

Effects of Growth Pressure and Temperature on Fatty Acid Composition of a Barotolerant Deep-Sea Bacterium

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The effects of pressure and temperature on the fatty acid composition in a barotolerant deep-sea bacterium that had branched-chain fatty acids were examined. The major fatty acids of the strain at atmospheric pressure were iso-C_{15:0}, C_{16:1}, iso-C_{17:0}, and iso-C_{17:1}. As the growth pressure increased, the proportion of unsaturated fatty acid increased because of an increase in the proportion of iso-C_{17:1}. On the other hand, as the growth temperature decreased, the proportion of unsaturated fatty acid increased because of the increase in the proportion of C_{16:1} and C_{18:1}.

In response to a change in ambient temperature, many organisms can regulate their fatty acid and lipid composition (4, 6, 15, 17). Such changes are explained by the regulation of the membrane fluidity that is necessary for the effective functioning of the biological membrane. This response, called homeoviscous adaptation, was originally reported for *Escherichia coli* (17). Although the deep-sea environment with high pressure is extreme for microorganisms from coastal waters, it is inhabited by barotolerant and barophilic bacteria (5, 7, 8, 21, 22). Recently, it was found that fatty acid compositions in barophilic bacteria vary in response to changes in pressure (2, 3, 20). The modulation is thought to be analogous to homeoviscous adaptation because high hydrostatic pressure raises the melting point of lipids. We observed the analogous modulation of fatty acid composition in a barotolerant deep-sea bacterium (10).

The effects of temperature on the fatty acid composition of bacteria that have straight-chain or branched-chain fatty acids have been widely investigated. Although pressure-induced changes in fatty acid composition have been reported, there are no reports on the effect of pressure in bacteria with branched-chain fatty acids as major membrane components. The change in fatty acid composition that regulates membrane fluidity under high pressure is worth studying to understand the biochemical basis of the bacterial adaptation to pressure.

In this report, we examine the effects of pressure and temperature on the fatty acid composition of a barotolerant bacterium that contains branched-chain fatty acids as a major component. The adaptation of the bacterium to high pressure is discussed.

Sample collection and screening of barotolerant bacteria. Using grab and core samplers, sediment samples were collected in August 1989 within the vicinity of Izena Hole in Mid-Okinawa Trough at depths from 1,116 to 1,645 m, located at 27°15.6' to 27°18.4'N latitude and 127°04.2' to 127°16.3'E longitude. The exact temperatures of sediment samples were not measured. Internal subsections of the sediments were taken with a sterile spoon from mud immediately after being hauled on deck. The samples were stored at 4°C until the bacterial isolation was carried out. The screening procedures for heterotrophic and barotolerant bacteria were described in the previous report (10). Among

109 strains that were randomly isolated from bottom sediment samples, 6 strains were selected as barotolerant bacteria that could grow at 60 MPa. The strain RS103 was used in this study because it contains iso-C_{15:0}, iso-C_{17:0}, and iso-C_{17:1} as major fatty acids. The strain was isolated from a sediment sample collected at the depth of 1,350 m (27°15.8'N, 127°04, 7'E). The surface temperature of the sediment was estimated to be about 17°C by heat flow measurements.

Effect of pressure and temperature on fatty acid composition of barotolerant bacterium. Liquid medium was inoculated with exponentially growing cells to give about 1×10^6 cells per ml. A total of 20 ml of the medium was sucked into a disposable syringe containing 20 ml of Fluorinert (FC72; Samitomo 3M Co., Tokyo, Japan). For the pressure experiments, the samples in pressure cylinders were incubated under various pressures at an optimum growth temperature of 30°C measured at atmospheric pressure. For the temperature gradient, the samples were incubated at various temperatures under atmospheric pressure. After incubation for 48 h, the cells were harvested by centrifugation at $12,500 \times g$ for 20 min at 4°C.

Analysis of fatty acids. Whole-cell methanolysate was used throughout the study for fatty acid methylester preparation. Fatty acid methylester was prepared according to the method of Delong and Yayanos (3).

An OV-1 column (G205; 40 m long by 1.2 mm in diameter; supplied by Nihon-Kagakuseihin Kensa, Kyokai, Japan) fitted to a gas chromatograph equipped with a flame ionization detector and integrator (model A-5; Shimadzu Co., Tokyo, Japan) was used for the analysis of fatty acid methylester. Fatty acid methylesters were initially identified by comparing the retention time with known standards. The identification was confirmed by comparing the electron impact mass spectra of the sample with those of pure standards. Gas chromatography-mass spectrometry was performed with a Shimadzu model QP-2000 instrument with a Nutrabond-1 column (25 m long by 0.25 mm in diameter). The injection temperature was 300°C, and the oven was programmed at a starting temperature of 50°C for 2 min, from 50 to 200°C at a rate of 30°C/min, and from 200 to 290°C at a rate of 5°C/min. Electron impact mass spectrometry was performed at 70 eV.

