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Antioxidant Activity of Whey from Milk Fermented with *Lactobacillus* Species Isolated from Nigerian Fermented Foods

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Summary

Eight *Lactobacillus* isolates obtained from five indigenous fermented foods (ogi, ogi baba, wara, kunnu and ugba) were investigated. Wara is a dairy-based food while the others are not dairy-based. The bacteria were isolated on MRS agar and purified by successive streaking on the same medium. The whey fraction of skimmed milk fermented with each isolate was assayed for radical scavenging effects using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. All the whey fractions showed radical scavenging activities. The five isolates with the highest activities were selected. On the basis of Gram stain reaction, cellular morphology, biochemical tests and carbohydrate utilization profiles they were identified as strains of *Lactobacillus brevis*, *L. fermentum*, *L. plantarum*, *L. casei* and *L. delbrueckii*. The antioxidant activities of whey fractions from 24-hour fermentations with the selected organisms were investigated using both radical scavenging effects and lipid peroxidation inhibitory activity. The radical scavenging activity was generally higher than the lipid peroxidation inhibition, except in the *L. plantarum* strain, which did not show any significant difference in both activities. The probiotic potential of the isolates was evaluated by pH and bile tolerance. None of the selected isolates showed any growth at pH=2.0 but *L. casei* and *L. delbrueckii* survived at this pH. Four of the five selected isolates were able to grow in 0.5 % dehydrated bile, with *L. casei* strain showing the highest level of growth, followed by *L. delbrueckii*. *L. plantarum* strain was not bile tolerant. The ability of *L. casei* and *L. delbrueckii* strains to survive at pH=2 and grow in the presence of bile indicates that the isolates may be able to colonize the gastrointestinal tract. The findings of this study indicate that *Lactobacillus* strains isolated from indigenous Nigerian fermented foods could be useful as starter cultures to provide antioxidants in food and that fermented milk may serve as a delivery vehicle for antioxidative, probiotic lactobacilli from non-dairy sources.

Key words: antioxidant activity, $DPPH^{\bullet}$ scavenging activity, fermented foods, lipid peroxidation, probiotics, whey

Introduction

Fermentation is a commonly used food processing technology, and lactic acid fermentation is probably the simplest and safest means of preserving food. Many African staple foods are fermented with lactic acid bacteria, and lactic acid-fermented foods constitute a significant portion of indigenous diets in many developing countries (*1,2*).

Lactobacillus species are probably the most important bacteria in the food industry. They are widely used as starter cultures and have been reported to play significant roles in the production of fermented foods. In addition to their importance in food fermentation, *Lactobacillus* species are Generally Recognized as Safe (GRAS)

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and have been reported to have beneficial health properties, as a result of which they are also finding increasing use as probiotics in other health-related applications (*3, 4*). A myriad of beneficial activities such as immunomodulatory, antiallergenic, antimicrobial, antihypertensive and antitumourigenic effects have been reported (*5–8*). *Lactobacillus* sp. have also been shown to possess antioxidant activities (*6,9–12*).

Antioxidant research has become a major scientific pursuit because of the evidence linking oxidative stress with many diseases and because of potential food preservative applications. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are activated forms of oxygen and nitrogen, respectively. These include free radicals and non-radicals, which arise from endogenous and exogenous sources. ROS and RNS have been implicated in the aetiology of numerous pathological conditions such as malaria, diabetes mellitus, cardiovascular, gastrointestinal and neurodegenerative diseases. Oxidative stress results when the oxidant/antioxidant ratio tilts in favour of oxidant factors, it is involved in the aging process and also causes inflammation (*13–15*). Free radicals attack cellular components leading to the oxidation of lipids, proteins and DNA, thus causing structural and functional changes to these molecules.

Oxidation of food constituents is also a key event in food spoilage. This may reduce the nutritional value and safety of the food by producing undesirable flavours and toxic substances. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) added to foods have been reported to have side effects (*16,17*). It is therefore important to investigate natural alternatives devoid of the safety concerns.

The global interest in harnessing the beneficial properties of microbes and their metabolites for human health, coupled with the unique opportunity offered by fermented foods as vehicles for the delivery of bioactive agents produced by food-grade microbes make it important to explore potential uses of indigenous food-grade lactobacilli in the development of functional foods and probiotics.

