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Comparative Evaluation of Lysis Reagents for DNA Extraction from Gram-Positive and Gram-Negative Bacteria

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Article Details

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ABSTRACT

The extraction of high-quality genomic DNA is a fundamental requirement for a wide range of molecular biology applications, including PCR, sequencing, and genetic engineering. However, standardized and cost-effective DNA extraction protocols that yield high-quality DNA from both Gram-positive and Gram-negative bacteria remain a challenge, particularly in resource-limited laboratory settings. This study aimed to evaluate and optimize simple, low-cost lysis methods compatible with phenol-chloroform-isoamyl alcohol (PCI) extraction to improve DNA yield and purity. Four different lysis approaches—distilled water, TEN buffer, TEN buffer with sodium dodecyl sulfate (SDS), and TEN buffer supplemented with SDS and Proteinase K were tested on Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). DNA yield and quality were assessed using spectrophotometry and gel electrophoresis. The results demonstrated that the combined use of TEN buffer, SDS, and Proteinase K significantly enhanced cell lysis, producing the highest DNA yield and purity for both bacterial types. In contrast, distilled water and TEN buffer alone resulted in poor lysis efficiency and lower DNA recovery. This study presents a reproducible, time-efficient, and economically feasible DNA extraction protocol that eliminates the need for expensive commercial kits while maintaining high-quality output. The proposed method is particularly suitable for routine molecular work in academic, diagnostic, and environmental microbiology laboratories, and offers a practical solution for standardizing bacterial DNA extraction from diverse species.

INTRODUCTION

The extraction of high-quality genomic DNA from bacterial cells is a critical prerequisite in microbiological, clinical, and molecular biology research. Accurate DNA isolation underpins a wide range of downstream applications, including polymerase chain reaction (PCR), DNA sequencing, gene cloning, genotyping, and antimicrobial resistance analysis(Bull, Ward, & Goodfellow, 2000; Weerakkody & Witharana, 2024). In recent years, as the reliance on molecular diagnostics and microbial genomics has expanded, so has the demand for efficient, reproducible, and cost-effective DNA extraction protocols that are adaptable to various bacterial types, including both Gram-positive and Gram-negative strains(Chakraborty, 2024). Bacterial cells possess distinct structural features that influence their susceptibility to lysis. Gram-positive bacteria, such as *Staphylococcus aureus*, have a robust, multilayered peptidoglycan cell wall, making them inherently more resistant to chemical and enzymatic disruption compared to Gram-negative species like *Escherichia coli*, which possess a thinner peptidoglycan layer and an outer membrane(Nikolic & Mudgil, 2023). These structural differences necessitate tailored lysis strategies for effective DNA extraction, especially when using traditional methods such as phenol-chloroform-isoamyl alcohol extraction, which, despite its effectiveness, can be limited by inconsistencies in yield, labor-intensiveness, and chemical hazards(Reid, 2013).

Although commercial DNA extraction kits offer standardized protocols with improved reproducibility and reduced processing time, they remain expensive and are not always accessible in low-resource settings, such as academic teaching laboratories. This limitation is particularly apparent in settings like the Microbiology Laboratory at the University of Haripur, where undergraduate and graduate students face challenges due to the lack of optimized, protocolized DNA extraction procedures. Inconsistent lysis efficiency, reagent wastage, and variability in DNA purity significantly impact the success of downstream applications, especially PCR. Several approaches have been proposed to overcome these challenges, including the use of detergents like SDS to solubilize cell membranes, buffers such as TEN (Tris-EDTA-NaCl) to stabilize nucleic acids, and enzymes like Proteinase K to degrade proteins that could interfere with DNA purity(Sackey, 1995; Santos, Oliveira, Arruda, & Martins, 2018). However, a systematic comparative evaluation of these lysis strategies, especially when applied to phenol-chloroform-based extraction methods, is still lacking in the context of simple, low-cost, and broadly applicable laboratory protocols(Sakyi et al., 2023). The present study addresses this gap by evaluating four lysis strategies distilled water, TEN buffer, TEN buffer with SDS, and TEN buffer with SDS and Proteinase K on two model bacterial species, *Staphylococcus aureus* and *Escherichia coli*. These organisms were selected not only for their clinical and environmental relevance but also for their distinct structural and biochemical characteristics, which present unique challenges in DNA extraction. By optimizing and validating a DNA extraction protocol that is cost-effective, rapid, and adaptable to both Gram-positive and Gram-negative bacteria, this work aims to establish a practical methodology for routine use in molecular biology labs, especially in academic institutions. Such a protocol would improve reproducibility, reduce reagent wastage, and enhance the reliability of molecular assays. The findings also have broader implications for resource-limited laboratories, where access to commercial kits may be constrained, but where high-quality DNA remains essential for research, diagnostics, and teaching.

RESULTS

GROWTH OF *E. COLI* AND *S. AUREUS* ON EOSIN METHYLENE BLUE AGAR

E. coli and *S. aureus* were grown on EMB agar. After 24 hours incubation metallic green sheen and yellow colonies were observed on EMB agar.

