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Influence of Nutrients Utilization and Cultivation Conditions on the Production of Lactic Acid by Homolactic Fermenters

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Abstract: Homofermentative lactic acid bacteria isolated from retted cassava ware screened for the production of lactic acid, pH survival and influence of nutrients utilization and cultivation conditions on the production of lactic acid by fermentation. All the *Lactobacillus* species isolated produced little quantity of lactic acid when grown at 30°C in normal De Man Rogosa Sharpe (MRS) broth. However, a temperature of 40°C at initial pH of 5.5 in constituted MRS medium with 6% (w/v) carbon concentration of D-glucose and 4% (w/v) nitrogen concentration of yeast extract fermented for 48 h supported lactic acid production optimally with *Lactobacillus acidophilus* producing 18.4±0.01 g L^{-1} of lactic acid. *Lactobacillus casei* had the highest percentage cell destruction (53.93%) in phosphate buffered saline pH 3.0 while *L. acidophilus* had the least (18.87%). Lactic acid produced by all the *Lactobacillus* species inhibited at least two or more food spoilage and/or pathogenic microorganisms and can be used in the food industry for decontamination of meat and poultry carcasses.

Key words: Lactic acid, cultivation conditions, nutrients utilization, homolactic-fermentation

INTRODUCTION

Organic acid production by microorganisms is important in food, pharmaceutical and cosmetic industry. Among the organic acids, lactic acid is a widely used chemical that has found application in many industries and various commercial purposes (Narayanan *et al*., 2004) being most commonly used as an acidulant and preservative of food stuffs and a starting material for biodegradable polymers (Senthuran *et al*., 1999; Kibeom, 2005). Lactic acid fermented foods have proven their wholesome value for thousands of years and widely accepted and appreciated by the consumers. The Lactic acid bacteria starter culture for lactic products; thus fulfill an irreplaceable role in ensuring the structure, taste, conservation and healthfulness of these products (Ziadi *et al*., 2005; Do-Won *et al*., 2006; Serna and Rodriguez, 2006). The antimicrobial effects of lactic acid have been extensively reviewed (Smulders *et al*., 1980; Ogunbanwo, 2005). The effect is due to the undissociated form of the acids which can penetrate the membrane and liberate hydrogen ions in the neutral cytoplasm, thus leading to inhibition of vital cell function (Axelesson and Sei, 1990; Piard and Desmazeaud, 1991; Sanni *et al*., 1995).

Lactic acid can be manufactured by chemical synthesis or carbohydrate fermentation. The carbohydrate fermentation is the cheapest means of production and it's preferable because of the ability of homolactic microorganisms to rapidly produce lactic acid in a pure form as a syrup liquid (Mossel, 1989; Ahmad and Marth, 1989; Prescott *et al*., 2008). Lactic acid production capacity seems to be carbon and nitrogen source regulated; the choice of an organism primarily depends on the carbohydrate to be fermented. The Lactic Acid Bacteria (LAB), which produce lactic acid, are classified according to their ability to ferment glucose or other sugars solely to lactic acid or to additional products i.e., as homofermentative or heterofermentative (Pelczer *et al*., 1993). Homofermentative Lactic acid bacteria produce pure or almost pure (90%) lactic acid. However, up till now lactic acid of biological origin has been produced in batch processes with low productivity characterized by severe product inhibition of cell growth leading to low productivity (Senthuran *et al*., 1999). Since lactic acid behaves as a growth-linked metabolite, this study tried to improve the lactic acid yield further by favouring higher cell mass formation via manipulation of the carbon and/or nitrogen level and cultural conditions of the fermentation medium.

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MATERIALS AND METHODS

Study location: This study was carried out in the Department of Botany and Microbiology, University of Ibadan, Nigeria between August 2005 and March 2007 as part of present contribution to knowledge in the area of Lactic acid bacteria and its application.

Isolation of microorganisms: Lactic acid bacteria were obtained from a carbohydrate based fermentation product-retted cassava (fufu). The samples were collected in sterile McCartney bottles. Serial dilutions of 10 g of the samples were made in 90 mL of sterile diluents containing 0.1% peptone water and homogenized for 30 sec. From appropriate 10 fold dilutions, isolation of LAB was carried out on Mann Rogosa Sharpe (MRS) agar and incubated anaerobically at 30°C for 48 h. Repeated streaking purified the cultures. Strains were characterized using the API 50CH strips and API 50 CHL medium (API systems, Biomerieux Sa.France) and other complementary test when necessary. The food spoilage and pathogenic bacteria used as indicator organisms were obtained from the culture collection of Medical Microbiology and Parasitology Department of the University College Hospital, Ibadan, Nigeria.

