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Isolation and Characterization of Lactic Acid Bacteria from Nigerian Fermented Foods and their Antimicrobial Activity

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The aim of this study was to investigate the *in vitro* antimicrobial activities of sixteen lactic acid bacteria (LAB) strains isolated from Nigerian fermented foods. The isolates showed strong activity against pathogenic bacteria, yeasts and moulds. The 16S rRNA gene sequencing and biochemical test reveal that the LAB strains belong to the genera *Lactobacillus and Leuconostoc*. *In vitro* antifungal activity was initially investigated by dual-culture plate method for yeasts and moulds, followed by agar well diffusion method using 10x concentrated cell-free supernatants (CFS) of the LAB isolates against all the target pathogens. Among these sixteen LAB isolates, *Lactobacillus delbrueckii* subsp. *bulgaricus* YML013 showed broad inhibitory spectrum against all the target pathogens, given it priority to be selected for further screening. Characterization of the selected broad-spectrum antifungal LAB isolate (*L. delbrueckii* subsp. *bulgaricus*YML013) revealed that, the antifungal activity was thermostable and the suspected compound(s) may be lipid in nature. Additionally, the assessment of the haemolytic activity and antibiotics susceptibility of the selected strain to the most commonly used antibiotics was also studied, and the results of all these screening suggestedthe LAB strain YML013 could be considered antimicrobial candidate against both microbial spoilage and enteric infections caused by pathogenic microorganisms.

Key words: Antimicrobial activity, Broad inhibition spectrum, Lactic acid bacteria, Nigerian fermented foods.

Spoilage is characterized by any change in a food product that renders it unacceptable to the consumer from a sensory point of view. This may be physical damage, chemical changes (oxidation, colour changes) or appearance of off-flavours and off-odours resulting from microbial growth and metabolism in the product. Microbial spoilage is by far the most common cause of spoilage and may manifest itself as visible growth (slime, colonies), as textural changes (degradation of polymers) or as off-odours and off-flavours. Despite chill chains, chemical preservatives and a much better understanding of microbial food spoilage, it has been estimated that 25% of all foods produced globally lost*post-harvest*or*post-slaughter*due to microbial spoilage (Gram *et al*., 2002). Mycotoxin contamination of crops has been a worldwide problem for thousands of years; however, the significance of the mycotoxins present in foods, their effect on human or animal health and the impact on the economy has been assessed only over the last few decades (Moss, 2006). Hundreds of people in developing countries die every year after consuming grains contaminated with mycotoxins, which is a global concern as a health hazardmeanwhileplant fungal pathogens or food spoilage moulds are the major sourcesof mycotoxin production. In the same vein, the prevalent of enteric infections is increasing as a result of acquired resistance of pathogenic bacteria

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such as *Escherichia coli 0157:H7, Salmonella enterica* and others to antimicrobial drugs. World health leaders described antibiotic-resistant microorganisms as "nightmare bacteria" that "pose a catastrophic threat" to people in every country of the world (CDC, 2010). It has been discovered that about 2 million people acquire infections with antibiotic resistant bacteria in United States each year, and at least 23,000 people die as a result of these antibiotic-resistant infections (CDC, 2013). Over a decade many scientists have been working on lactic acid bacteria and proved them to be an alternative to chemicals and antibiotics used in preventing the outgrowth of both microbial spoilage and enteric infections caused by pathogenic organisms.

