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ANTAGONISM OF LACTIC ACID BACTERIA STRAINS AGAINST ISOLATES OF HAEMOLYTIC STREPTOCOCCI

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Abstract*. The article presents the results of studies of antagonistic activity of lactic acid bacteria strains in relation to clinical strains of St. pyogenes and St. pneumoniae and biofilms formed by them. It is shown that 20 strains out of 25 tested test-cultures have high antagonistic activity against all tested test-cultures. Also, it is shown that EPSs of tested lactobacilli prevent the formation of St. pneumoniae biofilm.*
Keywords*: haemolytic streptococci, growth suppression, biofilm formation.*

Streptococci are gram-positive aerobic organisms that cause many diseases including pharyngitis, pneumonia, wound and skin infections, septicaemia and endocarditis. Symptoms vary depending on the organ affected. Complications of infection caused by BHSA include rheumatic fever and glomerulonephritis [1]. Most strains are sensitive to penicillin, but macrolide-resistant strains have emerged. Many streptococci produce virulence factors, including streptolysins, DNAases and hyaluronidase, which promote tissue destruction and spread of infection. Certain strains produce exotoxins that activate certain T cells, causing the release of cytokines including tumour necrosis factor-alpha, interleukins and other immunomodulators [2]. These cytokines activate complement, coagulation and fibrinolytic systems, leading to shock, multi-organ failure and death. BHSA is the most common cause of acute bacterial pharyngitis. Other causes of bacterial origin include group C and G streptococci and *Fusobacterium necrophorum* [3].

Materials and Methods.

Method for determining the antimicrobial activity of lactic acid bacteria strains against isolates of haemolytic streptococci. Antimicrobial activity of Lactobacillus strains against isolates of haemolytic streptococci was investigated by the method of stain on agar [4]. Prior to the experiment, the viability of test cultures was restored by threefold resuspension in tryptone-soy broth containing red blood cell mass (5%) (HiMedia, India) and incubation at 25-28º C for 24 hours under anaerobic conditions. For testing, 25 Lactobacillus strains were grown in MRS broth at 37oC for 24 hours. Then 7 µl aliquot of the test culture was applied as a spot on the surface of a cup containing 20 ml of MRS agar. The seeded cups were incubated for 48 hours under anaerobic conditions at 37oC. The layer with the grown test culture was then covered with a second layer of 8 ml of soft agarised (0.75% agar) tryptone soya medium (HiMedia, India) containing 106 CFU/ml daily culture of indicator bacteria. After daily incubation at 25-28o C in the thermostat under aerobic conditions, the formation of a zone of growth inhibition of the indicator culture by the tested strains was observed.

Method for determining the antagonistic activity of biofilms of Lactobacillus cultures. The antagonistic activity of biofilms of lactobacilli cultures was studied according to the previously described method with some modifications [5]. Overnight cultures of all strains grown in MRS broth were diluted to an optical density of 0.1 in freshly prepared MRS broth medium. Five microlitres of inoculum was added to the wells of a sterile 96-well polystyrene flat-bottom plate filled with 100 ml of fresh broth. Seeds were cultured for 48 h at 37°C under aerobic conditions

and then unattached cells were gently removed using a pipette and biofilms visually present on the bottom and sides of the plate were washed with 2 ml of phosphate buffer at pH7.1 (10 mM Na2HPO4, 1 mM KH2PO4, 140 mM NaCl, 3 mM KCl) to remove planktonic and poorly attached cells. Absorbance (A600 nm) of suspensions of pathogenic bacteria in TSB was adjusted to 0.25 0.05 to standardise the number of bacteria (107-108 CFU/ml), added to biofilms and incubated at 30oC for 24, 48 and 72 hours. Every 24 hours, half of the broth in the wells was replaced with fresh broth. After incubation, the planktonic cultures were carefully removed and the biofilm cells were suspended by scraping and thorough shaking. To estimate the number of attached bacteria in the biofilm three wells of each strain were washed three times and scraped as previously described. The obtained suspensions were transferred into sterile tubes and mixed by vortexing for 30 s. Then dilutions in saline (0.85% (wt./vol.) NaCl) were prepared, seeded on dishes with Miller-Hinton agar medium, incubated at 37°C for 24-48 hours and bacterial counting was performed.

