Journal of Multidisciplinary and Translational Research (JMTR)

journal homepage: https://journals.kln.ac.lk/jmtr/

Assessment of the cellulose-based dipstick method for bacterial and fungal DNA extraction

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Abstract

The development of rapid, low-cost DNA extraction methods is crucial for advancing molecular diagnostics and biological research. Conventional silica column-based kits, while efficient, are limited by cost, complexity, time, and equipment requirement, particularly in resource-limited settings. This study evaluates a cellulose-based dipstick method as a low-cost and fast alternative for DNA extraction form bacteria and fungi. Cellulose dipsticks, made of Whatman Grade 1 filter paper, were used in combination with optimized mechanical lysis protocols using glass beads in a sodium dodecyl sulfate (SDS) detergent-containing buffer solution. DNA yield, purity, and compatibility with quantitative PCR processes were carefully compared with commercially available silica columns for nine bacterial and five fungal species. The dipstick method yielded DNA concentrations of 0.94 ± 0.29 µg/mL for bacteria and 0.50 ± 0.15 µg/mL for fungi, with A260/A280 ratios of 1.73 ± 0.07 and 1.45 ± 0.24, respectively. Although the yield and purity were lower than those obtained with commercially available column-based extraction kit, the quantity and quality of DNA obtained using the dipstick method were sufficient for most PCR-based downstream applications. The dipstick method required just 30 ± 5 seconds to carry out the extractions compared to the 30 to 60 minutes required for commercially available DNA extraction kits. Agarose gel electrophoresis verified the integrity or the quality of the extracted DNA, with efficient amplification of target genes in all the species examined. The simplicity, rapidity, and field-friendly nature of the method readily circumvent significant issues with sample transport, stability of the samples during storage and transportation, and the cost of extraction. Nevertheless, the limitations of the dipstick DNA extraction method are its low DNA yield and the fragility of dipsticks during washing steps, which might limit suitability for high throughput applications. Despite these restrictions, the cellulose-based dipstick method offers a practical, scalable solution for DNA extraction in low-resource environments, with significant potential for field diagnostics and ecological studies.

Keywords: Cellulose, DNA Extraction, Fungal ITS, 16S rRNA, Whatman filter paper

Article info

Article history:
Received 5th April 2025
Received in revised form 25th May 2025
Accepted 19th May 2025
Available online 30th June 2025

ISSN (E-Copy): ISSN 3051-5262
ISSN (Hard copy): ISSN 3051-5602
Doi: https://doi.org/10.4038/jmtr.v10i1.80
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Introduction

Nucleic acid extraction is a core process in molecular biology, enabling the isolation and purification of DNA and RNA from biological samples (Singh and Kashyap, 2012). The significance of nucleic acid extraction goes beyond the laboratory, being applied in medical diagnostics, environmental science, food technology, forensic studies, and biotechnology applications. In medicine, the diagnosis of pathogens relies on nucleic acid extraction as a method for identifying antibiotic sensitivity and viral titers, which aid in the detection of disease, monitoring for disease development, and the planning of treatment regimens (Keyaerts *et al.*, 2005). For environmental science, nucleic acid isolation improves the effectiveness of metagenomic analysis that furthers species identification and analysis of stress-response. Furthermore, it supports bioremediation studies by evaluating microbial roles in pollutant degradation (Bag *et al.*, 2016). Nucleic acid isolation is also the foundation of key research undertakings in microbial ecology, phylogenetics, and genetic engineering (Giraldo *et al.*, 2019). This enables the assessment of interspecies interactions, evolutionary relationships, and the planning of novel biotechnological applications. The effectiveness and reproducibility of the extraction procedures greatly influence the efficiency of downstream applications, including PCR, sequencing, and gene expression analysis.

Nucleic acid extraction has been a part of molecular biology ever since Friedrich Miescher's isolation of "nuclein" in 1869 (Lamm, et al., 2020). In the last century and a half, extraction procedures have developed from crude chemical treatments to very advanced procedures, each overcoming the issues of the prior methodologies (Dairawan and Shetty, 2020). Conventional methods for nucleic acid extraction, such as the phenol-chloroform extraction, while efficient, are frequently confronted with substantial obstacles that hinder their use in various environments. These methods tend to entail complicated protocols, sophisticated equipment and skilled operators, and frequently involve hazardous chemicals, rendering them inappropriate for environments with minimal facilities or for field use. The employment of toxic chemicals constitutes severe health risks for operators and demands specialized handling and disposal, further complicating the extraction process (Dairawan and Shetty, 2020). Additionally, the fact that these methods are time-consuming, taking between 30–60 minutes for each sample, and are prone to human error, can result in variable recoveries and possible cross-contamination. The numerous liquid handling steps that come with traditional approaches heighten sample loss and the risk of contamination, especially if dealing with a high number of samples. Furthermore, the relatively high cost per sample ($5–20) due to the use of specialized reagents and equipment renders these methods less feasible for high-throughput applications or in a resource-poor setting (Mason and Botella, 2020). The development of cellulose-based extraction techniques, including the cellulose-based dipstick approach, presents an exciting prospect for the resolution of the above difficulties (Dairawan and Shetty, 2020). The simplicity and speed of cellulose-based extraction techniques enhance accessibility for researchers and educators alike, facilitating molecular diagnostics in diverse environments, including classrooms and remote field sites. Moreover, the low cost of cellulose materials further democratizes access to nucleic acid extraction technologies, enabling broader participations in scientific research and education (Mason and Botella, 2020).

