ISSN 0026-2617, Microbiology, 2014, Vol. 83, No. 3, pp. 247–254. © Pleiades Publishing, Ltd., 2014.
Original Russian Text © O.N. Ilinskaya, Yu.V. Sokurenko, V.V. Ul'yanova, V.I. Vershinina, P.V. Zelenikhin, A.I. Kolpakov, E.S. Medvedeva, N.B. Baranova, M.N. Davydova, A.A. Muzykantov, O.A. Chernova, V.M. Chernov, 2014, published in Mikrobiologiya, 2014, Vol. 83, No. 3, pp. 320–327.

EXPERIMENTAL ARTICLES

Ribonucleolytic Activity of Mycoplasmas

O. N. Ilinskaya[a, 1], Yu. V. Sokurenko[a], V. V. Ul'yanova[a], V. I. Vershinina[a], P. V. Zelenikhin[a], A. I. Kolpakov[a], E. S. Medvedeva[b], N. B. Baranova[b], M. N. Davydova[b], A. A. Muzykantov[b], O. A. Chernova[b], and V. M. Chernov[b]

[a] *Kazan (Volga Region) Federal University, Kazan, Russia*

[b] *Kazan Institute for Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Sciences, Kazan, Russia*

Received August 19, 2013

Abstract—Mycoplasmas are incapable of de novo synthesis of nucleotides and must therefore secrete nucleases in order to replenish the pool of nucleic acid precursors. The nucleolytic activity of mycoplasmas is an important factor in their pathogenicity. Bacterial ribonucleases (RNases) may produce a broad spectrum of biological effects, including antiviral and antitumor activity. Mycoplasma RNases are therefore of interest. In the present work, the capacity of *Acholeplasma laidlawii* and *Mycoplasma hominis* for RNase synthesis and secretion was studied. During the stationary growth phase, these organisms were found to synthesize Mg^{2+}-dependent RNases, with their highest activity detected outside the cells. Localization of *A. laidlawii* RNases was determined: almost 90% of the RNase activity was found to be associated with the membrane vesicles. Bioinformational analysis revealed homology between the nucleotide sequences of 14 *Bacillus subtilis* genes encoding the products with RNase activity and the genes of the mycoplasmas under study. Amino acid sequences of 4 *A. laidlawii* proteins with ribonuclease activity and the Bsn RNase were also established.

Keywords: mycoplasmas, *Acholeplasma laidlawi, Mycoplasma hominis*, ribonuclease activity, localization, vesicles

DOI: 10.1134/S0026261714030072

Interest to mycoplasmas (class *Mollicutes*) is caused by the unique biology of these smallest prokaryotes and a range of practical issues. Most mycoplasmas are parasites of humans, animals, and plants; some are the agents of socially important diseases, contaminants of cell cultures and vaccine preparations [1]. Control over mycoplasma infections and contaminations is thought to be possible upon investigation of the basic mechanisms of mycoplasma adaptation to environmental conditions, which are responsible for their abundance in nature and pathogenicity. Successful realization of a series of genomic projects related to a number of mycoplasmas proved the possibility of application of the post-genomic technologies to the subject. The well-known contaminants of cell cultures *Acholeplasma laidlawii* (causing agent of phytomycoplasmoses) and *Mycoplasma hominis* (causing agent of respiratory and urogenital disorders in humans) [2, 3] are unique in terms of their adaptive capabilities. Transcriptome–proteome analysis and nanoscopy studies of these microorganisms allowed for identification of their stress-reactive proteins [4, 5]; adaptation and virulence of the mycoplasmas were shown to be associated to a considerable degree with secretion of extracellular membrane vesicles [6, 7].

[1] Corresponding author; e-mail: ilinskaya_kfu@mail.ru

Nuclease activity is an important factor of mycoplasma pathogenicity. In contrast to other eubacteria, mycoplasmas are incapable of de novo synthesis of nucleic acid precursors. Nuclease activity provides for the possibility of obtaining the nucleic acid precursors essential for the cells [2, 3]. Ribonucleolytic (RNase) activity may determine, to a considerable extent, the genotoxic properties of these bacteria [8]. Earlier, nuclease activity of mycoplasmas was demonstrated to be associated mainly with the membrane [9]. Meanwhile, data of proteomic profiling evidence that extracellular membrane vesicles of a number of bacteria mediate the RNase traffic [10, 11]. In this connection, analysis of mycoplasma vesicles for the presence of RNase activity is of interest.

Small size of the mycoplasma genome is associated with its high information capacity [12, 13]. Despite the characteristic structural features of the mycoplasma genes, considerable homology between the DNA nucleotide sequences coding for proteins, in particular, RNases involved in metabolism of these microorganisms and those of the phylogenetically related bacteria (bacilli) were revealed [14, 15]. For example, the *rnhC* gene—one of the three *B. subtilis* genes coding for intracellular nonspecific endoribonucleases cleaving the 3'-O–P bond in RNA of the DNA/RNA duplex—is coding for RNase HIII (33.9 kDa) and is homologous to the genes of