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Fermented Corn Waste Liquor as a Potential Source for Probiotic Lactic Acid Bacteria

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Abstract

Probiotic bacteria offer potential beneficial uses to man. The most common types are the Lactic Acid Bacteria (LAB) which is sourced mostly from fermented diary and vegetable products such as yoghurt, cheese and sauerkraut. Although some fermented cereals are known to contain LAB, limited information is available on the massive production of LAB from low cost fermented meals such as corn mash waste. Hence the efficiency of 72h-corn waste liquor as a rich source for probiotic LAB was evaluated. The liquor aseptically recovered from sieved, wet-milled 72h-fermented corn mash wastes was cultured into DeMan Rogosa Sharpe (MRS) medium, Yeast extract agar, MacConkey agar, Nutrient agar and Sabouraud dextrose agar, to obtain their mean microbial counts. The most common LAB colonies from MRS plates were identified as Lactococcus lactis, Pediococcus acidilactici and Lactobacillus plantarum by morphological and physiological tests. Equal concentrations of the LAB isolates at their determined peak growth periods (14h, 18h &22h), were screened for antibacterial potentials against $1x10^3 mgml^{-1}$ concentration of the test pathogenic organisms using cross-streak method. Results obtained revealed a much higher LAB counts ($>2.40x10^7 cfuml^{-1}$) than that of other organisms that ranged from $2.4x10^1$ to $2.1x10^3 cfuml^{-1}$. Coliforms were scantily recovered ($<3cfuml^{-1}$). The degree of susceptibility of the pathogens to the test LAB varies. However, the order of antibacterial potentials determined by the zone of inhibition was Lactobacillus plantarum> Lactococcus lactis > Pediococcus acidilacti which had slight antibacterial effect (<0.58mm) on the test pathogens. This gave an indication that corn waste liquor is a potential source of probiotics that can be utilized for the prevention of diseases that are caused by the susceptible pathogens.

Key Words: Probiotics, Lactic acid bacteria, Fermentation, Corn waste-liquor.

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Introduction

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit in the host (FAO/WHO, 2001). They are often regarded as dietary supplements containing potentially beneficial microorganisms especially bacteria and yeasts and have been used for centuries as natural components in health promoting foods. A range of potential benefits of probiotics which include prevention of harmful bacterial growth, prevention of antibiotic associated diarrhea, management of lactose intolerance, prevention of colon cancer and lowering of cholesterol level have been documented (Sanders, 2000; Ouwehand *et al*., 2002; Hamilton-Miller, 2003; Gibson and Roberfroid, 1995).

The most commonly known probiotic organisms are the LAB which is naturally found in some diary products, decaying plants, intestinal tract and mucous membranes of animals and humans (Nester *et al*., 1998). There are conditions (e.g. antibiotic stress, HIV/AIDS) when the load of LAB in the body system becomes very low or virtually absent that exogenous sources are required to improve the immune function and prevent infection. The functional food products such as diary products (yogurt) and probiotic fortified foods beyond their nutritional function, offer this benefit

(Anukam *et al*., 2008). However, tablets, capsules, powders and sachets containing the probiotic bacteria in freeze dried form can also serve. For instance, *Lactobacillus reuteri* ATCC 55730 and *Lactobacillus reuteri* SD 2112 produced by 'Biogaia' and 'Biologies' in Mexico have been reported to improve the immune function, prevent diarrhea and mitigation in children (Shorinikova *et al*.,1997).

These products are not usually affordable by the low income earners in Nigeria. Thus, there is need to source for available local food substances that can be rich in probiotic Lactic acid bacteria. The study therefore was aimed at evaluating the 72h-fermented corn waste liquor as a good supplement for probiotic LAB that possesses antimicrobial property. Corn waste liquor in this context, is the filtrate that results from sieved, milled 72h-fermented corn waste.

Materials and Methods

Laboratory Preparation of Corn Waste Liquor: The yellow corn variety purchased from Enugu main market in Nigeria was sorted, weighed (300g) and pre-washed severally with sterile water (pH 6.8) to remove debris and dust particles. Thereafter, the washed grains were subjected to 48h fermentation under aseptic measures according to the modified methods of George-Okafor *et al*. (2007).The grains were steeped in sterile stainless steel container with cover. At every 12h, the steeped water was decanted and the fermenting grains were re-washed with sterile warm water (20-25^{o}C) for 48h. The use of warm water of 20-25^{o}C is because it is the optimum temperature range for most LAB fermentation (Akunkee, 1973). Thereafter, the grains were drained, ground with sterile hand mill and sieved with sterile muslin cloth. The recovered waste was further subjected to 24h fermentation, after which it was milled and sieved. The resulting filtrate was utilized as the source for LAB.

