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Formation of Crystalline Deposits by Several Genera of the Family *Enterobacteriaceae*

WILLIAM E. KEEFE

Department of Microbiology, Virginia Commonwealth University, Richmond, Virginia 23298

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Several species of bacteria from the family *Enterobacteriaceae* formed crystalline materials containing calcium when grown in a defined culture medium. *Enterobacter aerogenes, Proteus vulgaris, Citrobacter freundii,* and *C. intermedius* produced calcium pyrophosphate crystals. *Edwardsiella tarda* and *Escherichia coli* formed calcite III crystals, whereas *Proteus mirabilis, Klebsiella pneumoniae, Providencia stuartii,* and *Serratia marcescens* produced hydroxyapatite crystals. Several of these bacteria have been isolated from the kidneys of patients with kidney stones, indicating that microorganisms may be involved in the enucleation process of kidney stone formation.

Recently, Ennever et al. (5) demonstrated that the bacterium *Bacterionema matruchottii* formed crystalline material when grown in a special medium. Because bacterial species of several genera of the family *Enterobacteriaceae* have been isolated from the kidneys of patients with kidney stones (2), it was of interest to determine whether crystalline deposits are unique to *Bacterionema* or are also characteristic of members of the family *Enterobacteriaceae*.

The medium was prepared by the method of Ennever et al. (5), then sterilized by filtration through membrane filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.), and dispensed into-250 ml flasks. Each flask was inoculated with one of the microorganisms under study, and the culture was allowed to grow from 6 to 11 days at 37°C. The cultures were harvested by centrifugation at 27,000 × *g* for 7 min at 5°C. The harvested cells were suspended in distilled water and centrifuged three more times. The washed pellet was placed in a porcelain crucible, air dried, and ashed either over a Bunsen burner or in a muffle furnace at 650°C. The residue was subjected to X-ray powder diffraction analysis (1) by means of a 114.59-mm Picker camera (Picker X-Ray Corp., Cleveland, Ohio) and a Cu K-alpha X-ray source operating at 36 keV and 10 mA.

Enterobacter aerogenes, Enterobacter cloacae, Proteus vulgaris, Citrobacter freundii, and *Citrobacter intermedius* all formed calcium pyrophosphate crystals (Table 1). Figure 1a shows the X-ray powder pattern for crystals from these bacteria. In comparison, an X-ray powder diffraction pattern of calcium pyrophosphate is presented in Fig. 1b. The X-ray diffraction pattern of the hydroxyapatite crystals formed by *Proteus mirabilis, Klebsiella pneumoniae, Providencia stuartii,* and *Serratia marcescens* is shown in Fig. 2a. Accompanying this is a pattern of a hydroxyapatite standard heated over a Bunsen burner (Fig. 2b). *Edwardsiella tarda* and *Escherichia coli* produced crystals of calcium carbonate type III (3). These crystals were identified using the Hannawalt method of

TABLE 1. *Crystalline material formed by Enterobacteriaceae*

Microorganism	Growth time (days)	Crystalline material
Enterobacter aerogenes[a]	16	Ca pyrophosphate
E. cloacae ATCC 15012	10	Ca pyrophosphate
Proteus vulgaris[a]	5	Ca pyrophosphate
Citrobacter freundii[a]	6	Ca pyrophosphate
C. intermedius[a]	7	Ca pyrophosphate
Serratia marcescens (wild type)[a]	7	Hydroxyapatite
S. marcescens (white mutant)[a]	7	Hydroxyapatite
Providencia stuartii[b]	7	Hydroxyapatite
Proteus mirabilis[a]	7	Hydroxyapatite
Klebsiella pneumoniae[a]	7	Hydroxyapatite
Edwardsiella tarda[a]	5	Calcite-III
Escherichia coli ATCC 10536	7	Calcite-III

[a] From the culture collection of the Department of Microbiology, Virginia Commonwealth University.

[b] From the culture collection of H. J. Welshimer of the Department of Microbiology, Virginia Commonwealth University.

