Antagonism and synergism in *Gardnerella vaginalis* strains isolated from women with bacterial vaginosis

G. S. Teixeira,¹ K. L. K. Soares-Brandão,¹ K. M. G. R. Branco,¹
J. L. M. Sampaio,² R. M. D. Nardi,¹ M. Mendonça,³ R. B. Almeida,⁴
L. M. Farias,^{1†} M. A. R. Carvalho^{1†} and J. R. Nicoli^{1†}

Correspondence

J. R. Nicoli

jnicoli@icb.ufmg.br

¹Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

²Laboratório Fleury, São Paulo, SP, Brazil

³Departamento de Ginecologia e Obstetrícia, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

⁴Departamento de Propedêutica Complementar, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Antagonistic and synergistic substances are important for interactions between micro-organisms associated with human body surfaces, either in healthy or in diseased conditions. In the present study, such compounds produced by *Gardnerella vaginalis* strains isolated from women with bacterial vaginosis (BV) were detected *in vitro* and the antagonistic ones were partially characterized. Among 11 *G. vaginalis* strains tested, all showed antagonistic activity against at least one of the 22 indicator bacteria assayed. Interestingly, for some of these strains, antagonism reverted to synergism, favouring one of the indicator strains (*Peptostreptococcus anaerobius*) when the growth medium was changed. Partial characterization of antagonistic substances suggested a bacteriocin-like chemical nature. Depending on growth conditions, *G. vaginalis* isolated from women with BV produced antagonistic or synergistic compounds for other bacterial components of the vaginal ecosystem. This is the first report to our knowledge of the production of antagonistic and/or synergistic substances by *G. vaginalis*. This ability may be a pivotal factor in understanding BV and the ecological role of this bacterium in the vaginal environment.

Received 17 February 2010

Accepted 11 May 2010

INTRODUCTION

Bacterial vaginosis (BV) is a polymicrobial syndrome where indigenous *Lactobacillus* populations, which are usually dominant in the vagina of healthy women (Hillier, 2005), are replaced by a mixture of bacteria which generally includes *Gardnerella vaginalis*, *Prevotella/Bacteroides* species, *Peptostreptococcus* species, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Mobiluncus* species (Atassi *et al.*, 2006). All of these bacteria can be present in low populations in healthy women, but during BV their concentrations are generally increased 100–1000-fold over normal levels (Spiegel *et al.*, 1980). Which micro-organisms within the mixture associated with BV are essential for the syndrome or whether a single member may be the aetiological agent has never been resolved (Forsum *et al.*, 2005). Factors which lead to the decline of the lactobacilli and the overgrowth of an atypical

microbiota, as well as the sequence of bacterial population changes leading to BV, also remain unknown. Microbial interactions can probably modify the environment so that it becomes adverse for some micro-organisms by production of inhibitory compounds and suitable for other organisms by releasing specific growth factors (Pybus & Onderdonk, 1999).

Bacterial interactions, including synergism, commensalism and antagonism, are likely to play an important role in the ecology of the microbiota found on the human body surface. These phenomena may govern the atypical microbial population shifts observed during the development of BV. Pybus & Onderdonk (1999) described *in vitro* nutritional inter-relationships that can explain commensalism between *Prevotella bivia* and both *G. vaginalis* and *Peptostreptococcus anaerobius*. However, production of antagonistic substances may also be an important factor in the competitive colonization of the vaginal ecosystem. The lactobacilli metabolize glucose

†These authors contributed equally to this work.

Abbreviation: BV, bacterial vaginosis.

essentially to lactic acid, which contributes to the maintenance of a low vaginal pH (4.0–4.5) and reduces the growth of most pathogenic micro-organisms (Aroutcheva *et al.*, 2001). Among other antagonistic mechanisms, bacteriocinogenic activity is one of the most studied, and seems to contribute to the intra- and inter-regulation of the human microbiota, influencing microbial invasion and defence (Riley & Wertz, 2002). It has also been demonstrated that methodological factors such as medium composition, culture conditions (incubation atmosphere, pH, temperature) and bacterium growth phase may affect the expression of bacteriocin production (Lima *et al.*, 2002).

