FEMS Microbiology Letters 40 (1987) 321–324
Published by Elsevier

FEM 02688

Gliding movement of *Mycoplasma* sp. nov. strain 163K on erythrocytes

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Received 5 November 1986
Accepted 9 November 1986

Key words: *Mycoplasma* sp.; Motility; Erythrocyte; (Cattle; Sheep; Fish gill)

1. SUMMARY

Observations by both dark-field and differential interference contrast (DIC) microscopy revealed that *Mycoplasma* sp. nov. strain 163K, a mycoplasma isolated from the gills of a freshwater fish, is able to glide on bovine and ovine erythrocytes. Some organisms simply crossed the erythrocytes, some moved in irregular circles on the surface of the erythrocytes, and some circled around the erythrocytes one or more times. The ability to glide on living cells is discussed as a substantial factor in infection and pathogenicity.

2. INTRODUCTION

Gliding motility has been described for 5 *Mycoplasma* species: *Mycoplasma pulmonis* [1], *Mycoplasma pneumoniae* [2], *Mycoplasma gallisepticum* [3], *Mycoplasma genitalium* [4] and *Mycoplasma* sp. nov. strain 163K, which has recently been isolated from a freshwater fish [5]. The gliding movement of these mycoplasmas has been observed on liquid-covered glass or plastic surfaces, i.e., on artificial materials. We observed that *Mycoplasma* sp. nov. strain 163K is also able to glide on living cells. Its gliding movement on erythrocytes is demonstrated in the present paper.

3. MATERIALS AND METHODS

3.1. Organism

Mycoplasma sp. nov. strain 163K has been isolated from the gills of a tench (*Tinca tinca* L.) on modified Hayflick medium under aerobic conditions at 25°C [5]. The cells are flask-shaped, having a distinct head-like structure, and are able to attach to inert surfaces and living cells. They can glide on glass and plastic surfaces covered with liquid medium, moving always in the direction of their head-like structure. *Mycoplasma* sp. nov. strain 163K glides much faster than the other gliding mycoplasmas [6], and its movement is not interrupted by resting periods. Under appropriate conditions (suitable medium and suitable temperature), the average speed varies between 2.0 and 4.5 μm/s [6]. The gliding movement of strain 163K can be observed by microscopic examina-

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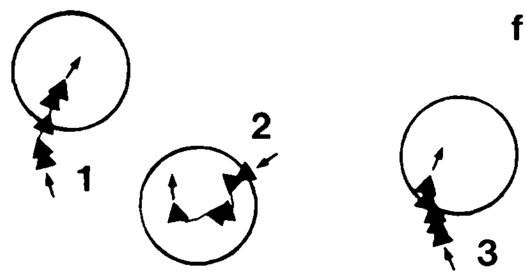

Fig. 1. Photomicrographs of interference-contrast microcinematographic frames (a–e) showing 3 erythrocytes and gliding organisms of *Mycoplasma* sp. nov. strain 163K. The motility tracks of the 3 mycoplasma cells (1, 2, 3) are followed from (a) to (e). The paths followed by the 3 cells are schematically presented in (f).

tion of a simple native preparation at room temperature, using a cell suspension in common liquid medium, whereas the demonstration of the movement of the other gliding mycoplasmas requires heated observation chambers, increased medium viscosity and time-lapse microcinematography.

3.2. Culture medium

Mycoplasma sp. nov. strain 163K was cultivated in the following medium: Bacto PPLO broth, 16.8 g; distilled water, 800 ml; bovine serum, 177.5 ml; fresh yeast extract (50%, w/v), 9.0 ml; Bacto DNA solution, 9.0 ml; penicillin solution (200 000 IU penicillin G/ml), 9.0 ml; pH 7.4. The solid medium was prepared by replacement of the Bacto PPLO broth by 28.0 g Bacto PPLO agar.

3.3. Erythrocytes

Erythrocytes were obtained from defibrinated bovine or ovine blood. They were washed 3 times with Alsever's solution (glucose, 20.5 g; NaCl, 4.2 g; citric acid, 0.55 g; sodium citrate, 8.0 g; distilled water to 1000 ml) and resuspended to a concentration of 10% (v/v) with PBS (NaCl, 8.5 g; Na_2HPO_4, 0.98 g; $NaH_2PO_4 \cdot 6\ H_2O$, 0.74 g; distilled water to 1000 ml; pH 7.2).

3.4. Observation of gliding on erythrocytes

Organisms of strain 163K were obtained by rinsing agar cultures of the 14th to the 22nd passage showing dense growth with culture medium. To the cell suspension containing about 10^8 colony-forming units (cfu), the same volume of a 2% erythrocyte suspension was added. A drop of 50 μl of the mixture was placed on a slide and covered with a coverslip. The preparations were examined by dark-field or DIC microscopy. For dark-field microscopy, a Leitz standard transmission microscope equipped with an oil-immersion dark-field condenser (numerical aperture 1.20) and an oil-immersion objective with an iris diaphragm (planachromat 100 ×, numerical aperture 1.10–1.30) was used. Photomicrographs were taken with a Leica IIIg set up camera using 35 mm Kodak Tri-X Pan (400 ASA) film. DIC microscopy was done with a Zeiss WL-microscope equipped with an Arri-Techno-camera. The gliding movement of the organisms was recorded microcinematographically, using a 35 mm color film (ECH 5294) with a frequency of 24 frames/s.

