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Full Length Research Paper

Bacteria population of some commercially prepared yoghurt sold in Enugu State, Eastern Nigeria.

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The demand for fermented dairies is on the increase in developing countries following consumer awareness of some of the health benefits attributed to prolonged intake. Eight samples of yoghurt produced from Enugu, Eastern Nigeria was collected from various locations. Samples were incubated using various media for the isolation, identification and enumeration of the bacteria population within the yoghurt. Reports indicated that total viable count of bacteria was in the range of 1.4 x 10^6 – 2.2 x 10^7 cfu /ml. Not all bacterial isolates were lactose fermenters. Members of the genus Staphylococcus, Aeromonas, Klebsiella, Pseudomonas, Bacillus, Streptococcus and Lactobacillus were isolated with 100, 25, 50, 33.3, 25, 12.5 and 25% frequency of occurrence, respectively. Viable counts of lactic acid bacteria were low indicating that probiotics effect following consumption may be poor. Occurrence of pathogenic organisms indicates improper handling and inadequate sanitary measures. More care should be taken during yoghurt fermentation.

Key words: Lactobacillus, fermentation, yoghurt, bacteria, contaminant.

INTRODUCTION

Milk is nutrient laden containing high concentration of protein, fat, vitamins and minerals. Milk fermentation is a classic method widely employed for milk preservation and conversion to value added products. Yoghurt production involves the fermentation of the lactose content of milk to yield lactic acid, CO_2, acetic acid, diacetyl, acetaldehyde, amongst others (Adolfsson et al., 2004; Tamine and Robinson, 2004) using *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

Fermentation of milk during yoghurt production reduces the pH from around 6.5 to 4.5 due to the production of organic acids. Fermenting organisms produce lactase, which hydrolyzes lactose to simple sugars, glucose, and galactose, making it more accessible to lactose intolerance patients (Wilton, 2004). Apart from easier digestibility, the ingested organisms enhance bioavailability of nutrients and ensure gastrointestinal balance, promoting colon health (Guarner and Schaafsma, 1998; Maltock, 2007; McDonagh et al., 1997).

Yoghurt consumption accelerates the healing of gastrointestinal tract disorder and reduces the incidence of vulvovaginitis as a result of candidiasis (Maltock, 2007). Following fermentation of milk to yoghurt, protein content is increased with concomitant reduction in cholesterol level. This is probably as a result of assimilation of cholesterol by probiotics or the binding of yoghurt to bile acids with a resultant decrease in cholesterol level. The high and easily assimilable nutritive value of yoghurt provides a suitable environment for microbial contamination, proliferation and spoilage. Yeasts and molds are the primary spoilage organisms of yoghurt and their role has been well documented (Canagenella et al., 1998; Cappa and Cocconcelli, 2001). The activities of yeast and molds in yoghurt gradually decrease the acidity of yoghurt making the food environment more susceptible to proteolysis and putrefaction by bacteria (Canganella et al., 1998).

There is currently a tremendous surge in the rate of

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Table 1. Total viable count of bacteria and presence of lactose fermenters in brands of yoghurt samples analyzed.

Sample	Total viable count (cfu/ ml)	Lactose fermenter
YW1	4.0×10^6	-
YW2	1.4×10^6	-
YS1	9.4×10^6	-
YS2	5.0×10^6	-
YE5	1.9×10^7	+
YE6	2.1×10^7	+
YN7	1.5×10^7	+
YN8	2.2×10^7	+

+ = Present, - = Negative.

yoghurt consumption in Nigeria leading to the establishment of many small-scale factories for yoghurt production. This is probably due to increased awareness of the benefits that accrue from constant consumption of yoghurt. Cases of food infections and intoxication have been attributed to poor and inadequate sanitary conditions observed in processing of many locally made foods (Stewart and Humphrey, 2002). The present study was undertaken with the aim to investigate the bacterial contaminants of yoghurt, to characterize and identify pathogenic bacteria present.

MATERIALS AND METHODS

Sample collection

Eight different brands of yoghurt manufactured in Enugu, Eastern Nigeria was randomly purchased from shops in different locations of the state. All samples were properly labeled and promptly processed.

