Published in final edited form as:
Int J Probiotics Prebiotics. 2008 ; 3(4): 219–230.

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

S. E. Dover[1,2], **A. A. Aroutcheva**[3,4], **S. Faro**[5], and **M. L. Chikindas**[2,4]

[1] The Royal Institute of Technology, School of Biotechnology, Valhallavägen 79, 100 44, Stockholm, Sweden

[2] Rutgers, The State University of New Jersey, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA

[3] Rush Medical Center, Chicago, USA

[4] Health Promoting Naturals, Inc., USA

[5] The Women's Hospital of Texas, Houston, USA

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.

Keywords

Bacterial vaginosis; Bacteriocins; Lactic acid; *Lactobacilli*; 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;

Corresponding Author Dr. M.L. Chikindas, Rutgers, The State University of New Jersey, 65 Dudley Road, New Brunswick, NJ 08901-8520, USA; Tel.: +1 732 932 9611 ext. 218; Fax: +1 732 932 6776; tchikindas@aesop.rutgers.edu.

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, *Enterobacterioceae*, 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; H_2O_2 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 H_2O_2 and a positive correlation has been made between women colonized by H_2O_2 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 *Lactobacilli* spp. isolated from women with BV produced H_2O_2 (Hillier et al., 1992 and Mijac et al., 2006).

The amount of H_2O_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). H_2O_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, *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 *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 *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 trichomonisis, eukaryotic yeast infections or bacterial infections such as BV where the normal flora is replaced by several pathogenic bacteria, of

which *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 *Lactobacillus* species (Hill, 1993). This condition of replacement of *Lactobacillus* spp. by pathogenic species is referred to as bacterial vaginosis (BV). Lactobacilli are mostly replaced by *G. vaginalis* as well as anaerobes such as *Prevotella*, *Bacterioroides* species, *Peptostreptococcus*, *Mobiluncus*, genital *Mycoplasma hominis* and *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; Leitich et al., 2003; Hay, 2004 and Schwebke 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 (Uehara et al., 2006). *In vitro* studies have shown that clindamycin and metronidazole inhibit *Lactobacillus* spp. at concentrations lower than doses topically applied for treatment (Aroutcheva 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 *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 *Lactobacillus* spp. growth. This treatment would be especially valuable to women with chronic or recurrent BV associated with reduction of vaginal *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

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 panry liners. Oral administration of probiotics may also improve the long-term effects of treatment of BV with vaginal suppositories containing *L. acidophilus* by balancing the vaginal and intestinal microflora (Delia et al., 2006). The introduced *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. fermentumRC-l4* and *L. rhamnosus* GU1 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_2O_2-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/Z. *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 menstruation 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 (Rönnqvist 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 ATR amino acids and ions, thereby depleting the transmembrane potential (**Δψ**) and pH gradient (for reviews see: Klaenhammer, 1988; Nissen-Meyer and Nes, 1997 and Sablone et al., 2000) (Oscáriz 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 haemotolytic 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 personel (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. (Aroutcheva et al. 2005, unpublished data).

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 (Aroutcheva 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* 5L08 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; Tansupasiri 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 lactobacilli recolonization 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, H_2O_2 is an oxidizing agent, toxic to catalase-negative bacteria such as most anaerobic microorganisms (Klebanoff et al., 1991b).

Several reports demonstrated that H_2O_2 might also be used as a treatment of BV. A 3% H_2O_2 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 (Winceslaus and Calver, 1996 and Cardone et al., 2003). Using H_2O_2 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; Forrer 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 Boziaris 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 ε-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 yeast (Maizel et al., 1964; Mandal et al., 2005 and Avato et al., 2006). Further, saponins from *Sapindus mukorosii*, 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).

Acknowledgments

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

References

Al-Mushrif S, Jones BM. A study of the prevalence of hydrogen peroxide generating lactobacilli in bacterial vaginosis: the determination of H_2O_2 concentrations generated, in vitro, by isolated strains

and the levels found in vaginal secretions of women with and without infection. Journal of Obstetrics and Gynecology 1998;18:63–67.

