Microbial diversity and activity in caves

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In recent times, there have been renewed interests in cave ecosystems for both economic and scientific reasons. This is because caves can contain fossils, artifacts, Palaeolithic paintings, ancient markings in form of finger flutings and beautiful speleothems (mineral deposits). These features are attractive and their presence has led to an increase in the number of people visiting caves (tourism) with associated economic benefits to the cave management authorities and the communities in which these caves are located. Unfortunately some of these cave features are susceptible to microbial damage by indigenous and foreign microorganisms, with this risk being exacerbated by unregulated human visitation. Therefore understanding microbial diversity and activities in caves is essential for cave conservation, restoration, safe and sustainable cave tourism.

Why study cave microorganisms?

Different groups of microorganisms such as bacteria, archaea, viruses and fungi are found in caves. However, increased human access (tourism) and cave modifications for tourism purposes (pavements and lighting systems' installation) can alter the natural microbial dynamics, introduce new microorganisms and change the caves' microclimatic conditions[1,2]. These changes can result in extensive damage of cave features such as Palaeolithic paintings and finger flutings over time. High numbers of human visitations can lead to increased health risks to cave visitors and workers via increased microbial load and exposure to opportunistic cave pathogens[3]. Caves can also be sources of novel microorganisms and biomolecules such as enzymes and antibiotics that may be suitable for biotechnological purposes.

Tools for studying cave microorganisms

Different culture dependent and independent methods have been used to study cave microorganisms. Culture dependent methods involve the use of either normal or oligotrophic or specialised culture media. Samples obtained from sediments, walls, atmosphere and other cave surfaces can be plated directly, or from diluents, on oligotrophic media such as 1/100 strength nutrient agar (bacteria) or media such as Potato Dextrose Agar (fungi)[4,5]. Counting, purification and identification of microbial isolates can then be carried out. Direct counting of microorganisms without plating is also possible using microscopic techniques.

Culture independent tools used for cave microbiology (taxonomy and metabolism) include polymerase chain reaction (PCR) based fingerprinting methods (DGGE and T-RFLP), clone library construction, quantitative PCR assays (including those targeting functional genes of interest), sequencing and the use of stable isotope probing methods[4–6]. In recent times, next generation sequencing tools (NGS) on a variety of platforms such as Illumina, SOLiD, Ion Torrent PGM and Roche FLX 454 and associated bioinformatics have been applied to the study of cave microorganisms[7]. NGS Data are of greater depth and higher quality than those obtained with other methods, although database limitations (poorly curated and annotated with regards to cave microorganisms such as fungi) may limit their usefulness.

Microbial diversity of caves

Caves can be terrestrial or aquatic and are usually oligotrophic in nature (nutrient limited) although some may be rich in specific minerals naturally or due to exposure to nutrient-laden sources. Therefore, different caves will have different groups of microorganisms occupying varying ecological niches and alongside cave fauna and environmental factors such as CO_2, temperature and organic matter content, define caves' biotic activities (formation/alteration of cave structures and nutrient cycling) (Figure 1). Microorganisms found in caves can be indigenous to the caves or introduced by humans, animals, water flow and wind action.

Bacteria in caves

Caves contain a broad variety of bacteria belonging to the Proteobacteria, Firmicutes, Actinobacteria and Acidobacteria. Proteobacteria appeared to be the major group detected through the use of PCR based molecular and NGS tools while most isolates from culture dependent assays belonged to Actinobacteria[3,7,8]. In open caves such as show caves, bacteria belonging to different genera such as *Cyanobacter*, *Pseudomonas, Bacillus, Micrococcus, Arthrobacter,*

Staphylococcus and *Mycobacterium* have been identified[4]. Some, like *Cyanobacter* are photoautotrophs found at the cave entrance or around light installations[9]. Others such as *Pseudomonas* and *Bacillus* are heterotrophs, degrading organic matter in the form of insects and animal droppings and extraneous matter. While these heterotrophic activities contribute to the biogeochemical cycle in caves, they can be a disadvantage in caves with Palaeolithic paintings (Figure 2). For example, the growth of bacterial species from genera such as *Aminobacter, Erythrobacter and Norcardioides*[10] on pigments from Palaeolithic paintings and cave walls may damage these paintings over time.

