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# Thioploca: Methylotroph and significance in the food chain*

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**Abstract**

*Thioploca* constitutes a great portion of the benthic biomass off the Chile-Peru coast. This organism is eaten by the higher organisms and constitutes a major input of organic carbon in the food chain in this region. *Thioploca* has been an enigma ever since its discovery in 1907 and the prefix ''thio'' in the genus name has led investigators to believe that hydrogen sulfide is the energy source necessary to synthesize *Thioploca* biomass. The results of this investigation indicate that methane is the energy and carbon source for the organism. The organism does not use radioactive labeled acetate, glucose, mixture of amino acids, thymine or bicarbonate as demontrated by autoradiography. Since the energy and carbon source is methane, it indicates that *Thioploca* is a methylotroph. Methane in this area is generated by microbial activity in reduced sediments and from seepage from coal seams that run under the seafloor. Methane, through *Thioploca*, represents a major new mechanism, other than photosynthesis, to add cellular carbon to the ecosystem off the Chile-Peru area. Because methane is the energy and carbon source, *Thioploca's* taxonomic position as well as its evolutionary position should be re-assessed.

## Introduction

*Thioploca*, since its discovery in 1907 by LAUTERBORN (1907) in the muds of Lake Constance, has been an enigma to microbiologists. Because it has not been isolated in pure culture, the information concerning this organism has been limited to gross morphology and description of its habitat. The current best description of this organism has been provided by MEIER and MURRAY (1965).

Large communities of *Thioploca* have been discovered off the Chilean coast (GALLARDO 1977). This marine *Thioploca* has also been found off the Peruvian coast. *Thioploca*, therefore, constitutes a large proportion of the biomass in these waters. Because it is known that bacteria in the marine environment can be used as a major source of food, the contribution of *Thioploca* in the environment off the Chilean-Peruvian coast is of major importance.

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The prefix, ''thio'', in the name of the organism, has probably mislead many investigators trying to study this organism in the laboratory. Because reduced sulfur compounds are not the energy and carbon source for methyltrophs, no isolation of the organism in pure culture was possible.

## Materials and methods

Sediment samples: Samples were collected with a Smith-MacIntyre bottom grab from sediments located at Concepcion Bay and adjacent areas. Uptake measurements: Samples of mud and *Thioploca* filaments from the grab samples were placed in 10 ml plastic syringes that had the needle end cut off so that the opening was the diameter of the barrel. These filaments were then incubated with the following radioactive substrates: 10 μCi [1,2–$^{14}$C] acetic acid (45–60 mCi/mM); 10 μCi D-[U-$^{14}$C]-glucose (304 mCi/mM); 10 μCi [methyl, 1,2–$^{3}$H] thymidine (90–110 mCi/μM); 10 μCi sodium bicarbonate [10 μg/μCi] purchased from New England Nuclear and 10 μCi [$^{3}$H]-amino acid mixture from Amersham Searle.

Syringes containing the mud and *Thioploca* filaments were injected with the different radioactive substances mentioned above so that there was a homogenous mixture of mud and substrates and then stoppered with a rubber stopper. The mud samples were then incubated at nearly in situ temperatures for 6 hours, 24 hours and 10 day periods.

The uptake was observed by means of microautoradiography stripping plates (Kodak AR-10).

After incubation, the filaments were washed three times with filtered sea water, fixed with 0.2% formaldehyde and placed onto a Nucleopore filter. The filaments were dissected under a stereomicroscope in order to open the sheath and obtain a better contact surface of the trichomes and sheath with the stripping film.

The plates were kept in contact with the *Thioploca* for 25 days at 6 °C before developing the Kodak D-19 developer.

Electron-transport system activity: The intracellular reduction of INT-tetrazolium salt (J.T. BAKER, Co.) was used to observe cell activity (ZIMMERMANN, ITURRIAGA and BECKER-BIRCK (1978).

*Thioploca* filaments were incubated in 10 ml filtered sea water at room temperature. The following substrates were added: 1 ml sodium acetate (1 g $l^{-1}$), 1 ml sodium citrate (1 g $l^{-1}$), and 1 ml of sodium thiosulfate (1 g $l^{-1}$). Control samples were incubated with INT solution only. In another set of samples, *Thioploca* filaments were placed in small serum bottles and incubated in 10 ml filtered seawater containing the INT solution. These samples were flushed with methane and tightly stoppered.

