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Thermal Tolerance of *Zymomonas mobilis*: Temperature-Induced Changes in Membrane Composition†

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The membrane composition of *Zymomonas mobilis* changed dramatically in response to growth temperature. With increasing temperature, the proportion of vaccenic acid declined with an increase in myristic acid, the proportion of phosphatidylcholine and cardiolipin increased with decreases in phosphatidylethanolamine and phosphatidylglycerol, and the phospholipid/protein ratio of the membrane declined. These changes in membrane composition were correlated with changes in thermal tolerance and with changes in membrane fluidity. Cells grown at 20°C were more sensitive to inactivation at 45°C than were cells grown at 30°C, as expected. However, cells grown at 41°C (near the maximal growth temperature for *Z. mobilis*) were hypersensitive to thermal inactivation, suggesting that cells may be damaged during growth at this temperature. When cells were held at 45°C, soluble proteins from cells grown at 41°C were rapidly lost into the surrounding buffer in contrast to cells grown at lower temperatures. The synthesis of phospholipid-deficient membranes during growth at 41°C was proposed as being responsible for this increased thermal sensitivity.

The new demand for ethanol as a fuel extender has created the need for more economic large-scale ethanol production and has sparked extensive research in the field of microbial ethanol production. Currently in the United States, *Saccharomyces cerevisiae* is the primary organism utilized for large-scale ethanol production. However, the bacterium *Zymomonas mobilis* appears to have tremendous potential for commercial ethanol production. *Z. mobilis* is an obligately fermentative gram-negative rod, capable of producing 1.9 mol of ethanol per mol of glucose (29). It has been reported to be more ethanol tolerant, glucose tolerant, and temperature tolerant than *S. cerevisiae* and to be capable of rates of glucose utilization and ethanol production three to four times higher than those of *S. cerevisiae*. In addition, *Z. mobilis*, a procaryote, may prove more amenable to genetic manipulation for strain improvement than *S. cerevisiae*.

In large-scale fermentations, one of the problems encountered is the increase in fermentor temperature owing to heat released during the metabolism of carbohydrate to ethanol. Such increases in temperature have been shown to decrease the efficiency of alcohol production and to limit its final accumulation (18, 19, 23). Increases in growth temperature have also been shown to induce changes in the membrane composition of many eucaryotic and procaryotic organisms, eliciting an adaptive response (11, 24). In this study, we examined the effects of growth temperature on the membrane composition and thermal tolerance of *Z. mobilis*, a potentially useful organism for commercial ethanol production.

MATERIALS AND METHODS

Organism and growth conditions. The organism used in these studies, *Z. mobilis* CP4, was generously supplied by Arie Ben-Bassat (Cetus Corporation, Berkeley, Calif.). Cultures were grown in either the complex medium described by Skotnicki et al. (29) or glucose minimal medium containing 100 g of glucose per liter. The minimal medium contained (grams per liter): monobasic potassium phosphate, 0.5; $Na(NH_4)HPO_4 \cdot 4H_2O$, 0.5; ferric ammonium citrate, 0.05; $MgSO_4 \cdot 7H_2O$, 1.2; calcium chloride, 0.015; biotin, 0.002; calcium pantothenate, 0.002. All studies employed complex medium except when specified otherwise. Stock cultures were maintained on complex medium solidified with 1.5% agar.

Overnight broth cultures were grown at 30°C and used as inocula unless stated otherwise. Batch cultures were grown in 250-ml Spinner bottles (50 rpm) vented by the insertion of a 25-gauge needle through a rubber serum stopper on the side arm. Cultures were incubated in water baths at 20, 30, 37, or 41°C. Bottles were inoculated to an initial optical density of 0.05 at 550 nm and were harvested in exponential growth at an optical density of 1.0 (250 μg of cell protein per ml). Growth was measured with a Bausch & Lomb Spectronic 70 spectrophotometer.

For studies examining cell survival, 16-h cultures were grown in complex medium at various temperatures and diluted into tubes containing 10 ml of complex medium to a final optical density of 0.4. At zero time, these were placed into a 45°C water bath. Serial dilutions of samples were plated on complex medium to determine viability after various times. Plates were incubated at 30°C for 3 to 4 days to allow colony formation.

Lipid analyses. Previous studies in our laboratory have described the methods used to identify and determined the lipid composition of *Z. mobilis* (5). Fatty acids were analyzed by gas chromatography, phospholipids were determined by thin-layer chromatography of $^{32}PO_4$ lipids, and lipid/protein ratios of isolated membranes were determined by colorimetric procedures for both lipid phosphorus (7) and protein (20).

Relative phospholipid-to-protein ratios of whole cells were determined as counts per minute of lipid phosphorus per milligram of cell protein, using cells which were prelabeled for five generations by growth in complex medium to which 0.4 μCi of $^{32}P_i$ per ml had been added. Complex medium contained 7.4 mM added phosphate in addition to the com-

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ponents of yeast extract (10 g/liter). Similar determinations of phospholipid/cell protein ratios were made by using cells grown in minimal medium containing 6.1 mM phosphate (0.2 mCi of $^{32}PO_4$ per mmol). The phospholipid values reported for the minimal medium experiments were converted to micrograms per milligram of cell protein assuming a phospholipid molecular weight of 750.