As shown in Table 1, strain RS103, which was grown at atmospheric pressure, contained iso-C_{15:0}, iso-C_{17:0}, C_{16:1}, and iso-C_{17:1} as major fatty acids. Branched-chain unsatur-

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TABLE 1. Fatty acid composition in strain RS103 cells grown under atmospheric pressure at 30°C

Fatty acid	Composition (%)
C _{15:0}	4.0
C _{15:1}	ND ^a
iso-C _{15:0}	32.5
C _{16:0}	5.7
C _{16:1}	12.2
C _{17:0}	3.2
C _{17:1}	3.7
iso-C _{17:0}	15.5
iso-C _{17:1}	12.2
C _{18:0}	3.4
C _{18:1}	7.8

^a ND, not determined.

ated fatty acid is not common in bacteria but had been reported in *Bacillus* species (1, 11, 12), *Flexibacter polymorphus* (9), and *Alteromonas putrefaciens* (13) in small quantities. Both iso- and anteiso-branched-unsaturated fatty acids ranging from C₁₁ to C₁₉ have been identified in *Staphylococcus aureus* (19). Recently, anteiso-C_{17:1} was identified in the lipid of some *Thermus* strains at low growth temperatures (14). Furthermore, iso-C_{15:1}, iso-C_{17:1}, and anteiso-C_{17:1} were found in *Streptomyces griseus* and *Brevibacterium fermentans* in small quantities at low temperature (18).

Strain RS103 was motile with a single flagellum and was an aerobic, gram-negative, and non-spore-forming bacterium. Glucose was not metabolized under both aerobic and anaerobic conditions. The GC content of DNA was 47.2 mol%. The bacterium grew well at 25 to 35°C but did not grow at 4°C under atmospheric pressure. Judging from these data, strain RS103 is likely to be identified under the genus *Alteromonas*. However, the strain had a unique fatty acid composition resembling those of the gram-positive bacteria and was markedly different from those of gram-negative bacteria. The specific characterization is under thorough investigation.

The effect of pressure on major fatty acid composition of strain RS103 is shown in Fig. 1. As the growth pressure increased from 0.1 MPa to 40 MPa, the proportion of unsaturated fatty acids increased from 34.5% to 49.5% because of an increase of iso-C_{17:1}. The proportions of iso-C_{15:0} and iso-C_{17:0} slightly decreased with the increasing pressure. The ratio of iso-C_{15:0} to iso-C_{17:0} changed between 1.84 and 2.33. The maximum ratio was obtained at 10 MPa. The proportions of C_{16:1}, C_{17:1}, and C_{18:1} did not change as a function of pressure. The proportion of iso-C_{17:1} in strain RS103 increased proportionally with the increasing pressure, and such an increase contributed to the increase in unsaturated fatty acid. In *Thermus thermophilus*, which contains branched-chain fatty acids with C₁₅ to C₁₇ as major components, the amount of shorter-chain fatty acids increases with the decreasing temperature and the fatty acids contribute to the maintenance of membrane fluidity (16). Nordström and Laakso (14) recently reported that in some *Thermus* strains, the proportion of anteiso-branched-chain fatty acids, namely, anteiso-C_{15:0}, anteiso-C_{17:0}, and anteiso-C_{17:1}, increases at lower temperature ranges. The increase is thought to contribute to the maintenance of membrane fluidity at low temperatures. In strain RS103, iso-C_{15:0} did not increase with the increasing pressure and the ratio of iso-C_{15:0} to iso-C_{17:0} changed a little. It is well known that bacteria adapt

