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**Exploring the global vaginal microbiome and its impact on human health**

Linda ABOU CHACRA<sup>1,2</sup> and Florence FENOLLAR<sup>1,2\*</sup>

<sup>1</sup>Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France

<sup>2</sup>IHU-Méditerranée Infection, Marseille, France

**\*Corresponding author:**

Prof. Florence Fenollar  
VITROME, Institut Hospitalo-Universitaire Méditerranée-infection  
19-21 Boulevard Jean Moulin  
13005 Marseille, France  
Phone: + 33 (0) 4 13 73 24 01  
Fax: + 33 (0) 4 13 73 24 02  
Email address: [florence.fenollar@univ-amu.fr](mailto:florence.fenollar@univ-amu.fr)

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## **Abstract**

Around the world, more than 175,000,000 women are diagnosed every year with gynaecological disease, in many cases contributing to high morbidity and mortality. For this reason, knowledge of the composition of the vaginal microbiome and its variations represents a real health challenge, as this is key to improving therapeutic management. This review traces the history of the poorly known vaginal microbiome and focuses on the latest findings concerning this ecosystem. Studies in the past decade have targeted complex bacterial communities within the vagina. However, due to the development of technology and the emergence of next generation sequencing (NGS), the exact definition of the vaginal microbiome has changed and can no longer be linked solely to the presence of bacteria. In order to reach a global view of the vaginal microbiome, it is essential to take into account all microorganisms that the vagina harbours, including fungi, viruses, archaea, and candidate phyla radiation. Although these communities represent only a minimal percentage of the vaginal microbiome, they may act as modifiers of its basic physiology and may play a key role in the maintenance of microbial communities, as well as metabolic and immune functions. Studies of the complex interactions between these different microorganisms have recently begun and are not yet fully understood. Results to date indicate that these microbial communities together constitute the first line of defence against infections. On the other hand, the slightest disturbance in this microbiome may lead to disease. For this reason, enhanced knowledge of these associations is critical to better identify predispositions to certain illnesses, which may open new therapeutic avenues. Currently however, only the tip of the iceberg is understood and current research on this ecosystem is revolutionising our knowledge and understanding of human health and disease.

## 1. Background

Although it is less studied than the intestinal ecosystem, the vaginal ecosystem is greatly significant for women's health. Given its impact on women's and infants' health, interest in this understudied topic is increasing [1]. Thus far, bacterial vaginosis has remained enigmatic in its pathogenesis, especially in recurrent forms, and it is not known whether a single condition or several conditions with overlapping diagnostic criteria are responsible [2,3]. Many questions arise: how does the change in lactobacilli flora manifest itself? What is the role of sexual intercourse? Is there a genetic predisposition? And most essentially, what is a healthy vaginal microbiome and how can it be characterised?

The vaginal microbiome includes a mixture of bacteria, eukaryotes [4], fungi [5], archaeobacteria [6] and viruses [7] (Figure 1). Most of these are commensal species that our bodies harbour from birth but which can cause infections in certain conditions. Many studies have focused on the bacteriome, which is considered to be the predominant component of the microbiome. While the mycobiome, virome, and human archaeome are still undervalued, they have a strong impact on human health and well-being [8].

In this review, we aim to comprehensively characterise the vaginal microbiome, beyond the standard bacterial characterisation, and explore the relationship between these microorganisms and human health. We began with a literature review targeting various databases (**Google, Google Scholar, PubMed, and Web of Science**) using the following keywords: vaginal microbiota/microbiome; bacteria; methanogens; virus; fungus; candidate phyla radiation, to highlight the latest findings on the vaginal microbiome.

## 2. Normal healthy bacterial vaginal microbiota

68 To understand the importance of the microorganisms present in the vagina and their effects on  
69 health, over the past decade the scientific community has studied the vaginal microbiota, which  
70 is a key issue towards better understanding and treating certain gynaecological conditions. The  
71 vagina is a moist biotope made up of a **changing and evolving** bacterial system according to  
72 different stages of life. Approximately 1 to 4 ml of vaginal fluid can contain  $10^8$  to  $10^9$  bacterial  
73 cells that play a major role in protecting the mucous membrane from infection and maintaining  
74 the physiological balance of the female genital tract [9]. Unlike other animals, women's vaginas  
75 are significantly acidic (pH of 4.5 or less) due to the presence of lactic acid-producing bacteria  
76 that are only found in weak quantities in other mammals [10].