G. vaginalis, the only species recognized in the genus *Gardnerella*, is a fastidious, β -haemolytic, catalase-negative, non-motile, non-capsulated, rod-shaped bacterium. This Gram-negative/variably Gram-staining facultative anaerobic bacterium depends on a 5% CO₂ atmosphere for *in vitro* growth (Jarosik *et al.*, 1998) and is commonly found in the vaginal mucosa of asymptomatic women, but shows high concentrations in BV (Ingiani *et al.*, 1997). There are no reports in the literature on production of synergistic or antagonistic compounds by *G. vaginalis*.

The present study focused on evaluating the *in vitro* production of antagonistic and synergistic compounds by *G. vaginalis* strains isolated from women with BV.

METHODS

Bacterial strains. Ten isolates of *G. vaginalis* isolated from women with BV and a reference strain (ATCC 14018) were tested for their inhibitory and/or stimulatory activity. The clinical isolates were identified according to Piot *et al.* (1982). The isolates were tested against themselves and against 22 indicator strains. Two groups of indicator strains were used: six strains pertaining to a eubiotic vaginal ecosystem (*Lactobacillus*); and 16 strains generally associated with a dysbiotic vaginal ecosystem (see Table 1).

***In vitro* antagonistic or synergistic assay.** Antagonism or synergism phenomena were detected by the double layer diffusion method (Booth *et al.*, 1977). To evaluate the influence of culture medium composition on these activities, *G. vaginalis* strains were grown in *Brucella* broth (BB) supplemented with 10% horse-blood serum, 3% meat extract and 1% proteose peptone for 48 h, at 37 °C, in an anaerobic chamber (Forma Scientific) containing an atmosphere of 85% N₂, 10% H₂ and 5% CO₂. Then the cultures were spotted with a Steers replicator onto the surface of the different culture media (see Fig. 1) using as base *Brucella* agar (BA), brain heart infusion agar (BHIA), Columbia agar (CA) and tryptic soy agar (TSA). After incubation at 37 °C for 48 h under anaerobic conditions, the strains were killed by exposure to chloroform for 30 min. Residual chloroform was allowed to evaporate and the test cultures were overlaid with 4 ml BB soft agar (0.75%) supplemented with 10% horse-blood serum, which had been inoculated with 0.2 ml of a 48 h BB culture of the indicator strain. Plates were incubated for an additional 24–48 h period at 37 °C under an appropriate atmosphere for the indicator strain, and then evaluated for the presence of growth inhibition or stimulation zone. Autoantagonism was defined as the inhibition of a *G. vaginalis* strain against itself; isoantagonism as inhibition of a *G. vaginalis* strain against another *G.*

Table 1. *In vitro* frequency of auto-, iso- and heteroantagonistic activity of 11 *Gardnerella vaginalis* strains against potentially pathogenic or *Lactobacillus* indicator strains

Antagonistic assays were performed on BHIA supplemented with 0.5% glucose under anaerobic conditions at pH 7.5. Reference dysbiotic indicator strains were: *Mobiluncus mulieris* ATCC 35239, *Bifidobacterium bifidum* ATCC 29521, *Bifidobacterium longum* ATCC 15707, *Peptostreptococcus anaerobius* ATCC 27337, *Prevotella intermedia* ATCC 25611, *Bacteroides fragilis* ATCC 25285, *Porphyromonas gingivalis* FDC 381, *Enterococcus faecalis* ATCC 4083, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Salmonella enterica* serovar Typhimurium ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 15303, *Bacillus cereus* ATCC 11778, *Proteus vulgaris* ATCC 6380 and *Streptococcus mutans* IM/UFRJ. Reference eubiotic indicator strains were: *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus fermentum* ATCC 9338, *Lactobacillus brevis* ATCC 367, *Lactobacillus plantarum* ATCC 8014, *Lactobacillus delbrueckii* subsp. *lactis* ATCC 7830 and *Lactobacillus casei* subsp. *rhamnosus* ATCC 7469. GRF, *G. vaginalis* ATCC strain. Results are the number of positive assays/total number of assays.

Strain	Antagonism (%)				Mean %
	Auto-*	Iso-	Hetero- (dysbiotic)	Hetero- (eubiotic)	
GRF	+	80	75	50	68
G1	+	30	75	50	52
G2	+	70	56	17	48
G3	+	40	69	17	42
G4	–	30	13	33	25
G5	+	80	56	0	45
G6	+	40	69	0	36
G7	+	70	19	50	46
G8	+	40	50	17	36
G9	+	50	69	50	56
G10	+	30	69	33	44
Mean %		51	56	29	45

* +/–, Presence/absence of growth auto-inhibition.

vaginalis strain; and heteroantagonism the inhibition of a *G. vaginalis* strain against a bacterial strain of another genus.

