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Full Length Research Paper

Bio-preservative activities of *Lactobacillus plantarum* strains in fermenting Casssava 'fufu'

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The growth of three pathogens, namely *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhii* were investigated in fermenting and non-fermenting cassava. The pH of the steeped cassava was also examined during fermentation. Antimicrobial effects of the *Lactobacillus plantarum* on the pathogens were also determined by agar diffusion method. All the pathogens were inhibited by *L. plantarum* strains with *Staph. aureus* having the highest inhibitory zone followed by *E. coli* and *S. thyphii*. However, in the fermenting cassava, the pathogens increased in population within the first 36 h of the process and decreased to complete extinction after the 96 h of fermentation. The *L. plantarum* exhibited high but varying degree of inhibition on the pathogens. The findings justify the bio-preservative roles of lactic acid bacteria in traditional cassava products.

Key words: Bio-preservation, *Lactobacillus plantarum*, fermentation, cassava and pathogens.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important food plants in West Africa and many parts of the tropics (Brujin and Fresco, 1989).Cassava has assumed the status of security and industrial crop in the tropics (Oyewole, 2002). Fermentation is one of the oldest and most economical methods of producing and preserving foods (Steinkraus et al., 1983; Cooke et al., 1987; Chavan and Kadam, 1989), particularly in the tropical countries where there is high temperature and high humidity, which favour food spoilage. In developed countries, most fermented foods are produced under controlled conditions. In developing countries, such foods are processed under uncontrolled conditions, using age-old techniques.

Investigations have been carried out to investigate the microorganisms involved in the fermentation of cassava products (Okafor, et al., 1984; Oyewole and Odunfa, 1988). In most case, *Lactobacillus plantarum* played an important role in the fermenting process. One important aspect of cassava products, which has not attracted much attention, is their microbiological safety. Cassava and its products like other food materials has potentials for supporting the growth of both pathogenic and spoilage microorganisms. These organisms may be introduced directly from workers or the environment during processing. There is therefore the need to confirm the abilities of some pathogenic organisms to survive in the fermenting cassava.

This study was designed to investigate the abilities of *Escherichia coli*, *Salmonella typhii* and *Staphylococcus aureus* to survive during the fermentation of cassava for fermented 'fufu'.

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MATERIALS AND METHODS

Culture media and chemical

The culture media used are peptone water, MacConkey agar and broth (Oxoid), Mann Rogosa and Sharpe (MRS) agar and broth (Oxoid). Other chemicals were of analytical grade.

Fermentation of cassava

Cassava tubers of the varietal clones TMS 30572 was obtained from the University of Agriculture, Abeokuta., Nigeria. The tubers were from 10 – 15 months. Cassava fermentation was carried out following the traditional method (Figure 1) as described by Oyewole

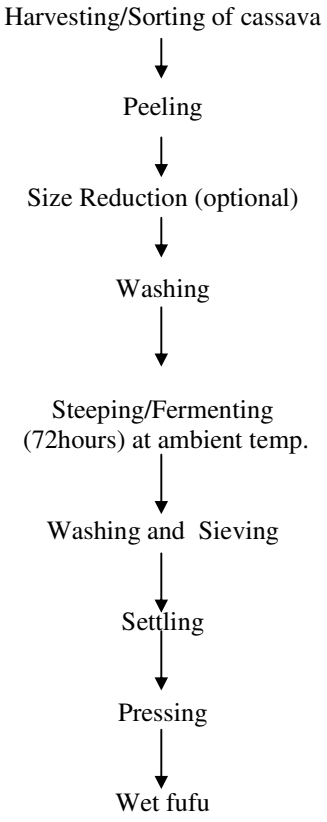

Figure 1. Flow chart for traditional "fufu" processing (Oyewole and Odunfa, 1989

and Odunfa (1989). Cassava roots (150 kg) were peeled and cut into cylindrical pieces (5 - 7 cm length) before being steeped in water (200 L) contained in back plastic bucket for 96 h. The resultant soft fermented cassava tubers were hand pulverized and sieved using plastic sieves of about 1.00 mm aperture. The sieved mash was allowed to sediment for 12 h before the top water was decanted. The sedimented mash was put into a cloth bag and squeezed to express out water. The resulting wet product is "fufu" which will be ready for consumption after being boiled and "turned" in boiling water.

