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ISOLATION AND CHARACTERIZATION OF POTENTIAL PROBIOTIC MICROORGANISMS FROM INDIAN TRADITIONAL FERMENTED FOOD PRODUCTS FOR THE PRODUCTION OF ANTIMICROBIAL SUBSTANCES AGAINST HUMAN PATHOGENS.

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ABSTRACT

Probiotic microorganisms or Probiotics are live and viable microorganisms predominantly present in the fermented food products, dairy food products and milk of various animal species, preserve the essential flora in the human intestinal gut. Probiotic microorganisms may produce various metabolites which are having inhibition ability against various human pathogens. Total 12 species of potential probiotic microorganisms were isolated from 16 different samples from different Indian traditional fermented food products e.g. Yogurt, Buttermilk, Cottage Cheese, Lassi, Kefir, sauerkraut and vegetable pickle. Isolated Species were characterized for identification and screened for their antimicrobial activity against eight human pathogenic strains such as Staphylococcus epidermidis, Helicobacter pylori, Escherichia Coli, Salmonella typhi, Streptococcus pyogenes, Neiserria gonorrhoeae and two fungal strains Aspergillus fumigates and Candida albicans. Observed antimicrobial potential of probiotics against selected human pathogenic strains. All pathogens were susceptible to the isolated probiotic strains and were exhibited best probiotic properties and tested for antibiotic susceptibility, acid tolerance and resistance against various concentrations of bile salt. We allowed for the use potential source of probiotic because of the ideal and novel properties of probiotic bacteria.

Keywords: - Probiotics, characterization, human pathogens, fermented foods, antimicrobials.

INTRODUCTION

In current days researchers have provided the experimental information about the probiotic microorganisms that are mostly involved in the treatment of many diseases caused due to various pathogens. Probiotics are live and viable microorganisms predominantly present in the fermented food products, dairy food products and milk of various animal species (Ram Kumar Pundir, Satish Rana, Neha Kashyap and Amandeep Kaur *et.al.* Feb- 2013). Nutritional complex wastes, aquatic regions and soil of regions are also the good sources for the probiotic microorganisms (D. Pelinescu, M.C. Chifiriuc. *et al.* 2011). Probiotics balance the intestinal flora of essential microorganisms in human or animal, this improves the health of human or animal species (Bussarin Kosin and Sudip Kumar Rakshit *et.al.*2006). In presence of probiotics reduce the use of excess antibiotics and other therapeutic agents (M. Heyman an S. Menard *et. al.* 2002). In many cases probiotics and their effects are predominantly prophylactic (preventive) in nature, rather than therapeutic purpose. Normally

probiotics are recognized to be mostly safe but sometimes they may cause undesired side effects in very rare cases. Probiotics are predominantly Lactic acid bacteria (LAB) is known as *Lactobacilli* may be the most common probiotics help in digestion of lactose sugar and *Bifidobacterium* mostly present in dairy products (Aayushi Mishra and Kanti Prakash Sharma *et.al*.2014) and helps in the Irritable bowel syndrome (IBS) and some other disease e.g. Inflammatory bowel disease (BID) and yeast such as *Saccharomyces boulardii* is used in the treatment of diarrhea. Probiotic microorganisms produce various antimicrobial substances such as organic acids (lactic acids and acetic acids), bacteriocins and reuterin. All antimicrobial compounds are having inhibitory action against various human pathogens. In this paper our study is focused on the isolation and characterization of probiotic microorganisms from Indian traditional fermented food products for the production of antimicrobial substances against various human pathogens. Total 16 samples were collected from different traditional Indian fermented food products e.g. Yogurt, Buttermilk, Cottage Cheese, Lassi, Kefir, sauerkraut and vegetable pickle for the isolation of probiotic microorganisms. We isolated total 12 probiotic bacterial species and biochemically characterized for the identification of isolated strains. Further we studied the production of antimicrobial substances and their effects against eight different human pathogens and acid tolerance with resistance against various concentrations of bile salt by probiotic isolates.

