

Full Length Research Paper

# Antibacterial activity of microorganisms isolated from the liquor of fermented maize *Ogi* on selected diarrhoeal bacteria

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The antibacterial activity of the microorganisms isolated from the liquor of fermented maize “ogi” (*Zea mays* Linn.), subjected to continuous natural fermentation at  $30 \pm 2^{\circ}\text{C}$  for 72h on some diarrhoeal bacteria was evaluated in this study using agar diffusion assay. The test bacteria used are *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Salmonella typhimurium*. The bioactive metabolites responsible for the antibacterial activity of the isolates on the test organisms were also evaluated. Three microorganisms were isolated and identified from the fermented liquor; these are *Lactobacillus plantarum*, *Lactobacillus brevis* and *Saccharomyces cerevisiae*. The *lactobacilli* isolated inhibited the growth of all the test organisms with *L. plantarum* exerting greater effect. *S. cerevisiae*, on the other hand, did not inhibit the growth of any of the test bacteria. The growth inhibition mediated by the *lactobacilli* is due to the synergistic activity of biometabolites; organic acid, hydrogen peroxide and bacteriocin produced by them on *E. coli*, *S. dysenteriae* and *S. typhimurium* and the synergistic effect of organic acid, bacteriocin but to a lesser degree, hydrogen peroxide on *S. aureus*. This work has been able to show that the growth inhibition mediated by the fermented liquor on the test organisms is attributable to the presence of bioactive metabolites such as organic acid, hydrogen peroxide and bacteriocin produced by *L. brevis* and *L. plantarum* present in the liquor.

**Keywords:** Fermented maize “ogi” liquor, microflora, antibacterial activity, diarrhoeal bacteria.

## INTRODUCTION

All over the world, diarrhoea is a serious health problem especially in children (Walderman, 1998; Kosek *et al.*, 2003; Black, 2004; Wendell *et al.*, 2008). Although diarrhoea is self limiting, but when it is as a result of bacterial infections, antibiotics therapy may be required. However, because most bacteria causing infections have developed resistance to most of the conventional antibiotics, there is the need to search for more effective antibacterial drugs to treat infections caused by these organisms.

In searching for alternatives to conventional antibiotics in treating bacterial diarrhoea, Adebolu *et al.* (2007) observed that the liquor of uncooked “ogi”, a Nigerian

fermented food has antibacterial activity against common bacteria that cause diarrhoea. Moreover, Adebolu (2007) observed that the fermentation duration of uncooked “ogi” plays a significant role in the growth inhibitory activity of the liquor on susceptible organisms. Furthermore, Adebolu and Adaramola (2010) observed that the mode of fermentation, whether continuous or discontinuous at every 24 h at  $30 \pm 2^{\circ}\text{C}$ , plays a significant role in the inhibition. Although, a lot of work has been done on the antibacterial activity of the liquor of “ogi”, more is still desired so that all necessary scientific intricacies will be taken care of for its usage to be maximally exploited. This present work therefore was done to determine the factors present in the liquor of fermented maize “ogi” responsible for its antibacterial activity on the selected diarrhoeal bacteria and which one of the factors is the most effective.

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**Table 1:** Growth inhibitory activity of the supernatant of the microbial isolates from the liquor of fermented maize “ogi” on selected diarrhoeal bacteria.

Organism	Diameter of zone of inhibition (mm)			
	OGI1	OGI2	OGI3	Control
<i>Escherichia coli</i>	0.0	7.0	8.0	19.0
<i>Staphylococcus aureus</i>	0.0	5.0	8.0	18.0
<i>Shigella dysenteriae</i>	0.0	7.0	9.0	18.0
<i>Salmonella typhi</i>	0.0	5.0	6.0	17.0

**Key:** OGI1, *Saccharomyces cerevisiae*; OGI2, *Lactobacillus brevis*; OGI3, *Lactobacillus plantarum*; Control, Liquor of maize “ogi” fermented for 72 h.

## MATERIALS AND METHODS

### Test Bacteria

Stock cultures of bacteria used in this study, *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhimurium* were collected from Microbiology Laboratory, Lagos University Teaching Hospital, Lagos State. They were confirmed in the laboratory through Gram’s staining, culturing on appropriate selective media and were also subjected to various biochemical tests.

### Maize grains used

Maize (*Zea mays*) grains used were purchased from Oba’s market, Akure, Ondo State, Nigeria.

### Preparation of “ogi” from maize grains

The maize grains used were sorted to remove pebbles, mouldy and deformed grains followed by washing in clean water to remove dirt and surface contaminants. Two kilograms of the clean grains were steeped in clean water that sufficiently covered the grains inside a clean plastic bucket with a cover and left at room temperature (30±2°C) for 72 h. The grains were washed in three changes of clean water and wet-milled using a local grinding machine. The resulting paste was sieved with a clean muslin cloth and the filtrate was collected into a clean plastic bucket with cover. The filtrate was allowed to settle at 30 ± 2°C for 72 h for natural fermentation to take place. The liquid on top after the filtrate has settled is referred to as the liquor while the sediment is referred to as the slurry.

### Isolation and identification of the microorganisms present in 72h fermented maize “ogi” liquor

The liquor was cultured on nutrient agar, Man de Rogosa and Sharpe (MRS) agar and MacConkey agar for the

isolation of bacteria and potato dextrose agar for fungi using standard microbiological techniques. Pure bacterial isolates were then subjected to tests such as Gram’s reaction and biochemical tests and identified according to Holt *et al.* (1994). The fungi, on the other hand, were identified according to Alexopoulos and Mims (1988).

