DNA Fingerprinting of Lactobacillus crispatus Strain CTV-05 by Repetitive Element Sequence-Based PCR Analysis in a Pilot Study of Vaginal Colonization

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Lactobacillus crispatus is one of the predominant hydrogen peroxide (H₂O₂)-producing species found in the vagina and is under development as a probiotic for the treatment of bacterial vaginosis. In this study, we assessed whether DNA fingerprinting by repetitive element sequence-based PCR (rep-PCR) can be used to distinguish the capsule strain of L. crispatus (CTV-05) from other endogenous strains as well as other species of vaginal lactobacilli. Vaginal and rectal lactobacilli were identified to the species level by using whole-chromosome probe DNA hybridization. The DNAs from L. crispatus, L. jensenii, L. gasseri, and an as-yet-unnamed H₂O₂-negative Lactobacillus species designated 1086V were subjected to rep-PCR. The results of gel electrophoresis and ethidium bromide staining of the DNA fingerprints obtained were compared. L. crispatus CTV-05 had a unique DNA fingerprint compared to all other lactobacilli. DNA fingerprints for 27 production lots of L. crispatus sampled from 1994 through 2001 were identical to that of the original strain isolated in 1993, suggesting strain stability. In a pilot study of nine women, this DNA fingerprinting method distinguished CTV-05 from other endogenous vaginal lactobacilli prior to and after vaginal capsule use. rep-PCR DNA fingerprinting is useful for strain typing and for evaluating longitudinal loss or acquisition of vaginal lactobacilli used as probiotics.

Lactobacilli, the predominant group of microorganisms of the vaginal flora of healthy women, function as endogenous microbicides through the production of lactic acid, which acidifies the vagina, and hydrogen peroxide (H₂O₂), which reacts with myeloperoxidase to form reactive molecules toxic to human immunodeficiency virus (HIV) and other pathogens (18, 19). Bacterial vaginosis (BV), which is characterized microbiologically by the reduction or absence of lactobacilli, has been associated with an increased prevalence of HIV in several cross-sectional studies (2, 6, 17, 22, 25, 34, 35, 39) and an increased acquisition of HIV in one longitudinal study (40). Further, BV has been linked with increased viral shedding among HIV-infected women (5). In a longitudinal study, women without vaginal lactobacilli had an increased risk of BV (13, 21). Lactobacillus species and L. jensenii, both H₂O₂-producing species (1), are the predominant vaginal Lactobacillus species colonizing women without BV in Europe (11), the United States (1, 41), and Japan (38). Lactobacillus-containing probiotic products have been proposed for the treatment of vaginal infections (3, 24, 29, 30, 37). Oral administration of a probiotic containing L. rhamnosus GR-1 and L. fermentum RC-14 once or twice daily for 28 days has been correlated with healthy vaginal flora (33). However, commercially available products sold as dietary supplements do not always contain the Lactobacillus species advertised on the label and do not always contain H₂O₂-producing lactobacilli (15). Furthermore, compared to vaginal lactobacilli, yogurt-derived lactobacilli poorly adhere to vaginal epithelial cells in vitro (44).

Studies evaluating the efficacy of some probiotic products containing lactobacilli remain inconclusive because the strain identification techniques have been inadequate, relying on classic phenotypic identification methods, such as sugar fermentation, to distinguish lactobacilli isolated before and after product use (7, 36). In one study, efficacy was revealed by comparing the clinical cure rate for BV in a group using the Lactobacillus product to that in a placebo group (12), while in another study, Nugent scoring for BV Gram staining was used to assess the outcomes for the treatment groups (32). Only one study to date has provided a DNA-based strain tracking method; in that study, random amplified polymorphic DNA-PCR analysis (8) was used to compare endogenous vaginal Lactobacillus strains to the probiotic strain.

