Multi Locus Sequence Typing: an Informative Approach to Molecular Ecology

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Abstract—The use of multi locus sequence typing in the investigation of Legionella pneumophila isolated in Russia allowed us to obtain new data for molecular-ecological analysis of the current situation. The strains from the groundwater of different distant regions of the country displayed similar allelic profiles. Mutation processes in cooling towers and in the areas of water stagnation in the autonomous water supply systems have a similar direction. The recombination mechanism was predominant in the process of clonal complex formation. An epidemiological assessment of the investigated strains on the basis of comparison of their allelic profiles with the EWGLI database is presented.

Keywords: multi locus sequence typing (MLST), allelic profile, clonal complex, single locus variation (SLV).
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Multi locus sequence typing (MLST) is an appropriate instrument of molecular ecology that allows the differentiation of strains within one species of microorganism. The development of this approach started in 1998 with the work of M.C.J. Maiden et al. [1]. It has been used for more than 25 groups of microorganisms.

Control of the legionella infection involves analysis of water quality in centralized and autonomous water supply systems and water condensation systems (cooling towers). The MLST scheme for the L. pneumophila strains was developed by The European Working Group for Legionella Infection (EWGLI) for effective control [2]. This scheme is used in the laboratories of 34 countries, including Russia. Assessment of the technique by a large number of researchers allows the correction of deficiencies in a timely fashion and the introduction of adjustments. For example, the system was supplemented with a seventh sequence target in 2007 to improve its differentiation ability [3]. Centralized control of results allows the introduction of newly tested alleles and allelic profiles. Information on the epidemiological significance of the strains that is accessible to registered users involved in L. pneumophila strain analysis helps one to evaluate strains isolated from a controlled object.

The MLST scheme has been employed in Russia since 2007, primarily for the investigation of the legionella outbreak in the town of Verkhnaya Pyshma in Sverdlovsk Oblast [4, 5]. We further analyzed 133 strains during the monitoring of objects in this town and other regions of Russia. The molecular-ecology analysis presented in this publication is based on the obtained multilocus sequencing data.

MATERIALS AND METHODS

Legionella DNA was isolated using the DNA-Extra-Sorb kit (Laboratory of Molecular Diagnostics and Gene-Engineered Constructs at the All-Russia Research Institute of Agricultural Biotechnology of the Russian Academy of Agricultural Sciences). Amplification and sequencing of the mip gene fragment was performed using the External Quality Assurance (EQA) scheme of the EWGLI based on the protocol of R.M. Ratcliff et al. [6] for the purpose of legionella species identification. Amplification and sequencing of the flaA, pilE, asd, mip, momp5, proA, and neuA gene fragments were performed according to the SBT EWGLI protocol [2] as described previously [7] using a 3130 Genetic Analyzer (Applied Biosystems/Hitachi) in order to determine the allelic profiles of the L. pneumophila strains.

Sequence analysis and alignment was performed with CLUSTAL W (1.83) software. Species identification of legionella on the basis of the mip gene sequence was performed using Basic Local Alignment Tool (BLAST) software and the EWGLI database [2]. The UPGMA algorithm from the PHYLIP ver. 3.68 software was used for the construction of the L. pneumophila phylogenetic tree on the basis of allelic profiles and Phylodendron ver. 0.8 beta software [8] was used for the visualization of the analysis results.

RESULTS AND DISCUSSION

A total of 133 strains of L. pneumophila that were analyzed using the MLST technique have been registered in the EWGLI database (EULV 0968–0973,

It should be noted that the strains isolated from the water supply systems in different, including distant, regions of Russia (Moscow, Tver, Sverdlovsk oblast, Khanty-Mansiysk Autonomous Okrug (KMAO)) had identical or similar allelic profiles. For example, strains with ST 114 and ST 191 were discovered both in Moscow and in Sverdlovsk oblast, and strains with ST 338 in Moscow and Tver oblast. Strains with ST 292 from the autonomous water supply systems in KMAO differ at only one locus of the allelic profile from the strains with ST 191 of the Moscow region and Sverdlovsk oblast.

Rapid divergence of the L. pneumophila strains from the cooling towers in the town of Verkhnaya Pyshma, especially during the period of high temperature and humidity in the summer, was shown by monitoring that included four stages of sampling. The loci of the pilE and momPS genes demonstrated the highest percent of exchanges. Analysis of the strains isolated from the autonomous water supply system in the Moscow region resulted in the identification of strains similar to the ones isolated from the groundwater, as well as strains with an allelic profile similar that is to that in the cooling tower strains. Strain Khimki-6, as an example, which was isolated from a water stagnation area, differed only in one locus of the allelic profile from the cooling tower strain with ST 321. These data suggest similarities in the divergence processes in the conditions in cooling towers and water stagnation.

Seventeen of the analyzed strains formed allelic complexes with a single locus variation (SLV) or variation at two loci (figure). In three cases the exchanges were related to the neu A gene, in the rest, to the fla A, pil E, asd, and pro A genes. The transition from ST 728 to ST 87 through the exchange of allele 3 for allele 13 of the neu A gene is caused by the exchange of a single base in the sequence, which can be explained by mutation. In the remaining examples SLV formation of new

UPGMA phylogenetic tree of the L. pneumophila strains that form clone complexes constructed on the basis of allelic profiles.