International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (2), pp. 001-006, February, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Influence of cultural conditions on hydrogen peroxide production by lactic acid bacteria isolated from some Nigerian traditional fermented foods

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Accepted 09 November, 2017

In this study, influence of cultural conditions on hydrogen peroxide production by lactic acid bacteria was investigated. *Leuconostoc mesenteroides* produced the highest quantity (0.024 L) of hydrogen peroxide while *Lactobacillus plantarum* produced the lowest amount (0.016 g/L) in normal MRS. The effect of temperature on hydrogen peroxide production was determined and the result showed that *Leuc. mesenteroides* produced the highest quantity (0.024 g/L) at 30°C while *L. brevis* produced the lowest amount (0.012 g/L) of hydrogen peroxide at 45°C. *Leuc. mesenteroides* produced the highest amount of hydrogen peroxide (0.032 g/L) when mannitol was used as the carbon source while *Lactobacillus delbrueckii* and *Lactobacillus fermentum* produced the lowest amount (0.020 g/L) when glucose was used as the carbon source. Furthermore, *Leuc. mesenteroides* produced the highest amount of hydrogen peroxide (0.033 g/L) when potassium nitrate was used as the nitrogen source while *L. fermentum* and *L. delbrueckii* produced the lowest amount (0.020 g/L) when yeast extract was used as the nitrogen source. Hydrogen peroxide produced by *Leuc. mesenteroides* has the highest zone of inhibition against *Pseudomonas aeruginosa* while hydrogen peroxide produced by *L. plantarum* had the lowest zone of inhibition against *Staphylococcus aureus*.

Key words: Hydrogen peroxide production, lactic acid bacteria, temperature.

INTRODUCTION

Various traditional fermented foods are produced in many African countries. The common substrates for fermentation are cassava and cereal grains such as maize, sorghum and millet. In Nigeria, ogi is a sour gruel produced from maize, sorghum and millet (Adesokan et al., 2010). It is normally prepared as a water suspension and cooked before consumption (Odunfa and Oyewole, 1998). Burukutu is an alcoholic beverage produced by fermentation of sorghum while palm wine is obtained from the fermented sap of palm trees. These beverages serve as inebriating drinks for fulfilling social obligations (Sanni and Lonner, 1993). Fufu and gari are the two main staple foods produced by submerged and solid state fermentation of cassava respectively (Oyewole, 1997). Lactic acid bacteria (LAB) are the predominant microorganisms during fermentation of these foods and beverages. However, yeasts are responsible for the alcoholic content of the burukutu and palm wine (Adesokan, 2005).

LAB possess the ability to produce hydrogen peroxide (Anders et al., 1970). The synthesis of hydrogen peroxide by lactic acid bacteria is believed to occur through oxidation of sugars or similar compounds (Kot et al., 1996).The involvement of flavoprotein and NADH oxidase in hydrogen peroxide synthesis have also been established (Anders et al., 1970).

Hydrogen peroxide produced by LAB may accumulate and reach a level that becomes inhibitory to the growth of

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other bacteria in some cultures. It kills sensitive bacteria by oxidation of cellular materials and destruction of basic molecular structures of cell protein (Zalan et al., 2005).

Today, consumers are very much concerned about the use of synthetic chemicals as food preservatives (Soomro et al., 2002). Therefore, hydrogen peroxide produced by LAB can be very useful for food preservation and prevention of growth of food borne pathogens. Few reports have been concerned with the conditions which affect hydrogen peroxide production (Villegas and Gilliland, 1998) . Therefore the present study was aimed at determining ways of optimizing hydrogen peroxide production.

MATERIALS AND METHODS

Sample collection

Samples of ogi (fermented maize gruel), cassava retting and burukutu (alcoholic beverage produced from sorghum) were obtained from Ibadan, South-western Nigeria. The samples were collected in sterile plastic containers and transported to the laboratory for immediate analysis.

