

Salt Resistance in Bacteria Isolated from Solar Salts

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ABSTRACT

Salt resistance in regard to the growth of halophilic bacteria isolated from solar salts was investigated. The isolated bacteria required Na⁺ for growth, and could not grow in a salt-free medium or in an environment made hypertonic by the non-electrolyte sorbitol. The bacteria exhibited an obligately halophilic character. Although some bacterial cells with rather high salt resistance were able to grow in NaCl concentrations from 0.1 M to 4.0 M, reaching full growth at 0.5 M to 3.0 M, optimal growth was obtained at 0.5 M NaCl. This suggests that they are a slightly halophilic bacteria of marine origin. The strains of bacteria were able to grow at temperatures from 25° to 42°C, but the optimal growth temperature was 25°C. These strains could also grow in 0.5 M NaCl, isotonic KCl, MgCl₂ and LiCl environments with BYP medium, but not in a hypertonic environment with sorbitol. This fact shows that they require salt as an osmotic environment for growth. These results show that slightly halophilic bacteria originating from a marine environment have considerable diversity in regard to salt resistance and temperature for growth.

INTRODUCTION

Large quantities of salt are imported to Japan from foreign countries, mainly Mexico and Australia. These salts consist of solar salts and rock salt. Solar salts usually originate from marine salt-erns in the Gulf of Mexico or Australia, etc., whereas rock salts are mined.

Historically, solar salts have been used to preserve fish, meat and hides. However, red discoloration of salted fish frequently occurred; this phenomenon, therefore, has long been of practical interest (Baase-Becking, 1931). This discoloration was shown by Harrison and Kennedy (1922) to be due to red halobacteria. Living halobacteria were entrapped within the fluid inclusion formed as the crystals developed (Norton and Grants, 1988). Solar salt easily forms hard rock crystals. The present authors attempted to isolate the bacteria from these solar salts, using BYP medium, containing 0.5 or 1.5 M NaCl concentration. The characteristics and salt resistance of the isolated bacteria were investigated.

METHODS

Bacterial strains were isolated from imported solar salts using BYP medium containing 0.5 and 1.5 M NaCl. BYP medium, used as a liquid or solidified with 1.5% agar, is composed of 1% polypeptone, 0.3%

yeast extract, 0.3% beef extract and 3 or 9% NaCl. The pH was adjusted to 7.2 with 1 N KOH solution.

Stock cultures were maintained on an agar slant BYP medium containing 1.5% agar. BYP medium was also used to prepare the cultures for the growth experiments. Pure cultures were obtained by colony formation with three repeats. The cells from liquid precultures were inoculated to growth media with 100-fold dilution. The cultures were shaken at 90 strokes per min at 30°C for various periods of time. Growth was estimated by measuring absorbance at 650 nm using a Bausch and Lomb Spectronic 20 photometer (Morishita and Takada, 1976).

RESULTS

Twenty-eight pure strains of halophilic bacteria were isolated using 0.5 M and 1.5 M NaCl BYP media. The growth responses of the strains to various NaCl concentrations were examined. These bacteria were able to grow in a NaCl environment and could not grow in a NaCl-free environment. The results of strains No. 8 and 10 are shown in Fig. 1. Maximal growth of both strains was obtained in 0.5 M NaCl concentration. Strain No. 10 could not grow in a 2 M NaCl environment. However, strain No. 8 showed salt-resistant growth in high NaCl concentration, such as 4 M.

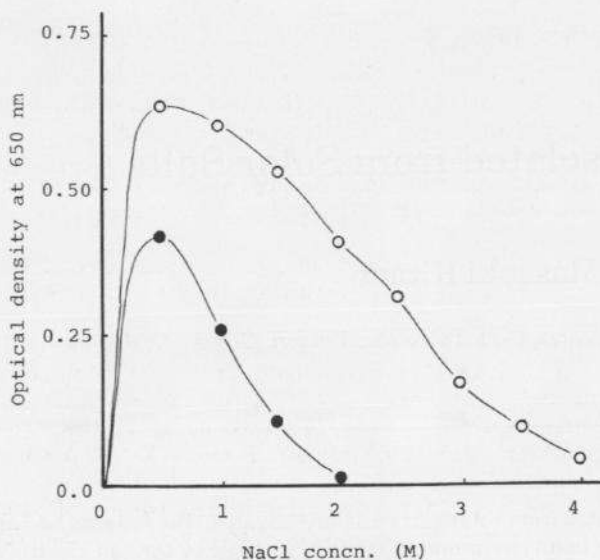


Fig. 1. Effect of NaCl concentrations on growth of isolated marine bacterial strains No. 8 (hollow circles) and No. 10 (filled circles).

