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COMPARATIVE EVALUATION OF PRIMERS AND DNA EXTRACTION TECHNIQUES FOR PCR AMPLIFICATION OF 16S rDNA OF BACTERIAL ISOLATES FROM PETROCHEMICAL INDUSTRIAL EFFLUENT

AJUZIE, C.U.*., OBUEKWE, I.S. AND ASO, R.E.

Department of Microbiology, University of Benin, Nigeria.

*Correspondence: Christopher.ajuzie@uniben.edu. 08073960603

ABSTRACT

The investigation delved into a comparative assessment of primers employed in the polymerase chain reaction (PCR) for amplifying bacterial DNA sourced from diverse sampling locations within the effluent of Eleme Petrochemical Industry. Two extraction methodologies, namely ethanol precipitation and guanidium thiocyanate ethylenediaminetetraacetate (EDTA) sarcosyl buffer (GES), were scrutinized. Comparative analysis of DNA yield and quality indicated a superior recovery with the GES method in contrast to ethanol extraction. The prominent bacterial isolates, *Pseudomonas* and *Bacillus*, exhibited yields of 611.6 and 615.5 ng/μl and DNA quality of 25.35 and 24.55 ng/μl (260/280), 28.99 and 31.43 (260/230) using GES. Conversely, ethanol precipitation yielded 90.2 and 0.2 ng/μl with DNA quality of 1.65 and 0.21 (260/280), 1.57 and 0.00 (260/230) for *Pseudomonas* and *Bacillus*, respectively. Successful amplification, validated through ethidium bromide on 1% agarose gel electrophoresis, was achieved. Genus-level identification of isolated bacterial organisms encompassed *Pseudomonas*, *Bacillus*, *Serratia*, *Micrococcus*, *Escherichia*, *Klebsiella*, *Vibrio*, *Proteus*, *Achromobacter*, *Citrobacter*, and *Flavobacterium*. The comparative assessment of PCR primers unveiled V6V8F and V6V8R as the most suitable for amplifying bacterial DNA. These primers facilitated the amplification of 16S rDNA fragments from all bacterial isolates with an anticipated size of 500 base pairs.

KEYWORDS: Primers, PCR, 16S rDNA, GES buffer, Effluent, EDTA, base pair (bp)

INTRODUCTION

Bacterial species of various diversities have been reported in crude oil and petroleum products (Goveas *et al.*, 2020; Sarfo *et al.*, 2023). Petrochemical industry effluents contain potential cultures of bacteria which show a capacity to utilize hydrocarbons and also have a resistance to heavy metals. Among the 92 isolates, four bacterial cultures namely; *Pseudomonas* sp., *Bacillus* sp., *Aeromonas* sp., and *Azotobacter* sp. grow well on petrochemicals such as benzene, butanol, ethylene glycol, n-heptane, methanol, phenol and xylene and show resistance to heavy metals such as cobalt, copper, lead, mercury, molybdenum and zinc. This suggests that they may play an important role in the purification of petrochemical industrial effluent (Xiang *et al.*, 2020; Saini *et al.*, 2023).

Efficiencies and accuracy in detecting bacteria involved in bioremediation hinge upon the quality of employed primers and the integrity of DNA (Brandt and Abertsen, 2018). The choice of primers significantly influences the specificity and sensitivity of bacterial identification, crucial for effective bioremediation processes. High-quality primers that precisely target the desired bacterial genes enhance the reliability of detection methods (Thij *et al.*, 2017). Simultaneously, maintaining DNA integrity is paramount, as degraded DNA can lead to false negatives or compromised accuracy in identifying bacterial communities. A meticulous approach in primer selection and preserving DNA integrity is essential for robust and trustworthy assessments in bacterial detection for bioremediation efforts ((Thij *et al.*, 2017; Brandt and Abertsen, 2018).

Studies have revealed that although effluents from Eleme Petrochemical Company Limited generally contain

relatively low concentrations of pollutants in the water and sediment, accumulation of these pollutants overtime can be fatal to aquatic and human life. Also, continued discharge of improperly treated effluent may further compound the environmental problems of communities living around this company (Israel *et al.*, 2008). This therefore makes imperative the need for early resolution of the problem of treatment for Eleme petrochemical effluent (Israel *et al.*, 2008). This dire need in recent times has found microorganisms quite instrumental. Microorganisms have been implicated in showing strong ability to bio treat petrochemical effluents. The potentials of microorganisms to catabolize and metabolize xenobiotic compounds has been recognized as potentially effective means of toxic and hazardous wastes disposal.