Screening for acid producing LAB: Mann Rogosa Sharpe (MRS) agar containing 0.05% (w/v) bromocresol purple indicator was used as medium for screening acid producing organisms. The test isolates were inoculated onto the agar and incubated at 30°C for 3-4 days. Colonies which developed yellow colouration indicated acid production.

Production of lactic acid by test isolates: The test organisms were grown on MRS broth for 72 h at 30°C. Cultures were centrifuged for 4 min at 18,000 x g. To the supernatant 5 mg mL^{-1} of catalase and proteinase K were added to eliminate the activity of hydrogen peroxide and bacteriocin, respectively. The quantity of lactic acid was estimated at 12 h interval except otherwise stated.

Antimicrobial activity of lactic acid: A well diffusion assay procedure was used (Shillinger and Lucke, 1989). Pre-poured indicator agar plates of 4 mm depth (1.5% agar) was overlaid with a 10 mL soft agar (0.7%) lawn to generate a potential mat of the indicator bacteria. The indicator lawn was prepared by adding 0.1 mL indicator organism (3.2×10^7 cfu) to 10 mL soft agar.

Wells of 6 mm diameter were cut into these agar plates by using a sterile cork borer and 100 µL of lactic acid fluid produced by the test isolates was placed into each well. The plates were incubated aerobically at 37°C for 24 h and then examined for zones of inhibition, which was scored positive if the clear zone was 0.5 mm or larger.

Estimation of lactic acid: To 25 mL of cell free supernatant broth cultures of the test organisms 3 drops of phenolphthalein were added as indicator and the quantity of lactic acid was determined by titration with 0.1 M NaOH until a pink color appeared. Each milliliter of 0.1 M NaOH is equivalent to 90.08 mg of lactic acid (AOAC, 1990).

Influence of growth conditions on the production of lactic acid

Temperature: The effect of incubation temperature on production of lactic acid was carried out. MRS broth was inoculated with 0.1 mL of an over-night 3.2×10^7 cfu culture of the test organisms, incubated at 4, 15, 30, 40 and 50°C for 48 h and lactic acid was estimated.

pH: To determine the effect of initial pH on the production of lactic acid, 100 mL of MRS broth was adjusted to initial pH values of 3.5, 5.5, 7.5 and 9.5 using 0.1 M HCl and 0.5 M sodium hydroxide. Each medium was inoculated with 0.1 mL of an overnight 3.2×10^7 cfu culture of the test organisms and incubated at 40°C for 48 h and lactic acid was estimated.

Influence of carbon source on the production of lactic acid: To test the influence of the type of carbon source, the test organisms were grown in modified MRS broth with different concentrations of carbon. The basal medium contained 0.2 g $MgSO_4.7H_2O$, 0.05 g $MnSO_4.4H_2O$, 5 g sodium acetate, 1.5 g KH_2PO_4, 1.5 g K_2HPO_4, 10 g peptone, 5 g yeast extract, 1 mL Tween-80 per litre of distilled water and various amounts of carbon such as D-glucose, lactose, starch and mannitol at 2 to 10% carbon concentration (w/v) were added. They were inoculated with 0.1 mL of an overnight 3.2×10^7 cfu culture of the test organisms and incubated for 48 h at 40°C with initial pH of 5.5 and lactic acid was estimated.

Influence of nitrogen source on the production of lactic acid: To determine the influence of different nitrogen sources, the test organisms were grown in basal medium containing 0.2 g $MgSO_4.7H_2O$, 0.05 g $MnSO_4.4H_2O$, 5 g sodium acetate, 1.5 g KH_2PO_4, 1.5 g K_2HPO_4 6% w/v glucose and 1.0 mL vitamin solution containing (per 100 mL 20% ethanol) 0.2 g vitamin B_6, 0.1 g niacin, 0.1 g calcium pantothenate, 0.1 g riboflavin and 0.1 g folic acid per litre of distilled water and various amounts of nitrogen sources such as yeast extract, casein, urea and $(NH_4)_2SO_4$ at 1 to 16% w/v nitrogen concentration were

added. They were inoculated with 0.1 mL of an overnight 3.2×10^7 cfu culture of the test organisms and incubated for 48 h at 40°C with initial pH of 5.5 and lactic acid was estimated.

