Single-molecule localization microscopy of structures formed by FtsZ protein in living *Mycoplasma gallisepticum* cells

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Abstract. The aim of this work was to visualize cell division process (in particular, structures formed by FtsZ protein) in living *Mycoplasma gallisepticum* cells using super-resolution microscopy. Utilization of FtsZ fusion with mMaple 2 fluorescent protein and single-molecule localization microscopy allowed us for the first time to obtain images of structures formed by this protein in vivo.

1. Introduction
It is well-known that FtsZ protein plays one of the main roles in division process in several well-studied bacteria [1]. In most well-studied bacterial species, including *E. coli*, FtsZ forms Z-ring and is an essential protein [2], so it is considered to be a promising target for new antibacterial drugs [3]. Despite intensive investigation, there are quite a lot of questions concerning the mechanisms of this protein functioning, even in such a well-studied model organism as *E. coli*. In species such as *Mycoplasma gallisepticum* (a member of the *Mollicutes* class, which is also called “mycoplasmas”), the role of the FtsZ protein is poorly understood and may differ significantly from that of *E. coli* FtsZ [4]. Mycoplasmas lack the cell wall, the synthesis of which is associated with the main role of the FtsZ protein in *E. coli* (if the Z-ring is considered as a framework for other division proteins). In addition, most of the homologs of known division proteins are absent in the reduced genomes of mycoplasmas, so they are interesting from the point of view of the concept of a minimal cell, namely the detection of a minimal set of proteins that ensure cell division. Particularly, for *M. gallisepticum* it is important because of its pathogenicity for poultry [5]. If we know how a division machinery works in *M. gallisepticum* cell, we will be able to develop drugs to block cytokinesis.

Localization microscopy (LM) is a type of super-high resolution fluorescence microscopy that allows overcoming the diffraction limit by temporal separation of the fluorescence of single molecules and their subsequent localization [6]. This method allows obtaining a resolution of about 10 nm in three dimensions, makes it possible to visualize fixed and living cells in a multi-color mode [7-9], so it has become one of the most popular methods of visualization in biology.

In this study, we used the LM method to study the FtsZ structures in living *Mycoplasma gallisepticum* cells.

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2. Materials and methods

*Mycoplasma gallisepticum* strain S6 cells were cultivated at 37 °C with aeration in a medium based on the Oxoid Mycoplasma broth base, supplemented with 10% horse serum, 5% yeast dialysate, 1% glucose, 100 μg / ml ampicillin, 2 μg / ml tetracycline and 1: 1000 phenol red indicator. Cultivation was carried out until the middle of the exponential phase, which was determined by the color change of the indicator due to acidification of the medium.

To observe under the microscope, the cells in the exponential growth phase (grown under aerobic conditions for maturation of the fluorescent protein) were placed in the chamber, consisting of a coverslip and a slide, connected by a double-sided adhesive tape. Visualization was carried out using custom-made set-up based on a fluorescent upright microscope. To implement the localization microscopy, the set-up was previously equipped with a set of solid-state and diode lasers, which are necessary to excite fluorescence, a highly sensitive cooled CCD camera with electronic multiplication, which was used to record the fluorescence of single molecules. To visualize the FtsZ protein in living *M. gallisepticum* cells, we used an additional copy of the *ftsZ* gene fused to the photoconvertible fluorescent protein mMaple 2. This additional copy was included in the chromosome of *M. gallisepticum* through transposon mutagenesis as in [10].

![Figure 1](image_url)

**Figure 1.** Images of structures formed by FtsZ protein in living *M. gallisepticum* cells, obtained using fusion of FtsZ with fluorescent protein mMaple 2. The super-resolution image of FtsZ (green) is combined with a conventional fluorescence image of DNA (magenta). Scale bar is 1 μm.
Figure 2. Conventional fluorescence images of structures formed by FtsZ protein in living M. gallisepticum cells, obtained using fusion of FtsZ with mMaple 2 fluorescent protein (green channel). To represent the cell morphology, the diffraction-limited fluorescent image of FtsZ is combined with the image in transmitted light (grayscale). Scale bar is 1 μm.

3. Results and discussion
Cells of any mycoplasma (200-600 nm in diameter) are too small to study the protein localization using only conventional fluorescent microscopy methods. Single-molecule localization microscopy method combined with endogenous fluorescent labeling allowed us to visualize for the first time the structures formed by the FtsZ protein in living M. gallisepticum cells. Preliminary data show that the
fusion protein of FtsZ with the mMaple 2 protein forms dense structures within the cell (see Figures 1 and 2). These structures appear to indicate the possible ability of the FtsZ protein to polymerize and thereby confirm the hypothesis that FtsZ retains its belonging to cytoskeletal components. It should be noted that the images of structures obtained by this method differ significantly from those obtained by immunofluorescence labeling. This contradiction can be explained by artifacts introduced by both methods. For example, the dense structures in Figures 1 and 2 can be interpreted as inclusion bodies due to the expression of the fusion protein. At the same time, the primary localization of these dense structures at one end of the cell looks intriguing and also suggests their interaction with the attachment organelle. Further study should clarify the actual role of FtsZ in mycoplasmas.

4. Acknowledgements
Authors would like to thank Dr. G.Yu. Fisunov (Federal Research and Clinical Center of Physical-Chemical Medicine, FMBA, Russia) for providing M. gallisepticum S6 strain and pTn4001mini_Recipient-MCS-M5 plasmid. The work was carried out using the unique installation “Laser tweezers”. The work was supported by Russian Science Foundation, research project No. 17-74-20065.

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