Sheath Formation by Strains of *Herpetosiphon* Species

V. B. D. SKERMAN, GILLIAN R. QUINN, L. I. SLY, AND J. V. HARDY

Department of Microbiology and Electron Microscope Center, University of Queensland, St. Lucia,
Brisbane, Australia

The recommendation to redefine the genus *Herpetosiphon* is disputed.

The genus *Herpetosiphon* and the type species *H. aurantiacus* were first described by Holt and Lewin in 1968 and defined as follows: "Produces unbranched, ensheathed filaments composed of procaryotic, Gram-negative, cylindrical, non-flagellated cells measuring 1.0 to 1.5 by 5 to 10 μm . The cells divide by transverse septum formation. Filaments attain lengths of 500 μm or more and exhibit a slow gliding motility on a solid surface; the sheath seems to move with the filament, the cells do not glide within the sheath. Separate cells are not released from filaments." Phase-contrast micrographs (1, 3) have been published of four species of *Herpetosiphon* showing the presence of a structure, visible only at the ends of filaments or where the cellular sequence is interrupted, which they interpreted as a sheath. Reichenbach and Golecki (4) regard these structures as the remains of cells which have lost their cytoplasm and are puzzled by the observation that "empty 'sleeves' at the ends of filaments are shorter than the length of an ordinary cell."

Reichenbach and Golecki (4) quote Gräf and Perschmann as having examined the structure of *Herpetosiphon* by using an electron microscope, without sectioning, and as having failed to reveal a sheath. Using thin-sectioning, freeze-etching, and negative-staining, Reichenbach and Golecki (4) claimed that *H. aurantiacus*, strain Hpa2, does not possess a sheath in the classical sense and, as a consequence, have recommended that the genus *Herpetosiphon* be redefined with the omission of reference to a sheath.

We isolated from lake waters and mosses in Southeast Queensland, Australia, a number of organisms which were morphologically indistinguishable from strains of *Herpetosiphon* obtained from H. Reichenbach and from the American Type Culture Collection. Some of these strains, when grown on agar composed of lake water solidified with 1.5% agar (Difco), showed clear evidence, under low-power ($\times 10$ objective), phase-contrast microscopy, of greyish sheathlike structures which had been vacated by the gliding filaments (Fig. 1). These structures were optically quite distinct from the

so-called slime trails which appeared as whitish trails left on the agar surface by the gliding filaments. The sheathlike structures could be lifted from the agar surface with a glass microtool (5) and repositioned on the agar surface leaving behind a so-called "slime trail" (Fig. 2). In aging cultures, quite extensive masses of the sheathlike structures accumulated. Mass evacuation of sheaths was also common where gliding filaments had formed dense concentric circles (Fig. 3, arrow).

Specimens that had been fixed in osmium tetroxide vapour, dehydrated through a graded series of alcohol, critical point-dried with CO_2 , coated with aluminium, and examined under a Cambridge Stereoscan IIA scanning electron microscope revealed the vacated sheath as a delicate tube (Fig. 4) which had collapsed into a flat ribbon with slightly raised edges. In the illustration, a receding loop in the filament had broken from the enclosing sheath.

Organisms with extensive sheath formation, grown on lake water plus 1.5% agar (Difco), were overlaid with molten lake water agar. Small blocks containing the embedded organisms were fixed in 1.2% glutaraldehyde in 0.07 M sodium cacodylate buffer (pH 6.5) for 16 h and then in 1.3% osmium tetroxide in 0.07 M sodium cacodylate buffer for a further 16 h. In preparations using ruthenium red, the stain (0.5%) was added at both of these steps (2). The blocks were then embedded in Spurr low-viscosity embedding medium (6). After sectioning and poststaining with saturated aqueous lead citrate and 5% aqueous uranyl acetate (Fig. 5), longitudinal sections showed clearly the continuation of the sheath beyond the end of the filament. The tapered end of the filament was due only to tangential sectioning. Transverse sections revealed the sheath as a delicate structure separated from the cells. Figure 6 shows cross-sections of a number of sheaths, some of which had been vacated.

The sheath had a distinct fibrillar structure, as noted by Reichenbach and Golecki (4).

The statement by Holt and Lewin (1) that the filaments do not move in the sheath is not correct. Figure 7 consists of micrographs taken

FIG. 1. *Herpetosiphon* sp. strain UQM 1435 growing on lake water agar. Phase contrast.

FIG. 2. Micromanipulation of vacated sheath of *Herpetosiphon* sp. strain UQM 1435 growing on lake water agar. Phase contrast.

FIG. 6. Transverse section through ensheathed filaments of *Herpetosiphon* sp. strain UQM 1552 grown on lake water agar. Stained with ruthenium red.

FIG. 7. *Herpetosiphon* sp. strain UQM 1552 grown on lake water agar, photographed under phase contrast at intervals during a period of 40 min.

-
- FIG. 3. Scanning electron micrograph of *Herpetosiphon* sp. strain UQM 1552 growing on lake water agar.
 FIG. 4. Scanning electron micrograph of *Herpetosiphon* sp. strain UQM 1552 growing on lake water agar.
 FIG. 5. Longitudinal section through an ensheathed filament of *Herpetosiphon* sp. strain UQM 1552, grown on lake water agar.

over a period of 40 min by low-power, phase-contrast microscopy which show a filament which has migrated inside the sheath. This migration occurred only under certain conditions of culture which, with studies on the mode of sheath formation, are currently under investigation.

The above work was conducted mainly with strain UQM 1552. Thus far vacated sheaths similar to those illustrated in Fig. 1 and 2 have been demonstrated also in UQM strains 1415, 1434, 1435, 1460, 1512, 1550, 1551, 1576, 1578, 1579, and two cultures of *H. aurantiacus* strain Hpa2 (UQM 1581) provided by H. Reichenbach.

On the basis of evidence presented in this communication, we do not support the recom-

mendation by Reichenbach and Golecki (4) to redefine the genus *Herpetosiphon*.

LITERATURE CITED

1. Holt, J. G., and R. A. Lewin. 1968. *Herpetosiphon aurantiacus* gen. et sp. n., a new filamentous gliding organism. *J. Bacteriol.* 95:2407-2408.
2. Leak, L. V. 1967. Fine structure of the mucilaginous sheath of *Anabaena* sp. *J. Ultrastruct. Res.* 21:61-74.
3. Lewin, R. A. 1970. New *Herpetosiphon* species (Flexibacteriales). *Can. J. Microbiol.* 16:517-520.
4. Reichenbach, H., and J. R. Golecki. 1975. The fine structure of *Herpetosiphon*, and a note on the taxonomy of the genus. *Arch. Microbiol.* 102:281-291.
5. Skerman, V. B. D. 1968. A new type of micromanipulator and microforge. *J. Gen. Microbiol.* 54:287-297.
6. Spurr, A. S. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26:31-43.