The influence of pH on the antagonistic or synergistic activity was determined on BHIA supplemented with 0.5% glucose (BHIA-G) at different pH values (from 4.5 to 7.5 with 0.5 pH intervals). The plates were incubated in an anaerobic chamber for 48 h at 37 °C. The antagonistic or synergistic activity was determined as described above.

Detection of interfering factors for antagonistic activity. To exclude the eventual interference of bacteriophage action on the antagonistic phenomenon, a piece (3 mm in diameter) of agar was removed aseptically from the inhibition zone, according to the method described by Turner & Jordan (1981). The agar block was placed onto BHIA-G and recovered with 4.0 ml BHI broth soft agar, containing the indicator strain. Plates were then incubated for 48 h at 37 °C under anaerobiosis, before evaluation for the presence of lytic zones.

Fig. 1. Aspect of *in vitro* antagonism (a) and synergism (b) of *G. vaginalis*. (a) Isoantagonism between a producer strain of *G. vaginalis* and other *G. vaginalis* strains used as indicator. (b) Synergism between a *G. vaginalis* producer strain and *Peptostreptococcus anaerobius* ATCC 27337 as indicator. In both cases, the medium used was CA, pH 7.0, under microaerophilic and anaerobic conditions, respectively. Large arrows show the *G. vaginalis* growth spot and the smaller arrows show the inhibition (a) or overgrowth (b) halo of *Peptostreptococcus anaerobius*.

To search for possible inhibition by long-chain fatty acids, tests were conducted on BHIA-G with and without the addition of 1% soluble starch (Walstad *et al.*, 1974). The pH on the inhibition zone and on the non-inoculated medium was measured using a surface micro-electrode (Microelectrode Inc.).

Finally, to exclude a possible interference of residual chloroform on the growth of indicator strains, the evaluation of antagonistic activity was performed without its use. After the growth of the producer strains, the indicator strains were inoculated with a loop near the

spots of the producer strains without previous exposure to chloroform. The cultures were incubated at 37 °C for 48 h, under anaerobiosis, and then examined for the presence of inhibition zones.

Sensitivity to four proteolytic enzymes was tested as described by Farias *et al.* (1994). Fifty microlitres of enzyme solutions [proteinase K (Boehringer; 50 µl ml⁻¹); type II trypsin (Sigma; 200 µl ml⁻¹); papain (Sigma; 10 mg ml⁻¹); α-chymotrypsin] was placed onto the agar surface around the growing spots and allowed to cover areas larger than the expected zones of inhibition. Incubation was carried out for 4 h at 37 °C under anaerobiosis. Two controls were run: one only with the enzyme, and the other only with the producing bacterium. Sensitivity to temperature was determined by exposure of the culture to temperatures of 37, 45, 60, 70 and 80 °C for 20 min before the assay. The effects of these conditions were determined on the basis of their activity toward the inhibitory substances produced against the indicator strain *Peptostreptococcus anaerobius* ATCC 27337.

Statistical analysis. All the experiments were performed in duplicate or triplicate. Data were analysed by Fisher's exact test. The level of significance was set at $P < 0.05$. Statistical analyses were performed using the SigmaStat software (version 1.0; Jandel).

RESULTS AND DISCUSSION

Antagonism of *Lactobacillus* species from the eubiotic vaginal ecosystem against pathogenic micro-organisms responsible for BV is a well-known phenomenon (Aroutcheva *et al.*, 2001; Atassi *et al.*, 2006). Among these pathogenic micro-organisms, a few synergistic interactions with each other have been demonstrated (Pybus & Onderdonk, 1999). However, there are few reports of antagonistic activity of the pathogens with each other as well as against *Lactobacillus* species, especially concerning *G. vaginalis*. The aim of the present study was to evaluate a possible production of antagonistic substances by *G. vaginalis* strains which could lead to the disappearance of lactobacilli or any other competitor and to potentially pave the way for establishment of a BV dysbiotic microbiota.

A total of 23 554 assays were performed to evaluate the production of synergistic or antagonistic substances by *G. vaginalis*. Fig. 1 shows the aspect of representative assays where antagonism (Fig. 1a) and synergism (Fig. 1b) were observed. Diameters of the inhibitory zones ranged from 2 to 16 mm, depending on the producer and indicator strains, as well as on the physico-chemical conditions of growth.

