

## Isolation and Properties of Ferromanganese-Depositing Budding Bacteria from Baltic Sea Ferromanganese Concretions

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Hyphal budding bacteria were observed by electron microscopy in thin sections of surface material from Baltic Sea ferromanganese concretions. Similar bacteria were also observed in and isolated from enrichment cultures prepared from the same concretion material. Three morphologically similar strains of Mn-Fe-depositing budding bacteria were isolated from the enrichment cultures. Strain B-4 possessed extracellular anionic polymers that accumulated Mn oxides. Mn deposition by B-4 was inhibited by elevated concentrations of Mn, 0.05% glutaraldehyde, 0.1 mM HgCl<sub>2</sub>, and heating at 93°C for 15 min, suggesting the participation of an enzyme protein in the Mn-depositing activity.

Budding bacteria occur in ferromanganese deposits from a variety of aquatic environments, including hydroelectric pipelines (16), water systems (12), thermal springs (13), and near deep-sea thermal vents (8). In a few cases, Mn-Fe-depositing budding bacteria have been isolated and studied in pure cultures (3, 5, 6, 13, 17). Based on observations made in one of these investigations (6), it was proposed that Mn-Fe deposition occurs in cultures by accumulation of the oxidized metals on anionic polymers that could be stained with ruthenium red (RR).

Electron microscopy (EM) of ferromanganese concretions from the western Baltic Sea (4) has revealed a myriad of Mn-Fe-encrusted bacteria in the surface layers of the concretions. Significant numbers of Mn-depositing bacteria were also detected in material scraped from the surfaces of these concretions (W. C. Ghiorse, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1979, N67, p. 190). These results suggested that the Mn-Fe-encrusted bacteria seen during EM analyses could have participated in the deposition of the metal oxides in the surface layers of the concretions. However, questions concerning which of the bacteria might be actively involved in the deposition of the metals remain unanswered. Additionally, although RR-staining polymers were detected in the surface material (4), suggesting the participation of anionic polymers in metal deposition, the actual mechanism(s) of metal deposition in the concretions remains unproven.

In the present investigation, evidence for the presence of hyphal budding bacteria in EM thin sections of Baltic Sea concretions is presented.

Additionally, the enrichment, isolation, and some of the morphological properties of Mn-Fe-depositing budding bacteria from surface material of the concretions are described. The presence of extracellular polymers and the inhibition of Mn deposition by protein alteration are also reported. Finally, the possible significance of the budding bacteria in the deposition of metals in Mn-Fe concretions is discussed.

### MATERIALS AND METHODS

**Description of ferromanganese concretions and sampling site.** Mussel shells and small stones encrusted with ferromanganese oxides were collected by scuba divers from sediments at depths of 23 to 25 m in the western Baltic Sea (10°5'E, 54°46'N). Samples from this location have been described previously (4). They consisted of concretions (encrusted mussel shells or stones), sediments, and water taken in sterile plastic (Twirl-Pak) bags on 4 February, 18 July, and 16 August 1977. All samples were either used immediately for EM studies, used for preparation of enrichment cultures as described below, or stored on ice until they could be processed in the laboratory (always within 24 h of sampling). Water temperatures at the bottom varied from approximately 2°C in February to 10°C in August. The salinity of the bottom water was variable, but on the average was approximately two-thirds of the salinity of open ocean water (i.e., ~24‰). More detailed chemical analyses of the ferromanganese concretions, sediments, and bottom water at this location have been reported (1).

**EM of ferromanganese concretions.** For EM analysis, pieces of surface material from fresh concretions were fixed in glutaraldehyde and processed for thin sectioning by using procedures designed for optimal preservation of bacterial cells. Some of the glutaraldehyde-fixed pieces were treated overnight with 5% (wt/vol)

oxalic acid to remove Mn and Fe oxides. The fixation and extraction procedures were previously described (4).

**Preparation of enrichment cultures.** For enrichment cultures, fresh concretions were removed from sample bags with sterile forceps and rinsed with sterile artificial seawater (9) that was diluted to two-thirds of its full-strength concentration (ASW). Outer layers of the concretions were then scraped off with a sterile scalpel and ground to a paste with a sterile mortar and pestle. Approximately 0.2 g of the resulting paste was added to test tubes containing 20 ml of a sterile mixture of ASW and 0.2% agar. The agar slurry was shaken to suspend the ferromanganese particles. Aliquots (0.1 ml) of the slurry were then added to flasks containing 100 ml of ASW plus either 0.4 g of methylamine-hydrochloride (MA) and 0.001 g of  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  (pH 7.0 to 7.4) (MA- $\text{MnSO}_4$  medium) or 0.1 g of  $\text{MnCO}_3$  without MA (pH 7.7) ( $\text{MnCO}_3$  medium). Each flask received 0.003 to 0.005 g (dry weight) of material from the original ferromanganese pastes. In one instance (B-4; see Table 1), the original test tube containing the ferromanganese paste suspended in the agar slurry was incubated as an enrichment culture.

Enrichments were normally incubated in the dark at room temperature (22 to 25°C). One enrichment culture was incubated at 4°C.

A total of eight enrichments were set up with material from seven different concretions (see Table 1). Enrichment cultures and strains of budding bacteria derived from them were assigned the same numbers as the concretions from which they were derived.

