Citrus Variegated Chlorosis Bacterium: Axenic Culture, Pathogenicity, and Serological Relationships with Other Strains of Xylella fastidiosa

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ABSTRACT


A xylem-limited bacterium serologically related to strains of Xylella fastidiosa has been associated previously with citrus variegated chlorosis, a new and potentially serious disease of citrus in Brazil. When isolated and grown on PW (periwinkle wilt) medium, this gram-negative bacterium measured 0.4 × 4 μm and was indistinguishable based on colony appearance from reference strains of X. fastidiosa obtained from the American Type Culture Collection, Rockville, MD. The bacterium also had a ridged cell wall typical of X. fastidiosa and induced symptoms typical of citrus variegated chlorosis in sweet orange after artificial inoculation. The bacterium was reisolated from petioles of symptomatically inoculated plants, and its identity was confirmed by membrane entrapment immuno-

fluorescence and Western blotting with antiserum UF-26 prepared against the original strain and extracts of petioles and midribs from inoculated plants. The organism was observed in large numbers in xylem vessels of diseased, but not healthy, plant petioles and in extracts of diseased, but not healthy, petioles, using a gold label with antiserum UF-26. The bacterium reisolated from symptomatic plant tissue was culturally, morphologically, and serologically indistinguishable from the strain used to inoculate the plants, completing Koch’s postulates. Antiserum UF-26 reacted most strongly with strains of X. fastidiosa that cause diseases of grapevines, almond, ragweed, and oak. The citrus strain of X. fastidiosa also reacted as strongly as the homologous strain from plum to antiserum 1609-P. This strain of X. fastidiosa may represent a new serological group intermediate between previously described serogroups of X. fastidiosa.

Additional keywords: Citrus sinensis, pecosita.

Fastidious, gram-negative bacteria with ridged cell walls cause diseases of numerous woody plants, including grapevine (14), peach and plum (11,45), almond (17), elm, sycamore, and oak trees (10,20), and mulberry (27). Similar bacteria cause periwinkle wilt (16) and ragweed stunt diseases (43). The bacteria are typically shown by electron microscopy to be tightly packed in the lumen of xylem vessels (32,33) and are transmitted in a persistent manner by various leafhopper vectors (7,35). Strains isolated from various host plants were considered genotypically and phenotypically similar and were classified as Xylella fastidiosa (44). The literature on this group of pathogens has been reviewed (24,38,42).

A new disease of sweet orange (Citrus sinensis (L.) Osbeck) has been associated with strains of X. fastidiosa in Brazil (8,40). The authors of initial reports suggested that the disease, citrus variegated chlorosis, posed an immediate threat to the Brazilian and world citrus industry (30,39). Pierce’s disease, caused by another strain of X. fastidiosa, already limits grapevine production in the southeastern United States and Central America (18). The preliminary epidemiological data presently available from Brazil are inadequate to forecast the future course of the citrus variegated chlorosis epidemic (19). However, it has been estimated that in the absence of remedial measures a disease incidence of 90% in a grove could occur 12 yr after introduction of a single infected tree (19). There are no data available concerning yield reductions that result from infection. Affected fruit, however, are hard and undersized and damage juicing machinery, causing entire lots of fruit to be rejected at processing plants (30). Symptoms also include a brilliant chlorosis, stunting, and canopy dieback. The disease has already spread widely within the citrus-producing region of Brazil (19,30). A similar disease associated with X. fastidiosa called ‘pecosita’ occurs in Argentina (2,5).

We now report the isolation and characterization of X. fastidiosa from infected citrus, as well as the completion of Koch’s postulates for X. fastidiosa as the causal agent of citrus variegated chlorosis. We also present serological data to characterize the relationships of the citrus variegated chlorosis strain to other strains of X. fastidiosa. Preliminary accounts of some of this work have been presented (3,29). Our results confirm those of Chang et al (9) who also completed Koch’s postulates for citrus variegated chlorosis and published while this manuscript was in preparation.

MATERIALS AND METHODS

Bacterial strains. Strains of X. fastidiosa were obtained from the American Type Culture Collection, Rockville, MD (Table 1), except for strain B, which was isolated in Beltsville, MD, from twigs collected from citrus variegated chlorosis-infected Pera sweet orange trees near Bebedouro, São Paulo, Brazil. Xanthomonas campestris pv. vesicatoria strain 084-1275 was provided by J. Miller, Division of Plant Industry, Gainesville, FL. Pseudomonas solanacearum strain K60, P. citricola strain Z1, Agrobacterium tumefaciens strain C58, and Clavibacter michiganense subsp. nebraskense strain PPF4 were provided by R. E. Stall, University of Florida, Gainesville.

