Malaysian Journal of Microbiology, Vol 10(1) 2014, pp. 15-23
http://dx.doi.org/10.21161/mjm.54113

Malaysian Journal of Microbiology
Published by Malaysian Society of Microbiology
(In SCOPUS since 2011)

Thermal stability of lactic acid bacteria metabolites and its application in preservation of tomato pastes

Samuel Temitope Ogunbanwo*, Ilesanmi Festus Fadahunsi and Anwuli Jennifer Molokwu

Department of Microbiology, Faculty of Science, P.O. Box 22346, University of Ibadan, Ibadan, Nigeria.
Email: st.ogunbanwo@mail.ui.edu.ng

Received 19 May 2013; Received in revised form 9 August 2013; Accepted 16 August 2013

Aims: Tomato (*Lycopersicum esculentum*) is one of the highly nutritious and perishable food ingredients. This work is intended to apply bio-preservation in extending the shelf life of tomato paste.
Methodology and results: Isolation of lactic acid bacteria (LAB) and spoilage tomato microorganisms was done from healthy and spoilt tomato samples respectively using De Mann Rogosa and Sharpe and nutrient agar and potato dextrose agar respectively. Antimicrobial metabolites of LAB (lactic acid, diacetyl and hydrogen peroxide) were determined and effect of treatment temperatures (60, 80 and 100 °C for 30 min) on its efficacy was also determined. The results obtained from this study revealed that *Lactobacillus brevis* and *L. plantarum* were isolated from healthy tomato samples while *Bacillus megaterium*, *B. laterosorum*, *Pseudomonas syringe*, *Aspergillus fumigates*, *Fusarium solani*, *F. acuminatum* and *F. funjikuroi* were isolated from the spoilt tomato samples. LAB isolated produced antimicrobial compounds with *L. plantarum* I recording the highest lactic acid (2.7 g/L), diacetyl (2.2 g/L) while 2.4 g/L of hydrogen peroxide was observed. Metabolite extract from *L. brevis* I (ML7) exhibited the largest broad spectrum activity with inhibition zones ranging from 10 mm to 18 mm against spoilage microorganisms except *F. funjikuroi*. At 60 °C and 80 °C metabolites produced by LAB species were inhibitory to some of the tomato spoilage microorganisms and at 100 °C no inhibition was detected. The shelf life of tomato paste treated with metabolite extracts from *L. plantarum* I, *L. plantarum* II, *L. brevis* I and *L. brevis* II (LAB3) and kept in refrigerator showed the longest shelf life of 20 days.
Conclusion, significance and impact study: Metabolite extracts from selected LAB were more effective than methyl propylparaben in preserving tomato paste stored at 4 °C and 25 °C against spoilage bacteria and the application of biopreservative should be encouraged in food processing industries.

Keywords: Lactic acid bacteria, tomato spoilage microorganisms, antimicrobial compounds, biopreservative, shelf life.

INTRODUCTION

Microbial spoilage of fruits and vegetable is known as rot, which manifests as loss of texture (Soft rot), changes in colour (black or grey) and often off odor (Trias *et al.*, 2008). Tomatoes which is the third most important vegetable crop on the basis of its market value (Law Ogbomo, 2011), coupled with high nutritive status and high water content which makes it very susceptible to spoilage bacteria and fungi during storage, harvesting and transportation (Spadaro and Gullino, 2004).

Fresh produce like fruits and vegetables, are normal part of the human diet and are consumed in large quantities in most countries. These products are rich in carbohydrates and poor in proteins with pH value from slightly acidic to 7.0 and provide a suitable niche to several bacteria, yeasts and moulds (Wiessinger *et al.*, 2000; Trias *et al.*, 2008). Tomato (*Lycopersicum esculentum*) is one of the highly nutritious food ingredient used in the preparation of food all over the world (Ogunniyi and Oladejo, 2011). It is a perishable fruit and its production in the world in 2008 was about 130 million tons. In Africa, Nigerian ranks highest in tomato production with about 829,000 tons/annum (Grubben and Denton, 2004). It is considered as the most important vegetable after onions and pepper (Fawusi, 1978). Its utilization as an ingredient in vegetable salads, other dishes and its processing into different products like puree, ketchups and juice is well documented. Nutritionally it contains a large amount of water, niacin, calcium and vitamins especially A, C, E which are important in the metabolic activities of man and protects the body against diseases (Taylor, 1987). Lycopene (acarotene) an essential component of tomato contributes in the prevention of cardiovascular disease and cancer of the prostrate (Clinton, 1998; Bernard *et al.*, 1999). The characteristics flavor of tomato is produced by the complex interaction of the volatiles and non-volatile components (Petro-Turza, 1987; Buttery, 1993). Among the common post-harvest fungal pathogens of tomatoes are *Pencillium expansum*, *Bobytis cinerea*, *Monilinia laxa* and *Rhizopus stolinifer* (Ogawa *et al*., 1995; Pla *et al.*, 2005). While that of bacterial origin are *Erwinia carotuvora* and *Xanthoman ascampestrispv* and