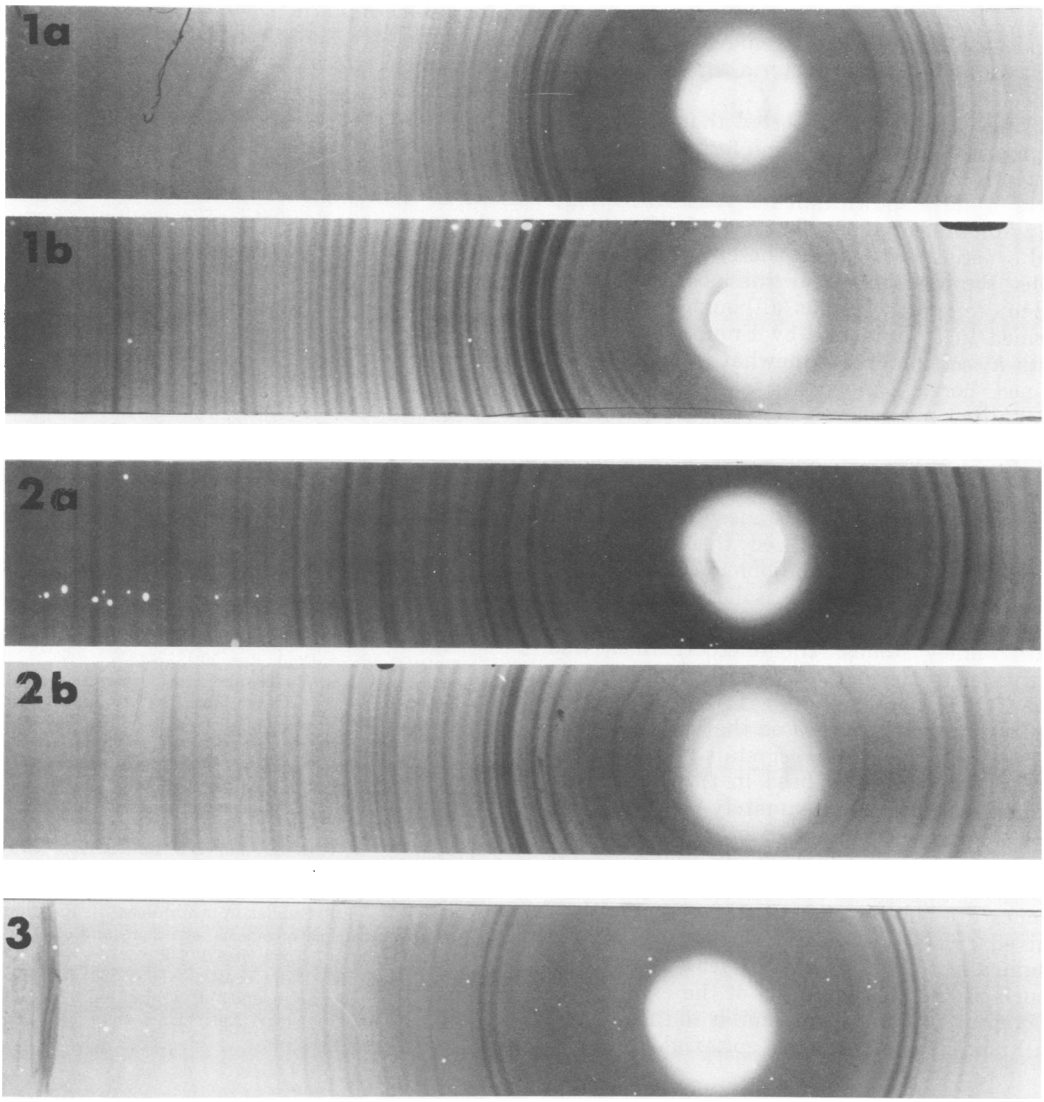

FIG. 1. *(a) X-ray diffraction picture of the material formed by C. intermedius. (b) X-ray diffraction picture of calcium pyrophosphate.*

FIG. 2. *(a) X-ray diffraction picture of the material formed by P. mirabilis. (b) X-ray diffraction picture obtained from a fused sample of hydroxyapatite.*

FIG. 3. *X-ray diffraction picture obtained from material formed by Edwardsiella tarda.*

comparing "d" values and line intensities of the unknown powder pattern with "d" values and line intensities of known compounds listed in the powder diffraction files published by the Joint Committee on Powder Diffraction Standard (Swarthmore, Pa.; see reference 9). The term "d" values refers to the interplanar spacing for a family of parallel planes of given indices in a crystal (1). The X-ray diffraction picture of the crystals formed by *Edwardsiella tarda* are shown in Fig. 3.

It should be noted that when these bacteria were cultured in nutrient broth, no crystalline residue was recovered. Repeated washing of the bacteria should have removed most if not all of the salts adhering to the surface of the bacteria. Moreover, approximately 200 ml of the culture medium was air dried and ashed, and the residue was subjected to powder diffraction analysis. The resulting pattern did not match those obtained from the bacterial residues. These observations confirm that the crystalline material

formed by the bacteria was different from that which would be produced from medium constituents adhering to the bacteria while they were collected and treated.

Ennever et al. (6) reported that under the appropriate conditions, *E. coli* forms crystalline material. Subsequently, Ennever and Summers (4) have shown that the yeast-like fungi, *Candida albicans*, also forms crystalline material when grown under these culture conditions. Parallel studies in this laboratory have led to similar conclusions, although the crystals formed in experiments by Ennever et al. (6) with *E. coli* differed somewhat from those obtained here. Ennever et al. identified the crystalline material as being hydroxyapatite, whereas in these studies *E. coli* formed calcite III. Because Ennever and his colleagues made use of a low-temperature ashing device, and in the present study both a Bunsen burner and a muffle furnace were utilized, the slight difference may be due to variations in the method of ashing. The *C. albicans* isolate used in this study was isolated from the urine of a patient with kidney stones. The microorganism was identified by the methods described in the *Manual of Clinical Microbiology* (11). Previous work in this laboratory indicated that *Pseudomonas aeruginosa* also forms crystals when grown under the conditions used in this study (unpublished data). Approximately 80% of all kidney stones are composed of either calcium oxalate or calcium phosphate (7, 10). Cell constants for the crystalline materials found in kidney stones are similar enough that the growth of mixed crystals in kidney stones may be explained in terms of epitaxial growth (8). In as much as the cell constants of the crystals observed in this study are similar to those found in kidney stone material, epitaxial growth of kidney stone material upon these bacteriologically formed crystals may be possible. Thus, because the bacteria *P. mirabilis*, *E. aerogenes*, *E. coli*, *P. aeruginosa* and the yeast-like fungus *C. albicans* have the capability for the development of crystalline materials and, since these microorganisms have been isolated from the urine of kidney stone patients (2), I suggest that crystalline deposits of microbial origin might act as loci for the formation of kidney stones.

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