Preserving African Food Microorganism for Green Growth

Optimisation of technological properties for selected cultures including establishment of pilot trials (M8 and O3.2)

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TECHNOLOGICAL PROPERTIES OF LACTIC ACID BACTERIA ISOLATES

Rate of Acidification by Lactic Acid Bacteria

The rate of acidification of millet dough by different Lactic Acid Bacteria isolated from fermenting millet dough which was monitored by changes in pH and titratable acidity are shown in Figures 1 and 2. During the first 4 h *L. brevis* exhibited the highest rate of acidification based reduction in pH value, in the next 4 hours *W.confusa* and final 4 hours *L. lactis ssp lactis.* With regards to increases in titratable acidity L. fermentum exhibited the highest rate of acidification in the four segments.

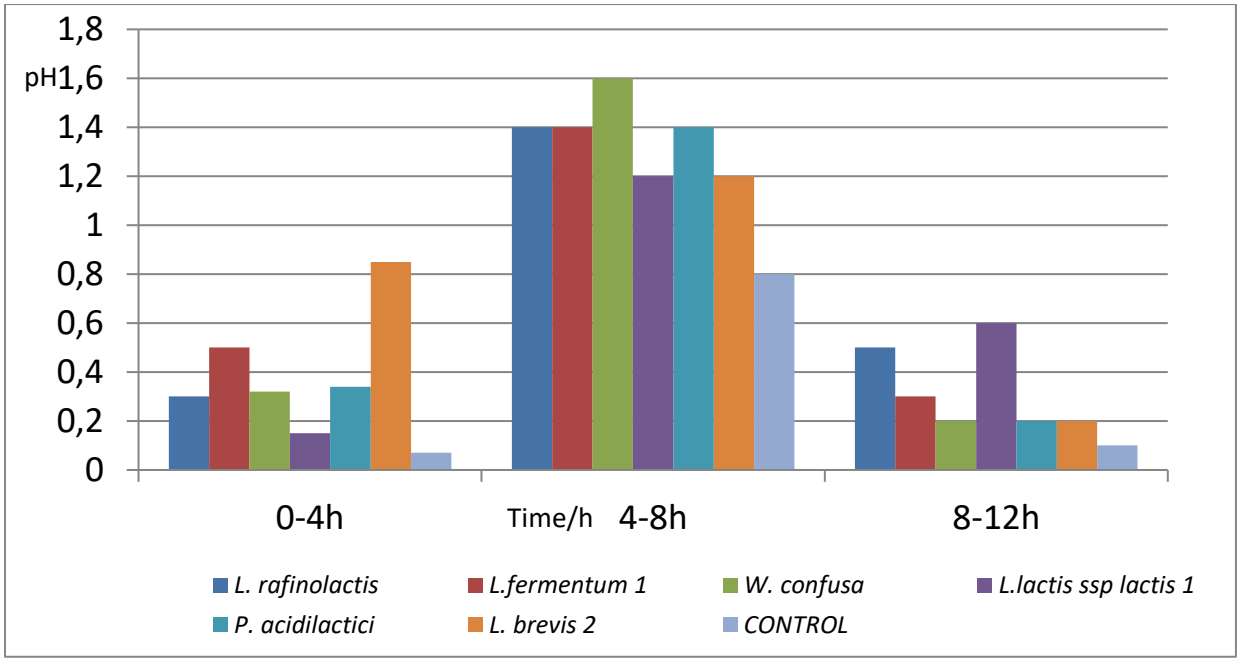

Fig. 1. Changes in pH during dough fermentation by Lactic Acid Bacteria isolates

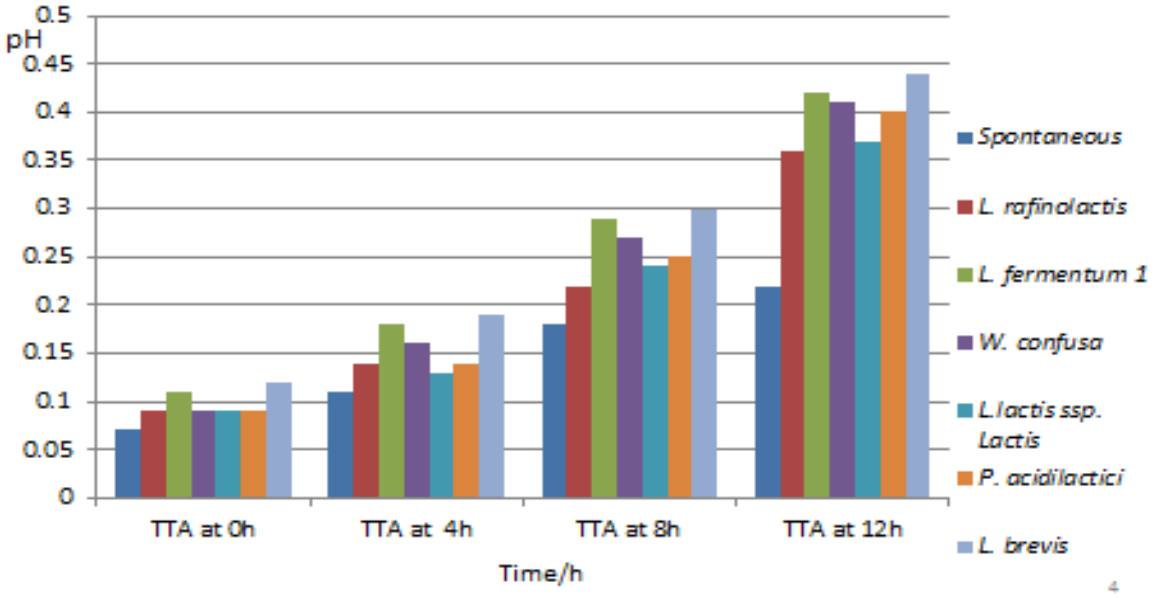

Fig. 2. Changes in titratable acidity during fermentation of millet dough by different lactic acid bacteria

Secretion of amylase by lactic acid bacteria isolates

Lactic acid bacteria isolates were screened for their ability to secrete amylase by growing them on a modified Nutrient agar containing 2 % starch and the result. The organisms tested were 30 isolates of *L. fermentum*, 15 isolates of *L. brevis*. 18 isolates of *W. confusa* and 12 isolates of *P. acidilactici*. Out of these isolates 13.33 % each of *L. fermentum*, and *L .brevis, 16.67*% of *W. confusa* and 8.33% of *P. acidilactici* produced clear zones ranging from 1mm to 2 mm, with 11.11% *W. confusa* producing clearing zones from 3mm to 4mm indicating amylase secretion (Table 1).

Protease secretion

The LAB isolates were streaked on Plate Count Agar (Oxiod CM325; Oxoid Ltd., Basingstoke, Hampshire, UK) supplemented with 0.5 % casein and incubated at 30^{o}C for 3 days. The plates were then flooded with 1M HCl. Protease positive was indicated by a clear zone around the

