NATURAL ANTIMICROBIALS AND THEIR ROLE IN VAGINAL HEALTH: A SHORT REVIEW

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ABSTRACT: Lactobacillus species maintain the vaginal ecosystem in a healthy condition by production of antimicrobial substances. Depletion of lactobacilli in the vagina results in bacterial vaginosis (BV), where the normal flora is replaced by several bacterial pathogens, usually Gardnerella vaginalis and obligate anaerobes. BV may cause complications such as premature labor, low birth weight and increased risk of HIV acquisition. The currently recommended antibiotic treatments for BV are not always effective and often lead to reoccurrence of the infection. In many cases, this is due to the antibiotic-resistant forms of the pathogens. Therefore, there is an interest in the development of treatments using antimicrobials derived primarily from Lactobacillus spp., such as ribosomally produced antimicrobial peptides (bacteriocins) and lactic acid. These substances effectively inhibit pathogenic bacteria, are safe and do not pose any threat to healthy vaginal Lactobacillus spp. It may be possible to find an effective treatment against BV while reducing the infection’s reoccurrence and the treatment-related complications through hurdle technology. This would be achieved by combining antimicrobials produced by Lactobacillus spp. with different natural antimicrobials obtained from plants or other non-pathogenic organisms.

KEY WORDS: Bacterial vaginosis, Bacteriocins, Lactic acid, Lactobacillus, Natural antimicrobials

Healthy vaginal microflora

Lactobacillus species are considered a natural part of the vaginal tract and beneficiary to its healthy status. They effectively protect the vagina against pathogens by producing antimicrobials such as hydrogen peroxide, bacteriocins, and weak organic acids like acetic and lactic acid (Aroutcheva et al., 2001a; Reid and Burton, 2002 and Valore et al., 2002).

Healthy vaginal Lactobacilli are active against several organisms including Gardnerella vaginalis and Escherichia spp. (McGroarty and Reid, 1988; McLean and McGroarty, 1996; McLean and Rosenstein, 2000 and Aslim and Kilic, 2006). A study also showed inhibition of G. vaginalis and Prevotella bivia in infected cervical epithelial HeLa cultured cells (Atassi et al., 2006).

Another recent study of 22 vaginal Lactobacillus strains isolated from healthy human subjects showed that 73% of the microorganisms were active against G. vaginalis (Aroutcheva et al., 2001a). A strain of L. iners, the most commonly isolated vaginal Lactobacillus spp. in healthy women, was found to disrupt Gardnerella biofilm surface area, density and depth (Saunders et al., 2007). Lactobacillus spp. derived from the GI tract have been reported as producing bacteriocins inhibiting anaerobic microflora, Enterobacteriaceae, and Gram-positive cocci but not other lactobacilli (Silva et al., 1987; Kawai et al., 1994 and Itoh et al., 1995). Also, bacteriocins produced by human vaginal Lactobacillus spp. inhibit a wide range of Gram-positive and, under certain conditions, Gram-negative bacteria (Aroutcheva et al., 2001c).

Lactobacillus spp. ferment glycogen secreted by vaginal epithelial cells into lactic acid, and colonization by these microorganisms correlates to the low pH in the vagina (Boskey et al., 2001 and Rönnqvist et al., 2006). The acidic environment protects the vagina against over-growth of pathogens and against HIV (Martin et al., 1985 and Hill and Anderson, 1992). Low vaginal pH is also beneficial for other antimicrobials; H₂O₂ is stable in this condition (Fontaine and Taylor-Robinson, 1990), and bacteriocins are highly active (Skarin and Sylvan, 1986). An increase of the vaginal pH leads to the decrease of the lactobacilli-associated antimicrobial activity (Pybus and Onderdonk, 1999 and Valore et al., 2006).

Almost 90% of Lactobacillus spp. found in the healthy vaginal microflora produce H₂O₂ and a positive correlation has been made between women colonized by H₂O₂ producing Lactobacillus spp. and an absence of BV (Hillier et al., 1993; Hawes et al., 1996;
Wilks et al., 2004 and Mijac et al., 2006). Only 4 to 26.5% of the \textit{Lactobacilli} spp. isolated from women with BV produced $\text{H}_2\text{O}_2$ (Hillier et al., 1992 and Mijac et al., 2006).

