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Oxidation of Manganese and Formation of Mn_3O_4 (Hausmannite) by Spore Coats of a Marine *Bacillus* sp.

S. MANN,[1] N. H. C. SPARKS,[1] G. H. E. SCOTT,[1] AND E. W. DE VRIND-DE JONG[2]*

School of Chemistry, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom,[1] and Department of Biochemistry, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands[2]

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Isolated spore coats of a marine *Bacillus* species were incubated in 25 mM $MnCl_2$ at pH 7.5. Manganese precipitates, formed on the coat surfaces, were analyzed by transmission electron microscopy, electron diffraction, and energy-dispersive X-ray spectroscopy. Initially, an amorphous manganese oxide was observed on the coats which recrystallized to hausmannite after prolonged incubation in the $MnCl_2$ solution. The spore coats catalyze the oxidation of Mn(II) and have no structural influence on the final mineral phase precipitated.

Although manganese-oxidizing bacteria have been known since the beginning of this century (2), many aspects of the mechanisms of manganese oxidation have remained obscure. The cellular components which catalyze the oxidation process have been identified in only a limited number of bacterial species (1, 3, 5, 9), and even less is known about the mineralogy of the manganese oxide precipitates.

Hastings and Emerson (7) have studied kinetic and thermodynamic aspects of manganese oxidation by spores of a marine *Bacillus* species. This species, called strain SG1, has been isolated from a near-shore manganese sediment by Nealson and Ford (10). Its mature dormant spores catalyze the oxidation of Mn(II) (11). The oxidizing activity is located on the spore coats (4). Hastings and Emerson used the nonequilibrium thermodynamic model for manganese oxidation of Hem and Lind (8) to predict the oxidation state of the manganese oxide formed by spores at defined pHs and free Mn(II) concentrations. At a pH of 7.5 to 7.6 and Mn(II) concentrations over 30 μM, formation of Mn_3O_4 (hausmannite) is thermodynamically favored. When the Mn(II) concentration drops below 30 μM, the Mn_3O_4 disproportionates to γ-MnOOH, and below circa 1 μM Mn(II) it eventually ages to MnO_2. Hastings and Emerson performed an experiment in which spores were allowed to oxidize Mn(II) at pH 7.6 and a constant free Mn(II) concentration of 0.2 μM. In the initial stage of the oxidation process, small octohedra characteristic of Mn_3O_4 were observed with electron microscopy. The aged precipitate consisted of X-ray-amorphous manganese oxide with an average oxidation state of 3.85. These observations corroborated the theoretical prediction very well.

In this study, we allowed isolated spore coats of *Bacillus* sp. strain SG1 to oxidize Mn(II) under conditions in which Mn_3O_4 is thermodynamically stable with respect to both γ-MnOOH and MnO_2. Cells were cultivated and spores were isolated from sporulated cultures as previously described (4, 11). Spore coats were prepared from isolated spores by the method of Goldman and Tipper (6). A stock spore coat suspension was made up in distilled H_2O. One milliliter of this suspension contained coats isolated from 0.1 g (wet weight) of spores (equivalent to 1.5×10^{10} spores; reference 4). One milliliter of a stock coat suspension was centrifuged (10,000 × *g*, 10 min) and suspended in 2.5 ml of 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). Portions of 0.5 ml each from this suspension were mixed with 0.5-ml portions of 50 mM $MnCl_2$ in 25 mM HEPES, pH 7.5. As demonstrated by Hastings and Emerson (7) and de Vrind et al. (4), spores and spore coats oxidize Mn(II) until the catalytic sites are covered with manganese oxide and biological manganese oxidation is blocked. Under the conditions used in the present investigation (spore coat concentration equivalent to 3×10^9 spores per ml), a maximum concentration of 1.3 mM Mn(II) can be oxidized (4). Consequently, the free Mn(II) concentration remains in the range at which hausmannite is thermodynamically stable.

At 3 h, 1 day, and 8 days after addition of the $MnCl_2$ to the spore suspension, preparations for electron microscopy were made by allowing a drop of the suspension to dry on an electron microscope grid. The samples were examined by selective-area electron diffraction, energy-dispersive X-ray analysis, and lattice imaging with a Jeol 2000FX transmission electron microscope. Simultaneously, a control solution of 25 mM $MnCl_2$ in 25 mM HEPES was checked for autooxidation of Mn(II). No oxides were detected in this control over the experimental period.

Energy-dispersive X-ray analysis spectra showed the presence of manganese on the spore coats after 3 h of incubation in 25 mM $MnCl_2$ (data not shown). These precipitates were amorphous, as shown by the absence of lines in the electron diffraction patterns. In contrast, the electron diffraction patterns of the manganese-containing phase observed on the spore coats after 1 day of incubation contained some diffraction spots, indicating the presence of crystallinity in the bacterial deposit. The manganese-containing phase

TABLE 1. Electron diffraction data for manganese-containing deposits present on spore coats after 8 days of incubation in 25 mM $MnCl_2$, pH 7.5, along with data for the mineral hausmannite

d spacing (nm)	
Spore coats	Hausmannite
0.389	0.406 (110)[a]
0.356	0.364 (102)
0.280	0.276 (103)
0.265	
0.194	0.203 (220)
0.179	0.179 (105)

[a] Miller index on the basis of a tetragonal unit cell (*a*, 0.5762 nm; *c*, 0.9469 nm).