Bacterial strains and cultures

Isolation of LAB strains was carried out from cassava retting, ogi and burukutu. Ten grams of each sample of ogi and cassava retting was added to 90 ml sterile diluent containing 0.1% peptone water and homogenized for 30 s while 10 ml of burukutu was added to 90 ml of the diluent. LAB isolation was carried out from appropriate 10-fold dilution on MRS agar and incubated anaerobically at 35°C for 48 h. Pure cultures of the isolates were characterized using API 50 CH and CHl kits (API system, Biomeriux Sa, France) . The identity of the isolates was confirmed by reference to Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986). The pathogenic bacteria employed as indicator organisms were obtained from culture collection of Department of Biology, The Polytechnic, Ibadan, Nigeria.

Quantitative determination of hydrogen peroxide produced by the LAB

The test organisms were grown in MRS broth for 72 h and centrifuged at 3000 xg for 15 min. 5 mg/ml of 0.1 N NaOH and protease was added to neutralize the influence of lactic acid and bacteriocin respectively. 25 ml of the supernatant fluid was obtained and 20 ml of diluted sulphuric acid were added. Titration was carried out with 0.1 M potassium permanganate. Each milliliter of 0.1 M potassium permanganate is equivalent to 1.79 mg of hydrogen peroxide. Solution and decolourisation of the sample was regarded as the end point (A.O.A.C, 1990).

Effect of growth conditions on the production of hydrogen peroxide

The effect of incubation temperature was determined. 18 h old culture of the LAB isolates were inoculated into normal MRS and incubated at 4, 30 and 45°C for 72 h. The quantity of hydrogen peroxide produced was determined as described above.

Effect of medium components on hydrogen peroxide production

The effect of carbon sources on hydrogen peroxide production was carried out. This was done by preparing three different batches of MRS containing 2% glucose, 2% mannitol or 2% maltose. The media were sterilized at 115°C for 15 min and then inoculated with 18 h old culture of the LAB isolates. Incubation was carried out at 37°C for 72 h and hydrogen peroxide produced was quantified described above. To determine the effect of nitrogen sources on hydrogen peroxide production, yeast extract, casein and potassium nitrate were employed as carbon sources. A basal medium containing 20 g dextrose, 1.0 ml tween 80, 2.0 g dipotassium hydrogen phosphate, 5.0 g sodium acetate, 0.2 g magnesium sulphate and 0.05 g manganese sulphate in 1 L distilled water. 5 g of each of the nitrogen sources was added separately into the basal media and sterilized at 121°C for 15 min. 18 h old culture of the isolates were inoculated and incubated at 37°C for 72 h. Hydrogen peroxide produced was quantified as described above.

To determine the effect of initial pH on production of hydrogen peroxide in MRS broth, the pH values were adjusted to 4.0, 5.5 and 7.0 using 0.1 N hydrochloric acid or 0.1 N NaOH. Each medium was inoculated with (1% v/v) 18 h old culture of the LAB isolates and incubated at 30°C for 72 h.

Antagonistic activity of hydrogen peroxide produced by the LAB isolates

The method employed for this test is a well diffusion assay. 18 h old culture of indicator organisms was used to inoculate nutrient agar plate. 6 mm diameter holes were created in the inoculated nutrient agar plate using a cork borer. Hydrogen peroxide produced by the LAB was dispensed into each of the holes.

RESULTS

In this study, lactic acid bacteria (LAB) were isolated from ogi, burukutu and cassava retting (fufu). The LAB isolated were *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus delbrueckii* and *Leuconostoc mesenteroides*. *L. plantarum* isolated from ogi had the highest frequency of occurrence of 30.56% while *L. brevis*, *L. fermentum* and *Leuc.mesenteroides* showed the lowest occurrence of 2.78% each (Table 1).