***Corresponding author**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Vesicatoria are most common (Pla *et al.*, 2005). In Nigeria, it is estimated that about 40–50 % of tomato are wasted at post-harvest stage every year (Okunoya, 1996; Olayemi *et al.*, 2010).

Antimicrobial agents are compounds used to extend the lag phase or kill microorganisms (Davidson and Harrison, 2002). Only a few naturally occurring antimicrobials, such as nisin, natamycin, lactoferrin and lysozyme have regulatory approval for application in the food industry. In recent times, the understanding of the preservation mechanisms of LAB is being exploited for industrial production of foods (Trias *et al.*, 2008) because of their natural acceptance as GRAS (Generally Recognized as Save) for human consumption and exhibit antimicrobial property (Aguirre and Collins, 1993).

The reported survival of these microorganisms in post-harvest conditions of tomatoes makes them most adequate to be selected as a candidate for the prevention of post-harvest spoilage (Trias *et al.*, 2008). In view of the use of chemical control agents which is of increasing public threat due to their toxicological risk which can cause health problems such as cancer and resistance development by spoilage microorganisms, there is need for an alternative method of preserving tomatoes during the period of abundance in Nigeria.

This work is designed to investigate the effectiveness of lactic acid bacteria metabolites in preserving tomato paste.

MATERIALS AND METHODS

Sample collection

Fresh and spoilt tomato samples were purchased from Shasha and Bodija markets in Ibadan, Nigeria. The samples were collected in separate sterile polythene bags and immediately transported to the laboratory for analysis.

Isolation procedure

The media used for isolation of microorganisms were MRS (De Mann Rogosa and Sharpe), nutrient agar, potato dextrose agar and plate count agar. Three grams of fresh healthy tomato and 3 g of spoilt tomato samples were separately weighed and differently transferred into sterile pestle containing 10 mL of sterile distilled water and grinded with mortal to dislodged the microorganism present in the tomato samples. The mixture obtained was serially diluted and 1 mL of 10^4 dilution was used to inoculate MRS and plate count agar plates. Incubation was carried out anaerobically at 37 °C for 48 h and 1 mL of 10^4 dilution of the mixture obtained from the spoilt tomato sample was used to inoculate nutrient agar, potato dextrose agar and plate count agar plates. Inoculation was carried out at 30 °C for 7 days for the PDA plates while the other plates were incubated at 30 °C for 48 h. The plates were later examined for growth of microorganisms which were repeatedly sub cultures to obtain pure cultures which were differently stored on agar slants in MacCanthney bottles.

Identification of isolates

The pure spoilage fungal isolates were examined Macroscopically and Microscopically and identified with reference to Alexopoulos and Mims (1969) while the characterization of the pure LAB cultures and spoilage bacterial isolates were carried out using API 50CH strips and 50CHL medium (API system, Montalieu, Vericeu, France) and API 20E and API 20NE respectively and identified with reference to Bergey's manual of systems bacteriology (Sneath, 1986).

Determination of proximate composition of healthy and spoilt tomato

The healthy and spoilt tomato samples were subjected to proximate analysis as follows:

Moisture content determination

The moisture content of the fermented samples was determined by weighing into moisture cans then weighed and then placed in an oven at 80 ℃ for 24 h to dry to a constant weight. Then brought out and allowed to cool in desiccators and then reweighed (A.O.A.C., 1995).

$$\text{Thus, moisture content} = \frac{\text{initial weight - final weight}}{\text{Initial weight}} \times 100$$

Ash content determination

Five mL of fermented samples were transferred into a pre-weighed porcelain crucible and weighed. The crucible was then placed into muffle furnace for 6 h at 600 °C to burn off all organic materials. The inorganic material does not volatilize at that temperature and is called ash. The furnace was allowed to cool below 200 °C and maintained at this temperature for 20 min. Then the crucible was placed in a desiccator with stopper top, allowed to cool and then reweighed to measure the ash content (A.O.A.C., 1995).

