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Isolation of Endophytic Bacteria and Amplification of 16SrDNA on Citrus in Zhaoqing Districts

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Abstract

Zhaoqing is the main source of Citrus in China. However, the reason of citrus Huanglongbing (HLB) is unclear and may be from endophyte. Therefore, the study of citrus endophyte is necessary. The experiment would be carried out by endophyte being isolated from citrus plant and amplifying the specific 16SrDNA fragment from endogenous bacteria to know the relation of citrus HLB to 16S rDNA of endogenous bacteria. The results showed that total 55 strains of endophytic bacteria were identified. And one specific fragment long of 1261 nt was got. Sequencing analysis showed for 91.3% homology to uncultured bacterium clone. And the 16SrDNA fragment was identified by PCR. The experiments would provide a theoretical basis for understanding the differences in pathomechanism among various HLB isolates.

Keywords: Citrus in Zhaoqing, endogenous bacteria isolated, 16S ribosomal (r)DNA fragment, sequencing and analysis

1. Introduction

Citrus Huanglongbing (HLB) is called as one of the most destructive diseases of citrus in world (Bové, 2006). And it infects citrus trees of almost all cultivars and causes substantial economic losses to the industry by adversely affecting the crop and shortening the lifespan of infected trees (Manjunath, 2008). HLB is caused by phloem-limited, unculturable alpha-proteobacteria of the genus Candidatus *Liberibacter* that are transmitted by plant-feeding psyllid vectors (Garnier, 1993; Gravena, 2004; Halbert et al., 2004). Although there are sparse reports on the cultivation of HLB bacteria on complex media (Sechler et al., 2009), the fact remains that HLB-causing bacteria are at present largely unculturable in the majority of laboratories world wide. This has severely limited our ability to examine carefully the bacteria's biology, making it difficult to develop effective, specific control strategies. Some reports have been published for specific detection and identification of individual *Xanthomonas* species (Palacio-Bielsa et al., 2004). However, specific diagnostic protocols for HLB have not yet been developed so that surveying a disease complex involving HLB-causing bacteria can be very useful to apply a tool detecting and correctly identifying all members of the genus at the same time. The 16S ribosomal DNA and multilocus sequence analyses have been widely used for studying phylogenetic diversity in microbial communities (Hauben et al., 1997; Vero et al., 2002; Almeida et al., 2001). More recently genome-based phylogeny of the genus *Xanthomonas* with species representing the major lineages within the group have been reported (Rodrigues et al., 2010). It is currently unclear whether this variation is caused primarily by differences among HLB-causing strains, differences in host genotype, environment, or some combination of these factors. Outside of the broad phylogeographic patterns previously described, it is also unclear how other worldwide HLB pathogens have evolved. The present study was conducted for molecular characterization and determining the sequence variabilities of the 16S ribosomal DNA and 16S-23S ribosomal intergenic region among five HLB isolates collected from different citrus cultivars from Zhaoqing district, a major citrus-growing belt in China, to establish their relationship within the isolates under present study as well as to compare them against previously reported relative HLB strains.

Zhaoqing districts of Guangdong Province is an important citrus-growing region in China. In the present study, we surveyed the citrus orchards to determine the extent of HLB disease in this region. HLB-like symptoms were present on a wide variety of citrus crops. Therefore, amplifying 16S rDNA from a large number of HLB citrus using primer sets OI1/OI2c and OI2/23S1 designed according to 16S rDNA and 16S/23S intergenic spacer

region of bacteria by PCR was carried out. In our studies, the 16S rDNA was cloned, sequenced and analized.

2.Materials and Methods

2.1 Plant Material Selection

Sample collection and culture maintenance A survey was conducted during October 2013 to record the incidence and distribution of HLB disease in two districts (Guangning and Huaiji) in Zhaoqing of China. The young twigs, leaves, and budstick samples from trees showing possible greening-related symptoms, were collected from a variety of citrus cultivars in Zhaoqing area (Figure 1).

