

schädlichen Pilzen dieses Gebiets eine grosse parasitische Aktivität. Beachtenswert ist ihre Angriffskraft gegenüber dem Hautpilz *Trichophyton gallinae*. Die Labyrinthulen zeigten auch bei *Rhizoctonia solani* einen deutlichen Effekt. Das Wachstum eines anderen Stammes dieses Pilzes wurde von Labyrinthulen aus der Ostsee kaum beeinflusst⁴. Die Hyphen von *Histoplasma capsulatum* waren in der Kultur so schmal, dass die Labyrinthulen nicht in sie eindringen konnten. Dieser negative Befund besagt jedoch nicht, dass Labyrinthula nicht doch Histoplasma in Felsenhöhlen vernichten kann, da der Pilz dort eine andere und für die Labyrinthulen vielleicht geeignetere Wachstumsform zeigt.

Summary. Labyrinthula could be isolated from marine *Cladophora* algae in Cuba. These strains were very active against several fungi, causing diseases of plants or skin, like *Fusarium oxysporum*, *F. moniliforme*, *F. vasinfectum*, *Pestalotzia* sp., *Colletotrichum gloeosporioides*, *Trichophyton gallinae* and destroyed the protoplasm of the hyphae by means of their parasitic activity.

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Is Fresh-Water *Hydra vulgaris attenuata* a Spirochaetales' Reservoir Host?

In the process of research on gametogenesis of *Hydra vulgaris attenuata*, by electron microscope, we found some spirochaetales, sometimes very numerous, in the mesoglea of many animals (Figures 1 and 2). The hydras, hosts of spirochaetes, were not in a suffering condition; in fact cultures were not depressed, although the infestation must have lasted a long time because the hydra specimens selected for examination were drawn from time to time for a period of several months.

The hydras were reared at 18–20 °C in a 5 l aquarium where aquatic plants (*Elodea* and *Myriophyllum*) were present, and fed every other day with *Daphnia*. Hydras, in different gametogenic stages, were fixed in 1% OsO₄ in veronal acetate buffer at pH 7.4. They were rapidly washed, dehydrated, infiltrated with uranylacetate and embedded in Epon 812 or in Araldite. The sections, obtained with a LKB ultramicrotome, were stained with lead salts according to MILLONIG's method¹ and observed with a Siemens Elmiskop 1 electron microscope².

The spirochaetales were always found in mesoglea of hydra, while, on the other hand, in epi- and gastrodermis no spirochaetes were present. This suggested that the presence of spirochaetales in hydra's mesoglea was not a fortuitous fact, because in the case of merely strong spirochaetal pollution of environment water and their occasional ingestion by hydras, gastrodermal cells should have shown spirochaetes in their food vacuoles.

Electron micrographs of sections of these spirochaetales revealed some morphological features (Figure 2). Their small spirals are 0.2 µ in length. Periplast membrane is present. The protoplast is spirally wound around a well-defined axial filament (Figure 3).

Morphological aspects do not allow us to determine with certainty either the genus or the species; nevertheless we are inclined to consider these spirochaetales as a *Leptospira*. In order to recognize the serotype, it would be essential to culture the spirochaetale³, but that has not been possible as yet, because only 'a posteriori' we found the pollution of hydra rearings and our present clones are no longer infested.

Mesoglea seems to be a good natural culture medium for spirochaetales. The mesolamella of hydra is an acellular layer between epi- and gastrodermis, consisting of an electron-lucent ground substance, within which tiny filaments of about 100 Å in diameter are randomly dispersed. In addition there are many granules identified as glycogen particles (SLAUTTERBACK and FAWCETT⁴) (Figure 2). The scattered fibrils have been considered

collagenous-like by some authors (CHAPMAN⁵), elastic-like or mixed by others (BOUILLON and VANDERMEERSCH⁶ BOUILLON⁷).

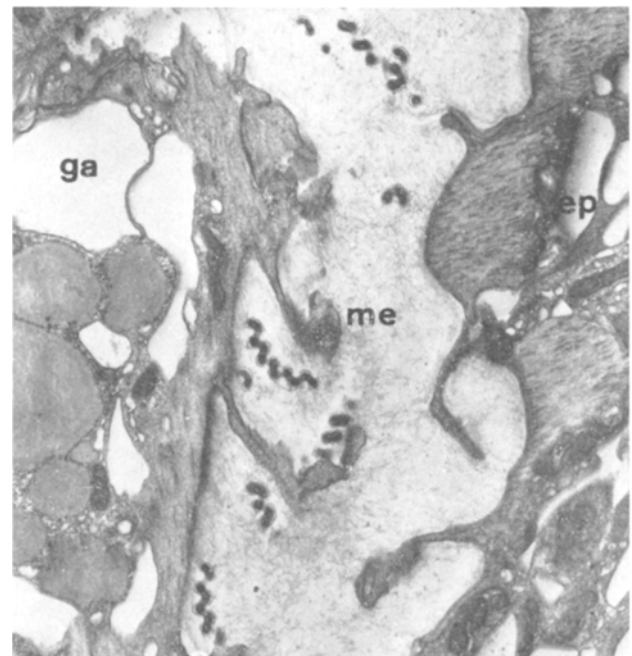


Fig. 1. Electron micrograph of a mesogleal tract crossed by cytoplasmic processes of epi- and gastrodermal cells. Many spirochaetales are present. Fibrils scattered. ga, gastrodermis; me, mesoglea; ep, epidermis. × 12,000.