Intracellular INT reduction was observed microscopically after incubation periods from 30 min to 24 hours.

## Results and discussion

*Thioploca* was found to be present in two types of sediments, both with abundant filaments. Brownish sediments taken from a depth of 75 m appeared in the oxidized state and no hydrogen sulfide could be detected organoleptically. Black sediments from a depth of 40 m definitely had hydrogen sulfide present. By visual observations, more *Thioploca* filaments appeared to be present in the brownish oxidized sediments but the filaments collected from the black sediments were larger.

Figure 1 illustrates the abundance of *Thioploca* in a grab sample. Figure 2 illustrates the amount of *Thioploca* that has been screened from a mud sample. In all experiments where *Thioploca* was incubated with radioactive substrates, no labelling of the trichomes was evident by microautoradiographic techniques. However, the epihytic bacteria on the sheath of the *Thioploca* demonstrated uptake of the radioactive substrates. All controls were negative.

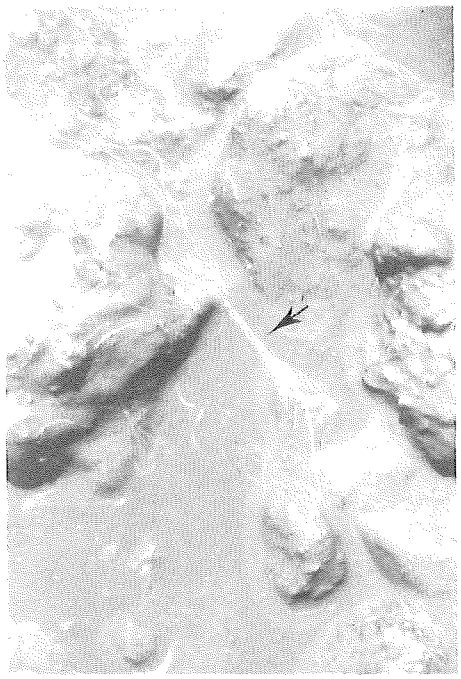


**Figure 1**

The occurrence of *Thioploca* on the surface of a sediment sample taken with a Smith-MacIntyre bottom grab sampler. The arrow points to one of the *Thioploca* sheath bundles.

**Figure 2**

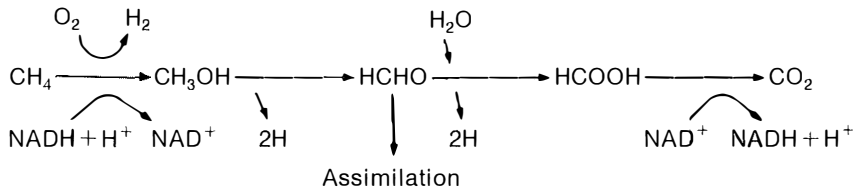
*Thioploca* screened from a mud sample. The mesh size of the screen is 1 mm.

No positive INT reaction was observed with acetate, citrate, or thiosulfate as the substrate with *Thioploca*. *Thioploca* from oxidized or reduced sediments incubated with methane as the energy and carbon source showed active intracellular INT reduction (as evidenced by the red coloration) for any of the incubation periods. Control samples with INT only (no substrates) showed no reduction of the INT. Bacteria associated with the sheath of the *Thioploca* showed a positive INT reaction.

*Thioploca* has never been isolated in pure culture and as a result, there is a paucity of data available to investigators. Previously, *Thioploca* was observed in only freshwater environments until GALLARDO (1977) reported large communities of *Thioploca* off the Chilean coast.

Because none of the radioactive substrates employed were used by *Thioploca*, the data suggested that organic matter probably was not the main carbon source for the growth of this organism. The presence of *Thioploca* in both oxidized surface sediments (no smell of hydrogen sulfide) as well as in the reduced sediments (black with hydrogen sulfide present) resulted in a search for an alternative carbon source. In this area, coal deposits are known to be under the local marine environment and methane is known to be released from coal deposits. Seepage of methane from coal deposits could explain the presence of methane in the area. On the other hand, methane is known to be produced microbiologically in reduced marine sediments and methane can then diffuse upwards to the sediment surface (CLAYPOOL and KAPLAN 1974; MARTENS and BERNER 1974, OREMLAND 1975, REEBURGH 1969). For the above reasons methane was employed as a substrate for *Thioploca*.