Table 1 shows that autoantagonism was noted for all the strains except G4. When used as indicator, this strain did not show sensitivity to isoantagonism, and when used as producer showed one of the lower antagonistic activities (30%), together with G1 and G10. Some of the strains, such as G6 (100%), G1 (91%) and G10 (91%), were highly sensitive to isoantagonism. Concerning isoantagonism, reference and G5 strains showed the higher activity (80%). All the *G. vaginalis* strains produced heteroantagonistic substances against at least one of the dysbiotic indicator strains. This heteroantagonistic activity was higher for the reference (GRF) and G1 strains (75%),

and again lower for the G4 strain. Among the 16 dysbiotic indicator strains, *Peptostreptococcus anaerobius* was the most sensitive as demonstrated by the frequency of inhibition (100 %) and the mean diameter of the inhibition zone (11.5 mm). High sensitivity was also observed for *Proteus vulgaris* (91 %), *Staphylococcus aureus* (82 %), *Bacillus cereus* (82 %) and *Bacteroides fragilis* (82 %). However, *Enterococcus faecalis* and *Streptococcus mutans* did not exhibit sensitivity under any of the conditions studied. Table 1 also shows antagonistic activity of *G. vaginalis* against eubiotic indicator strains (*Lactobacillus*). *Lactobacillus acidophilus* was the most sensitive indicator strain, whereas *Lactobacillus brevis* and *Lactobacillus plantarum* were totally resistant to the antagonistic activity of the *G. vaginalis* strains. Among these last strains, the highest frequencies (50 %) of antagonistic activity were produced by strains G1, G7, G9 and the reference strain (GRF). Two *G. vaginalis* strains (G5 and G6) did not show antagonistic activity against the *Lactobacillus* strains tested. Globally, the GRF strain showed the highest antagonistic activity (68 %) and G4 strain the lowest (25 %). Iso- (51 %) and heteroantagonism against dysbiotic indicator strains (56 %) were more frequent than heteroantagonism against eubiotic indicator strains (29 %).

Most women with BV are frequently and heavily colonized by *G. vaginalis*, but the exact role of this bacterium in the development of the syndrome is not well understood. It is accepted that the levels of *G. vaginalis* in the vaginal site may be controlled by antagonism or synergism interactions between the different microbial species present, but the production of inhibitory compounds has never been described for this bacterium. The present study demonstrated that various *G. vaginalis* strains produced an inhibitory diffusible substance(s) with auto-, iso- and

heteroantagonism against a wide range of bacteria. The selection of the indicator strains was based on their importance in the vaginal ecosystem. Additionally, *Salmonella* Typhimurium, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Bacillus cereus*, *Proteus vulgaris*, *Streptococcus mutans*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* were also included in the study to evaluate the spectrum of action of the antagonistic substance, and because some of them could eventually be transient organisms of the vagina. Apparently, antagonistic capacity was not indispensable for the development of BV, since among the 11 *G. vaginalis* strains isolated from vaginal ecosystem of women affected by the syndrome, some of them showed a high antagonistic activity (56 % for G9) whereas others presented a low capability (25 % for G4). Interestingly, the higher activity of G1 was associated with a high sensitivity to isoantagonism (91 %) whereas G4 was simultaneously less sensitive and less productive. Concerning the indicator strains, the higher sensitivity of *Peptostreptococcus anaerobius* to the antagonistic activity of *G. vaginalis* was also observed when oral *Fusobacterium* species (Oliveira *et al.*, 1998), *Actinobacillus actinomyces-comitans* (Lima *et al.*, 2002) and *Eikenella corrodens* (Apolônio *et al.*, 2007) were used as producer strains. The antagonism observed against the *Lactobacillus* species could explain the substitution of this predominant bacterial genus regularly found in the eubiotic vaginal ecosystem by the dysbiotic microbiota found in BV. However, as pondered above, this heteroantagonistic activity was not found for all the *G. vaginalis* strains tested.

