Microbiology, Vol. 69, No. 3, 2000, pp. 319–324. Translated from Mikrobiologiya, Vol. 69, No 3, 2000, pp 389–395.

EXPERIMENTAL ARTICLES

Peculiarities of Morphology and Ultrastructure in Bacteria of the Genus *Dethiosulfovibrio*

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Received September 20, 1999

Abstract—Cell morphology and fine structure were studied in two strains of rod-shaped, strictly anaerobic, gram-negative sulfidogenic bacteria: strain SR12^T (DSM 12538) and strain WS100 (DSM 12537) belonging to "*Dethiosulfovibrio starorussensis*." Cells of both strains, as well as cells of the type species of the genus *Dethiosulfovibrio, D. peptidovorans*, were found to possess multiple intracellular incomplete cross septa in all growth phases.

Key words: *Dethiosulfovibrio*, morphology, fine structure, intracellular incomplete cross septa

The type and sole species of the genus *Dethiosulfovibrio, D. peptidovorans,* is a strictly anaerobic gram-negative, thermophilic, neutrophilic bacterium using peptides and amino acids as carbon and energy sources and reducing elemental sulfur and thiosulfate to H_2S [1]. Morphologically, *D. peptidovorans* is a vibrio (3–5 by 1 μm), motile by means of 1–5 lateral flagella [1].

Several strains of vibrio-shaped strictly anaerobic sulfur- and thiosulfate-reducing bacteria were isolated from the so-called sulfur mats "Thiodendron" and described as a new species of the genus *Dethiosulfovibrio, "D. starorussensis"* [2]. These bacteria appear to be the components of a specific community being symbiotically associated with sulfur-oxidizing, aerotolerant, anaerobic spirochetes [3, 4]. All strains of *"D. starorussensis"* are mesophilic heterotrophs capable of fermenting proteins, peptides, amino acids, and some organic acids. During fermentation, they reduce elemental sulfur or thiosulfate to H_2S.

In this paper, we describe the morphology and fine structure of two strains of "*D. starorussensis*" and compare the peculiarities of their ultrastructure with those of *D. peptidovorans*."

MATERIALS AND METHODS

Two strains of *"Dethiosulfovibrio starorussensis"* used in this work were isolated from "Thiodendron" sulfur mats collected in mineral springs of the resort Staraya Russa, Novgorod oblast (strain SR12^T = DSM 12538) and in the littoral zone of the Kandalaksha Bay of the White Sea (strain WS100 = DSM 12537). Strain DSM 11002 of *D. peptidovorans* was obtained from DSMZ, Braunschweig, Germany.

Strains SR12^T and WS100 were grown on medium of the following composition (g/l of distilled water): NaCl, 20; $MgCl_2 \cdot 6H_2O$, 3; KH_2PO_4, 1; yeast extract (Serva), 5; peptone (Fluka), 2; sodium citrate, 10 mM; and resazurin, 1 mg.

The above basic medium was autoclaved (30 min, 125°C) in 500-ml glass bottles containing 400 ml of medium, cooled aseptically in the argon flow, and supplemented with the following sterile components (per liter): 10% $CaCl_2 \cdot 2H_2O$, 2 ml; trace elements [5], 10 ml; 1 M $Na_2S_2O_3$, 10 ml; and 1 M $Na_2S \cdot 9H_2O$, 1 ml. The pH was adjusted to 6.7–6.8. Inoculum was introduced at 1–2 vol %.

D. peptidovorans was grown in medium of the same composition without sodium citrate.

Strains were cultivated at the optimal temperatures of 28°C for SR12^T and WS100 and 40°C for *D. peptidovorans*.

To examine the ultrastructure, cells were collected from the early exponential, midexponential, and stationary growth phases (after 1, 2, and 7 days of growth, respectively).

In some experiments, a 6-month-old culture was used for inoculation. In this case, cells were collected in the early exponential phase.

