# Microbial precipitation of pedogenic calcite

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## ABSTRACT

**Pedogenic calcite in desert soils has become increasingly important as an indicator of paleoclimate, landscape stability, and landscape age. This study indicates that calcic and petrocalcic horizons in desert soils are not simply the result of inorganic precipitation of calcite. Soil microorganisms were found to be involved in calcite precipitation in a typical desert soil near Las Cruces, New Mexico. Fossilized remains of calcified fungal hyphae and *Microcodium* structures are abundant in the petrocalcic horizon. Soil bacteria and fungi precipitated calcite when cultured on a Ca-rich medium. In an experiment where soil columns were irrigated with Ca-rich solutions, calcite formed in soils containing soil microorganisms, but no calcite formed in sterile soils. Thus, biomineralization of calcite by soil microorganisms appears to be an important mechanism of unknown magnitude.**

## INTRODUCTION

The biomineralization of calcite by microorganisms has long been recognized in marine environments and more recently in lacustrine environments (Drew, 1911; Lalou, 1957; Thompson and Ferris, 1990; Buczynski and Chafetz, 1991). Calcite in arid soils, however, has generally been viewed as an inorganic process, whereby dissolved calcium carbonate crystallizes as the soil dries (Jenny, 1941; Doner and Lynn, 1989). Increasing evidence indicates that soil microorganisms may have played a substantial role in pedogenic calcite precipitation, in both modern soils and paleosols (Klappa, 1979; Callot et al., 1985; Phillips et al., 1987; Wright, 1986, 1990).

The focus on soil microorganisms has largely resulted from scanning electron microscopy (SEM) studies of calcareous terrestrial deposits. Such studies have revealed fossilized communities of soil microorganisms in samples from the Florida Keys (Kahle, 1977), the western Mediterranean area (Klappa, 1979), south Wales (Wright, 1986), southern Australia (Phillips et al., 1987), and the British West Indies (Jones, 1988).

Further evidence that microorganisms play a role in calcite precipitation is shown by ability of the bacteria to precipitate calcite in culture studies. Krumbein (1968) identified bacteria in a calcrete from Israel that could precipitate calcite when cultured in solid media. Boquet et al. (1973) found that many strains of bacteria, including *Salmonella* spp., *Bacillus pumilus*, and *Pseudomonas aeruginosa*, could form calcite crystals in 1 to 20 days on a medium containing calcium acetate.

In southern New Mexico, SEM and thin-section analyses revealed calcified filaments and *Microcodium* structures in petrocalcic horizons associated with soils of the lower La Mesa geomorphic surface (Chitale, 1986). These calcified filaments are similar in size and shape to filaments that Phillips et al. (1987) and Jones (1988) attributed to calcified fungal hyphae. The purpose of this study was to determine if soil microorganisms in a lower La Mesa soil are involved in calcite precipitation.

## METHODS

The soil is associated with the lower La Mesa surface in southern New Mexico (Fig. 1) and formed in noncalcareous alluvium deposited by the ancestral Rio Grande during middle Pleistocene time (Gile et al., 1981; Seager et al., 1987). The soil has a 55-cm-thick petrocalcic horizon that begins at 120 cm depth. The petrocalcic horizon has a stage IV morphology as defined by Gile et al. (1966). The soil is classified as a coarse-loamy, mixed, thermic Typic Haplargid, and is in the Rotura series.

Thin sections were made of epoxy-impregnated soil aggregates (peds) from 19 soil horizons extending to a depth of 389 cm. SEM analyses were made of broken peds, sputter coated with gold and viewed with a Philips scanning electron microscope operating at 7 to 15 kV.

The total number of soil microorganisms and the number of soil microorganisms that produced calcite were determined for three soil horizons by plating on a B-4 medium (Boquet et al., 1973). The B-4 medium consisted of 2.5 g calcium acetate, 4.0 g yeast extract, 15.0 g agar, and 10.0 g glucose per 1000 ml of distilled water. The pH was adjusted to 8.0 with NaOH. Triplicate spread plates were inoculated with soil-water dilutions ranging from $10^{-2}$ to $10^{-5}$. Plates were incubated at 32 °C and monitored for crystal formation by means of a stereo microscope. Microbially produced crystals were checked for effervescence with 1 *M* HCl and analyzed with X-ray diffraction (XRD). The total number of bacterial colonies

