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Isolation, Characterization and Identification of Lactic Acid Bacteria from Fermented Sorghum Dough Used in Sudanese *Kisra* Preparation

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Abstract: Forty two isolates were selected from 15 samples of traditionally fermented household starter dough from Khartoum state and also from laboratory-prepared fermented dough. Isolates were phenotypically characterized by their ability to ferment 21 carbohydrates using API 20 CHL kits and additional biochemical tests. Lactic Acid Bacteria (LAB) and yeasts were enumerated. The pH and lactic acid content were also determined. The LAB counts in household fermented dough varied between 7.1 and 9.4 log cfu ml^{-1} and yeast counts between 4.3 and 7.7 log cfu ml. The pH of household starter ranged from 3.7-4.5. The initial numbers of LAB in laboratory-fermented sorghum dough were increased from 5.5-9.0 log cfu ml during fermentation. Yeasts increased from 4.3-7.7 log cfu ml at 19 h. The pH declined from 5.04 to around 3.7. Lactic acid increased from 0.03-1.25%. Lactic acid bacteria isolated from both traditionally and laboratory prepared fermented dough were *Lactobacillus fermentum*, *Lactobacillus amylovorus* and *Lactobacillus brevis*. The yeast isolates were identified as *Saccharomyces cerevisiae*.

Key words: Lactic acid bacteria, yeasts, fermented sorghum dough

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Monesh] and finger millet are important food crops in arid and semi-arid regions of the world (Mohammed *et al*., 1991; Mukuru, 1992; Usha *et al*., 1996; Owuama, 1997).

Fermented foods and beverages constitute a major portion of people's diets in Africa (Sanni, 1993; Oyewole, 1997). Cereals grains including sorghum, maize and millet are common substrates for lactic acid. Fermented gruels and beverages known by different names (Odunfa and Adeyele, 1985). *Kisra* is widely produced in Sudan household levels from sorghum. Fermentation is spontaneous and uncontrolled thus resulting in a product of variable quality.

This study was undertaken to isolate, identify and characterize the Lactic Acid Bacteria (LAB) and yeasts present during sorghum fermentation. This information can contribute to the development of starter cultures with predictable characteristics, for use in small-scale and commercial production of *kisra* with stable and consistent quality.

MATERIALS AND METHODS

Fifteen samples of traditionally fermented sorghum dough made from dabar flour, local cultivar, were obtained from local households and markets in Khartoum State. Samples were kept in sterilized containers and stored in a refrigerator for further use as a starter.

Laboratory preparation and fermentation of sorghum dough: In the laboratory, sorghum fermented dough was prepared under sterile conditions; fermentation was performed according to the traditional way employed in Sudanese households. Sorghum flour was mixed with water in a ratio of 1:2 (w/v), then a starter was added to the dough at rate of about 10% (w/w) of the dough. The fermentation was usually completed in 12-19 h as pH 3.7 depending on the temperature and the amount of starter added. During fermentation, samples were aseptically withdrawn at 2 h intervals for pH determination and microbiological analysis.

Enumeration of Lactic acid bacteria and yeasts in fermented sorghum dough: Numbers of LAB were determined on selective media MRS agar. Appropriate dilutions were plated on MRS agar and incubated anaerobically using the anaerobic jars and the BBL, Gas Pak, anaerobic system envelopes (Becton, Dickinson, Cockeysville, USA) at 37°C for 48 h. Yeasts were enumerated by surface plating on malt extract agar (Oxoid) with 0.01% chloramphenicol as bacterial inhibitor and incubated aerobically at 25°C for 2-3 days.

Isolation of LAB from fermented sorghum dough: Lactic acid bacteria were isolated from traditional starter obtained from the households and from laboratory-prepared dough. MRS agar was used for isolation of lactobacilli. Surface-plated MRS agar was incubated anaerobically (BBL Gas pak plus Anaerobic System) at 37°C for 48 h. After counting, colonies on different plates were randomly picked from plates at higher dilutions (10^{-6}) and transferred into 10 ml test tubes with sterile MRS broth. The isolates were purified by successive streaking on the appropriate media (MRS agar) before being subjected to characterization.

