日植病報 65：140-146 (1999)
Ann. Phytopathol. Soc. Jpn. 65：140-146 (1999)

Detection of Fastidious Bacteria Causing Citrus Greening Disease by Nonradioactive DNA Probes

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Abstract

The greening fastidious bacteria (GFB), also tentatively named *Liberobacter*, cause citrus greening disease. They unevenly inhabit the sieve tubes of host plants in low concentration. A highly sensitive and specific DNA probe, developed with DNA cloning methods has been used to detect GFB in infected citrus hosts. One of the clones containing a 0.24-kb GFB-specific DNA fragment was labeled with biotinylated nucleotides by a PCR-labeling technique. A dot hybridization assay with the biotin-labeled DNA probe has been successfully used for detecting GFB in various citrus hosts including mandarins, tangors, sweet oranges and pummelos. This probe could specifically react with all GFB strains from several Asian countries, but not with those from South Africa. The probe developed has specificity and sensitivity sufficient enough to detect minute levels of GFB infection and, therefore, can be used in quarantine of the Asian greening disease.

(Received August 14, 1998; Accepted December 9, 1998)

Key words: greening fastidious bacteria, citrus greening, biotin-labeled DNA probe, dot hybridization.

INTRODUCTION

Citrus greening is a very important disease of citrus in the world, especially in Asia. It is caused by the greening fastidious bacteria (GFB) that inhabit the sieve tubes of host plants[3]. This pathogen causes a yellowing symptom on the leaves of citrus and retards the growth of its host plants. According to the influence of temperature on the symptom of disease, the GFB-induced disease can be categorized into two types, Asian and African. The Asian type, which is transmitted by the Asian psylla (*Diaphorina citri*), can develop symptoms at both warm (27-32°C) and cool temperatures. The African type, which is transmitted by the African psylla (*Trioza erytreae*), induces severe symptoms at cool temperatures (22-24°C), but not at temperatures above 30°C. The pathogens have not yet been successfully cultured *in vitro*, and a tentative genus name of *Liberobacter* has been proposed[4,10]. The disease can be diagnosed either by graft transmission to indicator plants or by electron microscopy, but both methods are time-consuming and inefficient. To develop more efficient diagnostic probes, radioactive DNA probes have been made previously by several researchers[2,15]. However, they are potentially hazardous and difficult to apply to the mass volume detection that is commonly faced in disease quarantine. For safety and convenience in quarantine, non-radioactive DNA probes have been developed and successfully used to the diagnosis of citrus greening disease by us since 1992[14]. Unlike several DNA probes of GFB prepared from the alternative host periwinkle (*Catharanthus roseus* L.)[15], we prepared our DNA probes directly after cloning DNA of GFB purified from diseased citrus host (*Citrus tankan* Hay.). Three recombinant clones containing specific DNA fragments of GFB were obtained by screening 836 transformants via cross-hybridization selection. One stable clone containing a 0.24-kb GFB-specific fragment was selected to develop a DNA probe. The DNA was labeled by biotin using PCR (polymerase chain reaction) technique. Based on our prior experiments, we found that the PCR-labeling probe is more efficient than the probe labeled by nick-translation or random-primer methods.

In this paper, we use dot hybridization with non-radioactive probes to successfully detect the pathogen causing Asian citrus greening in infected citrus hosts. Various methods of preparing DNA samples were compared for their efficiency in dot hybridization. A safe, specific and sensitive method suitable for large quantity diagnosis of citrus greening in plant disease quarantine has been described.

MATERIALS AND METHODS

Plant materials GFB-infected Tankan tangor (*Citrus tankan* Hay.) trees with characteristic symptoms of citrus greening were collected from northern Taiwan and used for GFB DNA cloning. The other GFB-infected citrus samples for detection were sampled from the

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fields of Okinawa (Japan), Kuangtung (China), Hanoi (Vietnam), Davao (Philippines), Sarawak (Malaysia), Chiang Mai (Thailand), Delhi (India), Al Mofaja (Saudi Arabia), Pretoria (South Africa) and Taiwan. These foreign samples were obtained from "The international cooperative project on the establishment of virus-free nursery system of tropical fruit trees in the Asia and Pacific area" supported by the Food & Fertilizer Technology Center for the Asian and Pacific Region. All GFB-infected seedlings were under good controls in a greenhouse. Healthy citrus plants for negative controls were obtained from pathogen-free seedlings through shoot-tip grafting technique and kept in a greenhouse[8,13].

