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Both the anaerobic pathway and aerobic desaturation are involved in the synthesis of unsaturated fatty acids in *Vibrio* sp. strain ABE-1

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Vibrio sp. strain ABE-1 is a unique marine bacterium in terms of its ability to synthesize *Δ9-trans*-hexadecenoic acid and *Δ7-cis*-tetradecenoic acid (14:1(7c); Okuyama, H., Sasaki, S., Higashi, S. and Murata, N. (1990) J. Bacteriol. 172, 3515–3518). The present study, involving labeling with [1-^{14}C]acetate, demonstrated that 14:1 is synthesized by the anaerobic pathway. When cells of this bacterium were grown in the presence of [1-^{14}C]myristic acid (14:0), this compound was converted to palmitic (16:0) and hexadecenoic (16:1) acids but not to 14:1, under aerobic conditions. These results suggest that the incorporated 14:0 was elongated to 16:0 and then converted to 16:1 by the aerobic desaturation of 16:0. It appears that the anaerobic pathway and aerobic desaturation are both involved in the synthesis of unsaturated fatty acids during aerobic growth of *Vibrio* sp. strain ABE-1.

Anaerobic pathway; Aerobic desaturation; Unsaturated fatty acid; *Vibrio*

1. INTRODUCTION

Anaerobic bacteria synthesize mono-unsaturated fatty acids via the anaerobic pathway, in which unsaturated fatty acids are synthesized by the type II FAS [1,2]. Most aerobic bacteria synthesize unsaturated fatty acids by the oxygen-dependent desaturation of saturated fatty acids. It has been considered, to date, that only one of these two pathways is operative in any given bacterium [2].

This communication describes for the first time observations indicating that both anaerobic pathway and aerobic desaturation are involved in the synthesis of unsaturated fatty acids in a facultative anaerobe, *Vibrio* sp. strain ABE-I (*Vibrio* ABE-1), grown under aerobic conditions.

Abbreviations: DAC, 3-decynoyl-*N*-acetylcysteamine; FAME, fatty acid methyl ester(s); FAS, fatty acid synthetase; HPCL, high-performance liquid chromatography; for fatty acids, the number before the colon indicates the length of the carbon chain and the number after the colon indicates the number of double bonds, for example, 16:1.

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2. MATERIALS AND METHODS

2.1. *Bacterium and growth conditions*

The psychrophilic bacterium, *Vibrio* ABE-1 [3], was grown at 15°C in a Tris-buffered synthetic medium [4] on a rotary shaker. Cells were washed with 500 mM NaCl and suspended at 1×10^7 cells/ml in 50 mM Tris-HCl (pH 7.5), 400 mM NaCl, 10 mM KCl, 5 mM $MgCl_2$ and 1.0 M glycerol.

2.2. *Labeling in vivo*

For the de novo synthesis of fatty acids, 50 μl of sodium [1-^{14}C] acetate (dissolved in water at 74 Bq/μl) was added to a 1-ml suspension of cells which was then incubated at 15°C for 30 min under aerobic or anaerobic conditions. The cells were harvested and lipids were extracted by the method of Bligh and Dyer [5]. In order to examine the aerobic desaturation of saturated fatty acids, 10 μl ethanol solution of [1-^{14}C]14:0 (2.24 kBq/μl) or [1-^{14}C]18:0 (0.37 kBq/μl) was added to a 1-ml suspension of cells. After shaking at 15°C for 5 min for aerobic conditions and after standing at 15°C for 15 min for anaerobic conditions, the total lipids were extracted as described above. Cells labeled under anaerobic conditions were heated for 1 min at 100°C, prior to extraction of lipids.

For anaerobic conditions, the cultures were first made anaerobic by the addition of 0.2% sodium dithionite (final concentration) and then radiolabeled materials were added to the cultures. The extent of anaerobiosis of cultures that contained sodium dithionite was ascertained from the coloration due to sodium resazurin in the cultures. DAC [6] and cerulenin [7] were added as ethanol solutions to inhibit the anaerobic synthesis of unsaturated fatty acids and the de novo synthesis of fatty acids, repectively, prior to labeling of cells. The concentrations were 3×10^{-4} M or 1×10^{-3} M for DAC and 10 μg/ml for cerulenin, respectively.

