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Association of filamentous epibacteria with *Tubificoides benedii* (Oligochaeta: Annelida)

N. Dubilier

Zoologisches Institut und Zoologisches Museum, Universität Hamburg; Martin-Luther-King-Platz 3, D-2000 Hamburg 13, Federal Republic of Germany

Abstract

A previously undescribed colonization of the marine oligochaete *Tubificoides benedii* (d'Udekum, 1855) from a sulfide-rich habitat by filamentous epibacteria was observed in 1984 and 1985. Electron microscopy showed that the bacteria were embedded in the cuticle of the posterior region of the worm. The association is apparently not pathogenic; possible forms of positive interaction are discussed.

Introduction

There are only a few descriptions of filamentous epibacterial colonizations of marine invertebrates. Prominent examples come from hydrothermal vent organisms: "terminal filaments" are attached to the posterior region of the polychaete *Alvinella pompejana* (Desbruyères and Laubier, 1980; Desbruyères *et al.,* 1983) and filamentous bacteria occur on the epithelial surface of an undescribed archeogastropod limpet (de Burgh and Singla, 1984). Some "nonvent" marine animals with filamentous epibacteria have been reported, but the association is either pathogenic as in benthic crustacea infested with *Leucothrix mucor* (Johnson *et al.,* 1971) or the bacteria do not penetrate the cuticle as in nematodes covered with blue-green bacteria (Wieser, 1959; Hopper and Cefalu, 1973; Ott *et al.,* 1982). This paper presents a description of filamentous epibacteria on a "nonvent" animal, in which the bacteria are embedded in the cuticle and the association is apparently not pathogenic.

Material and methods

Tubificoides benedii (d'Udekem, 1855) (formerly known as *Peloscolex benedeni*) was collected in October 1984 from an intertidal sand flat at the Island of Sylt (North Sea). The collection site was densely covered by algal mats which occur regularly in this area during summer and fall (Reise, 1983). The underlying sediment was anoxic and smelled strongly of hydrogen sulfide.

Individuals were collected with a 500-μm sieve and kept overnight in sediment from the collection site at 5 °C. Adult worms were fixed the next morning for 2 h in 5% glutaraldehyde buffered in 0.1 M HCl-cacodylate with 7% sucrose, $CaCl_2$ and $MgCl_2$ added (according to Pearse, 1972).

For transmission electron microscopy (TEM), the specimens were then postfixed for 1 h in 2% OsO_4 in cacodylic buffer, dehydrated in a graded aceton series and embedded in SPURR (Spurr, 1969). Ultrathin sections were stained with 40% uranyl acetate in methanol (1 min) followed by 0.25% lead citrate (5 s) and examined with a Zeiss EM 9S-2 electron microscope.

For scanning electron microscopy (SEM), the specimens were fixed as above, then dehydrated in a graded acetone series and transferred through a graded acetone-amyl acetate series into 100% amyl acetate. After critical point drying and gold coating, the worms were examined in a Cambridge S-4-SEM.

Results

The posterior segments of all *Tubificoides benedii* collected in the fall of 1984 were covered with fine white "hairs", ranging in length from ca 10 to 800 μm (Fig. 1 A). Analysis with TEM showed these "hairs" to be procaryotic filamentous cells embedded in the cuticle, with the basal bacterial cell of each filament very close to the apical end of the epidermal cells (Fig. 1 B). Bacteria were never found penetrating into the epidermis, nor was endocytosis of the bacteria, such as described in the hydrothermal vent limpet (de Burgh and Singla, 1984), ever observed.

