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Rapid Identification of *Ralstonia solanacearum* by the Direct Colony TLC and Simple TLC

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Rapid identification of bacterial wilt pathogen, *Ralstonia solanacearum* was conducted by the direct colony TLC and simple TLC methods. All fourteen isolates from different host plants in Bangladesh exhibited similar chromatograms with *R. solanacearum* ATCC 11696 the type strain by the direct colony TLC method. This result indicated that all the isolates were *R. solanacearum*. Physiological and biochemical tests on the fourteen isolates also verified that they were *R. solanacearum*. Seven strains collected in Bangladesh and Japan showed also identical chromatograms with the type strain by the simple TLC method. A benchmark spot under the common spot was detected in all isolates and strains at the direct colony TLC and simple TLC. Although the direct colony TLC and simple TLC methods showed the similar results, the simple TLC method was found less time consuming and easier than the direct colony TLC.

INTRODUCTION

Ralstonia solanacearum, the causal agent of bacterial wilt disease of crop plants is widely distributed in tropical and some warm temperate regions of the world (Kelman, 1953; Hayward, 1991) including Bangladesh (Talukdar, 1974; Miah and Hoque, 1987; Meah and Khan, 1987). For identification and characterization of *R. solanacearum* isolates, different physiological and biochemical tests such as growth studies, gram-reaction, oxidase, catalase, urease test, oxidation of glucose and gas production from nitrate are recommended (Hayward, 1964, 1992; Suslow *et al.*, 1982; Shekhawat *et al.*, 1992; Mehan *et al.*, 1994). These all tests are time consuming and laborious. Therefore, rapid methods for identification of phytopathogenic bacteria are require of times.

Rapid identification of phytopathogenic bacteria by the direct colony TLC method was conducted for the first time (Matsuyama *et al.*, 1993a,b; Matsuyama and Furuya, 1993c; Matsuyama, 1995). Identification of *R. solanacearum* at least at species level was possible by this method (Matsuyama and Furuya, 1993c; Matsuyama, 1995). Recently, Khan and Matsuyama (1998) invented simplified TLC method for identification of phytopathogenic bacteria. In this paper we describe the effectiveness of the direct colony TLC and simple TLC methods for identification of *R. solanacearum*.

MATERIALS AND METHODS

Bacterial isolates / strains

A total of 14 bacterial isolates and eight *R. solanacearum* strains were included in

this study. The 14 isolates were isolated from wilted chili (*Capsicum annuum*), eggplant (*Solanum melongena*), potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*) during 1992–94 and designated as C1, C2, E1, E2, E3, E4, E5, E6, P1, P2, T1, T2, T3, and T5. Streak–plate technique and a selective medium, tetrazolium chloride agar (TZC) were used to isolate the pathogens described by Kelman (1954). Virulent colonies of each isolate were selected based on cultural characteristics produced on the selective medium. After selection of colonies on TZC, the isolates were tested for confirmation of their pathogenicity in their respective host plants from which they were isolated as described by Winstead and Kelman (1952). The details of the isolates / strains are given in Table 1.

Table 1. Details of bacterial isolates / strains used in this experiment.

Bacterial isolate / strain	Host	Place of isolation / source
C1	Chili	Gazipur, Bangladesh
C2	〃	〃
E1	Eggplant	〃
E2	〃	〃
E3	〃	Munshigonj, Bangladesh
E4	〃	Gazipur, Bangladesh
E5	〃	〃
E6	〃	Narayongonj, Bangladesh
P1	Potato	Munshigonj, Bangladesh
P2	〃	〃
T1	Tomato	Gazipur, Bangladesh
T2	〃	〃
T3	〃	Munshigonj, Bangladesh
T5	〃	Gazipur, Bangladesh
Ralstonia solanacearum		
ATCC 11696^T	〃	ATCC
C319	Tobacco	KTES
8107	Eggplant	NIVOT
8224	〃	〃
6511	〃	NIAS
Ku 7502-1	Tomato	AKU
Z 9	Eggplant	IPSA
Z 10	Chili	〃

ATCC: American Type Culture Collection.
KTES: Kagoshima Tobacco Experiment Station, Kagoshima, Japan.
NIVOT: National Research Institute of Vegetables, Ornamental Plants and Tea, Morioka Branch, Japan.
NIAS: National Institute of Agricultural Sciences, Tokyo, Japan.
AKU: Faculty of Agriculture, Kyushu University, Fukuoka, Japan.
IPSA: Institute of Post Graduate Studies in Agriculture, Gazipur, Bangladesh.
Type culture: Small T at the shoulder of the isolate number indicates type culture.