* Corresponding author.

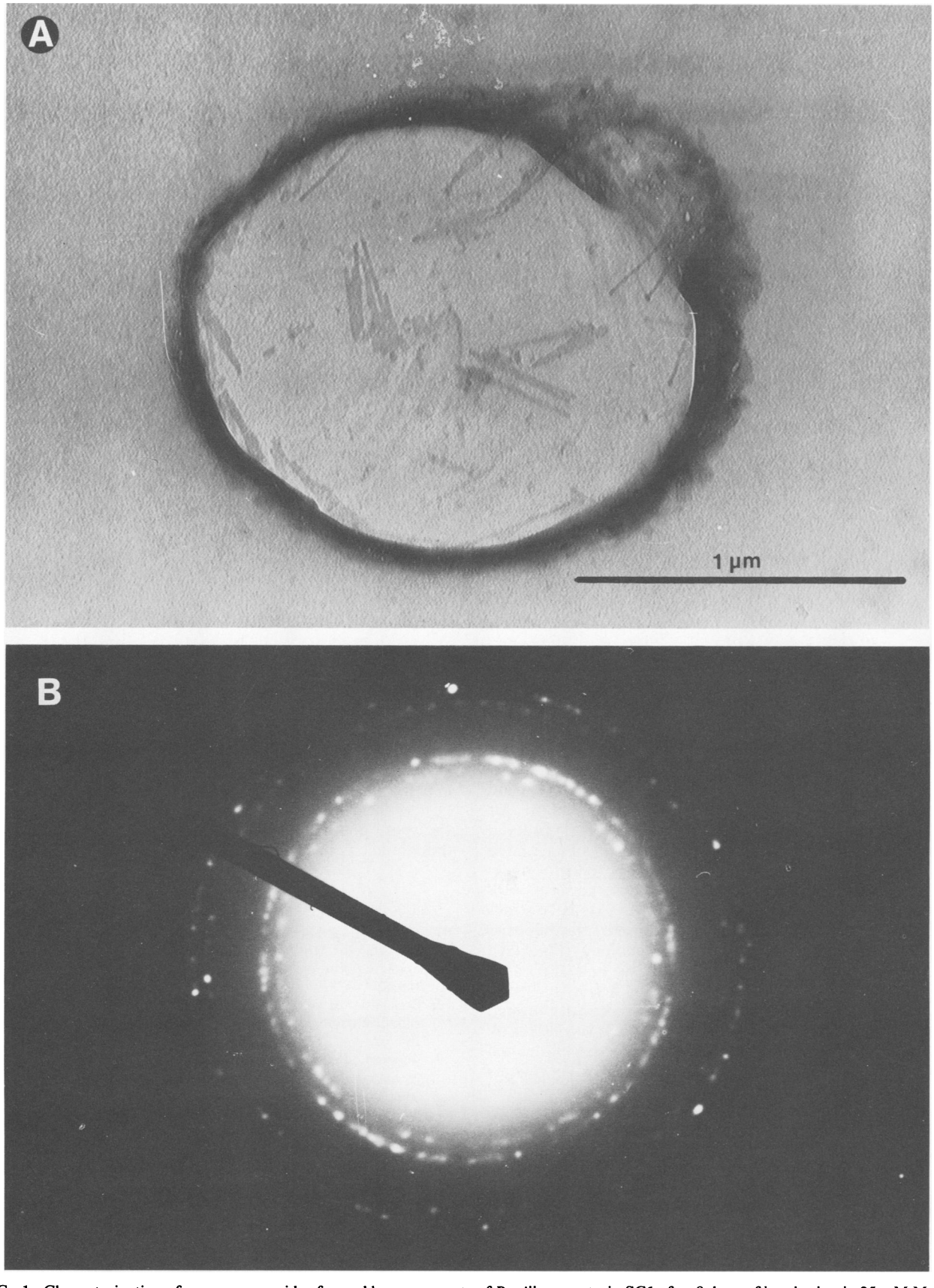

FIG. 1. Characterization of manganese oxides formed by spore coats of *Bacillus* sp. strain SG1 after 8 days of incubation in 25 mM $MnCl_2$, pH 7.5. (A) Electron micrograph of spore coat covered with manganese oxide. (B) diffraction pattern from sample shown in panel A (camera focal length, 410 cm).

