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Evaluation of Viability of Lactic Acid Bacteria in a Nigerian Commercial Yogurt and its Antagonistic Effects on Selected Strains of Diarrheagenic *Eschericia coli*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Yogurt is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy food supplements. It provides an array of nutrients in significant amount in relation to its energy, fat contents and other health benefits. The micro organism in the final product must be viable and abundant enough to confer health benefits to consumers.

Objective: The objective of this study was to determine the viability of lactic acid bacteria in selected yoghurt at refrigerated and room temperature storage conditions and antimicrobial properties of the selected yoghurt against diarrheagenic *E.coli.*

Methods: The viability of LAB was assessed in Quincy yogurt for one month using pour plate method at refrigerated and room temperature storage conditions. The antimicrobial activities of LAB in the yoghurt against 6 diarrheagenic *E.coli* strains were assessed by coculture method at lower (10^4 cfu/ml) and higher (2.1 x 10^6 to 1.64 x 10^8 cfu/ml) pathogen concentrations.

Results: The viable count of LAB in yoghurt decline from 5.3 log10 cfu/ml to 4.2 log10 cfu/ml and 5.3 log10 cfu/ml to 4.4 log10 cfu/ml for room temperature and refrigeration respectively. No *E. coli* strain survived in yoghurt samples after incubation for 24 hours at lower pathogen concentration while reductions of 2 log to total inhibition were observed at higher pathogen concentration.

Conclusion: This study reports interesting high antimicrobial properties of yoghurt against diarrheagenic *E. coli* strains. Storage of the tested yogurt at room temperature still retained relatively high log count of lactic acid bacteria over 1 month period, however, storage at refrigerated temperature retained higher quantities of lactic acid bacteria..

Keywords: Yogurt, Diarrhea, Probiotics, Intestinal Pathogens, Storage.

INTRODUCTION

Infectious diseases are still a cause of a large proportion of deaths and disabilities worldwide . Some infectious diseases are generally much higher in low-income than higher income countries. The poorest 20% of the world's population experience a higher burden of infectious disease compared to the remaining 80% (Gwatkin *et al*., 1999). Diarrhea is a state that can be caused by infectious agents e.g. virus, parasite, or bacterium. *Escherichia coli* is one of the cause of infectious diarrheagenic.manifesting as Enterotoxigenic *E.coli* (ETEC), Enteropathogenic *E.coli* (EPEC), Enteroaggregative *E.coli* (EAEC), Enteroinvasive *E.coli* (EIEC) and Shiga toxin-producing/Enterohaemorrhagic *E.coli* (STEC/EHEC). (Fuller *et al.,* 2011).

Yoghurt is a fermented milk product obtained from milk or its products through lactic acid fermentation by S*treptococcus salivarius subsp.thermophilus* and *Lactobacillus delbrueckii subsp.bulgaricus.*. Recently, other strains such as *Streptococcus lactis*, *Streptococcus cremoris, Lactobacillus bulgaricus,*

Lactobacillus plantarum as well as *Lactobacillus acidophilus* that also exerts beneficial health effects have been used *(*Hussain *et al*., 2010*)*. The main interest in yoghurt consumption used to be its nutritional properties however, it can also be a functional food. Hasler (2002) defined functional foods as whole, fortified, enriched or enhanced foods that provide health benefits beyond the provision of essential nutrients when they are consumed at efficacious levels. The functional food includes probiotics, prebiotics and synbiotics.Consumption of high population of functional foods contribute to good intestinal health. Beneficial organisms in the gut have the potential to stop undesirable bacteria from overgrowing in the gut. The human intestinal tract is home to complex microbial community which plays a central role in human health. Human gut contains 1000 bacterial species and 100-fold more genes than are found in the human genome *(*Ley *et al*., 2006*)*.

Although lactic acid bacteria (LAB) were used in yoghurt as a starter culture, if the numbers of viable bacteria are high, they can confer health benefits to consumers. Different combinations of starter cultures are used during yoghurt manufacturing to give a wide choice of therapeutic benefits. The viability of the starter culture in yogurt can be affected by various factors such as pH, acidity, storage temperature, microbial contaminants and others (Shah *et al*., 2000). Loss of yogurt effectiveness due to the losses of the viability of starter bacteria has been reported during storage, consequently preventing yoghurt from exerting any health effect in the gut (Mataragas *et al*., 2011, Machado *et al*., 2013) viability of bacteria in yoghurt in Nigeria could be affected by irregular power supply and high room temperature of the tropical region.

