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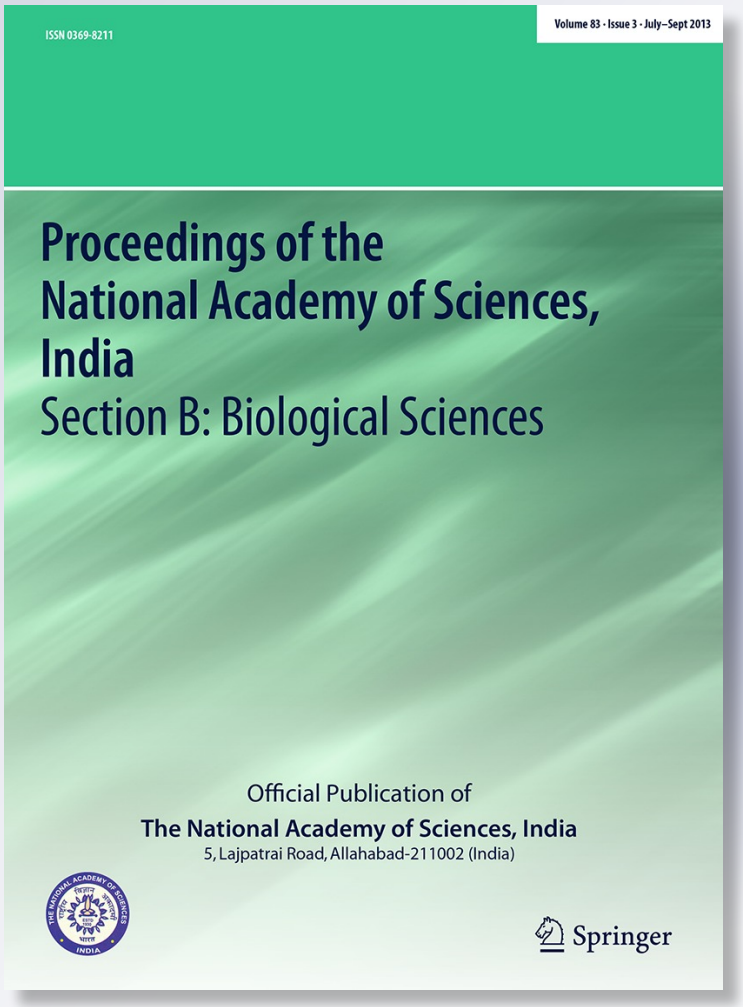

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REVIEW

Insights into Cave Architecture and the Role of Bacterial Biofilm

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Abstract Caves offer a stable and protected environment from harsh and changing outside conditions. They lend living proof of the presence of minute life forms that delve deep within the earth's crust where the possibility of life seems impossible. Devoid of all light sources and lacking the most common source of energy supplied through photosynthesis, the mysterious microbial kingdom in caves are consequently dependent upon alternative sources of energy derived from the surrounding atmosphere, minerals and rocks. There are a number of features that can be observed within a cave that may serve as evidence of microbial activity, for example, formation of biofilms comprised of multiple layers of microbial communities held together by protective gel-like polymers which form complex structures. Different bacterial biofilms can develop on the walls of the cave which can be visually distinguished by their colorations. Moreover, the pH generated by the metabolism of bacterial biofilm on the cave environment can lead to precipitation or dissolution of minerals in caves. Caves also offer an excellent scenario for studying biomineralization processes. The findings on the association of bacteria with secondary minerals as mentioned in this review will help to expand the existing knowledge in geomicrobiology and specifically on the influence of microorganisms in the formation of cave deposits. This paper reviews the current state of knowledge of biospeleology of caves and the associated bacterial biofilms. Recommendations for future research are mentioned to encourage a drift from qualitative studies to more experimental studies.

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Introduction

Life on earth has been microscopic for much of its 3.7 billion year history [1]. Life exists almost everywhere on earth. Presence of liquid water is a prerequisite for life. Microorganisms are ubiquitous in and on earth and can be found in almost any type of environment from clement to harsh [2]. In fact, the only places where they have not been found seem to be the places where researchers have not specifically looked for these microbes. Excluding those that live in or upon animals or plants, these organisms are also intimately tied into the geosphere, playing a major role in the dynamic processes that shape the earth. Such organisms that resist harsh physical and chemical conditions in their habitats are termed 'extremophiles' [3]. Nonetheless, the metabolic activity of these organisms has left its mark on every conceivable planetary structure, from isotopic fractionation of ore deposits in the deep subsurface to the oxygenation of the atmosphere [1, 4]. Such metabolic activities continue to be critically important in the maintenance of the biosphere, where microorganisms sustain higher forms of life through primary production, nitrogen fixation and organic carbon mineralization [5]. Despite the planetary evolution of bio- and geospheres, historically, researchers tended to ignore microbial activity in geological environments due to inability to explain many geochemical inorganic chemistries and the inability to culture microorganisms from these sites [6]. Eventually, these limitations were removed with the development of molecular scale geochemistry which allowed investigators to examine such environments without the need for cultivation [4, 7–9].

Microbial processes occurring in the absence of light have often been considered insufficient to support ecosystem level processes. The previous dogma was that life processes could not exist in complete darkness or without the input of photosynthetically generated energy [10]. However, since the discovery of the chemo-lithoautotrophic ecosystems in the hydrothermal vent systems in the ocean, it has become clear that the absence of light is not a limiting factor, since reactive mineral surfaces and solute-rich spring waters may provide energy sources sufficient for chemolithoautotrophs in the subsurface [11, 12]. Presently, chemolithoautotrophy is recognized as an important ecosystem-level process in aphotic terrestrial environments including deep aquifers [11] and caves [13–16]. Generally, complex microbial communities develop in natural environments. Recent methodological advances applying molecular techniques have permitted the survey of these communities and today, there has been acceptable knowledge of the major components of these communities. Nevertheless, in most cases, the consequences of the metabolism of these bacteria on the natural substrate (rock, soil, waters) remain to be proposed and understood. Seminal studies by Costerton et al. [17] have shown that microbial biofilms can greatly affect the substrates on which they grow by altering the microenvironments under the films to enhance corrosion and degradation of many materials.

