Research Article

www.ijrap.net

STUDY OF PROBIOTIC ATTRIBUTES AND POTENTIAL OF BACTERIA ISOLATED FROM INFANT FECES AND COMPARE WITH *L. RHAMNOSUS* AS CONTROL

Patial Vijeta [1], Bhardwaj Anupama [2]* and Pannu Shailpreet [3]

[1]Post Graduate Student, Department of Biotechnology, DAV College, Chandigarh, India

[2]Assistant Professor, Department of biotechnology, SGGS College, Chandigarh, India

[3]Scientific Officer, Orbit Biotech, Mohali, India

Received on: 17/07/15 Revised on: 18/08/15 Accepted on: 29/08/15

***Corresponding author**

Mrs Anupama Bhardwaj, Assistant Professor, Department of biotechnology, SGGS College, Chandigarh, India

E-mail: sharma.anu20@gmail.com

DOI: 10.7897/2277-4343.066141

ABSTRACT

In the present study Probiotic bacteria *Lactobacillus rhamnosus* R0011 isolated from lacidophil capsule was used as control and bacterial isolate isolated from infant feces used as test organism. Probiotic potential and attributes of both the bacterial strains were determined. The control and test strains were examined for their ability to grow in low pH conditions (3-6), sugar tolerance (5-30 degree brix). Thermal death point and thermal death temperature of both the strains were determined. Antimicrobial activity of both the strains was estimated against *E.coli* and *S.aureus.* The result showed that the probiotic bacteria *L.rhamnosus* R0011 and bacterial isolate were able to tolerate acidic pH, high temperature upto 70 ^{0}C and 5-6° brix sugar level. Both the strains were effective against *E.coli* and *S.aureus* species but more effective against *S.aureus*. It was observed, the strain *L.rhamnosus* R0011 and bacterial isolate acts as a promising probiotic supplements for the human consumption.

Keywords: Lacidophil, probiotic, TDT, TDP, *E.coli*

INTRODUCTION

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host[1] and has beneficial effects on gastrointestinal disorder, promotes lactose digestion and lowers the concentration of several metabolic products suggested to be injurious to health, including markers of carcinogenesis in the colon[2]. Probiotic microorganisms also exert several immune modulating effects[3]. The probiotic strain *L.rhamnosus* R0011 is a commercially available probiotic product that is broadly used in human dietary supplements and pharmaceutical products[4]. The infant feces isolated lactic acid bacteria have antimicrobial activity against (*S.aureus* and *E.coli*). The antimicrobial susceptibility of lactobacilli has received only little attention, and clinical isolates have rarely been studied[7].

The aim of the present study was to isolate the *L.rhamnosus* R0011 from Lacidophil capsule and promising probiotic bacterial isolate from infant feces and study their probiotic attributes and probiotic potential.

MATERIAL AND METHODS

Procurement of lacidophil capsule

Lacidophil capsule was procured from a chemist shop, Kitchener, Ontario, Canada.

Collection of feces sample

Sample was collected from human infant feces in

sterilized container using sterilized spatula.

Chemicals

MRS broth, MRS agar, Nutrient agar, Nutrient broth was procured from HiMedia Labs, Ahuja Agencies, Chandigarh.

Isolation of *L. rhamnosus* from Lacidophil capsule

To the 50ml MRS broth 1 lacidophil capsule was added. After overnight incubation one loopful culture was spreaded over the agar surface and then incubated for 48 h at 37 °C. Bacteria were isolated using streaking method. After incubation, bacteria were identified morphologically and microscopically.

Isolation of bacterial strain from infant feces

One gram of sample was thoroughly mixed with 9 ml of 0.9% saline Distilled water, serial dilutions being further prepared, from each dilution, an amount of 0.1ml was plated on MRS agar. After incubation, bacteria were identified morphologically and microscopically[5].

Determination of Probiotic attributes of control and test isolate

Thermal Death Point (TDP)

TDP test was performed to determine the stable temperature for the survival of bacteria. Five sterilized tubes of MRS broth (2 ml each) were prepared and each tube inoculated with the *L. rhamnosus* R0011 and bacterial isolate and then incubated at 37 °C for 24 h. Water bath was set at 40 °C, and then put one tube in water bath for 10 minutes. After 10 minutes culture was taken out and spread on MRS agar plate. Repeated the above-mentioned steps for the range of temperature 40, 50, 60, 70, 80 °C. After 24 h incubation period results were observed.

