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# Molecular characterization of the isolated microorganisms collected from local rice beer (Kiad)

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**Abstract**
The ribosomal RNAs, particularly 16S rRNA, have proven the most useful for establishing distant relationships because of their high information content, conservative nature, and universal distribution. The genomic DNA was isolated using the Wizard genomic DNA isolation and purification kit (PROMEGA) and 16SrRNA gene was amplified which was used for molecular characterization and phylogenetic analysis of the bacterial isolates.

**Keywords:** 16S rRNA, PROMEGA *etc.*

## Introduction
The molecular genetics is concerned with the current knowledge of the molecular nature of genes, their roles in controlling the function and development of organisms, their inheritance, and their evolution. All of the available molecular methods for evaluating phylogenetic relationships (e.g., DNA-DNA and DNA-RNA hybridization, 5S rRNA and protein sequencing, 16S rRNA oligonucleotide cataloging, enzymological patterning, etc.) have advantages and limitations. In general, macromolecular sequences seem preferred because they permit quantitative inference of relationships. Moreover, because they accumulate, sequences are most useful in the long term. Of the macromolecules used for phylogenetic analysis, the ribosomal RNAs, particularly 16S rRNA, have proven the most useful for establishing distant relationships because of their high information content, conservative nature, and universal distribution [1].

## Materials and Methods
### Isolation of DNA
DNA isolation was done by using the Wizard genomic DNA isolation and purification kit (PROMEGA) [3]. In this process, 1.5ml of the overnight culture was centrifuged at 13,000-16,000 rpm for 2 minutes. The supernatant (culture medium) was discarded completely. The pellet was suspended in 600µl of nuclei lysis solution and mixed. The suspension was incubated for 5 minutes at 80°C and then cooled to room temperature. 3µl of RNAse solution was then added and mixed well. The mixture was incubated at 37°C for 60 minutes. 200µl of protein precipitation solution was added. The mixture was vortexed properly for 15 seconds and then incubated in ice (4°C) for 5 minutes. Centrifugation was then followed at 13,000-16,000 rpm for 3 minutes. The supernatant was then transferred to clean tube containing 600µl isopropanol and mixed well. Centrifugation was done again at 13,000-16,000 rpm for 3 minutes and the supernatant was decanted. To the pellet, 600µl of room temperature 70% Ethanol was added, mixed and centrifuge for 2 minutes at 13,000-16,000 rpm. The supernatant was discarded and excess ethanol was then aspirated and the pellet was air-dried. The pellet was then rehydrated in 20-30 µl of rehydration solution for 1 hour at 65°C. The DNA obtained was resolved in agarose gel [4].

### Agarose gel electrophoresis of the isolated DNA
The presence of DNA in the sample was confirmed by performing agarose gel electrophoresis. Submerged gel electrophoresis was used for this purpose. In this process, 0.24g of agarose was weighed and dissolved in 30ml of TAE buffer by boiling it in the microwave oven. It was cooled to about 50-55ºC and 4µl of Ethidium bromide was added and mixed properly. The solution was poured into the gel tray fixed with the comb and then allowed to solidify.

After solidification, the comb was removed and the gel was transferred to the electrophoresis unit containing TAE buffer. The buffer was poured till the gel is submerged. 3µl of the loading dye and 7µl of the DNA sample of each isolate was mixed and then loaded into the wells. The gel was allowed to run for an hour at 80V. Migration of the DNA was observed by looking at the position of the tracking dye (bromophenol blue). After 1 hour, the gel was taken out and the DNA bands were observed under the Gel documentation system.

**PCR amplification of 16S rRNA gene**

The isolated genomic bacterial DNA was used to amplify the 16S rRNA by PCR. The amplification was done by using the Universal bacterial 16S rDNA primers; forward primer (BSF/20; 5'-AGAGTTTGATCCTGGCTCAG-3') and the reverse primer (BSR/20; 5'-AAGGAGGTGATCCAGCCGCA-3'). The Master mix/ Cocktail were prepared by adding the following reagents for a 25µl reaction volume which includes: dNTPs- 3µl, Taq buffer-2.5µl, Taq polymerase-0.3µl, forward primer-3µl and reverse primer-3µl. To this volume (11.8µl), 3µl of template DNA was added. The final volume was made up to 25µl by adding 10.2µl of double distilled water. The PCR cycle used is: Initial denaturation at 94ºC for 5 minutes, 30 cycles consisting of denaturation at 94ºC for 1 minute, annealing at 55ºC for 1 minute, extension at 72ºC for 1 minute, and the final extension at 72ºC for 5 minutes. The PCR product is then stored at 4ºC [5]. The PCR process was carried out in the PCR machine and the PCR products of 16S rRNA gene were visualized by running in 1% agarose gel electrophoresis [2].

**Results and discussion**

Genomic DNA from all samples was isolated; DNA bands were observed (Figure 1). Genomic DNA isolation for Samples D and F was performed twice until respective DNA bands were obtained.

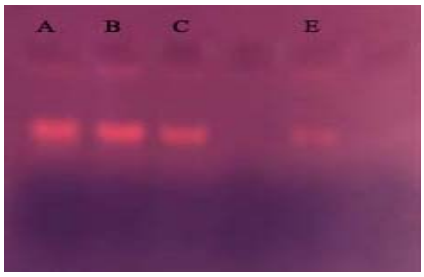


**Fig 1:** Agarose gel electrophoresis of isolated genomic DNA. (Lanes and samples: A, B, C and E)

16S rRNA genes were then amplified by PCR (Figure 2).

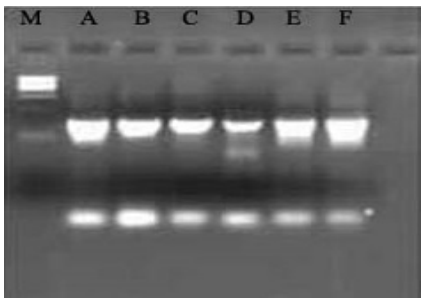


**Fig 2:** Agarose gel electrophoresis of the PCR product of amplified 16S rDNA gene. (M: DNA marker; Lanes and samples: A, B, C, D, E and F)

**References**

1. Reid MF, Fewson CA. Molecular characterization of microbial alcohol dehydrogenases. Critical Reviews in Microbiology. 1994; 20(1):13-56.
2. Pradhan D, Suri KA, Pradhan DK, Biswasroy P. Golden heart of the nature: *Piper betel* L. Journal of Pharmacognosy and Phytochemistry. 2013; 1(6):147-164.
3. Neeley ET, Phister TG, Mills DA. Differential Real-Time PCR assay for enumeration of lactic acid bacteria in wine. Applied and Environmental Microbiology. 2005; 71(12):8954-8957.
4. Hajal S, Begum SS. KIAD- A popular local liquor of *Pnar* tribe of Jaintia hills District, Meghalaya. Journal of Traditional Knowledge. 2007; 6(1):133-135.
5. Nagori K, Singh MK, Alexander A, Kumar T, Dewangan D, Badwaik H, Tripathi DK. *Piper betel* L.: A review on its ethnobotany, phytochemistry, pharmacological profile and profiling by new hyphenated technique DART-MS (Direct Analysis in Real Time Mass Spectrometry). Journal of Pharmacy Research. 2011; 4(9):2991-2997.