

A *trans*-Unsaturated Fatty Acid in a Psychrophilic Bacterium, *Vibrio* sp. Strain ABE-1

HIDETOSHI OKUYAMA,^{1*} SHOJI SASAKI,¹ SHOICHI HIGASHI,² AND NORIO MURATA²

Department of Botany, Faculty of Science, Hokkaido University, Sapporo 060,¹ and National Institute for Basic Biology, Okazaki 444,² Japan

Received 21 November 1989/Accepted 28 March 1990

A high level of a *trans*-unsaturated fatty acid was found in the phospholipids of a psychrophilic bacterium, *Vibrio* sp. strain ABE-1. This fatty acid was identified as 9-*trans*-hexadecenoic acid (C_{16:1}9t) by gas-liquid chromatography and infrared absorption spectrometry. C_{16:1}9t accounted for less than 1% of the total fatty acids in cells grown at 5°C and reached 12% of the total at 20°C. We suggest that the increase in the level of the *trans*-unsaturated fatty acid is related to the high growth rate of this bacterium at elevated temperatures. Possible biological roles of the *trans*-unsaturated fatty acid in the adaptation of the microorganism to the ambient temperature are discussed.

The phospholipids are major components of bacterial membranes (5, 9). Although the constituent fatty acids of phospholipids in most bacterial strains are saturated and monounsaturated with a *cis* configuration (5, 9), some bacterial strains contain substantial levels of monounsaturated fatty acids with a *trans* double bond (2, 6, 7, 11, 12). The origins of *trans*-monounsaturated fatty acids are diverse. *Escherichia coli* incorporates exogenously added *trans*-monounsaturated fatty acids into phospholipids (2, 12), even though it does not, itself, synthesize *trans*-monounsaturated fatty acids (10). However, it is likely that in *Vibrio cholerae* (6) and some strains of methylotrophs (11) certain *trans*-monounsaturated fatty acids are synthesized *de novo*. Tracer experiments have explicitly demonstrated that *trans*-monounsaturated fatty acids are synthesized from acetate in *Pseudomonas atlantica* (7). In all cases, the proportion of *trans*-monounsaturated fatty acids relative to the total amount of fatty acids tends to increase during starvation (6, 7, 18).

This communication reports the presence of a high level of a *trans*-monounsaturated fatty acid in the phospholipids of *Vibrio* sp. strain ABE-1 grown in a culture medium without free fatty acids. The synthesis of this fatty acid was markedly dependent on the growth temperature.

Growth conditions. *Vibrio* sp. strain ABE-1 was grown at 5, 15, and 20°C in a rotary shaker in a synthetic medium that contained sodium succinate as the sole source of carbon as described previously (8). Bacterial growth was monitored as the change in the turbidity of the culture at 600 nm. *Pseudomonas* sp. strain E-3 (19) was grown at 20°C in a rotary shaker in the same medium as that used for the culture of *Vibrio* sp. strain ABE-1 with the single exception that the concentration of NaCl was reduced from 400 to 50 mM (19).

Extraction and analysis of lipids. Total lipids were extracted from the cells by the method of Bligh and Dyer (3). Total phospholipids were separated from the total lipids by column chromatography on silicic acid as described by Nishihara et al. (13). Aliquots of the total lipids and the total phospholipids were subjected to methanolysis at 80°C for 3 h in 5% methanolic HCl. Resultant fatty acid methyl esters were extracted with hexane and analyzed with a gas-liquid chromatograph (model GC-8A; Shimadzu) equipped with a

flame ionization detector and a capillary column (Quadrex, CPS-1, 0.25 mm [inner diameter] by 50 m, 0.25- μ m film). The column temperature was maintained at 180°C, and both the injection and the detection temperatures were set at 200°C. The flow rate of the carrier gas, N₂, was 1.0 ml/min. The separated fatty acid methyl esters were identified by comparing their retention times with those of standards (no. AI59013; Gasukuro Kogyo Co., Tokyo, Japan) and were quantitated with a datum analyzer (Chromatopac C-R6A; Shimadzu).