If consumers are selecting yogurt for its health benefits, then the key to making the right choice is the assurance that it contains live and active cultures of lactic acid bacteria at high concentration with antimicrobial properties. Therefore this study is designed to determine the viability of lactic acid bacteria in selected yoghurt at different storage conditions and antimicrobial properties of the yoghurt against diarrheagenic *E. coli*.

METHODS

Yoghurt Samples

Different brands of yoghurt sold in Ibadan metropolis have been formerly investigated for the presence of viable lactic acid bacteria (unpublished data). A brand (Quincy) was selected for further studies due to high viable LAB in the yoghurt. The brand was obtained from shopping mall at Bodija in Ibadan. Details of the batch productions were recorded and the product was used within the stated expiry date. It is a full cream milk powder, sugar, flavoured yoghurt having on it bottle label a note of using a yoghurt culture that was not named. The shelf-life given by the product) manufacturer was 3 -4 months. The pH of the yoghurt was recorded as 3.91.The manufactured date of the batch used was 17th September 2016 and the expiry date was 14thDecember 2016.

Diarrheagenic Escherichia coli Strains.

Diarrheagenic *Escherichia coli* strains were collected from Molecular Microbiology division, Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan. The strains were designated as follows: Enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E.coli* (STEC), Enterohaemorrhagic *E.coli* (EIEC), Enteroaggregative *E. coli* (EAEC) and Enterotoxigenic *E. coli* (ETEC).

Isolation and Viable Bacterial Count of LAB in Yoghurt.

Two samples of Quincy yoghurt (one for room temperature and the other for refrigeration) were obtained. They were allowed to attain room temperature in order to prevent heat shock to the microbial content (Oranusi *et al*.,2011) and the samples were then shaken vigorously to disperse and suspend the microbial content. The stock, 10^2 and 10^4 dilutions of the yoghurts were plated out by pour plate method on De Man Rogosa Sharpe Agar (MRS, Oxoid, UK) and incubated under micro-aerophilic conditions at 37^0C for 48 hours. Plates were observed for bacterial growth and the total viable counts were counted. After the initial count, one opened bottle of yoghurt was stored at room temperature while the other was stored inside the fridge at 4^0C. The viable count was repeated weekly for both sets of yoghurts over a period of four weeks (i.e,at week 1,2,3 and 4).

Co-culture of Yoghurt Isolates and Gastrointestinal Pathogens

An examination of the interference of Quincy yoghurt with the growth of diarrheagenic *E. coli* was done by co-incubating EPEC,STEC, EIEC, EAEC and ETEC strains of *E.coli* with the yoghurt. Ninety nine milliliters (99ml) of yoghurt was transferred into a sterile bottle and 1ml of 10^6cfu/ml dilutionof overnight cultures of *E. coli* strains were added to get a final concentration of 10^4cfu/ml of pathogensin the

yoghurt. .Appropriate dilutions (from 10^2 to 10^4 dilutions) were plated out on MacConkey Agar at 0 hr to determine initial viable count of *E. coli* strains for the experimental and the control. In the control, the same dilution of *E. coli* used in experiment was added to normal saline. Both experimental and control cultures were incubated for 24 hrs at 37^0C aerobically. The viable counts of all surviving *E. coli* strains were determined for both experimental and control for the six strains of *E.coli* respectively (Drago *et al*., 1997). The above experiment was repeated for higher pathogen dilution of 2.1 x 10^6 to 1.64 x 10^8 cfu/ml for different *E. coli* strains at zero hour and the residual viable pathogen counts were obtained at 24 h. In the control, the same dilution of *E. coli* used in experimental was added to Nutrient Broth.

RESULTS

The initial pH of the yoghurt at the time of purchase was 3.91. The samples contained a substantial number of viable LAB at concentration of 10^5 cfu/ml. Lactic acid bacteria were isolated from Quincy yoghurt over a period of four weeks from a sample stored at room temperature and refrigerated sample. 1 log decrease in number of cfu/ml was obtained over a period of four weeks of study, which ranges from 1.84×10^5 cfu/ml to 1.69×10^4cfu/ml and 1.84×10^5 cfu/ml to 2.48×10^4 cfu/ml for both room temperature and refrigerated sample respectively (Fig 1).