Andersch B, Lindell D, Dahlen I, Brandberg A. Bacterial vaginosis and the effect of intermittent prophylactic treatment with an acid lactate gel. Gynecologic and Obstetric Investigation 1990;30:114–119. [PubMed: 2245947]

Antonio MA, Hillier SL. DNA fingerprinting of *Lactobacillus crispatus* strain CTV-05 by repetitive element sequence-based PCR analysis in a pilot study of vaginal colonization. Journal of Clinical Microbiology 2003;41:1881–1887. [PubMed: 12734221]

Anukam KC, Osazuwa E, Ahonkhai I, Ngwu M, Osemene GI, Bruce AA, Reid G. Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14: randomized, double-blind, placebo controlled trial. Microbes and Infection 2006a;8:1450–1454. [PubMed: 16697231]

Anukam KC, Osazuwa E, Osemene GI, Ehigiagbe F, Bruce AW, Reid G. Clinical study comparing probiotic *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. Microbes and Infection 2006b;8:2772–2776. [PubMed: 17045832]

Aranha C, Gupta S, Reddy KV. Contraceptive efficacy of antimicrobial peptide nisin: in vitro and in vivo studies. Contraception 2004;69:333–338. [PubMed: 15033410]

Aroutcheva A, Gariti D, Simon M, Short S, Faro J, Simoes JA, Gurguis A, Faro S. Defense factors of vaginal lactobacilli. American Journal of Obstetrics and Gynecology 2001a;185:375–379. [PubMed: 11518895]

Aroutcheva A, Simoes JA, Shott S, Faro S. The inhibitory effect of clindamycin on *Lactobacillus* in vitro. Infectious Diseases in Obstetrics and Gynecology 2001b;9:239–244. [PubMed: 11916182]

Aroutcheva A, Simoes JA, Faro S. Antimicrobial protein produced by *vaginal Lactobacillus acidophilus* that inhibits *Gardnerella vaginalis*. Infectious Diseases in Obstetrics and Gynecology 2001c;9:33–39. [PubMed: 11368257]

Aslim B, Kilic E. Some probiotic properties of vaginal lactobacilli isolated from healthy women. Japanese Journal of Infectious Diseases 2006;59:249–253. [PubMed: 16936344]

Atassi F, Brassart D, Grob P, Graf F, Servin AL. *Lactobacillus* strains isolated from the vaginal microbiota of healthy women inhibit *Prevotella bivia* and *Gardnerella vaginalis* in coculture and cell culture. FEMS Immunology and Medical Microbiology 2006;48:424–432. [PubMed: 17059467]

Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta M. Antimicrobial activity of saponins from *Medicago* sp.: structure-activity relationship. Phytotherapy Research 2006;20:454–457. [PubMed: 16619355]

Barnhart KT, Pretorius ES, Timbers K, Shera D, Shabbout M, Malamud D. In vivo distribution of a vaginal gel: MRI evaluation of the effects of gel volume, time and simulated intercourse. Contraception 2004;70:498–505. [PubMed: 15541413]

Bioflora. December 12. 2006 http://www.bioflora.co.za/vagiforte.htm

Boeke AJ, Dekker JH, van Eijk JT, Kostense PJ, Bezemer PD. Effect of lactic acid suppositories compared with oral metronidazole and placebo in bacterial vaginosis: a randomised clinical trial. Genitourinary Medicine 1993;69:388–392. [PubMed: 8244360]

Bonferoni MC, Giunchedi P, Scalia S, Rossi S, Sandri G, Caramella C. Chitosan gels for the vaginal delivery of lactic acid: relevance of formulation parameters to mucoadhesion and release mechanisms. AAPS PharmSci Tech 2006;7:104.

Boris J, Påhlson C, Larsson P. Six years observation after successful treatment of bacterial vaginosis. Infectious Diseases in Obstetrics and Gynecology 1997;5:297–302. [PubMed: 18476155]

Boris S, Suárez JE, Vázquez F, Barbés C. Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. Infection and Immunity 1998;66:1985–1989. [PubMed: 9573080]

Boris S, Barbés C. Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes and Infection 2000;2:543–546. [PubMed: 10865199]

Boskey ER, Cone RA, Whaley KJ, Moench TR. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. Human Reproduction 2001;16:1809–1813. [PubMed: 11527880]

Boskey ER. Alternative therapies for bacterial vaginosis: A literature review and acceptability survey. Alternative Therapies in Health and Medicine 2005;11:38–43. [PubMed: 16189947]

