International Journal of Scientific Research and Management (IJSRM)
||Volume||10||Issue||06||Pages||MP-2022-645-651||2022||
Website: www.ijsrm.in ISSN (e): 2321-3418
DOI: 10.18535/ijsrm/v10i06.mp01

Anti-Diarrhea Effects of OGI Produced Using *Saccharomyces Boulardii* As Starter Culture

ADIGUN, Musibau Adeleke, IBIKUNLE, Bukola Jelilat

Biological science department federal polytechnic offa kwara state Nigeria

Abstract
The antidiarrhea effect of 96 hr fermented 'Ogi' (Corn slurry) using *Saccharomyces boulardii* as starter culture against *Escherichia coli* was investigated in-vitro and in-vivo using Wistar rats. The pH and titratable acid of the slurry were determined, while the antimicrobial effect on diarrhea causing organisms, *Escherichia coli* was also determined. Using well diffusion method, 0.1 ml of the slurry effectively inhibited the growth of *E. coli* with zones of inhibition ranging from 2.4 (±0.14)a mm at 24hr of fermentation to 8.4(±0.11)d mm at 96hr of fermentation. The zones of inhibition increased with increasing period of fermentation of the filtrate which is Ogi. The titratable acidity of the fermenting Guinea corn increased with increasing period of fermentation and reached a peak at 96hr of fermentation (0.25 (± 0.02)c %). The pH however decreased with increase in the period of fermentation from 5.65 (± 0.01)a at 0hr to 3.54 (± 0.02)e at 96hr of fermentation. The antidiarrheal activity of Guinea corn slurry was investigated in-vivo using *E. coli* to induce diarrhea. The infected test groups of rats were fed orographically with fermented slurry of Guinea corn (Ogi), whereas positive control group was neither being infected nor fed with ogi while negative control group was infected with E. coli but not fed with Ogi. In test groups fed with fermented 'Ogi' recovered from diarrhea infection. The obtained results of the present study confirm the possibility of using fermented guinea corn slurry (Ogi) produced using *Saccharomyces boulardii* as starter culture for the treatment of diarrhea rather than the organisms in drug presentation which reduces compliance in diarrheal patients most especially children.

Key words: Antidiarrhea, Fermentation, Inhibition, Orographically, pH and *Saccharomyces boulardii*.

Introduction

Diarrhea is a gastrointestinal disorder which occurs when a person at equilibrium with microbial environment and diet is exposed to unfamiliar microorganisms such as viruses, parasites but mostly bacterial e.g. *Escherichia coli, Clostridium difficile*, etc. taking in through food or water orally which overwhelm the productive effect of the normal intestinal flora (Olatokuboh *et al*., 2014). Other factors such as carbohydrate mal-absorption (lactose intolerance) could also be responsible for diarrhea occurrence in infants.

Microbes act on the intestinal enterocytes directly or via the toxins they release. This is involved in the pathology leading to diarrhea. Pathophysiology of diarrhea involve increasing secretion of fluid into the intestine or/and reduced absorption of fluid from the intestine or rapid passage of stool through the intestine. It leads to the frequent passage of watery, loose stools or passing more than three liquid bowel movements daily.

This problem could be addressed by improving sanitation and adequate nutrition but an inexpensive and effective probiotic would have high value also.

Probiotic is defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO and WHO, 2011). Probiotic have demonstrated an ability to prevent and treat some infections confined to the gastrointestinal tract as its prophylactic ingestion inhibit pathogens, block their attachment to intestinal mucosal walls to mention a few (Tasteyre *et al*., 2012). Probiotics are viable microbial food supplements, which beneficially influence the health of the host (Schrezenmeir and de Vrese, 2011). The term probiotics was introduced by Lilly and Stillwell in 1965 to describe growth-promoting factors produced by microorganisms. Tissier (1906) first observed that children with diarrhea had in their stools a low number of bacteria characterized by a peculiar, Y shaped morphology. These "bifid" bacteria

were, on the contrary, abundant in healthy children. He later suggested that these bacteria could be administered to patients with diarrhea to help restore a healthy gut flora. Members of the genera, Lactobacillus and Bifidobacterium are mainly used, but not exclusively, as probiotic microorganisms and a growing number of probiotic foods are available to consumers (FAO and WHO, 2011). Probiotics can be classified as bacterial probiotic agents and non-bacterial probiotic agents (Marteau, 2012).

