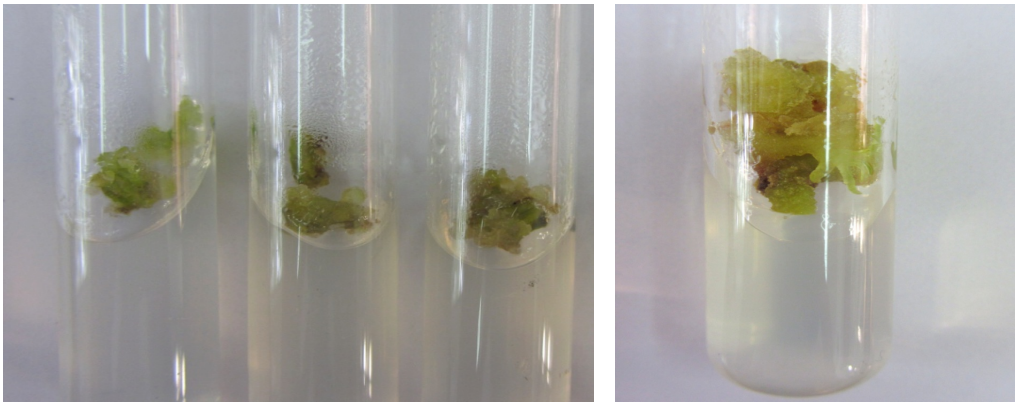

Рисунок 2 – Каллусогенез на модифицированной питательной среде МС

В целом, оптимальной питательной средой для индукции морфогенеза в культуре листовых и корневых эксплантов являлась среда Мурасиге-Скуга, содержащая 1 мг/л БАП, 0,1 мг/л НУК, 0,1 мг/л 2,4-Д. Для увеличения всхожести семян при получении стерильных проростков in vitro необходимо предварительно проводить стратификацию и обработку семян 0,1% тиомочевинной в течение 15 минут. Простерилизованные и обработанные тиомочевинной семена, культивируемые на безгормональной питательной среде МС дают хорошо развитые стерильные проростки.

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Extraction of lipopolysaccharide antigens from strains of *Salmonella typhimurium* and *Salmonella enteretidis*

There are results of lipopolysaccharide antigens water-phenol extraction of *S. typhimurium* and *S. enteritidis* in this article, antigenic and serological properties of these antigens were studied by the indirect enzyme linked immunosorbent assay.

Keywords: salmonella, lipopolysaccharide antigen, water-phenol extraction.

Тұрғымбаева А.М., Қауқабаева Г. К., Бәкірова Г.А., Сегізбаева Г.Ж.

Salmonella typhimurium және _Salmonella enteritidis_ штамдарынан липополисахаридті антигендерді бөліп алу

Бұл мақалада *S. typhimurium* және *S. enteritidis* липополисахаридті антигендерін сулы-фенолдық әдіспен бөліп алу нәтижелері сипатталған, сонымен қатар ИФТ жанама қойылымында ЛПС антигендердің серологиялық және антигендік қасиеттері зерттелінген.

Түйін сөздер: салмонеллдер, липополисахаридті антиген, сулы-фенолдық экстракция.

Тургимбаева А.М., Каукабаева Г.К., Бакирова Г.А., Сегизбаева Г.Ж.

Выделение липополисахаридных антигенов из штаммов _Salmonella typhimurium_ и _Salmonella enteritidis_

В данной статье описаны результаты выделения липополисахаридных антигенов *S. typhimurium* и *S. enteritidis* водно-фенольный методом, а также были изучены антигенные и серологические свойства ЛПС антигенов в непрямом варианте ИФА.

Ключевые слова: сальмонеллы, липополисахаридный антиген, водно-фенольная экстракция.

