INSTITUT PASTEUR

Research in Microbiology 162 (2011) 1006–1010

www.elsevier.com/locate/resmic

Demonstration of vaginal colonization with GusA-expressing *Lactobacillus jensenii* following oral delivery in rhesus macaques

Laurel A. Lagenaur [a,b,*], Peter P. Lee [b], Dean H. Hamer [a], Brigitte E. Sanders-Beer [c]

[a] *Vaccine Branch, National Cancer Institute, National Institutes of Health, 9000, Rockville Pike, Bldg37/Rm6002, Bethesda, MD 20892, USA*
[b] *Osel, Inc., 320 Logue Ave., Mountain View, CA 94043, USA*
[c] *BIOQUAL, Inc., 9600 Medical Center Dr., Rockville, MD 20850, USA*

Received 3 June 2011; accepted 16 August 2011
Available online 29 August 2011

Abstract

The vaginal microbiome, which harbors beneficial *Lactobacillus* strains, is believed to be a major host defense mechanism for preventing infections of the urogenital tract. It has been suggested that the gastrointestinal tract serves as a reservoir for lactobacilli that colonize the vagina. Using rhesus macaques, we examined whether oral delivery of human vaginal *Lactobacillus jensenii* 1153−1646, a GusA-producing strain, would result in colonization of the rectum and the vagina. Lactobacilli were identified from the vagina tracts of three macaques on the basis of β-glucuronidase enzyme production, 16S rRNA gene sequence and DNA homology using a repetitive sequence-based polymerase chain reaction.

Keywords: *Lactobacillus*; Colonization; Oral; Vaginal; Rectal

1. Introduction

The bacteria that colonize the vagina are collectively referred to as the vaginal microbiome. The amount and species of bacteria present have significant implications on a woman's overall health. The vaginal microbiome consists primarily of lactobacilli (Ravel et al., 2010), which may serve to protect the mucosa from the entry of pathogens (Klebanoff et al., 1991). Lactobacilli also acidify the vagina through lactic acid production, which may contribute to vaginal health (Boskey et al., 2001).

The normal rectal microbiome includes members of the *Lactobacillus* genus such as *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Lactobacillus paracasei* (Ahrne et al., 1998). Recent papers have suggested that the vagina and rectum may share common species of *Lactobacillus,* with *Lactobacillus crispatus*, *Lactobacillus gasseri* and *Lactobacillus jensenii* being those vaginal strains most commonly found rectally (Antonio et al., 2005; El Aila et al., 2009; Marrazzo et al., 2009). Using a repetitive element sequence-based PCR (rep-PCR) method, Marrazzo et al. showed that the same *Lactobacillus* strain could be detected from both the vagina and rectum of women (Marrazzo et al., 2009). Another group described the same *Lactobacillus* genotype in paired vaginal/rectal samples from women using randomly amplified DNA polymorphism analysis (RAPD) (El Aila et al., 2009; El Aila et al., 2011).

In an important predicate study, Reid et al. were the first to show that oral delivery of *L. rhamnosus* GR-1 and *Lactobacillus fermentum* RC14 could result in increased vaginal colonization by *Lactobacillus* (Reid et al., 2001). While this increase was likely comprised of the administered strains, the authors acknowledge that the ribotyping technique they used

* Corresponding author. National Cancer Institute, Vaccine Branch, Bldg37, Rm6002, 9000 Rockville Pike, Bethesda, MD 20892, USA. Tel.: +1 301 496 6855; fax: +1 301 402 5565.

E-mail addresses: llagenaur@mail.nih.gov (L.A. Lagenaur), ppl@oselinc.com (P.P. Lee), hamerd@mail.nih.gov (D.H. Hamer), bsanders@bioqual.com (B.E. Sanders-Beer).

0923-2508/$ - see front matter © 2011 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.resmic.2011.08.003

"lacks the precision that is necessary for accurate strain discrimination" (Zhong et al., 1998). In another study, the probiotic strains, *L. rhamnosus* GR-1 and *L. fermentum* RC14, were administered orally to healthy women for 60 days (Reid et al., 2003). Compared to placebo, women given *Lactobacillus* had a 0.9 $\log_{10}$ increase in *Lactobacillus* counts at day 28 and a 0.5 $\log_{10}$ increase at day 60 (Reid et al., 2003). However, the authors did not perform strain typing and thus could not definitively conclude that the increase was due solely to the administered strain; notably, the authors also found minor, but significant, increases in non-administered coliforms and yeast strains.

To determine whether the rectum can serve as a possible bacterial reservoir for colonization of the vagina, a GusA-tagged-*L. jensenii* strain was delivered orally via gavage or feeding to macaques and subsequently cultured from the rectum, feces and vagina. Using molecular techniques and DNA sequence, we definitively established that the rectal, fecal and vaginal colonizing lactobacilli were the orally administered strain. Our experiments verify that some strains of lactobacilli can traverse the gastrointestinal (GI) tract, and that the rectum may serve as a reservoir for vaginal colonization with *Lactobacillus* species.

