Note

Isolation of Free Ceramide from
Zymomonas mobilis

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Membrane lipids of *Zymomonas mobilis*, a Gram-negative bacterium having potential for commercial ethanol production, are made up of comparatively complicated components; in addition to diverse phospholipids,^{1,2)} hopanoids such as hopene, hopanol, tetrahydroxybacteriohopane, and glucosamine and ether derivatives of tetrahydroxybacteriohopane have been reported.^{3–7)} In the course of studying the membrane lipids of *Z. mobilis* to elucidate the relationship between the lipid composition and the tolerance to a high ethanol concentration and a high glucose concentration, we have found the occurrence of a new free ceramide in the membrane lipids of the organism. This paper describes the isolation and molecular species of the ceramide.

Zymomonas mobilis IFO 13756 was grown statically at 30°C in a medium containing 2% glucose, 0.5% peptone and 0.3% yeast extract, pH 5.5, as described previously.⁸⁾ The lipids were extracted by the method of Bligh and Dyer,⁹⁾ and developed on a silica gel G thin-layer plate with a solvent system of $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{CH}_3\text{COOH}$ (90:10:2, v/v).

The thin-layer chromatogram is shown in Fig. 1A. Spot c did not react with either ninhydrin or ammonium molybdenum, and the R_f value coincided with that of the ceramide standard, *N*-stearoyl-dihydrosphingosine (Sigma, St. Louis). This lipid also occurred at the same position on the chromatogram of the alkali-stable lipids (Fig. 1B). The infrared spectrum of the lipid showed the presence of a hydrocarbon chain (2930 and 2850 cm^{-1}), an amide group (1640 and 1550 cm^{-1}), and a hydroxy group (3400–3500 cm^{-1}), but no absorption band due to an ester group (1725 cm^{-1}). Acid hydrolysis of the lipid with 2N HCl in methanol at 100°C for 4 hr gave two compounds, a methyl ester of a fatty acid and a long-chain base. The methyl compound was extracted with hexane from the hydrolysate, while the long-chain base was done with ethyl ether from the residual aqueous phase of which the pH had been adjusted to 12 with concentrated KOH.

The occurrence of a long-chain base was first confirmed as a ninhydrin-positive compound on a silica gel G thin-layer plate with a solvent system of $\text{CHCl}_3/\text{CH}_3\text{OH}/$

Fig. 1. Thin-layer Chromatograms of Original (A) and Alkali-stable (B) Lipids from *Zymomonas mobilis*.

The plate was developed with $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{CH}_3\text{COOH}$ (90:10:2, v/v) and spots were made visible by charring after spraying with 50% H_2SO_4 . a, fatty acids; b, possibly hopanol; c, free ceramide described in the text; d, and e, unknown; f, tetrahydroxybacteriohopane.

$\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ (100:20:12:5, v/v). Then the long-chain base isolated was trimethylsilylated and analyzed by gas chromatography-mass spectrometry. The gas chromatogram of the trimethylsilyl (Me_3Si) derivative with 5% Silicone SE-30 on Chromosorb W AW-DWCS at 210°C gave only a single peak. In the mass spectrum, the molecular ion was at m/z 417, and prominent peaks were found at m/z 103, 132, 314 (M-103), and 285 (M-132), characteristic for the $\text{C}_1\text{--C}_2$ and $\text{C}_2\text{--C}_3$ bond cleavages of the Me_3Si ester of long-chain base.¹⁰⁾ To find whether the hydrocarbon chain is linear or branched, the long-chain base was oxidized with periodate, then reduced with NaBH_4 . The fatty alcohol generated was trimethylsilylated and analyzed by gas chromatography with 5% Silicone SE-30 at 180°C. The chromatogram of the Me_3Si -derivative showed a single peak corresponding to that of the Me_3Si ester of the tetradecanol standard, indicating that the long-chain base is 1,3-dihydroxy-2-aminohexadecane.

The fatty acid methyl ester was analyzed by gas chromatography with 5% DEGS on Chromosorb W AW-DMCS at 180°C. Only one peak was detected, agreeing with that of authentic palmitate. From the above results, it is obvious that the molecular species of the ceramide is *N*-2-palmitoyl-1,3-dihydroxyhexadecane.

Although sphingolipids are widely distributed in membrane lipids of animals and higher plants,¹¹⁾ bacterial sphingolipids, including free ceramides, are extremely limited. They have been reported to occur in some anaerobic rumen bacteria,^{10,12-14)} and in aerobic bacteria of *Bdellovibrio bacteriovorus*,¹⁵⁾ *Flavobacterium devorans*,¹⁶⁾ and *Acetobacter xylinum*.¹⁷⁾ Except for those of the latter two strains, these sphingolipids are involved in both sphingophospholipids and ceramides, of which the long-chain bases are mainly composed of *iso* or *anteiso* branched chains and the *N*-acyl fatty acids are almost all of the hydroxy and/or branched type. Sphingoglycolipids have only been found in *F. devorans* and only free ceramide in *A. xylinum*. Both the long-chain bases occurred as dihydrosphingosine with a linear hydrocarbon chain. The free ceramide from *Z. mobilis* described here was *N*-palmitoyldihydroxyhexadecane, which seems to be different in molecular species from other bacterial sphingolipids.

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