The antimicrobial activity of Quincy yoghurt against strains of diarrheagenic *E. coli* was initially determined at low pathogen concentration(the initial counts of pathogens in coculture at 0 hr were in range of 2.1×10^4 to 7.0×10^4cfu/ml). After coincubation, there was total inhibition of growth of the pathogens in the mixture (pathogens and LAB) with zero count.

The viable count of the pathogen monoculture after 24 hours ranged between 1.65×10^4cfu/ml to 4.72×10^6cfu/ml compare to the pathogens monoculture at zero hour which ranged between 2.1×10^4 to 7.0×10^4 cfu/ml. (Fig 2). Another co culture experiment was performed with same strains of *E. coli* but higher concentration of pathogens (2.1 x 10^6 to1.64 x 10^8 cfu/ml). The greatest log reduction (6 log) was observed in EIEC where there was total eradication of the bacteria from 6 log cfu/ml to no viable count after 24 h. EAEC has 5 log reduction from 8 to 3, EPEC has 2 log reduction from 6 to 4, ETEC has 4 log reductions from 6 to 2, STEC has 4 log reductions from 7 to 3 (Figure 3). The viable count of the pathogen monoculture after 24 hours ranged between 6.0 x 10^9 to 2.05 x 10^{11}cfu/ml compare to the pathogens monoculture at zero hour which ranged between 2.8×10^6 to 3.0×10^8 cfu/ml.

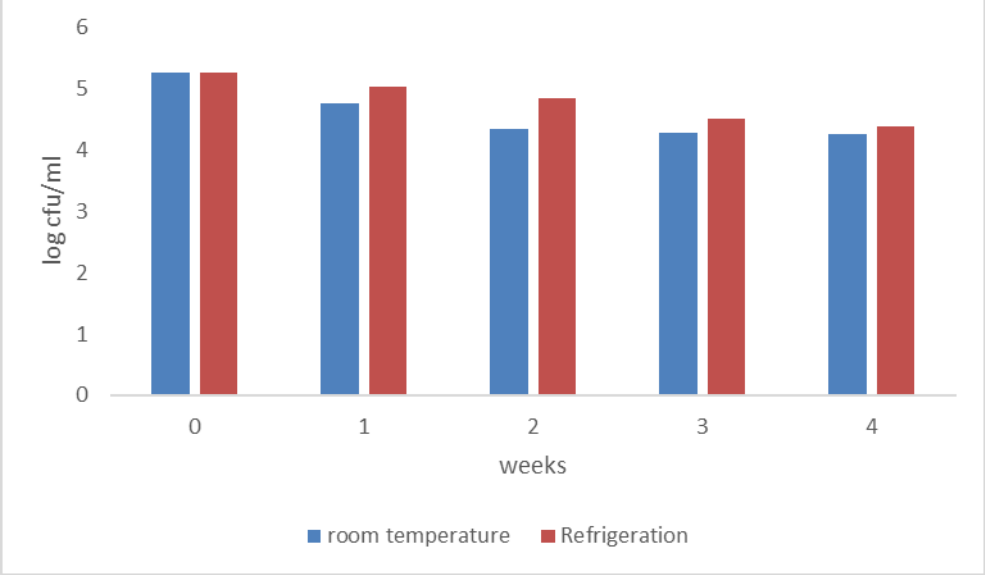

Figure 1: Viability of LAB from Quincy yoghurt at room temperature and refrigeration over a period of 1 month