L. crispatus strain CTV-05, a vaginally derived H₂O₂-producing strain, is a probiotic that is being evaluated for the treatment and prevention of BV. The vehicle for the probiotic is a gelatin capsule which is inserted vaginally. The purpose of this study was to evaluate whether repetitive element sequence-based PCR (rep-PCR), a DNA fingerprinting technique, can be used to distinguish probiotic L. crispatus strain CTV-05 from other L. crispatus strains and other endogenous vaginal Lactobacillus species. rep-PCR, which makes use of repetitive sequences dispersed throughout a bacterial genome for the direct amplification of genomic DNA, generates bacterial species- or strain-specific fingerprint patterns (42).
MATERIALS AND METHODS

Sources of strains. A total of 67 strains of L. crispatus, 41 strains of L. gasseri, 32 strains of L. jensenii, and 28 strains of an as-yet-unnamed species designated Lactobacillus sp. strain 1080V (1) were chosen and subjected to rep-PCR for comparison of their DNA fingerprints to the vaginal capsule L. crispatus CTV-05 DNA fingerprint. These lactobacilli were from a subset of previously identified lactobacilli isolated from vaginal and rectal cultures for 319 women visiting an adolescent medicine clinic and two sexually transmitted disease clinics in Seattle, Wash. (1). These species were chosen because of their vaginal prevalence (1). The DNA fingerprints for 24 Lactobacillus strains obtained from the American Type Culture Collection (ATCC) and representing 17 different Lactobacillus species of human origins were evaluated: L. acidophilus 4356, 4357, and 521; L. brevis 11577 and 14669; L. buchneri 11570 and 4005; L. casei subsp. casei 393; L. crispatus 33197; L. fermentum 23272; L. gasseri 9687 and 4963; L. jensenii 25258; L. johnsonii 33203; L. oris 49062; L. parabuchneri 49374; L. paracasei subsp. paracasei 27216; L. reuteri 23272; L. rhamnosus 7469 and 21052; L. ruminis 25644 and 27780; L. salivarius subsp. salivarius 11741; and L. vaginalis 49540. In addition, six other ATCC strains, L. alimentarius 29643, L. delbrueckii subsp. bulgaricus 11842; L. delbrueckii subsp. delbrueckii 9649; L. delbrueckii subsp. lactis 12315 and 4797; and L. plantarum 14917, were tested for comparison to strain CTV-05.

Lactobacillus identification. Lactobacilli were identified to the genus level by Gram staining, colony morphology, negative catalase test, and production of LTA1842, LTA25258, and six other ATCC strains, LTA23272; L. gasseri 9687 and 4963; L. jensenii 25258; L. johnsonii 33203; L. oris 49062; L. parabuchneri 49374; L. paracasei subsp. paracasei 27216; L. reuteri 23272; L. rhamnosus 7469 and 21052; L. ruminis 25644 and 27780; L. salivarius subsp. salivarius 11741; and L. vaginalis 49540. In addition, six other ATCC strains, L. alimentarius 29643, L. delbrueckii subsp. bulgaricus 11842; L. delbrueckii subsp. delbrueckii 9649; L. delbrueckii subsp. lactis 12315 and 4797; and L. plantarum 14917, were tested for comparison to strain CTV-05.

Small-volume DNA extraction for rep-PCR. The following DNA extraction procedure avoided the use of organic solvents described previously (1), were performed to identify lactobacilli to the species level. Briefly, this procedure involved bacterial lysis with various enzymes (see below), phenol-chloroform DNA extraction, ethanol precipitation for DNA purification, and slot blot DNA hybridization with whole-chromosome probes.

Small-volume DNA extraction for rep-PCR. The previously described DNA protocol (1) was modified to prepare DNA samples for rep-PCR. This modified protocol provided smaller volumes for easier manipulation of a large number of samples and avoidance of organic solvents. A 1.5-mL microcentrifuge tube containing 200 μL of lysis buffer (25% ultrapure sucrose, 50 mM Tris, 1 mM EDTA [pH 8.0]) was inoculated with a loopful of bacteria grown on BA plates. The loopful contained approximately 108 to 109 CFU. The sample was stored at 4°C for at least 20 h to ensure purity and vigor prior to experimental procedures and restocking.

Genomic DNA isolation and whole-chromosomal probe DNA hybridization, described previously (1), were performed to identify lactobacilli to the species level. Briefly, this procedure involved bacterial lysis with various enzymes (see below), phenol-chloroform DNA extraction, ethanol precipitation for DNA purification, and slot blot DNA hybridization with whole-chromosome probes.

