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Full Length Research Paper

Physiological Properties of a Microbial Community in Spontaneous Fermentation of Maize (*Zea mays*) for Ogi Production

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Based on the previous knowledge of spontaneously fermenting maize for the preparation of Ogi, four growth media were used for the isolation of the different common group of organisms implicated. Yeasts and Molds were identified as *Saccharomyces cerevisiae, Candida sp., Rhodotorula sp., Aspergillus niger and Penicillium sp.* The bacteria were *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus fermentum, Streptococcus lactis, Enterococcus feacalis, Escherichia coli, Staphylococcus aureus, Enterobacter sp., Citrobacter sp., Klebseilla sp., Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Aerobacter sp., and Corynebacteria sp.* A sharp decrease in pH of fermenting gruel from 5.7 to 3.5 was observed within 24 hours while titratable acidity increases within the first 48 hours with a further increase and decrease in pH and titratable acidity at 72 hours.These isolates were subjected to pH ranges of 3.5 – 6 and all were able to grow at optimum pH 6 with a reduction in the number of isolates as pH reduces. Growth in temperature ranging from 25^{0}C to 50^{0}C, salt (NaCl, $MgS0_4$, K_2HP0_4, $CuS0_4$ and $ZnS0_4$) and sugar (glucose, sucrose, lactose, melibiose and raffinose) concentrations of 0.2% to 1% were carried out on all the isolates. With increase in temperature and salt concentration, a reduction in the number of isolates that grew was observed. From this study, the optimum conditions that favours the growth of all the group of organisms was found to be pH 6, 30^{0}C, 0.2% of the salts used and a significant population of all the group of organisms utilized glucose as their best carbon source.

Keyword: Physiological characteristics, 'ogi' preparation, Spontaneous fermentation, microbial community, optimum conditions.

INTRODUCTION

Traditional fermented foods prepared from most common types of cereals (such as corn, rice, wheat or sorghum) are well known in many parts of the world. Some are utilized as colorants, spices, beverages and breakfast or light meal foods, while a few of them are used as main foods in the diet (Blandino *et al*., 2003). Several processing technologies which include cooking, sprouting, milling and fermentation have been put into practice to improve the nutritional properties of cereals, although probably, the best one is fermentation (Mattila-Sandholm, 1998).

"Fermentation" describes a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidized, and an organic carbohydrate acts as the electron acceptor (Adams, 1998). This leads to a general improvement in the shelf life, texture, taste, aroma as well as nutritional value (Odunfa and Oyeyiola, 1985; Uzogara *et al*., 1990). 'Ogi' is an acid fermented cereal gruel or porridge made from maize (*Zea mays*) or corn; sorghum (*Sorghum vulgare*) also known as guinea corn or millet (*Pennisetum typhoideum*) (Ohenhen and Ikenebomeh, 2007). The colour of 'ogi' depends on the cereal grain used: cream-

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white for maize, reddish brown for sorghum and dirty grey for millet (Banigo, 1993; Onyekwere *et al*., 1993). It is smooth in texture, has a distinctive aroma and a sour taste reminiscent to that of yoghurt (Banigo and Muller, 1972; Chavan and Kadam, 1989).

For the preparation of 'ogi', white maize, sorghum or millet grains are washed and steeped in clean water for 1-2 days. The softened grain is then wet milled into fine slurry which is subsequently sieved through a fine wire sieve. The pomace is retained on the sieve and later discarded for animal feed, while the starch which has been separated with the water is allowed to sediment in a pot. The sievate is allowed to ferment for 2-3 days during which it becomes sour. The fermented starch paste is ogi. For consumption, the paste is added to some quantity of water and boiled with continuous stirring to make gruel (Odunfa and Oyewole, 1998).

Assessments of the biochemical and related changes show that during fermentation, hydrolyzed starch is converted to organic acids at reduced pH (Akinrele, 1970). The concentrations of available lysine, methionine and tryptophan increase (Nanson & Field, 1984). The predominant acids produced are 0.6% of lactic acid, 0.1% of acetic acid, and butyric acid (≤0.02%). Altogether, 40 volatile acids were identified by gas chromatography (Banigo & Muller, 1972). The main acids contributing to the desired flavor of sourness of 'ogi' have been found to be directly affected by *Lactobacillus plantarum*, the predominating organism in the fermentation.

