

ANTIMICROBIAL ACTIVITY OF *LACTOBACILLUS FERMENTUM*, A VOLVO VAGINAL ISOLATE

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ABSTRACT

Objective: Lactobacilli are the dominant bacteria of a healthy human vagina. They have antagonistic effect on potentially pathogenic microorganisms and vaginal pathogens. The aim of the present study was to find probiotic isolate from vaginal samples which can inhibit the growth of pathogenic microorganisms.

Methods: A non-sporulating, catalase negative, Gram-positive bacteria was isolated from the vagina of a pregnant and identified women using 16S gene sequencing. The strain was identified to be *Lactobacillus fermentum*. The bacterium was grown in MRS broth for 24 hrs and the cell-free culture filtrate was used for antimicrobial assay. It has been found that minute quantity of culture filtrate (10 µl) exhibit inhibition against *Staphylococcus aureus* (MTCC 3160) and *Escherichia coli* (MTCC 1060).

Results: The cell free supernatant of bacterium identified as *Lactobacillus fermentum*, showed antimicrobial activity in minute doses (10µl) by well diffusion method.

Conclusion: It is suggested that this species of Lactobacilli could be considered for use in improving genital microfloral defense against Gram-positive and Gram-negative bacteria.

Keywords: *Lactobacillus*, Human vagina, Probiotic microorganism, 16s gene sequencing.

INTRODUCTION

Lactobacilli are the dominant bacteria of a healthy human vagina [1]. They are routinely found as part of a well-defined "normal" or "indigenous" microflora in the female reproductive tract [2]. *Lactobacillus acidophilus* complex has been isolated from the vaginal flora in 150 human samples [3]. There is no consensus on the argument that *Lactobacillus* species are the main inhabitants of in the human vagina, it is generally agreed that the number, of microbes, varies from about 107 to 108 CFU/g of fluid [1].

Lactobacilli are known to be present in the human vagina as probiotic microorganism. They have an antagonistic effect on potentially pathogenic microorganisms and vaginal pathogens. These bacteria play an important role in defending humans against pathogenic bacteria [4]. It is believed that, probiotic strains of *Lactobacillus* can be used for treatment of volvo-vaginal infections. The aim of the present study was to find probiotic isolate from vaginal samples which can inhibit the growth of both Gram-positive and Gram-negative organisms.

METHODS
Collection of samples

Vaginal samples were collected with permission of the Ethical Committee after obtaining informed consent of the volunteer who visited Health Care Centre of Red Cross Society, Karimganj, for health checkup. Healthy pregnant women who do not have vaginal infections were enrolled in this study. The vaginal swab samples were collected from the posterior fornix of the vagina of a woman of age 30 and second trimesters of pregnancy. The swabs were then brought to the laboratory and streaked over sterile MRS agar plates. The bacterial flora was studied after 48 hrs of incubation.

Morphological and biochemical study

The isolate was studied for Gram-staining, catalyze test and test for spore formation (Table 1).

Antibacterial assay

Antibacterial assay was done using well diffusion method. Mueller-Hinton agar plates were prepared, and the plates were seeded with *Escherichia coli* (MTCC 1060) and *Staphylococcus aureus* (MTCC 3160) separately using McFarland standard 0.5. Following this 5 mm well were prepared in each plate, and 10 µl of cell-free culture filtrate of the isolate was loaded in each well. The plates were incubated for 24 hrs at 35±2°C (Table 2).

DNA extraction and polymerase chain reaction (PCR) amplification

DNA isolation has been done, using the method described by Vural and Ozgun (2011) [5] with little modifications. For identification of the isolate, the 16S gene was amplified by using 27s forward primer and 1100 reverse primer (Table 3) and sequenced.

Each single reaction mixture (10 µl) contained 1 µl of template DNA, 1 µl of each primer (20 picomole), 5 µl of Master Mix 2X (HiMedia) and

Table 1: Morphological and biochemical characterization

Sample name	Gram-staining	Catalyze test	Test for spore formation
<i>L. fermentum</i>	Positive	Negative	Non-spore forming

L. fermentum: Lactobacillus fermentum

Table 2: Antimicrobial activity of the bacteria

Sample name	Zone size against test organisms (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
<i>L. fermentum</i>	6	5

L. fermentum: Lactobacillus fermentum, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus

Table 3: Primer sequence for PCR reaction

Name	Sequence	Reference
1100R	AGGGTTGCGCTCGTTG	Turner et al. 1999 [6]
27F	AGAGTTTGATCMTGGCTCAG	Lane et al. 1991 [7]

PCR: Polymerase chain reaction

2 µl of nuclease-free water. Reactions were run under the following conditions: Initial denaturation 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 49.3°C for 30 seconds, 72°C for 1 minute and final extension at 72°C for 3 minutes. 5 µl of each PCR product was run in 1% agarose gel for 2 hrs. The gel was visualized in GelDoc EZ imager (Bio-Rad). After sequencing, the data were analyzed using facilities of NCBI it has been found that the isolate was *Lactobacillus fermentum*.

RESULT AND DISCUSSION

It is known that, vaginal microbiota consists predominantly of Lactobacilli. In the first decade of the present century, phylogenetic analysis of vaginal samples (by 16s ribosomal RNA gene sequencing) have shown that bacterial flora in the vagina is very complex [8]. It has been also pointed out that, vaginal infection is associated with loss of lactobacilli and the introduction and/or overgrowth of some facultative anaerobes [9].

The Lactobacilli strain used in the present experiment was obtained from vaginal sample of a healthy woman the culture filtrate of this bacterium in a minute dose could show good antibacterial property. On Gram-staining the bacterium was identified to be a Gram-positive organism. The catalase test showed it to be catalase negative bacteria. This organism was also tested for its ability to form endospore, but obtained a negative result. On sequencing the partially amplified 16s gene of the organism matched with *L. fermentum*. It could potentially be used for the elaboration of probiotic products for vaginal application. It may be pointed out here that *L. fermentum* Ess1 has been reported to have unique growth inhibition of vulvo vaginal candidiasis pathogen [10]. The strain reported in the present paper may also be tested against candida for antifungal activity.

CONCLUSION

The central idea of the present experiment is selection of a *Lactobacillus* strain which may be used for isolation of treatment of infections caused by pathogenic bacteria. It is known that, *Lactobacillus*

and other related organisms of the group of lactic acid bacteria produce a protein called bacteriocin, which inhibits the growth of many microorganisms. In the present experiment *L. fermentum* isolated from the human vagina could inhibit the growth of *S. aureus* and *E. coli*, one Gram-positive and one Gram-negative organism, respectively. Further work may be done to observe the antimicrobial activity of this strain of *Lactobacillus* against pathogenic *E. coli* and *Candida* sp.

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