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Linda Abou Chacra, Florence Fenollar

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

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

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

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

7

8           **\*Corresponding author:**

9           Prof. Florence Fenollar

10          VITROME, Institut Hospitalo-Universitaire Méditerranée-infection

11          19-21 Boulevard Jean Moulin

12          13005 Marseille, France

13          Phone: + 33 (0) 4 13 73 24 01

14          Fax: + 33 (0) 4 13 73 24 02

15          Email address: [florence.fenollar@univ-amu.fr](mailto:florence.fenollar@univ-amu.fr)

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23

24 **Abstract**

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

## 46 **1. Background**

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

55 The vaginal microbiome includes a mixture of bacteria, eukaryotes [4], fungi [5], archaeobacteria  
56 [6] and viruses [7] (Figure 1). Most of these are commensal species that our bodies harbour from  
57 birth but which can cause infections in certain conditions. Many studies have focused on the  
58 bacteriome, which is considered to be the predominant component of the microbiome. While the  
59 mycobiome, virome, and human archaeome are still undervalued, they have a strong impact on  
60 human health and well-being [8].

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

## 67 **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].

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

95 Based on 16 rRNA gene sequencing, the vaginal bacterial community has been revealed to  
96 consist mainly of lactobacilli categorised into five groups, referred to as types of community  
97 states (CST), specifically I, II, III, IV and V [9]. Four of them are dominated by *Lactobacillus*.  
98 The first is dominated by *L. crispatus*, the second by *L. gasseri*, the third by *L. iners*, and the  
99 fifth by *L. jensenii*, while the fourth contains a lower proportion of lactobacilli but is composed  
100 of a polymicrobial mixture of strict and facultative anaerobes (*Gardnerella*, *Atopobium*,  
101 *Mobiluncus*, *Prevotella*, etc.) [9,25–28]. **The production of lactic acid, which is a major**  
102 **function of women’s health, appears to be maintained in all Lactobacillus-dominant**  
103 **communities.** Moreover, it has been shown that the frequency with which these communities  
104 occur differs according to ethnic origin, with type IV being more over-represented among Black  
105 and Hispanic women [9] in comparison to Asian and White cohorts (approximately 34% and  
106 40% compared to 18% and 9%, respectively) .

107 As shown in Figure 2, CST I has the lowest pH ( $4.0 \pm 0.3$ ) and CST IV has the highest pH ( $5.3 \pm$   
108  $0.6$ ) [9,27]. It is essential to note that *L. crispatus* is a stabilising element of the vaginal  
109 microbiota. This bacterium, in addition to producing lactic acid with a high content of D isomers,  
110 has many properties that hinder the **occurrence of dysbiosis**, particularly its ability to co-  
111 aggregate and produce bacteriocins and toxins. **Given the importance and characteristics of**  
112 **these lactic acid bacteria [29], the study of the human vaginal microbiota should be an**

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

118 Previous studies were conducted in the Vaginal Microbiome Project to characterize vaginal  
119 microorganisms [34], which identified, of the 2,776 bacterial species isolated in human  
120 beings [35], 581 species in the vagina. Of these, 122 different species were isolated using  
121 only a culture method, while 296 different species were detected using molecular biology.  
122 The remaining 163 species were identified by both methods [21]. The most predominant  
123 species belong to the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria  
124 [36,37].

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

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

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

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

136 In general, studies such as “The Integrative Human Microbiome Project” [46] can expand our  
137 knowledge of the composition of the vaginal microbial environment in health and disease and  
138 highlight the resulting differences for better management, especially for patients with bacterial  
139 vaginosis [47], in cases where in vitro fertilisation (IVF) has failed [48], for patients with cancer  
140 [49], and neonatal infections [50].

### 141 **3. Bacterial vaginosis**

#### 142 ***3.1 Vaginal microbiota***

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

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

#### 154 ***3.2. Epidemiology***

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



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

182 *Neisseria gonorrhoeae*, herpes simplex type 2, and human immunodeficiency virus infection  
183 [68,69]. In addition, the disease is linked to serious gynaecological and obstetric complications  
184 (infertility, endometritis, prematurity, and low birth weight) [70–73] as well as psychosocial  
185 disorders (malodour phobia, feelings of shame, rejection by sexual partner) [74–76].