Isolation and characterization of the pathogen. Symptomatic leaves were surface-disinfested with 10% bleach for 5 min, followed by two rinses in sterile distilled water. Strain B was obtained after midribs were excised and sap was expressed from 2-mm sections onto periwinkle wilt (PW)-medium plates (16). Plates were incubated at 28 C. Colonies were selected, and cells were
streaked on fresh plates to obtain single colonies. Identification of *X. fastidiosa* was based on in vitro fastidious growth habit, gram-stain reaction, size measurements made by light microscopy, electron microscopy, and serological assays. Cultures were stored at -70°C in PW broth + 40% glycerol. Strain B was subsequently used for plant inoculations. Reisolations from inoculated plants were done on PW or SPW plates that contained cychrome B and naladixic acid at 100 and 20 μg/ml, respectively. SPW is PW medium (16) supplemented with 5 g of malt extract, 10 g of sucrose, 0.1 g of myo-inositol, 0.01 g of thiamine HCl, 0.01 g of pyridoxine HCl, 0.005 g of nicotinic acid, and 0.002 g of glycine per liter. These ingredients are components of a citrus cell suspension culture medium that stimulated growth of the *X. fastidiosa* isolated from sweet orange (3).

**Cultural characteristics of strains of *X. fastidiosa***. Inoculum from 21-day-old cultures on buffered charcoal yeast extract (BCYE) (44) agar was dispersed in sterile distilled water and then streaked in triplicate onto different growth media reported to support growth of *X. fastidiosa*. Agar media tested included BCYE, Pierce's disease medium (2) (PD-2) (15), and SPW. The gram reaction of the inoculum also was determined, and the average length and width of each strain was estimated by bright-field microscopy and gram-stained cells. After 3 wk of growth at 27°C, diameters of 30 colonies, 10 on each of three SPW and PD-2 plates, were determined with a binocular microscope equipped with an ocular micrometer. Analysis of variance was performed on the data by the general linear model with SAS computer software (SAS Institute, Cary, NC). The entire experiment was repeated one time.

**Plant material and inoculations**. We initially maintained citrus plants for experimental inoculation in 15-cm pots containing Metromix with Peter's fertilizer in the irrigation water and Osmocote (Grace Sierra Horticultural Products Co., Milpitas, CA) with trace elements in the pots. Insecticides were used as necessary. Subsequently, plants were put in 25-cm pots of Metromix containing composted sewage sludge (2:1) to better control an unrelated root rot. Sixteen plants that included five Valencia sweet orange on sour orange (*C. aurantium L.*) rootstocks, seven self-rooted Madame Vinous sweet orange, and four Schaub rough lemon (*C. jambhiri Lush.) seedlings were inoculated as follows. Bacteria were scraped from PW plates or pelleted from PW broth and washed in phosphate buffered saline (20 mM sodium phosphate, pH 6.8; 0.85% NaCl; PBS) with 0.1% Tween 20 (PBST). Plants were inoculated by two methods. First, we applied 10 μl of a concentrated suspension (A<sub>600nm</sub> = 1.0) of bacteria to the surface of citrus branches and pierced the surface of the branches through the inoculum droplets with a 21-gauge syringe needle. Twenty-five inoculation sites were used per plant. We performed inoculations two times, 10 wk apart. To insure that sufficient inoculum reached the vascular system, in subsequent inoculations we used a razor blade to slash vertically part of the way through the branch to be inoculated, so the upper half of the branch could imbibe the inoculum solution from a microcentrifuge tube. In the bacterial cell suspensions used for these inoculations, A<sub>600nm</sub> = 0.1-0.3. The slashed branch was held in the microcentrifuge tube with laboratory tape, and after 2 h, the inoculum tubes were removed, and the wounds were wrapped with grafting tape. The volume of liquid taken up by the plants varied but averaged 200-300 μl. These inoculations were performed three times, 4, 8, and 22 wk after the second needle prick inoculations. We also inoculated 15 plants of the same varieties in a similar manner with PBST to serve as controls. These inoculations were repeated on a second set of Madame Vinous sweet orange, with an isolate of *X. fastidiosa* (BARC-5) from a symptomatic Valencia tree from the first inoculation series and distilled water instead of buffered Tween 20 as the suspension medium for the inoculum. During the course of this inoculation series, a second, fresh isolate of *X. fastidiosa* from citrus variegated chlorosis-diseased Brazilian Pera sweet orange was obtained (BARC-6) and was included in the inoculum for some of these plants. Five sweet orange plants received bud grafts from plant five, a symptomatic inoculated plant that was the source of strain BARC-5. Five plants received bud grafts from healthy citrus as a control.

