

Comparative Analysis of some DNA Extraction kits used for Molecular Analysis of Iron Ore Samples

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Abstract:

Bioleaching simply refers to the microbial-catalyzed process of conversion of insoluble metals into their soluble forms. As an applied biotechnology, it represents an extremely interesting field of research where genomic and proteomic techniques can be used in terms of knowledge development, but more in terms of process design, control, and optimization. Good quality DNA is a prerequisite for all experiments requiring DNA manipulation. DNA isolation is often the most fundamental and crucial in the entire process. As a result of different DNA isolation kits constantly becoming available, it has become imperative to compare their efficacies. This study compared some popular isolation kits used on bioleaching microorganisms from an Iranian Iron mine with that of Zymo research kit used on Agbaja Iron stones of Nigeria. The Iron mine of Agbaja in kogi State possess similar characteristics with those of the Iranian Iron mine-hence the choice of the comparative protocols. Modified CTAB method (Sigma) and kits of the QIAmp Investigator (Qiagen) and Accuprep Genomic DNA (Bioneer) were used to compare to that of ZR Genomic DNA (Zymo Research) used in this work. Spectrophotometric results showed that the highest concentration of DNA from 100µl of bioleaching bacteria culture was obtained by Qiagen kit (165.50µg/µl) followed by Zymo kit (50.40µg/µl), Bioneer kit (34.33µg/µl) and CTAB (23.65µg/µl). The Bioneer test took a total of 40 minutes to isolate bacterial DNA, followed by Zymo kit, 45 minutes, Qiagen, 50 minutes, and the CTAB 60minutes. These preliminary findings may serve as future baseline information in helping laboratories/Researchers make appropriate decisions in process optimization for isolating quality DNA especially from bioleaching microorganisms.

Keywords: Bioleaching, DNA Isolation, Iron Mine, Extraction Kits, Agbaja, Nigeria

Introduction:

The employment of microorganisms for metal recovery from low-grade ores, mineral concentrates, and secondary materials, has developed into a successful and expanding area of biotechnology (Appia-Ayme et al. 2006). In association with this interest, microbial communities of extreme acidophilic prokaryotes from bioleaching environments have long been the subject of active research; however, the components and interactions within these microbial communities' remains poorly understood. Recent acquisition of genomic data directly from organisms living in naturally extreme environments in combination with genome sequencing projects of individual species provides a novel

opportunity for prediction and exploration of the metabolic details that control both individual microorganisms and microorganism communities (Parro et al. 2007; Ram et al. 2005; Huggler et al. 2005). Acidophilic prokaryotes involved in metal recovery from sulfide minerals include members of the Bacteria and Archaea domains. Three species of chemolithotrophic bacteria are mainly involved: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum sp.*, all of which obtain energy primarily from iron and/or sulphur oxidation (Justice et al; 2014). *A. ferrooxidans* is capable of oxidizing reduced sulfur compounds and Fe²⁺ ions to form sulfate and Fe³⁺, respectively.

A. thiooxidans can only oxidize reduced sulfur compounds such as thiosulfate, tetrathionate, metal sulfides and elemental sulfur to form sulfate. *Leptospirillum* sp. is solely capable of oxidizing Fe²⁺ ions to form Fe³⁺. These autotrophic microorganisms utilize the energy and reducing power derived from iron and or sulfur oxidation for several metabolic processes, including CO₂ fixation and acquisition of several sources of nitrogen (Pandey and Pandey, 2011). In both *Acidithiobacillus* species, CO₂ fixation occurs via the Calvin-Benson-Bassham cycle whereas *Leptospirillum* sp. grows autotrophically; however the molecular mechanisms involved in carbon fixation remain obscure. In acidic bioleaching environments, dissolved inorganic carbon can reach levels below atmospheric concentrations average. Therefore, it is not surprising that CO₂ concentrating mechanisms have been identified in autotrophic prokaryotes present in such environments (Kenedy, 2015).