colonies as described by Almeida *et al*., (2007). Only 3.33% *L. fermentum* and 5.56% *W. confusa* secreted protease with clearing zones of 1-2 mm (Table 1).

Production of exopolysaccharride

Screening of isolates for EPSs production was carried out according to Guiraud (1998). Lactic acid bacteria isolates were streaked onto LTV agar and incubated at 30ºC for 48 h. The colonies were tested for slime formation using the inoculated loop method (Knoshaug *et al*., 2000). Isolates were considered positive for slime production if the length of slime was above 1.5 mm. Positive isolates were confirmed using MRS- Sucrose Broth without glucose and peptone as described by Pidoux *et al*., (1990). The positive isolates were noted according to the intensity of the opaque link. 46.67% of *L. fermentum*, 20% of L. brevis, 38.89% of *W. confusa* and 66.67% *P. acidilactici* produced a slime between 1 mm and 2 mm. 40% *L. fermentum* 60% of *L. brevis*, 61.11% and 25% of *W.confusa* produced a slime of 3-4mm whiles of 13.33% of *L. fermentum*, 20% L. brevis and 8.33% produced a slime above 5 mm (Table 1).

Table 1 Amylase Secretion, exopolysaccharide (EPS) production and protease secretion by Lactic Acid Bacteria Isolates

ISOLATE	TEST	ND	+	++	+++
		% of Isolate			
L. fermentum (n=30)	Amylase secretion	86.67	13.33	0.00	0.00
	EPS production	0.00	46.67	40.00	13.33
	Protease secretion	96.67	3.33	0.00	0.00
L. brevis 2(n=15)	Amylase secretion	86.67	13.33	0.00	0.00
	EPS production	0.00	20.00	60.00	20.00
	Protease secretion	100.00	0.00	0.00	0.00
W. confusa (n=18)	Amylase secretion	72.22	16.67	11.11	0.00
	EPS production	0.00	38.89	61.11	0.00
	Protease secretion	94.44	5.56	0.00	0.00
P. acidilactici (n=12)	Amylase secretion	75.00	8.33	16.67	0.00
	EPS production	0.00	66.67	25.00	8.33
	Protease secretion	100.00	0.00	0.00	0.00

ND: no clearing zone; +: 1-2mm clearing zone, ++: 3-4mm clearing zone, +++:5mm clearing zone.
For exopolysaccharaide production, ND: no slime; 1-2 mm length of slime, ++: 3-4 mm length of slime, +++:5mm length of slime.

