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SHORT COMMUNICATION

Phytopathogenicity of avian mycoplasma *Mycoplasma gallisepticum* S6: Morphologic and ultracytostructural changes in plants infected with the vegetative forms and the viable but nonculturable forms of the bacterium

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Abstract

The data obtained in this study proved that *Mycoplasma gallisepticum* S6 known as avian pathogen had a phytopathogenic potential. The vegetative forms and the viable but nonculturable (VBNC) forms of this mycoplasma could infect the plants via an assemblage of rootlets, invade different tissues, persist there and cause destructive events characteristic to phytomycoplasmoses. In comparison with the vegetative forms, the VBNC forms induced more prominent destructive changes. This phenomenon might be connected to increasing expression of proteins responsible for virulence in the bacterial cells. The fact that *M. gallisepticum* S6 could demonstrate virulent features (infectivity, invasiveness, persistence and toxigenicity) in regard to plants seems to require a development of new ways for controlling phytomycoplasmoses taking into account the probable presence of asymptomatic carriers of this bacterium.

Introduction

At present, many plant diseases have been reported to be connected with mycoplasma infec-

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tions, predominantly phytoplasmas (Bertaccini 2007). It is known, however, that other mycoplasmas, not only phytoplasmas, may be responsible for a variety of plant syndromes (Chernov et al. 2007). Previous research works showed that avian mycoplasma, *Mycoplasma gallisepticum*, is found in plants (Koromyslov et al. 1987). In the available literature, the analogous reports on phytopathogenicity of *M. gallisepticum* were absent up to now. Our previous experiments (Chernov et al. 2008a, b) demonstrated that adaptation of *M. gallisepticum* to unfavorable conditions was associated with entering the vegetative cell forms of the mycoplasma into the viable but nonculturable forms (VBNC). The latter have another morphophysiology, ultrastructure, DNA topology and gene expression. A specific objective of this work was to estimate a phytopathogenic potential of *M. gallisepticum* S6 cells – the vegetative forms and the VBNC forms of the mycoplasma in regard to *Vinca minor* L. and *Vigna radiata* L. (a specific indicator for phytomycoplasmoses and a plant that is not a specific indicator, respectively).

Materials and methods

M. gallisepticum S6 strain was obtained from the N.F. Gamalei Research Institute of Epidemiology and Microbiology (Moscow, Russia). *M. gallisepticum* S6 cells were grown in the liquid-modified Edward's nutrient medium at 37 °C (Chernov et al. 2008a). To obtain *M. gallisepticum* S6 culture adapted to unfavorable growth conditions, cells were grown in depleted medium and at subnormal temperature as described before (Chernov et al. 2008b). *Vinca minor* L. and *Vigna radiata* L. infecting was performed as previously described (Chernov et al. 2007).

Transmission electron microscopy was done according to Cole (1983). The material under study was fixed with 2.5% glutaraldehyde (Fluka, Germany) prepared on a 0.1 M phosphate buffer (pH 7.2) for 2 h. Then, the material was dehydrated using an acetone, ethanol and propylene series and post-fixed in 0.1% OsO_4 with addition of 25 mg/ml of saccharose. After treatment with epoxy resin (Serva, Switzerland), ultra thin sections were obtained using LKB-III ultramicrotome (Sweden). Then ultra thin sections were contrasted with uranyl-acetate (for 10 min) and lead citrate (for 10 min) and visualized using JEM-1200EX transmission electron microscope (Joel, Japan). Ultrastructure of cells of leaf conduction system were analyzed at transverse section.

DNAs from mycoplasma cells and plant tissues were isolated as reported (Sambrook et al. 1989). Mycoplasma detection in plant tissues was performed in PCR with specific primers (MgF1 5′-cagtggcttttcttttaggtt-3′ and MgR1 5′-tcgctgaatgtactggagtaa-3′) derived from the *himA/hup* gene of *M. gallisepticum* R (Papazisi et al. 2003). Electrophoretic separation of DNA fragments stained with ethidium bromide was performed according to Sambrook et al. (1989).