The positions of double bonds in the fatty acid methyl esters were determined by the pyrrolidide method (1). For the verification of *trans* and *cis* configurations of double bonds in the unsaturated fatty acids, the unsaturated fatty acid methyl esters were separated into two fractions by silver nitrate thin-layer chromatography on silica gel (no. 5721; E. Merck AG, Darmstadt, Federal Republic of Germany) with a solvent system composed of chloroform and ethanol (99:1 [vol/vol]). After the plate was developed in the solvent system, it was dried and sprayed with 0.01% primuline in acetone-H₂O (4:1 [vol/vol]). The spots of fatty acid methyl esters were detected under UV light (20). The separated methyl esters were extracted from the silica gel with chloroform-methanol-water (1:1:0.9 [vol/vol/vol]). The *cis* and *trans* configurations were determined by infrared absorption spectrometry (JASCO A-302 spectrometer) by the potassium bromide method (4) with authentic methyl esters of C_{16:1}9t and C_{16:1}9c (Gasukuro Kogyo Co.) as references.

Fatty acid nomenclature. Fatty acids are designated according to the number of carbon atoms and the number of double bonds, with the distance of the double bond from the carboxyl end and the geometry for *cis* indicated by "c" or for *trans* indicated by "t."

***trans*-monounsaturated fatty acid.** Gas-liquid chromatography of the fatty acid methyl esters of the total lipids from the cells (Fig. 1) demonstrated that the major fatty acids of the glycerolipids from *Vibrio* sp. strain ABE-1 were C_{16:0}, C_{16:1}9c, and C_{18:1}11c. A very similar chromatogram was obtained for the fatty acid methyl esters of the total phospholipids, which accounted for more than 96% of the total lipids in this bacterium (15). Between the chromatographic peaks of C_{16:0} and C_{16:1}9c, there were two additional com-

* Corresponding author.

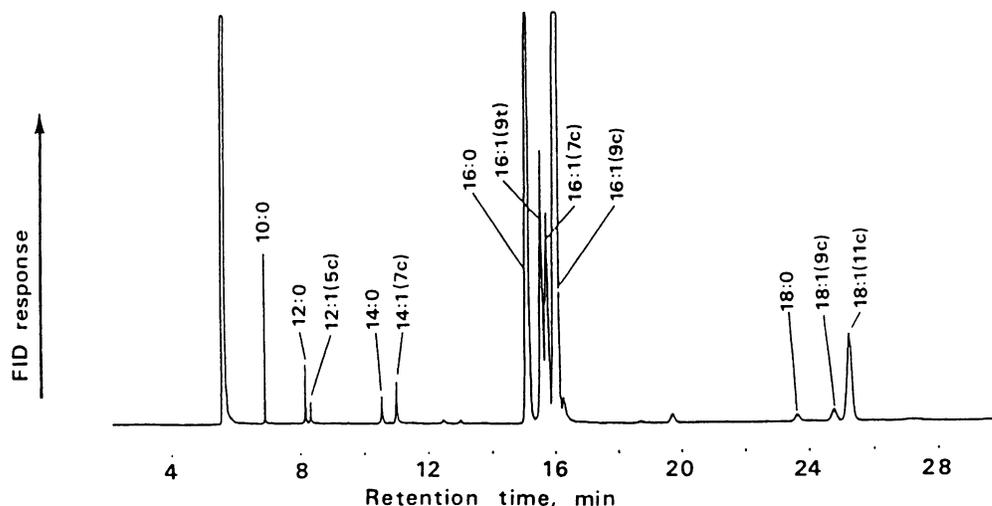


FIG. 1. Gas-liquid chromatogram of fatty acid methyl esters of total lipids from *Vibrio* sp. strain ABE-1 grown at 20°C. Conditions for the chromatography are described in the text. FID, Flame ionization detector.

ponents which were tentatively identified as $C_{16:1}9t$ and $C_{16:1}7c$ from their retention times.