Bourne N, Stegall R, Montano R, Meador M, Stanberry LR, Milligan GN. Efficacy and toxicity of zinc salts as candidate topical microbicides against vaginal herpes simplex virus type 2 infection. Antimicrobial Agents and Chemotherapy 2005;49:1181–1183. [PubMed: 15728922]

Boziaris IS, Adams MR. Effect of chelators and nisin produced in situ on inhibition and inactivation of Gram-negatives. International Journal of Food Microbiology 1999;53:105–113. [PubMed: 10634702]

Cardone A, Zarcone R, Borrelli A, Di Cunzolo A, Russo A, Tartaglia E. Utilisation of hydrogen peroxide in the treatment of recurrent bacterial vaginosis. Minerva Ginecologica 2003;55:483–492. [PubMed: 14676737]

Cherpes TL, Meyn LA, Krohn MA, Hillier SL. Risk factors for infection with herpes simplex virus type 2: Role of smoking, douching, uncircumcised males, and vaginal flora. Sexually Transmitted Diseases 2003;30:405–410. [PubMed: 12916131]

Cherrington CA, Hinton M, Mead GC, Chopra I. Organic acids: chemistry, antibacterial activity and practical applications. Advances in Microbial Physiology 1991;32:87–108. [PubMed: 1882730]

Chvapil M, Droegemueller W, Betts K, Heine W, Weinstein L. Preliminary testing of the contraceptive collagen sponge. Obstetrics & Gynecology 1980;56:503–506. [PubMed: 7191547]

Cina SJ, Downs JCU, Conradi SE. Hydrogen peroxide: a source of lethal oxygen embolism. American Journal of Forensic Medicine and Pathology 1994;15:44–50. [PubMed: 8166115]

Cohen CR, Duerr A, Pruithithada N, Rugpao S, Hillier S, Garcia P, Nelson K. Bacterial vaginosis and HIV seroprevalence among female commercial sex workers in Chiang Mai, Thailand. AIDS 1995;9:1093–1097. [PubMed: 8527084]

Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: safe, natural antimicrobials for food preservation. International Journal of Food Microbiology 2001;71:1–20. [PubMed: 11764886]

Colodner R, Edelstein H, Chazan B, Raz R. Vaginal colonization by orally administered *Lactobacillus rhamnosus* GG. Israeli Medical Association Journal 2003;5:767–769.

Decena DCD, Co JT, Manalastras RM, Palaypayon EP, Padolina CS, Sison JM, Dancel LA, Lelis MA. Metronidazole with Lactacyd vaginal gel in bacterial vaginosis. Journal of Obstetrics and Gynaecology Research 2006;32:243–251. [PubMed: 16594932]

Delia A, Morgante G, Rago G, Mustacchio MC, Petraglia F, De Leo V. Effectiveness of oral administration of *Lactobacillus paracasei* subsp. *paracasei* F19 in association with vaginal suppositories of *Lactobacillus acidofilus* in the treatment of vaginosis and in the prevention of recurrent vaginitis. Minerva Ginecologica 2006;58:227–231. [PubMed: 16783294]

Diep DB, Nes IF. Ribosomally synthesized antibacterial peptides in Gram-positive bacteria. Current Drug Targets 2002;3:107–122. [PubMed: 11958295]

Dover S, Aroutcheva A, Faro S, Chikindas ML. Safety study of antimicrobial peptide Lactocin 160, produced by vaginal Lactobacillus rhamnosus. Infectious Diseases in Obstetrics and Gynecology. 2007 Article ID: 78248.

Ellen, AB. December 5. 2006 http://www.ellenab.com/index_eng.htm

Eriksson K, Carlsson B, Forsum U, Larsson PG. A double-blind treatment study of bacterial vaginosis with normal vaginal lactobacilli after an open treatment with vaginal clindamycin ovules. Acta Dermato-Venereologica 2005;85:42–46. [PubMed: 15848990]

Ferris DG, Litaker MS, Woodward L, Mathis D, Hendrich J. Treatment of bacterial vaginosis: a comparison of oral metronidazole, metronidazole vaginal gel, and clindamycin vaginal cream. Journal of Family Practice 1995;41:443–449. [PubMed: 7595261]

Flynn CA, Helwig AL, Meurer LN. Bacterial vaginosis in pregnancy and the risk of prematurity: a meta-analysis. Journal of Family Practice 1999;48:885–892. [PubMed: 10907626]

Fontaine EA, Taylor-Robinson D. Comparison of quantitative and qualitative methods of detecting hydrogen peroxide produced by human vaginal strains of lactobacilli. The Journal of Applied Bacteriology 1990;69:326–331. [PubMed: 2246139]

Forrer JA, Woods W. Potential hazards of hydrogen peroxide within closed body cavities. The International Journal of STD & AIDS 1997;8:210.

Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: a review. The British Journal of Nutrition 2002;88:587–605. [PubMed: 12493081]

Grether JK, Nelson KB. Possible decrease in prevalence of cerebral palsy in premature infants? The Journal of Pediatrics 2000;136:133. [PubMed: 10636990]

Hashemi FB, Ghassemi M, Roebuck KA, Spear GT. Activation of human immunodeficiency virus type 1 expression by *Gardnerella vaginalis*. The Journal of Infectious Diseases 1999;179:924–930. [PubMed: 10068588]

Hashemi FB, Ghassemi M, Faro S, Aroutcheva A, Spear GT. Induction of human immunodeficiency virus type 1 expression by anaerobes associated with bacterial vaginosis. The Journal of Infectious Diseases 2000;181:1574–1580. [PubMed: 10823756]

Hay PE, Morgan DJ, Ison CA, Bhide SA, Romney M, McKenzie P, Pearson J, Lament RF, Taylor-Robinson D. A longitudinal study of bacterial vaginosis during pregnancy. British Journal of Obstetrics and Gynecology 1994;101:1048–1053.

Hay PE. Bacterial vaginosis and miscarriage. Current Opinion in Infectious Diseases 2004;17:41–44. [PubMed: 15090889]

Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-Hanssen P, Holmes KK. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. Journal Of Infectious Diseases 1996;174:1058–1063. [PubMed: 8896509]

Hill JA, Anderson DJ. Human vaginal leukocytes and the effects of vaginal fluid on lymphocyte and macrophage defense functions. American Journal of Obstetrics and Gynecology 1992;166:720–726. [PubMed: 1536258]

Hill GB. The microbiology of bacterial vaginosis. American Journal of Obstetrics and Gynecology 1993;169:450–454. [PubMed: 8357043]

Hillier SL, Krohn MA, Klebanoff SJ, Eschenbach DA. The relationship of hydrogen peroxide-producing lactobacilli to bacterial vaginosis and genital microflora in pregnant women. Obstetrics & Gynecology 1992;79:369–373. [PubMed: 1738516]

Hillier SL, Krohn MA, Rabe LK, Klebanoff SJ, Eschenbach DA. The normal vaginal flora, H_2O_2-producing lactobacilli, and bacterial vaginosis in pregnant women. Clinical Infectious Diseases 1993;16:S273–281. [PubMed: 8324131]

Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, Cotch MF, Edelman R, Pastorek JG, Rao AV, McNellis D, Regan JA, Carey JC, Klebanoff MA. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The New England Journal of Medicine 1995;333:1737–1742. [PubMed: 7491137]

Hiraki J, Ichikawa T, Ninomiya S, Seki H, Uohama K, Seki H, Kimura S, Yanagimoto Y, Barnett JW. Use of ADME studies to confirm the safety of ε-poly-lysine as a preservative in food. Regulatory Toxicology and Pharmacology 2003;37:328–340. [PubMed: 12726761]

Immucell. April 23. 2007 http://www.immucell.com/prod_mast.php#wipe

Itoh T, Fujimoto Y, Kawai Y, Toba T, Saito T. Inhibition of food-borne pathogenic bacteria by bacteriocins from *Lactobacillus gasseri*. Letters in Applied Microbiology 1995;21:137–141. [PubMed: 7576495]

Kawai Y, Saito T, Toba T, Samant SK, Itoh T. Isolation and characterization of a highly hydrophobic new bacteriocin (gassericin A) from *Lactobacillus gasseri* LA39. Bioscience Biotechnology & Biochemistry 1994;58:1218–1221.

