African Scientist Vol. 21, No. 2 June 30, 2020 1595-6881/2020 $10.00 + 0.00

Printed in Nigeria

http://journals.niseb.org.ng

AFS2020014/21205

Antagonistic Effects of *Lactobacillus* Isolates Against Diarrhogenic *Escherichia coli*

Oyedoh, P.O.,* Omonigho, S.E., Ebiala, I.F., Nwenarizi, C.J.

Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Edo State, Nigeria.

*Corresponding author; Email: oghoye.oyedoh@uniben.edu Tel: +234 (0) 815 661 7303

(Received June 12, 2020; Accepted in revised form June 29, 2020)

ABSTRACT: One of the bacterial pathogens implicated worldwide in intestinal illnesses is diarrheagenic *Escherichia coli* (DEC). This study was conducted to determine the antagonistic effects of *Lactobacillus* species isolated from soursop (*Annona muricata*) and pineapple (*Ananas comosus*) fruits against DEC isolates using agar well diffusion protocol. *Lactobacillus* species were isolated from a collection of pineapple and soursop fruits obtained from open markets in Benin City, Nigeria using culture-based methods. The antimicrobial-metabolites producing ability of *Lactobacillus* spp. were ascertained using a method described by the Association of Official Analytical Chemist (AOAC) methods and metabolites analyzed were lactic acid, hydrogen peroxide and diacetyl. *Lactobacillus* species that were isolated include: *Lactobacillus plantarum* and *L. acidophilus* from soursop; and *L. acidophilus*, *L. casei*, *L. delbrueckii*, *L. plantarum* were isolated from pineapple. The pineapple isolate - *L. acidophilus* showed a high antagonistic activity, with an inhibition zone of 18 mm. *Lactobacillus delbrueckii* and *L. plantarum* isolated from pineapple had an *in-vitro* concentration of hydrogen peroxide to be 23.70 g/l; diacetyl was 8.69 g/l and lactic acid concentration was 7.20 g/l, respectively. Results obtained from this study have shown that *Lactobacillus* species isolated from pineapple and soursop fruits are potential producers of antibacterial metabolites.

Keywords: Diarrheagenic *E. coli* (DEC), Antagonistic, *Lactobacillus* isolates, Pineapple, Soursop

Introduction

Diarrhea is the second cause of death among under 5 years old children, with nearly 1.70 billion disease cases and 760,000 death cases yearly worldwide (WHO, 2014). The most occurring case is as a result of viral, bacterial or parasitic infection of the Gastro-intestinal tract (Qadri *et al*., 2005). Diarrheagenic *E.coli* (DEC) is the commonest bacteria known to cause diarrhea both in developing and industrialized regions (Gomes *et al*., 2016). DEC infection involves adherence, colonization of GI surfaces, secretion of virulence factors and diarrhea as well as inflammation (Nataro *et al*., 1996). And gastrointestinal bacteria have been reported to affect the immune health of an individual (Rubio and Schmidt, 2018). Series of intestinal dysfunctions caused by diarrheagenic *E. coli* are self-limiting and solved in few days except for some rare cases that can proliferate to more severe diseases (Kaper *et al*., 2004). These dysfunctions (such as diarrhea, irritable, inflammatory bowel diseases and obesity) are caused by microbiota deviations which (Kalliomaki *et al*., 2001) could result from *E.coli* and other pathogenic invasion of the gastro-intestinal tract. This can be hindered by the ingestion of adequate amount of live microbes known to preserve health by ensuring maintained microbiota equilibrium (Reid, 2016). These live microbes are called probiotics and include: bacteria (*Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Bacillus*), yeast or mold (*Saccharomyces*, *Aspergillus* and *Candida*) (Reid *et al*., 2003). Microbes used and marketed commonly worldwide as probiotics are members of the genera *Lactobacillus* and *Bifidobacterium* (Champagne *et al*., 2011). *Lactobacillus* species are safe, Gram positive, rod shaped, catalase and oxidase negative strict microaerophilic to strict anaerobic organisms which confer health benefits to consumers if found in desirable number in their intestine (Vernoux *et al*., 2003). They enhance intestinal health by stimulating a healthy microbiota growth (Walter *et al*., 2008), inhibiting enteric pathogenic colonization of the intestine (Lee *et al*., 2012), producing antimicrobial substance, aid digestive capacity, stimulate antibody mediated immune response and reducing noxious faecal gas emission (Hong *et al*., 2002, Wang *et al*., 2012, Hou *et al*., 2015).