**Preparation of antiserum and serological assays**. Antiserum 948-LH, against the Pierce's disease bacterium (*X. fastidiosa*, ATCC 35881), and antiserum 1609-PP, against the phony peach bacterium (*X. fastidiosa* strain PPDB-5 [16]), were prepared previously in rabbits, using the method of Raja et al (37). Antiserum UF-26 was made against strain B similarly isolated from diseased citrus, except the bacterium was cultured on SPW agar plates. The immunoglobulin G (IgG) fraction of all antiserum was isolated by protein A column chromatography (31) and used in double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA) (13). In these assays, both the sample and conjugate buffer were PBS plus 0.1% Tween 20, 0.5% bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis), and 2.0% polyvinylpyrrolidone 40 (Sigma). IgG was conjugated with alkaline phosphatase, using glacial acetic acid. Immulon II plates (Dynatech, Chantilly, VA) were coated with IgG in buffer (5 μg/ml). Conjugate was diluted in conjugate buffer so that the homologous antigen/antiserum would give an A<sub>405nm</sub> of 1.0 in 1 h.

Strains of *X. fastidiosa* (Table 2) were harvested while actively growing 14 days after plating on SPW agar for use as antigens. Strain B bacteria were harvested 21 days after inoculation because of the slower growth rate of this strain. Bacterial cultures used for negative controls (Table 2) were harvested during log phase from agar plates of appropriate media. Bacteria were suspended in 2 ml of sterile distilled water and transferred to a centrifuge tube. They were washed three times with PBST. The A<sub>600nm</sub> was adjusted to 0.13 (1×10<sup>7</sup>/ml) and diluted 1×10<sup>5</sup> to sample buffer. ELISA data were summarized as the ratio of the A<sub>405nm</sub> of the tested strain to that of the strain used to prepare the antiserum (23).

Published procedures for dot immunobinding assays (DIBA) and Western blotting were used (28) with antiserum UF-26 as the primary antibody and with goat anti-rabbit conjugate. Extracts of diseased peletios from Brazil were included on each Western blot as positive controls. Serological identification and detection of *X. fastidiosa* was performed by membrane entrapment immunofluorescence (MEIF), as described previously, to detect *Xanthomonas campestris* pv. *citriumelo* and *Xanthomonas campestris* pv. *citri* (6) with the modification that single leaf petioles were used rather than leaf disks. Antiserum UF-26 to *X. fastidiosa* strain B was conjugated to tetramethylrhodamine isothiocyanate.
(TRITC) for this purpose. The negative-control antiserum used was prepared previously to Xanthomonas campestris pv. citri strain XC62 (12).

**Electron microscopy.** Citrus leaf petiole and midrib tissues were excised from symptomatic leaves and fixed with 3% glutaraldehyde in 0.066 M phosphate buffer (PB), pH 6.8. Pieces 4 mm in length were fixed for transmission electron microscopy (TEM) and larger pieces (8–10 mm) were fixed for scanning electron microscopy (SEM). Fixation was overnight at 4°C, and the specimens then were washed three times (20 min each) in PB. Samples for TEM were postfixed in 1% osmium tetroxide in PB for 2–4 h and then washed three times (20 min each) in PB. All samples were dehydrated in an ethanol series (10–100%). Samples for TEM were infiltrated and embedded in L. R. White resin, (Ted Pella Inc., Redding, CA), using the hard formulation and polymerization at 65°C. SEM samples were mounted on stubs, critical point-dried, sputter coated with gold palladium, and viewed on a Hitachi S-530 scanning electron microscope (Nissei Sangyo American, Ltd., Mountainview, CA) operating at 20 kV. Ultrathin sections (60–85 nm) were obtained from TEM-embedded samples, and immunogold labeling was done as previously described (4) with antiserum against strain B.