MATERIAL AND METHODS

Sampling for the isolation of probiotics

For the isolation of probiotics total 16 samples were collected from Indian traditional fermented food products e.g. yogurt, buttermilk, idli batter, cottage cheese, lassi, kefir, sauerkraut and vegetable pickles were present, two samples from each fermented food product. Samples were consisting of fermented food products were dried and mixed in liquid form. All fermented food product samples were collected in sterile zip-lock plastic bag in aseptic conditions and labelled them according to the source and name of fermented food products, stored at 4^0 C and kept away from all possible microbial contaminations (Azadnia, P. and Khan Nazer, A. H. *et.al.* 2009). Collected samples were transported to the laboratory for the isolation of probiotics and further analysis of probiotics.

Isolation of Probiotics from fermented food products

For the isolation of probiotics, collected samples were taken for serial dilution by serial dilution technique. In serial dilution technique, 1 gm of food sample was added to 10 ml sterile saline solution (stock) and mixed on a shaker for 2-3 minutes for homogenization of food products in saline solution (Tankeshwar acharya *et.al.*2010). After homogenization, 1 ml of stock solution transferred into 9 ml sterile saline solution and prepared 10^{-1} dilution, repeated the above same procedure of serial dilution and prepared 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions. All collected food samples were allowed for serial dilution and diluted up to 10^{-6}. Among the all dilutions 10^{-4} dilution was selected for inoculation into De Man Rogosa and Sharp (MRS) medium (highly selective medium for the growth of Lactic Acid Bacteria and other probiotics). An amount of 0.1ml of sample was used for inoculation, by spread plate technique on MRS agar plates and incubated at 37^0c for 24 to 48 hours under anaerobic condition (by using anaerobic jar gas packs). After incubation, bacterial colonies were observed on the inoculated MRS agar plates. Selected well isolated colonies from MRS agar plates, further inoculated into 100 ml MRS broth medium for enrichment of bacterial isolates and sub-cultured into MRS slants and maintained the pure culture of isolates in 30% glycerol at -20^0C (Vasiee A.R, Tabatabaei Yazdi, F. Mortazavi *et. al.* 2014)

Composition of MRS agar medium was used for the isolation of probiotics

Ingredients	Gms/Litre
Peptone	10
Beef extract	10
Yeast extract	4
Glucose	20
Sodium acetate	5
Tween 80	1
Dipotassium hydrogen phosphate	2
Ammonium citrate	2
Magnesium sulphate	1
Manganese sulphate	0.5
Agar	12
pH (at 25^0C)	6.5±0.2

Identification of bacterial isolates by morphological and biochemical characterization

Bacterial isolates were studied by colony characteristics and morphology features and further isolates were tested for gram staining reaction, motility testing, endospore staining. Biochemically bacterial isolates were assessed by catalase test, indole production test, H_2S production test, nitrate reduction test, arginine hydrolysis tests (Priti khemariya, sudhir singh, gopal nath and anil k. gulati *et.al.*2013), cytochrome oxidase test and glucose fermentation test. Gram positive, non motile and catalase negative cocci and bacilli were selected for the further characterization of probiotics (Ashwani Kumar, Dinesh KumarIndia *et.al.* 2014). Physiologically bacterial isolates were studied by antibiotic susceptibility test, antimicrobial production tests, resistance to low pH and resistance against various concentrations of bile salts.

Antibiotic susceptibility test

Antibiotic susceptibility of bacterial isolates was assessed by using the agar diffusion test or disc diffusion antibiotic sensitivity testing on sterile MRS agar plates (Nevijo Zdolec, Ivana Filipovic, Zeljka Cvrtila Fleck *et.al*.2011). For testing antibiotic susceptibility culture suspension was prepared from bacterial isolates on MRS agar medium and adjusted to 0.5 McFarland standards (10^8 CFU/ml) (Marta duskova and renata karpiskova et.al. 2013). A 0.1 ml freshly prepared bacterial cultures were spread on MRS agar plates. Various standard antibiotic discs were placed at the centers of surface of MRS agar plates and kept at 4^0 C for 30 minutes for diffusion of antibiotics onto agar medium. Each bacterial isolate was inoculated on separate MRS agar plate with antibiotic disc. After inoculation and diffusion all plates were incubated at 37^0 C for 24 to 48 hours. Incubated plates were observed for zone of inhibitions and measured the diameter zone of inhibitions in mm **(**Sarker, D., Roy, N. and Yeasmin, T. *et.al.*2010**).** Sensitivity pattern of isolates was performed by using Standard Penicillin G (10 Units), Gentamicin (30µg), Vancomycin (30µg), erythromycin (30µg) and Chloramphenicol (30µg).