The dynamics of growth, survival and biochemical activity of microorganisms in foods are the result of stress reactions in response to the changing of the physical and chemical conditions into the food micro-environment (e.g., the gradients of pH, oxygen, water activity, salt, and temperature) and the ability to colonize the food matrix and to grow with spatial heterogeneity (e.g., microcolonies and bio-films). Moreover, because in most food ecosystems microbial populations are assembled as a community, and because cells are generally immobilized and localized in high densities, food production (or degradation) is rarely the result of the activities of an individual but that of a group of microorganisms (Fleet, 1998). Therefore, the growth, survival, and activity of a given species or strain, whether it is an unwanted spoilage or pathogenic organism, or a desirable biocontrol of a probiotic agent, will, in most cases, be determined by the presence of other microorganisms and the in situ cell-to-cell ecological interactions which often happen in a solid phase(Giraffa, 2003).

In general, microorganisms from various sources have been studied based on their physiological conditions. The aim of this work is to determine the optimum physiological conditions of the microbial community in spontaneous fermentation of maize for 'Ogi' production with a view to establish the appropriate condition(s) for the development of highly nutritional and microbiologically safe ogi.

MATERIALS AND METHODS

Sample Collection

The cereal, SUWAN-Y (maize) used was collected from the Institute of Agricultural Research and Training (I. A. R. and T.) Moor-Plantation, Ibadan in clean sterile polyethylene bags and kept in the refrigerator until use.

Sample Treatment and Processing

Manual sorting and winnowing of the grains to remove stones, debris and defective seeds were carried out. The grains were weighed (500g), cleaned and steeped in sterile distilled water for 2 days in clean containers at room temperature (30 ± 2^0C). The water was decanted and the grains wet-milled using properly cleaned and surface sterilized blender. The resulting paste was sieved using sterile muslin cloth and the slurry was allowed to sediment during which spontaneous fermentation was allowed for 3 days (Odunfa and Adeyele, 1985).

Microbiological Analysis

The number and type of microorganisms per milliliter (mL) of the fermenting substrate was estimated daily for 72h by pour plate method using the serial dilution technique. The media used for microbial enumeration were Plate count agar (oxoid) for estimation of total viable bacteria; aerobic bacteria (Nutrient agar); for total lactic acid bacteria (MRS agar, oxoid) incubated at 37±2°C for 48h in anaerobic jars with anaerogen, Mac Conkey agar was used for total enterobacteria at 30±2°C for 48h and yeasts and moulds counts on Malt extract agar (oxoid) containing 0.5mg/l streptomycin sulphate (sigma) incubated at 30°C for 3-5 days. Twenty isolates were randomly picked from each of the growth media, (Malt Extract Agar, Nutrient Agar, MRS Agar and MacConkey agar) at each fermenting hour (0, 24, 48, 72 hours). These isolates were picked based on their macroscopic appearances which include shape, size, pigmentation and elevation, microscopic characteristics like Gram reactions, cellular shapes and arrangements, and catalase activity for lactics.

Chemical Analysis

The pH of the fermenting substrates was measured daily with a Jenway pH metre standardized with the

appropriate buffer. The amount of the lactic acid produced in the fermenting maize meal were determine by the standard titration procedure for total titratable acidity (TTA) according to A.O.A.C, (1990) .

$$\text{Lactic acid} = \frac{N \times V \times ME \text{ of lactic acid} \times 100}{V^2}$$

N = Normality of the NaOH used
V = Volume of the NaOH used
M.E = Equivalence factor
V_2=Volume of the sample used

Physiological Studies

Growth at different Temperatures

Growth of the isolates at 25^0C, 30^0C, 35^0C, 40^0C, 45^0C and 50^0C was assessed by inoculating in their appropriate broth with 24h old cultures, followed by incubation of the tubes at those temperatures. Growth at a particular temperature was determined quantitatively by observing turbidity of the broth (Gibson and Abd-El-Melek, 1945).

