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Characterization of a Broad-Spectrum Bacteriocin Produced by a Probiotic *Lactobacillus plantarum* and Assesment of the Strain's Stability in Palm Kernel Oil

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Abstract: The present investigation reports the characterization of the bacteriocin produced by the probiotic strain *Lactobacillus plantarum* 29V isolated from raw cow milk in the Western's highlands of Cameroon as well as the viability of this strain in the palm kernel oil. The antimicrobial compound synthesized by *Lactobacillus plantarum* 29V was sensitive to some proteolytic enzymes. It showed remarkable stability at high temperatures and in the presence of organic solvents, detergents and surfactants. It was also active in pH 2.0-10 and NaCl range of 1-7%. The neutralized cell-free supernatant of this bacterium inhibited the growth of several *Lactobacillus* sp., pathogenic and food spoilage microorganisms. The results of this study showed that palm kernel oil maintains the viable cell numbers of the probiotic strain *Lactobacillus plantarum* 29V, without any changes of peroxide and acid indexes of palm kernel oil.

Key words: Bacteriocin, probiotic, *Lactobacillus plantarum*, palm kernel oil, organic solvent

INTRODUCTION

The preservation of foods by natural and microbiological methods may be a satisfactory approach to solve economic losses due to microbial spoilage of raw materials and food products as well as to reduce the incidence of food borne illnesses (Galvez *et al*., 2008). The increasing demand for safe foods, with low level of chemical additives, has increased the interest in replacing these compounds by natural products which are not harmful to the host or the environment. Thus, biopreservation of food has emerged as an attractive and safe approach. The preserving effects of lactobacilli are due to the production of antimicrobial substance, such as organic acids, hydrogen peroxide and bacteriocins or related substances (Cocolin *et al*., 2007). Although, many bacteria can produce bacteriocins, those produced by lactobacilli are of particular interest to the food industry, since these bacteria have GRAS (Generally Regarded as Safe) status. Several bacteriocins from gram positive bacteria display bactericidal activity with fairly broad inhibitory spectra and may be useful as antibacterial agents for various practical applications (Lemos *et al*., 2008). The increasing interest in bacteriocins has stimulated the isolation of lactobacilli producers and the characterization of many novel peptides (Deraz *et al*., 2005). It is well documented that dairy products serve as best matrix for probiotic bacteria. However, a trend of non-dairy probiotics is growing due to some issues in dairy probiotics. The dairy matrix may contain potential allergens, such as casein and cold storage during the shelf life is required. Therefore, the demand for new non-dairy matrices and the trend of vegetarianism are increasing (Ranadheera *et al*., 2010). Also, producing probiotic products with foods and beverages which are part of the day-to-day life is encouraged. This leads to increased demand for non-dairy probiotic foods. In the development of novel probiotic products, the fermentability of the matrix should be considered since it can lead to an increase of the viable cell concentration. The expectation is to have a healthy product which can not induce lactose intolerance and allergy due to milk protein (Granato *et al*., 2010; Rivera-Espinoza and Gallardo-Navarro, 2010). Also, we are hoping to characterize a new food matrix which can carry probiotic culture at effective levels, in other to be used in developing countries since the storage conditions of already characterized food matrixes are sometimes inadequate. Therefore, it would be interesting to find other local food matrix for probiotics. Palm Kernel

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Oil (PKO) has large distribution in Southern Asia, Sub-Saharan Africa and South America countries and may be important as new matrix for functional food items. PKO and its hydrogenated and fractionated products are widely used either alone or in blends with other oils for the manufacture of food products. The non-food uses of PKO are very substantial and becoming of increasing importance because palm kernel oil and it's by products have many applications in pharmaceutical and cosmetic industries.

The present study reports the characterization of the bacteriocin produced by a probiotic strain *Lactobacillus plantarum* 29V isolated from raw cow milk from the Borroro's cattle breeders in the Western highlands of Cameroon as well as the assessment of the viability of this functional strain in the palm kernel oil.