The amount of $\text{H}_2\text{O}_2$ produced in the vaginal fluid of women with a healthy vaginal microflora was estimated to 1.0-15.5 g/ml (Al-Mushrif and Jones, 1998 and Strus et al., 2006). $\text{H}_2\text{O}_2$ seems to add to the antimicrobial defense of the vaginal environment, but is probably not a crucial factor (Strus et al., 2006). However, it creates an unacceptable environment for growth of anaerobes and a more difficult environment for HIV transmission (Klebanoff and Coombs, 1991a).

In addition to the production of antimicrobial compounds, \textit{Lactobacillus} spp. also possess other desired features that are common in many probiotics, including the ability to adhere to vaginal cells and mucus to make a barrier that excludes/reduces pathogens’ adherence, persistence and multiplication in the vaginal environment (Boris et al., 1988).

Vaginal probiotic \textit{Lactobacilli} are safe, non-invasive, and lack carcinogenicity. However, most of them are sensitive to common spermicides (Reid, 1999 and Boris et al., 2000). Benefits of \textit{Lactobacillus} and antimicrobials produced by these species for prevention and/or treatment of BV are the subjects of the current review: the authors decided to focus on natural antimicrobials to bring the scientific and medical communities’ attention to the use of natural antimicrobials and their combinations in addressing women’s health.

**Bacterial vaginosis**

The vaginal tract may be subject to infections of different nature. They may be viral, such as herpes and HIV, protozoan such as trichomoniasis, eukaryotic yeast infections or bacterial infections such as BV where the normal flora is replaced by several pathogenic bacteria, of which \textit{G. vaginalis} is the most prevalent. As this review focuses on control of bacterial communities, this review emphasizes natural treatments for BV.

The microecological niche of the vagina is a dynamic system and the pathogenic bacteria that are often found in small numbers in healthy women can far outgrow \textit{Lactobacillus} species (Hill, 1993). This condition of replacement of \textit{Lactobacillus} spp. by pathogenic species is referred to as bacterial vaginosis (BV). Lactobacilli are mostly replaced by \textit{G. vaginalis} as well as anaerobes such as \textit{Prevotella}, \textit{Bacteriroides} species, \textit{Peptostreptococcus}, \textit{Mobiluncus}, genital \textit{Mycoplasma hominis} and \textit{Ureaplasma} spp. (Hillier et al., 1995). BV is associated with high pH, a decrease in antimicrobial activity of the vaginal fluid compared to healthy women, and local impairment of the multiple innate immune pathways (Pybus and Onderdonk, 1999 and Valore et al., 2006). BV is not life threatening, but in pregnancy it is associated with premature labor, premature rupture of membranes and low birth weight, leading to high prenatal mortality. However, it is not known what mechanism(s) causes these adverse pregnancy outcomes (Hillier et al., 1995; Flynn et al., 1999; Leitch et al., 2003; Hay, 2004 and Schwebeke and Desmond, 2005). As BV is found in approximately 16% of pregnant women in industrial countries it poses a serious problem (Hay et al., 1994; Hillier et al., 1995; Svare et al., 2006; Wrong diagnosis, 2008).

Toxins from BV-associated microorganisms (such as lipopolysaccharides) may cross the placenta and cause brain injuries in fetuses. The toxins may cause permanent neurological brain damages such as cerebral palsy, a risk of developing Parkinson’s disease and schizophrenia (Grether and Nelson, 2000; Urakubo et al., 2001 and Ling et al., 2004). Studies have also associated BV with a higher risk of acquisition of HIV infection (Cohen et al., 1995; Sewankambo et al., 1997; Taha et al., 1998; Martin et al., 1999; Hashemi et al., 1999; Hashemi et al., 2000 and Sha et al., 2005), and herpes simplex virus type 2 infections (Cherpes et al., 2003). BV is usually treated with antibiotics, e.g. clindamycin or metronidazole, either taken orally or applied topically (WebMD 2006). However, antibiotics do not eradicate all vaginal pathogenic bacteria (Ferris et al., 1995 and Boris et al., 1997). This treatment of BV is only effective in 60% of cases, with the high reoccurrence rate of 30-40% (Paavonen et al., 2000; Eriksson et al., 2005 and Larsson and Forsum, 2005). Moreover, the healthy vaginal microflora is disturbed by antibiotics and the risk of developing antimicrobial drug resistance increases dramatically with overall increased use of antimicrobial (feminine hygiene and treatment) preparations (Uchare et al., 2006). \textit{In vitro} studies have shown that clindamycin and metronidazole inhibit \textit{Lactobacillus} spp. at concentrations lower than doses topically applied for treatment (Arouthcheva et al., 2001b and Simoes et al., 2001).

