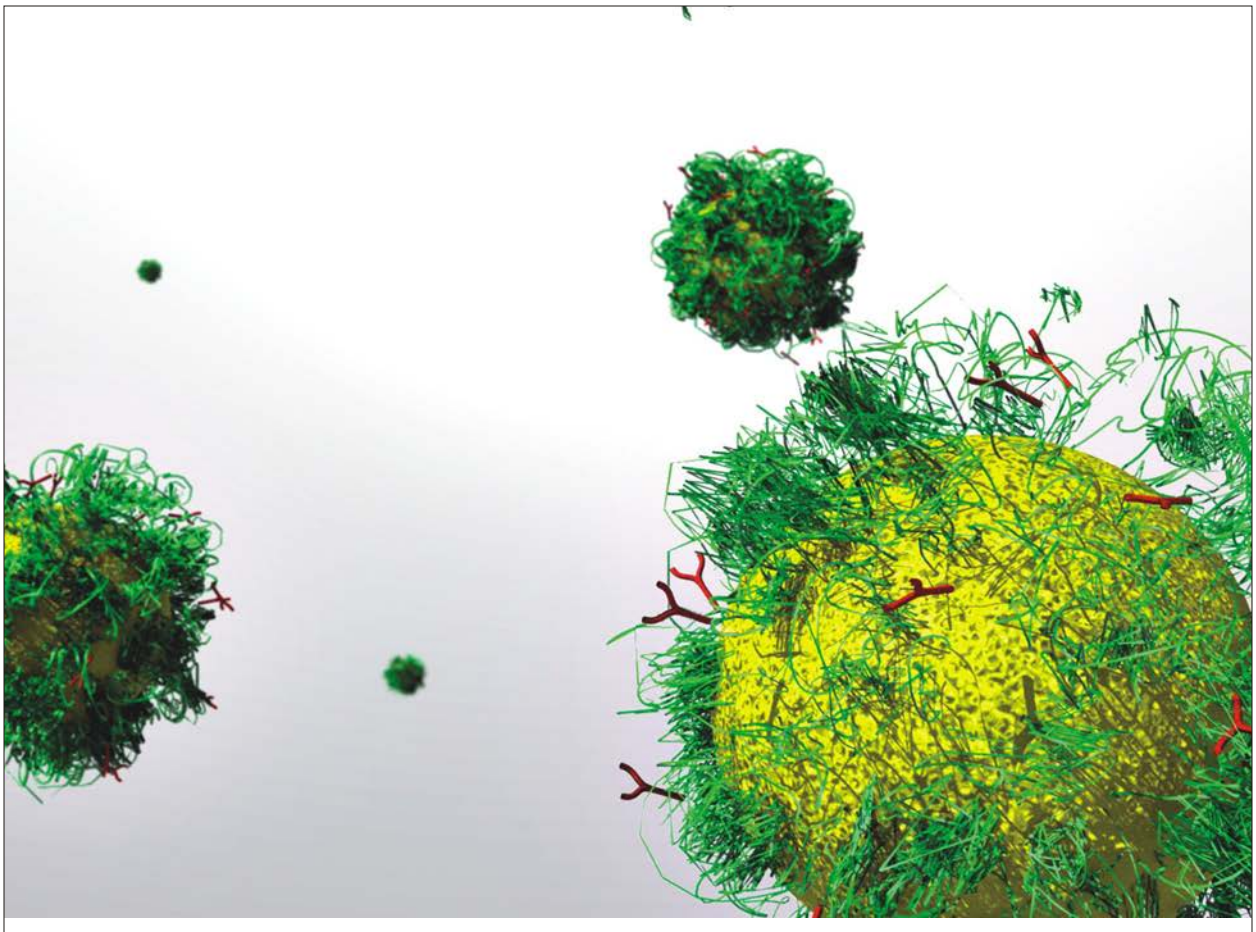

American Journal of Drug Discovery and Development

ISSN 2150-427X

Academic Journals Inc.

www.academicjournals.com

American Journal of Drug Discovery and Development 3 (1): 1-12, 2013
ISSN 2150-427x / DOI: 10.3923/ajdd.2013.1.12

Antimicrobial Spectrum of Anti-*Gardnerella vaginalis* Bacteriocin Producing *Lactobacillus fermentum* HV6b Against Bacterial Vaginosis Associated Organisms

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ABSTRACT

The aim of study was to isolate a Bacteriocin producing strain against *Gardnerella vaginalis* and other bacterial vaginosis causing organisms. For this purpose, about 100 bacteriocin producing strains Lactic acid bacteria were isolated from vaginal swabs of healthy and fecund females and evaluated for their antimicrobial activity. Nine isolates have shown anti-*G. vaginalis* activity. Out of nine HV 6b isolate have shown significant results against *G. vaginalis* so it has been selected for further study and it is identified as *Lactobacillus fermentum*. The antimicrobial activity of all nine strains including HV6b against other bacterial vaginosis associated organisms have also been studied. Mode of action of Bacteriocin produced from *Lactobacillus fermentum* was studied with scanning electron microscopy which showed the pore formation in the cell wall *Gardnerella vaginalis*. Antibiotic sensitivity profiles of *L. fermentum* HV6b was studied results showed that this isolated strain of lactic acid bacteria is resistant to most of antibiotics which have been currently used for treatment of bacterial vaginosis. Antibiotic sensitivity of *Gardnerella vaginalis* shows resistance of bacteria to Azithromycin, Co-trimoxazole, Metronidazole and Miconazole. Tetracycline and Erythromycin were required in more concentrated dose for inhibition. This study has shown that bacteriocin is a better alternative than antibiotics than the treatment of bacterial vaginosis. Viability loss of *Gardnerella vaginalis* was observed with different concentration of bacteriocin. Bacteriocin producing *L. fermentum* HV6b was found to be the responsible strain for formulating topical personal care and efficacy to the therapeutics aimed at prevention and treatment of bacterial vaginosis.

