

Antidiarrheal potential of *Lactobacillus* strains isolated from pharmaceutical formulations for the treatment of pediatric diarrhea

Syed Imran Ali^{1*}, Syed Baqir Shyum Naqvi² and Rabia Ismail Yousuf¹

¹Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

²Department of Pharmaceutics, Hamdard University, Karachi, Pakistan

Abstract: The consumption of probiotics in the prevention and treatment of diarrhea have been clinically justified, comprehensive studied and explored in many products around the world. In Pakistan, recommendation of probiotic formulations is being emerged to control the increased mortality and morbidity from diarrhea under 5 years of age children. The objective of the study was to evaluate the antimicrobial potential of isolated *Lactobacillus* strains against diarrheagenic *Escherichia coli*. Twelve strains were isolated from different probiotic pharmaceutical formulations available in Pakistan. Physiological and biochemical characteristics of isolates were analyzed. Selective media was used for the growth of probiotic isolates and *E. coli*. Agar spot and well diffusion methods were employed to evaluate the antimicrobial activity of isolates and measured as a zone of inhibition (mm). Changes in cell morphology was observed by Scanning Electron Microscopy. Statistical analysis was adopted with a level of significance $p < 0.05$. *L. reuteri* (28 mm) and *L. plantarum* (26 mm) showed significant inhibitory actions against *E. coli* due to increased organic acids and bacteriocins formations. Rest of isolates exhibited mild to moderate activity with an average inhibition (20 mm). *L. sporogenes* demonstrated weak antagonistic behavior. Use of multiple strains of *Lactobacillus* along with *L. reuteri* or *L. plantarum* as a therapeutic agent or in nutritional supplements could be a novel approach for the prevention and treatment of pediatric diarrhea.

Keywords: Pediatric diarrhea, probiotic pharmaceutical formulations, diarrheagenic *E. coli*, *Lactobacillus* strains.

INTRODUCTION

Globally, diarrhea is the second top listed infectious disease in infants and children under 5 years of age with the morbidity of 1.7 billion and mortality of 846,000 cases annually (WHO, 2016). In Pakistan, diarrhea after the respiratory infections is the second primary cause of mortality as infected 87 in every 1000 children below 5 years of age (Mumtaz *et al.*, 2014). Acute diarrhea in developing countries is significantly associated with a wide range of bacteria, viruses, and parasites (Nitiema *et al.*, 2011; Larson *et al.*, 2009). Global enteric multicenter (GEM) study which was conducted in South Asia including Pakistan, observed the involvement of causative pathogens such as *Rotavirus*, *Cryptosporidium*, *Shigella*, and *Enterotoxigenic E. coli* in moderate to severe diarrhea among infants and children aged below 5 years (Kotloff *et al.*, 2013). Diarrheagenic *E. coli* is more associated with infective diarrhea as it stimulates the secretion of water and electrolytes. The toxin secreted by *E. coli* causes destruction of absorptive cells located at the brush boarder of the small intestine (Nataro *et al.*, 2006; Navaneethan *et al.*, 2008).

The genus *Lactobacillus* is the largest group of non-pathogenic bacterial species belongs to probiotics and widely found in dairy products. Probiotics are defined as “live microorganisms which, when administered in an adequate amount, confer a health benefit to the host”

(FAO/WHO 2001). The US Food and Drug Administration classified probiotics as “live bio-therapeutics” intended for clinical use (Hoffman, 2008). The beneficial effects of *Lactobacillus spp.* were first observed by Eli Metchnikoff in 1907 when studied the longevity of Bulgarians (Metchnikoff, 1907).

Lactobacillus spp. are Gram-positive, rod-shaped, facultative-anaerobic, catalase negative, and non-spore-forming bacteria, grow well in microaerophilic conditions. *In-vitro*, they grow on MRS agar with white mucoid colonies. (Khalid, 2011; Makarova *et al.*, 2006). *Lactobacillus* are classified into homofermentative produce lactate and heterofermentative yield lactate with other end products such as acetate, ethanol and CO₂ (Zaunmüller *et al.*, 2006)