A very significant portion of Nigerian foods are fermented products. Fermentation processes are natural, dependent on the microbial flora of the substrate, fermentation vessels used and chance inocula from the environment. This offers a unique opportunity to discover new strains with a variety of properties and potential food biotechnology applications. In a study to determine the effect of fermentation by dairy starter cultures on the antioxidant property of milk, whey was found to show strain-dependent development of antioxidant activity (*12*).

The aim of the present study is to determine the effect of fermentation with lactobacilli isolated from indigenous Nigerian fermented foods on the antioxidative activity of milk and to assess the probiotic potential of the strains with significant antioxidant effects.

Materials and Methods

Materials

The *Lactobacillus* strains used in this study were isolated from fermented foods purchased from local markets in Lagos State, Nigeria or prepared at home in the traditional way by one of the authors. The foods include ogi (fermented corn gruel), which was made using the yellow maize (*Zea mays*), ogi baba (also fermented corn gruel) made from guinea corn (*Sorghum bicolor*), wara (an indigenous soft unripened cheese), kunnu (fermented millet, *Pennisetum typhoides*) and ugba (fermented African oil bean seed, *Pentaclethra macrophylla*). MRS (de Man-Rogosa-Sharpe) agar and broth were purchased from Oxoid Ltd, Basingstoke, Hampshire, UK, while API 50 CHL kits were from bioMérieux, Craponne, France. Peptone water was purchased from Difco Laboratories, Sparks, MD, USA, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co, St Louis, MO, USA. All other reagents used in this study were of analytical grade.

Preparation of the fermented foods

Ogi and ogi baba

Ogi is an acid-fermented cereal gruel or porridge popular throughout West Africa. It is made from maize (*Zea mays*), guinea corn (*Sorghum bicolor*) or millet (*Pennisetum typhoides*). The choice of grain used is dependent on ethnicity and individual preferences. The colour of ogi depends on the colour of the cereal used. The ogi made from guinea corn is also called ogi baba. Ogi is prepared by steeping the cleaned cereal grain into water for 48–72 h at ambient temperature. The grains are then wet milled and wet sieved, and the resulting slurry is called ogi. Ogi is made into a smooth paste with boiling water before consumption.

Wara

Wara is a soft unripened cheese produced by the addition of leaf extracts of Sodom apple (*Calotropis procera*) to unpasteurized whole cow's milk. Wara is an important source of protein in West Africa. It is eaten fresh, used as a meat substitute in stews, or fried and eaten as a snack.

Kunnu

Kunnu is a non-alcoholic fermented beverage with sweet and sour taste and a milky appearance. The traditional process for producing kunnu requires steeping of millet grains for 48 h, wet milling with spices (ginger, cloves, and pepper), wet sieving and partial gelatinization of the slurry, followed by the addition of sugar to taste and bottling.

Ugba

Ugba is a nutritious condiment and snack made by fermenting the African oil bean (*Pentaclethra macrophylla*) seeds. To make ugba, the seeds are first parboiled for about 4 h, followed by dehulling. The cotyledons are then sliced into thin strips, cooked for about 8 h, soaked in water overnight, washed and fermented for 3 to 5 days. The ugba has a grayish white colour when first sliced but is dark brown after fermentation.

Isolation and identification of Lactobacillus

Samples were taken from the foods under aseptic conditions, serially diluted with 0.1 % peptone water, plated on MRS agar and incubated under microaerophilic conditions at 37 °C for 48 h. To ensure purity, iso-

lates were randomly picked, reinoculated successively on fresh MRS agar and incubated under the previously stated conditions. The isolates were identified based on Gram stain reaction, cellular morphology and the results of biochemical tests and carbohydrate utilization with the API 50 CHL kit according to the manufacturer's instruction and with reference to Bergey's manual (*18*).

Selection of Lactobacillus isolates

The isolates were cultured essentially as described in a previous study (*12*). The isolate (10^5 CFU) was inoculated into 10-mL MRS broth and incubated at 37 °C for 24 h. The cultured broths were vortexed and used to inoculate sterile skimmed milk (sterilized at 121 °C for 20 min) at a 1 % (by volume) concentration, then incubated at 37 °C for 24 h to generate precultures. These precultures were used to inoculate fresh skimmed milk (pasteurized at 73 °C for 15 s) at 2 % (by volume) concentration. Fermentation was carried out in triplicate for 24 h at 37 °C. The radical scavenging activity of the whey fraction was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (*19*). Five isolates (OK1, OK3, OK5, OK6 and OK8) with the highest DPPH scavenging activity were identified and selected for this study.

