Таблица 2
Наличие характерных пиков у разных представителей сем. *Enterobacteriaceae*

Представители семейства *Enterobacteriaceae*	Пики *Enterobacteriaceae*		Представители семейства *Enterobacteriaceae*	Пики *Enterobacteriaceae*	
	4363±1 Da	6092±1 Da		4363±1 Da	6092±1 Da
Arsenophonus	-	-	*Morganella*	н/б	-
Biostraticola	н/б*	-	*Obesumbacterium*	н/б	-
Brenneria	-	-	*Pantoea*	-	-
Buchnera	н/б	-	*Pectobacterium*	-	-
Budvicia	-	-	*Phaseolibacter*	н/б	-
Buttiauxella	-	-	*Photorhabdus*	-	-
Cedecea	-	-	*Plesiomonas*	-	-
Citrobacter	-	-	*Pragia*	-	-
Cosenzaea	н/б	-	*Proteus*	-	-
Cronobacter	-	-	*Providencia*	-	-
Dickeya	4363	-	*Rahnella*	-	-
Edwardsiella	-	-	*Raoultella*	4364	-
Enterobacter	4364	-	*Saccharobacter*	н/б	-
Erwinia	4362	-	*Salmonella*	4363	6092
Escherichia	4364	-	*Samsonia*	-	-
Ewingella	4364	-	*Serratia*	-	-
Gibbsiella	н/б	-	*Shigella*	н/б	-
Hafnia	-	-	*Shimwellia*	4364	-
Klebsiella	4363	-	*Sodalis*	-	-
Kluyvera	-	-	*Tatumella*	-	-
Leclercia	-	-	*Thorsellia*	н/б	-
Leminorella	-	-	*Trabulsiella*	4363	6093
Lonsdalea	н/б	-	*Wigglesworthia*	н/б	-
Mangrovibacter	н/б	-	*Xenorhabdus*	4364	-
Moellerella	-	-	*Yersinia*	-	-
			Yokenella	4364	-

н/б — нет базы данных.

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Таблица 3
Протеомическая характеристика отдельных серотипов сальмонелл

Масса (m/z)	Характеристика серотипов (по данным R. Dieckmann и B. Malorny)	Идентификация изолятов *Salmonella* посредством MALDI Autoflex III Biotyper
6,008	*Virchow*	*Salmonella* Virchow свинина № 31 *Salmonella* Dublin «Рассвет»
6,022	*Choleraesuis*	*Salmonella* Choleraesuis свинина «Башкирия» *Salmonella* Choleraesuis «Ил» *Salmonella* Choleraesuis «Лен» *Salmonella* Choleraesuis «Мордовия» *Salmonella* Choleraesuis «Владимир»
6,036	*Enteritidis*	*Salmonella* Enteritidis «Ру 3» цыпленок *Salmonella* Enteritidis «Глеб» цыплята *Salmonella* Enteritidis мясо пельменное «Pel»
7,097	*Typhimurium*	*Salmonella* Typhimurium «комбикорм № 16»

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PROTEOMIC PROPERTIES
OF SALMONELLA ISOLATES

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SUMMARY
The paper presents results of Salmonella of studying *Salmonella* isolate properties by MALDI-TOF method using MALDI Autoflex III mass-spectrometer. Microorganism identification was performed using direct loading method followed by comparison of mass spectra with the database set in the apparatus. Peaks typical of many *Enterobacteriaceae* representatives and mass to charge ratio (m/z), characterizing *Salmonella* were determined.

Key words: *mass-spectrometer,* MALDI-TOF, *Salmonella* bacteria.

INTRODUCTION

Salmonella pathogenic bacteria are one of the major agents of enteric infections and present great problems for public health in developed and developing countries as they cause food toxicoinfections. 1.3 billion gastroenteritis cases and 3 million deaths caused by *Salmonella* infection are registered annually.

Salmonella are transmitted mostly by livestock animals. Besides, *Salmonella* can be transmitted from animals to humans and from humans to humans. The main source of human *Salmonella* infection is animal and plant products (meat, eggs, dairy products, fruit and vegetables). The modern area of foodstuff production, the so called "organic" farming, also increases risk of food poisoning including salmonellosis [1].