The environmental consequences of mining and quarrying are vast, for example habitat erosion, soil contamination and water contamination are some of the direct disadvantages. Due to an increase in consumerism, heightened dependence on technology, and an overall world population growth, more and more minerals and metals need to be extracted from the earth's surface. Even though the recycling industry is growing, it cannot meet these growing demands for minerals. Furthermore, with numerous accessible mining sites already exploited, the human race is in a constant struggle to find feasible mining methods.

One way to mitigate the environmental consequences of mining and quarrying is through the use of bioleaching microbes. These "rock munching microbes" such as the *Acidithiobacillus* and *Leptospirillum* bacterium, can clean up abandoned mine sites in a more environmentally friendly manner (Hettich, 2005). Kogi State of Nigeria is blessed with iron ore reserve, the main ones been Itakpe and Agbaja (about 2 billion tonnes of unexplored iron ore reserve). The economic gains that will arise from exploitation are very enormous that is in terms of employment generation and rapid industrialization.

Though the environmental aspect of these bacteria in bioleaching is very good, the efficiency of this process needs further studies, hence, the need to investigate the molecular basis of this challenge. The molecular characteristics of these isolated organisms may become the fulcrum for future cloning activities. However, all these begin with a carefully planned method for extracting DNA especially with the use of affordable and readily available kits. Good quality DNA is a prerequisite for all experiments of DNA manipulation.

The isolation and purification of DNA from cells is one of the most common procedures in contemporary molecular biology and embodies a transition from cell biology to molecular biology (in vivo to in vitro). The main aim of this work therefore, is to compare the efficiency of some already documented DNA isolation

kits with those of Zymo research practically applied in this work. This is with a view to investigating the comparative advantage or otherwise of the Zymo Kits for rapid DNA analysis of some bioleaching microorganisms.

Materials and Method:

Sample Collection:

Ore samples were collected from various drilled holes sites in Agbaja Iron Ore mine of Kogi state, Nigeria using sterile polypropylene bags and special geological forks. Samples were maintained on ice and immediately taken to the Laboratory for further work.

Bacterial growth and Culture Conditions:

The bacteria species used in this study were those previously isolated and identified in the preliminary stage of this research. They included *Acidithiobacillus* and *Leptospirillum* species. The bacteria were cultured in a modified 9k medium containing (per litre): KH₂PO₄ (0.4 g), CaCl₂.2H₂O (0.2 g), MgSO₄.7H₂O (0.4 g), (NH₄)₂SO₄ (0.4 g), FeSO₄.7H₂O (33.3 g), pH: 1.5-2 in 15g of nutrient Agar at 30°C and 180 rpm. The last bacteria were grown in LB medium at 37°C. Ore samples were treated with H₂SO₄ (0.5 N) for three weeks, then, crushed into small particles (0.2-0.5 µm in diameter). Cultures in 9k medium were incubated for 1 week until growth was observed microscopically or until a chemical change occurred in the medium compared with un-inoculated control. In cultures the growth was accompanied by a characteristics ferric precipitation and orange colouring of medium. Solid media were gelled with 0.6% (w/v) agarose.

DNA Extraction:

ZR fungal/Bacterial DNA Miniprep™ kit (Zymo Research) was used for the extraction of DNA from bacteria isolated from Agbaja Iron ore samples of Kogi state. The efficiency of the kit was then compared with those of AccuPrep kit from Biosensor (catalogue Number 6540-400), modified CTAB method, and QIAamp DNA Investigator Kit (Qiagen) recorded by some literature elsewhere (Sepalin et al. 2008) so as to look at the merit or demerit of our chosen protocol. DNA extraction procedures were performed by following the manufacturer's instructions. Extracted samples were stored at -20°C for further analysis.