This study focuses on DNA extraction from bacteria and fungi due to several reasons. The broader utility of DNA in microbial studies, particularly for species identification via conserved regions

like 16S rRNA genes justifies this choice. The higher stability and longer lifespan of DNA makes it suitable for field use and long-term storage, which are prerequisites for the development of portable extraction protocols (Matange et al., 2021). The physicochemical characteristics of DNA, particularly the negatively charged phosphate backbone of DNA, are highly compatible with cellulose-based extraction methods, which could increase the efficacy of the dipstick approach (Tan and Yiap, 2009).

The main aim of the current study was to determine the applicability of cellulose dipsticks as a rapid and cost-effective method for DNA extraction from bacterial and fungal samples. For this purpose, we compared both the yield and quality of DNA extracted by cellulose dipsticks from a variety of bacterial and fungal species using a commercial extraction kit as a comparison standard. We also compared the utility of DNA extracted by cellulose dipsticks in real-time quantitative PCR (qPCR) by contrasting amplification cycle threshold (Cq) values for bacterial 16S rRNA and fungal ITS regions with those resulting from a commercial extraction. Furthermore, the purpose of this research is to identify the most efficient cell lysis method for bacteria and fungi that can be effectively applied in the field without any equipment, thereby optimizing the DNA yield and quality. The present study provides a more convenient and efficient method for extraction of DNA to support research in many areas of molecular biology, especially in resource limited settings.

Methodology

Materials

All chemicals and reagents used were molecular biology grade (Sigma-Aldrich). Enzymes and kits included lysozyme solution (Thermo Scientific), lyticase (AG Scientific), proteinase K, DNeasy blood and tissue DNA extraction kit, and miScript SYBR Green PCR kit (all Qiagen). Luria-Bertani broth and Sabouraud agar (Thermo Scientific and Sigma-Aldrich, respectively) were used for microbial growth. Equipment included a Disruptor Genie cell disruptor (Scientific Industries), StepOne Real-Time PCR System (Applied Biosystems), and UV spectrophotometer (Thermo Scientific). Consumables included Whatman filter paper #1, Borosilicate Disruptor Beads (0.1 mm and 0.5 mm diameter), Stainless Steel beads (5/32, 6/32, and 7/32 inches), and generic laboratory plasticware. Liquid nitrogen was used for sample processing.

Bacterial and fungal species and cultivation from stock cultures

The research employed a wide variety of microbial species to evaluate the performance of the cellulose-based dipstick assay. The Gram-positive bacteria included *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (DSM 20044/ATCC 1228), *Lactobacillus plantarum* (ATCC 14917), *Lactobacillus acidophilus* (ATCC 4356), and *Bacillus subtilis* subsp*. spizizenii* (ATCC 6633). The Gram-negative bacteria included *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter cloacae* (ATCC 13047), and *Salmonella typhi* (ATCC 14028). The fungal isolates were *Aspergillus niger* (ATCC 16888) and *Candida albicans* (ATCC 10231) and *Mucor, Rhizopus,* and *Fusarium* isolates. The microbial strains were obtained from the Department of Microbiology, Faculty of Science, University of Kelaniya, Sri Lanka.

Microbial cultures were revived from -80°C glycerol stocks. In the case of bacterial species, approximately 100 µL of glycerol stock that had been thawed was inoculated into 5 mL of sterile LB broth and incubated at 37 °C for 18–24 hours. For fungal species, glycerol stocks were streaked onto Sabouraud agar plates and incubated at 25 °C for 3–5 days. All manipulations were conducted under sterile conditions in a biosafety cabinet to prevent contamination.

Producing the dipsticks and preparation of reagents

Cellulose dipsticks were prepared based on Mason and Botella's protocol (2020) with specific adjustments. Whatman filter paper was cut to the required size using DEPC-treated scissors, with sterile aluminum foil covering the binding zone. Paraplast Plus wax was melted and applied to impregnate the handling zone and provide an 8 mm^2 binding area. Dipsticks were stored at room temperature in airtight containers and remained stable for at least one year. Two buffers were prepared: extraction buffer (20 mM Tris-HCl, 25 mM NaCl, 5 mM EDTA, 0.1% SDS, pH 8.0) and wash buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Tris-HCl, NaCl, EDTA, and SDS stock solutions were combined and diluted to working concentrations. Buffers were stored at room temperature and remained stable for at least one year. Compared to the original protocol, three main adjustments were made: (i) 2% PVP-40 was omitted from the extraction buffer to reduce complexity and potential downstream inhibition; (ii) 1 mM EDTA was added to the wash buffer for nuclease protection; and (iii) nucleic acids were eluted into 35 µL nuclease-free water (five dips with gentle pressing) to enhance recovery and enable sample storage prior to analysis.

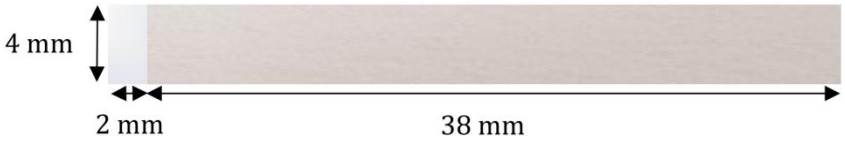

Figure 1. Schematic representation of a cellulose dipstick for nucleic acid extraction. The dipstick consists of two main zones: a nucleic acid binding zone (4 mm x 2 mm) at the left, and a wax-impregnated handling zone right. Double-headed arrows with labels indicate the dimensions of each zone. The total length of the dipstick is 40 mm, and its width is 4 mm throughout.

