

# Role of bacteria in the growth of cave pearls

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## Abstract

The growth of micritic cave pearls have been studied based on ones collected in Perlova Cave (Slovakia). The pearls display rough surfaces and irregular internal lamination. Several living bacteria have been detected inside the biofilm which covered still growing cave pearls. These bacteria produce organic matter from inorganic, gaseous  $\text{CO}_2$  dissolved in water and hence cause oversaturation with respect to calcite within the bacterial surroundings. Thus calcite precipitation is due to the bacterial metabolism. SEM investigations indicate that the precipitation proceeds upon the surfaces of the bacterial cells. This process results in mineral replicas of bacterial cells and finally causes almost complete obliteration of primary microbial structures. The bacteria uptake preferentially  $^{16}\text{O}$  and cause relative enrichment of heavier isotope ( $^{18}\text{O}$ ) in the bacterial surroundings and in precipitating calcite.

## Introduction

Cave pearls, known also as cave pisoids, have been reported in literature for a long time (HILL & FORTI, 1997). This group of speleothems includes a broad spectrum of grains varying in shape and internal structure. Best known are the forms with smooth, lustrous surface and regular concentric lamination. The smooth and shining outer surface of these pisoids is related to abrasion on contacts with neighbouring grains and substrate (BAKER & FROSTICK, 1947). Additional recognised prerequisites for their growth include the presence of suitable nuclei for the grain growth, supersaturated state of the solution from which the grains crystallise, and constant, balanced supply of water to the environment of their growth (GRADZIŃSKI & RADOMSKI, 1967; DONAHUE, 1969).

Many authors have pointed out that cave pisoids include also other forms, namely ones whose internal structure is much less regular (BAKER & FROSTICK, 1947; THRAIKILL, 1963, 1976; GRADZIŃSKI & RADOMSKI, 1967; DONAHUE, 1969; JONES & MACDONALD, 1989). Such pisoids have uneven, rough surfaces. No coherent concept of their origin has been hitherto proposed. The aim of this paper is to explain the origin of the irregular cave pisoids in the context of their growth environment.

## Material and methods

Of the 21 caves from which cave pearls have been studied, the best conditions for complex study on their growth were found in the recently discovered and relatively little visited Perlova cave (Perlová jaskyňa). Analyses made with the aim of explaining the conditions of pearl growth included chemistry and isotopic composition of water as well as chemical and mineral composition, stable isotopes ratios and internal structures of the pearls. Moreover, some pearls were aseptically collected and delivered to a microbiological laboratory, where micro-organisms were isolated and identified in biochemical tests.

## Speleological setting

Perlova Cave is situated at altitude of 910 m in the Mala Fatra mountain group (Central Carpathians, Slovakia; Figure 1) (MRÁZIK, 1987; HOLÚBEK & KLESKEŇ, 1993). The area above the cave is covered with deciduous forest, and the rock mass above the cave is about 10 m thick. The temperature in the cave varies between 5.1 and 6.8 °C. Water collects in stepped gour pools, from about 15 cm to more than 1 m wide, and a few centimetres deep (Figure 2). The water is supplied by drip from the ceiling and walls and by overflow from higher gour pools to the lower ones. Drops fall from the height of only 1.5 m or less, so the