Figure 1. Infected yellow leaves of Citrus

2.2 Isolation and Identification of Endogenous Bacteria

The samples were collected according to the Yellow shoot and leaves mottled in Citrus (Figure 1). And 24 samples of citrus HLB plant leaves from Zhaoqing districts in China The majority of strains were isolated from the samples by using normal bacteria isolating method, that was the leaves were immersed in sterile water for 5-6 times after surface disinfection with 75 alcohol, then the leaves of the xylem were removed and implanted in bacterial culture medium each dish 3-4 parts at 37 °C 1-2 d. And then the pure culture of bacterial colonies wuldbe obtained for studying. Pure culture is defined as a mass or group of cells arising from the same parent cell. Pure culture techniques, such as streak plate, pour plate and spread plate, isolate bacterial colonies from mixtures so that colonies comprising of the identical organisms can be studied. Isolation of pure culture is vital for characterizing a single species of bacteria Â otherwise presence of contaminants can lead to inaccurate observations (Jing et al., 2011). Then the isolated bacterial colonies undergo incubation at 370 °C for 24 hours for all the three plates, after which, a colony can be selected and isolated again to obtain pure cultures. Another step in the experiment is to perform a differential staining method known as Gram staining, which mainly differentiates the bacteria into two categories: Gram stain (stain Gram) not only can observe the morphological characteristics of the bacteria but also can be divided into two major categories of all the bacteria: blue purple is known as gram positive bacteria, G+ and red staining reaction (color) as gram negative G- (Campbell & Reece, 2005). One previously studied HLB isolate was maintained under equivalent conditions as a positive control.

2.3 Genomic DNA Isolation

DNA was extracted from the isolated bacterial strains. Starting material for DNA extraction consisted of liquid incubates after the surface of 48 h-old pure cultures grown on normal medium. Extraction of DNA was conducted using the Qiagen DNeasy Blood and Tissue kit (Qiagen) and performed according to the manufacturer's instructions. Extracted DNA samples were stored at 4 °C until use.

In order to compare the difference of total DNA between from the leaves with HLB and from the bacteria isolated in citrus with HLB, the Symptomatic leaves collected from the field were washed, blotted dry and cleaned with 70% ethanol to avoid surface contamination. Midribs and petioles were aseptically excised and ground with liquid nitrogen. DNA was extracted by using the DNA easy mini kit (Qiagen) as per the manufacturer's protocol. Briefly, 0.5 g of midribs was cut into small pieces with sterilized scissors, frozen with

liquid nitrogen then ground into powder with a mortar and pestle. Two milliliters of a 2% CTAB buffer were added, the mixture was transferred into a 1.5 ml Microcentrifuge tube and incubated in a water bath at 65 °C for 30 min with occasional agitation. After extraction with a phenol/chloroform/iso-amyl alcohol solution (24:24:1), DNA was pre-cipitated by the addition of 1/10 volume of 3 M sodium acetate (pH 5.2) and two volumes of alcohol and was collected by centrifugation. The pellets were dissolved in 100 µl of Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) with 0.5 µl of RNaseA (10 mg/ml) and incubated in a water bath at 37 °C for 30 min. DNA was stored at -20 °C for later useAnd normal leaves DNA of citrus was as the control.

2.4 PCR Amplification of 16S rDNA

Primer design and PCR reactions development of primers involved multiple sequence alignment and identification through BioEdit (Hall, 1999) of conserved consensus regions in genes common among *Liberibacter asiaticus* and xanthomonads in this study.

PCR amplification of 16S rDNA and 16S intergenic spacer region was carried out using common primer from bacteria sequences of these in the NCBI GenBank. As followed sets of F: 5′ GCGCGTATGCAATACGAGCGGCA 3′ and R: 5′ GCCTCGCGACTTCGCAACCCAT 3′ called OI1/OI2 primers respectively, in 25 µl reaction volume. Each reaction contained 100 ng of total DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of dNTP each, 0.4 mM of each primer, 1.25 U of GoTaq Hot Start DNA polymerase (Promega) and nuclease-free water. PCR amplification for OI1/OI2c primer set was carried out in Eppendorf Mastercycler Gradient PCR machine (Eppendorf, Hamburg, Germany) with one cycle of 4 min at 95 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, 1.30 min at 72 °C, and final extension at 72 °C for 10 min. For OI2/OI2 primer set PCR amplification was separately carried out in the same thermal cycler with one cycle of 4 min at 95 °C followed by 35 cycles of 0.45 min at 94 °C, 0.45 min at 55 °C, 1 min at 72 °C, and final extension at 72 °C for 10 min.

