Stamford Journal of Pharmaceutical Sciences

Effect of Proteinase-K on Genomic DNA Extraction from Gram-positive Strains

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Original Research Article

ABSTRACT

Direct extraction of DNA from natural environment and clinical samples has become a useful alternative for the phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. In this study, three different Gram positive microorganisms (*B. cereus, B. subtilis, and S. aureus*) were chosen for genomic DNA extraction. High salt SDS (Sodium Dodesyl Sulfate) based extraction method was followed to extract genomic DNA with addition of three different lysis protocols to observe the effect of proteinase-K on total genomic DNA yield, lysis steps were carried with SDS, SDS with 3 µl proteinase-K and SDS with 6µl proteinase-K. High molecular weight intact DNA bands were observed only for *Bacillus subtilis* when the extraction procedure was carried out in presence of SDS, SDS with proteinase-K (3µl) and SDS with increased amount of proteinase-K (6µl). In presence of SDS and increased amount of proteinase-K (6µl) the mean value of DNA concentration for *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus* were found to be 1.53±0.15, 1.36±0.10 and 1.65±0.10 µg/µl respectively. However, in absence of proteinase-K, the mean values of DNA concentration were found to be decreased (1.28±0.10, 1.34±0.15, 1.23±0.10 µg/µl for *B. cereus, B. subtilis*, and *S. aureus* respectively) for all these stains. Although in case of *B. subtilis* the overall effect of proteinase-K was not found to be significant in terms of DNA concentration and DNA band intensity, however, for *B. cereus*, and *S. aureus* sharp decrease in total extracted DNA concentration was observed suggesting the increased lysis effect of proteinase-K on the thick peptidoglycan layer of Gram-positive cell wall such as *B. cereus*, and *S. aureus*.

***Key words*:** Extraction, Genomic DNA, Lysis buffer, Gram positive organism.

INTRODUCTION

The development of molecular biological methods i.e. DNA based method involving DNA extraction and Polymerase Chain Reaction (PCR) have led to an array of new techniques that are not limited by the culturability of the microorganisms. Therefore, the extraction of DNA from natural environment has become a useful and an attractive alternative for the study of different microbial communities in soil (Amann *et al.*, 1995, Borneman *et al.*, 1997, Hugenholtz *et al.*, 1998). A large number of methods have been published for the extraction of total genomic DNA (Steffan *et al.*, 1988, Cullen *et al.*, 1998, Zhou *et al.*, 1996). Most of them are laborious. Variation in the efficiency of lysis yield and purity of the DNA can fundamentally affect the success of analytical technique, such as PCR and biases can be introduced in quantitative analysis. Therefore a suitable and selective method with a high extraction efficiency need to be developed that can be used as a routine basis.

The fundamental processes of DNA extraction from cells and tissues are sample lysis and the segregation of the nucleic acid away from

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contaminants. While DNA is more or less universal to all species, the contaminants and their relative amounts will differ considerably. The composition of fat cells differs significantly from muscle cells. Plants have to sustain high pressure, contain lipopolysaccharides that can interfere with extraction and cause toxicity problems when present in downstream applications. Fibrous tissues such as heart and skeletal muscle are tough to homogenize. These variations have to be taken into consideration while developing or selecting a lysis method. Detergents are used to solubilize the cell membranes. Popular choices are SDS, Triton X-100, and CTAB. CTAB can precipitate genomic DNA, and it is also popular because of its ability to remove polysaccharides from bacterial and plant preparations (Ausbel *et al.*, 1998).

Enzymes attacking cell surface components or components of the cytosol are often added to detergent-based lysis buffers. Lysozyme digests cell well components of gram-positive bacteria. Zymolase and murienase aid in protoplast production from yeast cell. Proteinase K cleaves glycoprotiens and inactivates RNase and DNase in 0.5 to 1% SDS solution. Heat is also applied to enhance lysis. Denaturants such as urea, guanidinium salts, and other chaotropes are applied to lyses cells and inactive enzymes, but extended use beyond what is recommended in a procedure can lead to a reduction in quality and yield. Sonication, grinding in liquid nitrogen, shredding devices such as rigid spheres or beads, and mechanical stress such as filtration have been used to lyse difficult samples prior to or in conjunction with lysis solutions.