MATERIALS AND METHODS

Description of Sampling Area

Eleme Petrochemical Company Limited (EPCL) is situated in Eleme, River state in the oil-rich Niger Delta area of Nigeria. It was established by the Federal Government of Nigeria in 1988. The major feedstock used in the company is delivered to it in liquid form via pipeline from the liquefied natural gas (LNG) plant located at Obiafu/Obrikom in Rivers state. The feedstock is free from methane, but composed of ethane, propane and butane with minor quantities of pentane and heavier hydrocarbons. The major products of the company are low density and linear low-density polyethylene (LLDPE), polypropylene (PP), vinyl chloride monomer, butane and mixture of other olefins. Effluents are usually treated with sulphuric acid, caustic soda, alum, urea, Di-ammonium phosphate, anionic polyelectrolyte and calcium hypochlorite. Thereafter, the treated effluent is directly discharged into receiving river bodies.

The Eleme River in Eleme kingdom took its source at Oyigbo and flows down Agbonchia farm settlement, Njuru, Okerewa and Aluto at which point the petrochemical effluent is discharged into it before entering into tidal creek by NNPC housing estate Aleto and flows down to Okrika. The river passes through sparse vegetation and its course flows across many roads and as such receives storm water runoffs from roads too.

Sample Collection

Samples were collected once a month between July 2020 and March 2021. Water samples were collected with a 2-liter plastic hydro-bios water sampler and transferred to clean 2-liter polyethylene containers and 250 ml capacity borosilicate glass bottles. The effluent samples include the process wastewater (PWW) (untreated effluent), clarified water (CW), retention pond gate (RPG), which is the industrial effluent that has undergone both chemical and biological treatment to eliminate or reduce waste contents to acceptable levels and the receiving river (RR) of Eleme kingdom. These were collected in polyethylene containers and borosilicate bottles of the same capacity. They were rinsed several times with water or effluent samples at the point of collection. The samples were transported to the laboratory using iced packed coolers after appropriate labeling.

Isolation and Characterization of Bacterial Isolates

Distinct colonies from mixed cultures in the Petri dishes were picked and transferred aseptically into sterile nutrient broth. The nutrient broth cultures were incubated at 37 °C for 24 h. The broth cultures were streaked on sterile nutrient agar plates and incubated at 37 °C for 24 h. Pure colonies were picked and transferred aseptically to nutrient agar slants, incubated for 24 h at 37 °C and stored in the refrigerator at 4°C for further characterization.

Pure cultures were presumptively identified on the basis of their morphological and biochemical characteristics by means of schemes of Buchanan and Gibbons (1974), Cowan and Steel (1985) and Harold (1990). The following tests were performed in duplicates using standard microbiological techniques; colonial morphology, cellular morphology, Gram reaction, motility, oxidase, catalase, urease activity, indole production, nitrate reduction, citrate utilization, carbohydrate metabolism (Hugh and Leifson's test), acid and gas production from various sugars, starch hydrolysis, methyl red test, Voges-Proskauer test and coagulase test.

DNA EXTRACTION FROM PETROCHEMICAL INDUSTRIAL EFFLUENT

Two different methods of DNA isolation were tried on the isolates from effluent samples to assess which would have a higher and quality yield of DNA. They include;

A. Ethanol Precipitation Method

The DNA extraction method described by Ercolini *et al.* (2003) was applied to extract DNA from pure isolates from petrochemical industrial effluent. Fifty microlitre (50 µl) sodium acetate (3 M pH 5.2) and 1 ml 100% ethanol were

pipetted into labeled Eppendorf tubes. Each isolate was then inoculated into respective tube and inverted to mix. It was then incubated at 20 °C for 2 hr, after which the tubes were centrifuged at 13000 rpm for 2 min. The supernatant was decanted and 1ml of 70% ethanol added and inverted to mix. The tubes were allowed to stand for 1 min and re-centrifuged at 13000 rpm for 2 min. The ethanol was decanted and re-centrifuged at 13000 rpm for 2 min. The supernatant was then pipetted off and the pellet allowed to air dry. The pellets were then re-suspended in 50 µl TE buffer and stored at 20 °C.

