Formation of bobierite (magnesium phosphate) crystal aggregates by *Acinetobacter* sp.

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Abstract

The formation of extracellular crystal aggregates of bobierite ($\text{Mg}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$) by *Acinetobacter* sp. is found with the use of B-17 medium. The crystal aggregates were observed in the colonies as deposits of brown-yellow coloration, and were identified by X-ray powder diffraction, chemical analysis, infrared spectra and scanning electron microscopy. The production of bobierite by soil bacteria may imply further versatility of the crystals formation by microorganisms.

Introduction

Bacteria are able to form extracellular crystals of calcium carbonate in liquid or solid media under certain growth conditions, (McCallum and Guhathakurta, 1970; Boquet *et al.*, 1973; Morita, 1980).

Intra and extracellular precipitation of crystalline phosphate compounds such as apatite (Ennever *et al.*, 1974; Streckfuss *et al.*, 1974) and struvite (Beavon and Heatley, 1962; Rivadeneyra *et al.*, 1983) have also been reported.

Bobierite is a magnesium phosphate with eight molecules of water ($\text{Mg}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$). This mineral was found by Bobierre in 1868, and was reported as a component of products which occurred in the different natural environments such as guano and kidney stones (Dana and Dana, 1960; Bastian and Gebhardt, 1976). However, the formation of bobierite by bacteria has never been reported. This paper reports the production of bobierite in a bacterial strain isolated from soil.

Materials and methods

Bacteria: The experiments were performed with 96 bacterial strains isolated from soil, all of these bacterial strains have the capacity to form calcium carbonate. Crystals formation in both solid and liquid B-17 medium was studied in all these strains.

Medium: The culture medium used in this study of crystal formation was the medium

B-17 designed by Rivadeneyra *et al.* (1983). It has the following composition per litre: yeast extract 4g, Mg acetate 2.4 g and K nitrate 0.5 g. The pH was adjusted to 8 using 0.1M NaOH and sterilized at 121°C for 20 min. The solid medium was prepared by adding 1.6% of Bacto-Agar (DIFCO).

The experiments, which were carried out in triplicate, were repeated 3 times which means that they were carried out 9 times for each medium (solid and liquid). After inoculation, the cultures were incubated aerobically at 27°C and the crystal formation was periodically observed macroscopically and microscopically up to 35 days. All experiments included two different kinds of control, one consisting of uninoculated culture medium and the other of medium inoculated with autoclaved bacterial cells.

Separation, purification and identification of crystals formed

The methods for separation and purification of crystals were the same as those used by Rivadeneyra *et al.* (1983).

The crystal structure was identified by the X-ray powder diffraction method and the purified crystals were also examined under an ISI Super Mini-SEM (scanning electron microscope), after being shadowed with gold.

The identification of the crystal was made by infrared spectroscopy and chemical analysis (both the chemical analysis and infrared spectra measurements were carried out in the Department of Analytical Chemistry, Faculty of Science, University of Extremadura, Badajoz. Spain by Dr. Berzas-Nevado).

Results and discussion

Only 21 out of the 96 strains studied were able to form crystal compounds, 20 of them formed struvite and only one strain, classified according to Bergey's Manual of Systematic Bacteriology (Juni, 1984) as *Acinetobacter* sp., showed extracellular aggregates of crystals, other than struvite, in both liquid and solid media and each time the experiment was carried out. These aggregates in solid medium are observed macroscopically in the colonies as deposit of brown-yellow color.

The crystal aggregates were identified as bobierite by X-ray diffraction (Fig. 1) according to the Joint Committee on Powder Diffraction Standards (Berry, 1974) criteria. However the diffraction peaks 6.75, 4.32, and 2.52 Å do not correspond exactly to those of bobierite, and neither do the diffraction intensities at 3.15, 2.68 and 2.41 Å fit with those in the J.C.P.D.S. card. These results show that the crystals may include some impure substances with bobierite.

The chemical analysis of the crystal showed the following composition in wt. %:

FIG. 1. X-ray powder diffraction pattern from the crystal aggregates formed by *Acinetobacter* sp. in B-17 medium. The crystals were identified as bobierrite, although the peaks marked by arrows on the diagram do not coincide with the J.C.P.D.S. pattern of bobierrite.

P_2O_5 32.6, MgO 28.9, CaO 0.12 and NH_4^+ 0.39. The infrared spectra (Fig. 2) also showed several bands which might be assigned to a hydrated phosphate compound. The bands at 1065, 1045 and 995 cm^{-1} may be assigned to the ν_3 vibration mode from orthophosphate groups, the band at 950 cm^{-1} might be assigned to the ν_1 mode of phosphate ions and the band at 590 cm^{-1} might be assigned to the ν_4 mode. The broad and strong bands, with the maxima at 3510 and about 3160 cm^{-1} and another one at 1630 cm^{-1} may be assigned to the water. The bands at 840 and 700 cm^{-1} cannot be assigned to the orthophosphate groups.

The scanning electron microscopy (Figs. 3a and b) showed that the precipitates are composed of microcrystalline aggregates. Bobierrite separated from both the liquid and solid media possessed identical characteristics.

FIG. 2. Infrared spectra taken from the crystal aggregates produced by *Acinetobacter* sp. in B-17 medium.

FIG. 3. Scanning electron micrographs of bobierrite. Bar in A and B correspond to 14.2 μm and 5 μm , respectively.

The formation of extracellular crystals in bacterial cultures has been reported by several authors (Greenfield, 1963; Oppenheimer, 1961; Shinano and Sakai, 1975; Rivadeneyra *et al.*, 1985). Studies in our laboratory have established the optimum culture conditions (media, pH, etc.) for crystal formations by soil bacteria, but the formation of bobierrite has never been observed before as a consequence of bacterial growth.

The B-17 laboratory medium is especially suitable for the formation of struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$), and is normally used here for this purpose. The production of bobierrite by soil *Acinetobacter* sp. implies further versatility of crystal formation by microorganisms.

It is known that bacteria play an important role in the calcium carbonate and struvite precipitation in natural environment, therefore the possibility of a similar role in the formation of bobierrite may be considered.

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