2.3. *Chromatography*

Extracted lipids were subjected to methanolysis with 10% acetyl

chloride in methanol for 3 h at 90°C [8]. Saturated and unsaturated FAME were separated by argentation thin-layer chromatography on silica gel plates with a solvent system of chloroform and ethanol (99:1, v/v) [9]. After the plate had been developed, it was dried and sprayed with a 0.01% primuline in a mixture of acetone and water (4:1, v/v) [10]. The spots were detected under UV light and by use of a radio tracer, AMBIS (K & M Co., Torrance, CA, USA). The FAME were extracted with hexane/methanol/500 mM NaCl in water (2:1:1, v/v/v) from silica gel that was scraped off from the plate, dried and then re-dissolved in a mixture of acetonitrile/2-propanol/water (8:2:1, v/v/v). The FAME were subjected to HPLC on a liquid chromatography system (model 510; Waters, Milford, MA, USA) equipped with a UV detector (model 481; Waters), a radioactivity flow monitor (model 7150; Packard, Downers Grove, IL, USA) and a column (TSKgel ODS-120T, 25 cm × 4.6 mm i.d.; Toso, Tokyo, Japan) which had been equilibrated with a mixture of acetonitrile/2-propanol/water (8:2:1, v/v/v). The FAME were eluted with the same mixture at a flow rate of 1.0 ml/min, and the eluate was monitored by absorption of light at 205 nm and by radioactivity. The identifications of FAME were based on comparisons of their retention times with those of authentic standards.

2.4. *Chemicals*

Sodium [1-^{14}C]acetate (1.95 GBq/mmol) and [1-^{14}C]18:0 (2.00 GBq/mmol) were purchased from Amersham International (Little Chalfont, UK). [1-^{14}C]14:0 (2.04 GBq/mmol) was purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA). DAC was prepared by the published procedure [6]. Authentic fatty acids used as internal standards and cerulenin were products of Sigma (St. Louis, MO, USA). The pre-coated thin-layer plates of silica gel were products of Merck (code no. 5721; Darmstadt, Germany). Solvents and water for HPLC were purchased from Kantoh Chemical (Tokyo, Japan).

3. RESULTS AND DISCUSSION

3.1. *Anaerobic pathway*

Vibrio ABE-1 has a type II FAS [11], which is responsible for synthesis of both saturated fatty acids and mono-unsaturated fatty acids [11]. Thus, it was expected that this organism would synthesize unsaturated fatty acids via the anaerobic pathway in vivo.

The cells of *Vibrio* ABE-1 incorporated sodium [1-^{14}C]acetate into fatty acid fractions under both aerobic and anaerobic conditions. As shown in Table I, under aerobic conditions, the rate of incorporation of

Table I

The incorporation of sodium [1-^{14}C]acetate into fatty acid fractions of *Vibrio* sp. strain ABE-1 under aerobic and anaerobic conditions

Growth conditions	DAC[a]	Incorporation of sodium [1-^{14}C]acetate into (pmol/30 min/10^7 cells)		
		Total fatty acids	Saturated[b] fatty acids	Unsaturated[b] fatty acids
Anaerobic	absent	153	72	81
Anaerobic	present	142	68	74
Aerobic	absent	319	121	198
Aerobic	present	289	101	188

[a]3-Decynoyl-*N*-acetylcysteamine at 3×10^{-4} M.
[b]Saturated and unsaturated fatty acids were separated by argentation TLC (see text).

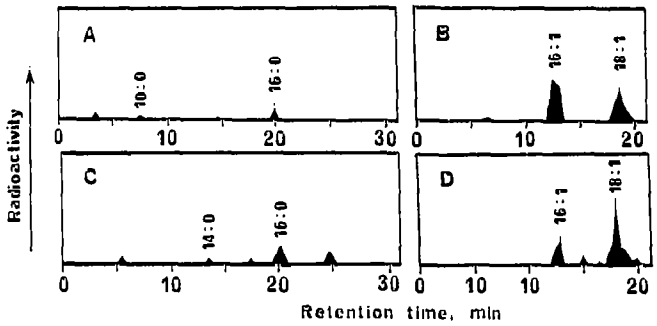

Fig. 1. HPLC profiles of radiolabeled FAME fractions from cells which were pulse-labeled with sodium [1-^{14}C]acetate in the absence (A and B) and presence (C and D) of DAC under anaerobic conditions. A and C, saturated fractions; B and D, unsaturated fractions.

