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Microorganisms in extreme environments

Introduction

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Usually the growth of microbes is described for optimal or ideal conditions, conditions which only occur in the laboratory: constant and optimal temperature, usually around neutral pH, all nutrients in saturating concentrations, optimal redox potential and gas phase. In nature, all these factors may vary widely with place and also with time, and therefore the growth behavior of an organism is far from the one observed under laboratory conditions. In recent years interest in really anomalous, so-called extreme environments has come mainly from the question of whether life might be possible on other planets, e.g. on Mars, where the environmental factors are completely different from the ones on Earth.

In an experimental system a distinction must be drawn between organisms that just tolerate certain extreme conditions for survival and others which show optimal growth and differentiation in an extreme environment. For our present consideration it is important that in extreme conditions cells not only remain viable, but that all important physiological reactions such as respiration, biosynthesis of proteins, or nucleic acids must have their optima there. For the molecular biologist it is a challenging goal to find the molecular mechanisms which stabilize cellular components and allow their synthesis under extreme conditions. Some of the most important environmental factors with the extremes observed in nature are summarized in the figure. While the important factors temperature, availability of water and oxygen are treated separately in the following four papers, a brief survey of some others is given here.

Acidity and *alkalinity*[6,13,20] are stress factors often observed in nature. Effluents of acid mines or volcanic springs may show a pH between 1 and 2, and fruit juices or acid soils are in the range of 3–4. In contrast, alkaline soils have a pH around 9, alkaline lakes are known to go up to pH 10, and surfaces of concrete have a pH of up to 11. Since most environments have a pH between 5 and 9, most microorganisms have their pH optimum within this range, and only few are able to grow outside these limits. In mine effluents organisms like *Thiobacillus thiooxydans* or *Th.ferrooxidans* and *Sulfolobus sp.* oxidize various sulfides to sulfuric acid, reducing the pH as far as 1. Large amounts of sulfuric acids are drained into rivers as a consequence of microbial sulfide oxidation.

Alkali-tolerant organisms are found among the nitrate and sulfate reducers, and some may survive at a pH of 13; species of *Bacillus, Flavobacterium,* and *Streptococcus* may also grow at a pH of 10–11. Since the internal pH of acidophiles as well as alkaliphiles is not far from neutrality there is a large pH difference across the membrane in these organisms. Ion pumps of high activity are necessary in acidophiles to export protons continuously. In alkaliphiles a large membrane potential has to compensate for the inverse pH gradient to allow ATP synthesis in these organisms.

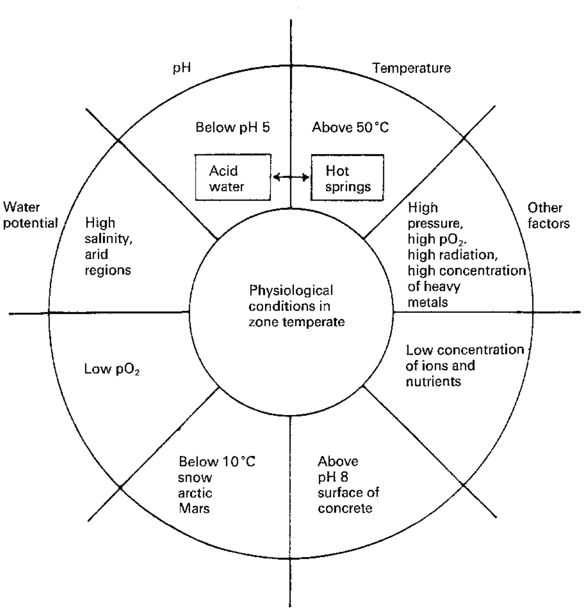
Radiation[3,9,24] is another environmental stress factor. Radiation in the UV range and ionizing radiation of shorter wavelengths are of special importance and cause cellular damage. The radiations are potentially destructive for life; however, cells can survive with a certain probability. Several mechanisms have been acquired for protection against irradiation. The most important natural source of radiation is the sun, although only a small percentage of the solar radiation, namely the UV region below 400 nm, causes cellular damage. The intensity of this UV radiation is dependent, among other factors, on the ozone concentration, clouds, and particle density in the atmosphere. A decrease in the ozone level of 1% will probably increase active radiation by 1–2.5%. Ionizing radiation may come as cosmic rays and is at a level of around 30 mrad/year depending on the altitude. Terrestrial radiation originates from nuclides in rocks. This type of radiation may be more hazardous since its source may be incorporated into cells. Man-made radiation up to the present accounts for only a few percent of that which occurs naturally; however, there may be high local accumulation with much higher levels of radiation.