Growth at different pH

The ability of the isolates to grow at various pH values was carried out by incubating the isolates in their corresponding broth media. The pH values of the 4 different media: MRS broth, MacConkey broth, Nutrient broth and Malt extract broth, was determined and adjusted to pH 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 using citrate phosphate buffer (Pearse, 1980). Ten mililiters of the media were dispensed into screw capped tubes and autoclaved at 121^0C for 15 minutes. The autoclaved media was inoculated with the test cultures before incubation at their various temperatures. Turbidity of the broth compared with the uninoculated controls was used as indicator of growth of the culture (Gibson and Abd-El-Melek, 1945).

Growth in different sugar concentrations

The test was carried out to investigate the ability of the isolates to utilize different sugars. The different sugars used were lactose, glucose, sucrose, melibiose and raffinose. Modified MRS fermentation broth medium from which meat extract and glucose had been omitted (De Man *et al*., 1960) and 0.05% (w/v) bromocresol purple indicator had been added were used in this test for lactic acid bacteria. Modified MacConkey fermentation broth medium from which lactose was excluded with 0.05% neutral red indicator were used for enteric bacteria isolates. Malt extract peptone broth with 0.05% bromothymol blue indicator was used for the growth of yeasts and moulds while Nutrient broth was used for the growth of aerobic bacteria. The media were dispensed into test tubes with inverted Durham tubes. After autoclaving, sterile test sugars were aseptically added to give a final concentration of 1%. Eighteen hours old culture were inoculated into 10ml of the basal medium containing the test carbohydrates and incubated for 3-5 days at 35 ± 2^0C. Fermentation of test sugar resulting in acid production (indicated by colour change) and displacement of liquid in Durham tubes were recorded as positive for gas production. No colour change was recorded as negative. Uninoculated tubes served as control.

Growth in different salt concentration

Overnight grown broth of test isolates were inoculated into Modified MRS broth, MacConkey broth, Malt extract peptone broth and nutrient broth from which all salts had been removed. The test salts were then added at concentrations (0.2, 0.4, 0.6, 0.8 and 1%) and incubated for 24 hours at appropriate temperatures. The salts used were NaCl, $MgSO_4$, K_2HPO_4, $ZnSO_4$, and CuSO4. Turbidity of the broth compared with the uninoculated controls was used as indicator of growth of the culture (Gibson and Abd-El-Melek, 1945).

RESULTS AND DISCUSSION

In this study, 80 isolates were selected from all the isolates present in spontaneously fermenting maize. They included twenty lactic acid bacteria, twenty yeasts and mould isolates, twenty aerobic bacteria and twenty enteric bacteria. These isolates were subjected to different physiological tests in order to construct a microbial community of the physiologically active microorganisms.

Table 1 shows the microbial load of cultivable microorganisms estimated during the fermentation of ogi. Cell counts increased with the fermentation time in MRS and MEA. The value ranges from 6.5 x 10^7 cfu/ml to 2.96 x 10^{14} cfu/ml on MRS plate. On MEA, cell counts increased from 3.0 x 10^7 to 2.7 x 10^{13} cfu/ml. The total microbial concentration on PCA increased within the first 48 hour with a value 2.48 x 10^{14} cfu/ml and later decreased by 72h to give a count of 1.72 x 10^{14} cfu/ml. No Enterobacteria were observed after 24 hour on MCA. Similar observation was shown in the work of Wakil *et al*. (2004). Apart from the flora present on the surface of the grains, microbial flora may have also developed during milling and malting process. The total lactic count, which includes both streptococci and lactobacilli, was higher than the total culturable (viable) count and yeast count.

