Available online at http://www.journalijdr.com

IJDR

International Journal of
DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 07, Issue, 06, pp.13208-13212, June, 2017

RESEARCH ARTICLE

PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM ANIMAL SOURCES

***Deepti D. Dhere, Manisha A. Dhotre and V.S. Shembekar**

Department of Biotechnology, Rajarshi Shahu Mahavidyalaya (Autonomous), Latur, (M.S.)

ARTICLE INFO

Article History:
Received 20th March, 2017
Received in revised form
09th April, 2017
Accepted 17th May, 2017
Published online 30th June, 2017

Key Words:
Probiotics,
Lactobacillus,
Antibacterial activity,
Bacteriocin.

ABSTRACT

In the present research study an attempt was made to isolate the probiotic bacteria from animal sources and their probiotic properties were determined. Total15 milk samples (from Cow, Buffalo, and Goat) were used as source of probiotic bacteria. Out 57 isolates 11 isolates were screened as prominent probiotics, among them 3 isolates from Cow, Buffalo and Goat were found with significant probiotic properties. The probiotic properties of bacterial isolates were evaluated by performing seven different tests e.g. catalase test, bacteriocin assay, organic acid production study, resistance to acidic conditions, tolerance to bile, auto aggregation study and co-aggregation study. The collective score of all probiotic properties was used to determine its probiotic potential, where, the probiotic potential of isolates (PB1) from milk of domestic animals (Cow) was found to be highest i.e. (66.6%) as compared commercial isolate (PB5), which was 60%. The strains isolated from milk of Goat and Buffalo (PB2 and PB3) shows equal probiotic potential. Study suggested that use of these isolated probiotic bacteria can be a remedy to treat the bacterial infections in aquaculture.

Citation: Deepti D. Dhere, Manisha A. Dhotre and V.S. Shembekar, 2017. "Probiotic properties of lactic acid bacteria isolated from animal sources", *International Journal of Development Research*, 7, (06), 13208-13212.

INTRODUCTION

Probiotic bacteria are usually associated with milk and milk products, which provide supplements in maintaining beneficial microbial balance intestine (Isolauri**,** 2001). Probiotic is the group of microbes that may help directly for enhancing resistance against intestinal pathogens and in prevention of diseases (Tambekar and Bhutada, 2010)**.** Studies suggest that there are about three prominent mechanisms by which probiotics bacteria works which are, 1) Probiotics block pathogenic bacterial effects by producing bactericidal substances and competing with pathogens and toxins for adherence to the intestinal epithelium; 2) Probiotics regulate immune responses by enhancing the innate immunity and modulating pathogen induced inflammation via toll-like receptor-regulated signaling pathways; and 3) Probiotics regulate intestinal epithelial homeostasis by promoting intestinal epithelial cell survival, enhancing barrier function, and stimulating protective responses (Vanderpool *et al.,* 2008). Probiotics have been applied as an alternative approach of prevention and therapy for several infections in fish.

****Corresponding author: Deepti D. Dhere,***
Department of Biotechnology, Rajarshi Shahu Mahavidyalaya (Autonomous), Latur, (M.S.).

The functional properties and safety of probiotics of particular strains of *L. casei, L. lactis, L. acidophilus* from various sources have been extensively studied. The commercial probiotic preparations also claim its efficiency for prevention of infectious diseases, but probiotics potential of bacteria from milk of domestic animals viz. Cow, Goat and Buffalo, with significance in prevention of enteric infection is less reported. Most of the probiotic bacteria used in food products to date have been of human or dairy origin as these probiotics possess the immunomodulatory effect and they are able to remain viable in the gastrointestinal tract (Masuda, Kimura, Okada, & Yasui, 2010). Therefore, an attempt was made to isolate *Lactobacillus* strains as probiotics from milk of domestic animals and its probiotics potential was compared with commercial probiotic preparations. The isolated probiotic bacteria can be used as a remedy to treat the bacterial infections in aquatic animals. Infectious diseases are the biggest problem in aquaculture and every year several infections are responsible for significant morbidity and mortality of fishes and lead to commercial loss for aquaculture business. Disease outbreaks are being increasingly recognized as a significant constraint on aquaculture production and trade, affecting the economic development of the sector in many countries. The massive use of antimicrobials for disease control and growth promotion in animals increases the

selective pressure exerted on the microbial world and encourages the natural emergence of bacterial resistance (Verschuere *et al.,* 2000). The resistant bacteria can proliferate after an antibiotic has killed off the other bacteria, and can also transfer their resistance genes to other bacteria that have never been exposed to the antibiotic (Verschuere *et al.,* 2008). In recent studies an attempt was made to isolate the probiotic bacteria from animal sources and their probiotic properties were compared with bacteria isolated from commercially available probiotic preparations. The isolated probiotic bacteria can be used as a remedy to treat the bacterial infections in aquatic animals.