Survival of LAB at low pH: The method of Conway *et al.* (1987) was employed. The cultures were grown in MRS broth (Oxoid) at 30°C overnight, then subcultured into 10 mL of fresh MRS broth and incubated fro another 24 h. Thereafter, the cultures were centrifuged at 2000 x g for 10 min at 4°C and the pellets washed twice in sterile phosphate buffered saline and re-suspended in 10 mL of PBS prepared by dissolving NaCl (9 g L^{-1}), $NaHPO_4$ (9 g L^{-1}) and KH_2PO_4 (1.5 g L^{-1}). Each suspension was then inoculated (1%) into PBS tubes with pH values 1, 2 and 3 and incubated at 30°C. Viable cells were enumerated at 0, 1 and 4 h on MRS agar plates, incubated anaerobically (Gas-Pak system BBL), at 30°C for 48 h.

Statistical analysis: Least Squared Means (LSM) of microbial populations and lactic acid were calculated from six experimental replications for each experiment. Statistical significance according to Duncans multiple range test was defined as $p \leq 0.05$, unless otherwise stated.

RESULTS

The homolactic *Lactobacillus* species isolated from retted cassava (fufu) were screened qualitatively and quantitatively for lactic acid production. All the *Lactobacillus* species namely *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus delbruekii* and *Lactobacillus casei* gave yellow colouration on MRS agar plate containing bromocresol purple as an indicator for acid production.

Lactobacillus acidophilus produced the highest quantity (5.2±0.01 g L^{-1}) of lactic acid at 48 h of fermentation while *L. casei* produced the lowest (2.4±0.03 g L^{-1}) at the same period of time. Although lactic acid production increased with time, the production peak for all the tested *Lactobacillus* species was reached at 48th h of growth, followed by gradual decline (Table 1).

The lactic acid produced by all the tested *Lactobacillus* species was able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. The growth of *Listeria monocytogenes* was also suppressed by lactic acid produced by all the isolates with the exception of that produced by *L. plantarum* and *L. casei* while *Bacillus cereus* was inhibited by lactic acid produced by all the *Lactobacillus* species, with the exception of that from *L. casei* (Table 2).

The influence of incubation temperature on the production of lactic acid revealed that the highest quantity of lactic acid was produced at 40°C by all the organisms with *L. acidophilus* recording the highest yield of 7.5±0.03 g L^{-1} while *L. casei* produced the lowest (1.1±0.01 g L^{-1}) at 50°C (Table 3).

The optimum initial pH for lactic acid production as shown in Table 4 was at pH 5.5. *L. acidophilus* produced the highest quantity (7.5±0.03 g L^{-1}) while *L. casei* produced the lowest (3.0±0.02 g L^{-1}).

The influence of different carbon sources on lactic acid production showed that all the organisms produced the highest quantity of lactic acid when D-glucose was used as the carbon source. As the quantity of D-glucose was increased from 2 to 6% concentration, the quantity of lactic acid produced also increased with the peak of production at 6% glucose concentration, after which there was gradual decrease as the concentration of D-glucose was increased to 10% carbon concentration. However, as

Table 1: Quantity of lactic acid (g L^{-1}) produced in MRS broth by test isolates of *Lactobacillus* species

	Time (h)					
Test isolates	12	24	36	48	60	72
Lactobacillus acidophilus	0.80±0.03^{a}	1.20±0.01^{b}	2.8±0.04^{b}	5.2±0.01^{c}	5.1±0.02^{c}	4.9±0.02^{c}
Lactobacillus plantarum	0.26±0.05^{ab}	0.58±0.01^{bb}	1.0±0.02^{bb}	2.6±0.03^{cb}	2.6±0.01^{cb}	2.3±0.04^{cb}
Lactobacillus delbrueckii	0.50±0.01^{ac}	0.90±0.03^{ac}	1.6±0.01^{bc}	4.0±0.01^{cc}	3.7±0.02^{cc}	3.5±0.01^{cc}
Lactobacillus casei	0.28±0.02^{ab}	0.65±0.01^{bb}	1.2±0.01^{bb}	2.4±0.05^{cb}	2.2±0.01^{cb}	2.1±0.03^{cb}

Values are mean (n = 6) ±SD. Means values in the same column followed by the same letter(s) are not significantly different according to Duncans multiple range test ($p<0.05$)

