SHORT COMMUNICATION

A RAPID, SENSITIVE AND RELIABLE METHOD FOR DETECTING *RALSTONIA SOLANACEARUM* USING FTA (WHATMAN) CARD

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SUMMARY

DNA isolation for the molecular characterization of *Ralstonia solanacearum* using conventional methods takes 2 to 3 days. The use of FTA (Whatman) card reduces the time for obtaining nucleic acid and large samples can be stored on these cards for several years. This paper presents a comparison of the use of genomic DNA obtained from FTA card with a conventional method. The FTA card method proved to be an efficient way of sampling and detecting *R. solanacearum* as it eliminates the need for multi-step extraction and purification procedures which often involve the use hazardous chemicals and require refrigeration. In this study bacterial ooze from infected plants of tomato, potato, pepper and eggplant was directly collected on this card and air-dried for 5 min. A small fragment of the card (<1 mm) was subjected to PCR with universal primers and the results were compared with those yielded by a conventional method. PCR products obtained with the FTA method were sufficient for reliable scoring and were comparable to genomic DNA isolation by conventional method. Our findings indicate FTA card technique as an appropriate method for the fast and reliable diagnosis of *R. solanacearum*.

Key words: genomic DNA, *Ralstonia solanacearum*, Flinders Technology Associates (FTA), diagnosis.

Ralstonia solanacearum is a widely distributed and economically important plant pathogen. The unusually wide host range, comprising over 450 host species in 54 botanical families (Allen *et al*., 2005), gives the pathogen an evolutionary advantage (Hayward, 1991). Rapid detection of *R. solanacearum* is important for scientific and practical purposes especially when performed with simple procedures. Flinders Technology Associates (FTA), a different method for collecting and storing biological material using a solid matrix was first applied to blood samples for diagnosis (Guthrie and Susi, 1963) and used in PCR for medical and forensic applications (Carducci *et al*., 1992). FTA card has proprietary (Whatman, 2004) chemicals impregnated into the paper which act to lyse cellular material, fix and preserve DNA and RNA within the fibre matrix. Thus, FTA filter paper-based DNA extraction is a simple technology that reduces the cost and time required to process a DNA ready for downstream application (Mbogori, 2006). The FTA card proved also useful in a nucleic acid diagnostic kit (Aneg *et al*., 2008). Experiments were therefore initiated to evaluate the efficiency, feasibility and applicability of these cards for *R. solanacearum* detection. DNA isolated by FTA card technology was compared to that obtained with a conventional method (Chandrashekara *et al*., 2011) and was then subjected to PCR amplification using the OLI1 and Y2 primers of Seal *et al*. (1993).

Solanaceous plants (tomato, eggplant, pepper and potato) showing typical bacterial wilt symptoms were collected from different commercial fields in south Karnataka (Bangalore, India). Stems of infected plants were cut, the bacterial ooze was directly applied to a FTA card by pressing for a few seconds, then the card was air-dried. A small section (<1 mm) from the centre of the dried sample area was cut using a Harris Micro Punch™. Bacterial DNA samples were purified according to the manufacturer's (Whatman FTA Technology) instruction.

A presumptive water streaming test was also carried out as described by Danks and Barker (2000). A bacterial suspension (100 µl) was serially diluted to 10^{-6} and spread onto a modified semi-selective medium (SMSA) (Elphinstone *et al*., 1996). The plates were incubated at 32°C and observed for the development of typical *R. solanacearum* colonies. These were subcultured and used for inoculation after incubation overnight in 100 ml of casein peptone glucose (casamino acid 1 g/l, peptone 10 g/l, glucose 10 g/l, pH 7.2) at room temperature. The cultures were centrifuged (7,000 rpm for 10 min) and pellets were frozen at -20°C for 2 h. Frozen bacterial pellets were thawed for 3 min at 37°C, suspended in 10 ml of lysis buffer (0.15 M NaCl, 0.05 M sodium citrate) and

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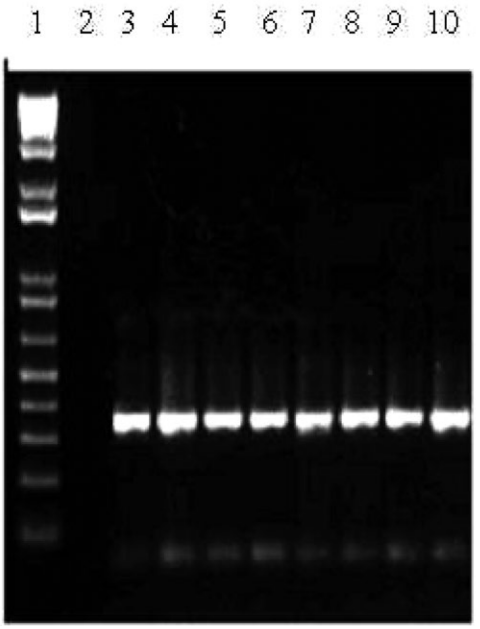

