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INHIBITION OF SOME INTESTINAL PATHOGENS BY *LACTOBACILLUS SPECIES* ISOLATED FROM OGI

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ABSTRACT

Twenty five strains of Lactobacillus species and yeast were isolated from ogi. They were identified as *Lactobacillus plantarum* (32.4%), *L. delbrueckii* (14.5%), *L. casei* (16.2%), *L. fermentum* (10.3%) *L. acidophilus* (16.4%), L. *brevis* (7.4%). The lactic acid strains were tested for bacteriocin and the strains that were tested positive were not used for further investigation while catalase was added to growth medium to eliminate hydrogen peroxide production. The organisms inhibited to varying degrees the growth of various intestinal pathogens of human, the typed strains collected from the University College hospital (UCH), Ibadan, Nigeria were used as indicator organisms. These are *Salmonella typhii*; *Shigella dysenteriae*; *Escherichia coli*; *Salmonella typhimurium*; and *Staphylococcus aureus*. Generally, gram positive bacteria were more inhibited than gram negative bacteria. Acidic pH supported inhibition while temperature (30- 35^{0}C) had negative effect on inhibitory activity of all the isolates except *L. delbrueckii* which tolerated temperature of 45^{0}C. We detected that ogi may be useful in preventing gastroenteritis caused by the aforementioned intestinal pathogens.

Keywords: Ogi, fermentation, Lactobacillus, weaning food, Inhibition

INTRODUCTION

Ogi is a fermented infant weaning and adult food produced from maize (*Zea mays* L) and sorghum (*Sorghum bicolor*) varieties. The gruel is used in western part of Nigeria to prevent stomach upset. Its preparation as described by various researchers (Akinrele, 1970; Banigo and Muller, 1972; Odunfa and Adeyele, 1985), is still a traditional family art.

Lactic acid bacteria have been used as a probiotic for many years. They are also recognised for their health and nutritional benefits (Gilliland, 1990), and equally play a fundamental role in microbial ecology, synthesizing a variety of antimicrobial compounds such as organic acid, hydrogen peroxide, diacetyl and bacteriocin (Klaenhammer, 1988).

Probiotic bacteria are used for treating intestinal harzardous micro flora and increased gut permeability characteristic to many intestinal disorders. Successful probiotics are able to survive gastric condition and colonize the intestine, at least temporarily, by adhering to the intestinal epithelium (Strum, 1997).

The ability of lactic acid bacteria to inhibit the growth of various gram positive or

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closely related bacteria is well documented. This inhibition could be through the production of organic acids, lactic and acetic acids (Sorrels and Speck, 1970; Gilliand and Speck, 1977). It could be as a result of hydrogen peroxide production (Price and Lee, 2001) or through the production of specific proteins or protein complexes called bacteriocin (de Vuyst and Vandamme,1994; Sanni *et al*., 1999). Lactic acid bacteria and their pro-active cellular substances exert many beneficial effects in gastro intestinal tracts. Some of these benefits are the prevention of the establishments and replication of several enteric mucosal pathogen through several antimicrobial mechanisms and the inhibition of intestinal mal absorption (Nandu *et al*., 2003).

However, information is scanty on intestinal bacteria sensitive to Lactobacillus inhibition. This paper therefore reports the inhibitory actions of *Lactobacillus spp* isolated from ogi on different intestinal pathogens of humans. This work was therefore carried out to investigate and determine the inhibitory activities of ogi micro flora.

MATERIALS AND METHODS

Samples collection

White maize (*Zea mays)* and red guinea corn (*sorghum vugare)* grains used for this study were obtained from retail market in Abeokuta, South Western Nigeria.

Traditional preparation of ogi

The cereal grains were cleaned and steeped in water for 2 days in earthenware pot (or any suitable container). The water was decanted and the grains were washed in several changes of clean water before wet milling and sieving with muslin cloth. The pomace was discarded and the starch suspension is allowed to sediment during which fermentation was carried out for 2-3 days by the natural micro flora of the grains (Odunfa and Adeyele, 1985).

Isolation of Lactobacillus species

This was done by weighing 1gram of each ogi samples into separate test tubes. The samples were serially diluted separately with sterile distilled water, from the diluted samples, 1ml was plated out in duplicates from 10^{-3}, $10^{-7}$and 10^{-9} solutions of each sample. MRS (Degman, Rogosa, Sharpe) agar was used, this was incubated for 24 – 32 hrs at 37^0C.