Sensitivity to radiation damage is dependent on the stage in the cell cycle as well as on the growth conditions and even more dramatically on species and strain. Differences in tolerance up to a factor of 200 have been observed even within the same species. Also, adaptation to radiation is possible, a fact that raises the question whether sterilization by irradiation will induce radiation-resistant organisms. In fact, radioresistant strains of various bacteria such as *E. coli* or *Salmonella* have been obtained already over 30 years ago, and at least some of these seem to be the result of single gene mutations.

How can a cell protect itself from radiation damage? While pigments, especially carotenoids, are effective in protecting against visible light, they do not protect the cell against UV or ionizing radiation. It also seems that no metabolic agent or cellular compound exist that could protect the cells against irradiation; this also holds true for higher levels of catalase or of DNA, or of different composition of DNA, which have all been suggested in this context.

It is thought that cellular damage occurs at the level of the DNA. This seems clear for UV light since the action spectrum is similar to the absorption of the nucleic acids. Several observations such as DNA content and base composition suggest that DNA is also the target for ionizing radiation. The lethal effect of radiation is mostly due to dimer formation among and between C and T, and crosslinking for UV radiation, while DNA breakage and free radical formation leading to chemical modifications results from ionizing radiation. Specific repair mechanisms are responsible for radiation-resistance. UV damage is controlled by the long known photoreactivation phenomenon where a specific enzyme cleaves the pyrimidine dimers. Light-independent, specific repair enzymes remove modified DNA structures and replace the fragment with the original base sequence, a process which involves several different steps. There is a close correlation between radiation resistance and the efficiency of the repair system. In this respect *Micrococcus radiodurans* and *M. radiophilus* are of special interest since they are by far more radiation-resistant than any other organism. Their repair system is clearly more efficient and dimers formed are replaced before DNA synthesis restarts.

In some environments *heavy metals*[4, 11] and other *toxic elements*[15] may restrict growth of organisms. Toxic levels of elements such as As, Ag, Cd, Co, Hg, Pb, U, or Zn are in the range of 10^{-5}–10^{-4} M. Some heavy metals like Co, Zn, or Mo are necessary for growth as trace elements in nutrient solutions, they are needed in the range of 10^{-9}–10^{-7} M. Toxicity is manifested by a change in morphology, in altered cell metabolism, bacteriostasis, or lethality. Several mechanisms are responsible for toxicity; often these elements react with functional groups such as – SH groups of enzymes or membranes. They may also cause the precipitation of substrates. Resistance against heavy metals may be due to a cellular detoxification system or a change in permeability. Often special metal binding proteins are found (metallothioneins) which drastically reduce activity of free heavy metals. Metal-tolerant microbes are of considerable technical importance in the process of metal leaching.



Environmental factors, which cause inhibition of growth and metabolism beyond certain limits. Inner circle: so-called physiological range, conditions which are acceptable for men and in which most of the higher organisms and a lot of microorganisms show the highest activities of life.

Mercury has a high affinity to –SH groups and is thus a strong enzyme inhibitor. Some microorganisms are capable of forming methyl- or dimethyl-mercury which are volatile and therefore may be regarded as a detoxification mechanism in soil and water. On the other hand methylmercury is highly toxic for vertebrates and furthermore methyl- and phenylmercury are again decomposed to elemental mercury and hydrocarbon by light or by certain microorganisms.