Antioxidant activity

The precultured strains were used to inoculate fresh pasteurized (73 °C for 15 s) skimmed milk (2 % by volume) and fermented at 37 °C for 24 h. Aliquots were taken at the start and during fermentation for the determination of antioxidant activity. To determine the antioxidant activity of the isolates and its development during fermentation, nonhydrolyzed casein was removed and the whey fraction was assayed for DPPH radical scavenging activity and inhibition of plasma lipid peroxidation. The fermentations and analysis were carried out in triplicates, with fresh pasteurized skimmed milk without bacteria used as control.

Preparation of whey fraction from fermented milk

The whey fraction was prepared essentially as described by Virtanen *et al.* (*12*) and used immediately after the preparation. Nonhydrolyzed casein was removed. Aliquots (15 mL) were collected from the fermented milk and the pH was adjusted to 4.6 with 1 M HCl. The suspension was centrifuged (10 000×*g* for 20 min at 5 °C in a Sorvall RC-2 centrifuge, Sorvall Ltd, Hertfordshire, UK) and the supernatant was filtered on a 0.45-μm filter (Millipore Corp, Billerica, MA, USA).

DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated using the method of Son and Lewis (*19*). DPPH was used as a stable radical. A volume of 2 mL of DPPH in ethanol (500 μM) was added to 2 mL of the whey fraction, mixed vigorously and allowed to stand in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. Ethanol was used as a blank, while DPPH solution in ethanol served as the control. The radical scavenging activity of the samples was expressed as % inhibition of DPPH absorbance:

$$\text{Inhibition}=[(A_{\text{control}}-A_{\text{test}})/A_{\text{control}}]\times 100 \qquad /1/$$

where A_{control} is the absorbance of the control sample (DPPH solution without whey fraction) and A_{test} is the absorbance of test sample (DPPH solution plus whey fraction).

Inhibition of lipid peroxidation

The lipid peroxidation inhibition activity was determined as described by Ou *et al.* (*14*). Plasma 0.4 mL, $FeSO_4$ solution (50 μM) 0.1 mL and whey 0.2 mL were mixed and incubated at 37 °C in a water bath for 12 h, after which 0.375 mL of 4 % trichloroacetic acid (TCA) and 75 μL of butylated hydroxytoluene (BHT) were added, mixed and placed in an ice bath for 5 min. The upper phase was obtained by centrifugation at 3000×*g* for 10 min in a Surgifriend SM902B centrifuge (Surgifriend Medicals, Essex, UK). To this, 0.2 mL of 0.6 % thiobabituric acid (TBA) were added, incubated at 100 °C for 30 min, allowed to cool and the absorbance was measured at 532 nm. In the control samples, the whey was replaced by deionized water. The results were expressed as % inhibition of plasma lipid peroxidation:

$$\text{Inhibition}=[1-A_{\text{test}}/A_{\text{control}}]\times 100 \qquad /2/$$

Acid and bile tolerance

To determine acid tolerance, the organisms were grown in MRS broths with pH adjusted to 2 or 4 using 1 M HCl. Bile tolerance was determined by growing the organisms in MRS broth supplemented with 0.5 % dehydrated fresh bovine bile, which was prepared by lyophilizing fresh liquid bile. Cells were inoculated at 2 % (by volume) and incubated under microaerophilic conditions at 37 °C. Aliquots of the cultures were taken over 30 h and the ability to survive or grow was determined by measuring the absorbance (*A*) at 600 nm using a Jenway 6102 spectrophotometer (Bibby Scientific Ltd, Straffordshire, UK). Each test was carried out in triplicate, with organisms inoculated in MRS as control.

Statistical analysis

Mean values of triplicate determinations and standard deviations were calculated. Results are shown as the mean values±standard deviation. Pearson's correlation coefficients were also determined to evaluate relationships between radical scavenging activity and lipid peroxidation inhibition.

Results

Antioxidative activity of Lactobacillus isolates

Preliminary screening of the whey fraction of skimmed milk fermented for 24 h with the eight isolates obtained from the fermented foods using the DPPH radical scavenging assay showed that all the isolates had antioxidant activity (Table 1). The whey fractions scavenged between (2.8±0.1) and (31.5±2.1) % DPPH radical (after correcting for the activity of unfermented skimmed milk). The five isolates with the highest activity (OK1, OK3, OK5, OK6 and OK8) were selected for further studies.

