



Antimicrobial activities of lactic acid bacteria isolated from traditionally- fermented maize (ogi) against *Candida albicans*

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ABSTRACT

Objectives: *Candida* species are among the most important and frequent opportunistic microorganisms causing nosocomial infection. The study aim was to assess the antimicrobial potentials of lactic acid bacteria (LAB) against *Candida albicans*.

Methodology and results: Four lactic acid bacteria (*Lactobacillus plantarum* ATCC 25927, *L. plantarum* isolated from ogi, *L. bulgaricus* and *Streptococcus thermophilus* from yoghurt) and an antibiotic (- clotrimoxazole) were evaluated. Disc diffusion method was used by seeding blood agar plate with *Candida albicans* containing 10⁶ cfu/ml LAB and 20µg/ml of clotrimoxazole. Zones of inhibition of *L. plantarum* (22mm), *L. bulgaricus*(19mm) and clotrimoxazole(21mm) were not significantly different at P< 0.05 while there was significant difference between clotrimoxazole and *S. thermophilus*.

Conclusion and application of findings: Antimicrobial activities of lactic acid bacteria isolated from fermented maize “ogi” were revealed. *Lactobacillus* species can provide some protection against candidiasis. Consumption of fermented foods such as “ogi” should be encouraged in women to increase *Lactobacillus* population in the gastrointestinal tract and subsequently in the female genital organs.

Key words: Candidiasis, lactic acid bacteria, fermented foods, Ogi, antimicrobials, women

INTRODUCTION

Candidiasis is a fungal infection mostly caused by *Candida albicans*. The fungus is a single-celled organism present in the genital and intestinal tracts. *Candida* species are found everywhere and represent the most common fungal species that affect humans. *Candida* accounts for approximately 15% of all hospital-acquired infections, more than 72% of all nosocomial fungal infections, and 8% to 15% of all nosocomial blood stream infections, 25-50% of nosocomial candidemia occurs in critical care units (Gudlaugsson et al. 2005).

Usually the immune system keeps yeast under control but during sickness or use of antibiotics, it can multiply and cause infections like- diaper rash, vaginitis and thrush (Balch and Balch, 2009). Candidiasis can affect various parts of the body and the most common being the mouth, ears, nose, toe nails, finger nails, gastrointestinal tract, vagina, eyes, lungs, brain and kidneys (Jones et al., 2004). It can be characterized by symptoms such as constipation, diarrhea, colitis, abdominal pain, itching, canker sores, sore throat, burning tongue, white spots in the tongue and mouth,

severe itching and even diabetes. Symptoms often worsen in damp or moldy places or after consumption of foods containing sugar and / or yeast (Balch & Balch, 2000). Studies conducted by Hidalgo & Vazquez (2005) show that candidiasis is not under control and in fact the situation is worsening. *Candida* species have their own ways of self defense that is well recognized. The main virulence factors include surface molecules that permit adherence of the organism to structures like human cells, extracellular matrix and prosthetic devices, acid protease and phospholipases that involve penetration and damage of cell envelopes and ability to convert to a hyphal form (phenotypic switching)(Hidalgo & Vazquez, 2005).

Lactic acid bacteria (LAB) are gram positive bacteria that produce acid as a major end product of their fermentative metabolism (Pelczar et. al., 1993). Lactobacilli are often present in decomposing plant material, milk and other dairy products and are important in production of fermented foods (Hidalgo & Vazquez, 2005). Lactic acid bacteria produce antimicrobial compounds that enable them to have competitive advantage over other organisms. Bacteriocins are produced by lactic acid bacteria and they exert a positive

influence on the host's health (Marteau et al., 2001) by stimulating the immune system and have the ability to activate macrophages and lymphocytes. They also improve level of immunoglobulin A (IgA) and production of gamma interferon. In addition to bacteriocins, LAB also produces organic acids, diacetyl, hydrogen peroxide and reduces the pH of the medium.

Both "ogi" and yoghurt contain lactic acid bacteria that produce nisin. Nisin has been found to have antimicrobial activity against several microorganisms (Guerra et. al., 2005).

Lactic acid bacteria are often recommended to replenish the population of friendly microorganisms that have been emptied out by course of oral antibiotics and supplementation has been suggested to prevent fungi infection.