Membrane isolation. For each growth temperature, four bottles of 250 ml each were harvested at an optical density of 1.0 (550 nm) at 5°C (8,000 × *g*, 10 min). These were combined and washed once in 10 mM Tris hydrochloride buffer (pH 8.1). The resulting pellet was resuspended in 20 ml of Tris buffer and disrupted with an ultrasonic disruptor (Heat Systems Ultrasonics, Inc., Plainview, N.Y.), with eight 30-s exposures in an ice bath. The resulting preparations were centrifuged at low speed (5,000 × *g*, 10 min) to remove unbroken cells and large debris. A mixed membrane fraction was obtained by centrifugation at 100,000 × *g* for 1 h at 5°C. The yield of this final preparation was somewhat variable and may be due in part to the varying susceptibility of cells grown at different temperatures to ultrasonic disruption. This mixed membrane fraction would be expected to contain a mixture of both inner and outer membranes along with ribosomal components and small wall fragments. Attempts to further purify the plasma membrane on sucrose gradients did not result in a distinct plasma membrane band in our hands. Thus, the mixed membrane preparation was used for polarization measurements and for colorimetric determinations of phospholipid/protein ratios.

Fluorescence depolarization. Fluorescence depolarization measurements were made by a modification of our previous procedure (1). Membranes were suspended in 10 mM Tris hydrochloride (pH 7.5) at a protein concentration of 0.1 μg/ml. Equal volumes of membranes and a 2 μM microcrystalline dispersion of diphenylhexatriene (DPH) in the same buffer were mixed. These were equilibrated at the assay temperature for 45 min to allow probe insertion. Fluorescence measurements were made with an SLM series 4000 spectrofluorimeter (SLM, Urbana, Ill.) in the ratio mode. DPH was excited at 360 nm, and the emission was measured at 418 nm. Sample temperature was regulated with a Neslab circulating water bath. Fluorescence depolarization was used as a relative measure of bulk membrane fluidity. The values reported are inversely related to probe mobility (bulk fluidity) under the conditions of these experiments.

Cell leakage. Spinner bottle cultures were harvested at an optical density of 1.5 (550 nm) by centrifugation (8,000 × *g*, 10 min, 22°C) and washed with 0.5 volume of 50 mM potassium phosphate buffer (pH 6.0). The washed pellet was resuspended in 0.5 volume of buffer, and 10-ml samples were incubated at various temperatures. Duplicate 1-ml samples were removed and centrifuged for 2 min at 10,000 × *g* in an Eppendorf microcentrifuge, and the supernatants were frozen for subsequent analyses. Magnesium was measured with the 60-s magnesium reagents supplied by the American Monitor Corporation (Indianapolis, Ind.) designed for blood analysis. Nucleotide leakage was measured as A_{260} and included any nucleic acids which may have been released. Protein was measured by the Bradford (4) dye-binding assay (Bio-Rad Laboratories, Richmond, Calif.).

Chloroform was used as a control to disrupt cell membranes (0.5 ml/10 ml of suspension, shaking for 1 h at 23°C) and to determine the total releasable magnesium and protein. Greater than 95% of the total cellular magnesium was released into the supernatant by this procedure, accompanied by 70% of the cellular protein. Magnesium leakage and protein leakage at different temperatures were expressed as a percentage of that released by chloroform. Results with nucleotides were expressed as A_{260}.

TABLE 1. Effects of growth temperature on phospholipid-to-protein ratio of cells and membranes

Growth temp (°C)	Medium	Sample	Phospholipid/mg of protein[a]		
			cpm	μg	% of 30°C cells[b]
20	Complex	Membranes[c]		560	124
30	Complex	Membranes		450	100
37	Complex	Membranes		300	67
41	Complex	Membranes		270	60
20	Complex	Cells[d]	2,580		114
30	Complex	Cells	2,260		100
37	Complex	Cells	1,970		87
41	Complex	Cells	1,330		59
20	Minimal	Cells[e]	13,500	21.9	95
30	Minimal	Cells	14,200	23.1	100
37	Minimal	Cells	9,400	15.3	66
41	Minimal	Cells	9,000	14.6	63

[a] Results represent averages from three experiments.
[b] Values are expressed as a percentage of that obtained at 30°C, the optimal growth temperature.
[c] Colorimetric determinations of the mixed membrane fraction.
[d] Cells were uniformly labeled in complex medium containing 10^8 cpm of $^{32}PO_4$ per ml. The radioactivity was determined in lipid extracts.
[e] Cells were uniformly labeled in minimal medium containing 6.1 mM phosphate (0.2 mCi/ml). A molecular weight of 750 was used to compute micrograms of phospholipid.

Chemicals. Complex medium components and agar were obtained from Difco Laboratories (Detroit, Mich.). Radioactive phosphate was purchased from Amersham Corp. (Arlington Heights, Ill.). The fluorescent probe, DPH, was obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wis.).