**Isolation of Mn-Fe-depositing budding bacteria.** The enrichment cultures were examined by phase-contrast microscopy at regular intervals over a period of 6 months. In most flasks after 2 to 4 months of incubation, surface films that contained Mn and Fe oxides and budding bacteria formed. When budding bacteria were observed by phase-contrast microscopy, samples were removed with a loop and streaked on MA- $\text{MnSO}_4$  medium solidified with 1.5% agar (MA- $\text{MnSO}_4$  agar). Some samples were streaked on a medium containing 4 g of MA, 1 g of  $\text{MnCO}_3$ , and 15 g of agar per liter of ASW (pH 7.7) (MA- $\text{MnCO}_3$  agar). Colonies that appeared on the plates after 1 week of incubation were examined by phase-contrast microscopy for the presence of budding bacteria. The colonies that contained budding bacteria were picked and restreaked on the same medium. Axenic cultures were obtained by introducing isolated colonies into fresh MA- $\text{MnSO}_4$  medium or into a medium containing 0.025 g each of peptone, yeast extract, and glucose per liter of ASW (pH 7.0 to 7.4) plus a mixture of vitamins (PYGV medium) (15).

**Tests for Mn and Fe oxides and catalase.** To test for deposition of Mn oxides, 0.01 g of  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  was added per liter of PYGV medium (PYGV-Mn medium). For Fe, an Fe-containing paper clip that weighed approximately 0.25 g was added to 100 ml of PYGV medium (PYGV-Fe medium). Mn and Fe oxides on cells in enrichment cultures or in colonies were detected with acidified (pH 3.5 to 4.0) leucoberbelin blue (LBB reagent) for oxidized Mn and with the Prussian blue reactions for oxidized Fe as previously described (5).

Catalase activity was detected by adding drops of 3%  $\text{H}_2\text{O}_2$  to cover colonies growing on PYGV agar and

then observing  $\text{O}_2$  formation. Tests for catalase activity in colonies growing on PYGV-Mn agar were considered invalid because Mn oxides in the colonies were reduced by  $\text{H}_2\text{O}_2$  at neutral pH and evolved  $\text{O}_2$  nonenzymatically.

**Light microscopy.** A Zeiss Photomicroscope II equipped with phase-contrast objectives and condenser was used throughout this work. Photomicrographs were recorded on Kodak Plus-X film.

**EM of strain B-4.** For EM studies, cultures of strain B-4 were grown in liquid PYGV or PYGV-Mn medium for 7 days at 22 to 25°C. For negative staining, equal portions of the culture fluid, 1% potassium phosphotungstate (pH 7.0), and 0.05% bovine serum albumin were mixed together in a test tube. Drops of the mixture were placed on Formvar-coated EM grids, blotted, air dried, and examined in a Phillips EM 300 operated at 80 kV.

For thin sectioning, cells were prefixed by adding 25% glutaraldehyde to achieve a final concentration of 0.5% glutaraldehyde in the culture flask. After 30 min at room temperature, the prefixed cells were harvested by centrifugation and washed in Veronal-acetate buffer (pH 6.2) (11). After being washed in Veronal-acetate buffer, some cells were further treated with RR to stain extracellular polymers. This was done by resuspending cells in 0.5 ml of the Veronal-acetate buffer and then adding 0.5 ml each of 2%  $\text{OsO}_4$  and 0.15% RR, both dissolved in distilled water. Cells not treated with RR were resuspended in the same manner except that distilled water replaced the RR solution. Fixation with  $\text{OsO}_4$  or  $\text{OsO}_4$ -RR lasted for 4 h at room temperature. Samples were then enrobed in agar, postfixed in 0.5% uranyl acetate, dehydrated in ethanol, and embedded in Spurr medium (14).

Thin sections were obtained with a diamond knife on an LKB ultramicrotome, mounted on EM grids, and double stained with saturated uranyl acetate and 0.03% lead citrate (18) solutions before examination in the Phillips EM 300.

Electron photomicrographs were recorded on Kodak Electron Image film.

**Tests for the effects of initial Mn(II) concentrations on the deposition of Mn(IV) oxides by strain B-4.** Duplicate Erlenmeyer flasks, each containing 20 ml of sterile PYGV medium, were inoculated with 1.0 ml of a 7-day-old PYGV-grown culture of strain B-4 to give a final cell density equivalent to 3.0 mg of protein per liter. Various amounts of sterile 1%  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  solution were also added to the flasks to give final concentrations of 3.2, 6.4, 9.6, and 12.6 mg of Mn(II) per liter. After 12 days of incubation at room temperature, duplicate samples were removed from each flask and analyzed for Mn oxides with LBB reagent and for protein by the biuret method as previously described (5).

**Tests for the effects of bactericidal treatments on Mn(IV) oxide deposition by strain B-4.** Suspensions of strain B-4 cells were prepared by centrifuging the contents of a 7-day-old PYGV culture at  $10,000 \times g$  for 10 min. The resulting pellets were suspended in fresh, sterile PYGV medium to a final cell density equivalent to 62 mg of protein per liter. Aliquots (20 ml) of the suspension were then added to duplicate, sterile 125-ml Erlenmeyer flasks containing enough sterile 1%  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  solution to give a final concentration of 3.2 mg of Mn(II) per liter. Identical flasks containing

