Note

A SIMPLE METHOD FOR DNA ISOLATION FROM *Xanthomonas* spp.

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ABSTRACT: A simple DNA isolation method was developed with routine chemicals that yields high quality and integrity preparations when compared to some of the most well known protocols. The method described does not require the use of lysing enzymes, water bath and the DNA was obtained within 40 minutes The amount of nucleic acid extracted (measured in terms of absorbancy at 260 nm) from strains of *Xanthomonas* spp., *Pseudomonas* spp. and *Erwinia* spp. was two to five times higher than that of the most commonly used method.
Key words: DNA, *Xanthomonas*, RAPD

MÉTODO SIMPLIFICADO DE ISOLAMENTO DE DNA DE *Xanthomonas* spp.

RESUMO: Foi desenvolvido um método simplificado de isolamento de DNA utilizando substâncias de uso rotineiro facilitando a metodologia e obtendo-se DNA de alta qualidade e integridade quando comparado com os métodos tradicionais. O método descrito não utiliza enzimas líticas, nem banho-maria, e o DNA é obtido em 40 minutos. As quantificações dos DNAs extraídos (medido em D.O. a 260 nm) das dez linhagens de *Xanthomonas* spp., uma de *Pseudomonas* spp. e outra *de Erwinia* spp. mostraram-se de duas a cinco vezes maiores que os métodos conhecidos e suas integridades, testadas em RAPD, apresentaram-se satisfatórias.
Palavras-chave: DNA, *Xanthomonas*, RAPD

INTRODUCTION

Several procedures are described for *Xanthomonas* DNA isolation (Alvarez et al., 1996; Hartung & Civerolo, 1987), as well as for *Pseudomonas* spp. and *Erwinia* spp., where the bacteria are grown in Wilbrink's agar plates for 72h and, cells are treated with egg white lysozyme for 30 min on ice. The lysis solution (0.5% SDS, 50 mmol L^{-1} Tris-HCl, pH 7.5, 400 mmol L^{-1} EDTA, 1 mg pronase) is added to cell suspension and incubated at 50°C in water bath for 3-5 h and then deproteination is carried out.

Pan et al. (1997) used a simpler method where bacterial cells are grown in 1.5 ml of XAS broth, centrifuged for 8 min at 1,500 x *g* , washed in 0.5 ml of STE buffer (100 mmol L^{-1} NaCl, 10 mmol L^{-1} tris, pH 8.0, 1 mmol L^{-1} EDTA) and lysed in 400 µl of lysing buffer (20 µl of 1 mol L^{-1} Tris, pH 8.0, 40 µl of 0.5 mol L^{-1} EDTA, 120 µl of 10% SDS, 4 µl of 2-mercaptoethanol and 216 µl of H_2O. The lysate mix is incubated at 65°C for 2 h and deproteinated. The method described by Ausubel et al. (1992) has been widely used (Lopes et al., 1998; Louws et al.,1994) and cells after and centrifuged for 2 min at 13,000 x *g*, the pellet is resuspended in 567 µl of TE buffer, 30 µl of 10% SDS and 3 µl of proteinase K (20 mg mL^{-1}) and incubated at 37°C for 1.5 h. Then 100 ul of 5 mol L^{-1} NaCl and 80 µl of CTAB buffer (10 g CTAB, 4.1 g NaCl in 100 ml of water) are added, incubated 10 min. in a 65°C water bath. Deproteination is done and isopropanol is used to precipitated the DNA. Other similar methods are cited by Boucher et al. (1985) in the work of Restrepo & Verdier (1997) and other procedures (Cupples & Morris, 1990; Kuflu & Cupples, 1997).

All the protocols cited are able to give large amounts of high quality DNA.

MATERIAL AND METHODS

The strains used came from the Tropical Culture Collection (CCT - Fundação Tropical de Pesquisas e Tecnologia "André Tosello"- Campinas, SP) (*Xanthomonas campestris* pv. *manihotis*; *X. campestris* pv. *vesicatoria*; *X. campestris* pv. *campestris*; *X. maltophilia*) and from our laboratories (*Erwinia* spp., *Pseudomonas* spp.; *X. albilineans and X. axonopodis* pv. *phaseoli*).

The cultures were grown in 5 ml nutrient broth (Difco) with 10% glycerol v/v (suggested by Maringoni et al. (1988) to avoid xanthan formation) for 72h at 27°C. One tube of 1.5 ml was used to centrifuged the cells at 13,000 x g for 5 min and the pellet was suspended in 200 µl Tris 0.1 mol L^{-1} and added with 200 µl of lysis solution (NaOH 0.2 N and 1% SDS), mixed and deproteinazed with 700 µl of

phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v), homogenized and centrifuged 10 min at 13,000 x *g* .To precipitate DNA, 700 µl of cold 95% ethanol was added and spinned, washed in 70% ethanol and centrifuged. Precipitated DNA is dried at room temperature and suspended in 100 ul of water. The method described by Ausubel et al. (1992) was performed comparing twelve strains. The samples from the both methods were electrophoresed on 0.8% agarose gels, stained with ethidium bromide and photographed under UV light . The RAPD was performed according to Williams et al. (1990) using primer OPL 11 (Operon Technologies, USA).

