UDC 579. 864

COLONIZATION OF THE RAT VAGINA BY LACTOBACILLI

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Colonization ability of 4 Lactobacilli strains was tested on a rat model. Among the investigated strains, *Lactobacillus gasseri* TL 093c is considered to be the best colonizer of rat vagina. This strain was still isolated after 25 days posterior to instillation. Introduced Lactobacilli decreased quantity of indigenous gram — negative rods and gram-positive cocci.

Key words : colonization, Lactobacilli, intravaginal instillation, inhibition.

Lactobacilli are the dominant bacterial species of the healthy human vagina and are routinely found as normal flora organisms in a wide variety of animals, for example monkeys and rodents [2] . Many studies have shown a correlation between loss or disruption of normal genital microflora, in particular *Lactobacillus* species, and an increased incidence of genital and bladder infections [4]. These observations have led to scientific investigations of the strains and properties of Lactobacilli which might be responsible for maintenance of non — infectious vaginal ecology [7] . It has been suggested that such properties may include adhesive and competitive interference abilities, production of bacteriocin — like substances which inhibit the growth of pathogens, hydrogen peroxide production, and a capacity to coaggregate with other urogenital bacteria to form a balanced integrated microbial community [1, 6]. Due to the functional role of endogenous vaginal flora, it is important to gain a better understanding of the composition and regulation of this system [3].

Thus, the present study was undertaken to (i) colonize epithelium of the rat vagina by Lactobacilli, (ii) observe relationship between instilled Lactobacilli and rat microbiota.

Materials and Methods

Determination of indigenous flora of the albino rat vagina. Vaginal samples have been taken from 4 rats three times using sterile swabs, three days apart each sampling. The samples were streaked on blood agar and MRS agar plates and were incubated at the appropriate conditions, and the microorganisms present on different days in the vagina were evaluated. The most dominant microorganisms present were noted. Lactobacilli were identified to the genus level by colonial and cellular morphology and catalase reaction [8].

Lactobacilli preparation and intravaginal instillation. Four different strains of *Lactobacillus* : *Lactobacillus jensenii* La1 (isolated from probiotic yogurt), *Lactobacillus delbrueckii* subsp. *lactis* TL 059a, *Lactobacillus sp.* UL 009b, and *Lactobacillus gasseri*

TL 093c (all isolated from human vagina) have been grown in MRS broth for 24 hours at 37° C, harvested and resuspended in phosphate — buffered saline (PBS, pH 7.1) and then 200 μl suspension (10^8 cells) was injected intravaginally. On 4-th, 5-th, 7-th, 8-th, 13-th, 16-th, and 25-th day vaginal samples have been taken using cotton — tipped swabs. The swabs were placed into 1 ml of PBS and inoculated on MRS agar and blood agar. Rat general microbiota and lactic acid microbiota have been studied after Lactobacilli instillation.

Estimate of colonization ability of instilled Lactobacilli on the vaginal mucosa of experimental rats. Vaginal specimens were obtained prior to the commencement of the study and subsequent to Lactobacilli instillation. To screen the duration of Lactobacilli stay in the rat vagina, whole cell protein extracts were obtained from all strains of Lactobacilli isolated from rats after instillation. Comparative analysis of the cell protein extracts was provided by means of sodium dodecyl sulphate polyacrilamide gel electrophoresis of proteins (SDS—PAGE), method by Laemmli [5].

Results and Discussion

Pretreated rat vaginal microbiota contained mostly gram-negative oxidase positive rods (56—85%), gram-positive rods (7—22%), staphylococci (2—24%), and streptococci (7—20%), as presented in Table 1. It is evident from these data that after Lactobacilli instillation, the quantity of gram-negative rods significantly decreased: 2 times for rat No. 4, 10 times for rat No. 2, and even disappeared for rats Nos. 1 and 3. Such effect probably may be caused by antimicrobial activity of the instilled strain of Lactobacilli. The introduced Lactobacilli were also effective towards gram-positive cocci and caused 2—9 time decrease of the latter, and for rat No. 3 at the end of the observed term the mentioned organisms were not isolated at all.

