

in a suitable position to grip the tubes. Thus, the hand holding the tubes is relaxed while the tubes are being observed. A series of tubes can be compared in sequence at one time, with one hand being free to record results. The ability to observe a series of 8 to 12 tubes at one time eliminates the necessity of checking back on tubes already replaced in the rack, a common occurrence for tubes lifted by hand.

The rubber lining assures a firm grip for each tube and allows for slight deviations in diameter. The lifter will work for any group of tubes having the same diameter and height, with or without plastic caps. The only limiting factor is the space between the rows of tubes, which might prevent insertion of the rubber-lined blades. The number of tubes that can be lifted at one time by the 8- to 9-in. blades will vary according to the tube spacing in the rack. Our racks were designed to

hold 121 test tubes (13 × 100 mm), with 11 rows of 11 tubes. Three of these racks efficiently fill a double-size water bath (inside measurement, 10.5 × 27 in.). Standard Wasserman, Kolmer, or U.S. Army Medical racks have holes spaced farther apart. It appears the holder would grip eight or nine tubes from any one of these racks.

Slippage of tubes from the holder has not occurred. A slight twist of the wrist is sufficient to shake the contents of all tubes being observed. Since the tubes do not move in the holder, they are readily replaced in the holes of the rack from which they were removed.

The holder also can be utilized for rapid removal of tubes to be placed elsewhere, with the open ends facing in one direction for ease in sterilizing and washing.

This study was supported in part by research grant E-3144 from the U.S. Public Health Service.

## FLAGELLATION OF *FLAVOBACTERIUM PISCICIDA*

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Received for publication 15 April 1963

*Flavobacterium piscicida* was first described by Bein (Bull. Marine Sci. Gulf Caribbean **4**:110, 1954) as a gram-negative rod with peritrichate flagellation. Recent studies have shown that the organism does not produce carotenoid pigments (Weeks et al., J. Bacteriol. **84**:1118, 1962), and the organism is being studied further in our laboratory. The present investigation indicates that the organism is polarly flagellated and, therefore, may not belong in the genus *Flavobacterium*.

Cultures used for this investigation were two strains of *F. piscicida* (Miami F-1 and a transplant of the original isolate, now designated Original) which were obtained from the University of Miami, Marine Laboratory: the first from S. P. Meyers and the second from S. J. Bein. The strains were grown on 1% Difco peptone agar prepared with Lyman and Fleming's (J. Marine Res. Sears Found. Marine Res. **3**:134, 1940) artificial sea water. Cultures were incubated and stored at 28 C.

Motility was determined by the use of a phase microscope before smears were made of the two

strains. Slightly turbid distilled-water suspensions were made from cultures 12 to 15 hr old.

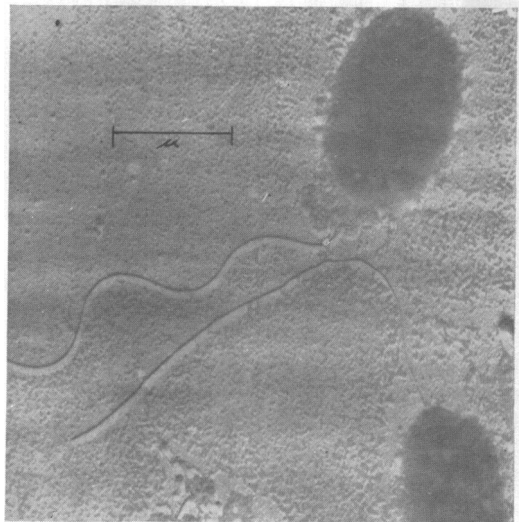


FIG. 1. Electron micrograph of *Flavobacterium piscicida* strain F-1, polar flagellum and fimbriae.

