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Relationships Between Heat Resistance and Phospholipid Fatty Acid Composition of *Vibrio parahaemolyticus*

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Vibrio parahaemolyticus was grown in tryptic soy broth (TSB) containing NaCl levels of 0.5, 3.0, and 7.5% (wt/vol). Cultures incubated at 21, 29, and 37 C were harvested in late exponential phases and thermal death times at 47 C ($D_{47\ C}$; time at 47 C required to reduce the viable population by 90%) were determined in phosphate buffer containing 0.5, 3.0, and 7.5% NaCl. At a given NaCl concentration in the growth medium, $D_{47\ C}$ values increased with elevated incubation temperatures and with elevated levels of NaCl in the heating menstrua. Differences in thermal resistance of cells cultured at a particular temperature were greater between those grown in TSB containing 0.5 and 3.0% NaCl than between those grown in TSB containing 3.0 and 7.5% NaCl. $D_{47\ C}$ values ranged from 0.8 min (grown at 21 C in TSB with 0.5% NaCl) to 6.5 min (grown at 37 C in TSB with 7.5%, heated in 7.5% NaCl buffer). Methyl esters of major phospholipid fatty acids extracted from cells were quantitated. The ratio of saturated to unsaturated fatty acids in cells grown at a given NaCl concentration increased with elevated incubation temperature. At a particular growth temperature, however, saturated to unsaturated fatty acid ratios were lowest for cells grown in TSB containing 3.0% NaCl.

Heat resistance and injury of food-borne pathogens such as salmonellae, *Staphylococcus aureus*, and clostridia have been studied extensively. Less attention has been given to the thermal stability characteristics of *Vibrio parahaemolyticus*, a gram-negative halophilic estuarine bacterium annually reponsible for the majority of food poisoning outbreaks in Japan, and recently incriminated as a causative agent of gastroenteritis in Western nations. The organism is reported to be extremely sensitive to thermal inactivation when heated in either laboratory media or shrimp substrates (3, 8, 25), and its resuscitation after thermal assault is influenced substantially by nutrients provided in recovery media (26). To our knowledge, nothing has been reported regarding the effects of NaCl concentration in growth media and temperature of growth on thermal resistance of *V. parahaemolyticus*.

Temperature of growth (15, 18, 19, 21, 23) and nutrient supply in growth media have been shown to affect the fatty acid composition of gram-negative bacteria. Fatty acid composition (13) and growth temperature (14), in turn, have been reported to influence thermal stability of bacteria. Although fatty acid profiles of lipopolysaccharides from *V. parahaemolyticus* have been studied (9, 22), little is known of the fatty acid composition of phospholipid components of the cytoplasmic membrane.

This study was initiated to determine the effects of growth temperature and concentration of NaCl in growth media on heat resistance and phospholipid fatty acid composition of *V. parahaemolyticus*. Effects of NaCl in heating menstrua on thermal stability were also studied.

MATERIALS AND METHODS

Cultural and harvesting conditions. *V. parahaemolyticus* T-3765-1 (03:K7) was obtained from H. Zen-Yoji, Tokyo Metropolitan Research Laboratory of Public Health. Identical procedures were followed to culture cells for both thermal resistance studies and phospholipid fatty acid analysis. Cells were grown in tryptic soy broth (TSB, pH 7.2, Difco) containing 3.0% (wt/vol) NaCl at 29 C for 16 h. A 1-ml inoculum from this culture was transferred to 500-ml Erlenmeyer flasks containing 200 ml of TSB (pH 7.2) with added NaCl to give final concentrations of 0.5, 3.0, and 7.5%. The inoculated media were then incubated at 21, 29, or 37 C on a New Brunswick gyrotory shaker (New Brunswick Scientific, New Brunswick, N.J.) at 100 rpm. Cells in late exponential growth phases were examined for thermal resistance. Growth curves had previously been determined by plating the growing cultures on TSB containing 1.5% agar and 3.0% NaCl.

Cells in late exponential phases were also ana-

lyzed for phospholipid fatty acid content. Organisms were collected by centrifuging at 8,000 × *g*. Pellets were washed in 0.1 M potassium phosphate buffer (pH 7.2) containing 3.0% NaCl, collected again, lyophilized, and stored at −30 C until analyzed. Dried-cell preparation were pulverized with a mortar and pestle just before phospholipid extraction. Cells collected from as many as 54 individual flasks representing four independent trials were pooled for a particular medium/growth temperature test combination.