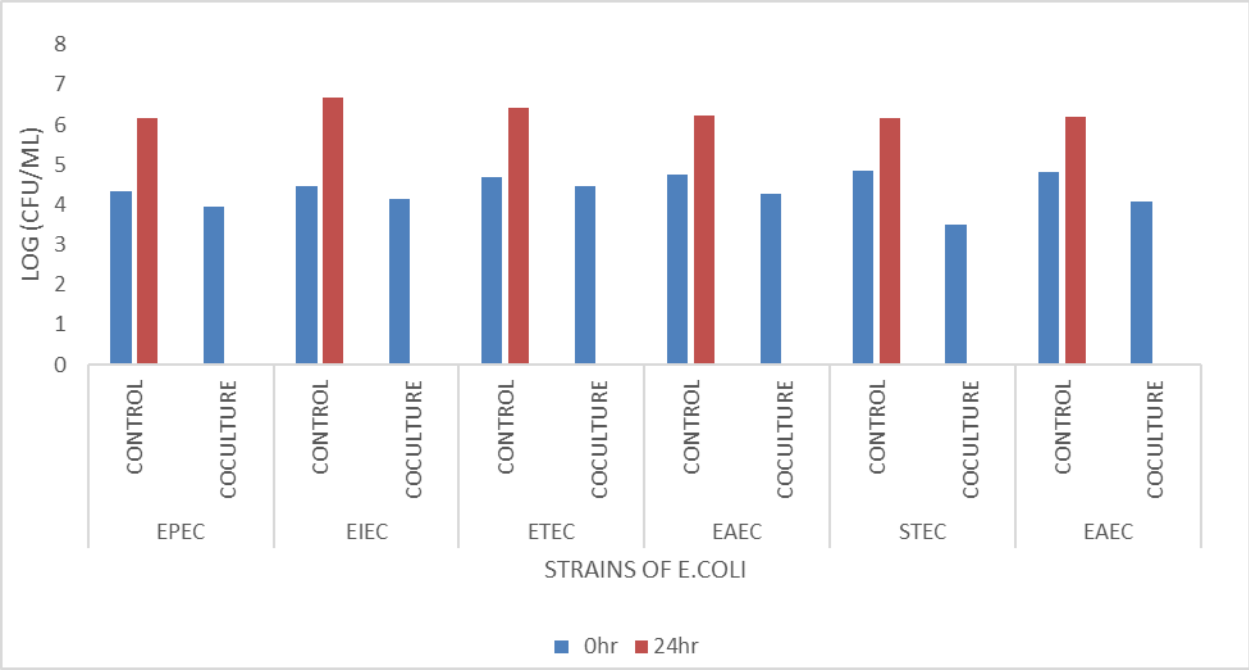

Figure 2: The Antimicrobial activities of LAB in Commercial Yoghurt (Quincy) Against Strains of *E.coli*(at Lower Concentration in Co-culture