RESULTS

Effects of incubation temperature on growth. Preliminary experiments were performed to determine the maximal growth temperature of *Z. mobilis* as 42°C. Previous studies by other investigators have reported that the optimal temperature for growth and alcohol production was between 30 and 35°C (18). A plot of the specific growth rates for *Z. mobilis* CP4 at different temperatures indicated that the optimal growth temperature was approximately 30°C, with 37 and 41°C failing to cause the increase in growth rate predicted by kinetic considerations. Based on these results, four growth temperatures were chosen for investigation: 20, 30, 37, and 41°C. The generation times for *Z. mobilis* at these temperatures were 6 , 2.4, 2.2, and 2.1 h, respectively.

Effects of growth temperature on membrane composition. Growth of cells at different temperatures resulted in major changes in the cellular phospholipid content (per milligram of protein) and the phospholipid/protein ratios of a mixed membrane fraction (Table 1). The lipid/protein ratio of the mixed membrane fraction decreased from 560 μg of phospholipid per mg of membrane protein at 20°C to 270 μg of phospholipid per mg of membrane protein at 41°C, with 30 and 37°C being intermediate. Since this was not a highly purified membrane preparation and such changes could be due in part to variations in purity and recovery, the phospholipid content was also examined in whole cells grown in minimal and complex media at these temperatures. In both cases, cells grown at 41°C contained approximately 60% as

TABLE 2. Effects of growth temperature on phospholipid composition[a]

Growth temp (°C)	Phospholipid composition (% total)				
	CL	PG	PE	PC	LPE + unknown
20	0.8	27.0	59.4	7.2	5.6
30	3.9	23.7	60.0	7.0	5.6
37	11.7	7.6	50.8	26.7	3.4
41	10.7	7.8	42.4	35.3	3.9

[a] Approximately 5,000 cpm was applied to each lane, and the phospholipids were separated by thin-layer chromatography. These were scraped into scintillation vials and counted. Values reported represent averages of duplicate determinations. Abbreviations: CL, cardiolipin; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPE, lysophosphatidylethanolamine.

much phospholipid per mg of cellular protein as those grown at 30°C, with 37°C being intermediate. With the exception of the value for whole cells grown at 20°C in minimal medium, the results with whole cells agree well with those obtained by analysis of the mixed membrane fractions.

Table 2 shows the effects of growth temperature on phospholipid composition. The proportion of cardiolipin (including trace amounts of phosphatidic acid) and phosphatidylcholine increased with increasing growth temperature. These increases were accompanied by a decrease in the relative abundance of phosphatidylglycerol and phosphatidylethanolamine consistent with a precursor-product relationship (25). A decrease in the proportion of lysophosphatidylethanolamine plus an unknown (probably an intermediate in the synthesis of phosphatidylcholine) was also observed.

Both the total fatty acid composition and that of the polar lipid fractions were analyzed at different growth temperatures (Table 3). Although the compositions of these differed somewhat, similar trends in temperature-related changes were observed with both. Increasing growth temperature resulted in a decrease in the proportion of vaccenic acid and an overall increase in the proportion of saturated fatty acids (primarily myristic acid). The relative amounts of palmitic acid and palmitoleic acid were lowest in cells grown at 20°C and also tended to increase with incubation temperature, although this trend was not evident in all intermediate temperatures.

TABLE 3. Effects of growth temperature on fatty acid composition[a]

Growth temp (°C)	Fatty acid composition (% total)						
	14:0	14:1	16:0	16:1	18:0	18:1	Sat FA
Total lipids							
20	12.5	1.8	8.7	2.6	1.4	71.5	22.9
30	17.3	1.1	12.5	2.6	Tr	65.6	30.1
37	19.5	2.4	10.5	4.9	Tr	61.6	30.3
41	22.8	3.3	9.9	9.7	Tr	52.1	33.4
Polar lipids							
20	13.0	Tr	8.9	1.9	Tr	75.6	22.0
30	19.5	Tr	14.1	1.0	Tr	65.3	33.6
37	17.3	Tr	18.8	1.8	Tr	61.7	36.2
41	21.3	Tr	14.9	5.5	Tr	55.1	37.4

[a] Fatty acid composition was computed as a percentage of total peak areas and represents an average from two experiments. Polar lipids were separated from neutral lipids with a silicic acid column. Abbreviations: 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:1, vaccenic acid; Sat FA, saturated fatty acids.

Effects of growth temperature on bulk membrane fluidity. The fluorescence depolarization of DPH was determined as a comparative measure of bulk membrane fluidity by using mixed membrane preparations from cells grown at different temperatures (Table 4). For comparison, polarization was examined at a common assay temperature, 30°C, and at the respective growth temperature. When measured at 30°C, membranes from cells grown at 20°C were more fluid (lower polarization value) than those from cells grown at 41°C, with membranes from cells grown at 30°C being intermediate, consistent with the increase in abundance of saturated fatty acids with increasing growth temperature (Table 3). When each membrane preparation was examined at its respective growth temperature, the trends were reversed, with the bulk membrane fluidity (lower polarization) of membranes from cells grown and assayed at 41°C being the lowest. These results suggest that the changes in membrane composition at different growth temperatures are insufficient to maintain a constant membrane fluidity at these growth temperatures.

