Rapid identification method in lactic acid bacteria and molecular identification of Korean vaginal *Lactobacillus* spp.

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Selective culture media and phenotypic tests enable lactobacilli to be differentiated from morphologically similar bacteria. However, accurate identification of *Lactobacillus* species can be accomplished by reference to 16S rRNA gene sequences. A number of DNA-based molecular typing methods including PCR primers that target the 16S rRNA region are available for *Lactobacillus* spp.. Lactobacilli play an important role in maintaining healthy vagina and they produce various inhibitory compounds which can prevent the growth of anaerobic pathogenic bacteria. However, in bacterial vaginosis(BV) patient, the number of lactobacilli decreases while the number of anaerobic bacteria increases for unknown reasons. To treat BV antimicrobial agents were used to eliminate the pathogenic bacteria but they can also eliminate vaginal lactobacilli. Therefore, the antimicrobial agent which can selectively inhibit the growth of anaerobic pathogenic bacteria while not inhibiting the growth of lactobacilli is needed for effective treatment of BV. Over two-year period 108 vaginal lactobacilli were isolated from Korean women and characterized in terms of their antagonistic activity, hydrogen peroxide production, cell surface hydrophobicity, antibiotics susceptibility, and PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). The *in vitro* antibiotics susceptibilities of 108 vaginal isolates to 13

antimicrobial agents were determined by broth dilution method based on NCCLS reference protocol. High rates of resistance were demonstrated for gentamicin, kanamycin, metronidazole, and streptomycin whereas all the isolates were susceptible to erythromycin. The concentrations of gentamicin, kanamycin, metronidazole, streptomycin, and erythromycin at which 90% of the vaginal isolates were inhibited(MIC_{90}) were 100 μg/Mℓ, 200 μg/Mℓ, >200 μg/Mℓ, 200 μg/Mℓ and 0.39 μg/Mℓ, respectively. For molecular identification, PCR-RFLP analysis was employed where the 16S rDNA was amplified by PCR and the PCR products were digested with 8 different restriction endonucleases prior to being electrophoresed in agarose gels. Based on PCR-RFLP results, almost half of the isolates were identified as *Lactobacillus crispatus*. Several isolates were further identified by sequencing of their 16S rRNA genes.