EFFECTS OF TEMPERATURE AND SALT ON LIPID AND FATTY ACID COMPOSITIONS OF A BACTERIUM ISOLATED FROM THE BOTTOM LAYER OF LAKE VANDA, ANTARCTICA

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A Gram-negative, aerobic bacterium isolated from the bottom layer of Lake Vanda, Antarctica, can grow in a synthetic medium containing biotin as a growth factor. The lipid and fatty acid compositions of the cells grown in the fatty acid-free medium were analyzed. Phosphatidylethanolamine, phosphatidylglycerol and wax esters were identified as major components of the chloroform-methanol extractable lipids. Palmitoleic and oleic acids comprised more than 90% of the total fatty acid of the lipids when the bacterium was grown at low temperature (5°C). Increasing the growth temperature induced an increase in the average fatty acyl chain length of the phospholipids and a decrease in the unsaturation degree of the fatty acyl chain of the wax esters. Increasing the NaCl concentration in the medium also induced an increase in the average fatty acyl chain length of the phospholipids. The effects of temperature and NaCl were compared further at the level of phospholipid molecular species. The *sn*-2 position of phosphatidylethanolamine and the *sn*-1 position of phosphatidylglycerol were the most sensitive sites to changes of temperature and NaCl concentration, respectively. An aerobic desaturase system was demonstrated to be the only pathway for unsaturated fatty acid synthesis in this bacterium.

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Lake Vanda is a saline lake located in the McMurdo Dry Valleys in Antarctica. The water of the upper layer (above 50 m) of this lake has low temperature (5–7°C) and low salinity, whereas that of the bottom layer (69 m) has high temperature (about 24°C) and high salinity (76.5 g/kg, as chlorinate). Some microbiological studies including isolation of microorganisms from the water samples of the Lake and analyses of fatty acid composition of several of the species found there have been reported (12, 14, 17, 19). Previously, Nagashima et al. isolated a Gram-negative, aerobic bacterium (they designated it as strain 13A) from a bottom water sample of the Lake and reported that this bacterium grown in a complex nutrient medium contains a small but significant amount of polyunsaturated fatty acids, linoleic and linolenic acid (19).

Recently, other bacterial isolates from the sea environment have also been shown to contain polyunsaturated fatty acids (1, 34, 35). However, many of these results were obtained with the cells grown in undefined complex nutrient media. Since some bacteria can readily use exogenous fatty acids and incorporate them into cellular constituents directly or after some modifications, fatty acid composition of such bacteria may be greatly influenced by exogenous fatty acids, in particular the bacteria deficient in fatty acid synthesis (29). The content of unsaturated fatty acids in phospholipids is altered by changing the environmental conditions including temperature (25), salinity (26) and hydrostatic pressure (10). So, in order to investigate the physiological roles of unsaturated fatty acids and evaluate the microbial interactions in nature, it is important to understand whether these acids are synthesized *de novo* or whether they are exogenous derivatives.

In order to evaluate the microbial interactions and nutrient fluxes in Lake Vanda, biochemical analyses on the bacterial isolates should be carried out in a chemically defined system. In this study, we analyzed the lipid and fatty acid compositions of the Gram-negative, aerobic rod bacterium, strain 13A, which was previously isolated from a bottom water sample of Lake Vanda (19), grown in a chemically defined fatty acid-free medium, and we discuss the changes in positional distribution of fatty acid in phospholipids in relation to changes in environmental temperature or salinity. Unsaturated fatty acid synthesizing systems of this bacterium were also investigated.