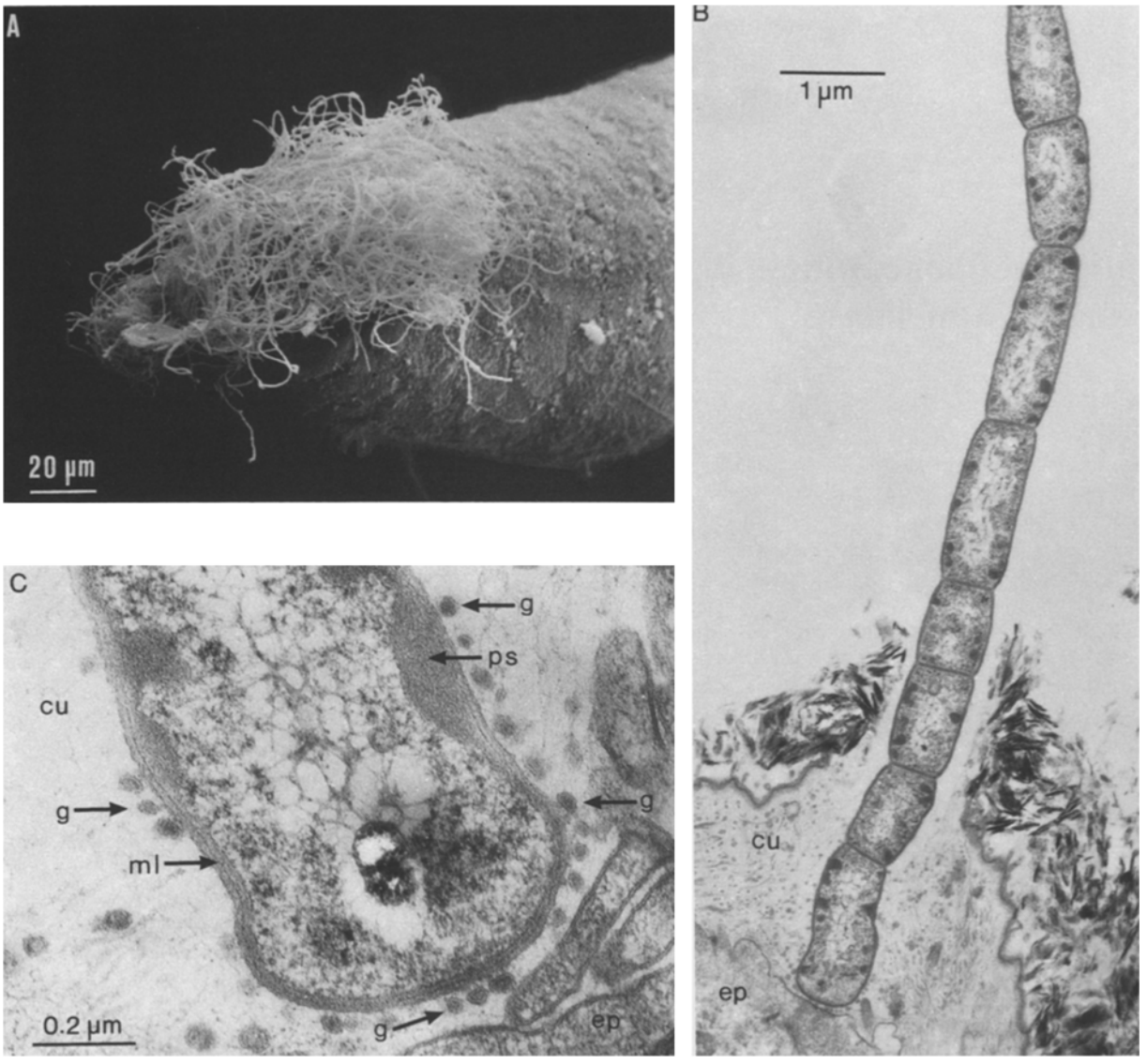

Fig. 1. *Tubificoides benedii.* (A) SEM of tail with filamentous epibacteria. (B) TEM of bacterial filament embedded in cuticle. Notice dark globular structures in the peripheral cytoplasm of each bacterial cell. (C) TEM of basal cell of bacterial filament showing globules that surround the bacterial cells in the cuticle. Abbreviations: cu = cuticle, ep = epidermis, g = globules, ml = middle layer of cell wall, ps = periplasmic space

All the endocuticular bacterial filaments were morphologically similar and consisted of cells with a diameter between 0.4 and 0.8 μm. Each individual cell contained an electron-light area in the center (the nuclear region) and a granular cytoplasm along the cell wall. In the periphery of the cytoplasm globular electron-dense inclusions of 60 to 100 nm diameter were regularly found (Fig. 1 B). The cell envelope was multilayered, resembling that of gram-negative bacteria; the middle layer could be clearly seen as a thin, dark line (Fig. 1 C). In the basal cells of the filaments the region between the cell membrane and the outer wall layers, known as the periplasmic space, often intruded into the cytoplasm (Fig. 1 C).

The bacterial cells in the cuticle were regularly surrounded by small spherical globules of a diameter between 24 and 40 nm (Fig. 1 C). These structures were bound with what, in some cross sections, resembled a unit membrane. On all cross sections the globules appeared spherical, which excludes their interpretation as microvilli of the epidermis.

Other bacteria could also be found in the slime layer covering the cuticle, but were never observed in the cuticular layer itself. These epicuticular bacteria occurred less frequently and were morphologically distinct from the endocuticular bacteria.

All worm specimens of the October sample examined were colonized by the endocuticular bacteria; the manner in which the tails were colonized differed from specimen to specimen. On some worms only the last segments were densely covered; on others, the filaments were spread over the last third of the worm. However, endocuticular bacteria were never found anteriorly, on co-occurring species

or living freely in the sediment. While *Tubificoides benedii* from the fine, detritus and sulfide-rich sediment between mussel beds in the same area of the collection site were also colonized by the endocuticular bacteria, 20 specimens collected from the Island of Norderney (North Sea) in April 1985 did not carry any bacteria.