To verify the *trans* configuration in $C_{16:1}9t$, we subjected the methyl ester of this unsaturated fatty acid to infrared absorption analysis. First, the fatty acid methyl esters from the total lipids were separated into three fractions (fractions 1, 2, and 3) by silver nitrate thin-layer chromatography. These three fractions seemed to correspond to saturated (fraction 1), *trans*-monounsaturated (fraction 2), and *cis*-

monounsaturated (fraction 3) fatty acid methyl esters, as judged from a comparison of their R_f values with those of standards (data not shown). The infrared absorption spectrum of fraction 2 (Fig. 2A, trace a) showed a peak at $1,170\text{ cm}^{-1}$, which is characteristic of a methyl ester, and another peak at 966 cm^{-1} , which is characteristic of a *trans* double bond (Fig. 2B, trace c). The spectrum of fraction 3 showed a peak at $1,170\text{ cm}^{-1}$, but no absorbance at 966 cm^{-1} was evident (Fig. 2A, trace b). Therefore, fractions 2 and 3 were

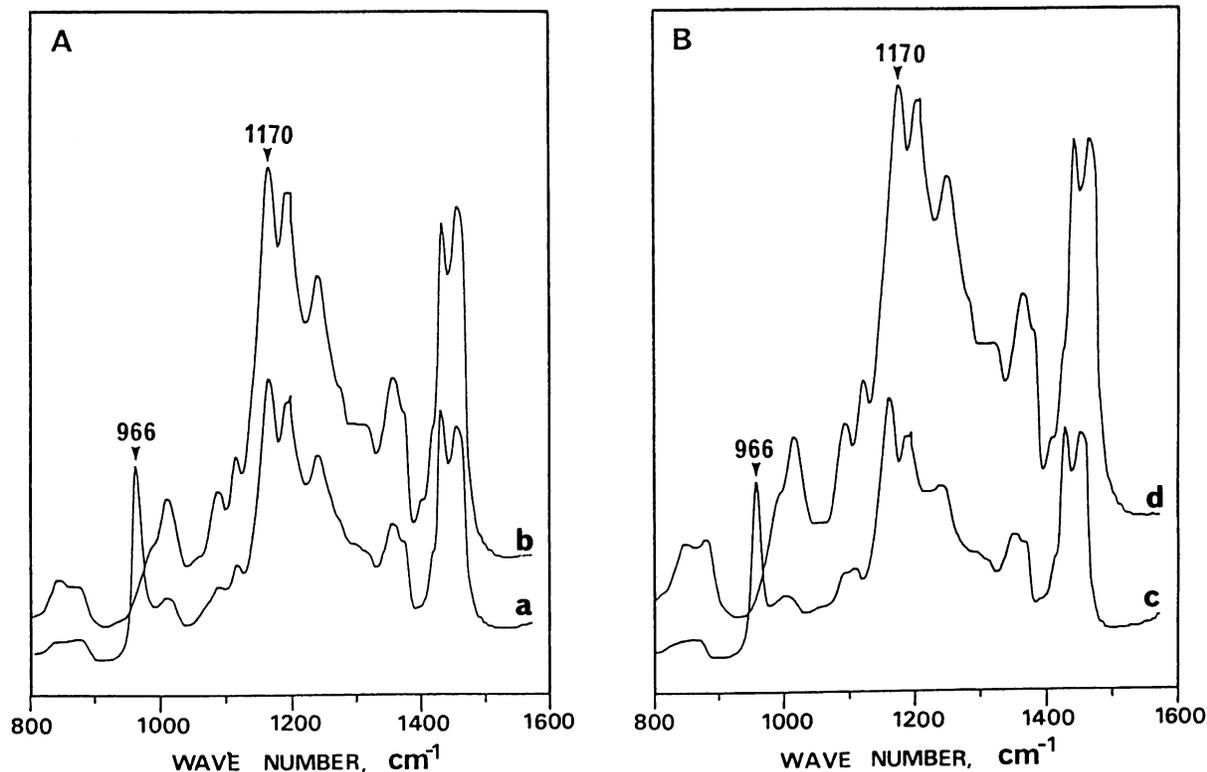


FIG. 2. Infrared absorption spectra of fatty acid methyl esters. (A) Fatty acid methyl esters of the total lipids from *Vibrio* sp. strain ABE-1. Traces a and b (fractions 2 and 3, respectively) were separated by silver nitrate thin-layer chromatography (see the text). (B) Methyl esters of authentic standards; traces c and d represent $C_{16:1}9t$ and $C_{16:1}9c$, respectively.

TABLE 1. Fatty acid compositions of the total lipids from *Pseudomonas* sp. strain E-3 and *Vibrio* sp. strain ABE-1

Organism and growth temp (°C)	% of:												
	C _{10:0}	C _{12:0}	C _{12:1} 5c	C _{14:0}	C _{14:1} 7c	C _{16:0}	C _{16:1} 9t	C _{16:1} 7c	C _{16:1} 9c	C _{18:0}	C _{18:1} 9c	C _{18:1} 11c	Unknown
<i>Pseudomonas</i> sp. strain E-3													
20	0	0	0	Tr ^a	0	20	2	1	49	Tr	Tr	26	0
<i>Vibrio</i> sp. strain ABE-1													
5	2	1	Tr	1	3	20	Tr	6	60	Tr	Tr	2	3
15	2	1	Tr	1	2	23	2	6	55	Tr	Tr	3	3
20	Tr	Tr	Tr	1	1	26	12	7	46	1	1	4	2

^a Tr, Trace (<0.4%).

identified, respectively, as having a double bond in the *trans* and *cis* configurations. The gas chromatographic analysis of fraction 2 indicated that C_{16:1}9t was the sole *trans*-unsaturated fatty acid in *Vibrio* sp. strain ABE-1 (data not shown).

