Plant Archives Vol. 21, No. 2, 2021 pp. 355–358 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no2.055

ANTAGONISTIC EFFECT OF *LACTOBACILLUS ACIDOPHILUS* AGAINST ENTEROPATHOGENS AND ITS UTILIZATION FOR DEVELOPMENT OF PROBIOTIC FOOD MIXTURES

Sharon C.L., Usha V., Aneena E.R., Lakshmy P.S. and Seeja Thomachan
Department of Community Science, College of Agriculture, Kerala Agricultural University,
Vellanikkara, Thrissur – 680 656, Kerala, India
Email: sharonlohi@gmail.com

(Date of Receiving : 10-04-2021; Date of Acceptance : 21-06-2021)

ABSTRACT

Probiotics are live microbial supplement, which beneficially affect the host by improving the intestinal microbial balance. *Lactobacillus acidophilus* is one of the most common probiotic bacteria which have beneficial effects on the microbiota of the gastrointestinal tract. In the present study, *L. acidophilus* was used as the probiotic culture. The antagonistic effect of *L. acidophilus* against the enteropathogens strains of *E. coli* (MTCC 40), *Salmonella enteritidis* (MTCC 3219), *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 430) and *Shigella flexineri* (MTCC 1457) was studied. The results showed that *L.acidophilus* was able to inhibit the growth of some of the selected pathogens in varying degrees. It was found to be most effective with a zone of inhibition of 24 mm recorded against *Staphylococcus aureus*. The antagonistic effect may be due to the production of organic acids, bacteriocins and hydrogen peroxide. Later, an attempt wasmade to develop probiotic food mixtures containing banana flour, soya flour, tomato, mango and papaya involving *L acidophilus.* The food mixture (25g) was mixed with 150ml water and stirred to obtain uniform slurry. Adjusted the pH to 4.5 and autoclaved at 121°C (1.5 kg cm^{-2}) for 15 minutes. After cooling this was inoculated with 300µl (8.07log cfu ml^{-1}) liquid culture of *L. acidophilus* (24 hour old culture) and incubated at 37° C for 24 hours. After fermentation it was freeze dried. The viability of the developed food mixtures were assessed for 6 months and it showed good viability which was within the recommended level of probiotic organism to assure health benefits.

Keywords: *Lactobacillis acidophilus,* Zone of inhibition, Probiotic, Banana, Viability

INTRODUCTION

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). A good probiotic should be a strain, which is capable of exerting a beneficial effect on the host animal(e.g. increased growth or resistance to disease). It should be non-pathogenic and non-toxic, should be present as viable cells, preferably in large numbers, should be capable of surviving and metabolising in the gut environment (e.g. resistance to low pH and organic acids), it should be stable and capable of remaining viable for periods under storage and field condition and the strain should be safe and tested for human use (Fuller, 1989).

It is also important that probiotic strains to be antagonistic against carcinogenic and pathogenic bacteria either by antimicrobial substances production or competition exclusion and supporting this, Dave and Shah (1997) reported that lactic acid bacteria produce hydrogen peroxide, diacetyl and bacteriocin as antimicrobial substances which create hostile environments for food borne pathogens and spoilage organisms.

The survival of probiotic organisms in the gut depends on the colonization factors that they posses, organelles which enable them to resist the antibacterial mechanisms that operate in the gut and need to avoid the effects of peristalsis (which tend to flush out bacteria with food) which can be achieved either by immobilising themselves or by growing at a much faster rate than the rate of removal by peristalsis and the strains need to be resistant to bile acid (Seo *et al*., 1989). Probiotic organisms exhibit antagonistic action towards enteropathogens such as *Escherichia coli, Shigella, Salmonella, Staphylococcus, Bacillus, Proteus* etc

For many years, dairy products have been recognized as valuable products to human health. If a staple based food mixture is developed from the commonly used foods in a community and then fermented with probiotic organism, it may have a better profile of nutrients, acceptability and therapeutic value.

