



THE BACTERIOSTATIC POTENCY OF STEEPED CORN-PASTE BEVERAGE ON SOME GRAM-NEGATIVE BACTERIA

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ABSTRACT

Food items that have undergone fermentation do exhibit the ability to be antimicrobial in nature and have been reported to be characterized by their health-promoting properties such as the control of gastro-enteritis in mammals. The core objective of this research work is to determine the potential bacteriostatic efficacy of steeped corn-paste beverage on some Gram-negative strains of bacteria. Salmonella-Shigella agar and McConkey agar were the media of choice used to isolate the target cultures of bacteria while nutrient agar was used to test for purity and for the agar-well diffusion assay. Standard bacteriological tests including sugar fermentation were the techniques employed for their identification. The corn paste was submerged in excess water in order to get the beverage and it was evaluated for purity, pH and alcohol. 24-day-old cultures of the pure bacterial isolates were utilized for the agar-well diffusion assay. The three target bacterial strains are *Salmonella enterica*, *Shigella dysenteriae* and *Escherichia coli*. Appreciable growth was observed from the impure beverage. It measured 3.3 on the pH metre and recorded an alcohol-by-volume of 5.3%. The control, without the beverage but instead distilled water, showed no bactericidal or bacteriostatic activity. The repressive characteristic of the steeped corn-paste beverage ranged from 5.50mm against *E. coli* (least susceptible) to 13.40mm against *S. dysenteriae* (most susceptible) between the 2nd and 4th day after inoculation, at the 100% concentration. The steeped corn-paste beverage showed a significant antibacterial capability against the three Gram-negative bacteria. Its prospects as an organic antibacterial diet should be expansively exploited.

Keywords: Fermented products, inhibitory property, inoculation, prospects, significant

INTRODUCTION

Food items that have undergone the metabolic process of fermentation do exhibit the ability to be antimicrobial in nature (Maruggi, 1991). The traditional processing of cereals such as maize, sorghum or millet does produce a local starch-cake called 'ogi' from fermentation. *Lactobacillus plantarum* is the principal and the most abundant bacterium used for the production of 'ogi' from corn fermentation. The bacterium is also responsible for the taste and flavour of 'ogi' due to lactic acid production, which is the principal constituent of those two aforementioned parameters. Prior to the ingestion of 'ogi', it is simmered into a gruel also known as pap, which has a smooth feel or texture. Foods transformed by fermentation have been reported to be characterized by their health-promoting properties such as the control of gastro-enteritis in mammals.

Diarrhoea is a watery stool for two or more consecutive days (Vrese and Marteau, 2007). Adebolu, Olodun and Ihunweze (2007) reported that antibiotics may be occasionally needed for the treatment of diarrhoea even though it can be self-resolved. The majority of the causal agents of

diarrhoea, particularly bacteria, have already established resistance to the majority of the routinely-used antibiotics. Antibiotic-induced diarrhoea, which is induced diarrhoea arising from the use of some of these antibiotics, may also occur.

Corn is one of the staple food items in most African countries and other developing countries as well, thereby making it important in their diets and a bonus if its by-product, in terms of its corn-paste steep beverage, is indeed somewhat medicinal by being antibiotic in nature. It is also a source of pap, which is a popularly-consumed fermented semi-solid gruel. Corn-paste steep beverage is readily available and cheaply too and that would make it an inexpensive alternative to orthodox medicine especially in the case of antibiotic resistance, particularly by Gram-negative bacteria. Gram-negative bacteria are usually more worrisome and challenging to combat than their Gram-positive bacteria counterparts due to their structural and physiological differences and intrinsic properties that proffer them greater adaptability and immunity, predominantly to beta-lactam antibiotics such as



penicillin and cephalosporins which mainly target bacterial cell wall, due to the presence of lipopolysaccharide outer membrane and porins. Diarrhoea and dysentery are two of the commonest bacterial infections ravaging developing countries, especially occupants of rural areas where those diseases are more endemic and also among children and adults with poor food hygiene, caused by *Salmonella enterica* and *Shigella dysenteriae* respectively. Unfortunately, both diseases are caused by Gram-negative bacteria! Although, they are both acute and may be self-limiting but they become bothersome when they require antibiotic therapy due to loss of body fluid or water (dehydration) and electrolytes such as chloride, sodium and potassium or when antibiotic therapy just induces diarrhoea or dysentery.

The core objective of this research work is to determine the potential bacteriostatic efficacy of steeped corn-paste beverage on some Gram-negative strains of bacteria.

MATERIALS AND METHODS

Collection of Sample

The research work was partly conducted at the Federal Polytechnic, Ilaro's Microbiology laboratory. Some wholesome corn grains were procured from an indigenous market and then processed into corn paste. A soil sample containing faecal matter (fowls' droppings) was obtained from a poultry farm into a clean polythene bag; for the isolation of some Gram-negative bacteria, using a spatula. In the course of this research work, disposable hand gloves were worn as a cautionary procedure.