2.5 Cloning of DNA and Sequence Analysis

The amplified PCR products were separated by horizontal gel electrophoresis in 1.5 % agarose gels in 0.5x TBE (Tris-borate EDTA) buffer at 50 V/cm for 45 min.The pre-stained gel with ethidium bromide (0.5 µg/ml) was then visualized under UV GelDoc system (Bio-Rad, USA). The expected PCR products were cloned into pMDt~8-T (TaKaRa) (Sambrook, 1989) and sequenced by Shenggong, China (Ding et al., 2005).

The sequence analysis started by searching the identity in NCBI Blast, then used the DNAman (Lynnon BioSoft, 5.2.2.0), Clustal X (EMBL-EBI, 1.81) and DNAstar software (Install Shield Software Corporation, InstallShield, 5.00.221.0) for multiplex alignment and phylogenetic tree construction. Bootstrapping (10,000 replications) was performed to estimate stability and support for the inferred clusters (Edgar, 2004).

2.6 Detection of Citrus by PCR

In order to identify the usage of the primers from 16SrDNA, the healthy green leaves and fruits with similar symptoms to huanglongbing according to the the symptoms Figure 1 showed were collected from Huaiji and Guangning county of Zhaoqing City orange sugar producing areas and stored at -80 °C ~ -20 °C. The DNA amplication was carried out by using primer sets OI1/OI2c. PCR amplification parameters and conditiongs were as mentioned before. The amplified DNA was visualized in UV GelDoc system (Bio-Rad, USA) after electrophoresis with ethidium bromide at a constant voltage of 100 V for 90 min in a 1.5% agarose in 1X TAE buffer.

3. Results

3.1 Isolation of Endogenous Bacteria in Citrus

Endophytic bacteria isolated from the branches and leaves of citrus plant were detected by microscopy after pure incubation according to colony morphology and Gram staining method for bacteria (Figure 2), Then total 55 strains of endophytic bacteria including 20 strains of healthy plants and 35 strains of disease plants belong to 5 genera finally were got in corresponding physiological and biochemical reactione referring to Berger'sManual of Systematic Bacteriology classification and identification. The citrus leaves with HLB were lack of genus of Bacillus by staining, morphological and physico-biochemical identification, and the total bacteria species were less than healthy plants (Tables 1 and 2).

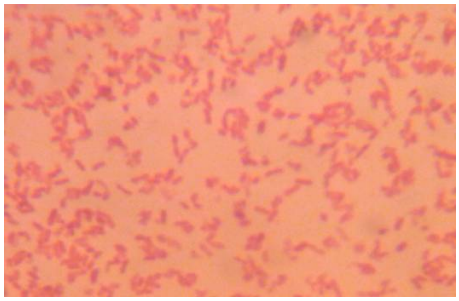

Figure 2. Plant endophytic bacteria Gram staining

Table 1. Isolation and identification of endophytic bacteria in citrus plants

	No	Strains	Characteristics	Identification
Healthy	A1	12	Gram positive, rod-shaped bacteria, spore,colony smooth, moist, smoothedge, lawn light yellow, translucent, contact enzyme test positive	Bacillus
	A2	8	Rod-shaped, gram negative, rough, oxidase test negative	Soil bacillus
Diseases	A3	9	Straight rod, gram negative, asporous, round and bacillussmooth, edge nonsmooth, lawn light yellow, transparent, oxidase and catalase test positive	*Xanthomonas*
	A4	6	Straight rod-shaped, gram negative, asporous, rough, thick sand cell, pale yellow, translucent, negative oxidase test, contact enzyme test positive	Erwinia
	A5	15	Rod-shaped bacteria, gram negative, asporous, colony drier, flat edge, lawn light white, translucent, oxidase test positive	*Pseudomonas* sp.
	A6	5	Rod-shaped bacteria, gram negative, rough, thick sand, pale yellow, translucent, negative oxidase test	Soil bacillus