Comparison of cell lysis methods for diverse biological samples

Several cell lysis protocols were compared in terms of their efficiency in DNA extraction from bacterial (*E. coli*) and fungal (*A. niger*) samples. *E. coli* cultures with an optical density of approximately 0.8 at 600 nm (OD600) were used, while 20 mg *A. niger* biomass was recovered from agar surfaces. Physical disruption strategies included liquid nitrogen maceration, bead beating (using borosilicate glass and stainless-steel beads), and blunt-end micropipette tip crushing. Enzymatic treatments included Proteinase K and Lysozyme for *E. coli*, and Proteinase K and Lyticase for *A. niger*. Maceration with liquid nitrogen involved freezing samples for 1–2 minutes followed by slow grinding. Bead beating was performed with a Disruptor Genie for two 30-second bursts or by manual shaking. Stainless steel ball beating involved the use of three sizes of balls (5/32, 6/32, and 7/32 inches) with manual shaking. Enzyme treatments were: Bacteria: Lysozyme (2 mg/mL, 37°C, 30 minutes); Fungi: Lyticase (10 U/mL, 37°C, 60 minutes); Both: Proteinase K (2 mg/mL, 56°C, 30 minutes). All the samples were then subjected to the dipstick DNA extraction procedure.

Comparative analysis of DNA extraction methods

Two DNA extraction methods were used: a cellulose dipstick method and a commercial kit based on silica columns. In the dipstick method, bacterial cultures or fungal biomass served as the samples. Bacterial samples consisted of cultures with an OD600 of approximately 0.5–0.6, from which 200 µL were subjected to the extraction process together with 500 µL of buffer and 0.1 mm glass beads. For fungal samples, 20 mg biomass was gently scraped off from the surface of the agar with a sterile scalpel and resuspended in 200 µL of 1X PBS and supplemented with 500 µL of extraction buffer and 0.5 mm glass beads. The tubes were shaken manually for 10 seconds to lyse cells. Nucleic acids were captured by immersing the cellulose dipstick in the lysate five times, washing in 800 µL wash buffer, and eluting into 35 µL nuclease-free water. For silica column method, a commercial kit was used following the manufacturer's recommendations. All microbial species analyzed were extracted in triplicate. The experimental design employed a paired approach, with matching measurements from both extraction methods obtained for each sample and its replicates to allow direct comparison while controlling for sample-to-sample variability. The quality and quantity of DNA extracted was determined spectrophotometrically at 260 nm and 280 nm.

qPCR amplification of DNA extracted using dipsticks

The efficiency of cellulose-based dipsticks for DNA recovery was evaluated by qPCR amplification of target genetic markers. Bacterial DNA was amplified using primers targeting the 16S rRNA gene: 16s-F (5'-AGAGTTTGATCCTGGGTCAG-3') and 16s-R (5'-TACGGCTACCTTGTTACGACT-3'), whereas fungal DNA was amplified using primers targeting the ITS region: Fungal-ITS 1-F (5'-TCCGTAGGTGAACCTGCGG-3') and Fungal-ITS 4-R (5'-TCCTCCGCTTATTGATATGC-3'). The PCR protocol for bacterial samples involved an initial denaturation at 95°C for 15 minutes, followed by 40 cycles of 94°C for 15 seconds, 59°C for 30 seconds, and 72°C for 1 minute 30 seconds, with a final extension at 72°C for 10 minutes. For fungal samples, the following conditions were used: 35 cycles of denaturation at 94°C for 1 minute, annealing at 59°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 1 minute. The qPCR was conducted using the miScript SYBR Green PCR kit at an annealing temperature of 59°C. Each 20 µL reaction mixture contained 10 µL of miScript SYBR Green PCR Mix, 2 µL of each forward and reverse primer (10 µM), 2 µL of template DNA, and RNase-free water. All reactions were performed in triplicate with a StepOne Real-Time PCR System. Amplification efficiency was determined to be more than 80% by the standard curve analysis. Specificity of target amplification was determined by no-template controls (NTC) and melting curve analysis. PCR products were separated on 1.5% agarose gels stained with ethidium bromide and visualized under UV light. The effectiveness of the dipstick method was validated by comparing PCR and qPCR results to those of a commercial extraction kit.

Statistical analysis

Results obtained from the cellulose-based dipstick method and the commercial DNA extraction kit were tested for normal distribution by the Shapiro-Wilk test. As the data were not normally distributed, the Kruskal-Wallis test followed by Dunn's post-hoc analysis was performed to compare differences in DNA concentration and quality (A260/A280 ratio) between the two methods. The Wilcoxon signed-rank test was performed to compare differences in DNA yield and

PCR amplification efficiency (Cq values) between methods for each species. Statistical significance was determined at $p < 0.05$. Results are presented as median ± median absolute deviation (MAD) or median (IQR). Analyses were performed using IBM SPSS statistics (version 27) and R Studio (R version 4.4.2) with the ggplot2 package for data visualization.

Results

Comparative analysis of cell lysis methods for *E. coli*

The Kruskal-Wallis test revealed significant differences between the lysis methods for both A260/A280 ratios and DNA concentrations ($p < 0.05$). Dunn's test with Bonferroni correction revealed specific differences between the lysis methods. For A260/A280 ratios, significant differences were observed between maceration in liquid nitrogen and steel balls 6/32 inches ($p = 0.04$) and between bead beating by hand with 0.1 mm beads and both steel balls 6/32 inches ($p = 0.02$) and blunt end micropipette tip crushing ($p = 0.03$). For DNA concentration, liquid nitrogen maceration exhibited significant differences with steel balls 7/32 inches ($p = 0.01$) and blunt end micropipette tip crushing ($p = 0.03$).