Within the last decade microbial identification of protein profiles or direct protein profiling has been used more frequently together with classical, and molecular and biological methods of microbial identification. This method is competitive in such criteria as identification accuracy and specificity but it is more rapid and cost effective [2, 3, 9, 10].

Time-of-light mass spectrometry MALDI (MALDI-TOF) is based on matrix assisted laser desorption and ionization of the tested substance followed by ion separation using time-of-light mass-analyzer. When exposed to laser the matrix crystallized with the tested material actively absorbs laser irradiation which leads to its desorption. When transiting to gas-phase the matrix carries molecules of the tested substance and facilitates their ionization with formation of singly charged ions [7]. The method enables to perform direct mass-spectrometry of microbial cellular protein fraction (direct protein profiling), i.e. without fractionation and protein purification, and obtain highly accurate and high resolution mass-spectra, unique for this species, characterizing the tested object on the "fingerprint pattern" principle [4].

Spectra comprising a mass-range of 2–20 kDa are usually used for microbial identification. Analysis of mass-spectra of *E. coli* in this range demonstrated only 30 out of 2000 proteins, calculated basing on *E. coli* sequencing data, in spectra [11]. Most of the obtained peaks were referred to ribosomal proteins, and the rest to DNA-binding proteins and cold shock proteins. Ribosomal proteins are quite conservative and that makes them taxonomically specific. Besides, many ribosomal proteins are located in cell cytoplasm – up to the half mass of a growing cell, and their set remains unchanged not depending on external conditions and growth stage which ensures mass-spectrum reproducibility. Studies of intra- and inter-laboratory reproducibility demonstrated high reliability of MALDI-TOF method [2].

The study was aimed at proteomic properties of *Salmonella* isolates recovered from food products and feeds.

MATERIALS AND METHODS

Isolates. 27 *Salmonella* isolates recovered from foodstuffs and feeds in the Russian Federation by the FGBI «ARRIAH» microbiology laboratory in 2006–2010.

Matrix. Saturated CHCA solution (alpha-cyano-4-hydroxycinnamic acid) and organic solvent (basic organic solvent) –50% acetonitrile solution/2,5% trifluoroacetic acid.

Data base for «Bruker» bacteria identification contains spectra of 4,111 microorganisms including spectra of 15 *Salmonella* strains.

Cultivation. All used isolates were grown in Columbian agar (*Columbia agar base*) within 24 hours at 37ºC.

Sample preparation. There was used a direct loading method where single colonies of the fresh culture were added to wells of a metal «Ground steel» Bruker plate using a sterile loop. Then matrix solution was added. Drying process took 15 minutes. The plate was put in the apparatus.

Mass-spectrometer *calibration* was performed prior to each experiment according to the manual [6], using *Bruker Bacterial Standard* («Bruker» Daltonik, Germany) as a calibrant.

Mass-spectrometry was performed using «MALDI Autoflex III Biotyper» (Bruker Daltonik, Germany) mass-spectrometer in linear mode. Analysis parameters were optimized for mass range of 2000–20137 m/z (mass/time), spectrum obtained as a result of summing 10 singular spectra was recorded. Method for microorganism detection, «MBT_MC» software, was used for *Salmonella* identification. FlexControl, MALDI biotyper version 3.0 and MALDI Biotyper RTC («Bruker» Daltonik, Germany) were used for recording, processing and analysis of obtained mass-spectra.

RESULTS AND DISCUSSION

Bacteria from the FGBI «ARRIAH» strain museum identified as *Salmonella* bacteria according to bio-chemical characteristics and serotyped according to the Kauffman-White classification were selected for proteomic property studies (Table 1).

