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Differentiation of xanthomonads causing the bacterial leaf spot of poinsettia in China from the pathotype strain of *Xanthomonas axonopodis* pv. *poinsettiicola**

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Abstract: In October 2003, a new bacterial disease with symptoms similar to those caused by *Xanthomonas axonopodis* pv. *poinsettiicola* was observed on poinsettia leaves at a flower nursery in Zhejiang Province of China. Three *Xanthomonas* strains were isolated from infected plants and classified as *X. axonopodis*. They were differentiated from the pathotype strain LMG849 of *X. axonopodis* pv. *poinsettiicola* causing bacterial leaf spot of poinsettia by comparison of pathogenicity, substrate utilization and BOX-PCR genomic fingerprints.

Key words: Bacterial leaf spot of poinsettia, BOX-PCR, Hypersensitive reaction, Pathogenicity, *X. axonopodis* pv. *poinsettiicola*
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INTRODUCTION

In recent years poinsettia cultivation has blossomed from a small garden business into a multimillion-dollar industry in China. Some fungal diseases, such as Rhizoctonia stem and root rot, Pythium root rot, Fusarium wilt, and Botrytis blight have been reported to cause considerable damage during propagation (Sun *et al*., 2003). A new bacterial disease characterized by leaf spot appearance was first observed on poinsettia in October 2003, in the Xiaoshan district of Zhejiang Province, China, one of the major poinsettia-production areas. Three bacterial strains have been isolated and classified as *X. axonopodis*. However, they have not been characterized yet on the pathovar level.

Bacterial leaf spot of poinsettia with symptoms similar to those caused by *X. axonopodis* pv. *poinsettiicola* (formerly *X. campestris* pv. *poinsettiicola*) was first reported by Patel *et al*.(1951) in India. DNA-DNA hybridization studies indicated that *X. axonopodis* was the largest and most heterogeneous genomic group (Vauterin *et al*., 1995). Phenotypic characterization including hypersensitive reaction, pathogenicity, and substrate utilization is essential for the characterization of phytobacteria, but insufficient for differentiating strains at or below the pathovar level. The rep-PCR technique has proved to be a rapid, simple and reproducible method to classify and differentiate *Xanthomonas* strains (Louws *et al*., 1994). The objective of the present study was to elucidate the relationship between the causal agent and the pathotype strain of *X. axonopodis* pv. *poinsettiicola*.

MATERIALS AND METHODS

Three strains, R22578, R22579 and R22580, isolated from symptomatic leaves of poinsettia in China and the pathotype strain LMG849 of *X. axonopodis* pv. *poinsettiicola* originally from India

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were used in this study. Cultures were stored in a freezer at −80 °C; and were checked for purity and grown routinely on Tryptic soy agar (Mew and Misra, 1994) at 28 °C. All strains were deposited in the BCCM^{TM}, Belgium and Institute of Biotechnology, Zhejiang University, China. Hypersensitive reactions of the 4 strains on tobacco as described by Schaad *et al*.(2001) were observed and pathogenicity test was done on pot-cultivated poinsettia seedling (cv. Qian-xi, Bi-de and Fu-xing) in greenhouse by pricking the leaves with a needle and placing a 10 μl droplet of bacterial suspension (approximately 10^8 cfu/ml) over the wounded site.

BIOLOG test was performed following manufacturer's instructions (BIOLOG, Inc., Hayward, CA) to compare the substrate utilization of the three strains with those of strain LMG849 (Xie *et al*., 2003). For genotyping, total DNAs of the 4 strains were extracted by the CTAB method (Ausubel *et al*., 1992) and repetitive PCR genomic fingerprints were obtained by using the BOX A1R primer described by Louws *et al*.(1994). All PCRs were performed in a total volume of 25 μl using 30−50 ng DNA, 500 μmol/L each dNTP, 2 μmol/L BOX A1R primer and 2.0 U *Taq* polymerase. Cycling conditions were: one cycle of 95 °C for 7 min, and 35 cycles of 94 °C for 1 min, 53 °C for 1 min and 65 °C for 8 min with a single final extension cycle at 65 °C for 15 min and a final incubation at 4 °C. PCR products were examined by electrophoresis in 1% agarose gel and DNA was visualized with a gel imaging system.