B. Guanidium Thiocyanate EDTA Sarcosyl Buffer (GES) Method

Modified rapid extraction of bacterial genomic DNA with guanidium thiocyanate ethylenediamine tetraacetate acid (EDTA) sarcosyl buffer (GES) described by Pitcher *et al.* (1989) was used to extract DNA from pure bacterial isolates of petrochemical industrial effluent. Pure bacterial cultures of 24 h were inoculated into brain heart infusion (BHI) broth and incubated overnight at 37 °C. One and half millilitres of each culture was pipetted into an Eppendorf tube and centrifuged at 13000 rpm for 1 min. It was repeated until enough pellets were obtained. The pellet was re-suspended in 1 ml of ice-cold lysis buffer. The cell suspension was re-centrifuged and the pellet re-suspended in 100 µl of same lysis buffer supplemented with 50 mg ml⁻¹ lysozyme. It was then incubated at 37 °C for 30 min. Zero point three millilitres of GES reagent was added, mixed well and incubated at room temperature (RT) for about 10 minutes until solution clears. The lysate was then cooled on ice for 2min. Zero point two five millilitres of ice-cold 7.5 M ammonium acetate was added, vortexed briefly and incubated on ice for 10 min. Half a millilitre of 24:1 CHCl₃ (chloroform) isoamyl alcohol was added, vortexed briefly and centrifuged for 10 min at 13000 rpm and 800-850 µl of supernatant was removed into a clean Eppendorf tube. Precisely 459 µl of ice-cold isopropanol was added, mixed gently for 1min, after which the pellet was clearly visible. It was then centrifuged at 13000 rpm for 1min to bring down the DNA. The supernatant was then removed from the pellet. The pellet was then washed thrice in 500 µl of 70% ethanol (EtOH) and air-dried. It was then re-suspended in 50µl of Tris HCl and EDTA (TE). The DNA were then stored at -20 °C.

QUANTIFICATION AND QUALITY OF DNA EXTRACT FROM ISOLATES

DNA quality and quantity was assayed using the Nanodrop ND-1000 spectrophotometer. The nucleic acid module was selected and the lens cleaned by pipetting 1 µl of water after which DNA samples were then read by pipetting 1 µl each into lens and again cleaned with 1µl of water after each read. The ratio of absorbance at 260 and 280 nm is used to assess the purity of DNA and RNA. A ratio of approximately 1.8 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

This is a secondary measure of nucleic acid purity. The 260/230 values for “pure” nucleic acid are often higher than the respective 260/280 values. They are commonly in the range of 1.8-2.2. If the ratio is appreciably lower, this may indicate the presence of co-purified contaminants.

PCR AMPLIFICATION OF 16S rDNA FOR PETROCHEMICAL INDUSTRIAL EFFLUENT BACTERIAL ISOLATES

Different regions of the 16S rDNA were amplified with different primers in order to determine the primers that provided the best DGGE differentiation of the microbial community responsible for decontaminating petrochemical industrial effluent.

V3 PCR Amplification

One set of primers amplifying the variable V3 region (V3F and V3R) as described by Cocolin *et al.* (2001) was used to amplify 16S rDNA from the petrochemical industrial effluent bacterial isolates. The V3 region of the 16S rDNA was amplified using V3 Reverse primer (518R-primer V3 16S MWG-Biotech, 5'-ATT ACC GCG GCT GCT GG-3') and V3 Forward primer (338F-primer, 5'-ACT CCT ACG GGA GGC AGC AG-3') with a GC clamp (CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC G) (Muyzer *et al.*, 1993) in order to increase DGGE separation.

Amplification was performed in a programmable heating thermocycler (C1000 Thermal Cycler).