Effects of growth temperature on thermal tolerance. We examined the effects of shifts in incubation temperature on the growth of *Z. mobilis* CP4 (Fig. 1). Shifting cultures growing at 20°C to higher temperatures (30 and 41°C) resulted in an immediate increase in growth rate to that appropriate for the higher temperature with no detectable lag (Fig. 1A). However, shifting cultures grown at 41°C to lower temperatures (30 and 20°C) resulted in significant growth lags of approximately 1.5 and 4 h, respectively (Fig. 1B). No such lags were observed upon shifting cells grown at 30°C to either 20 or 41°C (data not shown). These results indicate that a shift in growth temperature per se does not induce a growth lag. The growth lag observed upon shifting cells growing at 41°C to lower temperatures appears to reflect a unique property rather than a universal consequence of changes in growth temperature.

The effect of growth temperature on the survival of cells at 45°C was also examined (Fig. 2). Cells grown at 30 and 35°C were the most resistant to heat inactivation. Cells grown at higher temperatures and lower temperatures were more sensitive to heat inactivation, with cells grown at 41°C being the most sensitive.

Effects of temperature on membrane leakage. Figure 3 shows the effects of assay temperature on the leakage of cells grown at 30°C. As expected, the leakage of magnesium was much more rapid than that of proteins. The leakage of magnesium, nucleotides, and proteins increased with incubation temperature. At 45°C, substantial leakage of proteins occurred. It is likely that A_{260} measurements at this temper-

TABLE 4. Effects of temperature on fluorescence depolarization of DPH membranes from strain CP4

Growth temp (°C)	Assay temp (°C)	Polarization[a]
20	30	0.356
30	30	0.363
41	30	0.373
20	20	0.392
30[b]	30	0.363
37	37	0.350
41	41	0.342

[a] Values listed represent an average of three samples measured 30 times each; standard deviations were less than 0.001.

[b] Repeated from above for comparison.

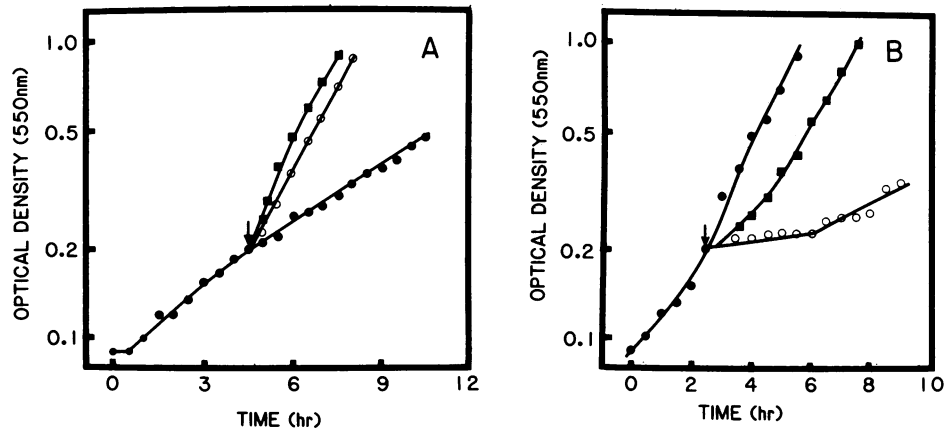

FIG. 1. Effects of a shift in incubation temperature on growth. An overnight culture (30°C) was diluted 1:100 into fresh medium and allowed to reach an optical density of 0.2 (550 nm) at either 20°C (A) or 41°C (B). The culture was then subdivided, and samples were shifted to 41, 30, and 20°C. The time of the shift is indicated by the arrows. Symbols for A: ●, 20°C to 20°C; ○, 20°C to 30°C; ○, 20°C to 41°C. Symbols for B: ○ , 41°C to 20°C; ■, 41°C to 30°C; ●, 41°C to 41°C.

ature included proteins and small nucleic acids which were released.

Figure 4 shows a comparison of the leakage of cells grown at 20, 30, and 41°C during incubation in buffer at 45°C, the temperature used for thermal inactivation studies (Fig. 2). Magnesium was lost most rapidly and at the same rate from all cells regardless of growth temperature. The leakage of A_{260} material (nucleotides plus nucleic acids) and protein leakage were most rapid in cells grown at 41°C. Protein leakage and leakage of A_{260} material were slowest in cells grown at 30°C, with cells grown at 20°C being intermediate, analogous to the trends observed for the thermal inactivation of cells grown at these temperatures (Fig. 2).

