Chapter 1
Helge Larsen (1922–2005) and His Contributions to the Study of Halophilic Microorganisms

Aharon Oren, Antonio Ventosa, and Yanhe Ma

1.1 Introduction

In 1972, the late Helge Larsen was honored by The Netherlands Society for Microbiology to deliver the fourth A.J. Kluyver memorial lecture. The somewhat enigmatic title of his presentation – "The halobacteria's confusion to biology" (Larsen 1973) was based on "The microbe's contribution to biology," the title of the famous series of essays by Kluyver and van Niel (1956). It was Albert Jan Kluyver who had encouraged Larsen to start a thorough study of the halophiles: "In the early fall of 1954 I had the good fortune that Professor Kluyver came to Trondheim upon an invitation from Norway Institute of Technology, to take part in my promotion to the status of *doctor technicae*. At that time, I had just been approached by representatives of the salt fish industry in Norway, who had inquired of me whether I would look into the problem of microbial deterioration of salt fish. I happened to discuss this matter with Kluyver and I clearly remember the enthusiasm with which he recommended me to take up work on the microbes of salt and salted products" (Larsen 1973).

Helge Larsen's diverse studies of the world of microorganism inhabiting hypersaline habitats made him the greatest expert of his time on the halophiles. His chapter on "Halophilism" in "The Bacteria" Vol. IV (Larsen 1962) was the first comprehensive review written on halophilic microorganisms and their modes of adaptation to life at high salinity, and this visionary article introduced many to the fascinating world of the halophiles. Later general articles (Larsen 1981, 1986) and

A. Oren (✉)
Department of Plant and Environmental Sciences, The Institute of Life Sciences,
The Hebrew University of Jerusalem, 91904 Jerusalem, Israel
e-mail: orena@cc.huji.ac.il

A. Ventosa
Department of Microbiology and Parasitology, University of Sevilla, 41012 Sevilla, Spain

Y. Ma
Institute of Microbiology, Chinese Academy of Sciences, Tianjin Institute of Industrial Biotechnology, 1 Beichen West Road, Beijing 100101, China

A. Ventosa et al. (eds.), *Halophiles and Hypersaline Environments*,
DOI 10.1007/978-3-642-20198-1_1, © Springer-Verlag Berlin Heidelberg 2011

specialized reviews on the biochemistry (Larsen 1967) and ecology (Larsen 1980) provided timely updates on the field of halophile microbiology.

1.2 Helge Larsen's Life and Studies

Helge Larsen (Fig. 1.1) was born in Ålesund, Norway on 25 April 1922. He graduated as a chemical engineer from the Norwegian Institute of Technology in Trondheim in 1944. From 1947 until 1950 he worked with Cornelis van Niel at the Hopkins Marine Station, Pacific Grove, CA, where he studied the biology of the green phototrophic sulfur bacteria. He obtained his Ph.D. degree in 1954 based on a thesis "On the microbiology and biochemistry of photosynthetic green sulfur bacteria." In 1955, he was appointed professor of biochemistry at the Norwegian Institute of Technology (now the Norwegian University of Science and Technology) in Trondheim, where he remained until his retirement in 1992.

In Trondheim, he worked on a number of projects of applied nature, developing modern fermentation technology in Norway. Early in his career he devised an efficient process for the industrial production of itaconic acid using *Aspergillus terreus*, and in the last years of his work he devoted part of his time to the development of an industrial process for the production of lysine.

His interest in the halophiles was also aroused because of applied aspects. From his early youth he was acquainted with the discoloration and spoilage of salted fish. By 1955, he had recognized that the color phenomena on dried cod known as brunmidd ("brown mite") and rødmidd ("red mite") were, respectively, caused by a halotolerant fungus and by an extremely halophilic prokaryote (Larsen 1986). It was realized that spoilage of the fish by the red microorganisms could be prevented by using rock salt instead of traditionally used sea salt which turned out to be the source of the red halophiles. Soon Larsen started to ask fundamental

