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Antimicrobial activity of *Lactobacillus* against microbial flora of cervicovaginal infections

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PEER REVIEW

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Comments

The results of the present study are very interesting and suggest that the *Lactobacilli* isolated from healthy vagina may serve as probiotics. This is a good study in which authors explained the role of *Lactobacilli* as probiotic to cure cervicovaginal infections.

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ABSTRACT

Objective: To assess the probiotic nature of *Lactobacillus* in preventing cervical pathogens by studying the effectiveness of antimicrobial activity against vaginal pathogens.

Methods: *Lactobacilli* were isolated from healthy vaginal swabs on selective media and different pathogenic bacteria were isolated by using different selective media. The *Lactobacillus* strains were tested for the production of hydrogen peroxide and antimicrobial compounds along with probiotic properties.

Results: Of the 10 isolated *Lactobacillus* strains, strain 1, 3 and 6 are high hydrogen peroxide producers and the rest were low producers. Results of pH and amines tests indicated that pH increased with fishy odour in the vaginal fluids of cervicovaginal infection patients when compared with vaginal fluids of healthy persons. The isolates were found to be facultative anaerobic, Gram-positive, non-spore-forming, non-capsule forming and catalase-negative bacilli. The results of antimicrobial activity of compounds indicated that 280 and 140 µg/mL was the minimum concentration to inhibit the growth of both pathogens and test organisms respectively.

Conclusions: The results demonstrated that *Lactobacillus* producing antimicrobial compounds inhibits the growth of cervical pathogens, revealing that the hypothesis of preventing vaginal infection by administering probiotic organisms has a great appeal to patients, which colonize the vagina to help, restore and maintain healthy vagina.

KEYWORDS

Antimicrobial compounds, Cervical infection, Hydrogen peroxide, Lactic acid bacteria, Minimal inhibitory concentrations

1. Introduction

The cervico-vaginal infections are one of the female morbid circumstances that account for the most frequent gynecological disorders and may cause serious abnormalities (tumors) associated with some viruses like human papilloma virus (HPV). HPV is one of the most common sexually transmitted pathogens and is strongly associated with pre-neoplastic and neoplastic lesions

of the uterine cervix. It has been suggested that HPV infection alone may not be sufficient to promote cervical carcinogenesis and other co-factors could be involved, such as smoking, oral contraceptives, immuno suppression, vitamin deficiency, bacterial vaginosis and other sexually transmitted diseases[1]. A variety of microbial flora can be found in the vagina and cervix at different stages of health, growth or diseases[2]. An important group is the *Lactobacillus* bacteria, predominantly present in the

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urogenital microflora of healthy women and the obliteration of *Lactobacilli* in patients who develop urinary tract infections and also bacterial vaginosis and many other genital infections including cervical cancer, which has led to a focus on these bacteria^[3,4]. *Lactobacilli*, primarily facultative or strict anaerobes generally has a fastidious growth requirement. They prefer an acidic environment by producing lactic and other acids. In general, *Lactobacilli* have not been associated with disease and have been regarded as nonpathogenic members of the intestinal and urogenital flora^[5]. *Lactobacilli*, through the antagonistic interaction with pathogenic bacteria, maintains the vaginal ecosystem in a healthy state. Regulatory processes are carried out by species of *Lactobacillus* that produces antibacterial compounds, such as lactic and other organic acids, hydrogen peroxide (H₂O₂) and bacteriocins. Bacteriocins are biologically active, low molecular-weight proteins or peptides that inhibit the growth of a variety of pathogenic bacteria which appear in the bacterial vaginosis or cervicovaginal infections.