DISCUSSION

The various factors involved in limiting bacterial growth and metabolism at elevated temperatures are currently poorly defined. Thermophilic organisms have in many cases been shown to contain thermostable enzymes and to have evolved specialized modifications in membrane composition (17). Changes in temperature have been shown to have dramatic effects on the organization of biological membranes (24). The maintenance of an intact and functional membrane is of vital importance to the cell since this serves as a primary barrier to prevent the loss of metabolites and as a site for transport systems and the organization of many enzyme functions. Microorganisms have been shown to undergo temperature-inducible changes in their membrane composition, and these are widely regarded as being adaptive (12, 14, 28). Thus, our attempts at understanding the mechanisms involved in the thermal sensitivity of *Z. mobilis* focused on the composition of the cell membrane.

The best characterized changes in the membranes of microorganisms occur in fatty acid composition. With increasing temperature, the proportion of saturated fatty acid esterified into membrane lipids increases at the expense of unsaturated acyl chains (6, 11). This increase in saturated fatty acid (primarily palmitic and myristic acids) has been well documented in *Escherichia coli* (8, 21, 28) and also occurs in *Z. mobilis* CP4. However, the changes observed with *Z. mobilis* are much smaller than those reported for *E. coli* and involve the replacement of an 18-carbon monounsaturated fatty acid with a 14-carbon saturated fatty acid. This reduction in acyl chain length in *Z. mobilis* may be maladaptive. Increases in growth temperature also caused shifts in the phospholipid composition of *Z. mobilis*. The proportions of cardiolipin and phosphatidylcholine increased with reductions in phosphatidylglycerol and phosphatidylethanolamine. In other systems, phosphatidylglycerol is a precursor of cardiolipin (15, 25), and phosphatidylethanolamine is a precursor of phosphatidylcholine via the methylation pathway (3). Thus, the membrane composition of *Z. mobilis* was progressively shifted toward the ultimate prod-

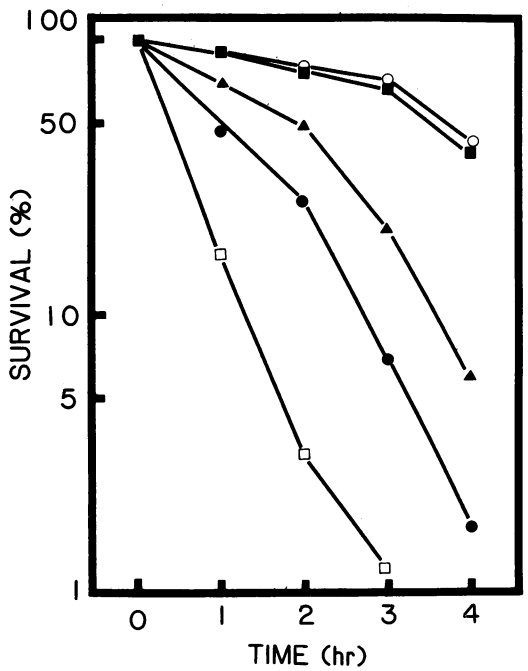

FIG. 2. Effects of growth temperature on cell survival at 45°C. Overnight cultures (30°C) were diluted 1:100 into fresh medium, grown at different temperatures to an optical density of 0.4 (550 nm), and shifted to 45°C. Percent survival was measured by plating serial dilutions of each culture at 1-h intervals. Data presented are average values for three independent trials. Symbols for growth temperatures: □, 41°C; ▲, 37°C; ■, 35°C; ○, 30°C; ●, 20°C.

