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Full Length Research Paper

Influence of starter culture of lactic acid bacteria on the shelf life of agidi, an indigenous fermented cereal product

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A total of eight lactic acid bacteria were isolated from various fermented cereal gruels (ogi). They were identified as *Lactobacillus plantarum*, *Lactobacillus casei*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus* and *Pediococcus acidilactici*. *P. acidilactici* in OG3 produced the highest quantity (12.0 g/l) of lactic acid after 48 h, while *L. acidophilus* in OG3 produced the least (3.6 g/l). All the tested isolates produced hydrogen peroxide with *L. mesenetroides*, OG2 having the highest yield of 54 mg/l after 36 h. Diacetly was also produced by all the test organism with *L. fermentum*, OG2 having the highest yield of 6.5 g/l, while the lowest producer was *L. casei* with 3.2 g/l. Bacteriocin production was observed in cell free supernatant of all the test organisms except *L. fermentum* in OG2, *L. casei* in OG3 and *L. brevis* in OG4. The bacteriocin produced by *L. plantarum* in OG3 and *L. casei* in OG3 had the highest antagonistic activity against *Aspergillus flavus* and *Aspergillus niger* used as indicator organisms. Agidi was produced using both bacteriocin producing (BP) *L. plantarum* OG3 and *L. casei* ogi made from white sorghum (OG1) and non bacteriocin producing (NBP) *L. fermentum* OG2 and *L. brevis*.OG4 The study showed that agidi produced with BP mixed starter cultures had a better shelf life of 6 days before spoilage occurred, relative to 4 days observed for agidi produced using non-bacteriocin producing starter culture and 2 days for the traditionally produced agidi.

Key words: Lactic acid bacteria, bacteriocin, fermentation, *agidi*, shelf life.

INTRODUCTION

Lactic acid bacteria (LAB) constitute a group of bacteria that have morphological, metabolical and physiological similarities, and they are also relatively and closely related phylogenetically. They are gram-positive, non-sporulating, non-respiring cocci or rods, which ferments carbohydrate to produce lactic acid as their major end product (Axelsson, 1998).

LAB were used in food and feed fermentation processes long before any knowledge of the presence of the bacteria themselves. Old Egyptian murals showed the production of spoilage several thousand years ago and fermentation as a preservative measure for food has been present since historic times. The use of fermentation has increased during the centuries and includes many different kinds of animal feed and food (Ross et al., 2002). However, it was only during the last century that we began to understand the metabolic processes behind the preserving effect of LAB. During the last century, it was realized that LAB are responsible for the fermentation and the biopreservative effect utilized in many food and feed processes. Biopreservation refers to extended

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Abbreviations: LAB, Lactic acid bacteria; **MRS,** de Man, Rogosa and Sharpe; **PDA,** potato dextrose agar; **MEA,** malt extract agar; BP-Ag, bacteriocin producing starter organism; **NBP-Ag,** non bacteriocin producing starter organism; **PCA,** plate count agar; **T-Ag**, traditional fermented agidi; **OG5,** ogi made from millet; **OG4,** ogi made from red sorghum; **OG3,** ogi made from white maize; **OG1,** ogi made from white sorghum; **OG2,** ogi made from yellow maize.

shelf life and enhanced safety of foods using the natural or added microflora and their antimicrobial products (Ross et al., 2002)

LAB are found in many nutrient rich environments and occur naturally in various food products such as diary and meat products and vegetables (Carr et al., 2002). Beside carbohydrates, they also need to be supplied with amino acids, peptides, salts and vitamins among others (Hammes and Weiss, 1992; Carr et al., 2002). They have, by tradition, been established as a natural consumer and environmentally friendly way of preserving food and feed. The primary preservative effect achieved by LAB is due to the production of lactic acid, which lowers the pH and directly inhibits many microorganisms (Brul and Coote, 1991). Besides the production of lactic acid, several other antimicrobial compounds such as hydrogen peroxide, formic acid, propoinic acid, acetone, diacetly, reuterin and bacteriocins are produced, which play an important role in their preservative capabilities (Lindgreen and Dobrogosz, 1990; Caplice and Fitgerald, 1999). The precise mechanism of antimicrobial action can often not be defined because of a complex interaction between compounds. Synergistic effects are often seen between the compounds involved in the antimicrobial action (Corsetti et al., 1998; Nikupaavola et al., 1999). Much research has been directed towards identifying different antimicrobial substances, primarily antibacterial, in simple *in vitro* systems, but little is known about the overall mechanism of complex preservation system within food and feed environments (Earnshaw, 1992).