MATERIALS AND METHODS

Isolation of probiotic bacteria from milk of domestic animals

As per need of our experimental study, total of 15milk samples (05 each from Cow, Goat, and Buffalo) were inoculated into MRS broth (Himedia) directly from animal teat to reduce loss of microbial load. The enrichment and isolation was done as per protocol given by D.H. Tambekar and S. A. Bhutada (2010) and all isolates were confirmed as catalase negative and then maintained on MRS agar slants for characterization and further studies.

Preparation of bacteriocin assay

The probiotic bacteria show antagonistic activity against many pathogens by producing Bacteriocin Like Inhibitory Substance (BLIS) which was determined by modifying the method described by Vivekanand *et al.* (2008). All the isolated bacteria were spotted on the MRS soft agar (0.8%) and were incubated at 37^0C for 16h then overlaid by indicator bacteria suspended in Nutrient agar (0.6%) and kept for incubation for next 12h. After incubation results were recorded in terms of zone of inhibition (mm).

Organic acid production

The probiotic bacteria show antagonistic activity by lowering the pH of the surrounding medium hence, organic acid production study of isolated bacteria was done by using protocol described by Hoque *et al.* (2010).

Resistance to acidic conditions (pH 3)

Resistance to acidic condition were studied by harvesting 16-18h active cultures of isolated bacteria by centrifugation for 10min at 5000 rpm and 4^0C, each pellet was washed with phosphate buffer saline (PBS) (pH 7.2) and re-suspended in PBS (pH 3) followed by incubation at 37^0C.Viable bacteria were enumerated at 0, 1, 2 and 3 hours with pour plate technique and plates were incubated at 37^0C for 48h (Prasad *et al.,* 1998).

Tolerance against Bile

For determining bile tolerance, MRS agar medium containing 0.3 to 1% bile was inoculated with 16-18h active culture by using spread plate technique. After the incubation, viable colonies were enumerated. MRS media without bile was maintained as negative control. (Srikanjana *et al.,* 2009). The percent tolerance was calculated by using the formula-

$$\% \text{ Growth} = \frac{\text{No. of CFU on MRS with Bile}}{\text{No. of CFU on MRS without Bile}} \times 100$$

Auto aggregation study

For auto-aggregation studies isolates were grown overnight at 37^0C in MRS broth and centrifuged at 6000 rpm for 15 minutes and cell pellet was re-suspended in PBS-7.2 to obtain an optical density (O.D.) of 0.6 at 600nm. (Natalia F. Gil *et. al*; 2009). O.D. of PBS was measured after every 1h up to 4h incubation. Auto-aggregation was inversely correlated with O.D.

Co- aggregation study

Co-aggregation studies were carried out by using the protocol described by Natalia F. Gil *et al.* (2009) against 4 pathogens namely *E. coli, V. cholerae*, *Pseudomonas sp.* and *S. aureus*.

Calculation of probiotics potential

The collective score of all probiotic properties was used to determine its probiotic potential which was compared with probiotic potential of bacteria from commercial probiotic preparations. Each property of isolates from milk of domestic animals and commercially available probiotics preparation were scored by using observation table-1. Probiotic potential is sum of score of acid, bile tolerance, organic acid production, catalase activity, auto-aggregation and co-aggregation and it is calculated as, observed score divided by maximum score into hundred.

$$\text{Probiotic potential} = \frac{\text{Observed Score}}{\text{Maximum Score}} \times 100$$

RESULTS AND DISCUSSION

In present study, total 15 milk samples (05 each, from Cow, Goat, and Buffalo) were analyzed from which 03 isolates shown strong positive result for production of BLIS against four indicator bacteria, namely *E. coli*, *Pseudomonas sp.*, *S. aureus* and *L. bulgaricus.* The isolate PB1 PB2 and PB3 from Cow, Buffalo and Goat respectively was recognized as excellent probiotics on the basis of their acid and bile tolerance, antibacterial activity, organic acid production potential, catalase activity, auto-aggregation and co-aggregation and on comparison with probiotic bacteria isolated from commercially available probiotics preparation PB4 and PB5.