The field of geomicrobiology has grown dramatically over the last decade and a true review of the work done would encompass several thick volumes. In this context, caves offer examples of possible past or present geomicrobiological interactions [18]. The present review, therefore, is intended to highlight the most recent and exciting works of significance in the field within the context of the founding studies. The study encompasses appropriate literature of earlier studies and details of the basic principles regarding involvement of biofilm bacteria with the cave architecture and mechanisms of interaction between microorganisms and minerals. In addition, this review focuses mainly on bacteria, since bulk of the research has been done in this group.

Cave and Its Architecture

The term of "cave" is defined as any natural space below the earth's surface that extends beyond the twilight zone, and is accessible to humans [19]. Caves have always haunted the imagination of mankind from the start of human history. These natural formations provided shelter to early man and were sought after earnestly, to provide a 'safe home' [20]. They host a wide spectrum of fascinating life forms, such as cave-dwelling spiders, leeches, mites, beetles, scorpions, fishes, snails and worms along with thick beds of bacteria and fungi that live on the rocks [21]. Caves, with limited exception, form through the erosional processes of water [22]. By the time caves are enlarged sufficiently to allow human access, the water has (generally) departed, leaving the cave exposed to an oxygenated atmosphere [23]. Entering a cave, one goes through a series of zones, beginning with an entrance zone that is strongly impacted by surface conditions. Deeper into a cave is the twilight zone, where limited light penetrates and surface conditions are ameliorated by cave conditions. In the deep cave, there is absence of light, temperature that is at or near the MAST (mean annual surface temperature) for the region, and high humidity [24].

Most of common types of caves in karst regions are those formed in limestone and other calcareous rocks, and as lava tubes in basaltic rock. Remaining types, including those formed in gypsum, granite, talus, quartzite, ice, and sandstone are usually limited in extent [25]. The adjoining geophysical architecture and climatic conditions always act as proximate factors that regulate the internal environmental conditions of any cave. Nevertheless, the cave (hypogean) ecosystem remains more or less stable compared to its ambient epigean environmental niche. Perpetual darkness, high humidity, almost constant temperature, low airflow and higher CO_2 concentrations altogether make the subterranean ecosystem a unique niche. The biota of the caves stays tuned with its ecological regime for successful survival. The biodiversity of the karst ecosystem is highly restrictive, i.e. species existing in a particular cave system may be restricted to that ecosystem only and perhaps completely absent in other adjacent caves. Caves are generally considered as extreme environments for life [26]. They are mostly resource limited due to the prevailing aphotic conditions and subsequently there is no photosynthesis, thus making most cave ecosystems to depend on allochthonous organic materials for energy [27, 28]. The entry of dripping, seeping and flowing water brings energy into the caves. Groundwater discharges as springs into the passages of some caves [13, 29–31] also serve as high energy yielding substrates for some microorganisms. Any natural/unnatural disturbances that can alter/modify the originality of the cave ecosystem could become a major deciding factor to destroys/alters its complete biodiversity. Natural geological disasters (i.e. collapse, wall dislocations, etc.), climatic abnormalities (long period of droughts, inundations) and mostly human encroachments (mining/quarrying, tourism and waste disposal) are some of the factors that are responsible for the same [32]. The entrance corridors where light penetrates the cave only has life supported by photosynthetic activity [33]. Alternatives to carbon fixation include chemoautotrophy and ammonium-, nitrite-, sulphur-, manganese- or iron-oxidizing chemolithoautotrophy [24].

Role of Bacterial Diversity in Caves

In the past decade, cave microbiology has emerged as one of the frontier areas of geobiology research involving the coordinated efforts of microbiologists, chemists and geologists to address challenging questions regarding microbial metabolism, biogeochemistry and their role in mineral precipitation/dissolution [20]. Bacterial communities in caves are known to acquire energy by transforming aromatic compounds, fixing gases, and oxidizing reduced metals within rocks. By their interaction with minerals, microbial species play an important role in reshaping the mineral environment of caves and helps form features such as stalactites, stalagmites and various cave wall deposits [20]. Speleothems are formed by a physicochemical reaction from primary mineral in a cave [34–36]. Reduced compounds in cave wall rock can be microbially oxidized to form secondary mineral deposits on top of the biofilm, dissolved rock underneath the biofilm, and acidic microenvironmental waters [36]. The metabolic processes of sulfur-, iron- and manganese-oxidizing bacteria [37] can generate considerable acidity, dissolving cave walls and formations [16, 38]. This leads to the formation of sharp redox boundaries at the microbe–mineral interface as the microorganisms use elements from the geological matrix of the cave wall to produce energy in this organic nutrient-limited environment [38]. These biogenic minerals range from carbonates (moonmilk), silicates, clays, iron and manganese oxides, to sulfur, and saltpeter (potassium nitrate) at scales ranging from microscopic to macroscopic sizes [39]. Many microbes have been identified to be related to dissolution and precipitation reactions that involves carbonates, moonmilk, silicates, clays, iron, manganese, sulfur, and saltpeter [24].

Calcium carbonate speleothems dominate in most known caves of the world and a number of geomicrobiological studies have been reported in literature on stalactites, stalagmites, helictites, moonmilk, pool fingers and cave pearls [20]. Microorganisms impact significantly their environments and in turn microbial communities are affected and shaped by the geochemistry of their environment [40]. One convincing evidence of microbial involvement comes from the formation of moonmilk which is a generic term for a soft, wet, plastic, fine grained speleothem present on the walls of many caves [40]. Biotic [41] and abiotic [42] processes have been postulated for the origin of moonmilk. Moonmilk from carbonate speleothem types has been frequently considered to be of microbial origin [43], either by direct precipitation of calcite by microorganisms or by formation of a nucleation surface on which minerals precipitate [24]. Evidence of microbial activities related with calcite moonmilk deposits have been reported [41]. However, the role that microbes play in moonmilk genesis is an unresolved issue in geomicrobiology. Calcite moonmilk is often composed of fine, microcrystalline fibers [40]. These calcite fibers are similar to those described in calcretes, eolianites, beach-rock cements, and other vadose settings and are reported in the literature with different descriptive terms such as lublinite, needle fibers, or calcite en aiguilles [44–46]. In these cases, fabrics usually appear altered by constructive and/or destructive diagenetic processes, hindering the study of their formation mechanism.