The predominance of *Lactobacillus spp.* in gut microflora is a key factor to protect the body against disease-causing microorganisms (Borchers *et al.*, 2009). The inhibitory compounds such as lactic acid, hydrogen peroxide and bacteriocins produced by *Lactobacillus* exhibit different antagonistic effects on diverse types of diarrheagenic pathogens (Itlo *et al.*, 2003)

MATERIALS AND METHODS

Collection of Samples

The Culture of enteropathogenic bacteria *E. coli* was obtained from the pathological laboratory of Hamdard

*Corresponding author: e-mail: imran4u5@yahoo.com

Hospital, Karachi. Isolates of probiotics were obtained from the antidiarrheal probiotic formulations containing *Lactobacillus* spp. (table 1). These probiotic samples were collected in airtight leak-proof, clean and sterile wide-mouthed container free from any disinfectant residue. Collected samples were immediately transferred to the laboratory for microbial analysis and refrigerated at low temperature (-4°C) to avoid any possible contamination and deterioration.

Isolation and enumeration of lactic acid bacteria

Selected strains of *Lactobacillus* were inoculated on MRS broth for 3 days at 37°C under anaerobic conditions (De Man *et al.*, 1960). Sub-cultured the broth samples on MRS agar for 3 to 6 days at 37°C under anaerobic conditions (Elizete *et al.*, 2005). MRS agar, which contains isolates of *L. acidophilus* and *L. dulbrueckii* also added with 0.5% salicin and 0.05% cysteine to improve the specificity of the medium (Shah, 2000; Hartemink *et al.*, 1997). Adjusted the pH of the media to 5.2 to 6.5 by the addition of 0.1 N solution of sodium hydroxide and hydrochloric acid guided through pH meter. The repeated streaking on MRS agar was done in order to get the pure colonies of each culture.

Preparation of enteropathogens test plates

Mueller Hinton agar susceptibility plates were prepared by pouring it into sterilized 100 ml of Petri dish with 100 mm diameter to get the even depth of 4 mm from the bottom surface which is obtained by adding 30-32 ml of Mueller Hinton agar into Petri dish of 100mm. Finally cooled the susceptibility plates to room temperature (Kihal *et al.*, 2006).

Growth of pathogenic bacteria

The inoculum of *E. coli* cultures was prepared under aseptic conditions to the test tube containing 2-3 ml of Mueller Hinton Broth. The test tubes were incubated at 37 °C for a few hours until the standard of turbidity achieved to McFarland 0.5 M standard. Broth suspension of *E. coli* was streaked evenly on the prepared Muller Hinton agar surface for their growth.

Physiological and biochemical characteristics of isolated lactobacillus spp.

Appeared colonies were elected for identification by Gram staining and biochemical techniques as per Bergey's manual included catalase, motility test and carbohydrate fermentation. The confirmed isolates of *Lactobacillus* were selected for the evaluation of antimicrobial activity against diarrheagenic *E. coli*.

Catalase and motility test

Many microorganisms other than lactic acid bacteria have an ability to break hydrogen peroxide into water and oxygen. Poured 3% solution of hydrogen peroxide into prepared MRS culture and colonies with no gas bubble formation were selected for further investigation (Nelson

et al., 1995). The motility of isolated strains was examined through the microscopic observation of slides prepared according to Hanging-drop wet method (McFadden *et al.*, 2000).

Fermentation test

Prepared MRS broth test tubes inoculated with 1% overnight fresh cultures incubated at 37°C for 5 days. Durham tubes were inserted into each culture tube and observed the CO₂ gas production to determine the homo and heterofermentative characteristics of isolates (Farhana *et al.*, 2013).

Acid production measurement

Titrate 10 ml of isolated culture in conical flask with 5 drops of phenolphthalein indicator with sodium hydroxide until the appearance of pink color. Volume of titration indicate the intensity of acid production by each isolate (Moulay *et al.*, 2006).

Tolerance to NaCl

To conduct NaCl tolerance test, isolated cultures of *Lactobacillus* grown on MRS broth were adjusted with different concentration of NaCl (1-9%). Each test tube was inoculated with 1% (v/v) fresh overnight culture of isolates and incubated anaerobically at 37°C for 24 h. After 24 h of incubation bacterial growth were observed by culture medium turbidity (Farhana *et al.*, 2013).

