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Evidence that gold crystals can nucleate on spores of *Bacillus cereus*

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Summary

During 1983, we learned that gold crystals can nucleate on micron-sized bacterial spores. When spores come into contact with aqueous gold, gold precipitates uniformly on their nearly spherical surfaces. As layers of crystalline gold progressively envelope a spore, the spore-centered crystal may gradually assume the shape of a 12-sided dodecahedron. The amount of gold deposited on Bacillus cereus spores from solutions of aqueous gold chloride, $AuCl_4^-$, increased with the time of exposure and in proportion to the concentration of gold in the aqueous solutions. Dodecahedral gold crystals grown on spores under laboratory conditions (fig. 2) closely resemble dodecahedral gold crystals found in nature (fig. 3).

Experimental

Spores of a laboratory isolate of Bacillus cereus (K-2) were grown on Plate Count agar at 30°C for at least one week, harvested into distilled water and stored at 5°C. Individual batches were washed as needed by repeated centrifugation from deionized water. Fisher atomic absorption gold reference standard, LOT 714840, 1000 $\pm$ 1 µg/ml, as chloride in distilled water, pH 2.65, was used at full strength or diluted with deionized water. X-ray specta were obtained using a Gandolfi x-ray diffraction camera. Chemical analysis (table 1) was carried out by means of inductively coupled argon plasma emmission spectrometry (ICP). Scanning electron microscopes (SEM) used were Model 180 and 250 Cambridge Stereoscan instruments.

Results

In one experiment, duplicate quantities of approximately 10^8 spores were suspended in 1000 µg/ml gold solution for 14 hours, washed free of soluble gold by repeated centrifuging from deionized water, and dried in a sterile laminar flow hood. X-ray diffraction analysis (fig. 1) confirmed that microcrystalline gold was present in the spore precipitate. SEM examination of the precipitate revealed nothing except recognizable bacterial spores. To learn if the deposition of gold on spores was proportional to the concentration of aqueous gold, aliquots containing approximately 10^8 spores were added to a series of solutions of gold ranging from 25 through 1000 µg/ml, permitted to react for 14 hours, washed free of soluble gold as above, and resuspended in deionized water. ICP analysis of the suspension (table 1) confirmed that the deposition of gold on spore surfaces was proportional to the concentration of gold in the solutions. Finally, approximately 0.25 g of washed spores were added to 20 ml of 1000 µg/ml gold solution and gently agitated for 36 hours on a wrist-action shaker along with a control containing no spores. In contrast to the control, a fine, gold-colored precipitation occurred in the culture tube to which spores had been added. After rinsing and drying, the precipitate was mounted on a new SEM stub with a conducting resin. SEM examination revealed many crystalline objects ranging in size from 1 µm to approximately 15 µm diameter. These objects were confirmed by energy dispersive x-ray diffraction analysis to consist of gold. Two such crystals (fig. 2 A-C; D) appear to have grown on bacterial spores, the latter (fig. 2 D) being at a slightly more advanced stage of growth. Other spores exhibiting irregular surface accumulations of gold are indicated by arrows (fig. 2 A and B).

Discussion

Watterson and others (1983) hypothesized that gold was initially replacing barium, calcium, and strontium in spore cortex material, which appeared from preliminary analytical data to be exposed to chemical interaction. More recent and detailed studies, however, do not bear out this hypothesis (table 1). We conclude that gold interacts primarily with the spore coat. The B. cereus spore coat consists of water-insoluble, sulfur-rich, keratin-like proteins rich in cystine and half-cystine crosslinkages (Aaronson and Pandy, 1977). These disulfide linkages presumably can act as local electron donors and specific binding sites for transition metal cations (Nieboer and Richardson, 1981). B. cereus spores have been shown to be inhibited by solutions of mercury, copper, chromium, and iron salts (Krishna Murty and Halvorson, 1957), apparently through binding to the spore surface. Beveridge (1978) demonstrated that tiny gold crystals form within purified Bacillus subtilis cell wall preparations after a few minutes exposure to 5 mM $AuCl_4^-$. This report, presented earlier in poster form (Watterson and others, 1983), is apparently the first to note the ability of bacterial spores to nucleate gold crystals.

Acknowledgment

The nugget shown in figure 3 was kindly donated by Mr. William L. Miller, Sacramento, California.

