PREFACE

> *Imagination is everything. It is the preview of life's coming attractions. Imagination is more important than knowledge.*
>
> Albert Einstein

Recently, I had the chance to see one of the largest exhibitions of Claude Monet's most famous series of paintings in London. As Kenneth Clark explains in his famous book *Civilization*, Monet attempted a kind of color symbolism to express the changing effects of light. For example, he painted a series of cathedral facades in different lights—pink, blue, and yellow—which seem to me too far from my own experience. The colors of these objects depend on the physical environment, such as sunlight, snow, the time of the day, the season etc.

Under different conditions, one object may show quite different properties. Who can be sure what is the absolute property? The biological world may have the same uncertainty.

Pioneering Studies of Extremophiles

THERMOPHILES

In June 1965, Thomas Brock, a microbiologist at Indiana University, discovered a new form of bacteria in the thermal vents of Yellowstone National Park. They can survive at near-boiling temperatures. At that time the upper temperature for life was thought to be 73 °C. He found that one particular spring, Octopus Spring, had large amounts of pink, filamentous bacteria at temperatures of 82–88 °C. Here were organisms living at temperatures above the "upper temperature for life." He isolated and collected many microbes from this geothermal area (Brock and Freeze, 1969). It is worth mentioning that the strain YT-1 was the first to be used as source of *Taq* polymerase (Brock, 1997). His findings paved the way for a new microbiology: taxonomy, physiology, enzymology, molecular biology, genetics, etc.

ALKALIPHILES

In 1968, I was looking at the Renaissance buildings in Florence in Italy which were so very different from Japanese architectures. About 500 years ago no Japanese could have imagined this Renaissance culture. Then suddenly a voice whispered in my ear, "There might be a whole new world of microorganisms in different unexplored cultures." "Could there be an entirely unknown domain of microorganisms at alkaline pH?" The acidic environment was being studied, probably because most food is acidic. Science, just as much as the arts, relies upon a sense of romance and intuition. Upon my return to Japan, I prepared an alkaline medium and inoculated it with small amounts of soil collected from various sites on the campus of the Institute of Physical and Chemical Research (RIKEN). To my surprise, after overnight incubation at 37 °C, various microorganisms flourished in all 30 test tubes. Here was a new alkaline world that was utterly different from the neutral world discovered by Pasteur. I named these microorganisms that thrive in alkaline environments "alkaliphiles." This was my first encounter with extremophiles (Horikoshi, 1971).

One of the enzymes isolated from alkaliphiles is cyclodextrin glycosyltransferase which produces very high yield of cyclodextrin (CD). CD can encapsulate many volatile compounds. For instance, Japanese horseradish (wasabi) is encapsulated in CD and its flavor and taste are stabilized for long times (Horikoshi, 2006).

Wasabi is one of the most important spices for Japanese food. Nowadays you may have typical Japanese food sushi all over the world. This means CD popularizes Japanese food culture throughout the world.

> *Time is the best appreciator of scientific work, and we know that an industrial discovery rarely bears all its fruits in the hands of its first inventor.*
>
> Louis Pasteur

Extreme Environments

Not too many years ago, almost all biologists believed that life could survive only within a very narrow range of temperature, pressure, acidity, alkalinity, salinity, and so on. Nature, however, contains many extreme environments, such as hot springs, saline lakes, deserts, alkaline or acidic lakes, and the deep sea. All of these environments would seem to be too harsh for life to survive.

However, in recent times many organisms have been found in such extreme environments. Moreover, some of them cannot survive in a so-called "moderate" environment. Thermophilic bacteria grow in environments with extremely high temperatures, but cannot grow at 20–40 °C. Some alkali-loving bacteria cannot grow in a nutrient broth at pH 7.0 but flourish at pH 13. These properties depend on growth conditions (pH values of media for thermophiles, culture temperatures for alkaliphiles and piezophiles, etc.). Thus, the idea of extreme environments is relative, not absolute. Clearly we have been too anthropocentric in our way of thinking. We should therefore extend our consideration to other environments having multi/poly extreme conditions for life. They metabolize inorganic sulfur or iron as their energy source. Some of them isolated from deep sea or sub-deep seafloor sediment have an entirely different metabolic pathway from conventional life. It is distinctly possible that very ancient life-forms may be in hibernation in the world's largest refrigerator.