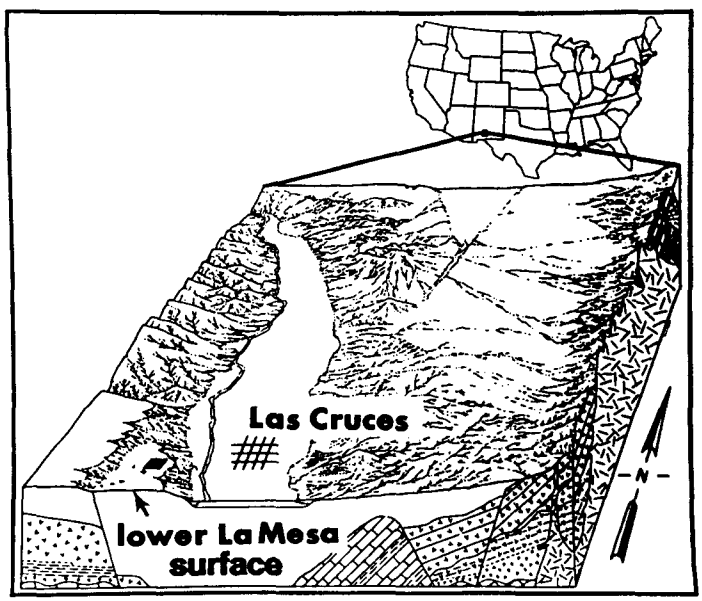


**Figure 1. Study area (black parallelogram) on lower La Mesa geomorphic surface (after Gile et al., 1981).**



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and the number of effervescent bacterial colonies (calcifying bacteria) were counted four days after inoculation. The total number of fungal colonies and the number of effervescent fungal colonies (calcifying fungi) were counted one month after inoculation.

A column experiment was designed to compare calcite precipitation in sterile soil with soil containing microorganisms (Fig. 2). Heated air (70 °C) was blown across the tops of four cotton-plugged columns to create a vapor-pressure gradient that caused $Ca^{2+}$ solutions to flow upward through the columns. Two solutions were used: a saturated $CaCO_3$ solution to simulate natural soil conditions and a 0.1 *M* $CaCl_2$ solution to supply larger amounts of $Ca^{2+}$. The columns were packed with sandy-loam soil taken from the Bk4 horizon (37 to 50 cm depth) of the lower La Mesa soil. The native calcite was removed with 0.5 *M* HCl, and the soil was adjusted to its natural pH of 8 with NaOH. A source of carbon for the microorganisms was provided by mixing 15 ml of a 1.6% nutrient-broth solution with the soil. The soil, solutions, and apparatus were sterilized by autoclaving twice at 121 °C for 90 min, 1 week apart. Soil microorganisms were added to two soil columns using 10 ml of a 1:10 soil-water dilution, whereas 10 ml of sterile water was added to the two sterile soil columns.

The column experiment ran for six weeks, during which time 92 ml of saturated $CaCO_3$ was used by both the inoculated and sterilized soil columns; 88 ml of 0.1 *M* $CaCl_2$ solution was used by the sterilized soil and 84 ml by the inoculated soil. After white crystals appeared, the soils were examined for effervescence with HCl, and the crystals were analyzed by XRD.

## RESULTS

Three lines of evidence were used to test if soil microorganisms are involved in calcite formation in the lower La Mesa soil: (1) the presence of calcified microorganisms in field samples, (2) the ability of present-day soil microorganisms to produce calcite when cultured on solid media, and (3) comparison of calcite formation in sterile and inoculated soil columns.

### Calcified Microorganisms in Field Samples

Calcified filaments, which were found in the calcic and petrocalcic horizons, are most common in the laminar zone of the petrocalcic horizon. In thin section, calcified filaments appear as needles, 1 to 5 μm in diameter and as much as 150 μm long (Fig. 3A). Calcified filaments were most common in voids, but in some cases filaments were also part of the micrite matrix in the petrocalcic horizon. SEM revealed that the filaments were tubular and coated with multiple microcrystals (Fig. 3B).