**RESULTS**

Isolation and cultural characteristics. A fastidious, gram-negative bacterium that reacted positively in DAS-ELISA and DIBA with antisera raised against X. fastidiosa isolated originally from diseased plum, grapevine, and ragweed was isolated from xylem-containing tissue of citrus showing citrus variegated chlorosis symptoms but not from asymptomatic citrus. The growth rate of this bacterium, as judged by colony size, was in the middle of the range of 10 reference strains tested for comparison on SPW and PD-2 (Table 1). Similar results were obtained on BCYE medium, although the charcoal in the medium made it impossible to precisely determine colony diameters. The average dimensions of the cells were $0.4 \times 2–4 \mu m$ after growth on BCYE, similar to that of other X. fastidiosa reference strains tested under the same conditions. On SPW medium, isolated colonies were clearly visible within 7 days after streaking, as compared to 10–14 days on BCYE and PW medium. Colony diameters comparable to that on SPW generally were not observed on PD-2 (Table 1) or PW (data not shown). BCYE also supported growth of strains B and BARC-5, but colony diameters also were consistently much smaller than on SPW (data not shown).

**Inoculation results.** Twelve of the 16 plants inoculated with X. fastidiosa and 10 of the 15 plants inoculated with PBST as controls in the first inoculation series survived 10 mo after the first inoculations described here. The remainder of the plants died of an unrelated root rot disease. Eight months after the initial needle-prick inoculations, one asymptomatic, inoculated plant (plant 5, Valencia on sour orange [C. aurantum] rootstock) tested positive in dot-immunobinding (data not shown). Western blot assays of this plant also gave a weakly positive reaction, consistent with a low concentration of strain B in this asymptomatic tree (data not shown). Ten months after the first needle-prick inoculations, and consistently thereafter, a bacterium with the same cultural, serological, and morphological characteristics was reisolated from plant 5. Plant 5 had mild margin leaf chlorosis at the time of the first isolation, which became progressively more pronounced (Fig. 1A). Symptoms were the same as observed on naturally infected trees in Brazil (Fig. 1B), including necrotic centers in chlorotic zones. X. fastidiosa was not isolated from other inoculated plants from the first inoculation series nor from any of the plants mock-inoculated with PBST as controls. No other plants gave a positive reaction in the DIBA or Western blot assays. In the MEIF tests, polycarbonate membranes containing petiole extracts from plant 5 contained gold-orange fluorescing bacteria when incubated with TRITC-labeled UF-26 antiserum (Fig. 2). Flourescing bacteria were not observed on membranes incubated with the control antiserum raised against Xanthomonas campestris pv. citri nor in petiole extracts from healthy control plants or from other inoculated plants when incubated with either labeled antiserum (data not shown).

In the second inoculation series, three of five plants that received bud grafts from plant 5 became symptomatic, and X. fastidiosa was detected in four of these plants by both Western blotting and MEIF of petiole extracts (Figs. 2 and 3). No symptoms of citrus variegated chlorosis or X. fastidiosa were detected in five

![Fig. 1. Symptoms of citrus variegated chlorosis on leaves A, from plant 5 artificially inoculated at Belleville, MD, and B, from a naturally infected tree from Brazil.](image-url)
plants that received bud grafts from a healthy Valencia sweet orange (Table 3). Eight of 17 Madame Vinous plants inoculated with strain BARC-5 or strains BARC-5 and BARC-6 became symptomatic 6 mo after the first inoculation by needle-pricking. Strains of *X. fastidiosa* indistinguishable morphologically and serologically from the strain used for inoculation were isolated from 14 of these plants, whereas 11 and 14 of these plants gave positive results in Western blotting and MEIF assays, respectively. No *X. fastidiosa* were isolated from the buffer-inoculated control plants, and none of these plants gave positive results in either the Western or MEIF assays (Table 3; Fig. 3).