Isolation of GFB from infected plants To isolate GFB, 50 g of infected leaf midribs were powdered in liquid nitrogen and resuspended in 150 ml GFB extraction buffer (300 mM mannitol, 50 mM Tris-HCl [pH 7.4], 5 mM EDTA, 4 mM 2-mercaptoethanol). Three rounds of low-speed centrifugation (4000×*g* for 15 min) removed most host debris. Crude GFB pellets were collected by high-speed centrifugation (12,000×*g* for 30 min). For reducing contamination, the crude GFB fraction was centrifuged in a continuous density gradient of sucrose (0.6 M to 1.6 M). The first and the second bands below the top of the gradient were collected, diluted with five volumes of GFB extraction buffer and centrifuged at 12,000×*g* for 30 min. The pellets were resuspended in 3 ml of DNA extraction buffer (1 M Tris-HCl [pH 8.0], 0.5 M EDTA, 5 M NaCl) and used for GFB-DNA cloning.

Extraction and cloning of GFB DNA The DNA was extracted according to the protocol of Lee *et al.*[5-7] with slight modification. In brief, GFB bodies resuspended in DNA extraction buffer, as described above, were solubilized with 1% *N*-lauroylsarcosine, incubated at 55°C for 1 hr and centrifuged at 4000×*g* for 15 min to remove debris. The supernatant was mixed with 1% CTAB (hexadecyl-trimethyl-ammonium-bromide) and incubated at 65°C for 10 min. The sample was clarified by adding an equal volume of chloroform : isoamyl alcohol (24 : 1) and centrifuging at 12,000×*g* for 5 min. The supernatant was clarified again by mixing with an equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1) mixture and centrifuging at 12,000×*g* for 5 min. The supernatant was mixed with 0.6 volumes of isopropanol and centrifuged at 12,000×*g* for 10 min. The pure DNA pellets were redissolved in 50 μl of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) containing DNase-free RNase (20 μg/ml).

For DNA cloning, the GFB DNA was digested with *Eco*RI restriction enzyme, then ligated with *Eco*RI linearlized pBS (Stratagene Biotec, La Jolla, CA) plasmid. The recombinant plasmids were used to transform competent cells of *E. coli* JM 83 by the method described by Sambrook *et al.*[11]. Ampicillin-resistant colonies were selected and screened by dot hybridization using biotin-labeled DNA preparations from GFB-infected Tankan tangor plants as well as from healthy Tankan tangor plants. The biotin-labeled DNA was prepared by Nick translation system (Gibco BRL, Gaithersbur, Md.) with biotin-7-dATP. Each colony was used in a minipreparation to isolate recombinant plasmids by alkaline lysis methods. Three microliters of plasmid DNA from each individual colony was spotted on nylon membranes after being suspended in 6 × SSC buffer (0.9 M NaCl, 0.09 M sodium citrate, pH 7.0). The membrane was baked, prehybridized and hybridized as described in a later section.

The clone which hybridized with the biotin-labeled DNA from diseased Tankan plants but not with the labeled DNA from healthy Tankan plant was selected as the candidate for developing GFB-DNA probes. The DNA from the candidate clone was labeled with biotinylated deoxyribonucleotides. The specificity and sensitivity of this biotinylated probe was verified by hybridization against DNAs isolated from either diseased or healthy citrus plants.