Fig. 2 shows the influence of culture medium on the mean frequency of antagonism for the 11 *G. vaginalis* strains, using *Peptostreptococcus anaerobius* as the indicator strain. Antagonist activity was best detected when BHIA and CA

Fig. 2. Influence of culture media on the antagonistic activity frequency of 11 *G. vaginalis* strains. The following media were used: 1, BHIA with 0.1 % glucose; 2, BHIA with 0.5 % yeast extract; 3, BHIA with 0.4 % sodium bicarbonate and 1 % yeast extract; 4, BHIA with 1 % glucose; 5, BHIA with 0.4 % sodium bicarbonate; 6, BHIA; 7, BHIA with 0.1 % yeast extract; 8, BHIA with 0.5 % glucose; 9, BHIA with 1 % yeast extract; 10, CA with 1 % proteose peptone; 11, CA; 12, CA with 1 % yeast extract; 13, CA with 0.4 % sodium bicarbonate; 14, BA; 15, BA with 1 % yeast extract; 16, BA with 1 % glucose; 17, CA with 0.1 % glucose; 18, CA with 0.5 % glucose; 19, BA with 0.5 % glucose; 20, BA with 0.4 % sodium bicarbonate; 21, BHIA with 1 % proteose peptone; 22, BA with 1 % proteose peptone; 23, CA with 1 % glucose; 24, BA with 0.1 % glucose; 25, TSA; 26, TSA with 0.5 % glucose. Different letters (a–m) indicate significant difference ($P < 0.05$).

were used. Supplementation with glucose, yeast extract and/or sodium bicarbonate did not show any consistent pattern of enhancement of the antagonistic activity. The best and similar ($P>0.05$) results were observed for BHIA and CA supplemented with 0.5 % and 0.1 % glucose, respectively. Antagonism was not observed when TSA was used, and a low frequency of this phenomenon was noted with BA.

During the experiments carried out to determine the influence of growth medium using *Peptostreptococcus anaerobius* as indicator strain, a reversion of antagonistic to synergistic inter-relationships was observed depending on the culture medium. Table 2 shows this synergistic activity of *G. vaginalis* strains enhancing the growth of *Peptostreptococcus anaerobius* on TSA medium. This phenomenon was enhanced when TSA was supplemented with 0.5 % glucose, except at a lower (5.0) and higher (7.5) pH.

It is well known that the production of antagonistic substance may be influenced by culture conditions such as medium composition, incubation atmosphere, pH, temperature and the growth phase of the micro-organism (Lima *et al.*, 2002). In the present study, among different combinations of media and supplements tested, higher production of antagonistic substance was observed when nutritionally rich culture media were used (BHIA and CA) when compared to media presenting lower nutritional value (TSA).

Another interesting and unexpected fact observed during the present study on growth conditions was their influence on the types of inter-relationships between the producer and the indicator strains. As described above, antagonism of *G. vaginalis* against *Peptostreptococcus anaerobius*

observed on BHIA and CA media was not found when TSA was used as growth medium. Surprisingly, in this last situation, a stimulation of *Peptostreptococcus anaerobius* growth was obtained around the *G. vaginalis* spot. However, these results are in agreement with data from the literature showing that production of inhibitory compounds, such as bacteriocin-like substances, needs nutritionally rich culture medium. Additionally, on a nutritionally poor culture medium, such as TSA (when compared to BHIA and CA), growth of more fastidious bacteria could be enhanced by growth factors produced by another bacterium. This might be the basis of the polymicrobial aetiology observed during BV with a clear association between *G. vaginalis* and various anaerobes (Pybus & Onderdonk, 1999; Cauci *et al.*, 2003; Eschenbach, 2007). Indeed, it is known that *G. vaginalis* produces amino and keto acids, such as succinate, which are considered growth factors for anaerobic bacteria.

The assay to detect the presence of bacteriophage in the inhibition zone was negative, indicating that antagonism was not due to the presence of a phage. The possibility of inhibition by long-chain fatty acids was also rejected because of the lack of the interference upon addition of soluble starch in the medium. No significant variations in pH values were detected inside and outside the inhibition zones, or in the non-inoculated medium. The experiments carried out without the use of chloroform gave the same results as those that included exposure to chloroform to kill the producer isolates.

Inhibitory activity was progressively reduced when exposed to temperatures of 37, 45 and 60 °C for 20 min, and was eliminated after exposure at 70 and 80 °C during the same period. The inhibitory activity produced by *G. vaginalis*

Table 2. *In vitro* synergistic activity of 11 *G. vaginalis* strains against *Peptostreptococcus anaerobius* ATCC 27337

Synergistic assays were performed on TSA supplemented or not with 0.5 % glucose and incubated at 37 °C under microaerophilic conditions. GRF, *G. vaginalis* ATCC strain; +, presence of growth stimulation; –, absence of growth stimulation.