Dry matter content

Five grams each of the samples was obtained and placed into pre-weighed crucibles and dried in at 100 °C for 12 h. The dried samples were weighed after cooling in a desiccator (A.O.A.C., 1984).

Crude protein determination

The Kjeldahl method which is the standard method for determining protein and other nitrogen containing compounds was used. Two grams of fermented samples were digested with sulphuric acid to decompose it and convert nitrogen to ammonium sulphate. The digestion

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

was speed up by adding Kjeldahl catalyst tablets to increase the boiling point. The solution was cooled and concentrated sodium hydroxide added to make the solution alkaline and distilled into a weak acid (boric acid) containing methyl red indicator until solution turned from red to green. Following distillation, the ammonia was trapped as ammonium borate and quantified by titrating with a strong standard hydrochloric acid (0.01 N) until solution turned from green to wine to measure the nitrogenous content. The amount of crude protein was calculated by multiplying the % nitrogen found by 6.25 (%) (CP = % Nitrogen × 6.25) (A.O.A.C., 1984).

Ether extract determination

Beaker was placed in oven at 80 °C for 10 min and then removed and placed in a desiccator to cool. Then the two grams dried fermented sample was weighed into the fat beaker, a glass thimble full of anhydrous diethyl either was added to the beaker and placed on the butt-type extraction apparatus. Then boiled on high temperature for approximately 4 h by moving heat under it to volatilize the ether, then condensed and allowed to pass through the sample, extracting ether soluble materials. The extract is collected in a beaker, allowed to cool and the porous thimble removed with contents saved for crude fiber determination. Ether was distilled and collected in another container until beaker was almost dry and the remaining ether extract was then dried in oven at 80 °C for 3 min, cooled in the desiccator and weighed to measure the ether extract content (A.O.A.C, 1995).

Determination of lactic acid, diacetyl and hydrogen peroxide production by LAB isolates

This was achieved based on the methods described by Ogunbanwo *et al.* (2008); Bamidele *et al.* (2011). For these measurements the test organisms were grown in MRS broth for 72 h and centrifuged at 3000 *g* for 15 min. Lactic acid was determined by transferring 25 mL of the supernatant fluid of the cell free of the test organisms into conical flasks and 3 drops of phenolphthalein were added as indicator. From a burette, 0.1 M NaOH was slowly added to the samples until a pink colour appeared. Each mL of 0.1 M NaOH is equivalent to 90.08 mg of lactic acid.

For hydrogen peroxide, 20 mL of 0.1 M diluted sulphuric acid was added to 25 mL of the supernatant fluid of the test organisms. Titration was carried out with 0.1 M potassium permanganate. Each mL of 0.1 M potassium permanganate is equivalent to 1.79 mg of hydrogen peroxide solution and decolourization of the sample was regarded to be the end point.

Diacetyl was determined by transferring 25 mL of the supernatant fluid of the test organisms into conical flasks and 7.5 mL of hydroxylamine solution were used for the residual titration. The flasks were titrated with 0.1 M HCl to a greenish-yellow end point using bromophenol blue as indicator. The equivalence factor of HCl to diacetyl is 21.5 mg.

Antagonistic activity of LAB metabolites against spoilage microorganisms

Lactic acid bacteria were grown in MRS broth for 72 h and the broth cultures of the LAB were centrifuged at 10,000 rpm for 30 min and the supernatant containing the metabolites were obtained and 100 µL of the supernatant was transferred into wells (6 mm diameter) bored in Muller Hinton and potato dextrose agar previously seeded with the spoilage bacteria cells and fungi spores obtained from spoilt tomato' after 'fungi spores respectively. The culture plates were incubated at 30 °C for 48 h and 7 days respectively and observed for zones of inhibition.

Effect of heat treatment on antimicrobial activity of LAB metabolites

One hundred milliliters of 72 h old cell free supernatant of the LAB isolates was separately transferred into 250 mL Erlenmeyer flasks and placed inside a water bath set at 60, 80, and 100 °C for 30 min at different occasions and tested against the spoilage microorganisms using the well diffusion method of Schilinger and Lucke (1989) and zones of inhibition were determined.