PCR-labeling of GFB-DNA probes The recombinant plasmid containing the GFB-specific DNA fragment was used as a template for PCR amplification to develop a biotin-labeled probe according to the PCR-labeling methods described by Panoud *et al.* with a slight modification[9]. Amplification was performed in 50 μl of reaction mixture containing 50 ng each of the two opposing primers (T3 primer : 5′-AATTAACCCTCACTAAAGGG-3′ ; T7 primer : 5′-GTAATACGACTCACTATAGGGC-3′), 100 ng of recombinant plasmids as templates, 0.2 mM each of three dNTPs (dTTP, dCTP dGTP), 0.18 mM of dATP, 0.02 mM of biotin-7-dATP (Gibco BRL) and 2.5 U of *Taq* polymerase in incubation buffer (100 mM Tris [pH 8.3], 500 mM KCl, 2 mM $MgCl_2$ and 0.01% gelatin, w/v). The reaction mixture was overlaid with 30 μl of mineral oil. The thermal cycles included two stages : the first stage was run one cycle (denaturation at 94°C for 4 min, annealing at 50°C for 1 min and extension at 72°C for 2 min) ; the second stage was run 30 cycles (denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min) and a final extension at 72°C for 10 min. All thermal cycles of PCR were performed at DNA Thermal Cycler 480 (Perkin-Elmer Cetus, Foster City, CA). The PCR product was purified by High Pure PCR Product Purification Kit (Boehringer Mannheim, Germany) and suspended in 50 μl of TE buffer (pH 8.0).

Preparation of DNA samples for hybridization Isolation of GFB from detected plants was conducted as described previously but deleting the sucrose density gradient centrifugation step. Briefly, about 5 g of leaf midribs was powdered in liquid nitrogen and suspended in GFB extraction buffer. After low speed centrifugation (4000×*g* for 10 min), the supernatant was centrifuged at 12,000×*g* for 15 min to pellet GFB. The GFB-DNA was directly extracted from the partially purified GFB. The partially purified GFB-DNA was suspended in 6 × SSC to make 3 μg/μl and was routinely used to detect the GFB.

For detection of GFB in dried citrus samples, leaf midribs were air-dried to about 50% of the fresh weight. The DNA samples from dried midribs were prepared as described above.

Two alternative DNA preparation methods were used to assess DNA purity and suitability for dot hybridization. One was highly pure DNA prepared from sucrose gradient-purified GFB (isolated from diseased plant midribs). Another one was a total DNA preparation that was much simpler, consisting of only one step. The total DNA was extracted from GFB-infected plants without the first step GFB isolation.

DNA hybridization For dot hybridization, DNA samples in 6 × SSC buffer were denatured by boiling for 10 min. Three microliters DNA, after two-fold serial dilution in 6 × SSC (starting concentration of 3 μg of DNA per microliter), were dot blotted onto nylon membranes. The membranes were air-dried, then baked at 80°C for 2 hr under vacuum. Prehybridizations were performed at 68°C for 1 hr in hybridization solution : 5 × SSC, 0.1% (w/v) *N*-lauroylsarcosine, 0.02% (w/v) SDS and 1% (w/v) blocking reagent (Boehringer Mannheim, Germany). Hybridizations were performed at 68°C for 12-16 hr in hybridization solution plus denatured DNA probes (50 ng/ml). The membranes were then washed twice for 5 min at room temperature with 2 × SSC containing 0.1% SDS and twice for 15 min at 68°C with 0.1×SSC containing 0.1% SDS. The membranes were rinsed for 1 min in buffer 1 (0.1 M Tris-HCl [pH 7.5], 0.15 M NaCl) and blocked at 64°C for 1 hr in buffer 2 (3% bovine serum albumin in buffer 1), then incubated in enzyme solution (0.1% streptavidin-alkaline phosphatase conjugate in buffer 1) at room temperature for 12 min. After washing twice at room temperature for 15 min with buffer 1, the membranes were rinsed for 2 min with buffer 3 (0.1 M Trsi-HCl [pH 9.5], 0.1 M NaCl, 50 mM $MgCl_2$) and incubated in substrate solution (buffer 3 containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate). The signals appeared after incubation for 30-45 min. The coloring reactions were stopped by TE buffer (pH 8.0).