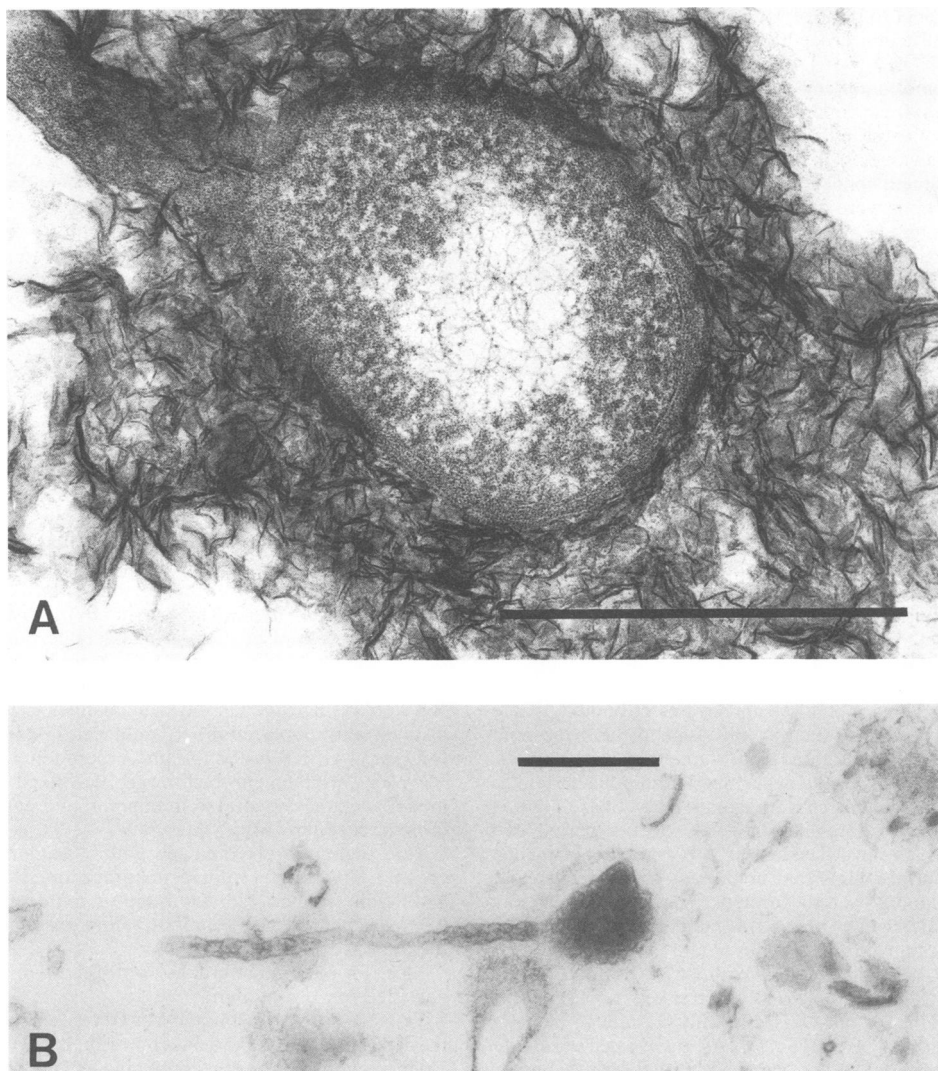


FIG. 1. Electron photomicrographs of thin sections of surface material from Baltic Sea ferromanganese concretions showing bacterial profiles that resemble budding bacteria. (A) Glutaraldehyde-OsO<sub>4</sub>-fixed concretion. (B) Concretion treated with 5% oxalic acid to remove Mn-Fe oxides after fixation. Bars = 0.5  $\mu$ m.

glutaraldehyde (0.05% final concentration) and HgCl<sub>2</sub> (0.1 mM final concentration) were also set up. Another set of flasks was heated for 15 min in boiling water (93°C) after addition of the cell suspension. A set of uninoculated flasks was prepared for all three treatments. After 50 h of incubation at 22 to 25°C, samples were removed from each flask and analyzed for Mn(IV) oxides and, when appropriate, for biuret-protein as previously described (5). Inoculated flasks were tested for the presence of viable cells by spreading 0.05 ml of the cell suspension on PYGV-MnSO<sub>4</sub> agar. Viable budding bacteria formed small, brown colonies within 1 week of incubation at 22 to 25°C. If no colonies were observed after a month of incubation, the flasks were considered to contain no viable cells.

## RESULTS

**EM evidence for budding bacteria in ferromanganese concretions.** Profiles of metal oxide-encrusted bacterial cells that resembled hyphal budding bacteria were observed occasionally in thin sections of glutaraldehyde-fixed surface material from the Baltic Sea concretions (Fig. 1A). In addition, hyphal budding bacteria were detected, albeit infrequently, in samples treated with oxalic acid to remove the metal oxides (Fig. 1B). The infrequent observations of hyphal budding bacteria in these samples may have been due, in part, to the fact that planes of sectioning were rarely parallel to hyphae. However, the

TABLE 1. Baltic Sea ferromanganese concretions and enrichment cultures from which Mn-Fe-depositing budding bacteria were isolated

Description	Concretion	Date collected	Enrichment medium <sup>a</sup>	Mn-Fe-depositing budding bacteria	
				In enrichments <sup>b</sup>	Isolated <sup>c</sup>
Encrusted mussel shell	B-4	4 February 1977	No additions	+	+
	B-7	18 July 1977	MA-MnSO <sub>4</sub>	+	-
	B-7	18 July 1977	MnCO <sub>3</sub>	+	-
	B-10	18 July 1977	MnCO <sub>3</sub>	+	+
	B-11	18 July 1977	MnCO <sub>3</sub>	-	-
Encrusted stone	B-14	16 August 1977	MA-MnSO <sub>4</sub>	+	+
	B-16	16 August 1977	MA-MnSO <sub>4</sub>	+ <sup>d</sup>	-
	B-17	16 August 1977	MA-MnSO <sub>4</sub>	+	-