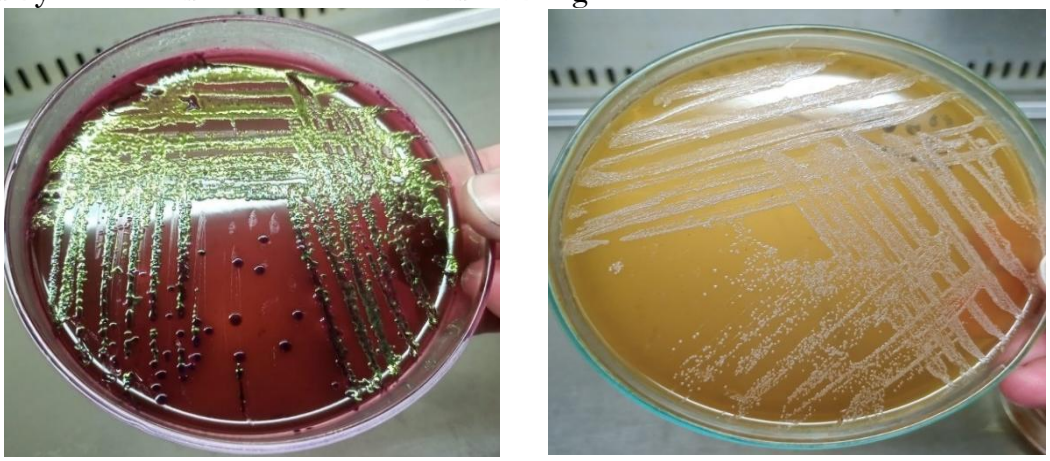

FIGURE 1: *GROWTH* OF E. COLI (A) AND S. AUREUS (B) ON EMB PLATE

MICROSCOPY

Colonies of *E. coli* and *S. aureus* were observed under a light microscope.

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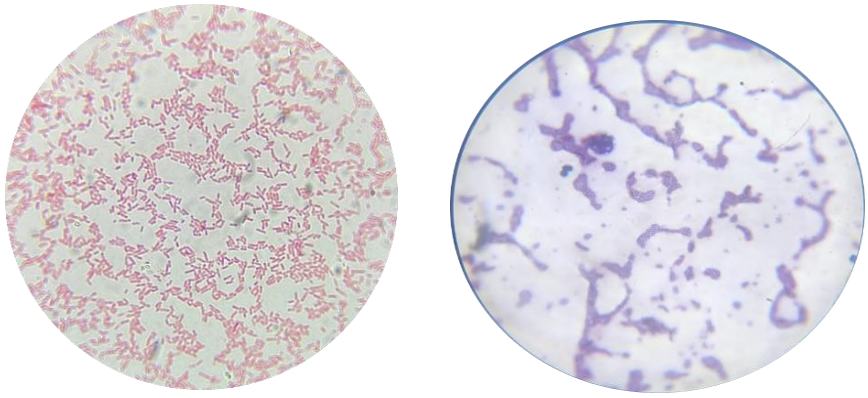


FIGURE 2: E. *COLI* AND *S. AUREUS* GRAM STAINING

CATALASE TEST

E. coli and *S. aureus* were catalase positive due to the immediate formation of bubbles which indicates the presence of catalase enzyme.

FIGURE 3: CATALASE TEST

OXIDASE TEST

S. aureus and *E. coli* were oxidase negative as no color change was observed which indicates the absence of cytochrome c oxidase.

FIGURE 4: OXIDASE TEST

COAGULASE TEST

The Coagulase test was performed for both *E. coli* and *S. aureus. E. coli* is coagulase negative and S. aureus is coagulase positive.

FIGURE 5: COAGULASE TEST

SPECTROPHOTOMETRY

Spectrophotometry was done for *E. coli* and *S. aureus* to measure the turbidity of both cultures. Spectrophotometry was performed on BIOBASE BK-UV100 spectrophotometer,

A B C

FIGURE 6: SPECTROPHOTOMETRY OF NUTRIENT BROTH(A), E. COLI (B) AND S. AUREUS (C)

AGAR PLATE NANODROP

TABLE 1: AGAR PLATE NANODROP RESULTS

	Concentration ng/µl			260/280 ratio		
Sample ID	**R1**	**R2**	**R3**	**r1**	**r2**	**r3**
APD-1	115.53	116.33	114.81	1.48	1.48	1.48
APT-2	154.75	160.86	158.16	1.45	1.46	1.47
APS-3	158.49	158.59	155.61	1.45	1.45	1.45
APK-4	179.03	180.23	179.45	1.5	1.5	1.5
APD-5	129.69	130.16	133.2	1.44	1.45	1.44
APT-6	145.21	144.16	147.05	1.46	1.46	1.45
APS-7	205.66	187.77	187.3	1.47	1.49	1.48
APK-8	201.47	202.53	201.74	1.45	1.45	1.45
AND-9	142.78	139.87	145.18	1.43	1.44	1.47
ANT-10	152.63	152.804	151.7	1.51	1.51	1.51
ANS-11	298.06	294.45	299.08	1.68	1.67	1.69
ANK-12	239.17	243.46	246.56	1.26	1.26	1.27
AND-13	14.84	143.09	136.25	1.48	1.45	1.44
ANT-14	148.75	152.77	153.03	1.49	1.49	1.49