Among the tested lysis methods, liquid nitrogen maceration yielded the highest DNA concentration (4.10 ± 0.10 µg/mL) with a favourable A260/A280 ratio (1.72 ± 0.02), indicating efficient cell lysis and acceptable DNA purity, although its use is limited in field settings. Manual bead beating with 0.1 mm glass beads produced relatively pure DNA (A260/A280 = 1.76 ± 0.04), while steel balls (6/32 inch) generated high DNA yield but lower purity (A260/A280 = 1.25 ± 0.02), suggesting a potential protein contamination. Enzymatic lysis with lysozyme and proteinase K resulted in high DNA concentrations with moderate purity. The crushing method using a blunt end micropipette tip was least effective. Detailed results for all methods are summarized in Table 1.

Based on these findings, and considering the compromise between DNA quality, quantity, and ease of use, manual bead beating using 0.1 mm borosilicate glass beads was selected as the optimum method for lysis of bacterial cells. This was subsequently paired with the cellulose-based dipstick DNA extraction procedure for a variety of bacterial species.

Table 1: Comparison of cell lysis methods for DNA extraction from *E. coli .*

Method	A260/A280 (Median ± MAD)	Concentration µg/mL (Median ± MAD)
Liquid nitrogen maceration	1.72 ± 0.02^{a}	4.10 ± 0.10^{a}
Bead beating by Disrupter (0.5)	1.42 ± 0.02^{ab}	1.55 ± 0.04^{ab}
Bead beating manually (0.5)	1.58 ± 0.03^{ab}	1.35 ± 0.04^{ab}
Bead beating by Disrupter (0.1)	1.58 ± 0.03^{ab}	2.15 ± 0.26^{ab}
Bead beating manually (0.1)	1.76 ± 0.04^{a}	1.86 ± 0.22^{ab}
Steel Balls (5/32 inches)	1.42 ± 0.02^{ab}	1.99 ± 0.22^{ab}
Steel Balls (6/32 inches)	1.25 ± 0.02^{b}	3.50 ± 0.31^{ab}

Steel Balls (7/32 inches)	1.33 ± 0.02^{ab}	0.86 ± 0.09^{b}
Lysozyme treatment	1.62 ± 0.02^{ab}	3.60 ± 0.10^{ab}
Proteinase K treatment	1.38 ± 0.02^{ab}	3.10 ± 0.10^{ab}
Crushing by blunt end micropipette tip	1.27 ± 0.02^{b}	0.95 ± 0.04^{b}

Multiple comparisons were performed using Dunn's test with Bonferroni adjustment for p-values. Different superscript letters (a, and b) within each column indicate statistically significant differences among methods ($p < 0.05$).

Comparative analysis of cell lysis methods for *A. niger*

The efficiency of various cell lysis methods in extracting DNA from *A. niger* was compared based on both qualitative and quantitative parameters. The Kruskal-Wallis test revealed that there were significant differences among the methods for both DNA concentrations and A260/A280 ratios ($p < 0.05$).

To locate specific differences between the methods, Dunn's test with Bonferroni correction was applied for pair-wise comparisons. For A260/A280 ratios, significant differences were detected between liquid nitrogen maceration and steel balls 7/32 inches ($p = 0.01$), and between bead beating by disruptor with 0.5 mm beads and steel balls 7/32 inches ($p = 0.04$). In DNA concentrations, liquid nitrogen maceration differed considerably from steel balls 7/32 inches ($p = 0.02$) and crushing with a blunt end micropipette tip ($p = 0.02$).

Liquid nitrogen maceration and bead beating with 0.5 mm glass beads provided the highest DNA yields and purity among the tested methods. Liquid nitrogen maceration resulted in the best overall performance (A260/A280 = 1.84 ± 0.03; 1.93 ± 0.11 µg/mL), while both the manual and disruptor bead beating with 0.5 mm beads gave comparable results.

In contrast, smaller beads (0.1 mm) produced moderate purity but lower DNA concentrations, suggesting insufficient cell disruption. Steel balls showed decreasing efficiency with increasing size, and enzymatic lysis (lyticase, Proteinase K) was moderately effective. The crushing method using a blunt-end micropipette tip was the least efficient. Full results are presented in Table 2.

Based on these results, manual bead beating with 0.5 mm glass beads was chosen for further experiments with fungal samples due to its efficiency and ease of use. The protocol was then merged with dipstick DNA extraction protocols to extract DNA from various fungal species.

Table 2: Comparison of cell lysis methods for DNA extraction from *A. niger.*

Method	**A260/A280 (Median ± MAD)**	**Concentration µg/mL (Median ± MAD)**
Liquid nitrogen maceration	1.84 ± 0.03^{a}	1.93 ± 0.11^{a}
Bead beating by Disrupter (0.5mm)	1.79 ± 0.03^{a}	1.69 ± 0.07^{ab}

Bead beating manually (0.5mm)	1.74 ± 0.03^{ab}	1.78 ± 0.01^{ab}
Bead beating by Disrupter (0.1)	1.71 ± 0.01^{ab}	0.80 ± 0.02^{ab}
Bead beating manually (0.1)	1.67 ± 0.01^{ab}	0.82 ± 0.00^{ab}
Steel Balls (5/32 inches)	1.76 ± 0.01^{ab}	1.15 ± 0.05^{ab}
Steel Balls (6/32 inches)	1.48 ± 0.01^{ab}	0.68 ± 0.03^{ab}
Steel Balls (7/32 inches)	1.26 ± 0.01^{b}	0.59 ± 0.02^{b}
Lyticase treatment	1.59 ± 0.03^{ab}	1.41 ± 0.03^{ab}
Proteinase K treatment	1.64 ± 0.03^{ab}	1.29 ± 0.08^{ab}
Crushing by blunt end micropipette tip	1.49 ± 0.03^{ab}	0.59 ± 0.05^{b}

Multiple comparisons were performed using Dunn's test with Bonferroni adjustment for p-values. Different superscript letters (a, and b)within each column indicate statistically significant differences among methods ($p < 0.05$).

