INTERRELATIONSHIP BETWEEN TEMPERATURE AND SODIUM CHLORIDE ON GROWTH OF LACTIC ACID BACTERIA ISOLATED FROM MEAT-CURING BRINES[1]

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ABSTRACT

GOLDMAN, MANUEL (American Meat Institute Foundation, Chicago, Ill.), R. H. DEIBEL, AND C. F. NIVEN, JR. Interrelationship between temperature and sodium chloride on growth of lactic acid bacteria isolated from meat-curing brines. J. Bacteriol. **85**:1017–1021. 1963.—An elevation of the temperature limit for growth of some *Pediococcus homari* (*Gaffkya homari*) and motile *Lactobacillus* strains could be effected by the addition of sodium chloride to the growth medium. At the optimal temperature for growth, sodium chloride was stimulatory, and as the temperature of incubation was increased a mandatory requirement for sodium chloride was manifested. At the optimal temperature for growth (30 C), the highest sodium chloride concentrations were tolerated; as the temperature was increased, this tolerance decreased, although the optimal sodium chloride concentration increased. No other substances were found that would replace the sodium chloride requirement at higher temperatures of incubation.

Individual bacterial species are often characterized by having specific minimal and maximal temperature limits of growth. These temperature limits are usually regarded as being rather constant characteristics of individual bacterial strains, although exceptions are known to occur. Little attention has been paid to the influence of the medium as it affects the temperature limits of growth.

Campbell and Williams (1953), Campbell (1954), and Long and Williams (1959) noted that Basamin, Armour's liver fraction L, and yeast extract (Difco) contained a substance which allowed growth at 36 C of an otherwise obligate thermophile, *Bacillus stearothermophilus* strain 2184-2. Also, Gjessing (1954) demonstrated that incorporation of a protein fraction from human plasma, or the addition of mercaptalbumin, resulted in a reduction of the minimal temperature limit for growth of a *B. subtilis* strain.

In connection with studies on a group of motile lactobacilli (Deibel and Niven, 1958) and a group of tetrad-forming cocci classified as *Pediococcus homari* (*Gaffkya homari;* Deibel and Niven, 1960), it was noted that the presence of sodium chloride in the growth medium significantly elevated the maximal temperature at which some strains of these bacteria would grow. This observation, and its ramifications, constitute the subject of this report.

MATERIALS AND METHODS

All strains employed in this study were isolated from meat-curing brines. Although the strains belonged to two separate genera, they possessed many characteristics in common. All were homofermentative, low acid-producing strains that were tolerant of high concentrations of salt but were not considered to be halophilic. Previously, they had been characterized as having low minimal and maximal temperature ranges for growth (Deibel and Niven, 1958, 1960).

All strains were transferred daily in APT broth (Evans and Niven, 1951). This medium was also employed as the basal medium for most of the experiments to be described. The pH of the medium was adjusted to 7.2 prior to the addition of sodium chloride and subsequent sterilization.

The test media were inoculated with one drop of a 24-hr culture and tempered in water baths at the appropriate temperatures before placing in

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air incubators. At the higher temperatures, water baths were employed for constant temperature control throughout the growth experiments. Screw-cap test tubes were used to prevent evaporation. Growth was estimated turbidimetrically in a Coleman spectrophotoemter at a wavelength of 660 mμ.

Results

In the initial characterization of the motile *Lactobacillus* and *P. homari* strains isolated from curing brines, the ability to grow at various temperatures in the presence of 0.5, 5.0, and 10% sodium chloride was tested. No growth occurred at 40 C with any of the strains, unless an appreciable amount of sodium chloride was added to the medium (Table 1). At this temperature, prolonged incubation of the cultures failed to evidence growth in media with no added sodium chloride. Without added sodium chloride in the medium, some strains of both the motile lactobacilli and the pediococci failed to grow at 37 C even after prolonged incubation. The effect of sodium chloride in promoting growth at temperatures above 40 C was not uniform. At 42 C, some strains failed to grow, and at 45 C all strains failed to grow, in spite of the presence of sodium chloride.

TABLE 1. *Growth responses of motile lactobacilli and Pediococcus homari strains at 10 and 40 C in different concentrations of sodium chloride*

Organism	No. of strains	NaCl (%) at 10 C			NaCl (%) at 40 C		
		0.5	5.0	10.0	0.5	5.0	10.0
Motile lactobacilli	14	3+*	3+	−	−	3+	2+
Pediococcus homari	9	3+	3+	2+	−	3+	2+

* Growth was estimated visually as follows: −, no growth; +, slight turbidity; 2+, moderate turbidity; and 3+, heavy turbidity. Readings at 10 C were made after 7 days; readings at 40 C were determined after 3 days.