In flooded or underwater caves, many bacterial groups playing different ecological roles have been detected. Bacterial activities in such caves range from organotrophic to chemolithotrophic activities. For example, in aquatic caves such as Nullarbor Caves (Australia) different bacteria genera such as *Pseudomonas, Nitrospira, Cytophaga, Thioalcalovibrio* and *Flavobacterium* have been detected[11]. Some of these microorganisms (*Pseudomonas, Cytophaga* and *Flavobacterium* spp) are organotrophs while others such as *Thioalcalovibrio* and *Nitrospira* spp are chemolithotrophs[11]. Chemosynthesis is especially prevalent in sealed caves with chemotrophs such as methanotrophs, methylotrophs and metal (iron, manganese and sulphur) oxidisers or reducers with species belonging to genera such as *Thiobacillus, Sulfurospirillum, Methylomonas*, *Pantoea* and *Hyphomicrobium* being detected[12].

Fungi in caves

Although cave systems such as terrestrial caves are usually nutrient poor biotopes, they contain different groups of heterotrophic fungi that exist in the form of mycelia or spores. Over 500 genera of fungi, slime moulds and fungus-like taxa have been reported in caves worldwide[13]. These belong to different taxa such as Ascomycota, Basidiomycota, Zygomycota, Mycetozoa, Oomycota and Chytridiomycota[13]. Ascomycota appears to be the most dominant group irrespective of whether culture dependent or independent tools have been used[5,7]. Commonly encountered genera include *Aspergillus, Penicillium, Mucor, Fusarium* and *Cladosporium*. In terms of pathogens, *Histoplasma capsulatum* (causes histoplasmosis in cavers) and *Pseudogymoascus destructans*[14], which was formerly known as *Geomyces destructans* (causes the devastating white nose disease in bats) are famous examples although other opportunistic pathogens such as *Trichosporon* spp. and *Microsporum gypseum* (dermatophytes) are known[3].

Cave fungi such as *Trichurus, Fusarium* and *Cladosporium* can function as decomposers of dead cave insects, fauna, animal, droppings and extraneous organic matter[5,15]. Some fungi such as *Isaria*

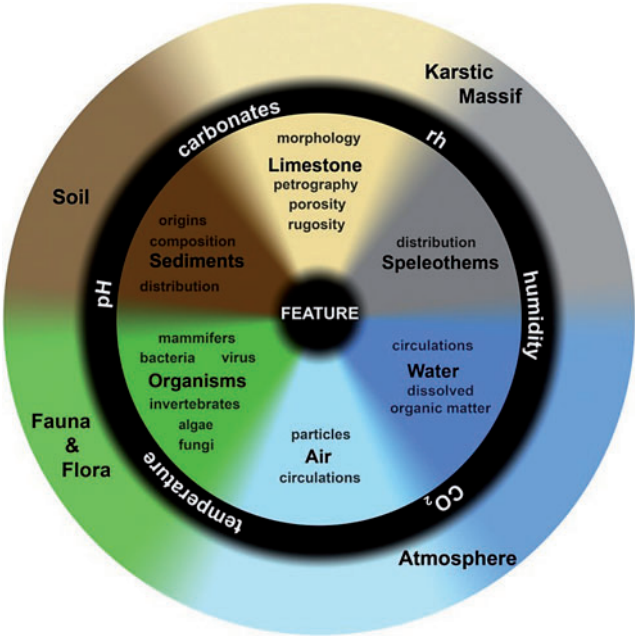

Figure 1. Ecosystem sketch of the evolution of a cave wall. Note that the inner zone refers to the endokarst, the outer zone to the karstic massif and exokarst, and the black circle to the shared parameters[22].