MATERIALS AND METHODS

Microorganisms: The strain *Lactobacillus plantarum* 29V used in this study was isolated from raw cow's milk during the period October to December, 2009. This strain was identified in the previous study on the basis of its morphological, carbohydrate fermentation profile by API 50CH kit and RAPD-PCR (Sieladie *et al*., 2011). Pathogenic indicator strains, such as *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (*MDR*, clinical isolate), *Enterococcus faecalis* ATCC 10541, *Escherichia coli* (MDR, clinical isolate), *Salmonella enterica* ssp. *enterica* serovare Typhi ATCC 6539, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* (clinical isolate) and *Klebsiella pneumoniae* (clinical isolate) were grown in tryptone broth at 37°C. All clinical isolates were obtained from the Centre Pasteur of Yaounde, Cameroon.

Antimicrobial activity detection and assay: The production of antimicrobial substance was detected using the triple-agar layer method (Todorov and Dicks, 2005). An overnight culture of the *L. plantarum* strain was spotted onto the surface of an MRS (Biolife, Italy) plate. Spotted plates were overlaid with a second layer of MRS Agar. The plates were incubated at 37°C (Incubator, Techmel TT9052, USA) for 48 h and then overlaid with active growing sensitive cells of *L. plantarum* 5S (10^8 cfu mL^{-1}), imbedded in a thin layer (7 mL) of soft MRS (MRS with 7.5% (w/v) agar). After anaerobic incubation during 24 h at 30°C, the bacterial lawn was examined for zones of inhibition surrounding producer colonies. Inhibition was recorded as positive if the width of the clear zone around the colonies of the producer was 2 mm or larger (Todorov and Dicks, 2005).

Screening of bacteriocin activity: For bacteriocin bioassay, the producer strain (*L. plantarum*) was grown anaerobically in MRS broth at 37°C for 6 h. Bacterial cells were removed by centrifugation (4000 g, 30 min, 4°C) to obtain cell-free supernatant. The supernatant was neutralized using 6 mol L^{-1} NaOH and heated at 80°C for 10 min. Soft MRS agar seeded with overnight cultures of the indicator strain of *L. plantarum* 5S (10^8 cfu mL^{-1}) was dispensed onto pre-poured MRS agar plates and 6 mm wells were punched after solidification. The wells were sealed at the bottom with sterile non-seeded agar. About 50 μL aliquots of sterile neutralized supernatant were placed into the agar wells in duplicates for each test. The plates were then incubated for 48 h at 37°C. Sterile un-inoculated MRS broth was used as control.

Bacteriocin activity: The bacteriocin activity was determined by the critical dilution method as described by Biswas *et al*. (1991). Neutralized cell-free supernatant of the *L. plantarum* strain grown in MRS broth at 37°C for 24 h was serially diluted (1:2-1:128) using sterile distilled water. A 10 μL portion in duplicate from each dilution was spotted directly onto MRS agar plates which were previously overlaid with soft MRS agar medium seeded with *L. plantarum* 5S cells (10^8 cfu mL^{-1} approximately). The plates were incubated at 37°C for 24 h and examined for zones of inhibition. The highest dilution that produced a clear zone of inhibition was multiplied by 100 (1mL/10μL) to obtain the arbitrary units per milliliter (AU mL^{-1}).

Effects of enzymes on bacteriocin activity: Sensitivity to proteolytic enzymes was checked when bacteriocin sample was treated with trypsin (Fluka Biochemika, Buchs, Switzerland) in 0.05 M Tris-HCl buffer, pH 8.0; Proteinase K (Merck, Darmstadt, Germany) in 0.05 M phosphate buffer, pH 7.0; α-Amylase (Sigma-Aldrich, Steinheim, Germany) in 0.05 M phosphate buffer, pH 7.0; lipase (Sigma-Aldrich, Steinheim, Germany) in 0.05 M phosphate buffer, pH 7.0 and lysozyme (Fluka Biochemika, Buchs, Switzerland) in 0.05 M phosphate buffer, pH 7.0. About 50 μL of the partially purified bacteriocin was lyophilized and resuspended in enzymes solutions at 1 mg mL^{-1} final concentration. Incubation was held at 37°C for 1 h. After incubation, the enzymes were denatured by heating the samples at 80°C for 10 min and the residual bacteriocin activity was determined as described before.