Definition of Extremophiles

Extremophiles are organisms that are adapted/evolved to grow optimally at or near the extreme ranges of environmental variables. R.D. MacElroy first coined the term "extremophiles" in a 1974 paper entitled "Some comments on the evolution of extremophiles," but definitions of extreme and extremophiles are of course anthropocentric. A much larger diversity of organisms are known that can tolerate extreme conditions and grow but not necessarily optimally in extreme habitats; these organisms are defined as extremophiles.

Distribution of Polyextremophiles

At the wider scale, extreme environments on Earth have arisen and continue to arise as a consequence of plate tectonic activity, the dynamic nature of the cryosphere, and the formation of endorheic basins. Plate boundaries occur wherever two tectonic plates collide and result in the formation of mid-ocean ridges, mountains, deep-ocean trenches, volcanoes, and other geothermal phenomena such as marine hydrothermal vent systems.

Hydrothermal vent systems are found in abundance worldwide and are presumed to have existed as soon as liquid water accumulated on Earth. Black smoker and carbonate chimney vents are different environments: black smokers arise at diverging plate boundaries above magma chambers, are highly acidic (pH 1–3), are very hot (up to 405 °C), with vent fluids rich in Fe and Mn, and CO_2, H_2S, H_2, and CH_4; carbonate chimneys in contrast are found off-axis (away from diverging boundaries), are highly alkaline (pH 9–12), moderately hot (up to 90 °C), and rich in H_2, CH_4, and low molecular weight hydrocarbons.

A high proportion of the Earth's surface contains water in solid form (sea ice, ice caps and sheets, glaciers, snowfields, permafrost), the longevity of which may be thousands or even a few million years. Cryosphere-climate dynamics are complex and influence precipitation, hydrology, and ocean circulation. Deserts develop in regions where precipitation is very low (or zero) and also unpredictable. Highly saline lakes and pans often develop under these circumstances.

The average depth of the world's oceans is about 3,800 m; high pressure generates yet another extreme environment. Oligotrophic environments are defined as those presenting very low nutrient concentrations; they include oceans deplete in iron, nitrate, phosphate, tropical laterite soils, and white sands. Finally, a range of environments are deemed to be extreme by virtue of chemically and/or physically caused toxicity (e.g., soils high in arsenic, lakes exposed to high incident radiation).

New extreme ecosystems continue to be discovered and investigated including the deep biosphere that exists at great depths in sub-seafloor sediments and in subterranean rock formations, and the carbonate chimney vent system. Extreme environments almost invariably are affected by two or more extreme conditions. Is our current knowledge of extremophile diversity comprehensive? It is highly possible that acidopsychrophiles, acidohalophiles, and thermohalophiles exist, and these should not be neglected by microbiologists!

It may appear overhasty to introduce the evolution and discussions of the origin of life in the preface. Assuming a thermophilic beginning, acidophily probably arose at an early stage, while alkaliphily evolved only after certain mineral precipitation and sufficient buffer concentration of CO_2 was established in the atmosphere. Moreover, halophily could have developed only after an arid climate was imposed on land and psychrophily only after a major temperature fall.

The question "what is life?" is precisely the question "what is evolution?"

Carl R. Woese

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Koki Horikoshi is Professor Emeritus of Microbiology at the Tokyo Institute of Technology, Toyo University, and RIKEN Institute, Tokyo. He was also Director of the Extremobiosphere Research Center of JAMSTEC. He received his Ph.D. from the University of Tokyo, Japan, and discovered alkaliphiles in 1969. His research on alkaliphiles, on which he has worked for over 40 years, has been published in more than 600 papers, and he is an ISI Highly Cited Person. His works has paved the way not only for an entirely new microbiology but also for novel applications of alkaline enzymes. Furthermore, he focused his interest on deep-sea microorganisms under extreme conditions (high pressure, high temperature, etc.), and he founded the Extremobiosphere Research Center at JAMSTEC. For these outstanding research activities, he has received many awards from various societies, one of the most prestigious being the "Award of the Japanese Academy" from the Emperor of Japan. Koki Horikoshi has published four monographs on alkaliphiles (1982, 1991, 1999, and 2006) and one *Extremophiles Handbook* (2011). In 1999, he launched the important journal *Extremophiles*, published by Springer, and he served as the Chief Editor for 13 years. He was a founder of the International Society of Extremophiles (ISE) (2001) and served as its first President. For these activities, ISE honored him with the award for lifetime achievement.

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