RESULTS AND DISCUSSION

Infiltration of tobacco leaves with cell suspensions of the 3 strains from China resulted in typical hypersensitivity reactions within 24 h and induced similar symptoms with field observations on pot-cultivated poinsettia seedling 2 weeks after inoculation. On the under leaf surface, new spots were dull grey to brown and slightly water-soaked. The spots later became chocolate-brown and visible on both surfaces. Lesions varied from circular to angular in shape and coalesced to form large, irregular, necrotic areas with a yellow halo. The lesions were often along the veins and midribs, although not confined to these areas. Bacterial exudates often formed on lesions, especially on the lower leaf surface. The ooze dried to form a shiny scale-like crest. Three cultivars (Qian-xi, Bi-de and Fu-xing) of poinsettia were all susceptible to the causal agent. A delayed hypersensitivity reaction on tobacco and weak pathogenicity symptoms on leaves of 3 poinsettia cultivars were observed for the pathotype strain LMG849.

Comparisons of metabolic activity on the carbon substrates, by which strains of *X. axonopodis* can be distinguished from those of other *Xanthomonas* species, revealed that the 3 strains had the similar utilization reactions as the type strain LMG849 (Table 1). Strains R22579 and R22580 showed indistinguishable reaction patterns were different from those of strain R22578 on the utilization of succinamic acid and *p*-hydroxyphenylacetic acid. The major difference between the 3 strains and the pathotype strain LMG849

Table 1 Partial substrate utilization profiles of the poinsettia bacterial strains R22578, R22579, R22580 and the pathotype strain LMG849

BIOLOG carbon sources	R22578	R22579	R22580	LMG849
Dextrin	+	+	+	+
Cellobiose	+	+	+	+
Gentiobiose	+	+	+	+
Maltose	+	+	+	+
D-psicose	+	+	+	+
D-trehalose	+	+	+	+
Monomethylsuccinate	+	+	+	+
Succinic acid	+	+	+	+
Bromosuccinic acid	+	+	+	+
Succinamic acid	−	+	+	+
D-alanine	+	+	+	+
L-alanine	+	+	+	+
L-alanylglycine	+	+	+	+
L-glutamic acid	+	+	+	+
Glycyl-L-glutamic acid	+	+	+	+
β-methyl-D-glucoside	±	±	±	−
L-rhamnose	−	−	−	−
Formic acid	+	+	+	−
D-galactonic acid lactone	−	−	−	−
D-galacturonic acid	−	−	−	−
D-glucuronic acid	−	−	−	−
p-hydroxyphenylacetic acid	−	±	±	−
α-ketovaleric acid	−	−	−	−
Quinic acid	−	−	−	−
Glucuronamide	−	−	−	−
L-phenyl-alanine	−	−	−	−
Thymidine	−	−	−	−

[1]: BIOLOG data are presented only for the reactions, in which the strains of *X. axonopodis* can be distinguished from other *Xanthomonas* species. +: Positive; ±: Weakly positive; −: Negative

was the utilization of formic acid and β-methyl-D-glucoside. The representative fingerprint patterns of the 4 strains were obtained using the BOX A1R primer (Fig.1), in which the bands, ranging in size from approximately 200 bp to over 2500 bp, were clearly differentiated by agarose gel electrophoresis. The three strains isolated from China showed a similar profile, which showed that a single species might be responsible for bacterial leaf spot of poinsettia in China. The genetic profiles of the 3 strains were clearly different from that of LMG849, which is consistent with the result of phenotypic characterization. The present study revealed the causal agent is different from that of the pathotype strain and confirmed that repetitive sequence-based polymerase chain reaction could be used as rapid, highly discriminatory screening techniques to determine the genomic diversity of *X. axonopodis*.

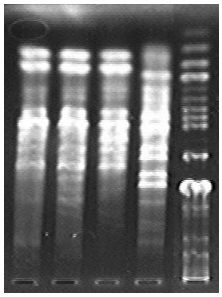

Fig.1 Genomic fingerprinting of bacterial strains amplified using primer BOX
Lane 1: R22578; 2: R22579; 3: R22580; 4: LMG849; M: 100 bp DNA molecular marker (Bioasia)

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