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List of Publications

Research Publications

1. **Das, S., Konwar, B.K.** Prophylactic application of vaginal lactic acid bacteria against urogenital pathogens and its prospective use in sanitary suppositories. *Int Microbiol* (2023). https://doi.org/10.1007/s10123-023-00376-8.
2. **Das, S., Konwar, B.K.** Influence of connatural factors in shaping vaginal microflora and ensuring its health. *Arch Gynecol Obstet* (2023). https://doi.org/10.1007/s00404-023-07200-8.
3. **Das, S., Konwar, B.K.** Inhibiting pathogenicity of vaginal Candida albicans by lactic acid bacteria and MS analysis of their extracellular compounds. APMIS. (2024) https://doi.org/10.1111/apm.13365

Conferences

1. **Oral Presentation, Das, S., Konwar, B.K.** (26th to 28th September, 2019), Role of Microbes in Reproductive Health and Hygiene of Women, *National seminar on application of nanotechnology and biotechnology in daily life*, Sibsagar College.
2. **Poster Presentation, Das, S., Konwar, B.K.** (13th and 14th November, 2019), Vaginal Health and Hygiene of Women, *National Seminar on Current Research in Drug Delivery and Development*, Dibrugarh University.
3. **Poster Presentation, Das, S., Konwar, B.K.** (24th and 25th February2022), Candida an opportunistic pathogen in uterine system, *National Seminar on Advances in Basic and Translational Research in Biology (ABTRiB)*, Tezpur University.
4. **Oral Presentation, Das, S., Konwar, B.K.** (27th February to 1st March, 2022), Bacteriostatic effect of isolated probiotic LAB on potential aerobic pathogens from vaginal swabs, *National Science Day Seminar on Biology is Fascinating*, Tezpur University.

APPENDIX

Tezpur University Ethics Committee
Tezpur: 784028 : Assam

Communication of Decision of Tezpur University Ethics Committee (TUEC)

IEC No: DoRD/TUEC/PROP/2022/02-R2

Protocol title: Isolation of culturable microbes from vaginal mouth and canal (5-8 cm depth)
Principal Investigator: Prof. Bolin Kumar Konwar
Name & Address of Institution: Tezpur University, Tezpur, Assam 784028
[ ] New review   [√] Revised review   [ ] Expedited review
Date of review (D/M/Y): 16-02-2023 Date of previous review, if revised application: 29-09-2022
Decision of the IEC/IRB: [√] Recommended   [ ] Recommended with suggestions [ ] Revision   [ ] Rejected
Suggestions/Reasons/Remarks: The proposal is recommended for approval.
Recommended for a period of: One (01) year with provision of extension subjected to submission of satisfactory report

Please note

-Inform TUEC immediately in case of any adverse events and serious adverse events
-Inform TUEC in case of any change of study procedure, site and investigator
-This permission is only for period mentioned above. Annual report to be submitted to TUEC
-Members of TUEC have right to monitor the trial with prior intimation

Date: 21/03/2023

Signature of Chairperson (with seal)

TUEC

Chairperson
Tezpur University Ethics Committee

TEZPUR UNIVERSITY
(A Central University)
Department of Molecular Biology and Biotechnology
NAPAAM, TEZPUR-784028, ASSAM, INDIA

Prof. B. K. Konwar
Professor, Dept. of MBBT

Ph. 03712-267007/8/9 Ext. 5402 (O)
Fax:03712-267005/267006(O)
E-mail: bkkon@tezu.ernet.in

Memo No. MBBT/ Rsc/59,

Date: 27.09.2021

To,
The Head
SAIF, IIT Bombay.

Sub: HR-LCMS FACILITY (Instrument name) Analysis of Samples at SAIF IIT Bombay.

Dear Sir,

Ms. Shreaya Das (Name of the user) PhD Scholar (Designation) studying/employed at our Institute under the guidance of Prof. B.K Konwar (Guide Name) would like to use the Instrument Facility at SAIF IIT Bombay.

We have 3 (three) (No. of Samples) for HR-LCMS QTOF (name of instrument). The Material Safety Data Sheet (MSDS) Payment proof are attached herewith for the said analysis.

Further we state that if any of the analysis data / results received from SAIF IIT Bombay is used in any Publication or Thesis, we agree to acknowledge SAIF, IIT Bombay in our Publication and Thesis. We shall submit a soft copy of the same to office.saif@iitb.ac.in for information.

