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Characterization of Surface Adhesins of Lactobacilli Used in Production of Probiotic Preparations

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We examined production of the protein-lipoteichoic complex detectable by concanavalin A in six lactobacillus strains. A correlation was found between detection of this complex by both reaction with concanavalin A and agglutination with strain-specific antiserum. The cultures were characterized by expression of different types of adhesins. Among them, strains were differentiated with low adhesion activity and intensive production of the protein-lipoteichoic complex and strains with wide adhesin spectrum not producing the complex. We assume that a combination of lactobacillus variants differing in production of the protein-lipoteichoic complex and adhesion potency can be a basis of efficient probiotic preparation.

Key Words: *probiotics; lactobacillus adhesins; concanavalin A*

The probiotics based on lactobacilli are intensively studied in laboratories and clinics. Two hypotheses explain the benevolent effect of these agents on the macroorganism.

According to the first hypothesis, lactobacilli chosen as the principal preventive and therapeutic agent adhere to the intestinal epitheliocytes and form an additional barrier on their surface, which impedes translocation of relatively pathogenic and manifestly pathogenic intestinal microflora into the internal medium of a macroorganism [9]. It is known that lactobacilli penetrating into the aggregated lymphatic follicles and contacting with circulating lymphocytes activate and modulate the immune system [10]. This is corroborated by the experiments, which demonstrated that intensive synthesis of interfe-ron-γ (IFN-γ) occurs after induction of splenocytes by concanavalin A (Con A). A similar result was obtained in experiments, where IFN-γ synthesis was induced by *L. casey* [7]. These data suggest that the signal inducing synthesis of IFN-γ can be transmitted to splenocyte membrane either by lectin or some lectin-like structure on the surface of *L. casey*.

The second hypothesis implies intensive reproduction of the probiotic cultures in the large intestine, which plays a role of "biological reactor" [3]. In the culture medium of this fermenter, the bacteriocins are accumulated together with metalloproteinases and the specific adhesins desquamating from the surface of lactobacilli. These substances inhibit and disintegrate opportunistic and pathogenic microflora thereby protecting the macroorganism from the intestinal bacteria potentially capable for translocation. Specifically, it is shown that the biomass of *L. fermentum* of RC-14 strain releases into the culture medium not only lactate, H_2O_2, and some enzymes, but also some surface adhesins, which prevent fixation of *S. dublin* on the intestinal epithelium [6,8]. Similar experiments carried out

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on the model *L. johnsoni* of La1 strain showed that the adhesin, which desquamated into the culture medium and protected the Caco 2 epithelial cells from fixation of *Listeria monocytogenes*, *E. coli*, and some species of *Salmonella* on their surface, is a protein component combined with lipoteichoic acid [5].

We previously showed that the protein-lipoteichoic complex (PLC) selectively reacting with Con A is accumulated in the filtrate of *L. fermentum* of 90-TS-4 strain. The same experiments demonstrated that PLC is located on the surface of *L. fermentum* cells of 90-TS-4 strain. Taking into consideration both modern hypotheses describing the mode of action of lactobacteria-derived probiotics, we hypothesize that the most efficient composition of the commercial preparation would be a combination of producer strains, which includes microorganisms carrying surface desquamating adhesion-active PLC and microorganisms, whose adhesion activity is determined by lectin-like structures tightly bound to the bacterial wall. Based on our previous study we used Con A as a marker of nondesquamating PLC for evaluation of the intensity of PLC production by various commercial strains of lactobacteria used in the manufacturing of modern probiotics.

MATERIALS AND METHODS

We used a collection of routine or perspective lactobacilli to be used as the efficient producents of probiotic preparations. The cultures were obtained from L. A. Tarasevich State Research Institute for Standardization and Control of Biomedical Preparations. The reference strains were the cultures of lactobacilli from National Microorganism Collection (USA) kindly provided by Prof. E. V. Sokurenko. This collection included 6 species of lactobacilli: *L. fermentum* 90-TS-4, *L. plantarum 8RA-3, L. acidophilus* K_3Sh_{24}, *L. casey* 37, *L. paracasey* ATCC 27216, *L. acidophilus* ATCC 4356, and *L. buchneri* PO.

The two-day cultures grown in MPC-1 medium at 37°C were washed from the biomass with buffered physiological saline (pH 7.2), diluted to a concentration of 10^6 microbial cells/ml using a turbidity standard, and tested for ability to agglutinate human IV(AB) and I(0) P+ erythrocytes, sheep and guinea pig erythrocyte.

At the same time, all cultures were tested in the agglutination reaction (AR) with Con A and immune serum to *L. fermentum* 90-TS-4. This serum was obtained from rabbits repeatedly immunized (4 times) with *L. fermentum* 90-TS-4 culture (intravenous injections once a week in doses increasing from 10^3 to 10^6 CFU). This serum diluted to 1:2500 agglutinated a homologous culture. The data were processed statistically with BIOSTAT software (McGraw Hill, Version 4.0.0.0).