Low nutrient levels[10, 17, 18], oligotrophic environments, are widely distributed but often neglected as an environmental stress factor. It is thought that in all natural habitats periods of growth alternate with periods of starvation, governed by the supply of nutrients. As nutrient concentrations are usually low in natural waters, freely suspended microorganisms must have mechanisms to overcome these limitations. Strains of various substrate affinity were selected in chemostat enrichment cultures with natural water depending on substrate concentration and dilution rate. Usually heavy wall growth was observed[21]. Attachment to suspended particles leads to rapid growth on these submerged solid surfaces. Attachment seems to be due to slime excretion, possibly polysaccarides, and is hardly observed in experiments with high nutrient levels. Solid surfaces include rocks and clays as well as living organisms, biological debris and furthermore the air-liquid interfaces are habitats not to be neglected in ecological studies.

A concentration of ions and macromolecules occurs at the interface of a solid surface and a solution due to adsorption to surface charges. This adsorption renders the surface more hydrophobic and changes the surface free energy. Organisms in an oligotrophic environment which adsorb at the interface accumulated with nutrients get a selective advantage for growth.

Finally, *hydrostatic pressure*[22, 25, 26] is also an environmental factor that affects life on earth. As with other parameters an optimum pressure for growth or for the activity of enzymes is observed for each organism. Often pressure sensitivity is coupled with another factor, e.g. it has been demonstrated that *E. coli* is much more sensitive to pressure when the temperature is suboptimal. Organisms containing gas vacuoles are extremely sensitive to changes in hydrostatic pressure, but also in the absence of gas vacuoles pressure affects the processes in rate and extent where changes in volume occur. The assembly of microtubules is one example of extreme sensitivity, it is a process which occurs with large volume changes.

Barotolerance often depends on the physiological state of the cells and thus is influenced e.g. by the substrate. *Streptococcus faecalis* is more sensitive to pressure during growth on pyruvate than on glucose or other sugars where maximal growth pressure may be increased up to 750 atm. High pressures occur not only in deep sea environments where the temperature is rather low, but also in soil where factors like high temperature, high salinity, and nutrient limitation may put further stress on living species. A severe problem may also be the narcotic or toxic effect of compressed gases with the exception of He which is observed with higher organisms. In microorganisms there appears to be a counteraction between narcotic gases and the effect of pressure, and small gas molecules like He or N_2 especially reduce growth inhibition due to high pressure.

All organisms need water to live. The *water availability*[14,28] in a certain environment is determined by the water activity or the water potential. In an aqueous system the most important factor in this respect is the solute concentration. Water binding to surfaces has to be taken into account in an inhomogeneous system such as soil or sediment. Life in hypersaline environments[5,19,27] is one aspect of water availability, a topic treated later by H. G. Trüper[31].

Another factor of the environment is the partial pressure of oxygen. On the one hand, oxygen is the electron acceptor in respiration and thus its presence determines energy transduction in aerobic organisms. On the other hand oxygen is clearly toxic[23] for microaerophilic or anaerobic organisms. While triplet oxygen is the normal form and non-toxic, singlet oxygen $^1O_2$, superoxide radicals O_2^-, and peroxide O_2^{-2}, formed in metabolism, are highly toxic. The enzymes catalase, peroxidase, and superoxide dismutase change these derivatives into non-toxic forms.

Interestingly enough, some substrates are better degraded by microoganisms under anaerobic conditions than in the presence of oxygen. Since the evolution of the anaerobic organisms must have gone on for a longer time, more metabolic pathways from unusual substrates may have been developed in anaerobic organims. Some aspects of anaerobic and aerobic metabolism are discussed later by A. Zehnder[33].