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TABLE 1. ICP analysis of selected elements in a deionized water suspension containing approximately 10^7 *B. cereus* spores per ml

[Spore aliquots were previously exposed for 14 hr to gold solutions ($AuCl_4^-$) of different concentrations.]

Au in test solution (μg/ml)	Analysis of spore suspension (ppm)			
	Au	Ba	Ca	Sr
0	.07	.006	2.0	.007
25	3.89	.006	2.0	.006
50	6.81	.004	2.0	.007
100	7.90	.002	2.0	.006
200	7.69	.002	2.0	.006
300	8.00	.001	2.0	.006
400	8.86	.002	1.0	.005
500	8.42	.001	1.0	.005
600	8.93	.002	2.0	.006
700	9.82	.001	2.0	.005
800	12.43	.002	2.0	.007
900	14.05	.002	2.0	.006
1000	13.84	.001	2.0	.006

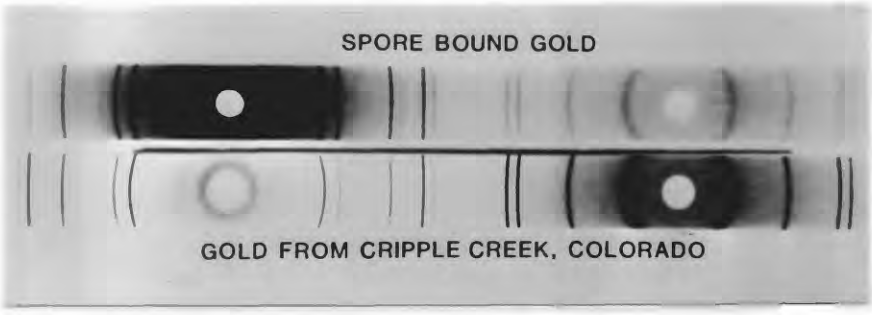

Figure 1.--X-ray diffraction pattern of B. cereus spores after overnight exposure to 1000 g/ml aqueous gold chloride ($AuCl_4^-$) solution: comparison with Cripple Creek gold standard.