Members of the *Lactobacillus sp.* are always catalase negative hence the production of catalase enzyme is considered as first step to detect its probiotics nature and all the isolates shown negative results. The antibacterial activity of the isolates due to production of BLIS showed that PB2 and PB3 exhibit similar activity against *E. coli* and *S. aureus.* PB2 showed maximum activity against *L.* bulgaricus while PB1 shows maximum activity against *E. coli* and least activity against *S. aureus*. PB4 and PB5 showed similar activity against *L. bulgaricus* while PB4 shown maximum activity against *S. aureus*. (Figure-1). Mami Anas *et al;* (2008) also carried out similar studies to evaluate antibacterial activity of *L. rhamnosus* isolated from goat milk showed inhibitory activity against *S. aureus, B. Cereus, E. coli and Salmonella typhi.*

Table 1. Score of probiotic potential of isolated bacteria

S.No	Probiotics properties	Indication	Score
1.	Acid Tolerance	CFU/ml at 4h. <50%to 0h.	0
		CFU/ml at 4h. >50%to 0h.	1
2.	Bile Tolerance	% Growth <40%at 0.3% bile salt	0
		% Growth >40%at 0.3% bile salt	1
3.	Organic acid production	Below 2%	1
		Above 2%	2
4.	Catalase activity	Positive	0
		Negative	1
5.	Auto-aggregation	No aggregation	0
		O.D.>0.3 at 4h.	1
		O.D. < 0.3 at 4h.	2
6.	Co-aggregation	No Co-aggregation	0
		With 2 pathogens	1
		With 4 pathogens	2
7.	Antibacterial activity	Average of Zone of inhibition against 4 indicator bacteria.	
		1-2cm	1
		2-3cm	2

Table 2. Organic acid (%) produced and pH in skim milk after 72h incubation

Source of Bacteria	Isolate	Incubation Temperature(^{0}C)	Organic acid (%)	pH observed
Cow Milk	PB1	37	2.448	3.7
Goat Milk	PB2	37	1.872	3.9
Buffalo Milk	PB3	37	1.118	4.0
CPP	PB4	37	2.205	3.8
CPP	PB5	37	2.341	3.7

Legend-CCP- Commercial probiotic preparations

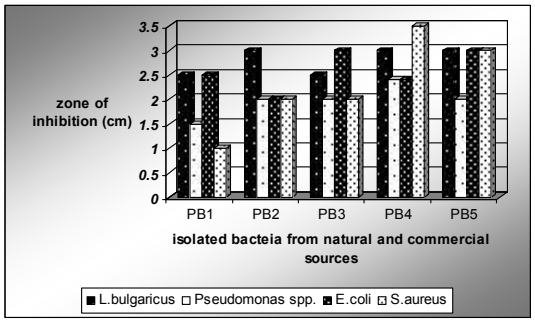

Figure 1. Antibacterial activity shown by isolated bacteria in terms of zone of inhibition

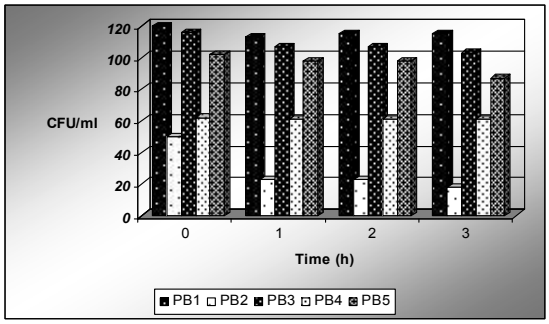

Figure 2. Acid tolerance shown by the isolated bacteria in terms of CFU/ml.

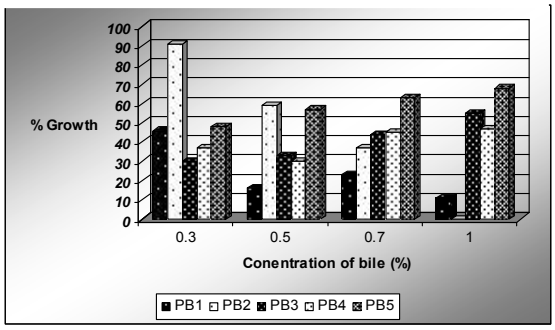

Figure 3. Bile tolerance shown by isolated bacteria in terms of % growth

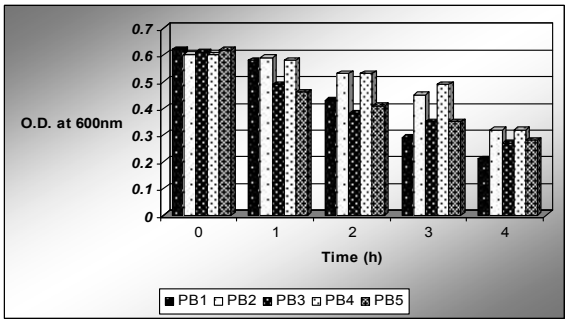

Figure 4. Auto aggregation shown by the isolated bacteria in terms of O.D. measured at 600nm

