STRUCTURAL FEATURES OF MANGANESE PRECIPITATING BACTERIA*

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Abstract. Studies of biological communities of the past (and their associated activities) are usually dependent upon preservation of fossil material. With bacteria this rarely occurs because of the absence of sufficient fossilizable cellular material. However, some bacteria deposit metabolic products that can, conditions allowing, be preserved indefinitely. In particular, manganese and iron depositing bacteria have the capacity to form preservable microfossils. In order to better understand these microfossils of the past, we have examined present day morphologies of manganese oxidizing bacteria. These bacteria are highly pleomorphic, depending on the growth medium, the age of the culture, and the extent of manganese oxidation. Transmission electron microscopy indicates that manganese may be deposited either intra- or extra-cellularly. The prognosis of the use of morphological information for the interpretation of ancient and modern manganese deposits is discussed.

1. Introduction

Since the time of Beijerinck (1913) it has been known that bacteria can catalyze the oxidation of soluble divalent manganese (Mn^{2+}) to tetravalent manganese as the insoluble oxide (MnO_2), although exact mechanism remain unknown.

Pure cultures of a wide variety of bacterial taxa can be isolated from diverse environments: soils, lakes and oceans (Table I). The association of such manganese precipitating bacteria with actively accreting manganese precipitates has led many workers to hypothesize that bacterial activity is causally involved with the genesis and/or growth of the precipitates. Such hypotheses have been put forth for marine manganese nodules (Ehrlich, 1972, 1975), freshwater manganese deposits (Klaveness, 1977; see Kuznetzov, 1970 for review), water pipe deposits (Tyler and Marshall, 1967a, b, c) and soil precipitates (van Veen, 1973; Bromfield, 1956).

The role of microbiota in the formation of fossil manganese deposits is even more difficult to assess; any associated organisms are long since dead, making culture methods impossible and identification of microfossils very difficult. However, bacteria-like colonies have been identified in the iron facies of the remarkably well-preserved Gunflint chert (Barghoorn and Tyler, 1965). Correlation of laboratory and micropaleontological investigations are expected to eventually elucidate the origins of manganese precipitates. The product of the manganese precipitating bacteria is the insoluble oxide, MnO_2, a stable mineral under aerobic conditions. The interaction of these bacteria with sediments may leave a morphologically distinctive record in the form of a precipitate. If the precipitates

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TABLE I
Bacterial genera that oxidize manganese[a]

Major group[b]	Class	Genus	Environment[c]	Reference
Pseudomonads		*Pseudomonas*	S	Zavarzin, (1962)
			M	Nealson (1978)
Facultative aerobic gram negative heterotrophs	Entero-bacteria	*Achromobacter*	M	Ehrlich (1966)
		Aeromonas	M	Ehrlich (1966)
		Brevibacterium	M	Ehrlich (1966)
		Oceanospirillum	M	Ehrlich (1979)
		Vibrio	M	Ehrlich (1966)
		Arthrobacter (Siderocapsa)	M	Ehrlich (1966)
		Flavobacterium	M	Nealson (1978)
	Sphaerotilus group	*Leptothrix (Sphaerotilus)*	F	Mulder and van Veen (1963)
		Clonothrix	F	Wolfe (1960)
		Metallogenium	F	Kuznetzov (1970)
		Kuznetzovia	F	Kuznetzov (1970)
	Prosthecate bacteria	*Pedomicrobium*	S	Gebers and Hirsch (1978)
		Hyphomicrobium	F	Tyler and Marshall (1967a)
Gram positive aerobic endospore formers		*Micrococcus*	M	Ehrlich (1963)
		Bacillus	M	Ehrlich (1963)
Actinobacteria		*Nocardia*	S	Schweisfurth (1968)

[a] This table, not all inclusive, merely illustrates the diversity of manganese precipitating bacteria.
[b] Phyla and classes of Margulis (1974)..
[c] Isolated from marine (M), soil (S) or fresh water (F) environments.

are well-preserved, then morphological examination of fossil and live microbes and their precipitates may provide insight into processes of past and present metal precipitation. Clearly, however, a prerequisite is the identification of the organisms and their precipitates formed under controlled conditions. This chapter, which describes manganese precipitates formed in the laboratory as the result of bacterial activity, discusses the prognosis of the use of such morphological information to interpret ancient and modern MnO_2 deposits.