Table 1. Preliminary screening of whey from milk fermented with lactobacilli isolated from Nigerian fermented foods for radical scavenging activity

Food source	Isolate	Inhibition of DPPH radical/%
ogi	OK1	16.5±3.3
ogi	OK2	2.8±0.1
ogi baba	OK3	6.5±1.8
ogi baba	OK4	2.9±0.1
wara	OK5	31.5±2.1
kunnu	OK6	22.5±1.4
kunnu	OK7	3.0±0.8
ugba	OK8	26.5±1.0

Results are mean values of triplicate analysis±S.D.

DPPH radical scavenging activity

The measurement of radical scavenging activity over a 24-hour fermentation period showed that the ability of the whey fraction increased with time for four of the five selected organisms, *L. casei* (OK1), *L. brevis* (OK5), *L. plantarum* (OK6) and *L. fermentum* (OK8). The radical scavenging activity of *L. delbrueckii* (OK3) did not show any significant increase after 4 h (Fig. 1). The highest development of DPPH scavenging activity was observed with the *L. brevis* strain isolated from wara, followed by a strain of *L. fermentum* isolated from ugba. The least activity was shown by *L. delbrueckii* strain from ogi baba (Table 2).

Inhibition of plasma lipid peroxidation

The pattern of the development of inhibition of lipid peroxidation of the whey fraction over a 24-hour fermentation period was similar to that of the radical scavenging activity, *i.e.* an increase over time for *L. casei*, *L. brevis*, *L. plantarum* and *L. fermentum*. There was no significant increase in the ability of the whey fraction from skimmed milk fermented with *L. delbrueckii* to inhibit peroxidation after 6 h (Fig. 2). The highest activity after 24 h was observed with *L. brevis* from wara although there was no significant difference in the activity of the whey fraction from milk fermented with *L. plantarum* isolated from kunnu. The *L. delbrueckii* strain from ogi baba had the lowest activity (Table 2).

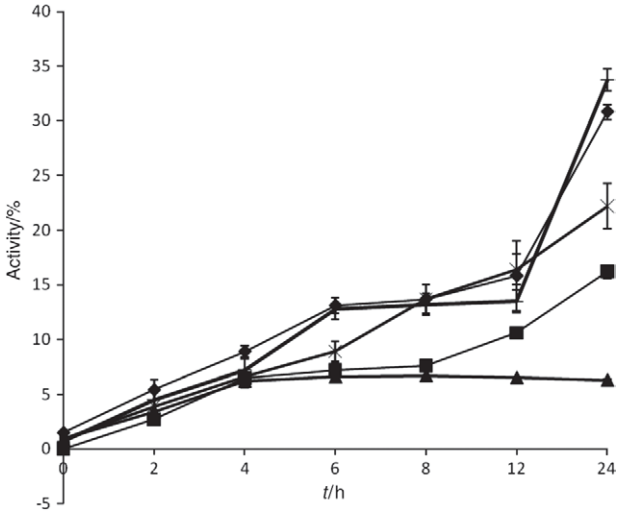

Fig. 1. Radical scavenging activity of whey from milk fermented with lactobacilli isolated from Nigerian fermented foods. (+) *L. brevis*, (♦) *L. fermentum*, (×) *L. plantarum*, (■) *L. casei*, (▲) *L. delbrueckii*. Bars indicate the standard deviation of triplicate determinations

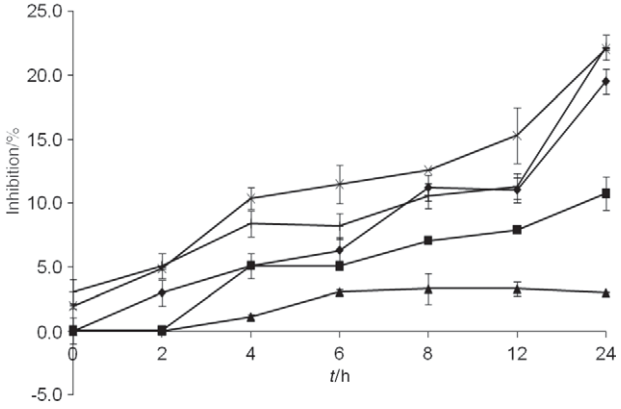

Fig. 2. Inhibition of lipid peroxidation of whey from milk fermented with lactobacilli isolated from Nigerian fermented foods. (+) *L. brevis*, (♦) *L. fermentum*, (×) *L. plantarum*, (■) *L. casei*, (▲) *L. delbrueckii*. Bars indicate the standard deviation of triplicate determinations