Assay for antimicrobial activity and substances

The inhibitory activity of isolated lactic acid bacteria against *E. coli* was obtained using the agar spot test. Overnight serially diluted cultures of probiotics bacteria of 10⁷-10⁹ CFU/ml was spotted on the surface of MRS agar plates and incubated further at 37 °C for 24 hours under anaerobic condition in order to allow further colonies. Subsequently the incubated plates were overlaid with a previously inoculated plate with pathogenic bacteria (10⁷-10⁹ cells per ml). All plates were incubated at 37 °C for 24 hours under anaerobic condition. Repeated the procedure for each isolated strains of probiotics. The bacteriocin and organic acid assay of isolates against *E. coli* was determined by agar- well diffusion method (Tejero *et al.*, 2012). For bacteriocin and organic acids, 5 ml of supernatant were treated with 1 mg/ml of pronase and 1N NaOH at pH 6.5 respectively incubated at 37°C for 24 h (Hoque *et al.*, 2010). Inhibitory potential of isolated *Lactobacillus* was estimated by measuring the diameter of a clear zone around the spotted LAB colonies through Vernier caliper.

Scanning electron microscopy of antagonistic activity of probiotics

Electron microscopy (SEM) was done at Centralized Science Laboratory, University of Karachi in order to find the morphological changes or cell rupture due to the effect of isolated *Lactobacillus* (Tareb *et al.*, 2013). Cultured colonies were selected under fume hood then

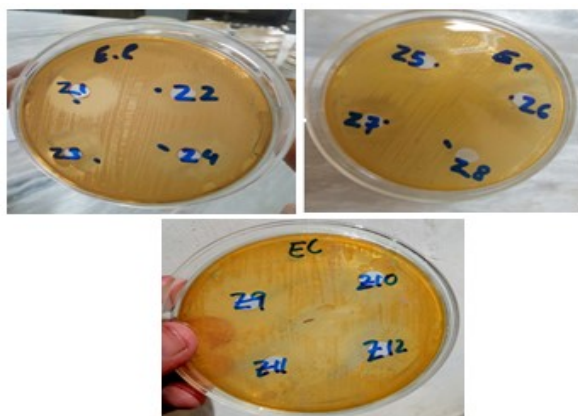
centrifuged at 5000 rpm for 15 minutes. The bacterial supernatant was discarded and bacterial cells were fixed in McDowell-Trump reagent at pH 7.2 for 2 hours then washed them with 0.1 M of phosphate buffer and centrifuged again for 10 minutes at 5000 rpm. The pellet was re-suspended in phosphate buffer solution and again centrifuged. Osmium tetroxide (1%) was used to fix the pellet for an hour. Washed the sample twice a time with distilled water for 10 minutes. Dehydrated the sample with ethanol at different concentrations of 50%, 75%, 95%, and 99% subsequently added 1 ml of hexamethyldisilazane in a sample tube for 10 minutes then allow the sample to dry at room temperature. Finally, a sample was coated with up to 300Å gold and observe the images under scanning electron microscopy.

STATISTICAL ANALYSIS

Results were analyzed with SPSS 24 version. Statistical analysis was done by comparing independent sample t-test, mean, and standard deviation at the level of significance $p < 0.01$ (table 4).

RESULTS

Twelve isolated strains were identified through Gram staining observed as Gram-positive, rod-shaped bacteria with white colonies under the microscope. Isolated *Lactobacillus* were catalase negative, non-motile, fermented glucose and lactose which confirm their origin to *Lactobacillus*. *L. reuteri* and *L. plantarum* showed more acid production compared to other isolates (table 1). All Isolated strains of *Lactobacillus* were resistant to different concentration of NaCl (1-9%), but *L. reuteri*, *L. rhamnosus*, *L. GG*, and *L. plantarum* were more tolerated to NaCl (table 2).



Z1: *L. acidophilus* LA-5, Z2: *L. dulbrueckii* spp. *bulgaricus* LB5-27, Z3: *L. rhamnosus* PXN-54, Z4: *L. paracasei* 431, Z5: *L. casei* 431, Z6: *L. bulgaricus*, Z7: *L. sporogenes*, Z8: *L. dulbrueckii* spp. *bulgaricus* PXN-27, Z9: *L. reuteri* DSM 17938, Z10: *L. rhamnosus* Rosell 343, Z11: *L. GG*, Z12: *L. plantarum* 299v.

