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# The human vaginal microbial community

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**Abstract**

Monopolization of the vaginal econiche by a limited number of *Lactobacillus* species, resulting in low pH of 3.5–4.5, has been shown to protect women against vaginal dysbiosis, sexually transmitted infections and adverse pregnancy outcomes. Still, controversy exists as to which characteristics of lactobacilli are most important with regard to colonization resistance and to providing protection. This review addresses the antimicrobial and anti-inflammatory roles of lactic acid (and low pH) and hydrogen peroxide (and oxidative stress) as means of lactobacilli to dominate the vaginal econiche.


*Keywords:* Vagina; Glycogen; Lactobacilli; Lactic acid; Hydrogen peroxide

## 1. Definition and dynamics of the normal vaginal microbial community

### *1.1. What is normal?*

It has recently been claimed that almost any type of vaginal microbial community (VMC) can be considered as normal, since even in the absence of lactobacilli there is frequently a lack of symptoms, there is organic acid production and there is long-term stability of the VMC [1,2]. Witkin et al. [1], for example, stated that in black women, the enhanced prevalence of asymptomatic bacterial vaginosis might merely reflect an increased likelihood that bacteria other than lactobacilli predominate, fulfilling the same protective functions, in agreement with the common function hypothesis (see 1.4).

Although science usually proceeds most quickly by paradigm shifts, in this case it may be wise not to throw overboard knowledge that has been gathered during more than a century, starting with Döderlein [3]. Indeed, numerous epidemiological studies continue to confirm that it is especially hydrogen peroxide ($H_2O_2$)-producing lactobacilli that are protective against sexually transmitted infections (STIs) and adverse pregnancy outcome (APO). In other words, the mere absence of these probiotic bacteria from the vaginal econiche is a health risk factor for women, their partners and their unborn and new-born children. Moreover, it is becoming clear that it is especially lactic acid [4,5], and probably even more, D-lactic acid, as produced by only some vaginal lactobacilli [6], but not any organic acid, that are health-promoting. Also, it is rather odd to consider stability as a hallmark of a healthy VMC, because the normal VMC fluctuates during the menstrual cycle (see 1.5) and because the most stable VMC is observed in cases of chronic bacterial vaginosis (BV). The notion that 10–42% of women whose vaginal microbial communities lack appreciable numbers of lactobacilli are asymptomatic is in fact long-established knowledge [e.g., [7]].

With regard to $H_2O_2$ production, it is not clear whether this in itself is a health-promoting characteristic rather than a biomarker for the presence of certain species of lactobacilli [4], i.e., lactobacilli other than *Lactobacillus iners* [5](see also 4).

Interestingly, a recent report indicates that the presence of lactobacilli is not jeopardizing anti-HIV medication, whereas colonization with *Gardnerella vaginalis* did impede the efficacy of the treatment [8].

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For all these reasons, it seems reasonable to consider only lactobacilli-dominated VMC as normal or health-promoting (healthy).

### 1.2. The predominant lactobacilli

Lactobacilli are the most aciduric bacteria among the acidophilic lactic acid bacteria, thriving at pH of 4.0 (and below, according to [4]), at which other species cannot grow. This makes them the favored bacteria in fermented food production, such as yoghurt, sauerkraut and fermented meat, since they are the last survivors when pH has sharply decreased. The same principle applies in the human vagina, although we wonder why, among mammals, this seems to be the case only in our species (see 1.4).

Based on DNA homology studies, the previous species *Lactobacillus acidophilus* sensu lato has been divided into six DNA homology groups that could not be distinguished biochemically [9], but that later could be characterized as six distinct species: *L. acidophilus* (*sensu stricto*), *Lactobacillus amylovorus*, *Lactobacillus crispatus*, *Lactobacillus gallinarum*, *Lactobacillus gasseri* and *Lactobacillus johnsonii* [10]. The L. *acidophilus* group now comprises 25 species, including *L. iners* and *L. jensenii*. tRNA-intergenic spacer length polymorphism analysis is another molecular approach that was extremely useful in identifying cultured lactobacilli [11] and that was applied for the identification of vaginal lactobacilli [12].

