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Full Length Research Paper

A basic and fast lysis system for arrangement of genomic DNA from Gram-negative microscopic organisms

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A very efficient, simple and economic lysis method for rapid preparation of genomic DNA from Gram-negative bacteria was developed. This method includes a novel step lysis by treatment with carvacrol followed by simple ethanol precipitation. The procedure was realised without using of detergents or enzymes. Moreover, the resultant genomic DNA was in good quantity and quality and can be used successfully for restriction endonucleases digestion, PCR amplification and others types of molecular biology manipulations.

Key words: Genomic DNA, lysis, carvacrol, Gram-negative bacteria, *Escherichia coli, Erwinia chrysanthemi*.

INTRODUCTION

Preparation of DNA from all organisms, including Gram-negative bacteria, is a key step in molecular biology studies. Several methods are developed to simplify this extraction while improving the quality of extracted DNA. However, all the existing methods invariably involve two important steps: Cell lysis and precipitation of the DNA. The lysis step is performed with detergents, enzymes, or organic solvents and consuming a long time (Marmur, 1961; Flamm et al., 1984; Trevors, 1984; Sambrook et al., 1989; Kuhnert et al., 1997; El Hassouni et al, 1999; Hai-Rong and Ning, 2006).

It has been previously shown that carvacrol; the major phenolic component of the essential oils of oregano and thyme (Lagouri et al, 1993; Juven et al, 1994; Ultee et al, 1999; Rhayour et al., 2003) can break bacterial cells. Due to its hydrophobic nature, carvacrol induces this effect by damage of biological membrane (Sikkema et al., 1992, 1994; Ultee et al., 1999; Rhayour et al., 2003; Bennis et al., 2004a, b; Chami et al., 2005a).

The main objective of this study was to develop an efficient, rapid and economic lysis method for preparation of genomic DNA from Gram-negative bacteria. This method includes a new lysis approach based on treatment with carvacrol followed by simple ethanol precipitation. Importantly, the purified DNA can be successfully used in different molecular biology manipulations, including PCR and restriction enzyme digestion.

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MATERIALS AND METHODS

Bacterial strains and culture medium

Escherichia coli TG1 (supE hsdΔ5thiΔ (lac-proAB) F' (traD36 proA+B+ lacq lacZΔM15) and *Erwinia chrysanthemi* VIII (LCB-CNR –equip BANNAS. Marseille) were cultivated in Luria-Bertani broth (LB) (Sambrook et al., 1989).

Carvacrol lysis method

Preparation of carvacrol emulsion

Carvacrol (Fluka. Chemika) was dispersed in sterile 0.2% agar suspension (Remmal et al., 1993).

Cells collection

E. coli and *E. chrysanthemi* were cultured for 16 to 18 h at 37°C on 3 ml LB broth. A 1.5 ml aliquot of culture was centrifuged at 2,000 × g for 3 min and the pellet was washed by 100 µl of GTE buffer (50 mM glucose, 25 mM Tris/Hcl, 10 mM EDTA, pH 8).

