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Isolation and Characterization of Lactic Acid Bacteria Associated with the Fermentation of a Cereal-Based Product for the Development of a Starter Culture

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ABSTRACT

Isolation and characterization of lactic acid bacteria (LAB) associated with the fermentation of maize for the production of *ogi* (a traditional weaning food) were evaluated for the development of starter cultures. Changes in pH, titratable acidity and LAB counts were investigated during the cereal based-product fermentation. A decrease in pH was associated with an increase in titratable acidity. The LAB were isolated, characterized and identified using morphological, physiological and biochemical methods with an API 50CHL system. The isolates were *Lactobacillus plantarum*, *Lactobacillus cellobiosus*, *Lactobacillus pentosus*, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus*. These isolates were capable of growing on sterilized wet milled maize with *L. plantarum* having the highest potential as a starter culture. Maize inoculated with a pure culture of *L. plantarum* showed the highest counts of 6.23×10^7 at 96 hrs of fermentation than the other lactic acid bacteria. There was a significant difference ($P > 0.05$) between the bacterial counts as well as the pH values obtained during the fermentation of maize by the different LAB.

Keywords: maize, microbial evaluation, physicochemical parameters

INTRODUCTION

Many African foods are fermented before consumption. Fermentation has been used as a means of improving the keeping quality of food (Holzapfel 1997; Ross *et al*. 2002; Lei and Jakobsen 2004). It inhibits spoilage and pathogenic microorganisms by a combination of factors, which include production of organic acids, hydrogen peroxide, antibiotic-like substances and the lowering of oxidation-reduction potentials (Nout and Rombouts 1992; Savadogo *et al*. 2004; Pan and Zhang 2008).

Spontaneous fermentations are difficult to control and are not predictable in terms of length of fermentation and quality of product and can produce unwanted products or are liable to contamination by pathogens (Nout 1994). To overcome this problem, the most predominant microorganisms found in an acceptable product are isolated and purified (Marshall 1993) while the medium used for the fermentation is pasteurized to exclude most unknown microorganism so that the purified microorganisms are allowed to initiate the fermentation. Such introduced cultures are termed starter cultures (Marshall 1993). By so doing, the fermentation can be manipulated in such a way that is possible to predict the amount and quality of product formed and the length of fermentation period (Chamunorwa *et al*. 2002).

Many African foods that are prepared by the action of diverse species of bacteria, mold and yeast are little known outside their native countries (Mathara *et al*. 2004). *Ogi* is a porridge prepared from fermented maize, sorghum or millet in Nigeria. It is a staple food and serves as a weaning food for infants. The traditional preparation of *ogi* involves soaking of corn kernels in water for 1 to 3 days followed by wet milling and sieving to remove bran, hulls and germ (Odunfa 1985). The pomace is retained on the sieve and later discarded as animal feed while the filtrate is fermented (for 2-3 days) to yield *ogi* which is sour, white starchy sediment. *Ogi* is often marketed as a wet cake wrapped in leaves or transparent polythene bags. It can be diluted and boiled into a pap, or cooked and turned into a stiff gel called *agidi* prior to consumption (FAO 1996).

In general, a wide spectrum of microorganisms is involved during the fermentation process but a few types usually determine the quality of the end products (Patrigani *et al*. 2006; Mathara *et al*. 2008). However, when adequate environmental conditions are provided, a particular microbial community can determine the quality of a specific food. Therefore, isolation, characterization and identification of microorganisms associated with fermentation of cereals with a view to select starter cultures that are adapted to cereal-based products production would be important to support the technical process and to obtain a predictable end-product with a desired quality. This may help in development of starter cultures and devising appropriate and affordable technology that could modernize cereal-based product production. Thus, there is a need for information on the systematic isolation and identification of lactic acid bacteria (LAB) involved in fermentation of cereal-based products. The objective of this work was therefore, to isolate LAB during the course of the traditional maize fermentation for the production of *ogi*, and to characterize and identify them with the aim of developing a starter culture.

