NEW BIOMEDICAL TECHNOLOGIES

The Use of Lactobacterin Based on the *Lactobacillus fermentum* Strain 90-TS-4 in Gynecological Patients

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Two subpopulations differing in adhesion to vaginal epithelium are isolated from *L. fermentum* producer strain 90-TS-4. Highly adherent variant contains concanavalin A-dependent nonprotein antigen in the cell wall. The relationship of this antigen with *L. fermentum* 90-TS-4 adhesin is discussed. It is concluded that this variant is the most promising for the use in gynecological patients.

Key Words: *lactobacterin; adhesion; antigen*

The efficacy of local application of lactobacilli (LB) in the treatment of vaginosis is now clearly demonstrated. Intravaginal applications of yogurts [8,10,12], lactate gels [5,9], and lyophilized LB preparations [6,11] are used. Lactobacterin, a Russian-made preparation [2,3] is based on two strains: *L. fermentum* 90-TS-4 and *L. plantarum* 8RA-3. The efficacy of these drugs depends on the colonization ability of the eubiotic, in particular adhesive properties of the strain. These properties depend on the surface structures of the cell wall. We studied possible associations of these structures with adhesion of *L. fermentum* 90-TS-4.

MATERIALS AND METHODS

Producer strain *L. fermentum* 90-TS-4 isolated from the female vagina and therefore more adapted to this microecological niche was used.

Antiserum to *L. fermentum* 90-TS-4 was obtained from rabbits immunized with LB suspension in ascending concentrations (alternating subcutaneous and intravenous injections at 7-day intervals, a total of 4 injections).

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Concanavalin A (ConA, Pharmacia Fine Chem.) was used in the initial concentration of 1 mg/ml.

Surface antigen of *L. fermentum* 90-TS-4 strain was obtained by phenol extraction [4]. The presence of this antigen on the cell surface was evaluated by agglutination on glass and in the advanced agglutination test with ConA and immune serum [1].

Adhesive properties were examined by LB adhesion to vaginal epithelial cells isolated from vaginal lavage of healthy women by centrifugation for 2 min at 1000 rpm. The supernatant was discarded, epithelial cells were washed three times with normal saline, and a standard suspension was prepared. The 24-h LB cultures were washed three times with normal saline and 1 ml suspension containing 4.7×10^8 cells/ml was added to 1 ml epithelial cells suspension. After 30 min incubation at 37°C on a shaker the cells were washed three times with normal saline, centrifuged at 1000 rpm for 2 min and smears were prepared. The smears were fixed and stained for 1 min with Gentian violet. Adhesion was assessed under light microscope, the number of adherent LB on 50 epithelial cell was determined.

RESULTS

Agglutination of *L. fermentum* 90-TS-4 with ConA revealed heterogeneity of the strain. Only one-third of

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TABLE 1. Agglutination of Isolated *L. fermentum* 90-TS-4 Colonies with ConA and Immune Serum

Colonies	Agglutinating with ConA	Nonagglutinating with ConA	Total
Agglutinating with serum	4	1	5
Nonagglutinating with serum	6	22	28
Total:	10	23	33

Note. χ^2=4.4 (p=0.05).

TABLE 2. Titers of Parent *L. fermentum* 90-TS-4 Strain and of Its Two Variants in Agglutination Test with ConA and Immune Serum

Agglutination variant	*L. fermentum*		
	90-TS-4 (parent)	90-TS-4(21)	90-TS-4(10)
ConA	0.125-0.00625	0.00625-0.00312	0
Serum to *L. fermentum* 90-TS-4	1:320-1:2560	1:2560-1:5120	0

colonies agglutinated in the presence of ConA. Agglutination with the serum also revealed agglutinating and nonagglutinating subpopulations in *L. fermentum* 90-TS-4 strain. We supposed that serum-induced agglutination of the culture is controlled by the surface layer of the cell wall containing a ConA-dependent antigen. In order to verify this hypothesis we compared immune serum- and ConA-induced agglutination on glass of individual *L. fermentum* 90-TS-4 colonies removed from solid medium. Our findings suggest that these reactions are probably interrelated (Table 1).

We selected two variants of the initial *L. fermentum* 90-TS-4 strain. One of them, *L. fermentum* 90-TS-4(21), was agglutinated by serum and ConA, the other, *L. fermentum* 90-TS-4(10) was not. Obviously the agglutinating variant possesses a pronounced glycoprotein surface layer, containing the ConA-dependent antigen, while the nonagglutinating variant lacks this structure. The agglutinating characteristics of these variants were not affected by long-term culturing, while their titers in the initial culture varied (Table 2).

Different titers in the parent strain are probably determined by the predominant subpopulation (agglutinating or nonagglutinating) in the mixed culture.

The nature of the studied antigen was examined by the "boiling" test and enzyme digestion (with trypsin and protease). One-hour heating at 60°C and treatment with proteolytic enzymes for 1 h did not decrease the agglutinating abilities of *L. fermentum* 90-TS-4(21), hence this structure is not a protein.

For determining the specificity of the antigen, a preparation containing surface components of the cell wall was prepared by phenol extraction. It precipitated with ConA and inhibited immune serum-induced agglutination of *L. fermentum* 90-TS-4(21). Decreased titer of bacterial agglutination in the presence of the phenol extract (1:640 *vs.* 1:2560 in the control) indicated the presence of a specific agglutinogen.

It was shown that ConA-agglutinating LB intensively adhere to chicken crop squamous epithelium, while monovalent ConA subunits block this adhesion [7]. We presumed that the *L. fermentum* 90-TS-4(21) variant containing ConA-dependent agglutinogen will be more adhesive than the nonagglutinating *L. fermentum* 90-TS-4(10) variant. Comparison of these variants in cultured female vaginal epithelial cells showed that epithelial cells from different women differ by the ability to attract LB. In one-third of women both LB variants adhered poorly (less than 2 bacterial cells per epithelial cell, Table 3).

Therefore, *L. fermentum* 90-TS-4(21) better adheres to vaginal epithelial cells than *L. fermentum* 90-TS-4(10).

Thus, the lactobacterin producer strain *L. fermentum* 90-TS-4 is heterogenous and consists of two sub-

TABLE 3. Adhesion of *L. fermentum* 90-TS-4(21) and *L. fermentum* 90-TS-4(10) to Vaginal Epithelial Cells, %

Variant of *L. fermentum* strain	Poor adhesion (<2 bacteria)	Medium adhesion (2-4 bacteria)	High adhesion (>4 bacteria)	% of epithelial cells adhesive for LB
90-TS-4(21)	37.5	37.5	25	82.8
90-TS-4(10)	62.5	25	12.5	76.3

populations differing in the cell wall structure, in particular the presence or abscence of a ConA-dependent antigenic component. This component is probably presented by teichoic acid or a polysaccharide involved in bacterial adhesion to the epithelium. The abscence or low content of this component in the *L. fermentum* 90-TS-4(10) cell wall reduces its adhesive properties in comparison with *L. fermentum* 90-TS-4(21). Hence, the drug for vaginal applications should be prepared from *L. fermentum* 90-TS-4(21) variant.

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