RESEARCH ARTICLE

# Lithium Acetate (LiOAc)-SDS Lysis DNA Extraction Method of Gram-positive Bacteria for PCR Templates

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## Abstract

**Objective**—DNA extraction assay using Lithium Acetate (LiOAc)-SDS lysis and lysozyme/SDS/proteinase K method for genomic DNA extraction of Gram-positive bacteria were compared.

**Materials and Methods**—Genomic DNA of eight Gram-positive bacteria species were extracted by LiOAc-SDS lysis method and lysozyme/SDS/proteinase K method. The DNA were evaluated for their purity and integrity using NanoDrop2000 and PCR reaction to amplify 16S ribosomal RNA (16S rRNA) gene and phenylalanyl tRNA synthetase (*phe*S) gene.

**Results**—Using LiOAc-SDS lysis, the DNA of seven bacterial species were amplified successfully except *Staphylococcus aureus*. All tested bacteria using template DNA from lysozyme/SDS/proteinase K method presented PCR products.

**Conclusion**—Therefore, LiOAc-SDS lysis method could be regarded as an alternative method for Gram-positive bacteria DNA extraction. The process of this extraction is safe, simple, rapid and cost effective for DNA preparation routinely.

*KKU Vet J. 2013;23(1):24-31.* http://vmj.kku.ac.th/

**Keywords**: LiOAc-SDS; DNA extraction; Gram-positive bacteria

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# การสกัดดีเอ็นเอจากแบคทีเรียแกรมบวกด้วยวิธี Lithium Acetate (LiOAc)-SDS lysis เพื่อใช้เป็นดีเอ็นเอต้นแบบในการเพิ่มปริมาณชิ้นดีเอ็นเอด้วยเทคนิค PCR

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## บทคัดย่อ

**วัตถุประสงค์** เพื่อศึกษาและประเมินคุณภาพของดีเอ็นเอแบคทีเรียแกรมบวกที่สกัดด้วย LiOAc และสารละลาย SDS เปรียบเทียบกับการสกัดด้วย lysozyme สารละลาย SDS และ proteinase K

**วัสดุ อุปกรณ์ และวิธีการ** สกัดดีเอ็นเอจากแบคทีเรียแกรมบวก 8 ชนิดด้วย LiOAc และสารละลาย SDS ประเมินความบริสุทธิ์ ความสมบูรณ์ของดีเอ็นเอต้นแบบโดยการเพิ่มปริมาณชิ้นดีเอ็นเอของยีน16S rRNA และยีน *phe*S ด้วยวิธี PCR เปรียบเทียบกับดีเอ็นเอต้นแบบที่สกัดด้วย lysozyme สารละลาย SDS และ proteinase K

**ผลการศึกษา** ดีเอ็นเอของแบคทีเรียแกรมบวก 7 ชนิดยกเว้นดีเอ็นเอของ *S. aureus* ที่สกัดด้วยLiOAc และสารละลาย SDS มีคุณภาพดีเมื่อวัดด้วยเครื่อง NanoDrop2000 สามารถเพิ่มปริมาณชิ้นดีเอ็นเอของยีน 16S ribosomal RNA และยีน *phe*S ได้เช่นเดียวกับดีเอ็นเอที่สกัดด้วย lysozyme สารละลาย SDS และ proteinase K

**ข้อสรุป** เทคนิคการใช้ LiOAc และสารละลาย SDS เป็นเทคนิคทางเลือกใหม่ในการสกัดดีเอ็นแบคทีเรียแกรมบวก เนื่องจากวิธีนี้ประหยัด ง่าย ปลอดภัย รวดเร็ว และได้ดีเอ็นเอที่มีคุณภาพและความบริสุทธิ์เหมาะสมแก่การใช้เป็นดีเอ็นเอต้นแบบสำหรับเพิ่มปริมาณชิ้นดีเอ็นเอด้วยเทคนิค PCR

*วารสารสัตวแพทยศาสตร์ มข. 2556;23(1):24-31.* http://vmj.kku.ac.th/

**คำสำคัญ**: LiOAc-SDS การสกัดดีเอ็นเอ แบคทีเรียแกรมบวก

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## Introduction

Molecular biological methods of bacteria are playing an important role in the field of clinical microbiology. DNA based methods involving DNA extraction and PCR are the techniques using for identification of bacteria routinely. A number of methods have been reported for isolation of bacteria DNA, for example Sodium Dodecyl Sulfate (SDS)/Cetyl Trimethyl Ammonium Bromide (CTAB)/ proteinase K [17, 20], SDS lysis [6], lysozyme/SDS [3], guanidium thiocyanate [14], lysosome/SDS/ proteinase K [10], beading-vortexing/SDS lysis [16], and mechanical lysis using high-speed cell disruption [11, 13]. Generally, after lysis of cells with detergent, enzyme and/or mechanical process, the genomic DNA are finally extracted with phenol-chloroform standard protocol that are harmful handling, time-consuming, and laborious manipulation. At least four to six changes of microcentrifuge tubes, incubation precipitation, elution or washing and drying steps must be introduced to complete the extraction.

