Full Length Research Paper

16S rRNA gene sequence and phylogenetic tree of lactobacillus species from the vagina of healthy Nigerian women

Kingsley C. Anukam1,2,4*, Emmanuel O. Osazuwa2, Ijeoma Ahonkhai2 and Gregor Reid1,3

1Canadian Research & Development Centre for Probiotics, Lawson Health Research Institute, 268 Grosvenor St., London, Ontario Canada.
2Department of Pharmaceutical Microbiology, Faculty of Pharmacy University of Benin, Nigeria.
3Department of Microbiology and Immunology and Surgery, University of Western Ontario, London, Ontario, Canada.
4Chidak Medical Diagnostic Laboratories, 200 M.M. Way, Benin City, Nigeria.

Accepted 23 May, 2005

Lactobacilli are ubiquitous in nature and in humans they play a very significant role in the general health maintenance of the host. Identification of lactobacilli has previously been based on culture-dependent methods and recently molecular techniques involving gene sequencing are now the ‘gold standard’. Scarce information exists in Africa on the real identity of Lactobacillus species, albeit phylogenetic distances among the species present in the human vagina. In this study, 185 vaginal swabs were collected from healthy premenopausal women (18 to 48 years). Bacterial DNA was extracted, amplified using PCR, with group specific Lactobacillus primers, and processed by denaturing gradient gel electrophoresis (DGGE). Phylogenetic tree was constructed with the sequences of the V2-V3 region of 16S rRNA gene. Results show two distinct divisions among the Lactobacillus species. The study presents a new understanding of the nature of the Lactobacillus vaginal microbiota of women in Nigeria, which may lead to the design of probiotic-lactobacilli for biotherapy.

Key words: Lactobacilli, gene sequence, phylogenetic tree, human vagina, Nigerian women.

INTRODUCTION

Lactobacilli play an important role in the maintenance of vaginal health of women (Redondo-Lopez, et al., 1990). In healthy women, lactobacilli are the dominant species in the vaginal microbiota (Keane et al., 1997). When lactobacilli are depleted due to antibiotics, douching or sexual practice, other pathogenic organisms such as Gram negative anaerobes takes over, which leads to a condition known as bacterial vaginosis (BV) (Sobel, 1997). The adverse effects of BV on pregnancy and acquisition of HIV is enormous to women in sub-Saharan Africa (Sewankambo et al, 1997). In the development of appropriate biotherapeutic remedy for BV such as probiotic-lactobacilli, the identification of the dominant vaginal lactobacilli present is very vital.

There have been significant advances in recent years in the characterization and identification of bacteria by molecular methods. Notably, Lactobacillus species that are commonly present in the human vagina have received considerable attention as a result of their probiotic properties (Reid, 1999). Most of the molecular techniques for lactobacilli identification are based on the nucleotide-nucleotide sequence of the V2-V3 region of the 16S rRNA gene. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) have been demonstrated to be suitable tools for the analysis of lactobacilli community of the human vagina because they allow the detection of species very quickly and economically (Burton et al., 2003). In the past, the nature of vaginal lactobacilli diversity has been limited due to reliance on culture-dependent methods. It has
been estimated that more than 99% of micro-organisms observable in nature are not cultivable with the available standard techniques (Amann et al., 1995). For thirty-two years between 1928 and 1960, the dominant lactobacillus species in the vagina was believed to be Lactobacillus acidophilus (Thomas, 1928; Rogosa and Sharpe, 1960). The taxonomy of lactobacilli has expanded as a result of genomic sequence analysis. Using DNA homology studies, L. acidophilus complex which previously cannot be distinguished biochemically has been subdivided into six distinct species; L. acidophilus, L. crispatus, L. gasseri, L. gillinarum, L. amylovorus and L. johnsonii (Du Plessis and Dicks, 1995). Previous studies indicated that colonization by L. crispatus or L. jensenii are the most common species of vaginal lactobacilli (Antonio et al., 1999). Only, recently has Lactobacillus iners been identified in the human vagina (Falsen et al., 1999). It was found in vaginal samples from Swedish women, but the most frequently occurring species were L. crispatus, L. gasseri, L. iners and L. jensenii (Vasquez et al., 2002).

The use of 16S rRNA phylogenetic sequence analysis is now accepted as a reliable molecular method for identification of microbial communities (Collins et al., 1991). In recent times, the concept of biodiversity in a particular ecosystem has been articulated through the use of phylogenetic tree maps based on gene sequences. Study on the diversity of vaginal lactobacilli from women in different countries, based on 16S rRNA gene sequences, has been done (Pavlova et al., 2002), and later washed by re-suspending cells in PBS and centrifuging at 13000 g for 3 min. The supernatant containing the DNA was stored at – 20°C.