Microbiological Evaluation: The recovered corn waste liquor was serially diluted with sterile de-ionized water up to 10^{-7}. Then, 1ml of each dilution was inoculated in duplicates into sterile plates of MacConkey agar (Lab^{M}), De Man Rogosa Sharpe' (MRS) agar (Oxiod), Nutrient agar (Lab^{M}), Yeast extract agar (Lab^{M}) and Sabouraud dextrose agar (Lab^{M}) respectively. The plates were incubated at 25^{o}C for 24-48h (for bacteria) and at 25-28^{o}C for 3-5days (for fungi). Some of the MRS plates were incubated at 37-40^{o}C for 48-72h under reduced oxygen content. The developed colonies were enumerated. The pure LAB isolates were identified at Bacteriology laboratory of University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu, Nigeria, using methods of Collins *et al.* (1991) and Cheesbrough (2000).

Inoculums Development of Lactic Acid Bacterial Isolates: The stock culture of each identified isolates of *Lactobacillus, Lactococcus* and *Pediococcus* species was activated by sub culturing a loopful (0.1g) into MRS broth (pH 5.5) and incubated at 25^{o}C for 24h. Then, 0.5ml of the diluted 24h culture of each isolate representing 1x10^{1}cellsml^{-1} served as the initial inoculum for the time-course determination.

Time-Course Determination of the Growth Phases of Lactic Acid Bacterial Isolates: Each identified LAB isolate (1x10cellsml^{-l}) was transferred into several test tubes, each containing 5ml of sterile MRS broth and incubated in a water bath at 25^{o}C. At onset, the first two tubes were removed at every 1hr interval and enumerated spectrophotometrically (LKB type) at 570nm wavelength. Subsequently, the time interval was increased by two-folds after every 4 tubes, until the death phase was observed. The first inoculated tube prior to incubation was the control. The cell density was expressed in relation to the difference between the initial growth (control) and the final growth. The peak growth period for each test LAB isolate was recorded.

Screening for Antimicrobial Potentials: The test organisms (*Staphylococccus aureus*, *Enterococcus faecalis*, *Klebsiella* sp, *Pseudomonas aeruginosa* and *Escherichia coli*) were obtained from Bacteriology Department of University of Nigeria Teaching Hospital (UNTH), Enugu, Nigeria. A cross-streak sensitivity method was adopted. Each LAB isolate (1x10^{3} cellsml^{-l}) was streaked vertically in a sterile Nutrient agar plate and pre-incubated at 25^{o}C for 14-15h (for *Pediococcus acidilactici*), 18h (for *Lactococcus lactis*) and 22-23h (for *Lactobacillus plantarum*). Thereafter, 1x10^{3} cellsml^{-l} of each test organism was streaked across and further incubated for 24-48h. Clear zones of inhibition around the contact points between the LAB isolates and the test pathogens were measured and the mean data recorded.

Results and Discussion

Lactic Acid Bacteria Recovery: The microbial evaluation of the 72h-waste liquor revealed a higher LAB counts (2.40x10^{7}-5.10x10^{7} cfuml^{-l}) than the other organisms (<2.10x10^{3}) at pH range of 5.0- 5.6

(Table1). The dominant property of LAB during corn fermentation has been previously reported (Edema and Sanni, 2006; George-Okafor *et al*., 2007). However, the utilization of fermented corn waste liquor in this study yielded more LAB (> $2.40x10^7$) and scanty coliforms (< 3cfuml-l) than that recovered from fermented corn mash (George-Okafor *et al*., 2007). It can be deduced from this result that the corn pericarp/testa and fibers that constituted the main part of the corn waste provided extra nutrients (minerals and vitamins) for the growth of the fastidious LAB. This view is in line with the report that filtrate of a by-product from distilled fermented maize is rich in nitrogen, minerals and growth factors (Cooney *et al*., 1979).

Table1. Mean Viable Counts (cfu/ml**) of Micro flora of 72h-Fermented Corn-Waste-Liquor**

pH value	Yeast load (x102)	Mold load (x101)	Total Bacterial load (x103)	Coliform load	Lactic Acid Bacterial load (x107)
5.6	1.04	1.15	2.10	2.00	2.40a,b.c
5.5	1.65	1.00	1.70	2.70	3.60a,b
5.3	1.62	1.13	1.30	1.30	5.10a,b,c
5.2	2.01	0.48	1.08	0.30	4.40a,c
5.0	2.40	-	1.01	-	4.55a,b,c

[a,b, c] Dominant LAB isolates
[a]*Lactobacillus plantarum* [b]*Pediococcus acidilactici* [c]*Lactococcus lactis*

The proliferation of the LAB lowers the carbohydrate content and the pH and this is due to the lactic acid production which is inhibitory to the growth of most other microorganisms (Fuller, 1992). However, other metabolic products such as hydrogen peroxide, carbon dioxide, bacteriocins and diacetyl must have provided additional antagonic action against the harmful organisms. The LAB recovered were diverse but *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Lactococcus lactis* were the predominant species. These predominant organisms are basically homofermenters. The implication of homofermenters as dominant LAB in the last stage of fermentation of cereal-based African foods has also been reported (Loner *et al.,* 1986). Contrary to this observation, is the report of Edema and Sanni (2006) which showed that both homo- and hetero-fermentative LAB were dominant at the end of the fermentation. The variation in micro flora dominance could be due to the steeping conditions (such as pH, time and temperature), type of cereal and recipe mixture (Edema and Sanni, 2006).

