First report *Emilia sonchifolia* wilt caused by *Ralstonia solanacearum*

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Abstract: *Emilia sonchifolia* bacterial wilt was severely occurred in the field in Guangxi, China. The pathogenic bacterium was confirmed by Koch postulate. Then Polymerase chain reaction(PCR) amplification was conducted using 16s rDNA universal primers to identify the pathogenic bacterium. PCR products sequencing results showed the pathogenic bacterium had 99% homology to *Ralstonia solanacearum*. This is the first report of *E. sonchifolia* wilt caused by *R. solanacearum*.

Key words: *Emilia sonchifolia* wilt; *Ralstonia solanacearum*

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Emilia sonchifolia is an annual or perennial compositae plant, widely distributed in China(Zhang *et al.*, 2005). *E. sonchifolia* is a traditional medicinal herb used to cure pneumonia, infectious hepatitis, diarrhea, acute conjunctivitis, etc(Li *et al.*, 1993). It is reported that *E. sonchifolia* contained flavonoids which showed stronger antibacterial activity against *Staphylococcus aureus* and weaker antibacterial activitiy against *Escherichia coli* and *Bacillus subtilis*(Li *et al.*, 2007). In recent years, *E. sonchifolia* is artificially planted widely in Guangxi of China. Surveys on *E. sonchifolia* diseases were conducted during 2004-2006 (Nong *et al.*, 2006) and a bacterial wilt disease was discovered which was severely occurred in the field. The disease incidence were 25% to 50%, causing significant economically loss. It is the first report of *E. sonchifolia* bacterial wilt occurred in the field and the pathogen identification was conducted in this paper.

1 Materials and methods

1.1 Bacteria isolation and pathogenicity testing

Diseased stems of *E. sonchifolia* were collected from the field and surface sterilizations were conducted using 75% alcohol for 1 min. Then the sterilized stems were cut into pieces about 5 cm in length. Put the stem pieces into sterile distilled water for 10 min then stirred for 1 min to obtain the bacteria suspension. Added 1 mL bacteria suspension into 10 mL sterile distilled water, diluted it into three concentrations. Removed 1 mL suspension of each concentration into culture dish, then poured into the 45 ℃ nutrient agar (NA, beef extract 3 g, peptone 10 g, glucose 20 g, agar 16 g, add water to 1 000 mL) medium and incubated for 3 d at 30 ℃. Different single colonies were selected for streaking cultivation. After 3 times of single colony purification, the purified bacteria were kept in sterile distilled water under the room temperature.

Cultivated the purified bacteria for 48 h, then added sterile distilled water to prepare 5×10^5 cfu/mL bacteria suspension. Inoculated two month old seedlings of *E. sonchifolia* by injecting the stem base or watering the roots. Each seedling was injected 1 mL bacteria suspension into stem base or watered 10 mL bacteria suspension into its rhizosphere soil. Sterile distilled

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water was inoculated as the control. Re-isolated and re-inoculated were conducted in accordance with Koch postulate(Fang,1998). Gram stain was conducted according to the reference(Dong *et al.*,2001).

1.2 Preparing bacteria DNA template

Single colony directly for PCR amplification method(Dai *et al.*,2000) was cited in this paper. Bacteria cells were cultured on NA plate for overnight at 28 ℃. Selected a single colony and removed the cells into a 1.5 mL microfuge tube containing 10 μL 1% SDS(sodium dodecyl sulfate) solution, then swirled it thoroughly. Added 300 μL TE(10 mM Tris-Cl, pH7.5, 1 mM EDTA) buffer to obtain DNA stock solution. Removed 10 μL DNA stock solution into a new 1.5 mL microfuge tube, which contained 9 μL sterile distilled water to dilute the solution. The diluted solution was used as PCR template directly.