Effects of pH, temperature, NaCl and chemicals on bacteriocins activity: The effect of pH on the bacteriocin was determined by resuspending the lyophilized partially

purified bacteriocin into buffer solutions (50 mM acetate buffer, pH 2.0 and 3.0; 50 mM potassium phosphate buffer, pH 4.0, 5.0, 6.0 and 7.0 and 50 mM Tris-HCl buffer, pH 8.0; 9.0 and 10.0. After 2 h of incubation at room temperature, the samples were readjusted to pH 6.5 with sterile 6 moL L^{-1} HCl or 6 moL L^{-1} NaOH and the activity determined as described previously (Tiwari and Srivastava, 2008). The effect of SDS, tween 20, tween 80, urea and triton X-100 was tested by suspending lyophilized partially purified bacteriocin in each of 1% (v/v or w/v) chemical solutions. NaCl was used at the final concentrations of 7% (w/v). After 5 h of incubation at room temperature the activity was determined. The effect of temperature on the bacteriocin was tested by heating the partially purified bacteriocin suspension at 80°C for 10 min, 100°C for 30, 60, 90 and 120 min and 121°C for 15, 20 and 30 min (Todorov and Dicks, 2005). After each treatment, residual bacteriocin activity was determined.

Growth rate and bacteriocin production: To evaluate the growth of strain *L. plantarum* 29V in MRS broth at 37°C, a turbidimetric method was used (Ignatova *et al*., 2009). Bacterial growth was measured as the absorbance of cell suspensions at 600 nm using UV/VIS spectrophotometer (Shimadzu UV-1208) at 0, 2, 4, 6, 8, 10 and 12 h. For determining bacteriocin production in relation with incubation time, the strain was inoculated into MRS broth and incubated at 37°C for 12 h. The samples were aseptically withdrawn, in duplicates from the culture media at 2 h intervals throughout the incubation period. The bacteriocin activities of each sample were recorded and the results were compared.

Assessment of the viability of the bacteriocin-producing *L. plantarum* 29V strain in Palm Kernel Oil (PKO): The Palm Kernel Oil samples (PKO) were purchased in the local market. Before use, it was heated at 63°C for 30 min and introduced in 100 mL sterile vials. In a separate experiment, *L. plantarum* culture were propagated twice in MRS broth and incubated at 37°C for 16 h. While 70 mL of broth media were inoculated with 10% overnight, activated culture of strain *L. plantarum* 29V. The growth of the culture in broth media was monitored by UV/VIS spectrophotometer (Shimadzu UV-1208) for 6 h. At the early stationary growth phase, bacterial cells were harvested from the media by centrifugation of the culture (4000 g, 30 min, 4°C). The pellet was then washed twice with 0.01 mol L^{-1} potassium phosphate buffer pH 7.0. The resultant pellet was suspended in 100 mL sterile potassium phosphate buffer 0.01 mol L^{-1} and stored at −20°C until used. The viable cell count of this suspension was determined by enumeration on MRS plate agar. About 100 μL of this concentrate (10^8 CFU mL^{-1}) was thoroughly homogenized and introduced into 100 mL of PKO. Non-inoculated PKO samples were used as control. Inoculated and non-inoculated PKO samples were stored at 25°C (room temperature) and 4°C, respectively for 30 days. From the inoculated PKO samples, an aliquot of 10 mL was taken at 10 days interval for viable cell counts of *L. plantarum*. Ten-fold serial dilutions were done in 0.1% DMSO (in physiological saline solution) while homogenizing by vortexing, followed by viable cell count on MRS agar using the pour plate technique. Plates were incubated anaerobically at 37°C for 48 h.