Direct colony TLC

The direct colony TLC was performed for the fourteen isolates as well as the type strain. Bacterial isolates were cultured on the slants of King's B agar medium (Eiken Chem. Co., Tokyo) at 30 °C for 3 days. One loopful of bacterial cells was taken from the slant and pasted on the silica gel TLC plate (Merck Co., Si 60, 0.25 mm in thickness) maintaining 1 cm distance from bottom end and 1.5 cm distance between the isolates. The pasted bacterial cells were dried completely using a hair drier. The TLC plate was first developed with chloroform–methanol (2:1, v/v) solvent system for 10 min. After drying the plate, bacterial cells were scraped out and the plate was developed in the same direction with chloroform–methanol–water (60:25:4, v/v/v) for 1.5 hr. The developments were carried out in an incubator at 25 °C. Thereafter, the plate was dried well and sprayed with ninhydrin (Ninhydrin spray, Tokyo Kasei Chem. Co.) followed by heating at 100 °C for 10 min. The spots appeared on the TLC plate were recorded by photocopy/photograph (Matsuyama *et al.*, 1993a,b; Matsuyama and Furuya, 1993c; Matsuyama, 1995).

Simple TLC

The simple TLC method was conducted for other eight strains including the type strain (except fourteen isolates). The growth conditions of strains were same as described in the direct colony TLC. The simple TLC method was conducted as described by Khan and Matsuyama (1998). One loopful of bacterial cells was suspended in 0.2 ml of chloroform–methanol (2:1, v/v) in a small glass vial and kept at least for 15 min at room temperature for lipid extraction. About 10 μl of lipid extract was spotted on the origin of silica gel TLC plate and completely dried by a hair drier. The plate was developed with chloroform–methanol–0.2% calcium chloride solution (55:35:8, v/v/v) for 1 hr. The development was conducted in an incubator at 25 °C. Lipid spots were detected by spraying ninhydrin followed by heating at 100 °C for 10 min and chromatograms were recorded by photocopy/photograph.

Physiological and biochemical tests like growth, gram–reaction, oxidase, catalase, urease, oxidation of glucose, carbohydrate oxidation, melanin production, hypersensitivity reaction on tobacco leaves and gas production from nitrate were conducted (Hayward, 1964, 1992; Suslow *et al.*, 1982; Shekhawat *et al.*, 1992; Mehan *et al.*, 1994; French and Sequeira, 1970; Lozano and Sequeira, 1970) for fourteen isolates in order to confirm the results of the rapid methods.

RESULTS AND DISCUSSION

All the fourteen isolates exhibited identical chromatographic profiles with *R. solanacearum* ATCC 11696 (type strain) by the direct colony TLC method. A benchmark spot (arrow–head in the figure) was founded at Rf 0.64 under the common spot (Rf 0.70) in all fourteen isolates as well as the type strain (Fig. 1). The similar type chromatographic profiles of *R. solanacearum* were reported earlier (Matsuyama and Furuya, 1993c; Matsuyama, 1995). The chromatographic profiles obtained by the direct colony TLC in this experiment suggested that all fourteen isolates from Bangladesh were *R. solanacearum*.

