LACTIC ACID BACTERIA IN FERMENTING MAIZE DOUGH

by W. F. K. CHRISTIAN

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Summary

Samples of maize dough at an advanced stage of fermentation have been analysed microbiologically. A mixed population of lactic acid bacteria and yeasts has been found in all the samples. Apart from the yeasts the most common species of bacteria found in all the samples examined is the homofermentative *Pediococcus cerevisiae*. This is associated with heterofermentative species which may be either *Leuconostoc mesenteroides* or *Lactobacillus fermenti*. The medium used in isolating micro-organisms from the fermented dough is important. On nutrient agar only yeasts have been isolated. The lactic acid bacteria tend to appear only on YDA and MRS agars, the latter media being more favourable for the lactobacilli.

Introduction

In Ghana fermented maize dough known locally as 'mbor' (Fanti) is used to prepare a number of dishes.

In the traditional preparation of the dough, the grain is washed and soaked for 12 to 24h. It is wet-milled into a meal which is made into a stiff dough by adding an adequate amount of water. The dough is then allowed to undergo natural fermentation for 1 to 3 days before use. During the process of fermentation, the dough becomes sour, rises and develops a characteristic aroma.

It appears little, if any, work has been done on the microbiology of fermented maize dough in the form it is prepared in Ghana. Whitby (1968) is of the opinion that the fermentation is of the acetic acid type. Work by Akinrele and Bassir (1967), however, suggests that the fermentation of dough may very likely involve lactic acid bacteria, fungi as well as yeasts.

The work reported in this paper was carried out to determine the types of bacteria found in fermenting maize dough.

Materials and methods

The samples of maize dough analysed were all prepared in Ghana in the traditional manner, sealed in plastic bags and sent by air for microbiological analysis in the United Kingdom. Four samples A, B, C and D were analysed. Sample A was bought in the market in Accra, Ghana. Samples B, C and D were prepared under more controlled conditions in the Food Research Institute.

When the samples were received for analysis they were all 7 days old and at an advanced stage of fermentation. The pH of sample A was 4.1, that of sample B 3.90 and those of samples C and D, 3.80 and 3.98 respectively.

Immediately on receipt, serial dilutions were aseptically prepared for each sample using Ringer's solution. Suitable dilutions of each sample were then plated using 1 ml amounts of inocula.

The media used in plating included MRS agar (pH 6.4) described by de Man, Rogosa and Sharpe (1960), MRS agar (pH 5.4) which before use was bufferred with acetic acid /acetate buffer; yeastrel dextrose agar (YDA), pH 7.2; Oxoid nutrient agar (NA), pH 7.2.

The yeastrel dextrose agar contained Oxoid Lab-Lemco, 10g; Oxoid bacteriological peptone, 10g; yeastrel, 3g; sodium chloride, 5g; glucose, 5g; Oxoid agar nitrate, 15g; distilled water, 1 litre. The medium was sterilized at 120°C for 15 min. For yeastrel dextrose broth the agar was omitted.

All plates except those of MRS (pH 5.4) were incubated aerobically at 30°C for 8 days. The MRS acetate buffer plates (pH 5.4) were incubated in anaerobic jars at 30°C also for 8 days.

After counting, a number of representative colonies were picked from different types of plates into yeastrel dextrose broth and

TABLE 1

Total number of bacteria appearing on different media

Sample	Medium			
	NA	YDA	MRS (pH 5.4)	MRS (pH 6.4)
A	30 x 10^6	160 x 10^6	90 x 10^6	No plating done
B	60 x 10^6	250 x 10^6	900 x 10^6	500 x 10^6
C	20 x 10^6	800 x 10^6	2310 x 10^6	1800 x 10^6
D	10 x 10^6	70 x 10^6	1000 x 10^6	700 x 10^6

TABLE 2

Representative organisms isolated from sample A

Medium	Cocci Gram + Catalase +		Cocco-bacilli Gram + Catalase —		Yeasts		Total no. of isolates examined
	No. of isolates	% occurrence	No. of isolates	% occurrence	No. of isolates	% occurrence	
YDA	16	61.5	3	11.5	7	27	26
MRS (pH 5.4)	9	64.3	4	28.6	1	7.1	14
NA	0	0	0	0	10	100	10

TABLE 3

Representative organisms isolated from sample B

Medium	Cocci Gram + Catalase +		Rods Gram + Catalase —		Yeasts		Total no. of isolates examined
	No. of isolates	% occurrence	No. of isolates	% occurrence	No. of isolates	% occurrence	
YDA	4	36.4	3	27.2	4	36.4	11
MRS (pH 5.4)	1	6.7	14	93.3	0	0	15
MRS (pH 6.4)	0	0	10	77.9	3	23.1	13
NA	0	0	0	0	12	100	12