Lactic acid bacteria (LAB) are known for their biopreservative action and may harbour both antibacterial and antifungal properties (Sarah *et al*., 2013; Adebayo and aderiye, 2011; Guo*et al*., 2011). LAB are naturally occurring in many food systems (Marilley and Casey, 2004; Tamminen et al., 2004; Mitra *et al*., 2005) and have been used for centuries for their fermentation and preservative properties. Protection of foods from spoilage and pathogenic microorganisms by LAB is through the production of organic acids and hydrogen peroxide (which reduce the pH of the medium), diacetyl, bacteriocins and other compounds as metabolites and therapeutic agents (Savadago*et al*., 2004; Mandal *et al*., 2009; Adebayo and Aderiye*et al*., 2010). In particular, theability of LAB to produce antibacterial peptides or bacteriocins has been discovered by many researchers (Cotter *et al*., 2005; Drider*et al*., 2006; Voulgari*et al*., 2010). Several low molecular weight compounds have been isolated with the ability to eliminate fungal growth either on their own or synergistically, which involves; organic acids (Cabo *et al*., 2002; Lavermicocca *et al*., 2003; Lind *et al*., 2007), phenyllacticacid (Lavermicocca *et al*., 2000; Gerez *et al*, 2009), fatty acids (Strom *et al*., 2002; Sjogren *et al*., 2003), proteinaceous compounds (Gerez *et al*., 2013; Rather *et al*., 2013),benzeneactic acid, 2-propenyl ester (Wang *et al*., 2012)and cyclic dipeptides (Yang and Chang, 2010). Thus, LAB isolated from traditional food starters and the bacteriocins they produced may be considered to be safe agents for preventing undesirable growth of pathogenic microorganisms (Yang *et al*., 2012).

This research evaluated the antimicrobial activity of lactic acid bacteria isolated from selected Nigerian fermented foods against foodborne pathogenic bacteria and fungi.

MATERIALS AND METHODS

Sample collection and preparation

Food samples such as Gari, Eba, Fufu from Cassava and Ogi from maize and sorghum used for this study were purchased from Nigerian women in Ibadan, South west of Nigeria in December 2012. The samples were collected in plastic bags and transported in box with ice packs to appliedMicrobiology and Biotechnology laboratory, Yeungnam University, South Korea for microbiological analysis.

Isolation of LAB strains

The samples were homogenized with autoclaved distilled water and serially diluted to 10^6 while dilutions 10^4 and 10^5 were chosen. The spread plate method was used for the isolation by aseptically transferring 100 µl of each dilution into set deMan Rogosa Sharpe (MRS) agar plate and incubated anaerobically for 24 h at 37 °C. After incubation, colonies were randomly selected and sub-cultured on MRS agar plates three times to obtain pure colonies.

Target organisms

The foodborne pathogenic bacteria and the moulds used in this research are KCTC, ATCC and KCCM strains (ST 1). Nutrient broth (NB) and brain heart infusion (BHI) broth were used for culturing bacteria at 37 °C for 24 h, while moulds and yeasts were grown on potato dextrose agar (PDA) and yeast extract peptone dextrose agar (YPDA), respectively at 25 °C for 7 days (Rather*et al.*, 2013).All the media used are Difco Laboratories products with high purity standard.

Antimicrobial activity by dual-culture assay

LAB isolates were screened for antifungal activity using a dual-culture overlay assay. LAB strains were inoculated in 2-cm lines on MRS agar plates and allowed to grow at 37 °C for 24 h anaerobically. The plates were then overlaid with 10 ml of YPD soft agar, (1% agar); containing 10^4 yeast cells or mould spores per ml. After 48 h of anaerobic incubation at 30 °C, the zone of inhibition was measured. The inhibition was graded by relating the inhibited growth area per inoculation

streak to the total area of the petri dish(Magnusson *et al*., 2003).

Preparation of cell free supernatants

The cell free supernatant was prepared as described previously (Rather *et al.*, 2013). 1 ml of cell free supernatant (CFS) was pipette into 1.5 ml eppendorf tube and concentrated by freeze-drying to achieve 10x concentration, 100 µl of autoclaved distilled water was added to each concentrated supernatant.

***In vitro* antimicrobial activity assay by agar well diffusion method**

Determination of antimicrobial activity of the isolated LAB strains wascarried out using agar well diffusion assay (Rather *et al.*, 2013). The antimicrobial effect was recorded by measuring the zones of inhibition around the well. Each assay was performed in triplicate to calculate intra-assay variation.