Therefore, there is an interest in developing alternative treatments against BV, such as selective antimicrobials, probiotics and acidification procedures that will inhibit BV pathogenic bacteria without killing healthy \textit{Lactobacillus} spp. Alternative treatments might be safer and more efficient than antibiotics. They would also reduce the risk of the infection’s reoccurrence by promoting healthy \textit{Lactobacillus} spp. growth. This treatment would be especially valuable to women with chronic or recurrent BV associated with reduction of vaginal \textit{Lactobacillus} spp. (Sobel, 1999 and Reid and Bocking, 2003a). Local treatment of BV with no systemic effects would be safer for pregnant women by preventing pre-term labor (Othman et al., 2007). It would also be less expensive and would thus provide a more affordable treatment for women in developing countries.

**PROBIOTICS IN VAGINAL HEALTH**

\textit{Lactobacillus} spp. that are non-pathogenic and produce antimicrobial substances are considered probiotic. This is defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (World Health Organization, 2005).

Probiotic treatment of BV has been put forward as an alternative treatment that could be administered either orally or topically to the vaginal tract in suppositories, tampons or panty liners. Oral administration of probiotics may also improve the long-term effects of treatment of BV with vaginal suppositories containing \textit{L. acidophilus} by balancing the vaginal and intestinal microflora (Delia et al., 2006). The introduced \textit{Lactobacillus} spp. would then colonize the vaginal tract and out-compete vaginal pathogens and
prevent new pathogen invasion. There have been several studies on the treatment of BV with probiotics with mostly positive results, although most studies have been small and often lacking control groups (Reid et al., 2001; Antonio and Hillier, 2003 and Uehara et al., 2006).

In a study of 64 subjects in Canada, BV was treated with *L. fermentum* RC-14 and *L. rhamnosus* GR1 ingested once a day for 60 days without any side effects. BV was cured in 37% of the probiotic-treated women, compared to 13% in the placebo group. However, 30 days after the final administration of probiotics, the *Lactobacillus* spp. counts were the same as those for day zero in the probiotic treated group (Reid et al., 2003b). It has also been shown that some *Lactobacillus* spp. do not colonize the vagina at all during administration (Colodner et al., 2003). However, one small study showed the colonization of introduced *Lactobacillus* spp. up to 12 weeks after the end of administration (Antonio and Hillier, 2003), and another one 7 days after administration (Delia et al., 2006).

Positive results of probiotic treatment were obtained from a placebo controlled study of 424 women, showing 55% satisfaction of treatment of BV with intravaginal capsules containing human-derived *H. peroxide*-producing *L. crispatus* (Marrazzo et al., 2006). Also, a trial in Nigeria, reported a 90% effective 5-day treatment of BV using vaginal capsules of *L. rhamnosus* GR-1/*L. reuteri* RC-14 (Anukam et al., 2006b). However, the authors consider a combined therapy of metronidazole (7 days) followed by probiotics (30 days) to be a more effective treatment of BV in an African environment (Anukam et al., 2006a).

A combined approach for treatment using antibiotics to eradicate BV associated organisms; followed by the introduction of *Lactobacillus* spp. to reconstitute and maintain a healthy vaginal flora, has also been under investigation with various degrees of success (Larsson and Forsum, 2005). In one study women were treated with clindamycin followed by a 5-day treatment using tampons containing a mixture of freeze-dried strains of *L. fermentum, L. casei* var. *rhamnosus* and *L. gasseri* during one menstrual period. There was no improvement in subjects using tampons containing *Lactobacillus* spp. compared to placebo tampons (Eriksson et al., 2005).

