

Lipid composition of a marine ammonium oxidizer grown at 5 and 25 °C

Ronald D. Jones* & Fredrick G. Prahl

Department of Microbiology and College of Oceanography, Oregon State University, Corvallis, Oregon 97331-3804, USA

ABSTRACT: The lipid composition of an ammonium oxidizing bacterium isolated from Alaskan waters was examined at 2 different growth temperatures (5 and 25 °C). The composition was surprisingly simple with palmitic acid (C_{16:0}) and a monounsaturated isomer, palmitoleic acid, comprising about 90 % of the total lipids. There was a > 4-fold increase in the degree of fatty acid unsaturation in the organisms grown at the lower temperature. Shifts in the cardinal growth temperatures of this organism may be explained by this observed change in lipid composition.

INTRODUCTION

An ammonium oxidizing bacterium has recently been isolated that is capable of growth at -5 °C (Jones & Morita 1985). This bacterium exhibits significantly different cardinal growth temperatures depending upon the temperature at which it is cultured. Cells grown at 5 °C have an optimum growth temperature of 22 °C with a maximum near 29 °C while cells grown at 25 °C have an optimum growth temperature of 30 °C and a maximum near 38 °C. In addition to this, cells grown at 5 °C oxidize significantly more ammonium at 5 °C than do 25 °C cells, and have generation times > 9 times faster than expected. It has been suggested that changes in either the enzymatic composition of the cell or the lipid composition of the cell membrane could explain these shifts in growth temperature and whole cell kinetics for this organism (Jones & Morita 1985).

Many bacteria have been shown to be able to alter their lipid composition as the growth temperature changes, thus maintaining fluidity of the membrane (Oshima & Miyagawa 1974, Souza et al. 1974, McElhaney 1976, Hasegawa et al. 1980). Temperature has also been demonstrated to significantly alter both the membrane lipid composition and growth characteristics of yeasts (Arthur & Watson 1976).

This paper examines changes in the lipid composition of *Nitrosomonas* sp. 4W30 grown at 2 different temperatures (5 and 25 °C).

MATERIALS AND METHODS

Organism and cultural methods. The marine ammonium oxidizer *Nitrosomonas* sp. 4W30 was used for these studies. A more complete description of this organism can be found in Jones & Morita (1985). Cells were grown in 2 identical 4 l batch culture units equipped with automatic pH controllers which maintained the pH at 7.8 ± 0.05 by the addition of 5 % K₂CO₃. The medium used was the same as described by Jones & Hood (1980) with the salinity adjusted to 30 ‰ using Instant Ocean Synthetic Sea Salts (Aquarium Systems, Inc.). Cultures were vigorously aerated and agitated by a magnetic stirring bar. The batch culture units were maintained at either 5 or 25 °C. Cells used to inoculate the 5 °C unit were adapted for growth at this temperature in a chemostat as described by Jones & Morita (1985). Cells were grown in these batch culture units until all of the ammonium was utilized. Cells were then harvested by centrifugation (5860 × g, 10 min, 5 °C), washed twice with 3 % NaCl and resuspended in 10 ml of 3 % NaCl. These suspensions were subjected to dry weight determinations and Petroff-Hauser direct counts. Cells grown at 5 °C had dry weight values of 5.7×10^{-9} mg cell⁻¹ and 25 °C cells had dry weight values of 4.1×10^{-9} mg cell⁻¹. These suspensions were then frozen at -20 °C until the lipid analysis could be performed.

Lipid analysis. Bacterial samples of *Nitrosomonas* sp. 4W30 (4.4 to 7.1 mg dry weight), grown at 5 and 25 °C, were analyzed in duplicate for lipid composition. Each sample was extracted with a solution of 2 %

* Present address: Department of Biological Sciences, Florida International University, Miami, Florida 33199, USA

aqueous potassium hydroxide (5 ml) and methanol (2 ml) under gentle reflux (1 h). Before each extraction, a known quantity of nC_{19} fatty acid was added as an internal recovery standard. Total nonsaponified lipids were separated from the basic extract by partitioning into 10% diethyl ether in hexane ($3 \times$, 5 ml). After acidification (pH 1) with concentrated HCl, total saponified lipids were isolated by again partitioning into 10% diethyl ether in hexane ($3 \times$, 5 ml). All fractions were dried over anhydrous sodium sulfate and concentrated by rotary evaporation. In preparation for gas chromatographic (GC) analysis, the saponified lipid fractions were methylated using 14% BF_3 in methanol and silylated using N,O -bis-trimethylsilyl-trifluoroacetamide (BSTFA) as described in the Pierce Chemical Handbook (1984). Nonsaponified lipid fractions were silylated also with BSTFA. A procedural blank was carried through the above analytical scheme as well.

