Science Arena Publications
Specialty Journal of Biological Sciences
ISSN: 2412-7396
Available online at www.sciarena.com
2018, Vol, 4 (1): 17-22

Isolation, Identification and Characterization of Lactic Acid Bacteria from Traditional Yogurt

Javid Ali, Arshad Hussain, Shamsur Rehman*

Food Technology Center, Pakistan Council of Scientific and Industrial Research Laboratories Complex, Peshawar, Khyber Pakhtunkhwa, Pakistan.

***Corresponding Author:**
Email: headftcplc @ gmail.com

Abstract : *The current study was carried out to isolate and identify the lactic acid bacteria and examine its antibiotic potential against human pathogenic bacteria. Twenty samples (n=20) of traditional yogurt were collected from local market. The species were isolated and identified through conventional standard procedures and then characterized for gastric transit, acidic pH, bile salt, viable ability at -20°C, 4°C and 25°C for five weeks. The isolated bacteria were identified as Peddiococcus pentosaccus, Lactobacillus delbrueckii, Lactobacillus helveticus, Lactobacillus plantarum and Pediococcus acidilactici. Agar well diffusion techniques were used for antibiotic potential of isolated bacteria inhibiting the growth of tested pathogenic bacteria. Maximum zone of inhibition in the range of 17-09 mm was observed for Lactobacillus delbrueckii against tested microbes. While the zones of inhibition for Peddiococcus pentosaccus (in the range of 16-02 mm), Lactobacillus helveticus (16-07 mm), Lactobacillus plantarum (13-01 mm) and Pediococcus acidilactici (12-03 mm) against the tested microbes were observed. At the end, the current findings showed that the five isolated lactic acid bacteria should be utilized as potential candidates against pathogenic bacteria.*

Keywords: *Milk Product, Infectious Diseases, Identification, Cell Morphology, Probiotic, Antibacterial Potential.*

INTRODUCTION

Probiotic bacteria are generally associated with milk and milk products, which gave supplements in retaining intestinal balance (Isolauri, 2001). Probiotics are live non-pathogenic organisms that alter the microflora of intestine and colonization in intestines, and their metabolic functions gave a positive influence on the host's health (Weizman et al., 2006). Various compounds such as reuterin, bacteriocins, lactic acids and acetic acids, were produced by probiotics. These compounds inhibit the growth of pathogens (Alvarez-Olmos and Oberhelman, 2001). In nature, lactic acid bacteria (LAB) are broadly distributed. The genus representation of this group includes Leuconostoc, Pediococcus, Lactococcus and Lactobacillus (Lilia et al., 2002). Probiotic addition to food gave many health advantages to the end user like reduced colon cancer, boosted up immune system, improved gastrointestinal function, and reduced cholesterol level (Berner and Donnell, 1998; Rafter, 2003; Saarela et al., 2002; McNaught and MacFie, 2001). Generally, for the manufacturers of commercial dairy food, probiotic products of LAB are consumed like precursor microbes (Heenan et al., 2002).

The biggest health problem of man is the infectious diseases, and each year worldwide, the gastrointestinal disorders are accountable for considerable mortality and morbidity (Culligan et al, 2009). It is estimated that

annually, more than 4 billion people are affected by diarrhoea, whereas due to enteric infection, 2.2 million deaths occurred, and generally it is the 5th most important death all over the world at all ages (WHO, 2004). The main enteric infectious agents are Staphylococcus aureus, Vibrio cholerae, Salmonella species, Shigella species, Proteus species, Klebsiella species, Pseudomonas species and Escherichia coli (Ballal and Shivananda, 2002). It was reported that probiotics are helpful in gastrointestinal diseases like typhoid, diarrhoea and dysentery (Tambekar and Bhutada 2010). The scientific community was awakened to the therapeutic and prophylactic uses of probiotic due to the rise of antibiotic resistant bacteria, and reflects them as alternative medicine (Ahmed, 2003).