Qualitative and quantitative DNA analysis across bacterial species

We compared the effectiveness of the cellulose dipstick method for bacterial DNA extraction with 0.1 mm glass beads for cell disruption versus a commercial DNA kit for nine species of bacteria (Table 3). The purity and yield of the DNA were determined by A260/A280 ratios and concentration, respectively. The median optical density (OD600) of the bacterial cultures was 0.55 ± 0.16, indicating a consistent mid-log phase growth across the diverse species tested.

The dipstick method overall produced lower A260/A280 ratios compared to the commercial kit, varying from 1.37 (*L. acidophilus*) to 1.84 (*S. aureus*). DNA concentrations obtained using the dipstick method were also lower than those obtained using the commercial kit, with the exceptions of *L. acidophilus* and *S. epidermidis*. There were notable differences between *B. subtilis* (1.58 vs 6.75 µg/mL) and *E. coli* (1.69 vs 8.91 µg/mL). DNA yields from commercial kits can vary widely depending on the kit and the bacterial strain. For instance, the DNeasy Blood & Tissue Kit and QIAamp DNA Mini Kit are reported to yield approximately 10 µg of DNA per $2x10^9$ bacterial cells (QIAGEN). The DNA yield obtained from our bacterial cultures using the commercial kit is consistent with expectations for high-density bacterial cultures.

Despite the seemingly obvious differences, the Wilcoxon signed-rank tests did not reveal any statistically significant differences ($p < 0.05$) between the two methods for A260/A280 ratios or DNA concentrations for any of the test species. This indicates that the cellulose-based dipstick method, combined with 0.1 mm glass bead lysis, has the potential to be a quick and inexpensive substitute for commercial extraction kits for extracting bacterial DNA.

Table 3: Quality and quantity of the extracted bacterial DNA

Species	**A260/A280**		**Concentration (µg/mL)**	
	Dipstick	**Commercial Kit**	**Dipstick**	**Commercial Kit**
Gram-positive bacteria				

B. subtilis	1.46 (1.41 - 1.51)	1.93 (1.88 - 1.98)	1.58 (1.48 - 1.63)	6.75 (6.73 - 6.95)
L. acidophilus	1.37 (1.32 - 1.42)	1.75 (1.71 - 1.80)	1.53 (1.48 - 1.63)	0.77 (0.72 - 0.82)
L. plantarum	1.68 (1.63 - 1.70)	1.98 (1.89 - 1.99)	0.58 (0.55 - 0.65)	2.11 (2.11 - 2.31)
S. aureus	1.84 (1.82 - 1.89)	1.95 (1.85 - 1.95)	0.60 (0.60 - 0.70)	1.33 (1.23 - 1.38)
S. epidermidis	1.74 (1.72 - 1.79)	1.87 (1.79 - 1.89)	0.35 (0.34 - 0.44)	0.34 (0.28 - 0.39)
Gram-negative bacteria				
E. cloacae	1.80 (1.62 - 1.99)	1.86 (1.82 - 1.92)	1.20 (1.10 - 1.26)	4.00 (3.89 - 4.10)
E. coli	1.76 (1.69 - 1.81)	1.81 (1.78 - 1.88)	1.69 (1.62 - 1.84)	8.91 (8.65 - 9.01)
P. aeruginosa	1.74 (1.69 - 1.82)	1.93 (1.84 - 1.94)	0.93 (0.89 - 0.99)	3.12 (3.04 - 3.22)
S. typhi	1.57 (1.56 - 1.66)	1.94 (1.85 - 1.95)	1.21 (1.19 - 1.29)	2.55 (2.51 - 2.65)

Values are presented as median (Interquartile Range). Wilcoxon signed-rank tests were performed to compare dipstick and commercial kit methods for each species. No statistically significant differences ($p < 0.05$) were found between the two methods for either A260/A280 ratios or DNA concentrations across all species tested ($n = 27$).

Qualitative and quantitative DNA analysis across fungal species

The performance of the dipstick technique using cellulose as the extractive agent and disruption by 0.5 mm glass beads for five fungal species was compared to that of a commercial kit for DNA extraction (Table 4). The dipstick method consistently provided lower A260/A280 ratios than the commercial kit, and median values ranged from 1.16 (*A. niger*) to 1.69 (*C. albicans* and *Mucor*). These lower ratios suggest the presence of contaminants, such as proteins or phenol, which can be absorbed at 280 nm to decrease the A260/A280 ratio. Despite the lower purity ratios, the dipstick method consistently produced higher DNA concentrations across all species examined. For example, *A. niger* gave a median concentration of 0.78 µg/mL using the dipstick method and 0.14 µg/mL using the commercial kit.

This discrepancy can be attributed to several factors. The dipstick method, which uses cellulose to capture DNA, may retain more nucleic acids, including potential contaminants, leading to higher overall DNA concentrations but lower purity ratios (Zou et al., 2017). Additionally, the dipstick method's efficiency in capturing DNA from fungal biomass might be enhanced due to its simplicity and rapidity, allowing for less loss of DNA during the extraction process compared to more complex commercial protocols.

Although some apparent differences in both A260/A280 ratios and DNA concentrations were observed, Wilcoxon signed-rank tests did not reveal any statistically significant differences ($p < 0.05$) between the two methods for all the species examined. This would indicate that cellulose dipstick methodology with 0.5 mm glass bead lysis has the potential to be a fast and low-budget substitute for the use of commercial extraction kits in fungal DNA extraction.