Production of antimicrobial substances by bacterial isolates and testing on human pathogens

Sterile MRS broth was used for the production of antimicrobial substances by bacterial isolates (Lihua Fan and Jun Song *et.al.* 2013). In this test we used 500ml Erlenmeyer's flasks; every flask was containing 200ml of MRS broth was sterilized by autoclave. Flasks were inoculated by colonies of well isolated bacteria were grown on MRS agar medium and incubated at 37^0C for 48-72hour. Incubated medium was centrifuged at 10,000 rpm for 15 minutes and culture supernatant was taken for testing of antimicrobial activity on human pathogens (Noordiana N. Fatimah A. B. and Mun, A. S. *et.al* 2013). Human pathogens or test microorganisms were grown separately according to their required respective nutrient medium. *Staphylococcus epidermidis, Helicobacter pylori, Escherichia Coli, Salmonella typhi, Streptococcus pyogenes, Neiserria gonorrhoeae* and two fungal strains *Aspergillus fumigates* and *Candida albicans* were used as test microorganisms were adjusted to 0.5 McFarland standards ($1.5x10^8$ CFU/ml). Supernatant of bacterial cultures were tested on human pathogens by agar well diffusion method protocol shown in below.

Protocol for Antimicrobial activity

20ml of respective sterile molten nutrient agar medium were mixed with 0.1ml test microorganisms or pathogens and poured in sterile empty Petri plates and allowed it to solidification.

↓

After complete solidification of sterile nutrient medium plates were allowed to dry and two agar wells (ideally 10mm in diameter) were prepared into each sterile nutrient agar medium plate by sterile borer.

↓

In each agar plate one well was poured with 100µl of supernatant of bacterial culture and 100µl of Sterilized distilled water (for negative control) and allowed for diffusion at 4^0C in refrigerator. Same procedure was done for all bacterial cultures supernatant.

↓

After diffusion all plates were incubated at 37^0C for 24-48 hours for testing on bacterial pathogens and 28^0C for 3-4 days for testing on fungal pathogens.

↓

After incubation of all plates were observed for zone of inhibitions and measured the diameter of zone of inhibition and recorded the results.

Resistance to low pH

Isolated bacterial isolates were tested for the acid tolerance or resistance against low pH value **(**Gamal Fadl M. Gad, Ahmed M. Abdel-Hamid *et.al.*2014). In this test we inoculated isolated bacterial cultures into sterile MRS broth test tubes of different pH values e.g. pH 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5,6, 6.7 and 7 and incubated at 37^0C for 24-48 hours. Incubated broth tubes were

observed for growth of bacteria at various pH values and determined the OD values at 620 nm and recorded the results by comparing the OD values of broth tubes.

Resistance against various concentrations of bile salt

Isolated bacterial isolates were assessed for the resistance against various concentrations of bile salt. In this test we used MRS broth tubes containing various concentrations of bile salt, e.g. 0.5, 1.0, 1.5, 2 and 2.5% (Lim, Sung-Mee and Dong-Soon Im *et.al.*2009). All tubes were inoculated by bacterial isolates and incubated at 37^0C for 24-48 hours. Incubated broth tubes were observed for the growth of bacteria and determined the OD values at 620nm and recorded the results.