<sup>a</sup> Except for strain B-4, all enrichment media were prepared by adding MA and MnSO<sub>4</sub> or MnCO<sub>3</sub> to ASW (see text). Each culture received the equivalent of 0.003 to 0.005 g of ferromanganese material scraped from the surface of the concretions. Strain B-4 was prepared by adding 0.2 g of wet ferromanganese paste to 20 ml of ASW plus 0.2% agar.

<sup>b</sup> Incubated for 2 to 6 months at 22 to 25°C.

<sup>c</sup> All isolates deposited both Mn and Fe oxides as shown by testing their colonies with LBB reagent for Mn and with the Prussian blue reactions for Fe.

<sup>d</sup> Incubated at 4°C.

low frequency of observations could also mean that hyphal budding bacteria were not predominant in the microbial communities inhabiting the surface material examined by EM. In contrast, a myriad of other morphological types of bacteria, many encrusted with metal oxides, have been observed in thin sections of the same surface material (4).

**Enrichment cultures.** Growth of budding bacteria and deposition of Mn and Fe was observed in all but one of the enrichment cultures that were prepared from the concretions (Table 1). The cultural conditions employed would be expected to encourage the growth of budding bacteria which are known to utilize one-carbon compounds and to grow at low concentrations of nutrients. Additionally, our enrichments were incubated for up to 6 months to allow slow-growing bacteria to appear. Thus, despite the infrequency of observation of hyphal budding bacteria in EM sections of surface material, such bacteria were observed frequently in the enrichment cultures.

Observations of the budding bacteria in the enrichment cultures were facilitated by their occurrence in metallic films that developed on the surfaces of the liquid and walls of the flasks. Phase-contrast light microscopy showed that mother cells of most of the budding bacteria in the films were covered with a refractile material (arrows, Fig. 2A) that was yellow-brown and turned blue upon addition of LBB reagent (Mn[IV] positive) and with Prussian blue reagents (Fe[III] positive).

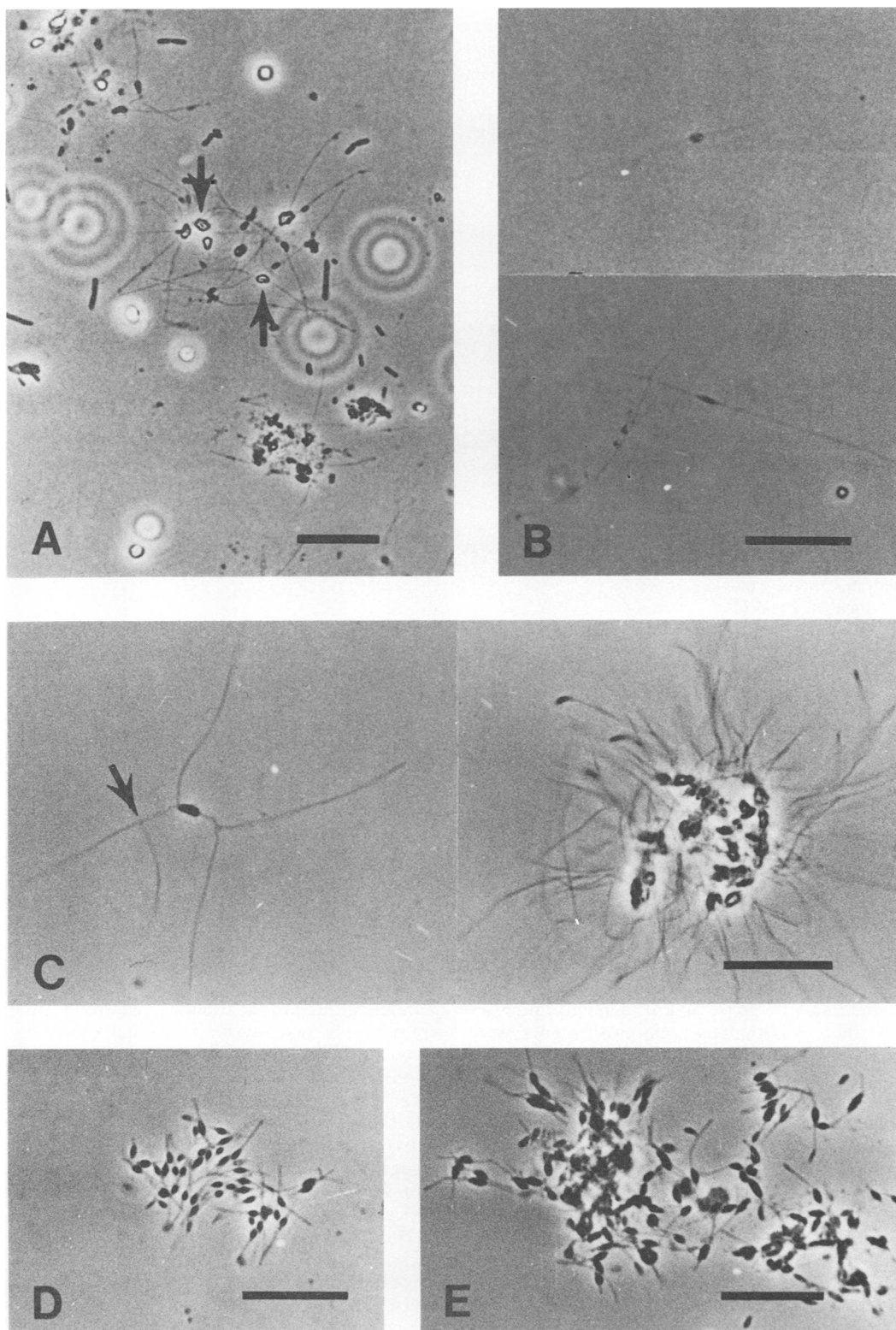
**Isolation and general properties of Mn-Fe-depositing budding bacteria.** Axenic cultures were

