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Short Communication

Isolation of total DNA from bacteria and yeast

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Many procedures in molecular biology require the isolation of high quality genomic DNA. This study investigated a new method to extract DNA from Gram-negative, Gram-positive bacteria, Mycobacteria and yeasts. Guanidine thioisocyanate present in DNAzol is capable of binding DNA to silica particle column. Subsequently the silica with adsorbed DNA is washed to remove impurities and the clean DNA eluted in appropriate buffer. Results indicated that the new extraction method is simple and reproducible. This isolation technique is faster and easier to perform than the other conventional extraction methods. Finally the recovered DNA is of high quality and suitable for downstream applications.

Key words: DNAzol, DNA isolation, bacteria, yeast.

INTRODUCTION

The isolation and purification of DNA is a key step for most protocols in molecular biology studies and all recombinant DNA techniques (Sambrook et al., 1989). Several DNA extraction method are widely used to isolate DNA from bacteria and yeast including phenol extraction but they often involve multiple, time consuming steps including the handling of toxic chemicals (Ausbel et al., 1995). DNAzol is a complete and ready to use reagent for the isolation of genomic DNA from solid and liquid samples of animal and plant origin. This reagent is an advanced DNA isolation method (Chomczynski et al., 1997) that combines both reliability and efficiency with simplicity of the isolation protocol. The DNAzol procedure is based on the use of a novel guanidine-detergent lysing solution that hydrolyzes RNA and allows the selective precipitation of DNA from a cell lysate. The protocol is fast and permits isolation of genomic DNA from a large number of samples of small or large volumes. Originally, the DNAzol procedure was proposed for DNA extraction from animal and plant tissues. In the present study we provide a modify DNAzol reagent protocol for the isolation and purification of total DNA from bacterial and yeast (Microbial DNAzol).

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MATERIALS AND METHODS

Strains and growth condition

Strains used in this study ar listed in Table 1. *Enterococcus faecalis*, *Enterococcus faecium*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Staphylococcus lugdunensis* was grown aerobically in trypticase soy broth (TSB) (Difco) at 37°C. *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were grown aerobically in Luria broth (LB) at 37°C. *Mycobacterium avium* and *Mycobacterium terrae* were grown on Lowestein-Jensen medium at 37°C for 10 to 14 days. *Candida albicans* was grown aerobically in yeast peptone dextrose (YPD) medium at 30°C overnight to stationary phase with a cell density of approximately $2X10^8$ cfu/ml. When starting with solid medium, a loop was used to take a small amount of bacteria (5-7 colonies) into a vial containing 100 µl TE (10mM Tris pH 8, 1 mM EDTA). Bacteria were collected and pelleted in a microfuge, washed with TE and resuspended in 100 µl TE containing specific cell wall-degrading enzyme prior to the addition of DNAzol.

Lysis treatment

Staphylococcal cell pellets were resuspended in 100 µl TE containing lysostaphin (20 µg/ml), enterococcal cells were resuspended in 100 µl TE containing a mixture of lysozyme (2mg/ml) and mutanolysin (50 µg/ml), Gram-negative listeria and mycobacterial cells were resuspended in 100 µl TE containing lysozyme (2mg/ml). Yeast cells pellet were resuspended in 100 µl sorbitol buffer (1M sorbitol, 0,1 M EDTA, 15mM β-mercaptoethanol,

Table 1. Bacteria and yeast strains from which genomic DNA was isolated using the modified DNAzol method.

Strains	Source
Escherichia coli	ATCC 35218
Pseudomonas aeruginosa	ATCC 27853
Staphylococcus aureus	ATCC 29213
Staphylococcus lugdunensis	ATCC 700328
Enterococcus faecalis	ATCC 29212
Enterococcus faecium	OCV SM
Enterobacter cloacae	OCV SM325
Klebsiella pneumoniae	ATCC 700603
Listeria monocytogenes	OCV SM160
Candida albicans	IM 43VR
Mycobacterium avium	ATCC 15769
Mycobacterium terrae	ATCC 15755

Strains labelled OCV were obtained from the Bacteriology Laboratory of Ospedale Civile of Verona city, reference ATCC and IM VR strains come from the collection of the Dipartimento di Patologia sez. Microbiologia of Verona, Italy.

pH 7.5) containing zymolyase (200U/ml). All enzymes were obtained from Sigma. The mixtures was incubated for 30 min at 37°C.

Column purification

500 µl DNAzol reagent (Invitrogen Life Technologies) was added to 100 µl of the lysed mixture. After incubation at 65°C for 5 min the cleared sample were transferred to the Concert Rapid PCR Purification System columns (Invitrogen Life Technologies) and centrifuged at 10,000 X g for 30-60 seconds. The columns were washed twice time by adding 0.75 ml of 70% ethanol followed by centrifugation for 30-60 s. The columns were further centrifuged for an additional 1 min at maximun speed and placed in a clean 1.5 ml microfuge tube. To elute the DNA, 55 µl of warm (65°C) TE buffer was added to the center of the membrane and allow it to interact with the resin for 1 min. The DNA was eluted by centrifugation at 10,000 X g for 1 min. Quantity and purity of DNA samples from new extraction procedures were checked by spectrophotometrical readings at 260 and 280 nm, as described by Sambrook et al. (1989). Table 2 shows DNA yields from various starting materials using this protocol.