Figure 1 : Location of Perlova cave

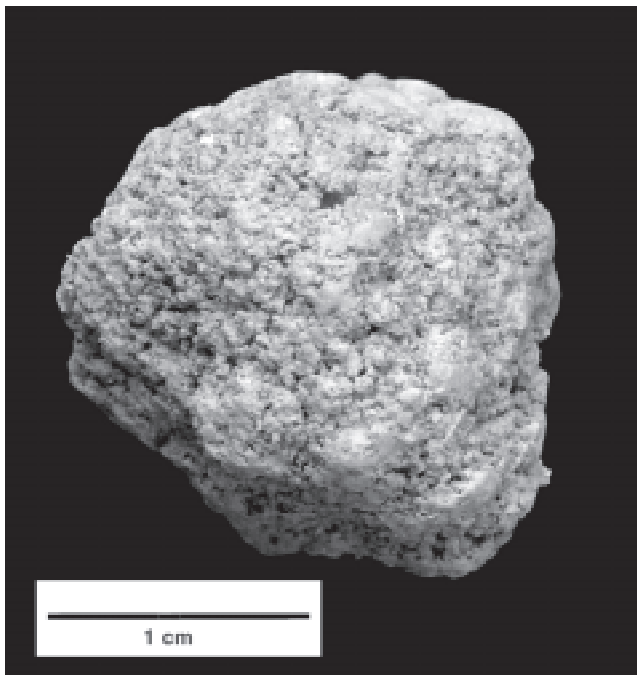


Figure 2 : Stepped gour pools with irregular cave pearls

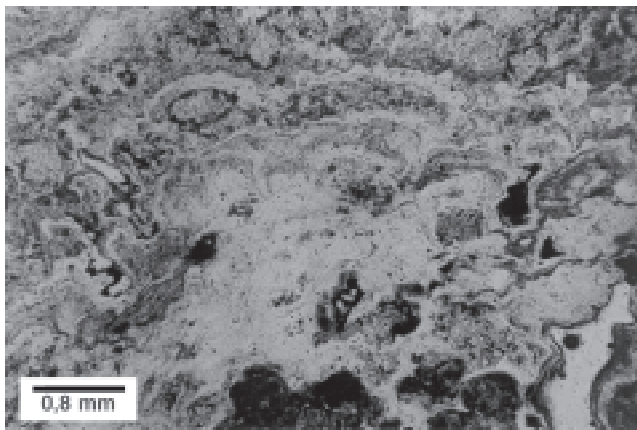
water in the pools is nearly stagnant. The intact fragile moonmilk gours testify that the flow of water is never violent. Calculations made on the base of the results of the chemical analyses, using WATEQ program, have shown that the water is supersaturated with respect to both, calcite and aragonite. Some of the gour pools contain more than a hundred pisoids, from a few millimetres up to more than 3 cm in size (Figures 2, 3). No pisoids were found cemented to the bottom.

## Results

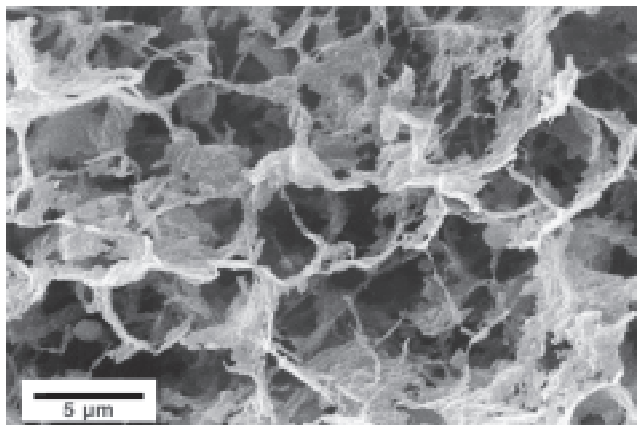
The irregular pisoids from Perlova cave have rough outer surface, no nuclei, subtle and irregular lamination and no corrosional surfaces in their internal structure (Figure 3, 4). They are built of low-Mg calcite and contain up to 5.5 weight % of non-carbonate admixture and up to 0.46 weight % of organic carbon. Observations in light microscope and SEM have shown that these pisoids are built mainly of micritic calcite. Abundant cavities between the crystals make for the high porosity (above 80%) and low density (up to 1.4 g/cm<sup>3</sup>) of the pearls of this kind.