Table 2. Comparisom of number and strains of bacteria isolated from citrus with HLB and health

Material	Strains	Genus
Healthy	20	The genus bacillus, *Agrobacterium* sp
HLB	35	*Xanthomonas* genus, *Pseudomonas* sp, *Agrobacterium*, Erwinia

3.2 PCR Amplication and Cloning of 16S rDNA

PCR amplification of 16S rDNA ntergenic spacer region was carried out using common primer from bacteria sequences of these in the NCBI GenBank. The expected PCR products of 1260 nt were cloned into pMD-T (TaKaRa) and identified by PCR. The obvious bands were amplified by PCR wherever the material with HLB or non HLB from Huaiji or Guangning area.

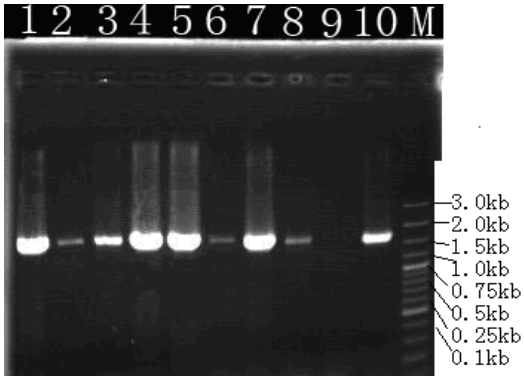

Figure 3. Selection of the positive clones by PCR

Note. 1. Clone from Huaiji leaf with HLB; 2. Clone from Huaiji plants with HLB; 3. Clone from Guangning plants with HLB; 4. Clone from Guangning plants with HLB; 5. Clone from Guangning healthy plants; 6. Clone from Guangning branch with HLB; 7. Clone from Guangning healthy plants; 8. Clone from Guangning branch with HLB; 9. dd H_2O; 10. Positive PCR, M.TaKaRaDL 3000 bp marker.

3.3 Sequence Analysis

Sequence of 1261nt long from citrus leaves amplified by PCR with the primers from endogenous bacteria 16SrDNA was identified as follows.

GGGCCTTGACTGATTACGATTCGAGCTCGGTACCCGGGGATCCTCTAGAGATTAA
CAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGG
GGGTTTCCGCCCCTCAGTGCTGAAGCTAACGCGTTAAGCACTCCGCCTGGGGAGT
ACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAACTCTTGACATCCC
ATTGACCGCTTGAGAGATCAAGTTTTCCCTTCGGGGACAATGGTGACAGGTGGTG
CATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCCTATCCTTAGTTGCCAGCATTTAGTTGGGCACTCTAGGGAGACTGCCGG
TGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCGCATGGCCCTTATGGGC
TGGGCCACACACGTGCTACAATGGCAATTACAATGGGAAGCAAGGCTGTAAGGC
GGAGCGAATCCGGAAAGATTGCCTCAGTTCGGATTGTTCTCTGCAACTCGGGAAC
ATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATATGTACC
CGGGCCCTGTACACACCGCCCGTCACACCCTGGGAATAGGTTTCGCCCGAAGCAT
CGGACCAATGATCACCCATGACTTCTGTGTACCACTAGTGCCACAAAGGCTTTTG
GTGGTCTTATTAGCGCATACCACGGTGGGGTCTTCGACTGGGGTGAAGTCGTAAC
AAGGTAACCCTAAATCGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGT
TTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCA
GCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCC
CTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTTC
TCCTTACGCATCTGTGCGTATTTCACACCGCATATGGTGCACCTCTCAGTACATCT
GCTCTGATGCGCATAGTAGCAGCCCGACACCCGCACACGGCTGACGCGCCTGAC
CGCTTGTCTGCTCCGCATCGCTACGAACAAAGCCTGGTGACGTTCGGACTGCATG
TGTTCCTAGTTCACCGTCATTCACACCGAACTGCTGCGAACCAGTAAAGGGCCG