2. Materials and Methods

Manganese oxidizing bacteria were isolated from enrichment cultures as previously described (Nealson, 1978). They were grown and maintained on K medium: 2 g bacto-peptone (Difco), 0.5 g yeast extract (Difco), 0.18 g $MnCl_2 \cdot 4H_2O$ and one liter of 75% seawater. The manganese chloride was made up as a 0.45% solution in distilled water, sterilized by filtration (0.2 μm Millipore membrane filter) and was added to the other components of the medium after they were autoclaved.

On solid medium (1.5% agar) and for qualitative manganese determinations, oxidation was detected by spot tests, using either Feigl's benzidinium reagent (1958), or leukoberbelin blue reagent (Krumbein and Altmann, 1973), both of which form characteristic blue-colored compounds upon reaction with manganese dioxide. The leukoberbelin blue (kindly supplied by Dr W. Ghiorse) offers the advantages that it is non-toxic, and reduces the oxidized manganese upon reaction, so that it can be used to gently remove MnO_2.

Samples were prepared for microscopy by fixation for up to three days with a 1% glutaraldehyde solution in sterile buffered seawater (75% seawater and 50 mM N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) buffer). During fixation, samples were kept at 4° and then washed in sterile buffered medium at 4°.

For scanning electron microscopy, seawater was gradually substituted by distilled water (50, 75, and 100%). Samples were then dehydrated in ethanol (50, 60, 70, 80, 90, and 100%) for 10 min at each concentration. After dehydration, freon 113 was gradually substituted for ethanol. Samples were then dried by the critical point method and attached to aluminum stubs with either double-stick tape or carbon paint. Once mounted, the samples were sputter-coated with gold to a thickness of 100 Å with a Technics coater, studied and photographed with a Cambridge S-4 scanning electron microscope. Energy dispersive X-ray (EDS) analysis to determine elemental composition of the samples was performed at the same time, using an ORTEC computerized system.

Samples for transmission electron microscopy were fixed in 1% glutaraldehyde in artificial sea water (ASW: NaCl 200 mM; $MgSO_4 \cdot 7H_2O$, 50 mM; KCl, 10 mM; $CaCl_2 \cdot 2H_2O$, 10 mM) for up to three days, washed three times (15 min each) in ASW, and post-fixed in 1% osmium tetroxide (OsO_4) in ASW for 1–2 hr. Samples were then dehydrated in a graded series of ethanol solutions to 100%, and finally in 100% propylene oxide. They were transferred to Spurr low viscosity embedding media without accelerator and rotated on a turntable for up to 12 hr. Samples were then placed in BEEM capsules with fresh Spurr's medium and allowed to sit in a desiccator for 6–12 hr. Samples were then polymerized for 6 hr to 70°. Thin sections (60–80 Å) were cut on an LKB Ultramicrotome III and studied and photographed using a Zeiss Transmission electron microscope (Model 9AS) at an accelerating voltage of 75 keV.

3. Results and Discussion

The taxonomic, morphological, physiological and ecological diversity of manganese oxidizing bacteria is shown in Table I. Are the differences in manganese precipitates correlatable with differences in the associated bacteria? A goal is to distinguish deposits formed by different groups of bacteria for eventual identification of the organisms and processes that form naturally occurring precipitates.

Several manganese oxidizing bacteria grown in culture demonstrate features of bacterial manganese deposits in nature. Figure 1 shows a pure culture of a strain of bacillus, SG-1. Also shown is a spontaneous mutant (SG-1W) of the same strain incapable of manganese oxidation. A crusty brown precipitate develops on the surface of the colony of the man-

