Comparative Study of Hot Beverages as Electrophoretic Buffers

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ABSTRACT

The pH of most beverages is relatively low, contributing to increased acidity levels. In this study, we report a rapid and cost-effective approach for nucleic acid characterization by employing various hot beverages as alternative running buffers for agarose gel electrophoresis. Commercially available DNA was subjected to electrophoretic analysis using different beverages after optimizing their pH values. The results indicate that Royal Stag exhibited minimal influence on nucleic acid migration and band intensity, whereas Peter Scot demonstrated enhanced resolution, concentration, and sensitivity in nucleic acid detection. Furthermore, parameters such as concentration, voltage, and electrophoretic time had negligible effects when using Royal Stag, while Peter Scot consistently yielded clearer and more defined bands. Overall, this approach presents a simple, efficient, and economical alternative to conventional TAE or TBE buffers for agarose gel electrophoresis.

Keywords: Antiquity, Peter Scot, Royal Stag, Bagpiper, TAE, Electrophoresis

INTRODUCTION

Agarose gel electrophoresis is a widely used technique for separating DNA fragments based on their size. The gel matrix is made of agarose, a linear polysaccharide derived from seaweed, which has a high gel strength that allows for the handling of low percentage gels suitable for separating large DNA fragments [1]. The size of the pores formed by the agarose bundles in the gel matrix is determined by molecular sieving, meaning that smaller DNA fragments can pass through the pores more easily than larger fragments. The performance of agarose gel electrophoresis is influenced by the properties of the electrophoresis buffer, such as pH, ion strength, and composition [2]. The choice of buffer can affect the migration of DNA fragments, and researchers often experiment with different buffer systems to optimize their results. Positive polarity buffer systems typically work over the entire pH spectrum, whereas negative net-charge nucleic acids are delayed on the gel surface in buffer systems with negative polarity

[3-5]. New methods have been developed to improve the resolution of agarose gel electrophoresis [6]. Multiple gradient-slab gel electrophoresis involves embedding several agarose gels with different concentrations within a single gel slab, allowing for more precise separation of DNA fragments. However, difficulties in using organic solvents with low buffer capacity can require buffer adjustments or the use of a buffer recirculation system [7]. Overall, agarose gel electrophoresis remains a widely used and versatile technique in biological sciences research [8]. With continued experimentation and development of new methods, it is likely to remain an important tool in molecular biology [9].

MATERIAL AND METHODS

Determination of pH in alcoholic beverages

Various drinks, including Bagpiper, Peter Scot, Sprite, Royal Stag, and Antiquity, were chosen from local market of Vijayawada, Andhra Pradesh, India, Ten millilitres of each sample were tested for initial pH and adjusted to typically a pH of 8.3 using either 1M NaOH or HCl. Using milliQ water or double-distilled water, the buffer was diluted to a concentration of 100 times, then stored at 40°C until needed [10].

Agarose gel electrophoresis

Genei's small mini-subcell (10 x 12 cm) was used for electrophoresis of the agarose gel and used beverages as a running buffer to classify nucleic acids. One microgram of commercial DNA (Salmon sperm DNA, SRL) is used as a loading sample along with a tracking dye. Beverages as buffers were measured using a DC-300 power supply (Genei Apparatus) with length, voltage, current and power of 300ml. Fifteen microliters of samples were prepared, and a CCD camera (UVI-Tech, Germany) stained gel with ethidium bromide for UV photography [11].

Quantification of gel band

ImageJ 1.38 (http:/rsb.info.nih.gov/nih-image/) is used as a method to measure the agarose gel band strength for the analysis of gel photos. Gel is coated with ethidium bromide and tested for UV-transilluminator band validation. Gels was put in a gel doc (UVI-Tech, Germany) and photographed. ImageJ is used for measuring the density profile, peak height, and peak intensity or band volume of the expected molecular weight [12].

RESULTS AND DISCUSSION

Determination of pH in beverages

Beverages are the most acidic according to American Dental Association. In fact, their acid content is in the same range as vinegar. Initial pH of all selected beverages was determined using pH meter (EuTech, Germany) after the pH sensor is calibrated as listed in table 1. Experiment was carried out for three times to obtain more accurate results.