Figure 4. 16S rDNA sequences of endophyte

3.4 Sequence Homology

There are many sequence homology bacteria showed, 11 sequences from homologic strains were selected for comparison by Blast searching of the sequence homology in Genbank. The comparison showed that the

16SrDNA sequences similar to known and many failed to clone bacteria (Uncultured bacterium) have a high similarity, the highest similarity is uncultured bacterium clone yf5-180 to 91.3% follow by uncultered bacterium AEC2582 to 90.5 and uncultered candidated bacterGS242 to 88.8 (see Figures 5 and 6). The homology of 16S ribosomal RNA gene was all lower, only it was just close to uncultered bacteriumAEC2582.seq (5), uncultered candidated bacterGS242.seq (6), uncultured *Aurantimonas* sp. (7)., Uncultured bacterium clone BH33-177 (8), Uncultured bacterium clone yf5-180 (10) and *Uncultured Methylobacterium* sp. (12). Therefore, it can be considered that this sequence may be a source of uncultured bacteria, because there is no isolated reports of Citrus Huanglongbing pathogen. However, they are not clearly identified the pathogen of Citrus Huanglongbing bacteria, this comparison column is likely to pathogenic bacteria related.

Percent Similarity in upper triangle

	1	2	3	4	5	6	7	8	9	10	11	12
1		83.4	82.6	81.4	90.5	88.8	88.5	88.4	83.4	91.3	84.0	88.5
2			98.5	96.9	86.5	75.8	81.2	77.4	99.0	80.8	99.7	81.0
3				95.9	85.7	75.1	80.2	76.3	97.9	80.8	98.2	80.1
4					84.3	73.7	79.0	75.6	96.6	80.5	97.4	78.9
5						84.4	88.2	83.6	86.8	85.3	87.4	88.6
6							86.7	83.4	75.9	87.6	76.3	85.5
7								84.0	81.2	89.8	82.1	96.1
8									77.7	89.1	78.3	83.9
9										80.8	99.6	81.1
10											80.9	89.3
11												82.0
12												

Figure 5. Distinguish of the nucleotides sequence among the different 16S region of bacteria isolate

Note. 1. 16srDNA.seq; 2. *Exiguobacterium acetylicum* strain VIT-S; 3. *Exiguobacterium sibiricum* strain BZa11; 4. *Proteobacterium* symbiont of Nilaparvata; 5. Uncultered bacteriumAEC2582.seq; 6. Uncultered candidated bacterGS242.seq; 7. Uncultured *Aurantimonas* sp; 8. Uncultured bacterium clone BH33-177; 9. Uncultured bacterium clone X671; 10. Uncultured bacterium clone yf5-180; 11. Uncultured *Exiguobacterium* sp. clone CD; 12. Uncultured *Methylobacterium* sp.

3.5 Phylogenetic Tree Analysis

Phylogenetic tree analysis shows that the endophytic bacteria 16S rDNA phylogenetic relationships were closer to the failed to produce bacteria (uncultured bacterium), and they were very close according to the genetic relationship. However, the relationship is far with uncultured *Exiguobacterium* sp. clone CD and uncultured *Methylobacterium* sp. strains (Figure 6).