Pseudomonas sp. strain E-3 is a psychrotrophic bacterium which can grow at temperatures from 0 to 30°C (19). The *trans*-monounsaturated fatty acid C_{16:1}9t was found in this bacterium also (Table 1), although it represented only 2% of the total fatty acids.

The biosynthesis of *trans*-monounsaturated fatty acids from acetate has been demonstrated in *P. atlantica* (7). In this bacterium, three kinds of *trans*-unsaturated fatty acid, namely, C_{16:1}9t, C_{17:1}9t, and C_{18:1}11t, are present. In contrast, *Pseudomonas* sp. strain E-3 contained only C_{16:1}9t as the *trans*-unsaturated fatty acid. Whether all *Pseudomonas* strains contain *trans*-unsaturated fatty acids is still in question. However, the occurrence of *trans*-unsaturated fatty acids is restricted to three genera: *Vibrio* (6), *Pseudomonas* (7), and *Eubacterium* (18). If *trans*-unsaturated fatty acids are distributed among only a few genera or species of bacteria, they might provide new criteria for classification of the bacteria.

Effect of growth temperature. Table 1 shows the effects of growth temperature on the relative levels of C_{16:1}9t in *Vibrio* sp. strain ABE-1. The level of the *trans*-unsaturated fatty acid was negligible in cells grown at 5°C, whereas it accounted for 12% of the total fatty acids in cells grown at 20°C. The increase in the relative level of C_{16:1}9t in cells grown at 20°C was accompanied by a decrease in that of C_{16:1}9c. It has been demonstrated in a number of bacterial strains and poikilothermic organisms that the fatty acid composition of membrane glycerolipids depends on the growth temperature (9). When growth temperature is decreased, the levels of *cis*-unsaturated, branched, and/or short-chain fatty acids are increased (9). These alterations are regarded as a mechanism for the thermoadaptive regulation of the fluidity of the membrane lipids. However, in *Vibrio* sp. strain ABE-1, the relative levels of C_{16:1}9t increased with the elevation of growth temperature. The temperature-dependent formation of a *trans*-unsaturated fatty acid in *Vibrio* sp. strain ABE-1 can thus be regarded as a new mode of thermoadaptive regulation for inducing changes in the fatty acid composition.