Microbial cultures

The pathogens used to determine the bio-preservative activity of *L. plantarum* were collected from the medical Laboratory of the University of Agriculture, Abeokuta, Ogun State. Nigeria. The *Staphylococcus aureus*, *Salmonella thypii* and *Bacillus substilis* cultures were stored frozen (–70°C in Trypticase soy broth [TSB; Oxoid] containing 20% glycerol). When needed, the cultures were thawed and incubated in Trypticase soy broth at 35°C for 24 h. *Escherichia coli* inocula from stock culture were activated for 24 h at 35°C in MacConkey broth.

Isolation of *Lactobacillus plantarum* strains

During the steeping period of cassava, the content of the vessel was thoroughly mixed. Samples (10 ml) of steep liquor were removed at 96 h of fermentation. The procedure of Banigo and Muller (1972a) was followed in the isolation and identification of *L. plantarum* strains.

Pathogen inoculation

The microbial inoculums (1 ml, 18 h old) was aseptically inoculated into all the steeped cassava (100 g) in all the five beakers containing sterilized distilled water (150 ml) and covered with aluminum foil. Four sets of the inoculated beakers were prepared and one of the beakers from each set served as a control. Control samples were not inoculated with the *L. plantarum.* One set of flasks was inoculated with *S. typhi* to an initial population of 1.0 x 10^3 cfu/ml; a second set was inoculated with E. *coli* to an initial

Table 1. Properties of *Lactobacillus plantarum* isolated during the fermentation of cassava at 96 h for "fufu" production.

Growth at	Positive reaction (%)
pH 3.9/4.0	100
pH 4.8	100
15°C	100
45°C	2
Co_2 from glucose	0
Fermentation of	
L – Arabinose	94
L – Arabitol	3
D – xylose	18
D – Mannose	100
D – Arabitol	78
D – Rafinose	92
D – Fructose	100
D – Glucose	100
Ribose	100
Galactose	100
Maltose	100
Lactose	98

samples were not inoculated with the *L. plantarum.* One set of flasks was inoculated with *S. typhi* to an initial population of 1.0 x 10^3 cfu/ml; a second set was inoculated with *E. coli* to an initial population of 4.0 x 10^2 cfu/ml, a third set with *Staph. aureus* to yield an initial population of 9.0 x 10^2 cfu/ml and fourth set with *B. substilis* to 4.6 x 10^2 cfu/ml

Determination of pH

The pH of the samples was measured by aseptically removing a 5-ml sample from the beakers and measuring the pH with a Fischer Accumet pH meter model 610A (Fisher scientific, USA)

Assessment of microbial growth

At 0 and 6 h intervals of growth, the inoculated pathogens were monitored during the steeping of the cassava. The samples were serially diluted under aseptic conditions. A portion (0.1 ml) of each dilution was aseptically inoculated into appropriate media plates

and each organism was spread by using spread plate method. The Petri dishes were incubated at 37°C for 24 h. Total counts of the colonies that developed on the plates were counted after 24 h incubation period and recorded at each interval to monitor their growth.

Antagonistic effect of *L. plantarum* strain

Antagonistic effects of *Lactobacillus plantarum* on *S. thyphii*, *Staph. Aureus*, *E. coli* and *B. substilis* were determined by the agar diffusion method (Sanni et al., 1999). Melted Nutrient agar (Oxoid) was inoculated with 1% of an 18-to 24-h old culture of the test organism and poured into sterile Petri dishes. Sterile disks (Whatman, 18.0 mm diameter, 0.88 mm thickness) were dipped into the slurry samples (product) into which was placed the (15 min, 5000 rpm) supernatant of the centrifuged products, and the 24-h-old culture of the *L. plantarum* in growth medium, separately. These disks were placed onto the surface of the agar. The plates were left at room temperature for 1 h to allow the test material to diffuse into the agar, and then incubated at the optimum temperatures of the test organisms for 24 h. After incubation, the inhibition zones were examined and measured in millimeters