Determination of thermal resistance. Exponential-phase cells (10 ml of NaCl-TSB culture) were transferred to 190 ml of 0.1 M potassium phosphate containing 0.5, 3.0, or 7.5% (wt/vol) NaCl (pH 7.2) at 47 ± 0.1 C. the initial viable cell populations in heating menstrua were approximately 10^8 for organisms grown in 3% NaCl-TSB and 10^7 for 0.5 and 7.5% NaCl-TSB cultures, regardless of incubation temperature. The suspension was heated under constant agitation. Samples were withdrawn at appropriate times, dispensed in sterile chilled test tubes, and serially diluted in 0.1 M potassium phosphate (pH 7.2) containing 3.0% NaCl. The recovery medium (tryptic soy agar) consisted of TSB containing 1.2% agar and 3.0% NaCl, and was tempered at 42 C before pouring. Colonies were counted after 18 to 24 h at 35 C and heat survivor curves of $\log_{10}$ viable number per milliliter versus time at 47 C were plotted. Decimal reduction times at 47 C ($D_{47\ C}$; time in minutes required to destroy 90% of the viable *V. parahaemolyticus*) were then read from the plotted data. $D_{47\ C}$ values reported in this article represent means from three or more independent trials.

Lipid extraction. Duplicate 1-g samples of lyophilized cells were rehydrated and immediately extracted with redistilled solvents by the procedure of Bligh and Dyer (5). This procedure effectively extracts neutral lipids and phospholipids from biological materials but does not extract nonlipid material or lipids that are bonded covalently to other cellular constituents. The extraction procedure has been successfully applied to the study of bacterial phospholipids (1, 12). After removal of organic solvent under a stream of nitrogen, extracted lipid was held at −18 C until analyzed.

Identification of neutral lipids. All samples were examined qualitatively for neutral lipids by thin-layer chromatography. A prepared silica gel G plate (Brinkman Instruments Inc., Westbury, N.Y.) was prewashed in hexane-diethyl ether-acetic acid (90:10:1; vol/vol), divided into 1-cm bands by scoring, and dried at 60 C for 20 min. Weighed lipid extracts were dissolved in hexane to give a concentration of approximately 200 μg/μl, and 1 μl of each extract was applied to the plate. As an aid in the identification of neutral lipids, a standard consisting of equal parts of cholesterol, octadecenoic (oleic) acid, triolein, methyl octadecenoate, and cholesteryl octadecenoate was applied to the center band and co-chromatographed with sample lipids in the hexane-diethyl ether-acetic acid solvent system. The developed plate was sprayed with the indicator solution described by Jones et al. (16) and viewed under ultraviolet light. The plate was further examined after spraying with sulfuric acid-potassium dichromate and charring at 100 C. Identification of neutral lipid components was based on a comparison of standard and sample R_f values.

Fatty acid analysis. Lipid fatty acids were converted to methyl esters with 1 ml of $NaOCH_3$ (0.2 M in anhydrous methanol, 100 C, 10 to 15 min) followed by 1 ml of BCl_3 in methanol (100 C, 5 min). After addition of 2 ml of 0.2 M KCl, methyl esters were extracted with petroleum ether and analyzed by gas-liquid chromatography with a MicroTek 220 chromatograph equipped with flame ionization detectors. Glass columns (6 feet by 1/4 inch [ca. 183 by 0.64 cm]) were packed with 10% Silar 5 CP (Applied Science Laboratories, State College, Pa.) coated on 80- to 100-mesh Chromosorb W that had been acid-washed and treated with dimethylchlorosilane. The oven, detector, and injection port temperatures were maintained at 200, 300, and 275 C, respectively, and the helium carrier gas flow was kept at 100 ml/min. Peak areas were measured with an Infotronics CRS 100 integrator, and major fatty acid values are reported as weight percentage of total fatty acids.

Sample methyl esters were examined for the presence of hydroxy fatty acids by gas-liquid chromatography before and after trimethylsilation with a 1:1 mixture of pyridine and *N*-trimethylsilylimidazole (27), and were examined for cylcopropane fatty acids by thin-layer argentation chromatography (10) and gas-liquid chromatography of the isolated saturated methyl esters. Peak identifications were based on retention time data obtained with the aid of authentic standards.