Kaewsrichan J, Peeyananjarassri K, Kongprasertkit J. Selection and identification of anaerobic lactobacilli producing inhibitory compounds against vaginal pathogens. FEMS Immunology and Medical Microbiology 2006;48:75–83. [PubMed: 16965354]

Kaminsky M, Willigan DA. pH and the potential irritancy of douche formulations to the vaginal mucosa of the albino rabbit and rat. Food and Chemical Toxicology 1982;20:193–196. [PubMed: 7200937]

Klaenhammer TR. Bacteriocins of lactic acid bacteria. Biochimie 1988;70:337–349. [PubMed: 3139051]

Klebanoff SJ, Coombs RW. Viricidal effect of *Lactobacillus acidophilus* on human immunodeficiency virus type 1: possible role in heterosexual transmission. Journal of Experimental Medicine 1991a; 174:289–292. [PubMed: 1647436]

Klebanoff SJ, Hillier SL, Eschenbach DA, Waltersdorph AM. Control of the microbial flora of the vagina by H_2O_2-generating lactobacilli. The Journal of Infectious Diseases 1991b;164:94–100. [PubMed: 1647428]

Larsson PG, Forsum U. Bacterial Vaginosis - a disturbed bacterial flora and treatment enigma. Acta Pathologica, Microbiologica et Immunologica Scandinavica 2005;113:305–316.

Leistner L. Basic aspects of food preservation by hurdle technology. International, Journal of Food Microbiology 2000;55:181–186. [PubMed: 10791741]

Leitich H, Bodner-Adler B, Brunbauer M, Kaider A, Egarter C, Husselein P. Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis. American Journal of Obstetrics and Gynecology 2003;189:139–147. [PubMed: 12861153]

Li J, Aroutcheva AA, Faro S, Chikindas ML. Mode of action of lactocin 160, a bacteriocin from vaginal *Lactobacillus rhamnosus*. Infectious Diseases in Obstetrics and Gynecology 2005;13:135–140. [PubMed: 16126497]

Ling ZD, Chang Q, Lipton JW, Tong CW, Landers TM, Carvey PM. Combined toxicity of prenatal bacterial endotoxin exposure and postnatal 6-hydroxydopamine in the adult rat midbrain. Neuroscience 2004;124:619–628. [PubMed: 14980732]

Maizel JV, Burkhardt HJ, Mitchell HK. Avenacin, an antimicrobial substance isolated from *Avena sativa*. I. Isolation and antimicrobial activity. Biochemistry 1964;3:424–426. [PubMed: 14155108]

Mandal P, Sinha Babu SP, Mandal NC. Antimicrobial activity of saponins from *Acacia auriculiformis*. Fitoterapia 2005;76:462–465. [PubMed: 15951137]

Marrazzo JM, Cook RL, Wiesenfeld HC, Murray PJ, Busse B, Krohn M, Hillier SL. Women's satisfaction with an intravaginal *Lactobacillus* capsule for the treatment of bacterial vaginosis. Journal of Women's Health 2006;15:1053–1060.

Martin LS, McDougal JS, Loskoski SL. Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy-associated virus. The Journal of Infectious Diseases 1985;152:400–403. [PubMed: 2993438]

Martin HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndinya-Achola JO, Bwayo J, Kreiss J. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. The Journal of Infectious Diseases 1999;180:1863–1868. [PubMed: 10558942]

McEntire JC, Montville TJ, Chikindas ML. Synergy between nisin and select lactates against *Listeria monocytogenes* is due to the metal cations. Journal of Food Protection 2003;66:1631–1636. [PubMed: 14503717]

McGroarty JA, Reid G. Detection of a *Lactobacillus* substance that inhibits *Escherichia coli*. Canadian Journal of Microbiology 1988;34:974–978. [PubMed: 3145135]

McLean NW, McGroarty JA. Growth inhibition of metronidazole-susceptible and metronidazole-resistant strains of *Gardnerella vaginalis* by lactobacilli in vitro. Applied and Environmental Microbiology 1996;62:1089–1092. [PubMed: 8975601]

McLean NW, Rosenstein IJ. Characterisation and selection of a *Lactobacillus* species to re-colonise the vagina of women with recurrent bacterial vaginosis. Journal of Medical Microbiology 2000;49:543–552. [PubMed: 10847208]

Mijac VD, Dukic SV, Opavski N, Dukic MK, Ranin LT. Hydrogen peroxide producing lactobacilli in women with vaginal infections. European Journal of Obstetrics, Gynecology and Reproductive Biology 2006;129:69–76.