The quantity of hydrogen peroxide produced in normal MRS by the selected LAB isolated is presented in Table 2. *Leuc. mesenteroides* produced the highest quantity (0.024 g/L) of hydrogen peroxide while *L. plantarum* produced the lowest amount (0.016 g/L) after 72 h incubation.

The influence of temperature on the amount of hydrogen peroxide produced by the LAB isolates was studied and the result is presented in Table 3. *Leuc*. m*esenteroides* produce the highest quantity (0.024 g/L) of hydrogen peroxide at 30°C while *L. brevis* produced the lowest amount (0.012 g/L) of hydrogen peroxide at 45°C.

The influence of pH on hydrogen peroxide production was determined and the result is presented in Table 4. *Leuc. mesentoroides* produced the highest amount

Table 1. Lactic acid bacteria species isolated from some fermented foods.

Lactic acid bacteria isolated	Fermented foods	Number	Percentage (%)
L. brevis	Ogi (maize)	1	2.78
	Burukutu	1	2.78
L. casei	Ogi (maize)	2	5.56
	Burukutu	1	2.78
	Ogi baba (guinea corn)	1	2.78
L. acidophilus	Ogi (maize)	2	5.56
L. plantarum	Ogi (maize)	11	30.56
	Burukutu	4	11.11
	Ogi baba (guinea corn)	3	8.33
	Fufu (retted cassava)	2	5.56
L. fermentum	Ogi (maize)	1	2.78
	Ogi baba (guinea corn)	1	2.78
	Fufu (retted cassava)	1	2.78
L. delbrueckii	Ogi (maize)	1	2.78
	Burukutu	2	5.56
	Ogi baba (guinea corn)	1	2.78
Leuc. mesenteroides	Burukutu	1	2.78
		36	100

Table 2. Quantity of hydrogen peroxide produced (g/l) by the LAB isolates.

Isolates	Incubation		
	24	48	72
L. plantarum	0.017*±0.01^{a}	0.022±0.02^{a}	0.016±0.02^{a}
L. brevis	0.020±0.04^{a}	0.022±0.01^{a}	0.021±0.03^{a}
L. fermentum	0.017±0.06^{b}	0.020±0.04^{a}	0.018±0.05^{b}
L. delbrueckii	0.017±0.03^{a}	0.020±0.05^{b}	0.018±0.03^{a}
L. mesenteroides	0.019±0.01^{a}	0.024±0.04^{a}	0.022±0.02^{a}

*Values are means (n = 5) ± standard deviation (SD). Mean values in the same column and row followed by the same letter are not significantly different according to Duncans multiple ranges test (P 0.05).

(0.024 g/L) of hydrogen peroxide at pH 5.5 while *L. delbrueckii* produced the lowest amount (0.009 g/L) at pH 7.0.

The influence of carbon sources on the amount of hydrogen peroxide produced is presented in Table 5. *Leuc. mesenteroides* produced the highest amount of hydrogen peroxide (0.032 g/L) when mannitol was used as the carbon source. *L. delbrueckii* and *L fermentum* produced the lowest amount (0.020 g/L) when glucose was employed as the carbon source.

The effect of nitrogen sources on the quantity of hydrogen peroxide produced is presented in Table 6. *Leuc. mesenteroides* produced the highest amount of hydrogen peroxide (0.033 g/L) when potassium nitrate was employed as the nitrogen source. *L. fermentum* and *L. delbrueckii* produced the lowest amount (0.020 g/L) when yeast exract was employed as the nitrogen.

The antimicrobial activity of the hydrogen peroxide produced by the LAB isolates was determined and the result is presented in Table 7. *Leuc. mesenteroides* had

Table 3. Effect of varied temperature on the quantity of hydrogen peroxide (g/l) produced by the LAB isolates.