Fig. 1. PCR for genomic DNA of *R. solanacearum* using specific primers (OLI1 and Y2) for both FTA card and conventional method of isolation. Lane 1: 1 KB Ladder (MBI Fermentas); Lane 2: blank. Lanes 3 to 6 comprise genomic DNA isolated using FTA card from tomato (lane 3); eggplant (lane 4); pepper (lane 5); potato (lane 6). Lanes 7 to 10 comprise genomic DNA isolated by the conventional method. Sample loaded in the same order as in lanes 3 to 6.

further incubated with 200 µl of lysozyme (10 mg/ml) at 37°C for 60 min. The cells were lysed with 500 µl of 20% SDS by gentle shaking, 15 ml of extraction buffer were added (2.5 ml of 5 M sodium perchlorate and 12.5 ml of a chloroform:isoamyl alcohol mixture 24:1). The purification and quantification of genomic DNA was carried out according to Chandrashekara *et al.* (2011). The genomic DNA from 4 bacterial isolates was amplified by PCR, essentially as described by Seal *et al.* (1993), using the above mentioned species-specific primers OLI1 and Y2 which detect the gene encoding the 16S rRNA. PCR products were analyzed by electrophoresis in 2% agarose gel and visualized under UV light (254 nm) after ethidium bromide staining. To determine the detection threshold of the FTA method, bacterial ooze of a *R. solanacearum* isolate from tomato was prepared by dilution at final concentrations from 10^{-1} to 10^{-4} CFU/ml, as determined by plating on SMSA. The samples were stored at room temperatures for evaluating amplification by PCR at different time intervals.

Traditionally, the bacterial wilt pathogen is identified in the field by the induction of tissue browning at the collar and vascular tissues, by the ooze streaming test, or by laboratory testing (ELISA or PCR). In our case the bacterial ooze was imprinted on the FTA card and the remaining sample was used for bacterial purification and isolation of genomic DNA by conventional methods. The bacterium was also isolated on modified SMSA (Elphinstone *et al.*, 1996) where it yielded well separated, irregular, slimy colonies with smooth margin, dull-white with pink to red centre, which very much resembled those of *R. solanacearum*. PCR amplification, to confirm the identification of the bacterial isolates as *R. solanacearum* was carried out using as template the genomic DNA isolated by both methods. All DNA samples yielded the expected product of 292 bp (Fig. 1). Thus, based on morphology, pathogenicity and PCR results it was ultimately ascertained that the wilted plants were affected by *R. solanacearum*

FTA card was evaluated for storage stability, showing that the DNA was stable even after 6 months, whereas with the conventional method the DNA stored at room temperature after 3 months did not yield any amplicon (Tables 1 and 2). As to sensitivity, using primers OLI1

Table 1. Comparison of FTA and the traditional method of genomic DNA PCR at different time intervals using the specific primers OLI1 and Y2.

Plant sample	CFU/ml of ooze	PCR results of genomic DNA stored at room temperature					
		FTA card method			Traditional method		
		0 month	3 month	6 month	0 month	3 month	6 month
Tomato	2.89×10^3	+	+	+	+	+	-
Potato	2.35×10^3	+	+	+	+	+	-
Pepper	3.83×10^2	+	+	+	+	+	-
Eggplant	2.15×10^3	+	+	+	+	-	-

Table 2. Sensitivity of FTA card at different dilutions of bacterial ooze using the specific primers OLI1 and Y2.

Tomato sample	CFU/ml of ooze	PCR results at different serial dilutions			
		10^{-1}	10^{-2}	10^{-3}	10^{-4}
Replication-1	2.89×10^3	+	+	+	-
Replication-2	2.89×10^3	+	+	+	-
Replication-3	2.89×10^3	+	+	+	-

and Y2, it was possible to detect on FTA card as little as 29 CFU/ml, estimated by plating on SMSA. These results were reproduced with bacterial ooze on FTA card after 3 and 6 month storage. The comparative PCR results showed that the FTA card method for genomic DNA isolation is simple, fast, reliable and sensitive, in agreement with its successful forensic applications (Zhong *et al*., 2001) and PCR-based genotyping (Tsukaya, 2004).

FTA is a paper-based technology designed for collecting and archiving nucleic acids, either in purified form or within pressed samples of fresh tissue. The results of this study reveal that this method is sensitive enough to detect plant pathogens with low population and that the presence of other organisms does not seem to decrease the sensitivity of the assay, as previously reported by Geoff *et al*. (2003). FTA cards yielded bacterial nucleic acids of a quality equivalent to that obtained with conventional chemical extraction methods. In perspective, this makes FTA technology liable of extension to other groups of plant pathogens, viruses in particular to facilitate and expedite diagnosis.

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