Bactericidal Activity of Culture Fluid Components of *Lactobacillus fermentum* Strain 90 TS-4 (21) Clone 3, and Their Capacity to Modulate Adhesion of *Candida albicans* Yeast-Like Fungi to Vaginal Epithelial Cells

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Antagonistic activities of *L. fermentum* strain 90 TS-4 (21), *L. casei* ATCC 27216, and *L. acidophilus* ATCC 4356 and bactericidal activity of lactobacillus culture fluid towards *E. coli* strain K12, *S. aureus*, and *S. epidermidis* test cultures were studied. The bactericidal effect of *L. fermentum* strain 90 TS-4 (21) clone 3 culture fluid preparation (pH 6.0) on the test cultures was dose-dependent. Adhesion of *C. albicans* yeast-like fungi to vaginal epitheliocytes was more pronounced for strains isolated from women with asymptomatic infection than for strains isolated from women with manifest forms. *L. fermentum* strain 90 TS-4 (21) clone 3 culture fluid preparation modulated adhesion of yeast-like fungi only if the fungal strain was initially highly adherent.

Key Words: *probiotics; L. fermentum; bactericidal activity; C. albicans; adhesion*

Candida yeast-like fungi (YLF) are an inseparable part of bacterial cenoses forming a monolayer on the surface of the terminal epithelium. Some YLF species, for example, *C. albicans*, in these monolayers penetrate as far as to the parakeratide epithelial layer. *C. albicans* YLF colonizing the vaginal epithelium have to combat for existence in the microecological niche with representatives of *Lactobacillaceae* family.

Candida YLF with their characteristic tropism to glycogen-rich tissues [2], most effectively adhere to vaginal epitheliocytes (VE) in pregnant and diabetic women. However, after preincubation of these cells with *Lactobacillus* strains freshly isolated from the vaginas of clinically healthy women, adhesion of *C. albicans* to the target cells decreases by 30-40%. The use of commercial lactobacterial preparations (acylact or lactobacterin) for the same purpose is little effective, because these preparations are based on strains of enteric origin; being introduced into the vagina, these probiotic lactobacillus cultures (not characteristic of this biotope) almost loose their capacity to adhere to VE and colonize them.

We previously showed that *L. fermentum* strain 90 TS-4 (21) expresses adhesin capable of detaching from the cell surface into the culture medium [1]. The components of the resultant culture fluid (CF) binding to VE membrane receptors compete with adhesins of other bacteria preventing their colonization on the mucosa. Clone 3 of *L. fermentum* strain 90 TS-4 (21) proved to be the most active producer of the component inhibiting adhesion

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of *C. albicans* reference strain, but not modifying adhesion of FimH-positive *E. coli* M17 strain [1].

Enrichment of probiotic preparations with components released into culture medium by *L. fermentum* strain 90 TS-4 (21) clone 3 is justified, because they prevent colonization of the mucosa in the terminal microecological niches by some opportunistic microorganisms. Obviously, lactocines exhibiting bactericidal effects should also be added to these probiotics.

We evaluated the effects of active components of *L. fermentum* CF in experiments with a representative collection of *C. albicans* YLF.

MATERIALS AND METHODS

Strains were isolated from the vaginas of women with candidal vaginitis (n=10) and asymptomatic infection (n=10). Cultures identified as *C. albicans* in Nickerson chromogenic medium (HiMedia) with parallel induction of the germinative tubule growth in the presence of the serum were selected for the experiment. *C. albicans* strains for further experiments were cultured in Saburo broth at 37°C for 24 h [1].

Donor VE washed from bacterial flora with buffered saline (pH 7.2) served as target cells in the adhesion test [1].

Culture fluid obtained by culturing lactobacilli (*L. fermentum* 90 TS-4 productive strain, *L. fermentum* strain 90 TS-4 (21) clone 2, *L. fermentum* strain 90 TS-4 (21) clone 3, *L. plantarum* 8 RA-3, *L. plantarum* 39) was purified from low-molecular-weight components by diafiltration in 200-ml cells (Amicon) using PM-30 membranes (Diaflo) with subsequent sterilization by filtration through 0.02-μ Millipore membranes [1]. Protein concentration in CF was evaluated by Lowry's method, reactivity of CF components with concanavalin A was evaluated in the precipitation test [1].

