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## DEEP BIOSPHERE OF SALT DEPOSITS

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### Definition

The *biosphere* is defined either as “the place on Earth’s surface where life dwells” (Eduard Suess’ original definition, 1875, in *The Origin of the Alps*), meaning the zone on Earth where life naturally occurs, or in a more narrow sense as just the organisms themselves. Recent advances in microbiology have demonstrated that microorganisms live deep beneath the Earth’s terrestrial surface and that the total mass of microbial life may, in biomass, exceed all animal and plant life on the surface (Whitman et al., 1998). Microorganisms live at such extremes that the “thickness” of the biosphere extends from 5,400 m above sea level to at least 9,000 m below sea level.

*Salt deposits* are accumulations of solid materials and are usually derived from evaporation of seawater. There are >70 elements dissolved in extant seawater, but only 6 ions make up >99% of all the dissolved salts (wt% in brackets): Na<sup>+</sup> (30.51), Cl<sup>−</sup> (55.08), SO<sub>4</sub><sup>2−</sup> (7.69), Mg<sup>2+</sup> (3.67), Ca<sup>2+</sup> (1.17), K<sup>+</sup> (1.10) (Hay et al., 2006).

### Introduction

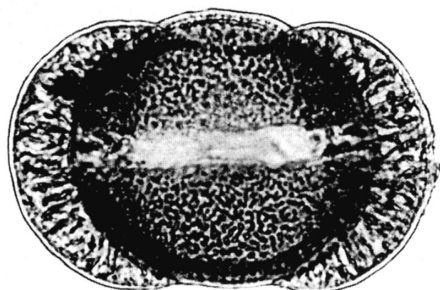
About 250 million years ago the continents on Earth were close together and formed Pangaea, a supercontinent, which persisted for about 100 million years and then fragmented. The land masses at that time were located predominantly in the southern hemisphere. The climate was arid and dry, and the average temperature is thought to have been several degrees higher than at present. This was one of the time periods in the history of the Earth, when

huge salt sediments formed. A total of about 1.3 million cubic kilometers of salt were estimated to have been deposited during the late Permian and early Triassic period alone (250–192 million years ago; Zharkov, 1981); newer research has discovered additional vast salt deposits, which were previously unknown – especially deposits below the Gulf of Mexico, and extensive Miocene salt (about 20 million years old) underlying the Mediterranean Sea, the Red Sea, and the Persian Gulf (Hay et al., 2006).

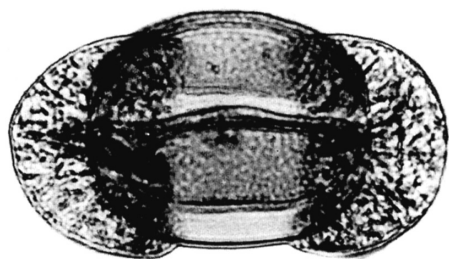
The thickness of the ancient salt sediments can reach 1,000–2,000 m. When Pangaea broke up, land masses were drifting in latitudinal and northern direction. Mountain ranges such as the Alps, the Carpathians, and the Himalayas were pushed up by the forces of plate tectonics. In the Alpine basin and in the region of the Zechstein Sea, which covered northern Europe, no more salt sedimentation took place after the Triassic period. Dating of the salt deposits by sulfur-isotope analysis (ratio of <sup>32</sup>S/<sup>34</sup>S as measured by mass spectrometry), in connection with information from stratigraphy, indicated a Permo–Triassic age for the Alpine and Zechstein deposits (Holser and Kaplan, 1966). This age was independently confirmed by the identification of pollen grains in the sediments (Klaus, 1974). Figure 1 shows pollen grains from extinct coniferous trees, which were found in Alpine Permian salt and also in Zechstein salt (Klaus, 1963).

### Microorganisms and signature sequences from salt deposits

As recently as 1981, Larsen (1981) described mined rock salts as free from bacteria, although isolations of halophilic microorganisms from ancient salt sediments had occasionally been reported since the early decades of the twentieth century (see Grant et al., 1998; McGenity et al., 2000). From Alpine Permian rock salt, which was collected from the salt mine in Bad Ischl, Austria, a haloarchaeon (see “*Halobacteria* – *Halophiles*”) was



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**Deep Biosphere of Salt Deposits, Figure 1** Photomicrograph of winged pollen grains of the genus *Lueckisporites*, found in Alpine Permian salt deposits and in Zechstein salt. *Upper panel*: *L. virkkiae*, dimensions  $60 \times 40 \mu\text{m}$ ; *lower panel*: *L. microgranulatus*, dimensions  $64 \times 35 \mu\text{m}$  (From Klaus, 1963 with permission.)

