

carious lesions, and identified as *S. mutans*^{5,6} were tested for hyaluronidase activity. These 8 strains were also negative for hyaluronidase activity.

An attempt was made to induce hyaluronidase activity by growing each of the reference cultures 3 times in brain heart infusion broth supplemented with 0.01% hyaluronic acid. The cultures remained negative for hyaluronidase activity, reducing the possibility that enzyme activity was lost through subculturing.

It may be concluded that *S. mutans* and the other cariogenic streptococci studied do not produce hyaluronidase under the conditions of this investigation⁷.

Zusammenfassung. Dreizehn Stämme *Streptococcus mutans* und 5 kariogene Streptokokken-Stämme wurden auf ihre Hyaluronidase-Aktivität untersucht. Alle 18 Stämme zeigten negative Hyaluronidase-Aktivität. Acht weitere Stämme von *Streptococcus mutans* wurden zusätzlich von kariösem Dentin isoliert und untersucht.

Auch hier könnte keine Hyaluronidase-Aktivität nachgewiesen werden. Hyaluronidase-Aktivität konnte nicht induziert werden und es wurde gefolgert, dass *Streptococcus mutans* und andere kariogene Streptokokken keine Hyaluronidase erzeugen.

L. G. SIMONSON, and I. L. SHKLAIR⁸

Microbiology Division, Naval Dental Research Institute, Naval Base, Great Lakes (Illinois 60088, USA), 26 June 1972.

⁵ S. EDWARDSSON, Archs. oral Biol. 13, 637 (1968).

⁶ H. V. JORDAN, B. KRASSE and A. MOLLER, Archs. oral Biol. 13, 919 (1968).

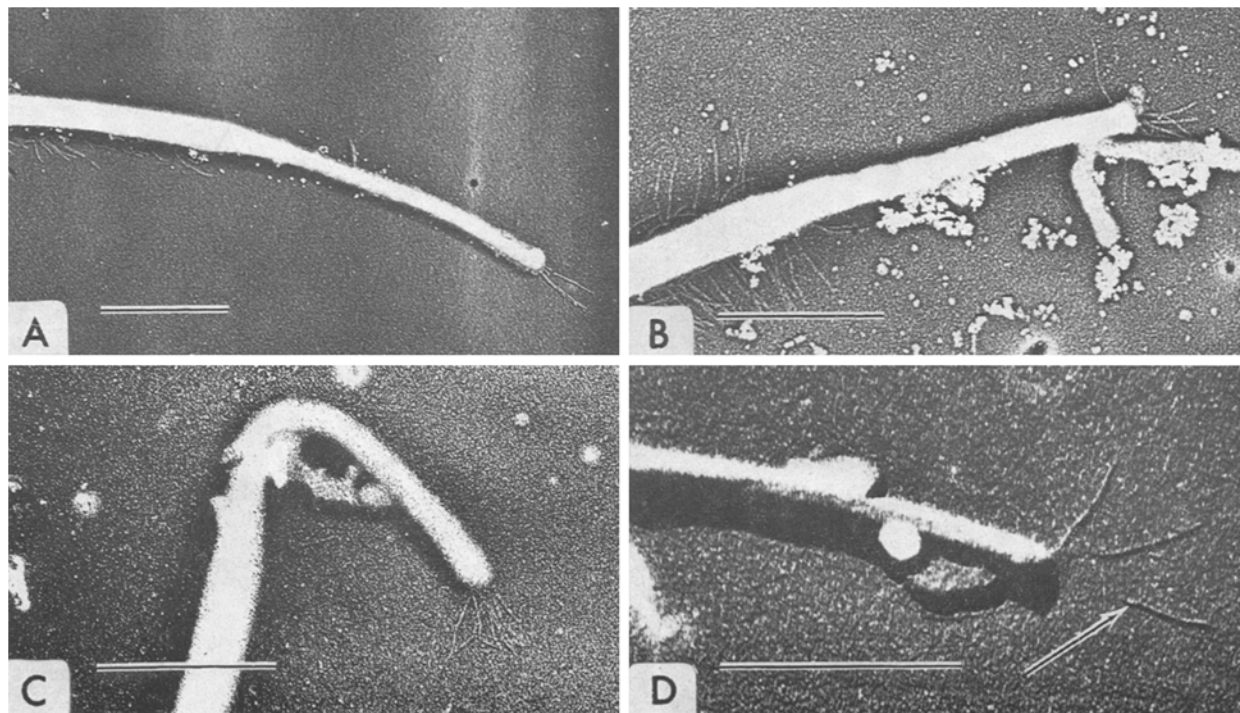
⁷ From Research Project No. MR005.20.01 6049A3JJ, Bureau of Medicine and Surgery, U. S. Navy Department, Washington, D. C. (USA).

