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# Unusual ultrastructure of bacterial endosymbionts in the bivalve *Thyasira sarsi* from Central Skagerrak

**R. Schmaljohann**
Institut für Meereskunde an der Universität Kiel
Düsternbrooker Weg 20, D-2300 Kiel, Germany

## Abstract

An ultrastructural investigation was made of the symbiotic bacteria in the gills of the bivalve *Thyasira sarsi* from the Central Skagerrak. The bacteria were characterized by sulphur globules in the periplasmic space and by peripheral intracellular membrane stacks. Apart from the membrane system they were very similar to previously described sulphur-oxidizing symbionts of the same species from Bergen, Norway. Stable carbon isotope data and lack of $C_1$-metabolism enzymes gave additional evidence for sulphur-oxidizing symbionts in *T. sarsi* in the Skagerrak, too.

## Introduction

The bivalve *Thyasira sarsi* occurs in various reduced marine sediments with high organic input, as Norvegian and Swedish fjords and seepage sites in the North Sea and Skagerrak. This species is known to harbour endosymbiotic bacteria in its gills. Enzymatic investigations on individuals collected near Bergen, Norway, gave evidence that these symbionts were chemoautotrophic sulphur-oxidizing bacteria (DANDO and SOUTHWARD 1986). This was confirmed by ultrastructural investigations (SOUTHWARD 1986) showing rod-like bacteria with typical membrane-bound sulphur globules. Other authors (WOOD and KELLY 1989), having isolated methylotrophic bacteria from the gills of *T. sarsi* from Oslofjord and Gullmarfjord (Sweden) suggested that environmental factors might determine whether a symbiosis based on methylotrophic or autotrophic bacteria is established.

In this study *T. sarsi* individuals collected in the Central Skagerrak, at the habitat of *Siboglinum poseidoni*, a pogonophore with methanotrophic endosymbionts (SCHMALJOHANN and FLÜGEL 1987), were compared to individuals from other locations. Ultrastructure, stable carbon isotope ratios and enzymatic activities were used to find out, which kind of symbiosis is established at this site.

## Material and methods

Specimens of *T. sarsi* were collected with a Van Veen grab in the Central Skag-

errak (58°02.85'N; 9°40.04'E) at 320 m water depth. Gill samples were fixed for electron microscopy within few hours after collection with 3 % glutaraldehyde in 0.06 M phosphate buffer with 7.5 % sucrose. After 2 hours fixation the gills were washed in buffer and postfixed with 1 % osmium tetroxide (2 hrs), dehydrated in ethanol series and embedded in Spurr's resin. Ultrathin sections stained with uranyl acetate and lead citrate were studied in a Zeiss EM 9 transmission electron microscope.

## Results and discussion

The bacterial gill symbionts of *T. sarsi* are located in specialized bacteriocytes, outside of the cytoplasmic membrane but enclosed by a "cuticle" of microvilli, as described by SOUTHWARD (1986). The bacteria are Gram-negative rods measuring 0.5-0.7 µm in diameter and up to 3.0 µm in length (Fig. 1a). There is only one morphological type of symbionts. Most cells show electron-transparent areas either in the periplasmic space or, bound with a double membrane, inside the cytoplasm (Fig. 1b). These structures probably correspond to sulphur globules more or less extracted during processing for electron microscopy. According to VETTER (1985) these globules occur only in the periplasmic space, are covered by an own protein membrane and, inside the cell, appear as double membrane-bound by the invaginated cytoplasmic membrane.

A varying portion of the bacteria is fitted with stacks of 4-6 paired membranes at the cell periphery which probably represent transverse sections through flattened vesicles (Fig. 1a). Each membrane is 9-10 nm wide, which is about the thickness of a unit membrane. In some cases vesicles of the membrane stack seem to be in contact with the cytoplasmic membrane (Fig. 1c). Carboxysomes were not found within the symbionts.

The ultrastructure of the *T. sarsi* symbionts is in its general features similar to that described by SOUTHWARD (1986) for Norvegian specimens. Only differences are the somewhat smaller size of the Norvegian symbionts (0.4-0.5 x 2.0 µm) and the presence of internal membrane structures in the Skagerrak symbionts. Size differences could be due to use of buffers with different osmolarity during fixation (14 % sucrose in contrast to 7.5 % for Skagerrak symbionts). Internal membrane systems, though not characteristic for sulphur-oxidizing bacteria, were found in similar extent also in cultures of *Thiobacillus thioparus* (HOLT et al. 1974) and in sulphur-oxidizing symbionts of the hydrothermal vent tubeworm *Riftia pachyptila* (CAVANAUGH et al. 1981).

The analysis of stable carbon isotope ratios is a valuable tool for elucidating the carbon source of an organism. Such data were published for *T. sarsi* from the Skagerrak by SCHMALJOHANN et al. (1990) and can be compared to data from other locations for this species (Tab. 1). Skagerrak specimens have the most $^{13}C$-depleted gill tissues of all, reflecting a nutrition strongly based on chemoautotrophic bacteria. Possible explanations for this are discussed in SCHMALJOHANN et al. (1990). Obviously this bivalve is very versatile in its nutrition. It has a reduced but functioning gut, and the proportion of symbiont dependant autotrophy varies according to environmental conditions (DANDO and SPIRO 1990). The $\delta^{13}C$ values of the Skagerrak specimens are within the range of animals living in symbiosis with sulphur-oxidizing bacteria. A symbiosis based on methane-oxidation, which could be speculated upon because of the presence of internal membranes within the symbionts and the neighbourhood to *Siboglinum poseidoni* with its methanotrophic symbionts, should be more $^{13}C$-depleted ($\delta^{13}C$

