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Isolation and Molecular Characterization of Anti-Helicobacter pylori Bacteriocin Producing *Pediococcus acidilactici* BA28

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Abstract

Bacteriocin producing strains of lactic acid bacteria were isolated from faecal samples of healthy individuals and evaluated for their antimicrobial activity against pathogens causing important human diseases such as peptic ulcers, gastrointestinal infections, nosocomial and skin infections. Faecal isolate BA28 is extremely attractive agent as it could be used to combat growing prevalence of sexually transmitted microbial infections and gastrointestinal infection which may be of great importance for future medicinal use. Bacteriocin shows maximum inhibition against *Helicobacter pylori* causing peptic ulcer disease. It was identified as *Pediococcus acidilactici* on the basis of biochemical testing and 16S rDNA sequencing. Based on the antimicrobial activity profiles of *P. acidilactici* BA28 is being suggested here as a candidate strain for formulating topical personal care therapeutics aimed at prevention and treatment of many human diseases especially peptic ulcers.

Keywords: Lactic acid bacteria; Peptic ulcer; *Helicobacter pylori*; *Pediococcus acidilactici*

Introduction

Peptic ulcers are produced by an imbalance between gastro-duodenal mucosal defence mechanisms and damaging forces of gastric acid and pepsin, combined with superimposed injury from environmental or immunologic agents. Counts of anaerobic *H. pylori* are very high in patients with duodenal and gastric ulcers. Most common symptoms of peptic ulcer disease are abdominal discomfort which is a dull, gnawing ache, comes and goes for several days or weeks, occurs 2 to 3 hours after meal, other symptoms includes weight loss, poor appetite, bloating, nausea, vomiting etc. Some time duodenal wall perforates and obstructs the path of food trying to leave the stomach [1].

Helicobacter pylori is a Gram negative, spiral-shaped, microaerophilic organism, that colonizes the mucosal layer of the gastric epithelium. In 1994, the International Agency for Cancer Research, an arm of the World Health Organization, classified *H. pylori* as a potential human carcinogen [2]. *H. pylori* weakens the protective mucous coating of the stomach and duodenum, which allows acid to get through to the sensitive lining beneath. Both the acid and the bacteria irritate the lining and cause a sore, or ulcer. *H. pylori* is able to survive in stomach acid because it secretes enzymes (urease, protease and phospholipases) that neutralize the acid [3]. This mechanism allows *H. pylori* to make its way to the "safe" area-the protective mucous lining. Once there, the bacterium's spiral shape helps it burrow through the lining.

Currently, preventive therapies for peptic ulcers rely almost exclusively on the use of antibiotics such as metronidazole, tetracycline, clarithromycin, amoxicillin and gives initial cure rates of approximately 90% or better [4,5]. Metronidazole becomes widely distributed in the body and undergoes oxidative metabolism in the liver, with the formation of several metabolites [6]. High concentration of this antibiotic therapy has been reported to impose several side effects such as diarrhea, dizziness, headache, loss of appetite, nausea or vomiting, stomach pain or cramps [7]. A number of reports have emerged that indicates emergence of drug resistance trait in peptic ulcers [8,9]. That's why more effective and safe therapeutics are desired to control peptic ulcers.

Lactic acid bacteria (LAB) play a major role in maintaining a healthy ecosystem and prevent overgrowth of pathogenic bacteria in stomach ecosystem [10]. Through the production of H_2O_2, organic acids and antimicrobial proteins called bacteriocins, probiotic LAB prevent many pathogenic bacteria from overgrowing and thereby creating a condition called duodenal ulcers [11,12]. Several investigators have isolated and partially purified bacteriocins from various strains of lactic acid bacteria [13,14]. Moreover, emergence of antibiotic resistant phenotype in ulcer associated pathogenic bacteria; it has become essential to develop alternative therapeutics/prophylactic measures against these pathogens. Present study was undertaken with the aim to isolate and characterize probiotics LAB from faecal samples of healthy individuals and evaluated for their antimicrobial activity against a panel of Gram-positive and Gram-negative pathogens causing various human diseases such as peptic ulcers and other gastro intestinal infections. Based on the antimicrobial spectrum of the strains isolated, it is suggested as a novel therapeutic against many human diseases especially peptic ulcer disease and help in improving human health.