Co-cultivation method

In order to study the complex activity mediated by YML013,10x CFS of*L. delbrueckii* sub sp. *bulgaricus* YML013 was co-cultivated with (10^4) fungal spores of *A. flavus* in 250 mlconical flask containing 100 ml potato dextrose brothusing the following ratio 1:1, 1:2 and 1:3 %.In the control experiment, only1% of(10^4) fungal spore suspension of *A. flavus* was added to the PDB. The flasks were incubated at 30 °C for 48 h. The dry weight of the myceliawas determined by filtering the culture using, and then dried at 70 °Cin a hot air oven to a constant weight. The percentage of inhibition was determined by comparing the growth of the control with the treated fungus using the formula: GI (%) = C0 – CF / Co x 100 (Hoda*et al*., 2011). Where, C0 was the dry weight of (control), and CF represented the dry weight of treated fungal mycelium.

Growth of*L. delbrueckii* subsp.*bulgaricus* YML013

The growth of the isolate was studied by measuring optical density (OD) at » = 600 nm andpH value.Optical density was determined using spectrophotometer (U-2000, Hitach Ltd, Tokyo, Japan) and pH of the isolate using pH meter (Mettler-Toledo group, Switzerland). Measured values of optical density and pH were plotted on growth curves.

Characterization of the antimicrobial compounds

The CFS of YML013was subjected to different treatments in order to determine the nature of the antimicrobial compound(s): exposure to varying degrees of temperatureand subjected to the action of the following enzymes: catalase, ±-amylase and lipase (with 1ml of 50 mM sodium phosphate pH 7.0), proteinase K (with 1 ml of 100 mM sodium phosphate pH 8.0) and trypsin (with 1 ml 50mM Tris HCL pH 8.0).This procedure was used for enzyme test;briefly 1 mg of each enzyme/ml of CFS was incubated 3 h at 37 °Cfollowed by heat inactivated for 5 min at 100 °C.Buffer solution, enzyme and 10x CFS served as controls. The antimicrobial activity of treated 10x CFS was determined by agar well diffusion assay as described above. All enzymes used are the product of Sigma-Aldrich, St. Louis, MO, USA.

16S rRNA gene sequencing and biochemical characterization

The isolates showing highest antimicrobial activity after the screening were identified by 16S rRNA gene sequencing. The sequences were compared with those in GenBank at the National Centre for Biotechnology Information (NCBI) using the BLAST program. While *L. L. delbrueckii* subsp. *bulgaricus*YML013 was also characterized biochemically using the API 50 CH strip and API CHL medium system according to the manufacturer's instructions (API bioMerieux, Durham, NC, USA) and as described earlier (Rather *et al*., 2013).

Haemolysis test

L. delbrueckii subsp. *bulgaricus*YML013 was cultured in MRS broth and then spot(5 µl)on tryptone soya agar (Oxoid) with an addition of 5% sheep blood for haemolytic activity tests. The plates were incubated anaerobically at 37 °C for 48 h, and then examined for the heamolytic reaction.Haemolytic activity was detected as the presence of a clear zone aroundbacterial colony. Strains that produce green-hued zones around the spots (α-haemolysis) or do not produce any effect on the blood plates (γ- haemolysis) are considered non haemolytic(Maragkoudakis *et al*., 2009). Strains displaying blood lysis zones around the spots were classified as haemolytic (β- haemolysis).

Antimicrobial susceptibility test

The antimicrobial susceptibility assay was carried out according to ISO, 2012 and European Food Safety Authority (EFSA) guidelines (ET ISO, 2012; EFSA, 2012)). According to EFSA

guidelines the following antibiotics were test in the concentration rages (mg/l) given in parenthesis : gentamicin (0.5 to 256 mg/l; Tokyo chemical industry co; Ltd, Tokyo, Japan), kanamycin (2 to 1024 mg/l;Biopure reagent, Seoul Korea), streptomycin (0.5 to 256 mg/l;Tokyo chemical industry co; Ltd, Tokyo, Japan), tetracycline (0.125 to 64 mg/l; Tokyo chemical industry co; Ltd, Tokyo, Japan), erythromycin (0.016 to 8 mg/l; Tokyo chemical industry co; Ltd, Tokyo, Japan), clindamycin (0.032 to 16 mg/l; Sigma-Aldrich, St. Louis, MO, USA), chloramphenicol (0.125 to 64 mg/l; Sigma-Aldrich, St. Louis, MO, USA), and ampicillin (0.032 to 16 mg/l;Biopure reagent, Seoul Korea). Irrespective of the bactericidal or bacteriostatic mechanism of the tested agent, the MIC was defined as the lowest antimicrobial concentration for which at least 80% visual reduction in growth was reported (Geert *et al*., 2010). The test was conducted in triplicate.