Characterization of Lactobacillus species

This was carried out by using API 50 CH strips (API System Biomerieux Sa, France). The strains were maintained as frozen stocks at -20^0C in Hogness freezing medium and propagated twice in MRS medium (Oxoid; Onipath Ltd, Basingstoke, Hampshire, England) before use.

Staphylococcus aureus used as indicator organisms was cultivated in brain heart infusion medium (Oxoid, UK). *Salmonella typhi* and, *Shigella dysenteriae* ,were cultivated in Desoxychocolate-Citrate agar (DCA) medium, while *E. coli* was cultivated in tryptic soy broth medium (Oxoid). One millilitre of the broth culture of the aforementioned isolates was used for the entire test except where otherwise stated.

Production of crude bacteriocin samples: *Lactobacillus species* were propagated in 1000 ml MRS broth (pH 7.0, glucose, 0.25% w/v, peptone, 0.5% w/v) for 72 h at 30^0C anaerobically (Oxoid Gas Generating

Kit) in triplicate (Sanni *et al.*, 1999). For extraction of bacteriocin, a cell-free solution was obtained by centrifuging (10,000 rpm for 20 min. at 4^0C with Beckman L5050B) the culture and was adjusted to pH 7.0 by means of 1M NaOH to exclude the antimicrobial effect of organic acid, followed by filtration of the supernatant through a 0.2 _m pore-size cellulose acetate filter. The supernatant was dialysed for 24 h at 4^0C (Schillinger and Lucke, 1989). Inhibitory activity from hydrogen peroxide was eliminated by the addition of 5 mg/ml catalase (C-100 bovine liver, Sigma) according to Daba et al. (1991). The bacteriocin producing strains were not used for further investigations.

Detection of antagonistic activity

For the detection of inhibitory activity of the test isolates, the well diffusion assay was employed (Schilinger and Locke, 1989; Takahiro *et a*l., 1991). This was carried out by using a sterile cork borer to punch six wells on the agar plates containing separately seeded indicator organisms. Two millilitres of the test isolates [treated with 5mg/ml catalase to eliminate inhibitory activity from hydrogen peroxide (Daba et al., 1991)] were introduced separately into each well and incubated anaerobically at 37^0C for 25hrs .The diameter of inhibitory zones $\geq$ 6 mm was defined as the positive result or it was taken as the well defined inhibition. The strains with well defined inhibitory observation on the plates were selected after 3 consecutive assays. While the pH was increased to 7 in order to eliminate acidity effect.

Effect of temperature on inhibitory activity of the test isolates

Set of plates was incubated 12hrs. at the following temperatures: 25, 30, 35, 40, 45, 50, and 55^0C, in order to know the optimum temperature for the reaction.

Effect of pH on the Inhibitory activity of the test isolates

The assay medium was adjusted using $0.005Ml^{-1}$ of HCl. or $0.005Ml^{-1}$ NaOH. to initial pH values of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 using a model 52 – 32 electrode pH meter.

RESULTS AND DISCUSSION

25 strains of *Lactobacillus species* were isolated from ogi. They were identified as *Lactobacillus plantarum* (32.4%), *L. delbrueckii* (14.5%), *L. Casei* (16.2%), *L. fermentum* (10.3%) *L. acidophilus* (16.4%), *L. brevis* (7.4%).

Members of lactic acid bacteria have been implicated in a variety of fermented foods (Tannock 1996; Odunfa and Adeyele, 1985). Bacteriocin production was detected in *L. fermentum*, *L. acidophilus* and *L. brevis* hence were not used for further investigations (results not shown). However, *L .delbrueckii, L. Plantarum* and 3 species of *L. casei* did not produce bacteriocin, hence the organisms were used for inhibitory assay.

Lactobacillus delbrueckii inhibited the growth of *Salmonella typhi, Shigella dysenteriae*, and *Staphylococcus aureus* with inhibition zones of 14.0, 8.0, and 14.0 mm, respectively. *Lactobacillus casei* (strain 02) inhibited the growth of *Salmonella typhii* and *Staphylococcus aureus* with the zones of inhibition of 10.0 and 20.0 mm, respectively. *Lactobacillus casei* (strain 03) inhibited *Salmonella typhi,*

Shigella dysenteriae, Salmonella typhimurium and *Staphylococcus aureus* with zones of inhibition of 10.0, 6.0, 32.0 and 21.0 mm respectively. *Lactobacillus casei* (strain S1) inhibited *Salmonella typhi, Shigella dysenteriae, Salmonella typhimurium* and *Staphylococcus aureus* with zones of inhibition 8.0, 12.0, 7.0, and 36.0 mm, respectively. Finally, *Lactobacillus plantarum* inhibited the growth of *Salmonella typhi, Salmonella typhimurium* and *Staphylococcus aureus* with zones of inhibition of 16.0, 14.0, 16.0 mm, respectively (Table 1).