The introduction of a *trans* double bond into fatty acids alters the thermal properties of phospholipids by decreasing the phase transition temperature (16). However, this effect is not as significant as that of the *cis* double bond (16). Therefore, we propose that properties of the membrane can be fine-tuned rather more effectively by the formation of *trans*-unsaturated fatty acids than by the formation of *cis*-unsaturated fatty acids.

The range of growth temperatures, 0 to 20°C, of *Vibrio* sp. strain ABE-1 is lower and narrower (17) than that of psychrotrophic or mesophilic bacteria. However, it has been demonstrated (14) that phase transition temperatures of the total phospholipids from *Vibrio* sp. strain ABE-1 change with growth temperature and that these phase transition temperatures are always below the temperature at which the bacterium has been grown. Therefore, it appears to be advantageous for *Vibrio* sp. strain ABE-1 to have C_{16:1}9t as the fatty acyl moiety of phospholipids for the fine regulation of membrane fluidity at elevated temperatures.

Effect of growth phase. The level of C_{16:1}9t in *Vibrio* sp. strain ABE-1 cells grown at 5°C was very low during the entire growth period (Fig. 3A). In contrast, it increased with time in cultures of cells grown at 20°C and reached a plateau at the late-exponential phase (Fig. 3B). The amounts of the total lipids per culture volume increased in direct proportion to the turbidity of the culture (unpublished data). Thus, the increase in the levels of C_{16:1}9t with the age of the cultures of *Vibrio* sp. strain ABE-1 cells at 20°C is attributable to the de novo synthesis of this fatty acid.

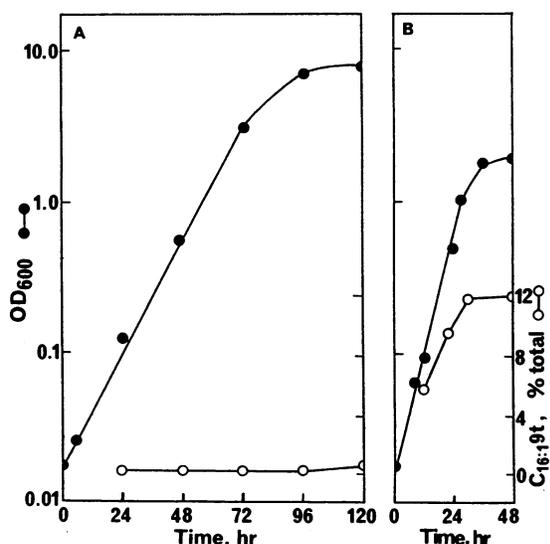


FIG. 3. Proliferation of *Vibrio* sp. strain ABE-1 at 5°C (A) and 20°C (B) and changes in the proportions of C_{16:1}9t relative to the total lipids. Aliquots of the culture were withdrawn at appropriate intervals and subjected to analysis of lipids and measurement of optical density (turbidity) at 600 nm (OD₆₀₀). Symbols: ●, turbidity; ○, C_{16:1}9t.

We gratefully acknowledge technical assistance received in infrared and gas chromatographic-mass spectrometric analyses from H. Hattori and H. Kajiura, Center of Analytical Instruments, National Institute for Basic Biology (NIBB). We also thank T. Tokumitsu, Hokkaido University, for helpful assistance in the distillation of organic solvents.

This work was carried out under the NIBB Cooperative Research Program (grants 87-161 and 88-136) and the NIBB Program for Biomembrane Research. This work was supported in part by a grant from the Akiyama Foundation to H.O. and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (grant 63480009), to S.S.