For Southern hybridization, DNA samples digested with *Eco*RI were electrophoresed in 1.4% agarose gel. The gels were then incubated in 0.2 N HCl for 10 min, soaked in denaturing solution (0.5 N NaOH, 1.5 M NaCl) for 35 min, equilibrated in transfer solution (1 M NH_4OAc, 0.02 N NaOH) for 10 min and then transferred onto nylon membranes by capillary-force transfer. The membranes were hybridized with biotin-labeled DNA probes and labeled 100-bp DNA Ladders prepared by Nick translation system (Gibco BRL). All the procedures of DNA hybridization and signal coloring were conducted as described previously.

RESULTS

Cloning of GFB DNA

GFB could be isolated from citrus plants by differential centrifugation and sucrose density-gradient centrifugation with poor efficiency. A total of 836 transformed *E. coli* colonies were obtained through cloning GFB DNA. All transformed colonies were screened individually by dot hybridization with biotin-labeled DNA preparations from both GFB-infected and healthy plants. Only three clones were GFB-specific. The recombinant plasmids were extracted individually from the three clones and biotinylated to make probes for dot hybridization tests. One stable clone with an insert of about 0.24-kb was selected to develop GFB DNA Probe 1 (GP1). Dot hybridizations of the GP1 consistently showed specific, sensitive reactions to nucleic acid extracts from GFB-infected citrus plants.

Detection of diseased citrus plants by dot and Southern hybridizaton tests with labeled GFB DNA probe 1 (GP1)

The results of dot hybridization of GP1 to the DNA preparations from diseased and healthy citrus plants in Taiwan are shown in Fig. 1. The citrus cultivars of Ponkan mandarin (*Citrus poonensis* Tanaka.), Tankan tangor (*C. tankan* Hay.), Luchen sweet orange (*C. sinensis* Osb.), Wentan pummelo (*C. maxima* f. *buntan* Hay.) and grapefruit (*C. paradisi* Macf.) with characteristic greening symptoms showed positive hybridization signals with GP1, whereas no hybridization signals appeared in healthy samples. Southern hybridization analysis also demonstrated that GP1 was specific and sensi-

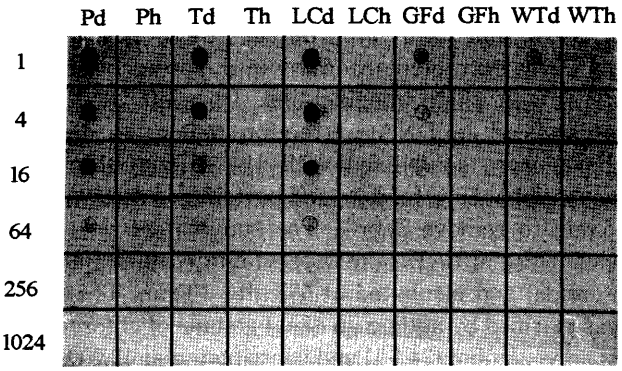

Fig. 1. Dot hybridization of the biotin-labeled GFB-DNA probe 1 (GP1) to DNA samples prepared from diseased (GFB-infected) or healthy (GFB-free) citrus samples. Samples from diseased citrus cultivars including Ponkan mandarin (Pd), Tankan tangor (Td), Luchen sweet orange (LCd), Grapefruit (GFd) and Wentan pummelo (WTd) were collected from orchards in Taiwan ; samples from healthy shoot-tip grafting citrus cultivars including Ponkan mandarin (Ph), Tankan tangor (Th), Luchen sweet oranges (LCh), grapefruit (GFh) and Wentan pummelo (WTh) were grown in an isolated greenhouse. Nucleic acids were applied to nylon membranes in 4-fold dilution series, 3 μl per spot ; the number 1, 4, 16, 64, 256 and 1024 indicate reciprocals of dilution from starting concentration at 3 μg/μl.

