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Fractionation of carbon isotopes in biosynthesis of fatty acids by a piezophilic bacterium *Moritella japonica* strain DSK1

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Abstract

We examined stable carbon isotope fractionation in biosynthesis of fatty acids of a piezophilic bacterium *Moritella japonica* strain DSK1. The bacterium was grown to stationary phase at pressures of 0.1, 10, 20, and 50 MPa in media prepared using sterile-filtered natural seawater supplied with glucose as the sole carbon source. Strain DSK1 synthesized typical bacterial fatty acids (C_{14-19} saturated, monounsaturated, and cyclopropane fatty acids) as well as long-chain polyunsaturated fatty acids (PUFA) (20:6ω3). Bacterial cell biomass and individual fatty acids exhibited consistent pressure-dependent carbon isotope fractionations relative to glucose. The observed $\Delta\delta_{FA\text{-}glucose}$ (−1.0‰ to −11.9‰) at 0.1 MPa was comparable to or slightly higher than fractionations reported in surface bacteria. However, bulk biomass and fatty acids became more depleted in ^{13}C with pressure. Average carbon isotope fractionation ($\Delta\delta_{FA\text{-}glucose}$) at high pressures was much higher than that for surface bacteria: −15.7‰, −15.3‰, and −18.3‰ at 10, 20, and 50 MPa, respectively. PUFA were more ^{13}C depleted than saturated and monounsaturated fatty acids at all pressures. The observed isotope effects may be ascribed to the kinetics of enzymatic reactions that are affected by hydrostatic pressure and to biosynthetic pathways that are different for short-chain and long-chain fatty acids. A simple quantitative calculation suggests that in situ piezophilic bacterial contribution of polyunsaturated fatty acids to marine sediments is nearly two orders of magnitude higher than that of marine phytoplankton and that the carbon isotope imprint of piezophilic bacteria can override that of surface phytoplankton. Our results have important implications for marine biogeochemistry. Depleted fatty acids reported in marine sediments and the water column may be derived simply from piezophilic bacteria resynthesis of organic matter, not from bacterial utilization of a ^{13}C-depleted carbon source (i.e., methane). The interpretation of carbon isotope signatures of marine lipids must be based on principles derived from piezophilic bacteria.

1. Introduction

Marine sediments, as the final repository of sinking matter and associated elements and compounds, contain perhaps the best long-term archive of surface—and deep ocean biogeochemical processes and paleoclimatic and paleoceanographic records in sedimentary organic matter (Meyers, 1997). Organic matter in marine sediments is a complex mixture of detrital material from water column transport and in situ bacterial synthesis (Laws et al., 1995; Harvey and Macko, 1997). Indeed, marine bacteria play an important role in the biogeochemical cycles in the ocean's interior (Azam et al., 1983; Cho and Azam, 1988; Wakeham and Lee, 1989; Deming and Baross, 1993; Harvey and Macko, 1997; Patching and Eardly, 1997). In fact, only a small fraction (0.1–1%) of the photosynthetically produced organic carbon reaches the deep-sea floor (De Barr et al., 1983; Jannasch and Taylor, 1984), suggesting the efficiency of the bacterial degradation processes (Harvey and Macko, 1997). Microbial decomposition and resynthesis of organic matter result in the input of microbial lipids into sedimentary organic matter (Wakeham and Beier, 1991; Sun and Wakeham, 1984; Teece

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et al., 1999). Thus, the interpretation of isotope data can be confounded by the fact that sedimentary lipids are contributed from both surface ocean input and deep-sea in situ bacterial production (e.g., Teece et al., 1999). In unraveling the myriad organic matter sources to marine sediments and the consequences of diagenesis and preservation, fatty acids and carbon stable isotopes can be useful tools. Fatty acid and carbon isotope signatures are used extensively as tracers in studying organic matter in oceanic environments and marine sediments to: (a) define the sources of marine organic matter in oceanic environments and in underlying sediments (Meyers, 1997; Pinturier-Geiss et al., 2001), (b) assess the production and export of organic matter in the photic zone (Gomez-Belinchon et al., 1988; Wakeham and Beier, 1991), (c) evaluate diagenesis and preservation of organic matter in marine sediments (De Barr et al., 1983; Wakeham et al., 1997, 1998, 2001; Meyers, 1997; Fahl and Stein, 1997), and (d) reconstruct paleoclimatic and paleooceano graphic conditions (Tolosa et al., 1999; Ratnayake et al., 2005).