Materials and Methods

Sample collection and isolation of lactic acid bacteria

Feacal samples from healthy individual were collected with informed consent and were transferred immediately to sterilized saline (0.85% NaCl). 1 ml sample was inoculated into sterile MRS broth (containing peptone 10 g/l, yeast extract 5 g/l, beef extract 10 g/l,

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dextrose 20 g/l, ammonium citrate 2g/l, sodium acetate 5g/l, $MnSO_4$ 0.05 g/l, $MgSO_4$ 0.01g/l, dipotassium phosphate 2g/l, tween-80 1ml/l, pH 6.5) for propagating healthy gut microflora and incubated for 18 to 24 h at 37°C. Samples were subcultured three times before proceeding with bacterial isolation and activity assays. LAB strains were isolated on MRS agar plates using pure culture techniques.

Bacterial strains and culture media used to study antimicrobial spectrum

The inhibitory spectra of bacteriocin producing LAB isolates was evaluated against important human pathogens especially indicated in peptic ulcer disease using spot-on-lawn [15] and well-diffusion methods [16]. Indicator cultures have been procured from Microbial Type Culture Collection, Chandigarh, Punjab, India, National Collection of Industrial Microorganisms, Pune, India, American Type Culture Collection and DSMZ, Germany. Growth requirements of indicator microorganisms are specified in table 1. Indicator strains were revived and maintained in growth media as prescribed by culture banks.

Bacteriocin activity assays

Isolated strains were subcultured thrice in MRS medium (pH 6.5) at 37°C for 24 h before proceeding with bacteriocin activity assays. 1ml

Sr. No.	Microorganism	Growth Media	Growth Conditions	Temperature / pH	Incubation Time	Sub-culture
Gram-positive Pathogenic indicator strains						
1.	*Bacillus subtillus* ATCC 6633	NB	Aerobe	37°C/ 7.4	24 h	15 days
2.	***Clostridium perfringens* MTCC 450**	NB	Anaerobe	37°C/ 7.4	24 h	15 days
3.	***Enterococcus faecalis* (isolate)**	MRS	F. anaerobe	30°C/ 7.4	24 h	30 days
4.	***E. faecalis* (NDRI isolate)**	MRS	F. anaerobe	37°C/ 6.5	24 h	30 days
5.	***E. faecalis* ATCC 29212**	MRS	F. anaerobe	37°C/ 6.5	24 h	30 days
6.	*Leuconostoc mesenteroides* MTCC 107	MRS	Aerobe	25°C/ 6.5	48 h	30 days
7.	***Listeria monocytogenes* MTCC 657**	BHI/NB	Aerobe	37°C/ 7.4	24 h	30 days
8.	*Micrococcus flavus* ATCC 10240	NB	Aerobe	30°C/ 6.5	24-48 h	3 months
9.	*Propionibacterium acnes* MTCC 1951	BHI	Anaerobe	25 / 7.4	5 days	30 days
10.	*P. acnes* DSMZ 16379	BHI	Anaerobe	25 / 7.4	5 days	30 days