RESULTS AND DISCUSSION

Twelve lactic acid bacteria strains were isolated during the 96 h period of cassava fermentation for fufu processing. These were classified on the basis of their morphological and biochemical characteristics according to the scheme of Sharpe (1979), Buchanan and Gibson (1979), Krieg and Holt (1984) and sheath et al. (1989). The characteristics exhibited by the strains were

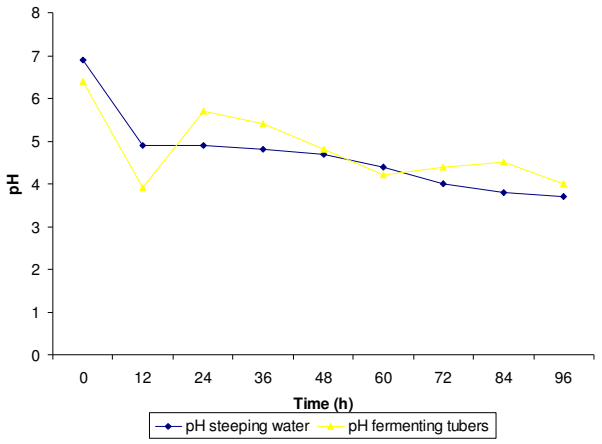

Figure 2. Changes in the pH of the steeping water and fermenting tubers during 'fufu' production.

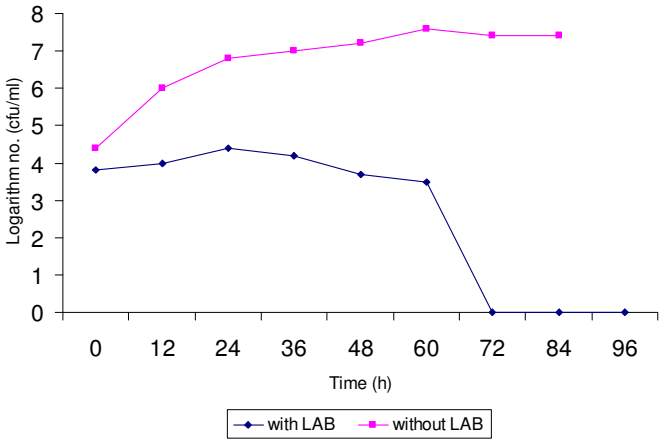

Figure 3. Growth of *E. coli* in the absence and presence of *L. plantarum* (lactic acid bacteria, LAB) during cassava fermentation.

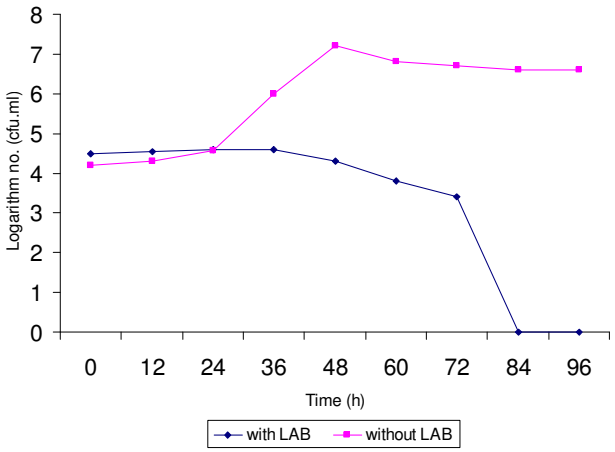

Figure 4. Growth of *S. thyphi* in the absence and presence of *L. plantarum* (lactic acid bacteria, LAB) during cassava fermentation.