Culture-based studies using DNA:DNA hybridization to identify the isolates revealed that *L. crispatus*, *L. gasseri*, *L. jensenii* and *Lactobacillus vaginalis* (more closely related to *L. fermentum* and *L. reuteri*) are the most common species found in the vagina [13–15], and confirmed an association between vaginal health and $H_2O_2$-producing species/strains as well [14,15]. Another species, *L. iners*, was first described in 1999 [16] and its presence (and its limited production of $H_2O_2$) was already established in the culture-based study of Antonio et al. [14], where it was listed as 'species L. 1086V' [Sharon Hillier, pers. comm.]. Another early study pointing to the prevalence of *L. iners* was the one by Tärnberg et al. [17].

Still, studies from around the world (using adequate identification methods) indicate that more species may be involved [18]. Predominant vaginal species were *L. plantarum* and *L. fermentum* in a Bulgarian study [19], *L. reuteri*, *L. fermentum* and *L. salivarius* in an Indian study [20] and *L. pentosus*, *L. fermentum*, *Enterococcus faecalis*, and also the strongly $H_2O_2$-producing *Weissella (Lactobacillus) viridescens* in a South African study [21].

It may be important to also consider rectal colonization with regard to the vaginal dominance of lactobacilli [22–24].

### 1.3. Is predominance of lactobacilli always indicative of health?

Cytolytic vaginosis and lactobacillosis, mostly considered together, also known as *Lactobacillus* overgrowth syndrome or Döderlein's cytolysis (and possibly as *Leptothrix vaginalis* [25]), are characterized by abundant growth of (frequently long and slender) lactobacilli in association with very low pH and numerous bare nuclei – easily confused with leukocytes, and possibly with debris cytoplasm – which may be confused with the type of bacterial cells that are characteristic of BV. Bare nuclei and debris cytoplasm result from the lysis of vaginal epithelial cells, which is generally assumed [e.g., [26]] to be due to the overgrowth of, and subsequent over-acidification by, lactobacilli. This assumption is corroborated by the cyclicity of the symptoms and their association with the luteal/secretory phase, when predominance of lactobacilli is usually most evident [27–29].

The prevalence of cytolytic vaginosis may be underestimated, because the clinical presentation is suggestive of vulvovaginal candidiasis (VVC) [30,31]. Cibley and Cibley [27] even state that most of the presumed chronic candidiasis cases seen at their practice were, in fact, cases of cytolytic vaginosis. A Bulgarian study, taking into account (and thus not biased by) confusion with VVC, reported 3.9% among 1152 women to present with cytolytic vaginosis [31].

Cytolytic vaginosis, despite the predominance of lactobacilli, cannot be considered as a healthy condition, because it frequently comes with symptoms, such as vaginal itching, burning and irritation [27,28], and thick or thin white cheesy vaginal discharge, pruritus, dyspareunia and vulvar dysuria [29], for which alkalinizing sodium bicarbonate treatment can be prescribed. The condition has even been linked to invasive carcinoma of the cervix [32] and as a risk factor for post-operative infection after first-trimester abortion [33].

### 1.4. The 'normal' human vaginal econiche is unique to humans and is highly abnormal when compared to other mammals

Under normal conditions, i.e., vaginal eubiosis, the vaginal econiche of humans is usually dominated by one or two out of only five major species of lactobacilli and characterized by a pH below 4.5. In fact, pH is as low as 3.5 [4]. It is mostly not realized that this situation of monopolization and concurrent low vaginal pH is extraordinary, considering that the vagina is not a sterile place, and moreover, that it is unique among mammals, because all available data on composition of VMCs in other mammals – including that of primates [e.g., [34–36]] – indicate the presence of a mixture of species, low levels of glycogen [35] and of lactic acid, and a neutral pH [37]. This was recently documented in more detail by Miller et al. [37], who found that, across 10 studies of human women, median vaginal pH was 4.5 (range = 4.0–4.9), while median vaginal pH across non-human mammals, including many primate species, was 6.8, with no species falling into the range of normal human pH. Furthermore, while the same *Lactobacillus* species as in humans were present, the average relative abundance of lactobacilli was only 1.1% in non-human mammals compared to 69.6% in human women.

Stumpf et al. [38] hypothesized that the specific protective role of lactobacilli in humans might have evolved because human females, in particular, may experience relatively high