Table 2. Radical scavenging activity and inhibition of lipid peroxidation of whey from milk fermented with selected lactobacilli

Food source	Isolate	Identity	DPPH scavenging activity/%	Lipid peroxidation inhibition/%	Pearson's correlation coefficient (r)
ogi	OK1	*L. casei*	16.2±0.6	10.8±1.3	0.98
ogi baba	OK3	*L. delbrueckii*	6.3±0.1	3.0±0.0	0.94
wara	OK5	*L. brevis*	33.7±7.8	22.2±4.4	0.76
kunnu	OK6	*L. plantarum*	22.2±2.1	22.0±0.1	1.00
ugba	OK8	*L. fermentum*	30.8±0.7	19.5±1.0	0.96

Results are mean values of triplicate determinations±S.D.

Correlation of the two activities

There was a strong positive correlation between the radical scavenging activity of the isolates and the inhibition of lipid peroxidation. The correlation coefficients were between 0.76 and 1.00.

Acid and bile tolerance

Growth analysis (evaluated by absorbance measurements at 600 nm) showed that two of the isolates, *L. casei* and *L. delbrueckii*, were able to survive at pH=2. None of the isolates was capable of growing at pH=2, while all were able to grow at pH=4. *L. casei*, *L. brevis*, *L. delbrueckii* and *L. fermentum* were able to grow in 0.5 % dehydrated bile. *L. casei* strain showed the highest level of growth followed by *L. delbrueckii*, while *L. plantarum* was the only strain not tolerant to bile (Figs. 3–7).

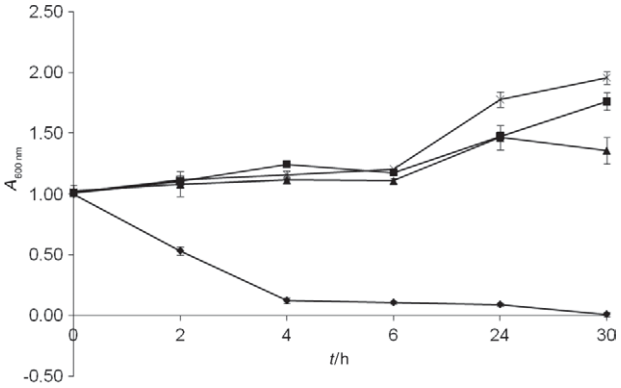

Fig. 5. Growth pattern of *L. brevis* strain isolated from wara in: (×) MRS broth, (♦) MRS broth with pH adjusted to 2, (■) MRS broth with pH adjusted to 4, (▲) MRS broth supplemented with 0.5 % dehydrated bile. Bars indicate the standard deviation of triplicate determinations

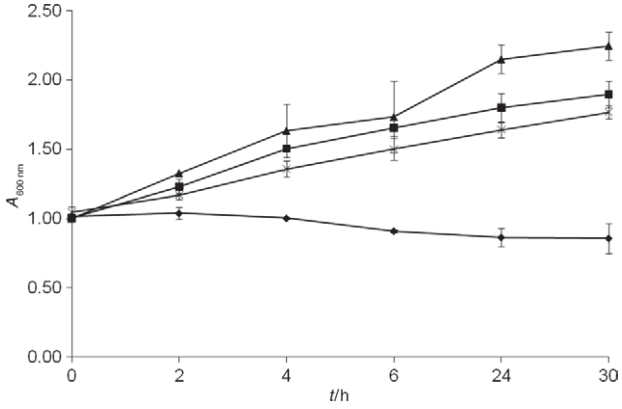

Fig. 3. Growth pattern of *L. casei* strain isolated from ogi in: (×) MRS broth, (♦) MRS broth with pH adjusted to 2, (■) MRS broth with pH adjusted to 4, (▲) MRS broth supplemented with 0.5 % dehydrated bile. Bars indicate the standard deviation of triplicate determinations