77 The history of describing the vaginal flora began in 1892 when German gynaecologist Albert  
78 Döderlein discovered the importance of bacteria that produce lactic acid in the vagina [11]. In  
79 1895, his collaborator Krönig then described immobile, non-sporing gram-positive rods [12],  
80 which were then cultivated by Curtis in 1913 [13]. The term "Döderlein's bacillus" was used in  
81 gynaecological publications until 1928, when Stanley Thomas classified the bacterium as  
82 *Lactobacillus acidophilus* [14]. In 1980, Lauer, Helming and Kandler were able to distinguish  
83 several species of *Lactobacillus* previously referred to as *L. acidophilus* through DNA-DNA  
84 hybridisation [15]. These lactic acid bacteria dominate within the **optimal** vaginal flora, ensuring  
85 the ecological balance of the vagina [16,17]. The composition of the vaginal flora is not fixed  
86 during a woman's lifetime. **The menstrual cycle, age, pregnancy, ethnicity, sexual partners,**  
87 **sexual hygiene, smoking, antibiotic treatment and the ecosystem in general, especially the**  
88 **surrounding microbes such as those from the intestines,** have an influence on the microbial  
89 composition of the vaginal flora [18–21].

However, in most studies, only a few species have been duplicated, including *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus gasseri*, and *Lactobacillus jensenii* in terms of their vaginal abundance in most women of childbearing age, while other lactobacilli have been less isolated; e.g., *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius* and *Lactobacillus vaginalis* [9,22–24].

Based on 16 rRNA gene sequencing, the vaginal bacterial community has been revealed to consist mainly of lactobacilli categorised into five groups, referred to as types of community states (CST), specifically I, II, III, IV and V [9]. Four of them are dominated by *Lactobacillus*. The first is dominated by *L. crispatus*, the second by *L. gasseri*, the third by *L. iners*, and the fifth by *L. jensenii*, while the fourth contains a lower proportion of lactobacilli but is composed of a polymicrobial mixture of strict and facultative anaerobes (*Gardnerella*, *Atopobium*, *Mobiluncus*, *Prevotella*, etc.) [9,25–28]. **The production of lactic acid, which is a major function of women's health, appears to be maintained in all Lactobacillus-dominant communities.** Moreover, it has been shown that the frequency with which these communities occur differs according to ethnic origin, with type IV being more over-represented among Black and Hispanic women [9] in comparison to Asian and White cohorts (approximately 34% and 40% compared to 18% and 9%, respectively) .

As shown in Figure 2, CST I has the lowest pH ( $4.0 \pm 0.3$ ) and CST IV has the highest pH ( $5.3 \pm 0.6$ ) [9,27]. It is essential to note that *L. crispatus* is a stabilising element of the vaginal microbiota. This bacterium, in addition to producing lactic acid with a high content of D isomers, has many properties that hinder the **occurrence of dysbiosis**, particularly its ability to co-aggregate and produce bacteriocins and toxins. **Given the importance and characteristics of these lactic acid bacteria [29], the study of the human vaginal microbiota should be an**

essential step for diagnosis as well as for treatment with probiotics and prebiotics [30]. The development of metagenomics has expanded our ability to better understand the human microbiota [31]. This technology can provide a description of the composition of bacterial communities, but it has not resulted in a comprehensive and complete view of microbial characteristics and interactions [32,33].

Previous studies were conducted in the Vaginal Microbiome Project to characterize vaginal microorganisms [34], which identified, of the 2,776 bacterial species isolated in human beings [35], 581 species in the vagina. Of these, 122 different species were isolated using only a culture method, while 296 different species were detected using molecular biology. The remaining 163 species were identified by both methods [21]. The most predominant species belong to the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria [36,37].

Despite the fact that many microbes are resistant to culture, which is a serious obstacle to a better understanding of microbial interactions and pathogenesis, the use of this technique has made a strong comeback in recent years [38,39].

A study done in 2016 led to the identification of 101 species of bacteria. Among them, 17 had never been reported in the vagina, and 11 new species [40].

Culture, combined with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF) as a tool for bacterial identification, termed “Culturomics” [41], can increase the repertoire of microbial species naturally present in the vaginal flora.

Interestingly, among these 122 cultivated bacteria, 53 had never been previously reported in the vagina. This work also identified three new species: “*Vaginimicrobium propionicum*”, “*Vaginella massiliensis*” [42,43], and “*Ezakiella massiliensis*” [44,45].

In general, studies such as “The Integrative Human Microbiome Project” [46] can expand our knowledge of the composition of the vaginal microbial environment in health and disease and highlight the resulting differences for better management, especially for patients with bacterial vaginosis [47], in cases where in vitro fertilisation (IVF) has failed [48], for patients with cancer [49], and neonatal infections [50].

### **3. Bacterial vaginosis**

#### ***3.1 Vaginal microbiota***

Bacterial vaginosis is the result of a disturbance of the vaginal ecosystem, i.e., a significant depletion of lactobacilli following a strong proliferation of strict or facultative anaerobes previously in the minority in the vagina [51], such as *Gardnerella vaginalis*, *Atopobium vaginae*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Prevotella*, *Peptoniphilus*, *Megasphaera*, *Mobiluncus*, and other fastidious or hitherto uncultivated bacteria [36,52,53].

