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Genotoxic Effects of Mycoplasma Cells (*A. laidlawii* PG8, *M. gallisepticum* S6, *M. hominis* PG37) Formed in Different Growth Conditions

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Abstract: In this work, the analysis of genotoxic features of the vegetative cells and the viable but nonculturable cells of *A. laidlawii* PG8, *M. hominis* PG37 and *M. gallisepticum* S6 and their cultural liquids was performed for the first time using the SOS chromotest allowing to reveal multiple genome alterations resulting in the formation of a single-stranded DNA and expression of the SOS operon. It was shown that the vegetative cells and the viable but nonculturable cells of *A. laidlawii* PG8, *M. hominis* PG37 and *M. gallisepticum* S6 as well as cultural liquid of the latter have genotoxic features. The values of the induction factor of the SOS response of the tester *E. coli* PQ37 strain exceed the parameter of the standard supermutagen (3.07) – 5.27, 3.79, 20.65 and 8.4 for the above-mentioned samples, respectively. The viable but nonculturable cells of *A. laidlawii* PG8, *M. hominis* PG37 and *M. gallisepticum* S6 as well as their cultural liquids have no DNA-disturbing action toward cells of the tester strain. Adaptation of the above-mentioned mycoplasma cells to unfavorable conditions of the environment is associated with the attenuation of their genotoxicity. The presence of mutagenic potential in the mycoplasma cells points out the necessity for the development of a new way to control mycoplasma infections and investigation of interactions between the microorganisms and higher organisms.

Key words: Mycoplasma % Adaptation % Genotoxicity % The SOS response % The VBNC cells% Vegetative cells

INTRODUCTION

Mycoplasmas (class *Mollicutes*) are causative agents of persistent infections in humans, animals, plants, the main contaminants of cell cultures including those used in biotechnology for production of viral vaccines [1]. Numerous literature data suggest cytopathogenic action of mycoplasmas towards infected cells [2,3]. There are data on mutagenic effect of mycoplasmas [4-6]. However, systematic investigations of genotoxic effects of these bacteria are absent. Performing these investigations seems very actual from fundamental and practical points of view – in order to reveal mechanisms of mycoplasma influence on eukaryotic cells and to find a way for controlling mycoplasma infections.

It was found in our studies [7,8] that adaptation of ubiquitous mycoplasma *Acholeplasma laidlawii*, infecting humans, animals, plants and being the main contaminant of cell cultures [9] and *Mycoplasma gallisepticum*, mycoplasma known as causative agent of avian diseases and contaminant of viral vaccines based on the basis of chicken embryos [10,11] to unfavorable conditions was associated with entering the vegetative cells into the viable but nonculturable (VBNC) cells. The vegetative cells and the VBNC cells differ in cell and molecular biology as well as in pathogenicity [7,12]. Recently, the analogous data were also obtained in our studies for *Mycoplasma hominis* (unpublished results)-mycoplasma associated to urogenital and extragenital human diseases and being a contaminant of cell cultures [13,14].

It is known that major alterations in protein expression, morphophysiology, ultrastructure, proliferation and virulence of some asporogenic bacteria may occur in unfavorable conditions [15]. It was presented that adaptation of microorganisms stress factors may be accompanied by synthesis of specific metabolites with mutagenic and antimutagenic activities

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[16,17]. The use of the standard SOS chromotest allows to reveal multiple genome alterations leading to formation of single-stranded DNA and expression of the SOS operon in cells of the tester *E. coli* PQ37 strain [18].

The aim of the present work was analysis of *A. laidlawii*, *M. gallisepticum*, *M. hominis* cells formed in different conditions – the vegetative cells and the VBNC cells on their ability to induce genome alterations in cells of the tester *E. coli* PQ37 strain.

MATERIALS AND METHODS

Acholeplasma laidlawii PG8, *Mycoplasma gallisepticum* S6 and *Mycoplasma hominis* PG37 strains were obtained from the N.F. Gamalei Research Institute of Epidemiology and Microbiology (Moscow, Russia) and *Escherichia coli* PQ37 strain (*Sfi A::mud (Ap lac)cts, lacA, U169, mal*$^+$, *uvrA, galY, phoC, rfa*) expression of $-galactosidase gene is under control of SOS operon) obtained from the N.I. Vavilov Institute of General Genetics of Russian Academy of Sciences (Moscow, Russia).

The vegetative cells and the VBNC cells of the mycoplasmas were obtained as described previously [7,8]. Culture suspensions were centrifuged (the vegetative cells at 3000 g, the VBNC cells at 8700 g) for 20 min. Supernatant was taken into a separate tube, precipitate was diluted in 1mL of the corresponding medium. Each sample was tested in the standard SOS chromotest [19] in triplicate. The expression level of the constitutive alkaline phosphatase and the induction level of the SOS response were used to evaluate genotoxic effect of the vegetative cells, the VBNC cells and cultural liquids of *A. laidlawii* PG8, *M. gallisepticum* S6 and *M. hominis* PG37 [19]. The magnification of the induction factor (IF) more than 2 was considered significant to confirm genotoxic properties of the tested samples [20]. Ethylmethane sulphonate (supermutagen) was used as a positive control (50 µg/mL).

The data were presented as mean±standard deviation. A p value of <0.05 was considered significant.