Table 1. Microbial load (cfu/ml) estimated during spontaneously fermenting maize

Fermentation Time (hrs)	MEDIUM/ MICROBIAL LOAD(cfu/ml)			
	PCA	MRS	NA	MEA	MCA
0	1.01×10^{8}	6.5×10^{7}	9.8×10^{7}	3.0×10^{7}	5.8×10^{7}
24	1.89×10^{14}	1.7×10^{14}	1.75×10^{14}	1.2×10^{13}	1×10^{13}
48	2.48×10^{14}	1.73×10^{14}	1.2×10^{14}	1.5×10^{13}	-
72	1.72×10^{14}	2.96×10^{14}	9.5×10^{13}	2.7×10^{13}	-

Key:
PCA- Plate Count Agar MRS- de Mann Rogosa Sharpe MEA- Malt Extract Agar
MCA- MacConkey Agar NA- Nutrient Agar

Table 2: pH and total titratable acidity (TTA) of spontaneously fermenting maize

FERMENTATION TIME(hrs)	pH	TOTAL TITRABLE ACIDITY(g/l)
0	5.65± 0.05	0.2065 ± 0.0005
24	3.5 ± 0.05	0.2425 ± 0.0005
48	3.9 ± 0.05	0.265 ± 0.005
72	3.8 ± 0.05	0.188 ± 0.001

N.B: values are mean ± SD, Where SD = Standard Deviation

These results were in accordance with the findings of Chavan and Kadam (1989) while the dominance of lactic acid bacteria in spontaneous fermentation of cereals has been reported by some researchers (Odunfa and Adeyele 1985; Halm *et al.,* 1993; Olukoya *et al*., 1993). The inability to detect enterobacteriaceae after 24 hours was due to low pH of the fermenting media. This observation was similar to those reported by earlier workers (Odunfa and Adeyele, 1985; Sanni *et al.,* 1994; Michodjehoun-Mestres *et al.,* 2005).

Table 2 shows the pH and the total titratable acidity of the fermenting maize obtained at different fermentation time. A gradual increase in lactic acid production was noticed within the first 48h which was 0.2065g/l and it reduced to 0.188g/l at 72 hours. There were changes in the pH of the fermenting maize at different fermenting time. The highest value of 5.65 was recorded at 0hr and it reduced within 24 hrs to give the least pH value of 3.5. A further increase and decrease in pH and titratable acidity at 72 hours was observed. Such a decrease in pH and increase in acidity due to microbial activity has been well documented (Aliya and Geervani, 1981; Achi, 1990). Effective inhibition of competing microorganisms appears to depend on achieving numbers of lactic acid bacteria sufficient to decrease the pH rapidly to levels where the growth of the pathogen is prevented. Production of the primary metabolite, lactic acid and the resulting pH decrease is the main preserving factor in food fermentation (Ogunbanwo *et al*., 2003).

The biochemical characterization tests carried out on the isolated microorganisms from the spontaneously fermenting ogi revealed their probable identity as *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus fermentum*, *Streptococcus lactis, Enterococcus feacalis, Saccharomyces cerevisiae, Candida sp., Rhodotorula sp., Escherichia coli, Staphylococcus aureus, Enterobacter sp., Citrobacter sp., Klebseilla sp., Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Aerobacter sp and Corynebacteria sp.* The mold isolated were *Aspergillus niger* and *Penicillium sp.* The most predominant organism is *Lactobacillus plantarum* which helps in the souring of the maize. *Corynebacterium* hydrolyses the corn starch to form organic acids while *Saccharomyces cerevisiae* and *Candida mycoderma* contribute to the flavor development (Odunfa *et al*., 1998; Caplice and Fitzgerald, 1999). The presence of these organisms in 'ogi' has been reported in the works of Akinrele, (1970); Odunfa *et al*. (1998); Ohenhen, (2002) and Ogunbanwo *et al.* (2003). The cultural and biochemical properties of the isolates agreed

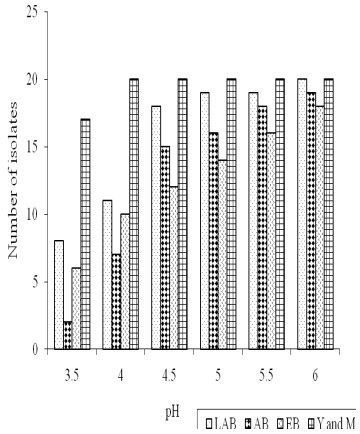

Figure 1: Effect of pH on the growth of isolates from fermenting maize

Key:
LAB :- Lactic acid bacteria EB :- Enteric bacteria
AB :- Aerobic bacteria Y and M :-Yeast and moulds