Different species and strains of species display diverse level of efficacy as probiotics (Newbold *et al*., 1995). Implicated species include: *Lactobacillus acidophilus*, *L. casei*, *L. debrueckii*, *L. brevis*, *L. cellobiosus*, *L. carvatus*, *L. fermentum*, *L. plantarum*, *L. reuteri*, *L. salivarius* and *L. gasseri* (Sekhon and Jairath, 2010). Research findings have certified their capacity of producing high molecular mass antibacterial bacteriocin-like substances and low molecular mass antagonistic compounds like organic acids (as an exclusive metabolic end-product), carbon dioxide, hydrogen peroxide and diacetyl (2,3, butanedione) (Piard and Desmazeaud, 1992; Fijan, 2014). Pringsulaka *et al*. (2015) reported their ability to exhibit bacteriocidal or bacteriostatic properties by releasing organic acid, hydrogen peroxide, lactoferrin and bacteriocin which prevent the proliferation of coliform bacteria. Studies have also revealed that consumption of probiotics carried through food to children and infants can also reduce antibiotics prescription. As the distortive ability of intestinal microbiota balance and other side effects posed by antibiotics therapy have resulted to a search for such alternative antibacterial agents (Finegold, 1986). Asides intestinal health improvement, probiotics improve lactose digestion, stabilize bone health (Amorim *et al*., 2018), make fruits and vegetables functional properties such as antioxidant and antihypertensive Y- aminobutyric acid (GABA) accessible to human (Su *et al*., 2015). In terms of probiotics transfer to man, the main vehicle in diverse world regime used as a carrier is fermented dairy products (Mishra *et al*., 2018). But the high cholesterol, lactose and animal protein that may limit consumption to some population group spun the need for non-dairy probiotic sources (Panghal *et al*., 2018).

Fruits lack these aforementioned drawbacks and their cellular content are rich in minerals, vitamins, sugars and other nutrients which are ideal substrates for probiotics bacterial growth (Oliveira *et al*., 2011). Furthermore, they contain prebiotics known to encourage the proliferation of probiotics (Awaisheh, 2016). Soursop and pineapple fruits are often harvested in an immature state and ripen at post-harvest stage (Biale and Barcus, 1970), producing off-flavour due to low phenol, lower organic acid and some fermentation (Paull *et al*., 1983). Therefore, it is imperative to ransack these non–dairy sources for the presence of Lactobacilli, a known fermenter and probiotics, in order to meet the increase demand for functional food (Salvetti and O'Toole, 2017). This preliminary study was designed, to assess the presence of *Lactobacillus* species in commonly consumed fruits and evaluate their antagonistic potency against diarrhea causing *Escherichia coli.*

Materials and methods

Sample and test isolates collection: Ready-to-eat pineapple (*Ananas comosus*) and soursop (*Annona muricata*) fruits purchased from open markets were transferred to the laboratory in sterile polythene bags. These fruits were identified in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The fruits were thoroughly washed, dissected with a sterile knife and the juice from the pulp was aseptically extracted. It was extracted by holding tightly and twisting to squeeze out the juice into a sterile empty Petri-dish. Diarrheagenic *Escherichia coli* (DEC) test isolates *E. coli* 834, *E. coli* 838, *E. coli* 634 and *E. coli* 638 obtained from the Medical Microbiology Unit, Department of Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria were selected for antagonistic activity of these fruits.

Physicochemical analysis of fruit samples: To 5.00 ml fruit juice placed in sterile beaker, a pH meter rod was dipped to measure the pH value. The titratable acidity was determined by acid/base titration method by titrating 0.10N NaOH against 5.00 ml fruit juice containing 2 drops of phenolphthalein used as an indicator. The moisture content was assessed by oven-drying to a constant weight in the method described by AOAC (1990).