This story began in 1953, when Leopold first isolated *Gardnerella vaginalis* (formerly known as *Haemophilus vaginalis*) [54]. Two years later, Gardner and Dukes reported the presence of this microorganism in women with non-specific bacterial vaginitis [55]. In 1983, the term “bacterial vaginosis” was established as opposed to the more classic term “specific vaginitis” caused by *Candida albicans* or *Trichomonas vaginalis*, because local inflammatory markers of this disease are weaker [56].

#### ***3.2. Epidemiology***

Bacterial vaginosis is the most common cause of vaginal dysbiosis in women of childbearing age. However, it is very difficult to determine its exact prevalence, because figures vary greatly depending on geographical location, patient age, socioeconomic background, type of consultation, and pregnancy status [3]. Most cases of bacterial vaginosis occur in women



between the ages of 15 and 44, and the incidence is very low in prepubertal and postmenopausal women [57]. Estimates of worldwide prevalence vary from 4% among European women to more than 50% among women in Eastern or Southern Africa and among homosexual women [58–60]. Despite its description in 1954-1955, bacterial vaginosis remains an enigma. Its exact aetiology remains elusive. The hypothesis of faecal transplant has been raised [61]. Indeed, the microbial population detected in bacterial vaginosis is very similar to the digestive microbiota, with the presence of bacteria belonging to the genera *Prevotella*, *Peptoniphilus*, and *Clostridium*. The presence of a gastro-intestinal flora associated with bacterial vaginosis may be linked to their inoculation during certain sexual practices, **according to a prospective study performed to investigate the induction of BV [62]. We noticed an initial decrease in the abundance of protective species of Lactobacillus, a marker of a healthy vaginal microbiota, and the appearance of a mixed flora in which A. vaginae, G. vaginalis, P. bivia and Megasphaera type I are the best indicators, causing an evolution of the vaginal microbiota from a state of eubiosis to dysbiosis [63], subsequently leading to a loss of vaginal equilibrium [64].** Five decades of intense research has revealed a number of risk factors for acquiring bacterial vaginosis. They may be endogenous factors such as hormonal changes, menstruation, or stress, but may also be exogenous, such as those related to intimate hygiene, sexual relations, including the number of partners, the administration of certain drugs, and education and socio-economic level [65,66]. This dysbiosis currently represents a public health problem. It is most often manifested by an abundant, greyish/greenish, malodorous leucorrhoea from the vagina. It should be noted that bacterial vaginosis can be asymptomatic in up to 50% of women, depending on the authors [67]. Studies have also shown that it is associated with an approximately two-fold increased risk of contracting sexually transmitted infections, including *Chlamydia trachomatis*,

*Neisseria gonorrhoeae*, herpes simplex type 2, and human immunodeficiency virus infection [68,69]. In addition, the disease is linked to serious gynaecological and obstetric complications (infertility, endometritis, prematurity, and low birth weight) [70–73] as well as psychosocial disorders (malodour phobia, feelings of shame, rejection by sexual partner) [74–76].

### 3.3. *Diagnosis*

Diagnosis is problematic and difficult due to its polymicrobial nature and a wide range of clinical features. The Amsel criteria [77] and the Nugent score [78] are the most commonly used diagnostic methods for bacterial vaginosis. These diagnostic tools present different problems. The Amsel criteria lack specificity and the Nugent score, even though it shows a higher specificity than the **Amsel criteria**, is largely limited by the lack of conclusion when assessing an intermediate flora. Some authors consider the intermediate flora as a transitional flora, while others consider it as a heterogeneous group of normal flora and bacterial vaginosis. The score is also strongly dependent on the technical experience of the operator and the quality of the smears. Several molecular tools have been developed to rationalise the diagnosis of bacterial vaginosis [79]. In particular, a quantitative real-time PCR has been developed to measure the bacteria present in bacterial vaginosis, such as *A. vaginae* and *G. vaginalis* [80–82].

### 3.4. *Treatment*

The main objective in treating bacterial vaginosis is to suppress the symptoms and signs of infection [83]. **The classic therapeutic approach is antibiotics as the recommended first-line treatment, 5–7 days of topical or oral metronidazole or seven days of intravaginal clindamycin, depending on personal choice and circumstances** [84,85], often associated with oestrogens [86], prebiotics and probiotics [28,87,88] to correct lactobacillus deficiency, although

to date few studies have been carried out. These strategies are indicated in symptomatic women and women who undergo certain surgical procedures [89].

Given that 50% of cases recur after three months of treatment [89,90], new strategies based on the transplantation of vaginal microbiomes from healthy women into patients with symptomatic and recurrent bacterial vaginosis are being considered as a therapeutic alternative [91,92]. In a recent study, five patients were thus treated, four of whom achieved long-term complete remission until the end of follow-up, between 5 and 21 months after transplantation of vaginal microbiomes. The results of the transplantation of vaginal microbiomes in women with bacterial vaginosis seem promising, but clinical trials will have to be conducted on larger cohorts of patients [54].