Thermal Death Time (TDT)

TDT test was performed to determine the survival time of bacteria at a given temperature. Five tubes of MRS broth were prepared and each tube was inoculated with a culture to be tested and incubated for 24 h. After TDP test killing temperature for strains were determined, then culture tubes were kept at that temperature in the water bath for 2, 4, 6, 8, and 10 min respectively. The tubes were taken out after the given time period and culture was spreaded on MRS agar plate. Plates were incubated overnight and results were observed after incubation period.

pH resistance of lactic acid bacteria

Acid tolerance of *L.rhamnosus* R0011 and bacterial isolate was studied to check the survivability at low pH. Four test tubes (having 10 ml MRS broth in each) were taken and pH was adjusted with 1N HCl and NaOH to pH 3, 4, 5, 6. After calibration the MRS tubes were inoculated with 1 ml of revived culture and incubated for 24h. After incubation period, 15µl of each test tube were spreaded on agar plates and plates were incubated overnight (24h) and results were observed.

Sugar tolerance

To the Six Test tubes having 5ml nutrient broth, glucose were added i.e. 5, 10, 15, 20, 25, 30 degree brix respectively amid help of refractometer. After autoclaving, 30µl of culture containing bacteria *L.rhamnosus* R0011 and bacterial isolate was added in each test tube and then incubated overnight. After incubation 15µl of culture was taken from test tube and spreaded on the nutrient agar plate. Result was observed after 24h. Repeat the step for different test tubes of 10, 15, 20, 25, 30-degree brix glucose.

Probiotic potential of control and test isolate

The strains both *L. rhamnosus* R0011 and Bacterial isolate were tested for their probiotic potential inhibitory effects. Antimicrobial effect of control and selected isolate against indicator bacteria was determined by agar well diffusion method[6, 8]. *E.coli* (MTCC No.1687) and *S. aureus* (MTCC No.737) were used as indicator bacteria inoculated on nutrient agar. A volume of 100µl of cell free supernatants was filled in 7-mm diameter wells cut in the nutrient agar. The diameter of inhibition of zone was measured after 48 h[7].

Table 1: TDT for control and bacterial isolate

Temp. (°C)	Time (mins)		2	4	6	8	10
80	C	CFU	$.13\times10^2$	$.07\times10^2$	$.04\times10^2$	$.02\times10^2$	0
76	T	/ml	0.17×10^2	$.10\times10^2$	$.06\times10^2$	$.03\times10^2$	0

C= control; T= bacterial isolate; mins= minutes; temp. = Temperature

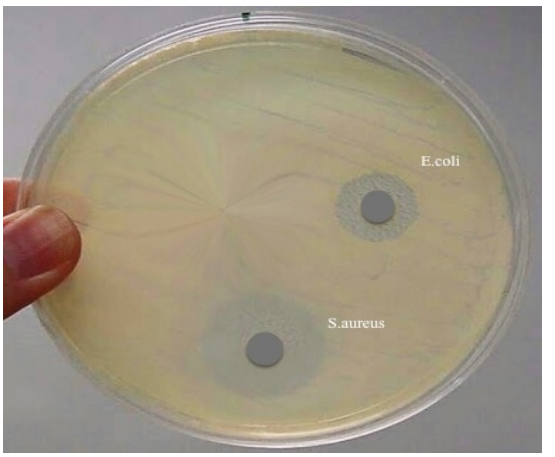

Figure 1: Zone of Inhibition against *E.coli* and *S.aureus* shown by Bacterial Isolate

RESULTS AND DISCUSSION

In this study, we first aimed to isolate promising bacterial isolate from infant feces and then evaluate some probiotic characteristics of a panel of lactic acid bacteria. To be considered as probiotic, microorganisms had to meet some selection criteria. Among all the in vitro parameters defined, we chose to test resistance to acidic, sugar level, determination of their TDT and TDP and antimicrobial activity as defined previously.

Isolation of control and test bacterial strain

The bacteria isolated from lacidofil capsule and bacterial isolate from infant feces. Both control and bacterial isolate was identified using gram staining, catalase test, MRVP test, and citrate test. Control and bacterial isolate was gram positive, rod shaped and negative for Catalase test.