The acid and peroxide indexes of PKO samples were determined according to standard NFT60-204 of the French Association for Standardization. These analyses were performed at 10 days interval.

Statistical analyses: Concerning the physico-chemical characteristics of PKO samples, data representing means and standard deviation of three independent experiments were subjected to Analysis of Variance (ANOVA) and multiple comparisons were done by Student-Newman-Keuls test, using the software GraphPad InStat (GraphPad Software Inc, V3).

RESULTS AND DISCUSSION

Screening of bacteriocin activity: The zones of inhibition observed in initial screening using triple-agar layer test and agar well diffusion assay showed the presence of antibacterial activity against indicator strain *L. plantarum* 5S. The neutralized cell-free supernatant of *L. plantarum* 29V culture demonstrated inhibitory activity of 1600 AU mL^{-1} which decreased to 800 AU mL^{-1} after partial purification using 60% ammonium sulfate precipitation. This is in agreement with the findings of Todorov *et al*. (2004) reporting the loss of plantaricin ST31 activity from 6.4×10^5 to 2.5×10^5 AU mL^{-1} after 60% ammonium sulfate precipitation. Since, inhibition was observed when the pathogens were grown in the presence of near-neutral supernatant (pH 6.5), inhibition effects could not be attributed to organic acids production but probably to hydrogen peroxide or bacteriocin like substances production. However, the antimicrobial activity of the inhibitory substance appeared unrelated to hydrogen peroxide as their activity was not lost after triple-agar layer test (under anaerobic conditions).

Effects of enzymes, pH, temperature, NaCl and chemicals on bacteriocins activity: The sensitivity of the inhibitory substance to trypsin, proteinase K, α-amylase, lysozyme

Table 1: Effect of enzymes, temperature and pH on the activity of the bacteriocin produced by strain *L. plantarum* 29V

	Bacteriocin produced by strain *L. plantarum* 29V		
Treatments	Activity (mm)	Activity (AU mL^{-1})	Residual activity (%)
Control	8	800	100.0
Effect of enzymes			
Trypsine	0	0	0.0
Proteinase K	0	0	0.0
Amylase	7	800	87.5
Lipase	8	800	100.0
Lysozyme	7	800	87.5
Heat treatment			
80°C, 10 min	8	800	100.0
100°C, 30 min	8	800	100.0
100°C, 60 min	6	400	75.0
100°C, 90 min	6	400	75.0
100°C, 120 min	4	200	75.0
121°C, 15 min	6	400	75.0
121°C, 20 min	6	400	75.0
121°C, 30 min	4	200	50.0
Effect of pH			
2	8	800	100.0
3	8	800	100.0
4	8	800	100.0
5	8	800	100.0
6	8	800	100.0
7	8	800	100.0
8	8	800	100.0
9	8	800	100.0
10	8	800	100.0

Residual activity was expressed as the percentage of diameter of inhibition zone of the test by the diameter of inhibition zone of the control

and lipase was determined in controlled and reproducible conditions as shown in Table 1. The inhibitory substance was fully inactivated by proteolytic enzymes. α-amylase, lysozyme and lipase had no effect on the activity thus confirming its proteinaceous nature. This bacteriocin (named P29V) was not inactivated by amylase or lipase indicating that it lacks carbohydrate and lipid moieties. The bacteriocins produced by this group of bacteria are considered as potent bio-preservative agents and their application in food is currently the subject of extensive research (Mojgani *et al*., 2009). According to Fricourt *et al*. (1994), lactic acid bacteria synthesize bactericidal agents that vary in their spectra of activity. Many of these agents are bacteriocins with a proteinaceous active moiety while others are non-protein agents (Saavedra *et al*., 2004). During the investigations, complete loss of the antagonistic activity after exposure to proteases, owing to the proteinaceous nature of the bacteriocins. Similar results were reported by Tiwari and Srivastava (2008) on plantaricin LR/14.