LITERATURE CITED

1. **Andersson, B. A., and R. T. Holman.** 1974. Pyrrolidides for mass spectrometric determination of the position of the double bond in monounsaturated fatty acids. *Lipids* **9**:185-190.
2. **Beacham, I. R., and D. F. Silbert.** 1973. Studies on the uridine diphosphate-galactose:lipopolysaccharide galactosyltransferase reaction using a fatty acid mutant of *Escherichia coli*. *J. Biol. Chem.* **248**:5310-5318.
3. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**:911-917.
4. **Chapman, D.** 1965. Infrared spectroscopy of lipids. *J. Am. Oil Chem. Soc.* **42**:353-371.
5. **Fulco, A. J.** 1983. Fatty acid metabolism in bacteria. *Prog. Lipid Res.* **22**:133-160.
6. **Guckert, J. B., M. A. Hood, and D. C. White.** 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. *Appl. Environ. Microbiol.* **52**:794-801.
7. **Guckert, J. B., D. B. Ringelberg, and D. C. White.** 1987. Biosynthesis of *trans* fatty acids from acetate in the bacterium *Pseudomonas atlantica*. *Can. J. Microbiol.* **33**:748-754.
8. **Hakeda, Y., and N. Fukunaga.** 1983. Effect of temperature stress on adenylate pools and energy charge in a psychrophilic bacterium, *Vibrio* sp. ABE-1. *Plant Cell Physiol.* **24**:849-856.
9. **Harwood, J. L., and N. J. Russell.** 1984. Lipids in plants and microbes, p. 7-12. George Allen and Unwin, London.
10. **Kass, L. R., and K. Bloch.** 1967. On the enzymatic synthesis of unsaturated fatty acids in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **58**:1168-1173.
11. **Makula, R. A.** 1978. Phospholipid composition of methane-utilizing bacteria. *J. Bacteriol.* **134**:771-777.
12. **Mavis, R. D., and P. R. Vagelos.** 1972. The effect of phospholipid fatty acid composition on membranous enzymes in *Escherichia coli*. *J. Biol. Chem.* **247**:652-659.
13. **Nishihara, M., H. Morii, and Y. Koga.** 1982. Bis(monoacylglycerol)phosphate in alkalophilic bacteria. *J. Biochem.* **92**:1469-1479.
14. **Okuyama, H., N. Fukunaga, and S. Sasaki.** 1986. Homeoviscous adaptation in a psychrophilic bacterium, *Vibrio* sp. strain ABE-1. *J. Gen. Appl. Microbiol.* **32**:473-482.
15. **Okuyama, H., H. Itoh, N. Fukunaga, and S. Sasaki.** 1984. Separation and properties of the inner and outer membranes of a psychrophilic bacterium, *Vibrio* sp. strain ABE-1. *Plant Cell Physiol.* **25**:1255-1264.
16. **Silvius, J. R.** 1982. Thermotropic phase transitions of pure lipids in model membranes and their modification by membrane proteins, p. 239-281. *In* P. C. Jost and O. H. Griffith (ed.), *Lipid-protein interactions*, vol. 2. John Wiley & Sons, Inc., New York.
17. **Takada, Y., T. Ochiai, H. Okuyama, K. Nishi, and S. Sasaki.** 1979. An obligately psychrophilic bacterium isolated on the Hokkaido coast. *J. Gen. Appl. Microbiol.* **25**:11-19.
18. **Verhulst, A., G. Parmentier, G. Janssen, S. Asselberghs, and H. Eyssen.** 1986. Biotransformation of unsaturated long-chain fatty acids by *Eubacterium lentum*. *Appl. Environ. Microbiol.* **51**:532-538.
19. **Wada, M., N. Fukunaga, and S. Sasaki.** 1987. Effect of growth temperature on phospholipid and fatty acid compositions in a psychrotrophic bacterium, *Pseudomonas* sp. strain E-3. *Plant Cell Physiol.* **28**:1209-1217.
20. **Wright, R. S.** 1971. A reagent for the non-destructive location of steroids and some other lipophilic materials on silica gel thin-layer chromatograms. *J. Chromatogr.* **59**:220-221.