MATERIALS AND METHODS

Source of maize grains

White maize (*Zea mays*) grains were purchased from a local market in Vom, Plateau state, Nigeria.

Fermentation of maize for ogi production

The maize grains were thoroughly washed with clean water and thereafter soaked in water for 48 hr. Then grains were wet milled and sieved and the filtrate was allowed to undergo fermentation naturally for a period of 96 hr. At 12 hr intervals during the fer-

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Table 1 Changes in lactic acid bacteria (LAB) (cfu/ml^{-1}), pH and titratable acidity (TTA) during fermentation of maize.

Time (hrs)	LAB	pH	TTA (%)
0	$7.05 \times 10^6 \pm 19.50$ b	6.05 ± 0.01 a	0.02 ± 0.01 d
12	$6.05 \times 10^6 \pm 4.05$ bc	6.00 ± 0.01 a	0.75 ± 0.04 a
24	$5.50 \times 10^6 \pm 15.00$ bc	5.75 ± 0.01 a	0.73 ± 0.01 a
36	$5.45 \times 10^6 \pm 14.50$ bc	4.65 ± 0.01 b	0.64 ± 0.01 b
48	$5.55 \times 10^6 \pm 15.50$ bc	4.10 ± 0.01 d	0.60 ± 0.01 b
60	$4.80 \times 10^6 \pm 18.00$ bc	4.95 ± 0.01 b	0.60 ± 0.01 b
72	$4.50 \times 10^6 \pm 14.00$ bc	4.66 ± 0.09 c	0.51 ± 0.01 bc
84	$5.10 \times 10^6 \pm 19.00$ bc	3.90 ± 0.01 d	0.41 ± 0.01 c
96	$6.70 \times 10^6 \pm 12.00$ bc	3.63 ± 0.01 d	0.36 ± 0.01 c
108	$9.65 \times 10^6 \pm 3.50$ a	3.26 ± 0.01 d	0.43 ± 0.01 c

Means in the same column with different letters are significantly different at P=0.05

mentation, samples were taken for bacteriological and chemical analyses.

Bacteriological analysis

Exactly 1 ml of sample was introduced into 9 ml of peptone water and serially diluted. From the appropriate dilution, 0.1 ml was inoculated onto sterilized de Man, Rogosa, Sharpe agar (Oxoid) plates for the isolation of lactic acid bacteria and incubated for 48 hr at 37°C ± 2. Thereafter the colonies were counted and recorded as colony forming units per ml (cfu/ml). Experiments were performed in replicates for each sample collected.

In order to identify the LAB, representative colonies were purified on nutrient agar (Oxoid) plates by repeated sub-culturing. Preliminary identification of strains obtained in pure cultures was based on Gram staining and spore staining, nitrate reduction, and the ability of the bacterial isolates to produce enzymes such as catalase, oxidase, urease (Cheesbrough 2000) and growth at different temperatures (10, 15, 45°C), as well as growth in the presence of different concentration of NaCl (6, 8, 10%). Biochemical tests were performed with the API bacterial identification system (API 50CHL, BioMérieux, France). Complete identification of LAB was achieved by use of the tests in *Bergey's Manual of Determinative Bacteriology* (Holt *et al*. 1994).

Chemical analysis

The changes in pH of fermenting samples were monitored every 12 hr by using a pH meter. For solid samples, 10 g were mixed with 20 ml of distilled water prior pH measurement and the liquid sample was measured directly. In order to determine the titratable acidity (TTA) 10 ml of sample was transferred to a 50 ml measuring flask and filled up to 50 ml with distilled water. After mixing, 10 ml of the diluted sample was titrated with 0.1 ml l^{-1} NaOH using 1% phenolphthalein as indicator and the TTA calculated (Amoa-Awua *et al*. 1996).