obtained by streaking metallic surface film material on MA-MnCO<sub>3</sub> agar. Small (~0.5 mm), brown colonies that appeared on plates after 1 month of incubation were examined by phase-contrast light microscopy. Those colonies that contained budding bacteria were introduced into flasks containing MA-MnSO<sub>4</sub> medium. Typically, after another 1 to 5 months of incubation, budding bacteria which usually possessed very long, often branched hyphae appeared (Fig. 2B and C). Eventually, aggregates of cells encrusted with phase-dense or refractile Mn oxides (Fig. 2C) were observed.

During 1978 and 1979, strains B-4, B-10, and B-14 were isolated from enrichments of three different concretions (Table 1). The three strains were morphologically similar to each other when grown in PYGV media, and all three strains deposited Mn or Fe oxides in their colonies when grown on PYGV-Mn or PYGV-Fe agar. All strains were weakly positive for catalase activity. Strains B-4, B-10, and B-14 were assigned the numbers IFAM-1210, IFAM-1211, and IFAM-1212, respectively, in the culture collection at Kiel.

The three strains all resembled *Hyphomicrobium* spp. (7) superficially; however, the diversity of budding bacteria demonstrated by DNA-DNA hybridization studies (10) precludes the assignment of these strains to a genus until further cultural characterizations and macromolecular comparisons can be made.

**Morphological properties of strain B-4.** Strain B-4 (IFAM-1210), the first isolate to be obtained in axenic culture, was further investigated with respect to morphological properties. When ob-



**FIG. 2.** Phase-contrast light photomicrographs of budding bacteria derived from Baltic Sea ferromanganese concretions. (A) Primary enrichment culture of concretion B-10 (see Table 1) after 5.5 months of incubation. Note mother cells (arrows) encrusted with refractile material that contained both Mn and Fe oxides. (B) Budding bacteria (strain B-4) isolated from concretion B-4 after 2 months of cultivation in liquid MA-MnSO<sub>4</sub> medium. (C) Same culture depicted in (B) after 5 months of incubation. Note branched hypha (arrow) and phase-dense and refractile Mn oxide coatings on mother cells. (D) B-4 cells after 5 days of incubation in liquid PYGV medium. Note short hyphae on these cells grown in PYGV medium. (E) B-4 cells after 5 days of incubation in liquid PYGV-Mn medium. Phase-dense Mn oxides in aggregates of cells and Mn oxide coatings on mother cells were not seen in cultures grown in PYGV medium without Mn (D). Bars = 10 μm.