### 186 **3.3. Diagnosis**

187 Diagnosis is problematic and difficult due to its polymicrobial nature and a wide range of clinical  
188 features. The Amsel criteria [77] and the Nugent score [78] are the most commonly used  
189 diagnostic methods for bacterial vaginosis. These diagnostic tools present different problems.  
190 The Amsel criteria lack specificity and the Nugent score, even though it shows a higher  
191 specificity than the **Amsel criteria**, is largely limited by the lack of conclusion when assessing  
192 an intermediate flora. Some authors consider the intermediate flora as a transitional flora, while  
193 others consider it as a heterogeneous group of normal flora and bacterial vaginosis. The score is  
194 also strongly dependent on the technical experience of the operator and the quality of the smears.  
195 Several molecular tools have been developed to rationalise the diagnosis of bacterial vaginosis  
196 [79]. In particular, a quantitative real-time PCR has been developed to measure the bacteria  
197 present in bacterial vaginosis, such as *A. vaginae* and *G. vaginalis* [80–82].

### 198 **3.4. Treatment**

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

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

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

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

##### 215 ***4.1. Methanogens***

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

273 viruses in the vaginal samples was also associated with a large diversity of anaerobic bacteria  
274 [123]. **In contrast, a metagenomic study showed that there was no difference in viral**  
275 **composition among 180 participants. A distinct viral community was not identified among**  
276 **the four distinct cervicovaginal bacterial community types [124].**

277 **Additionally, a second metagenomic study showed the presence of six phage families in the**  
278 **vagina of most second-trimester pregnant women. Members of the Siphoviridae family**  
279 **were the most prevalent (69.2%). The other families were Myoviridae, Microviridae,**  
280 **Podoviridae, Herelleviridae, and Inoviridae, as well as six additional phages whose families**  
281 **could not be identified [125]. Diverse viruses of Caudovirales, the tailed bacteriophages,**  
282 **have also been identified [124]. Interestingly, the composition of the vaginal phages, as well**  
283 **as the viral community, varied considerably from one woman to another but independently**  
284 **of the vaginal bacterial composition and pregnancy outcome.** Furthermore, Jakobsen et al.  
285 performed a metagenomic sequencing of purified virus-like particles in vaginal specimens from  
286 48 patients undergoing treatment for non-female factor infertility [126]. Most of the vaginal  
287 viruses found belonged to the class of double-stranded DNA bacteriophages, with eukaryotic  
288 viruses constituting 4% of the total reads. It should be noted that eukaryotic viral communities  
289 were also correlated with bacterial vaginosis. A further study assayed the presence of nucleic  
290 acids of six families of human DNA viruses in vaginal samples from pregnant women, including  
291 *Papillomaviridae, Polyomaviridae, Herpesviridae, Poxviridae, Adenoviridae, and Anelloviridae*  
292 viruses [126]. In addition, the presence of a very diverse virome may increase the risk of  
293 premature delivery. Changes within the vaginal virome have the same effects as vaginal bacterial  
294 changes during pregnancy, suggesting that pregnancy may be regulated not only by bacterial  
295 communities but also by viral communities [4].

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

### 302 **4.3. Fungi**

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

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

319 patient health may be poorly evaluated. **To date, two studies have presented the vaginal**  
320 **mycobiome apart from culture, targeting 18S ribosomal DNA (rDNA) with small subunits**  
321 **and 28S rDNA with large subunits, termed internally transcribed spacer regions [133,134].**  
322 Nevertheless, the role of *Candida* spp. has long been recognised in the vagina as colonisers of  
323 the genital epithelium without causing infection, and several studies of the vaginal mycobiome  
324 using culture or sequencing have demonstrated that *Candida* spp., especially *C. albicans*, is  
325 found in the vaginal tracts of 20–70% of asymptomatic healthy women [5,135,136]. These yeasts  
326 are often transported asymptotically, but after intense antibiotic use or when the host's  
327 immune system is attenuated, the balance between these fungi and the host can be disturbed, and  
328 the shift from the commensal phase (yeast morphology) to a pathogenic phase (hyphae form)  
329 becomes possible, making it the key element in the pathogenesis of vulvovaginal candidiasis.  
330 In addition to immune abnormalities and dysbiosis of the flora, *Candida* spp. also expresses  
331 other virulence factors that lead to vulvovaginal candidiasis, such as extracellular hydrolytic  
332 enzymes and heat shock proteins, and the formation of microbial biofilms may play a role in this  
333 infection. It has been consistently shown that there is a correlation between vulvovaginal  
334 candidiasis and the luteal phase, leading to a high oestrogenic level [137–140]. However,  
335 *Candida* is able to adapt to changes in the nutritional microenvironment and use lactate as a  
336 dietary source [131]. This is very important in the vaginal context, given the abundance of  
337 glycogen and lactic acid, and *Candida* may have evolved to slow immune responses in order to  
338 facilitate its own persistence and commensalism [8,141].  
339 A recent study of 294 healthy Estonian women of childbearing age was carried out on the  
340 vaginal microbiome to clarify its fungal composition. ITS1-2 sequencing was performed on 251  
341 of these samples [128]. In total, *Ascomycota* constituted 58% of the human fungi and