ANS-15	444.89	491.04	450.72	1.7	1.7	1.73
ANK-16	356.82	355.61	357.22	1.63	1.63	1.63

BACTERIAL BROTH CULTURE

TABLE 2: BROTH CULTURE NANODROP RESULTS

	Concentration in ng/µl			260/280 Ratio		
Sample ID	**R1**	**R2**	**R3**	**r1**	**r2**	**r3**
BPD-1	253.569	244.954	232.962	1.54	1.51	1.47
BPT-2	420.204	407.823	409.343	1.42	1.38	1.38
BPS-3	449.094	438.052	437.154	1.35	1.31	1.3
BPK-4	575.16	553.271	543.404	1.38	1.33	1.31
BPD-5	374.171	381.38	378.479	1.44	1.45	1.45
BPT-6	361.139	361.325	370.399	1.52	1.52	1.56
BPS-7	661.365	656.297	657.477	1.25	1.24	1.25
BPK-8	685.891	663.898	652.218	1.33	1.32	1.32
BND-9	388.107	374.417	380.34	1.49	1.43	1.48
BNT-10	856.09	825.977	814.625	1.4	1.36	1.36
BNS-11	526.443	522.496	547.994	1.38	1.39	1.39
BNK-12	708.319	711.539	714.72	1.32	1.29	1.3
BND-13	340.225	332.748	331.888	1.45	1.44	1.43
BNT-14	1068.99	1010.8	983.21	1.32	1.32	1.3
BNS-15	665.918	661.95	659.293	1.3	1.3	1.49
BNK-16	1045.45	1040.82	1058.54	1.3	1.3	1.31

STATISTICAL ANALYSIS

These Nanodrop results were analyzed statistically, by performing ANOVA and Post-Hoc test.

ANOVA

ANOVA was done to determine the significant differences between the means of three groups.

TABLE 3: DNA CONCENTRATION OF S. AUREUS GROWING ON AGAR PLATE

d. H_2O	TEN	TEN+SDS	TEN+SDS+PK
115.53	154.75	158.49	179.03
116.33	160.86	158.59	180.23

114.81	158.16	155.61	179.45
129.69	145.21	205.66	201.47
130.16	144.16	187.77	202.53
133.2	147.05	187.3	201.74

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
d.H20	6	739.72	123.286667	73.38690667
TEN	6	910.19	151.698333	51.10677667
TEN+SDS	6	1053.42	175.57	434.05788
TEN+SDS+PK	6	1144.45	190.741667	150.0370567

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15623.047	3	5207.68229	29.39749326	**1.572E-07**	3.0983912
Within Groups	3542.9431	20	177.147155			
Total	19165.99	23				

TABLE 4: 260/280 RATIO OF S. AUREUS GROWING ON AGAR PLATE

d.H2O	TEN	TEN+SDS	TEN+SDS+PK
1.48	1.45	1.45	1.5
1.48	1.46	1.45	1.5
1.48	1.47	1.45	1.5
1.44	1.46	1.47	1.45
1.45	1.46	1.49	1.45
1.44	1.45	1.48	1.45

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
d.H2O	6	8.77	1.46166667	0.000417
TEN	6	8.75	1.45833333	5.67E-05
TEN+SDS	6	8.79	1.465	0.00031
TEN+SDS+PK	6	8.85	1.475	0.00075

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00093333	3	0.00031111	0.811594	0.50238147	3.09839121
Within Groups	0.00766667	20	0.00038333			
Total	0.0086	23				

TABLE 5: 260/280 RATIO OF S. AUREUS GROWING ON AGAR PLATE

d. H20	TEN	TEN+SDS	TEN+SDS+PK
142.78	152.63	298.06	239.17
139.87	152.804	294.45	243.46
145.18	151.7	299.08	246.56
14.84	148.75	444.89	356.82
143.09	152.77	491.04	355.61
136.25	153.03	450.72	357.22

ANOVA: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
d.H20	6	722.01	120.335	2680.58811
TEN	6	911.684	151.947333	2.66659467
TEN+SDS	6	2278.24	379.706667	8424.48167
TEN+SDS+PK	6	1798.84	299.806667	3869.55655

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	270905.68	3	90301.8933	24.1170133	7.524E-07	3.09839121
Within Groups	74886.465	20	3744.32323			
Total	345792.14	23				

TABLE 6: 260/280 RATIO OF E. COLI GROWING ON AGAR PLATE

d. Water	TEN	TEN+SDS	TEN+SDS+PK
1.43	1.51	1.68	1.26
1.44	1.51	1.67	1.26
1.47	1.51	1.69	1.27
1.48	1.49	1.7	1.63
1.45	1.49	1.7	1.63
1.44	1.49	1.73	1.63

ANOVA: SINGLE FACTOR

Summary

GROUPS	*Count*	*Sum*	*Average*	*Variance*
D.H2O	6	8.71	1.4516666	0.0003766
TEN	6	9	1.5	0.00012
TEN+SDS	6	10.17	1.695	0.00043
TEN+SDS+PK	6	8.68	1.446666	0.0403466

ANOVA						
SOURCE OF VARIATION	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
BETWEEN GROUPS	0.246166667	3	0.0820555	7.952404027	0.001100	3.098391
WITHIN GROUPS	0.206366667	20	0.0103183			
TOTAL	0.452533333	23				

TABLE 7: DNA CONCENTRATION OF S. AUREUS IN BROTH CULTURE

d. H2O	TEN	TEN+SDS	TEN+SDS+PK
253.569	420.204	449.094	575.16
244.954	407.823	438.052	553.271
232.962	409.343	437.154	543.404
374.171	361.139	661.365	685.891
381.38	361.325	656.297	663.898
378.479	370.399	657.477	652.218