The main objectives of this study were to quantify the amount of extracted DNA with quality and purity using high salt SDS based technique and to observe the effect of different types of lysis agents (SDS and SDS + proteinase-K) on extracted DNA yield.

MATERIALS AND METHODS

Collection of Microorganisms

Gram positive organisms (Bacillus cereus, Bacillus subtilis and Staphylococcus aureus) were collected from department of microbiology, Dhaka University. These organisms were used in this study because these are highly available in natural environment (Alexander 1997), easy to culture and is of interest of many microbial applications. After collection, the above strains were allowed to grow in nutrient broth for 24 hour at 25°C for Bacillus strains and 37°C for S. aureus in a shaker at 100 rpm. Cell suspension was centrifuged at 4500 rpm for 5 minute at 4°C and then used for DNA extraction.

Genomic DNA Extraction

For genomic DNA extraction high salt SDS based method (Zhou *et al.* 1996) was followed. Reagents used for genomic DNA extraction were TE buffer (Tris-EDTA), 10% (w/v) sodium dodesyl sulfate (SDS), 20 mg/ml proteinase K, 5M NaCl, CTAB/NaCl solution, 24:1 chloroform/ isoamyl alcohol, 25:24:1 phenol/chloroform/isoamyl alcohol, isopropanol and 70% ethanol.

DNA Extraction Procedure

The test tubes containing the cell pellet (approximately 500µg each test tube) were used for DNA extraction. The cell pellet was resuspended in 567µl TE buffer by repeated pipetting. 30µl of 10% SDS was added in all tubes. However to observe the effect of different types of lysis agents on the overall extraction procedure of Gram positive bacteria, lysis steps were carried out with 0, 3 and 6µl of proteinase-K respectively. For all the step, DNA extraction was carried out in triplicate. 100µl 5M NaCl was added and mixed thoroughly and 80µl CTAB/NaCl solutions were added to each tube. The mass was mixed and incubated for 10 min at 65°C. Equal volume chloroform/isoamyl alcohol was added, mixed and microcentrifuged (MIKRO 2, Hettich, Germany) at 13000 rpm for 4 min. The supernatant was then transferred to a fresh tube. 0.6µl of isopropanol was added and mixed gently & kept for 1 day at -20°C for DNA precipitation. DNA pellet was then obtained by centrifuging at 13000 rpm for 4 min, DNA pellet was then dried and resuspended in 100µl TE buffer.

Qualitative Analysis of Extracted DNA

For qualitative analysis of extracted DNA gel electrophoresis was carried out. The quality of