To extract bacterial DNA, the rigid cell wall of Gram-positive bacteria make the process more complicated than to resolve in Gram-negative bacteria [18]. LiOAC is a chaotropic agent [4] which commonly used in yeast transformation protocols to weaken cell wall and plasmid DNA extraction [16]. Both yeast and Gram-positive have thicker cell walls than Gram-negative bacteria. The yeast cell wall comprises of about 85-90 % polysaccharide and 10-15 % protein, whereas the Gram-positive bacteria cell wall consists of a thick layer of peptidoglycan which attached with teichoic acid, teichuronic acid, polyphosphates, or carbohydrates [15, 18]. In 2011, Lõoke *et al*. [8] have reported the successful extraction of genomic DNA from yeast with LiOAc-SDS lysis method. The assay is rapid, safety and especially inexpensive comparing to commercial test. Therefore, this study was to apply the LiOAc-SDS lysis method for Gram-positive bacteria DNA extraction and the yield DNA were used as templates for PCR amplification.

## Meterials and Methods

### *Bacterial samples and culture conditions*

Eight species of Gram-positive bacteria including *Bacillus* spp., *Corynebacterium* spp., *Micrococcus* spp., *Rhodococcus* spp., *Streptococcus sui*s, *Staphylococcus aureus*, *Staphylococcus hyicus*, and *Staphylococcus epidermidis* were derived from Kamphaeng Sean Veterinary Diagnostic Center, the Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Sean Campus. A single bacterial colony of each species was inoculated in brain heart infusion broth (Difco) for overnight at 37 °C. The concentration of bacterial suspension was measured at 0.5 McFarland and the bacterial cells were harvested from 1 ml of the culture suspension at 12,000 x g for 1 min. The cell pellet was used for DNA extraction.

### *LiOAc-SDS lysis DNA extraction method*

Bacterial cell pellet was resuspended in 100 µl of 200 mM LiOAc 1% SDS solution, and incubated for 15 min at 70 °C. A volume of 300 µl of 96% ethanol was added for DNA precipitation.

Samples were mixed briefly and DNA was collected by centrifugation at 15,000 x g for 3 min. The supernatant was removed and washed with 500 µl of 70% ethanol. The DNA pellet was dried at 56 ºC and then resuspended in 100 µl TE. The cell debris was removed by centrifugation at 15000 x g for 5 min and the supernatant was used for PCR template.

***Lysozyme/SDS/proteinase K DNA extraction method***

Bacterial cell pellet was resuspended in 200 µl of TE and vortexed. An 8 µl of lysozyme at the concentration of 50 mg/ml was added and the solution was incubated at 37 °C for 30 min. A volume of 4 µl of proteinase K at the concentration of 20 mg/ml and 20 µl of 10% SDS were added in the tube, gently mixed, and incubated at 56 °C for 1 h. A volume of 70 µl of 5 M NaCl and 55 µl of 10% CTAB (10% CTAB/0.7M NaCl) were added and the mixture was incubated at 56 °C for 10 min. The samples were cooled down at room temperature. The DNA extraction with phenol/chloroform/isoamyl alcohol (25:25:1) according to standard procedure was performed twice. The DNA was precipitated by adding an equal volume of 96% ethanol at room temperature for 10 min followed by centrifugation at 12,000 x g for 5 min. The supernatant was discarded and the DNA pellet was washed twice with 75% ethanol. The DNA pellet was dried and dissolved in 100 µl of TE buffer.

***Determination of purity ratio of extracted DNA.***

Purity determination of extracted DNA could be evaluated by the calculation of the ratio of A260/A280 for absorbed DNA and protein at 260 nm and 280 nm, respectively. The ratio of A260/A280 in this study was detected by NanoDrop2000 (Thermo Scientific, USA).

