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GROWTH OF *THIOPLOCA INGRICA* IN A MIXED CULTURE SYSTEM[1]

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Abstract. Previously, *Thioploca ingrica* had not been grown in the laboratory and observation of the organism was limited to specimens taken directly from natural mud samples. The method described here makes laboratory cultivation of *T. ingrica* in mixed culture possible. The medium consists of low concentrations of extracted hay, water, and mud with its natural bacterial flora. Four weeks after the hay and mud were mixed, the medium was inoculated with *T. ingrica* washed from Lake Erie mud. It was found that the higher the concentration of hay, the lower the success rate and the longer the elapse of time before the organism established itself. Conversely, longevity of a successful culture improved with higher concentrations of hay. The growth of the organism in the flasks was observed *in situ* with an inverted microscope.

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From its discovery, *Thioploca* has been compared to *Beggiatoa* (Lauterborn 1907). Both are filamentous and gliding, contain sulfur inclusions, and share ultrastructural similarities (Lauterborn 1907 and Maier *et al* 1965). However, Kolkwitz (1912) noticed that the free filaments of *Beggiatoa* tolerated higher levels of oxygen (O_2) and hydrogen sulfide (H_2S) than the sheathed bundles of *Thioploca* filaments. The Bass Island area of Western Lake Erie was found to possess a rich mud population of *T. ingrica* (1960, Randles, personal communication). Such mud samples, kept in glass jars in the laboratory, supplied living organisms for years. In recent years, the population in Lake Erie has declined to the point where active bundles are no longer detectable.

The hay enrichment method, successfully used for *Beggiatoa* (Faust *et al* 1961), was modified in this study to provide lower levels of H_2S by varying the concentration of finely ground (60 mesh) extracted hay from 0.2 to 0.5 g/60 ml tap water in 125 ml Erlenmeyer flasks. Estimated with lead acetate paper, the level of H_2S rose to a maximum in about 10 days after inoculation with 2 or 4 ml of *Thioploca*-free mud from Western Lake Erie and then declined slowly. Inoculation of the mud-hay flasks with active bundles of *Thioploca ingrica* (washed from aliquots of native Lake Erie mud maintained in jars in the laboratory) was delayed for 2 to 6 weeks in order to avoid the period of maximum H_2S levels. The flasks were incubated at room temperature up to 52 weeks and examined at 4 week intervals for the presence of healthy filament bundles with an inverted microscope through the bottom of the flasks. When preliminary examination indicated that 4 and 5 week delays seemed best, the 4 week delay was used routinely.

Table 1 shows that the lower the concentration of extracted hay, the higher the percentage of positive cultures when inoculation with *T. ingrica* was delayed for 4 weeks. Hay concentrations of 0.2 and 0.3 g were about equally successful with 93 and 92% respectively. Simultaneous inoculation with mud and *T. ingrica* was 67% successful in the lowest concentration of hay, but failed in all higher concentrations. Since lower concentrations of hay produced lower H_2S levels, the data were in agreement with previous observations of the lower H_2S tolerance of *Thioploca* (Kolkwitz 1912). *Beggiatoa* was simultaneously inoculated with its associated flora (Faust *et al* 1961) and thrived in the maximum H_2S levels

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produced from 0.5 g hay, but not if inoculation with *Beggiatoa* was delayed 4 weeks.

The lower tolerance of *T. ingrica* also is indicated in table 1, which relates the latent time (mean incubation time which had elapsed when the first signs of growth were detected) to the concentration of hay. Again, the lower the concentration of hay, the lower the concentration of H_2S, and the sooner the organism could establish itself. However, when there was no delay in the inoculation of *T. ingrica*, the highest latent time of 10 weeks was obtained, suggesting that only a few survivors of the temporary period of maximum H_2S levels recovered, though slowly, to establish eventual positive cultures. As indicated above, survivors established themselves in only 67% of all trials by this method. The large deviations from mean values can be expected since cultures were examined at 4 week intervals only and since non-uniform in-

FIGURE 1. The appearance of a hay enrichment culture of *Thioploca ingrica*, photographed *in situ* through the bottom of the flask using a Zeiss inverted microscope equipped with a 6.3 X objective and a 10 X ocular, using oblique reflected illumination. The bar is 50 μm.

TABLE 1

Relationship of Positive Cultivation of Thioploca ingrica, Latent Time and Longevity to Concentration of Extracted Hay in Medium.

		Concentration of Extracted Hay (g/60 ml)							
		0.2		0.3		0.4		0.5	
Inoculation delay (weeks)		0	4	0	4	0	4	0	4
% positive cultures		67	93	0	92	0	50	0	28
number of trials		3	14	6	24	6	18	6	18
latent time,*	mean	10	4	–	5	–	8	–	9
	range	8–12	4	–	4–12	–	4–12	–	8–12
longevity,**	mean	14	12	–	18	–	19	–	33
	range	12–16	4–16	–	8–36	–	4–40	–	20–52

*Time between inoculation and detection of first sign of growing in weeks.
**Time between inoculation and detection of last sign of growth in weeks.

ocula had to be used. The latter cannot be changed yet since long, tangled, sheathed bundles of organisms fished out of native mud without control over age and physiological state prevent attainment of a uniform, standardized suspension.

Conditions conducive to a high percentage of positive cultures and fast establishment permitted shorter periods of viability (table 1). The longevity of a culture (mean incubation time which had elapsed when the last signs of growth were detected) increased with increasing concentrations of hay. The few successful cultures obtained with 0.5 g hay (fig. 1) showed the greatest longevity: a mean of 33 weeks (table 1). One would expect this result, for the higher concentrations of hay did produce small amounts of H_2S for longer periods.

The growth of *T. ingrica* occurred predominantly in the very bottom of the flasks below the hay and mud, a circumstance possibly resulting from a lower O_2 tolerance, as compared to *Beggiatoa* (Kolkwitz 1912) which grows above the hay and mud (Faust *et al* 1961). Growth below the hay and mud made it possible to check for growth *in situ* with an inverted microscope (fig. 1). Several ramifying sheathed bundles of different width were apparent, and the braiding of these bundles seemed to be rather common. The filaments within the sheath also were frequently braided, a fact responsible for the naming of the organism, *Thioploca*, the sulfur braid (Lauterborn 1907).

The hay enrichment method has been used to quantitate filaments of *Beggiatoa* in river and lake sediments by a MPN procedure (W. R. Strohl *et al* 1977). The hay enrichment was possible for the laboratory cultivation of *T. ingrica* when the inoculum consisted of bundles containing many active filaments. If growth of *T. ingrica* also could be achieved from single or a few free filaments, the method could be used for the detection of low concentrations of *T. ingrica* in mud samples.

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