Isolation and enumeration of bacteria

The content of each yoghurt container was uniformly mixed with a 10 ml sample which was aseptically withdrawn, mixed in a flask containing 90 ml of 0.1% sterile peptone solution, and then diluted for counting purposes. Decimal dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} etc) were made from this solutions by adding serially 10 ml of solution from proceeding concentration of 90 ml of the diluent. Each dilution was plated over nutrient agar, Mac Conkey agar, Simmon citrate agar, Sim agar, blood agar and cystine lactose electrolyte deficient agar (CLED).At the end of the various incubation periods, plates were counted using the digital illuminated colony counter (Gallenkamp). Colony counts were expressed as colony forming units per gram of sample. All counts were done in triplicate and average values were reported.

Characterization and identification of isolates

Bacterial isolates were characterized based on microscopic appearance, colonial morphology and biochemical test. The motility of the isolates was examined by the “hanging drop” technique. Gram reactions and cell morphology were examined from heat-fixed smears. The microorganisms were identified by the methods described by Cheesebrough (2003), Gordon et al. (1973), Cowan and Steel (1974) and Bergey’s Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

RESULTS AND DISCUSSION

Yeasts are considered as natural contaminants of yoghurt. Isolation of numerous yeasts strains and molds from yoghurt are well documented (Al-Tahiri, 2005; Fleet, 1990; Fleet and Mian, 1987; Green and Ibe, 1987; Li and Li, 1998; Moreira et al., 2001; Mayoral et al., 2005; Viljoen et al., 2003). However few reports exist on bacterial contaminants of yoghurt. A total of eight samples from various locations of Enugu in Eastern Nigeria were analyzed. There was remarkable variation in the mean total viable count of bacteria expressed in colony forming units (cfu) / ml. Mean viable counts varied from 1.4×10^6 to 2.2×10^7 cfu/ ml (Table 1). The current findings were lower compared to the report of Oyeleke (2009), yoghurt samples isolated from Minna City; Northern Nigeria had total viable bacteria counts varying from 1.0×10^7 to 9.4×10^7 cfu/ ml. Similarly Khan et al. (2008) recorded total viable count is in the range of 1.3×10^4 to 7.0×10^7 cfu/ ml from yoghurt samples collected from Karachi Mega City, Pakistan. The disparity in mean value of total viable count of bacteria is a reflection of variation in manufacturing practices and preservation protocols. Yoghurt is best kept at refrigerator temperature during storage, but unfortunately inadequate power supply makes this very difficult and unattainable. Yoghurt is therefore stored mostly at room temperature in Nigerian cities. Nigeria is located in the tropics with even higher climatic temperatures in the Northern part of the country. This may partly explain the reason for higher number of bacteria contaminants in yoghurt samples from Northern Nigeria. A higher temperature will make conditions even

Table 2. Morphological characteristics of bacterial isolates from yoghurt samples.

Isolates	Gram	Shape	Spore stain	Motility	Gelatin Liquefaction	Probable Genus
BY1	-	rods	+	+	-	Aeromonas
BY2	+	cocci	-	-	-	Staphylococcus
BY3	-	rods	-	-	-	Klebsiella
BY4	-	rods	-	+	+	Pseudomonas
BY5	+	rods	+	+	-	Bacillus
BY6	+	cocci	-	-	+	Streptococcus
BY7	+	rods	-	-	+	Lactobacillus

+ = Positive, - = Negative.

Table 3. Biochemical reactions of bacteria isolates from yoghurt samples.

Genus	Catalase	Coagulase	Oxidase	H_2S	Methyl Red	Voges Proskeur	O/F
Aeromonas	-	NA	+	+	-	+	F
Staphylococcus	+	-, +	-	+	-	-	F
Klebsiella	+	NA	-	-	-	+	F
Pseudomonas	+	NA	+	+	-	-	O
Bacillus	+	NA	-	-	+	-	F
Streptococcus	-	NA	-	+	-	+	F
Lactobacillus	-	NA	-	+	-	+	F

+ = Positive, - = Negative, O = oxidative, F = Fermentative, NA = Not applicable.

more favorable for the contaminating flora and facilitate proliferation. Indeed Moreira et al. (2001) reported that warmer weather and inadequate refrigeration are the principal causes of higher levels of contamination, increased species diversity and alteration in microbial flora. Although sugar content of yoghurt is mostly in the form of lactose, not all bacterial isolates were lactose fermenters. Interestingly, four of the yoghurt samples had no lactose fermenter among their microbial population. The presence of non-lactose fermenters is probably due to the abundant carbons sources for bacterial growth in the form of glucose and galactose sugars produced from lactose conversion by lactase enzyme. Moreover, sucrose applied as sweetener can serve as carbon source for some microbial species. According to the report of Fleet and Mian (1987), lactose fermentation is not a pre-requisite for the ability of yeast to contaminate yoghurt. This may also be applicable to bacterial contaminants of yoghurt.