Shelf life study of tomato paste

Healthy tomatoes were surface sterilized with 3 % solution of sodium thiosulfate and blended to pulp. The tomato paste was boiled for 10 min and dispensed in 20 g amount separately into three pre-sterilized containers. Ten milliliters of LAB metabolites, methyl propylparaben (positive control) were added differently to the tomato paste and stored at 4 °C and 28 °C respectively (Safdar *et al.*, 2010). Microbial load of each treatment was monitored by determining the colony forming unit (CFU/ g).

Statistical analysis

The experimental data were analyzed using Analysis of Variance (ANOVA) to determine significant difference between the means and these were expressed as mean ± standard deviation (SD). The level of significance was set at $p \leq 0.05$. The data were analyzed using t-test and SPSS version 17.0.

RESULTS

L. brevis and *L. plantarum* were isolated from healthy tomato sample with percentage occurrence of 45.4 % and 54.6 respectively while *B. megaterium*, *B. laterosorum*, *Pseudomonas syringe*, *Aspergillus fumigates*, *Fusarium solani*, *F. acuminatum* and *F. funjikuroi* were isolated from the spoilt tomato sample showing percentage of occurrence of 6, 8, 10, 13, 19, 21, and 23 respectively (Table 1).

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 1: Percentage occurrence of microorganisms isolated from healthy and spoilt tomato samples.

Healthy tomatoes		Spoilt tomatoes	
Organisms	Percentage Occurrence	Organisms	Percentage Occurrence
Lactobacillus brevis	45.4	*Bacillus megaterium*	6
Lactobacillus plantarum	54.6	*Bacillus laterosorus*	8
		Pseudomonas syringae	10
		Aspergillus fumigatus	13
		Fusarium solani	19
		Fusarium acuminatum	21
		Fusarium funjikuroi	23
Total	100	Total	100

Table 2: Proximate and mineral composition of tomato samples.

Samples	Dry matter	Moisture	Ash	Ether Extract	Protein	Ca	Mg	K	Na
Healthy	9.2±0.1^{a}	90.0±0.1^{a}	7.6±0.10^{a}	1.5±0.1^{a}	5.6±0.1^{a}	2.6±0.1^{a}	1.0±0.1^{a}	0.7±0.1^{a}	0.6±0.1^{a}
Spoilt	6.4±0.2^{b}	90.0±0.1^{a}	7.6±0.3^{a}	12.0±0.2^{b}	5.1±0.2^{b}	1.2±0.1^{a}	0.4±0.1^{b}	0.3±0.1^{a}	0.2±0.2^{a}

Mean values with different superscript down the column are statistically significant using t-test ($p \leq 0.05$).

Table 3: Quantity of antimicrobial compounds produced by Lactobacillus species at 72 h of incubation.

Isolates	Lactic acid (g/L)	Diacetyl (g/L)	Hydrogen perioxide (g/L)
Lactobacillus brevis II	2.3	1.8	2.4
Lactobacillus plantarum I	2.7	2.2	2.4
Lactobacillus brevis I	2.1	1.5	2.6
Lactobacillus plantarum II	2.7	1.2	1.6

The results of the proximate and mineral composition analyses of both fresh and spoilt tomato samples are shown in Table 2. The percentage dry matter, ether extract, protein, calcium, magnesium, potassium and sodium contents decreased from 9.2±0.1, 1.5±0.1, 5.6±0.1, 2.6±0.1, 1.0±0.1, 0.7±0.1 and 0.6±0.1 respectively in the healthy tomato sample to 6.4±0.2, 12.0±0.2, 5.1±0.2, 1.2±0.1, 0.4±0.1, 0.3±0.1 and 0.2± 0.2 respectively in the spoilt tomato sample.

Quantity of antimicrobial compounds produced by LAB species at 72 h of incubation is displayed on Table 3. All the LAB species produced lactic acid, diacetyl and hydrogen peroxide. *L. brevis* II produced 2.3 g/L of lactic acid, 1.8 g/L of diacetyl and 2.4 g/L of hydrogen perioxide while *L. plantarum* II showed a production of 2.7 g/L lactic acid, 2.2 g/L of diacetyl and 2.4 g/L of hydrogen peroxide. In addition, *L. brevis* I recorded 2.1 g/L of lactic acid, 1.5 g/L and 2.6 g/L of diacetyl and hydrogen peroxide respectively. Quantity of lactic acid, diacetyl and hydrogen peroxide produced by *L. plantarum* II were 2.7 g/L, 1.2 g/L and 1.6 g/L respectively.