Cells lysis by carvacrol

The washed cells were resuspended into 300 µl of carvacrol

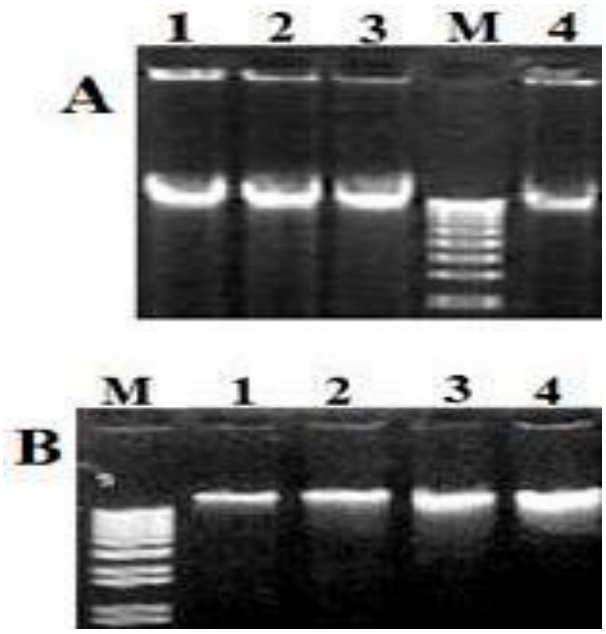

Figure 1. The pattern of DNA electrophoresis on a 0.7 % agarose gel. Lanes 1, 2, 3: The genomic DNA was extracted from *E. coli* (A) and *E. chrysanthemi* (B) by carvacrol lysis method at 0.325 mM, 0.65 mM and 1.3 mM respectively. Lane 4: The genomic DNA prepared by method of Marmur (1961). The ladder 1kb plus marker (Gibco/BRL) was used as size marker.

emulsion diluted in sterile distilled water at a final concentration of 0.325, 0.65 and 1.3 mM. The suspension was vigorously vortexed and incubated at 37°C for 5 min

DNA precipitation

After adding of 200 µl of 5 M sodium acetate (pH 7), the mixture was placed on ice for 15 min and centrifuged at 12,000 × g for 15 min. The genomic DNA was precipitated by adding 2 volume of ice cold ethanol absolute to the supernatant and was centrifuged at 12,000 × g for 20 min. The DNA pellet was washed by ethanol 70% and resuspended in 50 µl of TE buffer (10 mM Tris, 1 mM ETDA; PH8). Finally, RNase was added at 50 µg/ml and incubated at 37°C for 1 h to digest RNA.

A treatment step by phenol/chloroform/isoamyl alcohol was performed when the DNA was used for restriction analysis as described by Sambrook et al. (1989).

For standard agarose gel electrophoresis, 4 µl of DNA samples were separated in 0.7% (w/v) gel stained with ethidium bromide. Electrophoresis conditions were 100 V for 45 min in 0.5x TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM EDTA). In comparison, the genomic DNA was extracted from *E. coli* and *E. chrysanthemi* according to the method of Marmur (1961).

Quantification and qualification of DNA

Quantification and qualification of the DNA obtained from both methods were determined by reading absorbance at 260 and 280 nm. An OD of 1 corresponds to approximately 50 µg/ml for double-stranded DNA. The ratio A260/A280 provides an estimate of the purity of the nucleic acid (Sambrook et al., 1989).

Restriction enzyme digestion

4 µl genomic DNA obtained from *E. coli* or *E. chrysanthemi* by both methods were incubated with 5 U *Eco*RI or *Sal*I in a final volume of 20 µl for 1 to 2 h at 37°C and applied to 1% agarose gel electrophoresis.

PCR amplification

Two primers, P143 (5'-CACCCGTGGTTGTAAAACACGTTTCGG-3') located at 137 nt upstream of the initiation codon of msrA and 3' reverse complementary P145 (5'-GCGGTTTCCTGCTCTGTGGTT-3') were used for MsrA amplification. MsrA is a gene encoding for peptide methionine sulfoxide reductase. It is a virulence determinant of the plant pathogen *E. chrysanthemi*. PCR primers that target were the conserved sequences of MsrA gene. PCR with MsrA specific primers conserved sequences results in amplification of the P143-145 regions, which is specific to *E. chrysanthemi* (El Hassouni, 1999).

The 40 µl reaction mixture contained 2 µl of DNA extract, 4 µl of 10 x PCR buffer without $MgCl_2$; 2.4 µl of 25 mM $MgCl_2$; 8 µl of deoxinucleoside triphosphate (dNTP) mix (1 mM each dNTP); 0.4 µl (2 U) of Taq DNA polymerase (5 U/µl) (all Promega); 4 µl of each primer. The volume was made up to 40 µl with sterile distilled water. Amplification was performed with a DNA thermal cycle (TECHNE GENIUS) programmed as follows: a cycle for 3 min at 94°C followed by 35 cycles for 30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C. One cycle for 3 min at 72°C was conducted after the 35 cycles.