RESULTS AND DISCUSSION

About 44% of the isolates were isolated from maize Ogi, 25% from sorghum Ogi, 12.5% from Eba (cassava), 12.5% from Gari (cassava), while the remaining 6.25% was isolated from Fufu (cassava).*Lactobacillus fermentum* was the predominant strain followed by *Leuconostoc latis* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. After comparing the strength of inhibition among 16 strains, strain YML013 was selected. The strain was identified and named *L. delbrueckii* subsp. *bulgaricus* YML013after 16S rRNA gene sequencing. The molecular identification of YML013 was also supported by the biochemical analysis (Table 4). The API CHL 50 system was used to assess the metabolic profile of the new isolate YML013. Carbohydrates could be fermented by strain YML013 were D-ribose, D–glucose, D-mannose, N-acetylglucosamine, Esculin, D-lactose (bovine origin),D–trehalose and Potassium 5–ketogluconate. The carbohydrate fermentation profile was analyzed with the software apiweb (bioMerieux). The strain YML013 has 80.4 % identification. It shared 82.6 % metabolic profile similarity with *Lactobacillus subsp. bulgaricus* ATCC 11842.

Approximately 70% of LAB isolates showed antifungal activity (+) against all the targeted moulds.Broad inhibition zones were demonstrated by *L. fermentum* YME16 against *Aspergillus flavus* and *Aspergillusniger*.About 20% of the total number of screened isolates (*L.fermentum and Leuconostoc lactis*) had moderate activity (++) against *Penicillium expansum*, *Aspergillus flavus* and *Aspergillusniger*.On addition, no antifungal effect was shown by *L. delbrueckii* subsp. *bulgaricus*YML013 (10%)against any target pathogen, showing the inability of cell culture (live cell) of YML013 to produce antifungal compound. All the LAB strains demonstrated antimicrobial activity against the targeted organisms which ranged from mild to broad spectrum by agar well diffusion method. The different degrees of

Table 1. Antimicrobial activity of 10x cell free supernatant (CSF) of *L. delbrueckii* subsp. *bulgaricus* YML013

Target organisms	Source	Inhibition zone spectrum (mm)
Esherichia coli	0157:H7	31 ± 0.58
Staphylococcus aureus	KCTC 1621	32 ± 1.00
Bacillus subtilis	KCTC 1021	30 ± 1.15
Salmonella enterica	ATCC 29628	28 ± 1.15
Listeria monocytogenes	KCTC3569	28 ± 0.00
Candida albicans	KCTC 17485	17 ± 1.15
Zygosaccharomyces rouxii	KCTC 7880	16 ± 0.58
Aspergillus flavus	KCTC 16682	24 ± 1.73
Aspergillus niger	KCTC 16683	20 ± 1.00
Penicillium expansum	KCTC 6434	22 ± 2.88

The measurements expressed in (mm) are the mean of three replicates ± SD, and indicate a zone of inhibition around the well.

Table 2. Effect of temperature on *L. delbrueckii* subsp. *bulgaricus*YML013

Temperature	Inhibition zone (mm)		
^{0}C	15 min	30 min	60 min
50	24	22	23
70	24	22	24
100	24	24	24
121	24	-	-

Heating of CFS of YML013 with varying temperature and time, showed no significant different in the antifungal activity.

inhibition zones were expressed in mm (diameterof inhibition zone). In this test, zones of inhibition produced were found in the range of 10 to 35 mm (Table 1). *L. delbrueckii* subsp *bulgaricus* YML013 exhibited the highest level of inhibitory effect against all the targeted organisms, being considered and selected for further screening.

