Study of Heterogeneity of *Coxiella burnettii* Strains by Analysis of *groEL* Gene Restriction Fragment Length Polymorphism

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Restriction fragment length polymorphism was used for evaluation of genetic heterogeneity of *Coxiella burnettii* strains. A 594-b. p. fragment of *groEL* gene was cleaved with MN1I, HpaII, and Hin6I endonucleases. Genetic homogeneity of *Coxiella burnettii* strains from the Russian collection and their relation to the group of European and North American strains were detected.

Key Words: *Coxiella burnettii; restriction; groEL gene; genetic heterogeneity*

Analysis of restriction fragment length polymorphism (RFLP) is one of the methods used for molecular genetic characterization of *Coxiella burnettii* (CB) strains. After evaluation of the restriction profiles, the strains can be divided into genome groups with different degree of evolution relationship. The study of NotI endonuclease restriction patterns of total DNA of 80 CB strains isolated in Europe, Asia, USA, and North Africa resulted in distribution of these strains into geographic groups with evolutional relationship within the groups [5]. On the other hand, certain degree of polymorphism is possible within one geographic group [11]. For example, analysis of North American strains showed that one geographic group includes 6 genome groups [6].

Restriction analysis can be carried out not only on total DNA, but also on shorter PCR-amplified fragments of CB genome. Various genetic events within the genetic marker can lead to the loss of restriction sites and modification of the length of fragments obtained in the reaction, which can be detected by RFLP. By the present time, several marker genes were studied, which can be used for evaluation of the degree of CB genome polymorphism [2,8,9], including that for strains isolated in the same country [7].

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MATERIALS AND METHODS

The study was carried out on DNA from 14 strains of CB from the collection of strains of Pasteur Institute (Table 1). The agent was routinely cultured in chicken embryo yolk sac membranes and then purified from yolk sac tissues by repeated centrifugation [1].

DNA was isolated using Vecto-DNA-extraction kit of reagents (Vector-Best Firm) according to manufacturer's instruction.

Original primers were created using PRIMER3 software on the basis of the data on the structure of Nine Mile I strain nucleotide sequence (RSA493, GeneBank Database, access No. NC 002971). The sequence of the synthesized primers was submitted as an application for invention registration (priority certificate No. 2007118461 of May 17, 2007). The expected *groEL* amplicon length is 594 b. p. Theoretically optimal temperature of primer annealing is 59°C, experimentally determined temperature is 57°C.

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PCR was carried out in the automated amplification mode on a Tertsik thermocycler (DNA Technology Company) using DNA Amplification kit (Sileks Company) according to the instruction. DNA (2 µl) of each sample was added to the reaction mixture (30 µl). The PCR protocol included preheating of the reaction mixture at 94°C for 90 sec, which was followed by 37 cycles: denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and synthesis at 72°C for 90 sec; final elongation at 72°C for 10 min. PCR products were analyzed by electrophoresis in 1.5% agarose gel stained with ethidium bromide in a concentration of 0.5 µg/ml.

Amplified DNA was treated with HpaII, Hin6I, and Mn1I endonucleases (MBI Fermentas) in appropriate buffers. The mixture for restriction contained 10 µl PCR product, 6-8 µl sterile water, and 2-3 µl appropriate buffer (depending on the enzyme), and 0.5 µl endonuclease in a concentration of 20 U/µl. Restriction was carried out in the following mode: incubation at 37°C for 90 min; arrest of reaction at 65°C for 20 min, and exposure at -20°C for 10 min. The results were evaluated by electrophoresis in 2% agarose gel. The size of DNA fragments was evaluated visually by comparing with Gene Ruler 100 bp DNA Ladder molecular weight marker (MBI Fermentas).

Sequencing of the amplified site of *groEL* gene of Ixodes-3-Luga strain was carried out on ABI PRISM 310 Genetic Analyzer (Applied Biosystems) using Big Dye Terminator Kit.

RESULTS

The *groEL* gene encoding Hsp60 heat shock protein (*groEL)* [4] was selected for the analysis of CB complete genome sequence due to its highly conservative nature; the identity of *groEL* gene nucleotide sequence in CB species is 99-100%, which allows typing of CB strains for detecting variations for this gene. The product of *groEL* gene, Hsp60 chaperone protein, exhibits antigenic activity.