AccuPrep Genomic DNA Extraction Kit (Bioneer), (as described by Sepalin et al. 2010):

The AccuPrep Genomic DNA Extraction Kit was purchased from Bioneer Corporation. The procedure was as follows: 20µl of Proteinase K was added to a 1.5 ml micro centrifuge tube followed by 100µl of bacterial cell culture and 200µl of Binding buffer. The sample was mixed immediately by vortex mixer. After 10 minute incubation at 60°C, 100µl of Isopropanol was added to the sample

and mixing performed by pipetting. The lysate was transferred to a filter column in a micro centrifuge tube. After centrifugation at 8,000 rpm for 1 minute, the filter was washed with Wash buffer 1 and then Wash buffer 2. Finally, the genomic DNA was eluted by use of 200 µl elution buffer. According to the manufacturer, the AccuPrep Genomic DNA Extraction Kit can isolate an average of 6µg of total DNA from different sources, such as 200µl of whole blood, 5 x 10⁶ leukocytes and 25-30µg bacterial and mammalian tissues. PCR was carried out according to the manufacturer's instruction.

Cetyltrimethyl ammonium bromide (CTAB) method (as described by Sepalin et al. 2008):

An equal volume of CTAB dilution precipitation buffer was added to the solution containing crude DNA from the bacterial culture of 100µl. The CTAB-nucleic acid salts were pelleted by microcentrifugation at 8000 rpm for 10 minutes and the precipitate was washed 2-3 times with 300ml of cold wash solution to remove any free CTAB. The CTAB-nucleic acid pellet was dissolved in 100ml salt dissolving solution and the DNA was precipitated with 2 volumes of 95% ethanol and microfuging for 10 minutes. The resulting DNA pellet was washed with cold 70% ethanol and resuspended in deionized water.

The crude DNA solution was made up to 0.5 ml with distilled water and an equal volume of phenol-chloroform-isoamylalcohol was added, inverted and microcentrifuged at 11000 rpm for 5 minutes. The aqueous phase was removed and added to an equal volume of chloroform-isoamylalcohol, inverted and again microcentrifuged for 5 minutes. The aqueous phase was removed and the DNA was precipitated from it with either an equal volume of propan-2-ol, or 2 volumes of ethanol and 0.1 volumes of sodium acetate and chilled at -20°C. The resulting DNA pellet was washed with cold 70% ethanol and re-suspended in deionize water and the resulting DNA from the extractions/purifications were visualised for quality on 1 % agarose gels. PCR was the performed as described by the same Author above.

QIAamp DNA Investigator Kit (as described by Sepalin et al. 2008, Saiyed et al. 2008):

The QiAamp DNA Investigator Kit was purchased from Qiagen Company. Isolation of DNA began with the addition of 10µl of proteinase K to 100µl liquid broth bacterial culture and 100µl Buffer AL. After 10 minutes of incubation at 56 °C, 50µl ethanol was added. The mixture was incubated for 3 minutes at room temperature and then transferred to a QIAamp MinElute column and centrifuged at 8,000 rpm for 1 minute. The column was washed with 500µl W1 buffer and also with 700µl W2 buffer by centrifugations at 8,000 rpm for 1 minute each. The column was washed with 700µl ethanol. The filter-bound DNA was eluted with 50µl of triethyl (TE) buffer. Yields and quality of the DNA isolated by the three kits were measured by use of a Nano Drop ND-1000 Spectrophotometer (Thermo Scientific). DNA quality was assessed

using the absorbance ratio at 260:280 nm. DNA and protein absorb differently at these wavelengths. An A₂₆₀/A₂₈₀ ≥ 1.8 is generally accepted as "pure" for DNA; a ratio of ≥ 2.0 indicates that the preparation is contaminated with RNA; a ratio of ≤ 1.8 indicates a possible contamination with proteins or other contaminants that absorb strongly at or near 280 nm. The quality of the isolated DNA samples were also analyzed by electrophoresis on 1.0% agarose (wt/vol) gels using Tris, Borate EDTA pH 8.3 buffer and ethidium bromide staining. Gel imaging was done by using a Fluor Chem Q Analyzer from Alpha Innotech Company. PCR then followed according to the manufactures instruction.