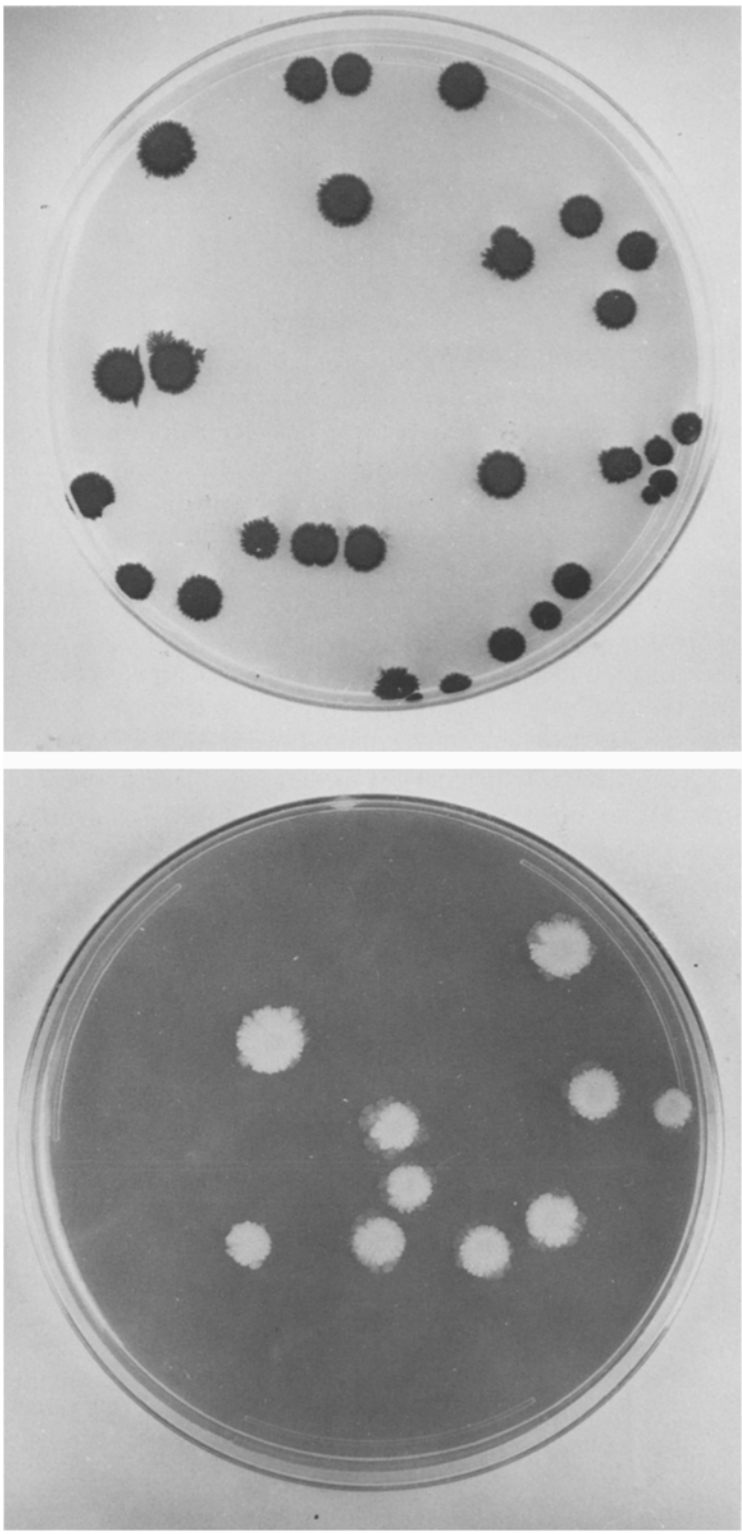

Fig. 1. A marine bacillus, strain SG-1 which rapidly oxidizes manganese (top) and its spontaneously derived mutant which is incapable of manganese oxidation (bottom). Two-week-old colonies; the black precipitate appears within a few days.