Strain	Culture medium and pH value											
	TSA						TSA + 0.5 % glucose					
	5	5.5	6	6.5	7	7.5	5	5.5	6	6.5	7	7.5
GRF	–	–	–	–	–	–	–	+	+	+	+	–
G1	+	+	+	+	+	+	–	+	+	+	+	–
G2	+	+	+	+	+	+	–	+	+	+	+	–
G3	+	+	+	+	+	+	–	+	+	+	+	–
G4	+	+	+	+	+	+	–	+	+	+	+	–
G5	+	+	+	+	+	+	–	+	+	+	+	–
G6	+	+	+	+	+	+	–	+	+	+	+	–
G7	+	+	+	+	+	+	–	+	+	+	+	–
G8	–	–	–	–	–	–	–	+	+	+	+	–
G9	–	–	–	–	–	–	–	+	+	+	+	–
G10	+	+	+	+	+	+	–	+	+	+	+	–

was lost after exposure to proteinase K and α -chymotrypsin, but not when papain and trypsin were tested.

Concerning the possible chemical nature of the inhibitory substance, various studies have described the production of organic acids, hydrogen peroxide and bacteriocins by vaginal Gram-positive bacteria such as *Lactobacillus* species (Ocaña & Nader-Macías, 2004; Kaewsrichan *et al.*, 2006), *Enterococcus faecium* and *Lactococcus lactis* (Todorov *et al.*, 2007). Based on the results of the assays performed to test the influence of growth conditions, BHIA supplemented with 0.5% glucose was selected to characterize the antagonistic activity expressed by *G. vaginalis*. The antagonism observed here for the *G. vaginalis* isolates was not due to chloroform action, bacteriophage, long-chain fatty acids or acidic pH, as demonstrated by the experiments on interfering factors. The antagonistic substance or substances detected were sensitive to high temperatures and exhibited loss of activity after exposure to some of the tested proteases, which suggest a protein nature. These results also suggest a bacteriocin molecule as being responsible for the observed inhibitory effects. The new antagonist activity described in the present study for *G. vaginalis* also showed a wide spectrum, inhibiting both Gram-positive and Gram-negative bacteria, and for this reason seems to be bacteriocin-like. Moreover, various producer strains exhibited a different inhibition spectrum depending on the medium, pH and incubation atmosphere employed in the assay, which could be explained by the production of more than one antagonistic substance with different physico-chemical and biological properties.

In conclusion, the present study is the first report, to our knowledge, describing the production by *G. vaginalis* of either antagonistic or synergistic compounds depending on the growth conditions. This and further studies may be of taxonomic and epidemiological interest and might also contribute to the understanding of the role of these substances in vaginal microbial ecology. For this purpose, purification and characterization of the substances detected and others eventually produced by this bacterium will be important, and are currently under way in our laboratory.

ACKNOWLEDGEMENTS

This work was supported by grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Pró Reitoria de Pesquisa da UFMG (PRPq/UFMG). The authors are indebted to Luzia R. Rezende and José Sérgio B. Souza for their technical assistance.

REFERENCES

Apolônio, A. C. M., Carvalho, M. A. R., Ribas, R. N. R., Sousa-Gaia, L. G., Santos, K. V., Lana, M. A., Nicoli, J. R. & Farias, L. M. (2007). Production of antagonist substance by *Eikenella corrodens* isolated

from the oral cavity of human beings with and without periodontal disease. *J Appl Microbiol* **103**, 245–251.

Aroutcheva, A., Gariti, D., Simon, M., Shott, S., Faro, J. & Simoes, J. A. (2001). Defense factors of vaginal lactobacilli. *Am J Obstet Gynecol* **185**, 375–379.

Atassi, F., Brassart, D., Grob, P., Graf, F. & Servin, A. (2006). *Lactobacillus* strains isolated from the vaginal microbiota of healthy women inhibit *Prevotella bivia* and *Gardnerella vaginalis* in coculture and cell culture. *FEMS Immunol Med Microbiol* **48**, 424–432.

Booth, S. J., Johnson, J. L. & Wilkins, T. D. (1977). Bacteriocin production by strains of *Bacteroides* isolated from human feces and the role of these strains in the bacterial ecology of the colon. *Antimicrob Agents Chemother* **11**, 718–724.