RESULTS

Heat resistance. Values for *V. parahaemolyticus* grown and heated in menstrua containing various levels of NaCl are summarized in Table 1. Thermal survivor curves were often diphasic, especially for cells grown at 29 and 37 C in TSB containing 7.5% NaCl and heated in 7.5% NaCl buffer. $D_{47\ C}$ values were calculated from the first segments of inactivation curves, which always had the greatest slopes and transcended at least 5 log reductions in viable population. The second segments of inactivation curves were noted to occur when the viable populations were reduced to 10^2 to 10^3 per ml. Increased temperature of growth resulted in increased heat resistance of *V. parahaemolyticus*. This was true for all tests wherein the percentages of NaCl in growth media were considered separately and the NaCl concentrations of heating buffers were unchanged. At a particular growth temperature, addition of NaCl to TSB generally yielded cells displaying more resistance to thermal destruction. This resistance was most pronounced when cells were heated in buffer containing 7.5% NaCl. Cells grown at a

TABLE 1. *Decimal reduction times for V. parahaemolyticus*

Growth temp (C)	NaCl concn (%)		$D_{47\,C}$ value
	Growth medium[a]	Heating medium[b]	
21	0.5	0.5	0.8
		3.0	2.9
		7.5	6.9
	3.0	0.5	0.8
		3.0	5.3
		7.5	9.4
	7.5	0.5	0.8
		3.0	5.5
		7.5	10.2
29	0.5	0.5	1.1
		3.0	7.1
		7.5	9.9
	3.0	0.5	1.2
		3.0	7.5
		7.5	16.1
	7.5	0.5	2.1
		3.0	17.6
		7.5	32.8
37	0.5	0.5	3.6
		3.0	15.3
		7.5	17.0
	3.0	0.5	2.6
		3.0	48.2
		7.5	64.5
	7.5	0.5	3.0
		3.0	50.2
		7.5	65.1

[a] TSB (pH 7.2).

[b] 0.1 M potassium phosphate (pH 7.2).

particular temperature in a medium with a given NaCl concentration had increased resistance to heat in buffer containing increased levels of NaCl.

Lipid analysis. An examination of the thin-layer chromatogram revealed small amounts of neutral lipid that consisted primarily of hydrocarbons and traces of free fatty acids in all extracts and traces of triglycerides in those cultures grown in 3.0% NaCl-TSB at 21 and 29 C. The combined neutral lipids were estimated to represent no more than 10% of total lipid.

Intense spots were observed at the point of application of each sample, indicating a high proportion of phospholipids (and possibly other polar lipids) in all extracts. That lipopolysaccharides were not extracted by the Bligh and Dyer (5) procedure was indicated by an absence of hydroxy fatty acids as discussed below under fatty acid analysis.

Fatty acid analysis. Gas-liquid chromatograms of total lipid fatty acid methyl esters revealed major peaks with retention times corresponding to methyl esters of saturated and monoene fatty acids. Major peaks were identified as dodecanoic (12:0), tetradecanoic (14:0), tetradecenoic (14:1), hexadecanoic (16:0), hexadecenoic (16:1), octadecanoic (18:0), and octadecenoic (18:1) acids (Table 2). Minor fatty acids comprised less than 3% of the total acids and were identified as dodecenoic (12:1), pentadecanoic (15:0), heptadecanoic (17:0), heptadecenoic (17:1), octadecadienoic (18:2), and eicosenoic (20:1) acids. Treatment of methyl esters with pyridine-*N*-trimethylsilylimidazole did not change fatty acid spectra, thus indicating an absence of hydroxy fatty acids previously identified in lipopolysaccharides of *V. parahaemolyticus* (9, 22). Gas-liquid chromatography of the saturated fatty acid fraction obtained by thin-layer argentation chromatography showed peaks corresponding to 12:0, 14:0, 16:0, and 18:0. Cyclopropane fatty acids, which behave as normal saturated acids in thin-layer argentation chromatography, were not detected. These data, in addition to those showing low quantities of hydrocarbons, free fatty acids, and triglycerides, indicate that fatty acid methy esters were primarily of phospholipid origin.

The relative amounts of saturated and unsaturated fatty acids incorporated into the phospholipid of *V. parahaemolyticus* were influenced by both NaCl content and temperature of growth media (Table 2). Cultures grown in 7.5% NaCl-TSB generally produced higher levels of 14:0 than those grown in lower concentrations of NaCl. Those cultures grown in 7.5% NaCl-TSB at 21 C produced unusually large quantities of 14:1 and much smaller quantities of 18:1 than cultures grown under other test conditions. The saturated to unsaturated (S/U) weight ratios of fatty acids in phospholipid of cells cultured at 21, 29, and 37 C in TSB containing 0.5, 3.0, and 7.5% NaCl are shown in Fig. 1. At any of the three NaCl levels, increased temperature of growth resulted in an increased S/U ratio. At a given incubation temperature, however, the S/U ratio was not linear with NaCl concentration.