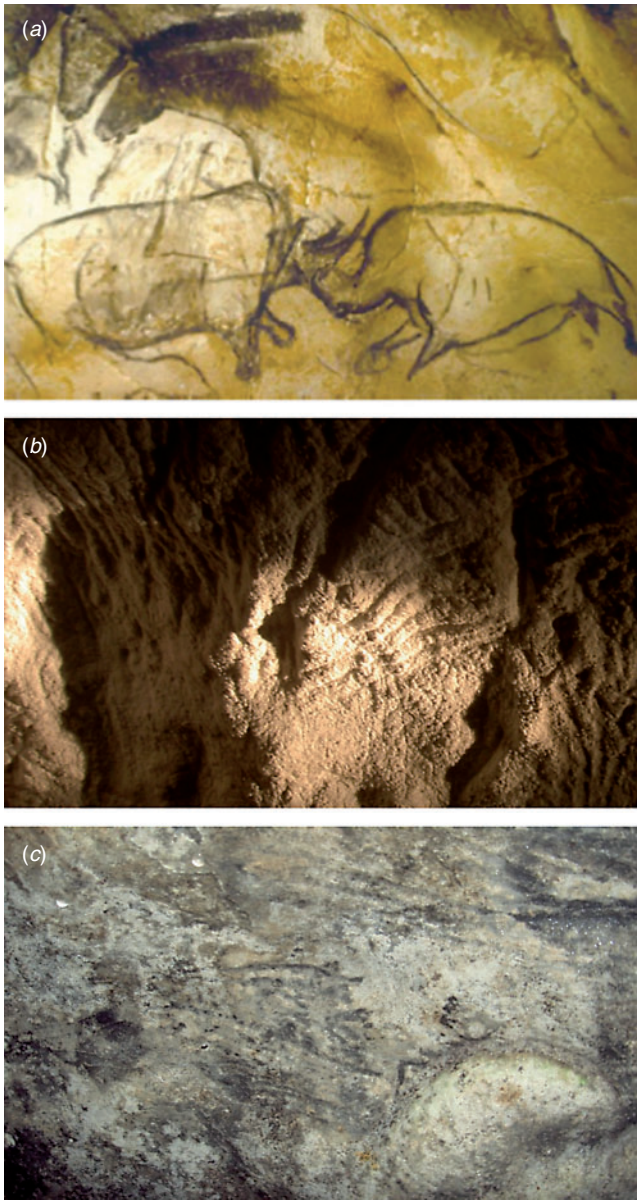

Figure 2. Horse panel from the Hillaire chamber of the Chauvet Cave in Vallon-Pont-d'Arc, France showing a rhinoceros drawn 30,000 years ago (*a*)[23], intact (*b*) and faded finger flutings (*c*) in Australian caves.

farinosa are parasites of cave insects[16] while others are food sources to cave invertebrates and protozoa. Fungi growing on cave surfaces alongside bacteria and archaea may be involved in speleothem formation[13]. Fungal solubilisation of the rocky substrata contributes to the caves' inorganic nutrient pool[17] and this process can severely damage rock art or Palaeolithic paintings. Fungal species such as *Fusarium solani* and *Ochroconis lascauxensis*[18] have being implicated in rock art damage; *F. solani*, colonisation of the famous Lascaux Cave Palaeolithic art being a good example[9,17].

Other cave microbial groups

Archaea are also found in caves (although in lesser numbers) with members of the Euryarchaeota, Crenarchaeota, Thaumarchaeota, Korachaeota and Nanoarchaeota being detected. Either Euryarchaeota or Crenarchaeota appear to be the most dominant phyla in molecular (DGGE and NGS) assay results[19,20]. Some members of the Euryarchaeota and Crenarchaeota groups are heterotrophs while others are thought to be chemolithotrophs involved in the formation of iron and manganese oxides in mineral rich caves[20]. Some members of these groups alongside with bacteria and fungi are also involved in speleothem formation[19].

Viruses are also found in caves and have become important given the recent outbreak of Ebola virus in some parts of the world. Most cave viruses of health concern are borne by bats (as reservoir hosts) from which these viruses can spread to cave visitors (animals and humans). Fruit bats are natural hosts of Marburg viruses (deadly haemorrhagic fever) while some African bats are hosts of the lethal Ebola virus with no known cure. Bat guano is rich in other viruses such as Adenoviruses, Astroviruses and herpesviruses[21].

In conclusion, cave microorganisms are metabolically versatile and are able to acquire energy independently through photo- and chemo-autotrophic activities or through heterotrophic activities. Different microbial groups also interact or work co-operatively in the formation of cave features and as part of the biogeochemical cycle. Understanding these interactions in terms of microbial diversity and function is important for the maintenance of this unique ecosystem especially those that contain features of scientific, archaeological and tourist values. This will allow for sound assessment of the impact of human access on caves and health risks associated with cave visitations and is crucial for sustainable management of cave resources.

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Biographies

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The biography for **Professor Ball** is on page 182.