Results of the effect of temperature revealed that bacteriocin from *L. plantarum* 29V (P29V) showed strong heat stability as 75 and 50% activity could still be recorded upon treatment at 100°C for 120 min and 121°C for 30 min, respectively (Table 1). Bacteriocin from

Table 2: Effect of chemicals on the activity of the bacteriocin produced by strain *L. plantarum* 29V

	Bacteriocin produced by strain *L. plantarum* 29V		
Treatments	Activity (mm)	Activity (AU mL^{-1})	Residual activity (%)
Control	8	800	100.0
Effect of organic solvent			
Acetone	8	800	100.0
Acetonitrile	8	800	100.0
Chloroforme	8	800	100.0
Ethanol	8	800	100.0
isopropanol	8	800	100.0
Methanol	8	800	100.0
Effect of detergent and surfactant			
SDS	6	400	75.0
Triton X-100	8	800	100.0
Tween 20	8	800	100.0
Tween 80	8	800	100.0
Urea	8	800	100.0
Effect of NaCl (%)			
1	8	800	100.0
2	9	800	112.5
3	9	800	112.5
4	9	800	112.5
5	9	800	112.5
6	9	800	112.5
7	11	1600	137.5

Residual activity was expressed as the percentage of diameter of inhibition zone of the test by the diameter of inhibition zone of the control

L. plantarum F1 is reported to be stable at 121°C up to 10 min (Ogunbanwo *et al*., 2003). Plantaricin TF711 retained 70% activity after boiling and no activity upon autoclaving (Hernnadez *et al*., 2005). The retention of activity by P29V after heating at 121°C for 30 min, place it within heat stable group of bacteriocins. This quality of the bacteriocin makes it superior in processed food stuffs where high heat is applied.

The bacteriocin P29V produced by *L. plantarum* 29V was not sensitive to pH. It showed stable activity between pH 2.0 and 10.0. Similar results were reported by Hernnadez *et al*. (2005) on bacteriocins produced by *L. plantarum* TF711 on the one hand. On the other hand, the bacteriocins produced by *L. plantarum* F1 was active between pH 2.0 and 12.0 but with only 50 and 6.2% activity at pH 8.0 and 12.0, respectively (Ogunbanwo *et al*., 2003). P29V showed good stability against the tested solvents and surfactants (Table 2). Additionally, high stability of P29V was observed at different NaCl concentrations, similarly to the findings of Tiwari and Srivastava (2008) on plantaricin LR/14.

Growth rate and bacteriocin production: The strain *L. plantarum* 29V showed a typical sigmoidal growth response (Fig. 1), consisting of a short lag phase of ~2 h and reaching the stationary phase by 8-10 h. Bacteriocin production followed a growth-associated pattern. Production of bacteriocin occurred throughout logarithmic growth with the highest activity

recorded at the end of logarithmic and during stationary growth (8-10 h). Thereafter, antimicrobial activity decreased. These characteristics are common to most known bacteriocin producing lactic acid bacteria (De Kwaadsteniet *et al*., 2005). *Staphylococcus warneri* FM20 and *L. plantarum* TF711 showed similar trend of growth and bacteriocin production (Hernnadez *et al*., 2005). Such a decreased in bacteriocins production could be assigned to the sensitivity of bacteriocin to proteolytic enzymes secreted by the producer bacterium, given that the production of other bacteriocins, such as plantaricin TF711 by *L. plantarum* LR/14 was reported to be maintained after late log phase (Tiwari and Srivastava, 2008).