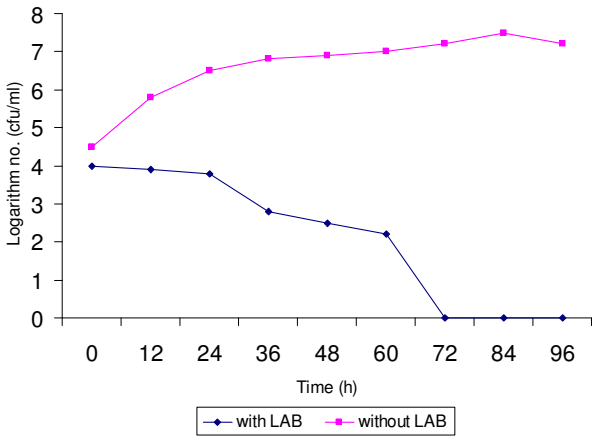

Figure 5. Growth of *B. substilis* in the absence and presence of *L. plantarum* (lactic acid bacteria, LAB) during cassava fermentation.

compared with those of standard strains described in the schemes. Table 1 shows the characteristics of the strains identified as *L. plantarum*.

The changes in the pH of the fermenting tubers and steeping water during 'fufu' production are shown in Figure 2. The pH of the tubers decreased from 6.35 at 0 h to 4.78 within the first 48 h of fermentation. The pH decreased further to 4.00 after 96 h of fermentation. The pH of the steeping water decreased from 6.95 at 0 h to 3.78 after 96 h of fermentation period, the pH of the steeping water was lower than the fermenting water.

The growth patterns of *S. typhii*, *E. coli, B. substilis* and *Staph. aureus* inoculated into non-fermenting and fermenting cassava are presented in Figures 3, 4, 5 and 6. All the pathogens grow uninhibited in the non-

Table 2. Antimicrobial effects of *Lactobacillus plantarum* on *Salmonella thyphii*, *Escherichia coli* and *Staphylococcus aureus*.

Lactobacillus plantarum	**Inhibition zone[a] (mm)**		
	C	**P**	**S**
Salmonella typhii	15.0	24.0	24.5
Staphylococcus aureus	17.0	27.0	25.0
Escherichia coli	16.0	26.0	26.5

[a]Antimicrobial activity expressed on the basis of the diameter of the zone of inhibition.

C, Culture; P, product, i.e. fermented slurry; S, supernatant of the product).

fermenting cassava. However, in the fermenting cassava the pathogens increased in population within the first 36 h of the process and decreased to complete extinction at 60 h for *E. coli and B. substilis*, and 72 and 96 h for *Staph. aureus* and *S. typhii*, respectively.

This observations shows that the fermentation process which involve *L. plantarum* caused a reduction in the level of pathogens. Similar investigation on the fermentation of maize showed that enteropathogenic *E. coli* were inhibited during the fermentation period (Monsah et al., 1990). Maria et al. (1991) also showed a reduction in the level of *Clisteria monocytogenes* present in regional cheeses by *Enterococcus faecalis* CRL 268 AMD.

L. plantarum had a broad antimicrobial inhibitory spectrum, with activity against several strains of bacteria pathogens (Table 2). In this work *L. plantarum* (culture, product and supernatant) inhibited the growth of the pathogens. However, products and the supernatants of the products were more inhibitory than the culture. Lactic acid bacteria are known to produce antimicrobial substances, but these are mainly in the form of organic acids and metabolites. Very few reports have been published about the bio-preservative activities of *L. plantarum* strains. Sanni et al. (1999) clearly documented the antibacterial activity of bacteriocin produced by *L. plantarum*. Other study shows that *L. plantarum* is inhibitory against both spoilage and pathogenic bacteria and to a lesser extent, against spoilage fungi (moulds). However, more studies will need to be carried out in these regards.

In summary, products fermented with *L. plantarum* cannot be assumed to be free from pathogens. However, an appropriate sanitation procedure and successful fermentation processes combined with the addition of *L. plantarum* produced antimicrobial metabolites can give reasonable assurance of the control of pathogenic microorganisms.

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