Finally, extremes of temperature, life above boiling point[1,8,30,32] and below melting point[2,7,16] are treated in the reviews by A. Gounot[12] and K. O. Stetter[29].

In summary, microbes have developed various strategies to live in environments which do not seem to allow life at first sight. It will be a challenge for scientists to find all the mechanisms which allow nature to overcome these situations.

1 Amelunxen, R. E., and Murdock, A. L., Microbial life at high temperatures: mechanisms and molecular aspects, in: Microbial Life in Extreme Environments, pp. 217–278. Ed. D. J. Kushner. Academic Press, London 1978.

2 Baross, J. A., and Morita, R. Y., Microbial life at low temperatures: ecological aspects, in: Microbial Life in Extreme Environments, pp. 9–71. Ed. D. J. Kushner. Academic Press, London 1978.

3 Bridges, B.., Survival of bacteria following exposure to ultraviolett and ionizing radiations, in: The Survival of Vegetative Microbes, pp. 183–208. Eds. T. R. G. Gray and J. R. Postgate. Cambridge University Press, Cambridge 1976.

4 Brierley, C. L., Brierley, J. A., Norris, P. R., and Kelly, D. P., Metal-tolerant micro-organisms of hot, acid environments, in: Microbial Growth and Survival in Extremes of Environment, pp. 39–51. Eds G. W. Gould and J. E. L. Corry. Academic Press, London/New York 1980.

5 Brock, T. D., Ecology of saline lakes, in: Strategies of Microbial Life in Extreme Environments, pp. 29–47. Ed. M. Shilo. Verlag Chemie, Weinheim 1979.

6 Brown, M. H., Mayes, T., and Lelieveld, H. L. M., The growth of microbes at low pH values, in: Microbial Growth and Survival in Extremes of Environment, pp. 71–98. Eds. G. W. Gould and J. E. L. Corry. Academic Press, London/New York 1980.

7 Cameron, R. E., Honour, R. C., and Morelli, F. A., Antarctic microbiology – preparation for Mars life detection, quarantine and back contamination, in: Extreme Environments, Mechanisms of Microbial Adaptation, pp. 57–82. Ed. M. R. Heinrich. Academic Press, London 1976.

8 Castenholz, R. W., Evolution and ecology of thermophilic microorganims, in: Strategies of Microbial Life in Extreme Environments, pp. 373–392. Ed. M. Shilo. Verlag Chemie, Weinheim 1979.

9 DeSerres, F. J., Some aspects of the influence of environment on the radiosensitivity of microorganims, in: Microbial Reaction to Environment, pp. 196–216. Eds G. G. Meynell and H. Gooder. University Press, Cambridge 1961.

10 Dow, C. S., and Lawrence, A., Microbial growth and survival in oligotrophic freshwater environments, in: Microbial Growth and Survival in Extremes of Environment, pp. 1–20. Eds G. W. Gould and J. E. L. Corry. Academic Press, London/New York 1980.

11 Ehrlich, H. L., How microbes cope with heavy metals, arsenic and antimony in their environment, in: Microbial Life in Extreme Environments, pp. 381–408. Ed. D. J. Kushner. Academic Press, London 1978.

12 Gounot, A. M., Psychrophilic and psychrotrophic microorganisms. Experienta *42* (1986) 1192–1197.

13 Grant, W. D., and Tindall, B. J., The isolation of alkalophilic bacteria, in: Microbial Growth and Survival in Extremes of Environment, pp. 27–38. Eds G. W. Gould and J. E. L. Corry. Academic Press, London/New York 1980.

14 Griffin, D. M., and Luard, E. J., Water stress and microbial ecology, in: Strategies of Microbial Life in Extreme Environments, pp. 49–63. Ed. M. Shilo. Verlag Chemie, Weinheim 1979.

15 Hugo, W. B., Survival of microbes exposed to chemical stress, in: The Survival of Vegetative Microbes, pp. 383–413. Eds T. R. G. Gray and J. R. Postgate. Cambridge University Press, Cambridge 1976.