Inoculation of sterilized wet milled maize with LAB

The wet milled maize was placed in air tight 1000 ml glass bottles and sterilized at 121°C for 15 min. After cooling to room temperature, 1 ml (approx. 10^6–10^7cfu/ml) pure culture of each LAB suspension was introduced separately into 100 ml of the sample and allowed to undergo fermentation for 96 hr. Total viable counts and pH changes during the controlled fermentation were monitored and determined as previously described. Control experiments contained only sterilized maize without inoculation of LAB.

Table 2 Percentages of lactic acid bacteria isolates obtained with the API 50CHL system.

Isolates	Identification by API 50CHL	% of total isolates
GG 20	*Lactobaccillus cellobiosus*	10
AJ 80	*Lactobacillus plantarum*	40
BF 40	*Leuconostoc mesenteroides*	20
KK 20	*Lactobacillus pentosus*	10
CD 40	*Pediococcus pentosaceus*	20

Letters represent the codes for group of isolates

Statistical analysis

The data obtained was analyzed by two-way analysis of variance (ANOVA) and the least significant difference (LSD) between means assessed at $P = 0.05$.

RESULTS

The changes in the counts of the LAB, pH and titratable acidity during the spontaneous fermentation of maize for the production of *ogi* are shown in **Table 1**. The LAB counts at 0 h (7.05×10^6) generally decreased gradually during fermentation. There was an increase in titratable acidity with a reduction in pH. Correlation between the LAB counts, pH and titratable acidity during the monitoring period showed no significant difference.

A total of 200 LAB were isolated. Isolates were characteristically Gram-positive, none sporing, none motile, negative for catalase and oxidase. The bacteria isolates were identified as *Lactobacillus plantarum*, *Leuconostoc mensenteroides*, *Pediococcus pentosaceus*, *Lactobacillus cellobiosus* and *Lactobacillus pentosus* by the aide of the API 50CHL bacterial identification system. The isolates were dominated by *L. plantarum*, followed by *L. mensenteroides* and *P. pentosaceus* (**Table 2**).

Changes in pH obtained during the fermentation of inoculated sterilized maize using pure cultures of LAB are shown in **Table 3**. The pH of the maize samples inoculated differently with pure cultures of each of *P. pentosaceus*, *L. plantarum* and *L. cellobiosus* dropped from an initial pH values indicating fermentation during the period. There was a significant difference between pH values of the different isolates during the fermentation period.

The result obtained when pure cultures of each of the three different LABs were inoculated into sterilized milled maize in order to determine the best for the production of *ogi* is shown in **Table 4**. Significant differences between bacterial counts of the different isolates during the fermentation existed. Maize inoculated with a pure culture of *L.*

Table 3 pH value of maize inoculated with different lactic acid bacteria.

Inoculum	Time (hrs)				
	0	24	48	72	96
Pediococcus pentosaceus	5.95 ± 0.01 a	4.92 ± 0.00 g	4.86 ± 0.01 h	4.88 ± 0.01 h	4.88 ± 0.01 h
Lactobacillus plantarum	6.00 ± 0.01 a	5.41 ± 0.01 c	5.16 ± 0.01 d	5.03 ± 0.01 d	4.83 ± 0.01 i
Lactobacillus cellobiosus	6.01 ± 0.00 a	4.48 ± 0.00 l	4.57 ± 0.01 k	4.64 ± 0.01 j	4.63 ± 0.01 j
Control without pure culture	6.03 ± 0.01 a	6.00 ± 0.01 a	5.56 ± 0.01 b	5.40 ± 0.10 e	5.40 ± 0.02 gh

Means in the same row having different letters are significantly different at P=0.05

Table 4 Bacterial counts of the different isolates in sterilized maize during fermentation.