Filamentous bacteria dominate subaqueous cave microbial mats, and from phylogenetic analyses, stable isotope evidence, and aqueous geochemistry surveys, populations are considered to be chemolithoautotrophic, aerobic to microaerophilic sulfur-oxidizing bacteria [13, 30, 31, 47]. Species composition of organic covers from the walls in the cave Planinska jama were identified by Megušar and Sket [48] and found that the majority of bacteria belonged to Gram positive cocci, rods and pleomorphic shaped cells. Several bacterial isolates such as *Bacillus subtilis, Bacillus cereus, Bacterium brevi, Proac-tinomyces polychromogenes* were retrieved in laboratory conditions. Based on the morphology and biochemical tests, Mulec et al. [49] identified different bacterial groups in silver flashing droplets known as cave silver, in a pond with calcite rafts and in weathered limestone. In all of the studied microenvironments, fluorescent pseudomonads seemed to be the prevalent bacteria which can be due to their versatile metabolic pathways [50]. Various types of mats in caves are largely made up of actinomycetes [51]. In permanent drips with high biodiversity of meiofauna in Škocjanske jame, Gerič et al. [52] established that bacterial communities contained low proportions of Gram-positive bacteria with high incidence of Enterobacteriaceae and Vibrionaceae and practically no culturable actinomycetes. Recently, two cultivation-independent studies based on the phylogeny of bacterial community 16S rRNA genes characterized filamentous microbial mats from the Sulphur River of Parker cave, Kentucky, and Cesspool cave, Virginia [30, 53]. In both 16S rRNA gene libraries, most clones were affiliated with uncharacterized environmental groups within the "Epsilonproteobacteria".

Bacterial Biofilms

Biofilms are collectives of one or more species of microorganisms [54]. They provide protection for growth, enabling microorganisms to survive hostile environments [55] and are significant in sediment stabilization and construction [56]. When microorganisms adhere to a surface, their immobilized cells grow, replicate and secrete extracellular polymeric substances (EPS) that engulf them in a gelatinous matrix [57]. Natural biofilms can be highly organized, may encompass one or several species, and can form a single layer, a three-dimensional structure or even aggregates [58, 59]. Caves with dim natural light, and

lighted hypogean environments, have been found to host diverse phototrophic microorganisms which group themselves into biofilms associated with rock surfaces [54].

Scanning electron microscopy of the surface of speleothems commonly reveals a variety of spheroid or filamentous features, interpreted as either microorganisms or biofilms (mucous/EPS) [14, 44, 60–62]. Mucus-like biofilms which are thick orange microbial mats with patches of yellow biofilms were reported floating on the spring waters on aphotic deep cave orifice in Borra caves, Visakhapatnam, India [18]. *Epsilon-* and *Gammaproteobacteria* were found to be important biofilm-forming groups in sulphidic springs and streams of Parker cave (Kentucky), Cesspool cave (Virginia), Lower Kane cave (Wyoming) and Movile cave (Romania) [30, 63–65]. The sulfide-rich Frasassi cave system is reported to host an aphotic, subsurface microbial ecosystem including extremely acidic (pH 0–1), viscous biofilms (snottites) hanging from the cave walls [66]. The snottites were composed primarily of bacteria related to *Acidithiobacillus* species. Other populations present in the snottites included *Thermoplasmata* group archaea, bacteria related to *Sulfobacillus*, *Acidimicrobium*, protists, and filamentous fungi [66]. Contos et al. [67] also demonstrated the presence of subaqueous calcite precipitates associated with microbial biofilms in Weebubbie cave, Australia. These deposits formed in waters well below the saturation index of calcite and demonstrated a unique structure, which could only be replicated in vitro with the addition of organic acids [68]. Such results led the investigators to conclude that the surface of the *Gammaproteobacteria* species found within the bacterial filaments of the cave [69] played a crucial role in calcite deposition [67].

Complex microbial communities producing colored colonizations on Altamira cave walls have been reported with white, yellow, and gray biofilms having been analyzed and distinguished based on the bacterial communities forming these colonizations [70–72]. In addition, a morphological study has been reported on the characteristics of these differently colored biofilms [73] suggesting that white and gray biofilms were associated with mineral deposits, while yellow colonies did not present associated mineral formations. The different coloration of these biofilms is the result of their distinctive composition of bacterial phylotypes [70]. Besides, a morphological description of these biofilms-forming colonies on the cave walls have been reported [73]. One of the critical parameters in a carbonate cave, such as Altamira cave, is pH because calcium carbonate precipitation is highly affected by pH. Basic pH (8.0 and above) have been reported to allow the precipitation of calcium carbonate in caves and other environments [74]. Consequently, pH measurements represent a simple indication that can provide useful information to assess the effect of bacterial growth and metabolism on cave walls.

Moonmilk, white and gray biofilms due to their alkaline pH values (above 8) can induce the formation of speleothems that can cover the actual wall of the cave which is a potential transformation resulting from bacterial metabolism [75]. Yellow biofilms lack associated carbonate precipitates [73], which is a result of their acidic pH. Although yellow biofilms are not able to induce the precipitation of carbonate, their acidic pH can result in the dissolution of carbonate from walls and speleothems in the studied cave. While yellow biofilms can induce carbonate dissolution, white and gray biofilms can result in the precipitation of carbonate minerals [75].