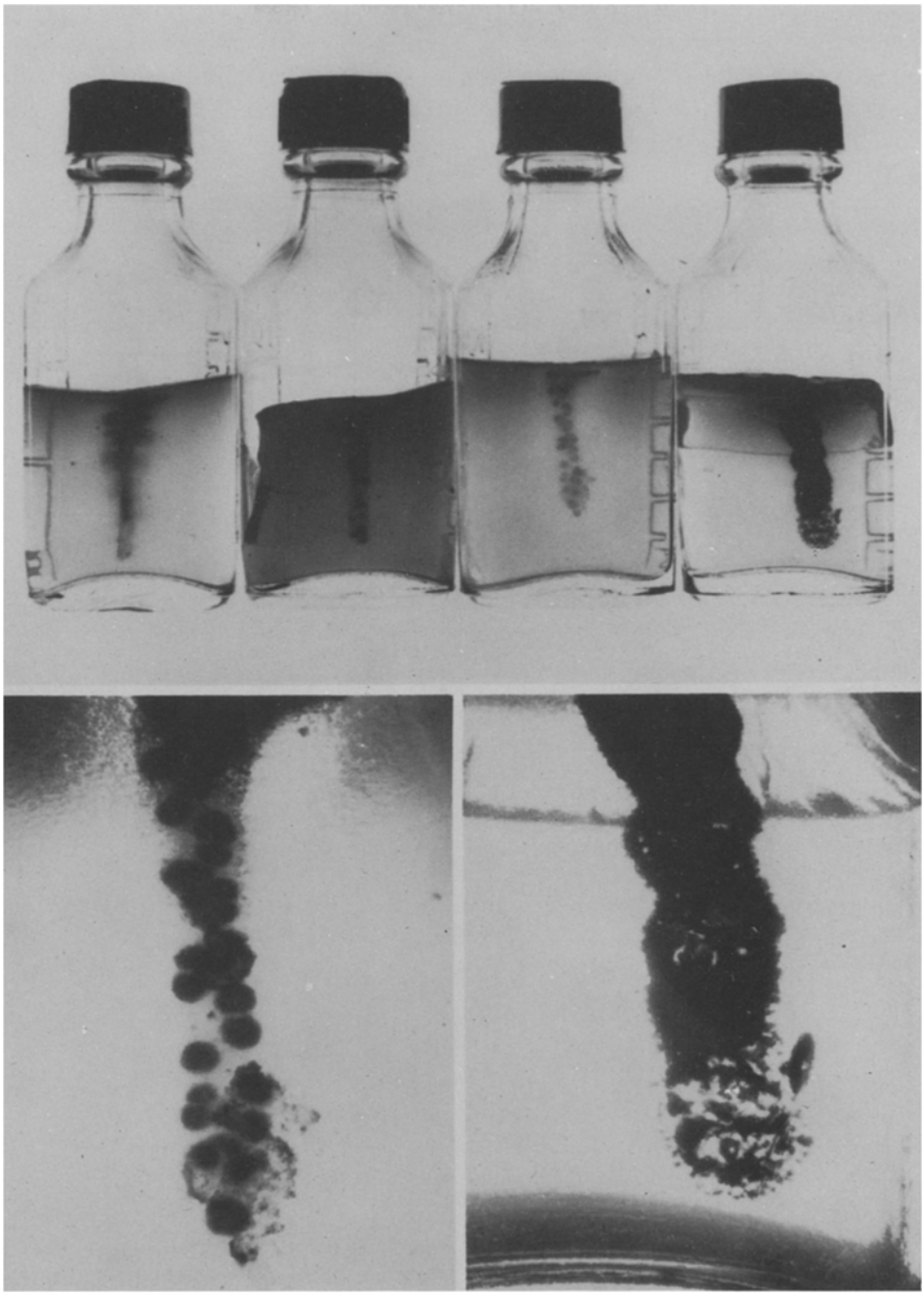

Fig. 2. Gram negative rod-shaped bacteria which oxidizes manganese slowly. These heavy precipitates took one year to develop in soft agar stab cultures. Manganese dioxide formation by these bacteria, which are probably microaerophilic, was originally detected on plates by benzidinium hydrochloride.

ganese oxidizing strain, while the mutant retains a characteristic white color, typical of many bacilli. However, for other bacteria, the low quantity and rate of manganese oxidation may preclude macroscopic precipitates; manganese oxidation is detected only when manganese test reagents such as benzidinium hydrochloride (Feigl, 1958) or leukoberbelin blue (Krumbein and Altmann, 1973) are used. After growth for at least one month,