TABLE 4

Representative organisms isolated from sample C

Medium	Cocci Gram + Catalase +		Rods Gram + Catalase —		Yeasts		Total no. of isolates examined
	No. of isolates	% occurrence	No. of isolates	% occurrence	No. of isolates	% occurrence	
YDA	8	61.5	0	0	5	38.5	13
MRS (pH 5.4)	3	23.1	8	61.5	2	15.4	13
MRS (pH 6.4)	2	13.4	8	53.3	5	33.3	15
NA	0	0	0	0	10	100	10

TABLE 5

Representative organisms isolated from sample D

Medium	Cocci Gram + Catalase +		Rods Gram + Catalase —		Yeasts		Total no. of isolates examined
	No. of isolates	% occurrence	No. of isolates	% occurrence	No. of isolates	% occurrence	
YDA	6	42.8	4	28.6	4	28.6	14
MRS (pH 5.4)	0	0	15	100	0	0	15
MRS (pH 6.4)	3	18.8	11	68.7	2	12.5	16
NA	0	0	0	0	10	100	10

incubated at 30°C for 2 days. They were then subcultured on yeastrel dextrose agar slopes and incubated for a day. The cultures were then Gram-stained and examined for the catalase reaction. All cultures were purified by streaking on MRS agar (pH 6.4) and picking single colonies.

Cultures were grouped into coccal, coccobacillary and rod-like forms and yeasts. From each group a number of cultures were selected for identification. Yeasts were identified solely on the morphology of Gram-stained preparations.

The media and methods of Gunther and White (1961) and Coster and White (1964) were used for the identification of *Pediococcus*, except that MRS broth with the meat extract omitted instead of tomato-juice broth was used as a basal medium for fermentation tests. Differentiation of species was based on the classification of Pedersen (1949), Gunther and White (1961), Coster and White (1964), and Diebel and Niven (1960). *Leuconostocs* were identified using the methods described by Abd-el-Malek and Gibson (1948) and Garvie (1960). The methods used in identifying the lactobacilli are those outlined by Rogosa and Sharpe (1959).

MRS broth (pH 6.4) was used in gas production tests, the determination of growth temperatures and, as already mentioned, as a basal medium for carbohydrate tests. In the test for gas production, a high glucose concentration of 4.5% and a heavy inoculum (1 ml) of a young culture in yeastrel dextrose broth were used to ensure satisfactory evolution of gas (Gibson and Abd-el-Malek 1945).

Fermentation tests were carried out using carbon sources at a concentration of 1.0%. All inoculations were made using young cultures grown in yeastrel dextrose broth or on yeastrel dextrose agar slopes, cultures from agar slopes being used mainly to inoculate carbohydrate fermentation substrates. The catalase test was carried out on a slide and confirmed by flooding with hydrogen peroxide a culture growing on an agar slant.

Results

Nutrient agar was the least favourable medium for the isolation of organisms in the fermenting grain mash. For all samples, the least number of colonies was counted on nutrient agar plates (Table 1). Of the colonies examined from nutrient agar plates, none were lactic acid bacteria, all being yeasts (Tables 2–5).

In the isolation of organisms from samples B, C and D, MRS agar (pH 5.4) incubated under anaerobic conditions was the most favourable medium allowing the greatest number of organisms, principally lactic acid bacteria, to grow (Tables 1, 3 and 5). Yeasts were unable to grow very well on this medium.

TABLE 6.

Characteristics of Leuconostoc mesenteroides *isolated from sample A*

Type of isolate	A	B	C
No. of isolates in total number examined	1	4	2
Gas from glucose	+	+	+
Growth at 15°C	+	+	+
Growth at 37°C	+	+	+
Growth at 45°C	—	—	—
Heat resistance at 55°C for 15 min.	+	—	—
Dextran formation	+	+	+
Diacetyl production	—	—	—
Litmus milk	—	—	Asl
NH_3 from arginine	—	—	—
Acid from:			
arabinose	—	—	—
xylose	+	+	+
maltose	+	+	+
sucrose	+	+	+
cellobiose	+	+	+
melibiose	—	—	—
lactose	—	—	+
trehalose	—	—	—
amygdalin	—	+	+
salicin	—	+	+
aesculin hydrolysis	+	+	+

Asl. = slight acid

In platings of sample A, however, fewer colonies appeared on MRS agar (pH 5.4) than on yeastrel dextrose agar. This may be accounted for by the tendency of the gram-positive, catalase-positive cocci, the pedio-

cocci, which predominated in sample A, to grow in greater numbers on yeastrel dextrose agar under aerobic conditions (Tables 2–5). In samples B, C and D, the pediococci again tended to appear on the YDA with yeasts while the rods which out-numbered the cocci appeared in greater numbers on the MRS agars (Tables 3–5).