MATERIALS AND METHODS

Bacterial strain and culture conditions. The Gram-negative, aerobic bacterium which was previously isolated from the bottom water-sample of Lake Vanda (77°32'S, 161°24'E) and designated as strain 13A (19), was used throughout in this study. The bacterium shows positive oxidase and catalase activities, and produces little acid from glucose. Nagashima et al. have described that this bacterium may be *Pseudomonas* sp. (19). A complex medium is composed of 1% peptone, 1% meat extract and an appropriate concentration of NaCl (pH 7.2). The composition of the chemically defined medium was as follows: Tris-HCl (pH 7.5)

50 mM, NaCl 0.4 M, KCl 10 mM, K_2HPO_4 3.3 mM, sodium citrate 3 mM, $FeSO_4$ 0.1 mM, $CaCl_2$ 1.0 mM, $MgSO_4$ 1.0 mM, NH_4Cl 25 mM, sodium succinate 0.1 M, and biotin 0.01 mg/l. To study the effects of exogenous fatty acids, the chemically defined medium was supplemented with 0.01% saturated or unsaturated fatty acid and 0.1% Triton X-100. The bacteria were cultured aerobically at temperatures ranging from 5 to 25°C. The growth of the cells were monitored by measuring turbidity at 600 nm.

Extraction and analysis of lipids. The cells in an early stationary phase of growth were harvested and washed twice with cold 0.5 M NaCl by centrifugation at $8,000 \times g$ for 10 min at 4°C. The washed cells were suspended in deionized water and total lipids were extracted by the method of Bligh and Dyer (2). Chloroform solution of the extracted lipids was evaporated under a stream of N_2 gas and the residue was dissolved in a small amount of chloroform. The total lipids were separated by thin-layer chromatography (TLC) on a silica gel plate using acetone/benzene/methanol/ H_2O (8:3:2:1, v/v) as a first solvent system and chloroform/methanol/28% ammonia (13:7:1, v/v) as a second solvent system. Lipids on the plate were made visible with iodine vapor and characterized with specific spray reagents. For further analysis, lipids were separated by TLC on a silica gel plate using a solvent system composed of chloroform/methanol/acetic acid/water (17:3:2:0.7, v/v). Individual lipid spots were scraped from the TLC plate and eluted from the silica gel with chloroform/methanol (2:1, v/v). Phospholipids were methanolized in 5% HCl, and the resultant fatty acid methyl esters were extracted with hexane as described previously (31). Phospholipids were estimated by measurement of phosphorus content, essentially according to the procedure described by Christie (5). Wax esters were saponified by refluxing in 3 M KOH-methanol at 70°C for 3 h. Wax fatty alcohols were extracted under alkaline conditions with light petroleum ether (bp 60 to 80°C) from the saponification mixture, which were then acidified below pH 2 with concentrated HCl and wax fatty acids were extracted with hexane. Methods for the conversion of fatty acids to methyl ester derivatives were the same as described previously (31). Alternatively, wax fatty alcohols and wax fatty acids (as methyl ester derivatives) were separated by TLC after extraction from the acidified saponification products and treatment with diazomethane, essentially similar methods as described by Russell and Volkman (27).

Gas-liquid chromatography. Fatty acid methyl esters were analyzed isothermally at 190°C by gas-liquid chromatography fitted with a 12 m $\times$ 0.2 mm capillary column of 10% EGSS-X on Chromosorb WAW DMCS (GL Science, Tokyo, Japan). Methyl ester of pentadecanoic acid was used as an internal standard. Wax fatty alcohols were analyzed by gas-liquid chromatography with a glass column packed with SE-30 at 100–200°C (4°C/min). Fatty acid methyl esters and wax fatty alcohols were identified by comparing the retention time with those of authentic samples. Chromatographic data were calculated with a data processor (Shimadzu Chromatopack C-R3, Shimadzu Co., Kyoto, Japan).

