APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2003, p. 5722–5725 Vol. 69, No. 9
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.9.5722–5725.2003

Precipitation of Barite by *Myxococcus xanthus*: Possible Implications for the Biogeochemical Cycle of Barium

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Received 6 November 2002/Accepted 16 June 2003

Bacterial precipitation of barite ($BaSO_4$) under laboratory conditions is reported for the first time. The bacterium *Myxococcus xanthus* was cultivated in a solid medium with a diluted solution of barium chloride. Crystallization occurred as a result of the presence of live bacteria and the bacterial metabolic activity. A phosphorous-rich amorphous phase preceded the more crystalline barite formation. These experiments may indicate the involvement of bacteria in the barium biogeochemical cycle, which is closely related to the carbon cycle.

The mechanism by which barite precipitates in undersaturated seawater is one aspect of the Ba biogeochemical cycle that still remains unknown. Considerable research has focused on this element over the last 2 decades, since it is known to be a reliable indicator for variations in marine biological productivity (for examples, see references 3, 6, 9, 11, 16, and 17). Chow and Goldberg (5) first proposed that barite formation is biologically mediated; Bishop (3) and Dehairs et al. (6) provided evidence that such precipitation occurs in the upper water column in microenvironments of decaying organic matter and other biogenic remains. Morphologies and sizes of marine barite crystals in the water column and in marine sediments (for examples, see references 6 and 16) also indicate a possible biogenic origin. Barite precipitation by living organisms (protozoa) has been demonstrated in lacustrine freshwater environments (4, 10, 19). In marine environments, intracellular barite crystals have also been found in vacuoles of unicellular *Exanthemachrysis gayraliae* (12) and in *Xenophyophorea* (14). However, these organisms do not appear to account for the abundance of barite crystals in the water column, and the living organisms which directly precipitate barite have not yet been identified in seawater (1). Thus, a reasonable proposal would seem to be that bacteria play a role in this process. As an initial approach to the investigation of bacterial barite production, an experiment was performed using *Myxococcus xanthus*, an abundant and ubiquitous heterotrophic soil bacterium (8). This microorganism has been demonstrated to induce precipitation of sulfates, phosphates, and carbonates (reference 13 and references therein; 20). Although myxobacteria are recognized mainly as soil bacteria (8), they are also found in marine environments (15).

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Barite production under laboratory conditions. Living and dead *M. xanthus* cells (strain 422 provided by the Spanish Type Culture Collection, Burjasot, Valencia, Spain) were inoculated on a solid culture medium with Ba (CM-Ba) (0.4% yeast extract, 2 mM $BaCl_2 \cdot 2H_2O$, 2% purified Difco agar-agar in distilled water [pH 7]). The inoculum was prepared by cultivating *M. xanthus* in liquid CT medium (7) for 72 h at 28°C with shaking (200 rpm) in order to obtain a density of $\sim 2 \times 10^9$ cells/ml. In living-cell experiments, 20-μl aliquots of the *M. xanthus* inoculum were deposited on the solid medium. Dead cells were obtained by using heat (by autoclaving at 120°C for 20 min) and UV light (by placing 20-μl cellular suspensions on the CM-Ba solid medium 28 cm under a Philips lamp EYE G15T8 for 15 min). For the dead-cell experiments, (i) 20-μl aliquots of heat-killed cell suspensions were inoculated on CM-Ba solid medium and (ii) the CM-Ba solid medium was inoculated with 20-μl aliquots of the *M. xanthus* culture and treated with UV light, as described above. Twenty-microliter aliquots of sterile CT medium and distilled water for controls were also plated on CM-Ba medium. Petri dishes were incubated at 28°C for 60 days. The petri dishes were observed once a day with light microscopy (magnification, ×100) to detect the presence of precipitates. Precipitates appeared in bacterial colonies only after 20 days of incubation, and crystals were abundant and easily detectable at 2 months. The final-stage pH was approximately 8 in the living-cell experiments. In CM-Ba culture medium, *M. xanthus* develops only a vegetative cell cycle. Precipitates were detected in neither the controls nor the dead-cell experiments. Precipitates were recovered by melting the solid medium in a microwave oven (600 W for 50 s) after washing with distilled water to eliminate culture medium remains and cellular debris.

