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Design of Oligonucleotide Primers for the Development of a Method for Typing Salmonella Isolates in the Russian Federation

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Abstract

This article presents the results of work on the creation of molecular genetic methods for the control of industrial and vaccine strains of microorganisms, including methods for typing salmonellosis pathogens to serovar. The technique is based on the sequence analysis of a fragment of a highly polymorphic region of the CRISPR1 locus by Sanger DNA sequencing. Using the reference genomes of Salmonella enterica from databases and our own whole genome sequencing data, we designed oligonucleotide primers and assessed their quality and specificity.

Disciplinary: Microbiology, Molecular Biology.

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1 Introduction

Typing of microorganisms is commonly understood as a phenotypic or genetic analysis of their isolates at the species or subspecies level, which is carried out to identify a set of characteristics specific to strains or clones [1]. For typing, the most promising is the use of molecular genetic methods, which allow obtaining information on the territorial distribution of certain genetic variants, on the reservoir of infection and pathogen transmission factors, establishing or excluding the relationship between individual cases of diseases, tracking the spread

of infection, etc. [2]. There is a wide range of genetic methods that can be used for typing microorganisms: DNA sequencing, restriction fragment length polymorphism (PCR-RFLP), multilocus sequence typing (MLST), plasmidotyping, ribotyping, IS200 element typing, pulsed-field electrophoresis (PFGE).), analysis of multiple tandem repeats (MLVA), PCR of randomly amplified fragments (RAPD-PCR), etc. [3].

As part of the work on the creation of test systems for the control of industrial and vaccine strains, we plan to develop a molecular genetic method for typing salmonellosis pathogens that cause one of the most common and economically significant diseases of animals and birds. Molecular genetic typing of the pathogen to the serovar will allow characterizing industrial strains, for example, in the process of depositing into the collection, to control vaccine preparations, and also to quickly identify the serovar of the pathogen in the livestock or poultry farm.

Phenotypic methods are widely used for subtyping Salmonella enterica, including serotyping with a large set of antigenic sera. The disadvantages of this method are labor intensity, insufficient reproducibility, and the need for the laboratory to have the entire set of adsorbed sera, as well as the presence of inagglutinable and monophasic Salmonella strains [4, 5].

At present, methods of molecular serotyping based on the evaluation of genetic markers have been developed: genes responsible for the production of specific O- and H-antigens, and other serotype-specific genetic determinants [4]. This includes commercial diagnostic test systems based on multiplex PCR or DNA microarrays. On the territory of the Russian Federation, the test systems "xMAP Salmonella Serotyping Assay" (Luminex) [6, 7], "Check&Trace Salmonella" (Check-Points) [8], SureFast ® Salmonella Species/Enteritidis/Typhimurium 4plex (R-Biopharm AG) are available [9]. However, these systems can only be used to identify the most common Salmonella serovars.

The PFGE method makes it possible to differentiate Salmonella strains belonging to the same serotype according to the profile of genomic DNA restriction fragments separated in a pulsed field. Due to the presence of an international standardized protocol, the PFGE method is used in many countries of the world for the molecular characterization of strains of most Salmonella serotypes in the surveillance of salmonellosis [10, 11]. At the same time, this method does not always allow one to reliably differentiate isolates of serotypes with a highly conserved genome. The PFGE subtyping data do not reflect the phylogenetic relationships of strains, but only represent the genetic diversity of circulating isolates [4].

Typing methods based on the sequencing of DNA fragments have a higher resolution, which makes it possible to identify a wide range of microorganism species depending on the selected target gene. However, the use of the standard technique for sequencing the Salmonella 16S ribosomal RNA gene fragment does not allow identification to the level of the serovar due to the high conservatism of the genome in the given region [12].