Key words: Lactic acid bacteria, bacterial vaginosis, *Gardnerella vaginalis*, bacteriocin, vaginal ecosystem

INTRODUCTION

Many scientist in the field of biology and chemistry have given significant contribution to the field of bio-medical and technology application by utilizing the natural resources (Chauhan and Kaith, 2012; Aan *et al*., 2011; Abd El-Hady and Abd El-Baky, 2011; Abdi *et al*., 2010; Raja and Thilagavathi, 2011; Issaoui *et al*., 2011; Abd El-Hady, 2011; Das *et al*., 2011; Rocco, 2011; Adedayo, 2012) yet many innovative minds are exploring the scientific means to confront the

disasters through infectious microbes. Bacterial Vaginosis is the common cause of vaginal discharge in women in reproductive age (Tolosa *et al*., 2006). *Gardnerella vaginalis* was first described by Gardner and Dukes (1955). BV has a complex microbiology (Hill, 1993; Fredricks *et al*., 2005) Healthy *Lactobacillus* populations of healthy microflora in women replaced by a group of organisms that includes *G. vaginalis*, anaerobic Gram-negative rods such as *Bacteroides* spp. (*Prevotella* species), *Peptostreptococcus* species, *Mycoplasma hominis*, *Ureaplasma urealyticum* and often *Mobiluncus*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, in Bacterial vaginosis (Ling *et al*., 2010; Lamont *et al*., 2011). BV is characterized by a milky or gray vaginal discharge with foul odor, the presence of clue cells and an increase in pH of the vagina to >4.5. Counts of anaerobic bacteria including *G. vaginalis* and others are drastically higher in diseased vagina. BV can cause adverse outcomes of pregnancy, including preterm delivery, premature labor, premature birth, infection of the amniotic fluid, infection of the uterus after delivery and death of the fetus or newborn. Susceptibility to viral infections by HIV, HSV type 2 and other sexually transmitted diseases increases in BV patients. Bacterial Vaginosis (BV) is associated with cervicitis endocervical mucopurulent discharge (Marrazzo *et al*., 2009). Key player in the pathogenesis of BV and the development of a biofilm is *G. vaginalis*. It was shown by a study of microbiota on the epithelial surfaces of vaginal biopsy specimens from women with BV showed that a biofilm adhered to part or all of the epithelium and *Gardnerella vaginalis* comprised 90% of bacteria in the biofilm (Swidsinski *et al*., 2005), *Gardnerella vaginalis*, a Gram-variable facultative anaerobe, is characterized by fastidious, beta-hemolytic, nonmotile, unencapsulated, nature of bacilli. It produces a pore forming toxin, vaginolysin, which affects only human cells. biofilms are also resistant to some forms of medical treatment (Verstraelen *et al*., 2009). Currently, preventive therapies for BV rely almost exclusively on the use of antibiotics such as metronidazole and gives initial cure rates of approximately 90%. Metronidazole becomes widely distributed in the body and undergoes oxidative metabolism in the liver with the formation of several metabolites. High concentration of metronidazole, could partially suppress healthy microflora of vagina (Simons *et al*., 2006). Metronidazole antibiotic therapy causes several side effects such as diarrhea, dizziness, headache, loss of appetite, nausea or vomiting, stomach pain or cramps (Lauritano *et al*., 2009). A number of reports have emerged that indicates emergence of drug resistance trait in *Gardnerella vaginalis* (Nagaraja, 2008). That is why more effective and safe therapeutics are desired to control BV.

Healthy vaginal ecosystem is maintained by Lactic acid bacteria that prevent the overgrowth of pathogenic bacteria in vaginal ecosystem by producing H_2O_2, organic acids and antimicrobial proteins called bacteriocins. The probiotic LAB prevents many pathogenic bacteria from overgrowing and thereby creating a condition called BV (Cribby *et al*., 2008). Several investigators have isolated and partially purified bacteriocins from various strains of lactic acid bacteria (Aslam *et al*., 2011). Emergence of antibiotic resistant phenotype in BV associated pathogenic bacteria has given the way to develop alternative therapeutics/prophylactic measures against these pathogens. Present study was undertaken with the aim to isolate and characterize anti-*Gardnerella vaginalis* bacteriocin producing isolate from human vaginal ecosystem and to study its mode of action that still remains unexplored.

MATERIALS AND METHODS

Sample collection and isolation of lactic acid bacteria: Samples of vaginal swabs from healthy and fecund females of reproductive age group were collected were transferred immediately to sterihzed saline (0.85% NaCl). The sample was inoculated into sterile MRS broth