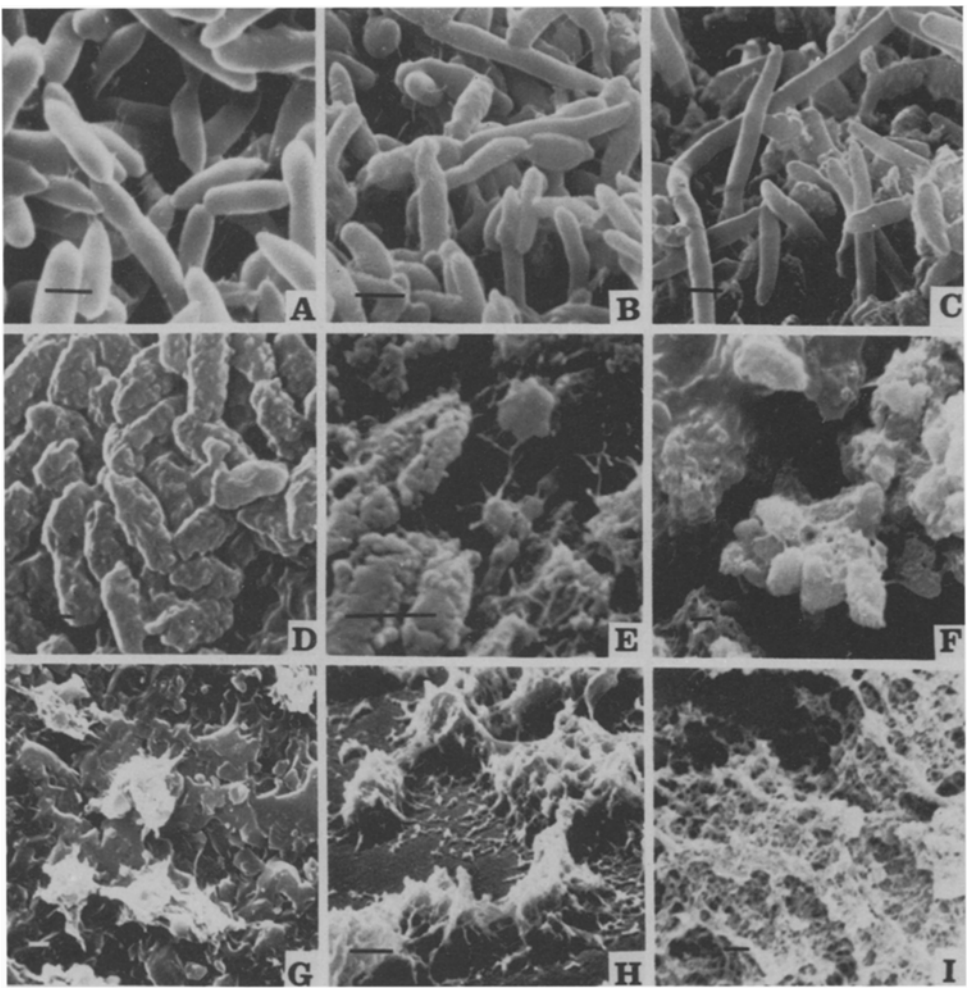

Fig. 3. Scanning electron micrographs of the same manganese oxidizing bacillus (strain SG-1) under different conditions. (Bars = 1.0 μm). (A) Agar medium (1.5%) lacking manganese, cells after one week of growth. (B) Cells after one day of growth in K medium (1.5% agar, 0.8 gm MnCl/liter). (C) Cells after one week of growth in K medium. (D) Cells taken from the top surface of the colony after one month of growth on K medium. (E) Cells taken from the bottom surface of the colony after one month of growth on K medium. (F) Clumps of manganese coated bacteria after several months of growth in manganese supplemented sea water. (G) Clumps of MnO_2 coated bacteria on sand grains in sea water after one week of growth. (H) Clumps of MnO_2 coated bacteria on glass slides after one week of growth. (I) Clumps of MnO_2 bacteria on glass slides after three months of growth.

however, nearly all manganese depositing bacteria become coated or associated with MnO_2. Figure 2 shows several cultures grown in stab cultures for one year. The subsurface precipitates are formed only when bacteria are present, and are associated with the bacterial cells.