10x CFS of *L. delbrueckii* subsp *bulgaricus* YML013was very effective in reducing fungal growth of *A. flavus* (Fig.1).The CFS of YML013 caused a great reduction in the mycelia mass of *A. flavus*.The percentage of inhibitionof YML013ranged from 55 to 77% based on the

Table 3. Effect of enzymes on *L. delbrueckii* subsp. *bulgaricus*YML013

Indicator strain	YML013 + Enzyme	Inhibition zones(mm)
Aspergillus flavus KCTC 16682	10x CFS without enzyme	24
	Catalase	24
	α-amylase	24
	Proteinase K	24
	Lipase	-
	Trypsin	24

percentage of CFS used. Comparing treatment with the control, more than 75% of *A. flavus* mycelium was inhibited by 10x CFS of YML013 in vitro.
The log phase of *L. delbrueckii* subsp. *bulgaricus* YML013 was experienced 4 x 10^8 at 18 h and started decreasing after 22 h and the pH values of YML13 ranged from 6 to 3.65 (Fig. 2) .

The antifungal activity of 10x CFS of *L. delbrueckii* subsp. *bulgaricus* YML013 remained unchanged after heating to varying degrees of temperature, which provedthe heat stability of YML013(Table 2). The result of enzyme test showed that *L. delbrueckii* subsp. *bulgaricus* YML013 possibly to produce lipid-like compounds as indicated by loss of activity after treatment with lipase(Table3).While no significant differences were observed in antifungal activity of YML013from the other enzymes tested.

After streaking the YML013 on tryptone soy agar with sheep blood and incubation at 37 °C for 48 h,no β-haemolysis(clear zones around colonies) was observed onthe plate agar.The results of this experiment showed non–haemolytic.YML013did not induce hemolysis as the agar under and around the colony remained unchanged after the incubation period,i.e., YML013 had no effect on the blood plate and considered γ-haemolytic (picture not shown).

The MIC values obtained for *L. delbrueckii* subsp.*bulgaricus* YML013 are given in Table 5. The YML013 was considered sensitive to a particular antibiotic used, when the MIC (mg/l) values obtained were lower or equal to the recommended breakpoint value defined at species level by the FEEDAP Panel (EFSA, 2012).YML013 was sensitive to all the antimicrobial agents used. In recent years, interest in lactic acid bacteria (LAB) has increased due to their probiotic and antimicrobial properties suggesting them to be a promising alternative to chemical preservatives in food and feed industries (Yang and Chang *et al*., 2010).In this study, a total of 16 LAB strains were isolated from Nigerian fermented foods; which were identified by 16S rRNA gene sequencing analysis and YML013 was further characterized biochemically using the API 50 CH strip and API CHL medium system. *L. fermentum* was the mostdominant LAB strain found in all the analyzed samples. However, *Lc. lactis* and *L. delbrueckii* subsp. *bulgaricus* were also found in Ogi and Eba fermented samples. Similar results on the presence of these LAB strains have been reported in various

Table 4. API50 CH of *L. delbrueckii* subsp. *bulgaricus* YML013

Active ingredients	Results
Control	-
Glycerol	-[1]
Erythritol	-
D- arabinose	-
L- arabinose	-
D - ribose	+[2]
D - xylose	-
L - xylose	-
D - adonitol	-
Methyl-β-D - xylopyranoside	-
D - galactose	-
D - glucose	+
D - fructose	-
D - mannose	+
L - sorbose	-
L - rhamnose	-
Dulcitol	-
Inositol	-
D - mannitol	-
D - sorbitol	-
Methyl – a- D - mannopytanoside	-
Methyl – a- D - glucopyranosside	-
N-acetylglucosamine	+
Amygdalin	-
Arbutin	-
Esculin	+
Salicin	-
D - cellobiose	-
D - maltose	-
D – lactose (bovine origin)	+
D - melibiose	-
D - saccharose	-
D - trehalose	+
Lnulin	-
D - melezitose	-
D - raffinose	-
Amidon (starch)	-
Glycogen	-
Xylitol	-
Gentiobiose	-
D - turanose	-
D - lyxose	-
D - tagatose	-
D - fuccose	-
L - fuccose	-
D - arabitol	-
L - arabitol	-
Potassium Gluconate	-
Potassium 2 - ketogluconate	-
Potassium 5 - ketogluconate	+