11.	*Staphylococcus albus* ATCC 11631	BHI	Anaerobe	25 / 7.4	5 days	30 days
12.	*S. aureus* MTCC 737	BHI/NB	Anaerobe	37°C/ 7.4	24 h	30 days
13.	*S. aureus* NCTC 7447	BHI/NB	Anaerobe	37°C/ 7.4	24 h	30 days
14.	***Streptococcus agalactiae* NCIM 2401**	MRS	Aerobe	37°C/ 6.5	48h	30 days
15.	***S. faecalis* MTCC 459**	MRS	Aerobe	37°C/ 6.5	48h	30 days
16.	*S. pyogenes* NCTC 10869	BHI	Aerobe	37°C/ 7.4	48h	30 days
17.	*S. thermophilus* MTCC 1928	MRS	Aerobe	40°C/ 6.5	28h	30 days
Gram-negative Pathogenic indicator strains						
1.	***Bacteriodes fragilis* MTCC 1045**	RCB	Anaerobe	37°C/ 6.8	5 days	30 days
2.	***B. ovatus* MTCC 3298**	RCB	Anaerobe	37°C/ 6.8	48 h	30 days
3.	***B. vulgates* MTCC 1350**	CMB	Anaerobe	37°C/ 6.8	72 h	30 days
4.	***Escherichia coli* BL 21 DE3 MTCC 1679**	LB	F. anaerobe	37°C/ 7.2	24 h	30 days
5.	***E. coli* DH 5α MTCC 1652**	LB	F. anaerobe	37°C/ 7.2	24 h	30 days
6.	***E. coli* KL 16 MTCC 1650**	LB	F. anaerobe	37°C/ 7.2	24 h	30 days
7.	***Helicobacter pylori* DSMZ 10242**	BHI	Anaerobe	37°C/ 7.4	48 h	30 days
8.	*Klebsiella pneumoniae* NCIM 2883	NB	F. anaerobe	30°C/ 7.2	24 h	30 days
9.	*K. pneumoniae* NCIM 2401	NB	F. anaerobe	30°C/ 7.2	24 h	30 days
10.	***Proteus mirabilis* NCIM 2387**	NB	F. anaerobe	37°C/ 7.2	24 h	30 days
11.	*Pseudomonas aeruginosa* ATCC 10662	NB	Aerobe	30°C/ 7.4	24 h	30 days
12.	***Vibrio cholrae* ATCC 14104**	NB	Aerobe	37°C/ 7.4	24 h	15 days
13.	***Yersinia enterocolitica* MTCC 861**	BHI/NB	F. anaerobe	30°C/ 7.2	12 h	30 days
Non-pathogen indicator strains						
1.	*Lactobacillus brevis* MTCC 1750	NB	F. anaerobe	30°C/ 7.4	24 h	30 days
2.	*L. bulgaricus* NCDC 253	MRS	F. anaerobe	37°C/ 6.5	24 h	30 days
3.	*L. casei* NCIM 2651	MRS	F. anaerobe	37°C/ 6.5	24 h	30 days
4.	*L. helveticus* NCIM 2126	MRS	F. anaerobe	37°C/ 6.5	24 h	30 days
5.	*L. leichmanni* NCIM 2027	MRS	F. anaerobe	37°C/ 6.5	24 h	30 days
6.	*L. pentosus* NCIM 2669	MRS	F. anaerobe	37°C/ 6.5	24 h	30 days
7.	*L. plantarum* NCIM 2912	MRS	F. anaerobe	37°C/ 6.5	24 h	30 days
8.	*Lactococcus lactis* subsp. Cremoris MTCC 1484	MRS	Aerobe	20°C/ 6.5	24 h	30 days
9.	*Pediococcus acidilactici* LB 42	MRS	Aerobe	37°C/ 6.5	24 h	30 days
Yeast indicator strain						
1.	*Candida albicans* MTCC 183	YEPD	Aerobe	30°C/ 7.2	48 h	60 days
2.	*C. albicans* MTCC 10231	YEPD	Aerobe	30°C/ 7.2	48 h	60 days
NB-Nutrient Broth; MRS- De Man's Ragosa Sharpe Medium; BHI-Brain Heart Infusion Broth; LB-Luria Broth; RCB- Reinforced Clostridial Broth; CMB- Cooked meat Broth; YEPD- Yeast Extract Peptone Dextrose						

