

# Extremophilic Microorganisms in Central Europe

Subjects: Area Studies

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“Extremophiles”, first introduced in 1974, describes organisms that thrive in environments where one or more physical or chemical parameters are beyond most organisms’ normal optimal range. They can be broadly separated into two categories: extremophilic organisms that require one or more extreme conditions to grow and extremotolerant organisms that can tolerate harsh conditions but grow optimally in a milder environment

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## 1. Caves and Karst Environments

### 1.1. Caves and Karst—Characteristics

Microorganisms can live underground in the soil, epikarst, and microcavities in rocks and fissures, many of which remain inaccessible and demand further research. Underground habitats in caves and other karst environments are generally characterised by low nutrient value and, in some cases, depletion of oxygen and extreme redox potential. Therefore, the cave habitat can change significantly from the surface to the rock base, resulting in many unknown microorganisms with unique physiologies <sup>[1][2][3][4]</sup>.

Worldwide, karst landscapes represent about 15% of the land area <sup>[5]</sup> while in Slovenia, this rises to almost 50% <sup>[6]</sup>. Caves generally consist of three zones. The first is the “entrance zone”, where the sunlight reaches, and consequently, there is green vegetation, and the temperature is variable. The second zone is the “twilight zone”, with less light and minimal phototrophic life, with minor temperature changes. As the light decreases, the phototrophic community changes from phanerophytes, pteridophytes, and bryophytes to algae and cyanobacteria <sup>[7]</sup>. The last zone is the “dark zone”, where there is no light, and the temperature remains constant <sup>[8]</sup>. The cave environments in the dark zone are also characterized by high humidity, low nutritional and energy resources, and sometimes extreme redox conditions <sup>[9]</sup>. However, the microbial communities in caves are still influenced by the Earth’s surface and atmosphere via the global element and energy cycles <sup>[4]</sup>.

### 1.2. Microorganisms in Caves and Karst Environments

Cave-inhabiting organisms are generally oligotrophic. However, additional anthropomorphic energy input allows the growth of other organisms, which thus compete for the same resources and space <sup>[9]</sup>. Different life forms have been found in caves, including viruses, archaea, bacteria and cyanobacteria, fungi, algae, protists, plants, and animals <sup>[10][11][12][13]</sup>. These can be present in different cave habitats and are associated with rock, cave walls, speleothems, springs, pools, and the air. Geochemical gradients of dissolved oxygen and sulphide can form ecotones where specialized archaeal and bacterial taxonomic groups thrive, depending on their metabolic and ecological requirements. Chemolithotrophic metabolism sustains the ecosystems of diverse bacterial and archaeal communities in oxygen-sulphide ecotones <sup>[14][15][16][17][18][19][20]</sup>.

Microbial mats from the Žveplenica sulfidic karst spring in Slovenia have been sampled to analyse the different taxa that are present <sup>[21]</sup>. Novel lineages of taxa were found in both the oxygenated and anoxic mats. The oxygenated mats contained undescribed and undifferentiated fungi, Annelida, Nematoda, Apicomplexa, and Gastrotricha. While in the anoxic mats, the most diverse taxa were Ciliophora, Nematoda, and Fungi-Ascomycota. The interconnections between bacterial and archaeal diversity with distinct microeukaryotes are likely related to the grazing options and the food-web structure that is within the karst system <sup>[21]</sup>.

## 2. Glaciers and Ice Caves

### 2.1. Characteristics of Frigid Environments

In frigid habitats, the availability of liquid water is what determines microbial activity. Frigid habitats include snow, surface ice, cryoconite holes, englacial systems, and the interface between the ice and the underlying rock and soil. Abiotic conditions and microbial composition are incredibly consistent throughout the world's glaciers and snow sheets [22].

## 2.2. Psychrophiles in Glaciers and Ice Caves

Psychrophiles are gaining recognition as the polar ice melts due to global warming, which uncovers and produces more niches for these microorganisms [23]. At the same time, ice-algal blooms in communities with fungi [24] and bacteria [25] can darken the surface of the ice and thus cause it to melt more rapidly, contributing adversely to the effects of global warming [23][24][26][27][28][29][30].

However, psychrophiles can also be found in moderate climates [31] in permanent glaciers, lakes [25], and ice caves. Glaciers and ice sheets are unique ecosystems since they are microbially driven and contain great diversity [32]. The habitats that are provided by different glaciers are remarkably similar, particularly in terms of their primary producers and ecosystem engineers. In aquatic and sediment systems, such as cryoconite holes, cyanobacteria are the dominant primary producers, while eukaryotic Zygonematales take on this role on ice surfaces and Chlamydomonadales within the snow. Chemolithotrophs that are associated with the sulphur and iron cycles and carbon transformation in subglacial ecosystems enable chemical transformation at the ice-rock interface that supports the delivery of nutrients to downstream ecosystems [22]. At the same time, samples from glacier cryoconite holes in the Austrian Alps contain bacteria, yeast, and hyphomycetes [33]. While aerobic heterotrophic bacteria were the most numerous, their cultivation showed that they were tolerant or secondary psychrophiles, as their optimal growth temperature was higher (20 °C). The yeast, however, grew most successfully at 2 °C.