Table 4: Quality and quantity of extracted fungal DNA

Species	A260/A280		Concentration (µg/mL)	
	Dipstick	Commercial Kit	Dipstick	Commercial Kit
A. niger	1.16 (1.12 - 1.21)	1.69 (1.55 - 1.74)	0.78 (0.72 - 0.82)	0.135 (0.088 - 0.185)
C. albicans	1.69 (1.40 - 1.74)	2.00 (1.96 - 2.20)	0.31 (0.26 - 0.34)	0.18 (0.15 - 0.20)
Fusarium	1.43 (1.36 - 1.48)	1.23 (0.75 - 1.25)	0.49 (0.45 - 0.52)	0.04 (0.035 - 0.043)
Mucor	1.69 (1.51 - 1.74)	1.98 (1.88 - 3.66)	0.65 (0.42 - 0.67)	0.11 (0.095 - 0.15)
Rhizopus	1.45 (1.34 - 1.50)	2.06 (2.01 - 2.35)	0.50 (0.40 - 0.53)	0.25 (0.22 - 0.27)

Values are presented as median (Interquartile Range). Wilcoxon signed-rank tests were performed to compare dipstick and commercial kit methods for each species. No statistically significant differences ($p < 0.05$) were found between the two methods for either A260/A280 ratios or DNA concentrations across all species tested ($n = 15$).

Amplification of extracted DNA using quantitative PCR

The quality of DNA extracted using the cellulose-based dipstick method for downstream application was evaluated against a commercial kit using qPCR for the nine bacteria and five fungi species (Table 5). The cycle quantification (Cq) values were used to check the efficiency of amplification and extraction. Smaller Cq values indicated higher amounts of target DNA void of PCR inhibitors. For the bacterial species, the commercial kit generally yielded lower Cq values, which meant more DNA yield from most of the tested species. For *P. aeruginosa*, however, the dipstick performed better, with a median Cq value of 25.8 compared to that of the commercial kit with a value of 36.2 ($n = 3$; paired samples). For the fungal species, the reverse was the case. The commercial kit performed better with *C. albicans* and *Fusarium.* The DNA extracted from the dipstick for *A. niger* amplified at earlier cycles.

Despite such differences observed, Wilcoxon signed-rank tests showed that there were no statistically significant differences ($p > 0.05$) between the two methods regarding Cq values for all the tested species. This shows that cellulose-based dipstick method attains comparable qPCR performance to commercial kit for bacterial and fungal DNA extraction.

Table 5: Cq Values for amplification of bacterial 16S rRNA region and fungal ITS region by qPCR

Species	Dipstick method	Commercial kit
Bacteria Species		
B. subtilis	17.9 (16.8 - 18.6)	17.4 (16.3 - 18.1)
E. coli	25.2 (23.9 - 26.0)	21.7 (20.5 - 22.5)
Enterobacter	38.2 (36.9 - 39.2)	38.0 (37.2 - 38.9)
L. acidophilus	27.7 (26.4 - 28.6)	20.3 (19.0 - 21.1)
L. plantarum	28.3 (27.0 - 29.2)	23.7 (22.2 - 24.6)

Pseudomonas	25.8 (24.5 - 26.6)	36.2 (35.0 - 37.0)
S. aureus	18.8 (17.6 - 19.6)	15.9 (15.1 - 16.5)
S. epidermidis	21.4 (20.2 - 22.2)	15.7 (14.9 - 16.3)
S. typhi	34.6 (33.4 - 35.4)	30.3 (29.2 - 31.0)
Fungal species		
A. niger	25.9 (23.1 - 27.4)	29.9 (24.0 - 31.4)
C. albicans	29.3 (26.2 - 30.7)	24.5 (21.6 - 25.9)
Fusarium	27.3 (24.2 - 28.7)	23.9 (21.2 - 25.2)
Mucor	29.4 (26.6 - 30.8)	29.2 (26.4 - 30.5)
Rhizopus	30.9 (27.3 - 32.5)	28.3 (25.3 - 29.7)

Values are presented as Median (Interquartile Range). Wilcoxon signed-rank tests were performed to compare Dipstick and Commercial Kit methods for each species. No statistically significant differences ($p < 0.05$) were found between the two methods for Cq values across all species tested. All PCR assays were performed in triplicate ($n = 42$).

Comparison of the amplification of DNA extracted using the dipstick and commercial column-based method by gel electrophoresis

Gel electrophoresis results (Figure 2) show successful amplification of the target regions of DNA (bacterial 16S rRNA region ~1500 bp and fungal ITS region 500–600 bp) extracted by both the cellulose dipstick method and the commercial kit.

For gram-positive bacteria, including *B. subtilis, S. aureus*, and *S. epidermidis*, both methods provided high-intensity bands. However, *L. plantarum* and *L. acidophilus* provided lower band intensities with the dipstick method compared to the commercial kit, and this could be due to variations in extraction efficiency for these species. This result is in agreement with the qPCR data presented in Table 5, where there were significant variations in Cq values for these microorganisms. Overall, Gram-negative bacteria had lower band intensities compared to gram-positive species. *P. aeruginosa* produced a higher intensity band with the dipstick method compared to the commercial kit, indicating potential species-related differences in extraction efficiency.

For the fungal organisms, both methods provided comparable band intensities for all the test organisms, and this suggests that the dipstick method is a suitable substitute for fungal DNA extraction. This is also supported by the statistical results in Table 5, where there are no significant differences in Cq values between the two methods.