Essential to the utilization of carbon isotope composition of lipids in biogeochemistry is our understanding and accurate characterization of isotope fractionation in biosynthesis of lipids in source organisms (e.g., Hayes, 2001). However, information on carbon isotope fractionation in piezophilic bacteria is non-existent. Our current understanding of microbial carbon isotope fractionation is based on studies on lipid biosynthesis in surface, warm temperature, and low (atmospheric) pressure bacteria. The models and parameters of lipid and carbon isotope biogeochemistry derived from surface bacteria may be significantly different from that of the deep-sea piezophilic bacteria, and may not applicable to solving marine biogeochemical problems. This lack of understanding of lipid biosynthesis and carbon fractionation by piezophilic bacteria hampers our interpretation of isotopic data of marine lipids (e.g., Schouten et al., 1998). It has been shown that piezophilic bacteria synthesize typical bacterial fatty acids (short-chain fatty acids) as well as polyunsaturated fatty acids (i.e., 20:5ω3 and 22:6ω3; DeLong and Yayanos, 1986), which were previously ascribed to surface phytoplankton. Schouten et al. (1998) showed that algal PUFAs were relatively enriched in ^{13}C relative to saturated fatty acids (16:0 and 18:0). A contrasting trend in $\delta^{13}C$ of fatty acid was observed in piezophilic bacteria. Fang et al. (2002) showed that the $\delta^{13}C$ of fatty acids varied significantly within and between two piezophilic bacterial species. Variations of the $\delta^{13}C$ values between fatty acids of these piezophiles were nearly 8‰ (DB21MT-5) and 14‰ (DB21MT-2). Despite the fact that the two bacterial strains were grown on the same medium and under the same temperature/pressure conditions, DB21MT-2 showed a systematic enrichment of ^{13}C in fatty acids compared to strain DB21MT-5 on a molecule-to-molecule basis. For both extremely piezophilic bacteria, the polyunsaturated fatty acids (20:5ω3 and 22:6ω3) exhibited the most depleted $\delta^{13}C$ values. It was concluded that the same type of microorganisms could have rather different $\delta^{13}C$ under the same growth conditions, and that sedimentary fatty acids with distinctive $\delta^{13}C$ values do not necessarily have to originate from different organisms.

Thus, in order to interpret the lipid and carbon isotope signatures of organic matter in oceanic environments and in underlying sediments, which is contributed by both surface primary producers and deep-sea piezophilic bacteria, determining carbon isotope fractionation in biosynthesis of fatty acids by deep-sea piezophilic bacteria is fundamentally important. In this paper, we examine the carbon isotope fractionation in fatty acid biosynthesis by a deep-sea piezophilic bacterium *Moritella japonica* strain DSK1 grown on a defined substrate (glucose) at a range of hydrostatic pressures.

2. Materials and methods

2.1. Growth of Moritella japonica DSK1

Moritella japonica DSK1 is a barotolerant species isolated from the Japan Trench sediment at 6356 m (Kato et al., 1995). This strain possesses both piezophily and psychrophily (Nogi et al., 1998) and can grow at pressures of 0.1–50 MPa (Kato et al., 1995; Nogi et al., 1998). Growth media was prepared using sterile-filtered natural seawater (Sigma Chem. Co.) supplemented with yeast extract (0.08%). Glucose (50 mM) was added as the sole carbon source. The media was distributed into airtight pouches. Fifteen milliliters of fluorinert™ (3M™ Corp., Minneapolis, MN) were added to the pouches to serve as a source of oxygen to the cultures (Kato et al., 1995). Fluorinert was saturated with oxygen (25% of the total volume) by bubbling high-purity oxygen for 2 h at 4 °C and filtered prior to use. The media was inoculated with DSK1 initially grown on agar plates (marine agar 2216, Difco, Detroit, MI) at atmospheric pressure. DSK1 cultures were then incubated at 0.1, 10, 20, and 50 MPa (megapascal). Cultures were removed from pressure vessels at stationary phase (based on optical density measurements at 600 nm; typically $OD_{600} = 1.2$ and 0.25 for cells grown at 0.1 MPa and higher pressures). Cell pellets were collected by centrifugation at 10,000*g* for 20 min for lipid and isotope analyses.