Table 1: Growth media and conditions of indicator microorganisms.

aliquot of broth culture was centrifuged at 10,000 rpm for 10 min and cell free supernatant (CFS) was collected in sterile micro-centrifuge tube. CFS was heat treated in boiling water bath for 20 min and allowed to cool at room temperature. Bacteriocin activity was assayed using spot-on-lawn [15] and agar well diffusion methods [16].

Biochemical and molecular characterization

Biochemical and 16SrRNA gene sequencing of isolate BA28 was carried out by MTCC, Chandigarh, India.

Statistical analysis of data

QI Macros ANOVA was used to calculate all statistical parameters. Statistical tools like ANOVA one factor was used to determine significance of the results obtained in duplicate experimental sets.

Results and Discussion

Isolation of bacteriocin producing LAB strains

In the present study, more than 100 bacterial strains were isolated

Indicator strains	DIAMETER OF INHIBITION ZONE (mm)													
	54	**69**	**90**	**75**	**76**	**59**	**19**	**28**	**40**	**16**	**80**	**BA 28**	**BA 30**	**BA 31**
Gram-positive pathogenic indicator strains														
B. subtillus ATCC 6633	-*	-	-	-	-	-	-	-	-	-	-	-	-	-
***C. perfringens* MTCC 450**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***E. faecalis* (isolate)**	23	24	20	19	21.5	12	14	17	18	19	19	**25**	23.5	21
***E. faecalis* (NDRI isolate)**	22	20.5	19	18	15	13	11	15	19	18.5	17.5	**23**	21.5	20
***E. faecalis* ATCC 29212**	21	**23.5**	21	22	16	14	15	16	20	20	16.5	21.5	14	13
L. mesenteroides MTCC 107	**15**	13	14	13	14	14	13	12	11	13	12.5	14	14	**15**
***L. monocytogenes* MTCC 657**	15	13	14	19	18	**24**	20	19.5	18.5	17	23	20	21	22
M. flavus ATCC 10240	20	21	19	22	24	24	23.5	24	12	13	12	22	24	**25**
P. acnes MTCC 1951	**15**	13	10	13	12	14	**15**	12	11.5	15	12	10	11	13
P. acnes DSMZ 16379	**14**	12	09	08	12.5	11.5	13	**14**	11.5	11	**14**	12	10	11
S. albus ATCC 11631	22	**23**	19	22	18	13	7	14	18	12	19	20	12	13
S. aureus MTCC 737	15	18	16	08	15	14	13	15	16	17.5	**18.5**	12	20	17
S. aureus NCTC 7447	**21**	20	19	15	16	17	20	19	18.5	13	14	17.5	16.5	17
***S. agalactiae* NCIM 2401**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***S. faecalis* MTCC 459**	23	21	19	18	14	15	12	13	18	19	20	**24**	23	22
S. pyogenes NCTC 10869	13	18	-	14	15	14	12.5	14	15	16	19	18	17.5	**20**
S. thermophilus MTCC 1928	**21**	20	19	15	16	17	20	19	18.5	13	14	17.5	16.5	17
Gram-negative pathogenic indicator strains														
***B. fragilis* MTCC 1045**	14	13	17	16	13	14	14	11.5	13	15	17	**18**	15	12
***B. ovatus* MTCC 3298**	15	18	16	12.5	15	14	13	13.5	12	16	17	**19**	18	16
***B. vulgates* MTCC 1350**	16	14	12	13	15	16	12.5	12	11	13	15	**17.5**	14	11
***E. coli BL 21* DE3 MTCC 1679**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***E. coli* DH 5 MTCC 1652**	18	**20**	19	14	14	11	12.5	12	12	15	16	14	13	16
***E. coli* KL 16 MTCC 1650**	17	**20**	13	14	08	14	16	17	15	12	10	12	13	14
***H. pylori* DSMZ 10242**	12	13	11	09	**16**	10	12	15	14	13	12	11	14	15
K. pneumoniae NCIM 2883	16	15	14.5	13.5	14	13	18	15	14	12.5	17	18	16	**20**
K. pneumoniae NCIM 2401	16	14	15.5	14	12	11	15	16	17	14	18	**19**	**19**	15
***P. mirabilis NCIM* 2387**	12	12	12.5	11	14	08	14	13	15	13	16	17	16	**18**
P. aeruginosa ATCC 10662	18	**20**	19.5	17	17.5	16	15	13	12	11	10	17	15	16
***V. cholrae* ATCC 14104**	12	12.5	11	13.5	14.5	15	16	17	18	13	15	**20**	19	21
***Y. enterocolitica* MTCC 861**	14	-	-	-	-	-	-	-	-	-	**15**	-	14	13
Non-pathogenic indicator strains														
L. brevis MTCC 1750	-	16	18	-	19	-	20	22	21	18.5	21	22	**23**	19.5
L. bulgaricus NCDC 253	-	12	-	-	-	14	-	-	-	-	**18.5**	15	-	17
L. casei NCIM 2651	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. helveticus NCIM 2126	17	18	19	17.5	**20**	13	17	12	14	16	15	19.5	18	19
L. leichmanni NCIM 2027	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. pentosus NCIM 2669	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. plantarum NCIM 2912	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. lactis subsp. Cremoris MTCC 1484	-	-	-	-	-	-	-	-	-	-	14	13	16	**19**
P. acidilactici LB 42	19	17	17	14	**20**	11	15	12	18.5	12	12	18	19.5	**20**
Yeast indicator strain														
C. albicans MTCC 183	18	15	16	14	12	17	13	15	12	14	15	18	**19**	18.5
C. albicans MTCC 10231	19	16	17	14.5	13	18	14	16	12.5	15	16	19	**20**	19.5
(-* negative reaction) Each data is an average of two samples; P value<0.05; Fcrit (1.982)<F value (23.837)														

Table 2: Antimicrobial spectrum of human LAB isolates.

from faecal samples and further screening was done on the basis of bacteriocin producing activity. Bacteriocin production trait was observed in only fourteen isolates designated as GI54, GI69, GI90, GI75, GI76, GI59, GI19, GI28, GI40, GI16, GI80, BA28, BA30, and BA31. Their antibacterial activity was tested against many indicator strains implicated in peptic ulcer, gastrointestinal, skin and lung diseases in humans. Isolate BA28 possessed extremely attractive antimicrobial spectrum which was highest among the tested indicators especially *H. pylori* DMSZ 10242 and therefore, was selected for further detailed study.

