Carbon Isotope Fractionation in Lipid Biosynthesis by Gram-Positive Piezotolerant Bacterium *Sporosarcina* sp. DSK25

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Current stable isotope geochemistry theory is not applicable to studying biogeochemical cycles in the deep ocean, as the theory was developed based on the biochemistry of microorganisms living on the surface. Recent studies have shown that carbon isotope fractionation in biosynthesis of lipids by piezophilic bacteria is pressure-dependent. However, these investigations have been focusing on gram-negative bacteria. Gram-positive bacteria in the deep ocean and the deep biosphere can be as important as their gram-negative counterparts in mediating global biogeochemical cycles. In this study, we examined carbon isotope fractionation in biosynthesis of lipids by *Sporosarcina sp.* DSK-25, a gram-positive piezotolerant bacterium. Strain DSK25 was isolated from the Japan Trench at a depth of 6500 m, and can grow at pressures of 0.1-60 MPa with an optimal growth condition of 35°C and 0.1 MPa.. Strain DSK25 was grown to stationary phase at pressures of 0.1, 10, 20, 30, 40 and 50 MPa in media prepared using natural seawater supplied with glucose as the sole carbon source. Bacterial cell biomass and individual fatty acids exhibited consistent pressure-dependent carbon isotope fractionations relative to glucose. Furthermore, precipitously increased carbon isotope fractionation was observed at 10 and 40 MPa. Given that most of the biosphere (including the hadal biosphere) lives under high pressures, our results have important implications for studying microbial metabolism and carbon cycle in the deep ocean and deep biosphere.