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Research Article

A MODIFIED METHOD FOR EXTRACTION OF HIGH QUALITY AND QUANTITY GENOMIC DNA FROM *XANTHOMONAS ORYZAE* PV. *ORYZAE*

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ABSTRACT
Xanthomonas oryzae pv. *oryzae* (*Xoo*) is a pathogenic bacteria causing bacterial leaf blight of rice and thus forms a major constraint in rice production. To study the pathogen virulence genes and pathogen population, genomic DNA from *Xoo* in good quality and quantity is a prerequisite. In the present study, a protocol for good quality with high yield genomic DNA from *Xoo* has been developed by incorporating initial washes of cell pellet with sterile water and Cetyl trimethyl ammonium bromide buffer for extraction, to do away with the excessive polysaccharides secreted by *Xoo* which interfere with isolation of pure genomic DNA. The genomic DNA isolated using the reported protocol was further tested for downstream applications like restriction and Southern blotting and found to be very amenable and reproducible.

Keywords: *Xanthomonas oryzae* pv. *oryzae, Genomic DNA, Southern Blotting, Rice*

INTRODUCTION
Xanthomonas oryzae pv. *oryzae* (*Xoo*), belonging to γ-subdivision of the Proteobacteria, is a major pathogen of rice (*Oryza sativa* L.) causing bacterial leaf blight disease (Zheng *et al.*, 2009). Rice being the staple crop for much of the world population including India (Khush, 2005), as well as a model for cereal biology, a better understanding of pathogenesis by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) remains a pressing goal both for control of bacterial blight and for fundamental understanding of bacterial-plant interactions. Considering the vast diversity of *Xoo* within India and other rice growing irrigated regions of Asia, typing of *Xoo* using molecular tools such as RFLP (Nelson *et al.*, 1994), PCR fingerprinting based on polymorphism in insertion sequences (IS) (Leach *et al.*, 1990; Mishra *et al.*, 2013) and Southern blotting for Transcription Activator Like Effector (TALE) gene content analysis (Yang and White, 2004) become critical to determine the pathogen population structure. For all of these, high yield of good quality genomic DNA of *Xoo* forms a preliminary requirement. Genomic DNA isolation appears to be very routine and easy but it still remains the point of congestion in case of *Xoo* strains. *Xoo* is a gram negative bacterium and produces polysaccharides as component of their wall structure as well as secretes them in form of extracellular macromolecules (Southerland, 1985). Excessive secretion of extra- cellular polysaccharides by *Xoo* cells makes it very difficult to isolate high concentration of good quality genomic DNA that is amenable to PCR, complete restriction, Southern blotting and other downstream applications like genome sequencing. Hence, optimising a protocol for high concentration of pure genomic DNA from *Xoo* becomes essential. Thus, we have devised a protocol using Cetyl trimethyl ammonium bromide (CTAB) in the extraction buffer, which is generally used for genomic DNA isolation from plants tissues rich in polyphenols and polysaccharides. Further, we have also added and modified few steps from the usual DNA extraction/precipitation based method to overcome the specific problems related to genomic DNA isolation from *Xoo*.

MATERIALS AND METHODS
Isolation of genomic DNA
The general procedure involving Sodium dodecyl sulphate mediated cell lysis; DNA extraction precipitation was modified to develop the reported protocol. *Xoo* isolate was grown in 50 ml PSB media (10 g/l of peptone, 10 g/l of sucrose, 1 g/l of L-glutamic acid, monosodium salt) and cells were pelleted at

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10,000 xg for 5 min. After discarding the supernatant, cell pellet was washed with sterile water for six times. Following six washes, pellet was resuspended in 1X TE buffer (10mM Tris-HCl, and 1 mM EDTA (pH-8.0)) after which 400 µl of lysozyme (conc. 100mg/ml) was added and incubated for 30 min. at 37°C. To this, 300 µl of Proteinase K (10mg/ml) was further added, mixed well and incubated for 3 hours at 56°C. Further, 3 ml of 5 M NaCl and 3 ml of CTAB/NaCl (100 mM Tris-HCl (pH8.0), 1.2 M NaCl, 20 mM EDTA, 2% CTAB, 0.2% β- mercaptoethanol, pre-heated to 65°C was added, mixed well and incubated at 65°C for 10 min. To this mixture, 15 ml chloroform: isoamyl alcohol (24:1) was added, briefly vortexed and spun at max speed for 10 min at room temperature. The upper aqueous phase was then carefully transferred to a clean microcentrifuge tube. This C: I purification step was repeated twice followed by precipitation of DNA with 0.6 volumes of isopropanol at -20°C overnight. Next day the sample was spun at max speed for 15 min at 4°C.The supernatant was discarded and pellet was washed with 70% ethanol, air dried at room temp and resuspended in 100 µl of DNase-free water. The isolated DNA was then treated with 3 µl of RNase (10mg/ml) and incubated at 37°C for 30 minutes. RNase was further heat inactivated at 65°C for 15 minutes. Concentration and purity of isolated DNA was determined by measuring its optical density on a nanodrop. DNA separation was carried out by loading 2 µl of isolated DNA on a 0.8 % agarose gel and photographed by an Alpha imager gel documentation system.