RESULTS

DNA fingerprints of L. crispatus strains were most distinguishable from each other in the 1,500- to 2,400-bp range (Fig. 1A). In this range, the fingerprint pattern of L. crispatus CTV-05 showed intense bands at approximately 1,500, 1,650, and 2,400 bp. Another band appeared at approximately 1,900 bp, and a doublet appeared at 2,100 bp (Fig. 1A, lane 2). In addition to the strains recovered from the nine women and organisms (43). The 25-μL PCR volume also consisted of 10% dimethyl sulfoxide; 1.2 μM each dATP, dGTP, dCTP, and dTTP; 7 mM MgCl2; 2.5 U of recombinant Taq DNA polymerase (GibcoBRL); and PCR buffer. Either 100 ng of phenol-chloroform-extracted DNA sample or 7 μl of small-volume DNA extraction sample was used.

The samples were placed in a GeneAmp PCR system 9600 thermocycler (Perkin-Elmer Cetus, Norwalk, Conn.). A modification to the method of Ver- salovic et al. (42) was the addition of ramp times. The PCR program started with an initial denaturation at 95°C for 7 min. This step was followed by 32 cycles of a 50-s ramp to 90°C for 30 s of denaturation, a 62-s ramp to 50°C for 1 min of annealing, and a 50-s ramp to 65°C for 4 min of extension. This step was followed by a final extension at 65°C for 16 min. The samples were stored at 4°C.

An 8-μl volume of each sample was electrophoresed in 0.8% SeaKem GTG agarose (FMC BioProducts, Rockland, Maine) in Tris-acetate-EDTA running buffer. Each gel was run with a 1-kb DNA ladder. The gels were stained with ethidium bromide and photographed.

The photographs were viewed for the intense bands observed at 1,500, 1,650, and 2,400 bp in the capsule strain L. crispatus CTV-05. When these three major bands were present, two additional minor bands (1,950 bp and a doublet at 2,100 bp) were identified. Identity to L. crispatus CTV-05 required the presence of all five bands. Positive identification of L. crispatus CTV-05 was reported only when two independent readers agreed. Samples yielding discrepant rep-PCR interpretations were restested. Discrepant results were unusual, with only 3 of 380 consecutively evaluated Lactobacillus strains yielding different interpretations between two readers. The reproducibility of the rep-PCR method was also quite high in that the fingerprint patterns from the ATCC type strains run on different dates by two different technicians had the same major bands.

Gelatin capsules containing L. crispatus CTV-05. Samples from various lots of gelatin capsules containing 109 CFU of CTV-05 were manufactured either at Chriseo Technologies, Lake Charles, La., or Gynelogix, Incorporated, Louisville, Colo. The capsules were sent to the laboratory for species identification and fingerprinting. Chriseo Technologies also supplied the gelatin capsules used in the human pilot study. The capsules were dissolved in a 1-mL solution of peptone yeast extract broth basal medium, 1% (wt/vol) dextrose, 1% (wt/vol) soluble starch, and 0.02% (vol/vol) Tween 80 (14). A drop of the broth was plated on a BA plate to check for purity. DNA was extracted from the lactobacilli and subjected to rep-PCR. The rep-PCR fingerprints were compared to that of original strain CTV-05.

Pilot study. Nine female volunteers 18 to 40 years old were recruited. The Human Subjects Committee at the University of Washington approved the protocol and consent documents. None of the women had clinical signs or symptoms of BV. Women who were currently menstruating, pregnant, or using systemic antibiotics were excluded. Vaginal smear samples were collected at study entry and at the two follow-up visits. These smear samples were Gram stained and evaluated with Nugent criteria (27). Each woman inserted one gelatin capsule containing 109 CFU of L. crispatus CTV-05 twice daily for 3 days.

Vaginal swab specimens were also collected at baseline and at 4 to 6 days and 9 to 11 days after enrollment. The specimens were used to inoculate one BA plate, one Rogosa agar (Difco Laboratories, Detroit, Mich.) plate, and two human blood bilayer Tween agar (PML Microbiologicals) plates. One of the human blood bilayer Tween agar plates and the BA plate were incubated at 36°C with 5 to 6% CO2, and the remaining plates were incubated at 36°C in an anaerobic chamber. Lactobacilli were identified to the genus level as described above. The isolates were stored at −70°C in reconstituted litmus milk until they were transported to the infectious disease laboratory at the Magee-Womens Research Institute.

Lactobacilli were identified from eight of the nine women at baseline and from all women at the follow-up visits. From the 27 visits, 66 lactobacillus cultures were recovered. All lactobacilli were tested for homology to L. crispatus by using whole-chromosomal probes as previously described (1). rep-PCR was performed for identification to the strain level.