Among the bacterial probiotics are; *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Enterococcus faecium etc. Lactobacilli* are important for the maintenance of the intestinal microbial ecosystem (Sandine, 2019). They are one of the early colonizer of the small intestine as they are found within the first week of life (Isolauri and Salminen, 2016). Several beneficial effects of these groups of bacteria in the gut had been reported. Such beneficial effects include protection from pathogens, alleviation of lactose intolerance, relief of constipation, anti-cholesterolaemic effect and immuno-stimulation (Casas and Dobrogosz, 2012). *Saccharomyces boulardii* is the only therapeutic yeast species proven to be beneficial as a probiotic (McFaland and Vandennplas, 2012). *Saccharomyces boulardii* is the sole probiotic that has proven a significant efficacy in treating relapsing *C. difficile*-associated diarrhoea (Collins *et al*., 2016). A potential probiotic organism must possess the following attributes: ability to survive the condition in the gut, antimicrobial production, and ability to adhere to the intestinal cell of the host (Collins *et al*., 2016).

Quality, safety and acceptability of traditional fermented foods largely depended on by people with low income have been substantially improved through the use of starter culture as reported by Wakil and Daodu, 2011. *Ogi*- acid-fermented cereal gruel is a staple food of several communities in Nigeria. Ogi is a cheap and popular weaning food in several West African countries. It is traditionally produced by lactic acid fermentation of maize, sorghum or millet. Several reports had identified steeping and souring as the two fermentation stages involved in the traditional process of *ogi*. Traditional fermented foods prepared from most common types of cereals (such as corn, rice, wheat or sorghum) are well known in many parts of the world. Some are utilized as colorants, spices, beverages and breakfast or light meal foods, while a few of them are used as main foods in the diet (Blandino *et al*., 2013). Several processing technologies which include cooking, sprouting, milling and fermentation have been put into practice to improve the nutritional properties of cereals, although probably, the best one is fermentation (Mattila-Sandholm, 2015).

The present study is therefore aimed at studying the anti-diarrhea effect of Ogi produced using strain of *Saccharomyces boulardii* as starter culture.

Materials and Methods

Collection of Materials

Test Bacteria:

The test organism used was *Escherichia coli* a clinical sample collected from the Microbiology Laboratory, University of Ilorin Teaching Hospital, Ilorin in Kwara State. The identity of the organism was confirmed in the laboratory using microscopic techniques, Gram's staining and biochemical tests.

Preparation of slurry "ogi" from Guinea corn Using *Saccharomyces boulardii* as starter culture

The sample was prepared from guinea corn (Sorghum bicolor) which was purchased from Owode market in Offa, Kwara State, Nigeria. Corn slurry (Ogi) was produced following the method of Wakil and Daodu, 2011. The guinea corn grain was screened and two kilograms of the clean grains was measured and steeped in clean water at room temperature (30+20C) for 24 h. The grains were wet-milled using a grinding machine and resulting paste was sieved through a clean muslin cloth and the filtrate was collected into a clean plastic bucket in which 5g of *Saccharomyces boulardii* was added. The mixture was allowed to settle at 30 + 20C for 96 h for fermentation to take place. During fermentation the slurry settled at the bottom of the bucket and the water on top is known as the liquor. Samples of the slurry and the liquor were taken after sieving and every 24 hours intervals during fermentation period for analysis.