Antimicrobial spectrum of isolated LAB strains

Antimicrobial activities of isolated LAB strains were comparatively investigated against a panel of microorganisms and highly significant results were obtained in the study (Table 2; Figures 1 and 2). A number of pathogenic and non-pathogenic tested Gram-positive bacterial strains were inhibited by the bacteriocin producing LAB isolates except species like *B. subtilis, C. perfringens, E. faecalis* (NDRI isolate), *L. casei*, *L. leichmanni*, *L. plantarum*, *L. pentosus*, *L. lactis* subsp. *cremoris* and *S. agalactiae*. Among non-pathogenic lactic acid bacteria *L. brevis, L. bulgaricus, L. helveticus* and *P. acidilactici* LB42 were strongly inhibited by isolate GI69 and GI76 respectively. Isolates produced anti-listerial bacteriocins as they successfully inhibited *L. monocytogenes* that causes spectic abortion, newborn and adult septicemia, listeriosis, meningitis and meningo-encephalitis in immune-deficient persons [17]. Other pathogens including food spoilage causing *L. mesenteroides* and *S. aureus* were successfully inhibited by most of the vaginal LAB isolates. *L. mesenteroides*, an epiphytic bacterium that plays an important role in several industrial food fermentations, is also responsible for nosocomial infections [18]. *S. aureus,* commonly found on nasal passages, skin and mucous membranes of humans, causes a wide range of suppurative infections, as well as food poisoning and toxic shock syndrome [19]. It is of interest to the food and pharma industry that LAB isolates exhibited broad inhibitory spectrum against food borne pathogens as well as spoilage organism. Bacteriocins produced by LAB isolates are effective against *E. faecalis* that can cause life-threatening infections in humans, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance contribute to its pathogenicity [20].

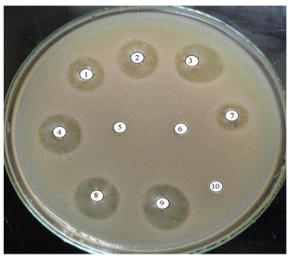

Figure 1: Spot-on-lawn assay showing growth inhibition in indicator lawn. Spots 1,2,3,4,7,8,9 shows bacteriocin activity.

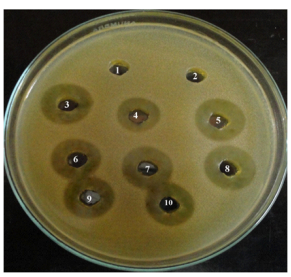

Figure 2: Well diffusion assay showing growth inhibition in indicator lawn. Wells 3-10 shows bacteriocin activity.

Opportunistic pathogens of humans including *M. flavus*, *P. aeruginosa* and *N. mucosa* were also moderately inhibited by LAB isolates obtained from human vaginal swabs. *M. flavus* is generally thought to be a saprotrophic or commensal organism, though it can cause disease in hosts with compromised immune status, such as HIV patients [21]. *P. aeruginosa* sometimes exists as a part of normal flora of humans. However, in immune-deficient persons, it causes chronic lung infections, burn and eye infections, pneumonia; thus being a serious problem in patients hospitalized with cancer, cystic fibrosis and burns [22]. Isolated bacteriocins have a great potential in formulating nasal and oral sprays and can be studied to control such infections in model systems. Growth inhibition of UTI pathogen *N. mucosa* by bacteriocins of vaginal LAB isolates makes them potential ingredient of anti-neisserial skin/mucosal formulations to eradicate such opportunistic UTI pathogens [23].

S. pyogenes, that causes many human skin diseases, ranging from mild superficial skin infections to life-threatening systemic diseases, is also susceptible to bacteriocins produced by human vaginal LAB isolates. *S. pyogenes* infections that begin in the throat or skin including pharyngitis [24] could be cured by using probiotic LAB which can colonize the buccal cavity or skin and slowly produce therapeutic agents for clearing infection. In accordance with earlier reports, the utility of bacteriocins to control these human diseases can be regarded as the best alternative to antibiotic therapy [25].