Isolates	Temperature								
	4°C			30°C			45°C		
	24	48	72	24	48	72	24	48	72
L. plantarum	0.013*+0.02^{a}	0.012±0.03^{a}	0.013±0.01^{a}	0.017±0.02^{b}	0.022±0.05^{ba}	0.016±0.03^{b}	0.015±0.04^{bd}	0.014±0.03^{a}	0.014+0.06^{a}
L. brevis	0.015±0.06^{ab}	0.017±0.02^{ab}	0.017±0.02^{ab}	0.020±0.03^{bb}	0.022±0.04^{bb}	0.021±0.02^{bb}	0.013±0.05^{bc}	0.016±0.04^{ab}	0.015±0.05^{ab}
L. fermentum	0.010±0.02ac	0.017±0.04ae	0.015±0.04ae	0.017±0.01be	0.020±0.02be	0.018±0.04bc	0.012±0.03bd	0.060±0.02ac	0.014±0.02ad
L. delbrueckii	0.014±0.03^{ad}	0.020±0.01^{ad}	0.018±0.03^{ac}	0.017±0.05^{bc}	0.020±0.04^{bd}	0.018±0.02^{bc}	0.013±0.02^{bd}	0.015+0.04^{ac}	0.015±0.03^{ac}
Leuc. mesenteroides	0.013±0.04^{ae}	0.017±0.03^{ac}	0.015±0.05^{ac}	0.019±0.02^{bc}	0.024±0.01^{br}	0.022±0.05^{bd}	0.013±0.03^{be}	0.014±0.01^{ae}	0.013±0.04^{ae}

* Values are means (n = 5) ± standard deviation (SD). Mean values in the same column and row followed by the same letter are not significantly different according to Duncans multiple ranges test (P 0.05).

Table 4. Effect of initial pH on the quantity of hydrogen peroxide (g/l) produced by the LAB isolates.

Isolates	pH								
	4.0			5.5			7.0		
	24	48	72	24	48	72	24	48	72
L. plantarum	0.014*±0.02a	0.017±0.03b	0.015±0.06b	0.017±0.03b	0.022±0.06c	0.020±0.04c	0.020±0.05a	0.015±0.04b	0.013±0.03a
L. brevis	0.015±0.06ab	0.017±0.04b	0.016±0.02b	0.020±0.02b	0.020±0.02c	0.022±0.02ca	0.010±0.03a	0.017±0.02b	0.015±0.02ab
L. fermentum	0.010±0.02a	0.017±0.01b	0.015±0.03b	0.017±0.04b	0.020±0.03c	0.018±0.03cb	0.010±0.02a	0.015±0.03b	0.013±0.04a
L. delbrueckii	0.012±0.03a	0.017±0.02b	0.015±0.04b	0.017±0.05b	0.020±0.01c	0.019±0.05cb	0.014±0.04a	0.017±0.01b	0.015±0.02ab
Leuc. mesenteroides	0.010±0.05ae	0.012±0.05bc	0.010±0.01bc	0.019±0.01b	0.024±0.04c	0.020±0.01c	0.009±0.03ab	0.014±0.05bc	0.012±0.01a

* Values are means (n=5) ± standard deviation (SD). Mean values in the same column and row followed by the same letter are not significantly different according to Duncans multiple ranges test (P 0.05).

the highest zone of inhibition against *Pseudomonas aeruginosa* while *L. plantarum* showed the lowest zone of inhibition against *Staphylococcus aureus*.

DISCUSSION

Different species of lactic acid bacteria were isolated from ogi, burukutu and fufu. The species isolated included *L. brevis*, *L. plantarum* and *Leuc. mesenteroides*. The isolation of LAB from these traditional fermented foods was reported in the literature (Ogunbanwo, 2005; Ogunbanwo et al., 2004; Caplice et al., 1999; Oyewole, 1997). *L. plantarum* had the highest frequency of occurrence compare to other species isolated. Sanni et al. (1999) reported the dorminance of *L. plantarum* isolated from ogi, an indigenous fermented food.