Cauci, S., Thorsen, P., Schendel, E. D., Bremmelgaard, A., Quadrifoglio, F. & Guaschino, S. (2003). Determination of immunoglobulin A against *Gardnerella vaginalis* hemolysin, sialidase, and prolidase activities in vaginal fluid: implications for adverse pregnancy outcomes. *J Clin Microbiol* **41**, 435–438.

Eschenbach, D. A. (2007). Bacterial vaginosis: resistance, recurrence, and/or reinfection? *Clin Infect Dis* **44**, 220–221.

Farias, L. M., Totola, A. H., Miranda, C. M. S., Carvalho, M. A. R., Damasceno, C. A. V., Tavares, C. A. P., Cisalpino, E. O. & Vieira, E. C. (1994). Extraction, partial purification and characterization of a bacteriocin (fragilicin) produced by a strain of *Bacteroides fragilis* isolated from *Callithrix penicillata*. *Res Microbiol* **145**, 9–16.

Forsum, U., Holst, E., Larsson, P. G., Vasquez, A., Jakobsson, T. & Mattsby-Baltzer, I. (2005). Bacterial vaginosis – a microbiological and immunological enigma. *APMIS* **113**, 81–90.

Hillier, S. L. (2005). The complexity of microbial diversity in bacterial vaginosis. *N Engl J Med* **353**, 1886–1887.

Ingianni, A., Petruzzelli, S., Morandotti, G. & Pompei, R. (1997). Genotypic differentiation of *Gardnerella vaginalis* by amplified ribosomal DNA restriction analysis (ARDRA). *FEMS Immunol Med Microbiol* **18**, 61–66.

Jarosik, G. P., Land, C. B., Duhon, P., Chandler, R., Jr & Mercer, T. (1998). Acquisition of iron by *Gardnerella vaginalis*. *Infect Immun* **66**, 5041–5047.

Kaewsrichan, J., Peeyananjarassri, K. & Kongprasertkit, J. (2006). Selection and identification of anaerobic lactobacilli producing inhibitory compounds against vaginal pathogens. *FEMS Immunol Med Microbiol* **48**, 75–83.

Lima, F. L., Farias, F. F., Carvalho, M. A. R., Alviano, C. S. & Farias, L. M. (2002). Influence of abiotic factors on the bacteriocinogenic activity of *Actinobacillus actinomycetemcomitans*. *Res Microbiol* **153**, 249–252.

Ocaña, V. S. & Nader-Macías, M. E. (2004). Production of antimicrobial substances by lactic acid bacteria II: screening bacteriocin-producing strains with probiotic purposes and characterization of a *Lactobacillus* bacteriocin. *Methods Mol Biol* **268**, 347–353.

Oliveira, A. A. P., Farias, L. M., Nicoli, J. R., Costa, J. E. & Carvalho, M. A. R. (1998). Bacteriocin production by *Fusobacterium* isolates recovered from the oral cavity of human subjects with and without periodontal disease and of marmosets. *Res Microbiol* **149**, 585–594.

Piot, P., Dyck, E. V., Totten, P. A. & Holmes, K. K. (1982). Identification of *Gardnerella* (*Haemophilus*) *vaginalis*. *J Clin Microbiol* **15**, 19–24.

Pybus, V. & Onderdonk, B. A. (1999). Microbial interactions in the vaginal ecosystem, with emphasis on the pathogenesis of bacterial vaginosis. *Microbes Infect* **1**, 285–292.

Riley, M. A. & Wertz, J. E. (2002). Bacteriocins: evolution, ecology, and application. *Annu Rev Microbiol* **56**, 117–137.

Spiegel, C. A., Amsel, R., Eschenbach, D., Schoenknecht, F. & Holmes, K. K. (1980). Anaerobic bacteria in nonspecific vaginitis. *N Engl J Med* **303**, 601–606.

Todorov, S. D., Botes, M., Danova, S. T. & Dicks, L. M. T. (2007). Probiotic properties of *Lactococcus lactis* ssp. *lactis* HV219, isolated from human vaginal secretions. *J Appl Microbiol* **103**, 629–639.

Turner, J. W. & Jordan, H. V. (1981). Bacteriocin like activity within the genus *Actinomyces*. *J Dent Res* **60**, 1000–1007.

Walstad, D. L., Resistz, R. C. & Sparling, P. F. (1974). Growth inhibition among strains of *Neisseria gonorrhoeae* due to production of inhibitory free fatty acids and lysophosphatidyl ethanol amine: absence of bacteriocins. *Infect Immun* **10**, 481–488.