A subject to be investigated is the possibility that different colored biofilms might preferentially develop on areas with slightly different organic nutrient supplies or environmental conditions. Different pH, organic and inorganic compounds can limit growth of bacterial biofilms on cave substrate [71, 76]. If this would be the case, the bacterial community of yellow biofilms might be processing different organic substrates than white and gray colonizations. Although this is a possibility to take into account, it is quite unlikely that relatively closed sites in a cave might receive a qualitatively different supply of organic nutrients and yellow, white and gray colonies in numerous cases develop in very close proximity [73]. Thus, it is expected that different bacterial communities composing the distinctively colored biofilms exert preferential metabolic processes with the available nutrients and the result is a different pH value in each of the types of samples under study. For example, the presence of a larger proportion of *Desulfovibrio*-related sulfate reducing bacteria as metabolically active cells in yellow biofilms than in biofilms with other colorations [70, 72] suggests a common presence of oxygen-deprived microenvironments, and consequently, an increase of fermentation processes by the dominant metabolically active *Gammaproteobacteria* detected in the yellow biofilms [70]. Fermentation of organic matter by *Gammaproteobacteria* is known to induce acidification of the medium [77]. The consequences of bacterial metabolism represent an important aspect to be monitored during the assessments of conservation strategies and the evaluation of potential for the transformation of cave environments [5].

A recent ongoing work (unpublished) by the authors includes the investigation of bacterial biofilms from two caves of Meghalaya in North-East India, Mawjyngbuin cave and Dam cave located in Mawjymbuin village under East Khasi Hills district, which have not attracted much attention of geomicrobiologists. Distinct biofilms of white and dark green color were encountered from the above two caves respectively (Table 1). It was found out that a few genera are prevalent in one cave but absent in the other under the selected sampling sites. The prevalence of *Sphingobacterium* in the Dam cave only can shed some light on the

Table 1 Bacteria characterized from caves around the world

Geographic location	Bacteria identified	References
Mawjymbuin cave, India	*Iodobacter fluviatilis, Bacillus cereus, B. amyloliquefaciens, Pseudomonas* spp., *P. alcaligenes, Lysinibacillus parviboronicapiens, Brevibacillus agri*	Authors (unpublished)
Dam cave, India	*Sphingobacterium kitahiroshimense, S. faecium, Bacillus* spp., *Brevibacillus agri, Achromobacter xylosoxidans*	Authors (unpublished)
Parker cave, USA	*Thiobacillus* spp., *Thiothrix* spp., *Thiomicrospira* spp.	[30]
Frasassi caves, Italy	*Thiobacillus* spp., *Sulfobacillus* spp.	[14]
Mawsmai cave, India	*Bacillus cereus, B. licheniformis, Micrococcusluteus, Actinomycetes*	[20]
Krem Phyllut, India	*Bacillus cereus, B. licheniformis, B. mycoides*	[20]
Borra caves, India	*Leptothrix*-like sheath forming bacterium, twisted spiral stalks of *Gallionella*-like organisms	[10]
An Earth-cave in Guizhou Province, China	Bacterial members belonging to *Proteobacteria*, *Acidobacteria*, *Planctomycetes*, *Chloroflexi*, *Bacteroidetes*, *Gemmatimonadetes*, *Nitrospirae*, *Actinobacteria*	[25]
Sahastradhara caves, India	*Bacillus thuringiensis* and *Bacillus pumilis*	[74]
Lower Kane cave, USA	Filamentous "*Epsilonproteobacteria*"	[60]
Altamira cave, Spain	High diversity of sulfate-reducing bacteria and other metabolically active anaerobic bacteria	[68]
Pajsarjeva Jama cave, Slovenia	Members of *Gammaproteobacteria*, *Actinobacteria, Nitrospira, Alphaproteobacteria, Betaproteobacteria* and *Deltaproteobacteria* as well as *Acidobacteria, Verrucomicrobia, Planctomycetes, Chloroflexi* and *Gemmatimonadetes*	[33]

uniqueness of this organism to adjust itself in this yet untouched cave. On the other hand, diverse species of *Pseudomonas* encountered in Mawjymbuin can be related to their versatile nature and ability to form biofilm which have been reviewed earlier by Sarma et al. [50]. A few of previous works documented on caves and the microorganisms involved are depicted in Table 1.

Bacteria and Mineral Precipitation in Caves

Caves offer excellent habitats for studying biomineralization processes (from active microorganisms to their mineral deposits), because they are stable environments where microbial fabrics can be preserved without extensive diagenetic modification or destruction. Initial work on cave microbiology prior to 1990 tended to concentrate on descriptive studies, with many investigators noting the presence of microorganisms in cave secondary mineral environments [5]. Generally, such observations were dismissed as the result of transport into the system through air movement or vectors (animal or human) [77–79]. Researchers suggested that due to geologic isolation from allochthonous surface energy input, microbial species would be limited to relatively few who are able to eke out an existence in this extremely starved environment [79]. Nonetheless, certain geochemical processes were difficult to explain by purely inorganic processes. At the beginning of the 1990s, new molecular techniques increased the number of environments that could be successfully studied by microbiologists [8]. Such techniques allowed researchers to examine the complex chemical interactions of microbial physiology with redox active minerals, in what had previously been considered abiotic, geological environments [4, 7]. Geologists likewise exposed biologists to the principles of mineralogy and novel techniques (X-ray powder diffractometry and energy dispersive spectroscopy) [7]. The introduction of new tools and techniques provided opportunities to pose novel questions in cave environments. To highlight the rapid evolution of cave geomicrobiology, studies on the major secondary deposits identified in caves carried out in the years will be reviewed in the following sections.

Calcite Precipitation

Microorganisms are found to be associated with carbonates and speleothems [80] and are able to produce similar crystals from organic calcium salts in the laboratory [62, 81–83]. Microbial carbonates are important in fluviatile, spring, cave and soil environments. Etching calcitic samples with weak acid sometimes reveals fossil microorganisms and/or biofilms that were entombed in the calcite [84, 85].The principal organisms involved are bacteria, particularly cyanobacteria, small algae and fungi, which form biofilms and/or microbial mats. Extracellular polymeric substances widely produced by microbes are important in facilitating sediment trapping, binding and providing nucleation sites for carbonate minerals [86].