The reaction mixture (50 µl total volume for each sample) for the PCR was composed as follows: 25 µl of 2×master mix (2×mm), 0.2 µl of forward and reverse primers, 1.5 µl of template DNA and 23.3 µl of nuclease free water. PCR amplification was performed as follows: 5 min at 94 °C to denature template DNA, 10 cycles decreasing by 1°C each round from 66 °C primer annealing, 20 cycles at 56 °C for primer extension, 3 min at 72 °C, and then the final extension was carried out at 72 °C for 10 min.

The PCR product was analyzed by electrophoresis on 1% TAE agarose (Agarose 3:1, Melford Laboratories Ltd., Ipswich, UK) gel containing ethidium bromide (0.2 µg/ml) in 1x TAE running (40 Mm Tris base, 20 mM acetic acid, 1 mM EDTA) at 75 volts for about 90 min. Samples contained 10 µl of PCR product and 2 µl of loading dye (Promega). The gel was viewed under a UV trans illuminator (GelDoc XR, Biorad) and the gel pictures were recorded by using the GelDoc system (The Quantity one 4.6.5 Basic software, USA).

16S PCR Amplification

One set of primers amplifying the 16S rDNA fragments as described by Stecher *et al.* (2007) was also used. The 16S rDNA fragments were amplified with primers 16S forward primer (5'-AGA GTT TGA TYM TGG CTC AG-3') and 16S reverse primer (5'-ACG GYT ACC TTG TTA CGA CTT-3'). Amplification was performed in a programmable heating thermocycler (C1000 Thermal Cycler).

The reaction mixture (50 µl total volume for each sample) for the PCR was composed as follows: 25 µl of Dream

Taq™ Green PCR Master Mix (2x), 2 µl of forward and reverse primers, 1 µl of template DNA and 21 µl of nuclease free water and 1 µl of MgCl₂. PCR amplification was performed as follows: 3 min at 95 °C for initial denaturation of template DNA, 1 cycle; 30 s at 95 °C for denaturation, 30 s at 5 °C for primer annealing, 1 min at 72 °C for extension, 25-40 cycles; and 15 min at 72 °C for the final extension, 1 cycle.

The PCR product was analyzed by electrophoresis on 1% TAE agarose (Agarose 3:1, Melford Laboratories Ltd., Ipswich, UK) gel containing ethidium bromide (0.2 µg/ml) in 1x TAE running (40 Mm Tris base, 20 mM acetic acid, 1 mM EDTA) at 75 volts for about 90 min. Samples contained 10 µl of PCR product and 2 µl of loading dye (Promega). The gel was viewed under a UV transilluminator (GelDoc XR, Biorad) and the gel pictures were recorded by using the GelDoc system (The Quantity one 4.6.5 Basic software, USA).

V6-V8 PCR Amplification

One set of primers amplifying the V6-V8 region of 16S rDNA was also used as described by Martin *et al.* (2007). The V6-V8 region was amplified with primers V6V8 forward primer (5'-AGC AGT AGG GAA TCT TCA-3') and V6V8 Reverse primer (5'-ATT TCA CCG CTA CAC ATG-3') with a GC clamp (CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC G) (Muyzer *et al.*, 1993) in order to increase DGGE separation. Amplification was performed in a programmable heating thermocycler (C1000 Thermal Cycler).

The reaction mixture (50 µl total volume for each sample) for the PCR was composed as follows: 25 µl of Dream Taq™ Green PCR Master Mix (2x), 2 µl of forward and reverse primers, 1 µl of template DNA and 20 µl of nuclease free water and 2 µl of MgCl₂. PCR amplification was performed as follows: 2 min at 95 °C for initial denaturation of template DNA, 2 cycles; 30 s at 95 °C for denaturation, 35 cycles; 40 s at 56 °C for primer annealing, 1hr at 72 °C for extension, and 5 min at 72 °C for the final extension. The PCR product was analyzed by electrophoresis on 1% TAE agarose (Agarose 3:1, Melford Laboratories Ltd., Ipswich, UK) gel containing ethidium bromide (0.2 µg/ml) in 1x TAE running (40 Mm Tris base, 20 mM acetic acid, 1 mM EDTA) at 75 volts for about 90 min. Samples contained 10 µl of PCR product and 2 µl of loading dye (Promega). The gel was viewed under a UV transilluminator (GelDoc XR, Biorad) and the gel pictures were recorded by using the GelDoc