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shown in Fig. 1A, 58 additional strains of *L. crispatus* evaluated by rep-PCR did not show identity to *L. crispatus* CTV-05. This result suggested that the *L. crispatus* strain chosen for development as a probiotic had a distinct fingerprint pattern not observed among 67 other vaginal *L. crispatus* strains.

DNA fingerprints of other endogenous vaginal *Lactobacillus* species, *L. jensenii*, *L. gasseri*, and *Lactobacillus* sp. strain 1086V (Fig. 1B to D) as well as 24 *Lactobacillus* ATCC strains (data not shown) were distinguishable from that of *L. crispatus* CTV-05. Typical fingerprints of isolates homologous to the *L. jensenii* whole-chromosomal probe are shown in Fig. 1B. Less heterogeneity was observed among a total of 32 *L. jensenii* strains than among the *L. crispatus* wild-type strains. Fingerprints of strains homologous to *Lactobacillus* sp. strain 1086V are shown in Fig. 1C. A total of 28 isolates of this H$_2$O$_2$-negative vaginal strain showed different rep-PCR fingerprints. Additionally, *L. gasseri* ATCC 4963 and 9857 and 41 clinical isolates having DNA homology to these two ATCC strains were distinguishable by rep-PCR fingerprinting. DNA fingerprints of 11 *L. gasseri* clinical isolates and the ATCC strains are shown in Fig. 1D. A total of 24 ATCC strains, 17 of which are human associated, and 168 clinical isolates were evaluated by rep-PCR, and none showed identity to *L. crispatus* CTV-05.

Various lots of *L. crispatus* CTV-05 produced between 1994 and 2001 had rep-PCR DNA fingerprints similar to that of the original strain (Fig. 2). This result suggested stability of the probiotic strain and reproducibility of the protocol. Changes in production processes for *L. crispatus* CTV-05 did not affect the genetic fingerprint of the microorganism.

In order to evaluate whether the use of *L. crispatus* capsules intravaginally resulted in colonization, nine women without genital infections were recruited for a pilot study. Women used the capsules intravaginally twice daily for 3 days. None of the nine women who were not colonized with *L. crispatus* CTV-05 at baseline (Table 1). Although four of the nine women were colonized by H$_2$O$_2$-producing *L. crispatus* at baseline, none of the nine women had strains of lactobacilli with DNA fingerprints similar to that of *L. crispatus* CTV-05. At the first follow-up visit, at 4 to 6 days after enrollment, all five of the women who were not colonized

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**FIG. 1.** rep-PCR DNA fingerprints generated from *Lactobacillus* ATCC strains and vaginal isolates. The vaginal isolates were previously identified to the species level by using whole-chromosome probes produced from *Lactobacillus* ATCC strains. Each gel is representative of isolates having DNA homology to one *Lactobacillus* species. Clinical isolates within a group of *Lactobacillus* species were from different women. *L. crispatus*, *L. jensenii*, *Lactobacillus* sp. strain 1086V, and *L. gasseri* were chosen because of their vaginal prevalence. Lane 1 of each gel contains the 1-kb DNA size ladder. (A) Isolates homologous to the whole-chromosome probe of *L. crispatus* ATCC 33197, *L. crispatus* CTV-05 and ATCC 33197 are shown in lanes 2 and 3, respectively. Lanes 4 to 11 show the rep-PCR patterns for *L. crispatus* isolates from eight women. (B) Isolates homologous to the whole-chromosome probe of *L. jensenii* ATCC 25258. ATCC 25258 was run in lane 2. Lanes 3 to 12 show the rep-PCR patterns for *L. jensenii* isolates, all from different women. (C) Isolates homologous to the *Lactobacillus* sp. strain 1086V whole-chromosome probe. The original *Lactobacillus* sp. strain 1086V is shown in lane 2. Lanes 3 to 12 show the rep-PCR patterns for *Lactobacillus* sp. strain 1086V-like isolates from nine women. (D) Isolates homologous to the whole-chromosome probe of *L. gasseri* ATCC 4963. *L. gasseri* ATCC 4963 and 9857 are shown in lanes 2 and 3, respectively. The rep-PCR DNA patterns for *L. gasseri* isolates from eight women are shown in lanes 4 to 11.
by *L. crispatus* at baseline were positive for *L. crispatus* CTV-05. At the second follow-up visit, at 9 to 11 days, four of the five women remained vaginally colonized by the capsule strain. Of the four women already colonized by H₂O₂-producing *L. crispatus* at baseline, two became colonized by the capsule strain, but only at the second follow-up visit. Thus, vaginal colonization by the suppository strain was somewhat less successful among women already colonized by H₂O₂-producing *L. crispatus*.

