

COMUNICACIONES

Ultrastructural view of the bacterium *Mobiluncus mulieris*: An approach to understand some problems for their isolation

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Resumen: Se hace una descripción ultraestructural de la bacteria *Mobiluncus*, uno de los agente anaerobios involucrados en la causalidad del síndrome conocido como vaginosis bacteriana. *M. mulieris* es un bacilo curvo de ca. 3 μm de longitud, lofotrico, cuyos flagelos son subpolares. A partir del primoaislamiento puede cocultivarse accidentalmente con un coco Gram positivo y un bacilo Gram negativo, cuyas colonias, semejantes a las de *Mobiluncus*, miden menos de 0.2 μm de diámetro (límite de resolución del ojo humano), por lo que se recomienda analizar las placas de cultivo bajo el microscopio de disección.

Key words: *Mobiluncus*, Bacterial vaginosis, anaerobic curved-shaped rod.

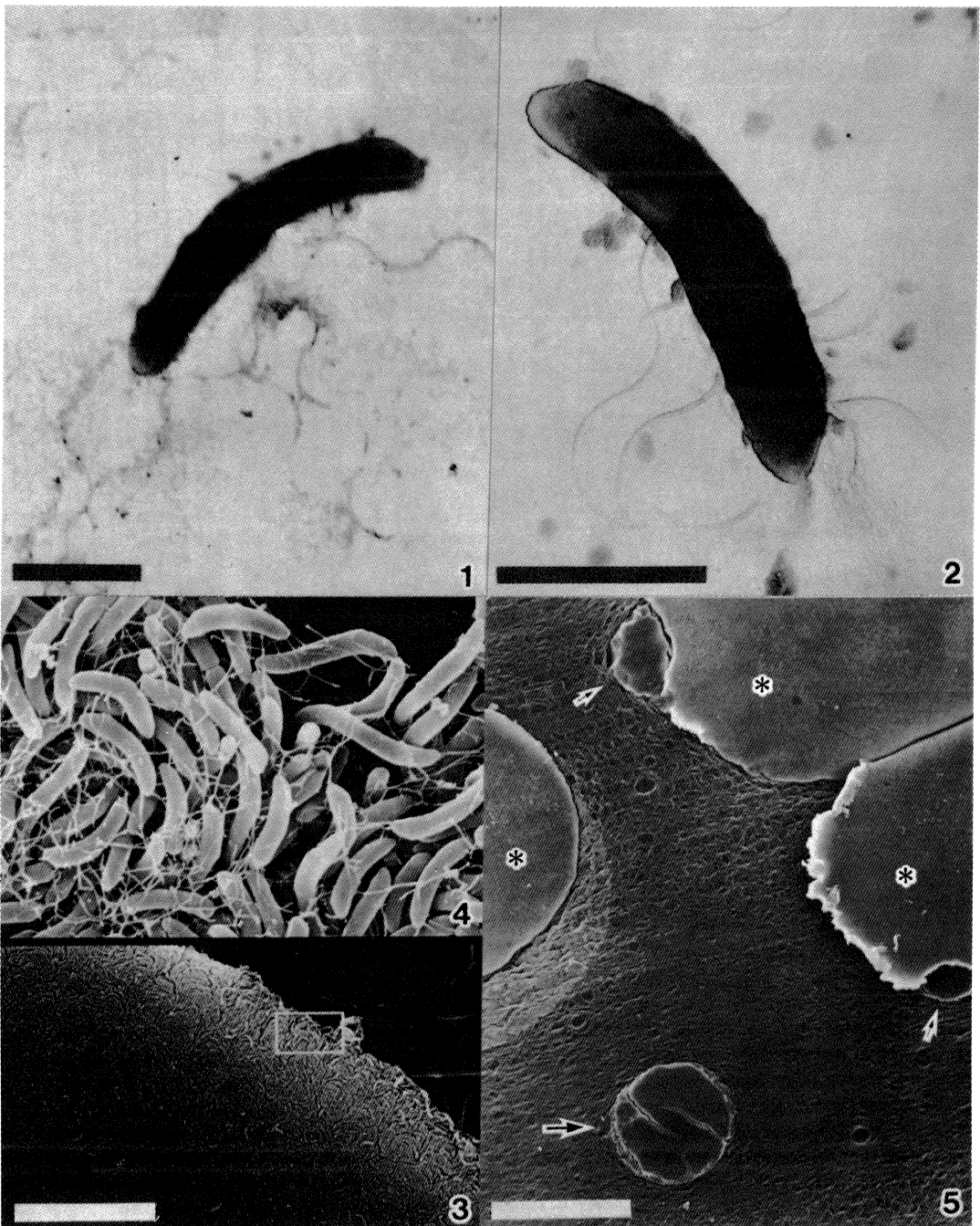
The genus *Mobiluncus* -from the Latin *mobilis* and *uncus*: motile hook - was described by Spiegel and Roberts in 1984, with two species: *M. curtisii* with the subspecies, *M. curtisii curtisii* and *M. curtisii holmesii*, so called in honor of A. H. Curtis and K. K. Holmes, respectively. The second species is *M. mulieris*, from the Latin *mulieris*: woman.