During the past few decades, interest has risen in the use of LAB as a natural food preservative to increase food safety and stability. This interest has been driven by the use of artificial preservatives which has given rise to concerns from consumers, and increase awareness of the microbiological safety of such foods (Onilude et al., 2005). Nowadays, consumers prefer foods with few chemical preservatives (Daeschel et al., 1993). As a result, there is increased interest in the preservation through LAB because of their safe association with human fermented foods. The common occurrence of LAB in foods and feeds coupled with their long lived use contribute to their natural acceptance as generally regarded as safe (GRAS) product for human consumption (Caplice and Fitgerald, 1999).

This present work is aimed at evaluating the influence of starter cultures of LAB to control spoilage of *agidi*; a cereal based African fermented food

MATERIALS AND METHODS

Collection of samples

Samples of maize (*Zea mays*), sorghum (*Sorghum vulgarae*) and millet (*Elusine caracana*), were bought from Bodija market in Ibadan metropolis, south western Nigeria. The agidi samples from which the spoilage moulds were isolated, were collected from different parts of Ibadan

Isolation and identification of lactic acid bacteria

LAB strains were isolated from traditionally fermented ogi. For all samples, 10 g were added to 90 ml of sterile dilute containing 0.1% peptone water and homogenized for 30 s. From appropriate 10 fold dilutions, isolation of bacteria was carried out on de Man, Rogosa and Sharpe (MRS) agar and incubated anaerobically at 30°C for 48 h. Isolates were identified according to Kandler (1983) and Smeath (1986) by cell and colony morphology, gram staining, catalase test, growth at 15 and 45°C, spore staining, motility test and other biochemical tests like oxidase, indole production, methyl red, Voges-proskauer, liberation of ammonia from arginine, growth in 4% broth, hydrogen sulphide production, growth at 4% NaCl, casein hydrolysis and carbohydrate fermentation.

Fungal isolation and identification

Mould isolates were obtained from the food samples following surface sterilization in 70% alcohol and plating in potato dextrose agar (PDA) medium. Pure cultures of each isolate were obtained after incubation at room temperature. Identification was effected by mounting fungal mycelium on lactophenol cotton blue and observed under x40 objective lens of the microscope, and colony colour, growth pattern on plates, details of philiades and spores were also used as identification parameters with reference to Domesch and Anderson (1970)

Preparation of fungal inocula

The isolated moulds were grown on malt extract agar (MEA) slants (oxoid) at 25°C for 7 days and then stored at 5°C. Inocula containing spores or conidia were prepared by growing the moulds on MEA slant for 7 to 10 days (or until sporulation). The spores were collected after shaking the slants with sterile peptone water (0.2%, w/v) and then filtered through Whatman No 2 filter to remove hypha fragment (Magnusson and Schnurer, 2001). The mould concentrations were then determined using a Buckner haemocytometer and adjusted to 10^4 spores per ml by adding distilled water (Krauss et al., 1988)

Determination of lactic acid, hydrogen peroxide, diacetyl and bacteriocin production

For these measurements, the test organisms were grown in MRS broth for 48 h and centrifuged at 3000 xg for 15 min.

Lactic acid

To 25 ml of the supernatant fluid of the test organism, three drops of phenolphthalein were added as indicator. From a burette, 0.1 ml NaOH was slowly added to the samples until a pink colour appeared. Each ml of 0.1 M NaOH is equivalent to 90.08 mg of lactic acid (AOAC, 1990).

Hydrogen peroxide

Twenty milliter of diluted sulphuric acid were added to 25 ml of the supernatant fluid of the test organisms. Titration was carried out with 0.1 M potassium permanganate. Each ml of 0.1 M potassium permanganate is equivalent to 1.79 mg of hydrogen peroxide solution and decolourization of the sample was regarded as the end point (AOAC, 1990).

Diacetly

This was determined by transferring 25 ml of the supernatant fluid of the test organism into conical flasks and 7.5 ml of hydroxylamine solution were used for the residual titration. The flasks were titrated with 0.1 M HCl to greenish-yellow end point using bromophenol blue as indicator. The equivalence factor of HCl to daicetyl is 21.5 mg (AOAC, 1990).