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

#### 347 **4.4. Candidate phyla radiation**

348 Candidate phyla radiation is a group of microorganisms that are abundantly distributed in many  
349 environments, including acidic, alkaline, and hypersaline habitats, and in the human body. In  
350 2016, Hug et al. presented a new version of the tree of life using culture-independent genomic  
351 methods. The genomes of the microorganisms were reconstructed without prior culture [142].  
352 The first genome projects for this group were reported in 2012. They appear to be monophyletic,  
353 corresponding to a significant fraction of the bacterial domain, with at least 74 lineages mainly at  
354 the phylum level. Therefore, it is possible that the root of the bacterial domain may be placed  
355 between well-known bacteria and new organisms known as candidate phyla radiation (CPR)  
356 [143,144]. Due to poor coupling of primers and/or introns, the detection of certain groups within  
357 the CPR is difficult. To date, we estimate the identification of 73 CPR groups. The term “CPR”  
358 is simply a representation of this considerable radiation. Several studies should be undertaken in  
359 order to better describe this radiation before proposing an official name for it. In fact, the phylum  
360 *Saccharibacteria*, formerly known as TM7, is part of the CPR.

361 Compared to bacteria, TM7x has a very small genome and ultra-small cell size, as well as an  
362 episymbiotic lifestyle on the surface of its host bacterium, and it is important to note that these  
363 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-

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

## 388 **5. Conclusions and perspectives**

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

402 In order to fully understand the whole iceberg rather than simply the top, it is time to revisit the  
403 subject in the light of technological progress. An improved use of biological tools allows for a  
404 better identification of the vaginal microbiota and their association with disease. Nevertheless,  
405 many questions remain unanswered. Future work concerning the structure of the complete  
406 vaginal microbiota, the physiological conditions of the vagina, immune regulation and, above all,  
407 the clinical picture, could lead to a comprehensive characterisation of the microbiome, as well as  
408 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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420 **Figure legend.**

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

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

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

432 [9,150]