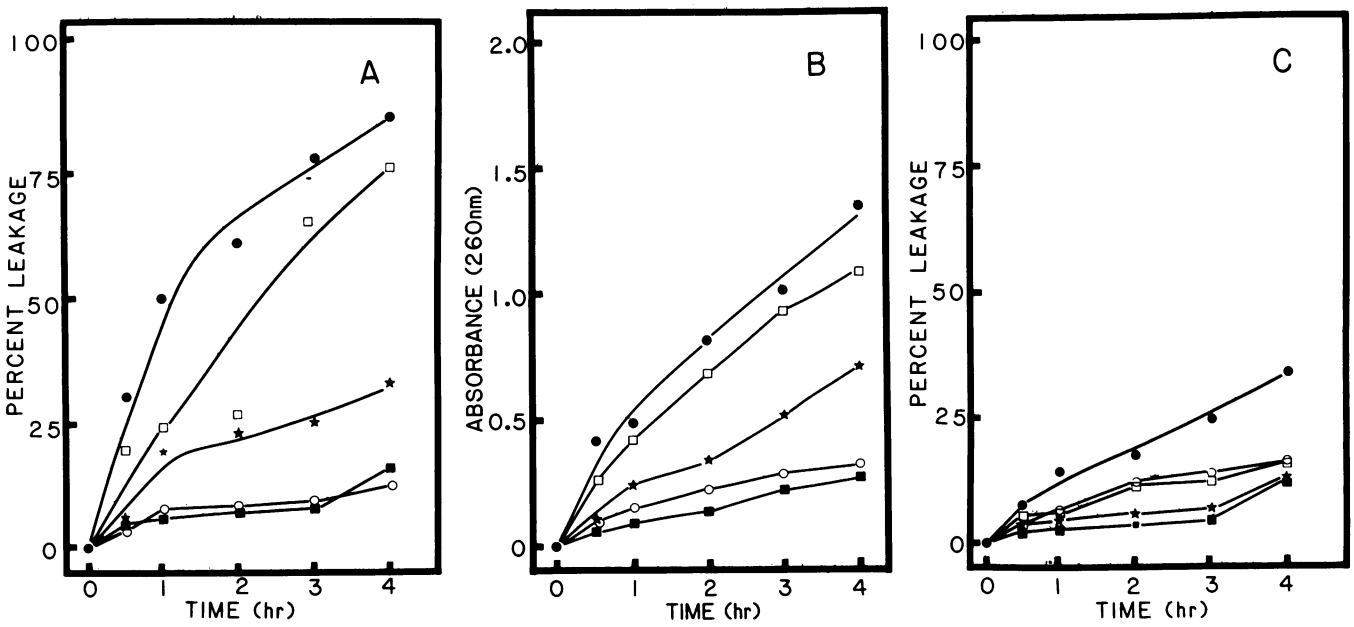

FIG. 3. Effect of assay temperatures on leakage of cells grown at 30°C. (A) Magnesium leakage; (B) A_{260} material leakage; (C) protein leakage. Data represent averages of duplicate experiments at each growth temperatures. Symbols for assay temperatures: ○, 20°C; ■, 30°C; ★, 35°C; □, 41°C; ●, 45°C.

ucts of phospholipid biosynthesis with increasing growth temperature.

The phospholipid content of *Z. mobilis* cells and membranes decreased dramatically with increasing growth temperature. The phospholipid-to-protein ratio of membranes from cells grown at 20°C was approximately twice that of membranes from cells grown at 41°C. Although such changes in other microorganisms have received little attention and are generally smaller than those found in *Z. mobilis*, they appear widespread. De Siervo (9) has shown that the phospholipid content (percent cell dry weight) of *E. coli* is increased upon shifting cultures from 37 to 27°C. Taneja et al. (30) and Khuller et al. (16) have shown that the phospholipid content (percent cell dry weight) of *Mycobacterium*

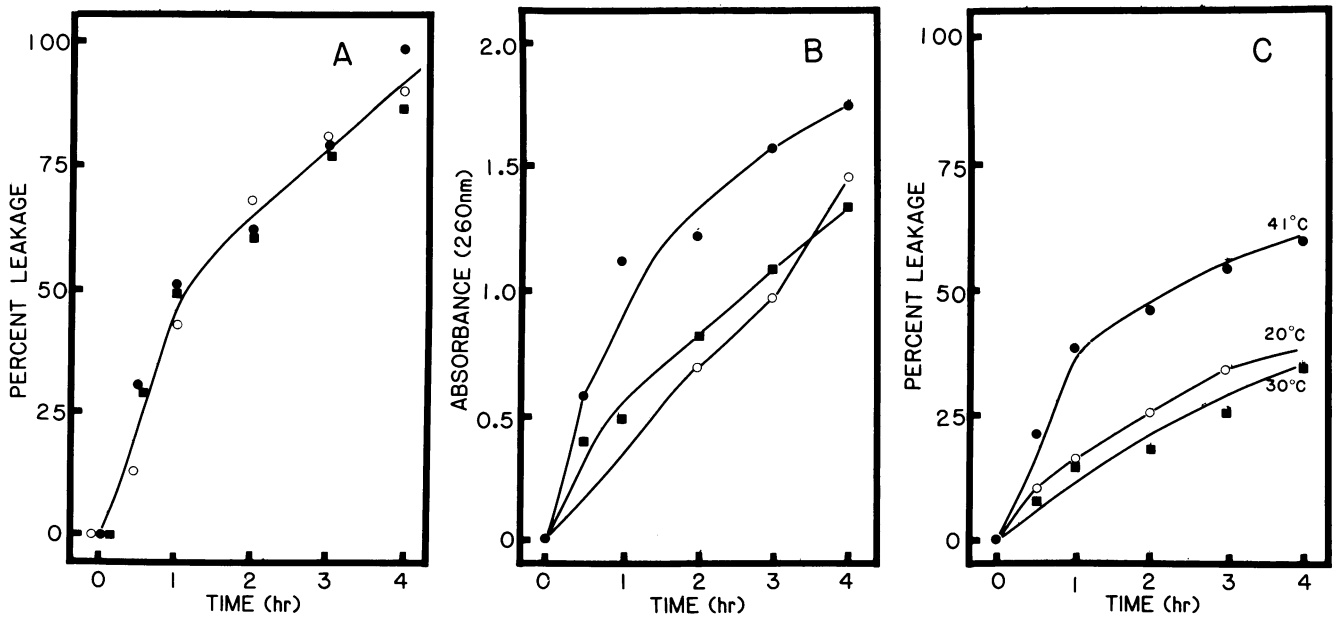

FIG. 4. Effect of growth temperature on leakage of cells incubated at 45°C. Cells were grown at 20, 30, or 41°C, washed, resuspended in buffer, and incubated at 45°C at zero time. Duplicate samples were withdrawn for the determination of magnesium leakage (A), leakage of A_{260} material (primarily nucleotides but may also include nucleic acids in some cases) (B), and protein leakage (C). Results are averages from three experiments. Values for magnesium leakage and protein leakage are expressed as a percentage of that released by chloroform disruption of the membrane. Symbols for growth temperatures: ○, 20°C; ■, 30°C; ●, 41°C.