⁸ We thank DT2 J. Mc CORMICK for technical assistance.

Flagellar Hairs on Zoospores of *Phytophthora* Species: Tip Hairs on the Whiplash Flagellum

The lateral hairs on the tinsel flagellum of the zoospores of *Phytophthora* species were observed by electron microscopy some time ago¹⁻³. Recently lateral hairs were reported on the whiplash flagellum of *P. erythroseptica* Pethybridge, *P. palmivora* (Butl.) Butl., and *P. parasitica* Dastur⁴⁻⁶. However, no reports of tip or terminal hairs on the whiplash flagellum of *Phytophthora* zoospores have been made. We report here the presence of tip hairs on the whiplash flagellum in 4 species of this genus, and lateral hairs on the whiplash flagellum of two species not previously reported to have them.

P. cinnamomi and *P. megasperma* var. *sojae* were grown in axenic cultures⁷; *P. palmivora* was grown on V-8 juice agar and *P. parasitica* on sterile alfalfa stem segments. Cultures with numerous zoosporangia were flooded with glass distilled water, and all except *P. parasitica* were chilled before allowing them to stand for 20–30 min to induce zoospore swarming. The swarming zoospores were concentrated, fixed, washed and prepared for electron microscopy as previously described⁸. The specimens were examined in either an RCA EMU-3B or Hitachi HU-12 electron microscope both with 50 μ m objective apertures.



a) Portion of whiplash flagellum of *P. megasperma* var. *sojae* unidirectionally shadow cast with palladium showing both lateral and tip hairs. b) Portion of whiplash flagellum of *P. parasitica* rotary shadow cast with platinum-palladium alloy showing both lateral and tip hairs. c) Rotary shadow cast tip of *P. megasperma* var. *sojae* flagellum with several hairs. d) Unidirectionally shadow cast tip of *P. megasperma* var. *sojae* flagellum showing detached tip hairs. All bars represent 1 μ m.

Tip hairs occurred on the whiplash flagellum of the zoospores of *P. cinnamomi* Rands, *P. megasperma* var. *sojae* Hildebrand, *P. palmivora* and *P. parasitica* (Figure 1, a–d). The length of the hairs varies from 460 to 500 nm on different species. The width as measured on shadow cast specimens is 11–12 nm. The tip hairs were not found on the whiplash flagellum of every zoospore observed as they are apparently quite fragile and probably break off during specimen preparation. Figure 1d, which shows 1 attached hair and 2 detached hairs, suggests that they are fragile and easily lost. Since the number of tip hairs varied within single species, this may be a further indication of their fragility. For example, many flagella of *P. megasperma* var. *sojae* had 7 or 8 tip hairs (Figure 1c) while others had only 1 or 2 (Figure 1a).

Lateral hairs were consistently found on the whiplash flagella of the zoospores of *P. cinnamomi* and *P. megasperma* var. *sojae*. The length and width of these hairs, as measured on shadow cast preparations, were about the same as those reported for *P. palmivora*⁵, i.e. ca. 500 nm long and 15 nm wide. Lateral hairs were also observed on the whiplash flagellum of *P. parasitica* (Figure 1b); the latter confirms the finding of REICHLÉ⁶ in his studies of negatively stained flagella of this species. These findings bring the total number of *Phytophthora* species with lateral hairs on the whiplash flagellum to 5, which suggests that their presence is a common phenomenon rather than an unusual one as previously suggested^{8,9}.

Although the presence of flagellar hairs (mastigonemes) is sometimes difficult to demonstrate because of their fragility, every effort should be made to carefully prepare specimens for electron microscopy before concluding that they are not present. Certainly all types of flagellar hairs are useful in taxonomic studies^{10,11}.

MANTON¹⁰, in discussing flagellar structure, has recommended that the term 'acronematic' not be used to describe the terminal structure of flagella since such a term suggests a terminal hair similar to lateral hairs on certain flagella. Perhaps the term could be validly used here to describe the tip hairs on *Phytophthora* whiplash flagella since they are indeed similar to the lateral hairs. Nevertheless for the sake of clarity we have simply designated them as tip or terminal hairs.