Isolation and Identification of Lactobacillus spp: Serial dilution of each fruit juice (1.00 ml) sample was aseptically carried out using sterile distilled water (9.00 ml) as diluent to make 10 fold dilutions. A 0.10 ml aliquot was taken from 10^{-4} & 10^{-5} dilutions and plated on sterile MRS agar plate with a glass spreader and incubated at 37ºC for 48 hrs. Having sub-cultured isolates of interest, pure and discrete colonies purified on nutrient agar plates were characterized culturally and morphologically (MacFaddin, 2000). Suspected small, dull or shiny and Gram positive bacilli colonies were further characterized using biochemical tests which include: catalase, oxidase, citrate, methyl red, Vogue-proskauer, ability to grow at 15ºC, 45ºC, to produce gas from glucose and few sugar fermentation test. Lastly, all seven isolates obtained tested negative for catalase, oxidase, citrate, methyl red, Vogue proskauer test**.**

Antibacterial activity against diarrheagenic E. coli: Agar well diffusion method was used to ascertain the antagonistic efficacy of *Lactobacillus* isolates against confirmed DEC isolates as described by Irobi *et al*. (1994). DEC isolates were inoculated in sterile TSB (Oxoid, UK) for 18 hrs. After which 0.10 ml of DEC suspension previously standardized to 0.5 McFarland standards was plated on Mueller Hinton agar plate (Oxoid, UK). On MHA plate, wells were aseptically bored using sterile 6.00 mm diameter cork borer. Approximately 100 µl of the cell free supernatant extract centrifuged at 4,000 rpm for 15 mins was introduced into the wells and allowed to absorb at room temperature (28 ± 2^{o}C) for 2 hrs and then incubated at 37^{o}C. The plates were observed for zone of inhibition 48 hrs later and measured in mm.
Antimicrobial production by bacterial isolates: Seven *Lactobacillus* isolates were cultured on MRS broth for 48 hrs and cell free supernatant was used for lactic acid, hydrogen peroxide and diacetyl production using methods described by AOAC. For the production of hydrogen peroxide, potassium permanganate (0.10 N) was titrated against a mixture (1:1) of broth culture of each isolate and diluted sulphuric acid until the mixture is decolourized (which is the end point). The amount of 0.10 N potassium permanganate used in mls is equivalent to 1.07 mg of hydrogen peroxide. Then lactic acid produced by each isolate was quantified by titrating 0.1 N NaOH against each culture broth (25 ml) containing 3 drops of phenolphthalein until a pink coloration is observed. 90.08 mg of lactic acid is equivalent to the amount of NaOH titrated in ml. Lastly, diacetyl produced was ascertained by titrating 0.10 N HCl against a mixture of 7.50 ml hydroxylamine, 25 ml broth culture and bromophenol blue indicator, until a greenish endpoint is observed. Every milliliter (ml) of HCl used is equivalent to 21.50 mg diacetyl.
Statistical analysis: All values obtained during the experiment were reported in mean ± standard deviation of triplicates as the case may be.

Results

Pineapple and soursop fruits were acidic, with pineapple displaying higher acidity with pH value of 3.92 ± 0.03, moisture content of 75.50 ± 0.13 % and titratable acidity of 1.77 ± 0.21 %. While soursop had the least pH, moisture content and titratable acidity were 3.89 ± 0.58, 23.50 ± 0.21 % and 0.95 ± 0.32 % respectively. The mean total *Lactobacillus* count (×105 cfu/ml) is 71.50 ± 3.85 for pineapple and 5.50 ± 0.71 for soursop. A total of 7 *Lactobacillus* isolates (3 from soursop and 4 from pineapple) were isolated from these fruit samples in this study (Table 2). Most of the bacterial isolates obtained from pineapple displayed zone of inhibition (mm) which ranged from 2.00 ± 0.10 to 18.00 ± 0.03 and produced more metabolites than soursop sourced isolates ranging from 2.63 ± 0.09 g/l to 23.70 ± 2.26 g/l (Tables 3 & 4).