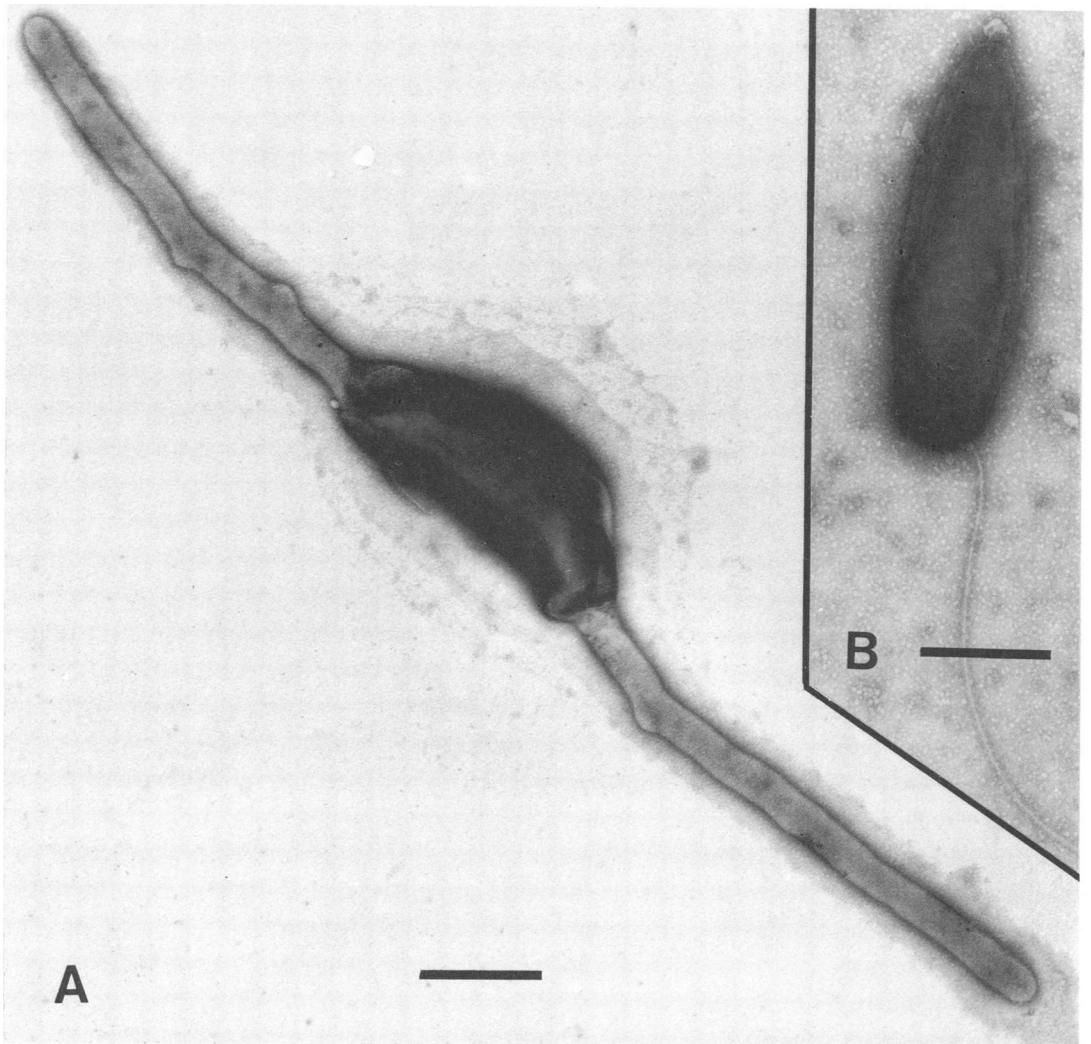


FIG. 3. Electron photomicrographs of negatively stained B-4 cells grown in PYGV medium. (A) Cell with two polar hyphae surrounded by material that appears to be composed of extracellular polymers. (B) Swarmer cell with a single polar flagellum situated at the blunt pole. Bar = 0.5  $\mu$ m.

served by phase-contrast microscopy, B-4 cells (Fig. 2B to E) possessed hyphae at one or both poles of the mother cells. Motile swarmer cells were produced at the tips of the hyphae. The cells attached easily to surfaces and to each other, forming aggregates. Shorter hyphae were observed on cells grown in PYGV medium (Fig. 2D and E) than on cells grown in MA-MnSO<sub>4</sub> medium (Fig. 2B and C). Cells growing in the presence of Mn appeared to be coated with phase-dense material that caused mother cells to appear larger in PYGV-Mn medium (Fig. 2D) than in PYGV medium (Fig. 2E). In PYGV-Mn medium, phase dense material also appeared between cells in aggregates (Fig. 2E). The dense material turned blue upon treatment with LBB

reagent, indicating that oxidized Mn was present.

EM observations on negatively stained B-4 cells grown in PYGV medium revealed mother cells and hyphae surrounded by a layer of material that appeared to consist of extracellular polymers (Fig. 3A). Swarmer cells were pointed at the pole where they had been attached to hyphae and possessed a single polar or subpolar flagellum at their blunt pole (Fig. 3B).

In thin sections of B-4 cells grown in PYGV medium and fixed in the presence of RR, electron-dense material (RR, Fig. 4A) was observed outside the cells associated with the extracellular polymers, suggesting that the polymers were acidic polysaccharides. In thin sections of cells