Representative gels are shown for two of the women in the pilot study in Fig. 3. For one woman, the *L. crispatus* strain establishing colonization of the vagina at baseline (Fig. 3A, lane 3) persisted over all three visits (lanes 4 to 6, 8, and 9). Nevertheless, *L. crispatus* CTV-05 established colonization, although it was not detected by culturing until the third visit (Fig. 3A, lanes 11 and 12). Interestingly, this woman also acquired colonization by *L. jensenii* (Fig. 3A, lane 7), which persisted at the third visit (lane 10). A second woman was initially colonized by an H₂O₂-producing strain (Fig. 3B, lanes 3 and 4) and remained colonized by that strain throughout the study (lanes 5 and 10). *L. crispatus* CTV-05 established colonization at the first follow-up visit (Fig. 3B, lane 7) and persisted through the third visit (lanes 8 and 9). An H₂O₂-negative strain was also detected at the second (Fig. 3B, lane 6) and third (lane 11) visits. These data suggest that colonization by *L. crispatus* CTV-05 does not necessarily result in displacement of other colonizing strains.

**DISCUSSION**

In this study, genomic fingerprinting based on rep-PCR was used to distinguish a probiotic strain, *L. crispatus* CTV-05, from other vaginal lactobacilli and 24 ATCC strains. The pilot study demonstrated successful colonization by *L. crispatus* CTV-05 in seven of nine women. Both women who were not successfully colonized by *L. crispatus* CTV-05 were colonized by H₂O₂-producing *L. crispatus* at baseline, suggesting that colonization by an exogenous strain of *L. crispatus* may be less successful in persons already having predominant H₂O₂-producing lactobacilli. Nevertheless, colonization by the probiotic strain did not result in displacement of other endogenous lactobacilli. This study demonstrates the feasibility of colonizing the vagina with a probiotic strain of *L. crispatus* and the value of rep-PCR for fingerprinting of the probiotic strain.

In a review of the use of probiotic lactobacilli, McGroarty addressed the need for DNA fingerprinting techniques for determination of whether a probiotic strain of lactobacilli colonizes the vaginal microflora (23). It is also necessary to differentiate the probiotic strain from endogenous lactobacilli colonizing the vagina. Although two studies reported the results of small clinical trials of a product containing a *Lactobacillus* strain of human origin, the authors did not present DNA fingerprinting data to support colonization by the strain of *Lactobacillus* found in the product (7, 12). Instead, evaluations were based on the number of cases of genital infections detected within a treatment group before and after use of the *Lactobacillus* preparations (7, 12).

In most instances, classic biochemical or phenotypic tests were used to identify lactobacilli found before and after product use (12, 36). Because of the lack of specificity of these phenotypic tests, genotypic techniques are now more frequently used, even for identification to the species level (4, 16). In one recent study, a ribotyping method (33) was used to identify *L. rhamnosus* GR-1 and *L. fermentum* RC-14 in a clinical trial. However, the ribotypes of endogenous vaginal lactobacilli were not compared to the ribotypes of *L. rhamnosus* GR-1 and *L. fermentum* RC-14. According to Zhong et al., ribotyping appears to distinguish isolates only at the species level (46). More recently, Gardiner et al. used random amplified polymorphic DNA-PCR to track three probiotic strains (8). However, it was necessary for them to implement pulsed-field gel electrophoresis to distinguish their *L. rhamnosus* probiotic strain from closely related strains of the species.

