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Isolation of Extreme Halophiles from Seawater

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Extreme halophilic bacteria were isolated from the ocean off the coast of Spain. All were gram-negative cocci. One isolate was compared to *Halococcus* sp. NCMB 757 and was found to have similar characteristics.

Extreme halophilic bacteria have been isolated from a broad diversity of hypersaline environments, especially those resulting from the evaporation of seawater (4). Although it is likely that these organisms could be dispersed throughout the world via ocean currents, little is known about this subject. On the other hand, the extremely halophilic bacilli are considered to be unable to survive in media with less than 10% NaCl (at this concentration the cell envelopes are dissolved), and although the cocci do not lyse below this limit, they do not show detectable growth at these concentrations (1, 5). The present study was designed to determine whether extreme halophilic bacteria could be isolated systematically from seawater.

Surface seawater samples were collected in plastic containers disinfected with alcohol. Samples were collected from the surface at a depth of 0 to 1 m and 5 km from the Mediterranean coast between Alicante and Santa Pola (southeastern Spain). They were taken from sites separated by about 5 km. Three samples of 5 liters were taken from each point. All were collected in January. The 5-liter samples were filtered through membrane filters (Gelman Instrument Co., Ann Arbor, Mich.; 0.2-μm pore size, 47-mm diameter) at ambient temperatures under strict aseptic conditions. The filters were then placed on petri plates containing either Eimhjellen or Sehgal and Gibbons media for extreme halophiles (3). The petri plates were placed in plastic bags which were sealed hermetically and incubated at 38°C.

After 5 to 7 days of incubation, each filter had between 10 and 25 nonpigmented colonies. Both gram-negative bacilli and gram-positive cocci were represented. Upon further incubation (15 to 20 days), 2 to 35 pink or red colonies also formed on each filter. The colonies were made up of gram-negative cocci. The bacteria from the nonpigmented colonies were able to grow in artificial seawater medium (6) supplemented with 1% yeast extract, but the bacteria from the pigmented colonies were able to grow only if the media contained at least 2 M NaCl. Thus, the latter were considered to be extreme halophiles.

Extreme halophiles were also isolated from seawater by the addition of 1,000 IU of penicillin per ml (final concentration) to the Eimhjellen medium. Since the extreme halophiles lack peptidoglycan in their cell wall (5), they could grow in the presence of penicillin. Under these cultural conditions, only pigmented colonies were observed, and all were gram-negative cocci. One of these isolates, hereafter referred to as F1-4, was selected for further study. It was compared with *Halococcus* sp. NCMB 757.

The ability of F1-4 and NCMB 757 to grow in various salt concentrations was determined. The media were composed of inorganic salts corresponding in proportions to the artificial seawater solution of Subov (6). Keeping these proportions, the concentration of all salts (except $CaCl_2$ and $NaHCO_3$, which were maintained at seawater concentrations) was increased over a range of concentrations. Yeast extract and antifoam were added to media to final concentrations of 1 and 0.5%, respectively. Media (100 ml) were dispensed into 500-ml Erlenmeyer flasks. The flasks were inoculated with 1-ml samples of cultures grown in media with 25% salt concentration that had attained an absorbance of 0.3 at 520 nm. The flasks were incubated at 38°C, and the media were continuously agitated via a magnetic stirrer. As shown in Fig. 1, F1-4 and NCMB 757 showed the same optimum and very similar spectra. Finally, we estimated the ability of the two strains for surviving in seawater by harvesting an exponentially growing population (same medium and incubation conditions as before) by centrifugation and suspending in sterile seawater at the original cell density. The suspension was kept at room temperature with shaking. Samples were taken at intervals and plated on Eimhjellen medium. Figure 2 shows that each microorganism could survive in this environment for at least 7 days, and a small percentage (10%) of the

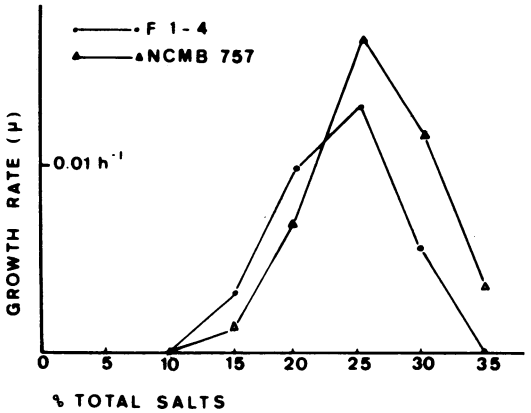

FIG. 1. *Growth rates of the strains F1-4 (isolated from seawater) and Halococcus sp. NCMB 757 at different concentrations of the seawater salts.*

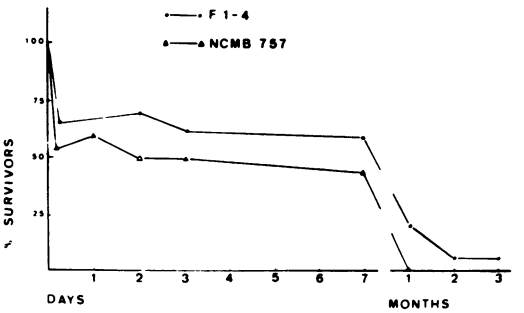

FIG. 2. *Percentage of survivors of the collection and marine strains of halococci suspended in sterile seawater. Death between 7 and 30 days occurred gradually.*

population of F1-4 was still viable after 3 months.

Our data indicate that extreme halophiles can be isolated from ocean water even though they are unable to grow at such low salinity. Although the salinity of the Mediterranean is considered to be slightly higher than most oceans, the salinity is considerably less than that required to support the growth of the extreme halophiles. Thus, it is apparent that the extreme halophiles can survive for periods of time in the ocean, which may play a role in their dispersal to hypersaline environments.

It is also interesting that all of the extreme halophiles that we isolated were cocci. This most likely is due to the fact that extreme halophilic bacilli have lower resistance to low salt concentrations than extreme halophilic cocci (2). Finally, although the origin of the extreme halophiles that we isolated is not known, it is possible that they originated from the salterns on the coast of Spain.

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