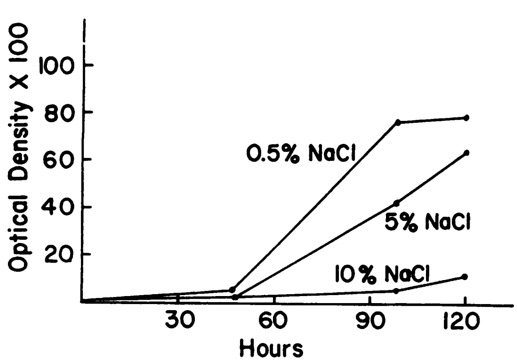

FIG. 1. *Growth responses of Pediococcus homari strain 7P2 with increasing sodium chloride concentrations at 10 C.*

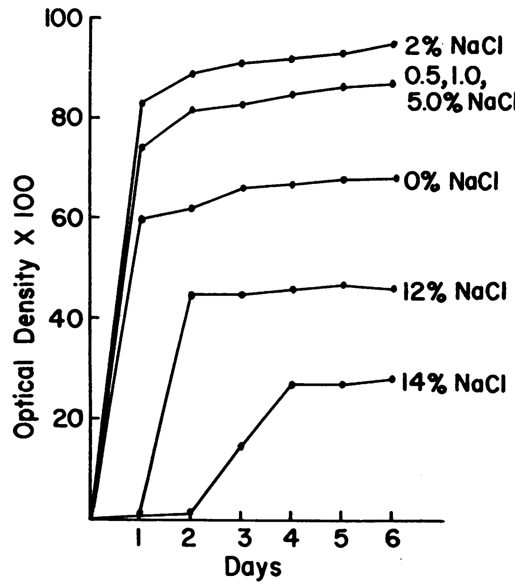

FIG. 2. *Growth responses of Pediococcus homari strain 7P2 to various sodium chloride concentrations at 30 C.*

Conversely, at 10 C the presence of high salt concentrations appeared to be inhibitory. None of the cultures of motile lactobacilli grew at 10 C in the presence of 10% sodium chloride (Table 1). Reduced growth also occurred with the *P. homari* cultures with 10% salt at 10 C. Both groups of microorganisms were able to grow at 5 C without added salt.

To gain more information on the effect of salt at various temperatures, one *P. homari* strain (7P2) was used to determine growth curves in APT broth having increasing concentrations of salt at 10, 30, 37, and 40 C (Fig. 1 to 4). This particular strain failed to grow at 41 C, regardless of the amount of salt added to the medium.

Confirming previous results, strain 7P2 grew satisfactorily at 10 C in the presence of only 0.5% salt (Fig. 1), and higher concentrations were inhibitory. Although not shown in the figure, the strain grew in the medium with no salt to approximately the same extent as with 0.5% salt.

At 30 C, considered optimum for the strain, low

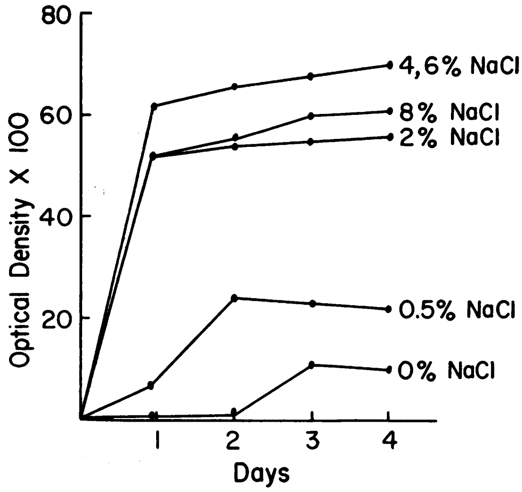

FIG. 3. *Growth responses of Pediococcus homari strain 7P2 with various sodium chloride concentrations at 37 C.*

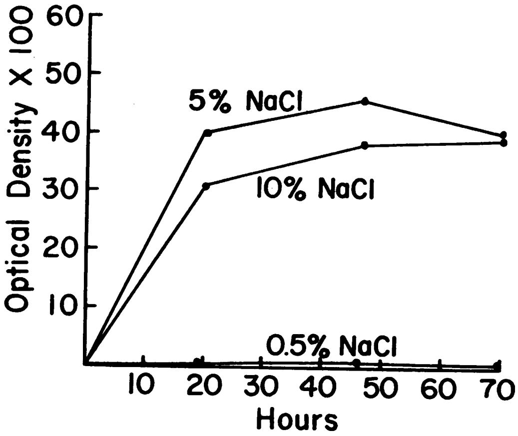

FIG. 4. *Growth responses of Pediococcus homari strain 7P2 with various sodium chloride concentrations at 40 C.*

concentrations of salt were stimulatory (Fig. 2). At this temperature, the culture was able to grow in the presence of 14% salt.

Figure 3 demonstrates that 37 C is near the maximal temperature for growth of strain 7P2 in a medium containing no sodium chloride. On the other hand, as little as 0.5% sodium chloride was greatly stimulatory, and a concentration of approximately 4% salt yielded maximal growth.