In our pilot study of an *L. crispatus* vaginal capsule, women were monitored for only 11 days. Vaginal insertion of CTV-05 gelatin capsules was done twice daily for only 3 days, so there

**TABLE 1. Detection of *L. crispatus* strain CTV-05 by rep-PCR DNA fingerprinting at various times**

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<th>Subject</th>
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* a, positive for strain CTV-05; b, negative for strain CTV-05.

b Positive for an H₂O₂-producing *L. crispatus* strain which had a DNA fingerprint different from that of *L. crispatus* strain CTV-05.
was only about a 1-week interval between the last capsule use and the follow-up visit. Vaginal colonization by CTV-05 was detected within 4 to 6 days. This time frame of colonization detection was also found in the study of the \textit{L. rhamnosus} GR-1 or \textit{L. fermentum} RC-14 probiotic (8). The vaginal capsule regimen of 3 days of use seems more amenable than either a regimen involving the oral intake of 3 ml of a probiotic suspension of either \textit{L. rhamnosus} GR-1 or \textit{L. fermentum} RC-14 twice daily for 14 days (33) or a 28-day regimen of a daily oral intake of one or two capsules of various combinations of \textit{L. rhamnosus} GR-1 and \textit{L. fermentum} RC-14 (32).

This pilot study suggests that a short duration of vaginal capsule use may be adequate to establish colonization, although the number of subjects tested was limited and the follow-up period was short.

Our decision to use rep-PCR DNA fingerprinting was based on the reported reliability of the method to type strains within a gram-positive species and to generate reproducible fingerprinting patterns (43). rep-PCR has been applied to the \textit{Lactobacillus} genus at the species level. However, different sets of rep-PCR primers have been used (10). An alternative strain typing technique considered was pulsed-field gel electrophoresis, which has been shown to be more costly, labor-intensive, and time-consuming than rep-PCR (20, 28).

rep-PCR DNA fingerprinting has been shown to be a useful tool for epidemiological typing (9, 28, 43). For example, rep-PCR DNA fingerprinting showed that strains of penicillin-resistant \textit{Streptococcus pneumoniae} classified by capsular serotypes were genetically heterogeneous (43). Using rep-PCR, we were able to determine whether different colony types had the same fingerprints.

\textit{L. crispatus} was chosen in this pilot study because this species has been reported to be prevalent among women with \textit{Lactobacillus}-predominant vaginal microflora in North America, Europe, and Asia (1, 11, 38). Colonization by H$_2$O$_2$-producing \textit{Lactobacillus} species has been associated with fewer diagnoses of gonorrhea (1, 13) and with a decreased acquisition of HIV (21). The production of lactic acid and catalase inhibitors by H$_2$O$_2$-producing lactobacilli was found to have cidal activity against \textit{Neisseria gonorrhoeae} in vitro (45). Thus, the establishment of a \textit{Lactobacillus}-predominant vaginal flora through the use of vaginal probiotic strains may have broad implications for public health in populations at high risk of sexually transmitted infections, including HIV.

Vaginal colonization by lactobacilli is dynamic, and loss of colonization by H$_2$O$_2$-producing lactobacilli has been associated with frequent sexual intercourse and antibiotic usage (41). \textit{Lactobacillus} species that produce H$_2$O$_2$, such as \textit{L. crispatus} and \textit{L. jensenii}, were more likely to persist over time in the vagina than H$_2$O$_2$-negative strains. Further, only one in three women who were not initially colonized by H$_2$O$_2$-producing lactobacilli became naturally colonized by H$_2$O$_2$-producing \textit{Lactobacillus} species over the 8-month follow-up period in that study. The authors suggested that the introduction of exogenous H$_2$O$_2$-producing lactobacilli may be the best option to help normalize the vaginal flora of many women.

If vaginal colonization is the intended consequence of probiotic use, selection of appropriate strains is critically impor-
tiant. Previous studies of *Lactobacillus* products for vaginal use reported the use of *L. fermentum* and *L. rhamnosus* (32, 33), both infrequent colonizers of the vagina (1, 11, 38). Although Wood et al. demonstrated that lactobacilli contained in yogurt did not adhere well to vaginal epithelial cells in vitro (44), dairy strains, such as *L. acidophilus* NCFM (31), are still being tested as potential vaginal probiotics. If colonization is desirable, then the use of strains capable of binding sites on vaginal epithelial cells is desirable.

This pilot study demonstrated that *L. crispatus* CTV-05 is able to persist for several days in the vaginal ecosystem of reproductive-age women and that the rep-PCR method can be used to fingerprint the probiotic strain. A larger study population will be needed to garner more information about the rate of colonization of strain CTV-05, especially among women with BV.

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REFERENCES