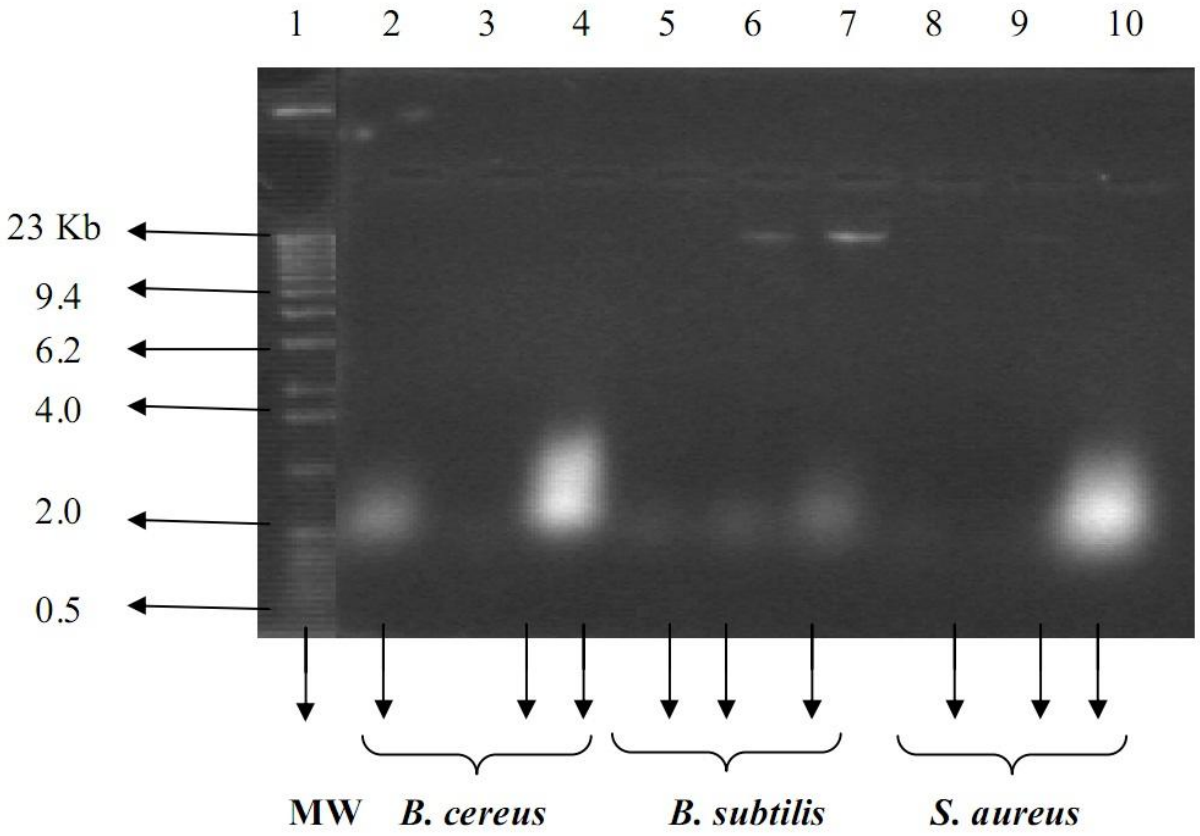

Figure 1. Agarose gel (1%) electrophoresis of total extracted genomic DNA from *B. cereus, B. subtilis,* and *S. aureus*. Lane 2, 5, and 8 represent genomic DNA bands in presence of SDS and Proteinase–K (3µl) for *B. cereus, B. subtilis,* and *S. aureus* respectively. Lane 3, 6, and 9 present genomic DNA bands in presence of SDS only for *B. cereus, B. subtilis,* and *S. aureus* respectively. Lane 4, 7 and 10 represent genomic DNA bands in presence of SDS and increased amount of Proteinase–K (6µl) for *B. cereus, B. subtilis,* and *S. aureus* respectively. Lane 1 represents the molecular weight marker.

the extracted DNA (absence of degradation) was estimated based on the size of the DNA fragments or the relative position of the DNA smears after electrophoresis of an aliquot of the DNA solution on 1% agarose gel. Photograph of a stained gel was taken directly on a UV transilluminator by gel documentation system (Kodak, Japan) (Fig. 1).

Quantitative Analysis of Extracted DNA

In order to know the DNA concentration, total extracted DNA was quantified using UV/VIS Spectroscopy (JASCO Ubest-55, Japan) at 260 nm and converted to concentration considering absorption of 1 OD (A) is equivalent to approximately 50µg/ml ds DNA (Sambrook *et al.*, 1989). Purity ratio (A_{260}:A_{280}) was also determined by determining the absorbance value at 280nm.