Results and discussion

It was detected that *V. minor* L., a specific indicator of phytomycoplasmoses, infecting with *M. gallisepticum* S6 resulted in total (100% of plants by 20th day) apparent infection. Chlorosis, necrosis, dwarfism, Hexenbesen, bulging of ribs were regis-

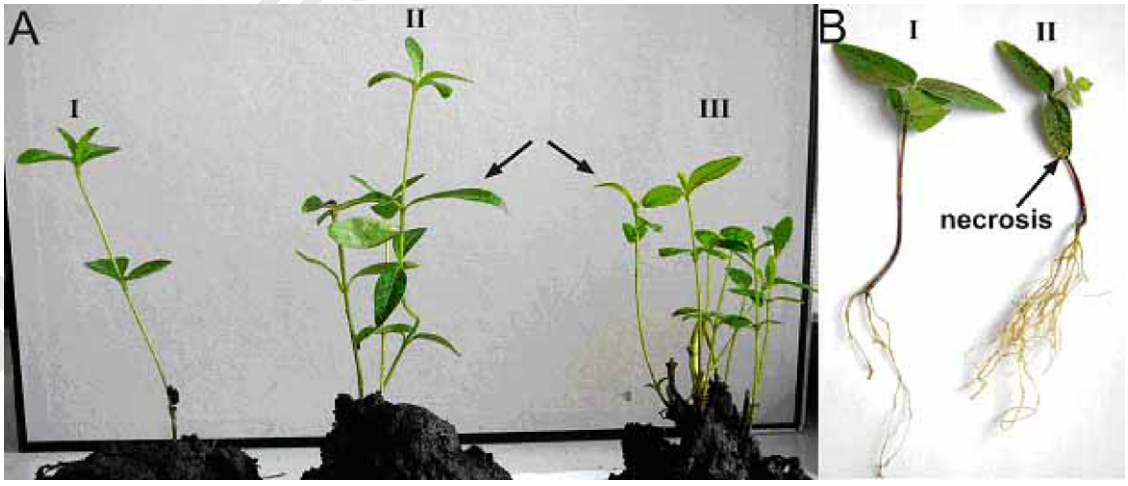

Figure 1. Plants *V. minor* L. (A) and *V. radiata* L. (B) noninfected (I) and infected with the vegetative forms and the VBNC forms of *M. gallisepticum* S6 (II and III, respectively). Specific morphoses (Hexenbesen) are indicated by arrows.

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tered in the infected plants (Figure 1A). As to *V. radiata* L. that is not a specific indicator of phytomycoplasmoses, in the *M. gallisepticum* S6-infected plants, apical necrosis, dwarfism, leaf roll, development of laterals were absent during the period of observation (21 days). Only in some *V. radiata* L. plants (8% of cases), there were necrosis of lamina and a weak chlorosis (Figure 1B). However, PCR data showed the presence of mycoplasma DNA in all tested samples (root, leaf, and hypocotyl) during 21 days (Figure 2).

Transmission electron microscopy analysis showed that *M. gallisepticum* S6-infected *V. radiata* L. plants have specific alterations in ultracytostructure in comparison with non-infected ones. It was visualized that in the non-infected cells phloem were presented by sieve tubes and by cells of parenchyma covering (Figure 3A), while cells of xylem – by tracheal elements and satellite cells that belong to cells of perivascular parenchyma (Figure 3B). Chloroplasts in cells of cancellous parenchyma of leaves occupy the prominent part of the cytoplasm and have a developed system of thylakoids, solid matrix and amyloid grains (Figure 3C). Nuclei have a parietal location, chromatin is uniformly distributed within all volume.