¹ G. MILLONIG, J. Biophys. biochem. Cytol. 11, 736 (1961).

² Preparation were carried out in the Centro di Microscopia elettronica of Bologna University.

³ On this problem we wish to express our thanks to Prof. BABUDIERI of the Istituto Superiore di Sanità di Roma for his suggestion.

⁴ D. B. SLAUTTERBACK and D. W. FAWCETT, J. Biophys. biochem. Cytol. 5, 441 (1959).

⁵ G. CHAPMAN, Q. Jl microsc. Sci. 94, 155 (1953).

⁶ J. BOUILLON and G. VANDERMEERSCH, Annls Soc. r. zool. Belg. 87, 9 (1956).

⁷ J. BOUILLON, Bull. biol. Fr. Belg. 93, 5 (1959).



Fig. 2. Electron micrograph of a mesogleal detail of *H. vulgaris attenuata*, in which some glycogen particles (gl) and a framework (f) of fibrils are present. A spirochaetale with periplast membrane and, in some area, axial filament, are shown. $\times 32,000$.

Anyway, mesoglea, owing to its proteic framework and its gelatinous consistence, is suitable as a semi-solid culture medium for spirochaetes.

We think that spirochaetales are perhaps 'inquilines' of fresh-water hydras. The question remains: Is fresh-water hydra a possible reservoir host of spirochaetes pathogenous for other animals?

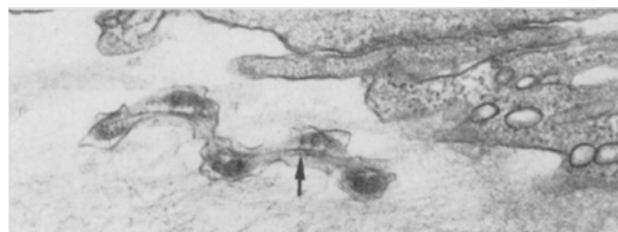


Fig. 3. Coils of a spirochaetale in mesoglea of *H. vulgaris attenuata* in which the axial filament is distinctly seen (arrow). $\times 38,000$.

Riassunto. Osservazioni al M.E. di esemplari di *Hydra vulgaris attenuata* hanno messo in evidenza la presenza di spirochetali nello strato mesogleale. Si fa l'ipotesi che le spirochete siano inquiline di questi polipi d'acqua dolce. Le idre potrebbero forse anche rappresentare un serbatoio di spirochete patogene per altri animali.

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Response of Different Amino Acids on the Sporulation of *Colletotrichum falcatum* Went on the Sugarcane Juice of Resistant Varieties

Extensive work has been done on the morphological and physiological studies of *Colletotrichum falcatum* Went, the causal organism of red rot disease of sugarcane on various solid and liquid media by various workers¹⁻⁴. It has also been reported that the sporulation of the fungus was more in the cane juice of resistant varieties, in comparison with that of the susceptible ones^{5,6}. Preliminary experiments suggested that the nitrogen content of the sugarcane juice, particularly organic nitrogen, supported the sporulation of *C. falcatum*. On the other hand, it suppressed the sporulation in the juice of susceptible varieties⁶. Hence it is worthwhile to investigate the effect of different amino acids on the sporulation of the organism on the sugarcane juice of the resistant varieties.

Materials and methods. Highly resistant variety (Co. 550) and highly susceptible variety (Co. 608) were grown at the experimental farm of the Indian Institute of Technology, Kharagpur, India, for 11 months. Isolate 404 of *C. falcatum* was selected for the present study. The methods followed for the extraction of juice, estimation of total-nitrogen content present in juice and the other details have already been described by the author in his previous report⁶. The cane juice agar medium used consists of 250 ml of cane juice and 20 g of agar in 750 ml of

distilled water. The pH was adjusted to 6.0 and the medium was sterilized at 10 lb pressure for 15 min. The 10-day-old culture was thoroughly mixed in the waring blender and the spores present in the homogenous suspension were counted in a haemocytometer.

The capacity of the fungus to utilize nitrogen from various amino acids was studied. To cane juice agar of resistant variety different amino acids were added. The total nitrogen content present in juice of resistant variety was adjusted to 18.00 mg of total nitrogen content per 100 ml of juice by substituting different amino acids. The results were statistically analyzed by following the methods given by SNEDECOR⁷.

¹ T. S. RAMAKRISHNAN, Proc. Ind. Acad. Sci. Sect. B 74, 395 (1942).

² E. V. ABBOTT, Sugar Bull. 129 (1946).

³ B. L. CHONA and M. K. HINGORANI, Phytopath. 40, 221 (1950).

⁴ B. L. CHONA and D. N. SRIVASTAVA, Proc. 2nd Bien. Conf. Sugar Res. and Dev. Workers in Indian Union (1954), p. 103.

⁵ K. V. SRINIVASAN, Sci. and Cult. 29, 2, 87 (1963).

⁶ K. V. B. R. TILAK, Phytopath. Z. 61, 286 (1968).

⁷ G. W. SNEDECOR, Statistical Methods, 4th edn (The Iowa State College Press, Ames, Iowa 1946).