Table 2: Inhibitory activity of lactic acid produced by homolactic *Lactobacillus* species against spoilage/pathogenic indicator organisms

	Producer strains (mm)			
Indicator organisms	*L. acidophilus*	*L. plantarum*	*L. delbrueckii*	*L. casei*
Staphylococcus aureus	+(20)	+(10)	+(13)	+(8)
Leuconostoc mesenteroides	+(15)	+(7)	+(9)	-
Escherichia coli	+(8)	+(10)	+(8)	+(5)
Listeria monocytogenes	+(12)	-	+(10)	-
Bacillus cereus	+(10)	+(8)	+(10)	-

+: Present, -: Absent

Table 3: Influence of different temperature on the production of lactic acid (g L^{-1}) in MRS broth

	Temperature (°C)				
Test isolates	4	15	30	40	50
Lactobacillus acidophilus	0.64±0.01^{a}	2.0±0.03^{b}	5.2±0.01^{c}	7.5±0.03^{c}	3.2±0.01^{b}
Lactobacillus plantarum	0.31±0.01^{ab}	1.3±0.02^{bb}	2.6±0.03^{cb}	3.9±0.01^{cb}	1.3±0.02^{bb}
Lactobacillus delbrueckii	0.47±0.02^{ab}	1.7±0.01^{bc}	4.0±0.01^{cc}	6.3±0.02^{cc}	2.1±0.03^{bc}
Lactobacillus casei	0.28±0.01^{ab}	1.1±0.03^{bd}	2.4±0.05^{ce}	3.0±0.02^{ce}	1.1±0.01^{bd}

Values are mean (n = 6) ±SD. Means values in the same column followed by the same letter(s) are not significantly different according to Duncans multiple range test ($p<0.05$)

Table 4: Influence of different pH on the production of lactic acid (g L^{-1}) in MRS broth

	pH			
Test isolates	3.5	5.5	7.5	9.5
Lactobacillus acidophilus	3.4±0.02^{a}	7.5±0.03^{b}	1.8±0.02^{a}	0.6±0.03^{c}
Lactobacillus plantarum	1.2±0.01^{ab}	3.9±0.01^{bb}	0.9±0.02^{ab}	0.03±0.01^{cb}
Lactobacillus delbrueckii	2.6±0.01^{ac}	6.3±0.02^{bc}	1.3±0.03^{ac}	0.04±0.01^{cc}
Lactobacillus casei	1.3±0.02^{ad}	3.0±0.02^{bd}	0.7±0.01^{ad}	0.02±0.02^{cd}

Values are mean (n = 6) ±SD. Means values in the same column followed by the same letter(s) are not significantly different according to Duncans multiple range test ($p<0.05$)

Table 5: Influence of carbon sources on lactic acid (g L^{-1}) production by *L. acidophilus* and *L. delbrueckii*

		Carbon concentration (% w/v)				
Test isolates	Carbon sources	2	4	6	8	10
L. acidophilus	D-Glucose	7.50±0.01^{a}	9.00±0.02^{a}	11.50±0.01^{b}	10.20±0.02^{b}	9.80±0.01^{b}
	Lactose	3.00±0.02^{ab}	4.80±0.02^{ab}	7.30±0.01^{bb}	6.50±0.01^{bb}	6.00±0.01^{bb}
	Starch	1.40±0.01^{ac}	1.60±0.01^{ac}	2.00±0.01^{bc}	2.00±0.01^{bc}	1.80±0.02^{bc}
	Inositol	0.06±0.04^{ad}	0.09±0.01^{ad}	0.09±0.03^{bd}	0.08±0.02^{bd}	0.06±0.01^{bd}
L. delbrueckii	D-Glucose	6.30±0.01^{a}	7.80±0.01^{a}	9.60±0.02^{a}	8.80±0.01^{a}	8.50±0.01^{aa}
	Lactose	2.20±0.01^{ab}	4.10±0.02^{ab}	6.80±0.01^{ab}	6.00±0.01^{ab}	5.80±0.02^{ab}
	Starch	1.00±0.03^{ac}	1.30±0.01^{ac}	1.80±0.01^{ac}	1.70±0.02^{ac}	1.60±0.01^{ac}
	Inositol	0.03±0.02^{ad}	0.09±0.04^{ad}	0.08±0.03^{ad}	0.08±0.02^{ad}	0.06±0.02^{ad}