The morphologies and compositions of precipitates were studied by scanning electron microscopy (SEM) (Zeiss-DSM 950) coupled with energy-dispersive X-ray (EDX) microanalysis (QX, 2000 Link) and by transmission electron microscopy

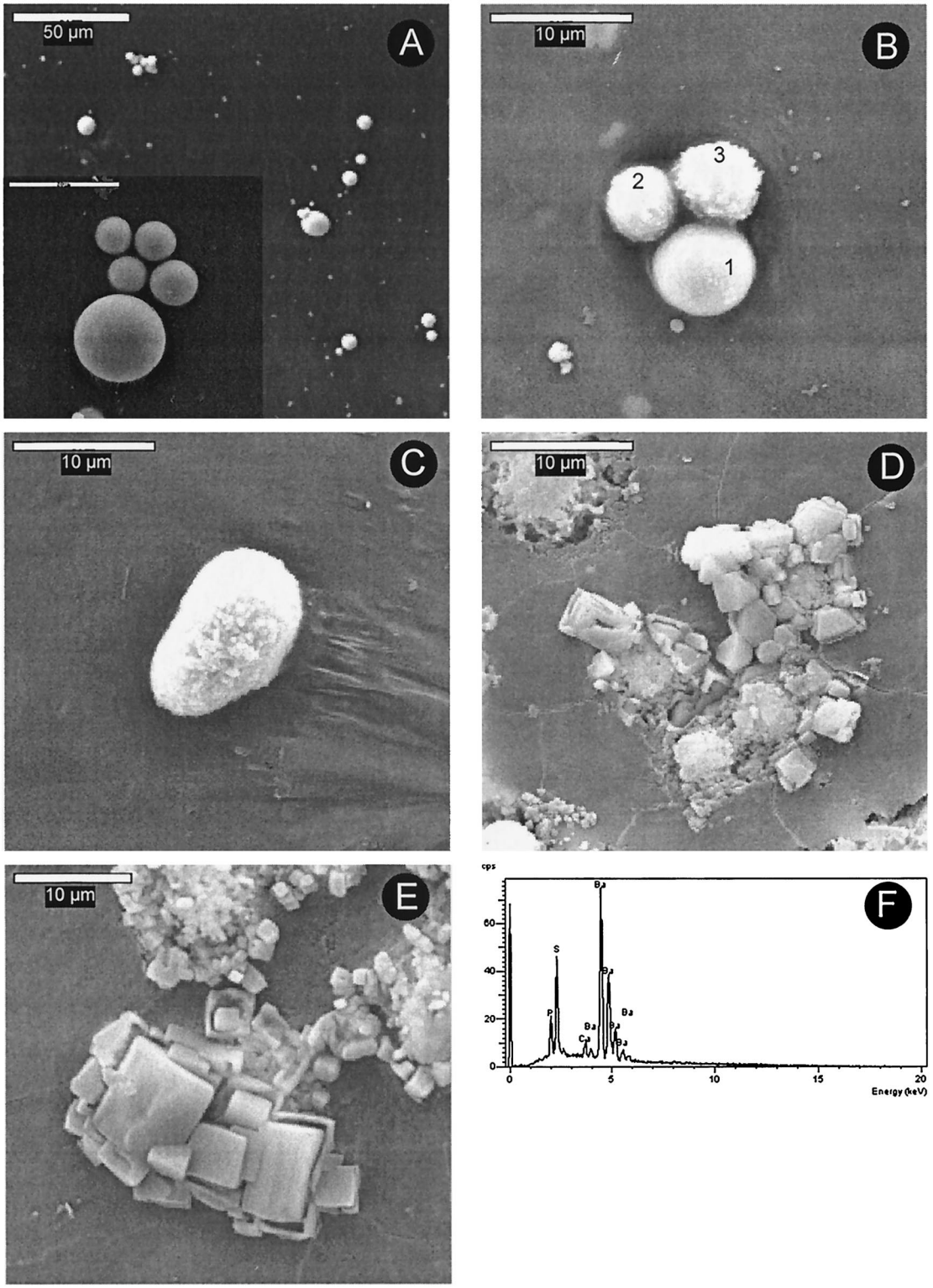

FIG. 1. (A to E) SEM photographs showing the evolution of barite crystals, from initial spherical aggregates to denser barite crystals. (F) Microanalysis of the barite crystal visible in panel D is shown graphically (see text).