The present research project was initiated to isolate, identify and characterize LAB from traditional yogurts and examine its antimicrobial activity against pathogenic bacteria.

Materials and Methods

Isolation of lactobacilli

Traditional yogurt samples (n=20) were collected from local market in 01litter sterilized glass bottle. One milliliter of yogurt sample was mixed with nine milliliter of sterilized phosphate buffer saline (PBS) for homogenization. Each sample was serially diluted tenfold in PBS. Afterward, pour plate method was employed in each sample to inoculate on de Man Rogosa and Sharpe (MRS) agar plates (Awan and Rahman, 2005). Incubation of inoculated MRS agar plates was carried out for 5 days at 32-40ºC. Well isolated and morphologically distinct colonies were chosen and shifted to fresh MRS agar plates through streaking. Lastly, pure cultural colonies were achieved.

Identification of LAB

Cultural, morphological, biochemical, and physiological characterizations were examined for all the colonies as described in (Bergey's Manual 1984). Species identification was verified with the help of commercial standard identification system (API-50 CHL Biomerieux®, France) as per manufacturer's guidelines. Maintenance of pure culture was carried out in MRS broth with 10% (v/v) glycerol at -20°C.

Screening of Probiotic Characteristics for Isolated Strains

Bile Salt Resistance

Isolated strains were grown in MRS broth in the presence of bile salt. MRS broth tubes were inoculated with 1.0, 0.5, 0.3 and 0.0% (w/v) of oxgall and were mixed with 5 log 10 CFU= 10^5 CFU of each strains (Dunne et al., 2001). After 24-hour incubation, the colony growth was assessed by plate count method (Awan and Rehman, 2005).

Tolerance to acidic pH

Isolated strains were checked on acidic tolerance by growing cultures in acidic MRS broth. Test tubes having MRS broth were adjusted to pH 2.0, 4.0 and 7.0 with the help of 0.5M NaOH and 1M HCl. In each isolated species, an amount of 5 log 10 CFU (10^5 CFU) was poured into each broth tubes. Incubation of test tubes was kept for 120 minutes at 37°C. Plate count method was used to evaluate the bacteria survival (Awan and Rahman, 2005).

Tolerance to stimulated gastric transit

Isolated culture was blended with one milliliter phosphate buffer saline and three milliliter stimulated gastric juice along with microbial amount of 5 log10 CFU (10^5 CFU). Survival of microbial cultures was checked after 120, 90, 60 and 30 minutes of incubation (Dunne et al., 2001).

Storage viability of isolated cultures

Bacterial cultures' viability at different storage duration was checked at different temperatures. Each culture suspension (10^5 CFU) was inoculated in test tubes. The cultured test tubes were kept at 40°C, 25°C and -20°C (with 10% V/V glycerol) for five weeks. Plate count method was used to monitor the microbial growth (Ashraf et al., 2009).

Antimicrobial Activity

Agar well diffusion method was used for antibacterial activities. Hundred microliters of bacterial cell free supernatants were poured in eight millimeters sterilized cut wells in the nutrient agar. The plates were kept in incubator at 35°C for 24 hours. The zones of inhibition diameter were measured with the help of vernier calipers (Ashraf et al., 2009).

Results

In the current study, Lactobacillus species were isolated from traditional yogurt. Out of 20 samples, six species of LAB were isolated and identified. Their biochemical, morphological, physiological and cultural properties are shown in table 1. All identified microbes showed resistance against various Oxgall concentrations, but viable count decreases as concentration increases. Tolerance capacity was observed dissimilar among the whole LAB (Table 2). All species of LAB were found of variable tolerance level to acidic environment. pH 4 revealed the most favorable medium as the viability increases, while the pH 2 showed the most inhibitory medium for LAB as the viability decreases (Table 3). Tolerance of LAB gastric transit studied at different incubation period showed variable among all microbes (Table 4). The findings showed that storage at 4°C and -20°C had no significant effect on viable count of LAB, and all the microbes have good viability subsequent to five weeks of storage. Generally, slight turn down in the viable colony was observed at -20°C and 4°C. Significant decreases were observed in viable count at 25°C storage condition (Table 5). The tested bacteria in the present study were generally found as food borne bacteria that cause gastroenteritis. The current study's results found that antimicrobial potential of the isolated LAB could inhibit all experimental pathogenic microbes at diverse inhibitory levels as given in table 6.