Cells were collected by centrifugation (4100 g, 75 min), washed with 2% NaCl solution in 0.1 M phosphate buffer (pH 6.7), and fixed in a 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 6.7) containing 2% NaCl.

Material for ultrathin sections was prepared by the standard methods [6, 7]. Ultrathin sections were obtained with an LKB III microtome (Sweden).

Cells from the midexponential growth phase were used to prepare specimens of whole cells for electron microscopy. Cells were fixed in a 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 6.7) and stained with a 2% $(NH_4)_2MoO_4$ solution (pH 7.0).

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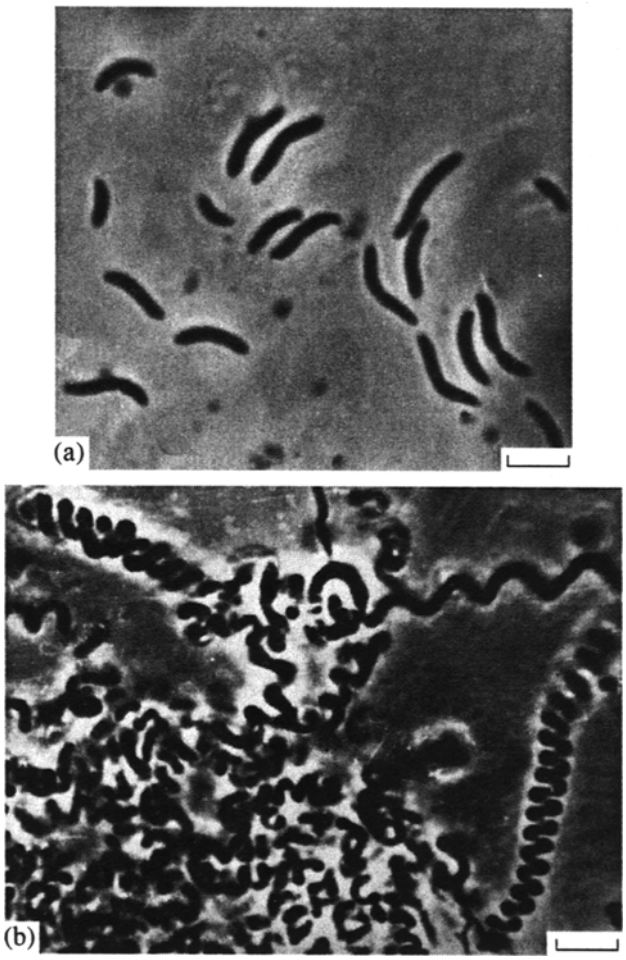

Fig. 1. Morphology of strain SR12^T. Exponential phase. Phase-contrast micrographs of (a) vibrioid cells and (b) helical filaments; bars represent 5 μm.