Table 1
Identification of *Salmonella* isolates using protein profiling method

№	Isolate name	MALDI result	Identification criteria, Ig*	Peak, intensity 100%, Da
1	*Salmonella* Typhimurium (beef № 4975)	*Salmonella* sp. *enterica*	2,344	6092
2	*Salmonella* Choleraesuis «Bashkiria»	*Salmonella* sp. choleraesuis	2,579	4363
3	*Salmonella* Typhimurium «dumplings No 1904»	*Salmonella* st anatum	2,511	6092
4	*Salmonella* Brezany	*Salmonella* st anatum	2,559	6092
5	*Salmonella* California	*Salmonella* st anatum	2,574	6092
6	*Salmonella* Enteritidis «Ru 3»	*Salmonella* st anatum	2,488	6092
7	*Salmonella* Choleraesuis «Len»	*Salmonella* sp. choleraesuis	2,574	4364
8	*Salmonella* Enteritidis «Gleb»	*Salmonella* st anatum	2,423	6092
9	*Salmonella* Newland duck	*Salmonella* st anatum	2,591	6093
10	*Salmonella* Choleraesuis «Il»	*Salmonella* st anatum	2,445	4364
11	*Salmonella* Choleraesuis «Yub»	*Salmonella* st anatum	2,442	6092
12	*Salmonella* Typhimurium «k/k16»	*Salmonella* st anatum	2,509	6092
13	*Salmonella* Typhimurium «k/k 7»	*Salmonella* st anatum	2,509	6096
14	*Salmonella* Virchow pork 31	*Salmonella* st anatum	2,506	6093
15	*Salmonella* Choleraesuis «Mordova»	*Salmonella* sp. *enterica* st Hadar	2,348	4364
16	*Salmonella* Enteritidis Meat for dumplings (chicken) «Ri 1»	*Salmonella* st anatum	2,473	6091
17	*Salmonella* Enteritidis Meat for dumplings «Pel»	*Salmonella* st anatum	2,482	6092
18	*Salmonella* Moscow «Radon» feedstuff	*Salmonella* sp. enteritidis	2,477	6091
19	*Salmonella* Dublin «Rassvet»	*Salmonella* sp. *enterica* st dublin	2,536	6093
20	*Salmonella* Saintpaul	*Salmonella* st anatum	2,649	6092
21	*Salmonella* Choleraesuis «Krasnodar»	*Salmonella* sp. *enterica*	2,247	4363
22	*Salmonella* S.W	*Salmonella* sp. enteritidis	2,408	6090
23	*Salmonella* Choleraesuis № 2 «Tambov»	*Salmonella* sp. *enterica*	2,457	6092
24	*Salmonella* Choleraesuis Pork «Tatarstan»	*Salmonella* sp. choleraesuis	2,528	6092
25	*Salmonella* Dublin «Kolchugino»	*Salmonella* st anatum	2,654	6092
26	*Salmonella* Choleraesuis «Ulyanovsk»	*Salmonella* st anatum	2,419	6092
27	*Salmonella* Choleraesuis «Vladimir»	*Salmonella* st anatum	2,523	4364

* 2,300–3,000 – high probability of species identification;
2,000–2,299 – guaranteed genus identification, probable genus identification;
1,700–1,999 – probable genus identification.

Due to the fact that microorganism database in the apparatus includes only 13 *Salmonella* serotypes and according to the Kauffman-White classification there are more than 2,600 of *Salmonella* serotypes in the present time the results of MALDI identification related to serotype determination can differ from results of classical serotyping.

Bacteria identification using direct protein method [5, 7] confirmed that all tested microorganisms belong to the *Salmonella* genus (Table 1). Herewith, microorganism identification criterion was within 2,236–2,649, which is indicative of high probability of identification.

Within the process of bacteria identification using MALDI-TOF method protein profiles were constructed for all *Salmonella* bacteria. They enabled determination of typical high intensity peaks.

It was determined that 6 out of 27 studied *Salmonella* isolates demonstrated a typical peak with 100% intensity and m/z 4364 Da in a protein spectrum. For all the rest isolates identification peak was at 6092 Da.

According to Zhou N. and Wang N. [12] a unique peak characterizing *Salmonella paratyphi*, is peak at m/z 6092 Da. In our test such peak was shown by all *Salmonella* isolates together with peak at 4363 Da.

Performed mass-spectrometry of *Enterobacteriaceae* family reprsentatives demonstrated in Maldi Biotyper database (Table 2) determined that peak 4363±1 Da is typical of many representatives of *Enterobacteriaceae* family, while peak at m/z 6092±1 Da is typical only of *Salmonella* и *Trabulsiella guamensis* bacteria. *Trabulsiella* bacteria were discovered in 1985 and originally were referred to *Salmonella* genus due to proximity of biochemical characteristics [8].

