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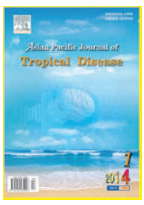

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Antimicrobial activity of *Lactobacillus* against microbial flora of cervicovaginal infections

Subramanyam Dasari[1], Raju Naidu Devanaboyaina Shouri[2], Rajendra Wudayagiri[3], Lokanatha Valluru[1*]

[1]*Department of Biotechnology, Dravidian University, Kuppam, A.P, India*

[2]*Department of Radiation Oncology, Government General Hospital, Guntur, A.P, India*

[3]*Department of Zoology, Sri Venkateswara University, Tirupati, A.P, India*

PEER REVIEW

Peer reviewer

Dr. G. Narasimha, Applied Microbiology laboratory, Department of Virology, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.
Tel: 9885964718
E-mail: gnsimha123@rediffmail.com

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 probiotc 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

*Corresponding author: Lokanatha Valluru, Department of Biotechnology,Dravidian University,Kuppam-517 426, A.P, India.
Tel: +919652840923
Fax: +91-08570278230
E-mail: lokanathav@yahoo.co.in
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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 (H_2O_2) 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 H_2O_2 by exposure of organisms grown anaerobically on agar containing horseradish peroxidises and tetramethyl benzidine (TMB) agar[4]. Colonies of H_2O_2-producing organisms form a blue pigment as horseradish peroxidases oxidize TMB in the presence of bacterial derived H_2O_2.

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