smegmatis is also higher in cells grown at 27°C than in those grown at 37°C. For *S. cerevisiae*, Hunter and Rose (14) have shown that the phospholipid content (percent cell dry weight) of fermenting cultures decreased from 4.2% at 15°C to 3.8% at 30°C. Similar decreases in phospholipid content may be a general feature of thermal adaptation in microorganisms.

Fluorescence depolarization studies indicate that the membranes of *Z. mobilis*, unlike those of *E. coli* (8, 28), do not maintain a constant membrane fluidity during growth at different temperatures. With increasing growth temperature, the membranes from *Z. mobilis* were progressively more rigid as indicated by the higher polarization values observed at the common assay temperature of 30°C. However, temperature-induced changes in composition were insufficient to compensate for the increase in fluidity which accompanied an increase in growth temperature. The observed changes in fluidity are consistent with the decrease in the proportion of unsaturated fatty acids during growth at the higher temperatures and with the reduction in the phospholipid/protein ratio (10). These changes in the fatty acid composition of *Z. mobilis* were relatively small in comparison with other organisms and primarily involved the replacement of vaccenic acid with a relatively low-melting-temperature 14-carbon saturated fatty acid at higher growth temperatures. The shifts from phosphatidylethanolamine to phosphatidylcholine and from phosphatidylglycerol to cardiolipin observed in *Z. mobilis* would not be expected to cause a major increase in rigidity (24). Thus, the decrease in phospholipid/protein ratios with increasing temperature appears to be the dominant event leading to an increase in membrane rigidity with increasing growth temperature.

Growth temperature had a major effect on the thermal tolerance of *Z. mobilis*. Since it is unlikely that the thermal sensitivity of soluble enzymes or macromolecules is affected by differences in the temperature of their biosynthesis, the thermal tolerance may be attributable to changes in membrane composition. Previous studies with *E. coli* have demonstrated a direct correlation between membrane fluidity and the rate of thermal inactivation at 47°C (8). With *Z. mobilis*, cells grown at 20°C were more sensitive to thermal inactivation at 45°C than cells grown at 30°C, consistent with their adaptation to a lower growth temperature. However, cells grown above 30°C, the optimal growth temperature for *Z. mobilis*, did not exhibit further increases in thermal tolerance and after growth at 41°C were uniquely sensitive to thermal inactivation. This hypersensitivity of cells grown near the maximum growth temperature, 41°C, was also apparent in the pronounced lags in growth upon shifting to a lower incubation temperature and in the rapid leakage at 45°C. Since the temperature-induced changes in phospholipid and fatty acid composition appear relatively minor, it is tempting to speculate that the decrease in the abundance of membrane phospholipid may be responsible for the unique thermal sensitivity of cells grown near the maximum growth temperature, 41°C.

The leakage of magnesium, nucleotides (A_{260}), and proteins from cells grown at 30°C increased with increasing assay temperature. The leakage of proteins into buffer at 45°C roughly correlated with the thermal inactivation of cells grown at different temperatures and provides a possible mechanism for cell inactivation. Protein loss from cells is interpreted as a disruption of membrane integrity. Protein leakage at 45°C was most rapid in cells grown at 41°C, consistent with their acute sensitivity to thermal inactivation. Protein leakage was minimal in cells grown at 30°C, with cells grown at 20°C being intermediate. Rapid loss of A_{260} material also occurred in cells grown at 41°C during incubation at 45°C.

Using mutants of *E. coli*, Bell (2), Henning et al. (13), McIntyre et al. (22), and Silbert et al. (26) have shown that *E. coli* can continue to grow for slightly less than one mass doubling in the absence of new phospholipid synthesis. This growth during lipid starvation was accompanied by cell lysis, attributed to defective membrane synthesis (13). The extremely low phospholipid content of membranes of *Z. mobilis* grown at 41°C may approach the minimum phospholipid requirement for membrane integrity in *Z. mobilis*. Indeed, the inability of phospholipid synthesis to keep pace with other macromolecular syntheses may be a major determinant of the maximal growth temperature in *Z. mobilis* and other organisms.