The calcified filaments appear to be fungal in origin. Their diameters are generally 4 μm, which is typical of fungal hyphae (Alexander, 1977). On the basis of the size of modern root hairs in the soil, which are generally 15 μm in diameter, the calcified filaments are too small to be calcified root hairs. Some calcified filaments branched (Fig. 3A) in a way characteristic of fungal hyphae (Trinci, 1984). The tubular nature of the filaments is probably the result of crystal formation on the exterior surfaces of hyphae, followed by the decomposition of the organic interiors (Klappa, 1979).

Circular structures that have bladed calcite crystals that radiate from center holes (Fig. 4) are common in the laminar zone of the petrocalcic horizon. These structures were interpreted by Chitale (1986) to be *Microcodium*. The *Microcodium* genus was created to designate these unusual calcite structures, thought to be organic in origin (see Klappa, 1978). Klappa (1978) interpreted *Microcodium* to be the product of mycorrhiza

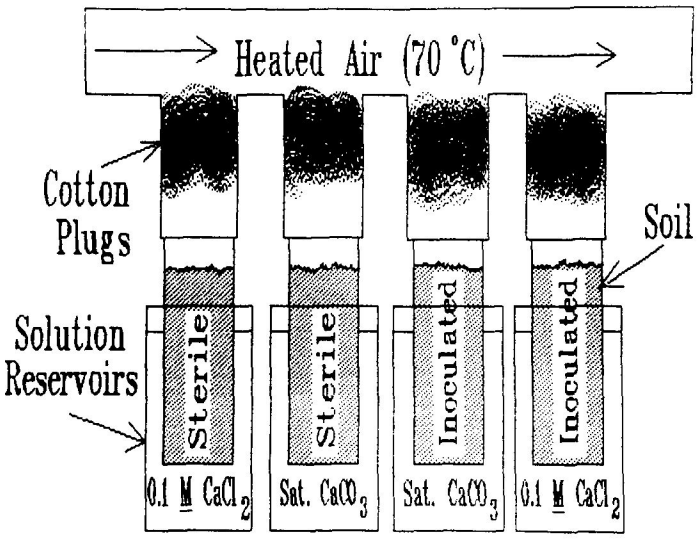


**Figure 2. Experiment apparatus used to compare calcite formation in sterile and inoculated soils. Bases of soil columns are immersed in two types of calcium-rich solutions: 0.1 *M* $CaCl_2$ and saturated $CaCO_3$.**

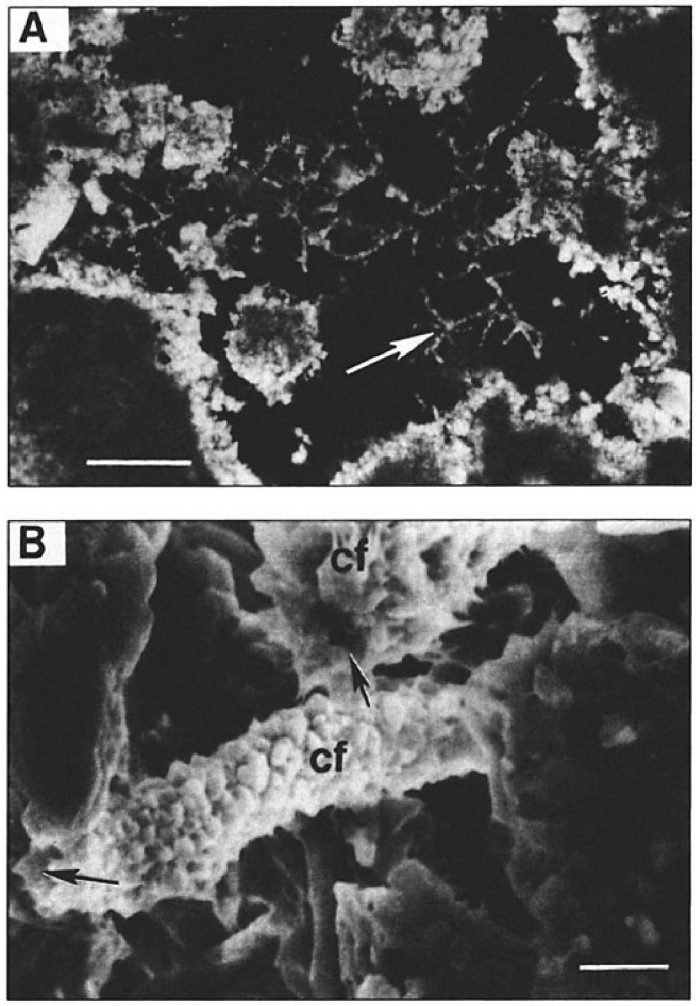


**Figure 3. Photomicrographs of calcified filaments in petrocalcic horizon. A: Thin section of hyphae-like calcified filaments (arrow; crossed polarizers; scale bar = 50 μm). B: SEM micrograph of calcified filaments (cf) illustrating their tubular nature (arrows; scale bar = 4 μm).**