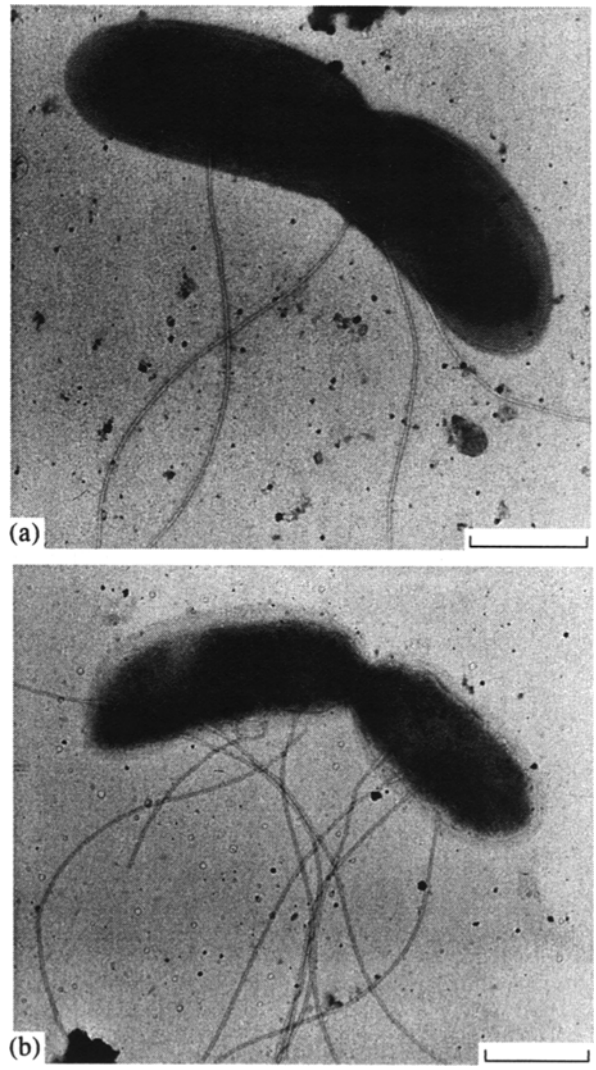

Fig. 2. Morphology of "*D. starorussensis*." (a) Strain SR12^T and (b) strain WS100. Electron micrographs of whole cells stained with ammonium molybdate; bars represent 1 μm.

Light microscopy was performed with a Zeiss-NU2 microscope equipped with a phase contrast device, and electron micrographs were obtained with a JEM-100C electron microscope (Japan) at an accelerating voltage of 60 kV.

To determine the influence of the organic acid concentration on the fine structure of the cells, strains SR12^T and WS100 were grown in medium without peptone and with lower contents of organic compounds: yeast extract, 0.1 g/l, and sodium citrate, 0.5 mM. In this case, cells were collected in the exponential phase.

RESULTS AND DISCUSSION

Morphology. Cells of both strains were vibrio-shaped or helical filaments. The shape and length of the helixes varied depending on the culture age and the medium composition. Early-exponential-phase cells of both strains were helical filaments 8–10 μm long with a wavelength of 5–8 μm. In the midexponential phase, slightly bent vibrioid-shaped cells of strain SR12^T measured 0.9 by 3–6 μm (Fig. 1a), and strain WS100 measured 0.9 by 5–7 μm.

Very long helixes were formed in the early exponential growth phase when an old (0.5–1-year) culture was used for inoculation. In this case, the size of the cells reached 0.8–0.9 by 100–110 μm in strain SR12^T and 0.8–0.9 by 60–70 μm in strain WS100; in both strains, the average length of the helical filaments was 30–40 μm with a wavelength of 5–8 μm. In transition to the midexponential phase we observed the formation of cross septa and the subsequent division into separate vibrioid-shaped cells.

The wavelength and the diameter of a helix differed markedly depending on the medium composition. When both strains were grown on deficient medium containing sodium acetate (1 g/l), yeast extract (1 g/l), and ferrous ammonium citrate (0.1 g/l) as the carbon and energy sources, the cells looked like tightly twisted

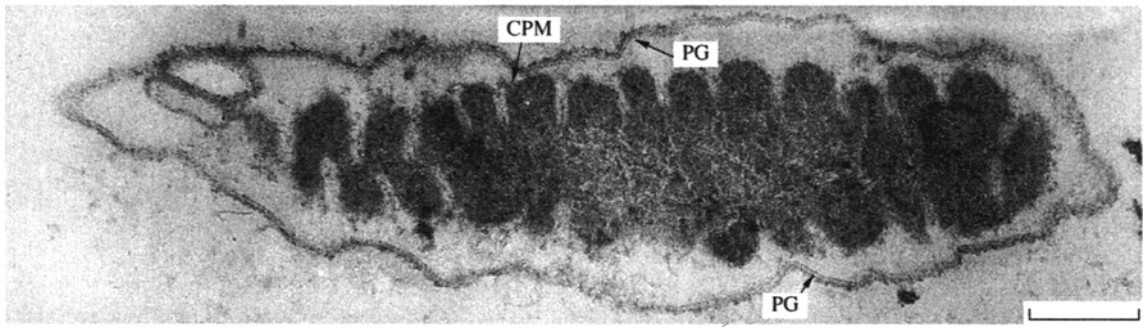

Fig. 3. Electron micrograph of an ultrathin longitudinal section of an vibrioid cell of strain SR12^T. Stationary phase. Hereinafter sections were stained with uranyl acetate and lead acetate. CPM is the cytoplasmic membrane; PG is a peptidoglycan layer; the bar represents 1 μm.