Discussion

The antibiotic properties exist because of the ability of Lactic acid's bacteria to synthesize bacteriocines and lactic acid. Probiotic bacteria produce peptides, which have antibiotic activities (Ashraf et al., 2009). Hydrogen peroxide and organic acids were produced by lactobacilli, were documented to stop pathogenic bacteria, while gram positive bacteria were inhibited by bacteriocin. The dissimilarity of antibacterial potential between tested bacteria was because of diverse intrinsic factors stimulated by numerous food natures (Srikanjana et al., 2008). The Lactobacilli species generally synthesize benzoic acid, H_2O_2, acetic acid and lactic acid (Schauss 1990). Bifidobacteria produced acid (propionic, butyric, acetic, lactic and formic) which are short-chain fatty acids (SCFAs). At maximum pH scale of these probiotics put forth numerous antimicrobial actions on organism's cell growth (Mark 1997).

Conclusion

The given output of this study regarding antagonistic activities of isolated bacteria on a broad range of pathogenic microbes has an important role in human health. These bacteria can be used for the synthesis of numerous type of pharmaceutical and food industry. New functional foods products can also be prepared from theses bacteria. As a result, rising utilization of dairy products especially yogurt having probiotic microbes of foods containing maximum and mainly effective lactobacilli are advised in day to day diet.

Acknowledgment

The authors are thankful to the Director General of PCSIR Laboratories Complex Peshawar-Pakistan for the support and encouragement regarding this study.

Conflicts of interest

There are no conflicts of interest.

References:

1. Ahmed FE. 2003. Genetically modified probiotics in foods. Trends Biotechnol. 21: 491–497.
2. Alvarez-Olmos MI, Oberhelman RA. 2001. Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. Clin Infectious Diseases. 32 (11):1567-1576.
3. Ashraf M, Arshad M, Siddique M and Muhammad G. 2009. In Vitro Screening of Locally Isolated Lactobacillus species for Probiotic Properties. Pakistan Vet. J., 29(4): 186-190.
4. Awan, JA and Rahman SU. 2005. Microbiology Manual. Unitech Communications, Faisalabad, Pakistan, pp: 49-51.
5. Ballal M and Shivananda PG. 2002. Rotavirus and enteric pathogens in infantile diarrhoea in Manipal, South India. Indian J Pediatr. 69:393-396.
6. Bergey's Manual of Systematic Bacteriology. 1984. N. Krieg (Ed), Vol. 1, 2. Baltirnore: Williams and Wilkins.
7. Berner, L., O Donnell, J., 1998. Functional foods and health claims legislation: applications to dairy foods. International Dairy Journal. 8: 355–362.
8. Culligan EP, Hill C and Sleator RD. 2009. Probiotics and gastrointestinal disease: successes, problems, and future prospects. Gut pathogens. 1(19):1-12.
9. Dunne, C., L. O`Mahony, L. Murphy, G. Thornton, D. Morrissey and S. O`Halloran, 2001. In-vitro selection criteria for probiotic bacteria of human origin: Correlation with in vivo findings. Amer. J. Clin. Nutr., 73: S386-S392.
10. Heenan, C.N., Adams, M.C., Hosken, R.W., Fleet, G.H., 2002. Growth medium for culturing probiotic bacteria for applications in vegetarian food products. Lebensmittel-Wissenschaft und-Technologie. 35: 171–176.
11. Isolauri E. 2001. Probiotics in human disease. Am J Clin Nutr, 73:1142 S-1146S.
12. Lilia T., Stanka S., and Tanya Y. 2002. Identification of Lactic Acid Bacteria Isolated from Katyk, Goat's Milk and Cheese. Journal of Culture Collections. 3:48-52.
13. Mark P. 1997. Choosing a Probiotic Supplement. Clinical Nutrition Insights. 6 (1). 1-4.
14. McNaught, C.E., MacFie, J., 2001. Probiotics in clinical practice: a critical review of the evidence. Nutrition Research. 21: 343–353.
15. Rafter, J., 2003. Probiotics and colon cancer. Best Practice and Research Clinical Gastroentrology. 17 (5): 849–859.
16. Saarela, M., Lahteenaki, L., Crittenden, R., Salminen, S., Mattila- Sandholm, T., 2002. Gut bacteria and health foods—the European perspective. International Journal of Food Microbiology. 78: 99–117.
17. Schauss AG. 1990. Lactobacillus acidophilus: method of action, clinical application and toxicity data. J. Adv. Med. 3(3):163-178.
18. Srikanjana K, Helmut V, Jakkapan S, Siriporn O. 2008. Probiotic Properties of Lactobacilli Isolated from Thai Traditional Food. Sci Pharm. 76: 485–503.
19. Tambekar DH and Bhutada SA. 2010. An Evaluation of Probiotic Potential of Lactobacillus sp. From Milk of domestic Animals and Commercial Available Probiotic Preparation of Enteric Bacterial Infections. Recent Research in Science and Technology. 2(10): 82-88.
20. Weizman Z, Facn, MD, Alsheikh A, MD. 2006. Safety and tolerance of a probiotic formula in early infancy comparing two probiotic agents: a pilot study. Journal of the American College of Nutrition. 25:415-419.
21. World Health Organization (2004). http://www.who.int/info/healthinfo/globalburdendis ease/GBD report part 2, pdf.