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(the symbiotic association of fungi with roots). In the lower La Mesa soil, *Microcodium* structures range from about 5 to 30 μm in diameter, and are most easily identified when thin sections are stained with Alizarin Red S.

In the laminar zone of the petrocalcic horizon, many of the laminae are wavy and have a stromatolitic appearance, which led some early investigators to interpret desert calcretes to be of lacustrine, rather than pedogenic origin (e.g., Price et al., 1946). Although the wavy laminae are not algal reefs, their wavy nature and the abundance of *Microcodium* structures probably reflect substantial biomineralization that occurs atop the petrocalcic horizon, which is a zone where roots and water tend to concentrate.

### Culture Experiment

An experiment similar to that of Boquet et al. (1973) was conducted to determine if microorganisms of the lower La Mesa soil could produce calcite. Two days after inoculation, many bacterial colonies had developed white crystals (Fig. 5). Crystals were collected and examined with XRD, which confirmed that the crystals were calcite.

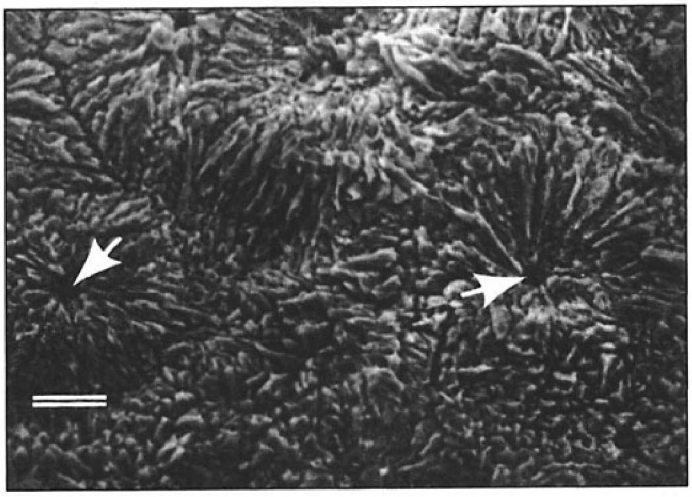

**Figure 4. SEM micrograph of *Microcodium* cross sections illustrating center holes (arrows) from which bladed calcite crystals radiate. Sample is from laminar zone of petrocalcic horizon. Scale bar = 8 μm.**

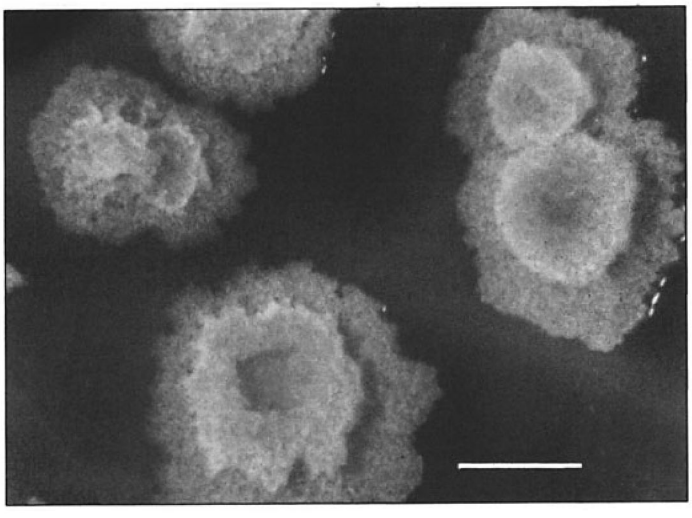

**Figure 5. Bacterial colonies covered with calcite crystals two days after inoculation with 1:1000 soil-water dilution. Scale bar = 0.25 mm.**

Table 1 shows the total number of bacteria and fungi and the number of calcifying bacteria and calcifying fungi. Thousands of microorganisms per gram of lower La Mesa soil produced calcite when grown on a calcium-rich medium. Even though less numerous, more fungi produced calcite than bacteria. However, fungal plates were incubated for one month because of their slower growth rate, whereas bacterial plates were incubated for only four days. Table 1 also shows that a larger proportion of bacteria and fungi in the petrocalcic horizon (Bkm2) were calcifying than in the overlying Bk1 and Bk4 horizons.