**Comparison to strains of *X. fastidiosa* by ELISA.** Strains of *X. fastidiosa* were used as antigens for DAS-ELISA assays (Table 2). When assayed at 10^7 cfu/ml, the average of four determinations of the A_405nm for the homologous antigen/antiserum assay was 0.92 for strain 35881 and antiserum 948-LH, 0.77 for strain B and antiserum UF-26, and 1.04 for strain 35871 and antiserum 1609-PP (Table 2). The data from two separate ELISA tests run at different times with different preparations of antigen were combined, with each antigen run in duplicate for each test. The strains were serologically compared by expressing the raw absorbance data as a ratio of that of the tested strain to the homologous strain for the test antiserum. The three antisera showed different reactivities against the different strains, but all the tested *X. fastidiosa* strains were serologically related, and no significant cross-reactions to other plant-pathogenic bacteria were observed for any of the three antisera (Table 2). A high serological relationship of the strain from sweet orange to strains of *X. fastidiosa* isolated from diseased grapevine and almond (group I; [34]) was evident when antisera UF-26, raised against strain B, isolated from diseased citrus was used as the test antiserum. The sweet orange strain did not react very strongly to antiserum against 948-LH (grapevine) in the reciprocal test but did react as strongly as strains 35870 (almond) and 35877 (grapevine) with this antiserum. Two of three strains of *X. fastidiosa* isolated from grapevine, one strain from elm, and one of two strains from mulberry were highly related when antiserum 948-LH was used, and strains from elm, plum, and sweet orange were highly related when antiserum 1609-PP was used. Similar results were obtained with test antigen adjusted to 10^6 cfu/ml. Reactions generally were weak when the test antigen was at 10^7 cfu/ml.

**Electron microscopy.** Bacteria were readily observed in the xylem vessels of petioles and midveins from symptomatic tissues of plant 5 when viewed with TEM (Fig. 4). The bacteria measured 0.4 × 2.0 μm. Using TEM, the bacteria were morphologically similar to those reported previously for citrus variegated chlorosis (8,40) (Fig. 5A). Immunogold labeling with UF-26 antiserum was positive (Fig. 5B) for these TEM sections. No bacteria were found in the xylem of control tissues or in tissues from other inoculated plants.

**DISCUSSION**

At 10-mo postinoculation and thereafter, a fastidious bacterium with the morphological and serological characteristics of the in-
ooculated strain B was consistently isolated on PW medium from a single Valencia plant, and MEIF assays gave positive results (Fig. 2). At this time, the plant presented a distinctive mild mottle similar to that observed on naturally infected sweet orange trees in Brazil (Fig. 1A and B). This mottle gradually became more distinct and spread throughout the plant. Similar symptoms were not observed on other inoculated or control plants, and none of the other plants gave a positive result in DIBA or electron microscopy assays. Bacteria of this type were not isolated from any other inoculated or control plants despite repeated attempts. Petiole sections from only this plant had xylem vessels that contained bacteria morphologically similar to X. fastidiosa when viewed by SEM (Fig. 4). The growth of this bacterium in xylem vessels (Fig. 5A) is typical of diseases caused by X. fastidiosa (24,42). In TEM sections, these xylem-limited bacteria were specifically labeled with gold-conjugated antiserum raised against the bacterium used for the inoculation (Fig. 5B), as observed in plants naturally showing symptoms of citrus variegated chlorosis (4). There is no possibility that this symptomatic and serologically positive plant was naturally infected with X. fastidiosa, because it was raised from seed in screened greenhouses in Beltsville, MD, and was isolated from other hosts of X. fastidiosa.

These results were confirmed in the second inoculation series, in which fresh isolates of X. fastidiosa were used as inoculum. X. fastidiosa was isolated from 82% (14/17) of these plants and infection was confirmed by symptomology, Western blotting, and MEIF assays (Table 3). These data demonstrate that the X. fastidiosa strain previously associated with citrus variegated chlorosis (4,8,40) is in fact the etiologic agent of this disease.

The inoculation efficiency in the initial experiment was, however, only 8% (1 diseased/12 inoculated survivors). Transmission efficiencies of less than 100% have been reported previously for artificial inoculations with X. fastidiosa. Inoculations with X. fastidiosa grown in vitro with susceptible ragweed (Ambrosia artemisifolia (L)) or red oak (Quercus rubra (L)) had efficiencies that varied from strain to strain and were usually less than 100% (10,43). High inoculation efficiency has been reported for the Pierce's disease organism and grapevines (Vitis vinifera (L)) (14) and, more recently, using strains of X. fastidiosa and elm (Ulmus americana (L)) and sycamore (Platanus occidentalis (L)) seedlings (41). Sherald attributed his success to several factors, including the short period that his isolates were maintained in culture prior to inoculation (24 days). X. fastidiosa strains that cause Pierce's disease of grapevines can lose pathogenicity in culture (21). Our isolate had been in culture for 1 yr prior to the initiation of the first inoculation series reported here. We also subsequently found that 0.1% Tween 20 was toxic to strain B of X. fastidiosa. We had included it in the initial inoculation buffer to obtain a uniform suspension, because strain B rapidly forms large aggregates in solution. It was omitted from the second inoculation series in which bacteria were suspended in sterile deionized water, in which the bacteria survive for more than 3 h without loss of viability. Our inoculation efficiency was apparently dramatically improved by the use of strains that have not been in culture for a long period of time and by the use of distilled water to prepare the inoculum. Chang et al (9) also easily obtained infections of inoculated sweet orange with this pathogen when using a fresh isolate, consistent with the results of our second inoculation series. These workers, however, used sweet orange seedlings rather than grafted plants and also inoculated with the bacterium in the PW growth medium. We are now performing a host-range study including several Citrus and non-Citrus species and varieties.