RESULTS AND DISCUSSION

Sampling and isolation of probiotics from fermented food products

Total 12 bacterial cultures were isolated from 16 samples were collected from different Indian traditional fermented food products. e. g. Yogurt, Buttermilk, Idli batter, Cottage Cheese, Lassi, Kefir, sauerkraut and vegetable pickle. Collected samples were serially diluted and prepared 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions. Among the all dilutions 10^{-4} dilution was selected for inoculation into De Man Rogosa and Sharp (MRS) medium. Incubated plates were observed for bacterial colonies. All the isolates were identified by cultural, morphological, and biochemical characteristics shown in the table no.1. Fermented food bacterial isolates were biochemically characterized and identified of bacterial species at the genus level. Selected well isolated identified bacteria were gram positive, non spore forming, non- motile, catalase negative bacilli and cocci, arginine hydrolysis, indole negative, H_2S negative, nitrate reductase negative, cytochrome oxidase negative and acid or gas producers from glucose sugar. We studied above all observations of selected bacterial isolates were belong to probiotic bacteria. For further characterization of probiotics, selected well isolated colonies inoculated into 100ml MRS broth for enrichment of cultures. Pure cultures of bacteria were maintained in 30% glycerol at -20^0C.

Table no.1. : Morphological and biochemical characterization of culture isolates from fermented food products.

Sample	Strain	Cultural/colony Characteristics	Morphology Features	Gram Staining	Endospore Staining	Catalase Test
Yogurt	PB1	Round, white, opaque, Moist, raised and entire	Rod shaped in Pair, chain	Gm +ve	- ve	- ve
Yogurt	PB2	Round, yellowish white, opaque, smooth, flat and entire	Rod shaped in chain	Gm +ve	- ve	- ve
Buttermilk	PB3	Round, creamy, opaque, Moist, raised and entire	Rod shaped in single	Gm +ve	- ve	- ve
Buttermilk	PB4	Round, yellowish white, opaque, moist, raised and entire	Rod shaped in single	Gm +ve	- ve	- ve

Idli batter	**PB5**	**Irregular, creamy, opaque, rough, raised and curled**	**Rod shaped in chain**	**Gm +ve**	**- ve**	**- ve**
Idli batter	**PB6**	**Round, white, opaque, smooth, raised and entire**	**Cocci in two pairs, rare in single**	**Gm +ve**	**- ve**	**- ve**
Cottage cheese	**PB7**	**Round, white, opaque, rough, raised and entire**	**Cocci in two pairs, rare in single and tetrads**	**Gm +ve**	**- ve**	**- ve**
Lassi	**PB8**	**Irregular, white, opaque smooth, raised and entire**	**Rod shaped in chain**	**Gm +ve**	**- ve**	**- ve**
Kefir	**PB9**	**Round, creamy, opaque, smooth, flat and entire**	**Cocci in pair, chain**	**Gm +ve**	**- ve**	**- ve**
Sauerkraut	**PB10**	**Regular, creamy opaque, Smooth, raised and curled**	**Rod in Single or chain**	**Gm +ve**	**- ve**	**- ve**
Vegetable pickle	**PB11**	**Round, yellowish white, opaque, smooth, flat and entire**	**Cocci in pairs or chain**	**Gm +ve**	**- ve**	**- ve**
Vegetable pickle	**PB12**	**Round, creamy, opaque, smooth, flat and entire**	**Rod shaped single, chain**	**Gm +ve**	**- ve**	**- ve**

Identification of bacterial isolates

Fermented food products isolates were characterized by morphological and biochemical analysis and based on Bergey's manual, bacterial isolated strains were identified. After identification of bacterial isolates, most of them isolated strains were Lactic acid bacteria (LAB) and were belonging to genus *lactobacillus* and rest isolates were represented to genus *Streptococcus, Pediococcus, Staphylococcus, Lactococcus and Bifidobacteria*. Further identified probiotic strains in their respective species, strain PB1 to strain PB5 were *L. acidophilus, L. rhamnosus, L. casei, L. plantarum, L. delbrueckii* respectively and strain PB 6 was *Pediococcus acidilactici,* Strain PB7 was *Pediococcus parvulus,* strain PB 8 was *L. fermentum,* strain PB 9 was *Streptococcus thermophilus* strain PB 10 was *L. brevis* strain PB11 was *Lactococcus lactis* strain PB12 was *L. helviticus.*

Probiotic isolates tested for Antibiotic susceptibility

Probiotic isolates were tested for antibiotic susceptibility by agar diffusion test or disc diffusion antibiotics testing on sterile MRS agar plates. Total 5 standard antibiotics discs were used such as Penicillin G (10 Units), Gentamicin (30µg), Vancomycin (30µg), erythromycin (30µg) and Chloramphenicol (30µg). 12 Probiotic isolates were used as antibiotic susceptibility cultures were adjusted to 0.5 McFarland standards (10^8 CFU/ml) and plated on MRS agar. Selected standard antibiotic discs were placed on agar plates with control, incubated at 37^0C for 24 to 48 hours.