Although Boquet et al. (1973) demonstrated that soil bacteria produced calcite in culture, we expanded on their study to show that soil fungi could also produce calcite in culture and that calcified hyphae grown in culture resembled calcified hyphae in field samples.

Many studies dealing with marine microorganisms indicate that aragonite is produced rather than calcite (e.g., Oppenheimer, 1961); soil microorganisms, however, tend to produce calcite (Krumbein, 1968; Boquet et al., 1973; Phillips et al., 1987). In our study, only calcite was found by XRD analysis of microbially produced crystals in the laboratory, and calcite was the only carbonate mineral found in field samples.

### Soil Column Experiment

Two weeks after the beginning of the column experiment, the inoculated soil irrigated with 0.1 *M* $CaCl_2$ began to turn white. After 6 weeks, when the experiment was terminated, the inoculated $CaCl_2$ soil column was much whiter than its sterile counterpart. When the apparatus was disassembled, neither sterile column effervesced with 10% HCl, evidence that no calcite had formed, but both inoculated soil columns effervesced. Soil from the column irrigated with 0.1 *M* $CaCl_2$ was strongly effervescent, whereas soil from the inoculated column irrigated with the less-concentrated saturated $CaCO_3$ solution was only slightly effervescent. These results were confirmed by XRD, which revealed calcite in the inoculated soil irrigated with $CaCl_2$, and no calcite in its sterile counterpart.

To check for contamination from the surrounding environment, pour plates (B-4 medium; Boquet et al., 1973) were made of the two sterilized soil columns. A few bacteria were found at the $10^{-3}$ dilution. The bacteria might have penetrated through the cotton plugs that covered the soil columns. Nevertheless, compared to the soil inoculated with soil microorganisms, the low-level bacterial contamination had little effect on calcite precipitation.

The column experiment, along with the culture experiment and the fossilized microorganisms in field samples, indicates that soil microorganisms are involved in calcite precipitation. The formation of calcite in the inoculated column was probably caused by the greater amount of bicarbonate generated by microbial respiration (Arnott and Pautard,1970).

## CONCLUSIONS

With the exception of the work of Chitale (1986), most research dealing with calcified fungal filaments has been conducted on subaerially exposed carbonates in coastal regions (e.g., Kahle, 1977; Wright, 1986;

TABLE 1. DISTRIBUTION OF MICROORGANISMS IN VARIOUS HORIZONS OF THE LOWER LA MESA SOIL

| | | Bacteria | | Fungi | |
|---|---|---|---|---|---|
| Horizon | Depth (cm) | Total | Calcifying | Total | Calcifying |
| | | (organisms/g oven-dry soil x $10^3$) | | | |
| Bk1 | 5-16 | 6100 | 8 | 1000 | 630 |
| Bk4 | 37-50 | 1600 | 2 | 630 | 540 |
| Bkm2 | 136-153 | 36 | 14 | 2 | 2 |



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Jones, 1988). Our study, along with that of Chitale (1986), indicates that calcified fungal filaments also form in desert soils that were initially noncalcareous, and whose calcite can be attributed to pedogenesis.

Our study provides reinforcing evidence that soil microorganisms play a fundamental role in the genesis of calcic soils. Biomineralization of calcite by soil microorganisms has important implications for identifying subaerially exposed sediments in the geologic column (Wright, 1986). Calcified soil microorganisms, in addition, may serve as useful microfossils that reflect paleoclimatic conditions.

The mechanism whereby microorganisms produce calcite is probably related to excess $Ca^{2+}$ being excreted by microorganisms, which concentrates $Ca^{2+}$ on their external surfaces (Phillips et al., 1987). In addition, production of $CO_2$ by microbial respiration contributes to formation of bicarbonate ions (Arnott and Pautard, 1970), which prompts calcite precipitation. That carbonates exist in soils as stage I filaments and stage II cylindrical nodules (Gile et al., 1981) may reflect the shapes of rooting systems where microorganisms are most concentrated.

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