Microbiology

Lactobacilli Bacteriocins: Promising Natural Therapeutic Agents

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Lactobacilli elaborate and secrete a variety of products used for their own specific protection; among these products are bacteriocins. Lactobacilli bacteriocins are peptides synthesized by ribosomes that possess strong antimicrobial activity. Consequently, these peptides epitomize an important defense mechanism against pathogenic bacteria. Multiple and diverse bacteriocins have been discovered and have been extensively studied. This short review will discuss the potential use of bacteriocins as antimicrobials, adjuvants to antibiotics, spermicides, and as antineoplastic agents for the treatment of bacterial vaginosis (BV).

Lactobacilli | Bacteriocins | Bacterial Vaginosis

Introduction

Most microorganisms including Lactobacilli elaborate and secrete a variety of products used for their own specific protection; among these products are antibiotics, bacteriocins, lytic agents, biofilm, and protein exotoxins.

Lactobacilli bacteriocins are peptides synthesized by ribosomes that possess strong antimicrobial activity. Consequently, these peptides epitomize an important defense mechanism against pathogenic bacteria.

Multiple and diverse bacteriocins have been discovered. These bacteriocins have been extensively studied; particularly their properties, their interactions with other microorganisms, their dealings with human sperm, and their interphases with epithelial cells present in the vaginal milieu.

This review will discuss the potential use of bacteriocins as antimicrobials, adjuvants to antibiotics, spermicides, and as antineoplastic agents for the treatment of bacterial vaginosis (BV).

Bacteriocins Classification

Lactobacilli secreted bacteriocins are a very efficient mechanism to control competing pathogenic micro-organisms and to maintain a stable vaginal milieu. [1, 2]

Bacteriocins, along with biofilms are the most promising alternatives to the current use of antibiotics. Bacteriocins have several advantages over antibiotics. Their mode of action is different and since they are synthesized in the ribosome, they display host cell immunity. [3]

Lactobacilli Bacteriocins' activity can be either related to inhibiting only those bacterial strains closely related to them or to inhibiting a different group of Gram-positive bacteria.

Bacteriocins significantly vary in their primary structures; almost all are cationic and very often are amphiphilic.

The majority of bacteriocins may be classified into one of three groups: (1) those with a high content of one (or two) amino acid(s) -- often proline; (2) those with intramolecular disulfide bonds -- often stabilizing a beta-sheet structure; and (3) those with amphiphilic regions when assuming an alpha-helical structure. [4]

Bacteriocins Modus Operandi

Lactobacilli mainly produce three classes of bacteriocins: (1) Lantibiotics (nisin being the most prominent); (2) small heat-stable non-lanthionine containing the membrane-active peptides: Lactococcus lactococcins, Lactobacillus sakacin A, and plantaricin A-bacteriocins; and (3) large heat-labile proteins. [5, 6]

Bacteriocins are considered to be small membrane-active compounds. They deploy their antimicrobial activity by producing pores in the target cytoplasmatic cell membranes causing membrane permeabilization. When the cell membrane becomes permeable there is a depletion of the amino acids and ions responsible for transmembrane potential and therefore a pH gradient disruption. [5, 6, 7, 4] For instance, Lactocin 160 disturbs the cellular membrane by "Deltapsi dissipation," inducing ATP efflux from pore formation**.** [8]

It is also well known that bacteriocins do not exert any direct cytotoxic or hematolytic activity. This fact was demonstrated using human vaginal epithelial HeLa cells and red blood cells in a rabbit model in invitro and vivo, in regards to nisin a bacteriocin produced by Lactococcus lactis. [9] Lactobacilli are not directly lethal to pathogens either. Nevertheless, by means of acidic growth inhibition stress induction in the pathogen cell membrane and vaginal milieu modification Make for a difficult environment for pathogenic bacteria to thrive in. [10]

Another bacteriocin, Lactosporin, seems to exert its antimicrobial activity by selectively dissipating the ΔpH and/or by producing leakage of ions from the targeted cells. It has also been shown to be safe and noncytotoxic when used in vaginal applications. [11]

In general, Lactobacilli are resistant to several antibiotics including metronidazole, aminoglycosides, and ciprofloxacin. Almost all of L. acidophilus are sensitive to penicillin and vancomycin. However L. rhamnosus and L. casei are resistant to metronidazole and vancomycin. [12]

Therapeutic Use of Bacteriocins

Bacteriocins do not cause vaginal redness or irritation, therefore they are appropriate for therapeutic use in humans. As a matter of fact, bacteriocins have been shown to not produce irritation or toxicity both in in vitro animal or human vaginal tissue models. [13]

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At present there are no publications regarding the approved use of bacteriocins for the treatment of BV. This promising investigation area has been ignored by researchers who are more focused on the study of probiotics instead of in the isolation, purification, and testing of bacteriocins.

1. Bacteriocins as Spermicides

There are few bacteriocins that have demonstrated spermicidal activity. Nisin is the only well-studied bacteriocin commercially available. Nisin is currently employed in food conservation and has been FDA-approved with the status: "generally recognized as safe." [3]

Affinity studies of nisin done by Reddy et al showed that spermatozoa were more susceptible to nisin than red blood cells and vaginal epithelial cells were. They suggested that nisin's spermicidal and antimicrobial properties could be used as a safe vaginal contraceptive regarding STDs treatment. [9]

Subtilosin has been shown to be a general spermicidal agent since it stopped human spermatozoa motility and forward progression in a dose-dependent fashion. [14]

2. Bacteriocins as Antibacterials

Bacteriocins have been shown to be effective against bacteria. Numerous Gram-positive and Gram-negative bacteria are susceptible to bacteriocin HV219 produced by Lactococcus lactis. [15] Vaginal L. reuteri produce a bacteriocin that has an antibacterial effect against methicillin-resistant Staphylococcus aureus. [16] Lactobacilli byproducts inhibit the development and virulence of E. coli. [10]

3. Bacteriocins and Bacterial Vaginosis

Bacterial vaginosis (BV) is a frequent and recurrent vaginal infection. BV has been associated with an increased incidence of preterm birth and sexually transmitted infections. A shift of the vaginal ecosystem milieu from typical Lactobacillus to multispecies is recognized as the most plausible cause. The most common species involved include G. vaginalis and Prevotella.

