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EVALUATION OF SOME PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM YOGHURT SAMPLES

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ABSTRACT

Yoghurt is obtained through fermentation of lactose in milk by relevant lactic acid bacteria (LAB) most of which are classified as Probiotics. This study aimed to assess eight yoghurts in Benin-city, Edo state in Nigeria. Isolation, identification and quantity of probiotic organisms; tolerance and antibacterial activity wereareas of emphasis.Isolation on selective media was followed by Gram staining; Catalase and Sugar fermentation tests; lactic acid bacteria enumeration; tolerance in pH and bile adjusted in selective media. Antibacterial activity was investigated using soft-agar overlay technique.Outcome of isolation and identification showed the presence of *Lactobacillus* species in four yoghurts, while *Streptococcus thermophilus* was absent in the yoghurts. Further identification showed *Lactobacillus acidophilus* in three and *Lactobacillus bulgaricus* in one of the yoghurts respectively. Three of the yoghurts contained *Lactobacillus* species ranging from 2.6×10^2 to 2.0×10^3cfu/ml and one contained 2.4×10^6cfu/ml. Tolerance in 0.3-2.0 % bile salt and 3.0-7.0 pH were within acceptable range. The Lactobacillus species demonstrated antibacterial activity. Non-compliance in the presence and quantity of probiotic microorganisms was seen in the yoghurts. However, antibacterial and gastrointestinal tolerance of the organisms was within limits.

Keywords: yoghurt, probiotics, tolerance, antibacterial activity.

INTRODUCTION

Yoghurt is a fermented product obtained through anaerobic fermentation of lactose in milk by relevant lactic acid bacteria (LAB) most of which are classified as Probiotics. (Tull, as cited in Sanful, 2009). Recapturing the definition of probiotics with respect to yoghurts can therefore be conceived as consumption of live lactic acid bacteria in adequate amounts which are able to confer both nutritional and health benefits.The relevantLAB used in yoghurt are preferably cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophiles* (Beal and Helinck, 2014). These two genera in the symbiotic production can be abridged as the acidification of milk and synthesis of relevant aromatic compounds (Hamann and Marth, 1984; Serra *et al*., 2009). Additionally, *Lactobacillus acidophilus* and bifidobacterium species may be present (Sri Lanka Standards Institution (SLSI), 1989; Vinderola and Reinheimer, 1999; Ranasinghe and Perera, 2016).

The acidification as a result of breakdown of lactose to lactic acid exacts a preservative attribute. The resultant reduction of the pH of cultured milk inhibits the growth of other bacteria. This attribute, thereby prolong the shelf life of yoghurt (Elagamy, 1992). Other components such as carbon dioxide, acetic acid, diacetyl, acetaldehyde is produced, that gives the yoghurt its characteristic fresh taste (Tamine and Robinson, 2004).

Yogurt has been known for its probiotic effects such as improved lactose assimilation and food digestibility, immune system boosting, anticarcinogenic activity and hypercholesterolemia, (Gayathri & Rashmi, 2016; Sharma *et al*., 2016), antimicrobial activity, prevention of food allergies (Richardson, 1996; Kailasapathy and Rybka, 1997; Mattila *et al*., 1999; Shah, 2000). It is specifically recommended the final product is required to carry live lactic acid bacteria in quantity 10^7-10^8cfu/g at the time of production. In addition the cultures must remain significantly alive at the end of the declared shelf life in order to exact its preservative and probiotic effects. (Chandan and Shahani, 1993; Fredua-Agyeman *et al*., 2016). In order to express its health benefits on the host, the relevant LAB in yoghurts must be able to survive and colonize the gastrointestinal tract (GIT), exhibit tolerance to both low pH and high concentrations of bile (Kirjavainen *et al*., 1998).