ANOVA: Single Factor

Summary

Groups	*Count*	*Sum*	*Average*	*Variance*
d.H2O	6	1865.515	310.9191667	5449.524
TEN	6	2330.233	388.3721667	725.5211
TEN+SDS	6	3299.439	549.9065	14140.21
TEN+SDS+PK	6	3673.842	612.307	3856.379

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	351123.8	3	117041.2558	19.36836	**3.93E-06**	3.098391212
Within Groups	120858.2	20	6042.909261			
Total	471982	23				

TABLE 8: 260/280 RATIO OF S. AUREUS IN BROTH CULTURE

d.H20	TEN	TEN +SDS	TEN +SDS+PK
1.54	1.42	1.35	1.38
1.51	1.38	1.31	1.33
1.47	1.38	1.3	1.31
1.44	1.52	1.25	1.33
1.45	1.52	1.24	1.32
1.45	1.56	1.25	1.32

ANOVA: Single Factor

Summary

Groups	*Count*	*Sum*	*Average*	*Variance*
d.H20	6	8.86	1.476666667	0.001587
TEN	6	8.78	1.463333333	0.006307
TEN +SDS	6	7.7	1.283333333	0.001907
TEN +SDS+PK	6	7.99	1.331666667	0.000617

ANOVA

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0.165979	3	0.055326389	21.24533	**1.98E-06**	3.098391212
Within Groups	0.052083	20	0.002604167			
Total	0.218063	23				

TABLE 9: DNA CONCENTRATION OF E. COLI IN BROTH CULTURE

d.H20	TEN	TEN+SDS	TEN+SDS+PK
388.107	856.09	526.443	708.319
374.417	825.977	522.496	711.539
380.34	814.625	547.994	714.72
340.225	1068.99	665.918	1045.45
332.748	1010.8	661.95	1040.82
331.888	983.21	659.293	1058.54

ANOVA: Single Factor

Summary

Groups	*Count*	*Sum*	*Average*	*Variance*
d.H20	6	2147.725	357.9541667	662.0926
TEN	6	5559.692	926.6153333	11640.86
TEN+SDS	6	3584.094	597.349	5155.721
TEN+SDS+PK	6	5279.388	879.898	34056.84

ANOVA

Source of Variation	***SS***	***Df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	1265315	3	421771.7694	32.74911	**6.52E-08**	3.098391
Within Groups	257577.6	20	12878.8778			
Total	1522893	23				

TABLE 10: 260/280 RATIO OF E. COLI IN BROTH CULTURE

d. H2O	TEN	TEN+SDS	TEN+SDS+PK
1.49	1.4	1.38	1.32
1.43	1.36	1.39	1.29
1.48	1.36	1.39	1.3
1.45	1.32	1.3	1.3

1.44	1.32	1.3	1.3
1.43	1.3	1.49	1.31

ANOVA: Single Factor

Summary

Groups	*Count*	*Sum*	*Average*	*Variance*
d.H2O	6	8.72	1.453333333	0.000667
TEN	6	8.06	1.343333333	0.001347
TEN+SDS	6	8.25	1.375	0.00499
TEN+SDS+PK	6	7.82	1.303333333	0.000107

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.072712	3	0.0242375	13.63572	**4.52E-05**	3.098391212
Within Groups	0.03555	20	0.0017775			
Total	0.108263	23				

POST-HOC TEST

After ANOVA, Post-Hoc test was performed to identify which specific group means are significantly different from each other.

TABLE 11: DNA CONCENTRATION OF S. AUREUS GROWING ON AGAR PLATE

Groups	P-VALUE (T-TEST)	Significant	TEST	ALPHA
			ANOVA	0.05
1V2	9.66323E-05	Yes	POST-HOC TEST (BONFERRONI CORRECTION)	0.008333
1V3	0.000202391	Yes		
1V4	6.29799E-07	Yes		
2V3	0.024118122	No		
2V4	5.08446E-05	Yes		
3V4	0.155142913	No		

TABLE 12: 260/280 RATIO OF S. AUREUS GROWING ON AGAR PLATE

POST-HOC TEST			**ALPHA**	
Groups	**P value (T-test)**	**Significant**	**TEST**	**ALPHA**
1v2	0.715279862	**No**	**ANOVA**	0.05
1v3	0.768179782	**No**	Post-hoc test (Bonferroni corrected)	0.008333
1v4	0.361524043	**No**		
2v3	0.413727797	**No**		
2v4	0.181149325	**No**		
3v4	0.469177003	**No**		

TABLE 13: DNA CONCENTRATION OF E. COLI GROWING ON AGAR PLATE

POST-HOC TEST:			ALPHA	
Groups	P-value (T test)	Significant	Test	Alpha
1v2	0.165821377	No	ANOVA	0.05
1v3	0.000127123	Yes	Post-hoc test (Bonferroni corrected)	0.008333
1v4	0.00028815	Yes		
2v3	0.000119215	Yes		
2v4	0.000168254	Yes		
3v4	0.108003173	No		

TABLE 14: 260/280 RATIO OF E. COLI GROWING ON AGAR PLATE

POST-HOC TEST:			ALPHA	
Groups	P-value (T test)	Significant	Test	Alpha
1v2	0.00034144	Yes	ANOVA	0.05
1v3	1.33999E-09	Yes	Post-hoc test (Bonferroni corrected)	0.008333
1v4	0.952801153	No		
2v3	0.53069979	No		
2v4	0.53069979	No		
3v4	0.53069979	No		