Hence, in this experiment an attempt was made to study the antibacterial activity of *L. acidophilus* (MTCC 447) against the enteropathogens strains of *E. coli* (MTCC 40), *Salmonella enteritidis* (MTCC 3219), *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 430) and *Shigella flexineri* (MTCC 1457) and later this probiotic organism was inoculated to banana based probiotic food mixtures and their viability was assessed for a period of six months.

MATERIALS AND METHODS

Antagonistic effect of *L. acidophilus* against enteropathogens

Pure cultures of *L. acidophilus* (MTCC 447) used in the study was obtained from Institute of Microbial Technology (IMTECH), Chandigarh. The enteropathogens strains of *E. coli* (MTCC 40), *Salmonella enteritidis* (MTCC 3219), *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 430) and *Shigella flexineri* (MTCC 1457), were also collected from IMTECH, Chandigarh.

The mode of inhibition of *L. acidophilus* was determined by agar well assay (Singh and Sharma, 1999). Saline suspensions (0.85%) of the pathogens were made using sterile cotton swab; lawn culture of the pathogen was made in nutrient agar in sterile plates by streaking the entire agar surface. Plates were allowed to set and dry. Wells of 5mm diameter were cut with sterile well borer in each plate. MRS broth of 25ml was prepared and distributed to each conical flask after adjusting the pH at 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0. The medium was sterilized at 121˚C for 15 mts. After cooling, 0.1ml of 24 hour grown inoculum was added to this and was incubated at 37˚ C for 24 hours.

From this *L. acidophilus* cultures, 200 µl (8.06 log cfuml^{-1}) were used to fill each well in the agar plate. The plates were then incubated at 37˚C for 24 hours. Diameter of the clear zone around wells was measured in mm.

Development of banana based probiotic food mixtures

Raw banana (Nendran *Musa* AAB) was purchased from the local market. This was peeled, washed, sliced and dried. The dried chips were powdered to a flour of 40 mesh size. This banana flour was used as a source of starch in all food mixtures. The foods selected for developing the probiotically fermented food mixtures were defatted soya flour (as source of protein in the food mixture), mango, papaya and tomato and these foods were purchased from the local market. The combinations of ingredients used in the study are detailed in Table 1

Table 1 : Combinations of ingredients present in the food mixtures

Food mixtures (Treatments)	Combinations
T_1	B (70%) + DS (20%) + M (10%)
T_2	B (60%) + DS (20%) + P (20%)
T_3	B (60%) + DS (20%) + T(20%)

B- Banana flour, DS- Defatted soy flour, M- Mango pulp, P- Papaya Pulp, T- Tomato pulp

After optimising the conditions for fermentation, the food mixture (25g) was mixed with 150ml water and stirred to obtain uniform slurry. Adjusted the pH to 4.5 and autoclaved at 121° C (1.5 kg/cm2) for 15 mts. After cooling it was freeze dried.In the freeze dried samples the viability of food mixtures were assessed for a period of six months.

Viable count of *L. acidophilus* in the food mixtures

Viable counts of *L. acidophilus* present in fermented food mixture were enumerated using MRS medium. One gram of the mixture was weighed and transferred to a tube containing 9 ml sterile distilled water (dilution 10 $^{-1}$). This was then serially diluted up to 10^{-7}. The samples were enumerated for microbial count by pour plate method using MRS agar and the results are expressed as log cfug^{-1}.