2.2. Lipid extraction and fractionation

Bacterial cells were extracted in test tubes using a one-phase solvent system consisted of dichloromethane (DCM), methanol, and phosphate buffer (potassium phosphate, dibasic, 50 mM, pH 7.4) (2:1:0.8) (Fang and Findlay, 1996). Crude lipids were collected after phase partitioning by adding DCM and deionized water to the test tube to the final ratio of methanol/dichloromethane/water 1:1:0.9. Total lipids were separated into neutral lipids, glycolipids, and phospholipids using miniature columns (Supelco, Inc., Bellefonte, PA) containing 100 mg silicic acid by sequential elution with 4 mL aliquots of chloroform, acetone, and methanol, respectively.

2.3. Analysis of fatty acids by gas chromatography/mass spectrometry

Phospholipid fatty acids were subjected to a mild alkaline trans-methylation procedure to produce fatty acid methyl esters (FAMEs) (Fang and Findlay, 1996). FAMEs were analyzed on an Agilent 6890 GC interfaced with an Agilent 5973 N mass selective detector. Analytical separation of the compounds was accomplished using a 30 m × 0.25 mm i.d. DB-5 MS fused-silica capillary column (J&W Scientific, Folsom, CA). The column temperature was programmed from 50 to 140 °C at 20 °C/min, then to 310 °C at 5 °C/min and held isothermally for 15 min. Individual compounds were identified from their mass spectra by comparison with standard or published spectra and from their relative retention times. Response factors were obtained for each compound using duplicate injections of quantitative standards at five different concentration levels. Concentrations of individual compounds were obtained based on the chromatography/mass spectrometry (GC/MS) response relative to that of an internal standard (C18:0 fatty acid ethyl ester). Double-bond position and geometry of monounsaturated fatty acids were determined by using methods described by Dunkleblum et al. (1985). Fatty acids are designated by the total number of carbon atoms:number of double bonds (i.e., a 16 carbon alkanoic acid is 16:0). The position of the double bond is indicated with a ω number closest to the methyl end of the fatty acid molecule with the geometry of either *c* (*cis*) or *t* (*trans*). The cyclopropyl group is indicated with cy.

2.4. Stable carbon isotope analysis of fatty acids

Isotopic ratios of fatty acid methyl esters were determined using a HP 6890 gas chromatograph coupled with a Finnigan Mat Delta Plus isotope ratio mass spectrometer via a Finnigan Mat combustion furnace containing Cu and Ni wires that were doped with oxygen and maintained at a temperature of 850 °C. The GC was equipped with a HP-5MS fused silica capillary column (30 m × 0.325 mm i.d., 0.25 μm film thickness). The GC oven and the injection port were programmed from 50 to 120 °C at 30 °C/min, from 120 to 300 °C at 5 °C/min, and maintained at 300 °C for 22 min, with injection port temperature 3 °C higher than oven temperature at all times. The flow rate for He carrier gas was set at 1.5 mL/min. Two microliters of sample solution spiked with cholestane as an internal standard of known isotopic composition were injected into an injection port. The isotopic composition of fatty acids is reported in the delta notation relative to the Pee Dee Belemnite (PDB) standard as follows:

$$\delta^{13}C‰ = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{PDB} - 1] \times 10^3$$

Isotope values reported were determined by averaging triplicate analyses. For compounds with a peak height of 0.5–5 V, precision was typically $\leqslant$0.5‰. Isotopic composition of individual fatty acids was obtained after the correction for the additional methyl carbon from methanol using a mass balance equation (Fang et al., 1993). The isotopic composition of methanol (−28.5‰) and glucose (−9.9‰) was determined using bulk stable isotope analysis.

3. Results

3.1. Fatty acid composition

The piezophilic bacteria biosynthesized typical bacterial fatty acids (FA): $C_{14\text{-}C19}$ saturated (SAFA), monounsaturated (MUFA), and cyclopropane (CYFA) fatty acids (Table 1). The long-chain polyunsaturated fatty acids (LC-PUFA) detected were *cis*-5,8,11,14,17 Eicosapentaenoic acid, EPA (20:5ω3) and *cis*-4,7,10,13,16,19-docosahexaenoic acid, DHA (22:6ω3). The fatty acid profiles were dominated by unsaturated fatty acids (UFA), mostly monounsaturated fatty acids. The concentrations of MUFA increased with pressure (Fig. 1), whereas the concentration of DHA decreased with pressure and was below detection limit at 50 MPa. The UFA/SAFA ratio consistently increased from 3.6 at atmospheric pressure (0.1 MPa) to 6.8 at 50 MPa (Table 1). A number of positional isomers of 16:1 and 18:1 with *cis* and *trans* configurations were detected, but 16:1ω5c and 16:1ω5t were detected only at 50 MPa. The total concentrations (TFA) of fatty acids were highest in cells grown at 0.1 MPa. TFA decreased substantially from 2996 μg g^{-1} dry wt 0.1 MPa to 366 μg g^{-1} dry wt at 10 MPa and then increased with pressure (Table 1).