***PCR amplification of extracted DNA***

PCR reaction was performed to determine the existence of inhibitor and interference during the DNA extraction process by amplification of 16S rRNA gene and *phe*S gene. The PCR amplification of 16S rRNA gene (1,500 bp) was performed with universal eubacteria primers designed by Giovannoni (1991) [5]. Eub B; 5´-AGAGTTTGATCMTGGCTCAG-3´was used as a forward primer and Eub A; 5´-AGAGTTTGATCMTGGCTCAG-3´ was used as a reverse primer (Science Pacific Co. Ltd.). The PCR amplification of *phe*S gene (723 bp) was performed with 5´- GATTAAGGAGTAGTGGCACG -3´ as a forward primer and 5-´ TTGAGATCGCCCATTGAAAT -3´ as a reverse primer (Science Pacific Co. Ltd.). These primers have been designed by Jones *et al*. (2003) [6]. Each reaction mixture contained of DNA template of DNA sample either from LiOAc-SDS lysis method or lysozyme/SDS/ Proteinase K method, 1.25 U Taq DNA polymerase enzyme (FERMENTAS®, Canada), 1X PCR buffer (FERMENTAS®, Canada), 3 mM $MgCl_2$ (FERMENTAS®, Canada), 0.2 mM of each dNTPs and 0.1 mM of each primers. The PCR reaction was carried out using DNA Engine PTC-200 thermal cycle (BIO-RAD, USA). The cycling conditions initialed denaturation at 94ºC for 4 min, followed by 40 cycles of denaturation at 94ºC for 30 s, primer annealing at 55ºC for 45 s and extension at 72ºC for 1 min. After amplification, PCR products were electrophoresed with the constant voltage of 90 voltages, 400 mA by 1.5% agarose gel, then stained with ethidium bromide and determined under UV light.

## Results

The results indicated the achievement of the genomic DNA extraction from seven out of eight bacterial species using LiOAc-SDS lysis method. The ratio of A260/280 nm of seven species excluded *S. aureus* were 1.95-2.0. This ratio revealed the preferable purity of extracted DNA (**Table 1**). The lower ratio (less than 1.8) of *S. aureus* was counted as unaccepted purity of extracted DNA.

**Table 1**. The Purity of Extracted DNA Using LiOAc-SDS Lysis Method and Lysozyme/SDS/ Proteinase K Method

| | Purity (A260/A280) : 1.8-2.0 | | | |
|---|---|---|---|---|
| | LiOAC-SDS lysis | | Lysozyme/SDS/proteinase K | |
| **Bacetria** | **N** | **Mean±SEM** | **N** | **Mean±SEM** |
| *Bacillus* spp. | 8 | 1.95±0.02 | 8 | 2.12±0.02 |
| *Corynebacterium* spp. | 8 | 2.01±0.01 | 8 | 2.11±0.02 |
| *Micrococcus* spp. | 8 | 2.00±0.02 | 8 | 2.08±0.01 |
| *Rhodococcus* spp. | 8 | 2.00±0.01 | 8 | 2.04±0.04 |
| *Staphylococcus aureus* | 8 | 1.7±0.02 | 8 | 2.12±0.01 |
| *Staphylococcus epidermidis* | 8 | 2.01±0.03 | 8 | 2.11±0.01 |
| *Staphylococcus hyicus* | 8 | 2.03±0.02 | 8 | 2.08±0.01 |
| *Streptococcus suis* | 8 | 2.04±0.01 | 8 | 2.15±0.01 |

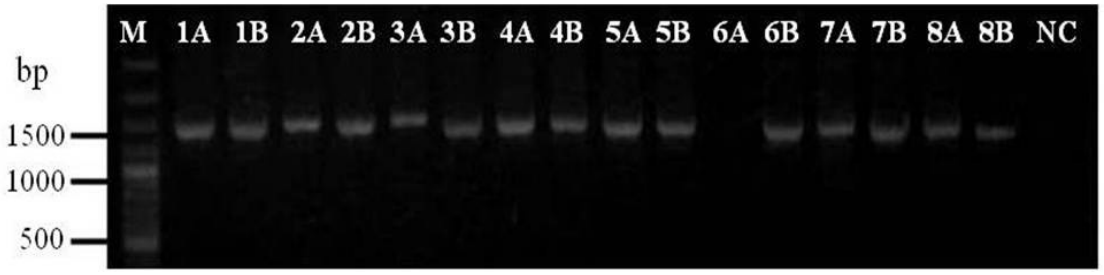
Abbreviation: N=number of replications

To confirm the quality of template DNA that were extracted in both methods, amplification of 16S rRNA gene and *phe*S gene which is a housekeeping gene encoded phenylalanyl tRNA synthetase, were performed. Lysozyme/SDS/proteinase K DNA extraction method gave the PCR products of 16S rRNA and *phe*S genes from all eight bacteria species whereas seven of eight bacterial species using LiOAc-SDS lysis method presented the PCR products (**Figure 1, 2**).