Antimicrobial Interaction between Lactic Acid Bacteria isolates

There was no microbial interaction between the lactic acid bacteria isolates as shown in table 2.

Table 2 Antimicrobial Interaction between Lactic Acid Bacteria isolates

ISOLATES (LAB)	INDICATOR STRAINS (LAB)					
	L. rafinolactis	*L. fermentum*	*W. confusa*	*L. lactis*	*P. acidilactici*	*L. brevis*
L. rafinolactis	-	-	-	-	-	-
L. fermentum	-	-	-	-	-	-
W. confusa	-	-	-	-	-	-
L. lactis ssp lactis 1	-	-	-	-	-	-
P. acidilactici	-	-	-	-	-	-
L. brevis 2	-	-	-	-	-	-

-: no inhibition zone

Antimicrobial Interaction between Lactic Acid Bacteria and Yeasts Isolates

There was neither a microbial interaction between the lactic acid bacteria isolates and *Saccharromyces cerevisiae* nor *C. krusei* (Table 3. There was however a weak interaction between *L. fermentum, W.confusa* and *L. brevis* against *C. albicans* and *C. membranifascians* as shown.

Table 3 Antimicrobial Interaction between Lactic Acid Bacteria and Yeasts Isolates

Lactic Acid Bacteria	*Saccharomyces cerevisiae*	*Candida krusei*	*Candida albicans*	*Candida membranifascians*
L. rafinolactis	-	-	-	-
L. fermentum	-	-	+	+
W. confuse	-	-	++	+
L. lactis ssp lactis 1	-	-	+	-
P. acidilactici	-	-	-	+
L. brevis 2	-	-	+	+

-: no inhibition zone, +: 1-2mm inhibition zone, ++: 3-4mm inhibition zone.

Antimicrobial Activity of Lactic Acid Bacteria against Some Common Enteric Pathogens

Table 4 shows the Antimicrobial activity of lactic acid bacteria against pathogen indicator- strains. All the isolates exhibited antimicrobial activity against all the pathogens tested (*Salmonella typhimurium, E. coli, Vibrio cholerae* and *Staphylococcus aureus*), except for *P. acidilactici* against *E. coli. L. fermentum* exhibited the strongest inhibition against *Staphylococcus aureus and Vibrio cholerae* with inhibition zones exceeding 5 mm while *Salmonella typhimuruim* and *E. coli* showed inhibition zones of less than 3 mm as shown in the table. This was followed by *L. brevis* which exhibited a strong inhibition zone of 3-4 mm against all the tested strains. *W. confusa* also exhibited a strong inhibition zone of 3-4 mm against *Salmonella typhimurium, E. coli* and *Staphylococcus aureus* but 1-2 mm inhibition zone against *Vibrio cholera.*

Table 4. Antimicrobial activity of lactic acid bacteria against pathogen indicator- strains

LAB ISOLATES	PATHOGENS			
	Staphylococcus Aureus	*E- coli*	*Salmonella Typhi*	*Vibrio cholera*
L. rafinolactis	++	+	+	++
L. fermentum	+++	++	++	+++
W. confuse	++	+	++	+
L. lactis ssp lactis 1	++	+	+	+
P. acidilactici	+	-	+	++
L. brevis 2	++	++	++	++

-: no inhibition zone, +: 1-2mm inhibition zone, ++: 3-4mm inhibition zone, +++:5mm inhibition zone

PILOT TRIAL OF THE STARTER CULTURE AT THE SME, SELASSIE FOODS

Washing of millet grains

Weighing of washed millet

Weighing the spices

The spices

Mixing the starter into a volume of water

Mixing the starter culture with a portion of the miiled millet

The starter culture was used to ferment the millet for the production of Hausa koko by the SME, Selassie Foods, because that is a product the company produces for both the local and diaspora market. No SME currently produces Fura so the work with the SMEs have been limites to Hausa koko which many SMEs produce and has similar unit operations to Fura. The trial was successful as it reduced the fermentation period of 3 days to 1 day for the SME. The SME has since resorted to using the starter culture through backslopping as we explained to him.