(Demann Regossa Sharpe number 1164 with 1 ml L^{-1}. tween-80 pH was adjusted 6.5) for propagating vaginal LAB microflora. After incubation of 18-24 h at 37°C. Samples were subcultured three times before proceeding with bacterial isolation and activity assays. Lactic Acid Bacteria (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 human vaginal LAB isolates was evaluated against important pathogens especially associated with Bacterial Vaginosis, using spot-on-lawn (Toba *et al.*, 1991) and well-diffusion methods (Pucci *et al.*, 1988). Growth requirements of indicator microorganisms Associated with BV are specified in Table 1. Indicator strains were revived and maintained in growth media as prescribed by culture banks. Indicator cultures have been procured from Microbial Type Culture Collection, Chandigarh, Punjab, India *Bacteroides fragilis* MTCC 1045, *Bacteroides ovatus* MTCC 3298, *Bacteroides vulgatus* MTCC 1350, *Candida albicans* MTCC 183, *Neisseria mucosa* MTCC 1772, *Staphylococcus aureus* MTCC 737, *Streptococcus faecalis* MTCC 459, *Streptococcus thermophilus* MTCC 1928, National Collection of Industrial Microorganisms, Pune, India *Proteus mirabilis* NCIM 2387, *Streptococcus agalactiae* NCIM 2401 and from American Type Culture Collection *G. vaginalis* ATCC 10418, *Micrococcus flavus* ATCC 10240, *Neisseria gonorrhoeae* ATCC 19424 *Candida albicans* ATCC 10231 *Staphylococcus albus* ATCC 11631 All other cultures including *Staphylococcus aureus* NCTC 7447, *Staphylococcus pyogenes* NCTC 1086914 were kindly provided by Orbit Biotech, Mohali, Punjab, India. *Gardnerella vaginalis* ATCC 14018 was revived and maintained in the Casman's medium containing *Gardnerella vaginalis* active supplement (constituting gentamicin sulphate, nalidixic acid and amphotericin B) and 5% w/v defibrinated human blood.

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. Out of 10 of 1 mL aliquot 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 and agar well diffusion methods.

Determination of viability loss

Turbidity method: Cell suspensions of *Gardnerella vaginalis* of organisms were grown as broth cultures (Casman broth+selective media) along with increasing concentrations of the bacteriocin to be screened and turbidity at 600 nm was measured after 24-48 h. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells. Thus, the increasing the turbidity of the broth medium indicates increase of the microbial cell mass.

Colony count method: The cell suspensions of *Gardnerella vaginalis* were used to prepare an experimental set of samples as follows: Untreated control, test samples treated with 0-500 μg mL^{-1} pure bacteriocin. After 24 h incubation at 37°C, experimental set was examined to enumerate CFU mL^{-1}. Enumeration was done by pour plating original suspension or serially diluting them with sterile saline and incubating them on MRS agar plates at 37°C for 24 h. Results of bacteriocin treated *G. vaginalis* suspensions were compared with untreated control. A reduction in cell counts mL^{-1} following bacteriocin treatment was regarded as cell viability loss (Kalchayanand *et al.*, 2004).

SEM analysis of bacteriocin-treated *G. vaginalis* to indicate its mode of action: The control and treated cells of the bacterial vaginosis pathogen *Gardnerella vaginalis* were examined by scanning electron microscope model-hitachi VP-SEM S-3400N, Europe, to visualize morphological change that occurred in the cells following the exposure to bacteriocin produced by human vaginal isolate HV6b. *Gardnerella vaginalis* cell suspension was prepared in distilled water and it was treated with pure bacteriocin at a concentration of 0.5 mg mL^{-1}. Suspension was kept for 90 min at 37°C in a hydrated chamber and then centrifuged at 10,000 rpm for 1 min at 4°C. Cell pellet was dissolved in minimum quantity of ethanol and a drop of it was transferred to poly-L-lysine-treated silicon wafer chips, that were kept for 30 min in a hydrated chamber for the cells to adhere. Chips were viewed at 10 kV accelerating voltage in a field emission scanning electron microscope and images of the bacteriocin treated cells for topography contrast were collected at different magnifications.

Investigation of antibiotic susceptibility: Antibiotic susceptibility *Lactobacillus fermentum* and *G. vaginalis* was studied against different concentrations (2-8 μg mL^{-1}) of some commonly prescribed antibiotics to BV patients including ampicillin, amoxicillin, amoxicillin and clavulanic acid, azithromycin, ciprofloxacin, co-trimoxazole, erythromycin, gentamicin, metronidazole, nalidixic acid, ofloxacin, penicillin, rifampicin, tetracycline, tinidazole and vancomycin by spot-on-lawn method using·.

Statistical analysis of data: QI Macros ANOVA statistical tools like ANOVA one factor was used to determine significance of the results obtained in duplicate experimental sets. Level of significance was adjusted at 0.05 and results were said to be significant if their p-value <0.05 and F_{crit}<F-value according to one way ANOVA.

RESULTS AND DISCUSSION

Isolation of bacteriocin producing LAB strains: One of the bacteriocin producing strain was selected from more than 100 bacterial strains that were isolated from vaginal swabs after screening on the basis of bacteriocin producing activity. Bacteriocin production was observed in only eight isolates designated as HV6, HV6b, HV75, HV76, HV54A, HV59A, HV59C, HV59D and HV69. Their antibacterial activity was tested against indicator strains implicated in BV (Table 1). Isolate HV6b possessed antimicrobial spectrum that was highest among the tested indicators especially *G. vaginalis* ATCC14018 and therefore, was selected for further detailed study.

Antimicrobial spectrum of the isolated LAB strains against BV associated organisms Antimicrobial activities of isolated LAB strains were investigated against a panel of microorganisms associated with bacterial vaginosis and highly significant results were obtained in the study (Table 2; Fig. 1, 2). Growth inhibition of Urinary Tract Infections (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 (Reid, 2001). *G. vaginalis* causing bacterial vaginosis in humans was strongly inhibited by human vaginal isolates 6, 6b, 76 and 59A. Several investigators have isolated and partially purified bacteriocin from different species of lactobacilli. Most of them were with nonhuman strains, predominantly isolated from food (Karaoglu *et al.*, 2003). Bacteriocin produced by LAB isolate HV6b has a great potential to control

G. vaginalis associated BV in humans. The use of human lactobacilli as probiotics assigned to restore and maintain a healthy urogenital tract represents a promising alternative to conventional chemotherapy (Kaur *et al*., 2010; Kumar *et al*., 2011). All the tested strains of *Bacteroides* were