**Figure 3 : Irregular cave pearl, note the distinct rough outer surface**



**Figure 4 : Cross section through irregular cave pearl, internal lamination is visible; transmitted light, II nicols**

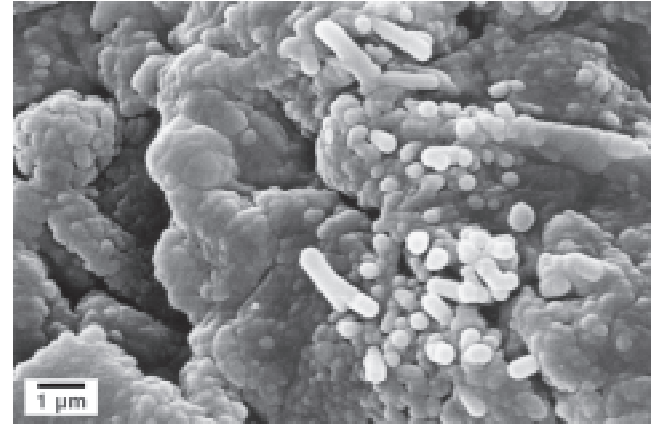


**Figure 5 : Biofilm covering the surface of irregular cave pearl, SEM image**

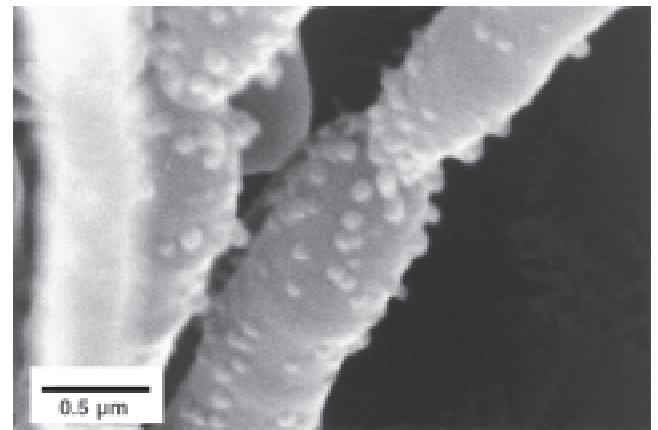
The outer surfaces of these pearls are covered with a thin mucous, spongy layer – biofilm (Figure 5). It is built of live microbial cells, their extracellular polymeric substances and a mineral fraction. The mineral fraction consists mainly of calcitic replicas of microbes. The replicas are identical in size and shape with the living cells (Figures, 6, 7). The living cells and their replicas occur together

with regularly shaped calcite crystals in the external parts of the pearls. Such crystals are the main component of the inner parts of the pearls.

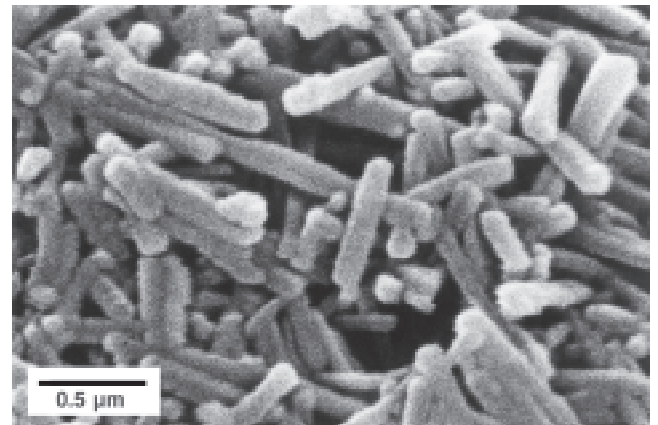
Microbiological study revealed the presence of various bacteria within the studied pearls. All studied samples included hydrogen-oxidising bacteria (knaegas bacteria) and dinitrogen-fixing bacteria. The most common of the first group were *Xanthobacter autotrophicus* and *X. flavus*, and of the second group – *Arthrobacter crystallopoietes*.