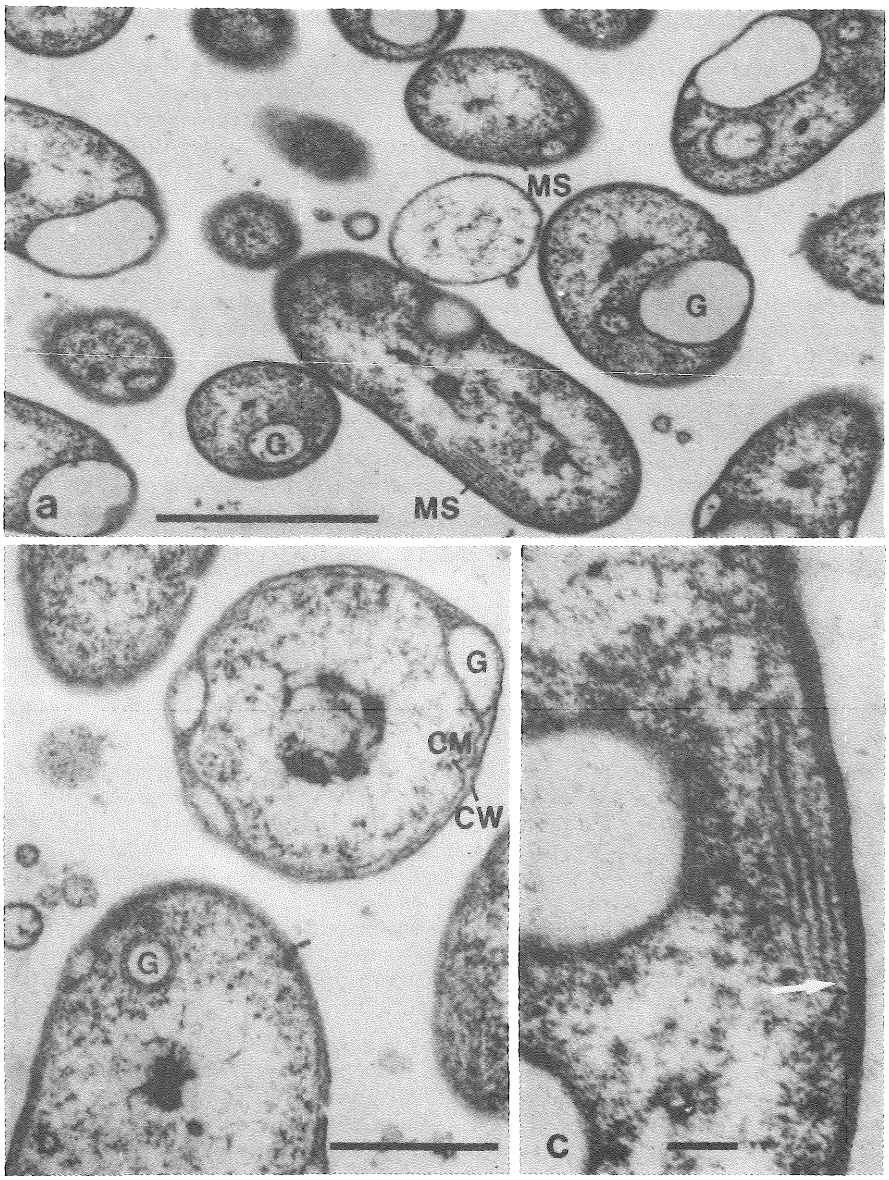


Fig. 1. Symbiotic bacteria in the gills of *Thyasira sarsi* from the Central Skagerrak. Transmission electron micrographs: a) Sulphur globules (G) in various sizes and stages of conservation are visible in most bacteria. Part of the cells show membrane stacks (MS) usually running along the periphery of the cell. Bar = 1.0 µm. b) Sulphur globules (G) are located in the periplasmic space, between cytoplasmic membrane (CM) and cell wall (CW), and appear as double membrane-bound vesicles within the cytoplasm. Bar = 0.5 µm. c) Intracellular membrane system in contact with the cytoplasmic membrane (arrow). Bar = 0.1 µm.

Table 1. Stable carbon isotope ratios of *Thyasira sarsi* from four sampling sites.

| Tissue | $\delta^{13}C$ (‰) | Origin | Depth | Reference |
|---|---|---|---|---|
| gills | -39.5 | Central | 300 m | SCHMALJOHANN |
| rest of body | -37.4 | Skagerrak | | et al. (1990) |
| gills | -34.3 | North Sea | 160 m | DANDO et al. |
| total animal | -31.4/-33.8 | pockmark | | (1991) |
| gills | -31.0 | fjord near | 90 m | SPIRO et al. |
| rest of body | -28.2 | Bergen | | (1986) |
| gills, 1986 | -22.2/-31.7. | Gullmarfjord | 110 m | DANDO and SPIRO |
| gills, 1988 | -17.1/-17.8 | (Sweden) | | (1991) |

<-50 ‰). A symbiosis of this type should also show activities of enzymes of $C_1$-metabolism as methanol dehydrogenase. The gills of *T. sarsi* from the Central Skagerrak did not contain this enzyme, in contrast to *S. poseidoni* from the same site.

The presence of typical membrane-bound sulphur globules in the periplasmic space, stable carbon isotope ratios and lack of $C_1$-metabolism enzymes indicate that the symbionts of *T. sarsi* use reduced sulphur compounds as an energy source, rather than $C_1$-compounds.

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