Table 1: Growth media and conditions of indicator microorganisms

BV associated pathogens	Gram test	Growth medium	Nature	Temp. (°C)	pH	Incubation time
Bacteroides fragilis MTCC 1045	-ve	RCB	Anaerobe	37	6.8	5 days
Bacteroides ovatus MTCC 3298	-ve	RCB	Anaerobe	37	6.8	48 h
Bacteroides vulgatus MTCC 1350	-ve	CMM	Anaerobe	37	7.2	72 h
Candida albicans ATCC 10231	Yeast	YEPD	Aerobe	30	7.2	48 h
Candida albicans MTCC 183	Yeast	YEPD	Aerobe	30	7.2	48 h
Gardnerella vaginalis ATCC 14018	+ve	CB	Anaerobe	37	7.2	48 h
Micrococcus flavus ATCC 10240	+ve	NB	Aerobe	30	7.4	24-48 h
Neisseria gonorrhoeae ATCC 19424	-ve	NB	Aerobe	37	7.4	24 h
Neisseria mucosa MTCC 1772	-ve	NB	Aerobe	37	7.4	24 h
Proteus mirabilis NCIM 2387	-ve	NB	F. anaerobe*	37	7.2	24 h
Staphylococcus albus ATCC 11631	+ve	BHI	Anaerobe	25	7.4	5 days
Staphylococcus aureus MTCC 737	+ve	BHI	Anaerobe	37	7.4	24 h
Staphylococcus aureus NCTC 7447	+ve	BHI	Anaerobe	37	7.4	24 h
Streptococcus agalactiae NCIM 2401	+ve	MRS	Aerobe	37	6.5	48 h
Streptococcus faecalis MTCC 459	+ve	MRS	Aerobe	37	6.5	48 h
Streptococcus pyogenes NCTC 10869	+ve	BHI	Aerobe	37	7.4	48 h
Streptococcus thermophilus MTCC 1928	+ve	MRS	Aerobe	40	6.5	28 h

F. anaerobe*: Facultative anaerobe, NB: Nutrient Broth, RCB: Reinforced Clostridial Broth, MRS: de Man's Ragosa Sharpe Medium, CB: Casman's Broth, BHI: Brain Heart Infusion Broth, CMM: Cooked meat media, YEPD: Yeast extract potato dextrose, BV: Bacterial vaginosis

Table 2: Antimicrobial spectrum of human vaginal LAB isolates against BV associated pathogens

	Inhibition zone (mm)								
BV associated pathogens	6	6b	75	76	54A	59A	59C	59D	69
Bacteroides fragilis MTCC 1045	12	12	15	14	12.5	*	-	12	-
Bacteroides ovatus MTCC 3298	15	15.5	14	14	12	7	-	9	7
Bacteroides vulgatus MTCC 1350	14	13	12	12	13.2	12	12	11	10
Candida albicans ATCC 10231	15	16	12	14	16	15	12	11	14
Candida albicans MTCC 183	16	17	15	14	15	14	13	12	11
Gardnerella vaginalis ATCC 14018	22	23	19	22	18	22.5	14	15	13
Micrococcus flavus ATCC 10240	20	21	19	15	16	15.5	13	18	14
Neisseria gonorrhoeae ATCC 19424	15	18	16	15	13	14	16	14	13
Neisseria mucosa MTCC 1772	15	18	16	8	15	14	15	16	17
Proteus mirabilis NCIM 2387	12	15	13	12	14	14	20	21	14
Staphylococcus albus ATCC 11631	20	22	15	11	16	20.5	13	12	14
Staphylococcus aureus MTCC 737	20	20	18	17	18	18	17	15	16
Staphylococcus aureus NCTC 7447	21	21	17	22	16	18	13	13	13
Streptococcus agalactiae NCIM 2401	-	-	-	-	-	-	-	-	-
Streptococcus faecalis MTCC 459	23	24	22	24	23.5	24	12	13	12
Streptococcus pyogenes NCTC 10869	18.5	19	17	17	14	20	11	15	12
Streptococcus thermophilus MTCC 1928	12	12	12	-	-	-	-	-	-

Each data is an average of two samples, p-value <0.05, F_{crit} (1.7184)<F-value (17.966), *: Negative reaction