Incorporation of exogenous fatty acids into cellular lipids. The cells grown at 25°C in the synthetic medium supplemented with 0.1% yeast extract (Difco, Detroit, MI, U.S.A.) instead of biotin were harvested and resuspended in 50 mM sodium phosphate buffer (pH 7.0). To this suspension (5 ml), was added 1.7 nmol [1-¹⁴C]palmitic or stearic acid, and incubated at 25°C for 10 min. The incorporation of labeled fatty acid into lipids was also determined in the presence of cerulenin (100 µg/ml). After the incubation, total lipid was extracted from the cells and transmethylated as described above. The methyl esters of saturated and unsaturated fatty acids were separated by silver nitrate TLC, and radioactivity distribution was analyzed by a radiochromatogram scanner (Aloka JTC-601). Radioactive spots which remained on the origin of the silver nitrate TLC plate were scraped off and extracted from the silica gel 3 times with hexane/ethyl ether (1:1, v/v). The extracted sample was further analyzed by TLC on a silica gel plate using hexane/ethyl ether/acetic acid (7:3:0.1, v/v) as a solvent system.

Fatty acid synthesizing system. Crude cell-free extract was prepared from the cells grown in the synthetic medium supplemented with yeast extract (0.1%) at 25°C as previously described (32). The soluble fraction was obtained from the crude extract by centrifugation at 100,000 × *g* for 30 min, and used as crude fatty acid synthase. In vitro [¹⁴C]malonyl-CoA driven fatty acid synthesis was analyzed as described previously (32) except that the reaction was carried out at 25°C in this study. After the incubation, lipids were saponified, and the free fatty acids were extracted with hexane. The fatty acids were converted to their methyl esters, and separated by a silver nitrate TLC plate as described above. Distribution of radioactivity in saturated and unsaturated fatty acid methyl esters was determined using a radiochromatogram scanner. The protein concentration was determined by the method of Bradford (3).

Positional distribution of fatty acid in phospholipids. Phospholipids were digested with *Rhizopus delemar* lipase, which cleaves the *sn*-1 acyl-ester bond of acylglycerol, by the procedures described by Christie (5). The digested products (free fatty acid, and lyso- and unreacted phospholipid) were separated by TLC on a silica gel plate using chloroform/acetone/methanol/acetic acid/H₂O (10:4:2:3:1, v/v) as a solvent system. The procedures used for analysis of the fatty acids were the same as described above.

Chemicals. Palmitic, stearic, and oleic acids, and Triton X-100 were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Pentadecanoic and palmitoleic acids, and oleoyl alcohol were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). The sodium salts of [1-¹⁴C]acetate and [1-¹⁴C]palmitic and stearic acids were products of ICN (Irvine, CA, U.S.A.). [2-¹⁴C]malonyl-CoA was obtained from Amersham (IL, U.S.A.). Acyl carrier protein was prepared from *Escherichia coli* K-12 cells by the method of Rock and Cronan (22). Cerulenin was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents used were of analytical grade.

RESULTS

Bacterial growth in a synthetic medium

Strain 13A, which has been isolated from Lake Vanda, is a Gram-negative, aerobic rod, and is positive for oxidase and catalase activities (19). Nagashima et al. (19) described that this bacterium may be *Pseudomonas* sp., however, the taxonomic status of this bacterium has not been established. Among the previously reported studies on this bacterium, the fatty acid composition of cells grown in undefined complex medium is intriguing, because this bacterium contains polyunsaturated fatty acids (19). To confirm whether polyunsaturated fatty acids are synthesized de novo or whether they are exogenous derivatives, we attempted to prepare a chemically defined synthetic medium for this bacterium. Tris-salts succinate medium (13), which has been used in our laboratory to culture several different bacterial taxa including a psychrophilic *Vibrio* sp. strain ABE-1, *Escherichia coli* K-12 and *Pseudomonas* sp. strain E-3, did not support the growth of strain 13A unless the medium was supplemented with a trace amount of biotin. Supplementation of unsaturated fatty acids such as palmitoleic or oleic acid (major fatty acid components of this bacterium) to the biotin-free synthetic medium sustained slight growth of this bacterium (data not shown). Table 1 shows the bacterial growth rates obtained under different temperatures and NaCl concentrations. The results confirm that the temperature and salt dependencies of this bacterial growth are not greatly altered in the chemically synthetic medium from those in the complex nutrient medium (19).