The morphology of SG-1 cells depends on several factors: composition of the growth medium, the age of the culture, the extent of manganese precipitation and the amount of surface available. Some of the morphological variations are shown in Figure 3. On agar plates, colonies with no manganese in the growth medium (A) never form precipitates; on manganese-containing medium, young colonies (B) precipitate less manganese relative to old (C–E). There are also differences between the top surface precipitates (D) and the bottom surfaces (E) of the same colonies grown on agar plates. Cells attached to glass show variation with age (H, 1 week; I, 3 months); and cells clumped from liquid (F) or growing on a sand grain (G) are also distinctive. Strain SG-1 even includes many morphologically variant forms that are scarcely recognizable as bacteria (Figure 3E–I). The morphological variation of such bacterial precipitates must be understood in great detail if they are to be used to gain insight into past processes. All the different morphologies in Figure 3 are produced by only one species of bacteria. A similar range of variation in other manganese precipitating microbes is likely to be seen. In fact, a complex life cycle displaying many different morphologies is characteristic of the manganese oxidizing bacteria, *Metallogenium*. *Metallogenium* and its precipitates is at least as variable as SG-1, if not more so. This organism, which has been studied by several Russian scientists (for review, see Kuznetzov, 1970), appears to be the modern counterpart of the genus *Eoastrion* of Barghoorn and Tyler (Barghoorn, 1977). Variable morphology of the budding bacteria *Pedomicrobium* has also been reported by Ghiorse and Hirsch (1977) and Gebers and Hirsch (1977). Such heterogeneity impedes and in some cases may even preclude identification of manganese oxidizing organisms by examination limited to morphology of their precipitates. Furthermore, most MnO_2-associated microorganisms have not even been examined in this context.

Although there are difficulties as discussed, it is hoped that, after a catalog of structures is obtained, the identification of causative organisms will be possible. Furthermore, if identification is achieved, since the bacterial deposition of manganese varies as a function of growth conditions, it may eventually be possible to infer the environmental conditions existing at the time of metal deposition.

Thin sections of the subsurface deposits of Figure 2 examined by transmission electron microscopy show that the precipitates are obviously of bacterial origin (Figure 4). In one case, the manganese precipitation is entirely extracellular. Ghosts or cellular depressions similar to those observed might have been left in fossil material by such a process. In another example, the manganese is precipitated inside the cells rather than outside; fossils of these cells would certainly be quite different from those of the first type. The precipitates around spores of SG-1 are also extracellular, as shown in thin sections of SG-1 that were scraped from a glass surface (Figure 4E). Similar thin sections of strain SG-1, after the removal of precipitated manganese by treatment with leukoberbelin blue reagent, are shown in Figure 4F.