#### **4. Vaginal microorganisms other than bacteria**

##### ***4.1. Methanogens***

Methanogens, previously known as archaeobacteria, are strict anaerobic prokaryotes in the field of archaea that use hydrogen to reduce carbon dioxide, formate, acetate, and various methyl compounds to methane [93]. **Based on analyses of the 16S ribosomal RNA (rRNA) gene, archaea were first classified as a distinct group of prokaryotes in 1977 by Carl Woese [94].** Initially thought to be found only in extreme environments, further research has revealed that these organisms inhabit a variety of ecological niches, including mesophilic habitats such as sewage sludge, marine and lake sediments, geothermal springs, deep-sea hydrothermal vents, and the human body [6]. Although archaea and bacteria are similar in morphology in terms of cell size, shape, and organisation, archaea possess certain genes and metabolic pathways closely related to those of eukaryotes, such as replication, repair, transcription, and translation [95]. In addition, archaea have many unique characteristics. While bacteria and eukaryotes contain

227 membranes composed of glycerol ester lipids, archaeal membranes are composed of glycerol  
228 ether lipids, chemically more resistant than those composed of esters [96,97], making it more  
229 stable as well as unique metabolic capabilities, including phototrophic, organotrophic, and  
230 lithotrophic activities [98].

231 The human archaeome, including the mycobiome and virome, remains little studied in the field  
232 of research, although it can influence the composition and function of the entire microbial  
233 community, which generally affects human health [99]. Compared to bacteria, the human body  
234 contains only a small amount of methanogens, represented by *Methanobrevibacter smithii*  
235 [100,101], *Methanobrevibacter oralis* [102], *Methanosphaera stadtmanae* [103],  
236 *Methanomassiliicoccus luminyensis* [104], *Candidatus* “*Methanomassiliicoccus intestinalis*”, and  
237 *Candidatus* “*Methanomethylophilus alvus*”, associated with the oral, intestinal, vaginal and,  
238 more recently, skin mucosa. Their presence often suggests an indirect correlation with pathology  
239 [93]. Recent studies have also provided evidence of the presence of groups of archaea other than  
240 *Methanomethylophilus alvus*, notably certain thaumarcheotal and halophilic species that appear  
241 to be part of the human microbiome [105–108]. Indeed, research on archaea faces  
242 methodological problems, including DNA extraction, selection of appropriate primers, low  
243 abundance of archaeological DNA, or incomplete reference databases of 16S rRNA genes,  
244 preventing the complete evaluation of this archaeome, and also lack of motivation from a  
245 medical point of view [109,110].

246 Methanogenesis is a key component in preventing acid accumulation, i.e., pH increase, which  
247 implies a disruption of the vaginal microbiota. **An initial study in 1990** reported the culture of  
248 *M. smithii* in vaginal specimens from patients with bacterial vaginosis. Methanogenic activity  
249 was not detected in specimens from healthy subjects [101]. **Another study in 2019 showed the**

250 **presence of a single methanogen, *M. smithii*, mostly from bacterial vaginosis samples [111].**

251 Other studies have been conducted, but did not detect archaea in samples from healthy or ill  
252 women [112], except for one metagenomics study **that recognized fewer than 2% of the reads**  
253 **in each sample as archaeal [113].**

254 Improving detection techniques will provide new knowledge about the diversity of archaea in  
255 humans and clarify their potential role in disease progression [114].

#### 256 **4.2. Viruses**

257 The virome is a group of viruses present in a specific environment. Thus, the virome differs in  
258 every part of the body [115,116]. Indeed, viruses are microorganisms with a highly variable  
259 genomic structure consisting of either single or double-stranded DNA or RNA. Unlike bacteria,  
260 they have no preserved genes that can be used to characterise them [117,118].

261 Despite the small size of the viral genome and the low level of viral genome material in samples,  
262 the development of high-throughput DNA and RNA sequencing methods has greatly improved  
263 the ability to detect viruses in the human microbiome, though this often appears difficult in  
264 practice [119–121]. Although few studies have identified the viral components of the  
265 microbiome, the eukaryotic vaginal virome has now been characterised.

266 In one study by the Human Microbiome Project, many eukaryotic viruses were found in vaginal  
267 samples from healthy women of childbearing age [122]. Only DNA viruses have been  
268 characterised. Most studies indicate that there are many members of the *Papillomaviridae*  
269 family, followed by *Herpesviridae*, *Anelloviridae*, and *Poliomaviridae* in vaginal samples [123].  
270 *Alphapapillomavirus* was detected in 38% of subjects, as well as several types of herpes virus  
271 [123]. In addition, a number of these were found in several sampling periods, leading to the  
272 assumption that the viruses were well established and replicating easily. Detection of these

viruses in the vaginal samples was also associated with a large diversity of anaerobic bacteria [123]. **In contrast, a metagenomic study showed that there was no difference in viral composition among 180 participants. A distinct viral community was not identified among the four distinct cervicovaginal bacterial community types [124].**