Lipid composition

Total lipid extracted from the cells grown in the synthetic medium at 25°C was analyzed by two-dimensional TLC on a silica gel plate. Phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and a neutral lipid were the major components and nearly equal amounts of these components were present. The neutral lipid was identified as a wax ester by cochromatography with authentic sample and analyses of saponified products by gas-liquid chromatography. The relative proportion of the three lipid components did not vary significantly with growth temperature,

Table 1. Effects of temperature and NaCl on the bacterial growth rate.

Temperature (°C) ^a			NaCl (%) ^b		
5	15	25	0	3	6
0.055	0.144	0.309	0.309	0.189	0.117

^a A preculture grown at 25°C was inoculated (1 to 100) into a fresh medium and cultured at indicated temperature.

^b A preculture grown at 25°C and in a medium without NaCl was inoculated (1 to 100) into a medium containing NaCl as indicated and cultured at 25°C.

Fig. 1. Effect of NaCl on lipid composition.

Lipid samples from the cells grown in a medium containing NaCl as indicated concentrations in the figure were fractionated on a TLC silica gel plate as described in MATERIALS AND METHODS. The amount of the individual lipid was calculated from the content of the total fatty acid determined by gas liquid chromatography. The values are expressed as mol %. Pentadecanoic acid was used as an internal standard. Average values of three independent experiments are plotted. ○, phosphatidylethanolamine; ●, phosphatidylglycerol; □, wax esters.

however the proportion changed greatly when the NaCl concentration in the medium was varied (Fig. 1). The content of wax ester remarkably decreased with increasing NaCl concentration, while that of phospholipids increased gradually.

Fatty acid composition

Changes in the fatty acid compositions of PE and PG with changes in the growth temperature are shown in Table 2. As previously analyzed by a gas chromatograph-mass spectrometer (19), palmitoleic (16:1) and oleic (18:1) acids comprise more than 90% of the fatty acids of the phospholipids except for PG extracted from the cells grown at 25°C. In contrast with the previous results (19), polyunsaturated fatty acid was not detected. Oleic acid was the most abundant fatty acid in the phospholipids at 25°C, but palmitoleic acid was the most abundant at 5°C. Thus, the degree of unsaturation of fatty acids in PE and PG did not change significantly with the growth temperature, while the average chain length of the fatty acids was shortened by lowering the growth temperature.

Temperature-dependent compositional changes in the fatty acyl chain of the wax ester is shown in Table 3. The wax fatty acyl chain was more saturated than that of the phospholipids. The effect of temperature on the fatty acyl composition

Table 2. Effect of growth temperature on fatty acid composition of the phospholipids.

	Temperature (°C)	Fatty acid (mol%)			
		16:0	16:1	18:0	18:1
PE	5	0.8±0.05	58.8±2.1	0.2±0.1	40.2±2.4
	15	1.0±0.4	38.4±2.2	1.9±0.4	58.7±1.7
	25	3.3±0.8	23.3±0.3	1.9±0.4	72.6±1.3
PG	5	3.5±0.1	52.8±3.4	1.1±0.7	42.7±2.8
	15	2.3±0.9	28.7±1.5	2.2±0.8	66.8±0.9
	25	6.6±0.2	16.8±1.9	5.7±0.5	70.9±2.1

Phospholipids were extracted from the cells grown at the indicated temperature. PE, phosphatidylethanolamine; PG, phosphatidylglycerol. Fatty acids are denoted by the chain length, and the number of double bonds. Values are averages of three individual experiments±SD.

Table 3. Effect of growth temperature on fatty acyl chain composition of wax ester.

Wax fatty acid				Wax alcohol			
Growth temperature (°C)				Growth temperature (°C)			
	5	15	25		5	15	25
16:0	4.8±0.1	8.4±2.9	11.4±0.8	16:0	11.5±1.0	9.6±0.2	17.2±3.2
t16:1 ^a	1.0±0.5	2.6±2.0	1.3±1.0	16:1	15.9±2.2	12.4±2.8	9.9±2.1
16:1	12.8±1.9	9.8±0.6	9.2±1.1	18:0	0.8±0.2	14.2±2.9	21.6±3.0
18:0	1.3±0.2	3.9±0.4	11.9±1.0	18:1	68.5±2.1	55.3±7.0	45.3±6.5
18:1	80.1±2.7	75.2±0.9	66.2±1.8	20:0	1.3±0.2	3.8±3.0	1.9±1.0
				20:1	2.1±1.0	4.9±3.7	4.1±1.7

^a Trans 16:1.