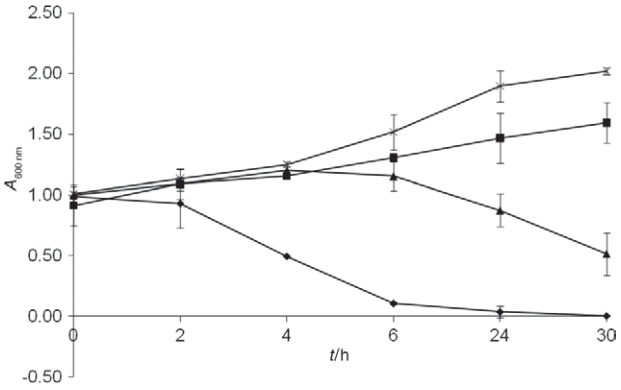

Fig. 6. Growth pattern of *L. plantarum* strain isolated from kunnu in: (×) MRS broth, (♦) MRS broth with pH adjusted to 2, (■) MRS broth with pH adjusted to 4, (▲) MRS broth supplemented with 0.5 % dehydrated bile. Bars indicate the standard deviation of triplicate determinations

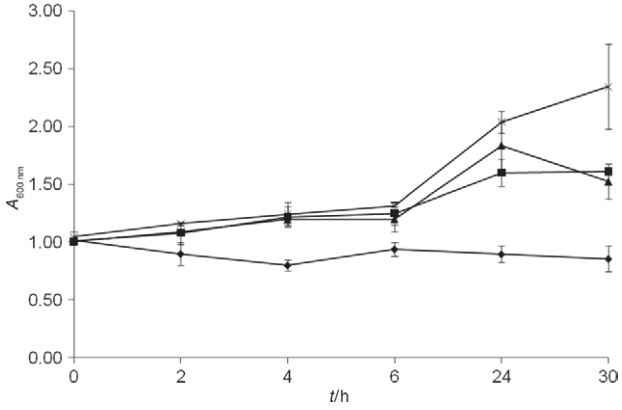

Fig. 4. Growth pattern of *L. delbrueckii* strain isolated from ogi baba in: (×) MRS broth, (♦) MRS broth with pH adjusted to 2, (■) MRS broth with pH adjusted to 4, (▲) MRS broth supplemented with 0.5 % dehydrated bile. Bars indicate the standard deviation of triplicate determinations

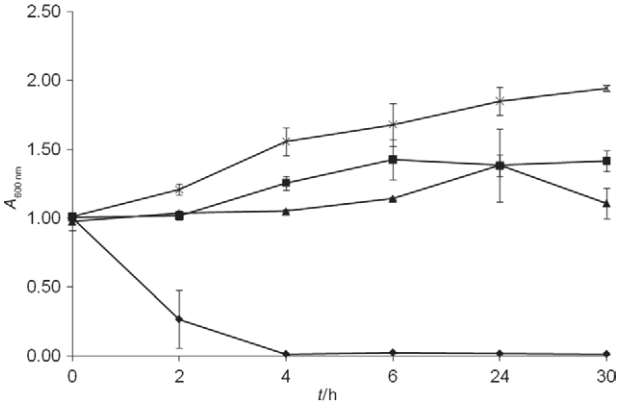

Fig. 7. Growth pattern of the *L. fermentum* strain isolated from ugba in: (×) MRS broth, (♦) MRS broth with pH adjusted to 2, (■) MRS broth with pH adjusted to 4, (▲) MRS broth supplemented with 0.5 % dehydrated bile. Bars indicate the standard deviation of triplicate determinations

Discussion

Natural defense mechanisms eliminate negative effects of the activity of free radicals. However, they are not always adequate to totally neutralize all endogenous and exogenous free radicals. Thus, scavenging properties of free radicals by food grade cultures can be useful in food manufacturing and can provide additional dietary sources of health enhancing antioxidants.

In this study, the antioxidative activity of whey from skimmed milk fermented with lactobacilli isolated from some indigenous Nigerian fermented foods was assessed. The probiotic potential of the strains was also investigated. Although most of the isolates were from non-dairy sources, they were used to ferment milk and then the development of antioxidant activity in the whey fraction of fermented milk was monitored. This was done because some of the products from which the isolates were obtained are subjected to heat treatment before consumption, which may inactivate labile substances. Indigenous fermented milk products are not usually heat treated and fermented milk products are more generally acceptable. In addition, a recent study has reported the development of antioxidant activity in whey during milk fermentation with lactic acid bacteria (*12*).