The observed low inhibitory activity trend against gram negative bacteria especially *E. coli* agreed with the reports of Gilliand and Speck, (1977), which stated that Lactobacilli showed strong antibacterial properties against gram positive bacteria than gram negative bacteria. The influence of temperature and other parameters were investigated using *Staphylococcus aureus* as indicator organism. The lower the temperature, the lower the Inhibitory activity was the trend observed in all the isolates, the optimum activity was recorded at 35^0C. Interestingly, *L. delbrueckii* was the only exception which showed a difference with high temperature, the highest inhibition was observed at 45^0C (Fig 1).

The inhibition increased steadily as the pH increases, however, the optimum activity was observed between 5.5 and 6.0, above this, there was a significant reduction of inhibition (Fig 2). This is highly advantageous because the acidic nature of the stomach as well as its temperature would not prevent the active organisms present in ogi from attacking the gastroenteritis.
Further studies on the specific antibacterial agent(s) produced by the isolates which is or are responsible for the action are in progress.

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Table 1: Radius of inhibition of various intestinal pathogens by Lactobacillus species at optimum conditios (mm)

			Salmonella Typhi	*Shigella dysenteriae*	*E. coli*	*Salmonella typhimurium*	*Staphylococcus aureus*
Isolates	Strain	Origin					
Lactobacillus *delbrueckii*	01	White maize Ogi	14.0	8.0	Nszi	Nszi	14.0
Lactobacillus casei	02	White maize Ogi	10.0	2.0	2.0	3.0	20.0
Lactobacillus Casei	03	Yellow Maize ogi	10.0	6.0	3.0	21.0	32.0
Lactobacillus Casei	S1	Red sorghum	8.0	12.0	4.0	7.0	36.0
L. plantarum	S2	Red sorghum	16.0	nszi	5.0	14.0	16.0

nszi = no significant zone of inhibition
* Conditions measured in mm

Table 2: Differences in fermentation of carbohydrates by LAB strains isolated from ogi

Carbohydratesa	Percentage of strains with positive reactions to API 50 CH					
	L. plantarum	*L .delbrueckii*	*L. casei*	*L. fermentum*	*L. acidophilus*	*L. brevis*
Arabinose	65 (13) b	57	80	40	0	80
Ribose	42	52(5)	60	100	100	57
Xylose	3	100	100	10	0	100
Galactose	3(65)	79(19)	100	100	100	100
Fructose	94	100	100	100	100	100
Mannose	94	100	100	100	100	94
Rhamnose	0	0	0	50	0	90
Mannitol	0	0(5)	0(5)	0	0	40
Methyl-D-mannoside	3	0	0	0(10)	0	0
Methyl-D-glucoside	3	0	0	0	0	0
Amygdalin	3(6)	86(10)	86(10)	100	0	0
Arbutin	3(3)	76(14)	76(14)	100	0	0
Esculin	3(3)	100	100	100	0	0
Salicin	0	81(14)	81(14)	100	0	0
Cellobiose	100	95(5)	95(5)	100	0	0
Maltose	0	100	100	100	100	100
Lactose	3	14(10)	14(10)	80(20)	80(20)	10(30)
Melibiose	94	0	0	40(10)	40(10)	70
Sucrose	0	90	90	0	0	90
Trehalose	0	0	0	90	90	0
Starch	0	0	0	0	0	0
Gentiobiose	0	95(5)	95(5)	100	100	90
D-Togatose	55	5	5	90	90	20
Gluconate	10	81(5)	81(5)	0(30)	0(30)	80(25)
5-Keto-gluconate	3	0	0	0	0	0

[a] all strains fermented glucose and N-acetyl glucosamine, but none of the strains fermented glycerol, erythritol, D-arabinose, L-xylose, adonitol, b-methyl xyloside, sorbose, dulcitol, inositol, sorbitol, inulin, melezitose, raffinose, glycogen, xylitol, D-turanose, D-lyxose, Dfucose, L-fucose, D-arabitol, L-arabitol, and 2-keto-gluconate.

