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Nitrocellulose Filter Blots for Species Identification of *Mobiluncus curtisii* and *Mobiluncus mulieris*

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Seventy strains of *Mobiluncus*, motile curved anaerobic bacteria associated with bacterial vaginosis, were correctly identified to species level by using bacteria fixed to nitrocellulose and hybridized with ^{32}P-labeled DNA.

Motile curved anaerobic bacteria isolated from the vaginas of women with bacterial vaginosis have recently been placed into the genus *Mobiluncus* (2, 3, 6, 7). These organisms are of interest because they are associated with bacterial vaginosis (formerly known as nonspecific vaginitis). These bacteria have been relatively difficult to culture and have only recently been studied in detail.

Two species have been characterized, *Mobiluncus curtisii* and *Mobiluncus mulieris*. They are differentiated on the basis of physical and biochemical properties. DNA homology studies have demonstrated that members of the same species have ≥75% of their DNA sequences in common. In contrast, the DNA relatedness between the two species was 9 to 25%. No significant DNA homology was found between the genus *Mobiluncus* and other succinate-producing organisms (6).

The isolation of *Mobiluncus* from clinical specimens takes 7 to 100 days in our laboratory. Identification by species requires another 7 to 14 days to complete (M. C. Roberts, S. L. Hillier, F. D. Schoenknecht, and K. K. Holmes, manuscript in preparation). Therefore, we examined the possibility of using clinical strains fixed to nitrocellulose paper hybridized against ^{32}P-labeled DNA from the type strains of each species, *M. curtisii* ATCC 35241 and *M. mulieris* ATCC 35243, to determine the species.

Seventy strains tested (39 of *M. curtisii*, 31 of *M. mulieris*) isolated in the United States and Europe were identified by species as described by Spiegel and Roberts (6). The strains were grown either in broth or on plates and then were coded and suspended in 0.5 ml of 1% (wt/vol) peptone water. Twenty microliters of bacterial suspension was placed on the nitrocellulose in duplicate. A total of 10^6 bacteria per ml were usually used, but the density was not critical as long as a minimum of 10^4 bacteria were spotted. The paper was treated as described by Fennell et al. (1), except pronase treatment was not used, to release and fix the DNA to the filters. The filters could be stored at room temperature for extended periods (4 to 8 weeks) before or after fixing of the DNA. Hybridization was performed as described by Wahl et al. (8) with ^{32}P-labeled DNA (4) from *M. curtisii* or *M. mulieris*. Each strain and controls were hybridized overnight at 42°C with the probes and dextran sulfate. The filters were then washed, dried, and exposed to Kodak X-Omat R film for 1 to 2 days at −70°C (1). The film was then developed.

The homologous probe always gave significantly stronger reactions than the heterologous probe (Fig. 1). We were able to identify all 70 *Mobiluncus* strains correctly as *M. curtisii* or *M. mulieris*. The presence of serum or growth medium did not interfere with the test, nor was there a difference between broth-grown and plate-grown cells. The method also allowed detection of mixtures of the two species, but identifying *M. curtisii* strains by subspecies was not possible. It was also possible to identify the strain correctly when it was mixed with other vaginal flora, thus allowing identification of species before purification of the strain. Using two probes allowed for easy identification of species, but using only one probe was not always adequate to differentiate the two. Other bacterial species which were biochemically or physically similar to *Mobiluncus* spp. were also tested. No reaction occurred with *Actinomyces odontolyticus*, *Actinomyces bovis*, *Anaerobiospirillum succiniciproducens*, *Anaerovibrio lipolytica*, *Succinivibrio dextrinosolvens*, *Campylobacter fetus* subsp. *veneralis*, *Campylobacter concisus*, *Wolinella recta*, *Wolinella* spp., *Vibrio succinogenes*, *Bacteroides gracilis*, *Eubacterium lentum*, or *Selenomonas sputigena*. Similar findings were obtained by DNA-DNA homology studies (6).

Recently, it has been possible to substitute biotinated DNA for radiolabeled DNA (5). Biotinated DNA was prepared and hybridized as described above. After washing, the filters were blocked and treated with either streptavidin and biotinated horseradish peroxidase or streptavidin and biotinated acid phosphatase as described by the manufacture (Enzo Biochem, New York, N.Y.). Both were capable of detecting and identifying by species the 20 *Mobiluncus* strains examined. Positive results were identified by the development of a pink-gray color. In our hands, the horseradish peroxidase test was easier to read. Neither gave false-positive reactions, although both were 100- to 1,000-fold less sensitive than the radiolabeled probes and required 10^5 to 10^6 bacteria for detection. However, the advantage of using a stable nonradioactive probe for the testing is attractive in a clinical environment.

Recently, we have tested 150 direct swab suspensions and found that the nitrocellulose filter blot assay was able to detect and identify *Mobiluncus* isolates by species in 60 to 70% of the culture-positive specimens. The filter blot required 2 to 7 days to complete, as compared with an average of 30 days for culture. Most of the blot-positive specimens had less than 25% *Mobiluncus* spp. in the flora as assayed by Gram stain and culture (Hillier et al., manuscript in preparation). No other organisms reacted with the probes.

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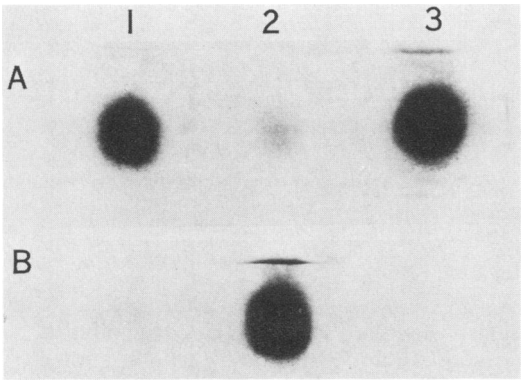

FIG. 1. Clinical strains of *M. curtisii* (columns 1 and 3) and *M. mulieris* (column 2), probed with ^{32}P-labeled *M. curtisii* (A) and ^{32}P-labeled *M. mulieris* (B).

Our data indicate that the nitrocellulose blot is a quick method of identifying *Mobiluncus* isolates by species. The advantage of this system is the ability to detect accurately and quickly the species by its DNA, even in a mixed population of bacteria. The potential uses for this method are just now being explored and hold great promise for the future.

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