In an attempt to introduce *L. plantarum* LB931 to the vagina, panty liners impregnated with the bacteria were used. The strain was isolated from 56% of vaginal samples after 4 months of panty liner use, but reduced to 6% after one month of discontinuing use. Although *L. plantarum* LB931 did not alter the vaginal microflora significantly or have effect on vaginal pH, the number of women lacking *Lactobacillus* spp. completely decreased, in contrast to the placebo group (Rönqvist et al., 2006).

There are a handful of various marketed probiotic products that claim to restore or maintain the vaginal microflora. For example, in Sweden and Norway the tampon Ellen® (RFSU AB, Kista, Sweden) is sold with a mixture of three *Lactobacillus* spp. (Ellen AB, 2006), Vagiforte® (Bioflora, Pretoria, South Africa) is distributed in South Africa with *L. acidophilus, Bifidobacterium bifidum* and *Bifidobacterium longum* in vaginal suppositories (Bioflora, 2006), and in India MYCONIP® (Unisankyo Ltd, Hyderabad, India) vaginal suppositories with Lactic Acid Bacillus (also known as *L. sporogenes*) in the form of spores are on the market (Uni-Sankyo, 2006). Whether these products have any effect is not clear, as there is little or no research data published.

An in vitro study of the commercial probiotic mixture of two *Lactobacillus* strains and one *Streptococcus spp.*, VSL#3® (Sigma-Tau Pharmaceuticals Inc., Gaithersburg, Maryland, USA) has shown that probiotic bacteria can grow in a culture system resembling that of a healthy vaginal environment and suppress the growth of *G. vaginalis* (Onderdonk, 2006).

With clear fluctuations in positive and non-effective treatments between groups of human subjects receiving probiotics, the question remains opened: how effective, reliable and reproducible is the probiotic approach in combating vaginal infections? The answer lies in complexity of the steps/obstacles the probiotic bacteria have to pass through in order to deliver their beneficial effect. The bacteria must first migrate to the surface of the epithelial cells, then adhere to the vaginal cells, successfully colonize them while competing with the pathogens for the environment, and finally, form a viable biofilm. Only if the bacteria succeed in all of these steps could they start producing sufficient amounts of antimicrobials to eradicate pathogenic bacteria. Therefore, there is a big challenge in finding a strain that will colonize the vagina, persist there after the end of treatment and confer continuous probiotic effects to prevent BV. Differences in success/failure rates in each of the processes that may occur in different human subjects, will contribute to possible significant variations in the outcome of the strain replacement approach.

**Natural products of *Lactobacillus* spp. as topical microbicides**

Antimicrobial peptides (bacteriocins) synthesized by *Lactobacillus* spp. and other bacteria are a defense mechanism against competitive microorganisms for the protection of their ecological niche (Diep and Nes, 2002). Their antimicrobial activity is usually due to permeabilization of the cell membrane, which causes an efflux of ATP, amino acids and ions, thereby depleting the transmembrane potential (ΔΨ) and pH gradient (for reviews see: Kleenhammer, 1988; Nissen-Meyer and Nes, 1997 and Sablone et al., 2000) (Oscariz and Pisabarro, 2001). Bacteriocins differ from antibiotics by their mode of action, being ribosomally synthesized and having host cell immunity (Cleveland et al., 2001). Bacteriocins do not have haemolytic or cytotoxic activity. This was shown for nisin, the bacteriocin produced by *Lactococcus lactis*, both in in vivo irritation studies on rats and rabbits and in in vitro research using human vaginal epithelial HeLa cells and red blood cells (Aranha et al., 2004 and Reddy et al., 2004). As bacteriocins do not induce vaginal irritation they are suitable for human use. There is currently no clinical data on the use of antimicrobial peptides for control of BV. In fact, this field of research is still in its infancy, being heavily overlooked by the majority of explorers predominantly focused on the use of probiotic bacteria rather than their isolated, purified and concentrated antimicrobials. The most recent reports indicate that vaginal probiotic-derived antimicrobials cause no irritation or toxicity as tested both in the animal vaginal model and on human vaginal tissue, in vitro (Dover et al., 2007).
So far, nisin is the only bacteriocin that is commercially available and well studied. Nisin is used for food preservation and has an FDA-approved GRAS (generally recognized as safe) status for certain applications (Cleveland et al., 2001). Nisin is also used in the product Wipe Out® Dairy Wipes (Immucell, USA), marketed as an antibacterial wipe for the udder prior to milking. The company also intends to further develop this product by making antibacterial wipes with nisin for workplaces, consumers and military personnel (Immucell, 2007). Due to its spermicidal activity, nisin has also been suggested as a contraceptive agent (Aranha et al., 2004 and Reddy et al., 2004). However, nisin may not be a good choice for vaginal application as it is strongly bactericidal for the healthy vaginal Lactobacillus spp. (Aroucheva et al. 2005, unpublished data).