TABLE 15: DNA CONCENTRATION OF S. AUREUS IN BOTH CULTURE

POST-HOC TEST:			ALPHA	
Groups	P-value (T test)	Significant	Test	Alpha
1v2	0.036413842	No	ANOVA	0.05
1v3	0.001880218	Yes	Post-hoc test (Bonferroni corrected)	0.008333
1v4	1.73296E-05	Yes		
2v3	0.008791005	No		
2v4	1.05188E-05	Yes		
3v4	0.281101585	No		

TABLE 16: 260/280 RATIO OF S. AUREUS IN BROTH CULTURE

POST-HOC TEST:			ALPHA	
Groups	P-value (T test)	Significant	Test	Alpha
1v2	0.720826991	No	ANOVA	0.05
1v3	1.16163E-05	Yes	Post-hoc test (Bonferroni corrected)	0.008333
1v4	1.91127E-05	Yes		
2v3	0.000656432	Yes		
2v4	0.003078471	Yes		
3v4	0.040167385	No		

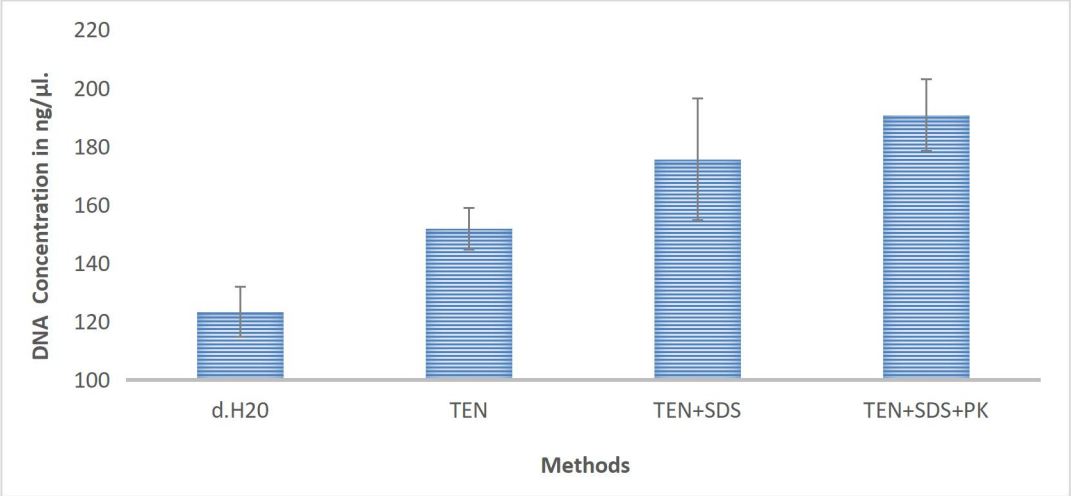
TABLE 17: DNA CONCENTRATION OF E. COLI IN BROTH CULTURE

POST-HOC TEST:			ALPHA	
Groups	**P-value (T- test)**	**Significant**	**Test**	**Alpha**
1v2	1.90298E-07	Yes	ANOVA	0.05
1v3	1.66555E-05	Yes	Post-hoc test (Bonferroni corrected)	0.008333
1v4	4.39642E-05	Yes		
2v3	9.84299E-05	Yes		
2v4	0.604134222	No		
3v4	0.005773802	Yes		

TABLE 18: 260/280 RATIO OF E. COLI IN BROTH CULTURE

POST-HOC TEST:			ALPHA	
Groups	**P-value (T- test)**	**Significant**	**Test**	**Alpha**
1v2	0.000131237	**Yes**	**ANOVA**	0.05
1v3	0.028805365	**No**	**Post-hoc test (Bonferroni corrected)**	0.008333
1v4	1.17584E-07	**Yes**		
2v3	0.352827967	**No**		
2v4	0.02788504	**No**		
3v4	0.033737036	**No**		