Fatty acids or alcohols are denoted by the chain length, and the number of double bonds. Values are averages of three individual experiments±SD.

of the wax ester was different from that observed with the phospholipids, i.e., the main change appeared in the unsaturation degree of the fatty acyl chain.

Effect of NaCl on fatty acyl chains of phospholipid and wax ester

Since the lipid composition of strain 13A was changed markedly by the NaCl concentration (Fig. 1), the effect of NaCl on the fatty acyl chain of the individual lipids was examined. As shown in Fig. 2, moderate changes were observed in the content of the two major fatty acid species, oleic and palmitoleic acids, in PE and PG. The content of oleic acid increased and palmitoleic acid decreased with increasing NaCl concentration. Changes of the fatty acyl chain of the wax ester (Fig. 2c and d) was markedly different from those of the phospholipids. The most pronounced effect of NaCl appeared in the changes of the acyl chain of the fatty alcohol. With increasing concentrations of NaCl, a drastic reduction and increase in the contents of oleoyl (18:1) and icosenoyl (20:1) alcohol occurred, respectively. The proportion of stearoyl alcohol also increased slightly in the presence of 6%

Fig. 2. Changes of percentage composition of fatty acyl chain in the phospholipids and wax esters by NaCl concentration.

Individual lipid sample was fractionated as described in the legend to Fig. 1. Wax ester was saponified with KOH, and resultant wax fatty acids and fatty alcohols were extracted as described in MATERIALS AND METHODS. a, PE; b, PG; c, wax fatty acid; d, wax fatty alcohol. ○, 16:0; ●, 16:1; ◇, 18:0; ◆, 18:1; □, trans 16:1; △, 14:0; ▽, 20:1.

NaCl. These results indicate that the acyl chain in the wax ester becomes more saturated and longer with increasing NaCl concentration.

Effects of temperature and NaCl on the positional distribution of fatty acid in the phospholipids

In order to investigate the effects of growth temperature and NaCl in more detail, the positional distribution of fatty acid in the phospholipids was examined as described in MATERIALS AND METHODS. The proportion of the two main fatty acids, palmitoleic and oleic acids, changed markedly with the growth temperature (Fig. 3). Increasing the temperature caused palmitoleic acid to be replaced by oleic acid. The pattern of the proportional change was similar at both the *sn*-1 and *sn*-2 positions. However, the degree of change in the *sn*-2 position was greater than that

Fig. 3. Positional distribution of fatty acids in the phospholipids from the cells grown at different temperatures.

Phospholipids were prepared from the cells grown at the indicated temperatures. Each phospholipid was digested with *Rhizopus delemar* lipase. The digested products were separated on a silica gel plate of TLC and the fatty acid methyl esters were prepared as described in MATERIALS AND METHODS. ○, 16:0; ●, 16:1; ◇, 18:0; ◆, 18:1.

in the *sn*-1 position. Furthermore, only the *sn*-2 position of PE could respond continuously to the temperature range examined, while the other positions responded scarcely to the change of temperature from 15 to 25°C.

Compared with the effects of temperature, the changes in the NaCl concentration induced smaller changes (Fig. 4). At both the *sn*-1 position of PE and the *sn*-2 position of PG, only a little change in the fatty acyl chain length was observed by changing the NaCl concentration (Fig. 4). In the *sn*-2 position of PE, a small but significant alteration was observed by increasing the NaCl concentration up to 3%, but further increases in the NaCl concentration did not induce a significant change. It was found that only the *sn*-1 position of PG could respond continuously to the whole range of NaCl concentrations examined.