[1] Un - used carbohydrate; [2] Used carbohydrates; ID: 80.4 %; *L. delbrueckii* subsp.*bugaricus*

Table 5. Antibiotic susceptibility test of *L. delbrueckii* subsp. *bulgaricus*YML013 with MIC values (mg/l)

Antibiotics		*L. delbrueckii* subsp. *bulgaricus*YML013						= MIC	<mic	mic	>mic	(mg/l)
	1[a]	2	3	4	5	6	7	8	9	10	11	12[b]
	Antibiotic concentration from 0.016 to 256 mg/l											
Gentamycin	P	0.5	1	2	4	8	16	32	64	128	256	N
Kanamycin	P	2	4	8	16	32	64	128	256	512	1024	N
Streptomycin	P	0.5	1	2	4	8	16	32	64	128	256	N
Tetracycline	P	0.125	0.25	0.5	1	2	4	8	16	32	64	N
Erythromycin	P	0.016	0.032	0.063	0.125	0.25	0.5	1	2	4	8	N
Clindamycin	P	0.032	0.063	0.125	0.25	0.5	1	2	4	8	16	N
Chloramphenicol	P	0.125	0.25	0.5	1	2	4	8	16	32	64	N
Ampicillin	P	0.032	0.063	0.125	0.25	0.5	1	2	4	8	16	N

[a] Positive Control well without antibiotics, but with the test strain containing solvent which is used to dissolve the antibiotics at the highest concentration; [b] Negative Control well without the test strain and the antibiotic, but with the medium.

fermented foods by others Nigerian scientists (Adebayo and Aderiye, 2010; Adebayo and Aderiye, 2007) with antimicrobial activities.

In dual-culture overlay assay, it was discovered that the cell culture of all the LAB isolates showed moderate inhibitory activity against entire group of targeted fungal pathogens. However, the cell culture of *L. delbrueckii* subsp. *bulgaricus* YML013 had adherent effect against the targeted fungal pathogens, this showedthe inability of cell culture of YML013to inhibit the pathogenic organisms meanwhilethe CFS of the same strain showed broad spectrum of inhibitory effect against all the targeted pathogens.

In addition, co-cultivation technique confirmed the effectiveness of 10x CFS of *L. delbrueckii* subsp. *bulgaricus* YML013 against *Aspergillus flavus*. In the agar well diffusion method; *L.delbrueckii* subsp. *bulgaricus* exhibited high level of zone of inhibition against all the targeted organisms. Therefore,*L. delbrueckii* sub sp. *bulgaricus*YML013 was recognized as the most effective strain among all the strains of LAB isolates.These results were concordant to the previously reported study (Tufail*et al.*, 2011; Zalan*et al.*, 2011). The growth of *L. delbrueckii* subsp.*bulgaricus* YML013 reached exponential phase after 18 h of incubation with 4 x 10^8 CFU/ml and stationary phase was observed after 26 h of incubation. The pH of the LAB isolate YML013 ranged from 6 at lag phase to 3.65 at both log and stationary phase. Furthermore, the strain YML013produced maximum antimicrobial activity during exponential and stationary phases, this suggested the production of secondary metabolites and these findings were in agreement with the study of Sathel*et al.* (2007).The haemolysis test of*L. delbrueckii* subsp. *bulgaricus*YML013 had no effect on the agar plate in accordance with the previous work (Maragkoudakis *et al.*, 2009; Sandra *et al.*, 2012; David *et al.*, 2012). This made YML013 a good candidate to serve as probiotic. In