Bacteriocins produced by vaginally isolated bacteria.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>BACTERIOCIN</th>
<th>BACTERIOCIN ACTIVITY AGAINST</th>
<th>REFERENCE</th>
</tr>
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<tbody>
<tr>
<td>Lactobacillus</td>
<td>Lactocin 160</td>
<td>Gardnerella vaginalis, Prevotella bivia, Peptostreptococcus anaerobius, Peptostreptococcus assacharoliticus, Micrococcus luteus, Listeria innocua, Proteus vulgaris, Pseudomonas aeruginosa</td>
<td>Li et al., 2005 and Aroucheva et al., 2001c</td>
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<td>rhamnosus 160</td>
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<td>Lactococcus lactis</td>
<td>Bacteriocin HV219</td>
<td>Enterococcus faecalis, Escherichia coli, Lactobacillus casei, Listeria innocua, Proteus vulgaris, Pseudomonas aeruginosa</td>
<td>Todorov et al., 2006</td>
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<tr>
<td>subsp. lactis</td>
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<td>HV219</td>
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<tr>
<td>Lactobacillus</td>
<td>Pentocin TV35b</td>
<td>Clostridium sporogenes, Clostridium tyrobutyricum, Lactobacillus curvatus, Lactobacillus fermentum, Lactobacillus sake, Listeria innocua, Propionibacterium acidipropionici, Candida albicans</td>
<td>Okkers et al., 1999</td>
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<td>salivarius CRL1328</td>
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<td>Lactobacillus</td>
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<td>pentosus TV35b</td>
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Several bacteriocins from vaginal isolates of Lactobacillus have been identified and further studied (Table 1). Lactocin 160 from L. rhamnosus 160 is active against G. vaginalis and acts by disturbing the cellular membrane, probably by pore formation, causing the efflux of ATP (Aroucheva et al., 2001c and Li et al., 2005). Bacteriocin HV219 from Lactococcus lactis subsp. lactis HV219 is active against several Gram-positive and Gram-negative bacteria (Todorov et al., 2006), and bacteriocin-like substances from L. salivarius and L. pentosus TV35b have also been studied (Ocana et al., 1999 and Okkers et al., 1999).

Further, an uncharacterized bacteriocin-like compound produced by the vaginal isolate L. jensenii 51.08 showed bactericidal effects on G. vaginalis, Candida albicans and E. coli (Kaewsrichan et al., 2006). The vaginal isolate L. reuteri was found to have antibacterial effect against methicillin-resistant Staphylococcus aureus, which was attributed to production of bacteriocin (Voravuthikunchai et al., 2006).

Lactic acid produced by Lactobacillus spp. lowers and maintains the vaginal pH in the range of 3.8-4.5. In its undissociated (protonated) form, lactic acid crosses the cell membrane and dissociates in the cytoplasm, thereby decreasing the intracellular pH, hindering vital cell functions and causing the death of pathogenic bacteria (Cherrington et al., 1991).