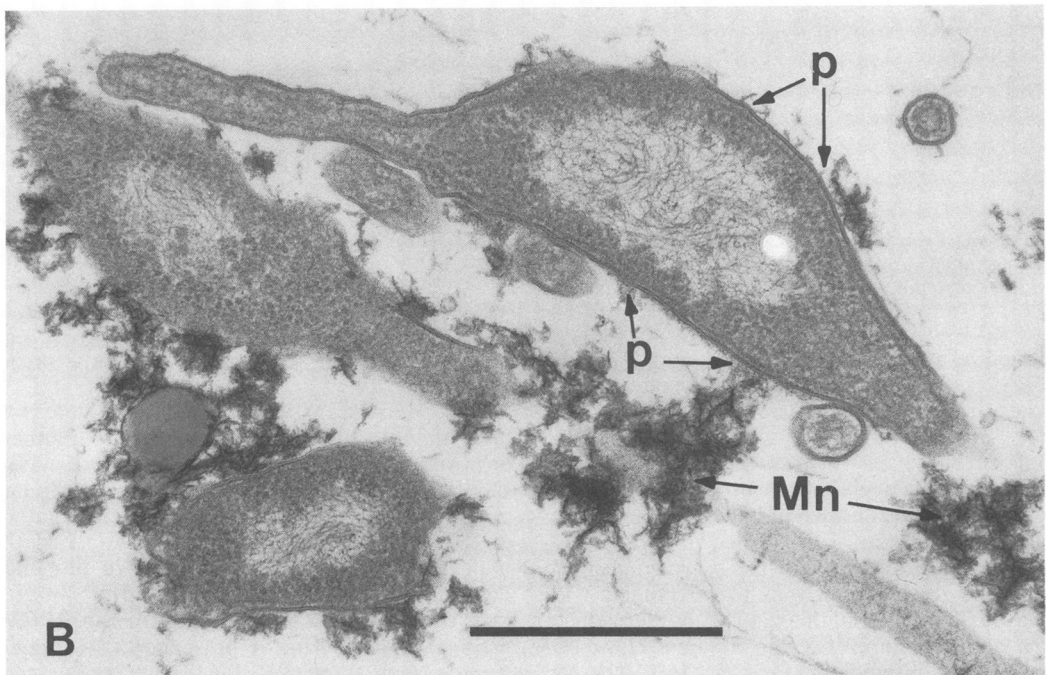
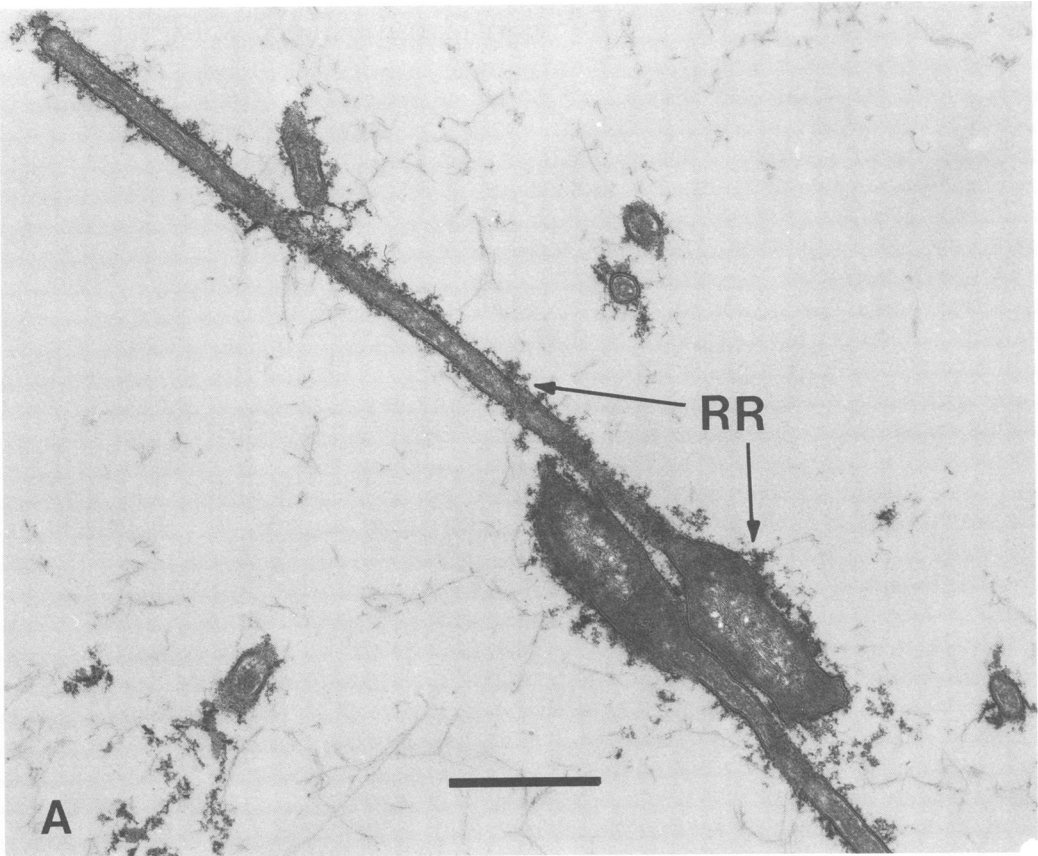


FIG. 4. Electron photomicrographs of thin sections of B-4 cells. (A) Cells grown in PYGV medium without added Mn and fixed in the presence of RR<sup>+</sup>, which stained the extracellular polymers. (B) Cells grown in PYGV-Mn medium and fixed without RR. Extracellular polymers (p) can be seen, as well as the Mn oxides (Mn) associated with them. Bars = 0.5  $\mu$ m.

grown in PYGV-Mn medium, electron-dense material (Mn, Fig. 4B) was observed in addition to the extracellular polymers (p, Fig. 4B). The additional electron-dense material can be assumed to be Mn(IV) oxides associated with the polymers, as has been demonstrated previously in Mn-Fe-depositing *Pedomicrobium*-like budding bacteria (6).

Other notable ultrastructural features observed in thin sections of these bacteria were a gram-negative-type cell wall, distinct 25-nm ribosomes, and relatively condensed nuclear regions of mother cells (Fig. 4B). Poly- $\beta$ -hydroxybutyrate bodies were observed occasionally (not illustrated).

**Effect of initial Mn(II) concentrations on Mn(IV) oxide deposition by strain B-4.** In cultures containing 3.2 mg of Mn(II) per liter, 1.45 mg of Mn was deposited on cells as Mn(IV) oxides and cellular protein increased from 3.0 to 66 mg/liter during 12 days of incubation (Table 2). At all higher initial concentrations [6.4, 9.6, and 12.8 mg of Mn(II) per liter], the amount of Mn(IV) deposited after 12 days was significantly less with each higher Mn(II) concentration, but the amount of protein was the same in each case (Table 2). These results show that increases in the initial Mn(II) concentration inhibited Mn(IV) oxide deposition. Similar results have been reported previously for other Mn-depositing budding bacteria (5; M. Rinck, Diplomarbeit, University of Saarland, 1980), as well as for other Mn-depositing bacteria (13). Although this is apparently a common property of Mn-depositing bacteria, the reasons for Mn inhibition remain unexplained.