The highest quantity of hydrogen peroxide was produced by *L. brevis* and *Leuc. mesenteroides* while the lowest amount was produced by *L. plantarum* at 30°C. Ogunbanwo (2005) reported that *Leuc. mesenteroides* had the highest yield of hydrogen peroxide while *L. plantarum* had the lowest yield among lactic acid bacteria isolated

Table 5. Effect of carbon source on the quantity of hydrogen peroxide (g/l) produced by the LAB isolates.

Isolates	Carbon sources		
	Glucose	Mannitol	Maltose
L. plantarum	0.022*±0.01[a]	0.029±0.03[a]	0.02±0.02[a]
L. brevis	0.024±0.02[a]	0.027±0.01[b]	0.024±0.04[a]
L. fermentum	0.020±0.05[b]	0.024±0.03[a]	0.020±0.02[b]
L. delbrueckii	0.020±0.01[a]	0.023±0.02[a]	0.022±0.01[a]
Leuc. mesenteroides	0.024±0.02[a]	0.031*±0.01[b]	0.023±0.02[a]

*Values are means (n = 5) ± standard deviation (SD). Mean values in the same column and row followed by the same letter are not significantly different according to Duncans Multiple Ranges Test (P 0.05).

Table 6. Effect of nitrogen source on the quantity of hydrogen peroxide (g/l) production.

Isolates	Nitrogen sources		
	Yeast extract	Casein	KNO_3
L. plantarum	0.022*±0.02[a]	0.029±0.01[b]	0.028±0.04[a]
L. brevis	0.024±0.03[a]	0.027±0.01[b]	0.031±0.02[b]
L. fermentum	0.020±0.01[b]	0.027±0.02[b]	0.029±0.01[b]
L. delbrueckii	0.020±0.02[a]	0.026±0.01[b]	0.029±0.02[b]
Leuc. mesenteroides	0.024±0.01[a]	0.025±0.02[b]	0.033±0.02[b]

*Values are means (n = 5) ± standard deviation (SD). Mean values in the same column and row followed by the same letter are not significantly different according to Duncans Multiple Ranges Test (P 0.05).

Table 7. Antagonistic activity of hydrogen peroxide produced by the LAB isolates against some indicator organisms.

LAB isolates	*P. aeruginosa* (mm)	*Staphylococcus aureus* (mm)	*Candida albicans* (mm)	*Escherichia coli* (mm)	*Proteus vulgaris* (mm)
L. plantarum	+12	+8	-	-	-
L. brevis	-	+10	-	-	-
L. fermentum	+16	+18	-	-	-
L. delbrueckii	-	+18	-	+17	-
Leuc. Mesenteroides	+22	+21	-	-	-

from ogi and fufu – two Nigerian fermented foods.

The highest yield of hydrogen peroxide was from *Leuc. mesenteroides* at pH 5.5. Rodriguez et al. (1997) reported that pH has a strong influence on the stability of hydrogen peroxide produced by LAB. The result obtained on the influence of carbon sources on hydrogen peroxide production indicated that mannitol was the preffered carbon source. The result also showed that glucose was not a good carbon source for hydrogen peroxide production. This agreed with the report of Berthier (1993) who stated that glucose did not enhance hydrogen peroxide production.

The experiment on the influence of nitrogen sources showed that potassium nitrate was the best nitrogen source for hydrogen peroxide production. When yeast extract was employed as a nitrogen source the lowest amount of hydrogen peroxide was detected. Yeast extract has been shown to exhibit catalase activity (Porubean and Sellars, 1979). The hydrogen peroxide produced by the LAB isolates inhibited some spoilage and pathogenic organisms to varying degrees. Lactic acid bacteria have been shown to posses ability to inhibit both spoilage and pathogenic organisms (Lewus et al., 1991; Ogunbanwo et al., 2004).

It could be concluded that *Leuc. mesenteroides* was the best hydrogen peroxide producer when potassium nitrate and mannitol were employed as the nitrogen and carbon sources respectively at 30°C in reconstituted MRS medium at pH 5.5 for 48 h.

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