Fig. 4. Positional distribution of fatty acids in the phospholipids from the cells grown in various concentrations of NaCl.

The phospholipids were extracted from the cells grown in the synthetic medium containing the indicated concentration of NaCl at 25°C. Other details and all symbols are the same as described in the legend to Fig. 3.

Pathway of unsaturated fatty acid synthesis

In some bacteria (18,31), there are two possible pathways for unsaturated fatty acid synthesis: the anaerobic pathway catalyzed by fatty acid synthase and the aerobic pathway involving desaturase. To determine which pathway is operating in strain 13A, we examined whether fatty acid synthase is able to produce unsaturated fatty acid in an in vitro system. Radioactivity in the reaction products was exclusively distributed in the saturated fatty acid fraction separated on a silver nitrate TLC plate, and no radioactivity was detected on the corresponding spot of unsaturated fatty acid (data not shown). This result suggests that unsaturated fatty acid was produced by the aerobic pathway. Indeed, unsaturated fatty acid was produced when the cells were incubated with [^{14}C]palmitic or [^{14}C]stearic acid even in the presence of cerulenin at the concentration (100 $\mu\text{g}/\text{ml}$) that completely inhibited fatty acid synthesis from [^{14}C]acetic acid (Fig. 5). As shown in Fig. 5, a

Fig. 5. Desaturation of stearic acid.

The cells grown in the synthetic medium supplemented with stearic acid (0.01%) was incubated with [$1\text{-}^{14}\text{C}$]stearic acid (1.7 nmol/5 ml of reaction mixture) in the presence of cerulenin (100 $\mu\text{g/ml}$) for 10 min at 25°C. Details of the extraction and transmethylation of the lipid are described in MATERIALS AND METHODS. The fatty acid methyl esters were separated on 20% silver nitrated-impregnated silica plate with toluene as the developing solvent. Radioactively labeled areas were located with a radiochromatoscanner. SFA, saturated fatty acid; UFA, unsaturated fatty acid.

considerable amount of radioactivity was detected also on the origin of the silver nitrate TLC plate. This compound was eluted from the silica gel and analyzed as described in MATERIALS AND METHODS, and it was found that this compound is a long-chain fatty alcohol (data not shown). These results indicate that long-chain fatty alcohols, a component of wax esters, can be produced in this bacterium.

DISCUSSION

The Gram-negative, aerobic bacterium, strain 13A from Lake Vanda, Antarctica, was able to grow well in a chemically defined medium containing biotin as a growth factor. Although this bacterium has been supposed to be *Pseudomonas* sp. (19), most species of *Pseudomonas* do not require organic growth factors. Because the sediment of Lake Vanda contains nutrients from algal origin (15), strain 13A can easily grow in the bottom layer of the Lake when sufficient oxygen is supplied.

Strain 13A may have a potential to synthesize polyunsaturated fatty acid, because unsaturated fatty acid is produced only by the aerobic fatty acid desaturase system (Fig. 5). However, the chloroform-methanol extractable lipids of this bacterium grown in a fatty acid-free synthetic medium did not contain a detectable amount of polyunsaturated fatty acid. A previous report of the presence of

polyunsaturated fatty acid in this bacterium (19) was based on the analysis of a lipid sample which was extracted from the saponified whole cells grown in a complex medium. The discrepancy between the present and previous results may be due to the difference in the procedures employed for the preparation of lipid sample and/or in the composition of the media. *Pseudomonas* has long been believed to synthesize unsaturated fatty acid by the anaerobic pathway (28). However, we showed that *Pseudomonas* sp. strain E-3 synthesizes unsaturated fatty acid by both the anaerobic and aerobic pathways (31). Although the mechanism for unsaturated fatty acid synthesis was taxonomically discussed in a group of Gram-positive bacteria (30), that of Gram-negative bacteria has not been studied from the taxonomic point of view. Further study is required to classify strain 13A.

Phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and wax esters were identified as the major components of the chloroform-methanol-extractable lipid of strain 13A. The presence of substantial amounts of wax esters is known in only a few bacterial species including the genera *Acinetobacter* (11), *Moraxella*, *Neisseria* (4), and in *Micrococcus cryophilus* (23). The presence of wax esters may be one of the useful characteristics for the classification of strain 13A.

Strain 13A grows in media supplemented with biotin at relatively wide ranges of temperature and salinity. It was found that the lipid composition of this bacterium was altered by changing the salinity but not the temperature. Increasing the concentration of NaCl induced the remarkable reduction and gradual increases in the proportion of the wax ester and phospholipids, respectively (Fig. 1). Since fatty acid is a common component of the above two lipids and serves as a precursor of wax fatty alcohol, the biosynthetic pathways for phospholipids and wax esters might compete for fatty acids. When a high concentration of NaCl is present in the medium, the phospholipid synthetic pathway is favored.

It is interesting that different effects of temperature on the fatty acyl chains were observed between the phospholipids and wax ester of strain 13A. By lowering the growth temperature, the fatty acyl chain length of the phospholipids was shortened, whereas the unsaturation degree of the fatty acyl chain of the wax esters was increased (Fig. 2). Such a different response to temperature between the fatty acyl chains of the phospholipids and wax esters was also reported with other psychrophilic bacterium, *M. cryophilus* (24,27). The fatty acid composition of the phospholipids of the Gram-negative bacterial strain 13A is similar to that of *M. cryophilus*. However, the effect of temperature on the positional distribution of fatty acid in the phospholipids is different between strain 13A and *M. cryophilus*. The phospholipids of *M. cryophilus* show preferences for the oleoyl and palmitoleoyl acyl chain for the *sn*-1 and *sn*-2 positions, respectively, even when the growth temperature is varied from 20 to 0°C (16). On the other hand, such a preference was not observed in the phospholipids of strain 13A. In the phospholipids of this bacterium, low temperature induced shortening of the fatty acyl chain length from oleic to palmitoleic acid at the *sn*-1 position as well as at the *sn*-2 position (Fig. 3). The difference observed between *M. cryophilus* and strain 13A may be due to the

different ability to cope with the environmental temperature. Strain 13A has to maintain suitable membrane fluidity at high temperature (25°C) at which *M. cryophilus* can not grow. Because the melting point of the phospholipids with *sn*-1 long and *sn*-2 short acyl chains is lower than that of *sn*-1 short and *sn*-2 long acyl chains (9), the presence of such phospholipids in the membrane as observed in *M. cryophilus* may be disadvantageous for strain 13A to maintain the proper membrane fluidity at high temperature. The fact that the phospholipids of most Gram-negative bacteria are those with *sn*-1 short and *sn*-2 long fatty acyl chains (33) provides the evidence for the above idea.

As shown in Figs. 3 and 4, the *sn*-2 position of PE and the *sn*-1 position of PG of strain 13A were the most sensitive sites to temperature and NaCl, respectively. Although the change of positional distribution by temperature was reported with *E. coli* phospholipids (20), the most sensitive site to temperature can not be determined because only two different types of data (high and low temperature) are available. It is well known that PE has a strong preference for a non-bilayer structure, hexagonal II, and the transition temperature from bilayer to non-bilayer structures is affected by the fatty acyl chains (7,8). Dioleoyl-PE adopts the hexagonal II phase at the temperatures above its transition temperature, $12 \pm 5^\circ\text{C}$. PG also has been shown to have a preference to form the non-bilayer structure in the presence of a high concentration of cations (6). Although the permanent presence of such a non-bilayer structure in the biomembranes is undesirable, the presence of phospholipids which tend to form non-bilayer structures is essential for the growth of *E. coli* (21). Thus, strain 13A may adapt to changes of temperature or salinity by changing the fatty acid composition of the phospholipids to maintain membrane fluidity as well as the balance between the bilayer and non-bilayer structures in the membrane.

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