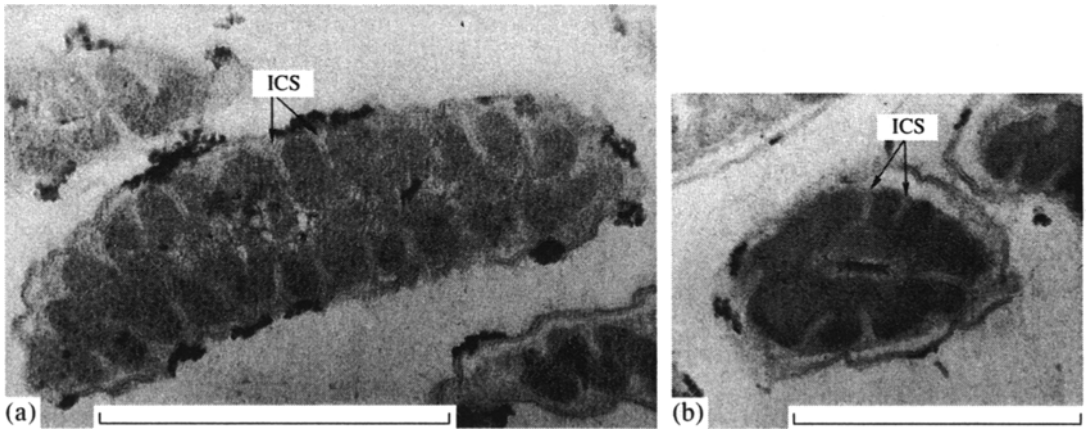

Fig. 4. Ultrastructure of stationary-phase cells of strain SR12^T. (a) Longitudinal and (b) transversal sections. ICS are incomplete cross septa; the bar represents 1 μm.

helices with a wavelength of 3–5 μm and a diameter of 3–3.5 μm (Fig. 1b).

Binary fission occurred through outgrowth of the cell septum.

Cells of both strains were motile by 1–4 flagella 4–6 μm long in strain SR12^T and by 3–6 flagella 5–10 μm long in strain WS100. The flagella were arranged along the concave side of the cells (Figs 2a and 2b), which explains the tumbling movement of the cells. In so moving, the cells formed X- and V-shaped figures, which is also characteristic of *D. peptidovorans*. Thus, by their morphology, the arrangement of flagella, and the type of movement, *D. peptidovorans* and "*D. starorussensis*" resemble representatives of the genera *Selenomonas* [8, 9] and *Pectinatus* [10, 11]. However, no formation of helical filamentous forms has been reported for *Selenomonas, Pectinatus*, or *D. peptidovorans* [1, 8–10, 11].

Ultrastructure. The fine structure examination of strains SR12^T and WS100 showed the presence of an outer membrane and a thin peptidoglycan layer typical of the cell wall of a gram-negative type. A periplasmic space was present. The width of the cytoplasmic membrane (CPM), the outer membrane, and the peptidoglycan layer was 9–11, 16–18, and 8–11 nm, respectively. Binary cell division occurs by ingrowth of the cell septum. A peculiar ultrastructural characteristic of both strains was the presence of numerous incomplete cross septa extending inside to one third of the cell diameter. Such incomplete cross septa were the result of CPM invaginations (Fig. 3) and were seen both on longitudinal and transversal cell sections (Figs. 4a and 4b). The space between the adjoining CPMs in each invagination was 26–30 nm. The number of invaginations varied, as a rule, from 10 to 20 on longitudinal sections and from 5 to 10 on transversal sections (Figs. 4a and 4b). These regularly arranged incomplete cross septa divided the cell into many compartments and conceivably served to increase the CPM surface. The formation of incomplete cross septa is not connected with the division of long filaments into separate cells. The fact that the incomplete cross septa showed not only on longitudinal but also on transversal cell sections indicated that by structure they more likely resembled a corn cob surface than an iris diaphragm formed by the flat ingrowth.