labeled acetate into the fatty acid fraction during pulse labeling was 319 pmol/30 min/10^7 cells, whereas under anaerobic conditions, the rate of incorporation of labeled acetate decreased to 153 pmol/30 min/10^7 cells. Furthermore, anaerobiosis decreased the rate of incorporation of labeled acetate into unsaturated fatty acids (Table I). By contrast, the addition of DAC at 3×10^{-4} M did not significantly decrease the rate of incorporation of labeled acetate into fatty acid fractions under aerobic and anaerobic conditions (Table I). Even under anaerobic conditions, DAC scarcely inhibited the synthesis of unsaturated fatty acids.

Fig. 1 shows HPLC profiles of radiolabeled FAME, prepared from cells that had been pulse-labeled with sodium [1-^{14}C]acetate under anaerobic conditions in the presence and absence of DAC. The saturated fraction contained 16:0 as the major component after incubations in the presence and absence of DAC (Fig. 1A,C). 16:1 and 18:1 were the major components of the unsaturated fatty acid fractions after incubations in the

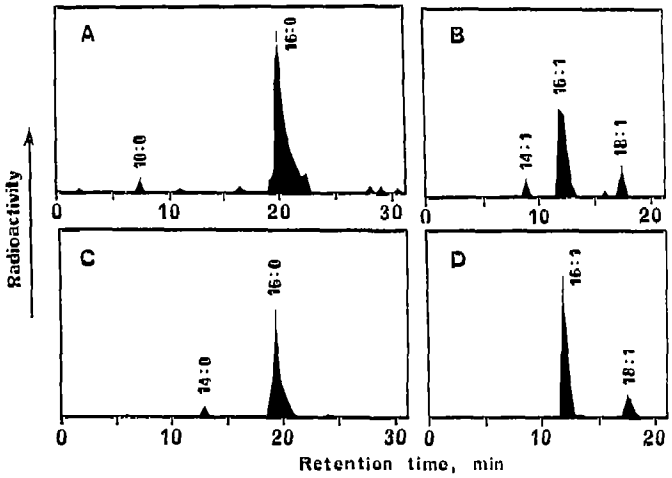

Fig. 2. HPLC profiles of radiolabeled FAME fractions from cells which were pulse-labeled with sodium [1-^{14}C]acetate in the absence (A and B) and presence (C and D) of DAC under anaerobic conditions. A and C, saturated fractions; B and D, unsaturated fractions.

presence and absence of DAC (Fig. 1B,D). 14:1 was not detected in the fraction of unsaturated fatty acids. As shown in Fig. 1B,D, relatively large amounts of 18:1 were synthesized under anaerobic conditions, as compared with those under aerobic conditions (Fig. 2B,D). It has also been shown in *Pseudomonas* sp. that fatty acids synthesized de novo under anaerobic conditions are longer than those synthesized under aerobic conditions [12]. This tendency is likely to be the cause of the absence of 14:1 in *Vibrio* ABE-1 as a product of de novo synthesis from labeled acetate under anaerobic conditions (see Fig. 1B,D). DAC at 1×10^{-3} M did not inhibit the synthesis of unsaturated fatty acids (Fig. 1D) under anaerobic conditions. Under such conditions, it is likely that DAC is not taken up by this bacterium.

Fig. 2 shows HPLC profiles of FAME prepared from cells pulse-labeled with sodium [1-^{14}C]acetate under aerobic conditions in the presence and absence of DAC. The saturated fraction contained 16:0 as the major component after incubations in the presence and absence of DAC (Fig. 2A,C). The unsaturated fatty acids synthesized in the absence of DAC included 14:1, 16:1 and 18:1 (Fig. 2B). Among these unsaturated fatty acids, the synthesis of only 14:1 was inhibit completely by the addition of 3×10^{-4} M DAC (Fig. 2D). These results suggest that 14:1 is the product of the anaerobic pathway, since DAC is a specific inhibitor of the anaerobic synthesis of unsaturated fatty acids [6], and that 16:1 and 18:1 may be synthesized by the anaerobic pathway (elongation of 14:1) and by aerobic desaturation.