Based on *groEL* gene sequence in strains, whose genome was sequenced, the following results of restriction for *groEL* gene site of 594 b. p. were predicted:

– 2 restriction sites for HhaI(Hin6I) endonuclease with fragment lengths 24, 122, and 450 b. p.;
– 1 restriction site for MnlI endonuclease, fragment lengths 80 and 516 b. p.;
– no restriction sites for HpaII endonuclease.

Restriction of the studied strains showed that their restriction profiles were identical and coincided with the profile predicted for *groEL* gene sequences of the strains, whose sequences were available in the GeneBank database.

No restriction sites were detected after treatment of *groEL* amplicon with HpaII restrictase.

The absence of appreciable differences between CB strains in studies of *groEL* gene by the PCR—RFLP method prompted sequencing of this amplicon for Ixodes-3-Luga strain. Comparison of the resultant sequence with the GeneBank data showed 99% identity of nucleotide sequences. The

TABLE 1. Characteristics of CB Strains Used in the Study

No.	Name	Year of isolation	Origin	Source
1	Dermacenter-Mongolia*	1984	Mongolia	*Dermacenter silvarum*
2	Ufa-1		Ufa	Blood from patient with Q fever
3	Cimex-1-Luga	1959	Luga region	*Cimex lecturarium*
4	*Apodemus flavicollis*-Luga	1958	Luga region (Leningrad region)	*Apodemus flavicollis* renal and hepatic tissues
5	*Apodemus agrarius*-Nevel		Pskov region	*Apodemus agrarius* spleen
6	Henzerling	1945	North Italy	Blood from patient with Q fever
7	Luga-1*	1962	Luga region (Leningrad region)	*Ixodes ricinus*
8	Kharkov	1987	Kharkov	*Ixodes ricinus*
9	Biryukov	1957	Leningrad	Blood from patient with Q fever
10	Ufa-2		Ufa	Sheep placenta
11	*Apodemus agrarius*-Luga	1959	Luga region (Leningrad region)	*Apodemus agrarius* kidneys
12	Kazakhstan-horseflies	1965	Kazakhstan	Blood-sucking flies
13	Ixodes-2-Luga	1959	Luga region (Leningrad region)	*Ixodes ricinus*
14	M-44 (vaccine)	1954	Italian-Greek	Grita strain selection

Note. *The strain is deposited at All-Russian Museum of Rickettsial Cultures, N. F. Gamaleya Institute of Epidemiology and Microbiology.

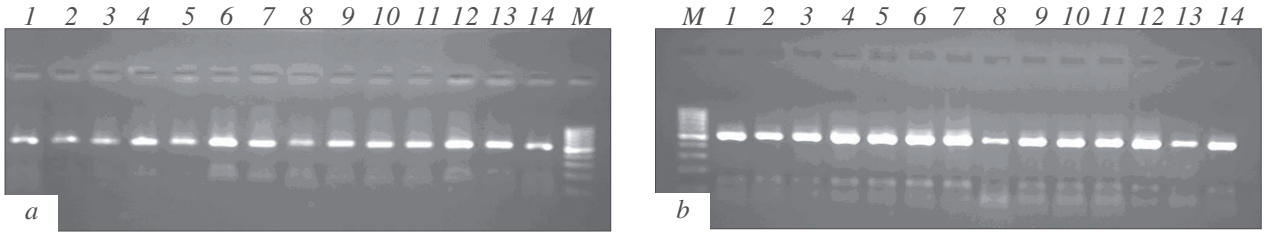

Fig. 1. Results of *groEL* amplicon restriction by Hin6I (*a*) and Mn1I (*b*) restrictases. *M*: marker.

resultant nucleotide sequence is published in the GeneBank database, access No. EF627450.

Restriction analysis of *groEL* gene amplicons of CB strains from collection of zooanthroponotic infections, isolated in the former USSR in 1954-1987 and differing by geographical origin, source and period of isolation, showed no appreciable genetic heterogeneity. The absence of differences confirmed findings of previous studies of these strains by molecular genetic methods, which showed genetic homogeneity of CB strains from the Russian collection and their relation to the group of European and North American strains.

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