Najjar MB, Kashtanov D, Chikindas ML. ε-poly-L-lysine and nisin A act synergistically against Gram-positive foodborne pathogens *Bacillus cereus* and *Listeria monocytogenes*. Letters in Applied Microbiology 2007;45:13–18. [PubMed: 17594454]

Nissen-Meyer J, Nes IF. Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis and mechanism of action. Archives of Microbiology 1997;167:67–77.

Nykänen A, Weckman K, Lapveteläinen A. Synergistic inhibition of *Listeria monocytogenes* on cold-smoked rainbow trout by nisin and sodium lactate. International Journal of Food Microbiology 2000;61(1):63–72. [PubMed: 11028960]

Ocana VS, Pesce de Ruiz Holquado AA, Nader-Macias ME. Characterization of a bacteriocin-like substance produced by a vaginal *Lactobacillus salivarius* strain. Applied and Environmental Microbiology 1999;65:5631–5635. [PubMed: 10584033]

Ojha P, Maikhuri JP, Gupta G. Effect of spermicides on *Lactobacillus acidophilus* in vitro-nonoxynol-9 vs. *Sapindus saponins*. Contraception 2003;68:135–138. [PubMed: 12954526]

Okkers DJ, Dicks LMT, Silvester M, Joubert JJ, Odendaal HJ. Characterization of pentocin TV35b, a bacteriocin-like peptide isolated from *Lactobacillus pentosus* with a fungistatic effect on *Candida albicans*. Journal of Applied Microbiology 1999;87:726–734. [PubMed: 10594714]

Onderdonk AB. Probiotics for women's health. Journal of Clinical Gastroenterology 2006;40:256–259. [PubMed: 16633132]

Oscáriz JC, Pisabarro AG. Classification and mode of action of membrane-active bacteriocins produced by Gram-positive bacteria. International Journal of Microbiology 2001;4:13–19.

Othman M, Neilson J, Alfirevic Z. Probiotics for preventing preterm labour. Cochrane Database of Systematic Reviews 2007;1:CD005941.

Paavonen J, Mangioni C, Martin MA, Wajszczuk CP. Vaginal clindamycin and oral metronidazole for bacterial vaginosis: a randomized trial. Obstetrics & Gynecology 2000;96:256–260. [PubMed: 10908773]

Pybus V, Onderdonk AB. Microbial interactions in die vaginal ecosystem, with emphasis on the pathogenesis of bacterial vaginosis. Microbes and Infection 1999;1:285–292. [PubMed: 10602662]

Reddy KV, Aranha C, Gupta SM, Yedery RD. Evaluation of antimicrobial peptide nisin as a safe vaginal contraceptive agent in rabbits: in vitro and in vivo studies. Reproduction 2004;128:117–126. [PubMed: 15232069]

Reid G. The scientific basis for probiotic strains of *Lactobacillus*. Applied and Environmental Microbiology 1999;65:3763–3766. [PubMed: 10473372]

Reid G, Bruce AW, Eraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. FEMS Immunology and Medical Microbiology 2001;30:49–52. [PubMed: 11172991]

Reid G, Burton J. Use of *Lactobacillus* to prevent infection by pathogenic bacteria. Microbes and Infection 2002;4:319–324. [PubMed: 11909742]

Reid G, Bocking A. The potential for probiotics to prevent bacterial vaginosis and preterm labor. American Journal of Obstetrics and Gynecology 2003a;189:1202–1208. [PubMed: 14586379]

Reid G, Charbonneau D, Erb J, Kochanowski B, Beuerman D, Poehner R, Bruce AW. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. FEMS Immunology and Medical Microbiology 2003b;35:131–134. [PubMed: 12628548]

Rönnqvist PDJ, Forsgren-Brusk UB, Hakansson EEG. Lactobacilli in the female genital tract in relation to other genital microbes and vaginal pH. Acta Obstetricia et Gynecologica Scandinavica 2006;85:726–735. [PubMed: 16752267]

Sablone E, Contreras B, Vandamme E. Antimicrobial peptides of lactic acid bacteria: Mode of action, genetics and biosynthesis. Advances in Biochemical Engineering/Biotechnology 2000;68:21–60. [PubMed: 11036685]

Saunders S, Bocking A, Challis J, Reid G. Effect of *Lactobacillus* challenge on *Gardnerella vaginalis* biofilms. Colloids and Surfaces B: Biointerfaces 2007;55:138–142.