**Additionally, a second metagenomic study showed the presence of six phage families in the vagina of most second-trimester pregnant women. Members of the Siphoviridae family were the most prevalent (69.2%). The other families were Myoviridae, Microviridae, Podoviridae, Herelleviridae, and Inoviridae, as well as six additional phages whose families could not be identified [125]. Diverse viruses of Caudovirales, the tailed bacteriophages, have also been identified [124]. Interestingly, the composition of the vaginal phages, as well as the viral community, varied considerably from one woman to another but independently of the vaginal bacterial composition and pregnancy outcome.** Furthermore, Jakobsen et al. performed a metagenomic sequencing of purified virus-like particles in vaginal specimens from 48 patients undergoing treatment for non-female factor infertility [126]. Most of the vaginal viruses found belonged to the class of double-stranded DNA bacteriophages, with eukaryotic viruses constituting 4% of the total reads. It should be noted that eukaryotic viral communities were also correlated with bacterial vaginosis. A further study assayed the presence of nucleic acids of six families of human DNA viruses in vaginal samples from pregnant women, including *Papillomaviridae*, *Polyomaviridae*, *Herpesviridae*, *Poxviridae*, *Adenoviridae*, and *Anelloviridae* viruses [126]. In addition, the presence of a very diverse virome may increase the risk of premature delivery. Changes within the vaginal virome have the same effects as vaginal bacterial changes during pregnancy, suggesting that pregnancy may be regulated not only by bacterial communities but also by viral communities [4].

As previously discussed, some eukaryotic viruses can be harboured in symptomatic and asymptomatic women and play a role in health and disease by influencing host physiology. Many of them induce effects through the direct interaction of viruses with the host, while others do so through the interaction of the virus with other components of the microbiome, especially bacterial-viral correlation [7,127]. It is likely that other effects of the viral presence, both acute and long-term, which may be associated with important outcomes, remain to be defined.

### **4.3. *Fungi***

Like bacteria, fungi are part of the normal flora of the epithelium and endothelium of the human body [128]. The vaginal mycobiome, defined as the fungal component of the vaginal microbiome, is an important part of the vaginal microbial ecosystem in many healthy women, but has not been the subject of a great deal of study in relation to bacterial communities [5]. The joint program of the National Institutes of Health Common Fund, the Human Microbiome Project and MetaHIT often prioritises the bacterial community and pays less attention to the fungal community [1,129,130]. In order to better understand contributions of the human fungal community to health and disease, several studies have been conducted. The first research on fungi residing in the vagina was based on culture [131,132].

With the emergence of genomic methods in the study of human fungi, which greatly expanded the ability to analyze human microbes, next-generation sequencing methods made it possible to determine the diversity of the characteristic commensal fungi in the vagina. However, despite advances in genomics, the characterisation of the mycobiome has been significantly limited due to the scarcity of databases used to classify fungal sequences, so many species cannot be classified and are difficult to analyse because the samples are extremely sensitive to environmental contamination. Therefore, the significant effects of the vaginal mycobiome on

patient health may be poorly evaluated. **To date, two studies have presented the vaginal mycobiome apart from culture, targeting 18S ribosomal DNA (rDNA) with small subunits and 28S rDNA with large subunits, termed internally transcribed spacer regions [133,134].** Nevertheless, the role of *Candida* spp. has long been recognised in the vagina as colonisers of the genital epithelium without causing infection, and several studies of the vaginal mycobiome using culture or sequencing have demonstrated that *Candida* spp., especially *C. albicans*, is found in the vaginal tracts of 20–70% of asymptomatic healthy women [5,135,136]. These yeasts are often transported asymptotically, but after intense antibiotic use or when the host's immune system is attenuated, the balance between these fungi and the host can be disturbed, and the shift from the commensal phase (yeast morphology) to a pathogenic phase (hyphae form) becomes possible, making it the key element in the pathogenesis of vulvovaginal candidiasis. In addition to immune abnormalities and dysbiosis of the flora, *Candida* spp. also expresses other virulence factors that lead to vulvovaginal candidiasis, such as extracellular hydrolytic enzymes and heat shock proteins, and the formation of microbial biofilms may play a role in this infection. It has been consistently shown that there is a correlation between vulvovaginal candidiasis and the luteal phase, leading to a high oestrogenic level [137–140]. However, *Candida* is able to adapt to changes in the nutritional microenvironment and use lactate as a dietary source [131]. This is very important in the vaginal context, given the abundance of glycogen and lactic acid, and *Candida* may have evolved to slow immune responses in order to facilitate its own persistence and commensalism [8,141].