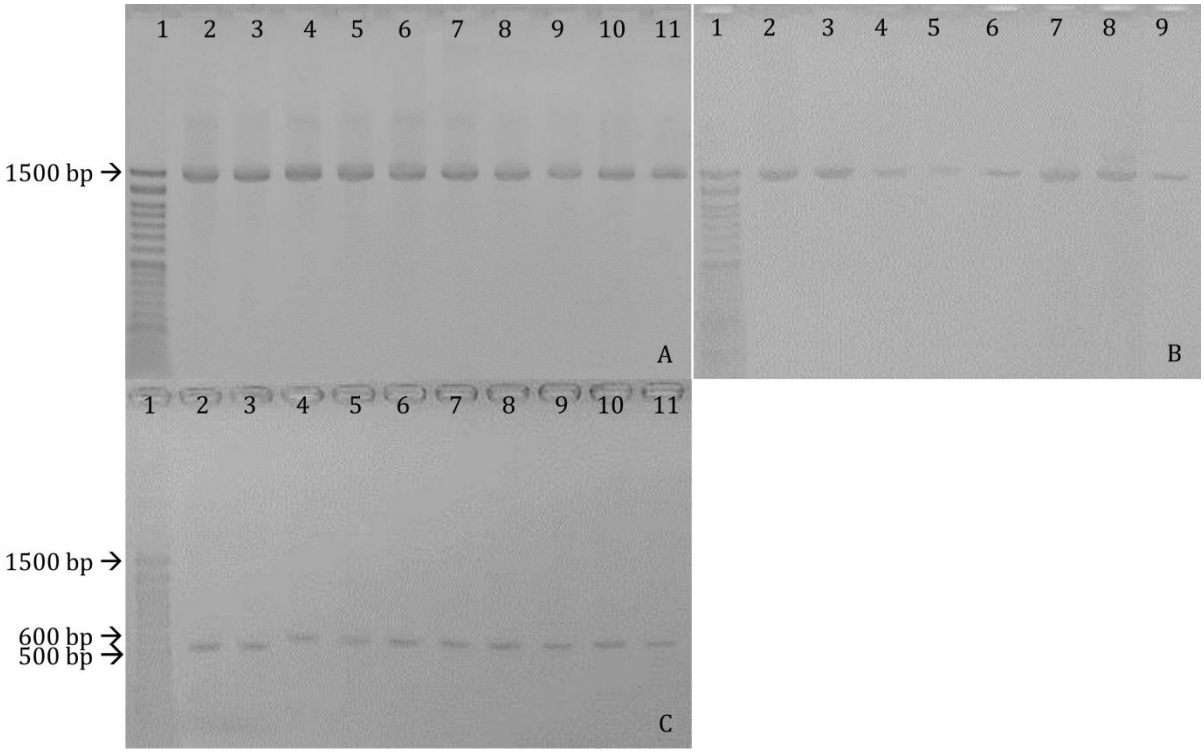

Figure 2: Agarose gel electrophoresis (1.5 %) of amplified bacterial 16S rRNA and fungal ITS region from extracted DNA. (A) gram-positive bacteria in Lanes 2-3: *B. subtilis*; 4-5: *S. aureus*; 6-7: *S. epidermidis*; 8-9: *L. plantarum*; 10-11: *L. acidophilus* (B) gram-negative bacteria in Lanes 2-3: *E. coli*; 4-5: *E. cloacae*; 6-7: *P. aeruginosa*; 8-9: *S. typhi*, and (C) fungi in Lanes 2-3: *C. albicans*; 4-5: *A. niger*; 6-7: *Mucor*; 8-9: *Rhizopus*; 10-11: *Fusarium*. Lane 1 in each panel: 50 bp DNA ladder. DNA extracted using the commercial kit is shown first for all species, followed by DNA extracted using the dipstick method. Bacterial 16S rRNA gene amplification produced ~1500 bp fragments, while fungal ITS region amplification resulted in 500–600 bp fragments. Successful amplification is observed for both methods across all tested microorganisms, with expected product sizes visible for each group.

Discussion

The development of rapid, cost-effective, and efficient methods for DNA extraction is crucial for the advancement of molecular diagnostics and research on biological samples including microorganisms. The current study compared the efficiency of a cellulose-based dipstick method for extracting bacterial and fungal DNA with a commercial extraction kit based on silica column methods (Qiagen). The optimized cell lysis and nucleic acid purification protocols that have been tested across a variety of microbial species demonstrate the flexibility of this method for both bacteria and filamentous fungi.

The efficacy of DNA extraction methods relies on intrinsic microbial characteristics, including the composition of cell walls, sample homogeneity, and the existence of inhibitors (Tongeren et al., 2011; Shin, 2012b). In this study, the mechanical disruption methods worked best in *E. coli*. Consistent with our results, the extreme cold temperature of liquid nitrogen (-196°C) has been shown to induce the formation of ice crystals within the cells, leading to mechanical disruption of both the cell membrane and wall, thus minimizing DNA degradation while maximizing extraction

efficiency (Ahari H. *et al.*, 2012). However, the practical application of liquid nitrogen maceration is limited in resource-constrained environments due to the need for specialized storage and handling equipment. Among bead-beating protocols, 0.1 mm glass beads worked more effectively than larger (0.5 mm) beads, in agreement with gram-negative bacterial structural requirements. Smaller beads cause greater mechanical stress from a greater number of contact points, more effectively lysing the cells without excessive shearing of the DNA. Manual bead beating has also been shown to optimize purity, possibly due to reduced mechanical shear compared to automated disruption (Islam et al., 2017).

The advantage of glass beads over steel highlights the importance of surface characteristics in DNA binding and elution. Borosilicate glass permits effective washing and virtually complete recovery of nucleic acid, which is critical for downstream applications requiring high-molecular-weight DNA. Enzymatic methods, while suitable for specific bacterial species, were less convenient due to enzyme stability issues and limited efficacy against diverse cell wall structures (Ali *et al*., 2017).