So, peak at m/z 6092 Da can be considered unique for *Salmonella* bacteria, and this information can be used when developing express-methods for microorganism detection immediately from material substance without preliminary isolation of pure cultures.

Besides, peaks used to characterize the microorganism's family and genus, according to data presented by Dieckmann R. and Marlony B. [5], peaks typical of 5 epidemically important Salmonella serotypes: Enteritidis, Typhimurium, Virchow, Infantis, Hadar, were determined. Furthermore, there were determined potential serovar-determining ions, which can be used as biomarkers for serotypes Choleraesuis, Heidelberg, Gallinarum (Table. 3). Obtained results confirm R. Dieckmann and B. Malorny's conclusion [5] as far as Choleraesuis (*Salmonella* Choleraesuis «Bashkiria», *Salmonella* Choleraesuis «Il», *Salmonella* Choleraesuis «Len», *Salmonella* Choleraesuis «Mordovia», *Salmonella* Choleraesuis «Vladimir») and Enteritidis (*Salmonella* Enteritidis «Ru 3», *Salmonella* Enteritidis «Gleb», *Salmonella* Enteritidis «Pel») serotypes are concerned (Table. 3).

It was noted that *Salmonella* Dublin «Rassvet» isolate showed peak at m/z 6008 Da, typical of Virchow serotype, and out of four studied isolates, determined as Typhimurium by agglutination test, only one *Salmonella* Typhimurium «K/K № 16» isolate showed typical peak at m/z 7097 Da.

CONCLUSION

As a result of performed tests it was determined that peak at m/z 4364 Da (typical of *Enterobacteriaceae* bacteria) and 6092 Da (unique for *Salmonella* bacteria) are typical of all studied Salmonella isolates. Results obtained in studying proteomic characteristics of different Salmonella serotypes confirm R. Dieckmann and B. Malorny's conclusion on peaks typical of Choleraesuis and Enteritidis serotypes but not typical of *Salmonella* Typhimurium isolates.

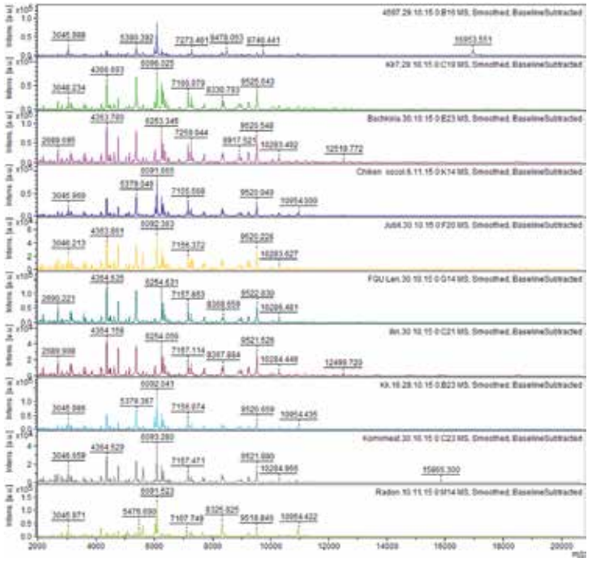

Fig. Protein profiles of Salmonella isolates

X axis shows m/z, Y axis shows relative peak intensity, recorded during mass-spectrometry analysis.

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Table 2
Typical peaks shown by different representatives of *Enterobacteriaceae* family