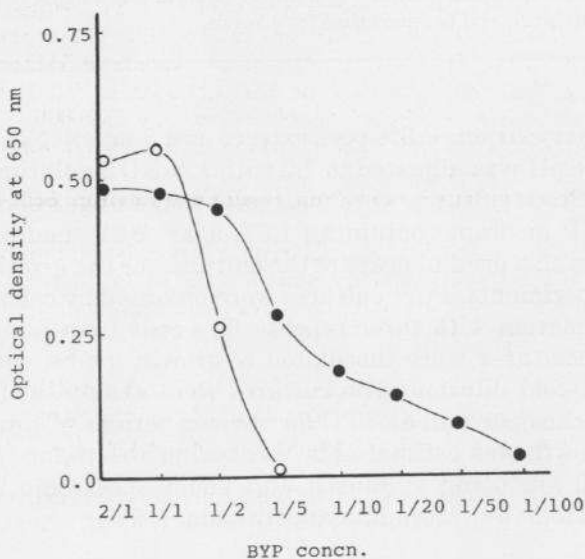


Fig. 1. Effect of nutritional concentrations on growth of isolated marine bacterial strains No. 2 (hollow circles) and No. 8 (filled circles).

The nutritional concentrations for growth of isolated bacterial strains were examined using BYP medium. The range of concentration for growth was 2/1–1/100 BYP medium with 0.5 M NaCl concentration. Results are shown in Fig. 2. Strain No. 2 could grow in 1/50 BYP medium but strain No. 8 did not grow even in 1/5 BYP medium. Salt-resistant types of isolated bacteria could not grow in low nutritional concentration medium, showing eutrophic charac-

ter. However, non-salt-resistant types of isolated bacteria were able to grow in low nutritional concentration medium, showing oligotrophic character. The temperatures for growth of isolated strains were examined. Results are shown in Table 1. All strains isolated showed the same tendency in growth temperature. Optimal growth temperature was 25°C in 0.5 M NaCl BYP medium. Strains No. 8 and 28 could not grow at 42°C; at 37°C, little growth was observed. However, strains No. 2 and 10 grew at 42°C.

The effects of salts other than NaCl on growth were examined in isolated strains. Results are shown in Table 2. Strains No. 2 and 10 also grew in KCl, MgCl₂ and LiCl, but not sorbitol, at 0.5 M NaCl isotonic environment in BYP medium. It is concluded, therefore, that these isolated bacteria require salt environment for growth. Strains No. 8 and 19, however, could not grow in salt environments other than NaCl; nor could they grow in a sorbitol environment.

TABLE 1

Growth in different temperature of isolated marine bacterial strains in 0.5 M NaCl environment

Strain No.	Temperature (°C)				
	15	25	30	37	42
2	0.0	0.340	0.260	0.260	0.245
10	0.0	0.560	0.310	0.280	0.150
8	0.0	0.149	0.110	0.040	0.0
28	0.0	0.141	0.129	0.020	0.0

Data were expressed as optical density at 650 nm in 0.5 M NaCl BYP medium.

TABLE 2

Growth in different salts and sorbitol environment in isolated marine bacterial strains

Strain No.	0.5 M NaCl isotonic					
	0	NaCl	KCl	MgCl ₂	LiCl	Sorbitol
2	0.0	0.315	0.495	0.440	0.283	0.0
10	0.0	0.490	0.110	0.520	0.427	0.0
8	0.0	0.460	0.0	0.0	0.0	0.0
19	0.0	0.490	0.0	0.0	0.0	0.0

Data were expressed as optical density at 650 nm in BYP medium.

DISCUSSION

Sea-adapted bacteria exhibit hereditary fitness to a salt environment. Hereditary adjustments have been observed in a salt environment (Morishita and Takada, 1976; Morishita, 1978b). Halophilic bacteria which could grow in a salt environment have the capacity of osmotic and ionic regulation (Presser and Brown, 1961; Hochachka and Somero, 1973); furthermore they require salt, especially Na⁺ as a nutrient for growth and metabolism (Drapeau and MacLeod, 1936; MacLeod et al., 1958; Morishita and Takada, 1976; Morishita, 1980b).

Slightly halophilic bacteria have been found in seawater and can be isolated from the marine environment; usually these are called marine bacteria. Marine bacteria require 0.5 M NaCl as an optimal concentration for growth (MacLeod, 1965; Morishita and Takada, 1976; Morishita, 1980b). It is supposed that bacteria isolated from solar salts are of marine origin, since solar salts are made from seawater.

Since the optimal concentration of the isolated bacteria was 0.5 M NaCl, these bacteria were identified as marine bacteria (MacLeod, 1965; Kushner, 1985), but salt resistance with regard to growth was not of the same pattern in all the bacteria. Presumably, some of the bacteria achieved salt resistance by adaptation. The typical marine bacteria isolated showed oligotrophic character, but could grow at 42°C and in KCl, MgCl₂ and LiCl environments in BYP medium. In contrast, salt-resistant types of marine bacteria isolated showed an eutrophic character, but could not grow at 42°C or in an environment with salts other than NaCl. Marine bacteria have shown a heterotrophic character (Wood, 1953; Morishita, 1980a).

From these results, it was concluded that the obligate, slightly halophilic bacteria isolated were of marine origin, and could adapt to a rock salt environment by acquiring salt resistance.

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