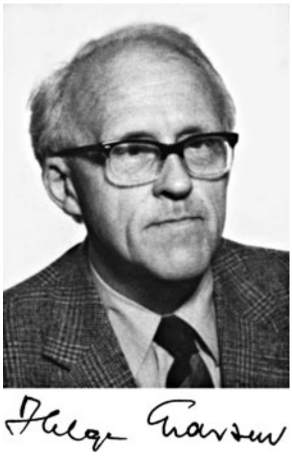

Fig. 1.1 Portrait of Prof. Helge Larsen (photograph: Det Norske Videnskaps Akademi; http://www.dnva.no/c27004/artikkel/vis.html?tid=27016)

questions about the nature of the halophilic microorganisms and their modes of adaptation to life in hypersaline environments. His extensive studies, described below, have contributed much to our understanding of the physiology, biochemistry, taxonomy, and ecology of the halophiles.

1.2.1 Studies on the Cell Envelope of Halophilic Microorganisms

Larsen's studies on the lysis of *Halobacterium salinarum* upon dilution with distilled water showed that Na^+ is specifically needed at high concentrations to keep the cell envelope intact. Attempts to demonstrate the presence of peptidoglycan in the cell wall failed, and Larsen and his coworkers showed that instead the cell is surrounded by an envelope composed of (glyco)protein subunits. The lysis phenomenon upon dilution was found to be due not to osmotic swelling and bursting of the cell, but to the disintegration of the envelope (Mohr and Larsen 1963a, b; Steensland and Larsen 1969). Different enzyme inhibitors tested did not affect lysis, excluding the possibility that enzymes are involved in the lysis phenomenon. In summary, "The observations support the conclusion that the globular lipoprotein particles, which constitute the bulk of the material of the cell wall of these bacteria, are bound together mainly by electrostatic forces and secondary bonds. When the cells are exposed to hypotonic solutions, or to ions which bind strongly to proteins, or to chemicals which are believed to break secondary bonds between protein molecules, the linkages binding the lipoprotein particles together are weakened so that the wall structure disintegrates. Only in the presence of high concentrations of sodium and chloride ions, or other ions which bind loosely to proteins, is it possible for the proteinaceous particles of the cell wall to associate in an orderly array" (Mohr and Larsen 1963b).

In contrast, the cells of the red extremely halophilic *Halococcus morrhuae* did not lyse in distilled water. Also, here the attempts to show presence of peptidoglycan failed, and instead the cells were shown to be surrounded by a thick wall composed of hexoses and hexosamines (Steensland and Larsen 1971).

At the time these studies were performed the concept of the Archaea as the third domain of life did not yet exist. Only in the late 1970s it became clear that the special nature of the envelopes of these organisms, and notably the lack of peptidoglycan, is also linked to their phylogenetic affiliation.

1.2.2 The Function of the Carotenoid Pigments of the Extreme Halophiles

To elucidate the function of the red carotenoid pigment of *Halobacterium salinarum*, Larsen compared the properties of the red wild type and a carotenoid-less white mutant. Both the red and the white cells survived exposure to the light intensity of full sunlight. However, when they were grown together at high light intensities, the pigmented wild type had a selective advantage and outcompeted the white mutant (Dundas and Larsen 1962). In the presence of the photosentitizer phenosafranine, the colorless mutant was rapidly killed at high light, while the red cells survived (Dundas and Larsen 1963). These experiments proved that the carotenoid pigments of many extreme halophiles provide protection against the damaging action of sunlight.

1.2.3 The Properties of the Gas Vesicles of Halobacterium

In a study of the gas vesicles of *Halobacterium salinarum*, Larsen characterized the conditions for production of new gas vesicles after collapse of the existing vesicles following application or pressure. He also devised a method for the isolation of gas vesicles from extreme halophiles, based on the lysis of the cell envelope in alkaline solution, a treatment that leaves the vesicles intact. These can then be collected from the surface of the solution (Larsen et al. 1967).