RESULTS

Tables 1 and 2 report the DNA quality and yields obtained from petrochemical industrial effluent isolates using two different DNA extraction methods: the Ethanol precipitation method and Guanidium thiocyanate EDTA sarcosyl buffer (GES). The extracted DNA were assessed for quality and quantity using nanodrop. The quantity of DNA in Table 1 using ethanol precipitation method ranged from -1.8-90.2 ng/µl with a quality of -3.68-26.89 (260/280) and -0.08-3.88 (260/230) while DNA extracted using GES had a better quantity and quality DNA with a range of -0.80-615.50 ng/µl

and -0.36-28.30 (260/280) and -0.08-3.88 (260/230) respectively as shown in Table 1.

Table 1: Quantity and Quality of DNA Extracted from Petrochemical Industrial Effluent Bacterial Isolates Using Ethanol Precipitation Method

| Isolates | Quantity (ng/μl) | Quality 260/280 (ng/μl) | Quality 260/230 (ng/μl) |
|---------------------------|------------------|-------------------------|-------------------------|
| <i>Bacillus</i> | 90.2 | 1.65 | 1.57 |
| <i>Serratia</i> | -0.3 | -3.68 | 0.02 |
| <i>Lactobacillus</i> | -1.0 | 0.68 | 0.08 |
| <i>Aeromonas</i> | -1.0 | 3.04 | 0.02 |
| <i>Enterococcus</i> | -0.1 | 26.89 | 0.02 |
| <i>Staphylococcus</i> | 0.0 | 0.04 | 0.00 |
| <i>Enterobacteriaceae</i> | 30.1 | 1.43 | 0.17 |
| <i>Proteus</i> | 1.2 | 0.98 | 0.05 |
| <i>Micrococcus</i> | 1.5 | 1.34 | 0.03 |
| <i>Escherichia</i> | -0.7 | 1.48 | 0.28 |
| <i>Klebsiella</i> | -1.8 | 5.70 | 0.04 |
| <i>Vibrio</i> | -0.2 | -0.36 | 0.00 |
| <i>Alcaligenes</i> | 54.4 | 1.31 | 0.76 |
| <i>Achromobacter</i> | 13.6 | 1.32 | 0.28 |
| <i>Citrobacter</i> | 0.9 | 1.36 | -0.08 |
| <i>Flavobacterium</i> | -0.6 | 0.88 | 0.39 |
| <i>Acinetobacter</i> | 0.5 | 1.81 | 0.16 |
| <i>Azotobacter</i> | 0.3 | 1.41 | 3.88 |
| <i>Salmonella</i> | 0.8 | 1.31 | 0.15 |
| <i>Pseudomonas</i> | 0.2 | 0.21 | 0.00 |

Figure 1 presents the diversity of the isolated and identified microorganisms from the study. The prevalence of Gram-negative bacteria was observed with Gram positive bacteria i.e. *Bacillus*, *Micrococcus*, *Staphylococcus* and *Lactobacillus* species making only 21.68% of the identified bacteria. *Pseudomonas*, *Bacillus*, and *Serratia* species were

the most predominant bacteria identified making up 10.33, 10.00 and 9% respectively. *Azotobacter*, *Lactobacillus* and *Salmonella* species were the least prevalent making up 1% each. *Aspergillus*, *Penicillium* and *Fusarium* species were the predominant fungi identified accounting for 19.58% each while *Cladosporium* and *Trichoderma* had the least frequency of isolation.

Table 2: Quantity and quality of DNA Extracted from Petrochemical Industrial Bacterial Isolates using Guanidium Ethylenediaminetetraacetate (EDTA) Sarcosyl Buffer (GES) Extraction Method