2. Materials and methods

2.1. Lactobacillus *preparation*

L. jensenii 1153−1646 used in the study was created at Osel, Inc. (Mountain View, CA) as an indicator strain. The DNA corresponding to the *L. gasseri gusA* gene encoding β-glucuronidase A and promoter was cloned. The *gusA* gene cassette was stably integrated by homologous recombination as a single copy gene into the *pox1* site of the *L. jensenii* 1153 genome as described in Liu et al. for the integration of the *cyanovirin-N* gene (Liu et al., 2006). The resolved strain, *L. jensenii* 1153−1646, secretes β-glucuronidase enzyme, which acts on the (5-bromo-4-chloro-3-indoxyl-beta-D-glucoronic acid, cyclohexylammonium salt) substrate turning the colonies blue on de Man Rogosa Sharp (MRS) agar plates (a selective agar for *Lactobacillus* sp.).

2.2. Lactobacillus *growth conditions, culture and identification*

L. jensenii 1153−1646 was cultured using BD Difco™ Lactobacilli MRS broth or agar (Becton Dickinson, Franklin Lakes, NJ). For inoculation of animals, lactobacilli were grown in 200 ml of MRS broth and incubated for about 20 h at 37 °C, 5% CO_2. Lactobacilli were pelleted by centrifugation at 1942x *g* for 10 min, then the supernatant decanted, and the pellet resuspended in 6 ml of PBS. The cell suspension was pelleted again at 1942x *g* for 10 min, and the supernatant decanted. The pellet was resuspended in 2 ml of PBS (1 ml per animal), containing approximately 1 x 10^9 colony-forming units (CFU) of *Lactobacillus*.

The vaginal and rectal/fecal microflora of each animal was sampled using the Port-A-Cul™ swab/tube collection system (Becton Dickinson, Cockeysville, MD). Swabs were first plated on MRS plates containing X-Gluc substrate (Gold Biotechnology, St Louis, MO), and incubated anaerobically using the Anaerobe Gas Generating Pouch System (Becton Dickinson). Lactobacilli were identified by selection of blue colonies and Gram stain for Gram-positive rods. Single colonies were grown on MRS and harvested with a sterile swab into API 50 CHL medium, which was used to inoculate an API 50CH Microorganism Identification Strip (Biomerieux, Inc, St. Louis, MO). After 48 h of anaerobic incubation, results were recorded. *Lactobacillus* DNA was extracted using a Mo Bio™ UltraClean Microbial DNA Isolation Kit (Mo Bio, Carlsbad, CA). The ~900 bp 16S rRNA gene was amplified using gene-specific primers, 8f (5′AGA GTT TGA TCC TGG CTC AG-3′) and 926r (5′-CCG TCA ATT CCT TTR AGT TT-3′), and a unique sequence of 214 bp spanning the *gusA* gene and *pox*1 cassette was amplified using primers F*gusA* (5′-GGA ATT TTT ACT CGC GAT CGT G-3′) and R cassette (5′-TGG CTC GCT ACA ATA TGC AC-3′). The 16S ribosomal DNA (rDNA) and the unique *gusA* cassette were cloned using a Topo TA kit (Invitrogen, Carlsbad, CA) and sequenced. The 16S rRNA gene sequence was analyzed using the Nucleotide Basic Local Alignment Search Tool (BLAST) and compared to the *L. jensenii* 1153 parental strain (Accession: NZ_GL545251.1), which has been fully sequenced. The *gusA* gene sequence was compared to the known sequence within the *Lactobacillus* cassette. Finally, strains were typed using a Diversilab® DNA Fingerprinting Kit for *Lactobacillus* and Diversilab® Analysis System (Biomerieux Inc.).

Due to poor recovery of *Lactobacillus* isolates from the rectum, selected rectal/fecal swabs were pre-incubated 12 h in MRS broth containing clindamycin at 20 μg/ml as a selection broth to enhance *Lactobacillus* growth.

2.3. Rhesus macaque studies

Four female Chinese-origin rhesus macaques (*Macaca mulatta*), ages 4−6 yrs, were used in this study. Animals were housed at BIOQUAL, Inc. in Rockville, MD. Non-human primate housing, care and treatments were performed in accordance with the standards of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), the Animal Welfare Act as amended, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002 and the NIH guidelines for Research Involving Recombinant DNA Molecules. For all procedures, animals were sedated with 10 mg/kg ketamine and 1 mg/kg acepromazine when required.

Prior to the start of the study vaginal and rectal cultures were taken from M4698 and M4701, and no *L. jensenii* 1153−1646 strain was isolated. In the first study, 5 ml sodium bicarbonate solution (130 mg/ml) followed 5 min later by 1×10^9 CFU *L. jensenii* 1153−1646 were administered to two macaques by intragastric gavage on days 0−3 and 6−8. Vaginal and rectal swabs (Port-A-Cul™ swab/tube collection