Numerous bacteria associated with BV are reported to develop multidrug resistance, therefore there is an increased need to developing new and alternative therapies.

Bacteriocins have been studied as prospective therapeutic alternatives to antibiotics against BV. Several of these natural antimicrobials demonstrated an inhibitory effect against BV-associated bacteria grown planktonically. Moreover, they do not usually affect the lactobacilli microflora.

A bacteriocin produced by L. acidophilus 160 inhibited the growth of nine isolates of G. vaginalis in a study performed by Aroutcheva and collaborators in 2001. [17]

In another study, Kaewsrichan et al selected two strains of L. crispatus (15L08 and 21L07) and one strain of L. jensenii (5L08) among 100 Lactobacilli obtained from the vagina of healthy premenopausal women. The researchers isolated their strains based on the properties of the Lactobacilli that were relevant to the vaginal mucosal colonization, the H2O2 production, and/or the bacteriocin-like compound. Their three isolated strains self-aggregated and adhered to vaginal epithelial cells. They displaced G. vaginalis and C. albicans. Moreover, L. jensenii 5L08A synthesized a bacteriocin which was bactericidal for G. vaginalis, C. albicans, and E. coli. They concluded that bacteriocins and H2O2 production were both essential for Lactobacilli to control pathogenic bacteria. [18]

In addition, Sutyak and his team evaluated the potential interaction of antimicrobial combinations against G. vaginalis and vaginal lactobacilli. They tried lauramidearginine ethyl ester (LAE), ε-poly-L-lysine, clindamycin phosphate, metronidazole, and the bacteriocin subtilosin A. They reported that Clindamycin,subtilosin (CS), metronidazole, and CS worked synergistically against G. vaginalis.

Additionally, clindamycin and polylysine (CP) worked antagonistically against L. acidophilus. Together clindamycin plus metronidazole (CM) were found to be antagonistically against L. vaginalis. The combinations of CP, clindamycin, LAE, CS, LAE, and CP worked antagonistically against G. vaginalis. [19]

Cavera et al also reported that subtilosin demonstrated antimicrobial activity against G. vaginalis while not being harmful to the lactobacilli present in healthy vaginal milieu.

Additionally the bacteriocin was safe to human cells based on an ectocervical tissue model. [14]

Lactocin 160, a bacteriocin produced by healthy vaginal lactobacilli specifically inhibits G. vaginalis and Prevotella bivia without affecting the healthy microflora. [20]

Fermenticin HV6b is a bacteriocin produced by L. fermentum HV6b MTCC 10770 that was isolated from human vaginal milieu. It has demonstrated inhibitory growth against several human pathogen bacteria associated with BV, including Bacteroides, G. vaginalis, Mobiluncus, Staphylococci, and Streptococci. It similarly possesses human sperm immobilization and spermicidal characteristics when tested against sperm, which make it a good candidate for BV treatment and for contraception. Even more interesting was the finding regarding in vitro studies in four different tissue models that fermenticin HV6b induces apoptosis in cancerous cells indicative of its possible use in cancer therapy. [21]

The effects of glycerol monolaurate (GML) on Lactobacillus, Candida, and G. vaginalis human vaginal microflora were studied by Strandberg et al. They reported that 6 months of GML vaginal treatment did not modify the number of lactobacilli in non-human primates. They also investigated the effects of GML on Lactobacilli, Candida, and G. vaginalis in symptomatic women. They concluded that GML was antimicrobial for Candida and G. vaginalis in vitro. Vaginal gels containing GML did not affect Lactobacilli but significantly reduced Candida and G. vaginalis. [19, 22]

4. Bacteriocins and Biofilms

Bacteriocins have been proposed as a valuable therapeutic alternative in the eradication of BV-biofilms.

Turovskiy reported that the bacteriocins: subtilosin, ε-poly-L-lysine, and lauramide arginine ethyl ester (LAE) selectively inhibit the growth of G. vaginalis.

Additionally, Turovskiy et al studied the susceptibility of G. vaginalis biofilms to bacteriocins. This group of researchers established in vitro that LAE possessed the strongest bactericidal effect against G. vaginalis biofilms. They proposed LAE as a potential natural agent capable of disrupting the very strong BV-biofilm. [23] Likewise, Algburi et al indicated that subtilosin and LAE worked synergistically with the antibiotics clindamycin and metronidazole to inhibit the biofilm produced by G. vaginalis without compromising any of the vaginal lactobacilli. These investigators also proposed a new strategy against the bacterial resistance of BV-associated pathogens biofilm. They stated that by acting synergistically with combined conventional antibiotics and natural bacteriocins the BV biofilms could be disrupted. [24]

These facts make evident that antibiotics, bacteriocins, and biofilm may synergistically be working to improve the cure rates of BV, especially in cases of recurrence or antimicrobial resistance. [25, 26]

Conclusion

The development of bacteriocins as adjuvants or alternative treatment to current antibiotics should be strongly pursued. Several bacteriocins have demonstrated advantages, their mode of action is different than antibiotics; they are host cell immune, and they are safe to the vaginal mucosa and vaginal milieu. Additionally they are potent agents against BV and other pathogen bacteria. Moreover, they should be explored for their spermicidal and antineoplastic capabilities.

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