For identification of lactic acid bacteria, overnight cultures of each isolate in MRS broth (Oxoid) were used. All isolates were initially tested for Gram reaction, catalase enzyme and production of acid from glucose in Hugh and Lelfsons medium by oxidation or fermentation reaction (Harrigan and MacCance, 1976). Only Gram positive bacteria with catalase negative reactions were observed (Schillinger and Lücke, 1987; Garvie, 1986; Kandler and Weiss, 1986) and the representative isolates were purified by successive streaking onto the same agar substrate. Representative yeast colonies on malt extract agar were examined microscopically, purified by successive streaking on malt extract agar and stored at 4°C. For the Gram-positive, catalase negative rods, growth at various temperatures 10, 15 and 45°C, tolerance of different salt levels (2, 4 and 6.5% w/v NaCl, hetero- and homo-fermentative activity (using MRS broth) with inverted Durham tubes in MRS broth were determined. Twenty isolates from household fermented dough and twenty isolates from laboratory-prepared fermented dough were then selected based on the above tests for further identification. The isolates were stored at -40°C in MRS broth containing 10% glycerol.

Characterization and identification of LAB and yeast isolates to species level: The carbohydrate fermentation profiles of the selected 20 isolates from household fermented dough and 20 isolates from laboratory-prepared fermented dough were investigated using API 20a and API CHL medium according to manufacture's instructions (API System, Bio-Merieux, France). The bacteria identified by the use of a computer programme, API LAB PLUS, version 3.2.2 software (BioMerieux) and reference to Bergey's Manual of Systematic Bacteriology. Pure isolates of yeasts were identified according to Lodder (1970); Barrnett *et al.* (1983) and Barrnett *et al.* (1990). The culture was examined microscopically after incubation at 28°C for 72 h. The shapes of the yeast cells and the form of budding were observed and registered (Barrnett *et al.*, 1983). Each isolate was then inoculated in- Potato Dextrose Agar (PDA) and incubated at 28°C for one to four weeks and then they were examined microscopically for ascospore formation (Barrnett *et al.*, 1983). YMA media was inoculated with fresh culture of yeast and incubated aerobically at 37°C and 42°C (Harrigan and MacCance, 1976). Resistance to cyclohexamide and the fermentation patterns among Glucose, Maltose, Galactose, Sucrose, Lactose and Fructose were carried out according to (Harrigan, 1998).

pH and titrable acidity: The pH was determined by using a pH meter model 7020 according to AACC (1983). The pH meter was calibrated using standard buffer solutions (Merck) at pH 4.0 and 7.0. Titrable acidity was determined according to the AOAC (1975) method and it was recorded as lactic acid percentages.

RESULTS AND DISCUSSION

Enumeration of LAB and yeasts: Table 1 shows the numbers of microorganisms in the household fermented starter samples. Lactic acid bacteria numbers varied between 8.4 and 9.0 log cfu ml^{-1} whereas yeasts varied between 4.3 and 5.5 log cfu ml^{-1}. The changes in microbial numbers during laboratory fermentation of starter are shown in Fig. 1. The LAB counts on MRS increased from 10^5-10^8 cfu g^{-1}. The largest increase in the numbers of LAB was noted during the 19 h of fermentation. These levels agree with Hamad *et al.* (1992) who found that, the total count of LAB in fermented dough was 9 x 10^8 cfu/g. The yeast counts increased from 10^4-10^7 cfu g^{-1}.

pH and titrable acidity: The pH ranges of fermented starter obtained from households are shown in Table 1. During laboratory fermentation, the pH of fermented dough decreased from 5.04-3.7 with in 10 h of fermentation (Fig. 1). These results in agreement with Hamad *et al.* (1992) who found that the pH in *kisra* fermentation dropped from about 5.4-3.4 in 10 h.