After the growth cycle YLF were washed from the culture medium and the suspension concentration was brought to 5 U by the opacity standart. *C. albicans* adhesion to VE was carried out at 37°C, the suspension of target cells was added to YLF at a ratio of 100 bacterial bodies per VE. An equal volume of *L. fermentum* strain 90 TS-4 (21) clone 3 CF (protein concentration 650 μg/ml) was added to VE suspension and the mixture was incubated for 30 min at 37°C, after which $^1/_2$ of YLF suspension volume was added to the test system (VE+ CF) and incubated at 37°C. After 15 min the mixture was washed 3 times in buffered saline, centrifuged at 1000 rpm for 2 min, and the preparation for microscopy was stained after Gram. Yeast-like fungi on EV surface were counted in 50 visual fields. The smears were examined under an MBI-15-2 light microscope (LOMO).

The results were evaluated by the percentage of "active" VE to which YLF adhered, mean number of YLF per "active" EV, and integral adhesion index (mean number of YLF on all VE).

The antagonistic activity of *L. fermentum* strain 90 TS-4 (21) culture was compared to that of lactobacilli from the American collection (*L. casei* ATCC 27216 and *L. acidophilus* ATCC 4356) in the delayed *E. coli* growth inhibition test. *E. coli* strains (JJB150, JJB4, JJF21, JJP114, and JJV4) were a gracious gift from by Dr. E. V. Sokurenko (USA).

Antagonistic activity of CF preparations free from low-molecular-weight components was evaluated by the size of test culture growth inhibition zone around the wells with the CF test sample. Bactericidal activity of CF sample was evaluated by the size of growth inhibition zone per mg protein (specific activity) towards *E. coli* K12 strain (National Collection of Microorganisms), *S. aureus*, *S. epidermidis* (clinical strains), and *Gardnerella vaginalis* CCUG 3717 (this culture was a gift from Dr. N. Nikolaichuk; Culture Collection, University of Goteborg).

RESULTS

L. fermentum strain 90 TS-4 (21) and *L. casei* ATCC 27216 strain exhibited high antagonistic activity (Fig. 1; Table 1).

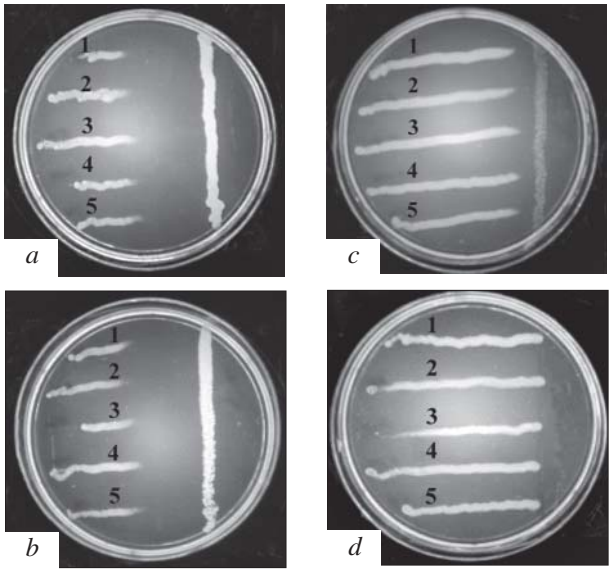

Fig. 1. Antagonistic activity of lactobacilli in *E. coli* growth inhibition delay test. *a*) *L. casei* ATCC 27216; *b*) *L. fermentum* 90 TS-4 (21); *c*) *L. acidophilus* ATCC 4356; *d*) control. 1) JJB150; 2) JJB4; 3) JJF21; 4) JJP114; 5) JJV4.