Several investigators have isolated and partially purified bacteriocin from different species of *Lactobacillus*. Most of the investigations were conducted with nonhuman strains, predominantly isolated from food^[6,7]. Human isolates of *Lactobacillus* species were found to have more antagonistic activity against other pathogenic microorganisms. A strain isolated from human faeces produced a substance with potent inhibitory activity against a wide range of bacterial species. It inhibited anaerobic bacteria (*Clostridium* sp., *Bacteroides* sp., *Bifidobacterium* sp.) and members of the family Enterobacteriaceae, *Pseudomonas* sp., *Staphylococcus* sp. and *Streptococcus* sp.; however, it did not inhibit other *Lactobacilli*. The inhibitory activity occurred between pH 3 and pH 5 and was heat-stable^[3]. *Lactobacillus gasseri*, the dominant species inhabiting human intestine^[8], was found to produce bacteriocin that exhibited a wide spectrum of bactericidal activity against enteric pathogens^[9]. Aroutcheva *et al.* isolated antibacterial proteins from *Lactobacillus acidophilus* obtained from urethral specimens that were active against *Gardnerella vaginalis*. A heat-resistant peptide was extracted from a vaginal isolate, *Lactobacillus salivarius*, which inhibited growth of *Enterococcus faecalis*, *Enterococcus faecium*, and *Neisseria gonorrhoeae*^[10]. Therefore the present investigation is to study the role of *Lactobacilli* in preventing the cervical pathogens by producing antimicrobial compounds.

2. Materials and methods

2.1. Isolation of *Lactobacillus*

Cervicovaginal smear samples were collected from 20 patients who had abnormal smears or cervicograms from pathology laboratory, Department of Obstructions and

Gynecology, Govt. Maternity Hospital, Tirupati, Andhra Pradesh, India. A total of four smears were also collected from healthy women who do not have any symptoms or vaginal diseases for the isolation of *Lactobacilli* species. Care was taken to avoid contamination of cervical smears and the swabs were immediately placed in thioglycolate transport medium.

2.2. Ethical approval

The present work was approved by the Institutional Ethical Committee (IEC), Sri Venkateswara Medical College, Tirupati along with the patient consent forms.

2.3. pH and amines test

Vaginal discharge and odour are frequent gynaecological complaints that result in women seeking medical care.

A portion of the undiluted vaginal material or one drop of the saline suspension was applied to the surface of a clean glass slide. One drop of 10% potassium hydroxide is added to the vaginal sample. The vapour layer above the surface of the slide was gently fanned to assess the presence of volatile amines which have a fishy odour^[11].

2.4. Culturing

The collected healthy swabs were placed on the selective media for *Lactobacilli*, De Man, Rogosa and Sharpe (MRS) agar. The plates were incubated at 37 °C for 24–48 h under anaerobic conditions like candle light jar. The *Lactobacillus* was presumptively identified by their ability to grow well on MRS medium^[5]. The pathogenic vaginal flora, isolated from the cervical swabs, were spread on different types of selective media like *Salmonella-Shigella* medium, *Gardnerella* selective agar with 5% Human Blood; Thayer–Martin agar medium, *Trichomonas* media and chocolate agar medium. The first three types of plates were incubated aerobically and the last one was incubated anaerobically in candle light jar for a period of 24–48 h at 37 °C^[12].

2.5. Hydrogen peroxide production

All the isolated *Lactobacillus* species were tested for the production of H₂O₂ by exposure of organisms grown anaerobically on agar containing horseradish peroxidases and tetramethyl benzidine (TMB) agar^[4]. Colonies of H₂O₂-producing organisms form a blue pigment as horseradish peroxidases oxidize TMB in the presence of bacterial derived H₂O₂.

2.6. Extraction of antimicrobial compound from broth

The isolated *Lactobacillus* grown on MRS agar for 24 h and the colonies were transferred into 15 mL of MRS broth and

incubated in an anaerobic chamber at 37 °C for 18 h. This culture was used to inoculate one liter of MRS broth and incubated in an anaerobic chamber at 37 °C for 18 h. The bacteria were harvested in the early exponential growth phase. Bacterial cells were separated from the broth culture by centrifugation at 9500 r/min for 25 min. The supernatant was filtered and the filtrate pH was adjusted to 5.5 with 12% ammonium hydroxide.