3.2. Stable carbon isotopic composition

The $\delta^{13}C$ values of bacterial cell biomass were −13.1‰, −14.8‰, and −17.6‰ at 0.1, 10, and 50 MPa (Table 2), suggesting a pressure-dependent carbon isotope fractionation relative to the carbon source ($\delta^{13}C_{glucose} = -9.9‰$). The associated fractionation was −3.2‰, −4.9‰, and −7.7‰ at 0.1, 10, and 50 MPa, respectively. Fatty acids exhibited a wide range of $\delta^{13}C$ values at the same pressure. The carbon isotope composition of fatty acids varied widely within a culture pressure (Table 2; Fig. 2). The difference in $\delta^{13}C$ among fatty acids was as much as −15.2‰, −23.0‰, −21.3‰, and −3.7‰ at 0.1, 10, 20, and 50 MPa, respectively. It is noteworthy that PUFA had much more negative $\delta^{13}C$ values than other short-chain saturated and monounsaturated fatty acids (Table 2).

A strong and consistent dependence of carbon fractionations on pressure was observed for individual fatty acids. Fatty acids became progressively more depleted in ^{13}C with pressure (Figs. 1 and 2). For instance, the $\delta^{13}C$ of 16:0 were −6.5‰, −13.7‰, −17.0‰, and −26.6‰ at 0.1, 10, 20, and 50 MPa, respectively (Table 2). The progressive depletion of ^{13}C was less pronounced from 10 to 20 and 50 MPa. Carbon isotopic fractionation between

Table 1
Concentrations (μg g^{-1} dry wt, percentage in parentheses) of fatty acids in *M. japonica* DSK1 grown at various pressures (MPa)

Compound	Culture pressure (MPa)			
	0.1	10	20	50
14:1	6.6 (0.2)	2.0 (0.5)	5.0 (0.9)	nd
14:1	2.3 (0.1)	0.4 (0.1)	1.5 (0.3)	nd
14:0	137.8 (4.6)	12.0 (3.3)	24.2 (4.4)	8.5 (1.3)
*i*15:0	1.3 (0.0)	0.1 (0.0)	nd	nd
a15:0	1.5 (0.0)	0.2 (0.0)	nd	nd
15:0	13.2 (0.4)	0.7 (0.2)	1.2 (0.2)	1.5 (0.2)
16:1ω7*c*	2118.0 (70.6)	248.3 (67.9)	411.8 (75.0)	21.7 (3.4)
16:1ω7*t*	14.2 (0.5)	1.8 (0.5)	3.9 (0.7)	472.1 (73.6)
16:1ω5*c*	nd	nd	nd	5.8 (0.9)
16:1ω5*t*	nd	nd	nd	4.7 (0.7)
16:0	477.3 (15.9)	45.1 (12.3)	54.8 (10.0)	67.3 (10.5)
17:1ω8	9.0 (0.3)	nd	0.9 (0.2)	2.1 (0.3)
*cy*17:0	6.1 (0.2)	15.2 (4.2)	1.0 (0.2)	13.1 (2.0)
17:0	2.2 (0.1)	3.5 (0.9)	2.0 (0.4)	0.3 (0.1)
18:1ω9*c*	29.2 (1.0)	3.0 (0.8)	5.1 (0.9)	6.5 (1.0)
18:1ω9*t*	81.3 (2.7)	29.6 (8.1)	31.4 (5.7)	35.2 (5.5)
18:1ω7	2.3 (0.1)	nd	nd	0.7 (0.1)
18:0	14.5 (0.5)	2.1 (0.6)	1.0 (0.2)	1.6 (0.2)
20:5ω3	1.9 (0.1)	nd	0.4 (0.0)	nd
22:6ω3	80.0 (2.7)	2.1 (0.6)	5.1 (0.9)	nd
SAFA	645.1 (21.5)	63.4 (17.3)	83.2 (15.2)	79.2 (12.6)
MUFA	2262.9 (75.5)	285.1 (77.9)	459.6 (83.7)	538.4 (85.4)
PUFA	81.9 (2.7)	2.1 (0.6)	5.1 (0.9)	0.0 (0.0)
CYFA	6.1 (0.2)	15.3 (4.2)	1.0 (0.2)	13.1 (2.1)
TFA	2996.0	365.9	549.0	630.6
UFA/SAFA	3.6	4.5	5.6	6.8

nd, not detected.