Apart from the yeasts, 3 main types of bacteria, all of the lactic acid group, were isolated from the 4 samples of fermented maize dough. These were the homofermentative *Pediococcus cerevisiae,* and the heterofermentative *Leuconostoc mesenteroides* and *Lactobacillus fermenti*. A single isolate of *Lactobacillus plantarum* which is homofermentative was also identified.

Pediococcus cerevisiae appeared with *Leuconostoc mesenteroides* in sample A and with *Lactobacillus fermenti* and *Lactobacillus plantarum* in samples B, C and D.

No *Leuconostoc* species were isolated from samples B, C and D. Lactobacilli were not isolated from sample A.

Leuconostoc mesenteroides (Table 6)

All the leuconostoc types appearing in the fermented maize sample A had characteristics similar to those of *Leuconostoc mesenteroides*. The cells which were slightly elongated and in pairs were Gram-positive and catalase negative. The elongated cells would appear to be a departure from the normal coccoid cells of leuconostocs. According to Garvie (1960), however, the cells of leuconostoc may be elongated.

All the isolates examined produced gas from glucose, grew at 15°C and 37°C and formed dextran from sucrose. No isolate grew at 45°C and produced ammonia from arginine. With the exception of isolate type D which produced slight acidity in litmus milk no isolate produced any change in this medium. No isolate produced diacetyl.

All isolates fermented xylose, maltose, sucrose, cellobiose and hydrolysed aesculin. Isolates types B and C, in addition, fermented salicin and amygdalin. Lactose was fermented by only isolate type C.

No isolate fermented arabinose, melibiose and trehalose. Isolate type A was the only organism which showed heat resistance at 55°C and failed to ferment salicin and amygdalin.

Pediococcus cerevisiae (Tables 7 and 8).

All the cocci isolated for identification had the morphology and physiological reactions typical of *Pediococcus cerevisiae*. The cells were round and grouped in tetrads and pairs. All cultures were Gram-positive and showed a positive catalase reaction. No isolate produced gas from glucose.

TABLE 7

Characteristics of Pediococcus cerevisiae *isolated from sample A.*

Type of isolate	D	E	F	G	H	I
No. of isolates in total number examined ...	1	1	1	5	2	1
Gas from glucose ...	—	—	—	—	—	—
Catalase reaction ...	+	+	+	+	+	+
Growth at 37°C ...	+	+	+	+	+	+
Growth at 45°C ...	+	+	+	—	—	—
Growth in NaCl 10%	—	—	—	—	—	—
Growth at pH 4.4 ...	+	+	+	+	+	+
Growth at pH 8.6 ...	+	+	+	+	—	—
Growth on Rogosa acetate medium (pH 5.4)	+	+	+	+	+	+
Litmus milk	—	—	—	—	—	—
NH_3 from arginine ...	+	+	+	+	+	+
Acetyl methyl carbinol from glucose ...	+	+	+	+	+	+
Acid from:						
arabinose	—	—	—	—	—	—
maltose	+	+	+	+	+	+
sucrose	—	+	—	—	—	+
raffinose	—	—	—	—	—	+
dextrin	—	—	—	—	—	—
sorbitol	—	—	+	—	—	—

Isolates G, H and I from sample A failed to grow at 45°C. Two of these isolates (types H and I) also did not grow at pH 8.6. Isolates from samples B, C and D were more nearly like *Pediococcus cerevisiae* in their growth characteristics. They all grew at 37°C and 45°C and only one (type M) failed to grow at pH 8.6 (Table 8).

All isolates failed to grow in 10% NaCl and to ferment arabinose and dextrin. No change was produced in litmus milk by any isolate.

Maltose was fermented by all isolates. In

addition sucrose was fermented by isolates types E, I, K, L and M, raffinose by isolates types I and K and sorbitol by isolates types F and N.

TABLE 8

Characteristics of Pedicoccus cerevisiae *isolated from samples B, C & D.*

Type of isolate	J	K	L	M	N
No. of isolates in total number examined ...	5	1	1	1	1
Gas from glucose ...	—	—	—	—	—
Catalase reaction	+	+	+	+	+
Growth at 37°C	+	+	+	+	+
Growth at 45°C	+	+	+	+	+
Growth in NaCl 10% ...	—	—	—	—	—
Growth at pH 4.4 ...	+	+	+	+	+
Growth at pH 8.6 ...	+	+	+	—	+
Growth on Rogosa acetate medium (pH 5.4) ...	+	+	+	+	+
Litmus milk	—	—	—	—	—
NH_3 from arginine ...	+	+	+	+	+
AMC from glucose ...	+	+	+	+	+
Acid from:					
arabinose	—	—	—	—	—
maltose	+	+	+	+	+
sucrose	—	+	+	+	—
raffinose	—	+	—	—	—
dextrin	—	—	—	—	—
sorbitol	—	—	—	—	+