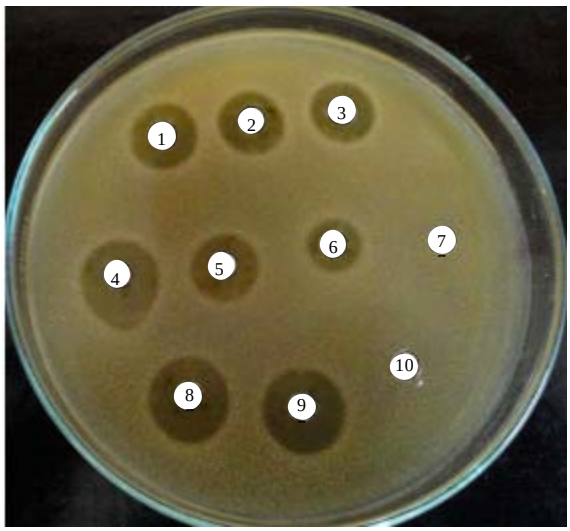

Fig. 1: Spot-on-lawn bacteriocin activity assay

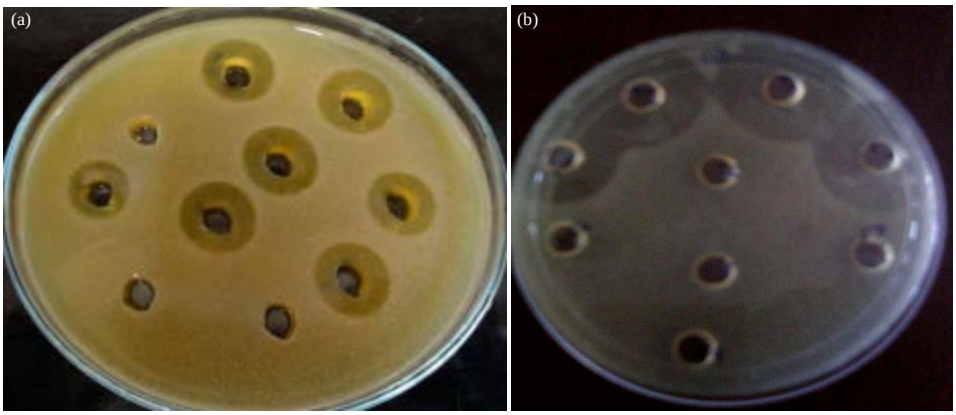

Fig. 2(a-b): Well diffusion bacteriocin activity assay against indicator strains (a) *Streptococcus faecalis* MTCC 459 and (b)*Gardnerella vaginalis* ATCC 14018 showing prominent inhibition zones

inhibited by isolate HV6b. *B. fragilis* is reported to cause obstetrics and gynecologic infections. *B. ovatus* infections can develop in all body sites, including the CNS, head, neck, chest, abdomen, pelvis, skin and the soft tissues (Sweet, 1985). *B. vulgatus* is commonly found in patients with ulcerative colitis. Inadequate therapy against these anaerobic bacteria may lead to clinical failure, thus probiotic therapy consisting of bacteriocin producing *Lactobacilli* is being suggested here (Bamba *et al.*, 1995).

C. albicans, *Mobiluncus*, *M. hominis*, *Peptostreptococcus* spp. and *P. bivia* are some other BV associated pathogens of urinary tract. Inhibition of *C. albicans* was also studied that indicated moderate to high inhibition by bacteriocins of LAB origin especially isolate HV6b. An acidic pH of vagina alone is not sufficient to inhibit vaginal pathogens and to prevent bacterial vaginosis. Thus, bacteriocin based therapeutics are urgently desired to cure such diseases and to overcome problems associated with antibiotic therapy such as recurrence of vaginal infections. There are many evidence that indicate potential of Generally Recognized As Safe (GRAS) lactic acid bacteria in

Table 3: Percentage growth of bacteria with bacteriocin by turbidity method

Bacteriocin conc. (μg mL^{-1})	Absorbance (600 nm)	Growth (%)
Control	1.236	100
100	0.785	63.51
200	0.491	39.72
300	0.285	23.058
400	0.123	9.951
500	-	-

Table 4: Changes in colony counts of *G. vaginalis* following treatment with bacteriocin produced by HV6b isolate

Bacteriocin conc. (μg mL^{-1})	Count ($\log_{10}$ CFU mL^{-1})
Control	5.24
100	5.13
200	4.95
300	4.65
400	3.95
500	nil

Each data is an average of two samples, p-value <0.05, F_{crit} (4.3877) <F-value (65.543)

maintaining and restoring gut homeostatic (Thirabunyanon, 2011). Use of live probiotic bacteria may have prophylactic applications but use of purified bacteriocins appears to be more attractive for eradicating an established infection (Lohans and Vederas, 2012). Ideally, anti-BV or anti-diarrheal therapeutics should specifically target disease causing microorganism and should have least interference with health promoting commensally microflora. In fact, the spectrum of activity of *L. fermentum* HV6b may be extremely well suited for targeting specific pathogens *in vivo*. In contrast to it, antibiotic prescribed frequently to cure gut and vaginal infections, strongly inhibit most of these beneficial microorganism at much lower concentrations (Le Blay *et al*., 2007).

Viability loss in bacteriocin treated *G. vaginalis* samples

Turbidity method: Cell suspensions of *G. vaginalis* of organisms grown as broth cultures (Casman broth+selective media) with increasing concentrations of the bacteriocin showed a sharp decrease in growth as at 330 μg mL^{-1} of bacteriocin 50% of turbidity was decreased and there was no growth of *G. vaginalis* with 500 μg mL^{-1} of bacteriocin turbidity at 600 nm is measured after 24-48 h. At the concentration of at 500 μg mL^{-1} no turbidity was found (Table 3).

Colony count method: The cell suspensions of *G. vaginalis* in MRS broth were treated with (0-500 μg mL^{-1}) different concentrations of pure bacteriocin and incubated at 37°C at 24 h. *Gardnerella* counts were determined and compared with respective control. Colony counts (log 10 CFU mL^{-1}) in the control samples were highest while reductions were observed in bacteriocin treated samples in a concentration dependent manner indicating the loss of viability. The count was reduced by 0.11 log cycles in *G. vaginalis* treated suspension with 100 μg mL^{-1} bacteriocin but by over 1.29 log cycles in suspensions of *G. vaginalis* treated with 400 μg mL^{-1} bacteriocin. There was a complete loss in viability and no CFU was detected in samples treated with 500 μg mL^{-1} bacteriocin. Viability loss of pathogenic bacteria exposed to various bacteriocins has been reported by other researchers (Ray *et al*., 2001). It has been shown that bacteriocin produced HV6b isolate is highly potent anti *G. vaginalis* agent with numerous therapeutic applications. If we colony forming units with control there was not a single colony at 500 μg mL^{-1} (Table 4).