isolated, which was recognized as a novel species and named *Halococcus salifodinae* strain BIp (Denner et al., 1994). This was the first isolate from ancient rock salt, which was formally classified and deposited in international culture collections. Two independently isolated strains, Br3 (from solution-mined brine in Cheshire, England) and BG2/2 (from a bore core from the mine of Berchtesgaden, Germany) resembled *Hc. salifodinae* strain BIp in numerous properties, including the characteristic morphology of coccoid cells arranged in large clusters (see Figure 2, right panel, in “*Halobacteria – Halophiles*”); in addition, rock salt samples were obtained 8 years later from the same site and several halococci were recovered from these samples, which proved to be identical to strain BIp (Stan-Lotter et al., 1999). The data suggested that viable halophilic archaea, which belong to the same species, occur in geographically separated evaporites of similar geological age. Another halococcal isolate from the Bad Ischl salt formation, which differed from the previously described strains, was identified as a novel species and named *Halococcus dombrowskii* (Stan-Lotter et al., 2002). *Hc. salifodinae* and *Hc. dombrowskii* have so far not been found in any hypersaline surface waters, or any location other than salt mines. Recently, a non-coccoid novel haloarchaeon, *Halobacterium noricense* was obtained from a freshly



**Deep Biosphere of Salt Deposits, Figure 2** Drilled bore cores, stored in a wooden box, from the Alpine salt mine in Altaussee, Austria obtained from about 500 m below surface. Pink portions represent halite (NaCl), containing traces of hematite; grayish portions contain mostly anhydrite ( $\text{CaSO}_4$ ).

drilled bore core (Figure 2) at the salt mine in Altaussee, Austria (Gruber et al., 2004). Other isolates from ancient salt deposits include a single rod-shaped *Halobacterium* from 97 000 year old rock salt in the USA (Mormile et al., 2003), which was deemed to resemble the well-characterized *Halobacterium salinarum* NRC-1 (see “*Halobacteria – Halophiles*”). From the Permian Salado formation in New Mexico, a novel strain, *Halosimplex carlsbadense*, was isolated (Vreeland et al., 2002).

Although the microbial content of ancient rock salt is low – estimates range from 1 to 2 cells/kg of salt from a British mine (Norton et al., 1993) to  $1.3 \times 10^5$  colony forming units (CFUs) per kg of Alpine rock salt (Stan-Lotter et al., 2000), and up to  $10^4$  CFUs per g of Permian salt of the Salado formation (Vreeland et al., 1998), equivalent to a range of 1 pg to 10  $\mu\text{g}$  of biomass per kg of salt-the reports showed that viable haloarchaeal isolates were obtained reproducibly by several groups around the world. The data support the hypothesis that the halophilic isolates from subterranean salt deposits could be the remnants of populations which inhabited once ancient hypersaline

seas; in addition, they provide strong evidence against the notion that the recovered strains could be the result of laboratory contamination, since the isolates were obtained independently from different locations.

The amplification of diagnostic molecules, such as the 16S rRNA genes of bacteria and archaea, by the polymerase chain reaction is now the standard method for obtaining material for subsequent nucleotide sequencing. For this technique it is not necessary to cultivate the microorganisms; the genes can be amplified by using DNA prepared from the material of interest. Analysis of dissolved Alpine rock salt with molecular methods was performed by extracting DNA and sequencing of 16S rRNA genes. The results provided evidence for the occurrence of numerous haloarchaea, which have not yet been cultured (Radax et al., 2001). Similarities of the 16S rDNA gene sequences were less than 90–95% to known sequences in about 37% of approximately 170 analyzed clones (Radax et al., 2001; Stan-Lotter et al., 2004); the remaining clone sequences were 98–99% similar to isolates from rock salts of various ages (McGenity et al., 2000) and to known haloarchaeal genera. In a similar experimental approach, using halite samples ranging in age from 11 to 425 millions of years, Fish et al. (2002) found haloarchaeal sequences and, in the older samples, also evidence for bacterial 16S rRNA genes which were related to the genera *Aquabacterium*, *Leptothrix*, *Pseudomonas*, and others. These data suggested the presence of a probably very diverse microbial community in ancient rock salt.