| Isolates | Quantity (ng/μl) | Quality 260/280 (ng/μl) | Quality 260/230 (ng/μl) |
|---------------------------|------------------|-------------------------|-------------------------|
| <i>Bacillus</i> | 615.50 | 25.35 | 28.99 |
| <i>Serratia</i> | 87.10 | 5.66 | 9.77 |
| <i>Lactobacillus</i> | 410.50 | 3.68 | 4.08 |
| <i>Aeromonas</i> | 66.00 | 3.04 | 1.02 |
| <i>Enterococcus</i> | 535.4 | 28.30 | 26.22 |
| <i>Staphylococcus</i> | 10.00 | 0.08 | 0.03 |
| <i>Enterobacteriaceae</i> | 130.10 | 21.43 | 20.17 |
| <i>Proteus</i> | 111.20 | 10.98 | 6.05 |
| <i>Micrococcus</i> | 11.50 | 3.34 | 1.03 |
| <i>Escherichia</i> | 2.37 | 1.48 | 1.08 |
| <i>Klebsiella</i> | 1.80 | 5.70 | 0.04 |
| <i>Vibrio</i> | 3.20 | -0.36 | 0.00 |
| <i>Alcaligenes</i> | 54.40 | 3.31 | 1.76 |
| <i>Achromobacter</i> | 11.6 | 1.32 | 0.28 |
| <i>Citrobacter</i> | 10.90 | 1.36 | -0.08 |
| <i>Flavobacterium</i> | 107.90 | 5.58 | 2.94 |
| <i>Acinetobacter</i> | 0.50 | 1.81 | 0.16 |
| <i>Azotobacter</i> | 0.30 | 1.41 | 3.88 |
| <i>Salmonella</i> | 0.80 | 1.31 | 0.15 |
| <i>Pseudomonas</i> | 611.6 | 24.55 | 31.43 |

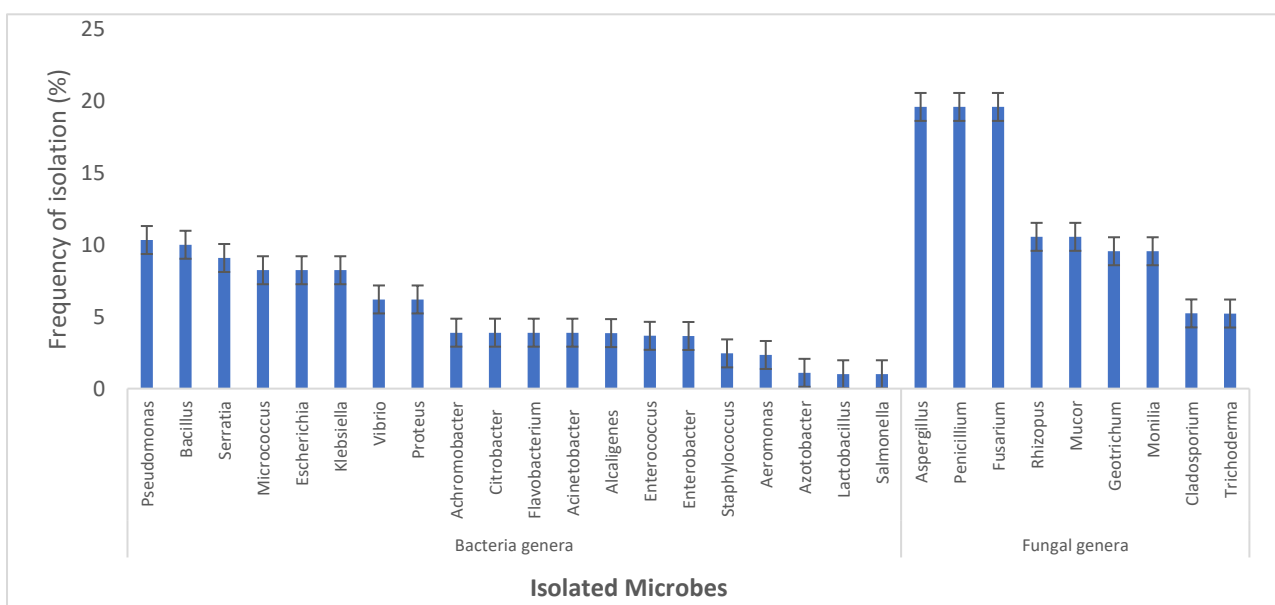


Fig. 1: Microbial isolates from petrochemical industrial effluent and their frequencies of isolation

Figures 2-4 show the results of three different sets of primers (16SF & 16SR; V3F & V3R & V6-V8) stained on 1% agarose gel to determine the primers that will provide the best DGGE differentiation of bacterial isolates from petrochemical industrial effluent. No amplification in Figure 2. There was partial amplification in Figure 3 as V3F&V3R primers partially enabled the 16S rDNA fragments of bacterial isolates amplified with a size of 200 bp. In Figure 4, V6V8F & V6V8R primers enabled all the 16S rDNA fragments of bacterial isolates amplified with the expected size of 500 bp.