The derivatized samples in isoctane were analyzed using a microprocessor controlled Hewlett Packard 5830 GC equipped with splitless injection, a $30 \text{ m} \times 0.25 \text{ mm}$ i.d. DB-5 fused silica capillary column (J&W Scientific) and flame ionization detection. Chromatographic separations were made using helium as the carrier gas and temperature programming (75 to 130°C at $10^\circ\text{C} \text{ min}^{-1}$, 130 to 290°C at $5^\circ\text{C} \text{ min}^{-1}$). Hexamethylbenzene was added to each sample as an internal GC injection standard prior to analysis. All reported data have been corrected for the recovery of nC_{19} fatty acid which was about 90% in all cases. Blank corrections were determined to be negligible.

RESULTS AND DISCUSSION

The lipid composition of *Nitrosomonas* sp. 4W30 is surprisingly simple (Fig. 1). Palmitic acid ($C_{16:0}$) and a monounsaturated isomer of palmitic acid comprise about 90% of the total lipid. The identities of these compounds were confirmed by GC/mass spectrometry. The monounsaturated C_{16} acid was derivatized using the methoxymercuration procedure described by Matsuda & Koyama (1977). GC/MS analysis of the derivative demonstrated that the $C_{16:1}$ acid in the bacterium is a single isomer with a Δ^9 double bond, i.e. palmitoleic acid. The remaining 10% of the lipid was also contained in the saponified fraction. These other lipids consisted of a monounsaturated isomer of palmitic acid (see shoulder on 16:1, Fig. 1) with an undetermined double bond position and several compounds that eluted after the C_{16} fatty acids. None of these latter compounds were identified from their electron impact (70 eV) mass spectra. Nevertheless, the spectra indicated the compounds were not simple fatty acids.

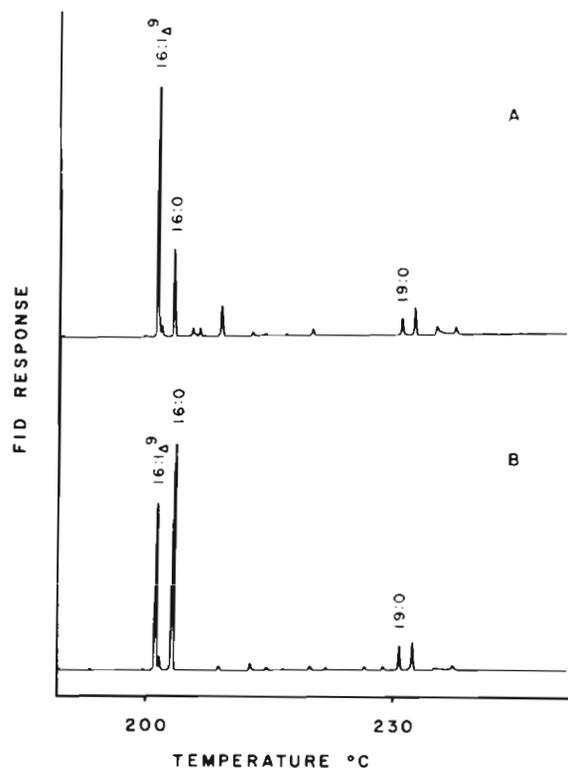


Fig. 1. Gas chromatograms of the total saponified lipid fractions from *Nitrosomonas* sp. 4W30 grown at: (A) 5°C and (B) 25°C . Peaks are labelled corresponding to palmitic acid ($16:0$), palmitoleic acid ($16:1 \Delta^9$) and the internal recovery standard ($19:0$; see 'Materials and Methods')

Further chemical characterization of these lipids is in progress. No lipids were detected in the nonsaponified fractions.

The concentrations of palmitic and palmitoleic acid in the bacterial cells grown at 5 and 25°C are given in Table 1. The data show a clear increase in the degree of fatty acid unsaturation for the organism grown at the lower temperature. While the total amount of C_{16} fatty acid remains relatively constant per cell, the ratio of $C_{16:0}$ to $C_{16:1}$ changes from 0.23 for cells grown at 5°C to 1.0 for cells grown at 25°C . This pattern reflects a well-established adaptation whereby an organism controls the physical properties of its membranes, e.g.