Observed the zone of inhibitions of various antibiotics and measured the diameter of zone of inhibitions and noted the sensitivity pattern of probiotic isolates by using standard antibiotics shown in the table no.2.

Table no.2. Sensitivity pattern of probiotic isolates by using standard antibiotics

	Diameter of zone of inhibition (in mm)				
Isolated Strain	**Penicillin (10 Units)**	**Gentamicin (30µg)**	**Vancomycin (30µg)**	**Erythromycin (30µg)**	**Chloramphenicol (30µg)**
PB1	**29**	**18**	**25**	**30**	**35**
PB2	**28**	**20**	**26**	**29**	**37**
PB3	**30**	**21**	**24**	**32**	**33**
PB4	**31**	**19**	**28**	**31**	**32**
PB5	**32**	**Resistance**	**26**	**30**	**33**
PB6	**28**	**22**	**24**	**29**	**30**
PB7	**26**	**20**	**24**	**28**	**31**
PB8	**29**	**21**	**Resistance**	**27**	**35**
PB9	**31**	**23**	**21**	**31**	**34**
PB10	**30**	**Resistance**	**22**	**26**	**32**
PB11	**27**	**18**	**Resistance**	**28**	**35**
PB12	**29**	**20**	**26**	**29**	**34**

Antimicrobial activity by probiotic isolates against human pathogens

Total 12 isolates were studied for the potential probiotics for the production of antimicrobial substances and observed their antimicrobial activity against 8 different human pathogens by agar well diffusion method. Probiotic isolates were inoculated into sterile MRS broth medium and allowed for proper incubation period, after incubation prepared culture supernatants were allowed for antimicrobial testing. Human pathogens or test microorganisms e.g. *Staphylococcus epidermidis, Helicobacter pylori, Escherichia Coli, Salmonella typhi, Streptococcus pyogenes, Neiserria gonorrhoeae* and two fungal strains *Aspergillus fumigates* and *Candida albicans* were adjusted to 0.5.MCfarland standards and grown separately according to their required respective nutrient medium. Test microorganisms or pathogens were inoculated in molten agar plates and prepared agar wells, culture supernatants were loaded in agar wells followed by diffusion then incubation at 37^0C for 24 – 48 hours. Incubated plates were observed for the zone of inhibitions and measured the diameter of zone of inhibitions in mm and studied antimicrobial activity of probiotic isolates shown in the table no.3. Among the all probiotic isolates PB1, PB2, PB3, PB4, PB5, PB8, PB10 and PB12 were showed maximum and effective zone of inhibition against selected 8 human pathogens. *Aspergillus fumigates* and *Candida albicans* were highly inhibited by above 8 strains of probiotics of shown 27mm-36mm diameter of zone of inhibition whereas minimally or minimum zone of inhibition by probiotic isolates were on *E.coli*, *Streptococcus pyogenes* and *Salmonella typhi* of 11mm diameter of zone of inhibition.

Table no. 3. Antimicrobial activity of probiotic isolates against human pathogens

	Diameter of zone of inhibition (in mm)							
Isolated Strain	*Staphylococcus epidermidis*	*Helicobacter pylori*	*E. Coli*	*S. typhi*	*Streptococcus pyogenes*	*Neiserria gonorrhoeae*	*Aspergillus fumigates*	*Candida albicans*
PB1	28	30	28	27	29	29	31	27
PB2	27	27	29	26	28	28	32	30
PB3	28	26	27	24	NA	NA	34	34
PB4	27	NA	29	26	29	23	31	31
PB5	24	26	27	26	27	21	36	33
PB6	14	13	15	12	NA	11	NA	NA
PB7	16	NA	17	14	NA	16	17	18
PB8	25	28	26	29	30	29	36	34
PB9	12	14	NA	11	13	12	NA	NA
PB10	26	27	26	25	28	26	29	27
PB11	21	17	NA	17	16	17	NA	NA
PB12	24	28	27	29	30	31	34	33