Antagonistic effect of bacteriocin produced by lab isolates against selected spoilage moulds

The agar well method of Schillinger and Lucke (1989) was used. Malt extract agar plates each containing 10^4 spores per ml was prepared, wells with a diameter of 5 mm were cut into the agar using a sterile cork borer. A single droplet of agar was added to each well in order to seal it to avoid leakage. 70 µl sample of each bacteriocin were added to the wells and allowed to diffuse into the agar during a 5 h pre incubation period at room temperature, followed by aerobic incubation at 30°C for 48 h. The antifungal effects were recorded by measuring the zone of inhibition around the well (Schillinger and Lucke, 1989).

Preparation of agidi

Maize grains (*Zea mays*) were sorted, cleaned and soaked in 2 L of disodium bisulphate solution for 24 h to eliminate microbial contamination. The grains were then drained and washed several times in sterile distilled water. The grains were soaked with sterile distilled water inside a 5 L glass fermenting vessel. This was inoculated with culture of bacteriocin producing and non bacteriocin producing (control) *Lactobacillus species* (singly and mixed) at about 5.0 x 10^6 cfu/ml and fermentation was allowed for a period of 3 days at ambient temperature. The water was drained and wet milling was done using a commercial milling machine. This was followed by wet sieving with the aid of a clean muslin cloth. The mixture was allowed to settle and the ogi slurry supernatant was decanted and packed in a jute bag (Ogiehor et al., 2005).

Shelf life study of agidi

Agidi was prepared by separately heating the ogi slurry fermented with bacteriocin producing (BP-Ag) and non bacteriocin (NBP-Ag) starter organism with occasional stirring at 78.5 + 2.5°C. The prepared agidi was packed into sterile polyethylene bag and was left to cool. It was sealed and stored at room temperature (28°C). Afterwards, the agidi samples were observed daily to determine when spoilage would start. The total microbial load was determined at the beginning of spoilage using modified pour plate method of Harrigane and Mccance (1966). Plate count agar (PCA) was used to enumerate the total viable count (bacteria), while PDA was used for fungi count. The plates (bacteria) were incubated at 37°C for 2 days and the colony forming units per gram of sample was determined. For fungi, the plates were at 30°C for about 3 - 5 days and counted.

Sensory evaluation of the agidi samples were carried out by 7 member panel consisting of fellow students of the department that are familiar with the products. The parameters used were appearance, colour, odour, taste and texture. The ratings were presented on a 9 point hedonic scale ranging from 9 = highly acceptability to 1 = low acceptability. Low acceptability indicates spoilage of products. Four samples of each agidi from each batch were used for the shelf life study and the results were subjected to Duncan's multiple range tests of variables.

RESULTS

A total of twenty five LAB isolates were obtained from the indigenously fermented cereal gruels, but eight of them were selected for the work. They were identified as *Lactobacillus plantarum*, *Lactobacillus casei, Leuconocstoc mesenteroides*, *Lactobacillus brevis, Lactobacillus fermentum*, *Pediococcus acidilactici*, *Lactobacillus acidophilus* and *Lactobacillus delbruecki*. Five fungi isolates were obtained from the spoilt agidi samples. They were identified as *Aspergillus niger, Aspergillus flavus, Rhizopus* spp., *Penicillum* spp. and *Aspergillus fumigatus*

Quantitative determination of the antimicrobial compounds produced by LAB isolates were carried out. *P. acidilactici* produced the highest quantitiy of lactic acid (12.0 g/l) after 48 h of incubation. This was followed by *L. plantarum* which produced 10.0 g/l after 48 h, while *L. acidophilus* produced the least concentration of 3.6 g/l. All the test organisms produced hydrogen peroxide with *L. fermentum* having the highest yield of 0.065 g/l, while *L. casei* had the least quantity of 0.032 g/l. All the organisms produced diacetly to varying degrees. After 48 h of incubation, *L. fermentum* and *L. brevis* had the highest yield of 6.5 and 6.0 g/l, respectively, while the lowest producer was *L. casei* (Table 1).