TABLE 9

Characteristics of Lactobacillus fermenti *(isolates types O, P, Q) and* L. plantarum *(isolate type R) isolated from samples B, C & D.*

Type of isolate	O	P	Q	R
No. of isolates in total number examined ...	6	1	5	1
Gas from glucose ...	+	+	+	—
Catalase reaction	—	—	—	—
Growth at 15°C	—	—	—	+
Growth at 45°C	+	+	—	+
Litmus milk	A	—	—	ACR
NH_3 from arginine ...	+	+	+	+
Growth in 0.4% teepol ...	—	—	—	+
Acid from:				
amygdalin	—	—	—	—
cellobiose	—	—	—	+
lactose	+	—	—	+
maltose	+	+	+	+
melibiose	+	+	+	+
raffinose	+	+	+	+
rhamnose	—	—	—	—

ACR = acid, coagulation and reduction.
A = acid only.

Lactobacillus fermenti (Table 9)

With the exception of isolate type R, all the lactobacilli isolated from samples B, C and D produced gas from glucose and were therefore classified under the sub-genus Beta-bacterium Orla-Jensen. All the gas-producing isolates (types O, P and Q) were identified as *Lactobacillus fermenti*. The cells were rods in pairs and short chains, Gram-positive, and catalase negative. They produced ammonia from arginine and failed to grow in 0.4% teepol. Isolates types O and P grew at 45°C but not at 15°C. Isolates of type Q failed to grow at both 15°C and 45°C.

All isolates fermented maltose, melibiose and raffinose, but failed to ferment amygdalin, cellobiose and rhamnose. Lactose was fermented by only isolate type O which was also the only organism producing acid in litmus milk.

The 5 isolates of type Q, which failed to grow at either 15°C or 45°C, were all identified as *L. fermenti* because of their inability to grow at 15°C. Although *L. fermenti* may be considered the one known species of the heterofermentative lactobacilli which is more thermophillic than the others, the really important differentiating characteristic of this species is its inability to grow at 15°C (Rogosa and Sharpe 1959).

Lactobacillus plantarum (Table 9)

Only one isolate (type R) of *L. plantarum* belonging to the sub-genus Streptobacterium Orla-Jensen, was identified among the isolations made from samples B, C and D. This isolate which was catalase negative had Gram-positive rod-like cells in pairs and short chains. It produced no gas from glucose, but grew at 15°C and 45°C and in 0.4% teepol. It produced ammonia from arginine and was very active in fermentation, fermenting all sugars tested except amygdalin and rhamnose.

Discussion

In all the samples of maize dough exa-

mined, the micro-organisms present consisted of a mixed population of lactic acid bacteria and yeasts. The lactic acid bacteria included both homofermentative and heterofermentative species. The homofermentative types consisted predominantly of *Pediococcus cerevisiae* which appeared in all samples analysed. The heterofermentatives varied according to the sample, being *Leuconostoc mesenteroides* in sample A and *Lactobacillus fermenti* in samples B, C and D. Only a single isolate of the homofermentative *L. plantarum* was identified, indicating that this organism was not present in great numbers in the maize mash.

It may, therefore, be said that in a maize dough which is at an advanced stage of fermentation the most important bacteria to be found, apart from the yeasts, are the homofermentative *Pediococcus cerevisiae* and the heterofermentative species which may include *Leuconostoc mesenteroides* and *Lactobacillus fermenti*. These bacteria are possibly those which are able to survive the high acidities produced in a maize dough which has been fermenting for a long time.

The presence of heterofermentative organisms in the maize dough would also account for the rise in volume which normally occurs during natural fermentation of the dough. This represents a leavening action also reported by Mukhergee *et al.* (1965) to take place in the fermentation of the Indian food Idli, a mixture of rice and black gram. The principal organism responsible for souring as well as for gas production in the fermentation of Idli is *Leuconostoc mesenteroides*.

The medium used and the conditions of incubation adopted in making primary isolations of micro-organism from the fermenting dough are important. On nutrient agar incubated aerobically only yeasts were isolated. Lactic acid bacteria appeared only on the YDA and the MRS agars, the latter media being more favourable for the growth of the lactobacilli especially under anaerobic conditions.

MRS agar of pH value 5.4 under anaerobic conditions tended to be more selective for lactobacilli. Under these conditions the yeasts and lactic acid bacteria other than lactobacilli appeared to be suppressed. The pediococci are, however, able to grow at pH 5.4. and in situations where they may predominate, as in sample A, may appear in large numbers on MRS agar of pH 5.4.

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