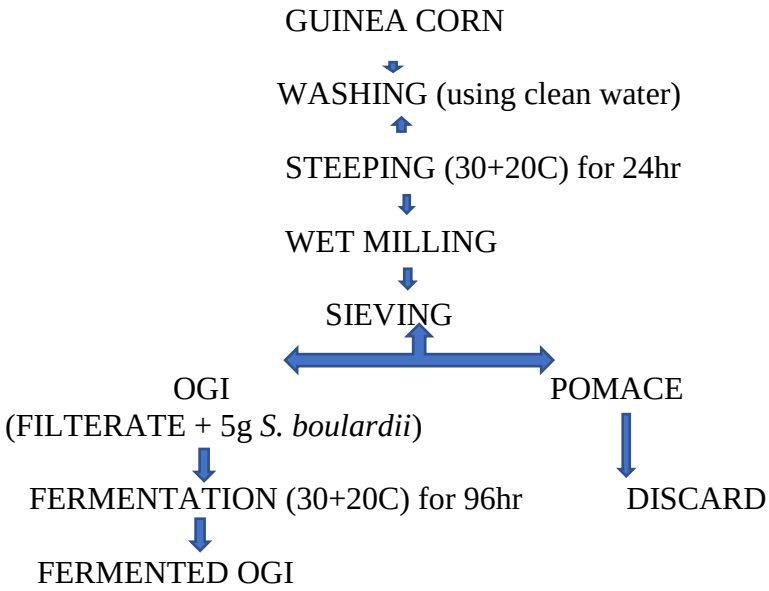

Figure 1: Production of fermented "ogi" using *S. boulardii* as starter culture (Wakil and Daodu, 2011).

Physicochemical properties

Measurement of pH of fermenting substrate

The method described by AOAC (2005) was used to determine pH of the fermenting medium. Samples of 10ml were taken every 24 hrs during the fermentation period and pH was measured using an Orion pH meter (Model 310, Orion Research Inc., Beverly, MA) equipped with glass electrode. The pH meter was calibrated with standard buffers of pH 4.0, 7.0 and 9.0 before the measurements.

Determination of titratable acidity of fermenting substrate

Aliquot (20ml) of the homogenized corn slurry sample was titrated against 0.1 M NaOH using phenolphthalein as indicator. Values obtained were expressed as percent acidity. All analyses were carried out in triplicates (AOAC, 2005).

Calculation

Titratable acidity (TA) = (volume of NaOH/volume of homogenized corn slurry) × 0.75 %.

Antimicrobial activity of slurry of guinea corn against *E. coli*

Agar well diffusion method was used in accordance to Adebolu, 2008; Abdus-Salaam *et al.*, 2014. One ml of 18hr old culture of the test organism (*E. coli*) was taken using a sterile pipette and placed into sterile Petri-dishes. Each plate was then overlaid with 20ml nutrient agar, carefully swirled to allow even distribution of the organisms within the agar and were allowed to gel before six wells (4mm in diameter) were bored in the agar with the aid of a sterile cork borer. The fermented slurry was put into the wells; 1ml per well, per three plates each respectively and sterile distilled water was used as control while chloramphenicol (2g:10ml distil water) was used as standard. The plates were incubated at 37^0C for 24 hours. The diameter zone of inhibition around the wells containing the 'Ogi' was determined and recorded. This was done for sample taken at every interval of 24 hour of fermentation from 0hr – 96hr.

Assessment of antidiarrheal effect of fermented Ogi in *E. coli* diarrhea-induced albino rats

Experimental Animals

Forty (40) albino rats (Rattus norvegicus) aged 5–6 weeks were purchased from IBK animal house in Offa, Kwara state. The animals were housed in steel cages and kept at room temperature; and were allowed to acclimatize for 21 days prior to the commencement of the experiment. All the rats were given water and pellet feed (Vital feed) by *ad libitum*.

Experimental Design

The rats were fasted 24 hours prior to the commencement of the experiments and were randomly divided into three groups of ten rats each. The rats were labeled indicating organism used for inducing diarrhea and feed sample. Escherichia coli suspension was prepared with physiological normal saline. The rats were

orogastrically dosed with 1 ml of 10^5 cfu/ml of *E. coli* suspension to induce diarrhea in them (Adebolu, 2008). The treatment above was repeated daily for 3 days. A post ingestion period of 20 days was observed after the administration of the culture. Time of change in fecal consistency was observed in grouped rats after the administration of *E. coli*. Therapeutic feeding with 96hr fermented liquor and corn slurry of guinea corn commenced as soon watery stool is noticed in the infected rats. Rats with watery stool were considered to have diarrhea. The rats were labeled CG, EG, ENL and ENO where CG is the group of rats fed with basal feed (Grower mash) only without being dosed with *E. coli*. EG is the group of rats dosed with 1 ml of 10^5 cfu/ml of *E. coli* and fed with grower mash. ENL is the group of rats dosed with 1 ml of 10^5 cfu/ml of *E. coli* and fed with fermented liquor while ENO is the group of rats dosed with 1 ml of 10^5 cfu/ml of *E. coli* and fed with fermented ogi. Feeding of the rats with sample after being infected was continued until there was change in their feacal products from watery stool to normal dried pallet-like feacal product.

