Selection of Lactobacillus Strains for Urogenital Probiotic Applications

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Almost 20 years ago, it was shown that lactobacilli expressed properties that appeared to play a role in protecting the host from urogenital infection [1]. Since then, studies have shown that exogenously applied lactobacilli or indigenous lactobacilli, can reduce the risk of urinary tract infection (UTI) [2–4]. The investigations have focused upon the large clinical problem of lactobacilli depletion being associated with UTI, bacterial vaginosis (BV), and yeast vaginitis, which together cause an estimated one billion episodes of disease among women each year.

The application of exogenous organisms, such as lactobacilli, to the host is termed probiotics, which is broadly defined as a living microorganism administered to promote the health of the host by treating or preventing disease. While many so-called probiotic products are available, the content and viability of most are unreliable [5, 6], leading to use of strains that do not colonize and therefore do not protect against recurrent UTI [7]. For probiotic applications to the urogenital tract to be successful, it is critical that a scientific basis be established for selection of strains. This should at least shed some light upon their mechanism of action in vivo. Several properties appear to be critical, namely the ability to colonize the host, to inhibit pathogen binding and growth, and to create a balanced flora that resists spermicidal killing [8–10].

Current Knowledge

Selection of Lactobacillus strains. An analysis of the scientific and dairy literature showed that around 7 strains had sufficiently substantial data published on their properties that are required of an antipathogen probiotic [8]. These Lactobacillus strains are L. rhamnosus GG, L. acidophilus NCFM, L. casei Shirota, L. reuteri MM53, L. casei CRL-431, L. rhamnosus GR-1, and L. fermentum RC-14. Other Lactobacillus strains, such as L. johnsonii LC1, appear capable of stimulating the intestinal immune response, and L. plantarum 299V may have some beneficial properties, but neither appear yet in extensive peer-reviewed publications.

The fact that not all lactobacilli possess properties that are required to colonize the vagina and inhibit urogenital pathogens needs to be emphasized. Until now, no studies comparing commercial strains have been done. Using methodologies published previously for adhesion [11], biosurfactant production [12], and hydrogen peroxide production [13], 7 commercial strains were found to lack at least one of the important properties required of a urogenital probiotic: L. rhamnosus GG (Valio), L. acidophilus NCFM (Rhodia), L. casei Shirota (Yakult), L. casei DN-114 001 (Actimel), L. acidophilus SIDU (SIDU Enterprises), L. johnsonii LJ1 (Nestle), and L. plantarum 299V (Probi) (table 1). These strains have been selected for intestinal activity, and clearly that is the environment to which they should be used unless substantial scientific evidence for other applications is produced.

Three Lactobacillus strains have emerged with excellent clinical potential for the urogenital tract: L. crispatus CTV05, L. rhamnosus GR-1, and L. fermentum RC-14 [8]. Of these, little is known about L. crispatus CTV05’s properties other than its ability to colonize the vagina and to produce hydrogen peroxide in vitro. This strain is being tested to prevent recurrence of BV, and while early data are promising, full clinical results will not be available for about 2 years.

The other 2 strains possess properties that make them potentially excellent candidates for the clinical treatment of the urogenital tract and the intestine. L. rhamnosus GR-1 is highly adherent to uroepithelial and vaginal cells, resistant to spermicide, able to inhibit growth and adhesion of urogenital and intestinal pathogens, and able to colonize the vagina [8, 14] and intestine [14a]. L. fermentum RC-14 is an adherent strain that produces hydrogen peroxide and a highly potent biosurfactant, and it also colonizes the vagina and the intestine [8, 14a]. An important component of L. fermentum RC-14’s anti-infective capacity is its biosurfactant, which inhibits adhesion of a range of uropathogens, including Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Group B streptococci, Providencia stuartii, and Staphylococcus epidermidis, and the genital pathogens Candida albicans, and Gardnerella vaginalis.

Several collagen-binding proteins have been isolated by surface-enhanced laser desorption/ionization studies (figure 1) [15]. Of particular interest is a 29-kDa protein that not only binds lactobacilli to collagen on vaginal epithelial cells but also inhibits significant numbers of pathogens from binding to surfaces [16]. Of note, another probiotic organism, L. casei Shirota, which is ingested by an estimated 24 million people each day,
Table 1. Major deficiencies in commercial probiotic organisms with respect to their selection for colonizing the urogenital tract and preventing bladder and vaginal infection.