Bacteriocin spectrum of inhibitory activity: As shown in Table 3, growth inhibition was tested against pathogenic gram-positive and-negative bacteria. The crude preparation of cell-free neutralized supernatant of *L. plantarum* 29V culture displayed a broad spectrum inhibition. Interestingly among the strains checked, some are strictly food-borne pathogens. Bacteriocin P29V exhibited a wider spectrum of inhibition against gram positive and negative pathogens as also reported by Todorov *et al*. (2004). The potential of this bacteriocin to inhibit the food-borne pathogens, such as *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (*MDR*, clinical isolate), *Escherichia coli* (MDR, clinical isolate), *Salmonella enterica* ssp. *enterica* serovare Typhi ATCC 6539, makes it of crucial interest, especially in processed foods where there is risk of food-borne pathogens. High thermostability and broad antimicrobial spectrum is further supplemented by its stability in the presence of NaCl, organic solvents, surfactants and detergents. This indicates that the antimicrobial compound from strain 29V could preserve its structure and bactericidal functions even under extreme conditions which is an important property in view of its potential application.

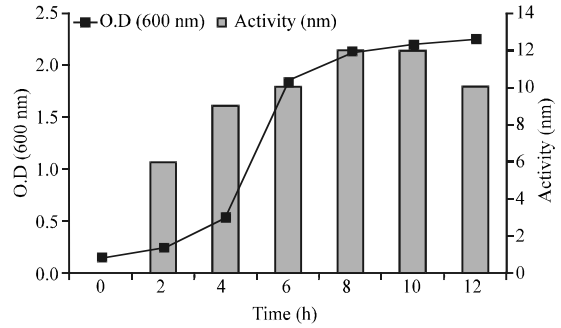

Fig. 1: Cell growth and bacteriocin (P29V) production at different time of incubation in MRS Broth

Viability of the bacteriocin-producing *L. plantarum* 29V strain in Palm Kernel Oil (PKO): As shown in Table 4, the results revealed that strain *L. plantarum* 29V maintains its stability in the palm kernel oil when stored at room temperature (25°C) as well as at refrigeration temperature (4°C) for 30 days. This could be due to the composition of palm kernel oil, mainly its high fatty acids

Table 3: Spectrum of inhibitory activity of the bacteriocin produced by strain *L. plantarum* 29V

Indicator strains	Sources	Growth conditions	Bacteriocin activity (mm)
Lactic acid bacteria			
Lactobacillus plantarum 5S	Our collection	MRS[c], 30°C	13.0±0.0
Lactobacillus plantarum 2S	Our collection	MRS, 30°C	0.0±0.0
Lactobacillus plantarum 3S	Our collection	MRS, 30°C	0.0±0.0
Lactobacillus plantarum 9S	Our collection	MRS, 30°C	0.0±0.0
Lactobacillus rhamnosus 1K	Our collection	MRS, 30°C	0.0±0.0
Lactobacillus plantarum 18V	Our collection	MRS, 30°C	11.0±0.0
Enterococcus faecium	DSM[a] 13596	BHI[d], 30°C	0.0±0.0
Enterococcus faecalis	ATCC 10541	BHI, 30°C	8.2±0.3
Gram positive pathogenic bacteria			
Listeria innocua	ATCC[b] 33090	T, PCA[e], 37°C	15.3±0.5
Staphylococcus aureus	ATCC 25923	T, PCA, 37°C	12.0±0.0
Staphylococcus aureus (MDR)	Clinical isolate	T, PCA, 37°C	8.0±0.4
Bacillus cereus	ATCC 11778	T, PCA, 37°C	0.0±0.0
Streptococcus mutans	DSM 20523	T, PCA, 37°C	14.3±1.1
Gram negative pathogenic bacteria			
Pseudomonas aeruginosa	ATCC 27853	T, PCA, 37°C	12.3±0.5
Escherichia coli	ATCC 13706	T, PCA, 37°C	13.6±1.1
Escherichia coli (MDR)	Clinical isolate	T, PCA, 37°C	7.3±0.5
Schigella flexneri	Clinical isolate	T, PCA, 37°C	14.3±0.5
Klebsiella pneumoniae	Clinical isolate	T, PCA, 37°C	0.0±0.0
Proteus mirabilis	Clinical isolate	T, PCA, 37°C	15.0±1.0
Salmonella Typhi	ATCC 6539	T, PCA, 37°C	13.6±1.1