Our results clearly indicate the development of antioxidant activity in the fermented milk whey. The fermented milk whey showed strain-dependent DPPH radical scavenging activity and inhibition of plasma lipid peroxidation. From the results of this study, the highest level of antioxidant activity was observed in the whey from milk fermented with the strain of *L. brevis* isolated from wara, a dairy product. This is probably due to the strain being better adapted to the milk substrate, in contrast to the other non-dairy isolates.

Although several studies have reported antioxidant activities in lactobacilli, a direct comparison of results is difficult because of the variety of assay methods used, the numerous ways in which the results are expressed, the use of nonstandardized inoculum size and various other discrepancies.

Various mechanisms have been adduced to the antioxidant activity in lactobacilli (*10–12*). This makes it difficult to assign one mechanism or compound to the effect. But regardless of the underlying mechanism, scavenging of the free radical terminates the oxidation chain reaction (*12*). Since the cells and casein were removed by centrifugation in this study, the observed antioxidant activities may be attributable to extracellular metabolites, hydrolyzed milk components and/or products of cell lysis (*10,12,20*).

A correlation between radical scavenging activity and lipid peroxidation inhibition supported the notion that radical scavenging activity is an indicator of antioxidant activity as there were strong correlations between the DPPH scavenging activity and the inhibition of plasma lipid peroxidation.

Oxidative stress is a key determinant of morbidity and mortality. Whereas increased inflammation and oxidative stress are predictors of mortality for haemodialysis patients, long-term elevated oxidative stress, in contrast to elevated inflammation, is a principal risk factor in the development of atherosclerosis and cardiovascular mortality in these patients (*21*). Although not conclusive, evidence indicates the potential protective effects of dietary antioxidants. These isolates may be useful in developing functional foods with high dietary antioxidant content with a potential to influence the functional level of antioxidants in the body.

Our findings may also indicate the need to develop indigenous *Lactobacillus* strains as starters for the food fermentation industry or as potential sources of substitutes to synthetic antioxidant food additives like BHT and BHA without the safety concerns, as the long history of consumption of the foods from which they were isolated proves the safety.

Good or potential probiotics, besides safety, must be able to survive in the extremely stressed, low pH and high bile concentration environment of the gastrointestinal tract (*22,23*). Evaluation of the acid and bile tolerance of the lactobacilli used in this study showed that *L. casei* and *L. delbrueckii* strains were able to survive at pH=2 and grow in the presence of bile. However, investigation to determine their value as potential probiotics is required.

Conclusions

The development of foods with beneficial effects in addition to the provision of nutrients is a growing niche. The aim of this study was to compare the development of antioxidant activity in the whey of milk fermented with strains of *Lactobacillus casei*, *L. delbrueckii*, *L. brevis*, *L. plantarum* and *L. fermentum* isolated from indigenous Nigerian fermented foods. The results show the development of strain-dependent antioxidant activity in the whey. These findings are suggestive of potential protective effects against oxidative damage by fermented products generated by these organisms. The tested lactobacilli may enhance the nutrition, shelf life and safety of fermented products.

References

1. G. Molin, Probiotics in foods not containing milk or milk constituents, with special reference to *Lactobacillus plantarum* 299v, *Am. J. Clin. Nutr.* (Suppl.), *73* (2001) 380–385.
2. V. Lei, M. Jacobson, Microbiological characterization and probiotic potential of Koko and Koko sour water, African spontaneously fermented millet porridge and drink, *J. Appl. Microbiol. 96* (2004) 384–397.
3. M. Kleerebezem, P. Hols, E. Bernard, T. Rolain, M. Zhou, R.J. Siezen, P.A. Bron, The extracellular biology of the lactobacilli, *FEMS Microbiol. Rev. 34* (2010) 199–230.
4. S. Anuradha, K. Rajeshwari, Probiotics in health and disease, *JIACM, 6* (2005) 67–72.
5. K. Ishida-Fujii, R. Sato, S. Goto, X.P. Yang, S. Hirano, M. Sato, Prevention of pathogenic *Escherichia coli* infection in mice and stimulation of macrophage activation in rats by oral administration of probiotic *Lactobacilus casei* 1–5, *Biosci. Biotechnol. Biochem. 71* (2007) 866–873.
6. S. Kapila, Vibha, P.R. Sinha, Antioxidative and hypocholesterolemic effect of *Lactobacillus casei* ssp. *casei* (biodefensive properties of lactobacilli), *Indian J. Med. Sci. 60* (2006) 361–370.