Results.

The antimicrobial activity of lactic acid bacteria strains against isolates of haemolytic streptococci. In our work we investigated the antagonistic activity of 25 local strains of lactobacilli in relation to clinical isolates of St. pyogenes and St. pneumoniae the investigated objects are presented in Table 1, low activity - 10-14.9 mm, medium - 15.0-19.9, high - more than 20 mm. The inactive group included cultures that did not inhibit the test strains. Most of the selected cultures showed a high value of antagonistic activity to St. pyogenes and St. pneumoniae pathogens: the diameter of growth suppression zones exceeded 20 mm (Table 1).

P. acidilactici OC1 cultures (from 20 mm to 30±0.18 mm) showed a high degree of antagonism to St. pyogenes and (20.2±0.3 mm) to *St. pneumoniae*, *L. plantarum* strain TK1 (from 20 to 30 mm and 40 mm, respectively). *L. salivarius* Pr and *P. acidilactici* B strains showed the lowest antagonistic activity against the test cultures used.

Three strains of *L. rhamnosus* were used, 2 of which were isolated from the oral cavity of healthy people (L. rhamnosus OC1 and L. rhamnosus OC2), 1 strain was isolated from cheese (L. rhamnosus 925). Among these strains against St. pyogenes and St. pneumoniae, antagonism rates were higher than average in the culture of L. rhamnosus 925. The lowest indices of antagonistic activity to the test strains were observed in L. rhamnosus OC1.

Also, among the studied 4 strains of *L. plantarum*, 2 strains: *L. plantarum* TK1 and *L. plantarum* mal showed high rates of antagonistic activity towards haemolytic Streptococcus (from 20 mm to 40 mm).

Low activity was recorded in *L. plantarum* B1 (from 14 mm). Thus, 20 strains out of 25 tested test-cultures have high antagonistic activity against all tested test-cultures.

The degree of biofilm formation in *Streptococcus pneumoniae* was reduced under the influence of EPS strain *St. salivarius* 17P and at all concentrations.

The activity of EPS of strain *St. salivarius* 17P against *Streptococcus pneumoniae* biofilm had an inverse correlation with EPS content, i.e. there was a decrease in activity with increasing EPS content and at 0.1 mg/ml the activity was 41 % (after 72 hours of incubation). Increase of EPS content up to 2 mg/ml involved a decrease of activity up to 28%.

The strains *P. acidilactici OC1, L. flantarum K2, L. plantarum mal, St. salivarius* 17P were selected for further studies as more promising for use as a probiotic culture among other identified strains of lactobacilli with antagonistic activity.

Table 1

Antagonistic activity of lactic acid bacteria to St. pyogenes and St. pneumoniae isolates

№	cultures	*St. pyogenes A4*	*St. pyogenes A7*	*St. pyogenes N1*	*St. pyogenes N2*	*St. pneumoniae 1*
	P. acidilactici OC1	30	30	20	25	20
	P.acidilactici OC2	24	26	22	26	25
	P. acidilactici OC3	15	36	40	20	30
	P. acidilactici 2	26	20	0	20	0
	P. acidilactici B	0	0	0	0	0
	P. sp.	26	18	20	24	35
	L. rhamnosus OC1	30	24	0	25	30
	L. rhamnosus OC2	30	30	28	26	25
	L. rhamnosus 925	30	15	32	26	40
	L. plantarum B1	28	16	38	14	34
	L. plantarum KA3	30	40	25	13	40
	L. plantarum TK1	30	30	26	20	40
	L. plantarum mal	26	25	28	28	30
	L. brevis OC1	25	36	40	20	30
	L. brevis 2	25	20	30	20	25
	St. salivarius OC1	24	32	30	0	40
	L. salivarius Pr	0	0	0	0	40
	E. durans	30	30	30	10	26
	L. reuteri 2	30	24	35	10	20
	L. fermentum F	0	0	25	0	28
	L. fermentum X	26	16	16	20	30
	L. kunkeei	25	26	30	18	20
	L. paracasei 2	22	24	24	18	30
	Leu. lactis Sh	26	24	26	26	19