Detection of PCR-amplified product was performed by electrophoresis on a 2% (w/v) agarose gel stained with ethidium bromide. A volume of 7 µl of PCR product was loaded in each lane, electrophoresis was conducted in 0.5x TBE buffer at 50V for 30 min. For all manipulations, DNA was visualized and photographed under UV light using a TCX-20-C Translluminator (VILBER LOURMAT).

RESULTS AND DISCUSSION

Evaluation of DNA extraction

DNA, using carvacrol lysis method was successfully extracted from *E. coli* and *E. chrysanthemi*. The DNA samples seen on agarose gel electrophoresis consisted of a large amount of chromosomal DNA with distinct and clear bands (Figure 1). In addition, 5 min of treatment by low concentration of carvacrol was widely sufficient for cells lysis.

Quantification of genomic DNA

The quantities of DNA were increased simultaneously with the carvacrol concentrations. In addition, the DNA concentrations extracted at 1.3 mM of carvacrol in both bacteria were greater than those obtained by method of Marmur (Table1). On the other hand, the A260/280 ratio varied from 1.78 to 1.98 for all DNA samples (Table1), which indicates that DNA preparations were sufficiently purified (Table1).

Table 1. Yields and ratio A260/A280 of *E. coli* and *E. chrysanthemi* genomic DNA obtained by carvacrol lysis method compared to method of Marmur.

Concentration of carvacrol	*E. coli*.		*E. chrysanthemi*	
(mM)	DNA concentration (µg/µl)	A_{260}/A_{280}	DNA concentration (µg/µl)	A_{260}/A_{280}
0.325	0.79 ± 0.045	1,98 ± 0.123	0.56 ± 0.04	1.78 ± 0.019
0.65	1.08 ± 0.09	1.87 ± 0.093	0.63 ± 0.0115	1.90 ± 0.001
1.3	1.47 ± 0.04	1.97 ± 0.083	1.34 ± 0.181	1.79 ± 0.057
Method of Marmur	1.28 ± 0.175	1.92 ± 0.049	0.62 ± 0.057	2.01 ± 0.011

Values are mean ± SD (standard error).

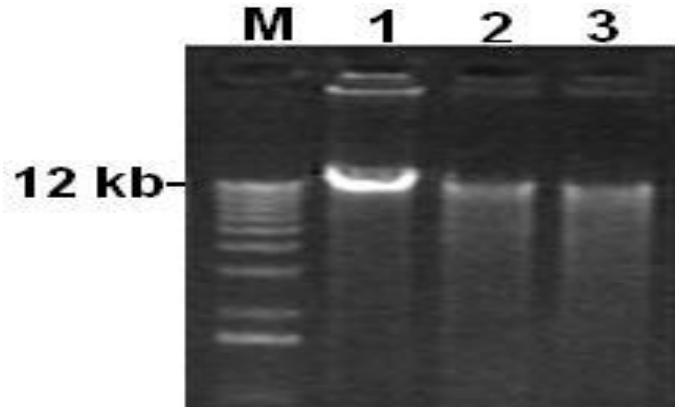

Figure 2.. Restriction digestion of genomic DNA extracted from *E. coli* by carvacrol lysis method at 0.65 mM. Lane 1: Undigested DNA, lane 2: *Eco*RI digested DNA, lane 3: *Sal*I digested DNA. The ladder 1kb plus marker (Gibco/BRL) was used as a size marker.