In the later growth phases of the culture, the invaginations looked more distinct, which was evidently correlated with the loosening of the cell wall components

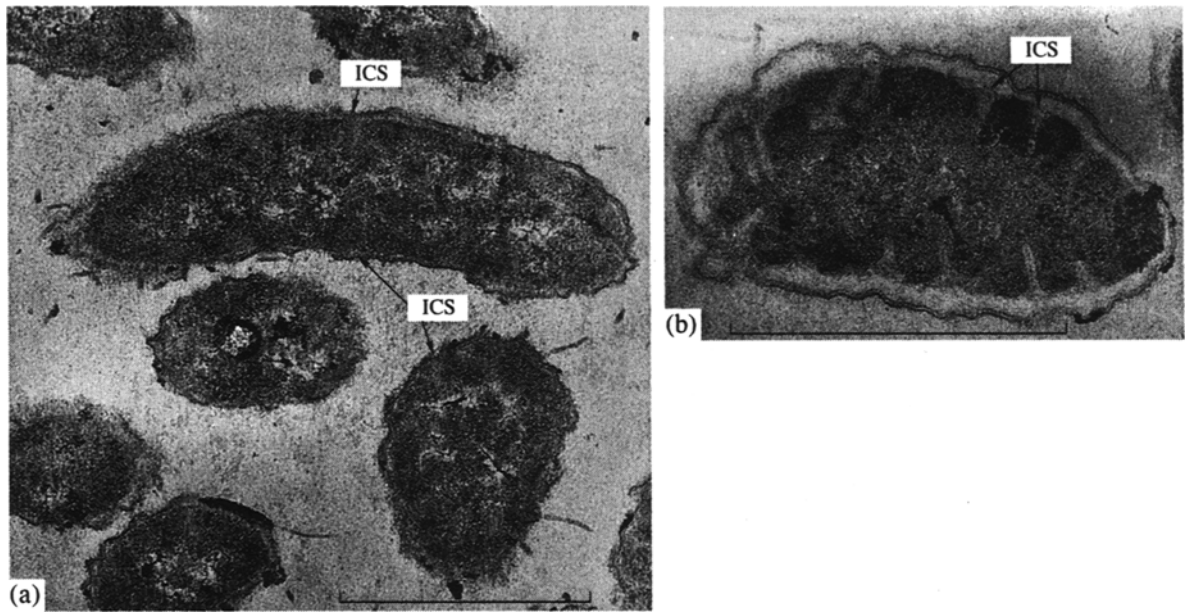

Fig. 5. Ultrastructure of (a) exponential-phase and (b) stationary-phase cells of strain WS100. ICS are incomplete cross septa; the bar represents 1 μm.