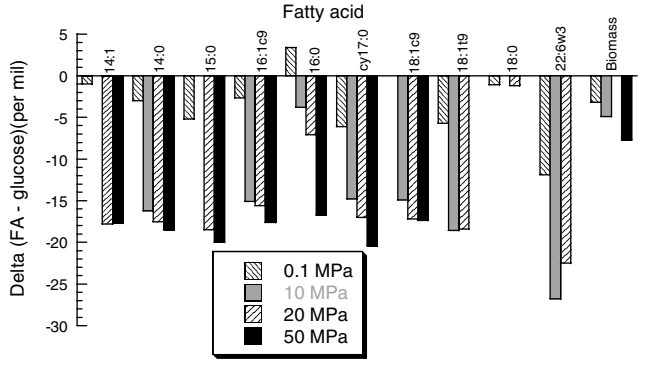

Fig. 1. Carbon isotopic fractionations of cell biomass and fatty acids (FA) relative to glucose ($\Delta\delta_{FA\text{-}glucose}$) in *M. japonica* DSK1 grown at 0.1, 10, 20, and 50 MPa.

Table 2
Stable carbon isotopic composition (‰) of fatty acids in *M. japonica* DSK1 grown on glucose at various pressures (MPa)

Compound	Culture pressure (MPa)			
	0.1	10	20	50
14:1	−10.9		−27.7	−28.3
14:0	−12.9	−26.1	−27.4	−28.3
15:0	−15.1		−28.4	−29.8
16:1ω7c	−12.6	−25.0	−25.5	−27.5
16:0	−6.5	−13.7	−17.0	−26.6
cy17:0	−16.0	−24.6	−26.8	−30.3
17:0	−19.5			
18:1ω9	−9.9	−24.8	−27.0	−27.3
18:1ω7	−15.6	−28.5	−28.3	
18:0	−11.0		−11.1	
22:6ω3	−21.7	−36.7	−32.4	
Biomass	−13.1	−14.8		−17.6

short-chain fatty acids (SC-FA; excluding DHA) and glucose ($\Delta\delta_{FA\text{-}glucose}$, average = −3.6‰) at 0.1 MPa was comparable to or slightly higher than fractionations observed on surface bacteria: e.g., *Escherichia coli* (Monson and Hayes, 1980, 1982) and *Shewanella putrefaciens* (Teece et al., 1999). However, the fractionation at high pressures was much higher than that for surface bacteria: average −13.9‰, −14.5‰, and −18.3‰ at 10, 20, and 50 MPa, respectively. A good linear correlation was observed between carbon isotopic fractionation and hydrostatic pressure (Fig. 2). R^2 was 0.965, 0.726, 0.966, and 1.000 for biomass, 15:0, 16:0, and 18:0 fatty acids.

Even carbon-numbered and odd carbon-numbered short-chain fatty acids and PUFA exhibited different extents of carbon isotopic fractionation. The average fractionation relative to glucose was −2.8‰, −13.9‰, −15.6‰, and −18.0‰ at 0.1, 10, 20, and 50 MPa for even-numbered FA, −5.6‰, −14.7‰, −17.7‰, and −20.1‰ for odd-numbered FA, and −11.8‰, −26.8‰, and −22.5‰ for DHA at 0.1, 10, and 20 MPa.