TABLE 1. *E. coli* Test Culture Growth Inhibition (mm)

Lactobacillus strain	JJB150	JJB4	JJF21	JJP114	JJV4
L. casei ATCC 27216	26	27	24	25	23
L. fermentum 90 TS-4 (21)	25	27	25	23	22
L. acidophilus ATCC 4356	7	9	11	7	6

The bactericidal effect of a culture can be due to the release of lactocines into the medium or capacity of lactobacilli to high acid production (acid production of *L. fermentum* strain 90 TS-4 (21) is about 250 Turner's degrees). It was therefore interesting to find out whether *L. fermentum* strain 90 TS-4 (21) clone 3 CF possessed bactericidal activity. *E. coli* strain K12, *S. aureus*, *S. epidermidis*, and *G. vaginalis* CCUG 3717 were selected as the test cultures. Bactericidal activity of CF preparation purified and free from low-molecular-weight components was not due to the level of acid production or presence of peroxide compounds. The bactericidal effect of CF preparation with pH 6.0, obtained by *L. fermentum* strain 90 TS-4 (21) clone 3 culturing, on the test culture was dose-dependent (Fig. 2). Changes in the preparation activity, depending on its protein concentration can be described by an equation:

$$y=b_0+b_1x+b_2x+\ldots+b_nx_n,$$

Fig. 2. Bactericidal activity of *L. fermentum* strain 90 TS-4 (21) clone 3 towards *E. coli* strain K12 test culture. Protein concentrations (μg/ml): *1*) 8450; *2*) 4225; *3*) 2112; *4*) 1056; *5*) 528.

where y is the diameter of test culture growth inhibition zone, x protein concentration in the preparation, and b the polynome value.

Calculation of specific bactericidal activity of *L. fermentum* strain 90 TS-4 (21), clone 3, CF preparation per mg protein for each test culture showed that the growth of *S. epidermidis* was inhibited most actively, that of *S. aureus* less so, and of *E. coli* strain K12 still less (the specific activities 10.40, 6.60, and 0.89, respectively). The preparation exhibited no activity towards *G. vaginalis* CCUG 3717 culture.

Lactocines released into culture medium were characterized by different spectra of bactericidal activities, depending on the producer species and strain (Table 2). For example, *L. plantarum* produced lactocines highly active towards *S. aureus* but inert for *E. coli* strain K12, while the spectrum of *L. fermentum* strain 90 TS-4 (21) clone 3 CF included this test culture.

On the other hand, bactericidal activity of CF preparations was not associated with the presence of active components in them. *L. plantarum* 39 strain (not agglutinating concanavalin A) less actively inhibited the growth of *S. aureus* than *L. plantarum* 8 RA-3, but its bactericidal activity was higher than that of highly agglutinable *L. fermentum* strain 90 TS-4 (21) clone 3.

Evaluation of adhesion of 20 clinical strains of. *C. albicans* to VE showed that the integral adhesion index varied from 2.02 to 0.10. Only two YLF cultures (04.1567 and 1156) in the group of cultures isolated from women with manifest infection had $X_2>1$. Seven of ten cultures from patients with asymptomatic infection had $X_2>1$. The number of active VE and number of YLF per adhesive cell were higher for strains isolated from patients with asymptomatic form of infection.

Incubation of VE with *L. fermentum* strain 90 TS-4 (21) clone 3 CF (protein concentration 650 μg/ml) significantly modified adhesion activity of YLF towards target cells. The integral index chan-

