

killing the yield of auxotrophs was considerably lower in comparison with bacteria<sup>18</sup>. The maximum number of auxotrophs was obtained only with 40 sec of irradiation. It is evident from these results that the response of *Rhodotorula* sp. to the mutagenic action of UV-light is similar to that of other species of yeasts<sup>19, 20</sup>.

Griseofulvin has long been known as an antifungal antibiotic, although a complete spectrum of sensitive organisms is not available. It is reported to inhibit the growth of mycelial fungi but has no effect on bacteria or yeasts<sup>21-24</sup>. Even among fungi, only those with chitinous cell have been shown to be sensitive<sup>18</sup>. It was therefore significant in the present study to show that *Rhodotorula* sp. cells are inactivated by griseofulvin, the rate of killing being proportional to the antibiotic concentration (Table I). The effective concentration of griseofulvin for increasing the yield of auxotrophs in the irradiated culture was 1 mg/ml which was below the lethal dose. The lower yields of auxotrophs with higher concentrations of antibiotic (viz. 4.7% and 3.1% for 5 and 10 mg/ml of griseofulvin respectively) was probably due to the inactivation of dormant mutant cells as well by mere penetration of the antibiotic. The results have also suggested that only actively growing cells of *Rhodotorula* sp. are sensitive to griseofulvin.

**Zusammenfassung.** Nach Vorbehandlung von Hefezellen mit Griseofulvin (selektive Eliminierung von Prototrophen) zu nachfolgender UV-Behandlung und Induktion von Mutanten, haben die bestrahlten Zellen der überlebenden *Rhodotorula*-Population fünfmal mehr Auxotrophe als die Kontrollen ohne das Antibiotikum.

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<sup>20</sup> S. E. REAUME and E. L. TAUTUM, *Arch. Biochem.* 22, 331 (1949).

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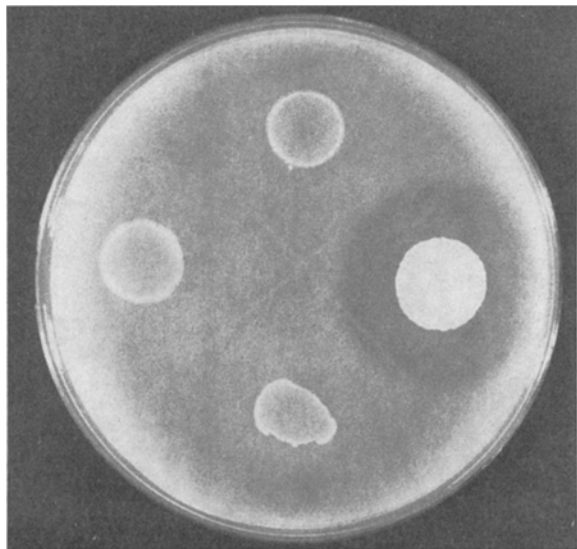
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<sup>23</sup> F. J. ROTH, B. SALLMAN and H. BLANK, *J. Invest. Dermat.* 33, 403 (1959).

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## A Survey of Some Cariogenic Streptococci for Hyaluronidase Activity

Among the oral streptococci, some strains of *Streptococcus mitis* and *Streptococcus salivarius* have been reported to produce hyaluronidase<sup>1</sup>. It has been reported that streptococci isolated from human carious dentin, having properties similar to those of *Streptococcus mutans*, could utilize hyaluronic acid as the sole source of carbon<sup>2</sup>. However, KJELLMANN<sup>3</sup> tested two strains of *S. mutans* and could not demonstrate any hyaluronidase activity. Since *S. mutans* has become increasingly associated with dental caries in recent years, it was thought important to survey for hyaluronidase activity in some reference strains of this organism and other known cariogenic streptococci.



Demonstration of the plate assay procedure for hyaluronidase activity. A clear zone around the inoculum indicates activity.

The oral streptococci surveyed in this study were: *S. mutans* strains FA-1, SL-1, HS-6, NTCC 10449, LM-7, AHT, BHT, GS-5, 6715, OMZ-176, Ingbritt, E-49, and K-1R; *Strep. sp.* nontypable strains 167 and 20; and *Strep. sp.* Lancefield group H strains F90A, SBE, and Challis. Four strains of *Staphylococcus aureus* were used as positive viable controls. Assay discs, each containing 115 USP units of hyaluronidase (Mann Research Labs.) were also used as positive controls in the test system.

The hyaluronidase activity of the microorganisms was determined by using a plate assay procedure described by SMITH and WILLETT<sup>4</sup>. Overnight broth cultures were inoculated onto the assay medium consisting of Brain Heart Infusion broth (Difco) supplemented with hyaluronic acid (Sigma Chemical Co.), bovine albumin (Sigma Chemical Co.) and 1% Noble agar (Difco). The plates were incubated in an atmosphere of 95% N and 5% CO<sub>2</sub> for 72 h at 37°C. After incubation, each of the agar plates was flooded with 2 N acetic acid for 10 min. The nondegraded substrate and albumin were precipitated by the acid, leaving a clear zone around only those colonies that degraded the hyaluronic acid substrate. Duplicate samples of each culture were tested on at least two occasions.