### Assessment of the growth inhibitory activity of the microorganisms isolated from the liquor of the fermented maize “ogi” on the test organisms

Each of the organisms isolated from the liquor of 72 h fermented maize “ogi” was grown separately in nutrient broth at 37°C for 24 h. After inoculation, the broth cultures were centrifuged at 3000 rpm for 5 min. The resulting supernatants (cell free extracts) were decanted and used immediately against the test bacteria using agar well diffusion assay. Fermented “ogi” liquor from which these three organisms were isolated was used as control. The effect of the bioactive metabolites in the supernatant of the isolates on the test organisms was then evaluated using the method of Jin *et al.* (1996).

## RESULTS

Three microorganisms were isolated from the liquor of the fermented maize “ogi” used in this study. These are *Lactobacillus brevis* and *Lactobacillus plantarum* which are bacteria and *Saccharomyces cerevisiae*, a yeast. The cell free extracts of *L. plantarum* and *L. brevis* inhibited the growth of all the test organisms with diameter zone of inhibition ranging from 6.0 - 9.0 mm and from 5.0 - 7.0 mm respectively but the cell free extract of *S. cerevisiae* did not inhibit the growth of any of the test organisms. The control, containing all the three isolates, on the other hand, had the highest growth inhibitory activity ranging from 17.0 - 19.0 mm in diameter on the test organisms (Table 1). When the metabolites present in these cell free extracts were selectively blocked to evaluate their relative contributions in inhibiting the growth of the selected organisms, there was no growth inhibition of the

**Table 2:** Effect of selective blockage of the different metabolites in the supernatant of *Lactobacillus plantarum* isolated from the fermented maize “ogi” on the selected diarrhoeal bacteria.

Organism	Diameter of zone of inhibition (mm)			
	A	B	C	D
<i>Escherichia coli</i>	0.0	0.0	0.0	8.0
<i>Staphylococcus aureus</i>	0.0	5.0	0.0	8.0
<i>Shigella dysenteriae</i>	0.0	0.0	0.0	9.0
<i>Salmonella typhimurium</i>	0.0	0.0	0.0	6.0

**Key:** A, Trysin treated supernatant; B, Catalase treated supernatant; C, NaOH treated supernatant; D Control, untreated supernatant.

**Table 3.** Effect of selective blockage of the different metabolites in the supernatant of *Lactobacillus brevis* isolated from the fermented maize “ogi” on the selected diarrhoeal bacteria.

Organism	Diameter of zone of inhibition (mm)			
	A	B	C	D
<i>Escherichia coli</i>	0.0	0.0	0.0	7.0
<i>Staphylococcus aureus</i>	0.0	2.0	0.0	5.0
<i>Shigella dysenteriae</i>	0.0	0.0	0.0	7.0
<i>Salmonella typhimurium</i>	0.0	0.0	0.0	5.0

**Key:** A, Trysin treated supernatant; B, Catalase treated supernatant; C, NaOH treated supernatant; D, Control, untreated supernatant.

test organisms except *S. aureus* whose growth was inhibited when hydrogen peroxide present in the cell free extracts of the isolated *lactobacilli* was selectively blocked with catalase (Tables 2 and 3). The inhibition however was not as wide as that mediated by the untreated supernatants of the isolated *lactobacilli* which were 5.0 and 8.0 mm respectively for the effect of *L. brevis* and *L. plantarum* on the organism.

## DISCUSSION

From this investigation, only *L. brevis* and *L. plantarum*, two of the three microorganisms isolated from the liquor of maize “ogi” subjected to continuous fermentation for 72 h, exerted growth inhibitory activity on all the test bacteria (Table 1). This shows that these organisms produce bioactive metabolites that have growth inhibitory activity on the selected diarrhoeal bacteria. These metabolites, according to Olukoya *et al.* (1994) and Ogunbanwo *et al.* (2003), include lactic acid, hydrogen peroxide and bacteriocin. These metabolites are produced by *lactobacilli*, which are reported to be part of the normal flora of “ogi” (Odunfa and Adeleye, 1985; Adebolu *et al.*, 2007).

In this study, when these metabolites were selectively blocked to evaluate their relative contributions in inhibiting the growth of the selected organisms, only the growth of *S. aureus*, which is a gram positive bacterium

was inhibited when hydrogen peroxide was selectively blocked with catalase (Tables 2 and 3). This shows that the inhibition mediated by *lactobacilli* isolated from the liquor of fermented maize “ogi” on this organism is majorly through the synergistic activities of bacteriocin and organic acid produced by them. Hydrogen peroxide, on the other hand, did not contribute much in inhibiting the growth of this organism. For the other organisms, however, the inability of the cell free extracts whose metabolites were selectively blocked to inhibit their growth shows that all the bioactive metabolites present in the cell free extracts are needed to inhibit the growth of these organisms. When these metabolites were not blocked, the cell free extracts containing them inhibited the growth of all the test organisms. This shows that synergistic effect of these metabolites was responsible for the inhibition of the growth of the test organisms. It is interesting to note that the three organisms in this category are gram negative bacteria which are known to have more complex cell wall components than the gram positive counterpart (Wiley *et al.*, 2008). Since the nature of the cell wall of bacteria has an effect on their level of susceptibility to antibiotics (Trias and Benz, 1994; Mann *et al.*, 1997; Rangel *et al.*, 2002; Dale and Mendelstam, 2008), it is possible that the cell wall of these organisms protected them from the effects of the selectively blocked bioactive metabolites. However, when the metabolites were not blocked, the cell free extracts were able to inhibit the growth of the test organisms. This might likely

be the reason for this observation.

From this study, it is concluded that the growth inhibition exerted by the liquor of fermented maize "ogi" on selected diarrhoeal bacteria is due to the synergistic effect of the different metabolites such as organic acid, bacteriocin and hydrogen peroxide produced by the *Lactobacilli* present in the "ogi".

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