2 Materials and Methods

The NCBI, ENA, EMBL, and DDBJ databases were used to search for DNA sequences. The search for homologous sequences was performed using BLAST [13]. Consensus sequences were

obtained using the Ugene program [14]. For multiple sequence alignment, the Ugene program and the Clustalomega algorithm [15] were used. The quality of the selected primers was assessed using the PCR PrimerStats [16] and OligoAnalysisTool [17] software. The specificity of primers was assessed using the Primer-Blast online resource located on the NCBI website [18].

3 Result and Discussion

To develop a method for typing Salmonella to serovarans, an analysis of the literature data was carried out, as a result of which a highly variable region of the CRISPR1 locus was chosen as a target for sequencing.

A family of repetitive DNA sequences called CRISPR has been found in the genomes of many prokaryotes [19]. This family is characterized by direct DNA repeats 24–47 bp long, separated by variable sequences 21–72 bp long, called "spacers" [17]. CRISPR seems to confer resistance to foreign DNA, such as plasmids and phages, in bacteria, and new integrated spacers originate from DNA that once entered the cell [11, 15]. Thus, the content of a spacer in the genome can provide information about the evolution of a bacterium.

Foreign researchers report the presence of two CRISPR loci in Salmonella [18, 19]. Fabre et al. (2012) showed that the sequences of CRISPR loci are highly polymorphic in Salmonella, and CRISPR polymorphism strongly correlated with both the serotype and the type of the multilocus sequence; therefore, different serotypes of Salmonella enterica can be effectively determined by the sequence and/or length of CRISPR loci [8].

To select oligonucleotide primers that allow amplification of a fragment of the CRISPR1 locus, we used the genome sequences of Salmonellaenterica bacteria of various serovars from public databases, as well as our own data of whole genome sequencing of industrial strains and Russian field isolates isolated both from biological material from animals and from food sources. products. The list of genomes used in the selection of primers is shown in Table 1.

Primer selection was performed by multiple alignment analysis of the CRISPR1 locus region sequences using the CLUSTALW algorithm. Using the online resources PCRPrimerStats and Oligo Analysis Tool, we evaluated the optimal annealing temperature, the possibility of dimer and hairpin formation. The criteria for selecting primers were as follows:

– average length – 16–25 nucleotides;

– GC composition 35-60%;

– annealing temperature from 50 °C to 72 °C, the difference in annealing temperature in a pair of primers should not exceed 6 °C;

– secondary structure (hairpin formation): to a minimum.

– dimers: undesirable, it is necessary to avoid self- and mutual complementarity between 3' ends;

– minimum G/C at the 3' end of the primers (no more than three of the last five nucleotides).

The sequences and characteristics of the selected primers are presented in Table 2. The selected primers meet all the declared quality criteria.

Table 1: List of genomes used in selecting primers for sequencing the CRISPR1 locus