Table 4 represents the result of antagonistic activity of LAB metabolites against tomato spoilage organisms. *L. brevis* II inhibited *B. megaterium*, *B. laterosporus*, *A. fumigatus* and *F. fujikuroi* with inhibition zones of 14 mm, 14 mm, 15 mm and 14 mm respectively but could not inhibit *P. syringae*, *F. solani* and *F. acuminatum*, but *L. plantarum* I inhibited *B. megaterium* (18 mm), *B. laterosporus* (22 mm), *A. fumigatus* (22 mm), *F. solani* (19 mm), *F. fujikuroi* (17 mm) but did not inhibit *P. syringue*, and *F. acuminatum*. However *L. plantarum* II inhibited all the spoilage microorganisms with the exception of *P. syringae* and *F. acuminatus*. The metabolites of *L. brevis* I inhibited all the spoilage microorganisms with the exception of *F. fujikuroi* but *B. megaterium*, *B. laterosporus*, *P. syringae*, *A. fumigates*, *F. solani* and *F. acuminatum* were inhibited showing 10 mm, 18 mm, 12 mm, 15 mm, 13 mm and 10 mm zones of inhibition respectively.

Table 5 represents the effect of heat treatment on LAB metabolite antagonistic property at different temperature for 30 min against fungal spoilage microorganisms of tomato. Metabolites of *L. brevis* inhibitory activity was only effective on *A. furmigatus* at 60 °C showing an inhibition zone of 9.0±1.00 mm, but the metabolite was not inhibitory against all spoilage fungi at

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 4: Antagonistic activity of LAB metabolites against spoilage microorganisms.

LAB metabolites	Spoilage microorganisms / Zone of Inhibition (mm)						
	B. megaterium	*B. laterosporus*	*P. syringae*	*A. fumigatus*	*F. solani*	*F. acuminatum*	*F. fujikuroi*
ML2	14	14	-	15	-	-	14
ML3	18	22	-	22	19	-	17
ML7	10	18	12	15	13	10	-
ML4	16	15	-	16	15	-	13

Key: ML2 = *L. brevis* II, ML3= *L. plantarum* I, ML4= *L. plantarum* II, ML7= *L.brevis* I

Table 5: Effect of heat treatment (30 min) on the antimicrobial activity of LAB metabolites against spoilage fungi.

LAB metabolites	Temperature											
	60 °C				80 °C				100 °C			
	A. fumigates	*F. solani*	*F. acuminatum*	*F. fujikuroi*	*A. fumigatus*	*F. solani*	*F. acuminatum*	*F. fujikuroi*	*A. fumigates*	*F. solani*	*F. acuminatum*	*F. fujikuroi*
ML3	10 ±0.1^{c}	11 ±0.1^{b}	0 ±0.0^{a}	9 ±0.2^{b}	9 ±0.2^{ac}	10 ±0.1^{d}	0 ±0.0^{a}	0 ±0.0^{a}	7 ±0.1^{b}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}
ML2	9 ±0.1^{ac}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}
ML4	8 ±0.0^{ab}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}
ML7	9 ±0.0^{ac}	8 ±1.0^{c}	0 ±0.0^{a}	0 ±0.0^{a}	0±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}	0 ±0.0^{a}

Zones of Inhibition (mm) mean values with different superscript down the column are statistically significant using LSD ($p \leq 0.05$).
Key: ML2, *L. brevis* II; ML3, *L. plantarum* I; ML4, *L. plantarum* II; ML7, *L.brevis* I.

80 °C and 100 °C. The same trend was exhibited by *L. plantarum* II, while metabolite from *L. brevis* I was inhibitory to *A. furmigatus* and *F. solani* with inhibition zones of 9.0±0.00 mm and 8.0±1.00 mm respectively at 60 °C while it could not inhibit other spoilage microorganisms at 60 °C, 80 °C and 100 °C.