In plants infected with the vegetative forms of the mycoplasma phloem cells (sieve tubes and cells

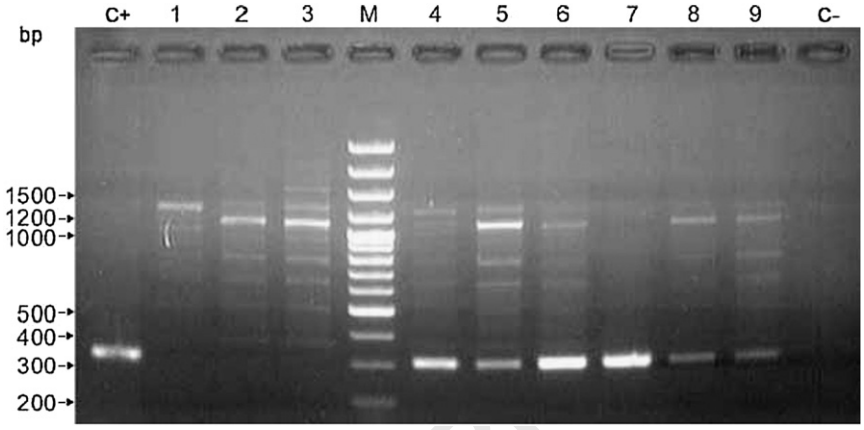

Figure 2. Electrophoregram of amplification products of *himA/hup* nucleotide sequences of *M. gallisepticum* S6 in *V. radiata* L. Note: 1–3 – non-infected roots, hypocotyls and leaves, respectively; 4–6 – plants infected with the vegetative forms; 7–9 – plants infected with the VBNC forms. C+ (DNA of the vegetative forms of *M. gallisepticum* S6) and C– (water) are positive and negative controls, respectively.

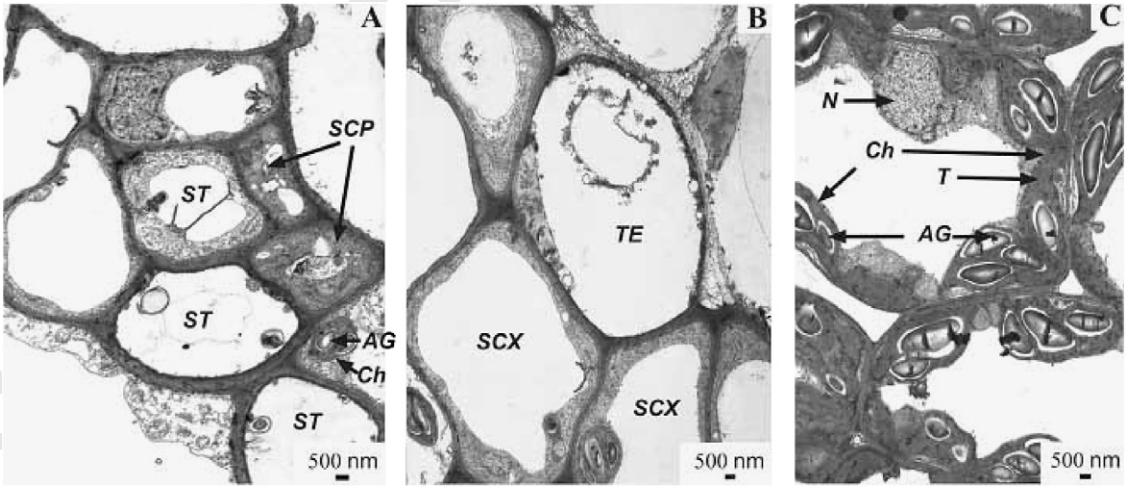

Figure 3. Transmission electron micrography of intact (non-infected) plants of *V. radiata* L. Note: A and B = cells of phloem and xylem, respectively; C = cells of cancellous parenchyma of leaves; SCX = satellite cells of xylem; SCP = satellite cells of phloem; AG = amyloid grains; M = mycoplasmas; ST = sieve tube; T = thylakoids; TE = tracheal elements; Ch = chloroplasts; and N = nucleus.