At 40 C, strain 7P2 failed to grow in the basal medium, and 0.5% salt was insufficient to allow growth (Fig. 4). However, the incorporation of higher concentrations of salt allowed some growth to occur, although the maximal growth response was reduced. At 41 C, the culture failed to grow under any conditions.

Since the requirement for sodium chloride had only been demonstrated in APT broth, a number of other complex media and medium constituents were screened for their ability to support growth in the absence of additional sodium chloride at 40 C. Tests were conducted at 30 C and 40 C, with and without 5.0% added sodium chloride at each temperature. These experiments failed to demonstrate a growth response at 40 C in the absence of supplementary sodium chloride concentrations.

The possibility that the requirement for sodium chloride at elevated incubation temperatures may have been due to osmotic effects was also investigated. Duplicate sets of various concentrations of glycerol and glucose were prepared in APT broth. Control cultures consisting of APT broth and APT broth with various sodium chloride concentrations, as well as the test series, were incubated at 30 and 40 C. No growth was observed at 40 C under any of the test conditions.

The specificity of sodium chloride was also investigated. Various salts containing common cations or anions as well as salts containing neither of the common ions were tested. After the toxicity levels at 30 C were determined, the various salts were tested for their ability to support growth at 40 C in the absence of sodium chloride. None of the following salts was able to replace the sodium chloride: sodium sulfate, sodium nitrate, sodium formate, sodium acetate, potassium chloride, choline chloride, ammonium acetate, ammonium formate, potassium nitrate, potassium sulfate, ammonium nitrate, ammonium sulfate, or lithium chloride.

DISCUSSION

The experiments described indicate the presence of an unusual mechanism operating in some of the lactic acid bacteria studied, in that these microorganisms tend toward halophilism as the temperature of incubation is increased above the optimal temperature for growth. It would appear that an osmotic pressure effect alone cannot account for the observation: nonelectrolytes or electrolytes, even those containing the cation or anion in common with sodium chloride, were unable to replace sodium chloride. In this respect, the specific requirement for sodium chloride is similar to that observed with the strict halophiles studied by Hess (1952), MacLeod, Onofrey, and Norris (1954), and Robinson and Gibbons (1952).

An interesting parallelism exists with the lactic acid bacteria studied in this present investigation and the halophile *Halobacterium halobium*. As discussed by Ingram (1957), the sodium chloride requirement for the growth of this microorganism is less exacting at lower temperatures of incubation. In general, the optimal temperature for halophiles is significantly higher as the sodium chloride concentration is increased, and Ingram (1957) stated that this may be a general phenomenon.

Gibbons and Payne (1961) also observed that growth at 50 C of the halophiles *H. cutirubrum* and *H. halobium* only occurred with 25 and 30% sodium chloride, and little or no growth occurred with 15 and 20% sodium chloride. However, the latter concentrations afforded growth at lower temperatures of incubation.

The mode of action of sodium chloride in elevating the temperature limit of growth is unknown. It may be speculated that sodium chloride inactivates an inhibitor at the higher incubation temperatures. Riley, Hobby, and Burke (1953) showed that low concentrations of salts could activate mammalian cytochrome oxidase by preventing combination of the enzyme with a naturally occurring inhibitor. Other areas for speculation could concern an altered permeability of the cell membrane and its reversal by sodium chloride, or the maintenance of critical levels of other intracellular ions by sodium chloride.

A number of investigators have observed a requirement for certain vitamins and cofactors when microorgansims are cultured at high temperatures, and, if the temperature is decreased, the requirement for an exogenous supply of the cofactor no longer exists (Mitchell and Houlahan 1946*a*, *b*; Begue and Lichstein, 1959; Maas, 1950). Recently, this generalization was extended to a streptomycin-dependent strain of *Escherichia coli* that could grow in the absence of the antibiotic at lower temperatures (Plunkett, 1962). These results are in accord with the hypothesis that a heat-labile enzyme system is involved, and circumvention of the inhibition is accomplished by decreasing the temperature of incubation.

Although the complex growth medium in the present study precluded extensive nutritional studies along these lines, the addition of other complex substances or the employment of other complex or synthetic media did not replace the growth-enhancing activity of sodium chloride. Thus, it may be concluded that further studies involving a nutritional approach to investigating the sodium chloride effect may not be fruitful.

Considering the environment from which they were isolated, it could be surmised that the lactic acid bacteria employed in this study represent microorganisms that are intermediate in their sodium chloride requirement. At the one extreme are the obligate halophiles which require very high concentrations of sodium chloride for growth, whereas at the other extreme are the nonhalophilic species, some of which are halotolerant. The brine lactic acid bacteria would ordinarily be considered halotolerant microorganisms; however, at their upper temperature limits of growth, they acquire characteristics similar to marine bacteria as defined by ZoBell (1947).

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