SCREENING OF BACTERIOPHAGES EFFECTIVE IN CONTROLLING *Ralstonia solanacearum*, THE WILT PATHOGEN OF TOMATO

R.A.C.D. DHARMASENA AND D.M. DE COSTA

Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya

Introduction

Among field diseases of tomato, bacterial wilt caused by *Ralstonia solanacearum* is a serious threat worldwide. Integration of cultural methods with host plant resistance has been recommended for the control of tomato bacterial wilt as chemical control or a single method of control has not been very effective. However, breeding for resistant varieties has not been durable because of genetic variability and wide host range of the pathogen. Moreover, cultural practices such as crop rotation and fallowing are not practically- or economically feasible. Existence of genomically-variable isolates of *R. solanacearum* infecting tomato has been reported from different locations of Sri Lanka (Gunathilake et al., 2004). Therefore, the present study was conducted to screen bacteriophages effective against a wide range of *R. solanacearum* isolates as a biological control option that can be incorporated in to an integrated management programme. Findings of the study would be helpful to design an effective management programme against bacterial wilt pathogens showing genomic variability and consequently variation in virulence.

Materials and methods

R. solanacearum was isolated from wilt-infected tomato plants collected from different fields in three agroecological zones (i.e. Mid Country Wet Zone, Mid Country Intermediate Zone and Low Country Dry Zone). Identity of the bacterium was confirmed by biochemical and molecular methods (Schaad, 1992; Lee and Wang, 2000; Lee et al., 2001) as well as *in vivo* pathogenicity assays (Williamson et al., 2002). Bacteriophages were isolated according to Crosse and Hingorani (1958) method from soil and compost samples collected from cultivated and uncultivated locations using a single isolate of *R. solanacearum* (i.e. isolate 6) as the bacterial lawn. Phage typing was done to determine the broad spectrum effectiveness of individual bacteriophages using all isolates of *R. solanacearum.*

Results

Seventeen isolates of *R. solanacearum* were obtained from stem ooze of wilt-infected tomato plants. Polyphasic identification approach including Gram staining, 3% KOH test, semi-selective media and PCR amplification using *R. solanacearum* specific primers (i.e. BP4-R (5'GACGACATCATTTCCACCGGGCG3')/ BP4-L 'GGGTGAGATCGATTGTCTCCTTG3' (Lee and Wang, 2000) and PS-1S-F(5'CGCAACGCTGGATGAACCC3')/PS-1S-R (5'CAGACGATGCGAAGCCTGAC3') (Lee et al., 2001)) confirmed 12 isolates as *R. solanacearum.* Three isolates resulted in a PCR product which is slightly different to the expected size as described by Lee and Wang (2000) and Lee et al. (2001) indicating a possible genomic variability. Out of the 17 isolates, five isolates caused bacterial wilt *in vivo* when used on the tomato variety T-245 (Figure 1). Plaques of different morphology were given by 11 samples tested (Figure 2). Results obtained for phage typing by different single plaques are shown in Table 1.

Discussion

Some of the isolates, though identified as *R. solanacearum*, did not cause wilt symptoms when inoculated to variety T-245. This could be due to variations of host-pathogen interactions of those isolates as they were originally isolated from different tomato

varieties. Therefore, as the next step, we intend to assess *in vivo* performance of these 17 isolates on a range of tomato varieties recommended by the Department of Agriculture. As findings of the PCR methods based on specific primers used for identification of different isolates revealed a genetic variation, the above pathogenicity variations could be expected. Despite the highly host-specific nature, a reasonable number of bacteriophages having a broad spectrum effectiveness against a majority of isolates of *R. solanacearum* could be identified from the present study. Future research will focus on mass culturing of the prospective bacteriophages, determination of their *in vivo* effectiveness and determination of the most effective method of application.

Conclusion

Bacteriophages with broad spectrum action against a range of *R. solanacearum* isolates were found based on *in vitro* investigations.