## 3. Adriatic Salterns

### 3.1. Characteristics of Hypersaline Habitats

Hypersaline habitats, i.e., habitats with increased NaCl content, differ significantly and range from salt lakes that are typical of Lake Tuz (Turkey) or the Great Salt Lake (USA) to the Dead Sea and the Red Sea and brine in tidelands and salterns. Each environment has its own unique mineral content, temperature, and pH characteristics, meaning that organisms have become adapted to various habitat requirements. They also vary in other physicochemical parameters such as chemical structure, average temperatures, conduction, pH, and mineral content. Additionally, the range of different parameters and their fluctuation in time is incredibly diversified, e.g., tropical salterns that operate all year have much more stable conditions and higher average temperatures than the Adriatic salterns with cold winters.

### 3.2. Adriatic Halophiles

Unlike psychrophiles where a few globally distributed species dominate, halophiles live in much more diverse environments. Hypersaline habitats are mainly dominated by prokaryotes (archaea and bacteria), with only a few eukaryotes reported. However, new research has shown that melanised fungi thrive in hypersaline saltern ponds [34][35][36].

The algae populations are heavily dominated by the unicellular algae *Dunaliella* (Chlamydomonadales) [37][38] and *Cladophora* (Cladophorales), as seen for the Sečovlje salterns in Slovenia [39] and Salin-de-Giraud saltmarshes in southern France [40]. As well as the unicellular green algae, these environments include cyanobacteria, anoxygenic anaerobic bacteria, sulphate reducers, sulphur oxidizers, nitrate reducers, and ammonia oxidizers [41][42]. In some cases, the density of the halophiles can be high enough to be visible, such as with the pink-red colour in the brine of salterns from the pigments (i.e., carotenoids) in archaea (e.g., *Haloquadratum*, Halobacteriales), bacteria (e.g., *Salinibacter* (Bacteroidetes)), and eucarya (e.g., *Dunaliella salina*) [41][43][44]. The cyanobacteria in Sečovlje salterns are mainly represented by *Microcoelus chtonoplastes* (Oscillatoriales) [39].

## 4. Thermal and Mineral Springs of South-Eastern Europe

### 4.1. Characteristics of High-Temperature Environments and Habitats with High Mineral Content

In addition to the dark and low-nutrient underground environments, hypersaline habitats like salterns and cold habitats like glaciers, there are other extreme environments in Slovenia and central Europe: mineral and thermal springs. Thermal springs represent extreme habitats due to their high temperature that is often accompanied by specific mineral content. Although the mineral springs range in temperature, they have high mineral content, e.g., magnesium and sulphides. They are often used as a source of mineral water, and hot springs are sometimes used for spas, depending on other characteristics.

## 4.2. Thermophiles in Central-European Thermal Springs

A condensed overview of the thermophiles that inhabit American hot springs was presented by Ashcroft (2002) <sup>[31]</sup>, albeit European examples were not included. Nevertheless, thermal springs (similar to extremely cold environments) worldwide appear to have unexpectedly similar chemical compositions and temperatures at their point of emergence <sup>[45]</sup>. Southern Europe is one of the areas with the highest density of hot springs <sup>[45]</sup>. However, not much is known about the microorganisms that inhabit these diverse habitats.

Slovenia has a lot of thermal springs and some mineral springs (13 large spas, with further smaller ones), which are mainly in the eastern and northeastern regions: Moravske toplice, Lendava, Radenci, Rogaška, Ptuj, Zreče, Topolšica, Dobrna, Laško, Olimia, Čatež, Dolenjske, and Šmarješke toplice, Banovci, Mala Nedelja, Rimske terme, Atomske toplice, Snovik, and Cerkno. Invanjševci ob Ščavnici and Strunjan are the only spas in the Primorska region. There are up to 60 natural thermal springs, such as those of Kostanjevica na Krki, Klunove Toplice, and Klevevž, and the thermal springs Ljubljana and Tolminka. The Slovenian thermal springs range in both temperature and geochemical conditions, and thus it is expected that different microbial communities will inhabit these different habitats.

## 5. Conclusions

Several types of extremophile microorganisms inhabit different environments in the central European moderate climate: oligotrophic microorganisms inhabit caves and other subsurface environments, while psychrophiles are found in snow and ice, halophiles in salterns and other hypersaline environments along the Mediterranean coast, and thermophiles in thermal springs. Their unique properties provide us with opportunities to study their different metabolic pathways and adaptive mechanisms. Their production of antimicrobial substances can be very useful in medicine and biotechnology, while the enzymes that they produce are thermostable and stable to high and low pH levels. Alternatively, extremophiles can also produce harmful compounds, which they use as an energy source. Lithoautotrophic extremophiles could be used to sequester atmospheric carbon dioxide and use it as an energy source.

The use of metagenomics has also provided enhanced information on microbial diversity, particularly for extremophilic environments, compared to previous cultivation-dependent and classical molecular techniques. However, gene sequencing cannot provide information on their metabolic pathways and ecology, as closely related organisms can have very different physiologies. Thus, we need to apply other “-omics” approaches to better understand their diversity, interactions, metabolic pathways, biogeochemical cycles, and dynamics, such as transcriptomics, proteomics, and metabolomics. This knowledge will provide greater insight into the roles of extremophiles in extreme environments and their potential biotechnological and medical applications.

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