Data analysis

The data gathered was processed in triplicate and mean value was determined and recorded. Results were presented in tables.

Results

Physicochemical changes in guinea corn slurry during fermentation

The physicochemical changes in guinea corn (GC) slurry during fermentation revealed that pH value reduced from 5.63(± .01)a at 0hr to 3.44(± .02)e at 96hr while there was increased in the titratable acidity from 0.13(± 0.02)a % at 0hr to 0.25(± 0.01)c % at 96hr as shown on Table 1.

Confirmatory test on the test organism

As shown on Table 2, the test organism was characterized and confirmed to be *E. coli* culture by showing positive activities to catalase, indole, nitrate, methyl red, presumptive and motility tests and negative activities to citrate, gelatin hydrolysis, urease, voges-proskaurer and oxidase tests.

In-vitro antibiotic activities slurry of fermented guinea corn against *Escherichia coli*

The susceptibility of *E. coli* to the liquor of GC slurry during fermentation was shown on Table 3. It revealed that liquor sample showed no activity against *E. coli* at 0hr of fermentation but the activity against *E. coli* by subsequent samples at 24hr to 96hr of fermentation showed increase in activities as the period of fermentation progressed, 4.1(±0.12)b mm zone of inhibition at 24hr of fermentation to 8.4(±0.11)d mm zone of inhibition at 96hr of fermentation).

Table 1: pH and titratable acidity during fermentation of ogi

Duration	pH	Titratable acidity (%)
0	5.63(± 0.01)a	0.13(± 0.02)a
24	4.82(± 0.02)b	0.16(± 0.01)b
48	4.52(± 0.03)c	0.18(± 0.01)a
72	4.48(± 0.02)d	0.22(± 0.01)b
96	3.44(± 0.02)e	0.25(± 0.01)c

Values are means of triplicate determination. Means having same alphabet along the column are not significantly different (P>0.05).

Table 2: Characterization of the test organism (*E. coli*)

Biochemical tests	Reactions
Catalase	+
Citrate utilization (Simmon's citrate Agar)	-
TSI Agar	G/A

Gelatin liquefaction (Nutrient Gelatin)	-
Indole Production	+
Nitrate Reduction	+
Urease (Urea Broth)	-
Voges-Proskaur	-
Methyl Red	+
Presumptive test (Lauryl sulphate Broth)	+
Motility (SIM Medium)	+
Oxidase	-
Organism	E. coli

Key: A/G- acid (yellow) and gas formation in butt of tube and acid (yellow) on slant surface

Table 3: In-vitro antidiarrhea activities of fermented Ogi against *Escherichia coli*

Susceptibility of *E. coli* to slurry of fermented 'Ogi'

Duration (hr)	Diameter of zones of inhibition (mm)		
	Slurry	Sterile distilled water	Chloramphenicol
0	0.0 (±0.00)a	0.0 (±0.00)a	10.4 (±0.12)a
24	4.1 (±0.12)b	0.0 (±0.00)a	10.4 (±0.12)a
48	6.2 (±0.11)c	0.0 (±0.00)a	10.4 (±0.12)a
72	7.3(±0.23)d	0.0 (±0.00)a	10.4(±0.12)a
96	8.4(±0.11)d	0.0 (±0.00)a	10.4(±0.12)a

Values are means of triplicate determination. Means having same alphabet along the column are not significantly different (P>0.05).

In-vivo antidiarrhea activities of fermented Ogi against *Escherichia coli*

Effect of fermented Ogi on *E. coli* induced diarrhea in Wistar rats

Effect of fermented Ogi produced using *S. boulardii* as starter culture on the *E. coli* induced diarrhea in Wistar rats is shown on table 4. The Albino rats that were infected with *E. coli* produced watery stools after four days of infection. In the rats to which fermented guinea corn liquor and slurry were administered, the stool started to thicken after 8 and 7 days of treatment respectively and produced pellet-like stools on the 19th and 17th day of experimental period respectively. The untreated rats started dying on the 12th day after infection and all were dead by the 16th day. The control rats that were neither infected nor treated produced pellet-like stools all through.