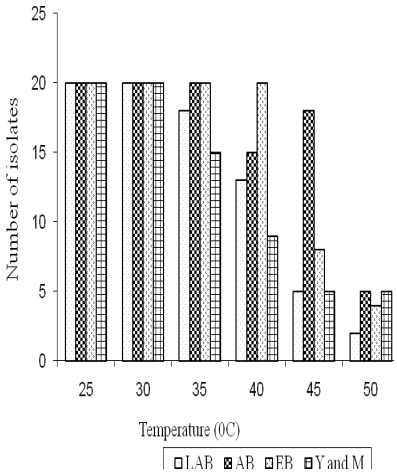

Figure 2: Effect of temperature on the growth of isolates from fermenting maize

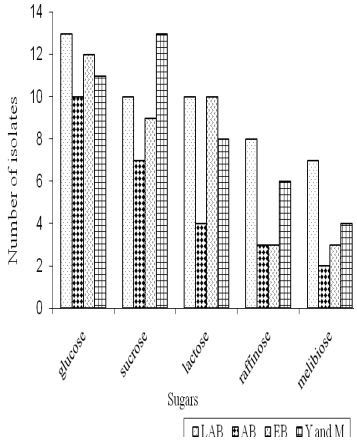

Figure 3: Effect of different sugars on the growth of isolates from spontaneously fermenting maize.

with the publication of Rogosa and Sharpe (1959); Kreger-van Rij, (1984) and Bergey's Manual of Systematic Bacteriology (Sneath *et al*., 1986).

All the selected isolates were subjected to different pH range and all grew at pH 6. There was a reduction in the number of organisms that grew as pH reduces with yeast and mould isolates having the highest number of growth at pH 3.5. The reduction in the number of lactic acid bacteria (LAB) isolates that grew is due to their varying sensitivity to acidity (Adams, 1995). Report has also shown that most bacteria cannot grow at low pH (Adebolu *et al.,* 2007) while Yeasts and moulds however has been seen to grow more at pH 3.5 (Cai, 1994). The variations in acid tolerance in all of the above examples can be attributed to their relative ATPase activities at low pH (Cotter, 2003).

All the isolates grew at temperatures of 25^0C and 30^0C and a decline in their growth number was observed as temperature increased (Figure 2). This might have been due to the high temperature stress seen to have caused thermal damage, which can disrupt hydrogen bonding and hydrophobic interactions, leading to general denaturation of proteins and nucleic acids. Cell viability has been reported to decline when temperature increases beyond growth-optimal levels (Kevin, 2005).

The ability of all the isolates to grow in 1% of the different sugars is seen in figure 3. Significant population of all the group of organisms utilized glucose as the best sugar with LAB having the highest number of growth in all the sugars used except for sucrose where yeast and mould showed the highest growth. Aerobic bacteria isolates were observed to show the least number of growth in all of the sugars tested. The growth of most fungi in sucrose has been demonstrated by Sati *et al*. (2006) and Bechem *et al*. (2007). High growth of all the group of organisms was also observed for sucrose and lactose while Raffinose and melibiose did not support the growth of most of the organisms.