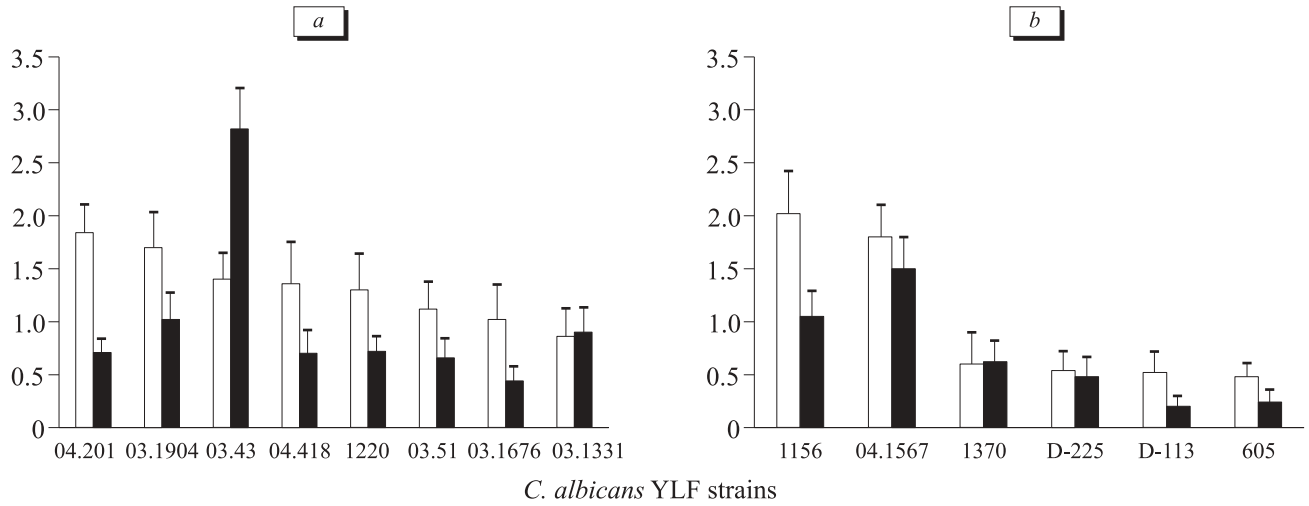

Fig. 3. Inhibition of *C. albicans* YLF adhesion to VE surface under the effect of *L. fermentum* strain 90 TS-4 (21) clone 3 CF. *a*) asymptomatic form; *b*) manifest form. Light bars: control; dark bars: experiment. Ordinates: mean number of *C. albicans* adhered to VE surface (integral index).

TABLE 2. Specific Bactericidal Activity of CF Preparations (mm/mg)

Test culture	Producer strain				
	L. fermentum			*L. plantarum* 8 RA-3	*L. plantarum* 39
	production strain 90 TS-4	90 TS-4 (21) clone 3	90 TS-4 (21) clone 2		
S. aureus	12.90	6.60	3.30	20.80	8.35
S. epidirmidis	7.80	10.40	2.38	3.80	4.40
E. coli K12	3.60	0.89	No result	No reaction	No reaction

ged in 25% of studied strains, the number of active VE in 40% cases, and the number of YLF adhered to "active" VE changed in 15% strains (Fig. 3). Incubation of CF preparation with target cells led to an increase of the integral index in 4 and decrease in 4 *C. albicans* strains. A statistically significant decrease in the number of "active" VE was observed in 5 cases and increase in 3 cases. Inhibition of YLF adhesion per "active" VE was detected for 2 strains and activation of adhesive characteristics was observed for 1 *C. albicans* strain. For the total sample of the strains, a pronounced effect with respect to one parameter of adhesion was noted in 50% cases, inhibition and activation being equally incident.

Hence, CF modulated the adhesive capacity of 14 *C. albicans* strains, the adhesion inhibition being observed in experiments with 13 *C. albicans* strains and only 1 strain exhibited a stronger adhesion. The degree of adhesion changes under the effect of CF was more pronounced for strains isolated from women with asymptomatic infection. Hence, the modulating effect of CF preparation obtained by culturing of *L. fermentum* strain 90 TS-4 (21) clone 3 on YLF adhesion to VE manifested only if YLF strains were initially highly adhesive. The components of *L. fermentum* strain 90 TS-4 (21) clone 3 CF changed the integral adhesion index of *C. albicans* strains, highly affine for VE. The same CF components of lactobacilli, released from low-molecular-weight substances, exhibited bactericidal effects towards *E. coli* K12, *S. aureus*, and *S. epidermidis* cultures.

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