Media Preparation and Isolation

Salmonella-Shigella agar and McConkey agar; selective medium and differential medium respectively, were the two media of choice to isolate the target cultures of bacteria while nutrient agar; a general-purpose medium, was used to test for purity and for the agar-well diffusion assay. The media employed were prepared according to the manufacturers' instructions (Chessbrough, 2004). The bacterial strains were isolated by the pour-plate technique from the soil sample (Chukwu *et al.*, 2010). A four serial ten-fold dilution from 1ml of the sample

was conducted using 1ml stock homogenate. 1ml stock homogenate was aseptically introduced into the 10⁻¹ test tube; the serial dilution was carried-out up to 10⁻⁴. The 10⁻⁴ diluent was used for the bacterial culturing. The target bacterial strains were repeatedly isolated during the period of study.

Cultural Identification

The bacterial isolates were identified according to Chessbrough (2004), Baker, Silverton and Pallister (2001) while Bergey's manual of systematic bacteriology was used as a reference (Cowan, 1974). Gram status, cultural and colonial characteristics and various biochemical tests including sugar fermentation were the techniques employed for their identification.

Production of Corn Paste

The method of Odunfa (1988) was utilized in the preparation of the corn paste accordingly;

- Corn grains sorting
- Cleaning/rinsing
- Sieving/discarding
- Wet milling
- Fermentation/steeping
- Storing

The corn paste was submerged in excess water in order to get the beverage. Pour-plate technique was utilized to incorporate the beverage into the nutrient agar medium, aseptically.

Evaluation of the Steep Beverage for Purity

The steeped corn-paste beverage obtained in this study was evaluated for purity. This was done by aseptically incorporating the product into sterile nutrient agar medium by pour-plate technique. The plate was incubated accordingly and then examined for possible growth of inherent fermentative bacteria, afterwards.

Test for pH and Ethanol

The pH of the steeped corn-paste beverage was tested using litmus paper and pH metre, to determine the alkalinity, acidity or neutrality of the product and the alcohol-by-volume of the beverage was tested using an alcohol meter, to determine the presence of ethyl alcohol (ethanol) in the product.

Agar-well Diffusion Assay



The inocula for this test were prepared using 24-day-old cultures of the pure bacterial isolates. Nutrient agar plates were prepared for the assay for each axenic culture. The plates were inoculated with the axenic cultures respectively and a sterile 5mm corkborer was used to make a well at the centre of each plate. 1ml of the product at 100% concentration was introduced into each well. The plates were then incubated at 37°C ± 2°C for 24hrs. The zones of inhibition were determined by using a pair of vernier calipers and then placed on a transparent ruler to take the exact measurement or reading. The zone-of-inhibition readings were taken daily for three consecutive days from the second day of incubation. A control, without the beverage but instead distilled, water was set-up. The potential ability of the beverage to inhibit the growth of the isolates was indicated by the appearance of clear zones within the medium and around the colonies for sensitivity or otherwise resistance (Oduwobi and Afuye, 2017).

RESULTS

The result for the bacteriological identification reveals the presence of three genera of bacterial strains

namely; *Salmonella*, *Shigella* and *Escherichia* which are all members of the family Enterobacteriaceae. The three target bacterial strains are *Salmonella enterica*, *Shigella dysenteriae* and *Escherichia coli*. Table 1 shows the identities of the isolates. The target bacteria are all Gram-negative rods and are non-spore forming, catalase positive, without capsule and non-gas-producing organisms which are able to ferment simple sugars differently. The steeped corn-paste beverage was tested for purity and after the incubation period, appreciable growth was observed and this suggests that the beverage contained inherent fermentative bacteria. The pH of the steeped corn-paste beverage showed a positive result for acidity which could potentially be lactic acid synthesized during fermentation of the corn grains, by those inherent fermentative bacteria. It measured 3.3 on the pH metre and that could be quite inhospitable to the presence and survival of non-acidophilic bacteria. The alcohol-by-volume of the beverage indicated a positive result with a value of 5.3%.