Values are mean (n = 6) ±SD. Means values in the same column followed by the same letter are not significantly different according to Duncans multiple range test ($p<0.05$)

Table 6: Influence of nitrogen sources on lactic acid (g L^{-1}) production by *L. acidophilus* and *L. delbrueckii*

		Nitrogen concentration (% w/v)				
Test isolates	Nitrogen sources	1	2	4	8	16
L. acidophilus	$(NH_4)_2SO_4$	0.34±0.02^{a}	1.20±0.01^{b}	2.50±0.02^{b}	1.40±0.01^{b}	1.20±0.03^{b}
	Yeast extract	13.20±0.01^{ab}	15.00±0.0^{lbb}	18.40±0.01^{bb}	18.00±0.01^{bb}	16.10±0.02^{bb}
	Urea	0.11±0.01^{ac}	0.11±0.03^{bc}	0.09±0.01^{bc}	0.04±0.01^{bd}	0.04±0.03^{bd}
	Casein	7.50±0.02^{ae}	8.30±0.01^{be}	10.00±0.01^{be}	10.00±0.03^{be}	9.80±0.02^{be}
	$(NH_4)_2SO_4$	0.22±0.01^{a}	0.80±0.02^{b}	1.80±0.02^{b}	1.00±0.03^{b}	0.70±0.01^{b}
L. delbrueckii	Yeast extract	10.00±0.02^{ab}	12.20±0.02^{bb}	14.60±0.01^{bc}	13.80±0.01^{bb}	12.00±0.01^{bb}
	Urea	0.08±0.01^{ac}	0.07±0.03^{ac}	0.04±0.01^{cc}	0.02±0.01^{bc}	0.01±0.02^{bc}
	Casein	4.10±0.01^{ad}	6.00±0.01^{bc}	8.20±0.01^{bc}	8.00±0.03^{bc}	7.60±0.02^{bc}

Values are mean (n = 6) ±SD. Means values in the same column followed by the same letter(s) are not significantly different according to Duncans multiple range test ($p<0.05$)

the complexity of the carbon source increased, the quantity of lactic acid produced by the test organisms decreased (Table 5). *L. acidophilus* produced 11.5±0.01 g L^{-1} of lactic acid as its highest quantity at 6% D-glucose concentration while the lowest (0.06±0.01 g L^{-1}) was produced by the same organism at 10% inositol concentration.

Of all the nitrogen sources used in this study, the medium containing yeast extract yielded the highest quantity of lactic acid followed by the medium that contained casein while low yield of lactic acid was observed in the media containing urea and ammonium sulphate. *L. acidophilus* produced the highest quantity of lactic acid (18.4±0.01 g L^{-1}) in medium supplemented with 4% (w/v) yeast extract as nitrogen source. However, when urea was used as nitrogen source, 0.09±0.01 g L^{-1} of lactic acid was produced by *L. acidophilus* at 4% (w/v) nitrogen concentration (Table 6).

Acid tolerance test results showed that none of the *Lactobacillus* species survived in phosphate buffered saline pH 1.0 and 2.0. However, in phosphate buffered saline pH 3.0 the cells viable count decreases as the

Table 7: Survival of *Lactobacillus* species in buffered saline (pH 3.0) at 30°C

LAB isolates	Viable counts (log_{10} cfu mL^{-1}) (h)			Percentage cell destruction (4 h)
	0	1	4	
Lactobacillus acidophilus	7.10±0.02^{a}	6.86±0.01^{a}	5.76±0.04^{a}	18.87
Lactobacillus plantarum	7.15±0.01^{ab}	6.94±0.02^{ab}	5.68±0.01^{ab}	20.56
Lactobacillus delbrueckii	7.12±0.01^{ac}	6.67±0.03^{ac}	3.28±0.02^{ac}	53.93
Lactobacillus casei	7.20±0.03^{ad}	6.63±0.01^{ad}	3.66±0.02^{ae}	49.17

Values are mean (n = 6) ±SD. Means values in the same column followed by the same letter(s) are not significantly different according to Duncans multiple range test ($p<0.05$)

incubation period's increases with *L. acidophilus* having viable count of 6.86±0.01 log_{10} cfu mL^{-1} after 1 h of incubation in phosphate buffered saline pH 3.0 which decreased to 5.76±0.04 log_{10} cfu mL^{-1} after 4 h of incubation. The percentage cell destruction varied from 18.87 to 53.93 among the tested *Lactobacillus* species as shown in Table 7. *L. casei* had the highest percentage cell destruction in phosphate buffered saline pH 3.0 while *L. acidophilus* had the least.