Varying the concentration of the salts was seen to affect the distribution of the number of isolates that could grow. Decrease in growth of isolates with increase in concentration of salts was observed (figure 4, 5, 6, 7 and 8). All the isolates were observed to grow at 0.2% and 0.4% NaCl while the highest number of organisms growing at the final concentration of 1% was recorded in Yeast and Mould group. A reduction in the number of isolates that grew was seen as concentration of $MgSO_4.7H20$ increases. At 1% concentration of K_2HPO_4, enteric bacteria, LAB, yeasts and moulds showed equal

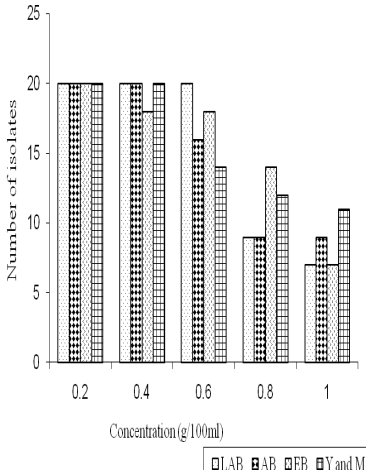

FIGURE 4: Effect of different concentrations of NaCl on growth of isolates from spontaneously fermenting maize

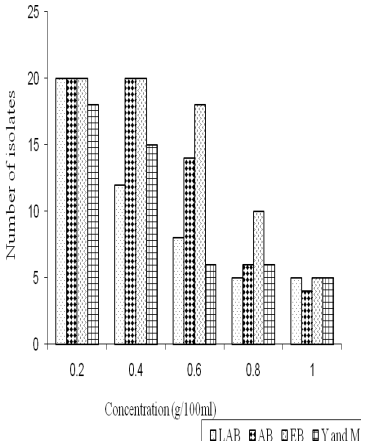

FIGURE7: Effect of different concentrations of $CuSO_4$ on growth of isolates from spontaneously fermenting maize

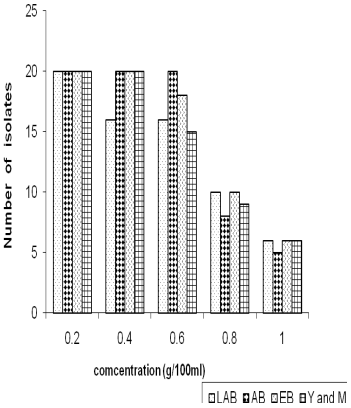

FIGURE 5: Effect of different concentrations of $MgSO_4.7H_20$ on growth of isolates from spontaneously fermenting maize

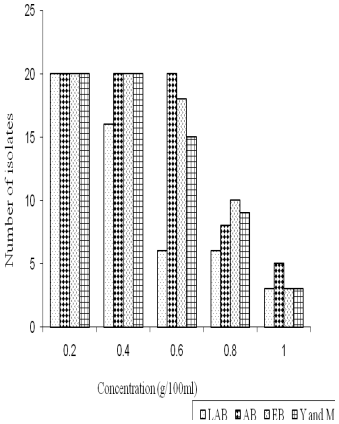

FIGURE8. Effect of different concentrations of $ZnSO_4$ on growth of isolates from spontaneously fermenting maize

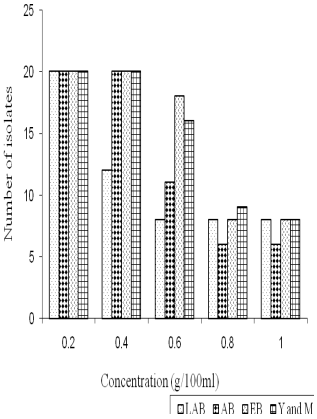

FIGURE 6: Effect of different concentrations of K_2HPO_4 on growth of isolates from spontaneously fermenting maize

number of growth of isolates (figure 8), while aerobic bacteria had the least number (figure 6). At 1% concentration of $CuSO_4$, aerobic bacteria showed the least growth (figure 4) and the highest number of growth at 1% concentration of $ZnSO_4$ was recorded in aerobic bacteria. The effect of salts on the various groups of organisms is seen in the works of Gunsalus and Charles, (1942), Tom *et al*. (1984), Fernandez *et al*. (1993), Nair (2006) and Marathe (2009).

In conclusion, the results of this study have shown that optimum pH and temperature that favours the growth of all groups of organisms in spontaneously fermenting maize was found to be pH 6 and 30°C. Significant population of all the groups of organism utilized glucose as the best sugar. Of the different salt concentrations used, 0.2% was the best to support the growth of all organisms from spontaneously fermenting maize.

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