Inoculum	Time (hrs)				
	0	**24**	**48**	**72**	**96**
Pediococcus pentosaceus	$3.60 \times 10^7 \pm 1.00$ c	$4.37 \times 10^7 \pm 3.21$ b	$6.30 \times 10^7 \pm 7.00$ a	$3.38 \times 10^7 \pm 7.64$ c	$4.10 \times 10^7 \pm 9.00$ c
Lactobacillus plantarum	$3.00 \times 10^7 \pm 1.01$ d	$4.43 \times 10^7 \pm 1.53$ b	$4.33 \times 10^7 \pm 15.28$ b	$3.63 \times 10^7 \pm 5.51$ c	$6.23 \times 10^7 \pm 19.76$ a
Lactobacillus cellobiosus	$4.10 \times 10^7 \pm 1.00$ c	$6.13 \times 10^7 \pm 3.51$ a	$3.90 \times 10^7 \pm 9.17$ c	$5.93 \times 10^7 \pm 26.86$ a	$5.17 \times 10^7 \pm 17.56$ a
Control (without pure culture)	$2.00 \times 10^2 \pm 1.00$ f	$2.30 \times 10^2 \pm 1.00$ e	$2.00 \times 10^2 \pm 1.00$ f	$1.30 \times 10^2 \pm 1.00$ h	$1.40 \times 10^2 \pm 2.00$ g

Means in the same row having different letters are significantly different at P=0.05

plantarum had the highest counts of $6.23 \times 10^{'}$ at 96 h of fermentation compare with other LAB.

DISCUSSION

The viable counts as well as isolation and identification of the LAB associated with the traditional fermentation of maize for the production of *ogi* (a weaning food) were investigated. Also the pH and titratable acidity were monitored during the fermentation period. In order to obtain a starter culture for production of *ogi* the potential of the different pure isolates of LAB were investigated.

Spontaneous fermentation usually results from the competitive activities of different microorganisms whereby strains best adapted and with the highest growth rate will dominate during the stages of the process. Among the bacteria associated with food fermentation, LAB are of predominant importance (Jay 2000).

Changes in counts of LAB, pH and titratable acidity during the fermentation of maize showed a general decrease in bacterial counts, marginal increase in titratable acidity with reduction in pH values. The decrease in LAB counts could be attributed to depletion of nutrients (Amoa-Awua *et al*. 2007). The reduction of the pH values towards acidity was possibly due to fermentation by the LAB (Abegaz 2007). An increase in acidity as the fermentation progressed was because of the accelerated growth rate of LAB (Inyang and Idoko 2006). The amount of acid produced increased with decrease in pH agreed with the findings of Mohammed *et al*. (1991). The correlation between pH and titratable acidity during the period of fermentation showed a negative correlation (not significant) which indicated that as pH was decreasing, titratable acidity was increasing (Omemu *et al*. 2007).

This study confirmed the presence of different types of LAB in cereal-based products. These isolates were *L. plantarum*, *L. pentosus*, *L. cellobiosus*, *L. mesenteroides* and *P. pentosaceus* with *L. plantarum* predominating. Most of the LAB isolated from the fermentation of maize for the production *ogi* had been isolated from other fermented foods. Julius *et al*. (2004) isolated *L. plantarum* and *L. mesenteroides* from maasai. Hounhouigan *et al*. (1993) isolated *L. brevis*, *L. curvatus* and *P. pentosaceus* during spontaneous fermentation of mawe from maize. Uchimura *et al*. (1991) reported *P. pentosaceus* in fermentation of Indonesian *ragi*.

The pH values recorded for the different starter cultures during fermentation of maize into *ogi* decreased towards acidity. Therefore using pH as an index of fermentation, it was noted that all the isolates of LAB used as starter cultures were capable of fermenting maize for *ogi* production under controlled laboratory condition. However the lower pH noted for the spontaneous fermentation could be attributed to the combined action of yeast and LAB (Khetarpual and Chauhan 1990).

L. plantarum used as pure cultures was noted to have the highest counts at the end of the 96 hrs monitoring of fermentation and this agreed with studies of Omemu *et al*. (2007). Thus it showed *L. plantarum* as the best starter because of increase in its bacterial counts as a single culture. This indicated that *L. plantarum* was better adapted than other LAB tested for use as starter culture for the fermentation of maize for the production of *ogi*. Therefore this study reviewed the potentials of *L. plantarum* as a starter culture for industrial production of *ogi*.

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