ECOLOGY AND BIODIVERSITY OF EXTREMELY ACIDOPHILIC MICROOORGANISMS

Douglas E. Rawlings
University of Stellenbosch, South Africa.

D. Barrie Johnson
University of Wales, Bangor, UK

Keywords: acidic environments, acid mine drainage, chemolithotrophs, extreme acidophiles, ferric iron respiration, iron cycling, microbial biodiversity, mineral biooxidation, iron oxidation, sulfur oxidation, thermophiles

Contents

Summary

Environments in which extremely acidophilic microorganisms (those able to grow optimally at pH 3 or less) are found occur in many places on Earth. Some are formed naturally while others are the result of human activity. Acidic environments formed outside of human intervention are typically areas with volcanic activity where inorganic sulfur compounds are brought to the surface where they are oxidized to sulfuric acid. Sulfide-containing minerals may also be exposed or brought to the surface as a result of mining activity and when oxidized these minerals give rise to acid and metal pollution known as acid mine drainage. Biological oxidation of reduced sulfur compounds is very much faster than chemical oxidation and, as a result, most extreme acid environments are created by biological activity. Although iron is an abundant element on the surface

of Earth, it is rapidly oxidized and forms insoluble precipitates in neutral environments. However, iron is readily soluble in low pH environments and the cycling of iron between its reduced ferrous and oxidized ferric forms plays an important role in the ecology of acidic environments. The energy released when reduced inorganic sulfur compounds and ferrous iron are oxidized permits the growth of a variety of carbon dioxide-fixing organisms. These autotrophic iron- and sulfur-oxidizing organisms form the backbone of most acid environment ecosystems in which a number of heterotrophic organisms also thrive. The temperature in many acid environments may be considerably higher than ambient, either because they are associated with volcanic activity (natural environments) or because the energy-producing reactions are exothermic (mining activity-linked environments). As a result, many extreme acidophiles are also thermophilic. In this chapter, most of the important extreme acidophiles, their characteristics and their interactions are described.

1. Definition of Extreme Acidophily

Natural and man-made environments with varying degrees of acidity are present on Earth. Given this continuous spectrum of acidic environments, any attempt to define an exact boundary between extreme and moderate acidophily will be somewhat arbitrary and open to debate. In this chapter, we have defined extreme acidophiles as those organisms that grow optimally at pH 3 or less. This definition includes a large number of autotrophic and heterotrophic organisms belonging to the three major biological lineages, *Archaea*, *Bacteria*, and *Eukarya*, however, it does not include organisms that are able to tolerate, but not grow optimally at, pH values of 3 or below. Organisms that are excluded by this definition are sulfur-oxidizing bacteria, such as *Thiomonas intermedius* (formerly *Thiobacillus intermedius*) and physiological relatives thatare able to lower the pH of their environment to below 3 but are not able to grow when inoculated into media at this pH. Also excluded are many intestinal bacteria and human pathogens that are able to temporarily tolerate the low pH environment of the stomach. Some of these bacteria, like *Helicobacter pylori*, exhibit some of the bioenergetic attributes of extreme acidophiles but have not been reported to grow in laboratory media below pH 4.

2. Low pH Environments

2.1.Geothermal and Volcanic Areas

High-temperature environments occur in zones of volcanism and in areas where Earth's crust is relatively thin. Examples of terrestrial and shallow marine locations include Yellowstone National Park (USA), Montserrat (West Indies), Whakarewarewa (New Zealand), Krisuvik (Iceland), the Kamchatka peninsular (Russia), Sao Michel (Azores), Volcano, Naples, and Ischia (all Italy) and Djibouti (Africa). Related to these are deep and abyssal submarine hydrothermal systems, such as the Mid-Atlantic ridge, the East Pacific Rise, the Guaymas Basin, and active seamounts (e.g., around Tahiti). Due to the influence of seawater, submarine hydrothermal systems are generally of pH 5–8 and saline. Acidic sites within geothermal and volcanic areas are most commonly located within solfataric fields. These are sulfur- and sulfide-rich areas where the potential for

acid production exceeds the buffering capacity of the basic minerals present. Elemental sulfur may be formed from condensation of volcanic gasses:

$$2H_2S + SO_2 \rightarrow 3S + 2H_2O \quad (1)$$

Biological oxidation of sulfur is mediated by acidophilic archaea (in the hotter zones within the solfatara) or bacteria (in the cooler fringes and run-off waters):

$$S + H_2O + 1.5O_2 \rightarrow H_2SO_4 \quad (2)$$

The extreme acidity and high temperatures cause the partial or complete destruction of minerals in the vicinity of the solfatara, leading to the formation of acidic mud pots. Oxygen solubility decreases with increasing temperature, so that the surface oxidized zone in a solfatarum often overlies an higher pH anaerobic layer, resulting in sulfur and iron cycling (involving changes in oxidation states of these elements) between the two layers.

Examples of extremely acidic environments found in geothermal and volcanic areas are shown in Figures 1–3.