1.2.4 Taxonomy of Halophilic Microorganisms

Larsen had a profound interest in the taxonomy of the red extreme halophiles, and he was an active member of the International Committee on the Systematics of Bacteria subcommittee on the taxonomy of *Halobacteriaceae* in the years 1986–1994. He named the order *Halobacteriales* (Grant and Larsen 1989a) and described *Halobacterium volcanii*, later renamed *Haloferax volcanii*, a species of pleomorphic flat cells, isolated from the Dead Sea. This organism requires less NaCl than do *Halobacterium salinarum* and *Halococcus morrhuae*, but shows a high tolerance to magnesium, the dominant cation in Dead Sea water (Mullakhanbhai and Larsen 1975).

For Bergey's Manual of Systematic Bacteriology Larsen wrote descriptions of the family *Halobacteriaceae* (Larsen 1984a; Larsen and Grant 1989), the genus *Halobacterium* (Larsen 1984b; Larsen and Grant 1989), the genus *Halococcus* (Larsen 1984c, 1989), the genus *Haloarcula* (Grant and Larsen 1989b), and the genus *Haloferax* (Grant and Larsen 1989c).

As thanks for Helge Larsen's contributions to the field, two recently characterized new species of extremely halophilic Archaea were named in his honor: *Halostagnicola larsenii*, an extremely halophilic archaeon from a saline lake in Inner Mongolia (Castillo et al. 2006) and *Haloferax larsenii*, an extremely halophilic archaeon from a solar saltern in China (Xu et al. 2007).

1.2.5 Studies on Dunaliella

During his stay in the laboratory of A. Duncan Brown as a guest professor at the University of Wollongong, NSW, Australia, Larsen studied a salt-sensitive mutant of *Dunaliella tertiolecta*. This mutant was shown to require high concentrations of carbon dioxide for growth and has low carbonic anhydrase activity (Brown et al. 1987).

1.2.6 Microbiological Studies of the Deterioration of Fish

Larsen's work on microorganisms associated with the spoilage of fish was not restricted to the extreme halophiles. Studies of post-mortem changes included the characterization of the release of ammonia and other malodorous compounds (Strøm and Larsen 1979). The formation of bad-smelling trimethylamine from trimethylamine oxide during the deterioration of fish was already known for a long time. Larsen was the first to recognize the true nature of the process as a novel mechanism of anaerobic respiration. Trimethylamine oxide, a compound that serves as an osmotic stabilizer in fish tissues, can serve as electron acceptor for respiration by *Proteus* and some other prokaryotes, enabling anaerobic growth on non-fermentable substrates (Strøm et al. 1979).

1.3 Final Comments

Helge Larsen passed away on 11 March 2005. It is sad to note that nobody within the community of halophile scientists was at the time aware of Helge's death, and the obituary in Norwegian published on the website of the Norwegian Academy of Sciences (Eimhjellen 2005) remained unnoticed until recently (Oren 2010). The portrait reproduced in Fig. 1.1 was derived from that website.

The first international symposium that dealt with all aspects of the life of halophilic microorganisms was held in Obermarchtal, Germany, in 1985. There, two of the editors of this book (A.V. and A.O.) had the pleasure meeting "Mister Halophile," as he was known at the time, discussing their work with him, and being stimulated by his enthusiasm. In view of Helge's great contributions to the field we dedicate this volume to his memory.

References

Brown AD, Goyal A, Larsen H, Lilley RMcC (1987) A salt-sensitive mutant of *Dunaliella tertiolecta*. Arch Microbiol 147:309–314

Castillo AM, Gutiérrez MC, Kamekura M, Xue Y, Ma Y, Cowan DA, Jones BE, Grant WD, Ventosa A (2006) *Halostagnicola larsenii* gen. nov., sp. nov., an extremely halophilic archaeon from a saline lake in Inner Mongolia, China. Int J Syst Evol Microbiol 56:1519–1524

Springer
http://www.springer.com/978-3-642-20197-4

Halophiles and Hypersaline Environments
Current Research and Future Trends
Ventosa, A.; Oren, A.; Ma, Y. (Eds.)
2011, XX, 387 p., Hardcover
ISBN: 978-3-642-20197-4