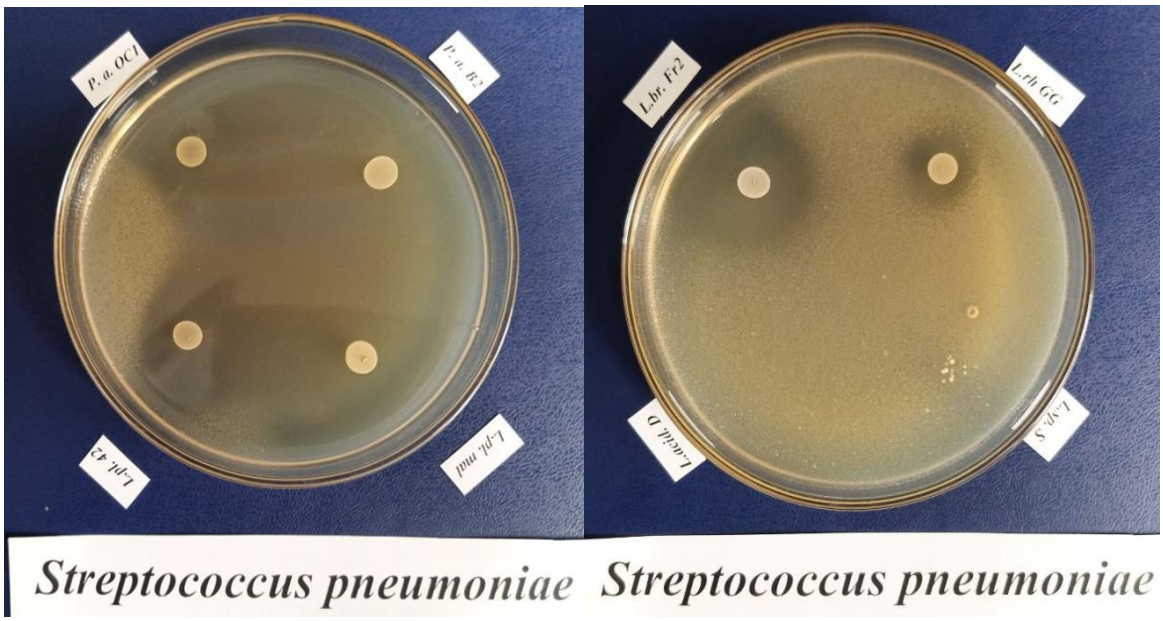

Fig. 1. Antagonistic activity of lactic acid bacteria to isolates Streptococcus pneumoniae

Table 2.

Antagonistic activity of lactic acid bacteria exopolysaccharides against biofilm formation by opportunistic bacteria.

	Biofilm forming pathogen strains	Incubation time	Antagonistik activity of EPS, %											
			St. saliv. 17 P			*L.pl.* 42			*L.pl.* K2			*Ped. acid.* OC1		
			0,1 мг	1 мг	2 мг	0,1 мг	1 мг	2 мг	0,1 мг	1 мг	2 мг	0,1 мг	1 мг	2 мг
	Streptococcus pneumoniae	24 h	15	11	4	14	15	18	28	22	10	1	14	5
		48 h	40	36	32	28	22	10	33	35	33	24	43	39
		72 h	41	38	28	27	28	29	40	35	35	1	0	1

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