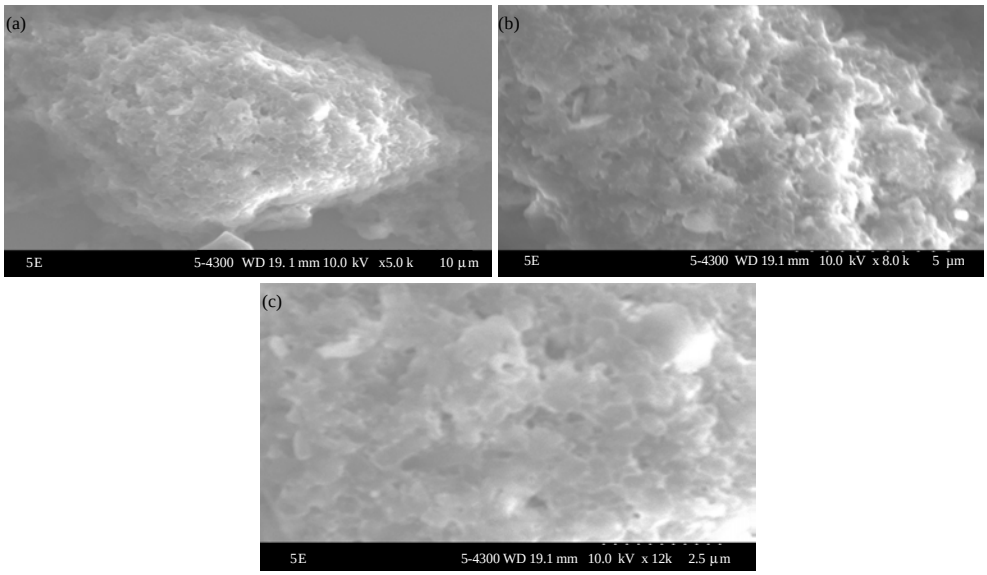

Fig. 3(a-c): Scanning electron micrographs of bacteriocin-treated *G. vaginalis* (a) Whole cell showing pore formation and (b) and (c) enlarged portions of the bacteriocin disrupted cell wall

SEM analysis of bacteriocin-treated *G. vaginalis* to indicate its mode of action: The SEM generated photo-micrographs of the control and treated cells of the pathogen *G. vaginalis* are presented in Fig. 3a-c; clear changes in cell morphology can be seen. The bacteriocin treated cells appeared collapsed that could be associated with the damage in the cell wall and cell membrane followed by lyses. Disruption of the cell wall and cell membrane is due to formation of a large number of pores (of small to moderate size) causing extensive damage to the cells and thus, inducing viability loss. These findings agree with the results of viability loss in bacteriocin treated *G. vaginalis* cells. The results clearly show that size of pores was increasing with increasing concentration of Bacteriocin.

Antibiotic susceptibility of vaginal *Lactobacillus fermentum* and *G . vaginalis*: Vaginal isolates displayed variations in their sensitivity to commonly prescribed antibiotics for treating BV. The safety investigation of the bacterial vaginal isolates revealed that they were sensitive to antibiotics viz. ampicillin, amoxicillin and clavulanic acid and penicillin but express a natural resistance phenotype to amoxicillin, azithromycin, erythromycin, gentamicin, metronidazole, nalidixic acid and tinidazole. Maximum inhibition zone was observed in case of isolate HV6 against ampicilhn (20 mm) followed by amoxicillin and clavulanic acid (19 mm). Antibiotic susceptibility of vaginal isolates against various antibiotics is given in Table 5. HV6b, HV54A and HV75 exhibited resistance to ampicillin. Strain HV69 showed its resistance to amoxicillin and clavulanic acid and its sensitivity to amoxicillin, azithromycin and metronidazole. On the other hand, *G. vaginalis* exhibited sensitivity to amoxicillin and clavulanic acid, ciprofloxacin, ofloxacin, penicillin, tetracycline, tinidazole and rifampicin at more than 2 µg mL^{-1} concentration, to cefixime, metronidazole and metronidazole H at a concentration more than 4 µg mL^{-1} and to co-trimoxazole and erythromycin at higher concentration (above 8 µg mL^{-1}). Resistances of HV6b isolate to most of these antibiotics except for penicillin and amoxicillin and clavulanic acid indicated its suitability as a component of triple therapy for bacterial vaginosis (Table 5) even we have compared the zone of bacteriocin HV6b against *Gardnerella vaginalis* along with the table of antibiotics which clearly

Table 5: Antibiotic susceptibility of *G. vaginalis* ATCC14018 and *L. fermentum* HV6b where R and ND refers to resistant and not defined

	Zone of inhibition in millimeters (mm)				
	G. vaginalis ATCC14018 (μg mL^{-1})				
Antibiotic used	2	4	6	8	*L. fermentum* HV6b (8 μg mL^{-1})
Amoxicillin	15	16	19	20	R
Amoxicillin+clavulanic acid	14	16	17.5	19	15
Azithromycin	-	-	-	-	R
Cefixime	-	17	19	22	nd
Ciprofloxacin	13	15	16	18	nd
Co-trimoxazole	-	-	-	14	nd
Erythromycin	-	-	-	13	R
Metronidazole	-	13	15	16	R
Miconazole	-	-	-	-	nd
Ofloxacin	15.5	16	19	24	nd
Penicillin	12	17	20	22	12
Rifampicin	14	16	17.5	20	nd
Tetracycline	9	12.5	13	14.5	nd
Tinidazole	15	16.5	18	23	R

