricted with *Xba*I, while the L africanum amplicon has two such restriction sites and yields three fragments (506 bp, 130 bp, 520 bp). Hence, the two liberobacter species can be easily identified from the *Xba*I restriction profiles of their 16S rDNA amplicons. Recently we have developed an alternative PCR detection method based on the sequence of the *rplKAJL-rpoBC* operon. In this case, the amplicon from L asiaticum measures 701 bp, while that of L africanum has 667 bp (HOCQUELLET, BOVÉ and GARNIER, unpublished results). With this technique the two liberobacter species can be identified directly from the sizes of their amplicons.

conclusion

CVC is a new disease of citrus caused by an 'old" pathogen known for many years: *X fastidiosa*. The CVC bacterium probably entered citrus through polyphagous sharpshooters which were initially contaminated from non-citrus plants. Coffee might have been one of these sources of contamination. It can, further be speculated that the coffee decline witnessed in the northern and northwestern parts of the State of São Paulo, 20 years ago, and which resulted in the replacement of coffee by citrus, could have been due to *X fastidiosa*. Transmission now occurs also from citrus to citrus, and some of the sharpshooters transmitting *X fastidiosa* in citrus occur also on coffee. Like Pierce's disease of grapevine, CVC has become a major problem for citrus in Brazil. It remains to be seen how important 'Requima do Cafeeiro' will become.

Brazil has, in addition, a sword of Damocles over its citrus orchards: the ubiquitous presence of *Diaphorina citri*, the Asian vector of the HLB liberobacter. It can only be hoped that no liberobacter-infected citrus plant material is introduced into the country.

The HLB organism was first seen in citrus by electron microscopy as early as 1970 and was soon found to be a walled bacterium, not a wall-less mollicute. To date, the organism has not yet been obtained in culture, but the development of molecular techniques has made it possible:

– to phylogenetically characterize the HLB bacterium as a genuine representative of the class Proteobacteria (most Gram- negative bacteria), and, more precisely, the alpha subclass,

– to designate the african HLB bacterium as '*Candidatus* Liberobacter africanum', and the asian bacterium as '*Candidatus* Liberobacter asiaticum',

– to devise molecular techniques, DNA hybridization and PCR to detect and identify the two HLB liberobacters.

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Trabajos recientes sobre las bacterias con membrana de los cítricos en los haces vasculares: *Xylella fastidiosa* y las liberobacters, proteobacteria patógenos de las plantas.

RESUMEN

INTRODUCCIÓN. La clorosis variegada de los cítricos o amarilleamiento (CVA) y el huanglungbin (HLB, antes greening) son dos graves enfermedades de los cítricos, debidas a bacterias con membrana, situadas en los tejidos vasculares. **LA CVA Y *XYLELLA FASTIDIOSA.*** Una bacteria, encontrada en los vasos del xilema de los cítricos con CVA en Brasil, se cultivó y se descubrió que era una forma de *X fastidiosa* que pertenecía a la subclase gamma de las *Proteobacteria.* Luego, se confirmó que dicha bacteria era el agente responsable de la enfermedad. Un método de detección sensible por serología mostró que la forma de *X fastidiosa* de la CVA estaba emparentada con otras formas de *X fastidiosa,* que provocan, en las plantas, numerosas enfermedades conocidas. **EL HUANGLUNGBIN Y LAS LIBEROBACTERS.** El HLB está causado por una bacteria situada en el floema que pertenece a la subclase alfa de las *Proteobacteria.* Se la denominó *Candidatus* Liberobacter. **DETECCIÓN DE LAS BACTERIAS RESPONSABLES DE CVA Y HLB.** Se han desarrollado una serie de métodos, específicos y sensibles de hibridación de ADN y de PCR, para detectar las liberobacters en los cítricos y en las dos psyllas vectores de esos organismos. **CONCLUSIÓN.** El declive de los cafetos notado en Brasil, hace 20 años, habría sido causado por *X fastidiosa,* agente de la enfermedad de CVA, que constituye, hoy en día, un importante problema sanitario en los cítricos. Treinta años después de que se evidenciara al agente responsable del HLB, se ha caracterizado a este organismo gracias al desarrollo de las técnicas moleculares.

PALABRAS CLAVES

Citricos, bacteria, chlorosis, filógenia, liberobacter, proteobacteria, xilema, floema.