H. pylori causing peptic ulcer in humans was strongly inhibited by all the 14 bacteriocin producing LAB isolates but maximum zone was observed in BA28. Several investigators have isolated and partially purified bacteriocin from different species of lactobacilli. Most of them were with nonhuman strains, predominantly isolated from food [26]. There are several antimicrobial peptides already known to inhibit *H. pylori* such as lacticin A164 produced by *Lactococcus lactis* subsp. *lactis A164* and lacticin BH5 produced by *L. lactis BH5*. Anti-*Helicobacter* activity of lacticin A164 was dependent on initial inoculum size as well as concentration of the bacteriocin added [27]. A study by Simova and coworkers [28] shows growth inhibition of *H. pylori* by bacteriocins viz. bulgaricin BB18 produced by *L. bulgaricus* BB18 and enterocin MH3 produced by *E. faecium* MH3. These bacteriocins are potential antimicrobial agents and in conjunction with their producers, may have use in applications to contribute a positive effect on the balance of intestinal microflora.

Biochemical characterization of LAB isolate

The strain consisted of Gram-positive cocci showing tetrad arrangement of cells with convex and smooth surface. The strain was found to be catalase negative and identified as *Pediococcus* species. The isolate was able to grow under alkaline environment (pH 9.0) at 10 and 42°C. The optimum temperature for growth of the organism is 35 to 38°C. It was able to ferment trehalose, xylose and mannose but could not utilize sorbitol, lactose and galactose. Strain did not produce gas on glucose containing medium. *Pediococcus* isolate BA28 did not show oxidase activity and indole production. *Pediococcus* isolate BA28 is salt as well as bile tolerant as it can grow in the presence of 12% NaCl and 30% bile salts in culture medium. It gave positive methyl red test, whereas a negative Voges-Proskaeur reaction. Strain was capable of producing ammonia from arginine that reflects presence of arginine

deaminase activity. It was unable to hydrolyze gelatin, starch, esculin, casein, urea and tween 20, tween 40, tween 60 and tween 80. Based upon carbohydrate fermentation profile and other physiological and biochemical tests, strain was classified as *Pediococcus*.

Molecular characterization of LAB isolate

Preliminary biochemical identification was confirmed and validated by molecular characterization. For molecular typing 16SrRNA sequencing was done by MTCC, Chandigarh, India. The genotypic analysis confirmed the isolated strain BA28 as *Pediococcus acidilactici*. 16SrRNA sequence of *Pediococcus acidilactici* BA28 is shown in figure 3.

Conclusions

In the last two decades, a variety of antagonistic bacteriocins, mostly produced by lactic acid bacteria, have attracted the attention of food and pharmaceutical sector for their potential use as natural food biopreservatives, probiotic formula foods and health care products [1,29]. Most of the LAB bacteriocins show a relatively narrow inhibitory spectrum, while only few of them could inhibit diverse groups of Gram-positive and Gram-negative bacteria [29,30]. A highly potent anti-*Helicobacter pylori* bacteriocin producing isolate from faecal sample was characterized for its antimicrobial spectrum. Bacteriocin production trait of *P. acidilactici* BA28 isolate was studied by spot-on-lawn and agar well diffusion methods against important human pathogens causing bacterial vaginosis, gastrointestinal infections, nosocomial and skin diseases. Based on the results obtained in this study, *P. acidilactici* BA28 are strongly recommended for treatment of peptic ulcer and other sexually transmitted diseases in combination with antibiotic therapy that could check recurrence of the disease after termination of antibiotic treatment.

Colonization of the gastric epithelium with probiotics LAB can particularly prevents gastric infection by bacteria and fungi. Moreover, bacteriocin based therapeutics are urgently desired to overcome undesirable side effects of antibiotics therapy. There is also an increasing body of evidence that indicate potential of the probiotics in cure and prevention of gut infections, various forms of diarrhea, enterocolitis, peptic ulcers and inflammatory bowel disease. Growing scientific evidences have proven efficacy of probiotics LAB strains in maintaining and restoring gut homeostasis. Antimicrobial properties of the selected isolate are supporting their potential therapeutic usage for formulating probiotic dietary supplements, yogurts, drinks, capsules and personal care products. Use of probiotic LAB may have prophylactic application, while the isolated bacteriocin appears to be more attractive for eradicating an established microbial infection. Pediococcal isolate BA28 showed inhibition of most of the tested pathogens. But it did not interfere with most of the Gram-positive probiotic lactic acid bacteria tested in the study. Therefore, there is a least possibility of its interference with normal human microflora, in contrast to frequently prescribed antibiotics. Isolate BA28 was identified on the basis of biochemical tests and 16S rDNA sequencing as *Pediococcus acidilactici*. This preliminary study has successfully revealed the desirable antimicrobial properties of *P. acidilactici* sp. BA28 as a therapeutic aid. It could be integrated and exploited with the state of art knowledge to fully explore their suitability as *in vivo* therapeutics.

CTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTTC
CGTTAATTGATTATGACGTGCTTGCACTGAATGAGATTTTAACACGAAGTGAG
TGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCCAGAAGCAGGGGATAA
CACCTGGAAACAGATGCTAATACCGTATAACAGAGAAAACCGCCTGGTTTTC
TTTTAAAAGATGGCTCTGCTATCACTTCTGGATGGACCCGCGGCGCATTAGCT
AGTTGGTGAGGTAACGGCTCACCAAGGCGATGATGCGTAGCCGACCTGAGAG
GGTAATCGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGT
GAGTGAAGAAGGGTTTCGGCTCCTAAAGCTCTGTTGTTAAAGAAGAACGTGG
GTGAGAGTAACTGTTCACCCAGTGACGGTATTTAACCAGAAAGCCACGGCTA
ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATT
TATTGGGCGTAAAGCGAGCGCAGGCGGTCTTTTAAGTCTAATGTGAAAGCCT
TCGGCTCAACCGAAGAAGTGCATTGGAAACTGGGAGACTTGAGTGCAGAAG
AGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAA
CACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAA
GCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGAT
GATTACTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTA
AGTAATCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAAGAATTGAC
GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAG
AACCTTACCAGGTCTTGACATCTTCTGCCAACCTAAGAGATTAGGTGTTCCCT
TCGGGGACAGAATGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAG
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTACTAGTTGCGAGCAT
TCAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGG
ACGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAA
TGGATGGTACAACGAGTCGCCAAACCGCGAGGTTTAGCTAATCTCTTAAAAC
CATTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGAATCG
CTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTAC
ACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGCCGGTGGGGTAA
CCT

Figure 3: 16S rRNA sequence of *Pediococcus acidilactici* BA28.

References

1. Kaur B, Balgir PP, Kumar B, Garg N (2010) *Helicobacter pylori* infection: efficacy of probiotics and role of genome wide association studies. Archives of Clinical Microbiology 1.
2. Dunn BE, Cohen H, Blaser MJ (1997) *Helicobacter pylori*. Clin Microbiol Rev. 10: 720-741.
3. Smoot DT (1997) How does *Helicobacter pylori* cause mucosal damage? Direct mechanisms. Gastroenterology 113: S31-S34.
4. Novosti B, Nicoline FT, Roland NN (2010) Increasing trend of metronidazole resistance in the treatment of *Helicobacter pylori* infection: A global challenge. African Journal of Biotechnology 9: 1115-1121.
5. Stupnicki T, Taufer M, Denk H, Ratschek M, Spath P, et al. (1996) Triple therapy with sucralfate amoxycilin and metrinidazole for healing duodenal ulcer and eradicating Helicobacter pylori infection. Aliment Pharmacol Ther 10:193-197.
6. Muscra MN, Pedrazzoli J, Miranda EL, Ferraz JG, Hofstatter E. et al. (1995) Plasma hydroxyl- metronidazole/metronidazole ratio in patients with liver disease and in healthy volunteers. Br J Clin Pharmacol 40: 477-480
7. Lauritano EC, Gabrielli M, Scarpellini E, Ojetti V, Roccarina D, et al. (2009) Antibiotic therapy in small intestinal bacterial overgrowth: rifaximin versus metronidazole. Eur Rev Med Pharmacol Sci 13: 111-116.
8. Lu H, Hsu PI, Graham DY, Yamaoka Y (2005) Duodenal ulcer promoting gene of *Helicobacter pylori*. Gastroenterology 128: 833-848.
9. Bang SY, Han DS, Eun CS, Kim JE, Ahn SB, et al. (2007) Changing patterns of antibiotic resistance of Helicobacter pylori in patients with peptic ulcer disease. Korean J Gastroenterol. 50: 356-362.
10. Rhee SJ, Lee JE, Lee CH (2011) Importance of lactic acid bacteria in Asian fermented foods. Microb Cell Fact 10: S5.
11. Wilson M (2005) The reproductive system and its indigenous microbiota, In Microbial inhabitants of humans: their ecology and role in health and disease, Cambridge University Press, The Pitt Building, Trumpington street, Cambridge, UK.
12. Kumar B, Balgir PP, Kaur B, Mittu B, Garg N (2012) Comparative analysis of antimicrobial and spermicidal activity of native andrec-pediocin CP2. Archives of Clinical Microbiology 3.
13. Ocana VS, de Ruiz Holgado AA, Nader-Macias ME (1999) Characterization of a bacteriocin-like substance produced by a vaginal *Lactobacillus salivarius* strain. Appl Environ Microbiol 65: 5631-5635.
14. Karaoulu SA, Aydin F, Kili SS, Kili AO (2003) Antimicrobial activity and characteristics of bacteriocins produced by vaginal *lactobacilli*. Turk J Med Sci 33: 7-13.
15. Pucci MJ, Vedamuthu ER, Kunka BS, Vandenbergh PA (1988) Inhibition of *Listeria monocytogenes* by using bacteriocin PA-1 produced by *Pediococcus acidilactici* PAC 1.0. Appl Environ Microbiol 54: 2349-2353.
16. Toba T, Yoshioka E, Itoh T (1991) Acidophilucin A, a new heat-labile