R: Resistance, nd: Not defined

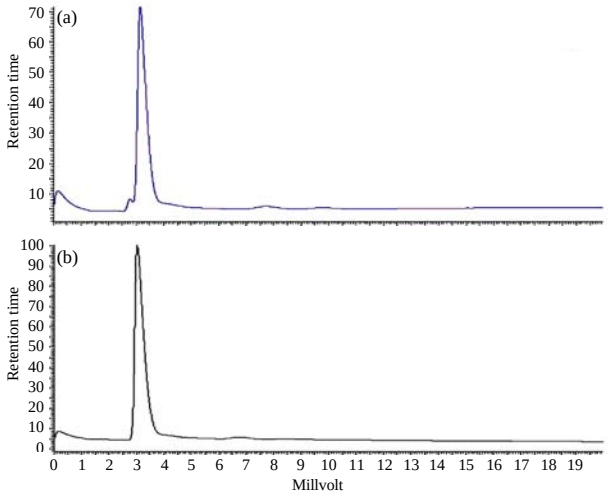

Fig. 4(a-b): Comparative GC chromatogram showing lactic acid production in (a) Cell free supernatant, (b) reference lactic acid

indicates better and safe therapy with bacteriocin. In a review on antibiotic sensitivity pattern of *Gardnerella vaginalis* which was done with cefuroxime and ceftazidime, ceftriaxone, cloxacillin, erythromycin, chloramphenicol and metronidazole. Metronidazole was used as the first-line drug of choice for the treatment of *G. vaginalis* (Adinma *et al.*, 1997). Nagaraja (2008) studied resistance of *Gardnerella vaginalis* with and recurrence of bacterial vaginosis after the treatment with metronidazole.

Characterization of *Lactobacillus fermentum*: In GC analysis, the polyethylene glycol (PEG) column was used. Lactic acid production was confirmed by gas chromatography by comparing it with a standard lactic acid (Fig. 4).

CONCLUSIONS

A highly potent anti-*Gardnerella vaginalis* bacteriocin producing isolate from human vaginal ecosystem was characterized based on its antimicrobial spectrum. Bacteriocin production trait of *L. fermentum* HV6b isolate was studied against important human pathogens causing bacterial vaginosis. This bacteriocin is a novel protein. A concentration dependent viability loss, tributary method and disruption of bacterial cells were observed in *G. vaginalis* culture exposed to bacteriocin produced by HV6B isolate that was further confirmed by SEM analysis. Antibiotic susceptibility of the isolated *Lactobacillus fermentum* and *G. vaginalis* was tested against many commonly prescribed antibiotics. Based on the results obtained in this study, human vaginal LAB isolates are strongly recommended for formulating anti-BV therapeutics/ointments/vaginal creams aimed at prophylaxes and treatment of BV and other sexually transmitted diseases in combination with antibiotic therapy that could check recurrence of the disease after termination of antibiotic treatment. Growing scientific evidences have proven efficacy of probiotics. Isolate HV6b 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. Preliminary experiments have established bacteriocin produced by *L. fermentum* sp. HV6b as an effective antimicrobial agent, with very impressive market value to formulate personal care products.

ACKNOWLEDGMENTS

Special thanks to Prof. Raj Bahadur Director Principal Govt. Medical College and Hospital (GMCH), Sector 32, Chandigarh, India, Prof. Anju Huria, Head of Department Obstt. and Gynae, GMCH, Chandigarh and Nurse Uma and Dr. Darshan Jot, Gynecologist, Phase 10, Mohali for their support in collecting samples under mutual consent. I also want acknowledge, Dr. Inderpreet Kaur, Scientist, Central Scientific Instrument Organization (CSIO), Chandigarh, India for extending facility of scanning electron microscopy. I also thankful to Jagvir Singh, Scientist (CSIO).

REFERENCES

Aan, M.P.S., M. Krishna, H.N.N. Murthy and S.K. Rai, 2011. Thermal, mechanical, morphological and flame retardance properties of DABA modified novolac/CFRP composites. Asian J. Mater. Sci., 3: 20-30.

Abd El-Hady, R.A.M. and R.A.A. Abd El-Baky, 2011. Enhancing the functional properties of sportswear fabric based carbon fiber. Asian J. Text., 1: 14-26.

Abd El-Hady, R.A.M., 2011. The effect of aesthetic beauty design on the performance properties of jacquard weft knitted fabrics. Asian J. Textile, 1: 74-86.

Abdi, S., Y. Ourova and O. Menchi, 2010. Development and characterization of metal matrix composite steel/Cu-Zn. Asian J. Mater. Sci., 2: 147-153.

Adedayo, A.V., 2012. Factors influencing semi-solid microsctructure. Asian J. Mater. Sci., 4: 1-12.

Adinma, J.I.B., N.R. Okwoli and C.N. Unaeze, 1997. Treatment of Gardnerella vaginalis infection. J. Obstet. Gynaecol., 17: 573-575.

Aslam, M., M. Shahid, F.U. Rehman, N.H. Naveed, A.I. Batool, S. Sharif and A. Asia, 2011. Purification and characterization of bacteriocin isolated from *Streptococcus thermophilus*. Afr. J. Microbiol. Res., 5: 2642-2648.

Bamba, T., H. Matsuda, M. Endo and Y. Fujiyama, 1995. The pathogenic role of *Bacteroides vulgatus* in patients with ulcerative colitis. J. Gastroenterol., 30: 45-47.

Chauhan, A. and B. Kaith, 2012. Accreditation of novel roselle grafted fiber reinforced bio-composites. J. Eng. Fibers Fabrics, 7: 66-75.

Cribby, S., M. Taylor and G. Reid, 2008. Vaginal microbiota and the use of probiotics. Interdiscip. Perspect. Infect, Dis., Vol. 2008.

Das, B.R., S.M. Istiaque and R.S. Rengasamy, 2011. Migration behavior of polyester/viscose blended rotar spun yarns. Asian J. Textile, 1: 27-34.

Fredricks, D.N., T.L. Fiedler and J.M. Marrazzo, 2005. Molecular identification of bacteria associated with bacterial vaginosis. N. Engl. J. Med., 353: 1899-1911.

Gardner, H. L., and C.D. Dukes, 1955. *Haemophilus vaginalis vaginitis*: A newly defined specific infection previously classified non-specific vaginitis. Am. J. Obstet. Gynecol., 69: 962-976.