Table 1

Comparison of the rat dominant microbiota before and after instillation of Lactobacilli

Source of isolation	Correlation to total bacterial quantity, %			
	Gram-positive rods	Gram-positive coccobacilli	Gram-positive cocci	Gram-negative rods
Rat No. 1 Before instillation	47	13	17	23
Rat No. 1 After instillation *L.gasseri* TL093c	84	12	4	0
Rat No. 2 Before instillation	45	2	29	22
Rat No. 2 After instillation *L.jensenii* La1	80	13	5	2
Rat No. 3 Before instillation	28	7	29	34
Rat No. 3 After instillation *L. sp.* UL009b	55	45	0	0
Rat No. 4 Before instillation	26	12	40	21
Rat No. 4 After instillation *L.delbrueckii* subsp. *lactis* TL059a	53	25	11	11

Along with the decrease of opportunistic microbiota in vaginal samples we have been constantly observing an increase of normal flora representatives. The index of gram-positive rods grew twice for all rats .

In this investigation *Lactobacillus* was not found to be the dominant bacterial genus of the albino rat vagina. Indigenous Lactobacilli have been isolated from rats Nos. 1 and 4. Lactic acid microbiota of rats Nos. 2 and 3 are represented only by coccobacilli. To exclude the possibility of antimicrobial activity of indigenous rat Lactobacilli against the introduced Lactobacilli, a bacteriocin assay has been perfomed. During this experiment no antagonistic activity of indigenous strains against the introduced strains was found.

To assess whether the introduced Lactobacilli adhered on the rat mucosal epithelium, and to determine how long they are maintained in the vagina, SDS-PAGE test was perfomed. One-dimensional SDS—PAGE of the whole cell protein extracts of 75 strains *Lactobacillus* isolated from 4 rats after Lactobacilli administration devided the rat isolates into 3 clearly defined groups corresponding to the band sets: first group consists of Lactobacilli strains which have high level homology with indigenous Lactobacilli, second — with introduced Lactobacilli and third — with Lactobacilli which were probably obtained from other rats as a result of horisontal transfer of microorganisms between rats. The data obtained during PAGE analisis affirmed that strains *Lactobacillus gasseri* TL 093c, *Lactobacillus jensenii* La1, *Lactobacillus sp.* UL 009b, retained in the rat vagina after Lactobacilli introduction for more than 25, 16 and 7 days correspondingly (Table 2).

Table 2

Duration of the instilled Lactobacilli stay in the rat vagina

Instilled organism	Number of days after instillatin
L. delbrueckii subsp. *lactis* TL 059a	0
Lactobacillus jensenii La1	16
Lactobacullus sp. UL 009b	7
Lactobacillus gasseri TL 093c	25

According to PAGE test, introduction of *Lactobacillus delbrueckii* subsp. *lactis* TL 059a was not successful.

The data presented in this study clearly support the view that Lactobacilli can be efficiently used for colonization of female reproductive tract. The rat model was established for investigation of this ability. With the help of this model it has been demonstrated that Lactobacilli could be introduced into the ecosystem were they could stably maintain population and eliminate a bacterial infection. Among the investigated strains, *Lactobacillus gasseri* TL 093c is considered to be the best colonizer of the rat vagina. This strain was still isolated after 25 days posterior to instillation. The results of this study indicate, that human original vaginal Lactobacilli and dairy Lactobacilli have an ability to colonize the rat vagina.

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КОЛОНІЗАЦІЯ ВАГІНИ ЩУРІВ ЛАКТОБАЦИЛАМИ

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Резюме

На моделі щурів показано принципову можливість колонізації вагіни лактобацилами. Встановлено, що ці мікроорганізми успішно приживлюються в репродуктивному тракті білих щурів із збереженням своєї життєздатності. Найбільш тривалий термін колонізації — 25 днів — спостерігався при інстиляції штаму Lactobacillus gasseri TL 093c. Відмічено, що інтродуковані лактобацили впливають на кількість грамнегативних паличок та грампозитивних коків ендогенного походження, суттєво знижуючи ці показники.

Ключові слова: колонізація, лактобацили, інтравагінальна інстиляція, інгібування.

КОЛОНИЗАЦИЯ ВАГИНЫ КРЫС ЛАКТОБАЦИЛЛАМИ

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Резюме

На модели крыс показана принципиальная возможность колонизации вагины лактобациллами. Установлено, что эти микроорганизмы успешно приживляются в репродуктивном тракте белых крыс с сохранением своей жизнеспособности. Наиболее длительный срок колонизации наблюдали при инстилляции штамма Lactobacillus gasseri TL 093c. Отмечено, что интродуцированные лактобациллы влияют на количество грамотрицательных палочек и грамположительных кокков эндогенного происхождения, существенно снижая эти показатели.

Ключевые слова: колонизация, лактобациллы, интравагинальная инстилляция, ингибирование.