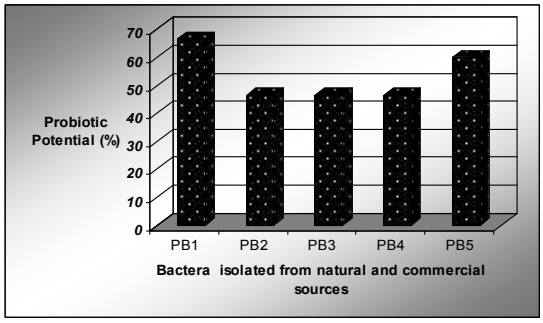

Figure 5. Probiotic potential of isolated bacteria based on cumulative probiotic score

The production of organic acid lowers the pH of the medium which shows the antagonistic activity for many pathogens. In the present study, the organic acid production found to increase with the incubation time, simultaneously pH of the media was decreased. Highest acidity after 72h incubation, 2.448% and lowest pH 3.7 was shown by PB1 (Table-2) Resistance to pH 3 is often used in vitro assay to determine resistance to stomach pH. As the food consumed stay into stomach at least for 3h, this time was taken in consideration**.** In the present study, the CFU/ml was recorded after every hour up to 3h the maximum tolerance to acid was shown by PB1 and PB3 as compared to PB2.The isolates from commercial probiotics preparations also shown tolerance to pH 3. (Fig.-2) Bile tolerance is one of the crucial properties required for probiotics bacteria to survive in small intestine. Bile salt destabilizes the membrane integrity of the bacterial cells. Potential probiotics strains suppose to survive in the presence of the bile salt and can colonize on the intestinal surface.

The concentration of bile used in this experiment was 0.3 to1% which is far greater than mean intestinal bile concentration (0.3%). The percent growth was calculated. Maximum growth was shown by PB2 while it does not show any growth on the 1% bile. Growth of PB1 decreased with increase in the bile concentration. PB3, PB4 and PB5 show increase in the growth along with the bile concentration. (Fig.-3) similar studies were carried out by the Tambekar and Bhutada, (2010) to evaluate the probiotic potential of isolated bacteria which showed acid tolerance up to pH 2 and bile tolerance up to 2%. The auto-aggregation is the intrinsic characteristic shown by the many probiotics bacteria. The auto-aggregation is the first step towards forming the biofilm. The biofilm formation is the characteristic shown by many probiotics bacteria which help them to colonize on the intestinal surface and subsequent inhibition of overgrowth and proliferation of pathogen. In the present study, the auto-aggregation of the isolates were observed by using PBS (pH 7.2), where PB1, PB3 and PB5 shows high rate of auto-aggregation, (Fig.-4). In a similar type of work by Kaushik *et al*. (2009), *L. rhamnosus* LA7 showed uppermost cell auto aggregation (46.5%). In another study by Hati *et al*; (2014), cheese isolate C6 exhibited 21.06 % cell auto aggregation at the end of 2 h of incubation. The co-aggregation is the property studied to know the ability of probiotic bacteria to inhibit the adherence of pathogens to intestinal surface by creating micro-environment where pathogens are blocked. The dissemination of the pathogens to tissue receptors is inhibited by probiotic bacteria and their inhibitory substance. In the current study the co-aggregation of the isolates was separately tested with 4 bacteria i.e. *S. aureus, Pseudomonas sp., E. coli.* and *Vibrio cholerae.* The PB1 shows co-aggregation with the four pathogens while PB4 shows with *S. aureus* only. PB2, PB3 and PB5 shows co-aggregation with *S. aureus* and *E. coli.*

The probiotic potential was calculated based on cumulative probiotic score of isolated bacteria from the milk of domestic animals and commercial probiotic preparations. The probiotic potential of isolates (PB1) from milk of domestic animals (Cow) was found to be highest (66.6%) as compared commercial isolate PB5, which was (60%). The strains isolated from milk of Goat and Buffalo (PB2 and PB3) shows equal probiotic potential. (Fig.-5) Seah Young Ng, *et al*. (2015) evaluated the probiotic potential of Lactic acid bacteria isolated from traditional Malaysian fermented Bambangan while Tambekar and Bhutada, (2010) also evaluated the probiotic potential of bacteria isolated from animal milk. Study suggested that use of these isolated probiotic bacteria can be a remedy to treat the bacterial infections in aquaculture. because infectious diseases are the biggest problem in aquaculture and every year several infections are responsible for significant morbidity and mortality of fishes and lead to commercial loss for aquaculture business.