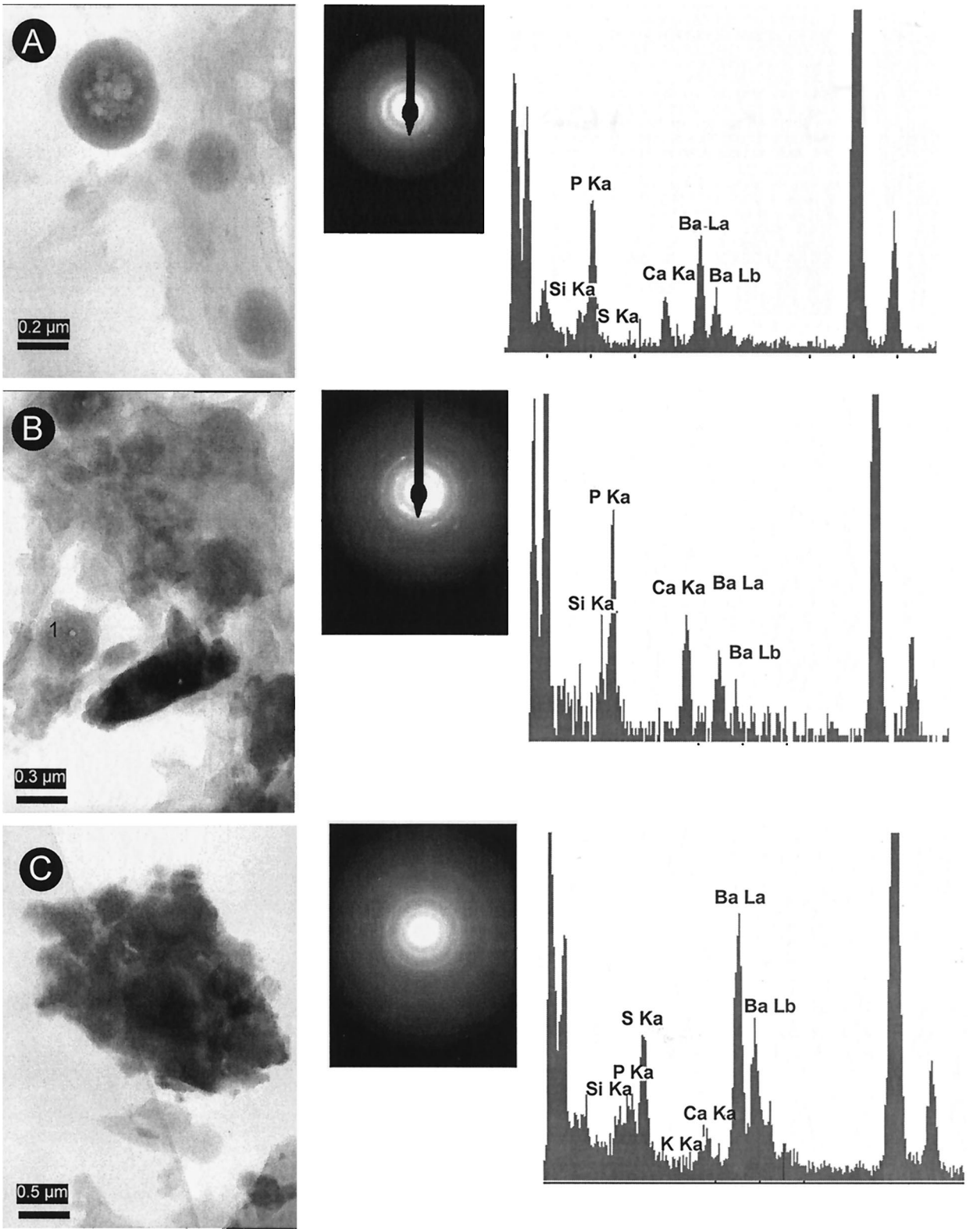

FIG. 2. TEM photographs and corresponding electron diffraction images and microanalyses of barite crystals (see text).