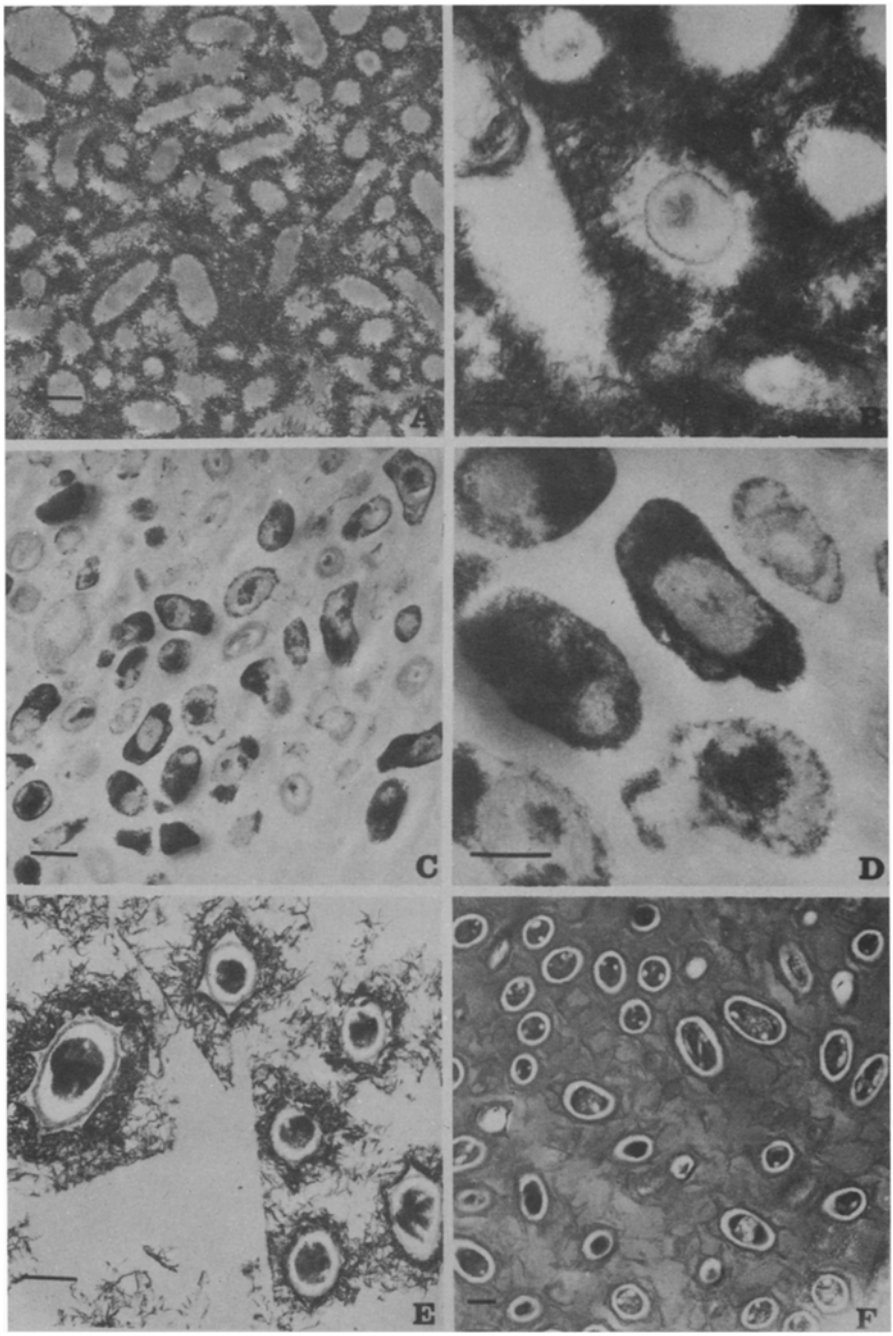

Fig. 4. Transmission electron micrographs of manganese precipitation by bacteria. (A) A gram negative rod (strain 56A) forms an extracellular precipitate of MnO_2. Bar = 0.5 μm. (B) Same as (A) but at higher magnification. Bar = 0.25 μm. (C) A second gram negative rod-shaped bacterium (strain 45B) that deposits MnO_2 inside the cells. Bar = 0.5 μm. (D) Same as (C) but higher magnification. Bar = 0.25 μm. (E) A third mode of MnO_2 deposition is seen in the bacillus (strain SG-1) in which the spores but not the cells become coated with MnO_2. Bar = 0.5 μm. (F) Spores of the bacillus like those in (E) which have been treated with leukoberbelin blue and the MnO_2 removed from the external surfaces of the spores Bar = 0.5 μm.

When precipitates are removed from the bacillus SG-1, characteristic bacterial structures remain (Nealson and Ford, 1980). Thus, with other manganese precipitates it may also be possible to identify organisms by removing the extracellular precipitates.

Further knowledge of the structure, metabolism, morphology and rates of production of precipitates of manganese oxidizing bacteria is prerequisite to understanding manganese accumulation in nature. Even after such information is available, determination of the origins of fossil and recent manganese precipitates may not be easy. Are bacterial precipitates really recognizable? How does the morphology of precipitates such as those seen in Figure 4 alter with continuing manganese deposition and diagenesis? Are unique or characteristic oxides or mineral phases produced by bacteria? What is their preservation potential? Are some precipitates only biogenic? Do certain oxides or mineral phases require conditions of temperature and pressure that preclude their biogenicity? Answers to these questions are intrinsic to an understanding of the biology and geology of the Precambrian environment in general, as well as to that of recent manganese concretions.

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