It has been stated that the lateral hairs on the tinsel flagellum of *P. infestans* arise from the 'axis' (presumably the fibrils) of the flagellum^{3,8}. However, this has not as yet been demonstrated in ultrathin sections of flagella⁸. Nothing is known about the point of origin of the hairs on the whiplash flagellum. With respect to the tip hairs

described in this report, one might at first wonder if they are extensions of the central fibrils of the flagellum. However, their width (11–12 nm) is less than the diameter of the whole fibril (30–36 nm) and greater than that of the filaments (4–5 nm) described by GRIMSTONE¹² and Ringo¹³ as forming the walls of the fibrils. This difference in diameters would seem to suggest that the tip hairs are not extensions of the flagellar fibrils¹⁴.

Résumé. Des mastigonèmes de 460–500 nm de longueur et 11–12 nm de largeur existent à l'extrémité du flagelle «whiplash» des zoospores des *Phytophthora cinnamomi*, *P. megasperma* var. *sojae*, *P. palmivora* et *P. parasitica*. Des mastigonèmes latéraux existent aussi sur le flagelle «whiplash» des 4 espèces. C'est la première mention de mastigonèmes latéraux sur le flagelle «whiplash» de *P. cinnamomi* et de *P. megasperma* var. *sojae*.

P. R. DESJARDINS, G. A. ZENTMYER, DAH-WU CHEN¹⁵,
T. A. DEWOLFE, L. J. KLOTZ and D. A. REYNOLDS

Department of Plant Pathology, University of California,
Riverside (California 92502, USA), 31 July 1972.

¹ V. R. FERRIS, *Science* 120, 71 (1954).

² V. R. FERRIS and H. H. LYON, *Phytopathology* 44, 487 (1954).

³ A. P. KOLE and K. HERSTRA, *Proc. K. med. Akad. Wet., Ser. C.* 62, 404 (1959).

⁴ R. VUJICIC, J. COLHOUN and J. A. CHAPMAN, *Trans. Br. mycol. Soc.* 57, 125 (1968).

⁵ P. R. DESJARDINS, G. A. ZENTMYER and D. A. REYNOLDS, *Can. J. Bot.* 47, 1077 (1969); *Mycologia* 62, 421 (1970).

⁶ R. E. REICHLÉ, *Mycologia* 61, 30 (1969).

⁷ D. W. CHEN and G. A. ZENTMYER, *Mycologia* 62, 397 (1970).

⁸ J. COLHOUN, in *The Fungus Spores* Proc. 18th Symp. Colsten Res. Sec. Bristol, England, (Ed. M. F. MADELIN; Butterworth, London 1966), vol. 18, p. 85.

⁹ C. E. BRACKER, *A. Rev. Phytopath.* 5, 343 (1967).

¹⁰ I. MANTON, *Adv. Bot. Res.* 2, 1 (1965).

¹¹ R. M. KLEIN and A. CRONQUIST, *Q. Rev. Biol.* 42, 105 (1967).

¹² A. V. GRIMSTONE, in *Formation and Fate of Cell Organelles* (Ed. K. B. WARREN; Academic Press, Inc., New York 1967), p. 219.

¹³ D. L. RINGO, *J. Ultrastruct. Res.* 17, 266 (1967).

¹⁴ Supported in part by NSF Grant No. GB-658 and the American Cocoa Research Institute and NSF Grant No. GB-8697.

¹⁵ Professor DAH-WU CHEN was on a Fulbright Fellowship in the Department of Plant Pathology, University of California, Riverside during 1968–69. His present address is the Department of Plant Pathology, Provincial Chung-Hsing University, Taichung, Taiwan, Republic of China.

COGITATIONES

Divalent Metal Ion Buffers with Low pH-Sensitivity

Introduction. Reaction rate and stability of numerous enzymes are dependent on the presence of certain, mostly divalent metal ions^{1–4}. Moreover, metal ions often play an important role in regulation of enzyme synthesis and activity *in vivo*⁵. It is, however, generally difficult to achieve, and exactly to reproduce, constant levels of free metal ion concentrations of less than about 10 μ M in a test medium. Impurities in the reagents often result in an uncontrolled level of divalent metal ions. Metal ions may be chelated by various components of a medium, such as buffer compounds (e.g. histidine), SH-compounds (e.g. cysteine, glutathione), substrates (e.g. ATP) and particularly proteins⁶.

The use of metal ion buffers, especially Ca²⁺-ion buffers, to circumvent these difficulties was proposed by many authors (e.g. Refs.⁷ and ⁸). It has been recognized^{7–10}, however, that the application of metal ion buffers poses some other problems, since most of the chelating agents of the polyaminocarboxylate type show considerable protonation.

Addition of a second divalent metal ion, which competes with protons and the primary metal ion Me²⁺, may lessen this pH-dependence. The application of this type of buffer provides an advantage for all investigations which require a constant level of the primary ion over a wide pH-range, e.g. for kinetic studies on the pH-