RESULTS AND DISCUSSION

Antibacterial activity of *L. acidophilus* on enteropathogens

Table 2 : Antagonistic activity of *L. acidophilus* (MTCC 447) at varying pH

pH / Test Organisam	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
	Inhibition zone in mm								
E.coli	20	16	17	17	16	16	15	13	12
Bacillus cereus	18	18	14	13	13	12	11	11	10
Staphylococcus aureus	15	14	14	13	12	12	11	11	10
Salmonella enteritidis	24	24	22	19	18	18	17	16	8
Shigella flexneri	5	5	5	5	5	5	5	5	5

- Zone of inhibition including the well diameter of 5 mm

Most probiotic strains are believed to have an ability to colonise the intestinal tract and thereby positively affect the mircoflora and perhaps exclude colonization by pathogens. *L. acidophilus* (MTCC 447) has also exhibited an antagonistic activity against some enterophathogens at different pH levels (Table 2). Among all the pathogens studied, *Salmonella enteritidis* was the inhibited by *L. acidophilus* most effectively with an inhibition zone of 24mm at pH 3.0, followed by *E. coli* (20mm), *Bacillus cereus* (18mm) and *Staphylococcus aureus* (15mm). *L. acidophilus* was not capable of inhibiting *Shigella flexneri* at any of the pH. Antagonistic activity of *L. acidophilus* was highest at pH 3.0 on all selected enteropathogens. A similar study by Liong and Shah (2005) indicated that *lactobacillu*s can be beneficial in food products because of their ability to produce hydrogen peroxide. This hydrogen peroxide produced enabled them to suppress the growth of S. *aureus, E. coli, C. botulinum* and other undesirable microorganisms. This was followed by inhibition by *E. coli* which showed a diameter of 20 mm at 3.0 pH but at 3.5 pH *Bacillus cereus* showed more inhibition than *E. coli.*

Earlier, in a study by Lin *et al*. (2009) *Lactobacillus acidophilus* RY2, *Lactobacillus salivarius* MM1 and *Lactobacillus paracasei* En4 were shown to significantly inhibit the growth of Entero aggregative *Escherichia coli* (EAggEC) and they suggested that *L. acidophilus* RY2 could be used as a probiotic organism against EAggEC. Gharaei-Fathabad and Eslamifar 2011 shown the inhibitory activity of *Lactobacillus paraplantarum* isolated from tea leaves against *S. typii* (65mm), *E.coli* (30 mm), *S. aureus* (56 mm), *E. faecalis* (55 mm) and *Citrobacter sps* (60 mm *Lactobacillus alimentarius*, *Lactobacillus sake* and *Lactobacillus collinoides* isolates showed moderate activity (inhibition zone <15 mm) except *L. collinoides* and *L. alimentarius* that had relatively strong activity (inhibition zone ≥15 mm) against *P. aeruginosa* and *B. subtilis*, respectively (Karami *et al.*, 2017).

The inhibitory effect is thought to be brought out by either due to competition for nutrients or due to presence of starter derived inhibitors such as diacetyl, lactic acid, hydrogen peroxide and bacteriocins (Abee, 1995).Studies have reported that *L.acidophilus* inhibits the pathogenic flora by production of certain antibiotics like Acidophilin, Lactocidin, Acidolin and Lactolin (Glodin, 1998). The strains vary in their ability to produce these substances and cultural conditions will influence the amount produced (Salminen *et al*., 1998). Organic acetic and lactic acids which are produced by lactic acid bacteria will lower intestinal pH and thereby inhibit the growth of many bacteria, especially pathogenic gram-negative types (Anand, 1984). These organic acids also increase peristalsis, thereby indirectly removing pathogens by accelerating their rate of transit through the intestine (Lario and Martin, 1990).

Carbon dioxide and diacetyl synthesized by lactic acid bacteria inhibit growth of pathogens. Numerous bacteriocins, such as nisin, lactobrevin, acidophilin, acidolin, lactobacillin, lactocidin and lactolin, have been reported to be produced by *lactobacilli.* Bacteriocins are active against a wide range of food-borne pathogens, depending on their specificity (Mishra and Lambert, 1996). Studies conducted by Coconnier (2000) has also established the secretion of antimicrobial substances by *L. acidophilus* strain isolated from human gut.