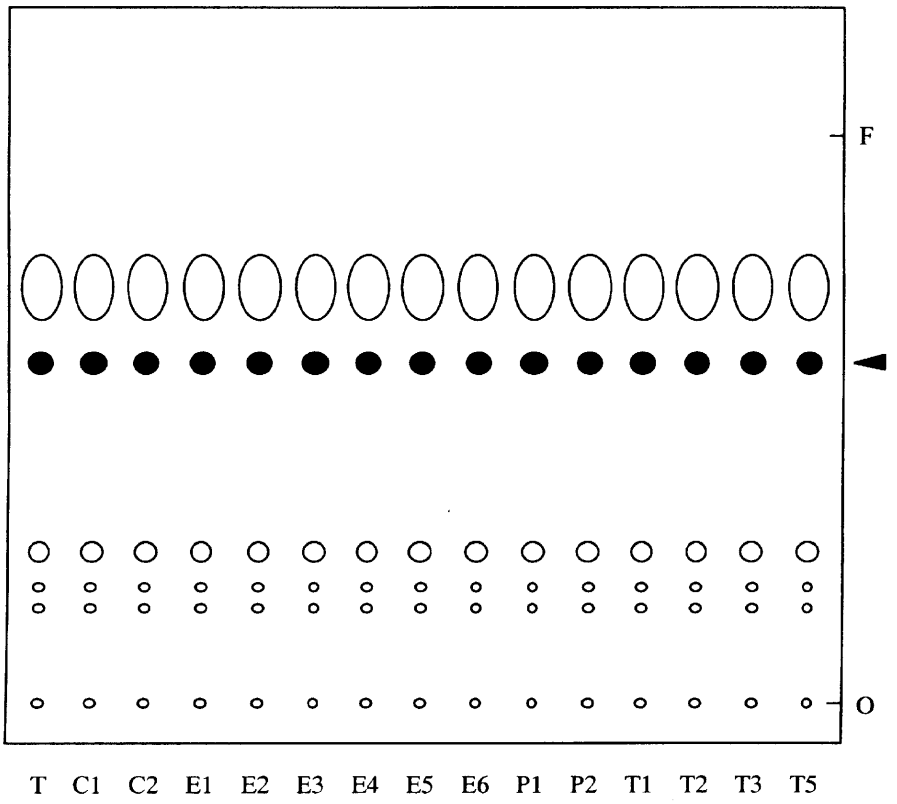

Fig. 1. Diagrammatic representation of TLC chromatogram of lipids from bacterial wilt pathogen by the direct colony TLC method[a].

T : Type strain, C1–C2 : Isolates from chili, E1–E6 : Isolates from eggplant, P1–P2 : Isolates from potato, T1–T5 : Isolates from tomato

O : Origin, F : Solvent front.
Arrow-head indicates the benchmark spot.

[a]The direct colony TLC method is described under materials and methods.

Physiological and biochemical tests for fourteen isolates also verified that all isolates were *R. solanacearum*. Four isolates namely, P1, P2, T1 and T2 were under biovar 2 and rest ten isolates were biovar 3. Three isolates viz. P1, P2 and T5 were under race 3 and other eleven isolates were under race 1 (Rahman *et al.*, 1996).

The other eight strains including the type strain also showed similar chromatograms by the simple TLC method, where the benchmark spot (arrow–head in the figure) was

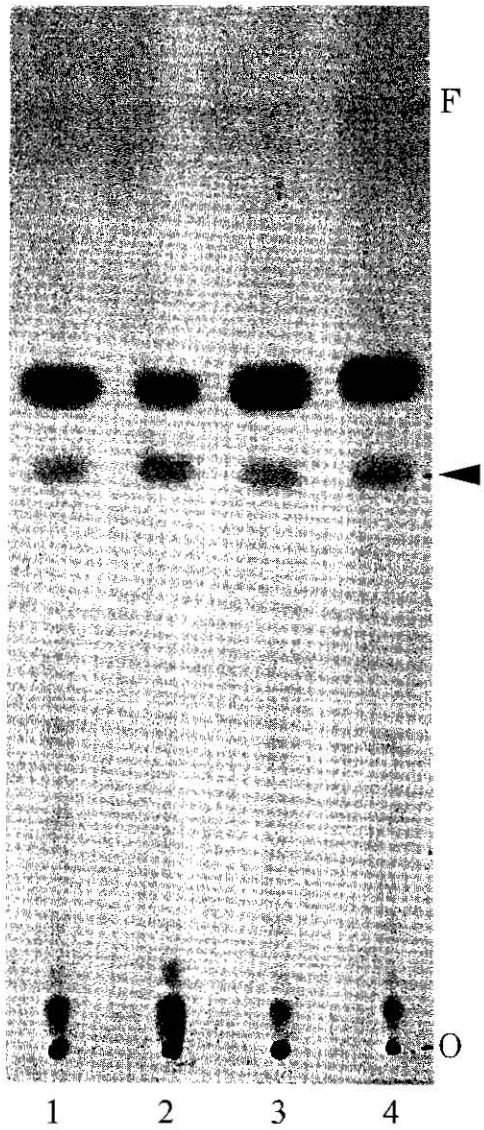

Fig. 2. TLC chromatogram of lipids from *Ralstonia solanacearum* strains by the simple TLC method[a].

1. *R. solanacearum* ATCC 11696[T]
2. 〃 C 319
3. 〃 Z 9
4. 〃 Z 10

O : Origin, F : Solvent front

Arrow-head indicates the benchmark spot of *R. solanacearum*.