The four strains of *Staph. aureus* and the control assay disc always gave a positive result for hyaluronidase activity. However, all the streptococcal strains tested were found negative for hyaluronidase, although growth was observed in all cases. A typical result is illustrated in the Figure. Eight additional strains, freshly isolated from

<sup>1</sup> H. GIBIAN, *The Amino Sugars IIB* (Academic Press, New York 1966).

<sup>2</sup> P. D. TOTO, M. V. SANTANGELO and J. V. MADONIA, *J. dent. Res.* 47, 1056 (1968).

<sup>3</sup> O. KJELLMAN, L. LINDER and G. FROSTELL, *Odont. Revy.* 22, 27 (1971).

<sup>4</sup> R. F. SMITH and N. P. WILLETT, *Appl. Microbiol.* 16, 1434 (1968).

carious lesions, and identified as *S. mutans*<sup>5,6</sup> 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 investigation<sup>7</sup>.

**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.

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

<sup>5</sup> S. EDWARDSSON, Archs. oral Biol. 13, 637 (1968).

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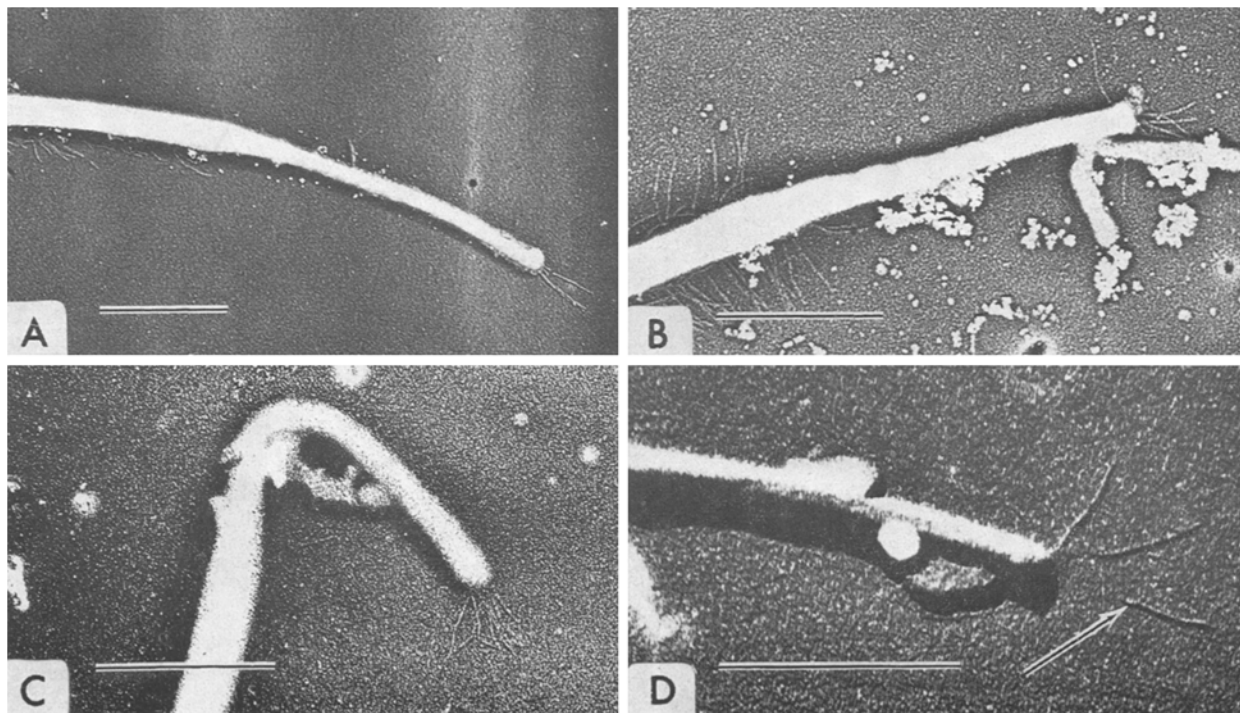
<sup>7</sup> From Research Project No. MR005.20.01 6049A3JJ, Bureau of Medicine and Surgery, U. S. Navy Department, Washington, D. C. (USA).

<sup>8</sup> 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 ago<sup>1-3</sup>. Recently lateral hairs were reported on the whiplash flagellum of *P. erythrosetica* Pethybridge, *P. palmivora* (Butl.) Butl., and *P. parasitica* Dastur<sup>4-6</sup>. 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 cultures<sup>7</sup>; *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 described<sup>8</sup>. The specimens were examined in either an RCA EMU-3B or Hitachi HU-12 electron microscope both with 50  $\mu$ 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  $\mu$ m.