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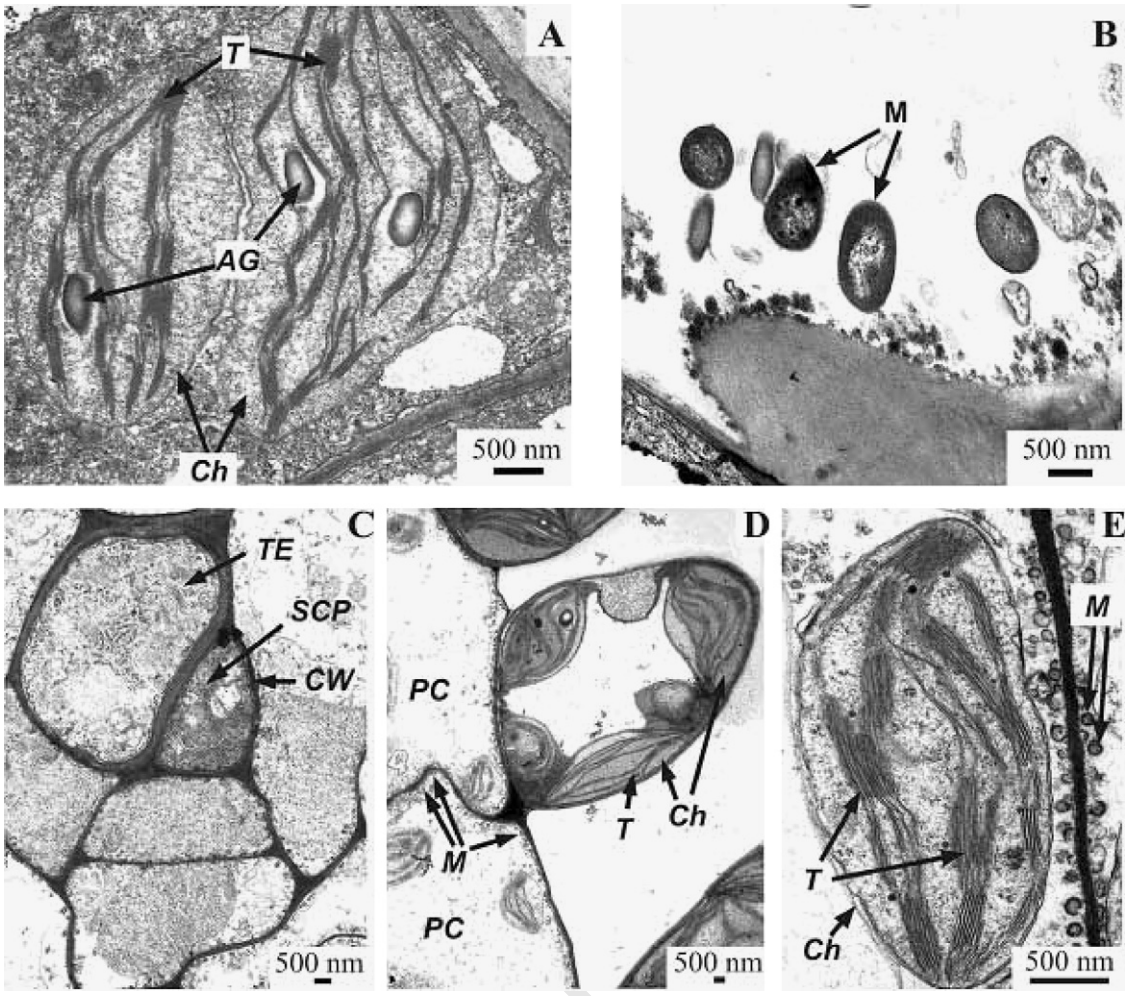

