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The swarming phenomenon of *Clostridium tetani*

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Resumen: El desplazamiento de algunas bacterias sobre medios de cultivo sólidos, conocido como fenómeno de "swarming", se ha estudiado principalmente en *Proteus* spp. En este informe se estudió ese fenómeno en *Clostridium tetani* y se encontró que, al igual que en *Proteus*, ocurre cuando la bacteria se transfiere de un medio de cultivo líquido a uno sólido y se asocia con cambios morfológicos, ya que los bacilos que miden *ca.* 10 µm de largo se transmutan en formas filamentosas hiperflageladas de más de 100 µm de largo.

Key words: *Clostridium tetani*, swarming phenomenon, flagella.

In bacteriology the "swarming" phenomenon is a complex behavior characterized by a growing film formation on a solid medium. It has been morphologically described in *Proteus* spp. (Williams and Schwarzhoff 1978, Belas 1992), *Clostridium tetani, Bacillus alvei* (Henrichsen 1972), and *Vibrio parahaemolyticus* (Belas and Colwell 1982). During swarming, short bacilli, usually less than 10 µm in length ("swimmer cells") become elongated and hyper-flagellated ("swarmer cells", Henrichsen 1972, Williams and Schwarzhoff 1978). The phenomenon is associated with the activation of genes that inhibit septation and liberate the expression of lateral flagella (Belas 1992). However, its role is unknown and may represent environmental adaptation (Belas 1992).

Swarming in *Clostridium* is basically mentioned regarding the isolation of *C. tetani* (Williams and Willis 1970, Smith and Williams 1984). This paper is a preliminary report of the changes associated with swarming in *C. tetani.*

C. tetani cultures (ATCC and Costa Rican strains) grown in nutrient broth under an anaerobic atmosphere for 24 hours at 35°C (Smith and Williams 1984), were inoculated in the center of blood agar plates (Trypticase soy base BBL, with 5% human blood), and on small squares (*ca.* 20 by 30 mm by 1-2 mm in depth) of trypticase soy agar on glass slides; these were examined with a phase-contrast microscope. To make the agar squares, a mask was made with masking tape on glass slides and sterilized in a Petri dish. Then, melted trypticase soy agar (*ca.* 0.5 ml) was dispensed aseptically over the mask.

Proteus mirabilis was used as a reference and was inoculated as per the above description. All the media were incubated for 18 to 24 hr at 35°C under either aerobic or anaerobic conditions (GasPak, BBL Microbiology Systems, Cockesville, MD 21030), as fit. Smears taken from the center and the periphery of the colonies were stained with the flagellar stain method of Kodaka *et al.* (1982). For transmission electron microscopy drops of 3 µl of distilled water were placed on formvar covered grids and bacterial samples were applied using a bacteriological loop (see Kodaka *et al.*