DISCUSSION

V. parahaemolyticus grew most rapidly in 3% NaCl-TSB. Slower growth rates were observed in hypotonic 0.5% NaCl-TSB and in hypertonic 7.5% NaCl-TSB, resulting in the for-

TABLE 2. *Phospholipid fatty acid composition of V. parahaemolyticus*

Growth temp (C)	NaCl concn in TSB growth medium (%)	Fatty acid composition[a]							
		12:0	14:0	14:1	16:0	16:1	18:0	18:1	Minor components
21	0.5	0.6	5.1	0.9	26.7	48.5	1.2	16.1	0.9
	3.0	0.3	3.7	0.6	23.2	48.8	1.4	21.6	0.4
	7.5	1.2	12.4	3.3	23.3	52.2	1.1	4.8	1.7
29	0.5	0.4	4.9	0.5	30.5	43.1	1.6	18.0	1.0
	3.0	0.3	4.5	0.4	27.1	42.8	1.8	22.1	1.0
	7.5	0.6	11.3	0.9	28.1	44.7	1.1	12.6	0.7
37	0.5	1.7	5.1	0.9	36.5	32.2	3.1	18.1	2.4
	3.0	0.4	5.5	0.4	31.3	39.6	2.4	19.4	1.0
	7.5	0.6	12.0	0.7	34.3	39.7	1.6	10.5	0.6

[a] Values are expressed as weight percentages of total phospholipid fatty acids. Fatty acids are designated as *x:y*, where *x* equals the number of carbon atoms and *y* equals the number of double bonds per molecule.

mation of swelled or filamentous type cells, respectively. Times required to reach late exponential growth ranged from 6 to 24 h, depending upon the incubation temperature and growth medium under study. Christian and Wiebe (7) also noted that temperature markedly affects the rate of growth of a *Vibrio* sp. Because of morphological differences, centrifuged cells that had been grown in 0.5 and 7.5% NaCl-TSB were extremely difficult to resuspend without clumping. Thermal survivor studies were therefore conducted using unwashed cell suspensions as they were withdrawn directly from culturing flasks. The change in NaCl concentration in heating menstrua as a result of this procedure should be noted; however, the magnitude of change is not considered great enough to markedly affect the relative range of $D_{47\ C}$ values observed. Nevertheless, the reader should realize that data presented for 0.5, 3.0, and 7.5% NaCl heating menstrua refer to initial concentrations and that these concentrations were, in some cases, modified by the addition of TSB cultures containing various levels of NaCl. Emphasis should be placed on comparisons of $D_{47\ C}$ values obtained from cells heated in menstrua containing low, medium, and high (0.5, 3.0, and 7.5%, respectively) NaCl.

The condition of *V. parahaemolyticus* cells before heating was affected both morphologically and biochemically by cultural techniques. Addition of NaCl decreases the water activity of TSB (4), thereby altering the osmotic sensitivity of cells produced in it. Cells grown at a particular growth temperature had increased thermal resistance when heated in a menstruum containing a higher percentage of NaCl

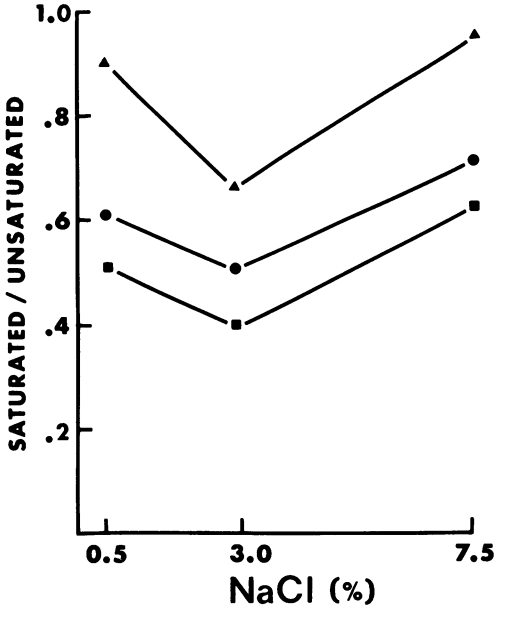

FIG. 1. *S/U phospholipid fatty acid weight ratios from V. parahaemolyticus cells grown in 0.5, 3.0, and 7.5% NaCl-TSB; (■) growth temperature of 21 C; (●) 29 C; (▲) 37 C.*

than the growth medium. This phenomenon is more pronounced at higher incubation temperatures. These data imply that the addition of NaCl to heating menstrua has a protective effect against destruction, regardless of the adaptation of the cells to osmotic conditions before thermal exposure. Calhoun and Frazier (6) noted similar behavior of *Escherichia coli*. Enhanced heat resistance was reported for cells that were both grown and heated in media

wherein reduced water activity was controlled through the addition of NaCl. The protective effect of NaCl in heating media against destruction of heat-sensitive salmonellae had also been demonstrated (2).