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

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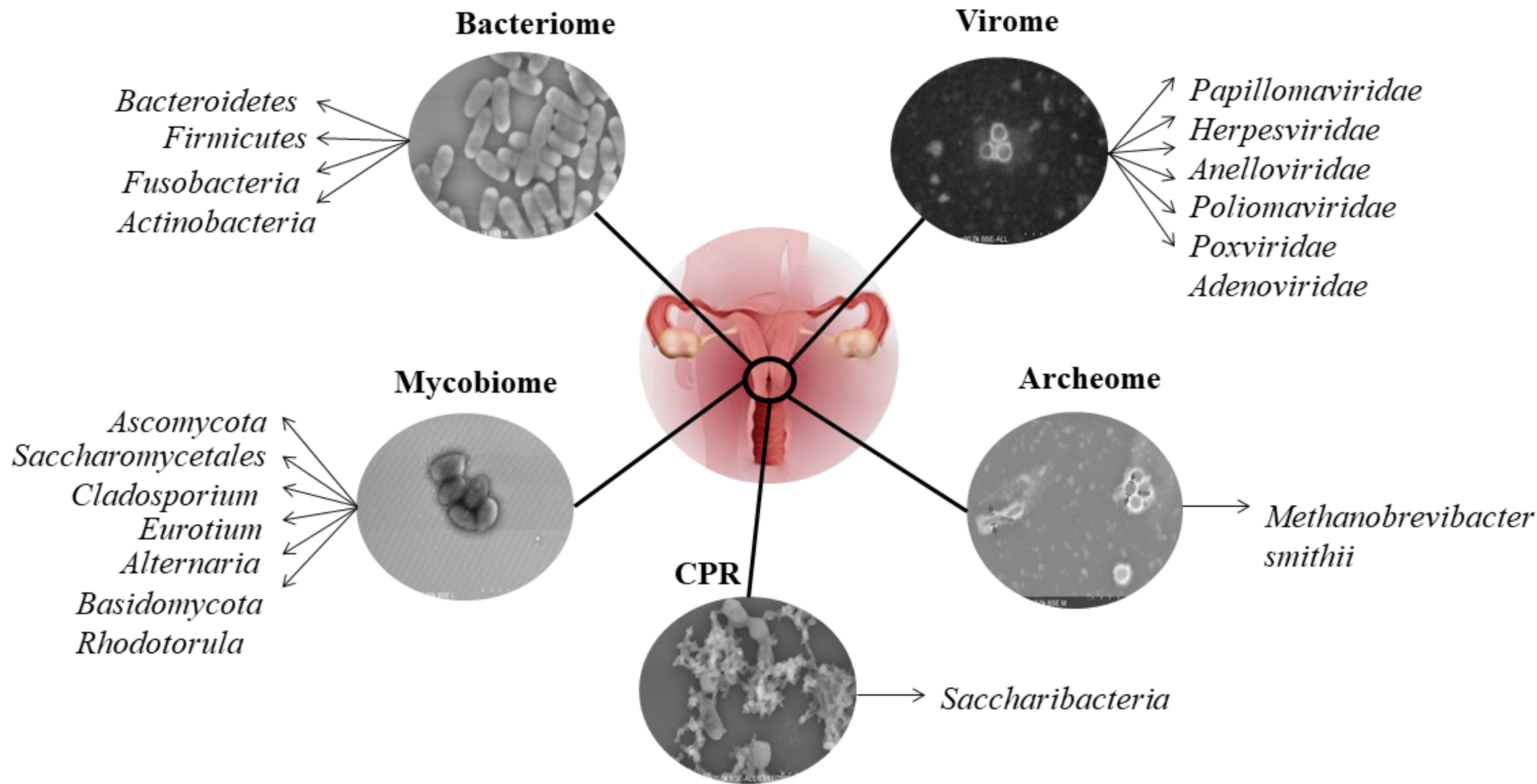
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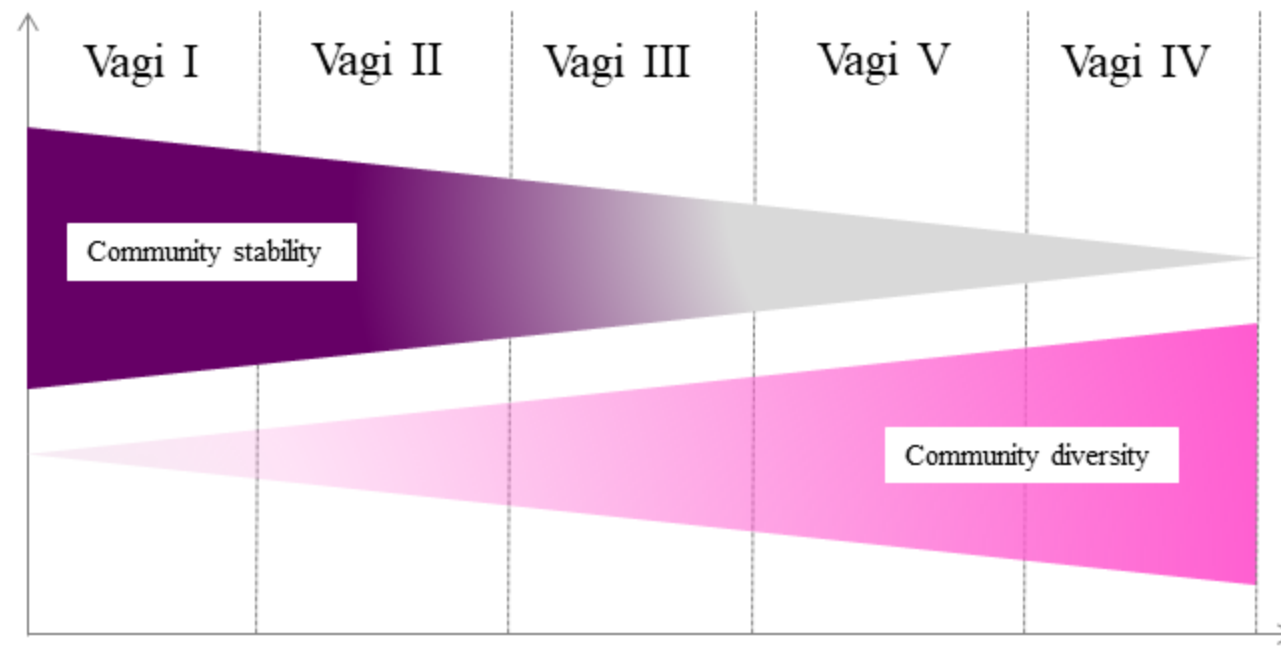


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**Figure 1. Schema of the vaginal microbiome.** The vagina comprises different microorganisms such as bacteria, fungi, archaea, viruses as well as candidate phyla radiation (CPR).



**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%                               |