Graphical representation of Results



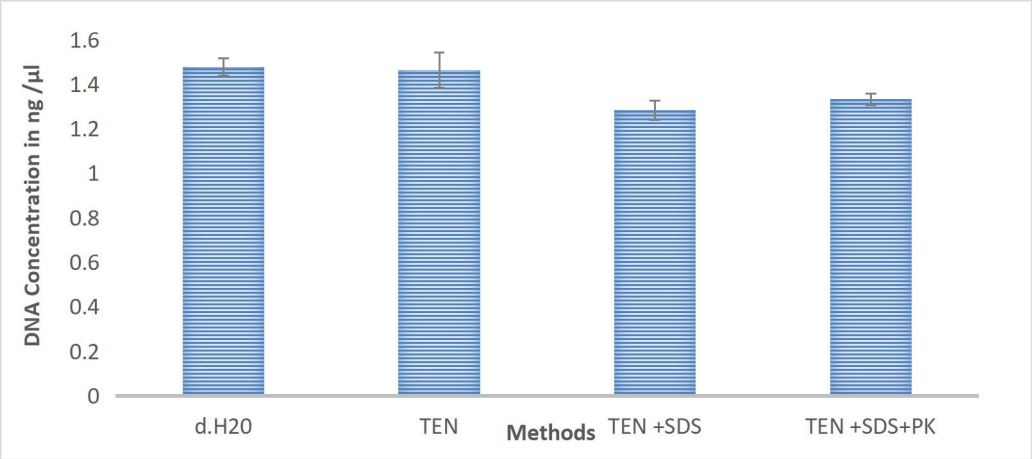


FIGURE 7: DNA CONCENTRATION OF S. AUREUS GROWING ON AGAR PLATE

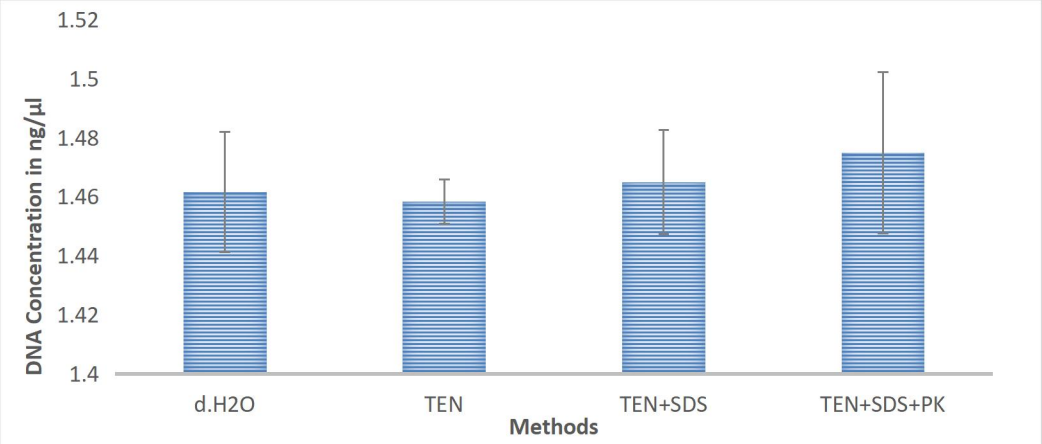


FIGURE 8: 260/280 RATIO OF S. AUREUS GROWING ON AGAR PLATE

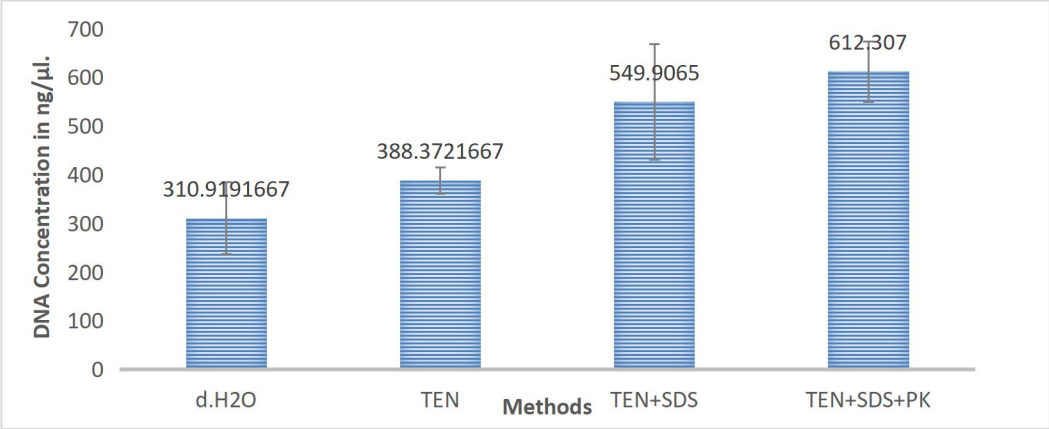


FIGURE 9: DNA CONCENTRATION OF S. AUREUS IN BROTH CULTURE

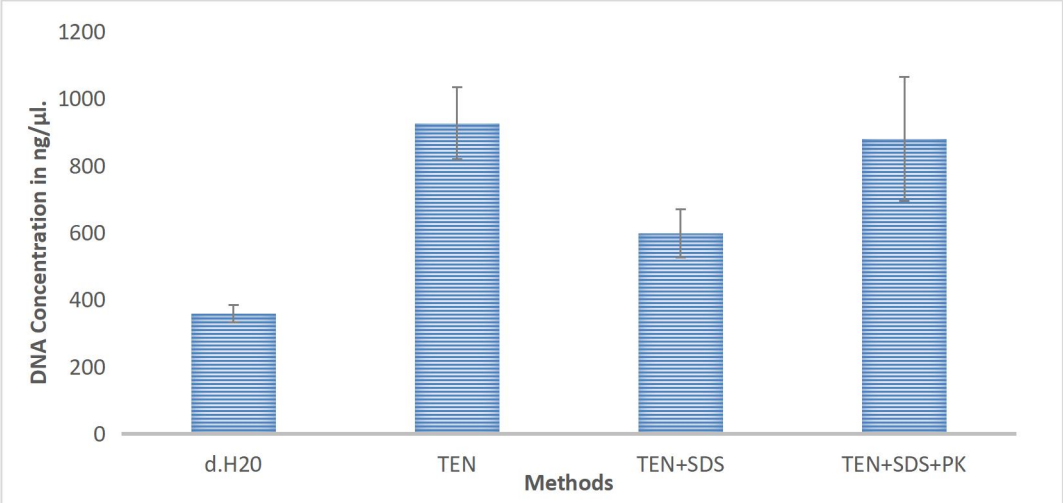


FIGURE 10: 260/280 RATIO OF S. AUREUS IN BROTH CULTURE

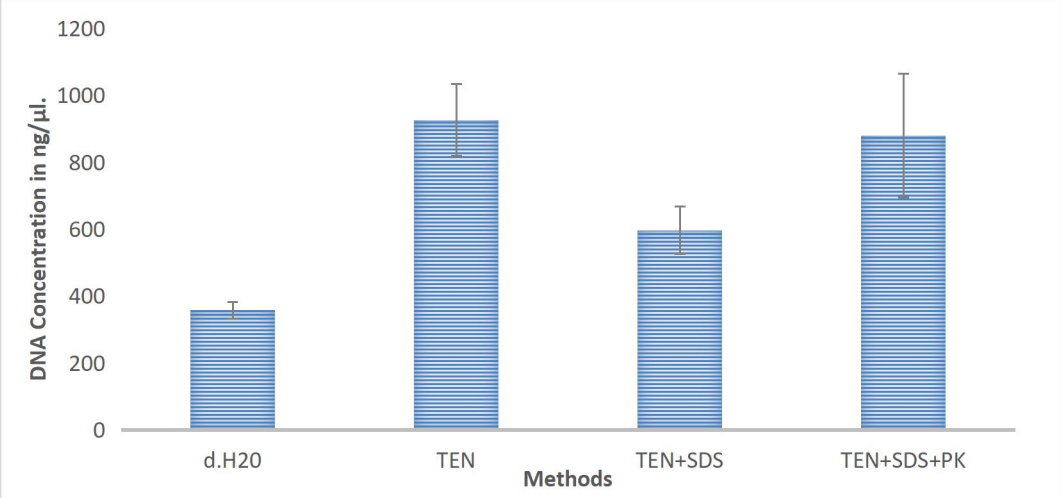


FIGURE 11: DNA CONCENTRATION OF E. COLI IN BROTH CULTURE

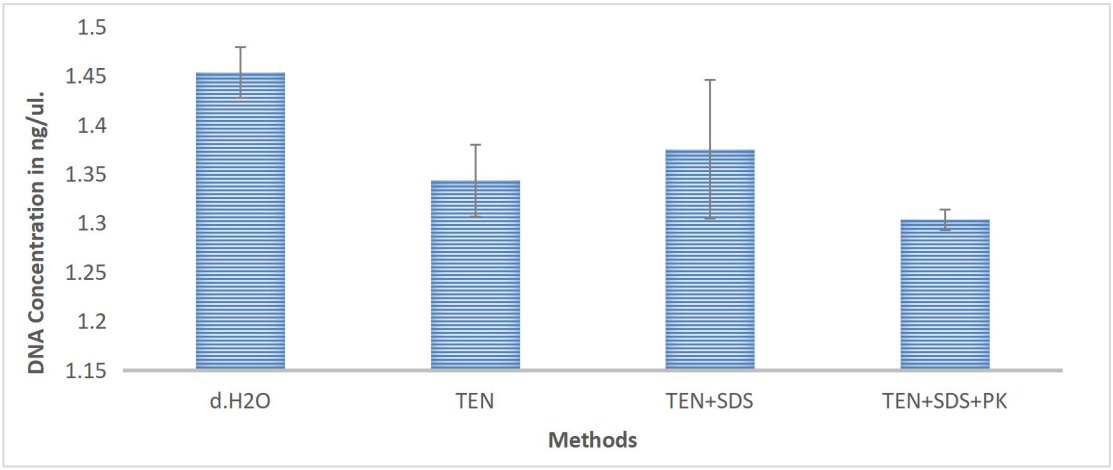


FIGURE 12: 260/280 RATIO OF E. COLI IN BROTH CULTURE

GEL ELECTROPHORESIS

Gel was run to analyze DNA fragments as well as ensuring the integrity and quality of DNA fragments.

AGAR PLATE RESULTS

Gel electrophoresis results from agar plate are given below.

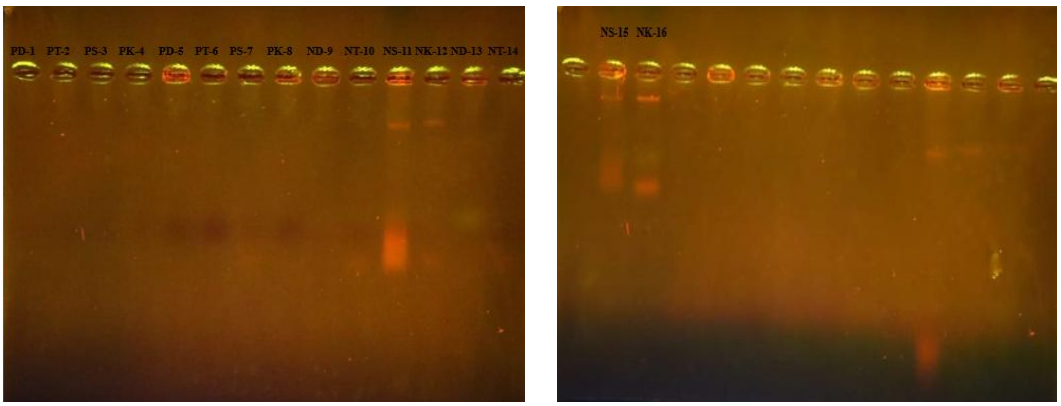


FIGURE 13: AGAR PLATE GEL ELECTROPHORESIS RESULTS

DISCUSSION

The extraction of high-quality genomic DNA is a foundational step in molecular biology and microbiological research, with applications spanning PCR, cloning, sequencing, genotyping, and microbial identification. In both clinical diagnostics and environmental monitoring, efficient DNA isolation from bacterial cells enables accurate pathogen detection, strain typing, and gene-based analysis(Moots, 2021). This study demonstrates a robust and cost-effective DNA extraction protocol using phenol-chloroform-isoamyl alcohol (PCI), optimized through the application of four distinct lysis strategies. The protocol was tested on both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, two taxonomically and structurally divergent organisms to assess lysis efficiency, DNA yield, and purity(Li et al., 2020). PCI extraction remains a gold standard for nucleic acid purification, particularly due to phenol's protein-denaturing capacity, chloroform's enhancement of phase separation, and