After serial transfers, other strains of X. fastidiosa adapt to culture and grow faster, and strains belonging to group II grow slowly on PD-3 medium. In contrast, strain B from citrus remains slow growing even after 18 mo of culture by serial transfer on PW medium. The additional nutrients in SPW stimulated growth in most of the strains tested compared to growth in PD-2 medium.

Fig. 3. Western blot of sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels developed with UF-26 polyclonal antiserum to strain B of Xylella fastidiosa. (Table 3 provides treatment details). Top: lanes 1–10, plants 64–74; lane 11, size standards; and lane 12, positive control. Middle: lanes 1–12, plants 102–113; lane 13 size standards; and lane 14, positive control. Bottom: lanes 1–12, plants 114–125; lane 13, size standards; and lane 14, positive control.

Fig. 4. Scanning electron micrograph of Xylella fastidiosa in xylem vessels. Magnification 13,500X.
which is similar in composition to PW medium. Perhaps significantly, this stimulation was particularly apparent with the freshly isolated strain BARC-5 compared with strain B (Table 1). This may relate to the adaptation to culture and loss of virulence reported previously for X. fastidiosa (21,41).

Based on the DAS-ELISA assays reported here, strain B is not easily assigned to either group I or II of X. fastidiosa as classified by Purcell (34). Group I strains react strongly with group I antisera but relatively weakly with group II antisera. The reverse is true for group II strains (16,43). The sweet orange strain tested appears to have greater serological affinity to the group I (34) strains than to the group II strains, although there was also a strong serological relationship to the strains from ragweed (group II) and oak (group II?) when antiserum UF-26 was used and to the plum strain (group II) when antiserum 1609-PP was used (Table 2). Our results confirm the close serological relationship observed (9) between the X. fastidiosa strain causing citrus variegated chlorosis and the strains that cause Pierce’s disease of grapes and almond leaf scorch. Our ELISA data, however, also showed a strong serological relationship to the type strain of the oak leaf scorch strain, whereas the others workers did not observe this relationship (9). The reason for this discrepancy is unknown. These serological reactions with antisera to groups I and II and citrus variegated chlorosis B strains can be used as a rapid method to place an unknown X. fastidiosa isolate in groups I and II or a possible intermediate group for citrus strains.

The origin of the citrus variegated chlorosis disease in Brazil remains undetermined. Because of the wide host range of X. fastidiosa, it is probable that the X. fastidiosa strains that cause citrus variegated chlorosis were originally in weeds or crops and were moved into citrus by a Homopteran vector (34,36,39). X. fastidiosa from citrus has been reported previously and has been tentatively proposed as the etiologic agent of citrus blight (22, 25,26). Extracts from trees affected by citrus blight have a characteristic set of blight related proteins that include a 12-kDa protein that may be diagnostic of the disease. However, extracts from trees affected by citrus variegated chlorosis lack all of these proteins (1). Alternatively, the citrus variegated chlorosis disease could have existed without identification for a period of time until it began to limit production in an area in which the environment promoted rapid spread and extreme symptom expression. A citrus disease, pecosita, associated with X. fastidiosa with serological relationships similar to citrus variegated chlorosis has been reported in Argentina (2,5), but the disease is not a limiting factor of production in Misiones or other areas of Argentina where it occurs.

LITERATURE CITED


Fig. 5. Immunogold labeling of Xylella fastidiosa strain B A, in xylem vessels and B, in petiole extracts from sweet orange plant 5. B, shows the rippled cell wall. Magnification 37,000X.