Protein from the supernatant was precipitated using the fractional precipitation method by adding increasing concentrations of ammonium sulfate (20%, 30%, 40%, 50%, 60% and 80%). After each precipitation, samples were centrifuged at 13000 r/min for 15 min (4 °C). The sample was desalted by dialyzing at 5 °C using dialysis tubing against one liter deionized water. The entire dialysis required four changes of deionized water over 3 d. The dialyzed protein solution was frozen at –80 °C and lyophilized. Dried samples were stored at 4 °C. To separate protein agglomerated with Tween–80, the sample was defatted three times with chloroform/methanol in a ratio of 2:16. The aqueous and chloroform/methanol layers were air dried, dissolved in a small amount of phosphate buffer solution, and tested for antibacterial activity.

2.7. Estimation of total protein concentration

The total protein concentration was estimated in the supernatant by the modified Lowry's method in micro titer assay^[13].

2.8. Demonstration of antimicrobial activity

2.8.1. Well diffusion method

The antimicrobial activity was determined by the well diffusion method. The isolated pathological strains were chosen as the target bacteria. The nutrient agar plates were inoculated with 100 µL of overnight cultures of pathogenic bacteria. Approximately 0.5 mm diameter wells were prepared by using borer and different concentrations (280, 140, 70, 35, 17.5, 71.5 and 8.6 µL) and standard streptomycin antibiotic for positive control of cell free supernatant was poured into the well and then incubated at 37 °C for 24 h.

2.8.2. Micro titer plate assay

Antimicrobial activity was assayed by determining the percentage of growth inhibition in pathogens by adding extracted compound^[14]. A total of 50 µL of overnight culture of test [*Escherichia coli* (*E. coli*) and *Bacillus*] and the pathogens (*Salmonella*–1; *Salmonella*–2; *Gardnerella*; *Chlamydia*; *Trichomonas*; *Neisseria*) organisms were inoculated into 50 mL of nutrient broth and incubated at 37 °C for 1 to 3 h at 200 r/min until they grown to about 0.2 to 0.4 OD at 630 nm. Each well was filled with 100 µL of culture.

Different concentrations of cell free supernatants were prepared by serial dilution of 100 µL with 2 fold dilutions (280, 140, 70, 35, 17.5, 71.5 and 8.6 µg/mL of broth) and 100 µL of culture was added to each well which was made up to 200 µL with sterile broth. Microbial growth was measured in positive control (culture alone) and negative control (treated with 0.4% formaldehyde) after 12 h of incubation,

read at 630 nm using ELISA reader (Bio–Rad, Germany). The percentage of reduction in growth of test bacteria due to the antimicrobial proteins was calculated as per the following formula mentioned below.

$$\% \text{ of reduction in growth} = \frac{\text{OD Value of Control well} - \text{test well}}{\text{OD Value of Control well}} \times 100$$

2.9 Tests for probiotic characteristics

Screening of potential probiotic *Lactobacillus* was done by sequential probiotic characteristics like pH, temperature, and salt tolerance *etc*^[15].

2.10. Morphological and biochemical tests for isolated strains

Isolates, grown on nutrient agar medium, were examined for Gram's staining, cell morphology and culture characteristics. Some biochemical characteristics of the cultures were studied by indole, methyl red, Voges Proskaur and citrate utilization tests, catalase, starch hydrolysis and lactose fermentation tests performed according to Bergey's manual of determinative bacteriology^[16].

3. Results

3.1. *Lactobacillus* isolation

The vaginal samples, collected from 4 healthy women, were diluted and cultured on MRS agar plates. Colonies (196) were primarily picked up from MRS agar surface. Most of them showed round, small colonies without any pigment, and white to cream color. All of them were later tested for Gram's staining, catalase production and checked for spore formation. Only 24 of them showed *Lactobacillus* properties with Gram–positive, catalase–negative and, non–spore formation. Finally 10 potent antimicrobial isolates were selected for further analysis.

