

water). Several drops of crystal violet are sufficient. Although crystal violet is satisfactory, almost any other stain can be employed. When excess dye is rinsed off with tap water, virus plaques stand out as sharply defined, clear areas in a blue cell sheet.

This procedure offers the following advantages: (a) plaques can be stained and observed immediately after they reach optimal size; (b) plaques are sharply defined, and can be counted accurately and easily (figure 1); (c) plaques can be preserved indefinitely after being fixed and stained, and thus can be counted at a convenient time. If desired, representative plaque bottles can be retained as permanent records of plaque size

and morphology for comparison with those of later experiments.

This procedure is useful with a wide variety of cell types and viruses. Primary cultures of human amnion and monkey kidney, as well as established human cell lines like HeLa, give good results. Although sharper plaques are obtained when viruses that produce complete cell destruction are used (e. g., poliovirus, Coxsackie A-9 and B-1), less virulent viruses such as vaccinia also yield suitable plaques by this method. Coxsackie viruses, ECHO viruses, and vaccinia virus require from 3 to 5 days for optimal plaque formation. Any standard overlay medium can be employed if the agar concentration is kept low.

MOTILITY OF *RHODOMICROBIUM VANNIELII*

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Received for publication June 11, 1959

The isolation, culture, and reproduction of the photoheterotrophic, budding bacterium, *Rhodomicrobium vannielii* has been described previously (Duchow and Douglas, *J. Bacteriol.*, **58**, 409, 1949; Murray and Douglas, *J. Bacteriol.*, **59**, 157, 1950). This organism was described as non-motile, but we have found that all of the original strains as well as a freshly isolated strain are motile in young cultures.

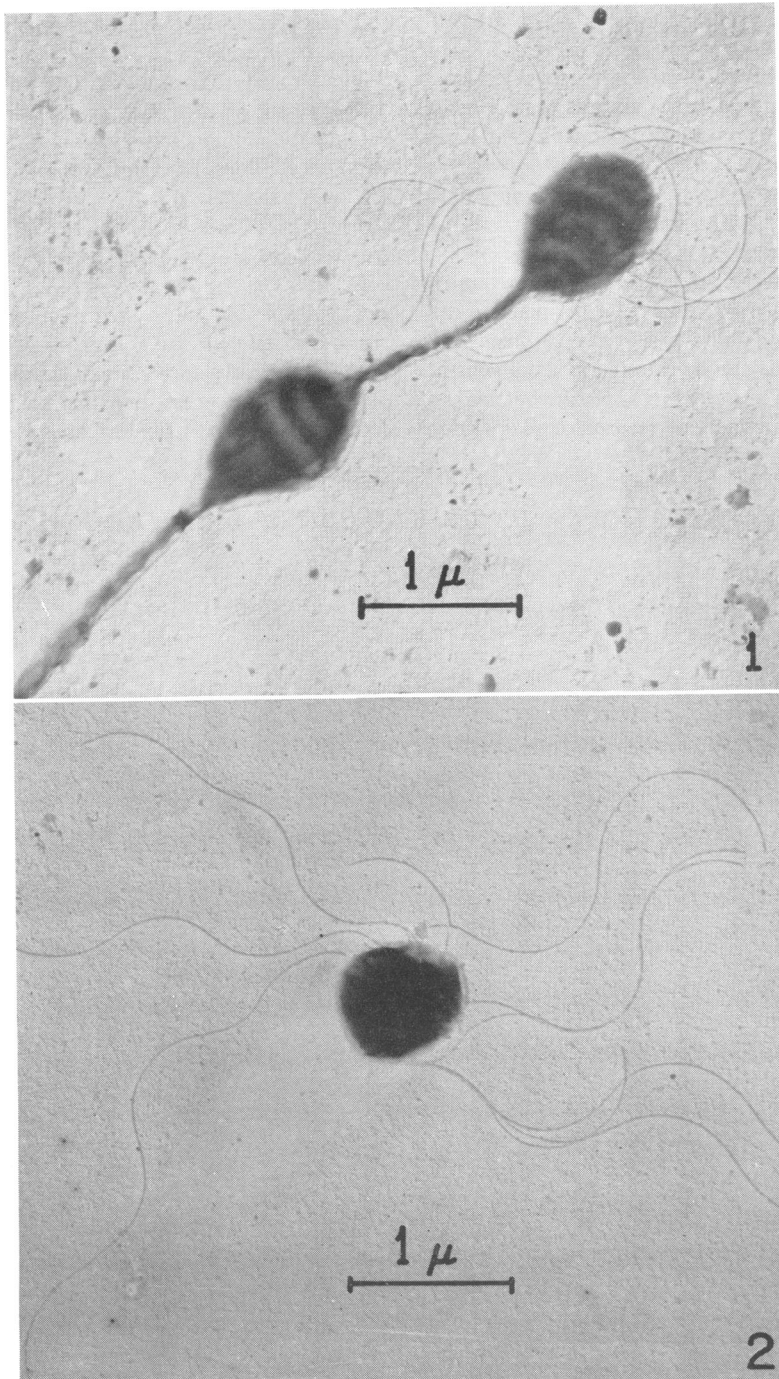
The substitution of 0.1 per cent sodium lactate for ethanol in the medium described by Murray and Douglas was found to enhance growth of the organism. Fluid cultures after 2 to 3 days of incubation exhibited actively motile clumps of 3 to 4 cells as well as motile single cells. The single cells were observed to arise from terminally

attached cells when the filament was disrupted through cell agitation caused by flagellar activity. Mevius (*Arch. Mikrobiol.*, **19**, 1, 1953) has observed that the single motile cells of *Hyphomicrobium vulgare* arise in the same manner.

Electron micrographs of attached and free cells observed in wet mounts are presented in the figures. Figure 1 illustrates an attached cell which possesses a number of flagella, whereas figure 2 presents a single, free-swimming cell with several flagella. Flagellar insertion is peritrichous, in contrast to the polar flagellation observed in the related forms, *Hyphomicrobium vulgare* (Mevius, *Arch. Mikrobiol.*, **19**, 1, 1953) and *Hyphomonas polymorpha* (Pograntz, *Schweiz. Z. allgem. Pathol. u. Bakteriologie*, **20**, 593, 1957). (See page 598 for figs. 1 and 2.)

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Acknowledgment. We are indebted to Dr. Velma Chambers for preparing the electron micrographs.



Figures 1 and 2. Electron micrographs of palladium-shadowed cells of *Rhodomicrobium vannielii*. Figure 1 shows a flagellated attached cell and figure 2 a flagellated free cell.