Fig. 1: Interactions of *Lactobacillus* Isolates against Diarrheagenic *E. coli*.

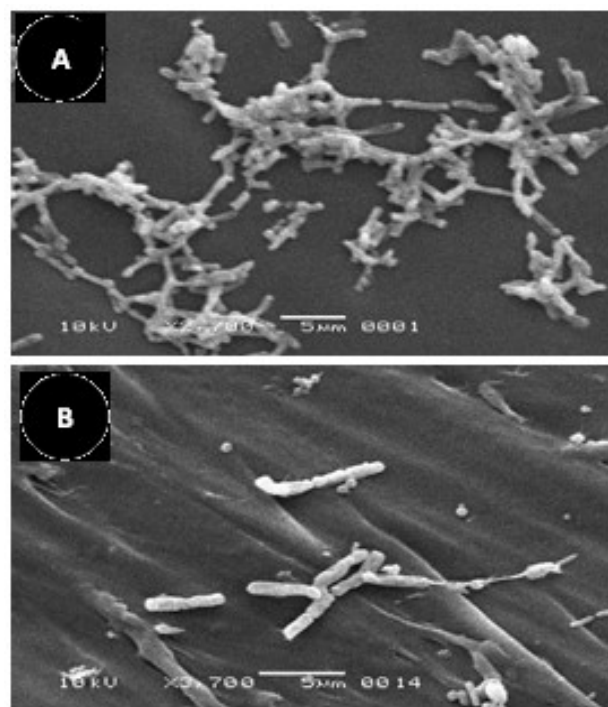


Fig. 2: Images: Scanning Electron Microscopy. (A) Control Untreated *E. coli* (B) *E. coli* disruption due to bacteriocin and organic acids.

Antimicrobial activity of isolated *Lactobacillus* against *E. coli*

Selected isolates of *Lactobacillus* were exhibited the antimicrobial effects against *E. coli* with an average inhibition 20 mm. However, *L. reuteri* and *L. plantarum* due to increased production of bacteriocins and organic acids produced maximum antagonistic activity with inhibition 28 and 26 mm respectively. The clear zone of inhibition on solid medium by the isolates against tested pathogen (table 3, fig. 1).

The above table depicted that an antimicrobial activity of *Lactobacillus* isolates is more significantly associated with the increased production of organic acids compared to bacteriocins.

Scanning electron microscopy

The images of *E. coli* treated with cell-free culture of isolated *Lactobacillus* were inspected by SEM. The pore formation or rupture in bacterial cell morphology could be one of the modes of action (fig. 2)

DISCUSSION

The purpose of the study was to investigate the probiotic potential of isolated *Lactobacillus* strains. During the study, twelve strains of *Lactobacillus* were isolated from the probiotic pharmaceutical formulations and assess their antimicrobial activity against diarrheagenic *E. coli*.

Table 1: Source and Characteristics of Isolated *Lactobacillus* Strains

Probiotic Strain	1	2	2	3	4	5	6	7	8	9
<i>Lactobacillus acidophilus</i> LA-5	Z1	PPF-1	+	*S Rod	-	Non-motile	+	+	-	++
<i>L. dulbrueckii ssp. bulgaricus</i> LBY-27	Z2	PPF-1	+	Rod	-	Non-motile	+	+	-	+
<i>Lactobacillus rhamnosus</i> PXN-54	Z3	PPF-3	+	Rod	-	Non-motile	+	+	-	+
<i>Lactobacillus paracasei</i> 431	Z4	PPF-4	+	*S Rod	-	Non-motile	+	+	-	++
<i>Lactobacillus casei</i> 431	Z5	PPF-4	+	Rod	-	Non-motile	+	+	-	+
<i>Lactobacillus bulgaricus</i>	Z6	PPF-7	+	Rod	-	Non-motile	+	+	-	+
<i>L. sporogenes</i>	Z7	PPF-9	+	Rod	-	Non-motile	+	+	-	+
<i>L. dulbrueckii ssp. bulgaricus</i> PXN-27	Z8	PPF-1	+	Rod	-	Non-motile	+	+	-	+
<i>Lactobacillus reuteri</i> DSM 17938	Z9	PPF-2	+	*L Rod	-	Non-motile	+	+	+	+++
<i>Lactobacillus rhamnosus</i> Rossell 343	Z10	PPF-5	+	Rod	-	Non-motile	+	+	-	++
<i>Lactobacillus GG</i>	Z11	PPF-6	+	*L Rod	-	Non-motile	+	+	-	++
<i>Lactobacillus plantarum</i> 299v	Z12	PPF-8	+	*S Rod	-	Non-motile	+	+	+	+++