For *A. niger*, a filamentous fungus possessing a strong polysaccharide-based cell wall (β-1,3-glucan and chitin), mechanical shearing with 0.5 mm glass beads was found optimal (Fraczek *et al.*, 2019). Larger glass beads provided the necessary force to break through the thick fungal cell wall without degrading DNA, a balance which was supported by research highlighting bead size as the key parameter in fungal lysis (Klimek-Ochab *et al.*, 2011). Enzymatic methods, while less efficient, has shown merit in situations demanding minimal mechanical shear (Goldschmidt *et al.*, 2014). Proteinase K, although efficient at hydrolyzing proteins, did not satisfactorily degrade chitin or glucan without additional agents (Klimek-Ochab *et al.*, 2011). Consistent with our findings, combining optimized bead-beating steps with dipstick purification has been shown to achieve rapid nucleic acid extraction (<30 seconds) without compromising compatibility with downstream applications such as PCR and sequencing (Mason and Botella, 2020).

The cellulose-based dipstick method exhibited apparent advantages and limitations compared with commercial DNA extraction kits for both fungal and bacterial species. For bacterial DNA extraction using 0.1 mm glass beads, the dipstick method yielded sufficient DNA concentrations for downstream applications, though at lower concentrations compared with commercial kits for most species. This is most likely because of bacterial cell wall structure differences: Gram-positive bacteria, with thick peptidoglycan layers, require more vigorous lysis, while Gram-negative species, with outer membranes, are more susceptible to mechanical disruption (Kushkevych, 2023). While yields were lower, the dipstick method produced DNA of equivalent purity for PCR amplification, as indicated by successful amplification of the 16S rRNA region in all species examined. Therefore, the developed method is applicable for routine sample or pathogen identification or diagnosis during field surveys.

For fungal species, the dipstick method coupled with 0.5 mm beads yielded more DNA than commercial kits, particularly for *A. niger* and *Mucor*, due to increased mechanical disruption of polysaccharide-rich cell walls (Rezadoost et al., 2016). Reduced A260/A280 ratios achieved with the dipstick method reflect a potential co-extraction of impurities such as β-glucans or chitin, which are inherent to fungal cell walls (Fernando et al., 2023). These findings are in agreement with studies on the challenge of recovering pure DNA from fungi, where recalcitrant cell wall material has a tendency to carry over during extraction processes (Kenjar *et al.,* 2021). Despite

these impurities, dipstick-extracted DNA was shown to be amplifiable for ITS regions, proving its functional utility in molecular assays.

No statistically significant difference in amplification efficiency between the dipstick method and commercial kits for either bacterial or fungal DNA was detected by qPCR analysis. While the commercial kit yielded lower Cq values for certain species (e.g., *E. coli*, *Lactobacillus spp.*), the dipstick method worked more effectively for *P. aeruginosa* and *A. niger*, revealing species-dependent extraction efficiencies. This could be resulted due to differences in cell wall composition and mechanical versus enzymatic lysis efficiencies. Proteinase K and chaotropic agents like guanidine HCl are used for lysis in commercial kits. Besides fungal DNA extraction, enzymes like lyticase are commonly used to break down β-1,3-glucan in fungal cell walls. Lysozyme is another enzyme that works well with Gram-positive bacterial cell walls. The ability of the dipstick technique to yield PCR-ready DNA, though of limited purity, highlights its suitability for field application and quick diagnostics where cost and simplicity are paramount.

To surmount purity and yield constraints, protocol adjustments can optimize the performance of the dipstick method. For fungal applications, the addition of enzymatic pre-treatments (i.e., chitinase or glucanase) can reduce polysaccharide contamination (Wilson et al., 2025). For bacterial DNA extraction, optimization of bead-beating duration or buffer composition (e.g., inclusion of chaotropic salts) can enhance the efficiency of lysis. These adjustments would maximize the performance of the method without compromising its intrinsic advantages: speed of processing, low equipment requirements, and cost-effectiveness. However, the method is limited in its applications due to low yield of DNA compared to commercially available silica-column kits and restrictions in washing steps due to the fragility of the dipsticks which can affect the purity of DNA yielded from inhibitor-rich samples. As for further development, the dipstick method should also be optimized for samples from different settings, such as patient samples, to broaden its applicability.

Manual bead beating followed by cellulose dipstick-based DNA extraction not only reduce the cost and time but also reduces the amount of single-use disposable plastic use significantly contributing to mitigating the environmental impact of molecular diagnosis (Aragaw and Mekonnen, 2022). At the beginning of 2025, the total reagent and consumable cost per reaction for the in-house manufactured dipstick method was approximately USD 0.14, compared to USD 4.5 for the Qiagen kit, demonstrating a clear cost advantage. This significant cost advantage, combined with comparable performance for PCR-ready DNA, highlights the dipstick method's suitability for routine and field-based molecular diagnostics, especially in resource-limited settings.

Conclusions

This study demonstrates that manual bead beating in the followed by cellulose-based dipstick extraction method is a rapid, low-cost, and field-compatible approach for microbial DNA purification. The cellulose dipstick technique offers a sustainable alternative to traditional silica column methods by minimizing single-use plastic waste, reducing chemical pollution, and mitigating exposure to hazardous reagents. This method yields DNA of sufficient purity for most PCR applications while aligning with green laboratory practices, particularly in resource-limited

settings where cost and sustainability are critical. Furthermore, this innovative approach has the potential to democratize molecular diagnostics, enhance environmental monitoring, and facilitate field-based studies, especially in regions with limited laboratory infrastructure.

Funding

This study is supported by the RP/03/SR/02/06/02/2016 Capital Block Grant, University of Kelaniya.

Conflict of interest statement

The authors declare no conflict of interest.

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