RESULT AND DISCUSSION

Figure 1 represents the quality of extracted bacterial genomic DNA from *B. cereus* (lane 2, 3 and 4); *B. subtilis* (lane 5, 6 and 7) and *S. aureus* (lane 8, 9 and 10) using three different lysis protocols. High molecular weight intact DNA bands were observed only for *Bacillus subtilis* when the extraction procedure was carried out in presence of SDS, SDS with proteinase-K (3µl) and SDS with increased amount of proteinase-K (6µl). Among these SDS with increased amount of proteinase-K showed strongest band (lane 7) and slight degraded DNA was also observed at the lower end of the gel.

However, for *Bacillus subtilis and Staphylococcus aureus* high molecular weight intact bands were not observed. Rather the DNA was found to be degraded and of low molecular weight. Both the

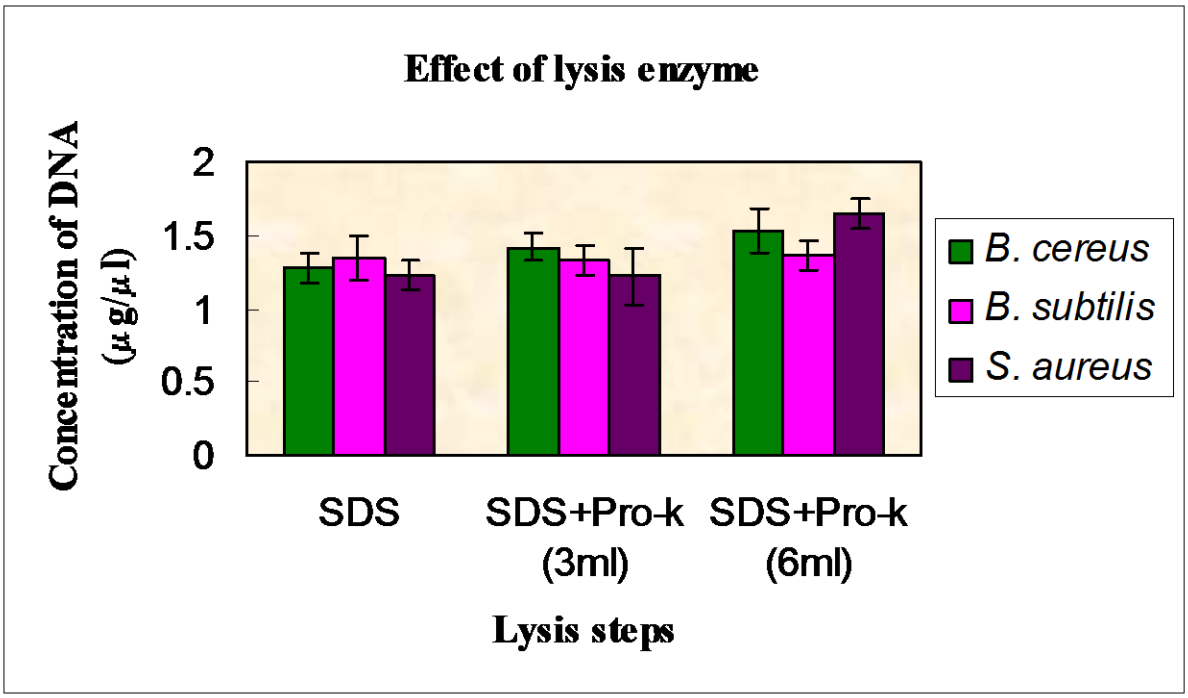

Figure 2. Effect of lysis enzyme on three different gram positive organism such as *B. cereus, B. subtilis and S. aureus*.

Bacillus subtilis and *Staphylococcus aureus* showed strongest & low molecular bands when the extraction process was carried out with an increased amount of proteinase-K in presence of SDS.

In case of both *Bacillus subtilis and Staphylococcus aureus* no band was observed in lane 5 and lane 8 suggesting that proteinase-K might have profound effect on the overall extraction pattern of Gram positive bacteria due to the presence of thick peptidoglycan layer.