A recent study of 294 healthy Estonian women of childbearing age was carried out on the vaginal microbiome to clarify its fungal composition. ITS1-2 sequencing was performed on 251 of these samples [128]. In total, *Ascomycota* constituted 58% of the human fungi and



*Saccharomycetales* 37%, including 16 different original taxonomic units of *Candida* spp., which were the most abundant (34% of the total). One percent of the total was *Cladosporium*, *Eurotium*, *Alternaria*, and *Basidiomycota Rhodotorula*. A large percentage of the total sequences were not classified in the fungal community [128]. Many sequences could not be mapped to specific taxa. This underlines the need to increase our knowledge in this field.

#### **4.4. Candidate phyla radiation**

Candidate phyla radiation is a group of microorganisms that are abundantly distributed in many environments, including acidic, alkaline, and hypersaline habitats, and in the human body. In 2016, Hug et al. presented a new version of the tree of life using culture-independent genomic methods. The genomes of the microorganisms were reconstructed without prior culture [142]. The first genome projects for this group were reported in 2012. They appear to be monophyletic, corresponding to a significant fraction of the bacterial domain, with at least 74 lineages mainly at the phylum level. Therefore, it is possible that the root of the bacterial domain may be placed between well-known bacteria and new organisms known as candidate phyla radiation (CPR) [143,144]. Due to poor coupling of primers and/or introns, the detection of certain groups within the CPR is difficult. To date, we estimate the identification of 73 CPR groups. The term “CPR” is simply a representation of this considerable radiation. Several studies should be undertaken in order to better describe this radiation before proposing an official name for it. In fact, the phylum *Saccharibacteria*, formerly known as TM7, is part of the CPR.

Compared to bacteria, TM7x has a very small genome and ultra-small cell size, as well as an episymbiotic lifestyle on the surface of its host bacterium, and it is important to note that these ultra-small bacteria have limited metabolic capacity. Having minimal amino acids, they are

364 unable to synthesise new nucleotides and lack the essential components to produce the  
365 membrane lipids required for the cell envelope, which other bacteria must parasitise [145–147].  
366 Depending on environmental situations, these enigmatic bacteria associated with humans can  
367 affect the structure and functionality of the bacterial host and lead to their destruction, resulting  
368 in disruption of the microbiome as well as induction of infectious diseases.

369 Following a study based on the analysis of vaginal secretions of women with bacterial vaginosis  
370 compared to women with normal flora by 16S rRNA PCR and fluorescence in situ hybridisation  
371 (FISH), the results show the presence of TM7 members of the uncultured bacteria in women with  
372 bacterial vaginosis [148].

373 In addition, recent research on the vaginal microbiome and prematurity based on extensive  
374 longitudinal analyses of 16S ribosomal rRNA, metagenomics, metatranscriptome, cytokine, and  
375 lipid profiles indicates that women who gave birth prematurely showed reduced levels of  
376 *Lactobacillus crispatus* and higher levels of TM7-H1 and other anaerobic bacteria [149]. In  
377 addition, identification of the same TM7-H1 taxon (i.e., BVAB-TM7) in samples from preterm  
378 infants was demonstrated. While TM7-H1 has been associated with adverse health conditions,  
379 this is the first reported association between preterm birth and TM7-H1 [149]. Indeed, with the  
380 ~0.72 megabase genome, TM7-H1 has been classified as Phylum *Candidatus Saccharibacteria*,  
381 and shows approximately 66% nucleotide identity with the recently described oral TM7x isolate.  
382 It denotes  $\alpha$ -amylase and can use glycogen as a carbon source. Like TM7x, TM7-H1 does not  
383 have new biosynthetic capabilities and may survive at the expense of other vaginal organisms  
384 that are yet unknown. However, this microorganism can produce pyruvate, acetate, l-lactate, and  
385 propionate. This production reduces antimicrobial activity and promotes the formation of pro-

inflammatory cytokines in the vagina, leading to disease. These results highlight new possibilities for assessing the risk of premature birth [149].

## **5. Conclusions and perspectives**

It has always been thought that the presence of microorganisms in the human body was unwanted due to their ability to cause infection, and many studies are part of the Human Microbiome Project, which aims to describe and analyse the different **microbiota** that make up our body and their impact on human health and disease. A global view of the vaginal microbiome represents a primordial issue in women's public health, namely that a better understanding of its composition allows for improved diagnosis and therapeutic management of patients. Various studies confirm that the term "normal vaginal flora" is relative and can be wrongly applied, depending on the case, given that the vast majority of these studies have always been oriented towards bacterial communities due to their abundance in our bodies and the challenges of searching for other organisms. It is therefore essential to consider all other organisms that exist in the vagina, including fungi, viruses, archaea, and CPR. Focusing on their identification is crucial in order to look for correlations between changes in the microbiome and human health, and may open new therapeutic avenues.

In order to fully understand the whole iceberg rather than simply the top, it is time to revisit the subject in the light of technological progress. An improved use of biological tools allows for a better identification of the vaginal microbiota and their association with disease. Nevertheless, many questions remain unanswered. Future work concerning the structure of the complete vaginal microbiota, the physiological conditions of the vagina, immune regulation and, above all, the clinical picture, could lead to a comprehensive characterisation of the microbiome, as well as a better elucidation of the balance of microbiome-host dynamics. Indicators of success will be a

409 new definition of the normal vaginal microbiome, based on the functional status and properties  
410 of the individual microorganisms that compose it.

