Adhesion Characteristics of Lactobacillus is a Criterion of the Probiotic Choice

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A sampling of lactobacilli from the German National Collection of Microorganisms and *L. fermentum* 90 TS-4 (21) reference strain clone 3 (Russian Federation) were studied. The results indicate that the receptors on the surface of lactobacillus strains from the German collection had no structures complementary to type 1 fimbriae, though adhesins of some of them reacted with mannose and galactose receptors. Adhesion on a monolayer of continuous cell cultures showed that adhesion activity of lactobacilli was a function of many derivatives, and hence, the choice of a model for evaluation of the adhesion characteristics of the strain should be based on adhesins exhibiting universal properties in different test systems. One of them can be lectin-binding adhesin; its expression on the surface of cultures of lactobacilli from the German collection varies within the same range as was shown previously for lactobacilli, studied by the same criterion. The molecular weight of lectin-binding adhesin is 25-30 kDa, and the corresponding receptors are frequently present on various eukaryotic cells, and hence, cell models can be considered as the most adequate for studies of the competitive interactions between lactobacilli and adhesins of pathogenic microorganisms.

Key Words: *lactobacilli; adhesin; type 1 fimbriae; Caco-2; T-24*

According to the modern concepts, lactobacilli used in the manufacture of probiotics can express conservative adhesins on their surface [1,2]. These structures, due to their complementary fixation on the target cell receptors, provide coating of the terminal mucosae with a biological film. Adhesins of another type, easily scaled from bacterial surface and transforming into the liquid phase, can shield the cell receptor, making it unavailable for not only lactobacilli, but also other bacteria. Hence, remaining outside the biological film lactobacilli inhibit colonization of the mucosa with foreign microflora.

Scaling adhesin proved to be a glycoprotein located on *L. fermentum* surface and reacting with concanavalin A (ConA) [3]. Released into culture medium, this lectin-dependent adhesin retains the capacity to inhibit *S. dublin* adhesion on epitheliocyte surface [4,6].

Hence, the effect of lectin-binding adhesin (LBA) is multifactorial. The aim of this work was to determine the criteria for the choice of the most perspective strains for the manufacture of lactobacillus-based probiotics.

MATERIALS AND METHODS

L. fermentum 90 TS-4 (21) clone 3 served as the reference strain; its culturing and adhesion characteristics were described previously [1,2]. We also used a sampling (Table 1) from the German National Collection of Microorganisms (Deutsche Sammlung von Mikroorganismen und Zollkulturen

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GmbH — DSMZ), received through Dr. E. V. Sokurenko (University of Washington, Seattle).

Two *E. coli* strains were obtained from the collection of Dr. E. V. Sokurenko. One of these strains (K-12) carried type 1 piles, the other (G1122) P-piles on their surfaces.

Adhesion activity of lactobacilli was studied by their capacity to bind chemically pure biosubstrates, pre-immobilized on the surface of 96-well polystyrene plates [7]. The wells were treated with FimH (type 1 pile binding domain protein, FimH-p), K-12 (type 1 pile protein, K-12 p), RNase A (monomannose receptor, RNase A), RNase B (trimannose receptor, RNase B), and pigeon ovalbumin (PEW; galactose receptor, PEW-r).

Adhesion activity of lactobacilli was evaluated by fixation on a monolayer of continuous Caco-2 (colonic adenocarcinoma) or T24 cells (vesical adenocarcinoma).

The expression of LBA on lactobacillus surface was detected by agglutination of suspensions, washed thrice in buffered saline (pH 7.2), in conA solution. The minimum concentration of conA, at which manifest agglutination was observed, was determined.

Adhesion activity of the culture on a monolayer of continuous tumor cells was evaluated by the index of the mean number of bacteria fixed to target cells in a visual field. The data on 100 fields were evaluated.

Culture fluid (CF) collected after culturing of *L. fermentum* strain 90 TS-4 (21) clone 3 served as the source of LBA [1,2]. Stepped filtration of CF through XM-300, XM-100, and PM-30 membranes (Diaflo) in Amicon cells showed that LBA was concentrated above the PM-30 membrane but not in any other fraction. The target product was isolated in pure form by affinity chromatography (batch method) on ConA-Sepharose [2].

The data were statistically processed using MS Office Excel software.

RESULTS

Adhesion screening of lactobacilli on immobilized substrates showed that none of the cultures from the sample presented in Table 1 adhered to the wells coated with FimH-6-p and K-12-p, which meant that these lactobacillus strains had no receptors complementary to type 1 fimbriae on their surface. Therefore, co-adhesion of lactobacilli from the sample presented in Table 1 and Fim-H-positive *E. coli* could hardly be expected in such a situation. Mixing of suspension of lactobacilli from this sample and *E. coli* strain K-12 did not lead to aggregation.

TABLE 1. Lactobacillus Strains Used in the Study

No.	Lactobacillus species	Catalogue No. of the strain
01	*L. oris*	4864
02	*L. vaginalis*	5837
03	*L. Johnsonij*	10,533
04	*L. mucosae*	13,345
05	*L. gastricus*	16,045
06	*L. apodemi*	16,634
07	*L. coryniformis*	20,001
08	*L. reuteri*	20,016
09	*L. fermentum*	20,055
10	*L. delbrueckii*	20,076
11	*L. bulgaricus*	20,081
12	*L. plantarum*	20,174
13	*L. gasseri*	20,243
14	*L. ruminis*	20,403
15	*L. vitulinus*	20,405
16	*L. kefiri*	20,485
17	*L. johnsonii*	20,553
18	*L. crispatus*	20,584
19	*L. animalis*	20,602
20	*L. graminis*	20,719
21	*Leuconostoc fructosum*	20,349

However, adhesins of some studied lactobacillus strains reacted with mannose and galactose receptors; for example, 9 strains adhered to monomannose receptor presented by RNase A, 4 to trimannose receptor (RNase B), and 2 to galactose receptor (PEW) (Table 2).