The frequent isolation of *L. plantarum* from most fermented cereal-based African foods (Onilude *et al.,* 2004; Amusa *et al*., 2005; Edema and Sanni, 2006), proved that the organism must be inherent in cereals and possesses strong adaptive features that enable it to survive all the fermentation conditions. The presence of *Lactococcus lactis* in this study could be linked to the waste liquor substrate utilized for the assay as the organism has not been frequently implicated as a dominant strain of homo fermentative micro flora in fermented maize-cereals.

Growth Pattern and Antimicrobial Potentials of Lactic Acid Bacteria (LAB): The antimicrobial activities of the LAB isolates varied (Table 2) as their optima growths differed (Fig.1). Their optima growth periods were 14h, 18h and 22h for *P. acidilactici*, *L. lactis* and *L. plantarum* respectively. The observed optima growth variation can be explained from their varying physiological characteristics. Their optima growth periods were determined in order to expose the LAB isolates to the pathogens when some or most of their antimicrobial agents must have been produced.

The results in table 2 show that *L. plantarum* exhibited the highest antimicrobial property by inhibiting most of the test pathogens (>0.75$\pm$ 0.5mm), with *Ent. faecalis* being the least affected. This result confirms both recent and previous reports that *L. plantarum* is very probiotic in nature (Brizuela *et al*., 2001; Anukam *et al*., 2008). For instance, *L. plantarum* was reported to perform many probiotic functions, including the eradication of *S. aureus* from fermented food (Neidzielin *et al*., 2001). However, there is limited information on its antimicrobial activity on *Klebsiella* sp and *Pseudomonas aeruginosa* which was observed in this study, thereby giving more credit to corn waste liquor as a potential substrate for probiotic organisms.

Table2: Sensitivity Pattern of the Test Pathogens to the Lactic Acid Bacterial Isolates at (1:1) ratio.

Test Pathogens	LAB Isolates and Zones of inhibition (mm) *Lactobacillus plantarum*	*Lactobacillus lactis*	*Pediococcus acidilactici*
Klebsiella sp	3.00±1.3	1.62±0.1	0.48±1.2
Escherichia coli	2.50±1.1	1.10±0.3	0.58±0.8
Pseudomonas aeruginosa	2.25±1.3	0.50±0.4	-
Enterococcus faecalis	0.75±0.5	-	-
Staphylococcus aureus	1.75+0.9	0.38+0.5	0.41+0.4

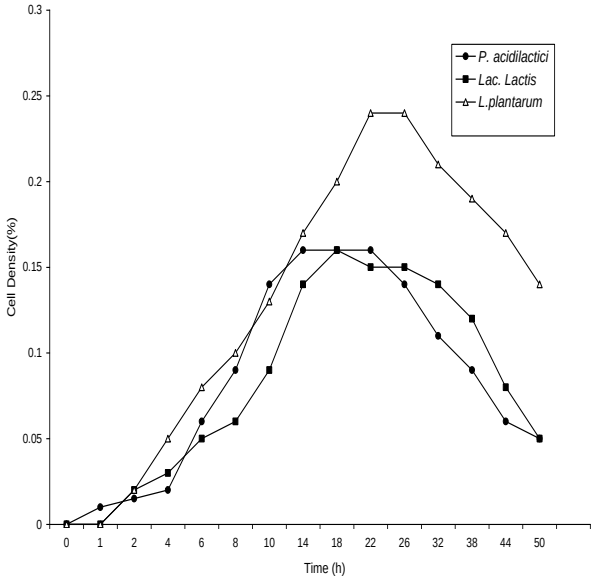

Fig.1. Batch growth pattern of the Lactic acid bacterial isolates

The high antimicrobial activity of *L. plantarum* could be attributed to the presence of plantaricin encoding genes which have been isolated from the organism and have also been shown to be lethal to most microorganisms including *Ent. faecalis* (Knoll *et al*., 2008). On the other hand, the resistance of *Ent. faecalis* to *Lac. lactis* and *P. acidilactici* could be explained from the fact that the pathogen might have possessed similar physiological features that enabled them resist the produced antimicrobial components.

However, it might be necessary to increase the LAB reacting dose as this may give room to a more susceptible result.

Conclusion

The results obtained from the study have shown that 72h-corn waste liquor is a rich source of pro-biotic LAB that have antimicrobial potentials. Its affordability will enable both children and adults consume it fresh as a dietary supplement when produced under aseptic condition. Its administration in sufficient amount as a prophylaxis will prevent human diseases especially those caused by the susceptible pathogens. Thus, this calls for a further study in order to determine the appropriate dose required for effective result.

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