### Long-term survival of cells

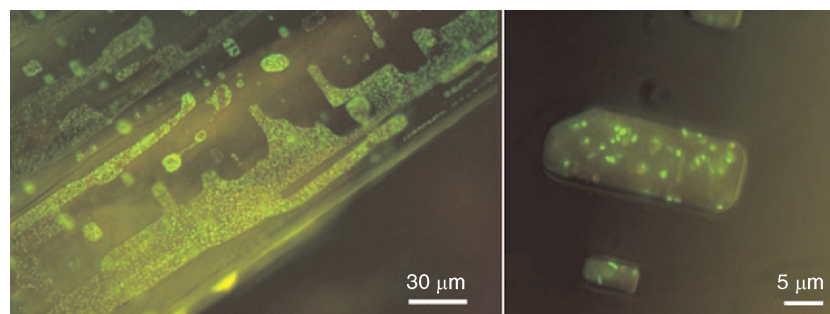
The reports cited above provide evidence that viable extremely halophilic archaea were isolated from salt sediments, which are thought to have been deposited about tens of thousands or even millions of years ago. The fluid inclusions in Permian rock salt were reported to contain cations and anions in a similar composition as today's seawater (Horita et al., 1991; Hay et al., 2006). While there is no direct proof that haloarchaea or other microorganisms have been entrapped in rock salt since its sedimentation, it would also be difficult to prove the opposite, namely that

masses of diverse microorganisms entered the evaporites in recent times (see also McGenity et al., 2000, for further discussion).

If a Permo–Triassic age is postulated for some of the haloarchaeal isolates and DNA signatures, then it becomes necessary to explain the biological mechanisms for such extreme longevity. Grant et al. (1998) discussed several possibilities, such as the formation of resting stages other than spores – since archaea are not known to form spores – or the maintenance of cellular functions with traces of carbon and energy sources within the salt sediments, which would imply an almost infinitely slow metabolism. At this time, there are no methods available to prove directly a great microbial age, whether it be a bacterium or a haloarchaeon. However, it can be shown, when simulating the formation of halite in the laboratory by drying salty solutions, which contained microorganisms that the cells accumulate within small fluid inclusions (Figure 3). The cells can be pre-stained with the fluorescent dyes of the LIVE/DEAD kit (Fendrihan et al., 2006), which provides information on the viability status of a cell (green fluorescence indicating viable cells); the procedure improves also the visualization of cells within crystals. The fluid inclusions were square or rectangular, as is common in the rectangular mineral halite, and the cells were rather densely packed within the fluid-filled spaces. From such experiments it appeared that the cells accumulated always in the fluid inclusions; there were no stained cells within the mineralic halite (Figure 3; Fendrihan et al., 2006). Suggestions have been made that fluid inclusions migrate within evaporites and thus, new nutrients might become accessible for the entrapped cells (McGenity et al., 2000).

### Extraterrestrial halite and conclusion

Traces of halite were found in the SCN meteorites (named after the locations where they were found – Shergotty in India, Nakhla in Egypt, and Chassigny in France), which stem from Mars (Treiman et al., 2000). The Monahans meteorite, which fell in Texas in 1998, contained macroscopic crystals of halite, in addition to potassium chloride and water inclusions (Zolensky et al., 1999). Recently,



**Deep Biosphere of Salt Deposits, Figure 3** Localization of pre-stained haloarchaea in fluid inclusions of artificial halite. Cells were stained with the LIVE/DEAD BacLight kit prior to embedding. Low (*left panel*) and high (*right panel*) magnification of *Halobacterium salinarum* NRC-1 cells. Cells were observed with a Zeiss Axioskope fluorescence microscope.



evidence for salt pools on the Martian surface was obtained (Osterloo et al., 2008). These results are intriguing – they suggest that the formation of halite with liquid inclusions could date back billions of years and occurred probably early in the formation of the solar system (Whitby et al., 2000). Could halophilic life have originated in outer space and perhaps traveled with meteorites, could haloarchaea have persisted in environments as they are found today on Mars?

Viable extremely halophilic archaea, representing novel strains, were isolated repeatedly from Permo–Triassic and other ancient salt sediments, suggesting their capacity for long-term survival under dry conditions. Together with the discovery of extraterrestrial halite it appears thus feasible to include into the search for life on other planets or moons specifically a search for halophilic microorganisms.

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## Cross-references

Archaea  
Astrobiology  
Bacteria  
Deep Biosphere of the Oceanic Deep Sea  
Extreme Environments

Halobacteria – Halophiles  
 Saline Lakes  
 Salinity History of the Earth's Ocean  
 Terrestrial Deep Biosphere

## DEEP BIOSPHERE OF SEDIMENTS

See entries “*Terrestrial Deep Biosphere*,” “*Deep Biosphere of Salt Deposits*,” “*Deep Biosphere of the Oceanic Deep Sea*,” and “*Basalt (Glass, Endoliths)*.”