Hydrochloric acid secreted by the gastric mucosa may kill many of the food-borne pathogens, as may both bile acids and pancreatic enzymes. The motility of the intestine, epithelial mucin secretion and the activity of microflora can act synergistically to kill pathogens and/or prevent their colonization and subsequent translocation across the intestinal mucosa. Several of these non-specific intestinal defense parameters may be modulated by diet.

Viable count of *L. acidophilus* in fermented food mixtureson storage

As presented in Table 3, there was a significant reduction in the viable count of *L. acidophilus* throughout the storage period. Initially, viable counts of *L. acidophilus* varied from 9.14 (T_2) to 9.45 (T_3) log cfu g^{-1}. After six months of storage, viable count was significantly reduced which varied from 8.95 (T_2) to 9.12 (T_3) log cfu g^{-1}. According to FSSAI, to realize the health benefits, probiotic bacteria must be viable and available at a high concentration, typically 10^8 cfu/ g of the product.

In the present study, the initial viable count of *L. acidophilus* was high in the food mixtures so that they retained high viable counts even after 6 months of storage. The cell count at the end of incubation must be sufficiently high to allow up to 90 per cent mortality of probiotic bacteria during storage and yet still leave their number above the desired minimum of 10^6 cfu ml^{-1} viable cells (Marshall, 1991). A common principle is that the higher the initial cell concentration, the longer the shelf life of the products (Costa *et al.,* 2002). Wang *et al.* (2007) developed a probiotic peanut flour fermented with 4 stains of *Lactobacillus* and found the cell population of *L. acidophiluus* of 9.48 log cfu/g after 72hr fermentation at 37 ^{o} C and after 28 days of storage no marked change in the viable count was observed. Nath (2015) in her study on probiotic honey beverage concluded that the chemical, nutritional, organoleptic and shelf life qualities and was shelf stable for 15 days at refrigerated condition and maintained viability. The cell viability depends on the strain used, interaction between species present, culture condition, oxygen content, final acidity of the product and the concentration of lactic acid and acetic acid in the food system.

Table 3: Viable count of *L. acidophilus* in fermented food mixtures on storage [log cfu g^{-1}]

FFM	Storage period in months						
	Initial	1	2	3	4	5	6
T1 (B+ DS +M)	9.17[a]	9.15[b]	9.12[c]	9.08[d]	9.05[e]	9.02[f]	8.98[g]
T2 (B+ DS + P)	9.14[a]	9.12[b]	9.09[c]	9.05[d]	9.03[e]	8.97[f]	8.95[g]
T3 (B+ DS + T)	9.45[a]	9.43[b]	9.41[c]	9.34[d]	9.31[e]	9.27[f]	9.12[g]

B- Banana flour, DS- Defatted soy flour, M- Mango pulp, P- Papaya Pulp, T- Tomato pulp
Values having different super script differ significantly at 5% level
DMRT row wise comparison

CONCLUSION

To provide health benefits, probiotics must overcome physical and chemical barriers such as acid and bile in GI tract and also exhibit antmicrobial properties. *L. acidophilus* (MTCC 447) showed antogonostic activity against enteropathogen strains of *E. coli*, *Salmonella enteritidis*, *Bacillus cereus* and *Staphylococcus aureus*. Application of probiotic culture in non-dairy products and environment represents a great challenge. Viability and probiotic activity must be maintained throughout the storage, handling and storage of the product containing probiotic and has to be verified at the end of shelf life. In the present study, three banana based food mixtures with good viability even after 6 months storage at room temperature was developed and was within the recommended level of probiotic organism to assure health benefits.

REFERENCES

Abee, T. (1995). Pore-forming bacteriocins of Gram-positive bacteria and self-protection mechanisms of producer organisms. *Microbiol. Lett*. 129: 1-10.

Anand, S.K.; Srinivasan, R.A. and Rao, L.K. (1984). Antibacterial activilt associated with Bifidobacterium bifidum I. *Cult. Dairy Prod. J.* 19: 6-8.