From cultural, morphological and biochemical reactions, a total of seven bacteria genera were isolated from the yoghurt samples namely: Aeromonas, Staphylococcus, Klebsiella, Bacillus, Pseudomonas, Lactobacillus and Streptococcus (Tables 2 and 3). This is in agreement with the findings of Oyeleke (2009), bacterial isolates from yoghurt were members of the *Bacillus* spp., *Staphylococcus* spp., *Enterobacter* spp., *Lactobacillus* spp. and *Streptococcus pyogenes*. Earlier, Canganella et al. (1998) reported the survival of *Enterobacter cloacae* and *Pseudomonas paucimobilis* in yoghurts manufactured from cow milk and soymilk during storage. The present report indicates that *Staphylococcus* sp had the highest frequency (100%) as shown in Table 4. This is in variation with the report of Oyeleke (2009) that bacterial contaminants were predominantly *Bacillus* sp with 70% frequency of occurrence. The presence of *Staphylococcus* spp. in all samples is probably as a result of the prevalence of the genus on parts of the human body such as hands, nose, skin and clothing (Prescott et al., 2004). Possibility of introduction of the organism into food during processing, handling and packaging through the human handler cannot be overruled. Coagulase positive *Staphylococcus aureus* is responsible for food poisoning as a result of food intoxication (Ahmed et al., 2009). Enterotoxin production by *S. aureus* is promoted by the presence of starch and protein (Wistreich and Lechtman, 1980). *Klebsiella* sp appeared in 50% of the samples tested with 19.04% rate of occurrence. *Klebsiella* sp is a coliform and may be an indicator of product contamination through feacal contaminated water or raw materials (Talaro and Talaro, 2006). Rate of occurrence of *Pseudomonas* sp was 14.29 with 33.3% occurrence. Pseudomonas is found in soil, water, plants and animal, and is present in small percentage in the

Table 4. Frequency of bacteria isolates in yoghurt samples.

Genus	Frequency of occurrence (%)	Rate of occurrence (%)
Bacillus	25.0	9.5
Pseudomonas	33.3	14.3
Klebsiella	50.0	19.0
Staphylococcus	100	38.1
Aeromonas	25.0	38.1
Lactobacillus	25.0	9.5
Streptococcus	12.5	4.8

normal intestinal flora and on the human skin (Jawetz et al., 1989). *Aeromonas* sp were even less in population, however they are free-living organisms isolated from fish, soil and food. Some strains are known pathogens of human. *Lactobacillus* sp and *Streptococcus* sp are desirable when isolates are the correct specie applied as starter cultures (Moreira et al., 2001). *S. thermophilus* and *L. bulgaricus* are probiotics that should be consumed life with the fermented food for health benefits to accrue. However, viable strains of these were not found in most of the yoghurt samples. Similar reports were shown by Oyeleke (2009), out of 20 yoghurt samples analyzed, Lactobacillus sp was isolated in three samples, while Streptococcus sp was in only one sample. Reports by Canganella (1998) indicated that substrates such as milk source could affect survival of Lactobacillus and Streptococcus in yoghurt. Soybean milk reportedly exhibited a protective effect on lactic acid bacteria as opposed to goat milk. Further studies are necessary to ascertain the pH and acidic content of the yoghurt, and presence of other microbial metabolites, which may explain why the LAB appears to be suppressed by contaminating microorganisms.

Conclusion

The yoghurt samples contain viable bacteria cells amongst which are pathogenic strains capable of causing various health complications such as gastrointestinal disorders. This indicates lack of good manufacturing practice (GMP) or inadequate storage. The paucity of probiotics cells in the dairy product implies that numerous health benefits and protection, which the food should provide to the consumer, will be lacking, with a resultant exposure to high risk of food borne infection and intoxication. There is therefore, the need for proper monitoring and quality control amongst local producers and health workers to ensure that correct guidelines and GMP for yoghurt is maintained. There is also the need to address storage problems in order to minimize the risk of food borne infections and intoxication through yoghurt consumption.

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