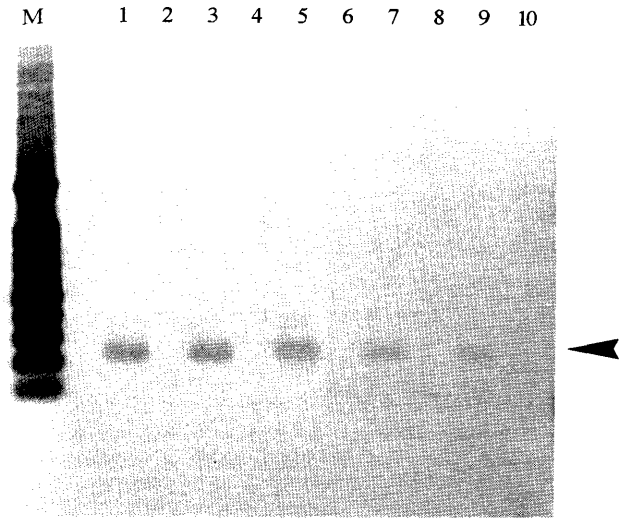

Fig. 2. Southern hybridization of the biotin-labeled GP1 to DNA samples prepared from diseased or healthy citrus samples. Lane M, the 100-bp DNA ladder as a marker ; lane 1-10, citrus cultivars as described in Fig. 1 : lane 1, Pd ; lane 2, Ph ; lane 3, Td ; lane 4, Th ; lane 5, LCd ; lane 6, LCh ; lane 7, GFd ; lane 8, GFh ; lane 9, WTd ; lane 10, WTh. The arrow indicates position of bands corresponding to size of the probe (about 0.24-kb).

tive in detecting GFB DNA in samples extracted from GFB-infected citrus plants. The hybridization signals of DNA extracted from healthy and diseased citrus samples were very different. The signal bands were located between 200-bp and 300-bp DNA marker (Fig. 2).

GP1 hybridized not only with GFB strains of Taiwan but also with several Asian strains collected from such locations as Okinawa (Japan), Kuangtung (China), Hanoi (Vietnam), Davao (Philippines), Sarawak (Malaysia), Chiang Mai (Thailand), Delhi (India) and Al Mofaja (Saudi Arabia). As shown in Fig. 3, the nucleic acid preparations from all Asian strains hybridized with GP1. However, samples from diseased plants collected from Pretoria (South Africa) did not hybridize with GP1.

The signal strength of dot hybridization from different samples was differentiated by serial dilution of the DNA extracts. The relative strength of signals is summarized in Table 1. Each spot of the dot hybridization produced a differential signal strength with its end point and marked as "+".

Comparative sensitivity of GFB detection between fresh and dried materials

Air-drying the citrus tissues before DNA extraction did not affect the sensitivity of dot hybridization with GP1 (Fig. 4). The signal strength from the dried, diseased pummelo sample was slightly stronger than that from the fresh, diseased pummelo sample, as demonstrated by a nucleic acid reciprocal dilution test.

Effects of the purity of DNA samples on dot hybridization sensitivity

DNA samples purified by three different methods were compared for their adequacy and sensitivity in a dot hybridization test. As shown by Fig. 5, the purity of the DNA samples affected the signal strength of the dot hybridization. The highly purified DNA preparation (through GFB-bodies isolation by sucrose density gradient centrifugation) produced a slightly better result with dot hybridization than did the standard preparation method. The crude DNA preparation (total DNA extraction) without the procedure of GFB isolation resulted in poor dot hybridization, especially in the case of pummelo samples.

DISCUSSION

The efficiency of GFB-DNA cloning in this study was very low ; only 3 out of 836 clones were specific. Low concentration and uneven distribution of GFB in the citrus tissue probably contributed to the low efficiency. Besides, separating the GFB from the organelles, *e.g.* host mitochondria which are similar in morphology and