Characterizations of LAB and yeasts: Among the 42 lactic acid bacteria isolated 52% (22 isolates) produced gas from glucose and were referred to as hetero-fermentative, whereas 48% (20 isolates) were homo-fermentative. All of the 22 hetero-fermentative lactic isolates were arginine positive. Six of these were able to grow at 15°C, fermented glucose, maltose and xylose and they were tentatively identified as *L. brevis*. The remaining 16 of the arginine positive hetero-fermentative lactic isolates were not able to grow at 15°C and fermented mannose tentatively identified as *L. fermentum*.

From 42 isolates obtained from the fermented sorghum (both traditionally and laboratory-prepared), 20 did not produce gas from glucose and showed homo-fermentative characteristics as mentioned above. These homo-fermentative rod shaped isolates were tentatively identified as *L. amylovorus*. (Table 2).

LAB and yeasts are common in a wide range of African traditional foods and beverage fermentations (Adegoke and Babalola, 1988; Steinkraus, 1996).

The number of LAB and yeasts differed among household samples and increased during laboratory fermentation. Gobbetti *et al.* (1994) and Steinkraus (1996) proposed that lactic acid bacteria create an acidic environment conducive to yeast proliferation while the yeasts provide vitamins and other growth factors such as amino acids for the lactic acid bacteria. The simultaneous increase in numbers of both LAB and yeasts may therefore be attributed to their symbiotic association. The results are in agreement with those reported by other authors (Mbugua, 1984; Odunfa and Adeyele, 1985; Mohammed *et al.*, 1991; Nche *et al.*,

Table 1: The pH and microbial counts (log cfu ml) of different household starter dough types

pH	Yeast counts	LAB counts	Number of samples	Product origin
4.1±0.42[a]	4.3±0.28	8.4±1.14	5	Khartoum
4.3±0.35	5.8±0.57	8.4±1.84	5	Omdurman
3.8±0.07	5.5±1.44	8.9±0.70	5	Khartoum North

[a]Results given as averages±SD

Table 2: Differential characteristics of four isolates from fermented sorghum based on API 20 A CHL analysis

	API 20 CHL Results*			
Isolate	A	B	C	D
Substrate tested				
Glucose	+	+	+	+
Mannitol	-	-	-	-
Lactose	+	-	-	+
Saccharose	+	+	+	+
Maltose	+	+	+	+
Salicin	-	+	-	-
Xylose	-	-	+	-
Arabinose	+	+	+	+
Esculin	-	+	+	-
Cellobiose	-	+	-	-
Mannose	+	+	-	+
Melezitose	-	-	-	-
Raffinose	+	-	-	+
Sorbitol	-	-	-	-
Rhaminose	-	-	-	-
Trehalose	-	+	-	-
API 20 CHL identification	*L. fermentum*	*L.amylovorus*	*L. brevis*	*L.fermentum*