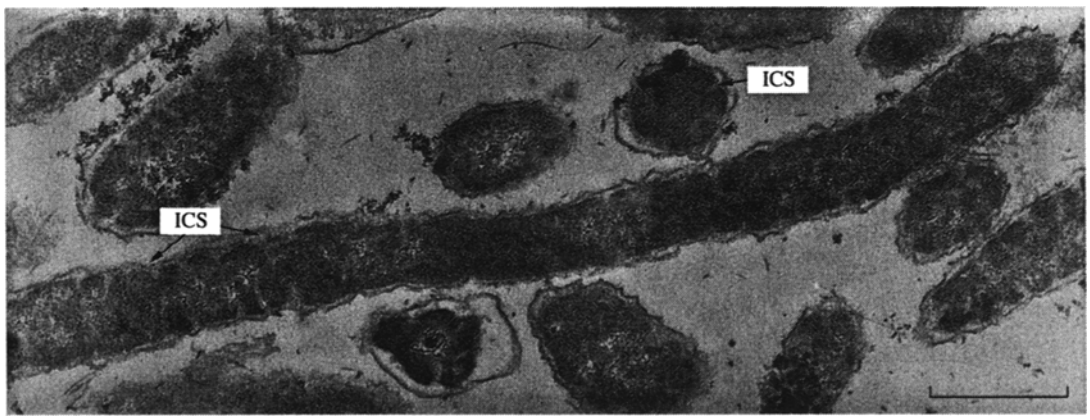

Fig. 6. Ultrastructure of helical filamentous cells of strain SR12^T. Exponential growth phase. ICS are incomplete cross septa; the bar represents 1 μm.

and the partial lysis of cells in the stationary phase (Figs. 5a and 5b). Incomplete cross septa were also observed on longitudinal and transversal sections of helical filamentous cells more than 10 μm long (Fig. 6).

In young early-exponential-phase cells grown in medium with a low content of organic compounds, incomplete cross septa were observed just as they were in the cells grown in the rich organic medium (Fig. 7). Therefore, the formation of incomplete cross septa in the cells of strains SR12^T and WS100 was not associated with the deficiency of the nutrient substrate in the medium and with the requirement for a greater CPM surface that could provide more intense processes of nutrient transport or cell metabolism under the limitation of nutrient substrates.

An examination of the cell fine structure of the type species of the genus *Desulfovibrio*, *D. peptidovorans*, also revealed the occurrence of incomplete cross septa formed by the CPM invaginations. They were invisible on longitudinal sections of exponential-phase cells (Fig. 8a) but were clearly seen in the stationary-phase cells of *D. peptidovorans* (Fig. 8b). They were similar in structure to the incomplete cross septa found in "*D. starorussensis*."

The occurrence of incomplete cross septa is a rather uncommon ultrastructural characteristic for the microbial cell. Similar septa are characteristic for just a few genera of gram-positive filamentous (*Caryophanon* [12] and *Oscillospira* [13]), gram-positive unicellular (*Sulfobacillus* [14]), and gram-negative (*Alysiella* [15],

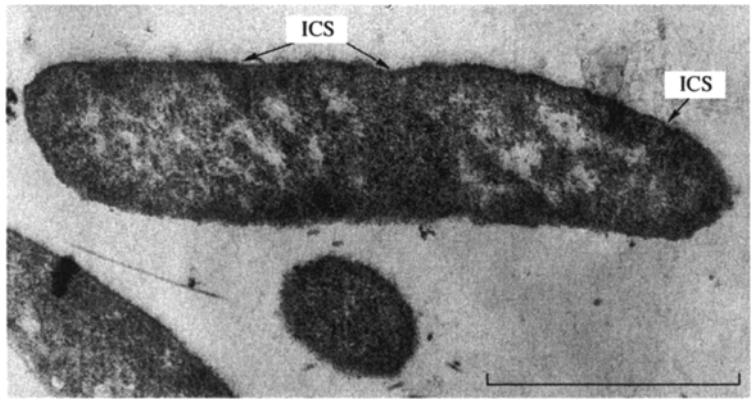

Fig. 7. Ultrastructure of strain WS100 grown on deficient medium. Exponential phase. ICS are incomplete cross septa; the bar represents 1 μm.