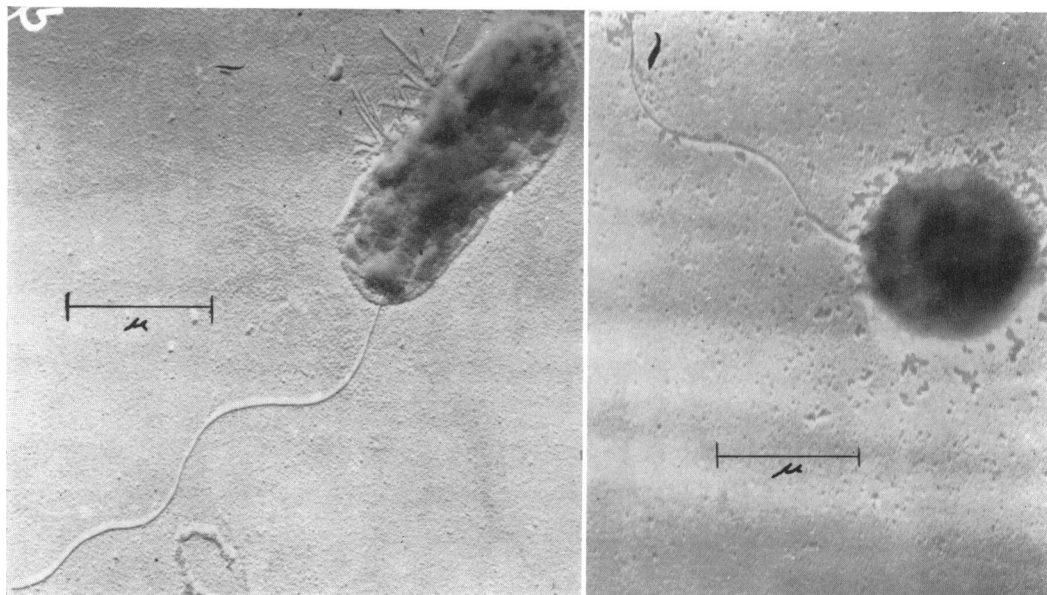


FIG. 2 (left). Electron micrograph of *Flavobacterium piscicida* strain Original, polar flagellum.

FIG. 3 (right). Electron micrograph of *Flavobacterium piscicida* strain Original, spherical cell and flagellum.

These were stained with Zettnow's silver ethyl amine reagent (Kingma-Boltjes, *J. Pathol. Bacteriol.* **60**:275, 1948) and by Leifson's (*J. Bacteriol.* **62**:377, 1951) method. Smears were allowed to air-dry and were not heat-fixed before staining. In Zettnow's method, the mordant was used for at least 15 min and the stain for 1 to 2 min.

Cultures used for electron microscope studies were 18 to 20 hr old. Turbid suspensions were allowed to stand for about 15 min and were then washed three times in distilled water to remove inorganic salts which otherwise interfered in shadowed preparations. A small drop of the turbid suspension of washed cells was placed upon Formvar-covered grids and shadowed with an alloy of gold and palladium (60:40).

Both strains of *F. piscicida* showed polar flagellation (Fig. 1 and 2). Neither the electron micrographs nor the stained preparations showed the peritrichate condition described by Bein. Further, the cellular motion is suggestive of polar rather than lateral attachment of flagella. Bein did not state the basis for his description of flagellation, and there is no certain resolution of

the contradiction. Some of the Leifson-stained preparations have shown very short, lateral appendages which could be due to the fimbriae shown in Fig. 2. It is possible that these structures explain Bein's description of flagellation.

Electron microscope studies showed that some of the cells of strain Original were spherical but still possessed a single flagellum (Fig. 3). To determine whether the spherical cells were preparative artifacts, crystal violet-stained smears were searched for spherical cells, and distilled-water suspensions were examined with phase contrast. The suspensions were studied after each stage of preparation for electron microscopy, and there was no evidence that spherical forms increased during preparation. Spherical forms were found in the stained smears taken directly from the cultures.

Electron micrographs were prepared by E. E. Spiker. The study was approved for publication by the Director, Idaho Agricultural Experiment Station, as Research Paper 571.