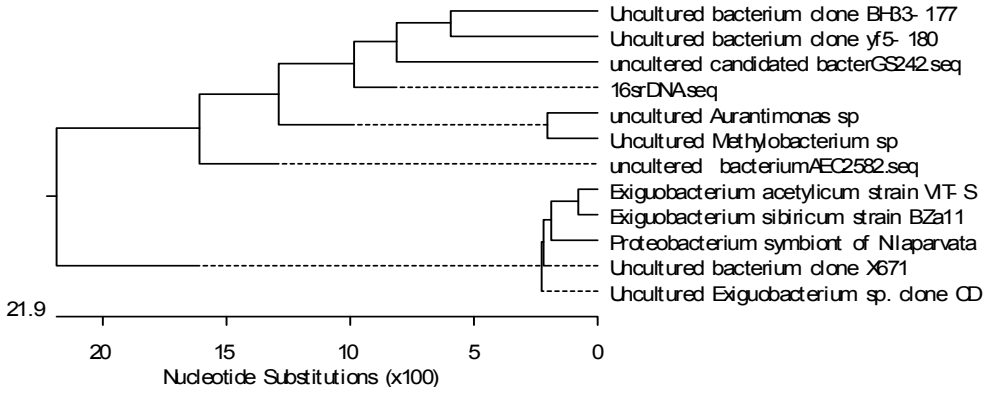

Figure 6. Phylogenetic tree analysis

3.6 Huanglong Pathogen 16SrDNA PCR Detection

The samples were collected from Zhaoqing HUAIJI and Quang Ninh, according to the phenotypic typical Huanglongbing and phenotypic health to pericarp DNA as a template and OI1 and OI2 primers for PCR amplification. 1167 bp specific band was amplified showed in Figure 7, including the yellow fruit typical Huanglongbing, and healthy non yellowing fruit phenotype. Healthy fruit PCR brightness and diseased plants compared to the lower, indicating that the diseased Huanglongbing infection is more serious, Huanglongbing pathogen content is relatively high, each sample showed positive samples were infected with Huanglongbing. In 4 samples, the positive rate was 100% (Figure 7). This PCR detection could not be used in citrus leaves for HLB because no specific band occurred but it could be as reference because the brightness of the electrophoretic bands is different. Brightness difference under the same quantity of samples added was verified not accidental, For the repeat detection for healthy leaves and sick leaves with brightness difference also could be observed.

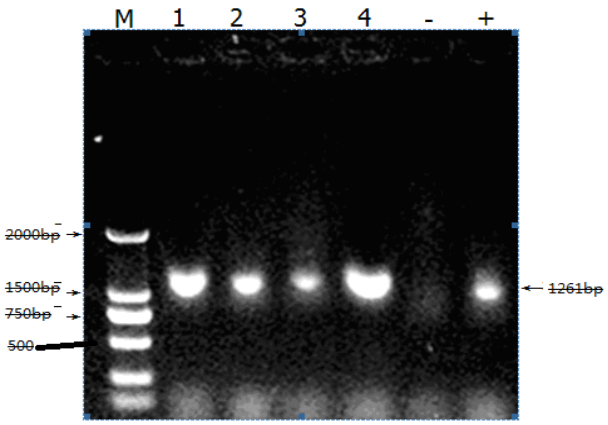

Figure 7. Huanglong pathogen 16SrDNA PCR detection

Note. M: DNA marker of DL 2000; +: the positive clone (from diseased plants); -: negative clones; 1: Source in HUAIJI diseased fruit; 2: Source in HUAIJI health fruit; 3: Source Guangning health fruit; 4: Source Guangning diseased fruit.

4. Discussion

Although the symptoms of HLB can be highly variable, it is currently unclear whether this variation is caused primarily by differences among HLB-causing strains, differences in host geno-type, environment, or some combination of these factors. Outside of the broad phylogeographic patterns previously described (Su et al., 2001). In this study, the number of endophytic bacteria species in healthy citrus plants were found less than in plantendophytic bacteria species. Among them, the *genus Bacillus* existed only in healthy citrus plants according to the relevant literature, and the *genus Bacillus* in multiple species had a low or moderate inhibitory effect on both *Escherichia coli* C83917, *Escherichia coli* bacteria C83901 and rat typhoid *Salmonella* bacteria such as