[b] the numbers in parenthesis indicate percent of strains that showed weak or delayed reactions

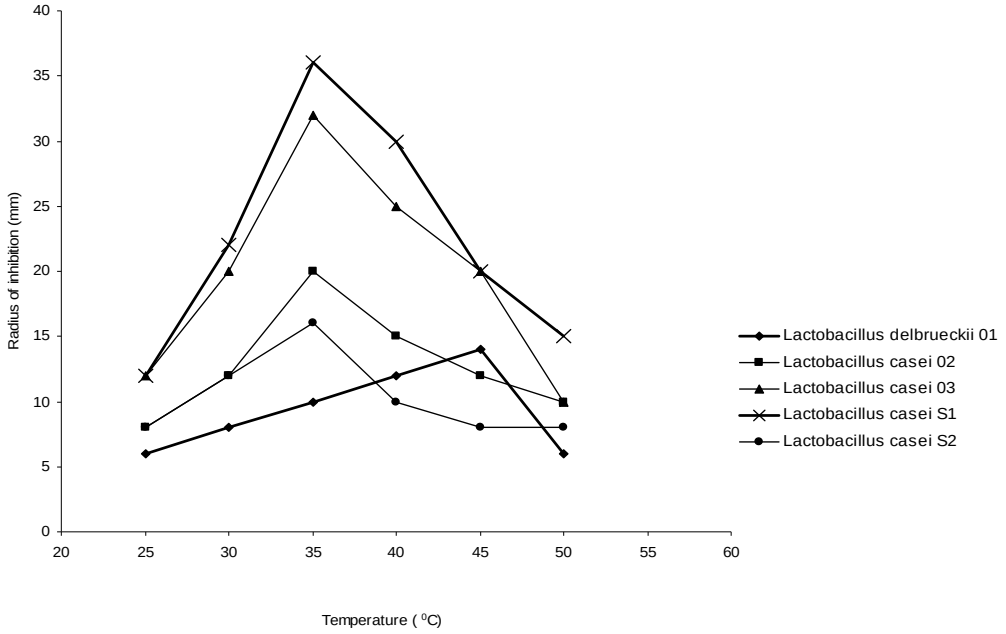

Fig. 1: Effect of temperature on inhibitory activities of the test isolates

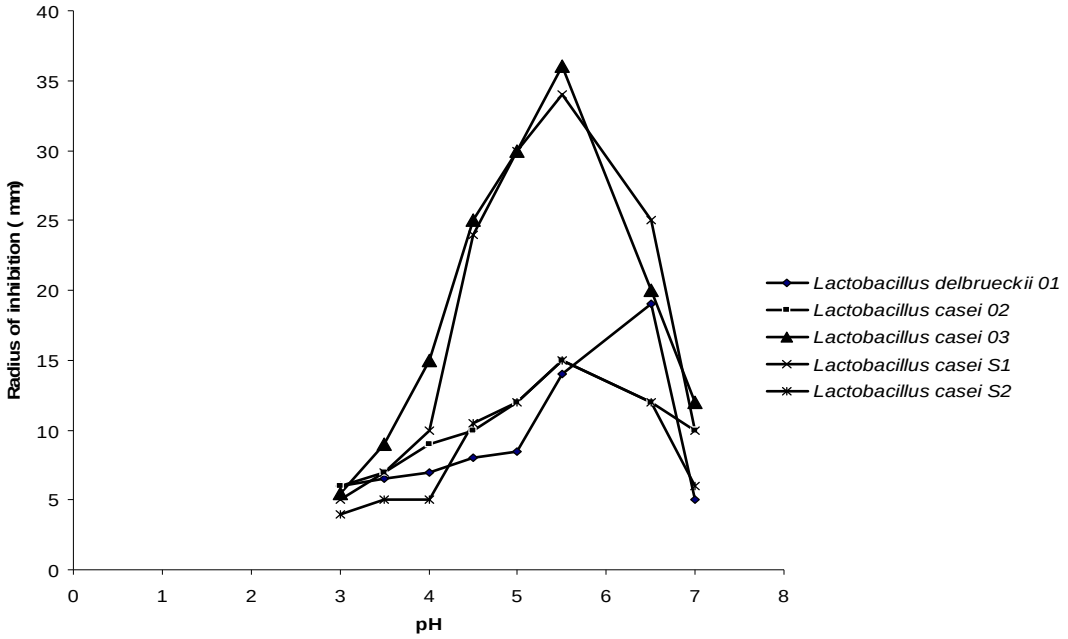

Fig. 2: Effect of pH on inhibitory activity of the isolates