Figure 4. Transmission electron micrography of *V. radiata* L. plants infected with the vegetative forms (A, B) and the VBNC forms (C–E) of *M. gallisepticum* S6. Note: SCP = satellite cells of phloem; AG = amyloid grains; M = mycoplasmas; T = thylakoids; TE = tracheal elements; Ch = chloroplasts; PC = parenchyma covering; and CW = cell wall.

of parenchyma covering) saved their ultrastructural organization. Changes of ultrastructure were detected only in cells of perivascular parenchyma; chloroplasts had a whitish matrix, and thylakoids formed stacks of disks consisting of 3–5 grana. Amyloid grains were localized between the grana (Figure 4A). In tracheides, there were single mycoplasma cells of 0.6–0.8 μm in diameter (Figure 4B). In *M. gallisepticum* cells cytoplasmic membrane is visualized; cytoplasm at periphery has electron-dense structure with electron-transparent area in the center. Along with mycoplasma cells, in the lumen of tracheides we observed cytoplasm islets with organelles that were not lysed during the formation of tracheides.

In *M. gallisepticum* S6 VBNC-infected plants, ultrastructure of cells of conducting bundle (xylem and phloem) and all organelles were totally destroyed in the area of necrosis (Figure 4C). Plasma membrane was not visualized, cytoplasma density was altered as well as integrity of tonoplast. Cells of cancellous parenchyma of leaves kept its ultrastructure but the adjoining parenchyma cells of fascicle facing were totally damaged and colonized with mycoplasmas (Figure 4D). A large amount of small coccoid structures including ones (about 0.15 μm in diameter) typical for mycoplasma nanocells forming at unfavorable conditions were observed at periphery of cell wall (Figure 4E). In chloroplasts of cells of perivascular parenchyma, amyloid grains were absent. There was a whitish stroma with a loose thylakoid packing. The structures of chloroplasts reflected decrease of its functional activity during chlorosis.

So far, *M. gallisepticum* was mainly considered as avian pathogen (mostly chicken and house finches) (Levisohn and Kleven 2000). The responsibility of this bacterium for contaminating viral vaccines

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including those used in humans was also reported (Benton et al. 1967; McGarrity et al. 1992). Our results showed that *M. gallisepticum* S6 cells have a phytopathogenic potential. This mycoplasma is able to demonstrate virulent features (infectivity, invasiveness, persistence and toxigenicity) in regard to plants *V. minor* L., a specific indicator for phytomycoplasmoses and *V. radiata* L. that is not a specific indicator for phytomycoplasmoses. As phytoplasmas may infect various plant organs (Christensen et al. 2004), *M. gallisepticum* S6 vegetative forms and the VBNC cells may invade assemblage of rootlets of plants, tissues of tige, and leaves: to persist there and cause destructive events characteristic for phytomycoplasmoses. This conclusion was confirmed by PCR analysis and transmission electron microscopy data.

The different degree of destructive events after infecting with the vegetative forms and the VBNC forms of *M. gallisepticum* S6 probably connected with features of cell and molecular biology of the vegetative forms and the VBNC forms of this bacterium (Chernov et al. 2008a, b) suggests a significant dissimilarity in toxigenicity of these mycoplasma forms. In the VBNC forms of *M. gallisepticum* S6, a significant increasing expression of proteins responsible for bacterial virulence was marked. It is not excluded, however, that specific factors responsible for phytopathogenicity of *M. gallisepticum* S6 also appear at interacting mycoplasma cells with plants. In this connection, transcriptome–proteome analysis of mycoplasma and plant cells during their interaction may be perspective.

Previously, the data analogous to ones of the present study were obtained in our experiments with *Acholeplasma laidlawii* PG8 cells (Chernov et al. 2007a). In this connection, a list of mycoplasmas with phytopathogenic potential seems to require specification, and virulent features of mycoplasma cells needs further investigation. The ability of *M. gallisepticum* S6 to cause inapparent infection in plants seems to require a development of new ways for controlling phytomycoplasmoses taking into account the probable presence of asymptomatic carriers of this bacterium.

Acknowledgments

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