NA= No activity was found against respective pathogen

Resistance or tolerance to low pH

In present study Probiotic isolates were tested for the acid tolerance or resistance against low pH values. Probiotic isolates were observed for their growth or they were able to grow at pH values e.g. pH 7, 6.7,6, 5.5, 5, 4.5, 4, 3.5, 3 and 2.5 but they were unable to grow at pH values 2, 1.5 and 1, shown in the table no.4. Isolated Probiotics were survived up to 2.5 pH or highly acidic pH of biological environment. Good probiotic strain shows higher tolerance or withstand at low pH range from 3.5 to 2. In this research isolated probiotics were exhibited good probiotic qualities in terms of acid tolerance ability of isolated strains were studied.

Table no.3. Resistance or tolerance pattern of probiotics against low pH

	Various pH values												
Isolated Strain	7	6.7	6	5.5	5	4.5	4	3.5	3	2.5	2	1.5	1
PB1	+	+	+	+	+	+	+	+	+	+	-	-	-
PB2	+	+	+	+	+	+	+	+	+	+	-	-	-
PB3	+	+	+	+	+	+	+	+	+	+	-	-	-
PB4	+	+	+	+	+	+	+	+	+	+	-	-	-
PB5	+	+	+	+	+	+	+	+	+	+	-	-	-
PB6	+	+	+	+	+	+	+	+	+	+	-	-	-
PB7	+	+	+	+	+	+	+	+	+	+	-	-	-
PB8	+	+	+	+	+	+	+	+	+	+	-	-	-
PB9	+	+	+	+	+	+	+	+	+	+	-	-	-
PB10	+	+	+	+	+	+	+	+	+	+	-	-	-
PB11	+	+	+	+	+	+	+	+	+	+	-	-	-
PB12	+	+	+	+	+	+	+	+	+	+	-	-	-

+= Growth of isolated bacteria, - = No growth of isolated bacteria

Resistance to various concentrations of bile salt

All acid tolerated potential probiotic isolates were shown their growth or they were survived at 0.5, 1.0, 1.5, 2 and 2.5% of bile salt concentrations shown in the table no.5. We observed the potential of probiotic isolates to tolerate or ability to resist the various concentrations of bile salt. All probiotic isolates were shown good ability of resistance against bile salts and strains PB1, PB2, PB3, PB4 and PB5 were exhibited excellent resistance ability to bile salt because of bile salt hydrolase activity, while rest isolates were satisfactorily resistance against bile salt (Silvia Simona Grosu-Tudor, Medana Zamfir *et.al.* 2012). Bile salt resistance its intrinsic ability of strain to tolerate bile and survive in the intestinal gut of human. In some times bile salt-resistant bacteria can also be obtained by selection toward other stress conditions, such as high acid or low pH. In our study bile salt resistance were shown by probiotic isolates were selected toward the other stress or extreme conditions such as high acidic or low pH condition.

Table no. 5. Resistance pattern of probiotics against various concentrations of bile salt.

	Concentrations of bile salt				
Isolated Strain	**0.5%**	**1%**	**1.5%**	**2%**	**2.5%**
PB1	+	+	+	+	+
PB2	+	+	+	+	+
PB3	+	+	+	+	+
PB4	+	+	+	+	+
PB5	+	+	+	+	+
PB6	+	+	+	+	+
PB7	+	+	+	+	+
PB8	+	+	+	+	+
PB9	+	+	+	+	+
PB10	+	+	+	+	+
PB11	+	+	+	+	+
PB12	+	+	+	+	+

+= Growth of isolated bacteria, - = No growth of isolated bacteria

CONCLUSION

In this paper we focused on probiotic bacteria isolated form Indian traditional fermented food products and they were produced antimicrobial substances were active against various human pathogens. Total 12 probiotic isolates were isolated from 16 samples of Indian traditional fermented food products and were morphologically and biochemically characterized and identified probiotic isolates. All probiotic isolates were studied for the production of antimicrobial substances effectively against various human pathogens. Further this study extended to the evaluation of potential of probiotics by acid tolerance or resistance to low pH with resistance against various concentrations of bile salt. All these were concerned of bacterial isolates were having ideal probiotic properties and considered as potential probiotic bacteria were actively producing antimicrobial substances inhibitory to all various human pathogens.