ZR fungal/Bacterial DNA Miniprep™ kit (Actually carried out in this work):

The ZR fungal/Bacterial DNA Miniprep™ kit was obtained from Zymo Research Company. The methodology used was described on the kit's manual. About 100mg (100µl) of the cell isolate was scooped under the laminar flow hood, suspended in 750µl lysis buffer and lysed. It was then vortexed for about 5 minutes and transferred to the centrifuge to spin at 12,000 rpm for about 3 minutes. The supernatant was transferred into the Zymo-spin™ TM spin filter; it was then spin at 10,000rpm for about 2 minutes. 1,200µl of Bacterial DNA binding buffer was added to the filtrate, thereafter, it was transferred to zymo spin IIC column and spin at 12,000rpm for 2 minutes. Then, DNA pre-wash buffer was added to column and centrifuge at 12,000rpm for 2 minutes. Also, fungal/bacterial DNA wash buffer was added and centrifuge at 12,000 rpm for about 2 minutes. The column was transferred into clean 1.5ml micro tube and 20-100µl of DNA elution was added to concentrate it, then it was spin at 12,000rpm for about 1 minute to elute the DNA. Finally, electrophoresis was carried out on the DNA sample.

Determination of concentration of nucleic acids:

The concentration and purity of nucleic acids aqueous solution was determined by measuring absorbance at 260 nm and 280 nm in a quartz cuvette of 1 cm path length against distilled water blank in a Hewlett Packard 8453 spectrophotometer. The nucleic acid concentration was determined on the assumption that an A₂₆₀ of 1.0 was equivalent to the following concentrations: oligonucleotides (20µg/ml), RNA (40µg/ml) and double-stranded DNA (50µg/ml), (Sinnott et al. 1998). The purity of nucleic acid samples was investigated by determining the ratio of Abs₂₆₀/Abs₂₈₀. A ratio of >1.5:1 indicated relatively pure DNA with no contaminating proteins or polysaccharides.

Results and Discussion:

The three DNA Extraction Kits were used to isolate genomic DNA from an Iranian iron ore samples whose characteristics were similar to those of Agbaja Iron ore of Kogi state and the yields were measured by uv-vis spectroscopy (all results were then compared to those of Zymo Research kits which was actually carried out in this project). The results in Table 2 showed that the

Table 1: Characterization of the DNA extraction kits used in comparison with the Zymo Kit

Method	Name of Kit	Vendor	Main Features
1	Accuprep Extraction kit	Bioneer	Proteinase K digestion and DNA binding to glass fibre
2	CTAB (Modified manual method)	Sigma	Combination of different lysing Chemicals for DNA extraction
3	Q1Amp Extraction kit	Qiagen	Proteinase K digestion and DNA binding to silica gel
*4	Zymo fungal/bacterial miniprep Ex- traction kit	Zymo Research	Genomic DNA lysis and special Zymo beads bind DNA and water elution

*Actual method carried out in this work and used as a standard for comparing with the others Informations from the other methods were based on their manufacturer’s manual

DNA extraction method	First Extraction (µg/µl)	Second Extraction (µg/µl)	Mean (X) (µg/µl)	StdDev (µg/µl)
Accuprep	34.43	36.55	35.49	1.5
CTAB	24.50	22.80	23.65	1.2
Q1Amp	150.35	180.65	165.50	21.43
*Zymo	48.68	52.12	50.40	2.4

Table 2: DNA yield for the different methods as compared to that of Zymo Research

Accuprep DNA concentrations were 34.43µg/µl, and 36.55µg/µl, respectively. The average concentration was 35.49µg/µl. The average total DNA yield from a cell culture of 1x10⁷cfu/ml from a starting cell culture of 6.4x10⁵cfu/ml (Table 4) is 6.554µg. The results of uv-vis spectrophotometry for DNA isolated with the CTAB modified method are shown in Table 3. The concentrations of the two extractions of DNA from Table 2 were 24.50µg/µl, and 22.8µg/µl. The average concentration was 23.65µg/µl. The total DNA yield from a starting cell culture of 5.3x10⁸ is 2.073µg. The results from uv-vis spectrophotometry of DNA isolated with the QIAamp DNA Investigator Kit are presented in Table 2. The results show that the concentrations of DNA isolated from cell culture samples were 150.35µg/µl, and 180.65µg/µl.