1 = Product code, 2 = Gram, 3 = Cell form (*S = Short, *L = Long), 4 = Catalase, 5 = Motility, 6 = Glucose, 7 = Lactose, 8 = Gas production, 9 = Acid production

Table 2: Tolerance of Isolated *Lactobacillus* to NaCl.

Con. NaCl	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9	Z10	Z11	Z12
1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3	+++	+++	++	++	++	++	++	++	+++	+++	+++	+++
4	++	+	++	++	++	+	+	++	+++	++	++	+++
5	+	+	+	+	+	+	-	+	+++	+	++	+++
6	+	+	+	+	-	-	-	+	+++	+	+	++
7	-	-	+	-	-	-	-	-	++	+	+	+
8	-	-	-	-	-	-	-	-	+	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-

Legend: Excellent Growth (+++), Good Growth (++), Least Growth (+), No Growth (-).

Table 3: Diameter of Zone of Inhibition (mm*) by Isolated Probiotics Strains on Diarrheagenic Pathogens, Bacteriocins and Organic Acids Incubated under Anaerobic Conditions at 37 °C for 24 h.

Code	Probiotic Strain	<i>E. coli</i>	Bacteriocins	Organic Acids
Z1	<i>Lactobacillus acidophilus</i> LA-5	22	10	14
Z2	<i>Lactobacillus. dulbrueckii ssp. bulgaricus</i> LBY-27	20	-	12
Z3	<i>Lactobacillus rhamnosus</i> PXN-54	20	7	10
Z4	<i>Lactobacillus paracasei</i> 431	20	-	12
Z5	<i>Lactobacillus casei</i> 431	18	-	10
Z6	<i>Lactobacillus bulgaricus</i>	18	7	10
Z7	<i>Lactobacillus sporogenes</i>	16	-	8
Z8	<i>Lactobacillus. dulbrueckii ssp. bulgaricus</i> PXN-27	20	7	14
Z9	<i>Lactobacillus reuteri</i> DSM 17938	28	23	21
Z10	<i>Lactobacillus rhamnosus</i> Rossell 343	24	16	16
Z11	<i>Lactobacillus GG</i>	24	16	14
Z12	<i>Lactobacillus plantarum</i> 299v	26	20	17

Table 4: Summary of Mean, SD, df and t-value for Inhibitory Substances Produced by Isolated *Lactobacillus* against *E. coli*

	Mean	Standard Deviation	df	t	p
Bacteriocins	8.83	8.26	11	3.70	0.003*
Organic Acids	13.16	3.63	11	12.53	0.000*

** Significant at 0.01

Isolates were inoculated under anaerobic conditions at 37°C MRS broth and agar. The grow cultures of isolates were Gram-positive, cocci and rod-shaped indicated their belonging to *Lactobacillus* (Elizete *et al.*, 2005). Based on catalase, motility, and sugar fermentation tests isolates revealed catalase negative, non-motile and fermented sugars confirmed as *Lactobacillus spp.* Production of gas during the fermentation showed the heterofermentative nature of isolated *L. reuteri* and *L. plantarum* (table 1).