(TEM)-analytical electron microscopy (Philips CM 200 equipped with a solid-state ultrathin-window EDX detector). Precipitates were identified as barite crystals ranging in size from 0.2 to 8 μm (Fig. 1 and 2).

Implications and perspectives of crystal analyses. SEM revealed that barite crystals initially appear inside spherical aggregates. This may indicate that crystal growth evolves from spheres (Fig. 1A and B, sphere 1) to aggregates in which barite crystals become visible (Fig. 1B, spheres 2 and 3, and C, D, and E), a hypothesis which is also supported by TEM observations (Fig. 2). Figure 2A shows the spherical aggregates in which barite crystals start to form. Electron diffraction images show the polycrystalline nature of these aggregates and the nearly random orientation of the crystals. In the EDX analyses, the composition of these phases can be seen to be dominated by P and Ba, in addition to Si and Ca (no sulfate was detected at this stage). Figure 2B shows an aggregate (sphere 1) similar to that in Fig. 2A and a denser barite crystal. Denser crystals can also be seen in Fig. 2C. EDX analysis again revealed that the lower-density aggregates are composed of P and Ba, as well as Si and Ca. In contrast, the denser barite crystals are mostly composed of Ba, S, and O (Fig. 2C). The presence of Si can be attributed to impurities from the nutritional culture medium. As the precipitates evolved to denser barite crystals (Fig. 2C), the composition changed to Ba and S, with minor amounts of Ca and P. All analyses generated results similar to those presented in Fig. 1 and 2.

The initial P-rich precursor phase suggests that phosphoryl and carboxyl groups in the structural polymers of the cell wall outer membrane may be sorbent constituents which play an important role in the precipitation process. Deprotonation of these groups provides discrete complexation sites for metals in solution. Sorption is enhanced as pH increases and as surface groups deprotonate. Sorption of large cations such as Ba^{2+} are particularly favored by PO_4^{3-} ligands, and this process enables the formation of high-coordination polyhedra (a coordination of 10 or higher). As the SO_4^{2-} content of the culture medium increases (likely due to degradation and oxidation of amino acids), the ions are captured by the Ba ions, thus giving rise to a barite growth nucleus.

This interpretation of the results is further supported by the absence of barium phosphate or barium sulfate production in dead-cell experiments. In live cultures, the production of metabolites and variations in pH may lead to small-scale saturation gradients within the culture which induce precipitation. Such gradients strongly affect the saturation level for nucleation (18). In a dynamic system, nucleation kinetics depend on the rate at which the system reaches supersaturation. Because mineral production occurs only in the living bacterial colonies, favorable conditions for crystallization occur in direct relation to bacterium presence and metabolism, both of which modify local conditions at the precipitation site. In biological processes, nucleation from supersaturated solutions does in fact take place on the charged points of substrates (2), e.g., lipopolysaccharides of the lipid bilayers of the bacterial membranes.

In summary, these results are the first to indicate that barite precipitates in bacterial cultures, and the results support the hypothesis that the origin of this mineral may be bacterially mediated. Such precipitation suggests that in marine environments, bacteria may enhance barite production by providing nucleation sites and by producing crystal growth. This is, however, only an initial approach for future investigation regarding the role of bacteria in the Ba biogeochemical cycle. Further research will be required in order to determine the exact role of bacteria in marine barite precipitation.

This work was supported by the Spanish "Dirección General de Investigación" (DGI BOS2001-3285) and by Research Group CVI 103 of the "Junta de Andalucía." Projects REN2000-0798 and REN2001-3868-CO3-01 and Research Groups FQM 195 and RNM0179 are also thanked.

The CIC personnel of the University of Granada assisted in the SEM and TEM analyses. Editing of the English manuscript was done by M. Bettini.

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