1.3 Polymerase chain reaction(PCR) amplification

16s ribosomal DNA (rDNA) sequence analysis methods were widely used on bacteria identification (Macrae,2000; Jiao *et al.*,2001). In this paper, 16s rDNA universal primers were used for PCR (P1: 5′ AGA GTT TGA TCC TGG CTC AG 3′; P2: 5′ AAG GAG GTG ATC CAG CC 3′). The PCR parameters were as follows: initial denaturation at 95 ℃ for 4 min, 35 cycles of 95 ℃ 30 s, 56 ℃ 40 s, and 72 ℃ 2 min. Final extension was 72 ℃ for 10 min. PCR product was sequenced by TaKaRa biotechnology(Dalian) Co., Ltd using PCR primers. Sequence alignments were conducted by BLAST online software.

2 Results and analysis

2.1 Symptom of *E. sonchifolia* bacterial wilt

In the field, diseased plant showed green wilt (Fig. 1), and then leaves became yellow wilt from bottom to above. Finally plant died and stem became black. In vertical section, black stem vascular tissues were showed, and in cross section, whitish bacterial exudates oozed. Artificially injecting bacteria suspension into stem base for 3 d, the leaves begin to appear green wilting, sometimes appear one-sided wilting. After inoculated for 5 d, the plant died and also appeared whitish bacterial ooze in the cross section. Re-isolated bacteria from diseased plant then re-inoculated health plant, the same symptom appeared.

Fig. 1 Symptom of *E. sonchifolia* bacterial wilt in the field

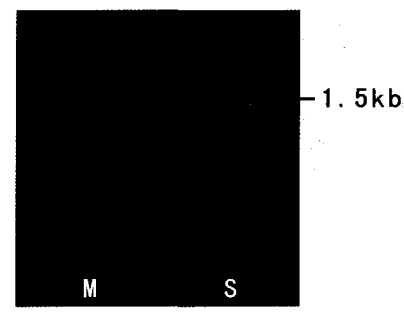

Fig. 2 PCR product electrophoresis pattern

M: 1kb DNA ladder; **S**: Sample PCR product.

2.2 Pathogenic bacteria

The colony of bacteria was milky white on NA plate, roundness or sub-round in shape, moist, smooth on surface. The colony turned brown after 5 — 8 d grown on medium and the medium nearby became deep brown. Gram stain was negative.

2.3 PCR amplification and sequencing

PCR product was about 1.5 kb(Fig. 2). Two terminals sequencing reactions were conducted using PCR primers respectively. 617bp of forward sequencing reaction showed 99% homology to *Ralstonia solanacearum* strain TW56(DQ924957.1)16s ribosomal RNA gene 5′ terminal fragment(from 24nt to 641nt). 788bp of reverse sequencing reaction showed 99% homology to *R. solanacearum* strain TW56 16s ribosomal RNA gene 3′ terminal fragment (from 1451nt to 663nt). According to the sequencing results, bacteria colony morphological character and negative gram stain, it can be confirmed that *E. sonchifolia* bacterial

wilt was caused by *R. solanacearum*.

3 Discussion

R. solanacearum, one of the world's most important phytopathogenic bacteria, causes lethal wilting diseases of over 200 plant species belonging to over 28 botanical families(Denny, 2000). Common crops affected by bacterial wilt include eggplant, capsicum, tomato, potato, tobacco, sweet potato, banana, ginger, onion, peanut, mung bean, cashew, papaya, cassava and sesame. *E. sonchifolia* usually grows wildly in the field and could be the potential host in original soil. In Guangxi of China, several important economical crops suffered severely by *R. solanacearum* wilt, such as tomato, tobacco, capsicum, ginger and so on (Huang, 2002). Due to *E. sonchifolia* growing wildly in the field, *R. solanacearum* could keep alive longer in the soil by parasitizing in the rhizosphere of *E. sonchifolia* after harvesting the crops. In recent years, *E. sonchifolia* is artificially planted widely in the field, occurrence of *R. solanacearum* wilt not only causes economical loss directly but also becomes the potential danger for the other economical plants growing nearby.

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首次报道一点红青枯病由茄青枯菌引起

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摘　要：一点红青枯病在广西种植区严重发生。通过柯赫氏法则证实病原菌为细菌。用16s rDNA通用引物进行PCR鉴定，测序结果表明，PCR产物与茄青枯菌有99%的同源性。首次报道一点红青枯病由茄青枯菌引起。

关键词：一点红青枯病；茄青枯菌