Inhibitory effect of bacteriocin was carried out using *A. flavus* and *A. niger* as indicator organisms. It was observed that bacteriocin produced by *L. plantarum* WM and *L. casei* RS showed a strong inhibition against *A. niger* and *A. flavus* (selected as test organisms), while bacteriocins produced by *L. fermentum*, *L. acidophilus*, *L. delbrueckii,* L. brevis and *P. acidilactici* showed no inhibition against both *A. niger* and *A. flavus* (Table 2).

Shelf life of traditional fermented agidi (T-Ag), agidi produced using NBP-Ag and the samples BP-Ag, were compared. It was observed that agidi prepared from maize fermented with the mixed culture of BP strains had the longest shelf life of 8 days. Agidi produced from maize fermented with single starter culture of BP *L. plantarum* had a shelf life of 6 days, while agidi prepared from maize fermented in the traditional way had a shelf life of 2 days. On the other hand, samples prepared from maize fermented with NBP mixed culture starters had a shelf life of 4 days before spoilage occurred. Agidi fermented with NBP mixed culture had a total bacterial load of 3.2 x 10^5 $\log_{10}$ cfu/g and fungi load of 1.6 x 10^4 $\log_{10}$ cfu/g when spoilage occurred after day 4. Agidi produced with single starter culture of BP *L. plantarum* had none on the same day. However, agidi produced with mixed culture of *Lactobacillus* strain had a bacteria load of 1.9 x 10^5 $\log_{10}$ cfu/g and fungi load of 3.3 x 10^6 $\log_{10}$ cfu/g when spoilage occurred after day 8, while the bacterial load and fungi load of agidi prepared from mixed culture of NBP *Lactobacillus* strain increased to 2.0 x 10^8 and 3.8 x 10^8 $\log_{10}$ cfu/g, respectively. In addition, samples prepared from traditionally fermented agidi increased to 1.0 x 10^8 and 3.0 x 10^8 $\log_{10}$ cfu/g, while those prepared using BP

Table 1. Quantity of antimicrobial compounds produced by lactic acid bacteria.

Organism	Lactic acid (g/l)	Hydrogen peroxide (mg/l)	Diacetly (g/l)
Lactobacillus plantarum in OG3	10.6	36	3.6
Pediococcus acidilactici in OG1	12	51	5.1
Lactobacillus fermentum in OG2	4.7	65	6.5
Lactobacillus brevis in OG4	4.6	60	6.0
Lactobacillus acidophilus in OG1	3.6	40	4.0
Lactobacillus casei in OG4	8.8	32	3.2
Lactobacillus delbrueckii in OG5	7.6	3.6	36
Lactobacillus mesenteroides in OG2	4.7	3.8	38

OG5: Ogi made from millet; OG4: ogi made from red sorghum; OG3: ogi made from white maize; OG1: ogi made from white sorghum; OG2: ogi made from yellow maize.

Table 2. Antagonistic activity of the bacteriocin produced by LAB isolates against selected moulds from agidi.

LAB isolates	*A. niger*	*A. flavus*
L. plantarum in OG3	++	++
P. acidilactici in OG1	-	-
L. fermentum in OG2	-	-
L. brevis in OG4	-	-
L. acidophilus in OG1	-	-
L. casei in OG3	+	++
L. delbrueckii in OG5	-	-
L. mesenteroides in OG2	-	-

-, No inhibition zone; +, inhibition zone ≤ 3 mm; ++, inhibition zone ≥ 3 mm.

L. plantarum increased to 1.0 x 10^6 and 2.8 x 10^7 $\log_{10}$ cfu/g after day 8, respectively.

Statistical analysis carried out using one way analysis of variance (ANOVA) and Duncan multiple range test (Table 3) indicated significant differences in the organoleptic attributes of the agidi samples produced using starter cultures

DISCUSSION

Cereal grains are the most important substrates for fermented foods in tropical Africa. These are sorghum (guinea corn and kaffir corn) which grows in most savannah regions, maize in the water forest regions and millets, the predominant grains in the dry savannas (Olasupo et al., 1997). The cereal grains are fermented to produce a variety of foods. The products are conveniently divided on the basis of their moisture content into alcoholic beverages, porridges, dumplings and baked products (Muller, 1980). The cereals are low in minerals but high in the B group vitamins. Porridges contain more cereals (about 90%) and are usually eaten with a spoon. In West Africa particularly, sour porridges are very popular. The cereal grain is fermented and milled to produce a gruel, which is known by various names in different parts of West Africa. In southwestern Nigeria, it is called ogi, while in Ghana, it is called koko or akasa. Other products of cereal include 'kaffir' beer brewed from sorghum; pito also brewed from sorghum and agidi mostly made from maize (Muller, 1980)