4. RESULTS

The observation by both DIC and dark-field microscopy showed that the cells of *Mycoplasma* sp. nov. strain 163K are able to glide on erythrocytes. Organisms which came into contact with the red cells entered their surface. Some cells simply crossed the erythrocytes, some moved in irregular circles on the surface of the erythrocytes, and some circled around the erythrocytes one or more times. The movements of three mycoplasma cells were followed in a series of enlarged interference-contrast microcinematographic frames, which are shown in Fig. 1. It is evident that the organisms enter the surface of the erythrocytes, and that they are gliding on it. Circling around the erythrocytes is shown in a series of negative prints of dark-field photomicrographs in Fig. 2.

5. DISCUSSION

With the exception of *M. genitalium*, the gliding mycoplasmas are inhabitants of the respiratory tract, where they are in close contact with the epithelial cells [7].

Mycoplasma sp. nov. strain 163K, though originally isolated from the gills of a fish [5], can also colonize the mammalian tracheal epithelium, as has been found in investigations with tracheal organ cultures from pigs and rats. Like the other

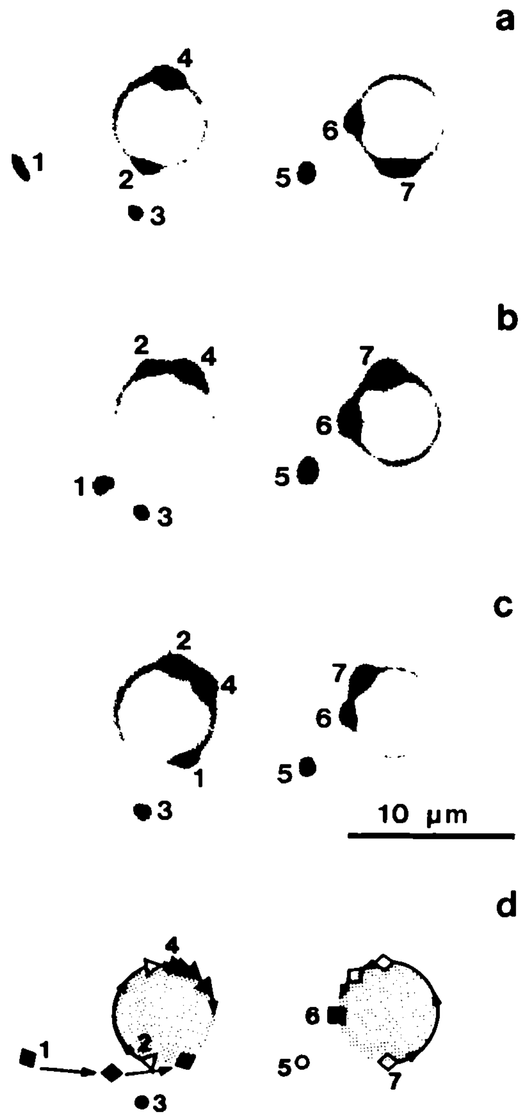

Fig. 2. A series of negative prints of dark-field photomicrographs (a–c) showing organisms of *Mycoplasma* sp. nov. strain 163K circling around the contours of 2 sheep erythrocytes. The left-hand erythrocyte is rounded by the cells 2 and 4, the right-hand one by cell 7. Cell 1 is entering the left-hand erythrocyte in (c). Cells 3, 5 and 7 did not move during the observation time. The paths followed by the mycoplasma cells are schematically presented in (d).

gliding mycoplasmas that occur in the respiratory tract, strain 163K can destroy the epithelial cells (unpublished results).

To reach the surface of the epithelial cells where they clearly have optimum living conditions, the mycoplasmas have to move down the cilia, and it is very probable that this is done by gliding.

The ability to glide on living cells has been demonstrated for strain 163K in the present investigation. It is very likely that the other gliding mycoplasmas are also able to glide on cells. However, the experimental proof of this will be difficult, because of the slow movement and the temperature requirements of these mycoplasmas, creating difficulties in observation (time lapse microcinematography, heated observation chambers).

It has been found recently that strain 163K also possesses chemotactic capabilities, and that it is attracted by mucus (unpublished data). It can be supposed that the stable concentration gradient in the mucus layer, which is generated by the steady secretion of mucus from the epithelial cells, enables the organisms to find the way to the surface of the epithelium.

The ability to glide on living cells is assumed also to be an advantage for strain 163K in the habitat in which it was originally found, i.e., on the gills of fish. In order not to be removed by the steady stream of water passing the gills, the organisms have to move to the base of the gill lamellae, and this is also probably achieved by gliding. Also the ability to glide on living cells have proved to be a substantial factor in infection and pathogenicity.

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