bacteriocin produced by *Lactobacillus acidophilus* LAPT 1060. Letters in Applied Microbiology 12: 106-108.

17. Ridgway EJ, Brown JM (1989) *Listeria monocytogenes* meningitis in the acquired immune deficiency syndrome-limitations of conventional typing methods in tracing a foodborne source. J Infect. 19: 167-171.

18. Bou G, Luis Saleta J, Sáez Nieto JA, Tomás M, Valdezate S, et al. (2008) Nosocomial outbreaks caused by *Leuconostoc mesenteroides* subsp. *mesenteroides*. Emerg Infect Dis. 14: 968-971.

19. Jamart S, Denis O, Deplano A, Tragas G, Vandergheynst A, et al. (2005) Methicillin-resistant *Staphylococcus aureus* toxic shock syndrome. Emerg Infect Dis 11: 636-637.

20. Larsen J, Schønheyder HC, Lester CH, Olsen SS, Porsbo LJ, et al. (2010) Porcine-origin gentamicin- resistant *Enterococcus faecalis* in humans, Denmark. Emerg Infect Dis16: 682-684.

21. Teacher JH, Kennedy AM, (1918) Meningitis Caused by The Micrococcus Flavus. The Lancet 192: 422.

22. Stotland PK, Radzioch D, Stevenson MM (2000) Mouse models of chronic lung infection with *Pseudomonas aeruginosa*: models for the study of cystic fibrosis. Pediatric Pulmonology 30: 413-424.

23. Reid G (2001) Probiotic agents to protect the urogenital tract against infection. Am J Clin Nutr 73: 437S-443S.

24. Okada N, Pentland AP, Falk P, Caparon MG (1994) M protein and protein F act as important determinants of cell-specific tropism of *Streptococcus pyogenes* in skin tissue J Clin Invest 94: 965-977.

25. Joerger RD (2002) Alternatives to Antibiotics: Bacteriocins, antimicrobial peptides and bacteriophages. Poult Sci 82: 640-647.

26. Pingitore EV, Salvucci E, Sesma F, Nader-Macías ME (2007) Different strategies for purification of antimicrobial peptides from lactic acid bacteria (LAB). Communicating Current Research and Educational Topics and Trends in Applied Microbiology 1: 557-568.

27. Kim TS, Hur JW, Yu MA, Cheigh CI, Kim KN, et al. (2003) Antagonism of *Helicobacter pylori* by bacteriocins of lactic acid bacteria. J Food Prot 66: 3-12.

28. Simova ED, Beshkova DB, Dimitrov ZHP (2009) Characterization and antimicrobial spectrum of bacteriocin produced by lactic acid bacteria isolated from traditional bulgarian dairy products. J Appl Microbiol 106: 692-701.

29. Kumar B, Balgir PP, Kaur B, Garg N (2011) Cloning and expression of bacteriocins of *Pediococcus* spp.: A review. Archives of Clinical Microbiology 2.

30. Klaenhammer TR (1988) Bacteriocins of lactic acid bacteria. Biochimie 70: 337-349.