## DEEP BIOSPHERE OF THE OCEANIC DEEP SEA

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### Definition and overview

Although used in many different ways, the term “biosphere” is principally defined either as zone in which life occurs, thereby overlapping the atmosphere, the hydrosphere, and the lithosphere, or as the entity of living organisms on Planet Earth. Both perceptions commonly focus on the Earth's near-surface environment, with all domains sharing solar energy used in the process of photosynthesis. The deep-sea realm takes a special position in this context, as deep-sea pelagic and the majority of benthic organisms live in the ocean's aphotic zone and inhabit the widespread abyssal plains, respectively. For a long time, their main food source has been considered to be based on particulate organic matter (POM) from the ocean's surface primary production and its sedimentation to abyssal depths (Gage and Tyler, 1991 and references therein, D'Hondt et al., 2002, 2004). With the discovery of “ocean vents” in the late 1970s (Corliss et al., 1979), this general perspective was broadened by the perception of the enormous potential of chemical energy through the reaction of seawater, rock material, and fluids rising from the Earth's interior. According to this concept of energy for life, the term ‘surface biosphere’ has been opposed to ‘sub-surface biosphere’ (also commonly found in literature as ‘deep biosphere’). Following this definition, the deep-seafloor with its highly diverse topography from heterotrophic to pure chemotrophic habitats has to be treated as a transition zone between both biospheres. Opposed to the “*deep hot biosphere*” (Gold, 1992), occurring by definition in oceanic as well as terrestrial subsurface environments, stands the “*deep cold biosphere*” as defined for permafrost sediments (Vorobyova et al., 1997) and ice cores from the depths of Lake Vostoc (Venter, 2001).

### Life in the deep sea

Comprising approximately 65% of the Earth's surface, the deep-sea environment is characterized by hyperbaric, aphotic, and low-temperature conditions and highly

diverse seascapes. Canyons, seamounts, ridges, fractures, and trenches, but also biogeochemical oases such as cold seeps, mud volcanoes, carbonate mounds, brine pools, gas hydrates, hot vent systems, and deep-water coral reefs provide ample niches for a highly diverse pelagic and benthic deep-sea community (Tyler, 2003). It was only during the construction of the transoceanic telegraphic communication network that people realized the ocean's topographic alterations and astonishing depths. In 1861, the repair of an overgrown cable from 1,800 m water depth in the Mediterranean finally aroused the scientific community which by then adhered to Edward Forbes' theory on a completely ‘azotic’ zone below a water depth of 550 m. Though, 11 years had elapsed before the first global, scientific expedition onboard the “*Challenger*” (1872–1876) finally convinced people that a flourishing life in fact exists in the deep-sea realm. Numerous, further expeditions and a rushing development of technical facilities allowed deep-sea researches in 1960 to reach even the ocean's deepest surveyed point, the Challenger Deep at 10,911 meters below sea level (mbsl), located at the southern end of the Mariana Trench within the western Pacific Ocean (Piccard and Dietz, 1961). Since then, several studies on large-scale patterns and the zoogeographical origins of deep-sea organisms evidenced a high macrobenthic diversity (Gage and Tyler, 1991 and references therein). These organisms display a depth-dependent zonation as a result of basin age, deep currents (as barriers or dispersal), topographic boundaries, disturbance processes, and sedimentation in connection with depth-related environmental patterns (for review see Levin et al., 2001; Stuart et al., 2003). Macro- and meiofauna are losing importance with increasing water and sediment depth, whereas microorganisms like bacteria, archaea, and fungi account for up to 90% of the deep-sea benthic biomass (Pfannkuche, 1992). Sinking particles may carry large numbers of microorganisms from upper zones ( $10^8$ – $10^{10}$  cells  $m^{-2} d^{-1}$ ), inoculating deep marine surface sediments with an autotrophic and heterotrophic microbial community, as demonstrated by results from sediment traps (Turley and Mackie, 1995; Danovaro et al., 2000; Vanucci et al., 2001) or the deep-seafloor (Lochte and Turley, 1988).

### Particulate organic matter (POM)

Due to the fact that most deep-sea benthic species are deposit feeders (Sanders and Hessler, 1969), the locally qualitatively and quantitatively, variable import of POM from the ocean's surface waters plays a crucial role for macro-, meio-, and microorganisms living in deep surface sediments (Gooday and Turley, 1990). Mainly consisting of phytoplankton, marine snow, fecal pellets, (dead) zooplankton and molts, this material undergoes different steps of degradation during its passage from the photic, epipelagic (0–200 mbsl), through the mesopelagic (200–1,000 mbsl) to the actual deep-sea zones, in particular the bathypelagic (1,000–4,000 mbsl), the abyssal

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