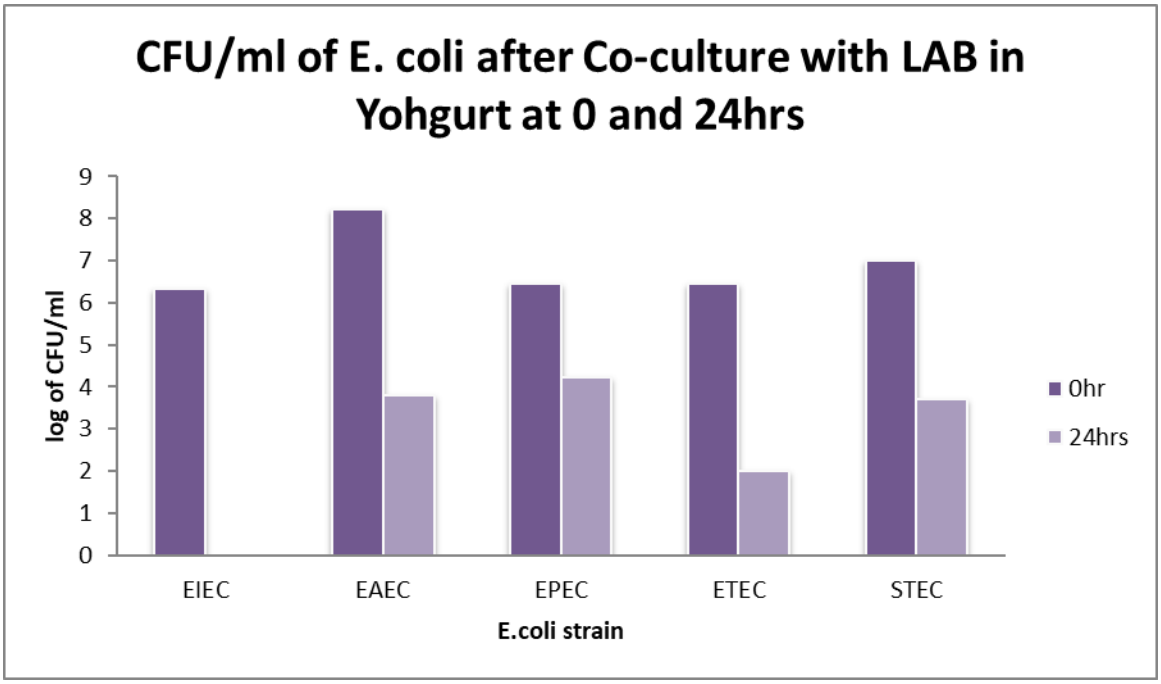

Figure 3: The Antimicrobial activity of LAB in Commercial Yoghurt (Quincy) Against Strains of *E.coli* (at Higher Concentration in Co-culture)

DISCUSSION

The assessment of viable LAB count in Quincy brand showed growth at concentration of 10^5cfu/ml of LAB. There was a decrease in the number of viable LAB present in the yogurt over a period of four weeks ranging from 5.3 log10 cfu/ml to 4.2 log10 cfu/ml and 5.3 log10 cfu/ml to 4.4 log10 cfu/ml for both yogurt samples stored at room temperature and refrigeration. Although the yoghurt were stored in the fridge under irregular power supply, it was better than when stored at room temperature thereby promoting storage of cultured yoghurt at reduced temperature. Although there was a decrease in the viable LAB count at the room temperature storage compared to the fridge storage, it shows that keeping yogurt at room temperature will still retain the viable count of LAB to an appreciable quantity. Ayeni *et al*.,(2011b) reported that the viability of most strains of LAB declined slowly during the cold storage of the dairy product and after 4 weeks. The need to monitor survival of yoghurt bacteria has often been neglected thereby resulting in a number of products reaching the market with few viable bacteria ranging from several hundreds to a few per gram of product. There are many factors affecting the viability of the starter culture in yogurt such as pH, acidity, storage temperature, microbial contaminates and others (Shah *et al*., 2000).

Interestingly, there was total inhibition of growth of the pathogens in the co-culture at lower pathogen concentration of 10^4 cfu/ml while the pathogens in the control grew with viable count between the range of 4.2 $\log_{10}$ cfu/ml to 6.7 $\log_{10}$ cfu/ml .There was total inhibition of enteroinvasive *E. coli* strains even at concentration of 10^6 cfu/ml and moderate inhibition of enteropathogenic *E. coli* strains at 2 log reduction.. The observed high E. coli inhibition by the yoghurt is encouraging and have the potentials of being a feasible potential for therapy of diarrhea caused by diarrheagenic *E. coli*. In a related study by Drago *et al*., (1997) *Lactobacillus* strains inhibited the in-vitro growth of *E.coli, Salmonella enteritis* and a cumulative effect was observed when *Lactobacillus* was combined in a culture against any of the pathogens. Beata *et al*.,(2012) also reported that six *Lactobacillus* strains inhibited the growth of *Salmonella seftenberg* after 48 hours of cultivation.

Antimicrobial action of LAB have been attributed to the production of main primary metabolites such as lactic and acetic acids, formic and benzoic acids, hydrogen peroxide (H_2O_2)., bacteriocin, ethanol and CO_2. (Ayeni *et al*., 2009, Ayeni *et al*., 2011a). Several investigations have demonstrated that various species of LAB exert antagonistic action against various pathogens in co-culture (Ayeni and Ayeni 2016, Adeoshun and Ayeni, 2016, Afolayan and Ayeni, 2017). LAB are capable of preventing the adherence, replication and pathogenic action of specific enteropathogens (Ayeni *et al*., 2011a).

This study showcases the potential of the use of yogurt containing sufficient amount of LAB in the local treatment of *E. coli* diarrhea. This has the potential application in Nigeria with absence of certified probiotics in the market.The refrigerated sample has an advantage of preserving the LAB for a long time and also reduces the rate of destruction of the LAB basically due to the cold temperate environment. The maintenance of such cold chain storage during the distribution of yoghurt products as well as passing the knowledge of good storage conditions to the consumer and retailers who stock the products for long before selling to the final consumers is essential.

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Limitation of the Study

The study investigated Quincy yoghurt. This viability and antimicrobial results obtained here cannot be used for any other brand of yoghurt.

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