isoamyl alcohol's prevention of foaming, which collectively contribute to the isolation of high-integrity genomic DNA(Tahir et al., 2024; Wahlberg et al., 2012). Among the evaluated lysis strategies, the combination of TEN buffer, SDS, and Proteinase K resulted in the highest DNA yield and purity. Spectrophotometric quantification (Nanodrop) and agarose gel electrophoresis confirmed that lysis conditions incorporating both a detergent and a proteolytic enzyme significantly improved cell disruption and DNA recovery(Qamar, Khan, & Arafah, 2017). The statistical analysis (ANOVA and post-hoc testing) further validated the superiority of SDS- and enzyme-assisted lysis compared to simpler methods involving only distilled water or TEN buffer. SDS, a well-known anionic detergent, disrupts the lipid bilayer and denature proteins, facilitating the release of intracellular contents(Arakawa, Niikura, Kita, & Akuta, 2024). Proteinase K digests contaminating proteins, including nucleases, thus protecting the integrity of DNA(Frazer et al., 2020). The components of TEN buffer Tris-HCl, EDTA, and NaCl play essential roles in maintaining pH, chelating divalent ions to inhibit nuclease activity, and stabilizing DNA during extraction. Together, these reagents provide a chemically favorable environment for efficient lysis and preservation of DNA integrity. The difference in DNA yield between Gram-positive and Gram-negative bacteria observed in this study reflects known structural differences in bacterial cell walls. *S. aureus*, as a Gram-positive organism, possesses a thick peptidoglycan layer embedded with teichoic acids, contributing to its resistance to lysis. In contrast, *E. coli* has a thinner peptidoglycan layer, and an outer membrane composed of lipopolysaccharides, making it more susceptible to detergent-mediated disruption(Kandaswamy, 2017; Whidbey, 2015). These structural variances explain the relative ease of DNA extraction from *E. coli* compared to *S. aureus*(Virtanen, Puljula, Walton, Woodward, & Karonen, 2023). Although Nanodrop readings showed high DNA concentrations across all methods, gel electrophoresis revealed significant differences in integrity. This discrepancy may be due to the presence of contaminants or partially degraded nucleic acids, which can influence absorbance readings but not support successful amplification or band clarity. For clear gel visualization, approximately 100 ng of DNA per well is typically required (Boudadi, EL Merzougui, Lachheb, Lachguer, & Serghini, 2025) underscoring the need for both spectrophotometric and electrophoretic validation when assessing DNA quality. Overall, the optimized protocol described in this study offers several advantages. It is simple, involves fewer extraction steps, avoids the need for expensive commercial kits, and yields high molecular weight DNA suitable for downstream applications. It is also broadly applicable to a wide range of bacterial species, making it especially valuable in academic, diagnostic, and low-resource settings. These findings are consistent with previous reports that emphasize the effectiveness of combining chemical lysis agents with enzymatic digestion to enhance DNA recovery from bacterial cells(Gautam, 2022). The integration of SDS and Proteinase K into TEN buffers, followed by PCI extraction, provides a reproducible, efficient, and economical method for isolating high-quality genomic DNA from both Gram-positive and Gram-negative bacteria(Weerakkody & Witharana, 2024). This method can serve as a standardized approach in microbiology laboratories for molecular applications requiring reliable DNA extraction.

CONCLUSION

In this study, a simple, rapid, and cost-effective DNA extraction protocol using the Phenol-Chloroform-Isoamyl Alcohol (PCI) method was successfully optimized for both Gram-positive and Gram-negative bacteria. The use of various lysis reagents distilled water, TEN buffer, SDS, and Proteinase K was systematically evaluated to enhance cell lysis and maximize DNA yield

and purity. Among these, the combination of TEN buffer, SDS, and Proteinase K proved to be the most efficient for extracting high-quality genomic DNA, as confirmed by spectrophotometric analysis and gel electrophoresis. The extracted DNA was of sufficient quality for a wide range of downstream molecular applications, including PCR, sequencing, gene cloning, and electrophoresis. This protocol not only reduces reliance on costly commercial kits but also provides a reliable alternative suitable for low-resource academic laboratories. Its simplicity and affordability make it particularly valuable for students and researchers in countries like Pakistan, where economic constraints often limit access to commercial molecular biology tools (Abid et al., 2023). This method can significantly enhance research quality and training in laboratories with limited access to commercial kits.

RECOMMENDATIONS

To further enhance the utility of DNA extraction methods in academic and clinical microbiology, future research should focus on:

- Optimizing lysis and purification protocols to further increase DNA yield and minimize contamination.
- Developing alternative, low-cost extraction reagents that are safer and more environmentally friendly than phenol-based methods.
- Standardizing protocols across laboratories, especially in resource-limited settings, to ensure reproducibility and efficiency.
- Exploring automation and miniaturization of PCI-based protocols for high-throughput applications.

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