Table 1: Physicochemical properties and bacterial enumeration of pineapple and soursop fruit juices samples

Parameters	Pineapple juice	Soursop juice
pH	3.92 ± 0.03	3.89 ± 0.58
Moisture content (%)	75.50 ± 0.13	23.50 ± 0.21
Titrateable acidity (%)	1.77 ± 0.21	0.95 ± 0.32
Bacterial count		
Lactobacillus spp (×10^{5}cfu/ml)	71.50 ± 3.85	5.50 ± 0.71
Other lactic acid bacteria (×10^{5}cfu/ml)	46.00 ± 1.66	2.50 ± 0.73

Values are mean ± standard deviation of triplicates.

Table 2: Cultural and biochemical characteristics of *Lactobacillus* species isolated from pineapple and soursop fruit juices samples

Characteristics	Presumptive Isolates with their Codes						
	L1SS	**L2SS**	**L3SS**	**L1PP**	**L2PP**	**L4PP**	**L5PP**
Cultural characteristic	7 0.2mm Moist Entire Raised	3 0.3mm Moist Entire Raised	1 0.2mm Moist Entire Raised	2 0.6mm Moist Entire Undulated	7 0.7mm Moist Undulated Raised	56 0.3mm Moist Entire Raised	6 0.2mm Moist Lobate Raised
Morphological characteristic	Gram positive bacilli (chains)	Gram positive bacilli (chains)	Gram positive bacilli (chains)	Gram positive bacilli (chains)	Gram positive bacilli (singly)	Gram positive bacilli (singly)	Gram positive bacilli (chains)
Production of gas from glucose	–	–	–	–	–	–	–
Growth at 15ºC	+	+	_	_	_	+	+
Growth at 45ºC	_	_	+	+	+	_	_
Ribose	+	+	+	+	+	+	+
Mannitol	+	+	+	+	_	+	+
Presumptive Isolates	*Lactobacillus plantarum*	*L. plantarum*	*L. acidophilus*	*L. acidophilus*	*L. delbrueckii*	*L. casei*	*L. plantarum*

Key: + positive, - negative. L1SS-first isolate from soursop L2SS-second isolate from soursop L3SS-third isolate from soursop L1PP-first isolate from pineapple L2PP-second isolate from pineapple L4PP-third isolate from pineapple L5PP-fourth isolate from pineapple

Table 3: Antibacterial activity of *Lactobacillus* isolates against diarrheagenic *E. coli* isolates (zone of inhibition - mm)

Presumptive Isolates	*E. coli* 834	*E. coli* 838	*E. coli* 634	*E. coli* 638
Soursop				
L. plantarum	8.00±0.11	7.00±0.10	5.00±0.20	5.00±1.20
L. plantarum	7.00±0.18	7.00±0.04	3.00±1.30	0.00±0.00
L. acidophilus	7.00±0.00	8.00±0.11	6.00±0.20	8.00±0.00
Pineapple				
L. acidophilus	18.00±0.03	14.00±0.11	7.00±0.50	6.00±0.10
L. delbrueckii	13.00±0.00	10.00±0.10	2.00±0.10	0.00±0.00
L. casei	16.00±0.10	6.00±0.04	4.00±0.20	2.00±0.30
L. plantarum	6.50±0.00	7.00±0.10	0.00±0.00	7.00±0.00

Values are mean ± standard deviation of duplicate.

Table 4: Antimicrobial metabolites produced by *Lactobacillus* isolates (g/l)

Presumptive Isolates	Hydrogen Peroxide	Lactic Acid	Diacetyl
Soursop			
L. plantarum	11.32 ± 1.10	1.48±0.05	1.24±0.03
L. plantarum	19.65 ± 1.34	1.20±0.02	5.36±0.30
L. acidophilus	15.81 ± 1.68	1.41±0.03	0.00±0.00
Pineapple			
L. acidophilus	10.81 ± 1.09	4.70±0.11	7.16±1.60
L. delbrueckii	23.70 ± 2.26	2.63±0.09	8.69±1.19
L. casei	12.56 ± 1.10	7.03±1.20	0.00±0.00
L. plantarum	4.10 ± 0.19	7.20±1.06	0.00±0.00

Values are mean ± standard deviation of triplicate.