Latin name	№ Genbank	Estimated amplicon size, bp
Salmonella enterica subsp. enterica serovar Typhimurium	NC_003197.2	1558
Salmonella enterica subsp. enterica serovar Saintpaul	CP083385.1	844
Salmonella enterica subsp. enterica serovar Enteritidis	CP085824.1	538
Salmonella enterica subsp. enterica serovar Gallinarum	CP077760.1	172
Salmonella enterica subsp. enterica serovar Newlands	CP082916.1	538
Salmonella enterica subsp. enterica serovar Infantis	CP082521.1	1917
Salmonella enterica subsp. enterica serovar Kentucky	CP082535.1	1167
Salmonella enterica subsp. enterica serovar Reading	CP082536.1	1917
Salmonella enterica subsp. enterica serovar Kiambu	CP082587.1	2370
Salmonella enterica subsp. enterica serovar Choleraesuis	CP051366.1	294
Salmonella enterica subsp. enterica serovar Newport	CP074606.1	965
Salmonella enterica subsp. enterica serovar Anatum	CP074670.1	538
Salmonella enterica subsp. enterica serovar Heidelberg	CP066851.1	1683
Salmonella enterica subsp. enterica serovar Bredeney_2105	own data	965
Salmonella enterica subsp. enterica serovar Bredeney_2097	own data	965
Salmonella enterica subsp. enterica serovar Enteritidis_S125	own data	538
Salmonella enterica subsp. enterica serovar Infantis_2099	own data	1918
Salmonella enterica subsp. enterica serovar Infantis_2108	own data	1857
Salmonella enterica subsp. enterica serovar Infantis_2132	own data	1918
Salmonella enterica subsp. enterica serovar Infantis_10033spb	own data	1918
Salmonella enterica subsp. enterica serovar Infantis_s10035spb	own data	1857
Salmonella enterica subsp. enterica serovar Infantis_11	own data	1918
Salmonella enterica subsp. enterica serovar Infantis_S5novosib	own data	1918
Salmonella enterica subsp. enterica serovar Infantis_s68302spb	own data	1918
Salmonella enterica subsp. enterica serovar Infantis_s68303spb	own data	1918
Salmonella enterica subsp. enterica serovar Infantis_s68304spb	own data	1918
Salmonella enterica subsp. enterica serovar Infantis_S7novosib	own data	1918
Salmonella enterica subsp. enterica serovar Infantis_S9319spb	own data	1918
Salmonella enterica subsp. enterica serovar Newport_1429	own data	1453
Salmonella enterica subsp. enterica serovar Rissen_2103	own data	1941
Salmonella enterica subsp. enterica serovar Typhimurium_2136	own data	1740
Salmonella enterica subsp. enterica serovar Enteritidis_R6	own data	538
Salmonella enterica subsp. enterica serovar Typhimurium 3	own data	1558
Salmonella enterica subsp. enterica serovar Abortusovis_372	own data	172
Salmonella enterica subsp. enterica serovar Typhimurium_371	own data	1497

Table 2: Characteristics of selected primers

Name	Subsequence, 5'->3'	Length, bp	Tm, °C	GC %	Self complementarity	Self 3' complementarity
SE_typ_CRISPR1-F	CTGGTGAAACGTGTTTAT	18	50.16	38.89	6.00	2.00
SE_typ_CRISPR1-R	(A/G)TAG(T/A/G)TTTAG(A/T)GTGTTCCC	19	50.49	36.84	2.00	0.00

Primer specificity was assessed using the Primer-Blast online resource located on the NCBI website. Figures 1 and 2 show the regions of the forward and reverse primers.

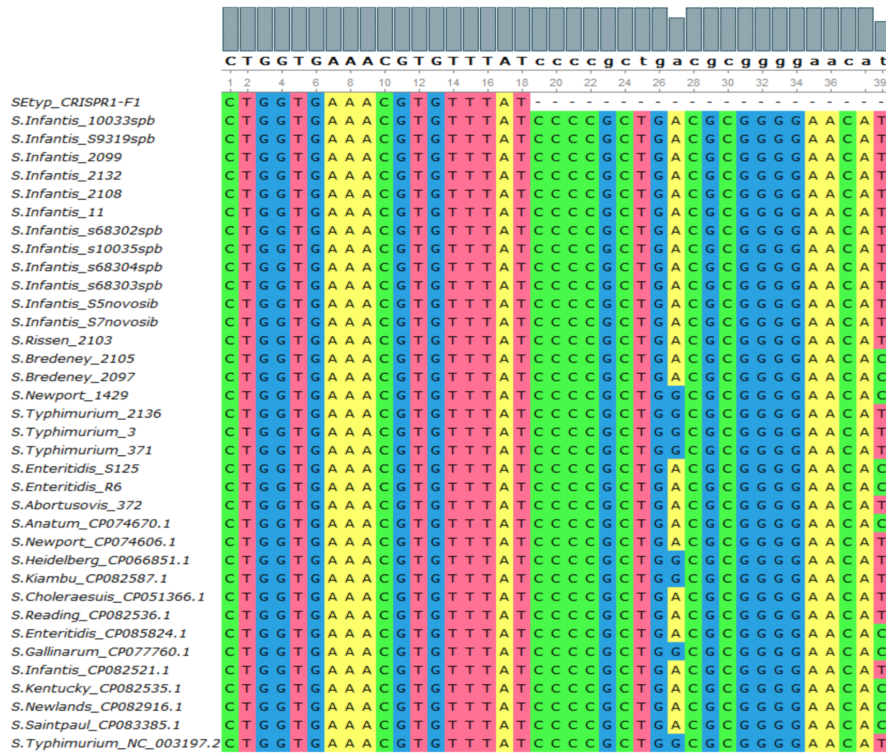

Figure 1: Multiple alignment of the CRISPR1 locus fragment with the forward primer sequence