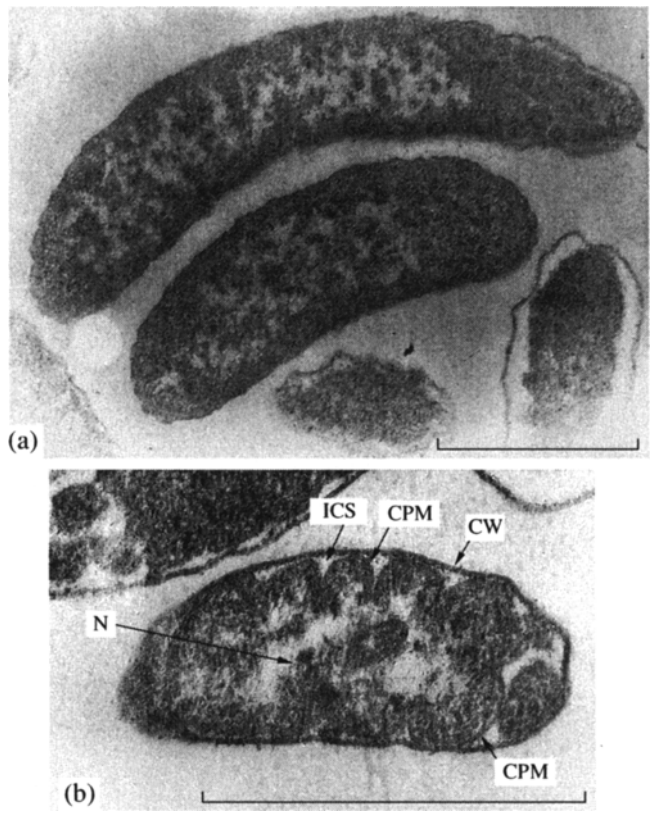

Fig. 8. Ultrastructure of *D. peptidovorans* cells. (a) Exponential and (b) stationary growth phases. ICS is an incomplete cross septa; CPM is the cytoplasmic membrane; CW is the cell wall; N is the nucleoid; the bar represents 1 μm.

Simonsiella [16]) bacteria. However, representatives of the genera *Caryophanon, Oscillospira, Alysiella*, and *Simonsiella* belong to multicellular trichomic microorganisms. The number of incomplete cross septa in each cell does not exceed 2–5 in *Caryophanon* and *Oscillospira* and is never more than one in *Alysiella* and *Simonsiella*. In distinction to the aforementioned bacteria, "*D. starorussensis*" does not belong to the trichomic bacteria. Longitudinal sections of vibrioid cells of "*D. starorussensis*" showed numerous incomplete cross septa, up to 20 per cell. In the early exponential phase of growth, long helical filaments, unseparated into single cells, also showed numerous incomplete cross septa. The breakage of long helical filaments into shorter ones occurred only by the middle of the exponential phase. As already noted, incomplete cross septa

were seen both on longitudinal and transverse sections, and the cytoplasm surrounded by the CPM invaginations looked like a corn cob.

Large, pocket-like CPM invaginations found in *Sulfobacillus thermosulfidooxidans* [14] have a similar structure but, in distinction to "*D. starorussensis*," are irregularly distributed in the cell.

It should also be noted that the ultrastructural peculiarities of CPM revealed in "*D. starorussensis*" differ in structure and function from those described in some prosthecate bacteria, in particular, in *Prosthecomicrobium polysphaeroidum*, which looks like a corn cob under certain growth conditions [17]. As distinct from the above prosthecate bacterium, in "*D. starorussensis*," the cell wall evenly surrounded the intracellular contents (Figs. 2–4) and did not follow the outline of the CPM invaginations. Furthermore, the development of incomplete cross septa or the CPM invaginations in "*D. starorussensis*" was not caused by the nutrient substrate limitation, but appeared to be a stable ultrastructural characteristic.

Thus, we have shown that the occurrence of numerous incomplete cross septa is not only peculiar to "*D. starorussensis*" strains, but also to the type species of the genus *Dethiosulfovibrio, D. peptidovorans*. This ultrastructural property appears to be an inherent characteristic of all representatives of the genus *Dethiosulfovibrio* and should be included in the description of the genus.

ACKNOWLEDGMENTS

We are grateful to V.I. Duda for discussion of the results. This work was supported by the Russian Foundation for Basic Research, project no. 99-04-48707, and by the State Scientific Program "Biodiversity," project no. 2.1.2.

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