PCR

The amplification reactions of the DNA sample were carried out in 0.2 ml PCR single tube (Diamed, Lab. Supplies, Mississauga, Ontario, Canada) with hinged flat cap in a Thermocycler (Eppendorf Mastercycler). Each PCR consisted of 5.0 µL of 10X buffer (10 mM Tris-HCl, 50 mM KCl), 2.5 µL of MgCl₂ (50 mM), 1.0 µL dNTPs (5 mM each), 1.25 µL of glycerol (80%) (Sigma), 4.0 µL of bovine serum albumin (BSA) (10 mg/ml) (Sigma), 50 pmol/µL of each primer, Lac-1 (AGCAGTAGGGATATCTTCTCCA) and Lac-2 GC(GCCCCGGGCGCCCAGGCGCCCCCGGGACCCGG GGATTYACCGCTTAC) (Invitrogen™, Life Technologies), 0.2 µL of Platinum® Taq DNA polymerase (5 U/µL (Invitrogen™, Life Technologies), 2.0 µL of the DNA template/sample, and sterile water (Fluka H₂O) to a volume of 50 µL. The PCR amplification has initial DNA denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 1 min and elongation at 72°C for 1 min, which was followed by a final extension at 72°C for 10 min. 5 µL PCR product was analyzed by electrophoresis (Bio-Rad) in 1.5% Ultrapure™ Agarose (Invitrogen, Life Technologies) gel, at 100 volts for 45 min, followed by staining with 1% solution of ethidium bromide (50 µL/L) and de-staining with TAE for 10 min. Gels were visualized by UV transillumination and recorded with Polaroid 667 instant film.

Denaturing gradient gel electrophoresis (DGGE)

In DGGE, the denaturing environment is created by a combination of uniform temperature, typically between 50 and 65°C and a linear denaturant gradient formed with urea and formamide. Preparation of gel gradients and electrophoresis was carried out according to the manufacturer’s instructions for the D-Code™ Universal Mutation Detection System (Bio-Rad Laboratories). 20µL of sample amplified PCR products loaded into the wells and gels were run at 130 V for 3.5 to 4 h in TAE. Gels were visualized as previously described.

Each DGGE fragment of interest was excised from the gels with a sterile scalpel and placed into a 1 ml Eppendorf tube for washing. Cut bands were washed in PCR buffer and incubated in 35 µL of the same buffer solution overnight at 4°C. 5 µL of the buffer solution containing the cut bands was used as template for PCR amplification. PCR re-amplification was conducted with Lactobacillus primers LGC-1 and LGC-2 (without the GC clamp). The amplification, annealing and extension, conditions were the same as described above. The DNA fragments from PCR reactions were purified using QIAquick purification Kit protocol (QIAGEN Inc. Mississauga, Ontario, Canada).

Sequencing and phylogenetic analysis

4 µL of the purified DNA mixture was added to 4µL of Big-Dye Terminator Reaction Mix (Applied Biosystems, USA), plus 1.6µL of primer and 0.4µL of de-mineralized H₂O. Sequences of the fragments, were determined by the automatic Big Dye (dideoxy chain terminator) sequencer ABIPRISM 3730x/ (Sequencing Facility, John P. Robarts Research Institute, London, Ontario).

Sequences were edited to exclude the PCR primer binding sites and manually corrected with Chromas 2.3 (Chromas version 2.3; www.techelysium.com.au.chromas.html). For identification of the closest relatives, newly determined sequences were compared to those available in the V2-V3 region of the 16S rRNA sequences using the GenBank DNA databases (Table 1) (www.ncbi.nih.gov) and the standard nucleotide-nucleotide BLAST algorithm (Altschul et al., 1990). The identities of the relatives were determined on the basis of the highest GenBank accession number.
<table>
<thead>
<tr>
<th>Lactobacillus species</th>
<th>GenBank Accession Number</th>
<th>Nucleotide sequences of the V2-V3 region of the 16S rRNA gene</th>
</tr>
</thead>
</table>
| L. iners clone FX181-4 | AY283275 | AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
RESULTS AND DISCUSSION

The edited nucleotide sequences shows that 118 (64%) of the samples were colonized by L. iners. This was followed by L. gasseri (8.3%), L. plantarum (7.0%), L. suntoryeus (7.0%), L. crispatus (4.0%), L. rhamnosus (2.7%), L. vaginalis (2.7%), L. fermentum (1.3%), L. helveticus (1.3%), and L. johnsonii (1.3%). Figure 1 show the phylogenetic tree based on the V2-V3 region of 16S rRNA gene sequences showing the relationships of the 10 Lactobacillus species from Nigerian women.