Table 1: pH in units of several beverages

Name of the beverage	**pH in units**
Bagpiper	2.79
Peter Scot	2.58
Royal Challenge	4.58
Royal Stag	4.89
Antiquity	2.44
Distilled water (Negative control)	7
TAE Buffer (Positive control)	8.3

Agarose gel electrophoresis

Commercial DNA of 15ul was loaded in each well, and electrophoresis was performed at 50 volts for one hour at 50 volts. Each band contains a large quantity of DNA fragments of the same size, all of which have travelled to the same position as a group. Gel is stained with a DNA-binding dye and placed under UV light, allowing us to see the DNA present at various positions along the length of the gel. Water at neutral pH as negative control of the reaction is used as running buffer, while TAE buffer as positive control. No bands were observed with water as choice and high-density bands were observed with TAE buffer as shown in figure 1.

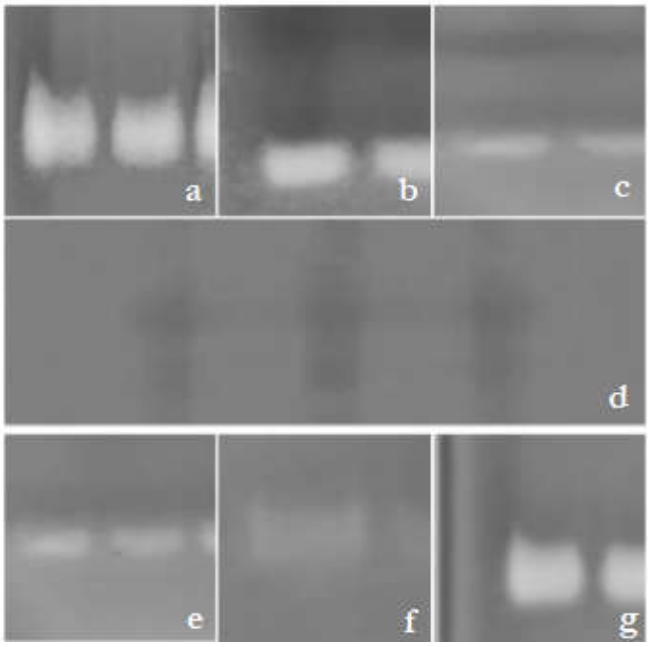

FIG. 1: Bands of DNA observed under UV-transilluminator and photographed using gel-doc. a-g: Bagpiper as buffer; Peter Scot as buffer; Royal Challenge as buffer; water as buffer; Royal Stag as buffer; Antiquity as buffer and TAE as buffer.

Quantification of gel band

The analysis of the ECL image was performed using the public domain ImageJ program (developed at the National Institutes of Health and available at http:/rsb.info.nih.gov / ij/), using the "Measure" function. The results of the analysis are a value for each band that is proportional to the Integrated Density Value (IDV) of the band. All photographs have been handled in a way that prevents the loss of information as shown in table 2.

Table 2: Quantification of band intensity using ImageJ

S.No	Label	Area	X	Y	Major	Minor	IntDen
1	Bagpiper	1548	81.500	27	145.561	13.541	266249
2	Peter Scot	3674	89.500	35	188.439	24.824	623466
3	Royal Challenge	1572	79.500	28	147.818	13.541	273148
4	Redbull	1449	84.500	25.500	181.669	10.155	227615
5	Antiquity	1056	93	29	198.595	6.770	168869
6	Water	380	30	33	42.878	11.284	64145
7	TAE	2700	95	19.500	203.108	16.926	389126.000

CONCLUSION

This study indicates that the described method is not intended to replace the conventional TAE buffer, but rather to serve as a preliminary approach for exploring potential trends in using beverages as alternative running buffers for nucleic acid characterization through agarose gel electrophoresis. Among the beverages tested—Peter Scot, Bagpiper, Royal Challenge, Antiquity, and Royal Stag—Peter Scot demonstrated the highest precision and sensitivity. However, these findings may vary with the inclusion of a broader range of beverages. The choice of running buffer and other experimental conditions should be carefully considered based on the specific research question and sample being analyzed. Nonetheless, the study provides some preliminary insights into the potential use of alternative running buffers for agarose gel electrophoresis in nucleic acid characterization.

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