Effect of heat treatment at different temperatures for 30 min on the antimicrobial activity of LAB metabolites against spoilage bacteria is shown in Table 6. Metabolite of ML2 at 60 °C inhibited only *B. lacterosporus* with an inhibition zone of 12±1.00, but did not inhibit other bacterial pathogens tested at 60 °C and 80 °C to 100 °C while the same metabolite inhibited *B. lacterosporus* exhibiting an inhibition zones of 13.0±1.00 mm, at 60 °C, inhibition zone of 12±2.64 mm at 80 °C and inhibition zone of 5.0±1.00 at 100 °C but did not inhibit other bacterial pathogen tested. The metabolite of ML7 only inhibited *B. laterosponrus* showing an inhibition zone 17±2.00 at 60 °C but did not inhibit other bacterial pathogen tested at 60 °C, 80 °C and 100 °C. The same trend observed with ML4 metabolites, intuiting only *B. laterosporus* with an intubation zone of 12±1.73 mm.

Result of the shelf life monitoring of tomato paste stored at 4 °C and 25 °C are shown in Table 7. The control sample at stored 4 °C showed a microbial load of 2.0×10^3 CFU/mL at the tenth day while the control sample stored at 25 °C showed microbial load of 4.0×10^3 CFU/mL at the fifth day. In the MP sample stored at 4 °C, microbial load of 2.0×10^5 CFU/mL was detected on the 15^{th} day while the same sample stored at 25 °C was observed to show a CFU/mL of 3.0×10^4 on 5^{th} day. LAB1 at 4 °C recorded 1.0×10^5 CFU/mL on the 20^{th} day while the same sample stored at 25 °C showed a microbial load of 2.0×10^3 on 5^{th} day. LAB 2 recorded a microbial load of 1.0×10^3 CFU/mL on the 15^{th} day of storage at 4 °C, while it recorded a microbial load of 3.0×10^3 CFU/mL on the 10^{th} day of storage at 25 °C. LAB3, when stored at 4 °C, microbial load of 2.0×10^2 CFU/mL was detected in the sample on the 25^{th} day while at 25 °C storage microbial load of 3.0×10^3 CFU/mL was observed on the 15^{th} day.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 6: Effect of heat treatment (30 min) on the antimicrobial activity of LAB metabolites against spoilage bacteria.

LAB metabolites	Temperature								
	60 °C			80 °C			100 °C		
	B. megaterium	*B. laterosporus*	*P. syringae*	*B. megaterium*	*B. laterosporus*	*P. syringae*	*B. megaterium*	*B. laterosporus*	*P. syringae*
ML2	0 ±0.0[a]	12 ±0.1[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]
ML3	0 ±0.0[a]	13 ±0.1[a]	0 ±0.0[a]	0 ±0.0[a]	12 ±0.3[a]	0 ±0.0[a]	0 ±0.0[a]	5 ±0.1[b]	0 ±0.0[a]
ML7	0 ±0.0[a]	17±0.2[c]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]
ML4	0 ±0.0[a]	12 ±0.2[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]

Zones of Inhibition (mm) mean values with different superscript down the column are statistically significant using LSD ($p \leq 0.05$).
Key: ML2, *L. brevis* II; ML3, *L. plantarum* I; ML4, *L. plantarum* II; ML7, *L.brevis* I.

Table 7: Shelf life monitoring of tomato paste stored at 4 °C and 25 °C (CFU/mL).

	Treatments	Time (Days)					
		1	5	10	15	20	25
Stored at 4 °C	BT	ND	ND	2.0×10^3	5.0×10^5	5.0×10^7	2.5×10^8
	MP	ND	ND	ND	2.0×10^5	4.0×10^7	1.2×10^8
	LAB1	ND	ND	ND	ND	1.0×10^5	2.0×10^7
	LAB2	ND	ND	ND	1.0×10^3	3.0×10^4	2.0×10^7
	LAB3	ND	ND	ND	ND	ND	2.0×10^2
Stored at 25 °C	BT	ND	4×10^3	1.4×10^3	2.5×10^8	5.7×10^7	1.05×10^8
	MP	ND	3.0×10^4	4.0×10^5	1.5×10^5	3.2×10^7	6.2×10^8
	LAB1	ND	2.0×10^3	3.0×10^5	8.0×10^5	2.5×10^7	5.5×10^7
	LAB2	ND	ND	3.0×10^3	7.0×10^3	2.3×10^7	4.8×10^7
	LAB3	ND	ND	3.0×10^3	5.0×10^3	2.1×10^7	4.5×10^2

Key: ND, not detected; BT, Control; MP, tomato + chemical preservative; LAB1, tomato + metabolite of *L. plantarum* 1 and *L. brevis* 1; LAB2, tomato + metabolite of *L. plantarum* II and *L. brevis* II; LAB3, tomato + metabolites of the four LAB.