3.2. Isolation of pathogenic bacteria

A total of 6 bacterial pathogens were isolated from cervicovaginal infection patients by selective media and the pure cultures were maintained on nutrient agar medium for further testing. The cultures include *Salmonella*–1; *Salmonella*–2; *Gardnerella*; *Chlamydia*; *Trichomonas*; *Neisseria*.

3.3. Hydrogen peroxide production test

All the selected 10 isolates were tested for their ability to produce H₂O₂. Results are shown in Table 1. Isolates 1, 3 and 6 produced blue and 4, 7, 10 produced bluish brown color and the remaining isolates could not produce hydrogen peroxides, suggesting that the first 3 isolates were high H₂O₂ producers, and the next 3 isolates were medium producers and remaining were low producers.

Table 1

The colony colour of isolated *Lactobacilli* strains grown on agar plates containing TMB for the detection of H₂O₂ Production.

Isolated strain	Colony colour	Level of H ₂ O ₂ production
Isolate-1	Blue	High
Isolate-2	White	Low
Isolate-3	Blue	High
Isolate-4	Bluish brown	Medium
Isolate-5	White	Low
Isolate-6	Blue	High
Isolate-7	Bluish brown	Medium
Isolate-8	White	Low
Isolate-9	White	Low
Isolate-10	Bluish brown	Medium

3.4. Extraction and estimation of total protein in cell free supernatant

Approximately 6 mL of cell free supernatant was obtained from the broth, and the supernatant was stored at -20 °C for further analysis. The total protein content of the supernatant was estimated 2.8 mg/mL.

3.5. Antimicrobial activity of extracted compound

Antimicrobial activity of the extracted compound was tested against the two laboratory organisms (Figure 1) and also tested against the isolated pathogenic bacteria (Figure 2).

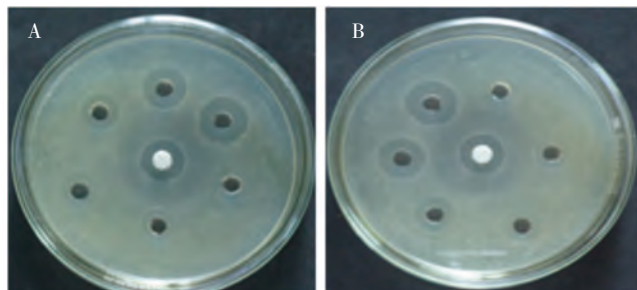


Figure 1. Antimicrobial activity of lactic acid bacteria against laboratory test organisms (A: *E. coli*; B: *Bacillus*).

Table 2

Antimicrobial activity of bacteriocin by disk diffusion and micro-titer assay.

Strains	Zone of Inhibition (cm)							
	Laboratory organisms		Pathogenic organisms					
	<i>E. coli</i>	<i>Bacillus</i>	1	2	3	4	5	6
Isolate-1	0.4±0.2	0.2±0.0	0.2±0.1	0.3±0.2	0.4±0.2	0.6±0.0	0.2±0.0	0.2±0.2
Isolate-2	0.6±0.2	0.8±0.4	0.9±0.3	0.6±0.3	0.2±0.2	0.8±0.2	0.1±0.0	0.2±0.0
Isolate-3	0.4±0.2	0.4±0.2	0.8±0.4	0.4±0.2	0.1±0.0	0.3±0.2	0.2±0.2	0.1±0.0
Isolate-4	0.3±0.2	0.5±0.2	0.4±0.2	0.4±0.2	0.5±0.2	0.1±0.0	0.2±0.0	–
Isolate-5	0.5±0.2	0.2±0.2	0.4±0.2	–	0.2±0.2	0.2±0.2	0.4±0.2	0.1±0.0
Isolate-6	0.5±0.2	0.1±0.0	–	0.4±0.2	0.4±0.2	0.3±0.2	0.4±0.2	–
Isolate-7	0.5±0.2	0.1±0.2	–	0.3±0.2	–	0.2±0.2	0.5±0.3	–
Isolate-8	1.0±0.3	0.9±0.3	0.9±0.3	0.4±0.2	–	0.2±0.0	0.1±0.0	–
Isolate-9	1.5±0.2	1.0±0.0	0.9±0.4	0.5±0.2	0.8±0.3	0.4±0.2	0.2±0.0	–
Isolate-10	1.0±0.2	0.8±0.5	0.4±0.2	1.0±0.0	0.2±0.1	0.2±0.2	0.1±0.0	–
Control	1.5±0.1	1.0±0.2	0.9±0.2	0.9±0.2	1.2±0.3	0.9±0.4	0.7±0.4	0.5±0.2