Figure 1. The Norris Geyser Basin, a solfatara field in Yellowstone National Park, Wyoming. This is a typical sulfur-enriched geothermal area, within which may be found geysers, hot springs, fumaroles, and mudpots.

Figure 2. A boiling (85 °C), acidic (pH3), hot spring ("Frying Pan") located at outer fringe of the Norris Geyser area, Yellowstone National Park. Thermoacidophilic archaea have been isolated from this and similar environments.

Figure 3. Gelatinous, filamentous, microbial growths in a stream draining the Frying Pan hot spring. The temperature in the stream is 30–50 °C, and the pH (at about pH 2.6) is lower than that of the hot spring itself, due to microbial oxidation of sulfur. The indigenous acidophilic microflora are more diverse than in the boiling hot spring, and include moderately thermophilic bacteria (e.g., *Sulfobacillus* spp.) as well as archaea. The green color of the growths is due to the presence of the moderately thermophilic and acidophilic rhodophyte, *Cyanidium caldarium.*

-
-
-

Bibliography

Goebel B.M., Norris P.R., and Burton N.P. (2000). Acidophiles in biomining. *Applied Microbial Systematics* (ed. Fergus G. Priest and Michael Goodfellow), pp. 293–314. Dordrecht: Kluwer. [This chapter covers the acidophiles that are used for the commercial recovery of metals from ores, including how they may be identified using molecular techniques.]

Johnson D.B. (1998). Biodiversity and ecology of acidophilic microorganisms. *FEMS Microbiology Ecology* **27**, 307–317. [A minreview on the diversity and ecological relationships found among acidophilic bacteria and archaea.]

Rawlings D.E. (1997). *Biomining: Theory, Microbes and Industrial Processes*. 302 pp. Berlin: Springer-Verlag. [This multiauthor book deals with the use of acidophiles in biomining and includes a section on acidophilic microorganisms, including their physiology and diversity.]

Wächtershäuser G. (1990). Evolution of the first metabolic cycles. *Proceedings of the National Academy of Science (USA)* **87**, 200–204. [This is an article concerning the possibility of "pyrite-pulled" early metabolic reactions. It contains references to earlier related work.]

Biographical Sketches

Douglas Eric Rawlings was born in East London, South Africa in November 1950. He completed BSc Hons (1972) and PhD degrees (1976) at Rhodes University in Grahamstown, South Africa. After two years as a research officer at the Leather Industries Research Institute and four years as a lecturer at the University of the Witwatersrand, he moved to the University of Cape Town (1982) where he was promoted to an *ad hominem* chair as Professor of Microbiology in 1988. In July 1998, he took up his present appointment as Professor of Microbiology at the University of Stellenbosch.

The research of Professor Rawlings has mostly concerned the bacteria involved in the extraction of minerals from ores. He was involved in the initial development of a commercially successful process for the biooxidation of difficult-to-treat gold-bearing arsenopyrite ores. The bulk of his research has focused on the molecular biology of the biomining bacterium, *Acidithiobacillus ferrooxidans* and more recently this work has been extended to include other biomining bacteria. The findings of this research have been published in over 80 peer-reviewed journal articles and book chapters. In 1997, he edited a book entitled *Biomining: Microbes, Theory and Industrial Processes*. He currently serves on the editorial board of the journals *Applied and Environmental Microbiology* and *International Deterioration and Biodegradation*, is General Secretary of the Royal Society of South Africa, and has served several terms on the council of the South African Society for Microbiology. Professor Rawlings is a life fellow and a distinguished teacher of the University of Cape Town, a recipient of a silver medal from the South African Society for Microbiology, the PanLab's award from the Society for Industrial Microbiology (USA), and is a fellow of the Royal Society of South Africa and a founder member of the South African Academy of Science.

David Barrie Johnson was born in New Tredegar, South Wales, in March 1954. He obtained a BSc (Hons) in Biochemistry and Soil Science from the University of Wales, Bangor in 1975 and a PhD (also from UWB) in 1979. He joined the academic staff at UWB as a demonstrator in 1977 and was appointment as a lecturer in 1979. In 1994, he was promoted to Senior Lecturer in the School of Biological Sciences at UWB.

Barrie Johnson's research activities have been in the field of environmental microbiology. In particular, he and his research team have been concerned with the microbiology of extremely acidic environments, both from pure and applied perspectives. His work has taken him to mining areas and geothermal sites in different parts of the world, including Yellowstone National Park, Wyoming, and the Caribbean Island of Montserrat. A major interest involves studying the biodiversity of acidophilic microorganisms, and he and his team have been very successful in isolating and characterizing novel acidophiles from natural and anthropogenic environments, in elucidating how these microorganisms with each other in nature. The applied aspects of his research include the use of acidophiles to accelerate mineral dissolution by oxidative and reductive mechanisms and investigating the use of these microorganisms to remediate acidic wastewaters. Outside of UWB, Barrie Johnson has worked at the Idaho National Engineering and Environmental Laboratory, USA and has lectured at universities in many different countries. He is currently serving on the editorial boards of *Biotechnology Letters* and *Resource and Environmental Biotechnology*. He has 90 publications as book chapters and in scientific journals.