DISCUSSION

The occurrence of *B. megaterium*, *B. laterosporus*, *P. syringae*, *A. fumigatus*, *F. solani*, *F. acuminatum*, and *F. funjikuroi* in spoilt tomato fruits had earlier been reported by Gosh (2009) and Ibrahim *et al.* (2011). This observation might be due to the ubiquitous nature of microorganisms and the ability to produce the required extra cellular enzyme to solubilise the tomato fruit into simpler components and utilize them for growth and metabolic activities (Salle, 1943; Allison *et al.*, 2009). The isolation of species of LAB from healthy tomato fruits is in accordance with findings of Sajur *et al.* (2007). The presence of LAB in tomato fruit is attributed to their high survival in post harvest conditions of tomatoes (Trias *et al.*, 2008).

The significant decrease in dry matter, ether extract, protein and mineral contents in the spoilt tomato corresponds with the report of Ibrahim *et al.* (2011). This might be due to microbial interaction in the spoilt tomato fruit which depleted the nutritional content of the fruit However, tomato is a perishable fruit with a high incidence of post-harvest loss (Adeoye *et al.*, 2009) caused majorly by spoilage bacteria and fungi. In preventing these spoilage microorganisms over the years, bactericides and fungicides have been used but the accumulation of chemical residue on the produce affects the environment and consumers' health (Trias *et al.*, 2008; Adeniyi *et al.*, 2011).

All the LAB isolated in this work produced lactic acid, diacetyl and hydrogen peroxide to varying degrees. This observation is in accordance with the reports of Ogunbanwo *et al.* (2004) and the ability of LAB to

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

produce antimicrobial compounds is due the absence of true catalyses to break down hydrogen peroxide generated which accumulates and becomes inhibitory to some organisms. The antagonistic activity of LAB metabolites against the spoilage bacteria and fungi agrees with the findings of Trias *et al.* (2008). The inhibitory effect of lactic acid is due to undissociated forms of the acids which penetrates the pathogen's membrane and liberate hydrogen ion in the neutral cytoplasm thus inhibiting vital cell functions (Corleh and Brown, 1980; Adeniyi *et al.*, 2006). Diacetyl is known to be very effective against fungi and this is due to the interference with the utilization of arginine (De Vyust and Vandamme, 1994) and in addition to a strong oxidizing effect on the organisms cell especially bacteria (Condon, 1987).

The stability of the LAB metabolites at temperatures of 60 °C, 80 °C and 100 °C revealed that as temperature increased, inhibitory activity decreased or was not detected (Assefa *et al.*, 2008). However, Muhialdin *et al.* (2011) reported stability of the LAB metabolites after heat treatment for 30 min at 90 °C and 121 °C.

The shelf life of tomato paste treated with LAB metabolites and stored at 4 °C showed a better preservation capability over the chemical preservative stored at 4 °C. This reveals a possible potential of the LAB metabolites in the retardation of food spoilage which agrees with the findings of Ogunbanwo *et al.* (2008). The biopreservative potential of LAB metabolites has been tested on other food product like suya (Adesokan, *et al.*, 2008) and chicken meat (Ogunbanwo and Okanlawon, 2006).

A major advantage in the use of lactic acid bacteria and their metabolites is that they are considered as GRAS (generally recognized as safe) and comply often with the recommendations for food products (Stiles and Holzapfel, 1997). Unlike some chemical preservatives, LAB metabolites have not been reported to have residual effect on the food product or the consumer's health.

CONCLUSION AND RECOMMENDATION

Lactic acid bacteria are known for the effective inhibitory activity of their metabolites against spoilage microorganisms which has been shown in other research work and in this study. Tomatoes are important, nourishing, highly perishable, widely used fruits and also serve as a source of income for producers in Nigeria. The metabolites produced by the LAB isolates in this study especially *L. plantarum* I were effective against the spoilage bacteria and fungi isolates, therefore can be used as biopreservative. Care should be taken to avoid mechanical damage in the skin of healthy tomato fruits after harvesting to prevent penetration of spoilage ENT microorganisms.

ACKNOWLEDGEMENT

The authors are grateful for the technical assistance of Mr. Jide of the Multidisciplinary Central Laboratory (MDCL), University of Ibadan and Mr. Popoola of IART Ibadan Oyo State Nigeria.

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