1: *Salmonella*-1; 2: *Salmonella*-2; 3: *Gardnerella*; 4: *Enterococci*; 5: *Neisseria*-1; 6: *Neisseria*-2.

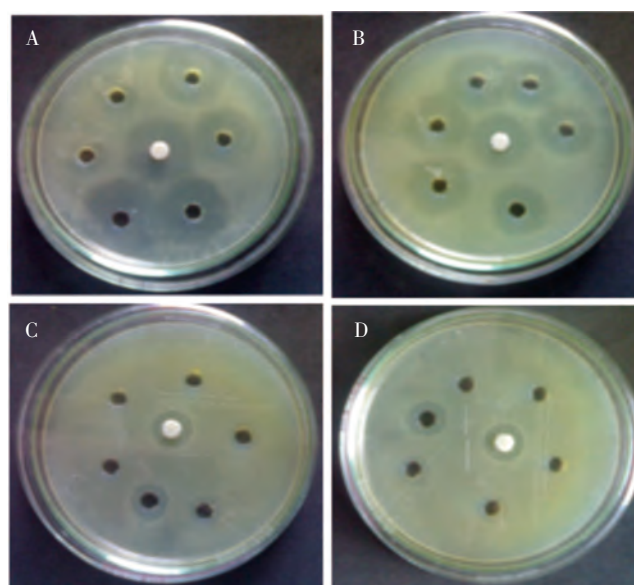


Figure 2. Antimicrobial activity of lactic acid bacteria against cervical pathogens (A: *Salmonella*; B: *Gardnerella*; C: *Enterococci*; D: *Neisseria*).

Results, presented in Table 2 and 3, demonstrated that 140 µg/mL was the minimum concentration to inhibit the growth of *E. coli* and *Bacillus* and 280 µg/mL was the minimum concentration to inhibit the growth of all pathogenic bacteria like *Salmonella*-1, *Salmonella*-2, *Gardnerella*, *Chlamydia*, *Trichomonas* and *Neisseria*, isolated from cervicovaginal infections. The same concentrations were also observed in micro-titer assay with a percentage of 96.2, 82.8, 91.5, 92.5, 79.2, 82.6, and 80.3, 97.1 for pathogens and laboratory organisms respectively.

3.6. Tests for probiotic characteristics

All the 10 isolates were tested for their ability to test the probiotic property, and the results were tabulated in Table 4. The optimum pH for all the isolates was ranged from 2–5, beyond which they could not grow luxuriantly. Similarly the optimum temperature for all the isolates was found to be 30–40 °C except for the isolates 6 and 7, which

Table 3

Reduction of microbial growth by the antimicrobial compounds extracted from isolated laboratory strains against pathogenic and laboratory test organisms.