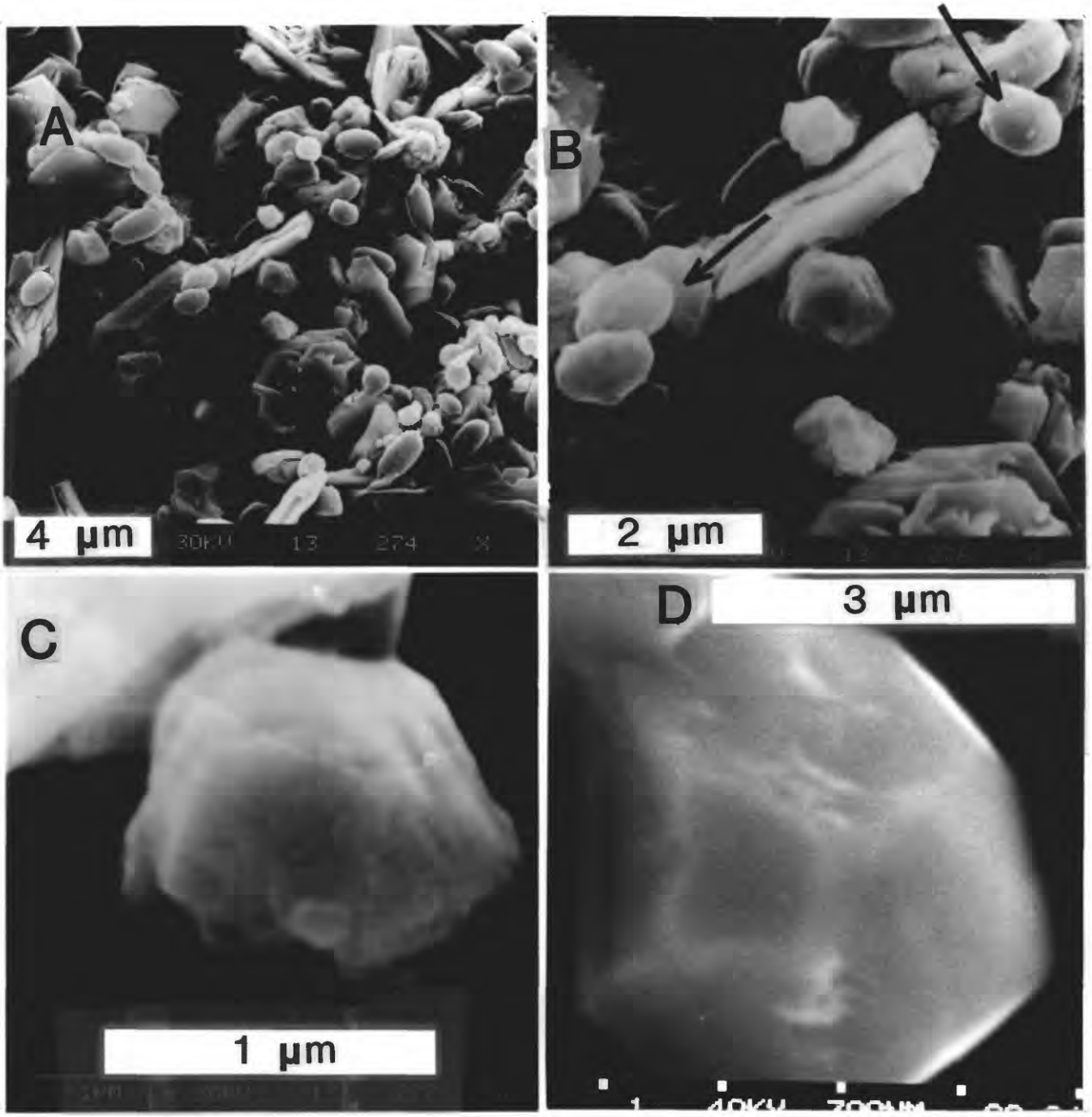

Figure 2.--A, B, and C: three SEM magnifications of a gold-coated B. cereus spore (center) after 36-hour exposure to 1000 g/ml aqueous gold chloride ($AuCl_4^-$) solution. Arrows point to other spores with surface gold accumulations. D--dodecahedral gold crystal from another microscope field.

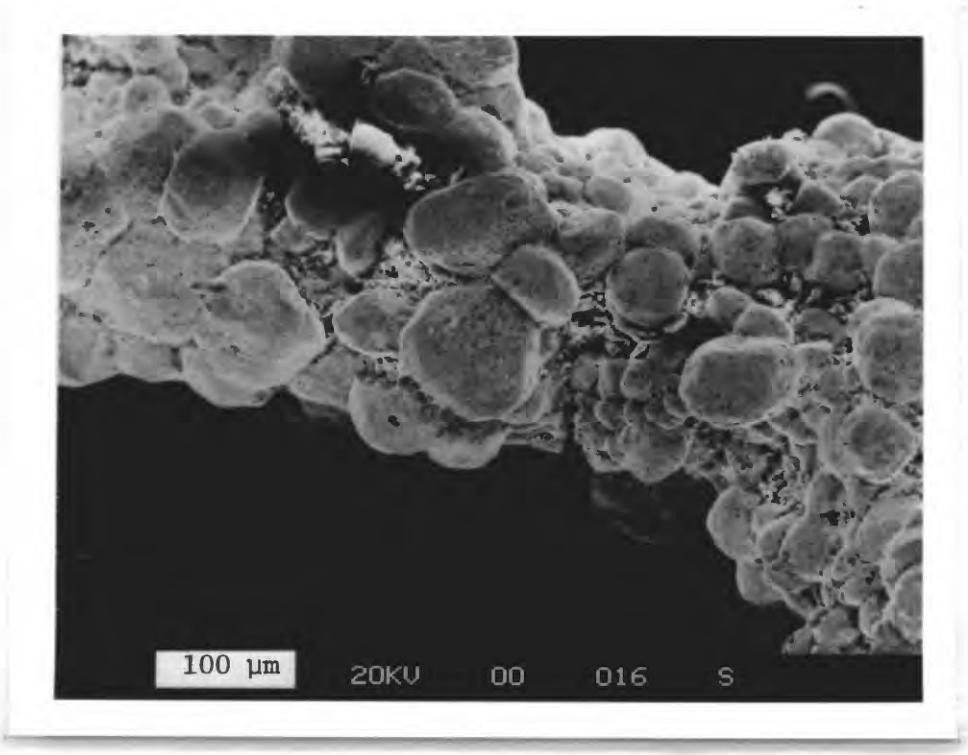

Figure 3.--SEM visualization of a tiny gold nugget found near Sacramento, California. The nugget consists of intergrown dodecahedra.