## Discussion

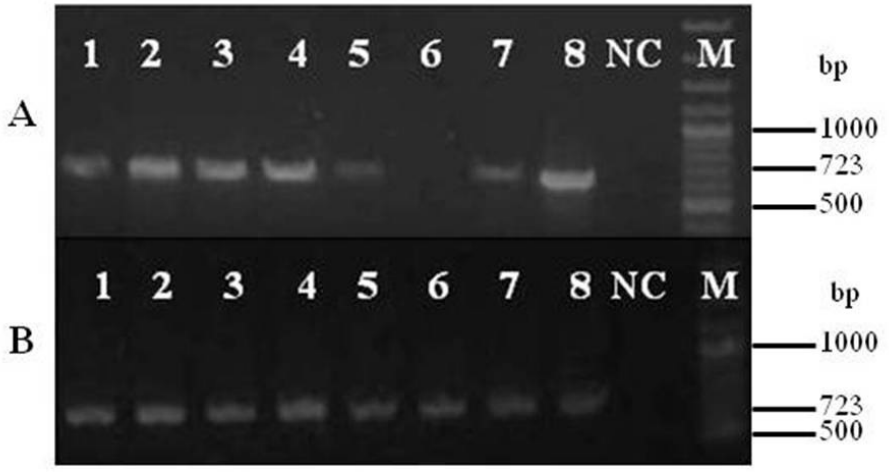
Gram-positive bacteria have the thick cell wall which composed of protein, peptidoglycan and secondary wall polymer such as teichoic acid [11, 15, 18]. The component of Gram-positive bacteria cell wall results to its hardness that leads to the difficulty of bacterial lysis and DNA extraction. The boiling method that is less cost and successful for lysis Gram negative DNA could not make the favorable yielded DNA in Gram positive [1, 9]. Enzymatic degradation method in DNA extraction poses the

**Figure 1**. PCR Products of 16S rRNA Gene (1500 bp) from Gram-positive Bacteria*




*PCR products of 16S rRNA gene (1,500 bp) from Gram-positive bacteria using DNA template extracted by LiOAc-SDS lysis method (A) and lysozyme/SDS/proteinase K method (B) were amplified from 8 bacterial species. Lane assignments: Lane 1, *Bacillus* spp.; Lane 2, *Corynebacterium* spp.; Lane 3, *Micrococcus* spp.; Lane 4, *Rhodococcus* spp.; Lane 5, *Streptococcus suis*; Lane 6, *Staphylococcus aureus*; Lane 7, *Staphylococcus epidermidis*; Lane 8, *Staphylococcus hyicus*; M, Marker and NC, Negative Control

**Figure 2**. PCR Products of *phe*S Gene (723 bp) from Gram-positive Bacteria*




*PCR products of *phe*S gene (723 bp) from Gram-positive bacteria using DNA templates extracted by LiOAc-SDS lysis method (A) and lysozyme/SDS/proteinase K method (B) were amplified from 8 bacterial species. Lane assignments: Lane 1, *Bacillus* spp.; Lane 2, *Corynebacterium* spp.; Lane 3, *Micrococcus* spp.; Lane 4, *Rhodococcus* spp.; Lane 5, *Streptococcus suis*; Lane 6, *Staphylococcus aureus*; Lane 7, *Staphylococcus epidermidis*; Lane 8, *Staphylococcus hyicus*; M, Marker and NC, Negative Control

expensive cost, complicated and time-consuming [10]. In general, lysozyme, proteinase K, mutanolysin and lysostaphin are used as the DNA extraction agents to lyse peptidoglycan of Gram-positive bacteria [1, 2, 10, 17, 19].

In this study, we found that the genomic DNA extracted from seven species of Gram-positive bacteria excluded *S. aureus* by LiOAc-SDS lysis method were good quality. PCR result gave the amplified products of 16S rRNA gene and *phe*S gene as well as the lysozyme/SDS/proteinase K extracted DNA [16, 17]. Likewise the previous report demonstrated the successful of genomic yeast DNA extraction by LiOAc-SDS lysis method [9]. However, *S. aureus* gave the ratio of A260/280 nm about 1.7 ±0.02. This ratio indicated the contamination of the extracted DNA in large amount and corresponded to the failure in PCR amplification derived from the insufficient quality of DNA template. The incomplete cell lysis of *S. aureus* might result from the more complicated of modified peptidoglycan than cell wall of other bacteria [1, 17, 20].

In summary, the LiOAc-SDS lysis method was successful in extraction of DNA of seven Gram-positive bacteria species tested. It showed the satisfied result by PCR product of designed amplicon from both 16S rRNA and *phe*S gene. The LiOAc-SDS lysis method would serve as an alternative DNA extraction technique that is rapid, cost effective, and environmental friendly.

## Acknowledgements

The authors would like to thank Associate professor Patamabhorn Amavisit for her helpful guidance. This research is supported by the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Education (AG-BIO/PERDO-CHE).

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