COMPARISON OF MEMBRANE ATPases FROM EXTREME HALOPHILES ISOLATED FROM ANCIENT SALT DEPOSITS*

HELGA STAN-LOTTER¹,‡, MICHAEL SULZNER¹, EVA EGELSEER¹, CYNTHIA F. NORTON² and LAWRENCE I. HOCHSTEIN³

¹ Institute of Microbiology and Genetics, University of Vienna, Dr. Bohrg. 9, A-1030 Vienna, Austria; ² Department of Biology, University of Maine at Augusta, Maine 04330, U.S.A.; ³ NASA Ames Research Center, Moffett Field, California 94035, U.S.A.

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Abstract. Halophilic microorganisms were isolated from Triassic and Permian salt deposits. Two were rods and grew as red colonies; another was a coccus and produced pink colonies. The rods lysed in solutions that lacked added sodium chloride. Growth of all isolates was inhibited by aphidicolin and their bulk proteins were acidic as judged from isoelectric focusing. Therefore, these organisms were tentatively identified as extreme halophiles. Whole cell proteins patterns of the isolates following gel electrophoresis were distinct and differed from those of representative type strains of halophilic bacteria. The membrane ATPases from the rods were similar to the enzyme from Halobacterium saccharovorum with respect to subunit composition, enzymatic properties and immunological cross-reaction, but differed slightly in amino acid composition. If the age of the microbial isolated is similar to that of the salt deposits, they can be considered repositories of molecular information of great evolutionary interest.

1. Introduction

Salt deposits which resulted from evaporation of sea water, particularly during the Triassic and Permian periods, are found throughout the world. The maps published by Zharkov (1981), which were constructed taking continental drift into consideration, show a large arid climatic zone, where present-day England, the Zechstein regions of Northern Germany and the alpine region were close to the equator. This zone contained all early and late Permian evaporitic basins. Microscopic studies reveal the presence of bacteria in thin sections or in residues of dissolved rock salt (Rippel 1935 and other literature cited by Sonnenfeld, 1984). Cultivation of viable bacteria from rock salt of Permian age (Zechstein) was reported by Reiser and Tasch (1960) and by Dombrowski (1963), although Bien and Schwartz (1965) were unable to confirm these results.

Recently, extremely halophilic bacteria were isolated from salt crystals from an English salt mine (Norton 1988, 1989) whose deposition appears to have occurred during the Triassic period (Arthurton 1973). Extreme halophiles have also been

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** Corresponding author.
isolated from a Permian age bedded salt deposit located in New Mexico (R. Vreeland, personal communication) and from rock salt from an Austrian salt mine near Bad Ischl, as will be described in this communication.

The extreme halophiles are members of the archaebacteria (recently renamed Archaea; Woese et al., 1990) which consist of organisms thought to have diverged early from the main line of prokaryotic evolution (Woese and Fox, 1977). Ribosomal RNA sequence comparisons suggest a slow rate of evolution for archaebacteria (Woese, 1987). Their cellular components could thus represent a repository of information with respect to the early evolution of life. The presence of microorganisms in salt crystals of great antiquity offers considerable promise, particularly if the in situ age of such organisms can be established.

The $F_1F_0$ATP synthases (F-type ATPases) catalyze the synthesis of ATP at the expense of a proton gradient either via respiration or photosynthetically (for a recent review see Fillingame, 1990). The ubiquitousness of the enzyme implies an early origin during the evolution of life. However, the enzyme’s structural and functional complexity suggests its development during a long evolutionary process. The membrane ATPase from the extreme halophile *Halobacterium saccharovorum* has been intensely studied by us (Kristjansson and Hochstein 1985; Hochstein et al., 1987; Stan-Lotter and Hochstein, 1989; Stan-Lotter et al., 1991) with the notion that it might provide information with respect to the evolution of F-type ATPases. A comparison between membrane ATPases from *H. saccharovorum* with those of bacterial isolates from rock salt is described here. In addition, the microbial isolates from rock salt were compared in several experiments with halobacterial type strains obtained from culture collections.

**Material and Methods**

**Bacterial Strains**

*H. saccharovorum* (ATCC 29252) was the original strain isolated from a solar evaporation pond located in the San Francisco Bay area (Tomlinson and Hochstein, 1976). Other extreme halophiles were obtained from the German Collection of Microorganisms (DSM), Braunschweig. Bacterial isolates from salt sediments were from two locations, the Winsford salt mine in Cheshire, England, and the salt mine (Salzberg) near Bad Ischl, Austria. Samples (ca. 0.5 g) from the Winsford mine were collected after blasting from newly exposed faces and kept in absolute ethanol for transport. After 6 h, the salt pieces were transferred to 10 ml of sterile complex medium that was 20% with respect to NaCl (Norton and Grant, 1988). Samples of rock salt, with varying contents of clay, were obtained from the salt mine at Bad Ischl. Sample pieces of about 2 g were dipped in ethanol and flamed. They were transferred to 50 ml of a similar culture broth (M2 medium; Tomlinson and Hochstein 1972) and incubated at 37 °C with shaking. After three to four weeks, the Winsford samples became turbid, indicating growth of microorganisms,