Quantitative Estimation of Total Extracted Genomic DNA

For quantitative estimation of DNA, absorbance value at 260 nm was considered. The absorption of 1 OD (A) is equivalent to approximately 50µg/ml ds DNA, approximately 33µg/ml ss DNA, 40µg/ml RNA or approximately 30µg/ml for oligonucleotides (Zhou *et al.* 1996). According to this, in presence of SDS and increased amount of proteinase-K (6µl) the mean value of DNA concentration for *B. cereus, B. subtilis,* and *S. aureus* were found to be 1.53±0.15, 1.36±0.10, and 1.65±0.10 µg/µl respectively (Figure 2).

However, in absence of proteinase-K, the values of DNA concentration were found to be decreased (1.28±0.10, 1.34±0.15, 1.23±0.10µg/µl for *B. cereus, B. subtilis,* and *S. aureus* respectively) for all these strains (Figure 2).

Although in case of *B. subtilis* the overall effect of proteinase-K was not found to be significant in terms of DNA concentration (Table- 1) and DNA band intensity (Figure 1). However, for *B. cereus,* and *S. aureus* sharp decrease in total extracted DNA concentration was observed suggesting the increased lysis effect of proteinase-K on the thick peptidoglycan layer of Gram positive cell wall such as *B. cereus,* and *S. aureus*.

Now if we consider the values of DNA concentration in absence of proteinase-k (SDS only) as 100% then the mean values of DNA concentrations increased from 110% (3µl proteinase-K) to 119% (6µl proteinase-K) for *B. cereus*; 99% (3µl proteinase-K) to 101% (6µl proteinase-K) for *B. subtilis*; and 100% (3µl proteinase-K) to 135% (6µl proteinase-K) for *S. aureus*.

Determination of Purity Ratio

Purity determination of DNA interference by contaminants can be recognized by the calculation of "ratio". The ratio A_{260}/A_{280} is used to estimate the purity of nucleic acid since protein

Table 1. Concentration and purity ratio of extracted genomic DNA from three different Gram positive organisms using three different lysis protocols.

Name of the organism	Lysis step	Absorbance		Concentration (µg/µl)	Ratio (260/280)
		260 nm	280 nm		
B. cereus	SDS + proteinase-K (3µl)	0.284±0.02	0.184±0.05	1.42±0.10	1.54
	SDS	0.255±0.02	0.171±0.03	1.28±0.10	1.49
	SDS + proteinase-K (6µl)	0.306±0.03	0.194±0.03	1.53±0.15	1.58
B. subtilis	SDS + proteinase-K (3µl)	0.265±0.02	0.177±0.02	1.33±0.15	1.50
	SDS	0.267±0.03	0.170±0.03	1.34±0.10	1.57
	SDS + proteinase-K (6µl)	0.271±0.02	0.193±0.02	1.36±0.10	1.40
S. aureus	SDS + proteinase-K (3µl)	0.244±0.04	0.165±0.03	1.22±0.10	1.48
	SDS	0.246±0.02	0.166±0.02	1.23±0.20	1.48
	SDS + proteinase-K (6µl)	0.330±0.02	0.213±0.03	1.65±0.10	1.55

absorbed at 280 nm. Although pure DNA should have ratio of approximately 1.8, in our case we could not get such a high ratio for any sample (Table-1), suggesting possible contamination with protein and phenolic substances.

The cell wall structure of Gram positive organism is more critical than the Gram negative cell wall structure because of the peptidoglycan layer. For the present study three different Gram positive strains (*Bacillus cereus*, *Bacillus subtilis and Staphylococcus aureus*) were chosen which are available in the environment sample such as soil, sediment and activated slugged. The present study emphasized to know the effect of proteinase-K on extracted genomic DNA of these Gram positive strains and found that *Bacillus cereus and Staphylococcus aureus* has significant effect where as *Bacillus subtilis* did not show any major impact on total DNA concentration. Our previous study revealed almost similar result in case of different Gram negative strains (Kabir *et al*., 2006).

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