Calcium carbonate can exist in three different polymorphic forms: calcite, aragonite and vaterite. Calcite is the most stable and common form of carbonate on earth's surface. It is also the most common constituent of sedimentary rocks. An equilibrium exists between insoluble (carbonate) and soluble (bicarbonate) forms in water.

$$CaCO_3 + CO_2 + H_2O \Leftrightarrow Ca(HCO_3)_2$$

The depletion of CO_2 from water favors the deposition of carbonate [83].

Recent studies have identified some of the factors that control the contribution of microbes to $CaCO_3$ precipitation [16, 18, 62, 65, 83, 87]. Bacteria and fungi can induce the precipitation of calcium carbonate extracellularly through a number of processes that include photosynthesis, ammonification, denitrification, sulphate reduction, and anaerobic sulphide oxidation [86]. Recent investigations carried out by the authors in Mawsmai cave, Meghalaya, India resulted in isolation of bacteria which formed crystals of calcite when inoculated on B4 agar medium supplemented with 2 % calcium carbonate. Morphology and size characteristics of both the crystals and the microorganisms were studied by scanning electron microscopy (Fig. 1).

Gypsum Precipitation

While most caves and karst features formed from carbonic acid dissolution of carbonate rocks, caves can also be formed by sulfuric acid dissolution [29]. Caves containing hydrogen sulfide-rich springs represent less than 10 % of all known caves globally [88]. Sulfidic caves are formed in carbonate rocks where sulfide-rich waters interact with oxygen at the water table or at subterranean springs [66]. One of the major problems interpreting caves formed by sulfuric acid speleogenesis is recognizing the mechanisms of sulfuric acid formation. However, in modern caves containing sulfidic waters, there is a wide range of microorganisms in these systems. A vast majority of them are sulfur-oxidizing bacteria that live within cave springs and streams, as well as growing on cave wall surfaces [89]. Some of these bacteria are reportedly acidophilic due to the production of sulfuric acid [90]. Therefore, sulfur-oxidizing bacterial populations can be examined as a means of understanding sulfuric acid speleogenesis. Additionally, studying the microbial communities in sulfidic caves can provide a better appreciation of other sulfidic habitats, relative microbial abundance and viability in the subsurface, and potential biogeochemical interactions pertinent to global sulfur cycling. Although sulfur-oxidizing microorganisms have been identified, a few studies have addressed the possible geomicrobiological impacts that the microbial communities may have on the cave environment. On the basis of observations of H_2S-bearing thermal springs, extensive gypsum deposits, and gypsum-replaced limestone cave walls in Lower Kane cave, Egemeier [29] proposed the original SAS (sulfuric acid speleogenesis) model to include the volatilization of H_2S from the groundwater to the cave atmosphere and H_2S oxidation (by microbial or abiotic sulfur oxidation) to sulfuric acid production on moist sub aerial cave wall surfaces, (Eq. 1). The sulphuric acid reacts with and replaces the carbonate host rock with gypsum and carbonic acid (Eq. 2). Gypsum easily dissolves into groundwater, and the net result is the removal of mass and an increase in void volume [16].

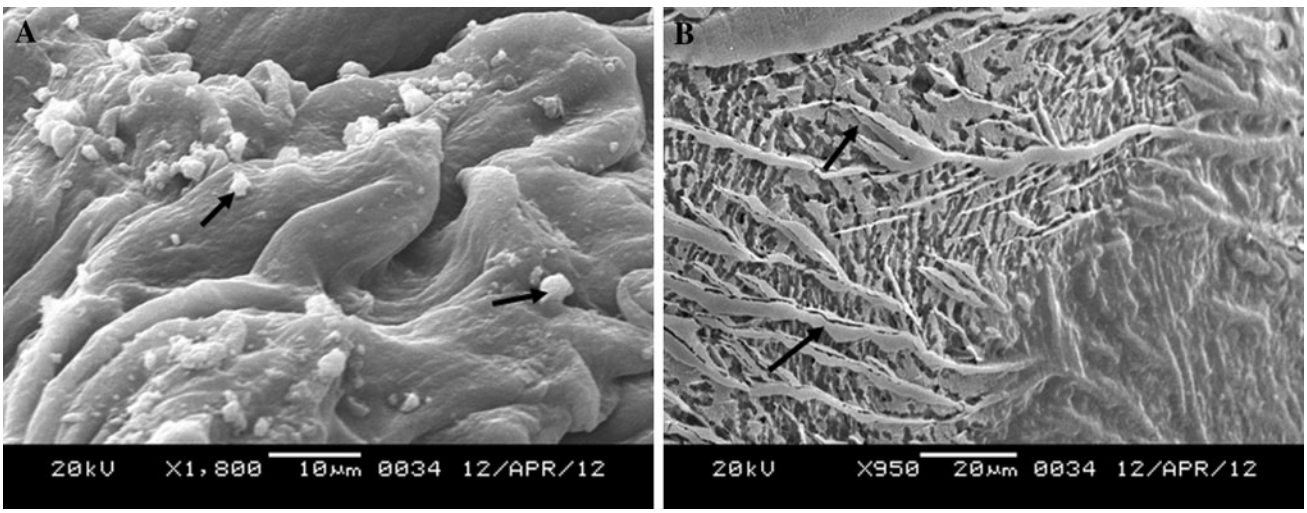

Fig. 1 Scanning electron microscopy images of calcite crystals precipitated in vitro on solid B4 media by bacteria isolated from Mawsmai cave, India after 40 days of incubation **a***arrows* indicate crystals of various sizes precipitated in vitro by bacteria, **b**fragment of a crystal precipitated by bacteria shows bacterial prints in the inner portion of the crystal at a higher magnification. Size and shape of the microbial rods can be observed with the prints on the crystal surfaces

$$H_2S + 2O_2 \rightarrow H_2SO_4 \quad (1)$$

$$CaCO_3 + H_2SO_4 \rightarrow CaSO_4 + H_2CO_3 \quad (2)$$

SAS is now recognized in several active sulfidic caves in the United States, Romania, Italy, and Mexico [13, 31, 91, 92], as well as in large ancient hypogene caves, e.g., Carlsbad Cavern, New Mexico [93]. In addition to subaerial processes, SAS in the Guadalupe Mountains has also been attributed to sulfuric acid dissolution at or just below the water table [88, 93, 94].