The aim of the study was to

- i. isolate and characterize lactic acid bacteria from fermenting maize liquor
- ii. determine the antimicrobial potentials of LAB against *Candida albicans*
- iii. determine the minimum inhibitory concentration of LAB against *C. albicans*

MATERIALS AND METHODS

Isolation of lactic acid bacteria: Lactic acid bacteria were isolated from fermenting maize liquor and commercial yoghurt samples. A 72hour-fermenting maize liquor was used for the isolation of LAB while yoghurt samples were serially diluted to 10^6 and dilutions 10^3 and 10^5 were chosen. One milliliter of each dilution was aseptically transferred into sterile petri dishes and 15ml of De Man Rogosa Sharpe media was added. The plates were replicated, incubated at 37°C for 48 hours under anaerobic condition.

After incubation, colonies were randomly selected and sub-cultured on MRS agar plates to obtain pure colonies. Standard biochemical tests such as gram stain, catalase, motility, indole, citrate, oxidase and sugar fermentation tests (Fawole & Oso, 1995) were carried out on the pure colonies of lactic acid bacteria.

Collection of test organism: The test organism (*Candida albicans*) was obtained from the Microbiology department of the Federal Medical Centre, Idi-Aba, Abeokuta and was confirmed using Germ tube test.

Germ tube test: A small portion of an 18-72 hours old isolate of *C. albicans* was suspended in 0.5ml of human serum in a test tube and incubated at 37°C for 2-3 hours.

A drop of the yeast suspension was dropped on a glass slide and viewed under the light microscope at x 100 magnification. A germ tube test is a filamentous extension from yeast cell, about half and up to 3 to 4 times the length of the cell (Ochei & Kolhatkar, 2000).

Determination of antimicrobial activities of lactic acid bacteria: LAB isolate was aseptically stabbed into 5ml of sterile MRS broth. Sterile peptone broth (2ml) was dispensed into five test tubes labeled $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$. From the LAB stabbed MRS broth, 2ml of the suspension from test tube $\frac{1}{2}$ was dispensed into test tube $\frac{1}{4}$ and subsequently till tube $\frac{1}{32}$. After which 2ml of test tube $\frac{1}{32}$ was discarded to give a uniform volume of 2ml per test tube.

Using the disc diffusion method, 0.5 ml of LAB suspension approximately 10^6 cfu was dispensed on to

sterile prepared discs. *Candida albicans* was evenly seeded on blood agar and each soaked disc containing a specified LAB was placed on the plate and incubated at 37°C for 18 hours. Zones of inhibition were measured using calibrated ruler. A control treatment using clotrimoxazole (20µg/ml) was done and zone of inhibition was measured.

Statistical analysis: Statistical analysis comparison between means was evaluated by student t-test and Analysis of Variance. A value of p< 0.05 was considered significant.

RESULTS AND DISCUSSION

The *Candida albicans* isolate obtained from the Federal Medical Centre, Idi-Aba, Abeokuta was sub-cultured

and tested. Results of the morphological and colonial characteristics are shown in Table1.

Table1: Morphological and colonial characteristics of *C. albicans*

Colour	Shape	Size	Edge	Elevation	Gram reaction	Germ tube test
Cream	Oval	Small	Smooth	Flat	Positive	Positive

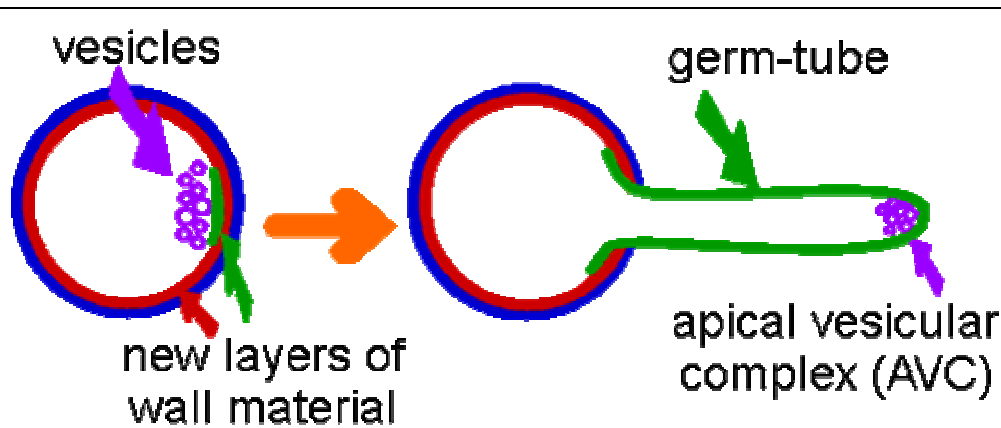


Figure.1: Diagram showing germ tube

Table 2 showed morphological and biochemical characteristics of lactic acid bacteria. Four isolates of LAB including *Lactobacillus plantarum* (ATCC 25927) and *L. plantarum* from ogi, *L.bulgaricus* and