[a]The simple TLC method is described under materials and methods.

observed at Rf 0.62 (Fig. 2). Khan and Matsuyama (1998) previously reported similar type chromatograms of *R. solanacearum* by this simple TLC method. In this experiment we used Bangladesh, American (type strain) and Japanese strains of *R. solanacearum* and observed all the strains exhibited similar type chromatograms. This indicated, the chromatographic profiles of *R. solanacearum* are identical by the direct colony TLC and simple TLC methods, even they are isolated from various host plants and different agro-ecological zones of the world.

The results of this experiment indicated that both the direct colony TLC and simple TLC methods showed similar results and are effective for rapid identification of *R. solanacearum* at species level. Furthermore, the simple TLC method was found less time consuming and easier than the direct colony TLC.

REFERENCES

French, E. R. and L. Sequeira 1970 Strains of *Pseudomonas solanacearum* from Central and South America; A comparative study. *Phytopathology*, **60**: 507–512

Hayward, A. C. 1964 Characteristics of *Pseudomonas solanacearum* . *J. Appl. Bact.*, **27** (2): 265–277

Hayward, A. C. 1991 Biology and epidemic of bacterial wilt caused by *Pseudomonas solanacearum*. *Ann. Rev. Phytopath.*, **29**: 65–87

Hayward, A. C. 1992 Identification of *Pseudomonas solanacearum*. Bacterial wilt Training Course (held on Oct. 5 to Nov. 16). *In SAVERNET, AVRDC*, 101 pp

Kelman, A. 1953 The bacterial wilt caused by *Pseudomonas solanacearum*. *Tech Bull. 99, North Carolina Agril. Exp. Sta., USA*, 194 pp

Kelman, A. 1954 The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology*, **44**: 693–695

Khan, A. A. and N. Matsuyama 1998 Rapid Identification of phytopathogenic bacteria by TLC. *J. Fac. Agr., Kyushu Univ.*, **42** (3,4) 281–287

Lozano, J. C. and L. Sequeira 1970 Differentiation of races of *Pseudomonas solanacearum* by a leaf infiltration technique. *Phytopathology*, **60**: 833–838

Matsuyama, N., I. H. Mian, A. M. Akanda and N. Furuya 1993a Application of the direct colony TLC method for identification of phytopathogenic bacteria. *J. Fac. Agr., Kyushu Univ.*, **37**(3,4): 281–285

Matsuyama, N., I. H. Mian, A. M. Akanda and N. Furuya 1993b Comparative studies on thin-layer chromatograms of lipids from various phytopathogenic bacteria. *Ann. Phytopath. Soc. Japan*, **59**: 528–534

Matsuyama, N. and N. Furuya 1993c Application of the direct colony TLC for identification of phytopathogenic bacteria (II). Chromatographic profile of *Erwinia* and *Pseudomonas* spp. *J. Fac. Agr., Kyushu Univ.*, **38** (1,2): 89–95

Matsuyama, N. 1995 Application of the direct colony TLC for identification of phytopathogenic bacteria (III). Distinction of the pseudomonads in the rRNA– homology group II (*Burkholderia* spp.). *J. Fac. Agr., Kyushu Univ.*, **40** (1,2): 189–196

Meah, B. H. and A. A. Khan 1987 Check list of fruit and vegetable diseases in Bangladesh. *Dept. of Plant Path., Bangladesh Agril. Univ., Mymensingh*, 22 pp

Mehan, V. K., B. S. Liao, Y. J. Tan, A. B. Smith, D. Mcdonald and A. C. Hayward 1994 Bacterial wilt of groundnut. *ICRIASAT Information Bull No. 35. ICRIASAT, Andhra Pradesh, India*, 23 pp

Miah, A. J. and M. O. Hoque 1987 Reaction of tomato cultivar against bacterial wilt (*Pseudomonas solanacearum*). *Bangladesh J. Plant Path.*, **3** (1,2): 51–54

Rahman, M. Z., I. H. Mian and N. Furuya 1996 Characterization of isolates of *Pseudomonas solanacearum* from three districts of Bangladesh. *Bull. Inst. Trop. Agr., Kyushu Univ.*, **19**: 25–31

Shekhawat, G. S., S. K. Chakrabati and A. V. Gadewar 1992 Potato bacterial wilt in India. *Tech. Bull. No. 38. Central Potato Res. Inst., Simla, India*, 52 pp

Suslow, T. V., M. N. Schroth and M. Isaka 1982 Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology*, **72**: 917–918

Talukdar, M. J. 1974 Plant diseases in Bangladesh. *Bangladesh J. Agril. Res.*, **1** (1): 61–83

Winstead, N. N. and A. Kelman 1952 Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. *Phytopathology*, **42**: 628–634