Vaginal gels containing lactic acid enhance the vaginal defense and have the advantages of being easy to manufacture, being able to spread out on the mucus and achieve an intimate contact with vaginal mucosa (Barnhart et al., 2004). In most cases, lactic acid is well tolerated in the vagina, as it does not induce irritation (Boeke et al., 1993; Tanssupasiri et al., 2005 and Decena et al., 2006); however pH-dependent irritation was seen in the rabbit vaginal irritation model. It is unclear how relevant this is, as the rabbit vagina has a pH of ~7.5, whereas in humans it is ~4.0 (Kaminsky and Willigan, 1982).

Lactic acid in topical vaginal gels for curing BV are available on the market e.g. Lactal (Trimedic AB, Kungsbacka, Sweden) and Replens (Jannsen-Cilag SpA, Milan, Italy). It was reported that women with BV treated with Lactal (Trimedic AB, Kungsbacka, Sweden) daily for 7 days, followed by application of Lactal or placebo for three days a month for six months, were clinically improved. Lactal reduced the symptoms of BV and promoted the reestablishment of the Lactobacillus spp. flora in 83% of the women compared to 16% in the placebo. However, after three months, only 28.5% were cured by Lactal treatment (0% in placebo group) (Andersch et al., 1990). A recent study of 90 Filipino women compared treatments of BV using either the antibiotic metronidazole administered orally, and local treatment with Lactacyd vaginal gel (LVG; Sanofi-Synthelabo Philippines, Makati City, Philippines) or a combination of these for 7 days. After 7, 14 and 56 days, the treatments were found as being equally effective, but the combination was better at promoting Lactobacillus spp. colonization (Decena et al., 2006). On the contrary, in a different study that compared a one-week treatment with metronidazole or lactic acid suppositories, it was concluded that lactic acid was not an effective treatment at all. After two weeks, the lactic acid treatment had resolved only 50%
of the BV cases, compared to 83% for metronidazole (Boeke et al., 1993).

Similar results were shown in a Brazilian pilot study using the lactic acid buffering formulation gel, Acidform/Amphora™ (Instead, La Jolla, CA, USA). The authors concluded that Acidform was much less effective, as only 23% were cured of BV in this group compared to 88% treated with 10% of topical metronidazole (CDC recommendation 0.75% metronidazol for topical administration). No data showed lactobacillirecolonization after metronidazol and Acidform application (Simoes et al., 2006).

There is a current research for optimal lactic acid concentration and improved delivery systems (Bonferoni et al., 2006). Lactic acid may be an efficient treatment if the dosage of the lactic acid is optimized, and the most favorable administration formulation is determined.

Hydrogen peroxide is yet another antimicrobial product of many Lactobacillus spp., contributing to the non-specific antimicrobial defense. As was mentioned above, H₂O₂ is an oxidizing agent, toxic to catalase-negative bacteria such as most anaerobic microorganisms (Klebanoff et al., 1991b).

Several reports demonstrated that H₂O₂ might also be used as a treatment of BV. A 3% H₂O₂ solution administered to women with BV by vaginal irrigation was able to cure BV in two studies. It also aided the restoration of normal vaginal bacterial flora and was tolerated in 100% of the women (Wincelshaus and Calver, 1996 and Cardone et al., 2003). Using H₂O₂ for vaginal application has the potential risk of gas embolism or subcutaneous emphysema, although no such events in the vagina have been reported (Cina et al., 1994; Forre and Woods, 1997 and Cardone et al., 2003).

Probiotics vs. probiotics-derived preparations: application challenges

As mentioned previously, most introduced Lactobacillus spp. do not continue to colonize the vaginal tract when exposure to these species is discontinued. Therefore, natural products secreted by Lactobacillus spp. can be used for development of topical microbicides and be considered as a choice for alternative treatment of BV. A combination of a bacteriocin with lactic acid could be more effective for prevention and treatment of BV, and at the same time promote the growth of indigenous Lactobacillus spp. instead of introducing new species. Natural antimicrobials can be delivered to the vagina in concentrations sufficient to kill vaginal pathogens. In the case of probiotic administration, it is difficult to control the number of Lactobacillus spp., which adhere to vaginal epithelial layer, and the amount of produced antibacterials.