NaCl being an inhibitory agent do antagonize the growth of certain types of bacteria. All the isolates were able to grow at 1-8% NaCl concentration, however, excellent growth was observed at 1-5% NaCl. *L. reuteri* grew at 1-8% whereas *L. rhamnosus*, *L. GG*, and *L. plantarum* were tolerated to 1-7% NaCl concentration (table 2). Ashwani *et al.* isolated the *Lactobacillus* from yogurt that were tolerated at 6.5% NaCl (Ashwani *et al.*, 2014). Farhana *et al.*, observed the tolerance of isolated *Lactobacillus* from human milk at 1-9% NaCl concentration (Farhana *et al.*, 2013)

The inhibitory effect of *Lactobacillus spp.* is mainly due to the accumulation of organic acids and from the production of antimicrobial substances such as bacteriocins and hydrogen peroxides. However, the production level of these compounds depends on strain and physical parameters (Khay *et al.*, 2011; Savadogo *et al.*, 2004). During the present study, antagonistic activity was mainly observed by the production of organic acids except isolates Z9 to Z12 inhibited *E. coli* due to the production of both organic acids and bacteriocins (table 3)

The antimicrobial activities of each isolate performed through agar spot and well-diffusion method by incubating them under anaerobic conditions measured by zone of inhibition. Similar techniques were used by the researchers (Yateem *et al.*, 2008; Farhana *et al.*, 2013). *L. reuteri* (28 mm) and *L. plantarum* (26 mm) showed significant inhibitory actions against *E. coli* followed by other isolates with an average inhibition (20 mm). Yateem *et al.*, studied a strain of *L. plantarum* isolated from camel milk demonstrated a strong inhibitory effect against *E. coli* (26 mm) due to the production of organic acids and bacteriocins (Yateem *et al.*, 2008). Yingchun *et al.*, suggested the strong antimicrobial activities of *L. GG*, *L. rhamnosus*, *L. paracasei*, and *L. casei* against *E. coli* (Yingchun *et al.*, 2011)

CONCLUSION

Isolated *L. reuteri* DSM 17938 and *L. plantarum* 299v with *In-vitro* characteristics of excellent NaCl tolerance, acid formation, sugar fermentation, and strong suppression of diarrheagenic *E. coli* are potential candidate for probiotic formulations. Moreover, all isolated *Lactobacillus* strains exhibited mild to moderate antagonistic effects except *L. sporogenes* demonstrated

weak antagonistic behavior. The present study also provides support for using the single or multiple strains of *Lactobacillus* in pharmaceutical probiotic formulations as well as in nutritional supplements for the treatment and prevention of diarrhea. *In-vivo* trails are needed for clinical evaluation of the potential of probiotic formulations in real-life situations.

ACKNOWLEDGEMENT

The author is thankful to the centralized science laboratory and department of pharmaceuticals, university of Karachi for providing facilities to execute research work.

REFERENCES

- Ashwani Kumar Singh, Abhishek Pandey, Megha Sharma, Komudi and Anupam Singh (2014). Probiotic activities of lactic acid bacteria isolated from human breast milk. *J. Biol. Engg. Res. & Rev.*, **1**(2): 7-12
- Borchers AT, Selmi C, Meyers FJ, Keen CL and Gershwin ME (2009). Probiotics and border of human enterocyte-like Caco-2 cells. *Cell Microbiol.*, **9**(9): 2254-66.
- De Man JC, M Rogosa and ME Sharpe (1960). A medium for the cultivation of *Lactobacillus*. *J. Applied Bacteriol.*, **23**(1): 130-135.
- Elizete DFRP and Carlos RS (2005). Biochemical characterization and identification of probiotic *Lactobacillus* for swine. *B CEPPA Curitiba.*, **23**(6): 299-310.
- FAO/WHO (2001). Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria: Food and Agriculture Organization of the United Nations and World Health Organization expert consultation report Rome.
- Farhana S Diba, Khondoker M Hossain, MA Azim and Md Moinul Hoque (2013). Isolation, Characterization and Determination of Antimicrobial Properties of Lactic Acid Bacteria from Human Milk. *JJBS.*, **6**(1): 111-116.
- Hartemink R, Domenech VR and Rombouts FM Lamvab (1997). A new selective medium for the isolation of *Lactobacillus* from faces. *J. Microbiol. Meth.*, **29**(1): 77-84.
- Hoffman F (2008). Development of probiotics as biologic drugs. *Clin. Infect Dis.*, **46**(2): 125-127.
- Hoque MZ, F Akter, KM Hossain, MSM Rahman, MM Billah and KMD Islam (2010). Isolation, identification and analysis of probiotic properties of *Lactobacillus spp.* from selective regional yoghurts. *World J. Dairy Food Sci.*, **5**(3): 39-46.
- Itlo A, Y Sato, S Kudo, S Sato, H Nakajima and T Tob (2003). The screening of hydrogen peroxide producing lactic acid bacteria and their application to inactivationg psychotropic food-borne pathogens. *Curr. Microbiol.*, **47**(1): 231-236.

