SUMMARY

Ralstonia solanacearum was isolated from wilting coleus plants (Coleus forskohlii) in six commercial nurseries around Bangalore (south India). Colonies of all isolates were irregular with smooth margins, slimy dull white with pink to red centre on SMSA medium, Gram-negative, non-capsulated and non-spore forming. Bacterial identity was confirmed by PCR with species-specific 16S rDNA-based primers OLI1+Y2 and DAS-ELISA.

The isolates could infect tomato, potato, and ginger, but failed to infect mulberry and banana. Six isolates were identified as race 1, based on pathogenicity tests, and as biovar 3, based on carbohydrate utilization.

Key words: coleus, PCR, Ralstonia solanacearum, race-1, biovar-3.

The medicinal plant coleus (Coleus forskohlii Brig.) is grown on 2,500 ha in India (Farooqi and Sreeramu, 2001), but in several parts of southern India its cultivation is limited by a bacterial wilt caused by Ralstonia solanacearum, a pathogen recently recorded from this area (Chandrashekara and Prasannakumar, 2010). Affected plants show yellow discolouration, loss of turgidity and drooping of the leaves, wilting and brownish vascular bundles (Fig. 1). A tentative identification of the causal agent as a bacterium was made by the ooze test (Danks and Barker, 2000), following which bacteria were recovered from symptomatic plants, characterized and identified as detailed below.

Six bacterial isolates (GCR-1, GCR-2, DCR-1, KCR-1, RCR-1 and BCR-1) were obtained from wilt-affected coleus plants collected in commercial nurseries in 5 locations around Bangalore (south India) [two isolates from Gouribidanur (GCR-1 and GCR-2), one each from Doddaballapur (DCR-1), Kolar (KCR-1), Rajankunte (RCR-1), and Bangalore BCR-1]. Bacteria were isolated on modified SMSA medium (Elphinstone et al., 1998), purified and stored in sterile water (Kelman, 1954; Schaad, 1988) for morphological, physiological, cultural, biochemical and pathogenicity studies along with three reference strains of race-1 biovar-3 (GSC-26, 134, JGS 3/118) provided by the Central Potato Research Institute, Shimla, Himachal Pradesh. Tests for starch hydrolysis, nitrate reduction, oxidase, indole production, esculin hydrolysis, arginine dihydrolyase, curdling of skimmed milk were carried out (Anonymous, 1957) and morphological characteristics of colonies were matched following Schaad (1988). Bacterial cells were observed with an electron microscope (JEOL-100S).

The six bacterial isolates from coleus (5×10^8 CFU/ml), suspected to be R. solanacearum and three reference cultures (R. solanacearum) were differentiated into biovars using carbohydrate fermentation discs (Hayward, 1964). Observations were recorded at 24 h and 48 h for change in colour from light red to white and then to yellow with whitish creamy growth around the disc.

The differential hosts tomato (Lycopersicon esculentum) cv. Avinash-II, banana (Musa acuminate) cv. Yallaki bale, ginger (Zingiber officinale), mulberry (Morus alba) cv. M-5 and potato (Solanum tuberosum) cv. Kufri jyothi were grown in a greenhouse and inoculated by pouring 20 ml of bacterial suspension (5×10^8 CFU/ml) on the root zone which was then covered with soil. Plants similarly inoculated with sterile water served as control.

The hypersensitivity (HR) test was done as described by Granada and Sequeira (1975). Bacterial suspensions (5×10^8 CFU/ml) were infiltrated into fully expanded tobacco leaves (Nicotiana tabacum cv. Samsun) and the reaction recorded after 24, 48, and 72 h. Purified bacterial cultures were inoculated and incubated overnight in 100 ml casein peptone glucose (casamino acid 1g/l, peptone 10g/l, glucose 10g/l, pH 7.2) at room temperature.

A newly developed technique was used for DNA extraction, i.e. bacterial cultures (100 ml) were centrifuged (7,000 rpm for 10 min) and pellets were frozen at -20°C for 2 h and thawed for 3 min at 37°C. The suspension in 10 ml lysis buffer (0.15 M NaCl, 0.05 M sodium citrate

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buffer) was incubated with 200 µl of lysozyme (10 mg/ml) at 37°C for 60 min. The cells were further lysed with 500 µl of 20% SDS by gentle shaking for 5 min. Thereafter 15 ml extraction buffer was added [2.5 ml of 5 M sodium perchlorate and 12.5 ml of chloroform: isoamylalcohol mixture (24:1)]. Preparations were incubated at –20°C for 2 h and allowed to thaw at room temperature by gentle shaking for 30 min and subsequently centrifuged at 5,000 rpm for 10 min. Supernatant was precipitated with an equal volume of isopropanol, washed with 70% ethanol. DNA was air dried and dissolved in 1 ml T10E1 buffer. After spectrophotometric quantification, the DNA was further analysed on 0.7% agarose gel.

Isolates were PCR-amplified using primers (OLI1 and Y2) corresponding to 16S rDNA (Seal et al., 1993). Further, serological confirmation was carried out by DAS-ELISA (Priou et al., 1999).

Bacterial colonies were highly fluid, irregularly shaped, convex, dark reddish with red center and whitish margin, i.e. morphologically similar to those of R. solanacearum (Elphinstone et al., 1998). Bacteria were Gram-negative, rod shaped, non-capsulated and non-spore forming. Biochemical tests were positive for oxidase and negative for indole production, were able to utilize various sucrose, glucose, rhamnose, maltose, mannoose, cellobiose, trehalose and mannitol as carbon source, were negative for indole and gelatin lignifications, positive for oxidase, levan production, nitrate reduction and did not produce fluorescent pigments on Kings-B medium. In the electron microscope bacterial cells appeared rod shaped, lophotrichously flagellated with one to three flagella at one end (Fig. 2).

Biovars of R. solanacearum are differentiated according to their ability to oxidize disaccharides viz., cellobiose, lactose and maltose and utilize sugar alcohols such as dulcitol, mannitol and sorbitol (Hayward, 1964). Since our isolates were able to utilize both disaccharides and sugar alcohols they could be assigned to biovar-3.

All isolates produced typical wilt symptoms on their respective host (6 on coleus and 3 reference cultures on potato) (Wang and Berk, 1997) and wilting within 4 to 5 weeks in tomato, potato and ginger, but not in mulberry and banana. These results support the identification of all bacterial isolates from coleus as R. solanacearum race-1, which is further supported by the hypersensitive reaction on tobacco leaves within 24 h (Granada and Sequeira, 1975).

Partial sequences of 16S rDNA genes are excellent targets for identification of bacteria at the species level and have been used for PCR amplification (Woese, 1987). The PCR-based diagnostic test using OLI1+Y2 primers specific to 16S rDNA (Seal et al., 1993) yielded 292 bp product expected for R. solanacearum (not shown).

Detection of R. solanacearum by CIP’s post-enrichment DAS-ELISA kit originally developed for identifying latent infection in potato seed tubers was adopted to authenticate the isolates. Coleus isolates that produced positive reaction with bright purple colour at a concentration of 10^4 CFU/ml on nitrocellulose membrane were positively compared with control strips provided with the kit (Priou et al., 1999).

In conclusion, the present study on bacterial wilt of coleus in south India constitutes the first record of R. solanacearum race-1 biovar-3 as the casual agent of the disease.

ACKNOWLEDGEMENTS

The authors are grateful to ICAR for providing funds to carry out the research in NATP-CGP grant. We
would like to thank Indian Institute of Horticulture Research, and University of Agricultural Science, Bangalore for facilities provided.

REFERENCES


Received March 29, 2010
Accepted November 7, 2011