Restriction analysis of genomic DNA

1 µg of genomic DNA samples isolated by the modified method were used for restriction digestion to check the quality of DNA. The gDNA sample was completely digested with *Bam*HI (Fermentas). Reaction tubes were incubated at 37°C incubator for overnight and sample was analysed on 1% agarose gel and photographed by an Alpha imager gel documentation system.

Southern blot hybridisation

Standard procedures for Southern blots were utilized (Ausubel *et al.*, 1988). The *Bam*HI digested DNA was migrated on 1% agarose gel at 50V for 36h. Filters were probed with non radioactive DIG DNA labelling and detection kit from Roche Applied science using 3' end conserved sequence of *avrXa7* cloned in our lab.

RESULTS AND DISCUSSION

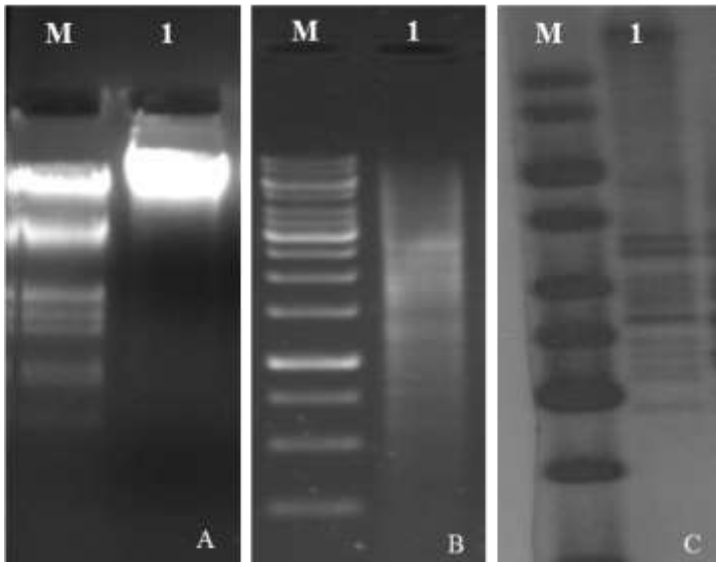

Figure 1: Isolation and analysis of genomic DNA from *Xoo* strains. A- Lane M- Lambda DNA digested with *Eco*RI and *Hin*dIII ladder (Fermentas), lane 1- Genomic DNA isolated from *Xoo*. B- Lane M-1kb Plus ladder (Fermentas); lane 1- Profile of *Xoo* genomic DNA digested with *Bam*HI. C- Lane M- 1kb Plus ladder (Fermentas); lane 1- Southern hybridisation of digested *Xoo* genomic DNA probed for TALE genes

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We have successfully isolated pure genomic DNA from the Xoo strains with high concentration with a yield 100 µg in 100 µl (1086 ng/µl) (Figure 1A). There was no contamination of RNA observed with just 30min of RNase treatment. The purity of DNA sample was further confirmed through complete digestion by the restriction enzyme *Bam*HI. For complete digestion, overnight incubation at 37 °C was done and no degradation during incubation with the enzymatic mix was seen (Figure 1B). The Southern blots as seen in Fig 1C had clean sharp bands, each band representing one TALE gene, with no background which confirms the absence of any kind of contaminating polysaccharide which often makes genomic DNA too viscous to be handled error free for restriction or southern blotting.

During isolation of genomic DNA, inclusion of six times washing step minimized the exopolysaccharides from bacterial cell pellet which was an inseparable contaminant earlier. The remaining contamination is taken care by CTAB. Any protein contamination was removed by chloroform and isoamyl alcohol based separation done twice here. In summary our protocol is reproducible and consistent. The isolated DNA can be completely digested with restriction endonucleases and further processed for clean Southern blots successfully. This confirms that isolated genomic DNA with high yield, free from polysaccharides is amenable for further research applications.

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