The subjects were most commonly colonized by the same Lactobacillus species, L. iners, as have also been observed in recent studies using PCR-DGGE and 16S rRNA gene sequence analysis in Caucasian women in Canada, USA and Sweden (Vasquez et al., 2002; Burton et al., 2003; Reid et al., 2004; Zhou et al., 2004). Indeed, this organism, which does not grow on Rogosa or MRS media traditionally used for lactobacilli recovery, was present in 64% of the samples. Our data revealed that L. iners, L. gasseri, and L. plantarum as the most frequently colonizing species, while the most frequently occurring species in Swedish were L. crispatus, L. gasseri, L. iners and L. jensenii (Vasquez et al., 2002). DNA homology studies of Antonio et al. (1999) and Song et al. (1999) also found that the most prevalent species of vaginal lactobacilli in women from Italy, United States (US) and Japan were L. crispatus, L. gasseri, and L.jensenii.

Previous studies conducted in the sixties to nineties had relied on biochemical and physiological properties to characterize Lactobacilli (Rogosa and Sharpe, 1960; Reid et al., 1996), or have depended on reference strains as a standard to identify clinical isolates by DNA-DNA hybridization (Antonio et al., 1999). These methods have limitations, as many of the reference strains were characterized with biochemical test, which may not be reliable. Besides, biochemical, physiological and DNA hybridization cannot establish the phylogenetic distances among the different lactobacillus species. Thus, it is
worth noting that the Lactobacillus species recovered from the Nigerian women were not unusual or different from those reported in recent studies of Caucasians. This would imply that if women in Canada, Finland, Sweden, Japan, respond favourably to probiotic lactobacilli administration, there should be no reason why Nigerian women could also not likewise benefit.

The phylogenetic tree depicted in Figure 1 shows the genetic distances of the various vaginal lactobacilli from the Nigerian women. There are two distinct divisions among the Lactobacillus species. L. iners, from both the cluster and topological algorithms, is closely related to L. vaginalis and L. fermentum. The work of Falsen et al. (1999) shows that L. iners was closely related to L. gasseri and L. johnsonii. L. gasseri, L. suntoryeus, L. johnsonii, L. plantarum, L. rhamnosus, L. crispatus and L. helveticus are in a more closely related. It should be noted that L. iners, L. gasseri, L. johnsonii and L. crispatus are homofermentative lactobacilli. These lactobacilli metabolize glucose only to lactic acid. While L. fermentum, L. vaginalis and L. rhamnosus are heterofermentative lactobacilli. They metabolize glucose to a mixture of lactic acid, acetic acid and carbon-dioxide. Based on the phylogenetic positions, our data shows that L. rhamnosus and L. crispatus are very much closely related than other species. The selection for a new probiotic L. jensenii vaccine against HIV was based on the fact that L. jensenii and/or L. crispatus was the most prevalent lactobacilli in Caucasian women (Chang et al., 2003). Although such remedy is highly needed in Africa, but our present study have shown that L. iners is the most prevalent among the women sampled in Nigeria, Africa's most populous country, and besides probiotic properties of L. iners are yet to be determined.

L. suntoryeus, usually found in malted grains, which has not been associated with the human vagina was identified in 7% of the samples. This species is closely related to L. plantarum and L. johnsonii in the phylogenetic tree. This present findings indicate cross species colonization phenomenon, which is inconsistent with previous studies reporting species specificity amongst microbes (Tannock et al., 1982).

In summary, the study presents a new understanding of the nature of the Lactobacillus vaginal microbiota of women in Nigeria. In addition, the application of lactobacilli probiotics from western countries to treat African women at risk of BV is worthy of testing. Two examples of such strains are L. rhamnosus GR-1 and L. reuteri (formerly fermentum) RC-14, both of which were isolated and shown to have probiotic properties, and both of which have been shown since to colonize the vagina of Canadian women and reduce the risk of BV recurrence (Reid et al., 2001). Animal safety studies have been successfully performed with these strains in pregnant and non-pregnant rats (Anukam et al., 2004; Anukam et al., 2005). Also encouraging animal data on a lactobacilli-based vaccine (Chang et al., 2003) lends credence to this approach.

ACKNOWLEDGMENTS

The support of Chidak Medical Diagnostic Laboratories, Benin City, Nigeria, and AST of Lawson Health Research Institute, London, Canada, is highly appreciated.

REFERENCES