Coconnier, M.H.; Lievin, V. and Lorrot, M. (2000). Antagonistic Activity of *Lactobacillus acidophilus* LB against Intracellular *Salmonella enterica* Serovar Typhimurium Infecting Human Enterocyte-Like Caco-2/TC-7 Cells. *Appl. Environ. Microbiol*. 64: 4573-4580.

Costa, E.; Usall, J.; Teixido, N.; Torres, R. and Vinas, I. (2002). Effect of package and storage conditions on viability and efficacy of the of the freeze-dried biocontrol agent Pantoe aagglomerans strain CPA-2. *J. Stored Products Res*. 38: 329-339.

Dave, R.I. and Shah, N.P. (1997). Vaibility of probiotic bacteria in yoghurts made from commercial starter cultures. *Int. Dairy J*. 7: 31-41.

FAO/WHO (Food and Agriculture Organisation /World Health Organisation). (2001). Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. WHO, Cordoba, Argentina. pp.168.

Fuller, R. (1989). Probiotics in man and animals. *J. Appl. Bacteriol*. 66: 365-378.

Gharaei-Fathabad E. and Eslamifar, M .(2011). Isolation and Applications of one strain of Lactobacillus paraplantarum from tea leaves (*Camellia sinensis*). *Am. J. Food Technol.* 6: 429-434

Goldin, B.R. (1998). Health benefits of probitoics. *Br. J. Nutr*. 80 : 203-207.

Karami, S.; Roayaei, M.; Hamzavi, H.; Bahmani, M.; Hassan Hassanzad-Azar, Leila, M. and Rafieian-Kopaei, M. (2017). Isolation and identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens. *Int. J. Pharm. Investig*. 7(3): 137-141.

Laroia, S. and Martin, J.H. (1990). Bifidobacteria as possible dietary adjuncts in cultured dairy products- A review. *Cult. Dairy Prod. J*. 25: 18-22.

Lin, P.P.; Hseih, Y, M.; Tsai, C.C. (2009). Antagonistic activity of *Lactobacillus acidophilus* RY2 isolated from healthy infancy feces on the growth and adhesion characteristics of entero aggregative *Escherichia coli. Anaerobe*. 15(4): 122-126

Liong, M. T. and Shah, N. P. (2005). Optimisation of cholesterol removal by probiotics in the presence of prebiotics by using response surface method. *Appl Environ. Microbiol*. 71 (14): 1745-1753.

Marshall, V. M. (1991). Gut-derived organisms for milk fermentations: Is Probiotics fact or fiction. *J. Chem. Technol. Biotechnol*. 51: 548-553.

Mishra, C. and Lambert J. (1996). Production of anti microbial substances by probitoics. *Asia Pacific J. Clin. Nutr.* 5: 20-24

Nath, A.H.; Ukkuru, M.P. and Kumari, M.K. (2015). Development of a probiotic honey beverage. *Int. J. Appl. Pure Sci. Agric.* 9:1-9.

Seo, G.; Shimizu, K. and Kono, M. (1989). Inhibition of growth of some enteropathogenic strains in mixed cultures of Streptococcus faecalis and Clostridium butyrium. *Microbios*. 40: 151-160

Singh, B.R. and Sharma, V.D. (1999). Characterisation and broad spectrum Klebocin produced by *Klebsiella pneumoniae* subspecies *aerogenes*. *Indain J Comp. Micrbiol. Immunol. Infect. Dis.*20: 116-120

Salminen, S.; Bouley, C.; Boutron, M.C.; Cummings, J.H.; Franck, A.; Gibson, G.R.; Isolauri, E.; Moreau, M-C.; Roberfroid, M. And Rowland, I. (1998). Functional food science and gastrointestinal physiology and function.*Br J Nutr*. 80: 147-171.

Wang, N.F.; Shi, Y.H.; Sun, J.; Le, G.W. (2007). Evaluation of peanut four fermented with lactic acid bacteria as probiotic food. Food Sci. Technol. Int. 13(6): 460-475.