The GSTIN Registration No. of our Institute is 18AAAJT2664F1Z1

Thanking You,

Signature : 27/09/2021
Name : B.K Konwar
Designation : Professor
Department : MBBT

Professor,
Dept. of Molecular Biology & Biotechnology
Tezpur University
Napaam, Tezpur- 784028

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RESEARCH

Prophylactic application of vaginal lactic acid bacteria against urogenital pathogens and its prospective use in sanitary suppositories

Shreaya Das[1] · Bolin Kumar Konwar[1]

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Abstract

Beneficial and pathogenic microbes coexist in the vaginal canal, where a diminishing population of lactic acid bacteria may cause recurring urogenital infections. Probiotic bacteria *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus vaginalis*, and pathogenic microbes *Enterococcus faecalis*, *Enterobacter cloacae*, *Shigella* sp., *Staphylococcus epidermidis*, and *Escherichia fergusonii* were isolated from vaginal swabs. *Lactobacillus* sp. and their probiotic culture free supernatant (PCFS) inhibited the growth of the above-mentioned urogenital pathogens. *L. crispatus* produced both lactic acid and hydrogen peroxide, exhibiting the best antimicrobial potential against the studied pathogens. Lyophilized *L. crispatus* had a shelf life of 12 months and the lyophilized PCFS also retained its antibacterial property with a minimum inhibition concentration of 1 μg/μL. Carboxy-methyl cellulose-alginate, a green alternative to super-absorbent polymers, was encapsulated with *L. crispatus* cells. The probiotic in its encapsulated state retained its viability for 21 days, and the bead showed 30% solvent absorptive capacity. PCFS-laced non-woven fabric displayed antibacterial property with no change in its physicochemical properties. These probiotic and postbiotic formulations have excellent prophylactic potential for urogenital infections. Such formulations can be exploited as additives in sanitary suppositories to enhance vaginal health.

Keywords Lactic acid bacteria · Probiotic · Postbiotics · Urogenital pathogen · Non-woven Fabric

Introduction

Microbiota encompasses a combined population of microorganisms inhabiting a particular niche. The vaginal canal (VC) plays host to a varied range of microorganisms post puberty (Larsen and Monif 2001). VC (3–5") is the outermost portion of the female reproductive tract, aiding in menstruation, parturition, and intercourse. The commonly found vaginal microbes are *Diphtheroid*, *Lactobacillus*, *Staphylococcus*, *Streptococcus*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, and *Pseudomonas* (Corbishley 1977; Chee et al. 2020). Some of these species are beneficial, some maintain commensalism, and few are pathogenic in nature (Ravel et al. 2011). On the onset of puberty, the estrogen hormone stimulates the vaginal epithelium to produce glycogen that is digested by α-amylase enzyme to form maltose (Gregoire et al. 1971); this sugar is utilized as a food source by microbes throughout the reproductive age (Sumawong et al. 1962; Nunn et al. 2020). The microflora shuffles throughout a menstrual cycle depending on the hormonal balance, self-hygiene, and sexual behavior until menopause (Taddei et al. 2018). The microflora post menopause shows similarity to the microbial flora prior to puberty (Larsen and Galask 1982; Das et al. 2023).

Beneficial microbes inhabit VC, and studies have shown their mutualistic relationship with the host. These microorganisms render protection to VC from pathogens throughout the reproductive age and are considered beneficial and termed probiotic (Pino et al. 2019). The menstrual cycle not only initiates the process of becoming reproductively active but also enhances the protection of VC by arranging a suitable environment for beneficial microbes to grow. Studies on the vaginal microflora of reproductive women at a genetic level have revealed various community state types (CST). *Lactobacillus* sp. fills up 70% of the microbial population in CST 1, 2, and 5 being a consistent candidate for a healthy vagina (Smith and Ravel 2017).