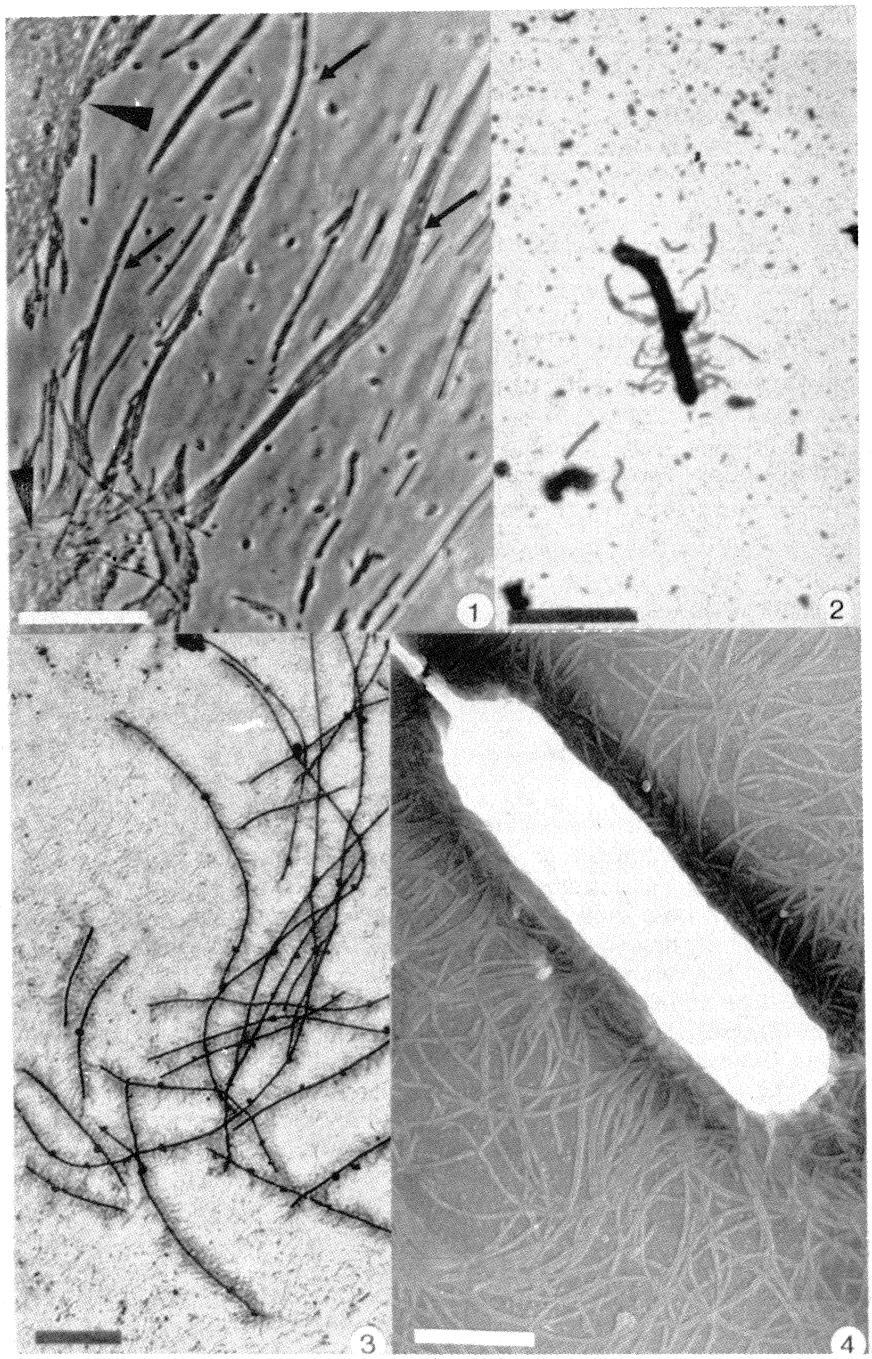

Fig. 1. Phase-contrast micrograph taken from the border of a colony of *Clostridium tetani*. Arrowheads show short bacilli (swimmer cells) near filamentous bacilli in peripheral projections of the colony (arrows). Bar = 50 μm.
Figs. 2 and 3. Light micrographs of a swimmer cell and a group of swarmer cells, respectively. The bacillum of Fig. 3 was taken from the center of a colony (bar = 10 μm), and the swarmer cells from one of the peripheral projections of a colony of *Clostridium tetani* (bar = 50 μm).
Fig. 4. Rotary shadow of a swarmer cell of *Clostridium tetani*, notice abundant flagella (bar = 0.5 μm).

1982). The grids were shadowed with platinum at a tilt angle of *ca* 10°.

Both, *P. mirabilis* and *C. tetani*, swarmed on agar surfaces. However, in the latter, the phenomenon was less evident. The strains of *C. tetani* isolated in Costa Rica expressed a more defined swarming area than the ATCC strains; nevertheless, this zone was visible only by the interference color gave by reflected light, which appeared pale gray. The growth of concentric rings characterictic of *P. mirabilis* was rarely seen in *C. tetani* and was obvious only in the center of the colony.

The colonies of *Proteus* showed peripheral projections of elongated bacteria, with active movement, resembling crawling nematodes. Morphologically similar projections were observed in the colonies of *Clostridium*, but they were made up of motionless bacteria (Fig. 1). Perhaps, this was caused by the negative effect of oxygen on this anaerobic agent.

The cells of *C. tetani* observed in the center of the colonies measure *ca.* 10 μm long (Fig.2). However, in their peripheral projections the cells reached more than 100 μm ($\bar{X} = 125 \pm 59$ in a range of 19-349 μm), and exhibited a copious amount of peritrichous flagella (Figs. 3 and 4). These flagella are apparently too fragile: many flagellar remainder were seen on the background of the light microscopy preparations and some cells lacked flagella. In the electron microscope the bacilli showed abundant flagella, forming arabesque images; nevertheless, some bacteria had fragmented flagella (Fig. 4).

These data corroborate that the displacement on solid media observed in *C. tetani* is a swarming phenomenon, ultrastructurally characterized by the presence of elongated and hyperflagellated cells.

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