Bacteria genus of *Salmonella* is one of the most common pathogen of food poisoning, that infects most types of food (meat, eggs, vegetables products). It is necessary to use innovative rapid methods of *Salmonella* detection for satisfaction of food manufacturers' requirements. Food manufacturers demand fast realization of finished products and reduction of storing costs [1]. According to WHO, in the USA more than one million people ill from *Sallmonella* every year. In 2011 salmonellosis killed 580 Americans. There is no clear statistics of *Salmonella* poisoning in Kazakhstan. By some estimates, in our country 30-40 people per 100 thousand of population become ill from *Salmonella* every year. Salmonellosis causes significant damage to the cattle breeding: high mortality of diseased young animals, stunting of convalescents, abort and expenses on organization of preventive and curative measures.

In epidemiological terms the most significant for human serotypes are *S. typhimurium* and *S. enteritidis*. *Salmonella* overcome nonspecific defense factors of mouth and stomach, and then these bacteria penetrate into the small intestine. There is *Salmonella* death and release of lipopolysaccharide (endotoxin). Endotoxin determs symptoms of intoxication in the small intestine.

Lipopolysaccharide (LPS) forms the outermost layer of gram-negative bacteria and serves to protect the cell from the environment. LPS is composed of three portions: lipid A, core oligosaccharide, and O antigen. In pathogenic bacteria, LPS plays an important role in the interaction between the bacterium and its host, having dramatic effects on the immune system. Whereas the lipid A moiety is the predominant cause of the endotoxic effects of LPS, the O antigen is the most immunodominant portion of the molecule [2]. Therefore, the structure and immunogenicity of O antigen can have significant effects on the ability of the humoral immune system to mount a response to an infection.

The aim of research was lipopolysaccharide antigens extraction of *S. typhimurium* and *S. enteritidis,* studying their antigenic and immunochemical properties.

Materials and methods

The cultivation of S. typhimurium and S. enteritidis strains. Stock cultures were plated from beef extract agar to 3-4 Petri dishes, incubation conditions: 18-24 hours, 37-37.5°C. Matrix hatch of strains was reseeded to Luria Bertani liquid medium for production of bacterial mass.

Extraction of LPS antigens. LPS antigens were extracted by method of O. Westphal and K. Jann [3]. Concentration of carbohydrates in lipopolysaccharide solution was measured by Molisch test (sulfuric acid method). Place 2 mL of a known carbohydrate solution in a test tube, add 1 drop of Molisch reagent (10% α-naphthol in ethanol). Pour 1-2 mL of conc. H_2SO_4 down the side of the test tube, so that it forms a layer at the bottom of the tube. Observe the color at the interface between two layers and compare your result with a control test. Concentration of contaminant proteins was detected by Bradford protein assay[4].

Immunization of BALB/c mice. Mice were immunized intraperitoneally with 100 μL LPS antigens by scheme of Fridlyanskaya I.I. [5]. BALB/c mice were 7-8 week old. On the first day of immunization mice were injected with LPS and 0.1 ml Freund's incomplete adjuvant (Gibco, USA), on 7^{th}, 11^{th}, 12^{th}, 13^{th} days they were immunized with LPS without adjuvant. Antigen injection dose was brought by phosphate buffered saline, pH 7,2-7,4.

The indirect enzyme linked immunosorbent assay (ELISA). Antibodies titers and antigen optimal concentration were studied by the indirect ELISA. The ELISA was based on interaction of extracted LPS antigens

of *S. typhimurium* and *S. enteritidis* with serum antibodies of mice immunized by same antigens. Mice antibodies were detected using by goat anti-mouse IgG-antiserum conjugated to peroxidase (Sigma, USA).

Results and discussion

Production technology of *S. typhimurium* and *S. enteritidis* bacterial mass has been established. That technology allowed to obtain a sufficient number of microbial cells. There were small-diameter colonies of *S. typhimurium* and *S. enteritidis* on beef extract agar after cultivation. Those smooth and convex colonies had rounded edges. Method of O. Westphal was quite suitable for LPS antigens extraction from bacterial mass of *S. typhimurium* and *S.enteritidis*.