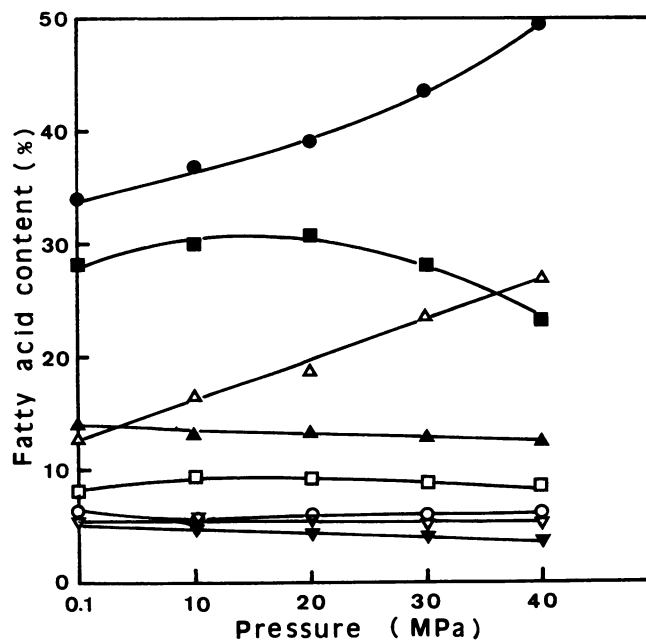


FIG. 1. Effect of pressure on fatty acid composition in strain RS103. ■, iso-C_{15:0}; ▼, C_{16:0}; □, C_{16:1}; ▲, iso-C_{17:0}; ▽, C_{17:1}; △, iso-C_{17:1}; ○, C_{18:1}; ●, total unsaturated fatty acid.

to low temperatures by increasing the production of fatty acids that have a low melting point. The melting temperatures of unsaturated fatty acids is lower than that of saturated ones. Levels of iso-C_{17:1} in strain RS103 significantly increased with increasing pressure; hence, it can be deduced that such increases contribute to the maintenance of membrane fluidity at high pressure.

The effect of temperature is shown in Fig. 2. As the growth temperature decreased from 40°C to 10°C, the proportion of unsaturated fatty acids increased from 27.6% to 51.9% because of the marked increase in C_{16:1} and a slight increase in C_{18:1}. Although the ratio of iso-C_{15:0} to iso-C_{17:0} increased from 2.13 to 4.0 with decreasing temperature (from 40°C to 10°C), the relative proportions of iso-C_{15:0} and iso-C_{17:0} decreased with decreasing temperature. iso-C_{17:1} levels that had increased with the increasing pressure decreased slightly with the decreasing temperature. Concerning the fatty acid composition in strain RS103, the response to temperature was different from the response to pressure. C_{16:1} in strain RS103 increased with decreasing temperature and contributed to the increase in the proportion of unsaturated fatty acid at low temperature. Therefore, it can be concluded that C_{16:1} contributes to the maintenance of membrane fluidity at low temperature.

We have already reported the effects of pressure and temperature on fatty acid composition in a barotolerant deep-sea bacterium, strain 4033-B (10). The effect of pressure on the fatty acid composition of the bacterium differed from the effect of temperature. In strain 4033-B, levels of C_{17:1} increased at high pressure and C_{16:1} increased at low temperature, while C_{17:1}, which increased at high pressure, did not change as a function of temperature. An analogous response was observed in strain RS103 in this report. The increase in iso-C_{17:1} with increasing pressure is suggested to be a strain-specific adaptation to pressure and apparently contributed to the barotolerant activity of strain RS103.

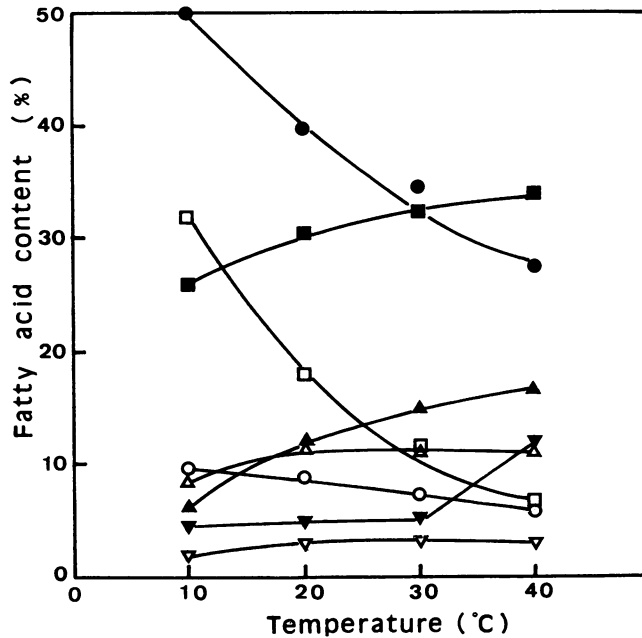


FIG. 2. Effect of temperature on fatty acid composition in strain RS103. ■, iso-C_{15:0}; ▼, C_{16:0}; □, C_{16:1}; ▲, iso-C_{17:0}; ▽, C_{17:1}; △, iso-C_{17:1}; ○, C_{18:1}; ●, total unsaturated fatty acid.