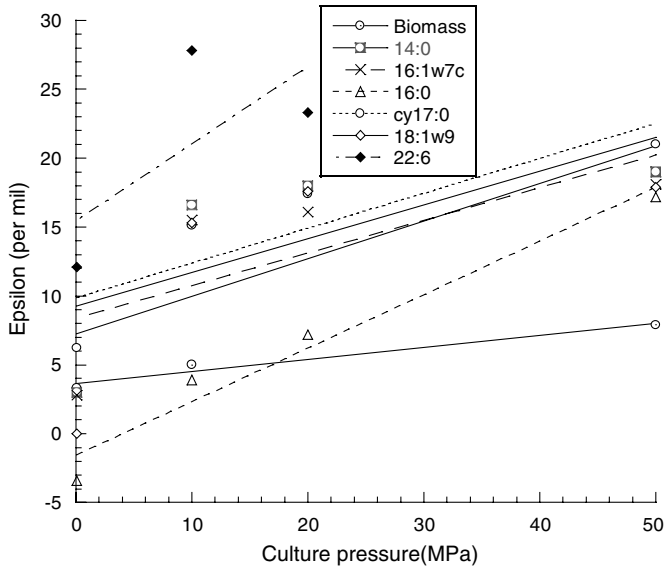

Fig. 2. The calculated carbon isotopic fractionation, ε ($\varepsilon = (\alpha_{s/p} - 1) \times 1000$), of cell biomass and selected fatty acids biosynthesized by *M. japonica* DSK1 at 0.1, 10, 20, and 50 MPa, where α is defined as $\alpha_{s/p} = (1000 + \delta_s)/(1000 + \delta_p)$, δ_s is the carbon isotopic ratio of substrate (glucose), δ_p is that of product (biomass and fatty acids).

4. Discussion

4.1. Pressure-dependent carbon isotope fractionations

We hypothesize that the observed isotope fractionation may be the result of the effects of high hydrostatic pressure on the kinetics of enzymatic reactions. Fatty acids are biosynthesized from the basic C_2 unit acetyl-CoA. The overall isotopic composition of an even carbon-numbered fatty acid, δ_{FA}, is given by $\delta_{FA} = (\delta_m + \delta_c)/2$, where δ_m and δ_c are $\delta^{13}C$ values of the methyl and carboxyl carbon in acetyl-CoA (Sakata et al., 1997). It has been demonstrated that the methyl carbon in acetyl-CoA is not fractionated relative to glucose in *E. coli* (Monson and Hayes, 1982). Only the carboxyl carbon is fractionated and the magnitude of fractionation is determined by a kinetic isotopic effect (ε_{PDH}). Assuming that the piezophilic bacterium *M. japonica* strain DSK1 utilized glucose using the same pathway as *E. coli*, the magnitude of substrate utilization can be calculated (Sakata et al., 1997) by:

$$\varepsilon_{FA-substrate} = (1 - f)\varepsilon_{PDH}, \quad (1)$$

where f is the fraction of pyruvate flowing to acetyl-CoA (Monson and Hayes, 1982). The average $\delta^{13}C$ value of the short-chain even carbon-numbered fatty acids at atmospheric pressure is $-12.7‰$, suggesting a depletion in ^{13}C of $2.8‰$ relative to glucose. Assuming that the kinetic isotopic effect (ε_{PDH}) associated with the pyruvate hydrogenase-catalyzed reaction is $23‰$ (Monson and Hayes, 1982), f is calculated to be 0.76 based on Eq. (1), essentially identical to 0.74 observed in *E. coli* (Roberts et al., 1955). However, carbon isotopic fractionation was substantially higher at high pressures, $-13.9‰$, $-15.6‰$, and $-18.0‰$ at 10, 20, and 50 MPa, respectively. As a result, f cannot be calculated ($f < 0$). This suggests that ε_{PDH} is greater than $23‰$ at high pressures. Given the low lipid content at high pressures (5- to 10-fold lower than at atmospheric pressure, Table 1), the f value may be as low as 0.1. Thus, the corresponding ε_{PDH} can be calculated: $31‰$, $35‰$, and $40‰$ at 10, 20, and 50 MPa for even carbon-numbered fatty acids. Therefore, carbon isotopic fractionation in biosynthesis of fatty acids is pressure dependent. It is possible that a negative volume change is involved in bacterial glucose metabolism and fatty acid biosynthesis. Hence, increasing pressure increases the reaction rate and the isotopic effect.

Another point for considerations is microbial stress and carbon isotope fractionation in biosynthesis of lipids. It is also possible that under the culture conditions used, *M. japonica* strain DSK1 might have been experiencing some stress at high pressure and that stress had an effect on carbon isotope fractionation in this organism. We are not aware of any previous studies on stress and carbon isotope fractionation in bacteria. Based on the inverse relationship between the amount of fatty acids and the ^{13}C depletion of lipids (Tables 1 and 2), as observed in different plants (Park and Epstein, 1966), we speculate that a lesser amount of pyruvate was oxidized to acetyl CoA for lipid biosynthesis at high pressures, so that there were correspondingly more significant fractionations at high pressures.