RESULTS

Tables 1 and 2 present results on the toxic and genotoxic features of the vegetative cells and the VBNC cells of the mycoplasmas. There were not detectable any changes in the activity of alkaline phosphatase due to action of the mycoplasma cells (*A. laidlawii* PG8, *M. gallisepticum* S6 and *M. hominis* PG37) as well as their cultural liquids. That is indicative for the lack of toxic metabolites in the mycoplasma cells.

The activity of $-galactosidase in *E. coli* PQ37 cells was increased due to action of the vegetative cells of *M. gallisepticum* S6 and its cultural liquid resulting in activation of the SOS response (Table 1). The values for the induction factor (IF) for *E. coli* PQ37 SOS response were 20.65 (the vegetative cells) and 8.4 (cultural liquid) that more than those for standard supermutagen (3.07).

Cells of *A. laidlawii* PG8 and *M. hominis* PG37 (but not their cultural liquids) also increased the activity of $-galactosidase in *E. coli* PQ37: IF 5.27 and 3.79, respectively (Table 1). The VBNC cells and their cultural liquids of all tested mycoplasmas did not influence the $-galactosidase activity in cells of the tester strain (Table 2).

Table 1: Induction of the SOS response in the tester *E. coli* PQ37 strain under the action of the vegetative cells of mycoplasmas and their cultural liquids

Variant		The activity of alkaline phosphatase, units	The activity of $-galactosidase, units	IF, units
M. gallisepticum S6	Cells	0.35±0.03	0.06±0.001	20.65
	Cultural liquid	0.43±0.05	0.03±0.001	8.4
A. laidlawii PG8	Cells	0.05±0.003	0.068±0.005	5.27
	Cultural liquid	0.096±0.008	0.027±0.005	1.09
M. hominis PG37	Cells	0.051±0.003	0.072±0.005	3.79
	Cultural liquid	0.039±0.005	0.024±0.004	1.55
Ethylmethane sulphonate		0.37±0.019	0.01±0.001	3.07

Table 2: Induction of the SOS response in the tester *E. coli* PQ37 strain under the action of the VBNC cells of mycoplasmas and their cultural liquids

Variant		The activity of alkaline phosphatase, units	The activity of $-galactosidase, units	IF, units
M. gallisepticum S6	Cells	0.38±0.05	0.13±0.02	0.9
	Cultural liquid	0.44±0.05	0.12±0.02	0.71
A. laidlawii PG8	Cells	1.98±0.002	0.56±0.008	1.12
	Cultural liquid	2.18±0.007	0.53±0.009	1.02
M. hominis PG37	Cells	0.054±0.005	0.025±0.004	1.2
	Cultural liquid	0.061±0.005	0.055±0.006	0.96
Ethylmethane sulphonate		0.37±0.019	0.01±0.001	3.07

DISCUSSION

For the first time, the analysis of genotoxic features of the vegetative cells and the VBNC cells of three mycoplasma species (*A. laidlawii* PG8, *M. hominis* PG37 and *M. gallisepticum* S6) and their cultural liquids was performed in this work. The obtained data showed the lack of toxic effects towards *E. coli* PQ37 in all tested samples. However, the significant increasing $-galactosidase activity due to influence of the vegetative cells of all tested mycoplasmas and cultural liquid of vegetative cells of *M. gallisepticum* S6 showed the presence of mutagenic potential in the samples. Very likely that the chromosome aberrations observed by some investigators [4,5] in *M. hominis*- and *A. laidlawii*-infected cells of mammals and humans are realizations of mutagenic potential of the mycoplasmas.

The presence of genotoxic features in the vegetative cells of *M. gallisepticum* S6 and its cultural liquid may be caused by the secreting genotoxic metabolites from the mycoplasma cells into environment and/or changes in the components of culture medium under the influence of the secreted metabolites of this bacterium.

The absence of genotoxic features in the cultural liquids of *A. laidlawii* PG8 and *M. hominis* PG37 may be indicative for the lack of the secreted mutagenic metabolites in the samples. It is not excluded, however, that the absence of the SOS response in *E. coli* PQ37 may be a result of very low concentrations of genotoxic metabolites and/or their localization in the plasmatic membranes. The detected changes in the genotoxicity levels in the vegetative cells of *A. laidlawii* PG8, *M. hominis* PG37 and *M. gallisepticum* S6 may reflect metabolic differences in these mycoplasmas belonging to the different phylogenetic groups [1].

The absence of the SOS response in *E. coli* PQ37 tester strain due to the influence of the VBNC cells of all tested mycoplasmas and their cultural liquids may be indicative for the lack of genotoxicity of the mycoplasma cells. It is known that a transition of some asporogenic bacteria into dormant (hypometabolic) state may be accompanied by the attenuated virulence in unfavorable conditions [15]. Probably, in the tested mycoplasmas, an attenuation of mutagenic activity also occurs during adaptation to unfavorable conditions.

Nevertheless, the presence of the VBNC cells of mycoplasmas not showing genotoxicity in a medium may present a potential risk taking into account the probability of reversion of the VBNC cells into the vegetative cells of bacteria [15]. However, the features of genotoxicity in the mycoplasma revertants need to reveal in the future.

Thus, it was shown for the first time in our work that cells of *A. laidlawii* PG8, *M. gallisepticum* S6 and *M. hominis* PG37 have genotoxic potential. The molecular principles of this phenomenon require further investigation. However, it is clear that the presence of mutagenic potential in the mycoplasma cells points out the necessity for the development of a new way to control mycoplasma infections and investigation of interactions between the microorganisms and higher organisms.

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