RESULTS

When testing the selected cultures for AR, we observed pronounced agglutination only with immune serum to *L. fermentum* 90-TS-4 diluted to 1:80 (the third dilution). This serum did not agglutinate other lactobacilli (Table 1). Therefore, this rabbit antiserum was species-specific and could be used as a marker for certain lactobacilli. Taking into consideration that the above lectin-dependent PLC was found in this lactobacillus species, AR with its antiserum was used as the marker test in the following experiments.

To detect lectin-dependent PLC in other lactobacilli, we carried out AR with Con A. Among the examined cultures, PLC was revealed not only in *L. fermentum* 90-TS-4, but also in *L. plantarum* and *L. buchneri.* By contrast, PLC was not detected in *L. acidophilus, L. casey,* and in *L. paracasey* (Table 1). We hypothesized that the degree of PLC expression can vary within *L. fermentum* species. To verify this hypothesis, the set of examined cultures was supplemented with clones of *L. fermentum* 90-TS-4 strain obtained from L. A. Tarasevich State Research Institute. As expected, these clones have different AR with Con A (Table 2).

Moreover, these clones demonstrated different AR with our original rabbit serum. There was a strong correlation between the intensity of AR of the culture with Con A and with the serum (r=0.84).

Thus, we could select variants of lactobacilli with pronounced expression of the lectin-dependent structures and variants that expressed no such structures. To screen these variants, one can use not only Con A, but also the antiserum to *L. fermentum* 90-TS-4.

On the next stage it was necessary to characterize the examined cultures by their adhesion activity. Since this property is determined by various types of adhesins and the degree of their expression, we examined the potency of the cultures to agglutinate four types of erythrocytes. It was established that a set of adhesins in 7 cultures of various types of lactobacilli and their activity in AR with the erythrocytes from sheep, guinea pig, and human IV(AB) and I(0) P+ blood groups are highly variable (Table 3). Among the examined species, *L. fermentum* 90-TS-4 strain had a narrow spectrum of adhesins: it moderately agglutinated

TABLE 1. Agglutination of Various Species of Lactobacilli with Serum *L. fermentum* 90-TS-4 and Con A

Lactobacullus species	Agglutination with serum		Agglutination with Con A	
	dilution order	titre	dilution order	Con A concentration mg/ml
L. fermentum 90-TS-4 production standard	3	1:80	8	3.5×10^{-4}
L. plantarum 8RA-3	—	—	6	15×10^{-4}
L. acidophilus K_3Sh_{24}	—	—	—	—
L. buchneri PO	—	—	5	30×10^{-4}
L. casey 37	—	—	—	—
L. paracasey ATCC 27216	—	—	—	—
L. acidophilus ATCC 4356	—	—	—	—

Note. Here and in Tables 2, 3: dash means the absence of the reaction.

TABLE 2. Agglutination of *L. Fermentum* Clones with Antiserum to *L. fermentum* 90-TS-4 and Con A

Clone *L. fermentum*		Agglutination with serum		Agglutination with Con A	
		dilution order	titre	dilution order	Con A concentration mg/ml
L. fermentum 90-TS-4	production standard	3	1:80	8	3.5×10^{-4}
L. fermentum 90-TS-4	clone 1	6	1:640	9	1.75×10^{-4}
	clone 2	5	1:320	10	0.875×10^{-4}
	clone 3	5	1:320	10	0.875×10^{-4}
	clone 4	5	1:320	9	1.75×10^{-4}
L. fermentum A4		—	—	5	30×10^{-4}
L. fermentum B2		2	1:40	8	3.5×10^{-4}

TABLE 3. Expression of Various Types of Adhesins on Lactobacilli (Culture Dilution Order)

Lactobacillus species	Agglutination activity with:			
	sheep erythrocytes	guinea pig erythrocytes	human erythrocytes	
			IV(AB)	I(0) P+
L. fermentum 90-TS-4 production standard	1	—	—	—
L. plantarum 8RA-3	1	—	—	—
L. acidophilus K_3Sh_{24}	—	—	—	1
L. buchneri PO	1	—	—	—
L. casey 37	1	—	—	—
L. paracasey ATCC 27216	—	4	—	—
L. acidophilus ATCC 4356	5	5	3	—

only sheep erythrocytes. By contrast, *L. acidophilus* K_3Sh_{24} actively agglutinated erythrocytes of all three types.

This study suggests that the design of probiotic preparation can include cultures with pronounced expression of various adhesins that cannot secrete lectin-dependent PLC and cultures of lactobacilli secreting this substrate but exhibiting moderate erythrocyte adhesion capacity. Screening of these variants can be based on AR with immune rabbit antiserum and Con A.

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