The yield of bacterial mass of *S. typhimurium* and *S. enteritidis,* as seen from table 1, from 1L medium was 22-28 g/L. The yield of LPS from cultures of *S. typhimurium* and *S. enteritidis* was 0,750-0,250 mg/ml, respectively.

Table 1 – Results of LPS antigens extraction of *S. typhimurium* and *S. enteritidis* by water-phenol method of O. Westphal

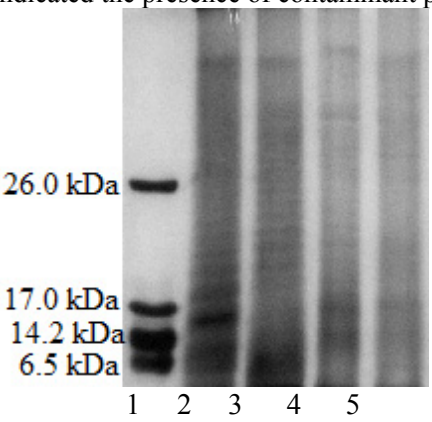
№	Culture	Yield of bacterial mass, g	Percentage of LPS yield		Presence of proteins in obtained LPS	
			mg	%	mg	%
1	*S. typhimurium*	28,0	0,750	0,0027	0,042	5,6
2	*S.enteritidis*	22,0	0,250	0,0036	0,042	2,8

LPS solutions of *S. typhimurium* and *S. enteritidis* contained 97,1% and 96,6% carbohydrates, the remaining 2.8% and 5.6% were contaminating proteins, respectively.

So, using of modified water-phenol method of O. Westphal for LPS extraction from bacterial mass of *S.typhimurium* and *S. enteritidis* allowed to obtain LPS antigens with maximum content of carbohydrates and minimum content of proteins.

SDS-polyacrylamide gel electrophoresis LPS antigens was carried out by the method of Laemmli [6] with 12% acrylamide. After separation by electrophoresis gel was stained by Silver Stain (Bio-Rad, USA) for detection and visual characteristic of LPS. Silver staining was a very sensitive method, that detected even 1 ng LPS and visualize bands of purified LPS (figure 1). Bands of LPS of *S. typhimurium* and *S. enteritidis* appeared as a dark staircase, as shown in figure 1.

Lipid A migrated very close to the dye, it appeared as a black area at the bottom of the gel. The research results of both strains showed different band contours of LPS. Staining gels with Comassie blue demonstrate bands, that indicated the presence of contaminant proteins.



1 – Marker Ultra-low (Sigma, USA); 2– LPS of *S. typhimurium*, phenol purification (Sigma, USA); 3 – LPS of *S. enteritidis,*phenol purification (Sigma, USA); 4– extracted LPS of *S. typhimurium*; 5– extracted LPS of *S. enteritidis*