Hill, G.B., 1993. The microbiology of bacterial vaginosis. Am. J. Obstet. Gynecol., 169: 450-454.

Issaoui, C., A.H. Said and S. Roudesli, 2011. A polyanizidine coated textile: Elaboration and characterization. Asian J. Textile, 1: 98-105.

Kalchayanand, N., P. Dunne, A. Sikes and B. Ray, 2004. Viability loss and morphology change of foodborne pathogens following exposure to hydrostatic pressure in the presence and absence of bacteriocins. Int. J. Food Microbiol., 91: 91-98.

Karaoglu, A.S., A. Faruk, S.S. Kilic and A.O. Kilic, 2003. Antimicrobial activity and characteristics of bacteriocins produced by vaginal lactobacilli. Turk. J. Med. Sci., 33: 7-13.

Kaur, B., P.P. Balgir, B. Kumar and N. Garg, 2010. *Helicobacter* pylori infection: Efficacy of probiotics and role of genome wide association studies. Arch. Clin. Microbiol., Vol. 1.

Kumar, B., P.P. Balgir, B. Kaur and N. Garg, 2011. Cloning and expression of bacteriocins of *Pediococcus* spp.: A review. Arch. Clin. Microbiol., Vol. 2.

Lamont, R.F., J.D. Sobel, R.A. Akins, S.S. Hassan, T. Chaiworapongsa, J.P. Kusanovic and R. Romero, 2011. The vaginal microbiome: new information about genital tract flora using molecular based techniques. BJOG, 118: 533-549.

Lauritano, E.C., M. Gabrielli, E. Scarpellini, V. Ojetti and D. Roccarina *et al.*, 2009. Antibiotic therapy in small intestinal bacterial overgrowth: Rifaximin versus metronidazole. Eur. Rev. Med. Pharmacol. Sci., 13: 111-116.

Le Blay, G., C. Lacroix, A. Zihler and I. Fliss, 2007. *In vitro* inhibition activity of nisin A, nisin Z, pediocin PA-1 and antibiotics against common intestinal bacteria. Lett. Applied Microbiol., 45: 252-257.

Ling, Z., J. Kong, F. Liu, H. Zhu and X. Chen *et al.*, 2010. Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. BMC Genomics, Vol. 11.

Lohans, C.T. and J.C. Vederas, 2012. Development of Class IIa bacteriocins as therapeutic agents. Int. J. Microbiol., Vol. 2012.

Marrazzo, J.M., M. Antonio, K. Agnew and S.L. Hillier, 2009. Distribution of genital Lactobacillus strains shared by female sex partners. J. Infect. Dis., 199: 680-683.

Nagaraja, P., 2008. Antibiotic resistance of *Gardrenalla vaginalis* in recurrent bacterial vaginosis. Ind. J. Med. Microbiol., 26: 155-157.

Pucci, M.J., E.R. Vedamuthu, B.A. Kunka and P.A. Vandenberg, 1988. Inhibition of *L. monocytogenes* by using bacteriocin PA-1 produced by *Pediococcus acidilactici* PAC 1.0. Applied Environ. Microbiol., 54: 2349-2353.

Raja, A.S.M. and G. Thilagavathi, 2011. Influence of enzyme and mordant treatments on the antimicrobial efficacy of natural dyes on wool materials. Asian J. Textile, 1: 138-144.

Ray, B., N. Kalchayanand, P. Dunne and A. Sikes, 2001. Microbial Destruction During Hydrostatic Pressure Processing of Foods. In: Novel Process and Control Technologies in the Food Industry, Bozoglu, F., T. Deak and B. Ray (Eds.). IOS Press, Amsterdum, Netherlands, pp: 95-122.

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

Rocco, F., 2011. Technical textile based on regenerated wool: Process and test of a new generation of fabrics. Asian J. Textile, 1: 42-49.

Simons, L.A., S.G. Amansec and P. Conway, 2006. Effect of *Lactobacillus fermentum* on serum lipids in subjects with elevated serum cholesterol. Nutr. Metabolism Cardiovas. Dis., 16: 531-535.

Sweet, R.L., 1985. Imipenem/cilastatin in the treatment of obstetric and gynecologic infections: A review of worldwide experience. Rev. Infect. Dis., 7: S522-S527.

Swidsinski, A., W. Mendling, V. Loening-Baucke, A. Ladhoff, S. Swidsinski, L.P. Hale and H. Lochs, 2005. Adherent bio films in bacterial vaginosis. Obstet. Gynecol., 106: 1013-1023.

Thirabunyanon, M., 2011. Biotherapy for and protection against gastrointestinal pathogenic infections via action of probiotic bacteria. Maejo Int. J. Sci. Technol., 5: 108-128.

Toba, T., E. Yoshioka and T. Itoh, 1991. Acidophilucin A, a new heat-labile bacteriocin produced by *Lactobacillus acidophilus* LAPT 1060. Lett. Appl. Microbiol., 12: 106-108.

Tolosa, J.E., S. Chaithongwongwatthana, S. Daly, W.W. Maw and H. Gaitan *et al.*, 2006. The International Infections in Pregnancy (IIP) study: variations in the prevalence of bacterial vaginosis and distribution of morphotypes in vaginal smears among pregnant women. Am. J. Obstetrics Gynecol., 195: 1198-1204.

Verstraelen, H., R. Verhelst, L. Nuytinck, K. Roelens and E. De Meester *et al.*, 2009. Gene polymorphisms of Toll-like and related recognition receptors in relation to the vaginal carriage of *Gardnerella vaginalis* and *Atopobium vaginae*. J. Reprod. Immunol., 79: 163-173.