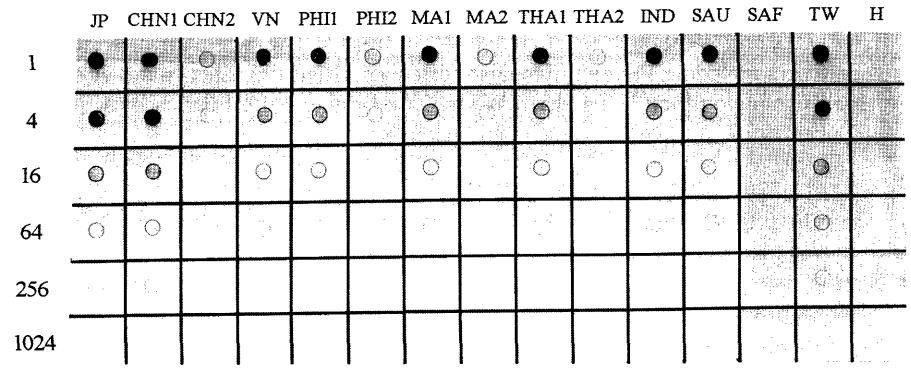

Fig. 3. Dot hybridization of the biotin-labeled GP1 to DNA samples prepared from diseased citrus cultivars collected from different countries including Okinawa of Japan (JP), China (CHN1, CHN2), Vietnam (VN), Philippines (PHI1, PHI2), Malaysia (MA1, MA2), Thailand (THA1, THA2), India (IND), Saudi Arabia (SAU), South Africa (SAF) and Taiwan (TW). Lane H indicates a nucleic acid sample from healthy citrus (Valencia sweet orange) for negative control. The number 1-1024 indicate reciprocals of 4-fold dilution from starting concentration 3 μg/μl of nucleic acid samples.

Table 1. Detection of greening fastidious bacteria (GFB) in citrus cultivars from different geographic origins by dot hybridization using GFB-specific DNA probe

Abbreviation	Cultivar	Country	Signal strength[a)]					
			1	4	16	64	256	1024
Pd	Ponkan mandarin	Taiwan	+++++	++++	+++	++	+	−
Td	Tankan tangor	Taiwan	+++++	++++	+++	++	+	−
LCd	Luchen sweet orange	Taiwan	+++++	++++	+++	++	+	−
GFd	Grapefruit	Taiwan	+++	++	+	−	−	−
WTd	Wentan pummelo	Taiwan	++	+	−	−	−	−
JP	Sikasya mandarin	Japan (Okinawa)	+++++	++++	+++	++	+	−
CHN1	Hungchiang sweet orange	China	+++++	++++	+++	++	+	−
CHN2	Peiyu pummelo	China	++	+	−	−	−	−
VN	Valencia sweet orange	Vietnam	++++	+++	++	+	−	−
PHI1	Zinkom mandarin	Philippines	++++	+++	++	+	−	−
PHI2	Local wild pummelo	Philippines	++	+	−	−	−	−
MA1	Lankat mandarin	Malaysia	++++	+++	++	+	−	−
MA2	Johor pummelo	Malaysia	++	+	−	−	−	−
THA1	Som-Keo-Wa mandarin	Thailand	++++	+++	++	+	−	−
THA2	Kao-Pean pummelo	Thailand	+	−	−	−	−	−
IND	Kino mandarin	India	++++	+++	++	+	−	−
SAU	Willow leaf mandarin	Saudi Arabia	++++	+++	++	+	−	−
SAF	Valencia sweet orange	South Africa	−	−	−	−	−	−
TW	Valencia sweet orange	Taiwan	+++++	++++	+++	++	+	−

Data are summarized from Figs. 1 to 3.

a) −, no hybridization signal; +, end point signal of serial dilution; ++～+++++, comparative signal strength. The numbers 1, 4, 16, 64, 256 and 1024 indicate reciprocals of dilution from starting concentration 3 $\mu g/\mu l$ of nucleic acid.