Isolated laboratory strains	% of growth reduction in micro-titre assay							
	Laboratory test organisms		Pathogenic organisms					
	<i>E. coli</i>	<i>Bacillus</i>	1	2	3	4	5	6
Isolate-1	51.6	97.1	96.2	80.0	84.0	85.6	66.9	71.1
Isolate-2	83.5	27.1	87.0	81.0	91.5	92.5	66.1	61.2
Isolate-3	30.5	80.6	21.0	82.8	79.5	91.5	77.7	41.4
Isolate-4	77.0	81.6	88.0	82.6	79.7	52.1	62.0	32.6
Isolate-5	30.5	82.8	81.0	12.4	87.9	79.4	53.4	80.2
Isolate-6	40.9	46.6	89.0	36.9	77.0	88.6	49.1	20.6
Isolate-7	80.3	82.6	21.0	66.0	7.0	36.1	60.1	22.4
Isolate-8	68.0	81.5	12.7	55.7	6.3	92.5	30.3	20.4
Isolate-9	21.6	84.9	49.0	65.3	63.5	81.6	26.5	17.9
Isolate-10	77.0	88.4	87.0	33.0	68.5	82.1	79.2	68.4

1: *Salmonella*-1; 2: *Salmonella*-2; 3: *Gardnerella*; 4: *Enterococci*; 5: *Neisseria*-1; 6: *Neisseria*-2.

Table 4

Probiotic characters of the isolated strains grown at different NaCl concentrations.

Strain	pH				Temperature (°C)				Concentration of NaCl (%)			
	2	5	7	9	30	40	50	60	3	4	6	7
Isolate-1	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve
Isolate-2	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve
Isolate-3	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve
Isolate-4	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve
Isolate-5	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve
Isolate-6	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Isolate-7	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	+Ve	+Ve
Isolate-8	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve
Isolate-9	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve
Isolate-10	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	+Ve	+Ve

+Ve: presence of growth; -Ve: no growth.

Table 5

Morphological and biochemical tests for isolated strains.

Strain No.	Test	1	2	3	4	5	6	7	8	9	10
1	Gram's staining	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
2	Catalase	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
3	Starch hydrolysis	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
4	Indole test	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
5	Methyl red	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
6	Voges Proskaur test	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
7	Citrate utilization test	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
8	Lactose fermentation	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
9	Spore formation	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
10	Capsule formation	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve

+Ve: positive; -Ve: negative.

grown even at 50 °C. Isolates 6, 7 and 10 showed high salt tolerance as evidenced by their conspicuous growth even at 6% NaCl where the remaining isolates grown at 4% NaCl concentration.

3.7. Morphological and biochemical tests

All the isolated *Lactobacillus* strains were tested according to Bergey's manual of determinative bacteriology, and the results were tabulated in Table 5, which shows that all of

the *Lactobacillus* strains were rod shaped, Gram-positive bacteria with presumptive test of catalase-negative.

4. Discussion

The presence of HPV in human cervical cells has been shown to be an initiating factor in the development of cervical dysplasia[17]. However, not all patients harboring HPV develop cervical dysplasia, indicating the presence

of additional promoting factors. Multiple studies have shown that patients with bacterial vaginosis have a higher incidence of cervical dysplasia^[18–20]. Bacterial infection or vaginosis is a polymicrobial syndrome involving the genital tract and cervix, characterized by the replacement of *Lactobacilli*–predominant flora with anaerobic pathogens^[21]. *Lactobacilli* have been shown to be the predominant bacteria, found in the normal vaginal microbial flora of women in reproductive age. *Lactobacilli*, principally the strains that produce H₂O₂ and other antimicrobial compounds, may have a protective effect against vaginal pathogens including gonorrhoea, *Staphylococcus* and *Shigella*^[22]. In the present study, of the 196 isolated strains, 10 had inhibitory effects on laboratory test organisms and produced H₂O₂ at different concentrations. All the 10 strains were tested for probiotic characteristics; isolates 6 could grow even at 60 °C, and 7% NaCl concentrations, isolates 7 and 10 grown at 40 °C, and 7% NaCl respectively. Positive results of probiotic treatment were obtained from the studies of Anukam^[23], showing 55% satisfaction of treatment of bacterial vaginosis with intravaginal capsules containing human derived H₂O₂–producing *Lactobacillus*. *Lactobacilli* prevent other bacteria by the production of H₂O₂, bacteriocin and some organic acids. The production of organic acids by *Lactobacilli* maintains the acidic pH and controls the other bacteria. In addition, the vaginal pH was identified as alkali ranging from 7–10, with fishy odour in patients suffering cervicovaginal infection. As stated above, *Lactobacillus* can produce lactic acid and some other organic acids which produces the antimicrobial activity of the microorganisms^[24]. Therefore the existence of antimicrobial substances present in the isolates studied would be partly due to organic acids, because the present investigation showed that the antimicrobial activity of the test isolates was more active at acidic pH than at alkali pH.