Table 4: Effect of fermented Ogi on the diarrhea induced by *E. coli* in Wistar rat

	Feacal product texture for period of 20 days after 4-day inoculation																			
Rat Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
CG (10)	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
EG (10)	p	p	p	p	w	w	w	w	w	w	w	D2	D3	D1	D1	D1	-	-	-	-
ENL (10)	p	p	p	p	p	w	w	w	w	w	w	w	w	rw	rw	rw	rw	rw	p	p
ENO (10)	p	p	p	p	p	w	w	w	w	w	w	w	rw	rw	rw	rw	p	p	p	p

Key: (10) = population of rats per group; p = pellet-like stool; w = watery stool; D = number of dead rat; rw = thicken stool.

CG= group of rats fed with grower mash only without being dosed with *E. coli*.

EG= group of rats dosed with 1ml of 10^5cfu/ml of *E. coli* and fed with grower mash.

ENO= group of rats dosed with 1ml of 10^5cfu/ml of *E. coli* and fed with fermented Ogi.

Discussion

During fermentation of guinea corn slurry, a decreased from 5.63(± 0.01)a to3.44(± 0.02)e in pH as the fermentation progressed was observed as shown on table 1. This was possible because of the accelerated growth rate of lactic acid bacteria (Inyang and Idoko, 2016). The decrease in pH, increase in Lactic acid bacteria and increase in titratable acid followed the same trend as reported for other natural fermented foods (Nduka *et al*., 2016). At the onset of fermentation, pH value was close to neutrality, but as fermentation progressed a drop in pH value was observed. This drop in pH agreed with the previous studies by Lei and Jakobsen (2014). Generally, acidity increased as fermentation advanced according to Akpinar-Bayizit *et al*., 2007. The reduction in pH and simultaneous increase in titratable acidity during fermentation could be attributed to the activities of lactic acid bacteria (Ojokoh *et al*., 2013). Lactic acid bacteria have been reported to degrade carbohydrates resulting in acidification (Ojokoh *et al*., 2013).

The antimicrobial activity of the slurry from guinea corn was investigated. It was found that the slurry from guinea corn showed no antibacterial activity at 0hr of fermentation and highest antibacterial effect 8.4 (±0.11)d mm at 96hr on *Escherichia coli* as shown on Table 3.

There was a progressive increase in the antibacterial effect of 'Ogi' sample on *E. coli* with increase in the fermentation period as shown on Table 3. This finding is in agreement with the report of Adebolu *et al*., (2007) who reported that the inhibitory effect of fermented ogi on the pathogenic bacteria (*E. coli*) confirms the traditional claims where corn liquors are used to treat diarrhea in many localities in Nigeria. This shows that raw fermented liquor and slurry has bioactive components responsible for the inhibitions recorded in the work against the test isolate. Lactobacilli were reported by Ogunbanwo *et al.,* (2013) to produce bioactive metabolites such as lactic acid, hydrogen peroxide and bacteriocin from sorghum ogi sample.

This studies on diarrhea, observing a significant reduction in stool frequency and stool consistency after fermented liquor and slurry of guinea corn therapy was conducted as shown in Table 4. The group of albino rats (ENL) that were induced with *E. coli* to have diarrhea stop stooling watery feacal product after 8 days of feeding them with 600ml of fermented liquor daily. The group of albino rats (ENO) that were induced with *E. coli* to have diarrhea stop stooling watery feacal product after 7 days of feeding them with 600ml of fermented slurry daily while the EG group of albino rats continue stooling and died of diarrhea. The CG group which served as control group had no symptom of diarrhea. The ameliorative effects in the group ENL and ENO showed the anti-diarrhea potentials of microorganisms present in fermented ogi and involved in its fermentation which eventually confer the therapeutic effect against diarrhea.

Conclusion

The slurry of fermented guinea corn (Ogi) inhibited the growth of *Escherichia coli* in-vitro and in-vivo but fermented slurry exhibited more effectiveness as the period of fermentation of Ogi increases.

Fermented guinea corn slurry produced using *S. boulardii* as starter culture could be used for the treatment of diarrhea. Further investigations on the antidiarrhea potential of African fermented cereals produced using other microorganisms of probiotic potential as starter culture are needed to establish possible beneficial effects such as probiotic treatment of diarrhea which is immense.

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