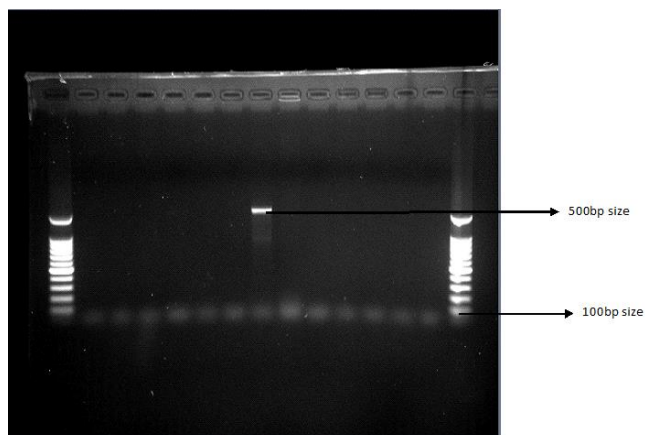


Fig. 2: Ethidium bromide-stained 1% agarose gel showing product size using 16SF and 16SR primers for amplification of 16S region of 16S rDNA of bacterial isolates from petrochemical industrial effluent.

DISCUSSION

The prominent bacterial genera identified in the effluent of Eleme Petrochemical Industrial were *Pseudomonas*, *Bacillus*, *Serratia*, *Micrococcus*, *Escherichia*, *Vibrio*, *Proteus*, *Achromobacter*, *Citrobacter*, *Flavobacterium*, *Alcaligenes*, *Enterococcus*, *Enterobacter*, *Staphylococcus*, *Aeromonas*, *Azotobacter*, *Lactobacillus*, and *Salmonella* species. This finding is consistent with the findings of Chikere and Okpokwasili (2004), who obtained similar isolates from the outfall site of petrochemical effluent in Eleme. It also aligned with other studies elsewhere (Goveas et al., 2020; Sarfo et al., 2023). *Pseudomonas* emerged as the predominant bacterial genus, constituting 10.33% of the total occurrences. The prevalence of *Pseudomonas* in petrochemical industrial effluent has been documented (Zhang et al., 2005). Additionally, Beal and Betis (2000) reported that *Pseudomonas* sp. possesses rhamnolipids capable of converting crude oil into both cell mass and biosurfactant.

Metagenomic DNA extraction from bacterial isolates in the petrochemical industrial effluent was crucial for the validity of this study. However, effluent DNA extraction presents challenges, including incomplete lysis, DNA adsorption to effluent surfaces, co-extraction of enzymatic inhibitors, yield loss, and DNA degradation or damage (Miller et al., 1999; Kumblathan et al., 2021).

This study compared two DNA extraction methods, ethanol precipitation, and guanidium thiocyanate

ethylenediaminetetraacetate (EDTA) sarcosyl buffer (GES), to identify a high-yield technique for obtaining PCR-quality DNA from isolated bacteria in petrochemical effluent. Results indicated a higher recovery of DNA with the GES method compared to ethanol extraction. The two most predominant bacterial isolates, *Pseudomonas* and *Bacillus*, exhibited higher yields and better DNA quality with the GES method than with ethanol precipitation.

The use of guanidium thiocyanate was advantageous in inactivating endogenous nucleases during DNA extraction. The de-proteinization with GES effectively separated DNA from proteins and cell debris. PCR experiments using various primers demonstrated successful amplification with V6V8F and V6V8R primers, confirming their suitability for PCR amplification of bacterial DNA from the effluent.

CONCLUSION

In conclusion, the study highlighted the diverse nature of indigenous bacteria in petrochemical effluent, attributed to its composition of process wastewater and office wastewater. The comparative evaluation of primers revealed that V6V8F and V6V8R primers were most suitable for PCR amplification of effluent bacterial DNA. Successful amplification was confirmed through ethidium bromide analysis on 1% agarose gel.

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