Inhibition zone diameters are means of triplicates; Wells (6 mm in diameter) were filled with 100 µL of NCFS (Neutralized Cell-free supernatant); MDR = Multi Drug Resistant.; [a]DSM = Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; [b]ATCC = American Type Culture Collection, Manassas, VA, USA; [c]de Man, Rogosa and Sharpe; [d]Brain Heart Infusion; [e]T, PCA = Tryptone 5%, Plate Count Agar

Table 4: Evolution of acid and peroxide indexes as well as the viable cells count of *L. plantarum* in Palm Kernel Oil (PKO) samples stored at different conditions

Parameters	Duration of storage (days)	PKO+*L. plantarum* 29V stored at refrigeration temperature (4°C)	PKO+*L. plantarum* 29V stored at room temperature (25°C)	PKO stored at refrigeration temperature (4°C)	PKO stored at room temperature (25°C)
Acid index (mgKOH g^{-1})	0	0.80±0.03	0.80±0.03	0.80±0.03	0.80±0.03
	10	0.82±0.04	0.83±0.00	0.82±0.00	0.82±0.11
	20	0.82±0.00	0.89±0.00	0.82±0.00	0.84±0.00
	30	0.83±0.00	0.87±0.00	0.87±0.00	0.87±0.00
Peroxide index (Meq O_2 kg^{-1})	0	10±0.69	10±0.69	10±0.69	10±0.69
	10	12±0.10	13±0.06	11±0.04	15±0.06
	20	27±0.16	31±0.16	25±0.48	31±0.95
	30	43±0.25	45±1.00	37±0.95	45±0.80
Viable cell counts of	0	10.0±0.56	10.0±0.56	0	0
L. plantarum (log CFU mL^{-1})	10	11.0±0.32	10.5±0.50	0	0
	20	10.0±0.21	8.5±0.70	0	0
	30	9.5±0.56	9.5±0.64	0	0

Values in this table are means and standard deviation of three independent experiments

content. Due to the composition of palm kernel oil, probiotics can not make the food matrix more acidic with their fermentative metabolism.

In their previous study, Kailasapathy (2002) reports that survival of probiotics in a suitable food matrix is affected by a range of factors including pH, post-acidification (during storage) in fermented products, hydrogen peroxide production, oxygen toxicity (oxygen permeation through packaging), storage temperatures, stability in dried or frozen form and lack of proteases. All these factors raise the stress on the microorganisms in keeping their physiological and biological functions or the functionality of cells within the matrix. The stress conditions may cause the probiotic cells a sub-lethal injury preventing the cell division (Oliver, 2005). Lactic acid bacteria have defense mechanisms, such as producing stress-induced proteins to regulate stress conditions and maintain the viability in food matrices (Van de Guchte *et al*., 2002). Under such conditions, cell could be non-culturable but still alive.

In addition, peroxide and acid indexes increase in all the samples during storage (Table 4). Lower temperatures slow down the oxidation and hydrolysis of oil. Similar results were reported by Kapseu showing that the peroxide values as well as the free acidity increase with temperature. The increase of free acidity, combined with the increase in peroxide value could be due to the hydrolytic activity of triglycerides in the palm kernel oil which releases free fatty acids subjected to oxidation. It is important to mention that the palm kernel oil was heated at 63°C for 30 min before use.

CONCLUSION

The present investigation has detected a bacteriocinogenic proteinaceous compound with excellent stability and fairly broad antimicrobial spectrum. The fact that this bacteriocinogenic activity is stable under a wide range of storage conditions should allow its usage in diverse food applications to prevent the growth of pathogenic and spoilage micro-organisms. Further studies, on the protein sequence and various applications on growth optimization and large-scale production are underway. Palm kernel oil maintains the viable cell numbers of *L. plantarum* 29V after 30 days of storage.

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