Table 1. Fatty acid concentrations in cells of *Nitrosomonas* sp. 4W30 adapted to different growth temperatures

Temperature ($^\circ\text{C}$)	Concentration ($\times 10^{-7} \mu\text{g cell}^{-1}$)		Concentration ($\mu\text{g}[\text{mg dry weight}]^{-1}$)	
	$C_{16:0}$	$C_{16:1}$	$C_{16:0}$	$C_{16:1}$
5	0.49	2.25	8.6	39.6
5	0.52	2.10	9.1	37.0
25	1.25	1.13	30.3	27.4
25	1.18	1.30	28.5	31.4

fluidity, by changing the composition of its structural lipids (VanDeenen et al. 1972).

The lipid compositional differences may explain why shifts in cardinal growth temperatures and changes in whole cell kinetics are observed in *Nitrosomonas* sp. 4W30 (Jones & Morita 1985). Although it is possible that other explanations exist (i.e. enzymatic changes), given the results reported in this paper and the similarities between enzyme function observed by Jones & Morita (1985), it seems likely that lipid compositional changes are a major factor. The ability to alter the relative proportion of palmitic acid and palmitoleic acid in the cell presents a very simple but highly effective method for this organism to adapt physiologically to the widely differing temperatures encountered in the environment from which it was isolated.

Blumer et al. (1969) examined the lipid composition of other species of marine nitrifying bacteria in an earlier study and documented the same simple fatty acid compositions. Recently, Karl et al. (1984) reported that chemolithotrophic production can contribute significantly to the downward particulate flux of organic carbon and nitrogen, ATP and RNA, in the mesopelagic zone of the North Pacific Ocean. In view of this recognized production term, a point originally made by Blumer et al. (1969) should be emphasized. The biomass of nitrifying bacteria, a type of chemolithotroph, in the oceans would be difficult to ascertain based on fatty acid information alone because these organisms synthesize chiefly those C₁₆ fatty acids which are common and most abundant in other living cells. The contribution of fatty acids from chemolithotrophic production to vertically transported particles, although quantitatively significant, could easily be masked by other biological sources of these common fatty acids. Further characterization of the more minor, presently unidentified, lipids of *Nitrosomonas* sp. 4W30 and other marine nitrifiers may demonstrate these compounds are sufficiently novel to allow their future use in assessing chemolithotrophic production on sinking particles in natural waters.

Acknowledgements. We thank R. Y. Morita for the use of his laboratory and the gas chromatograph. This material is based on research supported by National Science Foundation grants OCE 8410145 and OCE 8415482.

LITERATURE CITED

- Arthur, H., Watson, K. (1976). Thermal adaptation in yeast: growth temperatures, membrane lipid, and cytochrome composition of psychrophilic, mesophilic, and thermophilic yeasts. *J. Bacteriol.* 128: 56-68
- Blumer, M., Chase, T., Watson, S. W. (1969). Fatty acids in the lipids of marine and terrestrial nitrifying bacteria. *J. Bacteriol.* 99: 366-370
- Hasegawa, Y., Kawada, N., Nosoh, Y. (1980). Changes in chemical composition of membrane of *Bacillus caldotenax* after shifting the growth temperature. *Arch. Mikrobiol.* 126: 103-108
- Jones, R. D., Hood, M. A. (1980). The effects of temperature, pH, salinity and inorganic nitrogen on the rate of ammonium oxidation by nitrifiers isolated from wetland environments. *Microb. Ecol.* 6: 339-347
- Jones, R. D., Morita, R. Y. (1985). Low-temperature growth and whole cell kinetics of a marine ammonium oxidizer. *Mar. Ecol. Prog. Ser.* 21: 239-243
- Karl, D. M., Knauer, G. A., Martin, J. H., Ward, B. B. (1984). Bacterial chemolithotrophy in the ocean is associated with sinking particles. *Nature, Lond.* 309: 54-56
- Matsuda, H., Koyama, T. (1977). Positional isomer composition of monounsaturated fatty acids from lacustrine sediment. *Geochim. cosmochim. Acta* 41: 341-345
- McElhane, R. N. (1976). The biological significance of alterations in the fatty acid composition of microbial membrane lipids in response to change in environmental temperature. In: Heinrich, M. R. (ed.) *Extreme environments*. Academic Press, London, p. 255-281
- Oshima, M., Miyagawa, A. (1974). Comparative studies on the fatty acid composition of moderately and extremely thermophilic bacteria. *Lipids* 9: 476-480
- Souza, K. A., Kostiw, L. L., Tyson, B. J. (1974). Alterations in normal fatty acid composition in a temperature-sensitive mutant of a thermophilic *Bacillus*. *Arch. Mikrobiol.* 97: 89-102
- VanDeenen, L. L. M., deGier, J., Demel, R. A. (1972). Relations between lipid compositions and permeability of membranes. In: Ganguly, J., Smellie, R. M. S. (ed.) *Current trends in the biochemistry of lipids* No. 35. Academic Press, London, p. 377-382