Natural antimicrobials from Lactobacillus spp. can be further optimized using the multiple hurdle approach. Hurdle technology is the combination of different stress factors such as chemical or biological preservatives, low pH, temperature, oxidative compounds and competitive microorganisms to achieve a synergistic or additive effects to control pathogenic bacteria (reviewed by Leistner, 2000). Hurdle technology is often used in the food industry to preserve food.

These combinations would increase the effect of the treatment, as the bactericidal mechanisms are different between the antimicrobials, and decrease the risk of microbial tolerance of the preparation, since the risk of two resistance mutations occurring simultaneously against two different antimicrobials is very low.

Antimicrobial synergy is well studied for nisin in combination with other substances. Enhanced antimicrobial effect was seen in combinations of nisin with zinc, aluminum or sodium lactate against Listeria monocytogenes (Nykanen et al., 2000 and McEntire et al., 2003). Zinc salts have also been found to have contraceptive effect (Chvapil et al., 1980), and a zinc sulfate and usnic acid preparation has successfully been used as a post-surgical adjuvant therapy following treatment of Human Papillomavirus (Scirpa et al., 1999). Further, in a mouse vaginal challenge model, zinc salts demonstrated protection against herpes simplex virus type 2-infection (Bourne et al., 2005).

Hurdle technology may also broaden the antimicrobial spectra of bacteriocins. Gram-negative bacteria are rarely inhibited by bacteriocins, unless they have access to the inner membrane by the disruption of the outer membrane. Nisin in combination with metal chelators such as EDTA has shown to be effective against E. coli and Salmonella spp (Stevens et al., 1991 and Bozias and Adams, 1999).

In addition to ribosomally synthesized peptides, many secondary metabolites produced by microorganisms show antimicrobial activity that could also be utilized for this purpose. One such peptide is ß-poly-L-lysine, produced by Streptomyces albulus (Yoshida and Nagasawa, 2003 and Shih et al., 2006), which is mildly synergistic with nisin (Najjar et al., 2007). This polymer is active against Gram-positive and Gram-negative bacteria as well as some fungi and yeasts and is safe for human use (Shima et al., 1984; Hiraki et al., 2003 and Tarantino, 2004).

Another group of natural antimicrobials that might be considered for this purpose are saponins. These natural surfactants are glycosides of steroids and triterpenoids, produced by several plants and some marine organisms (Francis et al., 2002). Some saponins have antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as yeasts (Maizel et al., 1964; Mandal et al., 2005 and Avato et al., 2006). Further, saponins from Sapindus mukorossi, proposed to be used as a contraceptive agent, have insignificant activity against the healthy Lactobacillus spp. as opposed to the common spermicide nonoxynol-9 (Ojha et al., 2003).

CONCLUSION

The optimal treatment and prophylaxis against BV infection should preferably be natural, non-toxic for humans or healthy vaginal microflora, biodegradable and cost effective. Such a formulation could be made from antimicrobials produced by Lactobacillus spp. in combination with other safe and effective substances. Natural antimicrobials, while inhibiting growth of pathogens, will help to survive and promote the growth of the native Lactobacillus spp., thereby supporting the natural defense against pathogenic organisms. Hurdle technology that combines different antimicrobials with synergistic or additive activity may
enhance the treatment results. This will be more effective than
currently used antibiotics in the treatment of BV and more effective
against antibiotic resistant strains. Having mechanisms of action
different from antibiotics, these natural antimicrobials might even
kill antibiotic-resistant pathogens. To elucidate this hypothesis
further, more research is needed. BV is especially prevalent in low-
income countries, where HIV also poses a grave threat. It is important
to produce a BV treatment that is affordable for women in these
countries. There is also a strong interest amongst women in industrial
countries for alternatives to the antibiotic treatment of BV, and
many already use some kind of home remedy (Boskey, 2005).

ACKNOWLEDGEMENTS
This research was sponsored by the NIH grant “Natural
antimicrobials against bacterial vaginosis” NCCAM NIH
R21AT002897-01

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Natural antimicrobials and vaginal health


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