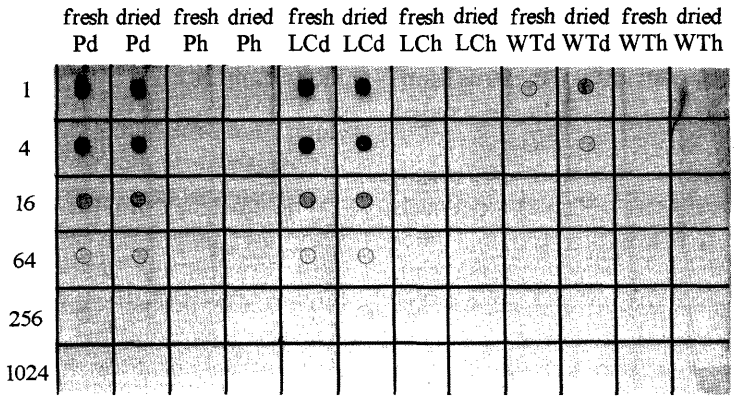

Fig. 4. Dot hybridization of the biotin-labeled GP1 to DNA samples prepared from fresh or air-dried tissues. Fresh tissues from diseased or healthy citrus cultivars including Ponkan mandarin (fresh Pd, fresh Ph), Luchen sweet orange (fresh LCd, fresh LCh), and Wentan pummelo (fresh WTd, fresh WTh); while air-dried tissues from diseased and healthy citrus cultivars including Ponkan mandarin (dried Pd, dried Ph), Luchen sweet orange (dried LCd, dried LCh), and Wentan pummelo (dried WTd, dried WTh). The number 1-1024 indicate reciprocals of 4-fold dilution from starting concentration 3 $\mu g/\mu l$ of nucleic acid samples.

size[12)], was difficult. Therefore, cloning GFB is somewhat more difficult than cloning other phytopathogens under such circumstances.

This study showed that GP1 was a stable and useful diagnostic DNA probe for detecting citrus greening organism. It could react with all Asian GFB strains from such countries as Taiwan, Japan (Okinawa), China, Vietnam, Philippines, Malaysia, Thailand, India and Saudi Arabia, but not with the strain from South Africa. Two types of citrus greening diseases have been recognized: one is the Asian type, which develops symptoms at temperatures above 30°C; the other is the African type with no symptoms above 30°C[1)]. More recently, GFB has been characterized and tentatively named *Liberobacter*. In 1997 Jagoueix *et al.* proposed two different species for the Asian and African GFBs from a comparison of their 16S/23S rDNA sequences[4)]. The Asian GFB was named *Liberobacter asiaticum*, and the African GFB was named *Liberobacter africanum*. Our results indicated that the probe (GP1) was specific to *Liberobacter asiaticum*, and could be used as a common probe for all GFB strains from various citrus species.