Discussion and conclusion

Diarrheagenic *E. coli* (DEC) is the most significant etiological agent of childhood diarrhea and represents a public health problem in developing nations (Nataro and Kaper, 1998). This pathogenic agent could be inhibited by probiotics harbored in ready to eat fruits, as the latter contain usable substrates like cellulose, fibre (Russo *et al*., 2014). In view of this, from pineapple and soursop fruits used in this study, Lactobacillus spp were isolated. Species isolated include: *Lactobacillus plantarum*, *L. acidophilus*, *L. casei* and *L. delbrueckii*. Their presence could be attributed to the acidic nature of the substrates. According to Sheehan *et al*. (2007), an acidic environment is a unique niche that encourages the proliferation and exploration of probiotics. Also, research conducted by Peres and co-workers (2012), on plant materials reported that numerous *L. acidophilus*, *L. casei* and *L. plantarum* strains can grow in fruits due to their acidic tolerance nature. Specifically this study revealed pineapple to be more acidic, with higher moisture content than soursop. It also had higher mean total *Lactobacillus* and lactic acid bacterial count as shown above. Zhang *et al.* (2011) did a study on the physicochemical parameters effect on bacterial and fungal communities and discovered that the higher the surface moisture the higher the bacterial activity. *Lactobacillus plantarum* and *L. acidophilus* were isolated from soursop, while *L. acidophilus*, *L. casei*, *L. delbrueckii* and *L. plantarum* were isolated from pineapple. All isolates reflected different degree of antagonistic activity against DEC isolates, with *L. acidophilus* having the highest zone of 18.00 ± 0.03 mm against *E. coli* 834. In a study on the antibacterial activities of some probiotics against common human intestinal pathogen conducted using same protocol, all species demonstrated different level of potency with *L. lactis* W58 displaying the highest zone of 12.00 mm against *E. coli* O157: H7 ATCC 35150 (Campana *et al*., 2007). This could be indicative of *Lactobacillus* species ability to produce antibacterial metabolites. As all isolates of pineapple produced highest lactic acid, hydrogen peroxide and diacetyl content, those of soursop produced least. Lactic acid bacteria are known to mainly produce organic acids as antimicrobial metabolites (Obadina *et al*., 2006). Some also produce hydrogen peroxide as reported by Collins *et al.* (1983) against *Pseudomonas fragi* and *Staphylococcus aureus*. The descending order for lactic acid production is *L. plantarum* (from pineapple) > *L. casei* > *L. acidophilus* > *L. delbrueckii* > *L. plantarum* (from soursop) > *L. acidophilus* (from soursop) > *L. plantarum* (from soursop), for hydrogen peroxide production is *L. delbrueckii* > *L. plantarum* (from soursop) > *L. acidophilus* (from soursop) > *L. casei* > *L. plantarum* (from soursop) > *L. acidophilus* > *L. plantarum* (from soursop) and for diacetyl production is *L. delbrueckii* > *L. acidophilus* > *L. plantarum* (from soursop) > *L. plantarum* (from soursop). Similarly, Kalalou (2004), reported in his study on the antagonistic effect of *Lactobacillus* strains that their potency against fecal pathogens was attributed to low pH activity and bacteriocin production. Therefore ready-to-eat pineapple and soursop fruits contain live beneficial *Lactobacillus* spp. with antagonistic efficacy against DEC and could serve as a potential vehicle for the transfer of these organisms into the GIT.

The health problems associated with GI deviations cannot be overlooked especially with the increase intake of antibiotics which could result to diarrhea in children and adults. As a result non-allergenic natural approach such as consumption of fermented food and foods with antibacterial live microorganisms has been introduced over the years.

This study has revealed that pineapple & soursop fruits also naturally contain live microbes (*Lactobacillus* species) with antagonistic activity against DEC. Making fruits a potential candidate for the transfer of antibacterial LAB when consumed and safely delivered into GIT.

References

Amorim JC, Piccoli RH, Duarte WF: Probiotic potential of yeast isolated from pineapple and their use in the elaboration of potentially functional fermented beverages. *Food Res Int* 107: 518-527, 2018.

Biale JB, Barcus DE: Respiratory patterns in tropical fruits of the Amazon Basin. *Trop Sci.* 12: 93-104, 1970.