The investigation of sulfur cycling is important in determining the redox conditions in a variety of chemical systems. The effects of microbes on these conditions are a significant part of studying the system. It is important to understand what microbes do in any environment and exactly how they are affected by environmental changes. Sulfur cycling is central to various environments worldwide and is significant for soil fertilization, transportation of nutrients, mobilization of metals and organic contaminants in wetlands and aquifers [95]. Understanding the role of specific microbial species in different environments and geochemical conditions could be applied to a variety of geochemical problems where microbes are key components of chemical cycling, such as contaminant remediation through bio-stimulation/biocogmentation [96]. Additionally, the activity of sulphate reducing bacteria has been shown to mediate the precipitation of dolomite [82, 97].

Iron Precipitation

Iron oxide minerals in several types of environments on the earth, from freshwater to marine systems, aquifers, caves and soils, known to be formed in close association with bacteria are referred to as biogenic minerals [10]. Fe-mineral precipitation includes reaction steps such as Fe(II) oxidation, Fe(III) hydrolysis and precipitation [98]. The organisms that are responsible for iron oxidation are therefore vital to understanding of iron cycling in natural cave environments.

X-ray diffraction (XRD) studies of an organic mat in Borra caves, Vishakapatnam, India showed the presence of Fe minerals dominantly hematite (Fe_2O_3), minor amounts of zinc gallium sulfide and nitrofuryl compounds where the minor organic and sulfidic compounds detected were related to the metabolic products of various thriving communities of bacteria living in the mat which indicated a biogenic origin [10]. The two major groups of bacteria identified by direct microscopy and scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDX) from these iron rich water samples were *Leptothrix*-like organisms and a few stalks of *Gallionella*-like organisms.

It is now established that microbes can precipitate iron either within the cell-interior or along the sheaths surrounding microbial cells and filaments [99]. These biogenic minerals are formed as a result of the direct metabolic activity of bacteria or as a result of passive sorption and nucleation reactions. Furthermore, the metabolic activity of iron-oxidizing bacteria under oxygenic conditions promotes the oxidation of Fe^{2+} to Fe^{3+} and the precipitation of biogenic iron oxides as extracellular precipitates near or on the bacterial cells [100, 101]. In addition, the surface reactivity of the bacterial surface confers a net negative charge to its cell wall, thereby, leading to the adherence of soluble iron on its cell wall and finally the precipitation of iron oxides under saturation conditions. The extracellular polymeric materials produced by bacteria can act as template for iron sorption and Fe-oxide nucleation [99]. There is a high probability that the major iron mineral deposits of the microbial mats are based on microbe–mineral interactions rather than a pure inorganic precipitation reaction [10].

Other Minerals

Cave nitrate (a.k.a. nitrocalcite or calcium nitrate) is the saltpeter commonly found in dry cave sediments and historically was an important component of gunpowder manufacture [102]. Studies in Mammoth cave demonstrated the presence of nitrifying bacteria, specifically *Nitrobacter* spp., in densities 100 times higher than surface soils, although no consensus was reached on a biogenic source for these nitrates [103]. The stable isotope work of Jameson et al. [104] demonstrated that saltpeter is enriched in the lighter isotope of nitrogen, supporting the hypothesis that microbial activity is involved in the formation of cave nitrates. Microbiologists have continued to debate the degree to which bacteria, such as *Nitrosomonas* and *Nitrobacter*, facilitate the creation of the cave saltpeter deposits and the origin of nitrogen [102]. Nonetheless, to date, no consensus exists to explain the formation of these minerals in caves, likely a reflection of the loss of any commercial value for such deposits with the advent of industrial chemistry [5].

Several early studies also proposed microbial participation in the formation of cave manganese deposits [105]. The presence of rods, sheets, strands, and smooth spheroid morphologies in the fossil remains of manganese precipitates in stalactites, karst breccia and root calcrete crusts in Grand Cayman caves led Jones [105] to conclude that some of these manganese precipitates were biogenic. The degree to which the phylogenetically diverse group of microorganisms known to oxidize reduced manganese can promote such oxidation, passively or enzymatically, is debated. Microorganisms can increase the rate of manganese oxidation by up to five orders of magnitude [106] and the large accumulations of manganese oxides that occasionally occur in caves represent potentially microbial mediated production. The work of Cunningham was the first to recognize an association between microbial species and ferromanganese