Positive INT reduction was observed when methane was employed as a substrate indicating that active electron transport does take place. As a result, energy is derived from methane oxidation by *Thioploca*. The oxidation pathway of methane to carbon dioxide proceeds via a series of two-electron oxidation steps where methane is oxidized according to the following scheme (COLBY, et al. 1979):




According to the above reactions, formaldehyde becomes the substrate that enters into further metabolic activity and therefore indicates that methane is also the carbon source. Our data have indicated that various other radioactive substrates are not incorporated into the cell. However, at this time it is impossible to state that $CH_4$ is the only carbon source for *Thioploca* since this may also be a mixotroph. *Thioploca* appears to be a methylotroph as defined by COLBY and ZATMAN (1978) and QUAYLE and FERENCI (1978). Its evolutionary relationship among methylotrophs becomes a very interesting question. Also there arises the question of whether or not it possesses nitrogenase like some of the other methylotrophs.

The utilization of methane as the main carbon and energy source instead of reduced sulfur compounds represents a drastic change from the older concepts about the energy source for *Thioploca*. Although the organism does have sulfur granules in its trichomes, the oxidation of hydrogen sulfide to sulfur probably does not yield energy to the cell. Since *Thioploca* lives mainly on the surface of the sediment in contact with slightly oxygenated water, the hydrogen sulfide may actually undergo abiological oxidation within the cells of *Thioploca* to form sulfur granules, since it is well known that hydrogen sulfide exposed to air will produce sulfur granules. This could be analogous to the sulfur granules found in *Beggiatoa* when exposed to hydrogen sulfide, the granules forming in a relatively few minutes (BURTON and MORITA 1964).

The presence of *Thioploca* in areas where methane is present also occurs in Walvis Bay. Walvis Bay has methane 2.6 to 440 times its solubility in sea water (SCRATON and FARRINGTON 1977) and one of us (VAG) has observed *Thioploca* in this region.

Bacteria are known to be a food source for many of the higher marine organisms. Bacteria are eaten directly by copepods, ciliates, flagellates, sponges, ect. and when associated with detritus, fecal material (mainly coprophagy), and particulate organic matter and may serve as a source of ectocrine compounds, and hormonal-like compounds (for overview see FENCHEL and BLACKBURN 1979). In the Peru-Chile Subsurface Countercurrent area, GALLARDO (1977) reported a large biomass of *Thioploca*. In repetitive quantitative grab sampling off Concepcion, Chile (36°35'20''S, 73°04'20''W) at 60 m depth, GALLARDO (1977) reported 106 g (wet weight) per 0.1 $m^2$ for the microbial component (mainly *Thioploca*, but also cyanobacteria and flexibacteria) while the benthic infaunal biomass in the same sample attained only 11.5 g (wet weight) by use of an 0.25 $mm^2$ sieve. This does not include the eubacterial organisms that could be in high numbers. One of us (VAG) has observed filaments of *Thioploca* in the mouthparts of shrimp (*Pleurocodes monodon*), a gastropod, (*Nassarius gayi*), and a polychaete (*Nephtys ferruginea*). An amphipod (*Ampelisca araucana*) will catch and ingest both the trichomes and sheaths of *Thioploca* when offered to them. *Thioploca* filaments have been found in the proximal part of the gut of the above mentioned polychaete and a snail (*Nassarius gayi*). Gut content analyses of *P. monodon* show the presence of *Thioploca* trichomes but most of the gut content is detritus. The standing crop measurements of the filamentous bacteria of this region strongly suggest that there is a trophic relationship between the shrimp (both penaeid and galatheid), hake and the *Thioploca* (GALLARDO 1977). However, the productivity, in relation to time, of *Thioploca* would give us a better idea as to the contribution of *Thioploca* to the trophic levels of this region. Nevertheless, the accumulated data presently on hand strongly indicate that *Thioploca* may be one of the main sources of organic C at the first trophic level (disregarding the classical definition of the first trophic level, but to include reduced carbon in cells of all forms).

The utilization of methane in areas where it is not produced in anaerobic environments but from seepage from coal deposits under the sea is a new means by which new reduced carbon can enter the trophic levels of the sea. The extent of this process has yet to be assessed.

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**Addendum**

Longeri and Gallardo were able to demonstrate approximately 60 and 75 nM of methane per liter of seawater which was taken from the sediment-seawater interface near Dichato, Chile on 10 June 1980 and 9 July 1980 respectively. The location from which the seawater was taken is approximately where the *Thioploca* was obtained for this investigation.

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