7. A.A. Osuntoki, O.R. Ejide, E.A. Omonigbehin, Antagonistic effects on enteropathogens and plasmid analysis of lactobacilli isolated from fermented dairy products, *Biotechnology*, *7* (2008) 311–316.
8. A.A. Osuntoki, E.A. Omonigbehin, S.O. Gbenebitse, U.E. Mojiminiyi, Prevention of salt induced hypertension in rats by oral administration of *Lactobacillus acidophilus* yoghurt, *Nig. Food J. 25* (2007) 185–190.
9. N.K. Lee, C.W. Yun, S.W. Kim, H.I. Chang, C.W. Kang, H.D. Paik, Screening of lactobacilli derived from chicken feces and partial characterization of *Lactobacillus acidophilus* A12 as an animal probiotics, *J. Microbiol. Biotechnol. 18* (2008) 338–342.
10. C.C. Ou, T.M. Lu, J.T. Tsai, J.H. Yen, H.W. Chen, M.Y. Lin, Antioxidative effects of lactic acid bacteria: Intact cells *vs.* intracellular extracts, *J. Food Drug Anal. 17* (2009) 209–216.
11. J. Sun, X.L. Hu, G.W. Le, Y.H. Shi, Lactobacilli prevent hydroxyl radical production and inhibit *Escherichia coli* and *Enterococcus* growth in system mimicking colon fermentation, *Lett. Appl. Microbiol. 50* (2010) 264–269.
12. T. Virtanen, A. Pihlanto, S. Akkanen, H. Korhonen, Development of antioxidant activity in milk whey during fermentation with lactic acid bacteria, *J. Appl. Microbiol. 102* (2007) 106–115.
13. N.R. Madamanchi, A. Vendrov, M.S. Runge, Oxidative stress and vascular disease, *Arterioscler. Thromb. Vasc. Biol. 25* (2005) 29–38.
14. C.C. Ou, J.L. Ko, M.Y. Lin, Antioxidative effects of intracellular extracts of yogurt bacteria on lipid peroxidation and intestine 407 cells, *J. Food Drug. Anal. 14* (2006) 304–310.
15. L.M. Sayre, G. Perry, M.A. Smith, Oxidative stress and neurotoxicity, *Chem. Res. Toxicol. 21* (2008) 172–188.
16. H. Abramovič, V. Abram, Effect of added rosemary extract on oxidative stability of *Camelina sativa* oil, *Acta. Agric. Slov. 87* (2006) 255–261.
17. R.V. Hedegaar, D. Kristensen, J.H. Nielsen, M.B. Frøst, H. Østdal, J.E. Hermansen, M. Kröger-Ohlsen, L.H. Skibsted, Comparision of descriptive sensory analysis and chemical analysis for oxidative changes in milk, *J. Dairy Sci. 89* (2006) 495–504.
18. O. Kandler, N. Weiss: Regular Non-Sporing Gram Positive Rods. In: *Bergey's Manual of Systematic Bacteriology*, *Vol. 2*, P.H.A. Sneath, N.S. Mair, J.G. Holts (Eds.), Williams and Wilkins, Baltimore, MD, USA (1986) pp. 1209–1234.
19. S. Son, B.A. Lewis, Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: Structure-activity relationship, *J. Agric. Food Chem. 50* (2002) 468–472.
20. B. Hernández-Ledesma, B. Miralles, L. Amigo, M. Ramos, I. Recio, Identification of antioxidant and ACE-inhibitory peptides in fermented milk, *J. Sci. Food Agric. 85* (2005) 1041–1048.
21. B. Bayés, M.C. Pastor, J. Bonal, A. Foraster, R. Romero, Oxidative stress, inflammation and cardiovascular mortality in haemodialysis-role of seniority and intravenous ferrotherapy: Analysis at 4 years follow-up, *Nephrol. Dial. Transplant. 21* (2006) 984–990.
22. S. Klayraung, S. Okonogi, Antibacterial and antioxidant activities of acid and bile resistant strains of *Lactobacillus fermentum* isolated from miang, *Braz. J. Microbiol. 40* (2009) 757–766.
23. Y. Duangjitcharoen, D. Kantachote, M. Ongsakul, N. Poosaran, C. Chaiyasat, Potential use of probiotic *Lactobacillus plantarum* SS2 isolated from a fermented plant beverage: Safety assessment and persistence in the murine gastrointestinal tract, *World J. Microbiol. Biotechnol. 25* (2009) 315–321.