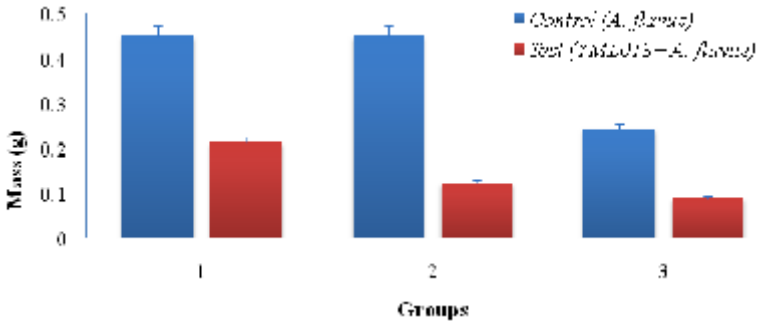

Fig. 1. Effect of *L. delbrueckii* subsp.*bulgaricus*YML013 on dry mass (g) of *Aspergillus flavus* after 48 h of co-cultivation with varying percentages

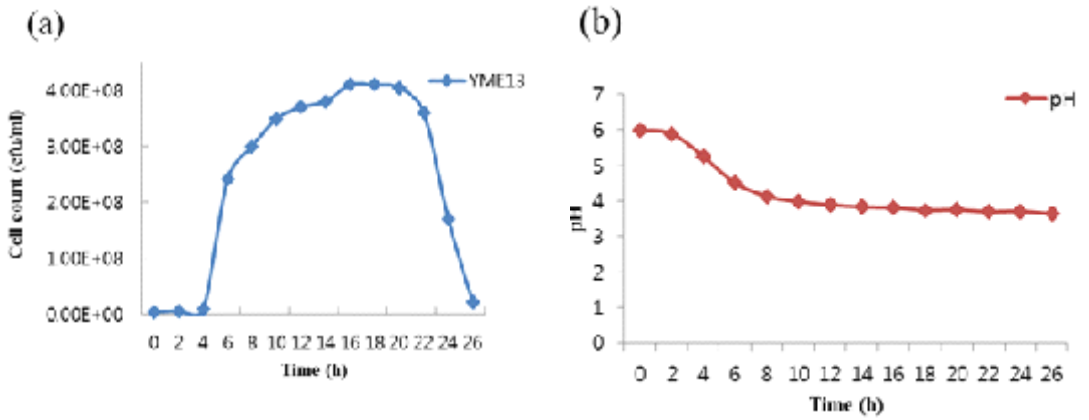

Fig. 2. Growth curve (a) and pH (b) of *L. delbrueckii* subsp. *bulgaricus*YML013 after 26 h of incubation

addition, YML013was sensitive against all antibiotics used.These results werein contrary with previous study (Maragkoudakis *et al.*, 2009)where low resistance of LAB was recorded against clindamycin and gentamycin.Moreover the effect of enzyme and heat treatment were also examined on the antagonistic activity of *L. delbrueckii* subsp. *bulgaricus* YML013against *Aspergillus flavus* in other to determine the nature ofantimicrobial compound(s) present in the LAB isolateYML013. Addition of various enzymes to 10x CFS of YML013against *A. flavus* had no effect on its antifungal activity, but the activity was lost by the addition of lipase which showed that YML013may produce biologically active compounds of lipid nature (Strom *et al.*, 2002; Sjogren *et al.*, 2003). This indicates that the inhibition observed was not due to hydrogen peroxide, nor peptide or carbohydrate. Also, the antifungal activity of 10x CFS of *L. delbrueckii* subsp. *bulgaricus* YML013was stable across a varying temperature range with similar supportive study previously conducted by others (Katerina *et al*., 2007; Flora *et al.*, 2013).

The results obtained in this research work demonstrated potential LAB strain isolated from Nigerian fermented foods (Cassava and Ogi) which significantly inhibited various pathogenic bacteria, yeasts and moulds *in vitro.* The LAB strain YML013 showed no haemolytic activity and absence of antibiotic characteristics (Sandra *et al*., 2012; Katerina *et al*., 2007).

In conclusion, LAB are generally recognized as safe (GRAS), therefore, it is supported that LAB or LAB derived compounds may serve as possible alternative for practical application in controlling various pathogenic microorganisms. Further investigations on nature and mode of action of YML013 and its biologically active compound (s) are in progress in order to confer their applications as a potential probiotic and a good biopreservative agent for controlling of microbial spoilage and enteric infections in the future.

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