Carlos A, Rubio R, Schmidt R: Severe defects in the macrophage barrier to gut macrophage in inflammatory bowel disease and colon cancer. *Anticancer Res* 38(7): 3811-3815, 2018.

Champagne CP, Ross PR, Saarela M, Hansen FK: Charalampopoulos D: Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrice *Int J Food Microbiol* 149(3): 185-193, 2011.

Cho IJ, Lee NK, Hahm YK: Characterization of *Lactobacillus* spp isolates from the feces of breast feeding piglets. *J Biosci Bioeng* 108(3): 194-198, 2009.

Coeuret V, Dubernet S, Bernardeau M, Gueguen M, Paul J: Isolation, characterization and identification of Lactobacilli focusing mainly on cheeses and other dairy products. *Milk* 83: 269-306, 2003.

Collins FL, Kim SM, McCabe LR, Weaver CM: Intestinal microbiota and bone health: the role of prebiotics, probiotics and diets. **In**: Bone Toxicology. Springer International Publishing, New York, USA pp417-443. 2017.

Collins MD, Farrow JA, Phillips BA, Kandler O: *Streptococcus garvicae* sp. nov. and *Streptococcus plantarum* sp. nov. *J Gen Microbiol.* 129(11): 3427-3431, 1983.

Daeschel MA: Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technol* 43:164-167, 1989.

Fijan S: Microorganisms with claimed probiotics properties: An overview of recent literature. Int J Environ Res Public Health 11: 4745-4767, 2014.

Finegold SM: Anaerobic infections and *Clostridium difficile* colitis emerging during antibacterial therapy. Scand J Infect Dis Suppl 49: 160-164, 1986.

Gomes TAT, Elias PW, Scaletsky ACI, Guth CEB, Rodrigues FJ, Piazza FMR, Feerreira SCL, Martinez BM: Diarrheagenic *Escherichia coli*. Brazi J Microbiol 47(1): 3-30, 2016.

Hong JW, Kim IH, Kwon OS, Kim JH, Min BJ, Lee WB: Effect of dietary probiotic supplementation on growth performance and fecal gas emission in nursing and finishing pigs. J Anim Sci Technol 44: 305-314, 2002.

Hou C, Zeng X, Yang F, Liu H, Qiao S: Study and use of the probiotics *Lactobacillus reuteri* in pigs- a review. J Anim Sci Biotech 6(1): 14-22, 2015.

Irobi ON, Moo-Young M, Anderson WA, Daramola SO: Antimicrobial activity of the bark of *Bridelia ferruginea* (Euphorbiaceae). Int J Pharmacogn 34: 87-90, 1994.

James P, Nataro SH, Alan D, Vial A, Sears L: T-cells in culture as a model for enteroaggregative Escherichia coli pathogenesis. Infect Immun 64(11): 4761-4768, 1999.

Kalliomaki M, Salminem S, Arvilommi H, Kero P, Koskinen P, Isolauri E: Probiotics in primary prevention of atopic disease: A randomized placebo-controlled trial. Lancet 357: 1076-1079, 2001.

Kaper JB, Nataro JP, Mobley HL: Pathogenic *Escherichia coli*. Nat Rev Microbiol **2**(2): 123-140, 2004.

Kato I, Yokokura T, Mutai M: Augmentation of mouse natural killer cell activity by *Lactobacillus casei* and its surface antigens. Microbiol Immunol **28**: 209-217, 1984.

Lee JHVD, Valeriano YR, Shin JP, Chae GB, Kim JS, Ham J: Genome sequence of *Lactobacillus mucosae* LM 1 isolated from piglet feces. J Bacteriol 194: 47-66, 2012.

Lee KW, Park Y, Jeong RH, Heo JH, Han SN, Kim HJ: Probiotics properties of Weissella strains isolated from human faeces. *Anaerobe* 18(1): 96-102, 2011.

MacFaddin JF: Individual biochemical test. **In**: Biochemical test for identification of medical bacteria. Philadelphia: Lippincott Williams & Wilkins 1-456, 2000.