REFERENCES

1. Ram Kumar Pundir, Satish Rana, Neha Kashyap and Amandeep Kaur (Feb- 2013) Probiotic potential of lactic acid bacteria isolated from food samples: an in vitro study. Ambala Haryana, India.

2. Aayushi Mishra, Kanti Praksh Sharma (June-2014) Isolation and Characterization of probiotic microorganism from fermented food products. Lakshamangarh, Sikar, Rajsthan, India.
3. Vasiee A.R., Tabatabaei Yazdi, (2014). Isolation, identification and characterization of probiotic *Lactobacilli* Spp. From Tarkhineh. Ferdowsi University of Mashhad, Mashhad, Iran.
4. Bussarin Kosin and sudip Kumar Rakshit (20 March 2006). Microbial and Processing criteria for production of probiotics: A review. Asian Institute Technology, Pathumthani, Thialand.
5. M. Heyman and S. Menard (2002) Probiotic Microorganisms: how they affect intestinal pathophysiology. Paris Cedex (france).
6. D. Pelinescu, M.C. Chifiriuc. Selection and characterization of the probiotic potential of some lactic acid bacteria isolated from infant feces.Belfield, Dublin , Ireland.
7. Nevijo Zdolec, Ivana Filipovic, zeljka Cvrtila Fleck , Aleks Marić , Dean Jankuloski3 (2011) . Antimicrobial susceptibility of lactic acid bacteria isolated from fermented sausages and raw cheese. Faculty of Veterinary Medicine in Skopje, Food Institute, Republic of Macedonia.
8. Marta Duskova and Renáta Karpiskova (2013). Antimicrobial Resistance of Lactobacilli Isolated from Food. Veterinary Research Institute, Brno, Czech Republic.
9. Gamal Fadl M. Gad , Ahmed M. Abdel-Hamid (2014). Antibiotic resistance in lactic acid bacteria isolated from some pharmaceutical and dairy products. El-Minia University, Egypt.
10. Lim, Sung-Mee and Dong-Soon Im (2009). Screening and Characterization of Probiotic Lactic Acid Bacteria Isolated from Korean Fermented Foods. Pusan National University, Busan. Korea.
11. Azadnia, P. and Khan Nazer, A. H. (2009). Identification of lactic acid bacteria isolated from traditional drinking yoghurt in tribes of Fars province. School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
12. Noordiana N., Fatimah A. B. and Mun, A. S.(2013). Antibacterial agents produced by lactic acid bacteria isolated from Threadfin Salmon and Grass Shrimp. Selangor, Malaysia.
13. Priti KHEMARIYA, Sudhir SINGH, Gopal NATH and Anil K. GULATI (2013). Isolation, Identification, and Antibiotic Susceptibility of nis+ Lactococcus lactis from Dairy and Non-dairy Sources. Banaras Hindu University, Varanasi (UP), India.
14. Lihua Fan and Jun Song (2013).Antimicrobial microbes-bacteriocin producing lactic acid bacteria. Canada
15. Silvia Simona Grosu-Tudor, Medana Zamfir (2012).Probiotic potential of some lactic acid bacteria isolated from romanian fermented vegetables. Romania .
16. Ashwani Kumar, Dinesh KumarIndia (2014).Isolation and characterization of bacteria from dairy samples of Solan in Himachal Pradesh for identification of *Lactobacillus spp*. Faculty of Biotechnology, Shoolini University, Solan, H.P.
17. Tankeshwar acharya (2010).Techniques of isolation and Enumeration of Bacteria.
18. Sarker, D., Roy, N. and Yeasmin, T.(2010). Isolation and antibiotic sensitivity of *Bacillus thuringinesis* strain from dump soil Department of Biochemistry and Molecular Biology Rajshahi University, Rajshahi-6205. Bangladesh.