Acknowledgment

Authors acknowledge the financial assistance by National Science Foundation, Sri Lanka (Grant no. RG/2007/BT/05)

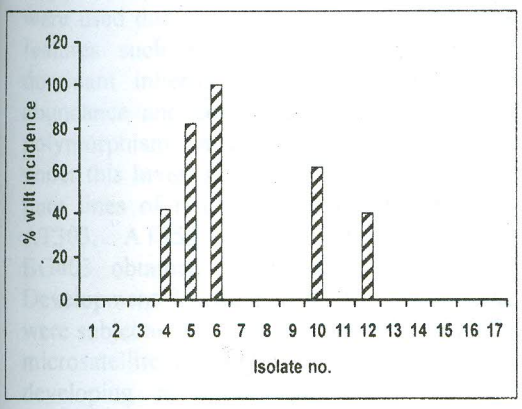

Figure 1. Percentage wilt incidence of *R. solanaceaum* inoculated tomato (variety T-245), 35 days after inoculation

References

Gunathilake PMPCK, Bandara JMRS and Samarajeewa PK (2004). Genetic diversity and pathogenicity of *Ralstonia solanacearum* E.F. Smith in tomato (*Lycopersicon esulentum* Mill.) in Kandy, Matale and Moneragala districts. Tropical Agricultural Research 16: 51-60.

Crosse JE and Hingorani MK (1958). A method for isolating *Pseudomonas mors-prunorum* phages from soil. Natrue, 181: 60-61.

Lee Y and Wang C (2000). The design of specific primers for the detection of *Ralstonia solanacearum* in soil samples by polymerase chain reaction, Bot. Bull. Acad. Sin. 41: 121-128.

Lee Y, Fan S, Chiu L and Hsia K (2001).Isolation of an insertion sequence from *R. solanacearum* race 1 and its potential use for strain characterization and detection. Applied and Environmental Microbiology, 67: 3943-3950.

Schaad, NW (1992) Laboratory guide for identification of plant pathogenic bacteria. APS Press, St. Paul, USA.

Williamson L, Nakaho K, Hudelson B and Allen C (2002). *Ralstonia solanacearum* race 3, biovar 2 strains isolated from geranium are pathogenic on potato. Plant Diseases, 86: 987-991.

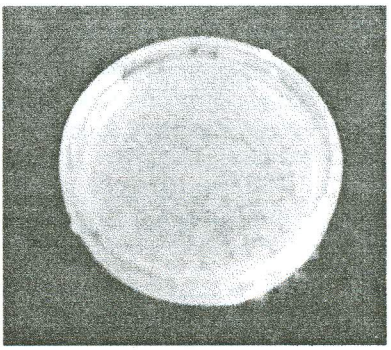

Figure 2. Plaques with different morphology on a lawn of *R. solanacearum* isolate 6.

Table 1. Effectiveness of different phages on different isolates of *R. solanacearum*

Bacterialisolate / Phage code	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
6B	x	√	x	x	√	x	x	x	x	x	x	x	x	x	x	x
7B	√	√	x	x	x	√	x	x	x	x	x	x	√	x	x	x
6C	√	√	x	x	√	x	x	x	x	√	x	x	x	x	x	x
7E	√	x	x	x	x	√	x	x	x	x	x	x	√	x	x	x
6F	x	√	√	√	√	√	√	x	x	x	x	x	x	x	x	x
J	√	√	√	√	√	√	√	x	x	x	x	√	x	√	x	x
K	x	√	√	√	√	√	x	x	x	x	x	x	x	x	x	x
N	√	x	x	√	√	√	√	x	x	x	x	√	√	√	x	x
O	x	√	√	√	√	√	√	x	x	x	x	√	x	x	x	x
P	√	x	√	√	√	√	√	√	x	x	x	√	√	x	x	x
V	x	√	√	√	√	√	x	x	x	x	x	√	x	x	x	x

√ - plaque formation present X – plaque formation absent