Response of bacteria to heat stress is dependent upon chemical makeup of the cells as well as the physical and chemical nature of the heating environment. Several reports show correlations between cellular fatty acid composition and heat resistance. Changes in growth medium composition have been demonstrated to be responsible not only for quantitative differences but, in some cases, for qualitative changes in fatty acid spectra of gram-negative cells (14, 20). In the present study, elevated levels of tetradecanoic acid were noted in phospholipid of *V. parahaemolyticus* grown in 7.5% NaCl-TSB, as compared with 0.5 and 3.0% NaCl-TSB cells cultured at the same temperature. Considering each NaCl-TSB medium individually, increased percentages of hexadecanoic acid were accompanied by decreased levels of hexadecenoic acid in cells as the growth temperature was increased. These changes were associated with higher heat resistance. Increased percentages of saturated fatty acids in phospholipids of *E. coli* (17-19, 23) and *Thermus aquaticus* (15) also have been correlated with elevated temperatures of growth. Hansen (13) demonstrated a clear relationship between increased S/U ratios in lipid of *E. coli* cultured at elevated temperatures and increased heat resistance. Studies were conducted using a single growth medium. He speculated that, since fatty acids are found principally in the cell envelope, increased ratios between high-melting and low-melting fatty acids significantly enhanced thermal stability of bacterial cells. Relative proportions of saturated and unsaturated fatty acids have been demonstrated to affect the fluidity (24) and permeability of cell membranes. Higher S/U ratios in lipsomes are associated with lower permeability (11, 12). The general correlation of higher heat resistance with higher growth temperature and high S/U ratios in phospholipid of *V. parahaemolyticus*, as shown in this study for cells grown in a given NaCl-TSB medium, would tend to strengthen the theory that cytoplasmic membrane fatty acid composition affects the thermal integrity of bacterial cells. However, data reported in this communication indicate that this correlation can be made only for cells grown at various temperatures in TSB containing identical concentrations of NaCl. For example, cells grown in 7.5% NaCl-TSB at 21 C have a higher S/U ratio than cells grown in 3.0% NaCl-TSB at 29 C. Yet the $D_{47\,C}$ values for cells grown in 7.5% NaCl-TSB at 21 C and heated in 0.5, 3.0, or 7.5% NaCl buffer are lower than the $D_{47\,C}$ values calculated for cells grown in 3.0% NaCl-TSB at 29 C and heated in corresponding buffers. Also, at a particular growth temperature, higher S/U ratios do not necessarily correlate with higher heat resistance. This is exemplified by the observation that cells grown at a given temperature in 3.0% NaCl-TSB have lower S/U ratios than cells grown in 0.5% NaCl-TSB, yet a comparison of the two types of cells heated in buffer with the same salt concentration indicates that 3.0% NaCl-TSB cells have greater resistance to inactivation. Therefore, in addition to fatty acid content, quantity and quality of other cellular components are undoubtedly affected by growth medium composition and are in turn influencing the heat resistance of *V. parahaemolyticus* cells.