DHA was significantly more depleted in ^{13}C relative to glucose than other short chain saturated and monounsaturated fatty acids. This depletion may be the result of a different biosynthetic pathway for DHA. Morita et al. (1999, 2000) and Metz et al. (2001) showed that the DHA-producing *Moritella marina* MP-1 has two independently functioning fatty acid biosynthetic systems: the FAS (fatty acid synthase)- and PKS (polyketide synthases)-based pathways. The former is for synthesizing the short-chain "bacterial" fatty acids and the latter biosynthesizes the long-chain polyunsaturated fatty acids (e.g., DHA). Wallis et al. (2002) suggested that the PKS pathway may be widespread in deep-sea bacteria. Examining the $\delta^{13}C$ values of fatty acids reveals that DHA is more depleted in ^{13}C than other fatty acids at all pressures, suggesting that biosynthesis of ^{13}C-depleted fatty acids may be an intrinsic property of strain DSK1 and perhaps of other piezophilic bacteria as well.

4.2. Geochemical implications

It has long been believed that bacteria are unable to produce polyunsaturated fatty acids (Erwin and Bloch, 1964; Tornabene, 1985). The chain length of bacterial fatty acids is believed to range from C_{12} to C_{19}. Polyunsaturated fatty acids in environmental samples are attributed generally to contributions of microeukaryotes in the interpretation of overall microbial community composition (Vestal and White, 1989). In the determination of the sources of organic matter in marine sediments, polyunsaturated fatty acids (e.g., 20:5, 22:6) have frequently been interpreted as bio-

markers of plankton (Gomez-Belinchon et al., 1988; Colombo et al., 1996). Previous studies and this work clearly show that piezophilic bacteria can synthesize as well as take up 20:5 and 22:6 from the environment (DeLong and Yayanos, 1986; Yano et al., 1998; Fang et al., 2002). This finding has important implications in marine biogeochemistry. Given the fact that PUFAs produced by plankton in surface waters are preferentially degraded in the water column (De Barr et al., 1983; Wakeham et al., 1997), piezophilic bacteria can be an important source of polyunsaturated fatty acids 20:5 and 22:6 to deep-sea sediments (Fang et al., 2000). Thus, the reconstructions of paleoceanographic environments and biological activity using fatty acid biomarkers must be approached with caution, because of the probable bacterial origin of polyunsaturated fatty acids (Mayzaud et al., 1976).

Isotope data are nearly always interpreted assuming that the isotopic composition of fatty acids indicates the isotopic composition of the producing organism and that, in turn, can reveal the carbon source utilized by the producer and thus its position within the paleo—or present ecosystem. Furthermore, carbon isotopic data of fatty acids isolated from marine environment have been interpreted based on theories derived from surface bacteria. Our results suggest that piezophilic bacteria fractionate carbon isotopes significantly (14–18‰) more than surface heterotrophic bacteria. Thus, the recycling and resynthesis of fatty acids by piezophilic bacteria utilizing organic matter from primary production will greatly alter the carbon isotope signature of both short chain bacterial and long-chain planktonic fatty acids in oceanic environments and marine sediments. For example, if the carbon isotopic composition of phytoplankton-derived organic matter is −22‰ (Teece et al., 1999), fatty acids synthesized by piezophilic bacteria would have $\delta^{13}C$ values of −36‰ to −40‰. These depleted $\delta^{13}C$ values of fatty acids could be falsely interpreted as terrestrial origin, or from bacteria utilizing isotopically light carbon source (e.g., methane). Furthermore, DHA biosynthesized by piezophilic bacteria would have much more negative $\delta^{13}C$ values compared to DHA produced by surface plankton (e.g., Schouten et al., 1998). Thus, our results provided important information for distinguishing organic matter from surface ocean primary production from that from deep-sea in situ bacterial secondary synthesis, which is useful for determining the source, diagenesis, and vertical transport of organic matter in oceanic environments.