The antimicrobial spectrum of Lactic acid bacteria containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in yoghurts display a wide array of

antimicrobial spectrum against: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fragi*, *Micrococcus flavus* (Singh *et al*., 1979); *Shigella flexneri*, *Shigella sonnei (*ZareMirzaei *et al*., 2018; Dave and Shah, 1997)

This product is considered a healthy product and enjoys a positive standing in the minds of consumers (Granato *et al*., 2010; Cruz *et al.,* 2013; Annuziata and Vecchio, 2013). In Nigeria, most people take yoghurt as a dessert, snack or as a probiotic drink to aid digestion and to re-establish a balance within the intestinal microbiota (Olatidoye *et al.,* 2017). This study aimed to assess eight yoghurts in Benin-city, Edo state in Nigeria. Isolation, identification, quantity of probiotic organisms, tolerance and antibacterial activity were areas of emphasis in this study.

MATERIALS AND METHODS

Sample collection of yoghurts

Samples of eight yoghurts were purchased from common supermarkets in Benin-city, Edo state in November, 2018 and were named P1, P2, P3, P4, P5, P6, P7 and P8. The samples were transported and stored in the refrigerator maintained at 2 – 8 ºC in Pharmaceutical microbiology research laboratory until used for these studies.

Isolation of lactic acid bacteria isolates on selective media

One (1 ml) of each yoghurt formulation was suspended in 9 ml of sterile Phosphate buffer saline (PBS). Serial dilutions up to 10^5 were made and 100 µl of each suspension were aseptically inoculated onto 5% Blood agar and deMan, Rogosa, Sharpe (MRS) agar plates using spread plate technique. The Blood agar plates were incubated aerobically while the MRS agar plates were incubated in a microaerophilic condition both at 37 ºC for 24 - 48 h. Discrete colonies were collected, stored and labelled appropriately (Weese and Martin, 2011; Stephens and Turner, 2015).

Identification based on Gram staining and Catalase test of lactic acid bacteria isolates

Gram status of the isolated organisms wasdetermined by the microscopic examination of Gram-stained isolates with a magnification of 100x. While the Catalase status was determined in presence of one drop of 3 % hydrogen peroxide in accordance with Kale, 2014.

Identification based on Sugar fermentation test of lactic acid bacteria isolates.

Representatives of the isolates from yoghurts were confirmed to genus and

species using conventional sugar fermentation tests. Lactic acid bacteria converting bromothymol blue to yellow are recorded as positive while those retaining the blue colour of indicator are recorded as negative (Cheesbrough, 2006). Sugar fermentation outcomes were compared with similar microorganisms in Bergey's manual (Holt *et al*., 1994).

Enumeration of lactic acid bacterial isolates

The yoghurt samples were aseptically opened and 1 ml of each of the sample was homogenized in 9 ml of sterile PBS (pH 7.4). A 1:10 serial dilutions of the mixtures were made and 100 µl were spread aseptically on MRS agar and 5 % blood agar plates. The inoculated MRS agar plates were incubated at 37 ºC in a microaerophilic condition for 48 h while the blood agar were incubated aerobically at the same time and temperature condition used for MRS agar (Fredua-Agyeman *et al*., 2016). Discrete colonies were counted at the end of the incubation period. The number of viable bacteria was presented as colony forming units per volume.

pH tolerance studies of lactic acid bacterial isolates

pH tolerance was determined by first culturing the isolates on appropriate broth (MRS broth and Nutrient broth) for 20 h at 37 ºC. Secondly, 1x10 ^{6}cfu/ml concentration of each isolate was added into 10 ml of appropriate broth adjusted to pH 1.5, 3.0 and 7.0 using 0.1 N HCl. The broth cultures were incubated for 24 - 48 h at 37 ℃ and survivial were determined based on turbidity. (Gore Paul and Bhagwat, 2016).