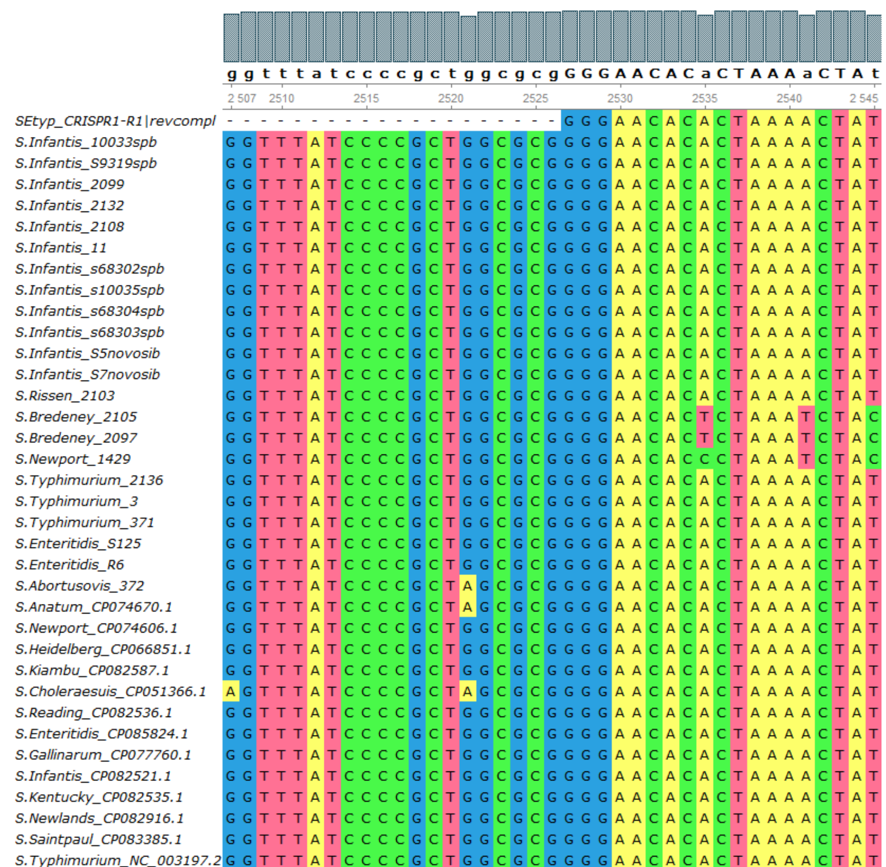

Figure 2: Multiple alignments of the CRISPR1 locus fragment with the reverse primer sequence. The reverse primer is shown as an inverted complementary sequence (reverse complement)

4 Conclusion

Oligonucleotide primers were designed for Salmonella typing to serovar in the highly polymorphic region of the CRISPR1 locus. Since the sequences of Russian strains and isolates were also used in the selection of primers, the use of the technique will allow them to be effectively

typed. The fragment of the CRISPR1 locus selected as a target has a specific length and nucleotide sequence for each serovar. Comparison of the sequence obtained by sequencing the target fragment with the fragments from the databases will make it possible to conclude that the studied Salmonella strain belongs to a certain serovar.

5 Availability of Data and Material

Data can be made available by contacting the corresponding authors.

6 Acknowledgement

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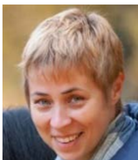

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