Schwebke JR, Desmond R. Risk factors for bacterial vaginosis in women at high risk for sexually transmitted diseases. Sexually Transmitted Diseases 2005;32:654–658. [PubMed: 16254538]

Scirpa P, Scambia G, Masciullo V, Battaglia F, Foti E, Lopez R, Villa P, Malecore M, Mancuso S. Azinc sulfate and usnic acid preparation used as post-surgical adjuvant therapy in genital lesions by human papillomavirus. Minerva Ginecologica 1999;51:255–260. [PubMed: 10479878]

Sewankambo N, Gray RH, Wawer MJ, Paxton L, McNaim D, Wabwirre-Mangen F, Serwadda D, Li C, Kiwanuka N, Hillier SL, Rabe L, Gaydos CA, Quinn TC, Konde-Lule J. HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. Lancet 1997;350:546–550. [PubMed: 9284776]

Sha BE, Zariffard MR, Wang QJ, Chen HY, Bremer J, Cohen MH, Spear GT. Female genital-tract HIV load correlates inversely with Lactobacillus species but positively with bacterial vaginosis and *Mycoplasma hominis*. The Journal of Infectious Diseases 2005;191:25–32. [PubMed: 15592999]

Shih IL, Shen MH, Van YT. Microbial synthesis of poly(epsilon-lysine) and its various applications. Bioresource Technology 2006;97:1148–1159. [PubMed: 16551535]

Shima S, Matsuoka H, Iwamoto T, Sakai H. Antimicrobial action of ε-poly-L-lysine. The Journal of Antibiotics (Tokyo) 1984;37:1449–1455.

Silva M, Jacobus NV, Deneke C, Gorbach SL. Antimicrobial substance from a human *Lactobacillus* strain. Antimicrobial Agents and Chemotherapy 1987;31:1231–1233. [PubMed: 3307619]

Simoes J, Aroutcheva A, Shott S, Faro S. Effect of metronidazole on the growth of vaginal lactobacilli in vitro. Infectious Diseases in Obstetrics and Gynecology 2001;9:41–46. [PubMed: 11368258]

Simoes JA, Bahamondes LG, Camargo RPS, Alves VMN, Zaneveld LJD, Waller DP, Schwartz J, Callahan MM, Mauck CK. A pilot clinical trial comparing an acid-buffering formulation (ACIDFORM gel) with metronidazole gel for the treatment of symptomatic bacterial vaginosis. British Journal of Clinical Pharmacology 2006;61(2):211–217. [PubMed: 16433875]

Skarin A, Sylwan J. Vaginal lactobacilli inhibiting growth of *Gardnerella vaginalis, Mobiluncus* and other bacterial species cultured from vaginal content of women with bacterial vaginosis. Acta Pathologica, Microbiologica et Immunologica Scandinavica 1986;94:399–403.

Sobel JD. Is There a Protective Role for Vaginal Flora? Current Infectious Disease Reports 1999;1:379–383. [PubMed: 11095812]

Stevens KA, Sheldon BW, Klapes NA, Klaenhammer TR. Nisin treatment for inactivation of *Salmonella* species and other Gram-negative bacteria. Applied and Environmental Microbiology 1991;57:3613–3615. [PubMed: 1785933]

Strus M, Brzychczy-Wloch M, Gosiewski T, Kochan P, Heczko PB. The in vitro effect of hydrogen peroxide on vaginal microbial communities. FEMS Immunology and Medical Microbiology 2006;48:56–63. [PubMed: 16965352]

Svare JA, Schmidt H, Hansen BB, Lose G. Bacterial vaginosis in a cohort of Danish pregnant women: prevalence and relationship with preterm delivery, low birth weight and prenatal infections. British Journal of Obstetrics and Gynaecology 2006;113:1419–1425. [PubMed: 17010117]

Taha TE, Hoover DR, Dallabetta GA, Kumwenda NI, Mtimavalye LA, Yang LP, Liomba GN, Broadhead RL, Chiphangwi JD, Miotti PG. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. AIDS 1998;12:1699–1706. [PubMed: 9764791]

Tansupasiri A, Puangsricharern A, Itti-arwachakul A, Asavapiriyanont S. Satisfaction and tolerability of combination of lactoserum and lactic acid on the external genitalia in Thai women. Journal of the Medical Association of Thailand 2005;88:1753–1757. [PubMed: 16518969]

Tarantino LM. CFSAN/Office of Food Additive Safety. Agency Response Letter. 2004 GRAS Notice No. CRN 000135.