On a quantitative basis, we can estimate the relative strength of carbon isotope signature of EPA and DHA from deep-sea piezophilic bacteria and surface phytoplankton. The total mass of marine primary producers is 2×10^{15} g (Garrison, 2005). Assuming that phytoplankton contains 5–15% fatty acids on a dry weight basis (Fisher and Schwarzenbach, 1978), the total amount of fatty acids produced by marine primary producers would be 0.1–0.3×10^{14} g (based on 90% water content of phytoplankton). The percentage of EPA and DHA in total fatty acids of phytoplankton is about 15% (Olsen, 1999). Thus, the total amount of EPA and DHA produced from marine primary production is $0.15–0.45\times10^{13}$ g. Because of the high reactivity and labile nature of EPA and DHA, these compounds are preferentially degraded in the water column during transport (De Barr et al., 1983; Wakeham et al., 1984), only a small proportion (0.01–0.02%) of the surface water-produced PUFA reaches the bottom water of the oceans (De Barr et al., 1983; Wakeham et al., 1984; Wakeham and Lee, 1993). Therefore, the amount of EPA and DHA that are produced in the surface ocean and that may potentially reach the sediment/water interface of the deep sea is $0.15–0.45\times10^{9}$ g.

The volumes of the deep sea and the top sediment layer (0–20 cm) are 1.028×10^{24} cm^3 and 7.356×10^{19} cm^3, respectively (calculated based on Garrison, 2005). Assuming that bacterial abundance is 4.6×10^{8} cells/ml in deep-sea surface sediment and 0.5×10^{5} cells/ml in the water column (Whitman et al., 1998), the total abundance of bacteria in the deep-sea surface sediment and water column is estimated to be 3.39×10^{28} and 5.14×10^{28} cells, respectively. The total number of cells in the deep sea is 8.53×10^{28} cells, which is equivalent to 2.39×10^{16} g dry weight (2.8×10^{-13} g/bacteria cell; Madigan et al., 2003). There is considerable uncertainty with respect to the proportion of marine bacteria that are piezophilic bacteria and the percentage of piezophilic bacteria that produce PUFA (Fang and Bazylinski, 2005). Assuming that 20% of the deep-sea bacteria are metabolically active piezophilic bacteria that produce EPA and DHA at a rate of 3.0 μg g^{-1} dry weight (Table 1), the total amount of EPA and DHA from piezophilic bacterial production is 0.143×10^{11} g. Thus, the amount of EPA and DHA from deep-sea piezophilic bacteria is nearly two orders of magnitude higher than that from marine primary producers. Therefore, the carbon isotope signature of fatty acids preserved in marine sediments may be derived mostly from piezophilic bacteria whose contributions may easily override that of surface phytoplankton.

Thus, our results provided important information for distinguishing organic matter from surface ocean primary production from that from deep-sea in situ bacterial secondary bacterial synthesis, which is useful for determining the source, diagenesis, and vertical transport of organic matter in oceanic environments.

5. Conclusions

Our experiments demonstrate that carbon isotope fractionation in fatty acid biosynthesis of piezophilic bacteria is pressure-dependent and that the fractionation increases linearly with pressure. The observed isotope effects may be attributed to the kinetics of enzymatic reactions affected by hydrostatic pressure and to different biosynthetic pathways in biosynthesis of short-chain and long-chain fatty acids. The carbon isotope imprint of fatty acids preserved in marine sediments may be derived mostly from piezophilic bacteria, not surface phytoplankton. Our results have

important implications for marine biogeochemistry. The increased carbon isotope fractionations at high hydrostatic pressures suggest that caution must be exercised in using $\delta^{13}C$ values of fatty acids in deducing sources of organic matter in marine environments. Specifically, sedimentary fatty acids with highly negative $\delta^{13}C$ values may be derived from marine bacterial secondary metabolism, not from bacterial utilization of a ^{13}C-depleted carbon source (i.e., methane). Thus, the interpretation of carbon isotope signatures of marine fatty acids must be carried out based on principles derived from piezophilic bacteria, not surface bacteria. Furthermore, the carbon isotopic composition of fatty acids from piezophilic bacteria are distinctly different other biological sources (e.g., marine phytoplankton) and can be useful in determining the source of organic matter in marine environments when fatty acid biomarkers alone do not allow source differentiation. More work is required to investigate carbon isotope fractionations in lipid biosynthesis by other piezophilic bacteria from deep sea and thus, to provide fundamental information in using $\delta^{13}C$ of fatty acids in determining the source and diagenesis of organic matter in marine sediments.

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