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LITERATURE CITED

1. Ames, G. F. 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli*: structure and metabolism. J. Bacteriol. 95:833-843.
2. Baird-Parker, A. C., M. Boothroyd, and E. Jones. 1970. The effect of water activity on the heat resistance of heat sensitive and heat resistant strains of salmonellae. J. Appl. Bacteriol. 33:515-522.
3. Beuchat, L. R. 1973. Interacting effects of pH, temperature, and salt concentration on growth and survival of *Vibrio parahaemolyticus*. Appl. Microbiol. 25:844-846.
4. Beuchat, L. R. 1974. Combined effects of water activity, solute, and temperature on the growth of *Vibrio parahaemolyticus*. Appl. Microbiol. 27:1075-1080.
5. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.
6. Calhoun, C. L., and W. C. Frazier. 1966. Effect of available water on thermal resistance of three nonsporeforming species of bacteria. Appl. Microbiol. 14:416-420.
7. Christian, R. R., and W. J. Wiebe. 1974. The effects of temperature upon the reproduction and respiration of a marine obligate psychrophile. Can. J. Microbiol. 20:1341-1345.
8. Covert, D., and M. Woodburn. 1972. Relationships of temperature and sodium chloride concentration to the survival of *Vibrio parahaemolyticus* in broth and fish homogenate. Appl. Microbiol. 23:321-325.
9. Deneke, C. F., and R. R. Colwell. 1973. Studies of the cell envelope of *Vibrio parahaemolyticus*. Can. J. Microbiol. 19:241-245.
10. Galanos, D. S., V. M. Kapoulas, and E. C. Voudouris. 1968. Detection of adulteration of olive oil by argentation thin-layer chromatography. J. Am. Oil Chem. Soc. 45:825-829.
11. DeGier, J., J. G. Mandesloot, and L. L. M. Van Deenen. 1968. Lipid composition and permeability of liposomes. Biochim. Biophys. Acta 150:666-675.
12. Haest, C. W. M., J. DeGier, and L. L. M. Van Deenen.

1969. Changes in the chemical and the barrier properties of the membrane lipids of *E. coli* by variation of the temperature of growth. Chem. Phys. Lipids 3:413-417.

13. **Hansen, E. W.** 1971. Correlation of fatty acid composition with thermal resistance of *Escherichia coli*. Dan. Tidsskr. Farm. 45:339-344.
14. **Hansen, E. W., and K. Skadhauge.** 1971. The influence of growth temperature on the thermal resistance of *E. coli*. Dan. Tidsskr. Farm. 45:24-28.
15. **Heinen, W., H. P. Klein, and C. M. Volkmann.** 1970. Fatty acid composition of *Thermus aquaticus* at different growth temperatures. Arch. Mikrobiol. 72:199-202.
16. **Jones, D., D. E. Bowyer, G. A. Gresham, and A. N. Howard.** 1966. An improved spray reagent for detecting lipids on thin-layer chromatograms. J. Chromatogr. 23:172-174.
17. **Kito, M., S. Aibara, M. Kato, and T. Hata.** 1972. Differences in fatty acid composition among phosphatidylethanolamine, phosphatidylglycerol and cardiolipin of *Escherichia coli*. Biochim. Biophys. Acta 260:476-478.
18. **Kito, M., M. Ishinaga, S. Aibara, M. Kato, and T. Hata.** 1973. Relation between cyclopropane fatty acid formation and saturation of phospholipid species in *Escherichia coli* K-12. Agr. Biol. Chem. 37:2647-2651.
19. **Marr, A. G., and J. L. Ingraham.** 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. 84:1260-1267.
20. **Modak, M. J., S. Nair, and A. Venkataraman.** 1970. Studies on the fatty acid composition of some salmonellas. J. Gen. Microbiol. 60:151-157.
21. **Ray, P. H., D. C. White, and T. D. Brock.** 1971. Effect of temperature on the fatty acid composition of *Thermus aquaticus*. J. Bacteriol. 106:25-30.
22. **Rietschel, E. T., W. J. Palin, and D. W. Watson.** 1973. Nature and linkages of the fatty acids present in lipopolysaccharides from *Vibrio metchnikovii* and *Vibrio parahaemolyticus*. Eur. J. Biochem. 37:116-120.
23. **Sinensky, M.** 1971. Temperature control of phospholipid biosynthesis in *Escherichia coli*. J. Bacteriol. 106:449-455.
24. **Sinensky, M.** 1974. Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 71:522-525.
25. **Vanderzant, C., and R. Nickelson.** 1972. Survival of *Vibrio parahaemolyticus* in shrimp tissue under various environmental conditions. Appl. Microbiol. 23:34-37.
26. **Vanderzant, C., R. Nickelson, and R. W. Hazelwood.** 1974. Effect of isolation-enumeration procedures on the recovery of normal and stressed cells of *Vibrio parahaemolyticus*, p. 111-116. *In* T. Fugino, G. Sakaguchi, R. Sakazaki, and Y. Takeda (ed.), International symposium on *Vibrio parahaemolyticus*. Saikon Publishing Co., Ltd., Tokyo.
27. **Worthington, R. E., and L. R. Beuchat.** 1974. α-Galactosidase activity of fungi on intestinal gas-forming peanut oligosaccharides. J. Agr. Food Chem. 22:1063-1066.