**Effect of bactericidal treatments on Mn(IV) oxide deposition by strain B-4.** Suspensions of untreated B-4 cells retained their viability, but their cellular protein did not increase significantly during 50 h of incubation in PYGV-Mn medium (Table 3). The untreated cell suspensions deposited significantly more Mn(IV) than did cells that were incubated in the presence of lethal concentrations of glutaraldehyde and HgCl<sub>2</sub>. Interestingly, the two chemical treatments did not completely inhibit Mn deposition; however, in heat-treated flasks, virtually no Mn(IV) oxides were deposited.

These results suggest that the Mn-depositing activity of these bacteria is similar to that of previously examined *Pedomicrobium*-like bacteria (6) in that it depends on a relatively stable protein.

## DISCUSSION

Mn-Fe-depositing budding bacteria were observed by EM in surface material from Baltic Sea ferromanganese concretions. Budding bac-

TABLE 2. Effect of initial Mn(II) concentrations on Mn(IV) oxide deposition during growth of strain B-4

Initial concn <sup>a</sup> (mg/liter)		Concn after 12 days (mg/liter)	
Mn(II)	Protein	Mn(IV) oxides <sup>b</sup>	Protein <sup>c</sup>
0	3.0	0	54
3.2	3.0	1.45	66
6.4	3.0	1.20	60
9.6	3.0	0.95	63
12.8	3.0	0.75	63

<sup>a</sup> Concentration added to reaction flasks containing PYGV medium.

<sup>b</sup> Measured with LBB reagent. Error of  $\pm 3\%$ .

<sup>c</sup> Error of  $\pm 5\%$ .

teria were also isolated from enrichment cultures of the surface material. These results support the argument that budding bacteria, along with other Mn-Fe-depositing microorganisms (4), participate actively in the formation of the concretions. Additionally, one of the isolates, B-4 deposited Mn oxides in a matrix of extracellular polymers in a manner similar to that previously demonstrated for *Pedomicrobium*-like budding bacteria (6). The depositional activity of B-4 was adversely affected by elevated levels of Mn and by treatments known to disturb enzyme proteins. Thus, it can be further argued that budding bacteria could participate in concretion formation by enzymatically oxidizing Mn and accumulating the oxides in a polymer matrix. Finally, as was shown for *Pedomicrobium*-like bacteria (6), Fe oxides would also be expected to deposit in the polymer matrix.

Other than the present report concerning brackish-water Mn-Fe concretions and the work of Tyler and Marshall (16) concerning budding bacteria in freshwater Mn-Fe encrustations, ob-

TABLE 3. Effect of bactericidal treatments on Mn oxide deposition by cell suspensions of strain B-4<sup>a</sup>

Treatment	Concn (mg/liter) at 50 h		Viability <sup>d</sup>
	Protein <sup>b</sup>	Mn(IV) oxides <sup>c</sup>	
None	63	1.10 (100)	+
Glutaraldehyde (0.05%)	60	0.65 (59)	-
HgCl <sub>2</sub> (0.1 mM)	57	0.30 (27)	-
Heat (90°C for 15 min)	62	0.04 (4)	-
Uninoculated <sup>e</sup>	0	0 (0)	-

<sup>a</sup> A 7-day culture was centrifuged and suspended in 20 ml of fresh PYGV-MnSO<sub>4</sub> medium to approximately 62 mg of protein per liter. Flasks were incubated for 50 h at 22 to 25°C.

<sup>b</sup> Error of  $\pm 5\%$ .

<sup>c</sup> Measured with LBB reagent. Error of  $\pm 3\%$ . Percentages are shown within parentheses.

<sup>d</sup> Growth on PYGV-Mn agar.

<sup>e</sup> All treatments.



servations of Mn-Fe-depositing budding bacteria in samples from nature have rarely been documented. Therefore, it is of considerable interest that recent studies of samples from deep-sea thermal vent regions showed budding bacteria among a variety of other bacteria inhabiting Mn-Fe deposits near the vents (8). As in the Baltic concretions, hyphal budding bacteria in the deep-sea deposits were observed by EM and isolated from enrichment cultures. However, unlike the Baltic samples, the budding bacteria in deep-sea deposits were not clearly associated with metal oxides (8), nor were any of the isolates capable of depositing Mn oxides in their colonies (J. S. Poindexter, personal communication). These results suggest that hyphal budding bacteria may not participate in the deposition of Mn and Fe in the deep-sea deposits. Alternatively, deposition may be mediated by other bacteria or be principally nonbiological. Such possibilities indicate a need for further microbiological investigation of these and other Mn-Fe-deposits, such as deep-sea ferromanganese nodules and lake ores, to determine the relative importance of Mn-Fe-depositing microorganisms in metal deposition.

The results of this work do not clearly indicate a particular enzymatic mechanism of Mn-Fe deposition. However, Dubinina (2) has proposed that *Leptothrix pseudo-ochraceae*, *Siderocapsa* spp., and *Metallogenium* spp. can deposit Mn and Fe by means of a catalase that performs a peroxidase function. In the proposed mechanism, H<sub>2</sub>O<sub>2</sub>, produced during metabolic oxidation of organic substances, serves as an oxidant for Mn at neutral pH, provided that catalase acts as a peroxidase. Significantly, all three of the isolated Baltic Sea budding bacteria were catalase positive. Thus, catalase (peroxidase)-mediated Mn-Fe deposition is a strong possibility that remains to be investigated for the budding bacteria, as well as for other Mn-Fe-depositing bacteria in the concretions.

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