✉ Bolin Kumar Konwar
bkkon@tezu.ernet.in

1 Department of MBBT, Tezpur University, Napaam-784028 Assam, India

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REVIEW

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Influence of connatural factors in shaping vaginal microflora and ensuring its health

Shreaya Das[1] · Bolin K. Konwar[1]

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Abstract

Vaginal canal (VC) is exposed to the external environment affected by habitual factors like hygiene and sexual behaviour as well as physiological factors like puberty, menstrual cycle, pregnancy, child birth and menopause. Healthy VC harbours beneficial microflora supported by vaginal epithelium and cervical fluid. Connatural antimicrobial peptide (AMPs) of female reproductive tract (FRT) conjunctly with these beneficial microbes provide protection from a large number of infectious diseases. Such infections may either be caused by native microbes of the VC or transitory microbes like bacteria or virus which are not a part of VC microflora. This review highlight's the role of hormones, enzymes, innate immunological factors, epithelial cells and vaginal mucus that support beneficial microbes over infectious ones thus, helping to maintain homeostasis in VC and further protect the FRT. We also discuss the prospective use of vaginal probiotics and AMPs against pathogens which can serve as a potential cure for vaginal infections.

Keywords Female reproductive tract (FRT) · Vaginal canal (VC) · Homeostasis · Antimicrobial peptides/proteins (AMP) · Probiotic · Lactic acid bacteria (LAB)

Introduction

The microenvironment of vagina post-puberty shuffles due to hormonal changes, diet, sexual and general habits [1, 2]. The FRT being an open system is highly prone to microbial attack. Two innate immunological factors confer protection in different ways:

Epithelial cells and mucus act as the first line of physical defence; these cells chemically confer protection by producing AMPs, chemokines and cytokines through pattern recognition receptors (PPRs) [3]. Toll-like receptors (TLRs), on the vaginal epithelial cells and neutrophils in mucus, recognise pathogenic molecules and release AMPs that destroy the pathogens through ionic disbalance by creating pores on pathogen cell membrane. The FRT has ten types of TLRs that can recognise virus, bacteria, lipopolysaccharide (LPS), flagellins, heat shock proteins, ss RNA and DNA. VC also has few immune cells; the highest ~20% population is T cells followed by dendritic cells, macrophages, innate lymphoid cells and granulocytes [4, 5]. On recognition of transitory pathogens, cytokines and chemokines activate secondary immunity.

VC supports the growth of native microbiota belonging from *Lactobacillaceae* family [6]. Transitory microbes belonging to *Mycoplasma, Chlamydiales and Neisseriaceae* family successfully invade and proliferate when a woman is immune compromised [7, 8]. On contrary, few native microbes like *Bacteroides* are inherent to the VC but are present in miniscule density. When the growth of native beneficial *Lactobacilli* sp is inhibited, the miniscule microbiota overgrows and tend to show virulence to the host by activating immune cells [9]. The vaginal environment is maintained by a subtle balance of large number of aerobic and anaerobic bacteria and fungi. Common vaginal aerobic Gram-positive bacteria are—*Diphtheroid, Lactobacilli, Staphylococcus, and Streptococcus*; and Gram-negative bacteria are—*Escherichia, Klebsiella, Enterobacter, Proteus, and Pseudomonas*. The anaerobic bacteria found in VC are *Bacteroides, Bifidobacterium, Clostridium, Eubacterium, Fusobacterium, Peptococcus, Peptostreptococcus, and Propionibacterium* [10, 11]. The vaginal flora has 90% prevalence of acid tolerant bacteria, out of which 60% is *Lactobacillus* sp., rest

✉ Shreaya Das
shreaya.das10@gmail.com; shreaya@tezu.ernet.in

Bolin K. Konwar
bkkon@tezu.ernet.in

1 Department of MBBT, Tezpur University, Napaam, Assam 784028, India

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Inhibiting pathogenicity of vaginal *Candida albicans* by lactic acid bacteria and MS analysis of their extracellular compounds

SHREAYA DAS and BOLIN KUMAR KONWAR

Department of MBBT, Tezpur University, Napaam, Assam, India

Das S, Konwar BK. Inhibiting pathogenicity of vaginal *Candida albicans* by lactic acid bacteria and MS analysis of their extracellular compounds. APMIS. 2024.