**Figure 6 : Bacterial fabrics of irregular cave pearls**



**Figure 7 : Living (?) bacterial cells overgrown with minute calcite crystals**

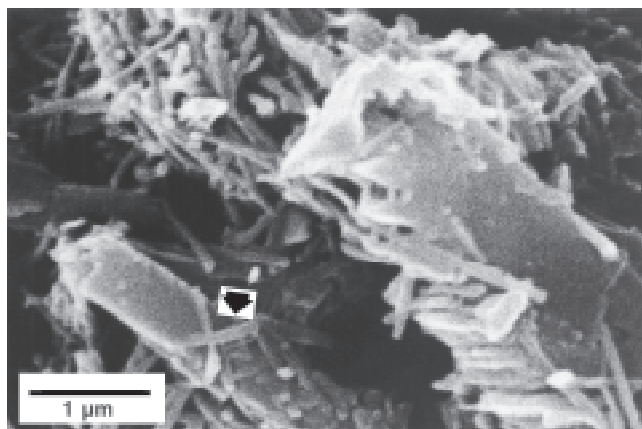


**Figure 8 : Calcified replicas of bacterial cells**

## **Growth of irregular cave pearls**

### **Process of calcification**

The presence of calcitic replicas of bacterial cells shows that calcification occurs in living cells or simultaneously with their death (cf. JONES & KAHLE, 1986). In the next stage, the replicas become



**Figure 9 : Micrite calcite crystal with “ghosts” of bacterial replicas (arrow)**

the nuclei of crystal growth and are successively overgrown with calcite crystals (cf. GUO & RIDING, 1994). The final stage of this process are regular crystals of calcite micrite with few „ghosts” of the microbial replicas. The process of calcification obliterates the primary bacterial fabric (cf. SZULC & SMYK, 1994, GRADZIŃSKI et al., 1997).

#### Mechanism of calcification

Calcification is caused by disturbance of chemical balance within the pearls and the its surrounding biofilm. This disturbance results from microbial physiological processes. The main role is played by chemolithoautotrophic hydrogen-oxidising bacteria (cf. ARAGNO & SCHLEGEL, 1992), which cause extracellular secretion of calcite by consumption of CO<sub>2</sub> (cf. SIMKISS, 1986). This process is active in conditions of high carbonate alkalinity and availability of Ca<sup>++</sup> ions (cf. KEMPE & KAŻMIERCZAK, 1990; MERTZ, 1992). The hydrogen-oxidising bacteria are indirectly dependent on the supply of organic matter because the hydrogen they use is a by-product of heterotrophic dinitrogen-fixing bacteria (EADY, 1992; ARAGNO & SCHLEGEL, 1992). The hydrogen-oxidising bacteria absorb preferentially the light oxygen isotope (<sup>16</sup>O) in CO<sub>2</sub> or O<sub>2</sub>. This results in a relative enrichment of their immediate surrounding, including the extracellular calcite, in the heavier isotope (<sup>18</sup>O).

#### The role of biofilm

The biofilm on the surface of growing pearls controls the rate of ion diffusion from the environment (cf. DECHO, 2000). It is due to this action that only calcite is precipitated though the water in which the pisoids grow is supersaturated with respect to calcite and aragonite (cf. BUCZYŃSKI & CHAFETZ, 1991). The biofilm causes also mechanical trapping and binding of mineral grains (CUNNINGHAM et al., 1995; JONES, 1995). As a result, many non-carbonate mineral grains are incorporated into the pearls. The biofilm insulates the pearl surfaces, protecting them in times when environmental conditions become unfavourable, for instance during drops in the pH of the environment, securing the uninterrupted growth of the pearls.

#### Growth conditions of the irregular cave pearls

The development of the irregular cave pearls proceeds in conditions of low energy of the environment, which enable growth of the delicate biofilm on their surfaces (cf. LORCH & OTTOW, 1985; PEDLEY, 1992). Growth of such pearls begins on a floating fragment of biofilm, i.e. a cluster of living bacterial cells (PAERL, 1975). Their further growth in the low-energy environment is due to a back-feeding process. Biogenic calcification controls the growth of porous internal fabric of the pearls, which, in turn, enables the growth of the pearls in the low-energy environment (cf. FOLK & CHAFETZ, 1983; VERRECCHIA et al., 1997).

#### Conclusions

1. Irregular cave pearls grow due to biogenic calcification caused by physiological processes in the hydrogen-oxidising bacteria.
2. The growth of these pearls proceeds in a low-energy environment.
3. Calcification requires availability of Ca<sup>++</sup> ions, organic carbon and high carbonate alkalinity.
4. Processes of biogenic mineralisation result in precipitation of calcite as the only mineral phase and control preferential enrichment of calcite in the heavy oxygen isotope.

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