Table 1: Characterization of Isolated LAB Strains

Characteristics	LJ-2	LJ-3	LJ-4	LJ-5	LJ-6
Cell arrangement	Group of 2 or 4 cocci	Thin, long rods	Medium-long rods rounded ends	Short, rounded rods	Cocci in tetrads
Gram stain reaction	G+	G+	G+	G+	G+
Spore formation	-	-	-	-	-
Milk curdling	+	+	+	+	+
Catalase test	-	-	-	-	-
pH optimum	6.5	5.8-6.5	5.8-6.5	5.8-6.5	6.5
Glucose fermentation	+	+	+	+	+
Growth at 10°C	ND	-	-	+	ND
Growth at 30°C	+	+	+	+	+
Growth at 45°C	+	+	+	-	+
Growth at 50°C	-	ND	ND	ND	+
Growth in 4% NaCl	ND	ND	ND	ND	+
Growth in 6.5% NaCl	ND	ND	ND	ND	+
Colony morphology	grey-white, 1-2 mm	small-sized, slightly convex, R, <1 mm	circular, irregular, "snowflake" type, 1-2 mm	circular, white, glistening, convex, 1 mm	Grey- white one – two mm
Identified LAB	Peddiococcus pentosaccus	Lactobacillus delbrueckii	Lactobacillus helveticus	Lactobacillus plantarum	Pediococcus acidilactici

Legend: G+ = Gram positive, ND= Not determined, - = Negative reaction, += Positive reaction.

Table 2: Plate Count (log_{10}) Value of Isolated Probiotic Bacteria at different Oxgall Concentrations

OC (%)	LJ-2	LJ-3	LJ-4	LJ-5	LJ-6
1.0	6.6±0.5	6.8±01	6.4±0.7	6.2±01	6.0±0.2
0.5	6.6±0.2	7.2±0.9	6.8±0.6	6.3±0.1	6.2±0.4
0.3	7.3±0.1	7.5±0.3	7.1±01	6.7±0.7	6.5±0.8
0.0	7.7±0.5	7.9±0.1	7.5±0.2	7.4±01	7.1±0.3

OC (%) = Oxgall concentration (%), each values represent mean ± SD.