Streptococcus thermophilus from yoghurt were screened

Table 2: Morphological, cultural and biochemical characteristics of lactic acid bacteria

	LAB 1	LAB 2	LAB 3	LAB 4
Cultural characteristics	Small, white, circular, smooth, glossy, slightly raised	Small, white, circular, smooth, glossy, slightly raised	Small, creamy, glossy, circular, smooth, flat, entire, glossy	Small, white, circular, smooth
Gram test	+ve	+ve	+ve	+ve
Cell shape	rod	rod	long rod	spherical, paired
Motility	-ve	-ve	-ve	-ve
Catalase	-ve	-ve	-ve	-ve
Indole	-ve	-ve	-ve	-ve

Citrate	-ve	-ve	-ve	-ve
Oxidase	-ve	-ve	-ve	-ve
Glucose	+/g	+/g	+/g	+/g
Fructose	+/g	+/g	+	+/g
Lactose	+/g	+/g	+/g	+/g
Maltose	+/g	+/g	+	+
Sucrose	+/g	+/g	+	+/g
Suspected organism	<i>Lactobacillus plantarum</i>	<i>Lactobacillus plantarum</i>	<i>Lactobacillus bulgaricus</i>	<i>Streptococcus thermophilus</i>

Key

LAB 1-----Stock culture ATCC 25927

LAB 2-----“Ogi” isolate

LAB 3-----Yoghurt isolate

LAB 4-----Yoghurt isolate

+/g----with acid production indicated by gas, + ----- no acid production

Table 3 showed various degrees of *Candida albicans* inhibition ranging from 22mm to 5mm. *L. plantarum* ATCC 25927 and *L. plantarum* from ogi did not differ in their inhibitory pattern as both isolates gave the highest

zones of inhibition. The least inhibitory organism was *S. thermophilus*. The range of the zones of inhibition for clotrimoxazole was 19-21mm.

Table 3: Zone of inhibition (mm) at different concentrations of *Lactobacillus* species against *C. albicans*

Concentration	Dilution	LAB1	LAB 2	LAB 3	LAB 4
10 ⁶	½	22	22	19	5
10 ⁵	¼	19	19	15	3
10 ⁴	1/8	15	15	12	1
10 ³	1/16	9	9	8	-
10 ²	1/32	4	4	5	-
Clotrimoxazole ((20µg/ml)	21	21	19	20

Key

LAB 1-----Stock culture ATCC 25927, LAB 2----- “Ogi” isolate, LAB 3----- Yoghurt isolate, LAB 4----- Yoghurt isolate

In the last decade, there has been extensive research into the use of lactic acid bacteria to control pathogenic microorganisms. LAB are known to have an inhibitory effect by producing acids (organic acids) and antimicrobial substances that prevent microbial growth. Results obtained in this study showed that some species of LAB especially *L. plantarum* had inhibitory potential as good as that of conventional antifungal agent such as clotrimoxazole. *L. plantarum* isolated from fermented food such as “ogi” compared favorably well with the American Type Collection. The results therefore indicate that some strains of LAB especially *L. plantarum* are capable of synthesizing inhibitive substances on *C. albicans* and these substances have been found to be mainly proteins(Klaenhammer, 1993;

Jimenez-Diaz *et al.*, 1993; Vandenberg, 1993). Millette *et al.*, (2004) reported that lactic acid bacteria produce nisin and it inhibits bacterial growth in semi synthetic media.

A number of theories have been advanced to explain the relationship between antibiotic therapy and candidiasis. Ouwenand (1998) was of the opinion that intestinal infections with *Candida albicans* might result from the removal of a normal inhibition of organisms such as *Escherichia coli*. Savadago (2004) emphasized the destruction of the intestinal flora by antibiotics resulting in B-vitamin deficiency might predispose to oral candidiasis.

The results obtained in their study in vitro gave an indication that under normal conditions, a kind of

counterbalance exists between candidas and lactobacilli, with the yeasts providing nutritional stimulation for the lactobacilli and the lactobacilli serving to prevent the excessive development of *C. albicans*. The destructive effect of lactic acid on other organisms has been emphasized by Chikthimma (2001), Savadago (2004). The lowered pH might have caused destruction of normal flora like *E. coli* and might play a contributory role in reduction of *Candida albicans*

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- observed in this study. Cabo et al., (2002) were of the view that organic acids present in the culture could be involved in the detected antimicrobial activity. They stated further the presence of both lactic acid and acetic acid in the medium was responsible for the antifungal activity. Antibiotic therapy was seen to destroy lactobacilli while *C. albicans* is well known to be resistant to conventional antibiotics.
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