DISCUSSION

Homofermentative *Lactobacillus* species were isolated from retted cassava and identified as *L. acidophilus, L. plantarum, L. delbrueckii* and *L. casei*. According to Tannock (1986), members of the lactic acid bacteria can be detected in a variety of habitats including fermented foods.

All the *Lactobacillus* species yielded yellow colonies on MRS agar containing bromocresol purple as an indicator of acid production. The ability to convert carbohydrate to lactic acid, acetic acid, alcohol and carbon dioxide in food components has made *Lactobacillus* species so important to mankind in the preservation of edible and nutritious food (Prescott *et al*., 2008).

The four species of *Lactobacillus* we studied produced little quantity of lactic acid when grown at 30°C in normal MRS broth with *L. acidophilus* producing the highest quantity at 48 h of incubation while *L. casei* produced the lowest.

Lactic acid produced by all the tested *Lactobacillus* species have inhibitory activity on two or more spoilage and/or pathogenic microorganisms used as indicator organisms in this study. This shows that lactic acid, especially the naturally produced one, will be of great medical importance in combating pathogens and spoilage organisms in food when the lactic acid producing *Lactobacillus* strain is used as starter culture for food production. In addition to the pH effect, the undissociated form of the organic acid mediates the antimicrobial effect of collapsing the electrochemical proton gradient causing bacteriostatic effect and eventual death of the susceptible bacteria (Eklund, 1989). The effect is more pronounced at pH values below the pk, value of the acid (Axelesson and Sei, 1990; Piard and Desmazeaud, 1991). The main application of lactic acid in the food industry is in the decontamination of meat and poultry carcasses (Triona and Colin, 1999).

Attempt was made in this study to optimize lactic acid production by improving cultivation conditions and nutrient utilization. Temperature of 40°C was found to be optimal for lactic acid production by homolactic fermenters while progressively low quantity of lactic acid was produced at temperatures lower than 40°C. This is in tandem with the findings of Oyewole and Odunfa (1992) that acid production was slow and low in cassava roots fermented at 20°C, while higher temperatures resulted in high rate of acidification. The best initial pH for the optimal production of lactic acid by the test isolates of *Lactobacillus* species is at pH 5.5. This is the optimal pH for the growth of lactic acid bacteria. According to De Man *et al.* (1960), a medium with an initial pH of 5.5-6.0 enhances better LAB cell growth. This could be attributed to the higher lactic acid production observed at this pH.

Effect of different carbon sources at a given substrate concentration on lactic acid production showed that maximum quantity of lactic acid was produced in constituted medium containing 6% (w/v) carbon concentration of D-glucose, while the least value was obtained in constituted medium containing inositol as carbon source. This could be as a result of the ability of Lactic acid bacteria to metabolize different carbon sources differently which is based on the specific activities of the enzymes involved in carbohydrate degradation (Jill and Glatz, 1998). It is remarkable that the highest quantity of lactic acid biosynthesis took place in the medium with 4% (w/v) nitrogen concentration of yeast extract. However, nitrogen sources such as urea and ammonium sulphate are typically slow catabolisable nutrients. Since, proteolytic activity is first required for their consumption, a state of nitrogen limitation is created because of this, resulting in the suppression of possible metabolic regulatory mechanisms such as the repression of catabolic enzymes and amino acid transport (Aharonowitz, 1980).

A slow rate of metabolism would also results in a low specific growth rate (De Vuyst and Vandamme, 1993) which could lead to reduction in lactic acid production.

Lactobacillus acidophilus and *L. plantarum* had high survival rates in phosphate buffered saline pH 3.0 when compared to *L. delbrueckii* and *L. casei*. This could be the reason why high quantity of lactic acid was produced by these organisms, since the cells did not surfer from the severe product inhibition experienced by other organisms.

A sharp increase in the production of lactic acid was observed when the test organisms were cultured in constituted medium supplemented with 6 % (w/v) carbon concentration of D-glucose and 4 % nitrogen concentration of yeast extract compared to the normal MRS broth. This may be due to ability of the medium to support the growth and production of lactic acid by homolactic fermenters at 40°C for 48 h. In the longer term, it will be possible to optimize starter cultures and to use them in industrial processes for a better control, higher yield and consistency of the quality of lactic acid for commercial purposes.

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