Many of these fermented cereal products have been produced traditionally by spontaneous microflora which results in unstable products. Therefore, starter culture studies could be useful to minimize the fermentation risks and to reach a standard production schedule (Ahmet and Hihat, 2009). The main purpose of this work is to isolate and identify potential starter cultures for the production of agidi, a traditionally fermented cereal product. Consequently, traditionally fermented cereal gruels were collected from Ibadan, south west Nigeria. More than 25 isolates were evaluated due to their antimicrobial activity. However, the ultimate selection was done employing agar spot antimicrobial activity tests against moulds used as indicators. The moulds isolated from spoilt agidi samples were characterized as *A. flavus, Penicillium spp, A. niger* and *Rhizopus spp*. Morphological and physiological characteristics of the isolates agrees with the findings of Ogiehor et al. (2005). Several authors have also detected and isolated most of theses spoilage

Table 3. Organoleptic attributes of *agidi* samples produced using different starter cultures.

Sample code	Taste	Aroma	Texture	Colour	Overall acceptability
A	3.50[a]	2.75[b]	3.25[a]	3.00[a]	3.50[b]
B	4.50[b]	3.75[ab]	2.75[a]	3.00[a]	2.50[a]
C	3.00[a]	3.50[a]	3.00[a]	3.25[a]	3.75[b]
D	4.75[b]	4.75[b]	4.75[a]	4.75[b]	5.00[b]

A: Agidi sample fermented with bacteriocin producing single starter culture of *L. plantarum;* B: agidi sample fermented with non bacteriocin mixed culture of *Lactobacillus* spp.; C: agidi sample fermented with non bacteriocin producing mixed culture of *Lactobacillus* spp.; D: agidi samples produced with cultures of bacteriocin *L. plantarum* and *L. Casei.* Means with same letters are not significantly different according to Duncan multiple test (P< 0.05).

organisms in fermented foods and beverages (Filtenborg et al., 1996; Corsetti et al., 1998; Pitt and Hocking, 1999).

L. plantarum ogi made from red sorghum (OG4) and *L. plantarum* ogi made from white sorghum (OG1) were observed to exhibit the highest activity in mycelia growth for most of the moulds tested, while *L. brevis* OG4 showed the lowest antagonistic activity. Other ogi used in the study include ogi made from yellow maize (OG2), ogi made from white maize (OG3) and ogi made from millet (OG5). The antagonistic effect of LAB isolates on fungi growth established in this work is in agreement with the earlier works of Magnusson and Schnurer (2001), who reported that *Lactobacillus coryneformis* have a strong inhibitory activity in dual agar plate against the moulds *Aspergillus fumigatus*, *Aspergillus nidulans*, *Penicillium roqueforti.* The biopreservative effects of LAB on different agidi samples were monitored. Starter culture of bacteriocin producing *Lactobacillus* strains were selected to aid in the control of spoilage and improve organoleptic properties of agidi. The shelf life of agidi samples produced using bacteriocin producing starters showed a relative improvement when compared with the ones fermented by the traditional method. This is thus an indication of the possible potential of bacteriocin and other metabolites produced by LAB in the retardation of food spoilage. The results of this study are comparable to those of Ogunbanwo who demonstrated the use of BP *Lactobacillus* strains to extend the shelf life of fufu*, a* traditionally fermented cassava product (Ogunbanwo et al., 2004). Stiles (1996) also demonstrated the use of BP *Lactobacillus* strains in food to control the growth of enterotoxigenic *Escherichia coli.*

The recent advances in biotechnology have significantly increased the production of high quality, nutritious and tasteful foods that remain fresh for long time and are completely safe and less reliant on artificial additives. The potential application of bacteriocins as consumer friendly biopreservatives either in the form of protective cultures or as additives is significant. Besides being less potentially toxic or carcinogenic than current antimicrobial agents, LAB and their byproducts have been shown to be more effective and flexible in several applications (Stiles, 1996). In conclusion, this study recommends that the characteristics of bacteriocins and other compounds with antimicrobial activity should however, be explored in more detail with the objective of defining controlled use of selected cultures which can assure safety and improve organoleptic qualities of traditionally fermented foods.

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