Table 1: The Target Bacteria from the Soil Sample

Characteristic test/Probable bacterium	<i>Salmonella Enterica</i>	<i>Shigella dysenteriae</i>	<i>Escherichia Coli</i>
Morphology	rod	rod	rod
Gram stain	-	-	-
Capsule	-	-	-
Spore production	-	-	-
Fructose	+	+	-
Lactose	-	-	+
Glucose	+	-	+
Sucrose	-	-	+
Mannose	+	+	-
Citrate	-	-	-
Oxidase	+	-	-
H ₂ S production	+	-	-
Urea utilization	-	-	-
Indole production	-	+	+
Catalase	+	+	+
Methyl red	+	+	+
Voges-proskauer	-	-	-
Motility	+	-	+

Keys: + = Positive, - = Negative

Table 2: The Antibacterial Profile of the Isolates to the Steeped Corn-paste Beverage



Zone of inhibition (mm)			
Day	<i>S. enterica</i>	<i>S. dysenteriae</i>	<i>E. coli</i>
1	0.00	0.00	0.00
2	6.70	8.81	5.50
3	10.10	12.29	8.20
4	12.75	13.40	10.50

The control, without the beverage but instead distilled water, showed no bactericidal or bacteriostatic activity as the isolates were all resistant to it by the conspicuous appearance of heavy growth. That is to say that the growth of the bacterial strains wasn't impaired by the control (distilled water). Table 2 shows the repressive characteristic of the steeped corn-paste beverage and it ranged from 5.50mm against *E. coli* (least susceptible) to 13.40mm against *S. dysenteriae* (most susceptible) between the 2nd and 4th day after inoculation, at the 100% concentration. The three bacteria were all sensitive to the product. *S. dysenteriae* was the most susceptible with a value of 13.40mm, followed by *S. enterica* with a value of 12.75mm and lastly *E. coli* with a value of 10.50mm. They were all resistant at the first day of inoculation. The steeped corn-paste beverage was eventually able to inhibit the growth of all the bacterial strains and also, the spectrum of its antibacterial activity varied.

DISCUSSION

This investigation revealed that the steeped corn-paste beverage could be an additionally very hopeful solution in the therapy and probable prophylaxis of ailments linked with entero-bacteria. The three targeted Gram-negative bacteria for this study had their growth inhibited by the steeped corn-paste beverage that was incorporated into the medium, as portrayed by their incapability to thrive well in the presence of the steeped corn-paste beverage and measured in term of the zones of inhibition. This suggests that pap prepared with its steeped corn-paste beverage could be leisurely consumed while simultaneously acting as a prophylactic or therapeutic beverage against bacterial food poisoning. By the 2nd day, the zones of inhibition appeared and started increasing. The plausible onset of the synthesis of

lactic acid and other antibacterial metabolites by the bacterial cultures inherent in the beverage could have been responsible for the zones of inhibition. It was determined that the beverage obtained was acidic and hence must have impeded the survival and proliferation of the bacteria.

Although, the microbial succession during the fermentation process was not investigated but those microorganisms, particularly bacteria, which started the conversion must have given way to others. During the fermentation of the corn grains, the pH lowered and climaxed at the last day of fermentation. The inhibitory property increased with duration in days. This observation is corroborated by Chuayana, Ponce, Rivera and Cabrera (2003). Kotz, Peterson, Moody, Savaiano and Levitt (1990) explained that the antimicrobial effect is due to the combined effect of acids and the production of other antimicrobial metabolites by lactic acid bacteria (LAB) which have significant effect on the bacterial growth as well as increasing the zones of inhibition. Caplice and Fitzgerald (1999) attributed the anti-microbial effect of lactic acid bacteria (LAB) to lactic acid and organic acids production causing the pH of a growth environment to decrease. In addition, Lindgren and Dobrogosz (1990) stated that low pH induces organic acids to become lipid soluble, which then diffuses through cell membranes and into the cytoplasm of bacteria.

Steeped corn-paste beverage is reported to contain lactic acid bacteria (LAB) and some other bio-active components which possess inhibitory effect on microorganisms. One of these bioactive components is bacteriocin (Ogunbanwo, Sanni and Onilude, 2003) and which acts by destroying bacterial membranes (Savadago, Outtara, Bassole and Traore, 2006). Studies of several bacteriocins have indicated that they



are non-toxic and non-immunogenic, as reported by Lindgren and Dobrogosz (1990).

CONCLUSION

It can thus be inferred that, under routine conditions, the steeped corn-paste beverage showed a significant antibacterial capability against the three Gram-negative bacteria, perhaps, mainly due to the presence of lactic acid and ethanol from fermentation. *Shigella dysenteriae* was the most susceptible bacterium, as it recorded the highest zone of inhibition while *Escherichia coli* was the least susceptible.

RECOMMENDATIONS

Pap is a product of lactic acid fermentation and thus potentially offers some therapeutic health benefits, therefore, its prospects as an organic antibacterial diet, together with its steeped corn-paste beverage, should be expansively exploited particularly against Gram-negative bacteria. The protein-fortified pap; Dogik, developed by the Federal Institute of Industrial Research (FIRO), Oshodi, Lagos, Nigeria, should be highly commercialized due to its therapeutic property on the basis of its ability to control diarrhoea, especially among infants in African countries since pap is used as their popular weaning food.

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