The average concentration of the isolated DNA was 165.50µg/µl. The total DNA yield in a starting culture of 1.4x10⁶ was 15.375µg. The results in Table 3 for DNA isolated by use of the Bioneer Accuprep Genomic DNA Extraction kit showed that the ratio of sample absorbance at 260 nm and 280 nm were: 1.42 and 1.40. The average A260/280 was 1.41. DNA isolated using the QIAamp DNA Investigator Kit gave ratios of sample absorbance at 260 nm and 280 nm as 1.81 and 1.83, respectively (Table 3). The average

ratio of A260/280 absorbance was 1.82. DNA isolated by using the CTAB modified Genomic DNA Extraction method yielded ratios of 1.93 and 1.88 with an average of 1.91 (Table 3). The results in Table 3 for DNA isolated using the Zymo Genomic DNA Extraction Kit showed A260/A280 nm ratios of 1.87 and 1.83 (Table 3).

Discussion:

Molecular methods have increasingly been used for the detection and quantification of diverse kinds of microbes. Moreover, the need for a rapid and easy molecular detection and quantification technique is imperative (Sinnet et al; 1998). An equally important step in nucleic-acid-based quantification of bacteria is sample processing preceding the actual assay, as has previously been mentioned in other literature. It was observed that the DNA obtained by QIAamp DNA Investigator Kit produced the highest concentration on an average of 165.50µg/µl, while the other two kits only yielded 50.40µg/µl (ZR Genomic DNA) and 35.49µg/µl (Accuprep) and the modified CTAB method yielded 23.65µg/µl from 100µl of the bacterial culture.

The significance of these results was seen in the large difference

DNA method	extraction First Extraction (std yield=1.8)	Second Extraction (std yield=1.8)	Mean (X) (std yield=1.8)	StdDev (std yield=1.8)
Accuprep	1.42	1.40	1.41	0.01
CTAB	1.93	1.88	1.91	0.01
Q1Amp	1.81	1.83	1.82	0.04
*Zymo	1.87	1.83	1.85	0.03

Table 3: DNA purity as a ratio of A260/A280 for the methods as compared to that of Zymo

Method	DNA conc(µg/µl)	DNA purity (std yield=1.8)	DNA yield per 1x10 ⁷ cfu/ml	Time for assay (minutes)
Accuprep	35.49	1.41	6.554µg	40
CTAB	23.65	1.91	2.073 µg	60
Q1Amp	165.50	1.82	15.375 µg	50
*Zymo	50.40	1.85	10.425 µg	45

Table 4: Cost benefit of the Extraction kits compared to that of Zymo Research

in the DNA concentration between ZR Genomic DNA kit and the other two kits along with the modified CTAB method. Measurement of DNA purity by using A260:A280 ratio showed an average DNA ratio of 1.82 for the DNA isolated with Q1Amp DNA Investigator Kit, and a ratio of 1.85 for the DNA isolated with ZR Genomic DNA extraction kit. A ratio of 1.41 and 1.91 was obtained for the DNA isolated by the Accuprep DNA extraction kit and CTAB respectively which indicated the presence of contaminants that absorb strongly at or near 280nm. Based on this criterion Q1Amp DNA Investigator Kit produced the highest purity DNA. Processing time varied considerably between the kits, ranging from 40 minutes for completion of the Accuprep Genomic DNA extraction kit to 60 minutes for the modified CTAB method (Table 4).

The results of this study confirm that DNA extraction is a key step in this process. Several strategies can be used for the extraction of DNA from microbial samples, such as enzymatic, chemical/thermal lysis, mechanical disruption of the cell wall by beads or sonication, or a combination of all of the above. A disadvantage of enzymatic lysis is that commercially available enzymes can be contaminated with microbial DNA. Highly sensitive and specific nucleic-acid-based methods for the detection of bacteria necessitate the use of DNA extraction reagents that are free from contaminating bacterial nucleic acids. In addition, the use of enzymes often requires special conditions and preservation requirements, such as refrigeration and buffer storage.