411

#### 412 **Authors'statements**

413 Linda Abou Chacra performed bibliographic search and drafted the manuscript, and Florence  
414 Fenollar designed the study, drafted and revised the manuscript.

#### 415 **Declaration of competing interest**

416 The authors declare no conflicting interest in the present study.

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**Figure legend.**

**Figure 1. The vagina comprises different microorganisms.**

The vaginal microbiome can be described by the microorganisms that compose it: bacteria, fungi, viruses, archaea and recently, candidate phyla radiation (CPR). To date, bacteria are the best known and described. The vaginal bacteriome contains mainly *Firmicutes* with a strong predominance of *Lactobacilli* as well as *Bacteroidetes*, *Actinobacteria*, *Fusobacteria*. The mycobiome is mainly composed of *Ascomycota* and *Saccharomycetales*, and one percent of the total is *Cladosporium*, *Eurotium*, *Alternaria*, and *Basidiomycota Rhodotorula*. The virome is composed of *Papillomaviridae*, *Polyomaviridae*, *Herpesviridae*, *Poxviridae*, *Adenoviridae*, and *Anelloviridae*. The archeome is composed of *Methanobrevibacter smithii*. Finally, the CPR contains *Saccharibacteria*.

**Figure 2. Main characteristics of the five types of vaginal bacterial community states**

[9,150]

There are five vaginal **bacterial** community states. The first is dominated by *Lactobacillus crispatus*, the second by *L. gasseri*, the third by *L. iners*, and the fifth by *L. jensenii*. While the fourth does not contain a significant number of *Lactobacilli*, it is composed of a polymicrobial mixture of strict and facultative anaerobes (*Gardnerella*, *Atopobium*, *Mobiluncus*, *Prevotella*). Indeed, it has been shown that the frequency of these communities differs according to ethnic origin, with type IV being more common in African and Hispanic women than in Asian and White women.