Table 2. DNA yields and purity from various starting materials isolated with the modified DNAzol method.

Source	Amount of starting material (ml)	DNA yield (µg)	Purity $A_{260/280}$
Gram-negative bacteria * (≅ $1X10^9$ cells/ml)	1.5 ml	10-15	1.9
Gram-positive bacteria * (≅ $1.5X10^8$ cells/ml)	1.5 ml	6.5-10	1.85
Yeast * (≅ $1X10^8$ cells/ml)	1.5 ml	4.5-6.5	1.82
Acid-fast bacteria * * (≅ $1X10^8$ cells/ml)	1.0 ml	5-7	1.8

RESULTS AND DISCUSSION

We found that treating microbial cells with specific cell-wall degrading enzymes greatly facilitates the susceptibility of these cells to DNAzol extraction procedures (data not shown). The total DNA extracted using the modified DNAzol method seen on agarose gel after electrophoresis and staining with ethidium bromide consisted of a large amount of unsheared chromosomal DNA with some strains containing plasmid DNA (Figure 1A). The recovered nucleic acids have an $A_{260/280}$ ratio of 1.8-2.0 (Table 2) and are suitable for direct restriction enzyme digestion (Figure 1B). The DNA is also suitable for Southern blot, molecular cloning, polymerase chain reaction (PCR), extra long polymerase chain reaction (XL PCR) and other molecular biology and biotechnology applications (data not shown).

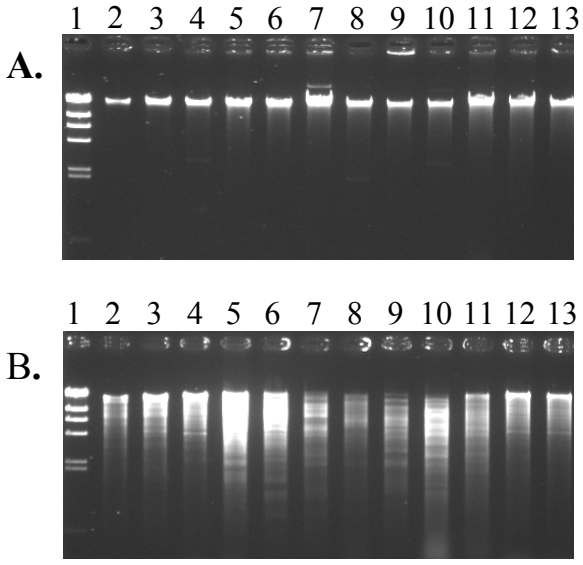

Figure 1. Agarose gel electrophoresis (0.8%) of the genomic DNA isolated by the modified DNAzol method (**A**) and digested with EcoRI endonuclease (**B**). Lane 1, λ Hind III molecular size (bp) standard (fragment length: 23.130, 9.416, 6.557, 4.361, 2.322, 2.027, 564); lane 2, *E. coli* ATCC 35218; lane3, *P. aeruginosa* ATCC 27853; lane 4, *E. cloacae*; lane 5, *K. pneumoniae* ATCC 700603; lane 6, *S. aureus* ATCC 29213; lane 7, *S. lugdunensis* ATCC 700328; lane 8, *E. faecalis* ATCC 29212; lane 9, *E. faecium* OCV SM; lane 10, *L. monocitogenes* OCV SM160; lane 11, *C. albicans* IM 43VR; lane 12, *M. avium* ATCC 15769; lane13, *M. terrae* ATCC 15755.

In conclusion, our results have demonstrated that the newly described method represents a rapid, simple and reproducible method for small scale isolation of high quality genomic DNA from bacteria and yeast, including those that are difficult to disrupt. The amount of genomic DNA isolated using the DNAzol method was the same as

other standard procedures using proteinase K and phenol extraction (data no shown). This technique also eliminates the need for time-consuming organic extractions and ethanol precipitation, because contaminants are removes without precipitation, solvent extraction, or other handling steps that can lead to loss or degraded DNA. The procedure using Microbial DNAzol requires about 0.75 h compared to 2 h or more using other procedures, and more samples can be processed in less time. Finally, the modified protocol increase laboratory efficiency and safety.

REFERENCES

Ausbel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1995). Current Protocols in Molecular Biology. John Wiley and Sons, 2.4.1.

Chomczynski P, Mackey K, Drews R, Wilfinger W (1997). DNAzol: A reagent for the rapid isolation of Genomic DNA. BioTechniques 22: 550-553.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A laboratory Manual (2nd Edition). Cold Spring Harbor Laboratory, New York.