Acknowledgement

The authors are thankful to UGC, New Delhi for providing financial support to the Major Research Project. The authors are also thankful to Principal, Rajarshi Shahu College, Latur, for providing laboratory facilities.

REFERENCES

Gil N.F., Rafael C. R. Martinez, Bruna C. Gomes Auro Nomizo, Elain C.P. De Martinis 2009. Vaginal *Lactobacilli* as Potential Probiotics against Candida spp., Brazilian Journal of Microbiology 41. pp:6-14.

Hati, S., Shilpa, V., Surajit, M., Dipika, Y. and Vandana. 2014. Evaluation of probiotic potentials of *L. plantarum* C6 isolated from cheese. Indian Journal of Dairy Sciences. 67(5): 413-420.

Hoque, M. Z., F. Akter, K.M. Hossain, M.S.M. Rahman, M. M. Billah and Islam K. M.D. 2010. Isolation, Identification and Analysis of probiotic Properties of *Lactobacillus spp*. from selective Regional Yoghurts, World Journal of Dairy and food Sciences.5(1) pp:39-46

Isolauri E. 2001. Probiotics in human disease. American Journal of Clinical Nutrition, 73:1142 S-1146S.

Kaushik, J., Kumar, A., Duary, R., Mohanti, A., Growth, S. and Batish, V. 2009. Functional and probiotic attributes of an indigenous isolate of *Lactobacillus plantarum.* PLoS One. 4: 80-99.

Klayraung S. and O. Siriporn 2009. Antibacterial and Antioxidant Activities of Acid and Bile Resistant Strains of *Lactobacillus fermentum* Isolated from Miang. 40. pp:757-766.

Mami A., Henni J.E. and Kihal M. 2008. Antimicrobial Activity of Lactobacillus Species Isolated from Algerian Raw Goat‟s Milk Against *Staphylococcus aureus*. World Journal of Dairy & Food Sciences. 3 (2): 39-49.

Mandal, V., S. K. Sen and M. N. Chandra 2008. Optimized Culture Conditions for Bacteriocin Production by *Pediococcus acidilactici* LAB5 and Its Characterization, Indian Journal of Biochemistry and Biophysics.Vol.45. pp:106-110

Masuda, T., Kimura, M., Okada, S., & Yasui, H. 2010. Pediococcus pentosaceus Sn26 inhibits IgE production and the occurrence of ovalbumin-induced allergic diarrhea in mice. Bioscience, Biotechnology, and Biochemistry, 74, 329–335. doi:10.1271/bbb.90656

Prasad J., Gill H., Smart J. and Gopal P. K. 1998. Selection and Characterization of *Lactobacillus* and *Bifidobacterium* strains for use as Probiotic, International Dairy Journal 8:993-1002.

Seah Young Ng, Siew Siew Koon, Birdie Scott Padam and Fook Yee Chye 2014. Evaluation of Probiotic potential of Lactic acid Bacteria isolated from traditional Malaysian fermented Bambangan (*Mangifera pajang*) J. of Food, Vol 13, (4), pp: 563-572

Tambekar, D.H., and Bhutada S.A. 2010. An Evolution of Probiotic potential of *Lactobacillus spp*. From Milk of

Domestic Animals and Commercial Available Probiotic Preparations in Prevention of Enteric Bacterial Infections, Recent Research in Science and Technology.2 (10) pp:82-88

Vanderpool, C., Fang Yan, and Polk D. Brent, 2008. Mechanisms of Probiotic Action: Implications for Therapeutic Applications in Inflammatory Bowel Diseases, Wiley Inter Science. Volume 14, Number II:1585-1596

Verschuere, L., G. Rombaut, P. Sorgeloos and V. Willy 2000. Probiotic Bacteria as Biological Control Agents in Aquaculture, Microbiology and Molecular Biology Reviews. pp:655-671.s

****