Bile salt tolerance studies of lactic acid bacterial isolates

Bile salt tolerance was assessed by first culturing the isolates on appropriate liquid media for 20 h at 37 ºC. Specifically, 20 µl of cell suspension to give 1 × 10 ^{6}cfu/ml concentration of each isolate was suspended into 10 ml of appropriate broth supplemented with 0%, 0.3% and 2% of bile salts. Thereafter the broths were incubated for 24-48 h at 37 ℃ depending on the organism of interest and viabillity were determined based on turbidity of the broth cultures. (Gore Paul and Bhagwat, 2016)

Antibacterial activity of lactic acid bacterial isolates

Antibacterial activity of all collected isolates against some representative test pathogens (*Bacillus subtilis, Escherichia coli Staphylococcus aureus* and *Klebsiella pneumonia*) were determined by soft agar overlay technique according to modifications of Ran *et al.*, 2012; Halder *et al.*, 2017. The MRS agar plates containing the confluent growth of the probiotic isolates in spot form (ranging from 4-5 mm diameter) were subsequently overlaid with soft Muller-Hinton agar (0.8 % agar) already pre-inoculated with test pathogens. The overlaid medium was allowed to set and subsequently incubated at 37 ºC for 24 h. The inhibitory zone diameter (IZD) was recorded as indication of antibacterial activity, while the absence of IZD was considered as lack of antibacterial activity.

RESULTS AND DISCUSSION

Outcome of isolation and identification showed the presence of *Lactobacillus* species in only four yoghurts: P2, P5, P6 and P8. While *Streptococcus thermophilus* was absent in all the eight yoghurts as shown in table 1. Further identification based on Sugar fermentation test showed *Lactobacillus acidophilus* in three individual yoghurts namely P2, P5 and P6. While P8 demonstrated the presence of *Lactobacillus bulgaricus* as presented in table 2. The presence of only a single specie pattern seen in outcome of this assessment is not in conformity with Adolfsson, Meydani and Russell (2004); Gonçalves, Freitas, Nero and Carvalho (2009). Where the dual presence of *Lactobacillus bulgaricus and Streptococcus thermophiles* with any other LAB is universally recommended in yoghurts.

Three of the yoghurts (P2, P6 and P8) contained total *Lactobacillus* count ranging from 2.6 x 10^2 to 2.0 x 10^3 cfu/ml and P5 total count was 2.4 x10^6 cfu/ml as shown in table 3. The low LAB in some of the preparation is not within the acceptable limit as proposed by Chandan andShahani, (1993) which is expected to be within 10^7-10^8cfu/ml (or cfu/g).

In the tolerance assessment 0 % bile concentration and pH 7.0 were considered as positive controls. Qualitatively all the isolate P2I, P5I, P6I and P8I exhibited tolerance in pH 3.0 and pH 7.0 with no tolerance in pH 1.5 as shown in table 4. A good source of probiotics such as yoghurts should withstand at least a pH value of 3.0 in accordance with Fernandez *et al.* (2003) which was established in these four yoghurts.

The four isolates showed tolerance in 0 %, 0.3 % and 2 % bile concentrations as shown

in table 4. Which is within and above the 0.3 % bile concentrations as recommended for selecting or identifying tolerance of probiotic organisms for human use as specified by Goldin and Gorbach (1992); Begley, Gahan and Hill (2005). Tolerance in varying concentration of bile salt and pH were within acceptable range for gastrointestinal consumption of lactic acid bacteria.

All the individual Lactobacillus species isolated from the yoghurts demonstrated antibacterial activity against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia* with IZD ranging from 7 – 11 mm as shown in table 5.Quantitatively, an improved antibacterial effect may have been recorded in the presence of dual or multispecies of these probiotic organisms according to Mchiouer, Bennani and Meziane (2016).

In summary, tolerance and the antibacterial activity as seen by the LAB in some of the yoghurts are consistent with some of the properties of Probiotics. Nevertheless, the non-compliance in dual or multiple species and quantity of these lactic acid bacteria need to be reviewed positively in Edo state.

CONCLUSION

This data showed non-compliance in the total presence and quantity of probiotic microorganisms in the yoghurts evaluated. However, antibacterial and gastrointestinal tolerance of the individual organisms was within limit.

CONFLICT OF INTEREST

This work had been previously included into an abridged form in “First International Conference on Biologics Research and Development book of abstract”. Published in July, 2019, p 59.