The increase in iso-C_{17:1} at higher pressure was clearly manifested; however, whether this occurrence is essential for pressure adaptation in strain RS103 remains unknown. What is certain is that pressure-induced specific regulators are involved in the regulatory mechanism of iso-C_{17:1} synthesis and that strain RS103 can be a useful bacterium for the clarification of the mechanism of bacterial adaptation to high pressure.

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REFERENCES

- Daron, H. H. 1970. Fatty acid composition of lipid extracts of a thermophilic *Bacillus* species. *J. Bacteriol.* **101**:145-151.
- DeLong, E. F., and A. A. Yayanos. 1985. Adaptation of the membrane lipids of a deep-sea bacterium to changes in hydro-

- static pressure. *Science* **228**:1011-1012.
- DeLong, E. F., and A. A. Yayanos. 1986. Biochemical function and ecological significance of novel bacterial lipids in deep-sea prokaryotes. *Appl. Environ. Microbiol.* **51**:730-737.
- Demendoza, D., and J. E. Cronan, Jr. 1983. Thermal regulation of membrane lipid fluidity in bacteria. *Trends Biomed. Sci.* **1983**:49-52.
- Deming, J. W., and R. R. Colwell. 1982. Barophilic bacteria associated with digestive tracts of abyssal holothurians. *Appl. Environ. Microbiol.* **44**:1222-1230.
- Fulco, A. J. 1983. Fatty acid metabolism in bacteria. *Prog. Lipid Res.* **22**:133-160.
- Jannasch, H. W., and D. C. Taylor. 1984. Deep sea microbiology. *Annu. Rev. Microbiol.* **38**:487-514.
- Jannasch, H. W., and C. O. Wirsen. 1984. Variability of pressure adaptation in deep-sea bacteria. *Arch. Microbiol.* **139**:281-288.
- Johns, R. B., and G. J. Perry. 1977. Lipids of the marine bacterium *Flexibacter polymorphus*. *Arch. Microbiol.* **114**:267-271.
- Kamimura, K., H. Fuse, O. Takimura, Y. Yamaoka, K. Ohwada, and J. Hashimoto. 1992. Pressure-induced alteration in fatty acid composition of barotolerant deep-sea bacterium. *J. Oceanogr.* **48**:93-103.
- Kaneda, T. 1968. Fatty acids in the genus *Bacillus*. II. Similarity in the fatty acid compositions of *Bacillus thuringiensis*, *Bacillus anthracis*, and *Bacillus cereus*. *J. Bacteriol.* **95**:2210-2216.
- Kaneda, T. 1971. Factors affecting the relative ratio of fatty acids in *Bacillus cereus*. *Can. J. Microbiol.* **17**:269-275.
- Matsui, Y. S., S. Suzuki, T. Suzuki, and K. Takama. 1991. Phospholipid and fatty acid compositions of *Alteromonas utrefaciens* and *A. haloplanktis*. *Lett. Appl. Microbiol.* **12**:51-53.
- Nordström, K. M., and S. V. Laakso. 1992. Effect of growth temperature on fatty acid composition of ten *Thermus* strains. *Appl. Environ. Microbiol.* **58**:1656-1660.
- 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.
- Oshima, M., and A. Miyagawa. 1974. Comparative studies on the fatty acid composition of moderately and extremely thermophilic bacteria. *Lipid* **9**:476-480.
- Sinensky, M. 1974. Homeoviscous adaptation: a homeostatic process that regulates the viscosity of the membrane lipids in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **71**:522-526.
- Suutari, M., and S. Laakso. 1992. Changes in fatty acid branching and unsaturation of *Streptomyces griseus* and *Brevibacterium fermentans* as a response to growth temperature. *Appl. Environ. Microbiol.* **58**:2338-2340.
- White, D. C., and F. E. Frerman. 1968. Fatty acid composition of the complex lipids of *Staphylococcus aureus* during the formation of the membrane-bound electron transport system. *J. Bacteriol.* **95**:2198-2209.
- Wirsen, C. O., H. W. Jannasch, S. G. Wakeman, and E. A. Canuel. 1987. Membrane lipids of a psychrophilic and barophilic deep-sea bacterium. *Curr. Microbiol.* **14**:319-322.
- Yayanos, A. A., A. S. Dietz, and R. van Bostel. 1982. Dependence of reproduction rate on pressure as a hallmark of deep-sea bacteria. *Appl. Environ. Microbiol.* **44**:1356-1361.
- Zobell, C. E., and R. Y. Morita. 1957. Barophilic bacteria in some deep-sea sediments. *J. Bacteriol.* **73**:563-568.