Chemical lysis often involves the use of aggressive and toxic chemicals, which is less desirable for on-site detection where laboratory safety conditions are absent (Butler, 2005). DNA extraction efficiency is limited by several factors (as shown in table 1). An important factor is cell concentration, which can be

a limiting factor for the quantification of natural samples where cell concentrations will vary. At low concentrations, method sensitivity will play a role and at high concentrations, overloading of the method can reduce DNA extraction efficiency. All the merits of the extraction methods compared with that of Zymo used in this work are summarized in tables 1-4 in the result section. For Accuprep extraction method, problems can arise for certain harder-to-lyse species at concentrations above 10⁷ cells ml⁻¹. A second factor is the type of bacterial species that is being subjected to the extraction. For instance, the constitution of the bacterial cell wall may impede cell lysis and the liberation of DNA from the cell. A third factor may be the physiological state of the cells that are subjected to cell lysis, in which context, it can be noted that the cells used in the present study were of liquid cultures in stationary phase. Another factor that may influence the outcome of quantification is the size of the DNA fragments generated. Long fragment sizes generated by the DNA extraction method may result in incomplete denaturation during the thermal cycling of qrtPCR, whilst, on the other hand, short fragment sizes may result in reduced amplification efficiency. The QIAamp® DNA Mini Kit was developed and commonly used for a variety of bacterial species. QIAamp® DNA Mini Kit(QIAGEN GmbH, Hilden, Germany), total DNA can be purified from a variety of biological, clinical and forensic specimens.

The method uses chemical lysis by undisclosed lysis buffers containing chaotropic salt, enzymatic digestion by treatment with proteinase K and thermal lysis (Gullsbj, 2008). The method uses a separate protocol for Gram-positive bacteria, with the additional use of enzymatic digestion by lysozyme or lysostaphin with Triton. DNA is bound to a silica-gel membrane in a spin-column, whilst PCR inhibitors are supposedly, not retained (Table 1). The bound

DNA is then washed, eluted in buffer and can then be stored at -20°C . overloading of the column of the QIAamp® DNA Mini Kit may lead to significantly lower yields than expected, according to the manufacturer's instructions.

The CTAB method was also developed for use with a broad spectrum of bacterial species. To our knowledge, the use of CTAB Elute for qualitative/quantitative bacterial estimation has been the most popular method till date. The main constituents and boiling procedure of the method have also been used for other species. However, as confirmed by our results, it must be taken into account that inter-species and even intra-species differences in DNA extraction efficiencies will exist, influencing the outcome of microbial quantification. In summary, most of the DNA extraction protocols described here have been found to be comparatively costly and time consuming and may lead to substantial loss of some DNA for molecular analysis.

However, they all have the following advantages: Average to Good yields ((Accuprep -CTAB-QIAmp and Zymo) of high quality genomic DNA, Circumvention of the use of liquid nitrogen for crushing of the bacterial biomass, Reduction in the extraction steps, Minimal requirement of chemicals and equipment needed for lysis/extraction and elimination of toxic and potentially hazardous substances. (Tables 1-4). High molecular weight DNA ($>1.0\text{kb}$, figure 2) resulted from the use of the Zymo method used in this work.

The ratio of the absorbance at 260nm and 280nm was found to be 1.85 indicating a good purity with very little smear on the DNA. The isolated DNA produced good banding patterns for *Acidithiobacillus* and *Leptospirillum* species indicating its good quality. The entire DNA extraction kit also worked well in releasing large quantities of DNA from the bacteria culture used (Table 4). The DNA isolated from the bacteria culture was well resolved on 1% agarose gel. This shows that the isolated DNA is free of polysaccharides and polyphenols which are known to inhibit taq DNA polymerase and restriction endonucleases. DNA eluted from the kits could ensure a very suitable DNA for amplification using PCR.

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