+, positive reaction; -, no reaction

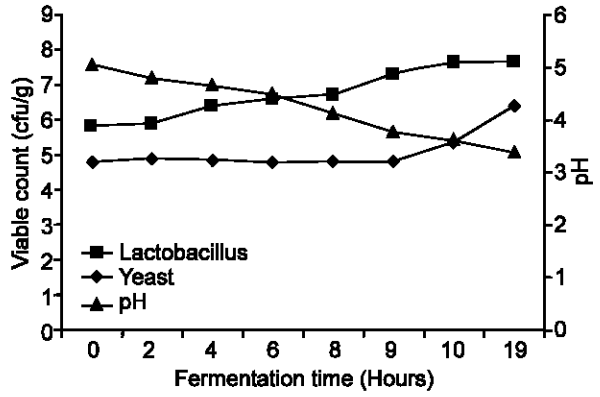

Fig. 1: Laboratory fermentation using traditional starter in dabar flour

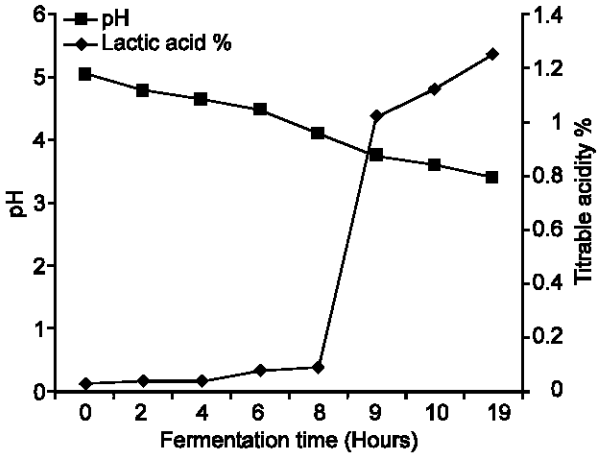

Fig. 2: pH and acidity during laboratory fermentation

1994). Melaku and Faulks (1988) also indicated that numbers of LAB increased during the first stages of the natural fermentation with a slight reduction in number during the later stages of fermentation.

The decrease in pH and increase in lactic acid followed the same trend as reported for other traditionally fermented foods (Mohammed *et al*., 1991; Choi *et al*., 1994; Dziedzoaze *et al*., 1996). The high levels of lactic acid might be attributed to the predominance of LAB. A slight decrease in lactic acid concentration was observed in the late stages of fermentation. Akinrele (1970) attributed the decrease in lactic acid concentration to the utilization of lactic acid by yeasts. In the present work, the acid found in highest concentrations in fermented dough was lactic acid. Banigo and Muller (1972) identified the main acids in ogi as lactic, butyric and acetic acids. The production of these acids during the fermentation of maize dough porridge and their ability to inhibit a variety of organisms has been reported (Mensah *et al*., 1991).

The lactic acid bacteria identified in fermented dough have been reported in other fermented foods. *L. fermentum* and *L. brevis* have been suggested to be the

predominating microorganisms during fermentation of fufu and ogi, two Nigerian foods (Adegoke and Babalola, 1988), *kisra* a Sudanese sorghum fermented flat bread (Mohammed *et al*., 1991; Abd Elmoniem *et al*., 1994), kenkey, a Ghananian fermented maize dough (Halm *et al*., 1993).

L. fermentum and *L. amylovorus* have been suggested to be the predominating microorganisms during fermentation of sorghum dough in Sudanese *kisra* (Halm *et al*., 1993). In togwa, a Tanzanian fermented food prepared from sorghum, maize, millet and maize-sorghum, the microorganisms were tentatively identified as *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus fermentum, Pediococcus pentosaceus* and yeast *Issatchenkia orientalis, Saccharomyces cerevisiae* and *Candida tropicalis* (Mugula *et al*., 2003) and in ogi, a fermented porridge prepared from sorghum in West Africa, the main microorganisms involved were *Lactobacillus spp*. particularly *Lactobacillus plantarum* and two yeasts *Candida krusei* and *Debaryomyces hensenii* (Odunfa and Adeyele, 1985).

These dominant microorganisms in *kisra* fermentation seem to be found in other indigenous fermented foods in the genus but not the species. Lei and Jakobsen (2004) reported that the dominating lactic acid bacteria from five Koko production sites in Northern Ghana prepared from millet were found to be *Weisella confuse* and *Lactobacillus fermentum*, followed by the less dominant *Lactobacillus salivarius* and *Pediococcus spp*. The yeast populations observed were *Saccharomyces cerevisiae*. The association of yeasts and lactic acid bacteria is known from a wide variety of traditional food and beverage fermentations (Soni *et al*., 1985; Adegoke and Babalola, 1988; Sakai and Caldo, 1985).

Yeasts have also been reported to make a useful contribution to the improvement of flavour and acceptability of fermented cereal gruels (Banigo *et al*., 1974; Odunfa and Adeyele, 1985; Akinrele, 1970) reported the contribution of S. cerevisiae and *Candida mycoderma* to the flavour acceptability of ogi. *S. cerevisiae* proliferated at the beginning while the latter was predominant at the end of fermentation.

By using lactic acid bacteria and yeast isolates as starter cultures, controlled fermentation studies can be carried out.

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