- Khalid, K. (2011). An overview of lactic acid bacteria. *Int. J. of Biosciences*, **1**(3): 1-13.
- Khay E, Idaomar M, Castro Bernarclez, PF and Abrini J (2011). Antimicrobial activities of the bacterial like substances produced by LAB isolated from maroccan dromedary milk. *Afr. J. Biotechnol.*, **10**(3): 10447-10455.
- Kihal M, DE Henni, H Prevost and C Divies (2006). A new manometric method for measuring carbon dioxide production by starter culture: A case of *Leuconostoc mesenteroides*. *African J. Biotechnol.*, **5**(1): 378-383.
- Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH and Panchalingam S *et al* (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. *Lancet*, pp.209-22.
- Larson CP, Henning L, Luby S and Faruque A (2009). Infectious childhood diarrhea in developing countries. *Modern Infectious Disease Epidemiology*, 291-308.
- Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E *et al.* (2006). Comparative genomics of the lactic acid bacteria. *Proc. Natl. Acad. Sci. USA.*, **103**(42): 15611-15666.
- McFadden J (2000). Biochemical tests for identification of medical bacteria. *J. Clin. Pathol.*, **34**(5): 572.
- Metchnikoff E (1907). The prolongation of life: Optimistic studies. William Heinemann, UK. *J. Clin. Micro.*, **31**(1): 572
- Moulay M, H Aggad, Z Benmecherne, B Guessas, DE Henni and M Kihal (2006). Proteolytic activity of cultivable lactic acid bacteria isolated from Algerian raw goat's milk. *World J. Dairy Food Sci.*, **1**(3): 12-18.
- Mumtaz Zafar and Mumtaz (2014). Knowledge attitude and practice of mothers about diarrhea in children under five years. *Dow Uni. Health Science*, **8**(1): 3-6.
- Nataro JP, Mai V, Johnson J, Blackwelder WC, Heimer R, Tirrell S, Edberg SC, Braden CR, Glenn Morris J Jr and Hirshon JM (2006). Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New Haven. *Connecticut. Clin. Infect.*, **43**(4): 402-407.
- Navaneethan U, Giannella RA (2008). Mechanisms of infectious diarrhea. *Nat. Clin. Pract. Gastroenterol. Hepatol.*, **5**(11): 637-647.
- Nelson G and George S (1995). Comparison of media for selection and enumeration of mouse fecal flora populations. *J. Microbial Methods*, **22**(3): 293-300.
- Nitiema LW, Nordgren J, Ouermi D, Dianou D, Traore, AS, Svensson L and Simporé J (2011). Burden of rotavirus and other enteropathogens among children with diarrhea in Burkina Faso. *Int. J. Infect Dis.*, **15**(4): 646-652.
- Savadogo A, Ouattara CAT, Bassole IHN and Traore AS (2004). Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. *Pak. J. Nutr.*, **3**(1): 49.
- Shah NP (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. *J. Dairy Sci.*, **83**(4): 894-907.
- World health organization (2016). News release media center death attribute to unhealthy environments, www.who.int/mediacentre/newsrelease/2016/death.
- Yateem, M.T, Balba, T. Al-Surrayai, B. Al-Mutairi and R and Al-Daher (2008). Isolation of Lactic acid bacteria with probiotics. *Int. J. Dietary Sciences*, **3**(4): 194-199.
- Yingchun, Zhanga L , Anwei Zhanga, Ming Dua, Huaxi Yia, Chunfeng Guoa, Yanfeng Tuoba and Xue Hana (2011). Antimicrobial activity against *Shigella sonnei* and probiotic properties of wild *Lactobacillus* from fermented food. *J. Microbiological Research*, **167**(3): 27-31
- Zaunmuller T, Eichert M, Richter H and Unden G (2006). Variations in the energy metabolism of biotechnologically relevant heterofermentative lactic acid bacteria during growth on sugars and organic acids. *J. App. Microbiol Biotechnol*, **72**(3): 421-429.