Mobiluncus infections account for over 50 % of the bacterial vaginosis, a common mild infection characterized by the presence of abundant vaginal discharge having a distinctive fishy odor (Cook *et al.* 1992, Spiegel 1991). This clinical entity, which affects about 20 % of adult women (Cristiano *et al.* 1989), is linked to complications such as pelvic inflammatory disease, preterm birth, premature rupture of membranes, chorioamnionitis, postpartum endometritis, cuff cellulitis, neonatal scalp abscesses, nonpuerperal breast abscesses, and umbilical and mastectomy wounds infections (Spiegel 1991).

This genus comprises motile, Gram variable, curved rods, of 1.5-3 μm in length, 0.4-0.6 μm in diameter, with 1 to 8 subterminal flagella, attached subpolarly or near the centre

of the concave side, with a common origin in *M. curtisii* or multiple origins in *M. mulieris*. They are considered fastidious, strict anaerobes, which produce colonies of less than one millimeter in diameter after 5 days of incubation at 35 °C. Rabbit -or horse-serum supplemented media are required to support their growth (Spiegel and Roberts 1984). The aim of this paper is an ultrastructural approach to some reasons why the isolation of *Mobiluncus* has some difficulties associated with other anaerobic agents from the human vagina.

The ultrastructural analysis of *Mobiluncus* was made with agar plates used for the prime isolation of the first strain of this agent isolated in Costa Rica (Acuña *et al.* 1994), and also from plates inoculated with the isolated bacteria. In the first case, vaginal secretions of a 34-year-old woman with bacterial vaginosis syndrome were inoculated on blood agar plates and incubated at 35°C under anaerobic atmosphere in a jar with a GasPak envelope (BBL) for 7 days. Squares of blood agar with isolated colonies of *Mobiluncus* were fixed with 2.5% glutaraldehyde (GA) in phosphate buffer (0.1M, pH 7.4) for two hours and postfixed with 1 %



Figs. 1 and 2: Negative staining and shadow casting of *Mobiluncus*, respectively. The curve shaped rod with at least three flagella, with different origin is showed in both micrographs (Bar = $1\mu\text{m}$).

Figs. 3 - 5: Scanning electron micrographs of colonies of *Mobiluncus*. Figure 3 corresponds to a low magnification and the square is ten times enhanced in figure 4 (Bar = $1\mu\text{m}$). The curve bacilli covered by an intricate embroidery of flagella is showed.

Figure 5 represents a panoramic view; three colonies of *Mobiluncus* (Asterisc), two of them grew over another colonies of small bacilli (Small arrow); also near of them, there is a small colonie of cocci (Arrow). Bar = 0.2 mm.

osmium tetroxide (in the same buffer) for one hour. The samples were then dehydrated through ethanol followed by critical point drying. Specimens mounted on aluminum stubs were coated with gold of approximately 200 nm in thickness using an ion sputter coater (Eiko IB-3) and observed with a scanning electron microscope (SEM). Also, GA fixed bacterial suspensions were mounted on formvar supported grids and negative stained with phosphotungstic acid or shadowed with platinum and observed under transmission electron microscopy (TEM).

After a 5 day period of incubation the colonies of *Mobiluncus* were small (less than 1 mm in diameter), convex, glossy, and colorless. The staining of smears from the colonies revealed the presence of Gram variable, usually Gram negative, curved rods of ca. 3.5 μm in length. At least four subpolar flagella with multiple origin were observed under electron microscopy (Figs. 1 and 2). With SEM the characteristic curved shape of this agent can be seen and the flagella appear as intricate embroidery on the colony. It was not possible to discern the flagellar pattern (Figs. 3 and 4).

Some authors report that long periods of time (up to 30 days) are required for isolation of *Mobiluncus* in pure culture. In our experience, the main obstacle to that purpose was the presence of two anaerobic agents, small Gram-negative bacilli and Gram-positive cocci; the colonies of which resemble those of *Mobiluncus*. Moreover, isolated *Mobiluncus* colonies showed occasional contamination with one of these agents. Figure 5 illustrates this situation: small colonies of less than 0.2 mm in diameter (about the resolution limit of the human eye) appeared very close to the *Mobiluncus* colonies.

The use of Gram smears has become the main diagnostic tool in bacterial vaginosis caused by *Mobiluncus* (Burns *et al.* 1992, Cristiano *et al.* 1989, Hay *et al.* 1992, Joesoef *et al.* 1991, Krohn *et al.* 1989, Nugent *et al.* 1991), because the isolation of this agent is made difficult by its requirements for strict anaerobiosis, slow growth, and interference by other anaerobic vaginal bacteria. These anaerobes can be accidentally caught with a bacteriological loop, and inoculated together with *Mobiluncus* spp. This coculture represents an important obstacle for the biochemical and

chromatographic identification of *Mobiluncus*. In order to avoid this problem it is recommended that the analysis of culture plates be done under a dissecting microscope.

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