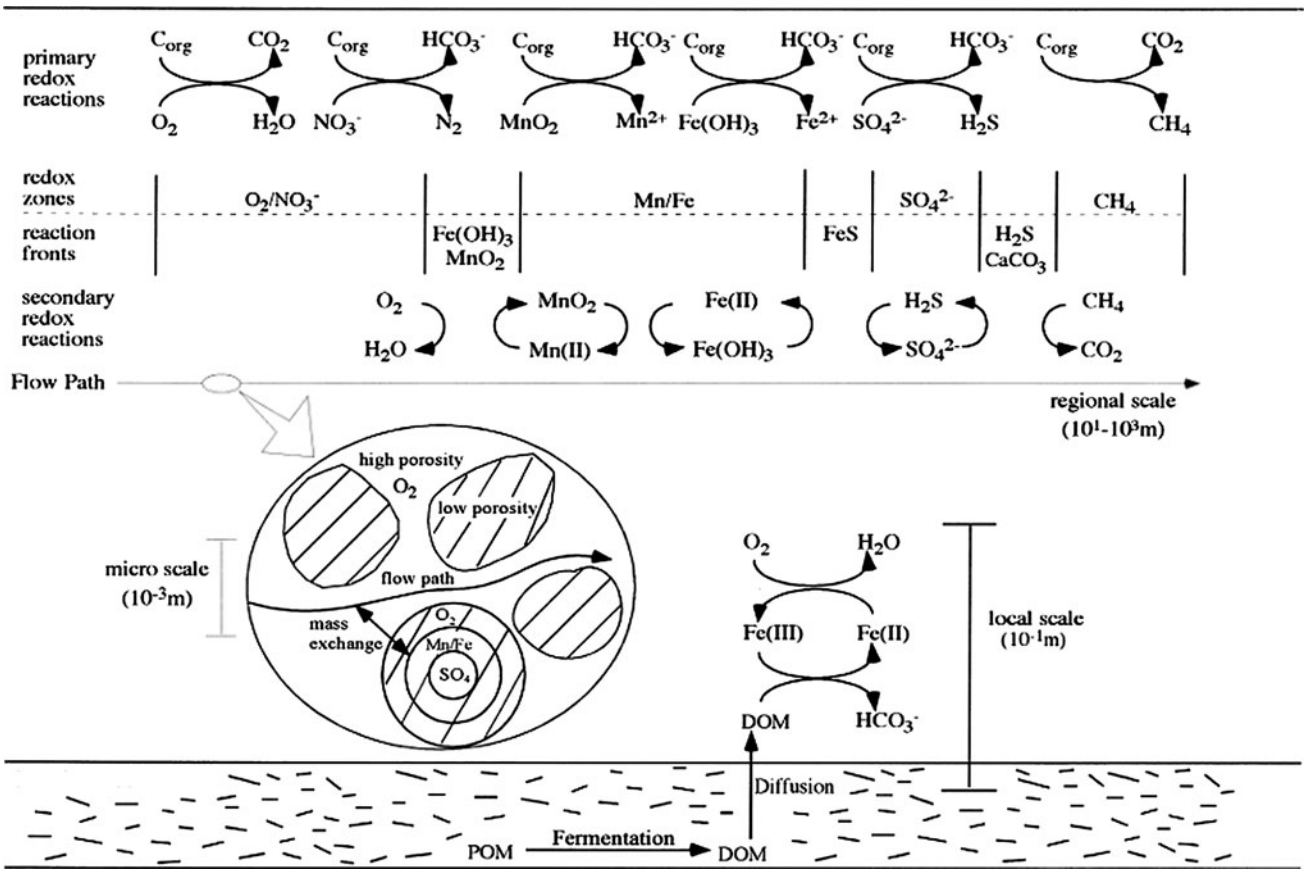

Fig. 2 A schematic diagram depicting various redox reactions and zonations at a variety of spatial scales. *POM* particulate organic matter, *DOM* dissolved organic matter (*Source* Huntera et al. [154])

deposits within Lechuguilla Cave [78]. Additional forms of poorly crystalline manganese oxides and hydroxides (pyrolusite, romanechite, todorokite, and rhodochrosite) have been described from caves [107, 108]. Figure 2 reveals a schematic diagram depicting various redox reactions and zonations at a variety of spatial scales.

Discussion

Niche separation has been documented for microbial clades at a variety of taxonomic levels [109]. As has been clearly established, the majority of bacteria in natural environments are present in biofilms at various types of interfaces [110]. Biofilms develop in a variety of natural or artificial habitats, including the air–water interface in aquatic environments and the solid–water interface of organic and mineral substrates in aquatic and terrestrial environments, cave ecosystems, on the surface of pipes and tanks in various types of industry, on the surface of implanted medical devices, and in the digestive tract of living organisms [14].

Despite rapid progress in the past decade, the deep subsurface remains one of the least explored microbial habitats on earth [109]. Trends in community composition within cave systems still remain unclear [111]. The amount of bioavailable energy from organic sources derived from primary production and detritus are critical to the structuring of ecological communities [112–114]. Many caves in temperate regions occupy the lower end of the range of carbon inputs seen globally and the communities that reside in them are generally energy starved [115–117]. While in situ energy production from chemolithoautotrophic microbes and autochthonous internal cycling of organic matter occurs within caves [13, 24], but in their natural state, most cave communities are supported by organic inputs that originate from outside or allochthonous sources. Studies on caves have demonstrated that the biodiversity and biomass of the microbial communities within caves are generally low as compared to surface habitats [118–120]. The putative reasons for this pattern include the low rate of inputs and low diversity of the in situ and outside organic substrates [115].

Enrichment-based and cultural investigations on typical heterotrophic microbes have shown that microbes grow in proportion to less than 1 % in an environment [6]. Culture-independent 16S rRNA gene sequence analysis has been employed to study bacterial communities in environmental

samples without prior cultivation. It has significantly revealed a broader diversity of 16S rRNA gene sequence types than culture-based studies [6, 9, 121]. The combination of phylogenetic sequence analysis with restriction fragment length polymorphisms (RFLPs) of PCR-amplified bacterial 16S rRNA genes has become a powerful tool to investigate natural bacterial communities [25]. Microbial diversity in selected habitats cannot be comprehensively studied by traditional approaches [62]. Hence laboratory-based culture experiments, as well as geochemical and molecular biological techniques are needed to be employed to understand the possible extent of microbial involvement in the formation of various cave architecture.

In this review, the potential effects of differently colored bacterial biofilms under development on cave walls were also distinguished and deduced their potential impact on calcium carbonate precipitation or dissolution. Thus, the consequences of bacterial metabolism in nature can be assessed, for instance, through pH measurements, to complement results on bacterial diversity surveys and distribution in natural environments of distinctive and complex bacterial communities. Acidophilic microorganisms control biogeochemical cycling in a variety of natural and anthropogenically influenced environments [122]. Acidophiles from high-temperature environments such as hot springs and fumaroles [123, 124], and iron-rich systems such as bioleaching operations and acid mine drainage (AMD) [125–127] have been the target of numerous studies linking microbial activity with geochemistry. Sulfidic caves have important similarities with nonthermal and moderately thermal zones around hydrothermal vents, including complete darkness, high sulfide concentrations, and food chains based on bacterial chemosynthesis [109]. However, the niche model emerging from studies of sulfidic caves is at least consistent with what is known about niches of cultivable mesophilic and moderately thermophilic vent chemoautotrophs [128–130].