Admitting that mannose and galactose receptors can be expressed on eukaryotic cells, we tested

TABLE 2. Adhesion of Some Lactobacillus Strains on Immobilized Substrates Containing Mannose and Galactose Receptors

Lactobacillus strain	Substrate		
	RNase A	RNase B	PEW-r
13,345	+	+	+
10,553	+	+	+
16,634	+	+	-
20,349	+	+	-
20,016	+	-	-
20,174	+	-	-
20,405	+	-	-
20,485	+	-	-
20,719	+	-	-

TABLE 3. Agglutination of Some of Lactobacillus Strains in the Presence of ConA

Lactobacillus strain	Concentration of conA solution, mg/ml
10,533	0.003
16,045	0.008
16,634	0.025
20,016	0.025
20,349	0.004
20,403	0.012
20,405	0.003
20,485	0.006
20,719	0.050

the lactobacillus cultures with two standard continuous cell strains (Caco-2 and T24). No correlation between adhesion activities of the studied lactobacillus strains towards these two cell strains was detected (r=0.02). Strains 10,533 and 20,584 were highly active towards Caco-2 and T24 cells, strains 16 045 and 20,349 to T24, but not Caco-2 cells. Strains 20,055, 16,634, 20,485, and 20,403 actively adhered to Caco-2 and poorly to T24 cells. It means that adhesion activity of lactobacilli is a function of many derivatives: a set of receptors on the surface of target cells and an assortment of adhesins coinciding or not with these receptors by complementarity. The choice of the model for evaluation of adhesion characteristics of the strain should be based on adhesins universal in different test systems. We hypothesized that one of them is LBA.

Therefore, lactobacillus strains from the studied collection were tested for ConA agglutination. Six strains were agglutinated with ConA in concentrations of 0.05 to 0.003 mg/ml, 6 strains were not agglutinated, and the rest strains could not be properly suspended in saline and therefore could not be tested. The expression of LBA on the surface of the first part of the sample (Table 3) varied within the same range as in a previous analysis of lactobacillus samples by the same criterion [1,2].

The incidence of LBA moderately correlated with adhesion of the cultures to Caco-2 and T24 cells. The tetrachoric coefficient of correlation was 0.62 for lactobacillus adhesion on Caco-2 and 0.59 for adhesion on T24. The relationship between adhesion index and agglutination activity was moderate (correlation coefficient r was 0.47 for Caco 2 and 0.45 for T24). This means that LBA makes an important contribution to adhesion activity of many lactobacillus strains, but participation of other adhesins in the analyzed process reduces the rigidity of this relationship. The functional characteristics of lactobacillus LBA were studied after its isolation and purification in experiments with the reference strain. Polyacrylamide gel electrophoresis on a Novex Mini-Cell device (Invitrogen) with concentrating gel 90 B and separating gel 150 B showed that the molecular weight of LBA was 25-30 kDa (Fig. 1); approximately the same parameters for LBA were reported previously [5].

The results indicate that LBA is a glycoprotein, incorporating mannose in the carbohydrate chain. A series of additional experiments showed that it did not inhibit the adhesion of *E. coli*, expressing type 1 pili, to Caco-2 and T24 cells, did not inhibit guinea pig erythrocyte agglutination with Fim-H-positive *E. coli* strains, but blocked the adhesion to eucaryotic *Candida* yeast-like target cells. Hence, LBA expressed by some lactobacillus species and easily detected by ConA is an adhesin differing from FimH. Receptors to it are often present on various eukaryotic cells, which makes cell models the most adequate for studies of competitive reactions of lactobacilli with adhesins of pathogenic microorganism.

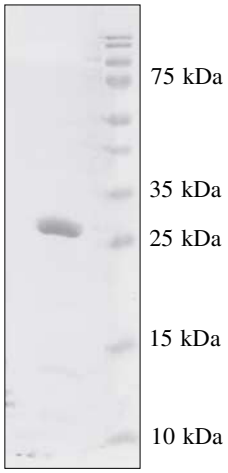

Fig. 1. Phoregram of purified LBA isolated from CF of *L. fermentum* strain 90 TS-4 (21) clone 3.

REFERENCES

1. I. V. Anokhina, E. G. Kravtsov, N. V. Yashina, *et al.*, *Byull. Eksp. Biol. Med.*, **142**, No. 11, 557-561 (2006).
2. I. V. Anokhina, E. G. Kravtsov, N. V. Yashina, *et al.*, *Vestn. Rossiisk. Universiteta Druzhby Narodov*, **35**, No. 2, 52-57 (2007).
3. R. J. Fuller, *Gen. Microbiol.*, **87**, No. 2, 245-250 (1975).
4. C. Heinemann, J. E. van Hylckama Vlieg, D. B. Janssen, *et al.*, *FEMS Microbiol. Lett.*, **190**, No. 1, 177-180 (2000).
5. A. Henriksson and R. Szewzyk, *App. Environmental Microbiol.*, 499-502 (1991).
6. K. Madsen, A. Cornich, P. Soper, *et al.*, *Gastroenterology*, **121**, No. 3, 580-591 (2001).
7. U. Sonnenborn and R. Greinwald, *Beziehungen zwischen Wirsorganismus und Darmflora*, 2nd ed., Stuttgart-New York (1991), P. 55-69.