TDP (Thermal death point) of control and bacterial isolate

The maximum growth of both *L. rhamnosus* R0011 and bacterial isolate was obtained at 40°C. At this temperature 5000 and 4300 colonies of control and bacterial isolate were obtained; at 70°C there was least growth i.e. 35 and 47 colonies.

TDT (Thermal death time) of control and bacterial isolate

Again this test was performed for the range of temperature i.e. 74°C, 76°C, 78°C, 80°C for 10 minutes each. After incubation period, it was found that the control was stable at 72°C to 76° C and unstable at 80°C for 10 mins while bacterial isolate was stable at 70-74°C and unstable at 76°C for 10 mins incubation period.

pH tolerance of control and bacterial isolate

Maximum growth of control was observed at pH 6 while bacterial isolate showed maximum growth at 4 pH. Being resistant to low pH is one of the major selection criteria for probiotics.

Sugar tolerance of control and bacterial isolate

The results showed that control and bacterial isolate had grown in 5 and 6-degree brix glucose concentration. High sugar level reduces the growth of the bacteria and thereby reduces its effectiveness.

Antimicrobial activity of control and bacterial isolate

Agar well-diffusion method, used in this test, proved to be useful for selecting probiotic isolate of *Lactobacillus spp*., that possessing the ability to inhibit or compete with harmful bacteria. In present study after 48h of incubation period, control had shown 6.2 mm and 4.8 mm of clear zone against *S.aureus and E.coli* respectively while Bacterial isolate had shown clear zone of 6.0 and 4.2 mm for *S.aureus* and *E.coli*.

CONCLUSION

After studying probiotic attributes and potential of bacteria isolated from infant feces, it was concluded that it could be used as probiotic. All characteristics show that *L.rhamnosus* R0011 and bacterial isolate strains has potential to act as probiotics.

REFERENCES

1. Schrezenmeir J, de Vrese M. Probiotics, Prebiotic, and synbiotics-Approaching a definition. Am J Clin Nutr 2001; 73:361-364.
2. Steer T, Carpenter H, Tiohy K, Gibson GR. Perspectives on the role of the human gut micro flora and its modulation by pro and prebiotics. Nutr Res Rev 2000; 13: 229-254. http://dx.doi.org/10.1079/095442200108729089
3. Kalliomaki M, Salminen S, Arvilommi H, Kero P, kosinen P, isolauri E. "Probiotics in primary prevention of atopic disease". Lancet 2001; 357: 1076-1079. http://dx.doi.org/10.1016/S0140-6736(00)04259-8
4. Foster LM, Tompins TA, Dahl WJ. A comprehensive post market review of studies on a probiotic product containing *Lactobacillus helveticus* R0052 and *Lactobacillus rhamnosus* R0011. Benef. Microbes 2011; 2 (4): 319-334. http://dx.doi.org/10.3920/BM2011.0032
5. Goderska K, Czarnecki Z. Characterization of selected strains from *Lactobacillus acidophilus* and *Bifidobacterium bifidum.* African J. of Micro. Res. 2007;1(6): 66-78.
6. Ashraf M, Arshad M, Siddique M, Mutiammad G. In vitro screening of locally isolated Lactobacillus species for probiotic properties. Pakistan Vet. J 2009; 29(4): 186-190.
7. Salminnen MK, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V, Järvinen A. Lactobacillus bacteremia, species identification and microbial susceptibility of 85 blood isolates. Clin. Infect. Dis. 2006; 42:35-45. http://dx.doi.org/10.1086/500214
8. Cyriac MB, Pai V, Varghese I, Shantaram M, Jose M. Antimicrobial properties of areca catechu (areca nut) husk extracts against common oral pathogens. Int.J.Res.Ayurveda Pharm 2012; 3(1): 81-84.

Cite this article as:

Patial Vijeta, Bhardwaj Anupama and Pannu Shailpreet. Study of probiotic attributes and potential of bacteria isolated from infant feces and compare with *L. rhamnosus* as control. Int. J. Res. Ayurveda Pharm. 2015;6(6):760-763 http://dx.doi.org/10.7897/2277-4343.066141

Source of support: Nil, Conflict of interest: None Declared