Table 3: Plate count (log_{10}) Value of Isolated Probiotic Bacteria at Various pH values

pH	LJ-2	LJ-3	LJ-4	LJ-5	LJ-6
2.0	5.6±0.1	5.8±0.3	5.3±00	4.8±0.2	4.2±0.3
4.0	6.2±0.5	6.5±00	5.9±00	4.4±0.4	3.2±0.2
7.0	1.8±00	2.0±00	1.7±00	1.5±00	0.0±00

Each values represent mean ± SD.

Table 4: Plate Count (log_{10}) value of Isolated Probiotic Bacteria at Various Gastric Transit

Period of Incubation	LJ-2	LJ-3	LJ-4	LJ-5	LJ-6
120 minutes	4.5±0.1	5.1±00	2.8±00	2.2±00	00±00
90 minutes	4.9±0.3	5.4±00	3.7±00	3.6±00	2.8±00
60 minutes	5.2±0.5	5.9±00	5.0±00	4.8±00	3.8±00
30 minutes	5.6±0.2	5.7±0.3	5.2±00	5.0±00	4.5±0.2

Each values represent mean ± SD.

Table 5: Plate Count (log_{10}) Value of Isolated Probiotic Bacteria at Various Storage Temperature

Storage Duration	Storage Temp.	LJ-2	LJ-3	LJ-4	LJ-5	LJ-6
1st week	-20°C	4.99±0.1	4.97±0.9	4.95±0.1	4.94±0.2	4.92±0.9
	4°C	4.90±0.3	4.95±0.7	4.91±0.3	4.93±00	4.96±0.1
	25°C	4.96±0.5	4.92±0.6	4.83±0.5	4.87±0.7	4.81±0.8
2nd week	-20°C	4.89±0.2	4.96±0.7	4.93±0.7	4.91±0.8	4.90±0.3
	4°C	4.79±0.3	4.76±0.5	4.90±0.3	4.88±0.1	4.93±0.2
	25°C	4.93±0.1	4.84±0.1	4.77±0.2	4.76±0.3	4.71±0.1
3rd week	-20°C	4.77±0.5	4.86±0.3	4.88±0.1	4.89±00	4.90±0.3
	4°C	4.77±00	4.67±0.4	4.85±0.2	4.60±0.1	4.85±0.4
	25°C	3.80±00	3.99±00	4.55±0.4	3.88±00	3.99±00
4th week	-20°C	4.65±0.3	4.71±0.2	4.72±0.8	4.70±0.3	4.80±0.5
	4°C	4.67±0.4	4.59±0.1	4.62±0.6	4.56±0.2	4.79±0.6
	25°C	3.50±00	3.76±00	3.88±00	3.66±00	3.77±00
5th week	-20°C	4.50±0.1	4.30±0.5	4.20±0.4	4.30±0.1	4.60±0.7
	4°C	4.56±0.2	4.44±0.4	4.40±0.2	4.48±0.4	4.68±0.2
	25°C	3.40±00	3.66±00	3.66±00	3.22±00	3.19±00

Each values represent mean ± SD.

Table 6: Antibacterial Activity of Isolated Lactobacilli

Test bacteria	Lactic Acid bacteria strains				
	LJ-2	LJ-3	LJ-4	LJ-5	LJ-6
Salmonella Typhi	12±0.2	17±01	11±01	06±00	05±01
Shigella flexneri	08±0.1	15±10.5	16±02	10±01	12±01
Staphylococcus Aureus	11±01	13±01	12±01	04±00	09±0.5
Escherichia coli	10±01	11±01	15±00	13±01	11±01
Bacillus cereus	14±00	14±02	NZI	05±00	03±00
Streptococcus pneumoniae	02±00	09±00	07±00	01±01	06±00
Klebsiella pneumoniae	16±01	12±0.4	11±00	03±00	NZI

Mean values (± SEM) of zone of inhibition (mm), NZI= No zone of inhibition.