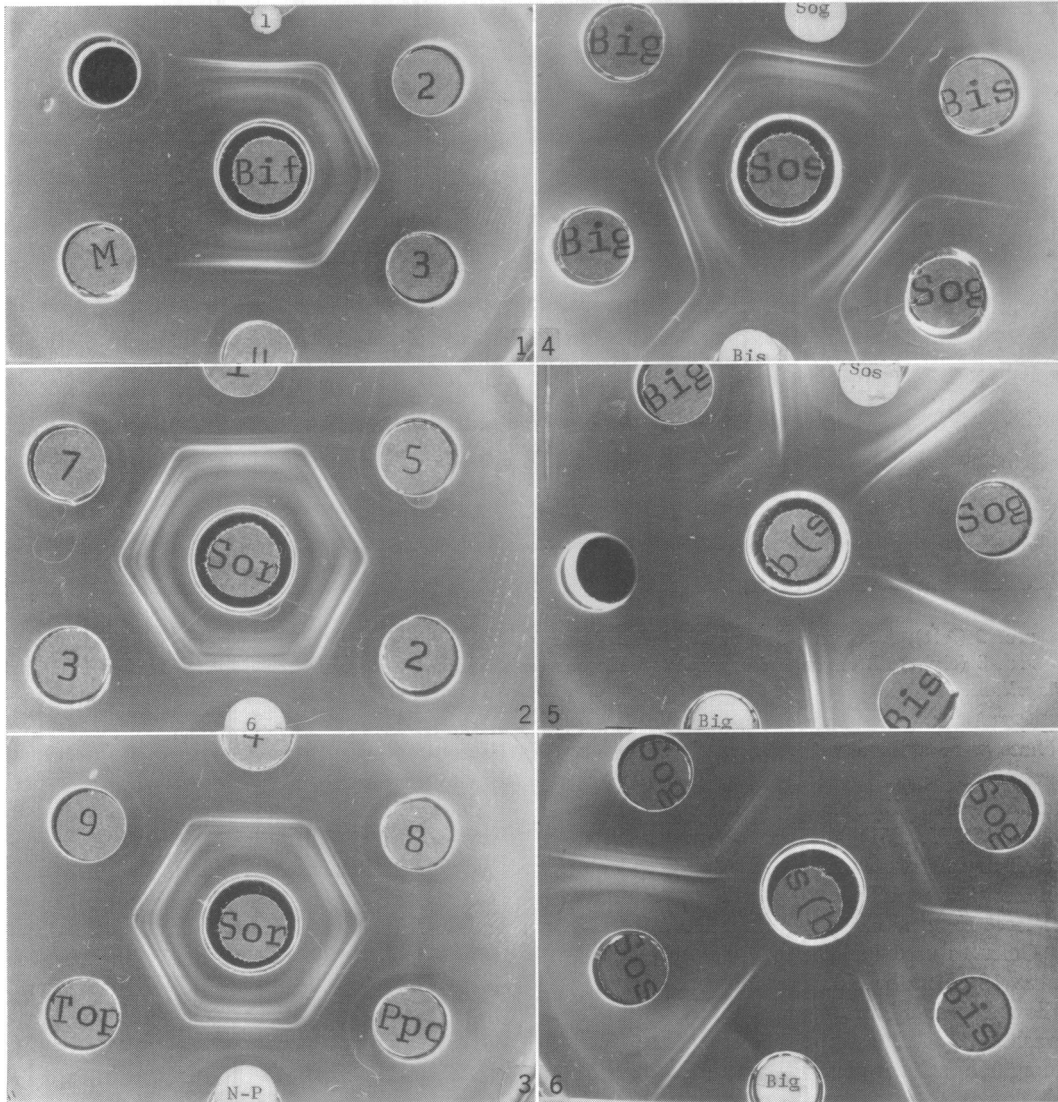


FIG. 1. *Clostridium bifermentans* antiserum (Bif) against four strains of *C. bifermentans* and uninoculated medium (M).

FIG. 2 and 3. *Clostridium sordellii* antiserum (Sor) against nine strains of *C. sordellii*, pool of pathogenic strains (Ppo), pool of nonpathogenic strains (N-P), and combined pool of all nine strains (Top).

FIG. 4. *Clostridium sordellii* antiserum (Sos) and *C. bifermentans* antiserum (Bis) against *C. sordellii* antigen pool (Sog) and *C. bifermentans* antigen pool (Big).

FIG. 5. *Clostridium bifermentans* antiserum absorbed with *C. sordellii* antigen pool [b(s)] against *C. bifermentans* antigen pool (Big), unabsorbed *C. sordellii* antiserum (Sos), *C. sordellii* antigen pool (Sog), and unabsorbed *C. bifermentans* antiserum (Bis).

FIG. 6. *Clostridium sordellii* antiserum absorbed with *C. bifermentans* antigen pool [s(b)] against *C. sordellii* antigen pool (Sog), unabsorbed *C. bifermentans* antiserum (Bis), *C. bifermentans* antigen pool (Big), and unabsorbed *C. sordellii* antiserum (Sos).