Seasonal variations in the colonization were encountered during the investigation period. In the fall of 1984 the filaments were extremely dense and could be easily seen in the dissecting microscope at 10- to 20-fold magnification. In the following winter and early spring only a few single filaments could be detected at the same magnification. In the early summer of 1985 the colonization increased to the density observed in the fall.

Discussion

The electron microscopic studies clearly show that the "hairs" covering the posterior region of *Tubificoides benedii* are bacterial filaments embedded in the cuticle of the worm. The morphological similarity of the filaments indicates that all endocuticular bacteria probably belong to the same genus. The structure and function of the globules that surround the bacteria in the cuticle remain puzzling. The globules only occur in the direct vicinity of the bacteria; if they are really covered by a unit membrane, they could originate from the epidermal layer and should then be considered as some sort of "reaction" of the worm to the bacteria.

The colonization of the posterior segments of *Tubificoides benedii* by endocuticular bacterial filaments is apparently a regular phenomenon. A "white fuzzy growth" on *T. benedii* has occurred frequently since 1974 (Dr. K. Reise, personal communication) in the sampling area. The colonization shows no evidence of being pathogenic. Bacteria were never observed in the vicinity of lesions in the cuticle, such as described in *Tubifex tubifex* by Fischer and Horváth (1977). The physical condition and behaviour of the worms did not differ from bacteria-free animals. Preliminary results of physiological experiments with *T. benedii*, still in progress, have shown no difference in enzyme activities between worms with endocuticular bacteria and those without them.

The observed variation in the colonization during the year suggests that seasonal factors have an influence on the growth of the bacteria. Strong hydrogen sulfide odours at the collection site during the summer and fall were concomitant with a high density of the colonization and could be indicative of sulfur bacteria. Recent reports on a white fuzzy growth on two marine tubificids from areas with high hydrogen sulfide concentrations in Scandinavia (Dr. C. Erséus, personal communication) and Puget Sound (Dr. P. Chapman, personal communication) support this speculation. Morphological descriptions of bacteria are insufficient for their identification, but the resemblance of the epibacteria with filamentous sulfur-oxidizing bacteria (Brock, 1981; Jannasch, 1983, 1985) should be mentioned.

The association between *Tubificoides benedii* and the endocuticular bacteria is apparently more than a coincidental colonization. The fact that the bacteria can only be found on the posterior end of the worm indicates some form of worm/bacteria interaction. Morphological barriers, such as the cuticular papillae (Giere and Rhode, in press), are improbable because these often extend into the area of the filaments. It seems rather that the tail of *T. benedii* offers advantages to the bacteria that could be explained by its position in the habitat.

Observations on the vertical distribution of the oligochaete at the collection site showed that the worm is orientated with its head in the sediment and its tail sticking out. Ninety percent of the population was always found within the first few cm below the sediment surface. This corresponds with other data on the distribution of *Tubificoides benedii* in sulfide-rich sediments (Hunter and Arthur, 1978). Laboratory observations confirmed the field studies. In addition, in the aquarium one could see the worms slowly moving their tails back and forth through the water.

Thus, the tail end of *Tubificoides benedii* could offer a suitable microhabitat for the bacteria:

The position of the worm's tail above the sediment surface would prevent the otherwise relatively immobile bacteria from being buried in anoxic sediments by wave action.

The described orientation of *Tubificoides benedii* in the sediment has led to the conclusion that the species most probably has an intestinal respiration (Dahl, 1960). The slow movements of the tail and the proposed intestinal respiration could also be of advantage during stagnant water periods at low tide by creating micro-oxic conditions which favour growth of sulfur-oxidizing bacteria.

Alongside the oligochaete's body in the sediment an enhanced exchange of porewater with the overlying tidal water results in an influx and efflux of O_2, SO_4^{2-}, H_2S, CO_2, NH_4^+, and low molecular organics, all of which could benefit many different forms of bactria. Investigations have shown that high concentrations of bacteria consistently occur at the anoxic-oxic interface of marine sediments (Yingst and Rhoads, 1980; Jørgensen, 1982).

Substrates such as CO_2 and NH_4^+ could also be supplied by worm excretion.

In short, the tail of *Tubificoides benedii* is an attractive microsite, because it communicates between the anoxic and oxic sediment layers and the worm excretes metabolites. At this point, one can only speculate on the nature of the association between *T. benedii* and the endocuticular bacteria. So far, an impact on the populations of *T. benedii* by the colonization could not be found, whereas possible benefits for the bacteria can be envisaged. Therefore, commensalism sensu lato (Odum, 1975) seems a suitable definition until further investigations can shed more light on this association.

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