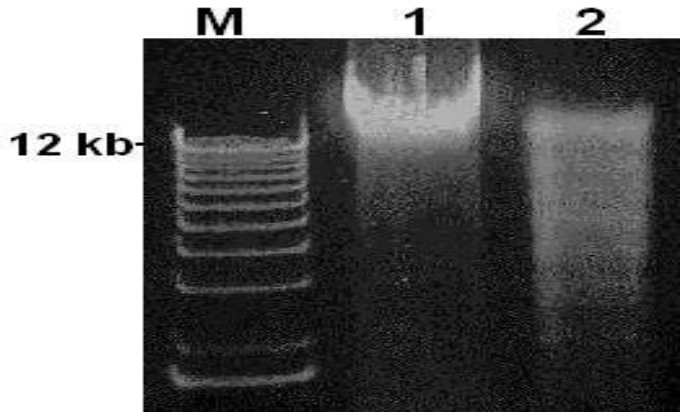

Figure 3.. Restriction digestion of genomic DNA extracted from *E. chrysanthemi* by carvacrol lysis method at 0.65 mM. Lane 1: Undigested DNA, lane 2: *EcoRI* digested DNA. The ladder 1kb plus marker (Gibco/BRL) was used as a size marker.

Endonuclease digestion

To further characterize the carvacrol-extracted genomic DNA and assess its purity so much, DNA was either digested with *Eco*RI or *Sal*I. Figures 2 and 3 show the

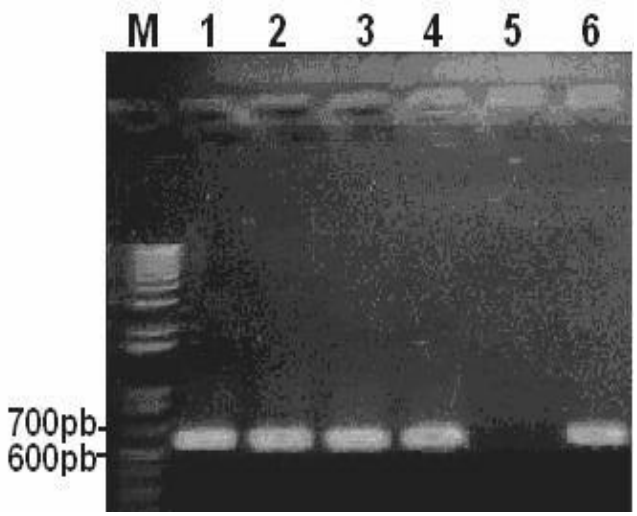

Figure 4. Direct PCR amplification products of genomic DNA extracted from *E. chrysanthemi*. Lanes 1, 2, 3, Amplification products of chromosomal DNA extracted by carvacrol at 0.325 mM, 0.65 mM and 1.3 mM. Lane 4: Amplification products of chromosomal DNA extracted by method of Marmur. Lane 5: H_2O contamination control. Lane 6, positive control. Lane M; Size markers 1kb ladder. Fragments sizes are indicated on the left.

treatment of DNA with either restriction enzyme which led to digestion of DNA and appearance of fragments of different sizes, with no clear band at the level of the non-digested DNA, indicating that the digestion is total. These results confirm the high purity of the genomic DNA and thereby the efficiency of the carvacrol-based method.

DNA amplification by PCR

Amplification of specific DNA fragments by PCR is a powerful and widely used technique. Therefore, it was important to show that the genomic DNA extracted with carvacrol can be amplified by PCR. Using the DNA extracted from *E. chrysanthemi*, by carvacrol lysis technique as target, the MsrA gene was successfully amplified. The amplified fragment had the expected size of 650 bp and the same fragment has been obtained with the method of Marmur (1961) (Figure 4). So the carvacrol-extracted

DNA can be amplified by PCR, indicating its high quality and purity.

In this study, *E. coli* and *E. chrysanthemi* were lysed by carvacrol in the aim to extract genomic DNA. However, the lysis step in standard method involves several steps with different temperatures and takes a long time (Marmur, 1961). Importantly, the carvacrol lysis method is very efficient in terms of time, volume of reagents and number of steps. Thus, 5 min of treatment by carvacrol was enough to lyse maximum of cells and to extract genomic DNA in high quantity and quality.

In conclusion, we have developed a new lysis method to extract the genomic DNA from Gram-negative bacteria, using only carvacrol in the lysis step. This method was easy, inexpensive and rapid, and more; the genomic DNA can be used successfully in PCR, restriction enzy-me and other molecular biology applications.

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