

Lactobacilli have been shown to inhibit *in vitro* growth of many pathogens and have been used as probiotics to treat a broad range of gastrointestinal and/or vaginal disorders. We sought to determine the *in vitro* inhibitory potential of lactobacilli of vaginal origin to some bacteria associated with bacterial vaginosis (BV), to characterize the inhibitory substances produced by these lactobacilli and to assess H₂O₂ production. Vaginal specimens were obtained by swabbing the lateral vaginal walls from 107 women two months following BV treatment. One hundred and fifty eight *Lactobacillus* spp. were isolated in 82 of the 107 women. *Lactobacillus jensenii* was the predominant strain isolated among these women (29/158; 18.4%). Among 158 culture supernatants tested for antibacterial activity against BV-associated bacteria, none inhibited the growth of *Bacteroides fragilis* while 23% (37/158), 28% (45/158) and 29% (46/158) inhibited the growth of *Prevotella bivia*, *Gardnerella vaginalis* and *Mobiluncus* spp. respectively. The lactobacilli produced supernatants with a pH range between 2.62 and 6.71; the highly acidic (pH 2–3.99) supernatants were more inhibitory to the indicator strains. There was significant reduction in the mean zones of inhibition following chemical and physical treatment of the supernatants ($p = 0.0025$). Acid, bacteriocins and H₂O₂ demonstrated potential for antagonism of the bacterial pathogens. These substances may augment each other rather than each working independently on the pathogens.