RESULTS AND DISCUSSION

DNA samples were quantified in spectrophotometer at 260/280 nm and the results can be seen in TABLE 1. The integrity of the samples are shown in Figure 1 and 2, and the quality can be observed in the RAPD shown on Figure 3 and 4, for both methods where in Figure 3 a higher number of polymorphic bands are shown than in Figure 4, using the same primer.

This work presents a simpler high performance and short time consuming procedure for *Xanthomonas* DNA isolation and for the two other bacteria, *Pseudomonas* spp. and *Erwinia* spp.. Samples 260/280 nm ratio was approximately 1.7 to 2.0 in both methods but the quantity and quality seen in agarose gel electrophoresis is higher in the first method.

TABLE 1 - Absorbancy (260 nm) for DNA from *Xanthomonas* species and from one *Pseudomonas* spp. and one *Erwinia* spp. isolated by both methods (Method 1 – method of this paper; Method 2 - method described by Ausubel (1992)).

	Method 1	Method 2
Strains	A_{260nm}	A_{260nm}
	---------- ng ul^{-1}	----------
X. campestris pv. citri	1771	330
Erwinia spp.	1418	444
Pseudomonas spp.	1754	361
X. albilineans	1672	807
X. albilineans (Copersucar)	1531	326
X. axonopodis pv. phaseoli	1737	462
X. campestris pv. manihotis - CCT 0088	1572	244
X. campestris pv. vesicatoria - CCT 0085	924	250
X. campestris pv. vesicatoria - CCT 0086	1145	377
X. campestris pv. campestris-CCT 0369	628	516
X. campestris pv. manihotis - CCT 1224	660	324
X. maltophilia - CCT 1897	602	392

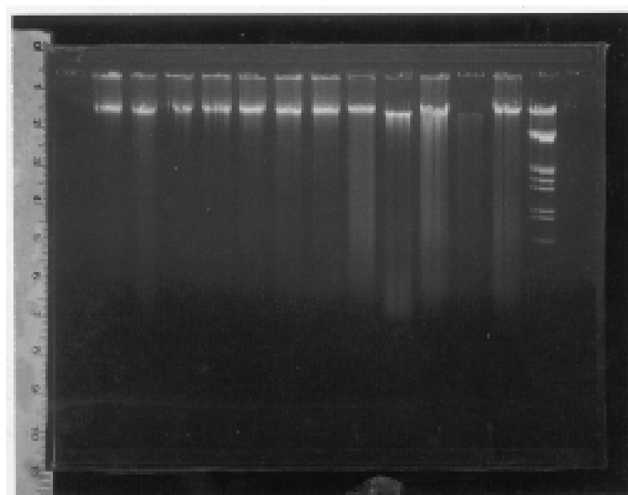

Figure 1 - *Xanthomonas* DNA isolated by the method proposed. From right to left, λ EcoRI-Hind III, *X. campestris pv. citri*, *Pseudomonas* spp., *Erwinia* spp., *X. albilineans, X. albilineans* (Copersucar), *X. axonopodis pv. phaseoli, X. campestris pv. manihotis* (CCT 0088), *X. campestris pv. vesicatoria* (CCT 0085), *X. campestris pv vesicatoria* (CCT0086), *X.campestris pv. campestris* (CCT0369), *X. campestris pv. manihotis* (CCT1224) and *X. maltophilia* (CCT1897).

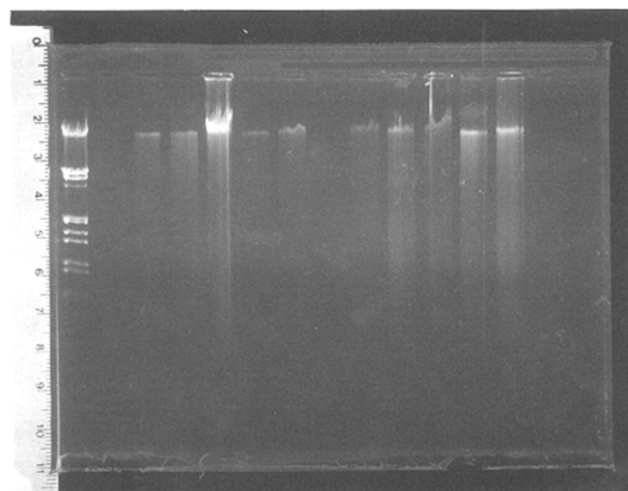

Figure 2 - *Xanthomonas* DNA isolated by the method on Ausubel (1992). From left to rigth, λ EcoRI-Hind III, *X. campestris pv. citri*, *Pseudomonas* spp., *Erwinia* spp., *X. albilineans, X. albilineans* (Copersucar), *X. axonopodis pv. phaseoli, X. campestris pv. manihotis* (CCT 0088), *X. campestris pv. vesicatoria* (CCT 0085), *X. campestris pv. vesicatoria* (CCT0086), *X. campestris pv. campestris* (CCT0369), *X. campestris pv. manihotis* (CCT1224) and *X. maltophilia* (CCT1897).