In the present investigations, the antimicrobial activity of probiotics was tested against the pathogenic bacteria isolated from vaginal swabs. The partially purified bacteriocin protein isolated from the culture medium inhibits the growth of all tested laboratory test organisms as well as pathogens. The present study correlates with many other studies which have revealed that *Lactobacillus* could produce organic acids, hydrogen peroxide and bacteriocins^[25]. It is also noted that the extracted supernatant contains 2.8 mg/mL of total protein in a 100 mL of production medium. The extracted compound was tested for their antimicrobial activity, using a micro-titer assay method. The compound was serially diluted in a two-fold dilution to obtain minimal inhibitory concentration (MIC) for the inhibition of bacterial growth. Of the tested concentrations, 140 µg/mL was found to be the MIC for both the *E. coli* and *Bacillus*. However a higher concentration of the antibacterial compound was needed to inhibit the pathogenic bacteria like *Gardnerella vaginalis* with 280 µg/mL MIC and *Chlamydia trachomatis* with 140 µg/mL MIC.

In recent years, the use of probiotics has received greater attention as an alternative, inexpensive and natural remedy to restore and maintain health^[26]. Two strains, *Lactobacilli* GG and *Lactobacilli* GR–1 appear to be effective in colonizing and protecting the intestine and urogenital tract respectively against microbial infection^[27].

In conclusion, antimicrobial compounds present in the *Lactobacillus* isolated from healthy vaginal swabs shows *in vitro* antimicrobial activity against cervicovaginal pathogens and are more active under acidic conditions. Hence, it might be used as bioprotective agents to control the bacterial infections and maintain the normal healthy vagina that may lead to decreased risk assessment.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The health of the female genital tract depends significantly upon the composition of the vaginal micro flora. These organisms produce acid substances to avoid other pathogenic microorganism including bacteria, fungi and other parasites from the vagina. Therefore only facts that decrease acidity of the vagina can cause a vaginal infections, and in most cases vaginal infections reach the cervix. the present paper clearly illustrates the role of *Lactobacilli* which are dominant in healthy vaginal micro flora on the pathogenic bacteria which causes cervicovaginal infections.

Research frontiers

The present study is being performed in order to determine the effect of healthy vaginal *Lactobacilli* on the bacteria which causes cervicovaginal infections. Nowadays use of probiotics has received great attention as an alternative inexpensive and natural remedy to restore and maintain health. In this paper the authors have experimentally proved that *Lactobacilli* isolated from healthy vaginal swabs showed an antimicrobial activity against cervicovaginal pathogen.

Related reports

In this study, heavy programme has been recorded

in terms of treatment of cervicovaginal infections by increasing numerous pharmaceutically active substances as well as broadening the knowledge on the pathogenic and physiopathogenic mechanisms of genital infections.

Innovations & breakthroughs

In this study the probiotic bacterial strain *Lactobacilli* isolated from vaginal swabs shows *in vitro* antimicrobial activity against cervicovaginal pathogen. This study is very useful and innovative for modern medicine.

Applications

This study is useful for controlling the bacterial infections and maintaining the normal healthy vagina that may lead to decreased risk assessment.

Peer review

The results of the present study are very interesting and suggest that the *Lactobacilli* isolated from healthy vagina may serve as probiotics. This is a good study in which authors explained the role of *Lactobacilli* as probiotic to cure cervicovaginal infections.

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