Figure 1 – Electrophoresis LPS of *S. typhimurium* and *S. enteritidis*

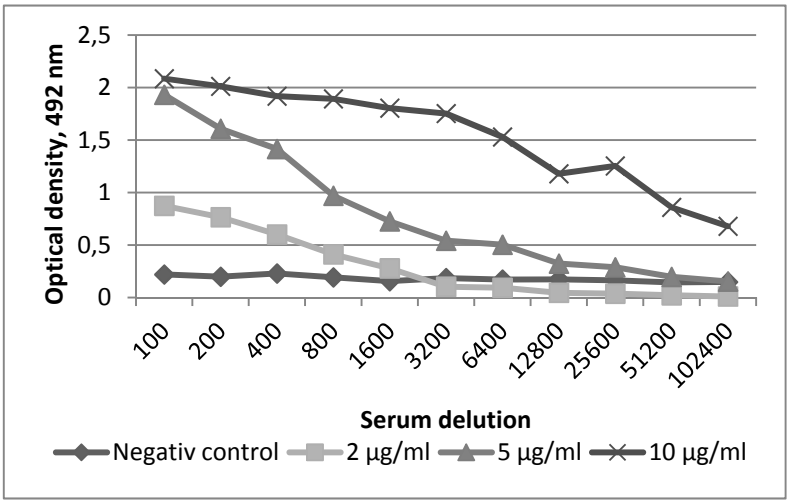


Figure 2 – Testing of the optimal concentration of LPS antigen by ELISA

LPS serological properties of *S. typhimurium* and *S. enteritidis* were studied by indirect ELISA. LPS antigens were immobilized at various concentrations from 2 mg/ml to 10 mg/ml on the surface of the polystyrene plates wells for optimal LPS antigen concentrations determination. As optimum we identified conditions, under which absorbance values of the highest dilution of immunized mice serum had exceeded the absorbance of negative serum two or more times (figure 2).

Optimal antigen concentrations for sensibilization of solid phase are 5 μg/ml and 10 μg/ml, the antibody titer reached 1:6400. When antigen concentration was 2 μg/ml, the antibodies titer decreased to 1:800. Increase of antigen concentration to 20 μg/ml led to intense background image.

LPS antigens of *S. typhimurium* and *S. enteritidis* were used to mice immunization for detection of LPS antigenic properties. Serum of immunized mice were tested on the content of specific antibodies against LPS antigens by the indirect ELISA.

The optimal combination of five injections facilitated the emergence of sufficient serum antibodies with high affinity to used antigens. Specific serum antibodies titers of immunized mice against LPS ranged from 1:6400 to 1:25600. This indicated an active induction of B-lymphocytes clones, which produced antibodies with a predetermined specificity.

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Молекулярно-генетическое маркирование пшеницы

Проведен скрининг сортов пшеницы селекции КазНИИЗиР на присутствие эффективных генов устойчивости к бурой и стеблевой ржавчине. С использованием молекулярных маркеров генов Lr 32 идентифицирован у 5 из 12 изученных сортов озимой пшеницы и у Lr 21 - 4 из 9 изученных сортов озимой пшеницы. Ген Sr 24 был выявлен у 10 сортов пшеницы из 13, ген Sr 36 не обнаружен у 5 сортов из 14 изученных. Полученные результаты будут использоваться для создания сортов устойчивых к бурой и стеблевой ржавчине с применением MAS-селекции.

Ключевые слова: пшеница, ПЦР, гены устойчивости, стеблевая ржавчина, молекулярные маркеры, праймер.

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Molecular genetic labeling of wheat

PCR analysis showed the presence of the desired gene in 5 out of 12 studied varieties of winter wheat: Naz, Arap, Myra, Farabi, sprouts - Lr primers 32 and 4 out of 9 studied varieties of winter wheat: Karasaj, Farabi, seedlings, Nurek primers Lr 21. We studied varieties are marked by the presence or absence of genes Sr36, Sr24. Sr 24 gene was identified in the following varieties: Vitreous 24 Almali; Zhetisu; Karasaj; Naz Sapaly, Farabi, Seedlings, Nurek, Arap, have been identified: Jubilee 60, Myra, Beauharnais - 56; gene Sr 36 is not found in the following varieties : Vitreous 24 Almali, Karasaj, Jubilee 60 and 56 rainfed

Keywords: wheat, PCR resistance genes, rust, molecular markers, primer.

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Бидайды молекулярлы-генетикалық маркерлеу

ПЦР- анализ зерттелген 12 күздік бидай сорттарының 5-де Lr 32 праймері бар екенін көрсетті, олар: Наз, Арап, Майра, Фараби, Рассад, ал Lr 21 праймерлері зерттелген 9 күздік бидай сорттарының 4-інде анықталды: Қарасай, Фараби, Рассад, Нуреке. ПЦР- анализ Sr 24 гені келесі сорттарда бар екендігін көрсетті: Стекловидная