All mentioned methods employ chemicals such as egg white lysosyme, pronase, proteinase K and require periods of water bath incubation. In addition, the method using pronase (Alvarez et al., 1996; Hartung & Civerolo, 1987) takes from 4 to 6 hours, the method described by Pan et al. (1997) is simpler but requires water bath and takes about three hours to DNA isolation the DNA. The last described protocol (Ausubel et al., 1992;

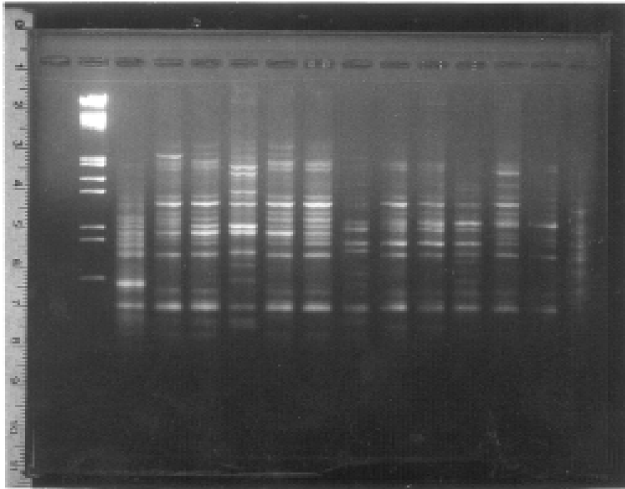

Figure 3 - RAPD from *Xanthomonas* DNA isolated by the method proposed. From left to right, λ EcoRI-Hind III, *X. campestris pv. citri*, *Pseudomonas* spp., *Erwinia* spp., *X. albilineans, X. albilineans* (Copersucar), *X. axonopodis pv. phaseoli, X. campestris pv. manihotis* (CCT 0088), *X. campestris pv. vesicatoria* (CCT 0085), *X. campestris pv. vesicatoria* (CCT0086), *X. campestris pv. campestris* (CCT0369), *X. campestris pv. manihotis* (CCT1224) and *X. maltophilia* (CCT1897).

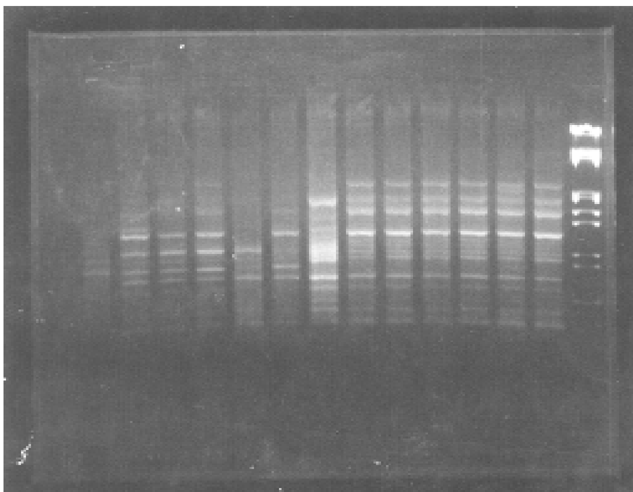

Figure 4 - RAPD from *Xanthomonas* DNA isolated by the method from Ausubel (1992). From right to left, λ EcoRI-Hind III, *X. campestris pv. citri*, *Pseudomonas* spp., *Erwinia* spp., *X. albilineans, X. albilineans* (Copersucar), *X. axonopodis pv. phaseoli, X. campestris pv. manihotis* (CCT 0088), *X. campestris pv. vesicatoria* (CCT 0085), *X. campestris pv. vesicatoria* (CCT0086), *X. campestris pv. campestris* (CCT0369), *X. campestris pv. manihotis* (CCT1224) and *X. maltophilia* (CCT1897).

Lopes et al., 1998; Louws et al., 1994) which was used in comparison to our method requires proteinase K, water bath and three hours for DNA isolation. The presented method has no need of lysis reagents other than NaOH and SDS and takes about 30 to 40 minutes to total DNA isolation showing that simple reagents can provide good results in DNA isolation, quality and quantity-wise for *Xanthomonas* species.

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