Representatives of *Enterobacteriaceae* family	Peaks of *Enterobacteriaceae*		Representatives of *Enterobacteriaceae* family	Peaks of *Enterobacteriaceae*	
	4363±1 Da	6092±1 Da		4363±1 Da	6092±1 Da
Arsenophonus	-	-	*Morganella*	n/d	-
Biostraticola	n/d*	-	*Obesumbacterium*	n/d	-
Brenneria	-	-	*Pantoea*	-	-
Buchnera	n/d	-	*Pectobacterium*	-	-
Budvicia	-	-	*Phaseolibacter*	n/d	-
Buttiauxella	-	-	*Photorhabdus*	-	-
Cedecea	-	-	*Plesiomonas*	-	-
Citrobacter	-	-	*Pragia*	-	-
Cosenzaea	n/d	-	*Proteus*	-	-
Cronobacter	-	-	*Providencia*	-	-
Dickeya	4363	-	*Rahnella*	-	-
Edwardsiella	-	-	*Raoultella*	4364	-
Enterobacter	4364	-	*Saccharobacter*	n/d	-
Erwinia	4362	-	*Salmonella*	4363	6092
Escherichia	4364	-	*Samsonia*	-	-
Ewingella	4364	-	*Serratia*	-	-
Gibbsiella	n/d	-	*Shigella*	n/d	-
Hafnia	-	-	*Shimwellia*	4364	-
Klebsiella	4363	-	*Sodalis*	-	-
Kluyvera	-	-	*Tatumella*	-	-
Leclercia	-	-	*Thorsellia*	n/d	-
Leminorella	-	-	*Trabulsiella*	4363	6093
Lonsdalea	n/d	-	*Wigglesworthia*	n/d	-
Mangrovibacter	n/d	-	*Xenorhabdus*	4364	-
Moellerella	-	-	*Yersinia*	-	-
			Yokenella	4364	-

n/d – no database

Table 3
Proteomic characteristics of *Salmonella* serotypes

Mass (m/z)	Serotype characteristics (according to R. Dieckmann и B. Malorny)	*Salmonella* isolate identification with «MALDI Autoflex III Biotyper»
6,008	*Virchow*	*Salmonella* Virchow pork № 31 *Salmonella* Dublin «Rassvet»
6,022	*Choleraesuis*	*Salmonella* Choleraesuis pork«Bashkiria» *Salmonella* Choleraesuis «Il» *Salmonella* Choleraesuis «Len» *Salmonella* Choleraesuis «Mordovia» *Salmonella* Choleraesuis «Vladimir»
6,036	*Enteritidis*	*Salmonella* Enteritidis «Ru 3» chick *Salmonella* Enteritidis «Gleb» chicks *Salmonella* Enteritidis meat for dumplings «Pel»
7,097	*Typhimurium*	*Salmonella* Typhimurium «feedstuff № 16»

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СРАВНИТЕЛЬНАЯ ОЦЕНКА КАЧЕСТВА ПИТАТЕЛЬНЫХ СРЕД ДЛЯ ВЫЯВЛЕНИЯ БАКТЕРИЙ РОДА САЛЬМОНЕЛЛА

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РЕЗЮМЕ
В статье представлены результаты оценки продуктивности и селективности хромогенных питательных сред (висмут-сульфитный агар и ксилозо-лизиновый дезоксихолатный агар) зарубежного и отечественного производств. Коэффициент продуктивности ксилозо-лизинового дезоксихолатного агара зарубежного и отечественного производств составил более 0,58, селективность не менее 2 lg. Коэффициент продуктивности висмут-сульфитного агара зарубежного производства был выше продуктивности висмут-сульфитного агара отечественного производства и составил более 0,19. Селективность данных сред одинакова и равна 5 lg. На основании полученных результатов для проведения исследований по выявлению бактерий рода *Salmonella* в пищевых продуктах рекомендовано использование висмут-сульфитного агара импортного производства, ксилозо-лизинового дезоксихолатного агара отечественного или зарубежного производств.

Ключевые слова: *Salmonella,* питательные среды, селективность, продуктивность.

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COMPARATIVE EVALUATION OF MEADIA USED FOR DETECTION OF *SALMONELLA* SPP.

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SUMMARY
The paper demonstrates evaluation results for productive and selective capacities of domestic and imported chromogenic nutrient media (Bismuth sulphite agar and *xylose lysine deoxycholate agar (XLD agar))*. The productivity ratio of imported and domestic XLD agar amounted to over 0.58 and selectivity ratio – to less than 2 lg. The productivity ratio of imported Bismuth sulfate agar was higher than the productivity of the domestic one and amounted to over 0.19. The selective properties of the both media are similar and amount to 5 lg. Based on the evidence found, imported Bismuth sulfate agar and domestic or imported XLD agar can be recommended for use in the experiments for detection of *Salmonella* spp in food products.

Key words: *Salmonella,* nutrient media, selective capacity, productivity.