Bacillus subtilis and *Bacillus licheniformis* (Tang et al., 2009). Kong Qingjun isolated a strain of Bacillus from cotton which could produce obvious antagonism against *Fusarium Wilt* of cotton and Cotton Pink bacteria in all test plates showed clear inhibition zone(Kun et al., 2009) He Hong et al isolated 2 strains of pepper from the Capsicum Seedlings with good effect against *Bacillus subtilis* cabbage in banana anthracnose of crop (He et al., 2003), Ou et al. (2009) isolated 143 strains endophytic bacteria from mangrove, and 18 strains of Phytophthora capsici also had strong antagonism, and the strongest antagonistic effect of strain RS261 was tentatively identified as Bacillus amyloliquefaciens. The studies have shown that polymyxin, mould, gramicidin and active substances produced in the growth of *Bacillus subtilis* had an obvious inhibition on pathogens or endogenous infection caused by the conditions bacteria. The *Xanthomonas* genus strains for tests would be gone on phylogenetic studies using 16S ribosomal DNA sequences in our isolated strains, and other isolated endophytic bacteria such as *Pseudomonas* sp, *Agrobacterium*, *Erwinia* from plants associated bacteria should be worth further study.

The unculturable bacteria could not be incubated and isolated by conventional isolation and identification methods. However, molecular biological techniques not subject to this restriction. The method by using PCR and denaturing gradient gel (PCR-DGGE) was applied to identify Citrus endophytic bacteria resulting in nine genera of 15 kinds of different bacteria through the Blast and alignment in GenBank (Li et al., 2011). It was proved that non cultured bacteria could be identified by molecular biological technique. In our experiment, 16SrDNA, a relatively high homology to the bacteria of Citrus endophytic bacteria can not be cultured that was uncultured bacterium. Although the technique could prove the microorganisms populations of flora existed, the understanding of microbial properties and potential value still need to use the conventional isolation and culture to solve. So for the *Xanthomonas* genus, Pseudomonas sp, Agrobacterium and Erwinia in the soil and plants and as the uncultured bacterium identified by 16SrDNAanalysis need to be further studied for their relationship with citrus Huanglongbing pathogen between them.

Though unclear world-wide HLB pathogens how to evolve,the, the present study conducted for molecular characterization and determining the sequence variabilities of the 16S ribosomal DNA and 16S-23S ribosomal intergenic region is an important work for further understanding HLB pathoen.

16S rDNA presents in all prokaryotic cells, and it is an important indicator of the study of bacterial classification, evolution and genetic relationship of using in phylogenetic studies, especially on uncultured bacteria for molecular classification is necessary. At present, it is known that as long as the 16S rDNA gene less than 3% in bacteria and plant phytoplasma would be set for a new species (Dan et al., 2008). Because there is no culture of Citrus Huanglongbing pathogen isolated reports, the molecular study on uncultured bacterium is meanful. It is reported that the proportion of failing to culture bacteria in Citrus Huanglongbing bacteria is accounted for bigger than 5% (Wang et al., 2010). In this study, the use of molecular biology methods of Guangdong Zhaoqing Guangning Citrus Huanglongbing plant 16S rDNA was amplified by PCR, and the PCR products were cloned, sequence determination and homology analysis, to determine the taxonomic status of the plants. At present, domestic and foreign scholars on Citrus Huanglongbing plant pathogens carried out a lot of research in order to provide the reference basis for the prevention and control of Citrus Huanglongbing. In this study, a fragment was found a special sequence, homology within NCBI database comparison, similarity with its highest is uncultered candidated bacterGS242.seq and uncultured bacterium clone yf5-180 to 90.5-91.3% through amplication of Huanglongbing phytoplasma 16S rDNA gene in Zhaoqing Citrus. Despite the report that Asian species of Huanglongbing pathogen could be successfully cultured in citrus leaf extract medium, only a isolated case, no repetition of successful reported again (Sechler et al., 2009). Therefore, this means that the sequence determination indicated an undiscovered species of bacteria. The bacteria likely pathogen is huanglongbing. And it will be a valuable subject for the study of the endogenous bacteria from citrus.

The study had shown that different symptoms in different parts of the same plant performance would occurred (Sun et al., 2012). The symptoms were more obvious so relatively easy to identify in the leaves other than in the fruit difficult to distinguish. It was induced that the endophytic bacteria strains in leaf and fruit maybe different. So that the symptoms and severity of Huanglongbing in different citrus species were different (Cheng et al., 2013; Zhao et al., 2013).

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