16 Innis, W. E., and Ingraham, J. L., Microbial life at low temperatures: mechanisms and molecular aspects, in: Microbial Life in Extreme Environments, pp. 73–104. Ed. D. J. Kushner. Academic Press, London 1978.

17 Jannasch, H. W., Microbial ecology of aquatic nutrient habitats, in: Strategies of Microbial Life in Extreme Environments, pp. 243–260. Ed. M. Shilo. Verlag Chemie, Weinheim 1979.

18 Koch, A. L., Microbial growth in low concentrations of nutrients, in: Strategies of Microbial Life in Extreme Environments, pp. 261-279. Ed. M. Shilo. Verlag Chemie, Weinheim 1979.

19 Kushner, D. J., Life in high salt and solute concentrations: halophilic bacteria, in: Microbial Life in Extreme Environments, pp. 318–368. Ed. D. J. Kushner. Academic Press, London 1978.

20 Langworthy, T. A., Microbial life in extreme pH values, in: Microbial Life in Extreme Environments, pp. 279–315. Ed. D. J. Kushner. Academic Press, London 1978.

21 Marshall, K. C., Growth at interfaces, in: Strategies of Microbial Life in Extreme Environments, pp. 281–290. Ed. M. Shilo. Verlag Chemie, Weinheim 1979.

22 Morita, R. Y., Survival of bacteria in cold and moderate hydrostatic pressure environments with special reference to psychrophilic and barophilic bacteria, in: The Survival of Vegetative Microbes, pp. 279–298. Eds T. R. G. Gray and J. R. Postgate. Cambridge University Press, Cambridge 1976.

23 Morris, J. G., Nature of oxygen toxicity in anaerobic microorganisms, in: Strategies of Microbial Life in Extreme Environments, pp. 149–162. Ed. M. Shilo. Verlag Chemie, Weinheim 1979.

24 Nasim, A., and James, A. P., Life under conditions of high radiation, in: Microbial Life in Extreme Environments, pp. 409–439. Ed. D. J. Kushner. Academic Press, London 1978.

25 Marquis, R. E., and Matsumura, P., Microbial life under pressure, in: Microbial Life in Extreme Environments, pp. 105–158. Ed. D. J. Kushner. Academic Press, London 1978.

26 Marquis, R. E., Microbial barobiology. BioScience *32* (1982) 267–271.

27 Rose, A. H., Osmotic stress and microbial survival, in: The Survival of Vegetative Microbes, pp. 155–182. Eds T. R. G. Gray and J. R. Postgate. Cambridge University Press, Cambridge 1976.

28 Smith, D. W., Water relations of microorganisms in nature, in: Microbial Life in Extreme Environments, pp. 369–380. Ed. D. J. Kushner. Academic Press, London 1978.

29 Stetter, K. O., Fiala, G., Huber, R., Huber, G., and Segerer, A., Life above the boiling point of water? Experientia *42* (1986) 1187–1191.

30 Tansey, M. R., and Brock T. D., Microbial life at high temperatures: ecological aspects, in: Microbial Life in Extreme Environments, pp. 159–216. Ed. D. J. Kushner. Academic Press, London 1978.
31 Trüper, H. G., and Galinski, E. A., Concentrated brines as habitats for microorganisms. Experientia *42* (1986) 1182–1187.
32 Welker, N. E., Microbial endurance and resistance to heat stress, in: The Survival of Vegetative Microbes, pp. 241–277. Eds T. R. G. Gray and J. R. Postgate. Cambridge University Press, Cambridge 1976.
33 Zehnder, A. J. B., and Svensson, B. H., Life without oxygen: what can and what cannot? Experientia *42* (1986) 1197–1205.