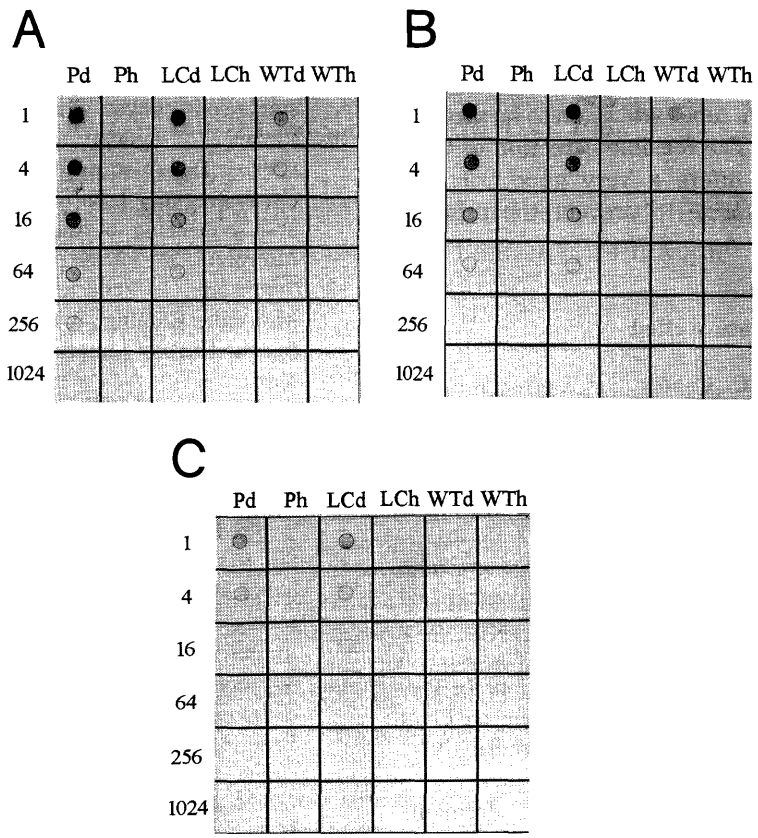

Fig. 5. The effect of nucleic acid extraction methods and samples from diseased or healthy citrus on the dot hybridization using biotin-labeled DNA probe (GP1) (Fig. A-C). Samples from diseased and healthy citrus cultivars including Ponkan mandarin (Pd, Ph), Luchen sweet orange (LCd, LCh), and Wentan pummelo (WTd, WTh). A, nucleic acid samples from high purity DNA preparation through a GFB isolation procedure by sucrose density gradient centrifugation (DGCS); B, nucleic acid samples from standard DNA preparation through partial purification of GFB by differential centrifugation without DGCS; C, nucleic acid samples from total nucleic acids of citrus tissues without GFB isolation and DGCS. The number 1-1024 indicate reciprocals of 4-fold dilution from starting concentration 3 $\mu g/\mu l$ of nucleic acid samples.

Under the same DNA preparation method, the relative strength of the hybridization signals could reflect the GFB-DNA contents. For differentiation of the strength of signals, reciprocal DNA dilutions were made. As shown in Table 1, all signals from dot hybridizations could be divided into five grades according to their dilution end points and signals. The DNA samples from diseased mandarins (including tangor) and diseased sweet oranges gave stronger signals; their original signals (undiluted) can be categorized as first (+++++) or second (++++) grade. The original signals from diseased grapefruit samples can be categorized as third grade (+++). The DNA extracts from diseased pummelos gave the weakest signals, fourth (++) or fifth (+) grade. The results suggest that the GFB population in pummelo hosts may be lower than that in mandarin or sweet orange. As we compared all local and Asian GFB strains, we found that these geographically different citrus strains did not show significant differences in hybridization signals, except that from South Africa which did not react with GP1.

For accuracy and consistency of GFB detection, the simplification and standardization of the dot hybridization procedure are indispensable. The DNA preparation methods affected the sensitivity of dot hybridization. High purity DNA preparations (through GFB isolation by density gradient centrifugation of sucrose) resulted in the best sensitivity of detection, but they were time-consuming. Low purity DNA preparations (total DNA extracts from citrus hosts) were rapid and simple, but gave no clear hybridization signals. Moderate purity DNA preparations (through partial purification of GFB by differential centrifugation) was ideal because it was less time consuming and was not significantly different in dot hybridization sensitivity from the high and moderate purity DNA preparations. Therefore, this method was chosen as the standard for preparation of DNA samples for GFB detection in our laboratory.

Interestingly, the GP1 probe could hybridize to DNA extracts from dried citrus tissues, which gave slightly stronger signals than those from fresh samples. The results indicated that GFB should still be intact in sieve tubes of dried tissues, and the GFB-DNA content in prepared DNA extracts would probably be more stable

because of the lack of nucleases in dried tissues. Drying tissues usually causes clumping of host cells, which interferes with the extraction of DNA from host cells of mesophyll. However, sieve tubes still seem to be protected in vascular boundles. The feasible detection of GFB in dried citrus samples offers a very good way for international GFB quarantine to prevent the possible spread of GFB.

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和 文 摘 要

Ting-Hsuan HUNG・Meng-Ling WU・Hong-Ji SU：非放射性標識DNAプローブを用いたカンキツグリーニング病の病原難培養性細菌の検出

Greening fastidious bacteria (GFB)（一時的に *Liberobacter* とも呼ばれている）は，カンキツグリーニング病を引き起こす病原体である。本病原細菌は宿主植物の篩部に低密度で存在する。本研究では，感染カンキツよりGFBを高感度で特異的に検出できるDNAプローブをクローニングによって得た。GFBに特異的に反応する0.24 kb断片を含むクローンをビオチン化ヌクレオチドを用いたPCR法によって標識し，この標識DNA断片をプローブとしたドットハイブリダイゼーションによって，マンダリン，タンゴル，スイートオレンジ，バメロなどの数種カンキツ植物中のGFBを検出することができた。本プローブはアジア諸国から蒐集したすべての感染植物のGFBと特異的に反応したが，南アフリカから得た植物のGFBとは反応しなかった。本プローブは感染植物中の微量のGFBを検出するために十分な特異性と感度を示し，特にアジア型グリーニング病の検疫に有効利用できる。