Todorov SD, Danova ST, Van Reenen CA, Meincken M, Dinkova G, Ivanova IV, Dicks LMT. Characterization of bacteriocin HV219, produced by *Lactococcus lactis* subsp. *lactis* HV219 isolated from human vaginal secretions. Journal of Basic Microbiology 2006;46:226–238. [PubMed: 16721875]

Uehara S, Monden K, Nomoto K, Seno Y, Kariyama R, Kumon H. A pilot study evaluating the safety and effectiveness of *Lactobacillus* vaginal suppositories in patients with recurrent urinary tract infection. International Journal of Antimicrobial Agents 2006;28:S30–34. [PubMed: 16859900]

Uni-Sankyo. December 12. 2006 http://www.pubergen.com/myconip.htm

Urakubo A, Jarskog LF, Lieberman JA, Gilmore JH. Prenatal exposure to maternal infection alters cytokine expression in the placenta, amniotic fluid, and fetal brain. Schizophrenia Research 2001;47:27–36. [PubMed: 11163542]

Valore EV, Park CH, Igreti SL, Ganz T. Antimicrobial components of vaginal fluid. American Journal of Obstetrics and Gynecology 2002;187:561–568. [PubMed: 12237628]

Valore EV, Wiley DJ, Ganz T. Reversible deficiency of antimicrobial polypeptides in bacterial vaginosis. Infection and Immunity 2006;74:5693–5702. [PubMed: 16988245]

Voravuthikunchai SP, Bilasoi S, Supamala O. Antagonistic activity against pathogenic bacteria by human vaginal lactobacilli. Anaerobe 2006;12:221–226. [PubMed: 16931064]

webMD. October 15. 2006 http://www.webmd.com/hw/std/hw53060.asp

Wilks M, Wiggins R, Whiley A, Hennessy E, Warwick S, Porter H, Corfield A, Millar M. Identification and H_2O_2 production of vaginal lactobacilli from pregnant women at high risk of preterm birth and relation with outcome. Journal of Clinical Microbiology 2004;42:713–717. [PubMed: 14766841]

Winceslaus SJ, Calver G. Recurrent bacterial vaginosis-an old approach to a new problem. The International Journal of STD & AIDS 1996;7:284–287.

World Health Organization, Food Safety Department. Modern food biotechnology, human health and development: an evidence based study 2005:9.

Yoshida T, Nagasawa T. ε-poly-L-lysine: microbial production, biodegradation and application potential. Applied Microbiology Biotechnology 2003;62:21–26.

Wrong diagnosis. January 20. 2008 http://www.wrongdiagnosis.com/b/bacterial_vaginosis/stats-country.htm

TABLE 1

Bacteriocins produced by vaginally isolated bacteria.

SPECIES	BACTERIOCIN	BACTERIOCIN ACTIVITY AGAINST	REFERENCE
Lactobacillus rhamnosus 160	Lactocin 160	Gardnerella vaginalis Prevotella bivia Peptostreptococcus anaerobius Peptostreptococcus assacharoliticus Micrococcus luteus Listeria monocytogenes	Li et al., 2005 and Aroutcheva et al., 2001c
Lactococcus lactis subsp. lactis HV219	Bacteriocin HV219	Enterococcus faecalis Escherichia coli Lactobacillus casei Listeria innocua Proteus vulgaris Pseudomonas aeruginosa	Todorov et al., 2006
Lactobacillus salivarius CRL1328		Enterococcus faecalis Enterococcus faecium Neisseria gonorrhoeae	Ocana et al., 1999
Lactobacillus pentosus TV35b	Pentocin TV35b	Clostridium sporogenes Closridium tyrobutyricum Lactobacillus curvatus Lactobacillus fermentum Lactobacillus sake Listeria innocua Propionibacterium acidipropionici Candida albicans	Okkers et al., 1999