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Concentrated brines as habitats for microorganisms

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Summary. Concentrated salt solutions (brines) occur widely in the natural form of coastal lagoons, salt or soda lakes as well as in the man-made form of salterns or saltworks. They are inhabited by a limited number of specialized microorganisms, which use different strategies of haloadaptation. Extremely halophilic archaebacteria (Halobacteriaceae) compensate the high osmotic pressure of brines by high cytoplasmic K^+ion concentrations. This requires appropriate adaptations of both the intracellular and extracellular functional macromolecules. Only some of the halophilic, halotolerant and extremely halophilic eubacteria follow this strategy; while the majority of them, and the halophilic algae, synthesize and accumulate in their cells organic compatible solutes (mono-, disaccharides, glycosylglycerols, sugar alcohols, amino acids, betaines). These compounds have besides their water-binding activity, protective functions for enzymes. In eubacteria the most important compatible solute is glycine betaine. Besides this substance, further new substances have been found, such as ectoine, for which functions as well as biosynthetic pathways have yet to be elucidated.

Key words. Brine microorganisms; saline habitats; halophilic bacteria; compatible solutes; water stress; *Halobacterium*; *Ectothiorhodospira.*

Salt, i.e. sodium chloride, has been in use as an appetizing food additive since very ancient times and by most peoples of the world. Also, in many countries and cultures, salt has been used for thousands of years for preserving meat, fish, raw hides, and – in ancient Egypt – even human bodies[53]. However, this useful and economically very important application has not always been successful; spoilage of salt-preserved foods and hides has been known for a long time, too.

The rise of bacteriology at the beginning of this century brought the explanation for such preservation failures, and the titles of the first bacteriological studies in this field nicely document the particular directions of studies and results: for example Klebahn[29] 'Die Schädlinge des Klippfisches, ein Beitrag zur Kenntnis der salzliebenden Organismen', Harrison and Kennedy[19] 'The red discoloration of cured codfish', Petter[45] 'Over roode en andere bacteriën van gezouten visch', Lochhead[35] 'Bacteriological studies on the red discoloration of salted hides', Gibbons[16] 'Bacteria associated with the reddening of salt fish'.

Spoilage of salted goods was thus found to be frequently accompanied by massive growth of red colored bacteria. As these bacteria were found to require salt for growth they could not have survived in the fish or hides before the salting process. Therefore they must have been associated with the salt itself. It is well known by fishermen, for example in Norway, that salt imported from marine salterns, e.g. those of Portugal or Mediterranean countries, is better suited for salting fish than rock salt, due to its smoothness. On the other hand, salt makers have known since ancient times that in solar evaporation basins brines turn red, and that this is important for the timing of crystallization sequences of salts from ocean water.

It is therefore surprising that thorough studies of the bacterial flora of such saltworks have only been made very recently. The scanty bacteriological studies of strong brines up to 1925 were summarized briefly by Baas-Becking[2], who also pointed out for the first time the principal difference between brines of concentrated sea water and those of desert lakes, i.e., the influence of the chemical constituents upon the spectrum of organisms to be found in the different kinds of brine. His findings were substantiated by Hof[21]. The first thorough study of a large natural highly saline lake was Volcani's excellent doctoral thesis on the microflora of the Dead Sea[64]. Since then, numerous salt lakes and lake systems have been studied with respect to their microfloras, e.g., Great Salt Lake, Utah[46], again the Dead Sea[40], lakes in the Kulundinskaya Steppe, USSR[26], in the Wadi Natrun, Egypt[23], in the East African Rift Valley[57], in the Great Plains, Canada[33,41] and in Australia[10].

Many of these natural salt lakes are also used as mineral sources for the chemical industry of the countries where they are situated. The chemical composition of brines depends on the minerals dissolved in the water before evaporation. As seawater has a rather constant composition worldwide, brines in marine salterns and coastal lagoons confirm this uniformity. Ecologists thus speak of 'thalassohaline' environments in contrast to 'athalassohaline' ones that show typically different chemical compositions and are usually landlocked lakes such as 'desert