Maintaining healthy vaginal microflora post-puberty is critical. In this study we explore the potential of vaginal lactic acid bacteria (LAB) and their extracellular metabolites against the pathogenicity of *Candida albicans*. The probiotic culture free supernatant (PCFS) from *Lactobacillus crispatus*, *L. gasseri*, and *L. vaginalis* exhibit an inhibitory effect on budding, hyphae, and biofilm formation of *C. albicans*. LGPCFS manifested the best potential among the LAB PCFS, inhibiting budding for 24 h and restricting hyphae formation post-stimulation. LGPCFS also pre-eminently inhibited biofilm formation. Furthermore, *L. gasseri* itself grew under RPMI 1640 stimulation suppressing the biofilm formation of *C. albicans*. The PCFS from the LAB downregulated the hyphal genes of *C. albicans*, inhibiting the yeast transformation to fungi. Hyphal cell wall proteins HWP1, ALS3, ECE1, and HYR1 and transcription factors BCR1 and CPH1 were downregulated by the metabolites from LAB. Finally, the extracellular metabolome of the LAB was studied by LC–MS/MS analysis. *L.gasseri* produced the highest antifungal compounds and antibiotics, supporting its best activity against *C. albicans*. Vaginal LAB and their extracellular metabolites perpetuate *C. albicans* at an avirulent state. The metabolites produced by these LAB *in vitro* have been identified, and can be further exploited as a preventive measure against vaginal candidiasis.

Key words: Lactic acid bacteria; candidiasis; probiotics; extracellular metabolites.

Bolin Kumar Konwar, Department of MBBT, Tezpur University, Napaam-784028, Assam, India. e-mail: bkkon@tezu.ernet.in

Human microbiota is established post-birth and influenced by various factors such as mode of birth, fetal care, and diet. Mycobiome is a broad class under the human microbiota comprising of fungi that colonize humans [1], and has been investigated inadequately due to their low population in the human body [2]. The culture-independent studies from vaginal canal (VC) of healthy women have reported several fungal species from Ascomycota, Basidiomycota, and Oomycota phylum. Multiple studies has reported the presence of fungal species like *Candida*, *Aspergillus*, *Saccharomyces*, *Rhinocladiella*, *Dothideomycetes*, and *Cryptococcusn* from VC of healthy adults [3]. *Candida albicans* of ascomycota family is the most prevalent fungal species found in the VC post-puberty. Other non-albican species found in VC are *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, and *C. psuedotropicalis* [4]. Overgrowth of *Candida sp.* in VC has reported to cause inflammation, redness, itching, and rash in the infected area leading to heavy discomfort [5]. *Candida* sp transits from yeast to hyphal form, initiates biofilm formation and adhere as well as invade the vaginal epithelial cells causing candidiasis [6]. 80–90% of candidiasis cases is caused by *Candida albicans*, whereas the remaining 10–20% of cases are caused by non-albican species [5]. 75% of women population are affected by vulvovaginal candidiasis (VVC) once prior to menopause and 15% suffers from recurrent VVC, making it the second most prominent vaginal infection after bacterial vaginosis (BV) [7].

Recent studies on vaginal microbiome of healthy women using modern sequencing techniques illustrate that LAB accounts up to 70% of the total microbial population in the VC [8, 9]. The VC

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Chapter 1: Introduction

1.1. Female Reproductive System

Female reproductive tract (FRT) in human comprises of the uterus, ovaries, fallopian tubes, cervix, and vagina. At the fourth week of gestation the pelvic region of an embryo has a wolffian duct, a mullerian duct, the metanephros, cloacae and gonadal ridge. Prior to fifth week of gestation the gonads of an embryo are indistinguishable. Post 5th week the primordial germ cells migrate from the endodermal lining of yolk sac through the dorsal mesentery of hindgut to the gonadal ridge. The development of female or male genitalia is determined by the presence of the Y chromosome. In the absence of the expression of the SRY gene, the primitive sex cord degenerates, leading to the development of the female ovaries. The epithelium cells in the ovary divides to form the cortical cord. The cortical cord matures and breaks eventually to form primordial cluster with oogonium covered by a layer of epithelium cells. In absence of testosterone hormone, the wolffian duct degenerates, whereas the mullerian duct forms in absence of anti-mullerian hormones. The duct later differentiates to form the uterus, cervix, and fallopian tubes [1, 2]. Health status of the FRT influences intermittent physiological processes like fertilization, implantation, foetal growth, and parturition. Additionally, the health of tract also has an impact on the sexual health and other physiological processes like menstruation and regular mucus outflow [3].

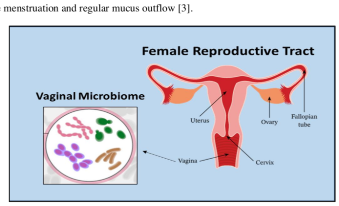

Fig1.1.: The female reproductive tract

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Role of microorganism in reproductive health and hygiene of women and the potential use of Lactobacillus

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