| Lactobacillus strain | Major deficiencies
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<tr>
<td><em>L. acidophilus</em> SIDU (SIDU Enterprises)</td>
<td>Poor adhesion to epithelial cells; poor inhibition of pathogen growth and adhesion</td>
</tr>
<tr>
<td><em>L. acidophilus</em> NCFM (Rhodia)</td>
<td>Poor adhesion to epithelial cells; poor inhibition of pathogen growth; does not produce hydrogen peroxide</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> GG (Valio)</td>
<td>Does not have 29-kDa biosurfactant protein that inhibits pathogen binding; weakly produces hydrogen peroxide</td>
</tr>
<tr>
<td><em>L. casei</em> Shirota (Yakult)</td>
<td>Does not have 29-kDa biosurfactant protein; does not produce hydrogen peroxide</td>
</tr>
<tr>
<td><em>L. casei</em> DN-114 001 (Actimel)</td>
<td>Does not have 29-kDa biosurfactant protein</td>
</tr>
<tr>
<td><em>L. johnsonii</em> LJ1 (Nestle)</td>
<td>Does not have 29-kDa biosurfactant protein</td>
</tr>
<tr>
<td><em>L. plantarum</em> 299V (Probi)</td>
<td>Does not have 29-kDa biosurfactant protein</td>
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NOTE. None of these strains has any reported efficacy against urogenital infections.

a Deficiencies were determined on the basis of in vitro experiments [8, 11–13].

Figure 1. Shown is the presence of an active anti-uropathogenic protein on *Lactobacillus fermentum* RC-14 and its absence in *Lactobacillus casei* Shirota. Aliquots of biosurfactants were prepared from the lactobacilli strains and spotted onto the surfaces of PS-1 protein chip arrays containing immobilized collagen type-III (Cn-III/PS-1) and analyzed by a system termed surface-enhanced laser desorption/ionization time of flight mass spectrometry [15]. The plotted spectra depict the relative peak intensities vs. mass-to-charge ratio (M/z) of proteins for each biosurfactant. Arrows highlight distinct collagen-binding peaks. Dual-charged peaks (M+2H⁺) and their parental single charged peaks (M+H⁺) are bracketed. The star-labeled arrows identify p29 CnB and its mature processed form (p26).
(sppK) and a response regulator (sppR) and which induces bacteriocin production [19]. Furthermore, expression signals have been found in L. brevis surface (S)-layer protein gene, which directs protein production [20]. Alternatively, lactobacilli might signal production of mucus in the vagina, which may act as a deterrent to pathogens [21].

The benefits of applying probiotics to the urogenital tract include the avoidance of the side effects associated with antibiotic use; such side effects can be substantial and even cause death and drug resistance, which is rapidly increasing for ampicillin, trimethoprim/sulfamethoxazole, and fluoroquinolones used to treat UTIs. Another advantage to patients is the “natural” approach to health maintenance, an approach that entails replenishment of depleted flora to create a microbial environment that is better able to fight off pathogens. While there are some clinical data to indicate that this defense occurs against sexually transmitted pathogens [22], more studies are needed to prove the effect conclusively.

The combination of Lactobacillus strains for urogenital application is potentially critical for several reasons. The production of H$_2$O$_2$ appears to be important in interfering with the growth of pathogens, particularly those causing BV. Therefore, a case can be made for a strain that produces H$_2$O$_2$, such as L. fermentum RC-14. The added effect of RC-14’s biosurfactants against urogenital pathogens makes it a good choice for inclusion. However, H$_2$O$_2$-producing strains are killed by low concentrations of the spermicide nonoxynol-9 (N-9) [23], and while studies in monkeys and humans are somewhat contradictory in their findings of the net effect on vaginal lactobacilli after exposure to N-9 [24–27], it would be an advantage to have a probiotic, such as L. rhamnosus GR-1, that resists high concentrations of N-9. Further support for combination therapy can be found in monkey studies showing that a cocktail of normal flora was better than a single strain at colonizing the vagina and interfering with uropathogens [28].

Critics have argued that randomized, double blind, placebo-controlled trials comparing probiotics with antibiotics are essential before the former can be considered a serious alternative to the latter in the clinical management of UTIs. The argument is that antibiotics are the reference standard for the prevention of UTIs. However, this assumption needs to be challenged. Daily, long-term antibiotic therapy is surely not optimal for the patient because of the risk of side effects, mounting drug resistance, and failure of this approach to restore the patient’s own natural defense against infection. Studies on long-term antibiotic use have shown a wide spectrum of UTI recurrence rates, from 0.13 to 2.3 infections per patient year [29–31], and with more resistant strains emerging, these figures may now be higher. Thus, what rate of UTI recurrence with the use of lactobacilli probiotic therapy would be regarded as equivalent to the rate of recurrence with antibiotics? A previous study achieved a recurrence rate of 1.6 per patient per year, using weekly vaginal therapy with a non-optimal preparation of L. rhamnosus GR-1 and L. fermentum B-54 [2]. This, at the very least, provides support for further pursuit and use of probiotics in the urogenital tract as a means of health maintenance.

Commentary

In conclusion, probiotics, selected on a scientific basis and proven to colonize the vagina and interfere with urogenital pathogen adhesion and growth potentially offer a natural, effective, and practical way to reduce the risk of UTI, yeast vaginitis, and BV. Urogenital infections have been described as a silent epidemic, and it is time for modern science to find ways to significantly decrease the incidence of these infections. Women would clearly prefer to maintain their health than be on a repetitive cycle of treating bladder and vaginal infections. It is with these patients in mind that alternatives to antibiotics should be sought.

Acknowledgments

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References