Mishra SS, Behera PK, Kar B, Ray RC: Advances in probiotics, prebiotics and neutraceuticals. **In**: Innovations in technologies for fermented food and beverage industries. Springer International Publishing, New York, USA pp121-141. 2018.

Nataro JP, Kaper JB: Diarrheagenic *E. coli*. Clin Microbiol Rev 11(1): 142-201. 1998.

Newbold CJ, Wallace RJ, Chen XB, Mcintosh FM: Different strains of Saccharomyces cerevisiae differ in their effects on ruminal bacteria numbers invitro and in sheep. J Anim Sci 73: 1811-1818, 1995.

Obadina AO, Oyewale OB, Sanni LO, Tomlins KI: Bio-preservative activities of *Lactobacillus plantarum* strains in fermenting cassava "fufu". Afr J Biotech 5(8): 620-623, 2006.

Official Method of Analysis*:* Association of Official Analytical Chemists, Inc., 15th edition, Arlington, USA. 1298 p, 1990.

Panghal A, Janghu S, Virkar K, Gat Y, Kumar V, Chikara N: Potential non-dairy probiotic products-A healthy approach. Food Biosci 21: 80-89, 2018.

Paull RE, Deputy JC, Chen NJ: Changes in organic acids, sugars and headspace volatile during fruit ripening of soursop (*Annona muricata*, L.). J Am Soc Holticul Sci 108: 931-934, 1983.

Peres CM, Peres C, Hernández-Mendoza A, Malcata FX: Review on fermented plant materials as carriers and sources of potentially probiotic lactic acid bacteria-With an emphasis on table olives. Trends Food Sci Technol 26: 31-42, 2013.

Piard JC, Desmazeaud M: Inhibiting factors produced by lactic acid bacteria 2. Bacteriocins and other antibacterial substances. Milk 72: 113-142, 1992.

Pringsulaka O, Rueangyotchanthana K, Suwannasai N, Watanapokasin N, Amnueysit P, Sunthornthummas S: *Invitro* screening of lactic acid bacteria for multi-strain probiotics. Livest Sci 174: 66-73, 2015.

Reid G: Probiotics: definition, scope and mechanisms of action. Best Pract Res Clin Gastroenterol 30(1): 17-25, 2016.

Reid R, Jana JM, Sebulsky T, John K, Cormick MC: Potential uses of probiotics in clinical practice. Clin Microbiol Rev 16(4): 658-672, 2015.

Russo P, Chiara MLV, Vernile A, Amodio ML, Arena MP, Capozzi V, Massa S, Spano G: Fresh-cut pineapple as a new carrier of probiotic lactic acid bacteria. Biomed Res Int 12: 1-9, 2014.

Saarela M, Lahteenmaki L, Crittenden R, Salminen S, Mattila-Sandholm T: Gut bacteria and health foods- the European perspective. Int J Food Microbiol 78: 99-117, 2002.

Sheehan VM, Ross P, Fitzgerald GF: Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. Innov Food Sci Emerg Technol 8(2): 279-284, 2007.

Su J, Wang T, Li YY, Li J, Zhang Y, Wang Y, Li H: Antioxidant properties of wine lactic acid bacteria, *Oenococcus oeni.* Appl Microbiol Biotech 99(12): 5189-5202, 2015.

Walter J: Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. Appl Environ Microbiol 74(16): 4985-4996, 2008.

Walter J, Schwab C, Loach DM, Ganzle MG, Tannock GW: Glucosyltransferase A (GtFA) and inolusucrase (Inu) *Lactobacillus reuteri* TMW1106 contribute to cell aggregation invitro biofilm formation and colonization of the mouse GIT. Microbiol 154: 72-80, 2008.

Wang J, Ji H, Wang S, Zhang D, Hui L, Wang Y, Shan D: Assessment of probiotic properties of *Lactobacillus plantarum* ZLP001 isolated from GIT of weaning piglets. Afr J Biotechnol 10(54): 11303-11308, 2011.

Wang Q, Dong J, Zhu Y: Necrotizing enterocolitis and mortality in preterm very-low-birth-weight infants: an update meta-analysis of 20 randomized controlled trials. J Pediatr Surg 47(1): 241-248, 2012.