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Letters to the Editor

Monitoring for Methylobacteria in Water Systems

Methylobacteria are slow-growing, pink-pigmented organisms that have been reported to be opportunistic pathogens in immunocompromised patients (3, 7, 8, 9). *Methylobacterium mesophilicum* and *Methylobacterium zatmanii* have been the two most commonly reported species isolated in clinical samples. Tap water has been implicated as a possible mode of transmission for these bacteria in the hospital environment. Methylobacteria have been reported to exhibit resistance to chlorination (6) and have been isolated from tap water in various clinical settings, including an investigation of a pseudo-outbreak (4), water from dental units (2), and blood bank purification units (11). The association of these organisms with tap water recently prompted Hornei et al. (7) to suggest that it may be helpful to monitor for these organisms in water distribution systems in hospital units for immunocompromised patients.

We have previously reported the occurrence of pigmented bacteria in potable water and noted the role of these organisms in nosocomial infections (12). In order to provide information to laboratories who may wish to monitor water for methylobacteria, we evaluated two procedures and several bacteriological media incubated at different temperatures. Two type strains, *M. mesophilicum* (ATCC 29983) and *M. zatmanii* (ATCC 43833), were grown in nutrient broth at 30°C, washed by centrifugation, resuspended in phosphate buffer (1), and inoculated separately into sterile dechlorinated tap water. Appropriate dilutions were assayed in triplicate by the spread plate procedure (SP) and the membrane filter technique (MF) using 0.45-μm-pore-size cellulose acetate filters (1). Eight media were evaluated in the study. Heart infusion agar (HIA), MacConkey agar (Mac), nutrient agar (NA), 5% sheep blood agar (SBA), and Sabouraud agar (Sab) were incorporated as representatives of media used in clinical laboratories. Buffered charcoal yeast extract agar (BCYE) was also included based on a recent report by Hornei et al. (7) citing this agar as the only medium capable of recovery of *M. zatmanii* upon initial isolation from blood cultures (7). Two media routinely used for heterotrophic plate count analysis in drinking water, plate count agar (PCA) and R2A medium, were also evaluated (1). All media were used in both methods (SP and MF) and incubated at 30 and 37°C. Appropriate dilutions for visualizing individual colonies were observed for growth and pigment production on each medium after 1, 2, 3, 4, 5, and 7 days of incubation.

Not unexpectedly, *M. mesophilicum* was not recovered by either method on any of the media incubated at 37°C. On HIA and Mac, growth was not detected by either method at 30°C. Using the MF procedure, pigmented pinpoint colonies of *M. mesophilicum* were observed on SBA after 7 days of incubation at 30°C. Similarly, pinpoint colony formation occurred after 7 days on BCYE agar incubated at 30°C, with very light pink pigmentation observable only on the MF plates. Growth levels on NA, Sab, PCA, and R2A media were similar at 30°C by both methods, producing 1- to 2-mm-diameter pink-pigmented colonies after 5 to 7 days of incubation.

M. zatmanii was capable of growing at both incubation temperatures. After 5 days of incubation on HIA, small pink colonies (<1-mm diameter) were observed at 30°C by both procedures. At 37°C after 7 days of incubation, pinpoint colonies were observed on the HIA plates but pigmentation was apparent only by the MF method. No growth was observed on Mac agar by either method at 30 or 37°C. After 5 to 7 days of incubation, colonies (1-mm diameter) were present on SBA incubated at 30 and 37°C by both methods, but like *M. mesophilicum*, pink pigmentation was visible only on the MF plates. Pinpoint pigmented colonies appeared on BCYE agar for both MF and SP procedures after 7 days of incubation at both temperatures. As with *M. mesophilicum*, growth levels on NA, Sab, PCA, and R2A media were similar, with 1- to 2-mm-diameter pink-pigmented colonies appearing on plates by both methods at both temperatures after 5 to 7 days of incubation.

Pink pigmentation is the primary diagnostic characteristic used in the initial isolation of methylobacteria. It should be noted that with extended incubation, pink colonies often become coral in appearance (5). Based upon these results, it would appear that a laboratory wishing to monitor water for methylobacteria would be advised to use an agar medium such as NA, Sab, PCA, or R2A incubated at 30°C for 5 to 7 days to allow for the recovery of these slow-growing, pink-pigmented organisms. Depending on the type of medium, both the SP and MF methods were capable of recovering the target organisms. The MF procedure allows the analyst to examine larger volumes of water (1), and the white membrane provides a good background for distinguishing pigment.

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