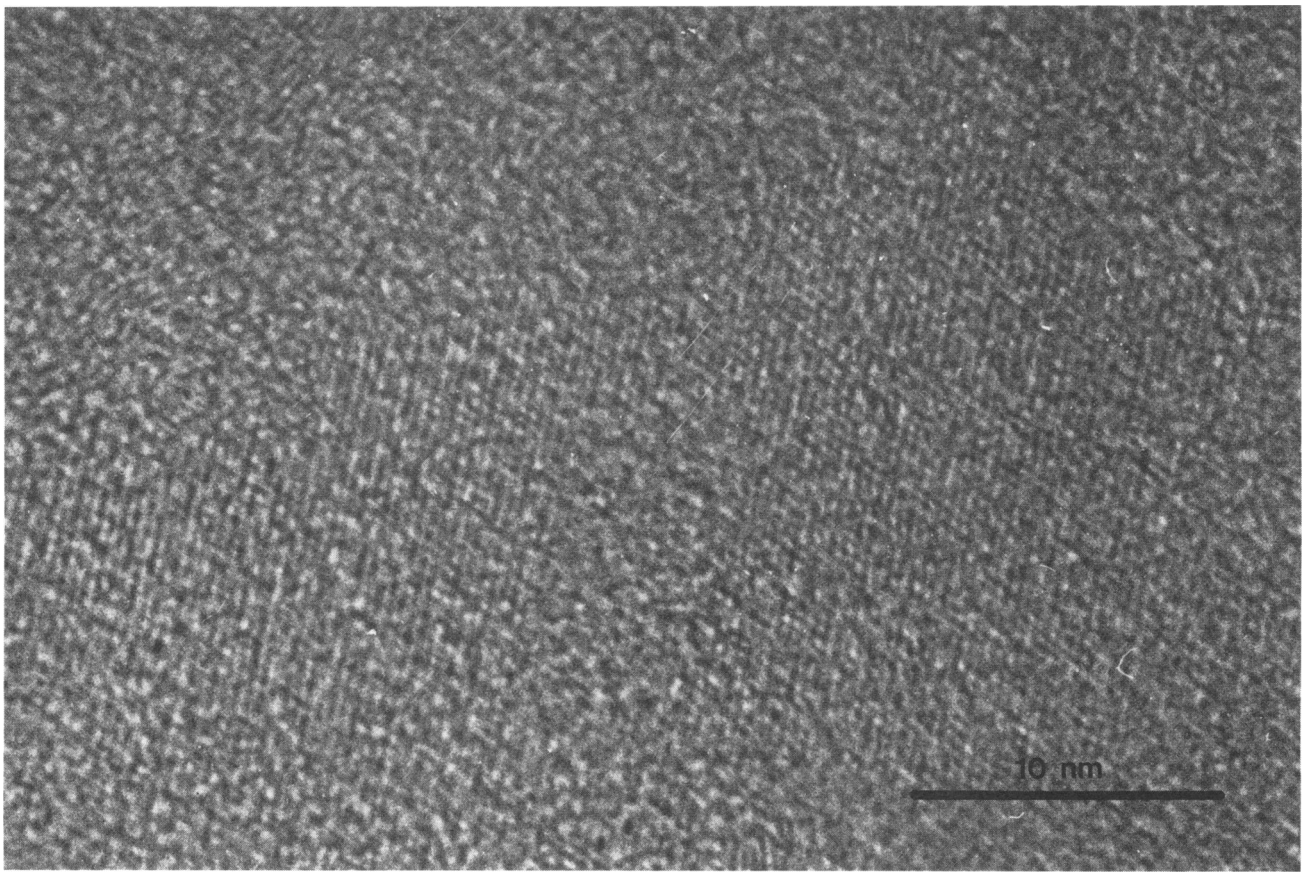

FIG. 2. High-resolution lattice imaging of manganese oxides formed by spore coats after 8 days of incubation in 25 mM $MnCl_2$, pH 7.5. Lattice fringes (0.4 nm) corresponding to the (110) planes of hausmannite were observed in localized domains on the spore coat surface.

after 8 days of incubation (Fig. 1A) yielded electron diffraction patterns which were more intense and contained well-defined powder diffraction rings (Fig. 1B). The patterns for 1- and 8-day samples were both identified as poorly ordered hausmannite (Mn_3O_4) (Table 1) on the basis of tabulated X-ray diffraction data for Mn oxides. The absence of many of the hausmannite diffraction lines in these patterns indicated that the spore coat deposits contained extensive structural disorder in the unit cell. Furthermore, the deposits tended to have no characteristic morphology (Fig. 1A), unlike well-ordered hausmannite, which is typically cube-shaped.

The structural nature of the spore coat deposits formed after 8 days of incubation was studied at high resolution by lattice imaging. Lattice fringes corresponding to well-defined hausmannite crystal planes were observed only over regions of limited size (20 to 30 nm) (Fig. 2). The surface of the spore coat was therefore covered in isolated domains of hausmannite separated by amorphous or extensively disordered manganese oxides.

Spore coats of *Bacillus* sp. strain SG1 apparently oxidized Mn(II) to a stable form of Mn_3O_4 at sufficiently high Mn(II) concentrations, in accordance with the model proposed by Hem and Lind (8). The crystal structure of hausmannite, however, forms from an initially amorphous manganese oxide which subsequently undergoes recrystallization after prolonged incubation in $MnCl_2$ solution. The formation of the amorphous precipitate is kinetically favored and is consistent with catalytic oxidation of Mn(II) by the spore coats. We therefore assume that subsequent recrystallization of the precipitate into hausmannite is not mediated by the spore coat surface but proceeds according to the thermodynamic conditions in the system.

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