To study cave microbiology, it is of crucial importance to preserve caves in their intact state and introduction of alien microbiota in caves must be reduced to minimum. Also, the biotechnological and bioremediation potential of cave inhabiting microorganisms is still underexploited [75]. Many microbes have the potential to harbor different important substances which can be effective under low cave temperature and thus interesting for industry such as those related to production of antibiotics and tumor suppression substances. For example, in Carlsbad Cavern novel species of microorganisms that can degrade complex hazardous aromatic compounds, such as benzothiazola and benezenesulfonic were isolated [131]. Microbes can use these compounds involved in the manufacture of plastics as an energy source for their growth [22]. Microorganisms isolated from cave-dwelling fauna are also a significant source of biotechnologically important substances. Some fungal isolates from the cave cricket *Troglophilus neglectus* [132, 133] showed high chitinolytic, lipolytic and proteolytic activities [134]. Biotechnological applications and uses of some microbes associated with hypogean environments as cited in this review are mentioned in Table 2.

Microorganisms by virtue of their metabolic activities have the ability to create localized microenvironmental conditions, e.g., by increasing alkalinity they induce supersaturation of $CaCO_3$ in aqueous phase and initiate bacterial mineralization and precipitation of crystals [40, 135]. After initial mineralization, the metabolic activity of bacteria could decrease or even stop completely, but the size of small fibre calcite crystals increase gradually [40]. Exopolysaccharides and mucus encapsulating the bacteria enhance diffusion gradients, whereby ions could diffuse through and enhance precipitation of minerals [82]. The findings on the association of bacteria with secondary minerals also will help to expand the existing knowledge in geomicrobiology and specifically on the influence of microorganisms in the formation of cave deposits. Cave geomicrobiology ought to be strongly supported as one of the new, revolutionary areas of knowledge at the boundary of geology and microbiology. Finally according to Forti et al. [43], it can be concluded that: "Speleothems definitely does not develop by vegetative growth…But without biogenic control caves should be very poorly decorated!!!"

Future Perspectives

Extremophiles can serve as models for extraterrestrial microbes that may live in celestial bodies. The most promising among these to contain habitable areas are Mars

Table 2 Biotechnological applications and uses of some microbes associated with hypogean environments

Microorganism	Application/uses	References
Polaromonas subterraneae	Degradation of complex aromatic compounds (benzothiazole and benezenesulfonic acid) for growth, which are compounds involved in the manufacture of plastics and are dangerous environmental contaminants	[22]
Streptomyces spp.	Antimicrobial activity against major human pathogens	[155]
Spirillospora, Nonomuraea	Antibacterial, antifungal and anti-cancer activities	[153]
Mucor spp.	High chitinolytic, lipolytic and proteolytic activities.	[134]

(where the Phoenix Lander recently discovered water) and the Jovian satellite Europa, also Titan (the moon of Saturn) has some features that resemble those that may have existed on earth during its earliest stages. From the characteristics of extremophilic microorganisms found on the present-day earth, some insights on the question of habitability of other planets can be derived, and learn about possible bioindicators that may be suitable when searching for extraterrestrial life [136].

The knowledge of the metabolic properties and environmental tolerance of closely related species could help us to understand the possible role of bacteria detected from caves. Snottites in sulfidic caves separated by vast geographical distances have strikingly similar community structures. The extreme and isolated nature of snottite habitats around the world provide an excellent opportunity to study ecological and geochemical controls on microbial diversity. The simplicity of snottite community structures also makes them ideally suited for genetic and metagenomic studies, currently in progress, aimed at mapping the spatial and temporal histories of microbial evolutionary events such as adaptations and gene transfers [53]. According to the closely related species, both of the studied biofilms could be characterized by phylogenetically diverse microbial communities [137]. It is now recognized that many important mineral transformations, originally considered to be inorganic in nature, can be mediated by microorganisms [5]; from the microbial precipitation of dolomite in groundwater [82, 138]; transformation of smectite to illite clay [139]; to the production of iron, uranium and even gold deposits [4]. Likewise, through a more thorough understanding of geochemistry, knowledge of the range of habitable environments on earth has been expanded like in the endolithic environments of extreme temperatures [140, 141] to the deep subsurface, where hydrogen produced from volcanism, serpentinization and even radiolysis provides sufficient energy to support microbial growth [142–144].

Since the emergence of geomicrobiology as a science, understanding of microbial interactions with minerals has evolved beyond a preliminary appreciation of their role in carbon, sulfur and nitrogen cycling. By understanding how microorganisms survive the extreme starvation of caves, human impacts on such hypogean environments can be better understood [145, 146]. In doing so, such work could preserve cultural treasures, such as Paleolithic paintings in the caves of northern Spain, where tourist activity altered the cave environment and brought in heterotrophic microorganisms that threaten to damage these images [50, 147, 148]. An understanding of such processes also facilitated the development of microbially precipitated calcite coatings, which can help to preserve historical monuments and sculptures [149–151]. Presently, it is hard to predict the similar outcome from the increasing number of microorganisms being cultured from cave environments, although they range from such beneficial activities as bioremediation to drug discovery [5].

Earth consists a staggering number of microbes, a majority of which have remained unknown and unexamined not only because most of them are yet uncultivable but also because many habitats have remained unexplored [152]. Cave geomicrobiology research can thereby generate fundamental knowledge about the subtle interplay between mutualism/competition and heterotrophy/autotrophy in terrestrial subterranean systems, which have numerous practical applications within medicine, human health and industry, and can aid in understanding the possibility of life elsewhere in the solar system. In conclusion, further multifaceted (microbiological, ecological, physiological, geological, molecular biological, etc.) research is needed to characterize the microbial ecosystem and yield new information regarding the metabolic functions of the different microorganisms inhabiting such a complex environment. Efforts have been initiated globally including India, to explore new habitats to look for microbes capable of making novel products and processes [152]. As the caves continue to be discovered, they present newer environments to be examined for life forms. As a result, the understanding of microbial activity in such subterranean systems can only continue to grow, as present questions are addressed and new questions are posed. This is a fascinating challenge for all the scientists working in caves, architecture and associated life forms.

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