



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?

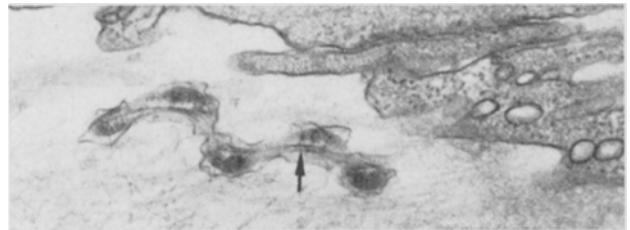


Fig. 3. Coils of a spirochaetale in mesoglea of *H. vulgaris attenuata* in which the axial filament is distinctly seen (arrow). $\times 38,000$.

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).

Effect of different amino acids on the sporulation of *C. falcatum* on the sugarcane juice of resistant variety

Cane variety	Total nitrogen content present in 100 ml of juice (mg)	Amount of total nitrogen adjusted to 18 mg/100 ml juice by substituting	Sporulation (mean of 3 replicates) 1×10^8
Co. 550 (resistant)	12.80	—	21.70
		DL-Alanine	22.50
		L-Arginine	23.25
		L-Asparagine	29.82
		L-Cysteine	18.11
		L-Glutamic acid	31.00
		Glycine	21.25
		L-Histidine HCl	22.00
		DL-Isoleucine	26.12
		DL-Leucine	24.00
		L-Lysine HCl	22.32
		DL-Methionine	17.10
		DL-Phenylalanine	20.55
		L-Proline	21.25
		DL-Serine	21.00
		DL-Threonine	21.75
		DL-Tryptophan	23.55
		DL-Valine	24.95
		S. Em.	± 0.0352
		C.D. at 5%	0.0884
		C.D. at 1%	0.1159
Co. 608 (susceptible)	22.50	—	14.85

Results and discussion. It was found, in general, that the sporulation was more in the juice agar of resistant variety as compared with that of the susceptible variety.

The juice of resistant variety had significantly lesser content of total nitrogen than that of the susceptible ones. There was a significant increase in sporulation in the cane juice agar of resistant variety due to the addition of various amino acids (Table). Further, the addition of L-asparagine and L-glutamic acid have remarkably increased the sporulation. This confirms the earlier observation that the nitrogen content in juice, particularly amino nitrogen, plays an important role in supporting the sporulation of the fungus and also the resistance of the cane towards the disease. The addition of L-cysteine and DL-methionine significantly decreased the sporulation. The sporulation was poor in the cane juice agar of susceptible variety due to the presence of high total-nitrogen content present in the juice⁸.

Zusammenfassung. Die Sporenbildung von *Colletotrichum falcatum* Went. wird bei der Kultur auf Zuckerrohrsaft durch den Zusatz von Asparagin und Glutaminsäure begünstigt, während Zystein und Methionin die Sporenbildung herabsetzen.

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Regulation of Host and Symbiont Population Size in *Paramecium bursaria*

The ciliate protozoan, *Paramecium bursaria* maintains within its cytoplasm several hundred green algal cells of a *Chlorella* species. Perpetuation of this association has been explained by proposing a steady state in number of algae per protozoan cell¹⁻³. This paper examines this proposal by comparing population growth of the 2 partners, alga and protozoan, throughout the growth cycle of cultures maintained in light and in darkness. Evidence is also presented bearing on the existence and nature of regulatory mechanisms for maintaining such a proposed steady state.

Materials and methods. The chlorella-bearing strain of *P. bursaria*⁴ was grown in a baked lettuce infusion⁵ modified by the addition of 3 mM phosphate buffer adjusted to pH 7.2 and inoculated with *Enterobacter* (*Aerobacter*) *aerogenes*. Stock cultures were maintained in continuous light of 150 ft-c produced by cool white fluorescent tubes. The temperature of stock and experimental cultures was $25 \pm 1^\circ\text{C}$. Experimental cultures (50 ml in 125 ml cotton-plugged Erlenmeyer flasks) were provided with a diurnal regimen of 20 h light + 4 h darkness. The concentration of protozoa in each experimental culture was determined by averaging the number counted in three 0.1 ml samples of animals immobilized by adding a bacteriological loopful of 10% aqueous formaldehyde. Populations of endogenous algae in the protozoan samples were determined by liberating the endoplasmic algae. This was accomplished by forcibly ejecting the animal

cells in a stream from a 2.0 ml standard glass hypodermic syringe with a No. 27 needle. Algal counts were then made with a hemacytometer. Endogenous algal cell population is expressed either as cell population per millilitre of culture; or the algal cell population divided by the protozoan cell population in a particular sample yields the 'symbiont index' representing the mean algal cell population per paramecium cell.

Results. Populations of both the ciliate host and its endosymbiont algae are capable of reproducing in continuous darkness on the bacterized lettuce medium used in this study (Figures 1a and 1b, lower curve), confirming the results of SIEGEL and others^{1,6,7}. The rate of increase and total yield of endogenous chlorella populations is greatly enhanced by light (Figure 1a, upper 2 curves). On the other hand, growth of host protozoan populations is also enhanced by light (Figure 1b, upper 2 curves). Since paramecia devoid of algae are unaffected by light, these results establish that algal photosynthesis is directly

¹ S. J. KARAKASHIAN, *Physiol. Zool.* 36, 52 (1963).

² S. J. KARAKASHIAN and M. KARAKASHIAN, *Evolution* 19, 368 (1965).

³ R. PADO, *Folia biol.* 13, 173 (1965).

⁴ R. W. SIEGEL, *Am. Nat.* 92, 253 (1958).

⁵ T. M. SONNEBORN, *J. expl. Zool.* 113, 87 (1950).

⁶ E. PRINGSHEIM, *Arch. Protistenk.* 64, 289 (1928).

⁷ R. W. SIEGEL, *Expl. Cell Res.* 19, 239 (1950).