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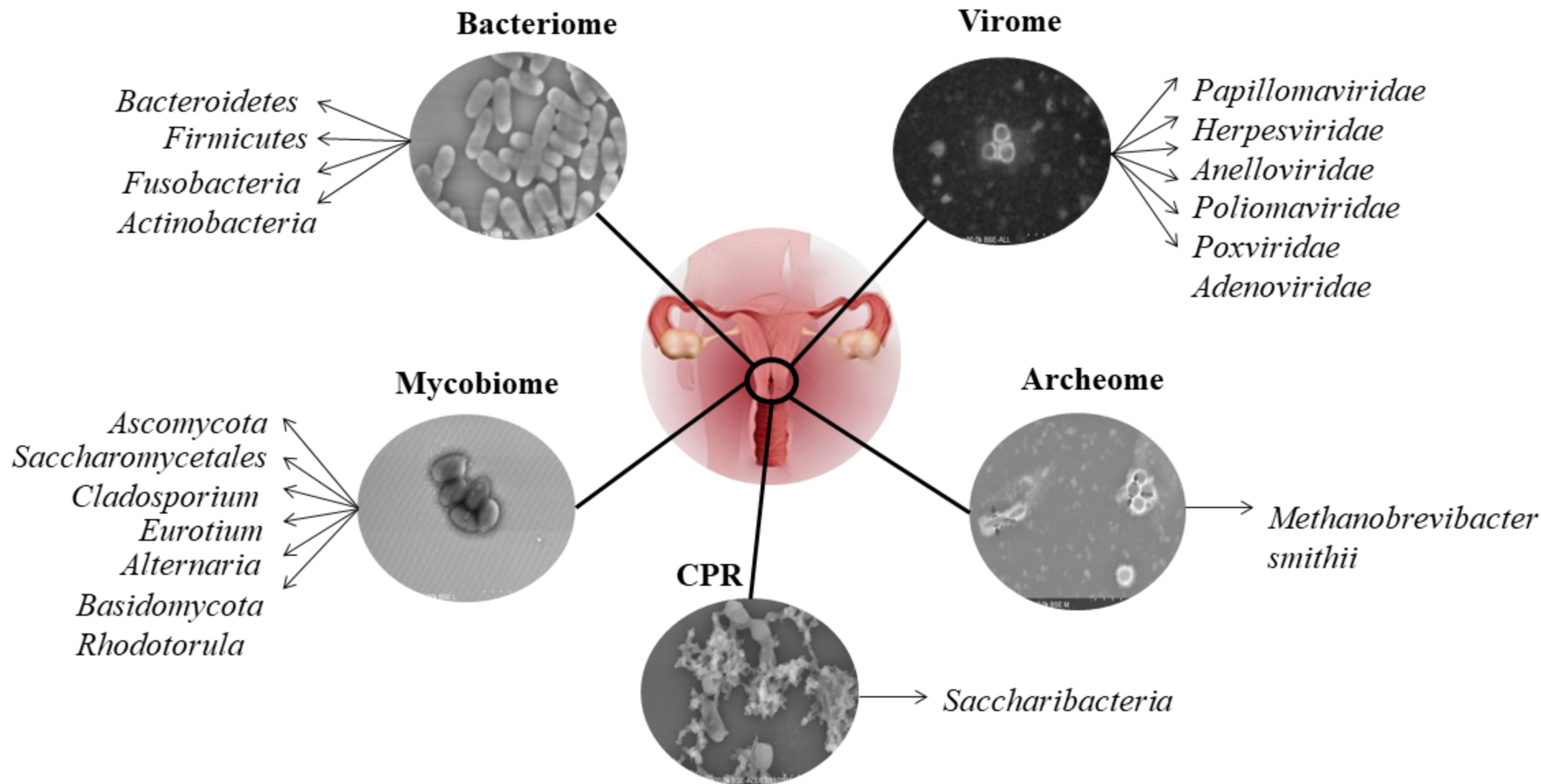
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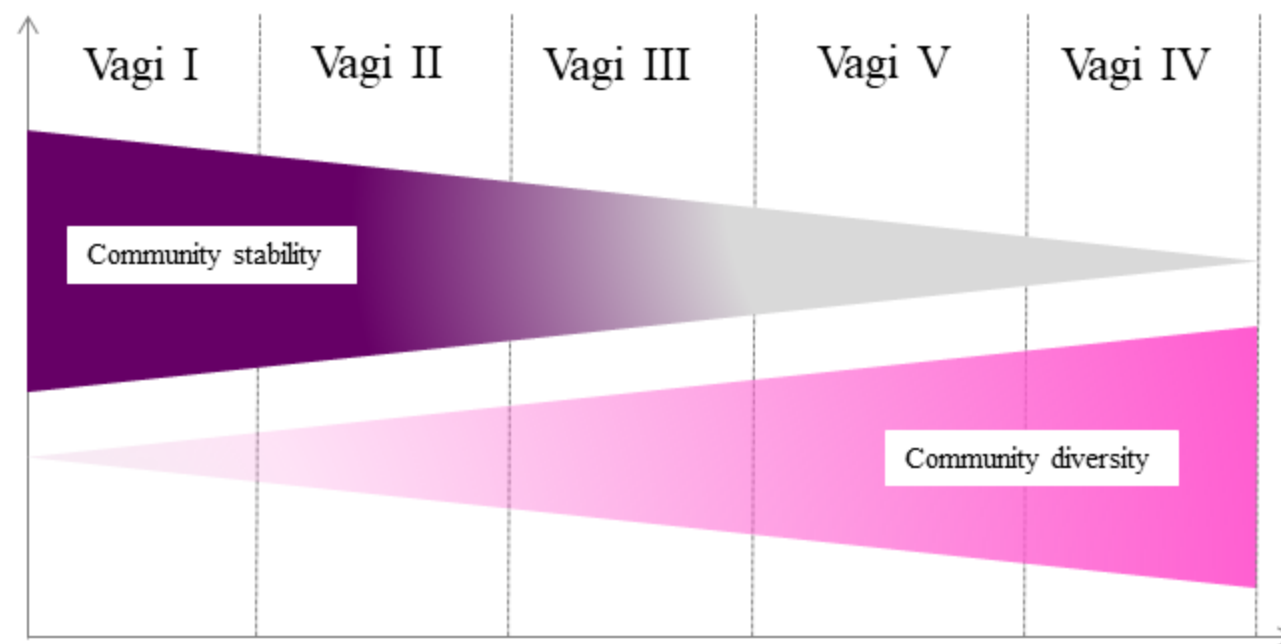


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**Figure 1. Schema of the vaginal microbiome.** The vagina comprises different microorganisms such as bacteria, fungi, archaea, viruses as well as candidate phyla radiation (CPR).



**Figure 2. Main characteristics of the five types of vaginal bacterial community states [9,150] according to lactobacillus diversity, pH, ethnicity.**

<b>Bacteria</b>	<i>L. crispatus</i>	<i>L. gasseri</i>	<i>L. iners</i>	<i>L. jensenii</i>	<b>Mixture of anaerobic bacteria</b>
<b>pH</b>	4.0 ± 0.5	5.0 ± 0.7	4.4 ± 0.6	4.7 ± 0.4	5.3 ± 0.6
<b>Ethnic background</b>					
<b>Caucasian</b>	30-50%	<10%	15-30%	<10%	<10%
<b>Hispanic</b>	<10%	<10%	30-50%	<10%	30-50%
<b>Asian</b>	15-30%	<10%	30-50%	<10%	15-30%
<b>African</b>	15-30%	<10%	30-50%	<10%	30-50%