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LITERATURE CITED

1. **Abruzzini, F. A., and L. O. Ingram.** 1982. Temperature mediated processes in teleost immunity: homeoviscous adaptation in teleost lymphocytes. Proc. Soc. Exp. Biol. Med. **169**:12–18.
2. **Bell, R. M.** 1974. Mutants of *Escherichia coli* defective in membrane phospholipid synthesis: macromolecular synthesis in an *sn*-glycerol 3-phosphate acyltransferase K_m mutant. J. Bacteriol. **117**:1065–1076.
3. **Bell, R. M., and R. A. Coleman.** 1980. Enzymes of glycerolipid synthesis in eukaryotes. Annu. Rev. Biochem. **49**:459–487.
4. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
5. **Carey, V. C., and L. O. Ingram.** 1983. Lipid composition of *Zymomonas mobilis*: effects of ethanol and glucose. J. Bacteriol. **154**:1291–1300.
6. **Cronan, J. E., and P. R. Vagelos.** 1972. Metabolism and function of the membrane phospholipids of *Escherichia coli*. Biochim. Biophys. Acta **265**:5–60.
7. **Dawson, R. M. C.** 1960. A hydrolytic procedure for the identification and estimation of individual phospholipids in biological samples. Biochem. J. **75**:45–53.
8. **Dennis, W., and M. Yatvin.** 1981. Correlation of hyperthermic sensitivity and membrane microviscosity in *Escherichia coli* K1060. Int. J. Radiat. Biol. **39**:265–271.
9. **De Siervo, A. J.** 1969. Alterations in the phospholipid composition of *Escherichia coli* during growth at different temperatures. J. Bacteriol. **100**:1347–1349.
10. **Dombek, K. M., and L. O. Ingram.** 1984. Effects of ethanol on the *Escherichia coli* plasma membrane. J. Bacteriol. **157**: 233–239.
11. **Finnerty, W. R., and R. A. Makula.** 1975. Microbial lipid metabolism. Crit. Rev. Microbiol. **4**:1–40.
12. **Haest, C. W. M., J. DeGier, and L. L. M. Van Deenen.** 1969. Changes in the chemical barrier properties of the membrane lipids of *Escherichia coli* by variation of the temperatures of growth. Chem. Phys. Lipids **3**:413–417.
13. **Henning, U., G. Dennert, K. Rehn, and G. Deppe.** 1969. Effect of eleate starvation in a fatty acid auxotroph of *Escherichia coli* K-12. J. Bacteriol. **91**:784–796.
14. **Hunter, K., and A. H. Rose.** 1972. Lipid composition of *Saccharomyces cerevisiae* as influenced by growth temperature. Biochim. Biophys. Acta **260**:639–653.
15. **Kanfer, J., and E. P. Kennedy.** 1963. Metabolism and function of bacterial lipids. J. Biol. Chem. **238**:2919–2922.

16. **Khuller, G. K., R. Taneja, and N. Nath.** 1983. Effect of fatty acid supplementation on the lipid composition of *Mycobacterium smegmatis* ATCC 607, grown at 27 and 37°C. J. Appl. Bacteriol. **54**:63–68.
17. **Kushner, D. J. (ed.)** 1978. Microbial life in extreme environments. Academic Press Inc. (London), Ltd., London.
18. **Laudrin, I., and G. Goma.** 1982. Ethanol production by *Zymomonas mobilis*: effect of temperature on cell growth, ethanol production and intracellular ethanol concentration. Biotechnol. Lett. **4**:537–542.
19. **Lee, K. J., M. L. Skotnicki, D. E. Tribe, and P. L. Rogers.** 1981. The effect of temperature on the kinetics of ethanol production by strains of *Zymomonas mobilis*. Biotechnol. Lett. **3**:291–296.
20. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
21. **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.
22. **McIntyre, T. M., B. K. Chamberlain, R. E. Webster, and R. M. Bell.** 1977. Mutants of *Escherichia coli* defective in membrane phospholipid synthesis. J. Biol. Chem. **242**:4487–4493.
23. **Ohta, K., K. Supanwong, and S. Hayashida.** 1981. Environmental effects on ethanol tolerance of *Zymomonas mobilis*. J. Ferment. Technol. **59**:435–439.
24. **Quinn, P. J., and D. Chapmann.** 1980. The dynamics of membrane structure. Crit. Rev. Biochem. **8**:1–117.
25. **Raetz, C. R.** 1978. Enzymology, genetics, and regulation of membrane phospholipid synthesis in *Escherichia coli*. Microbiol. Rev. **3**:614–659.
26. **Silbert, D. F., R. C. Landerson, and J. L. Honegger.** 1973. The unsaturated fatty acid requirement in *Escherichia coli*. Biochim. Biophys. Acta **311**:349–361.
27. **Sinensky, M.** 1971. Temperature control of phospholipid biosynthesis in *Escherichia coli*. J. Bacteriol. **106**:449–455.
28. **Sinensky, M.** 1974. Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **71**:522–525.
29. **Skotnicki, M. L., K. J. Lee, D. E. Tribe, and P. L. Rogers.** 1981. Comparison of ethanol production by different *Zymomonas* strains. Appl. Environ. Microbiol. **41**:889–893.
30. **Taneja, R., U. Malik, and G. K. Khuller.** 1979. Effect of growth temperature on the lipid composition of *Mycobacterium smegmatis* ATCC 607. J. Gen. Microbiol. **113**:413–416.