3.2. *Aerobic desaturation*

When the cells of *Vibrio* ABE-1 were labeled with [1-^{14}C]14:0 under aerobic conditions, the label was incorporated into the phospholipid fraction. The FAME prepared from the phospholipids were separated into saturated and unsaturated fractions (Fig. 3A). The FAME fraction from lipid fractions other than the phospholipids contained no unsaturated fatty acids. By contrast, label from 14:0 was not incorporated into the phospholipid fraction under anaerobic conditions. Furthermore, the FAME fraction prepared from the total lipids of cells labeled under anaerobic conditions did not contain any unsaturated fatty acids (Fig. 3B). These results suggest that cells of *Vibrio* ABE-1 are not able to desaturate 14:0 via the aerobic pathway.

Fig. 4 presents HPLC profiles of radiolabeled saturated and unsaturated fatty acid fractions from phospholipids of cells of *Vibrio* ABE-1 incubated with [1-^{14}C]14:0 under aerobic conditions. The high level of radioactivity in 16:0 and 16:1 suggests that 14:0 was readily converted into 16:0 and 16:1 under aerobic conditions. By contrast, the FAME fractions prepared from total lipids of cells labeled with [1-^{14}C]14:0 and [1-^{14}C]18:0 in the presence of cerulenin under aerobic conditions did not contain any unsaturated fatty acids

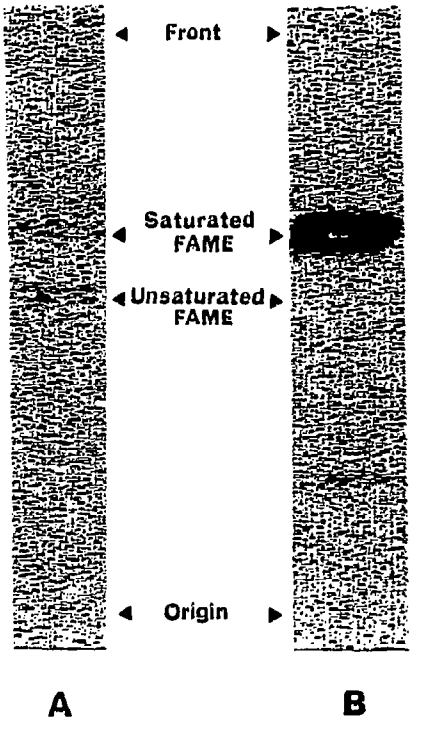

Fig. 3. TLC profiles of radiolabeled FAME fractions from cells labeled with [1-^{14}C]14:0. (A) FAME were prepared from the phospholipid fraction of cells labeled under aerobic conditions. (B) FAME were prepared from total lipids of cells labeled under anaerobic conditions. A minor spot in (B) was not determined.

(Fig. 5). These results show that 16:1 is synthesized by aerobic desaturation of 16:0 in this bacterium and that it is very likely that 18:1 is formed by elongation of 16:1.

Vibrio ABE-1 has *Δ5-cis*-12:1, *Δ7-cis*-14:1, *Δ7-cis*-16:1, *Δ9-cis*-16:1, *Δ9-trans*-16:1, *Δ9-cis*-18:1 and *Δ11-cis*-18:1 as unsaturated fatty acyl components of membrane phospholipids [9]. The positions of double bonds, *Δ5* in 12:1, *Δ7* in 14:1, *Δ9* in 16:1 and *Δ11* in 18:1, suggest that these unsaturated fatty acids are synthesized via the anaerobic pathway. Although the positions of double bonds of labeled fatty acids derived from sodium [1-^{14}C]acetate were not determined, it is likely that 14:1 and some fraction of 16:1 are *Δ7-cis*-14:1, and *Δ9-cis*-16:1, respectively, that is, products of the anaerobic pathway.

As discussed by Erwin and Bloch [2], in terms of their

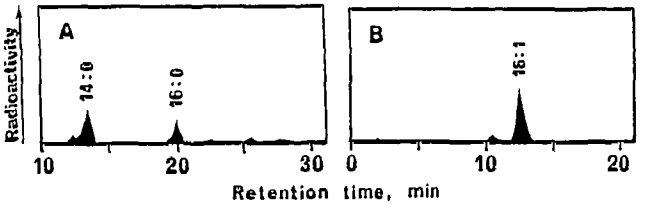

Fig. 4. HPLC profiles of radiolabeled FAME fractions of phospholipids prepared from cells labeled with [1-^{14}C]14:0 under aerobic conditions. (A) Saturated fraction. (B) unsaturated fraction.

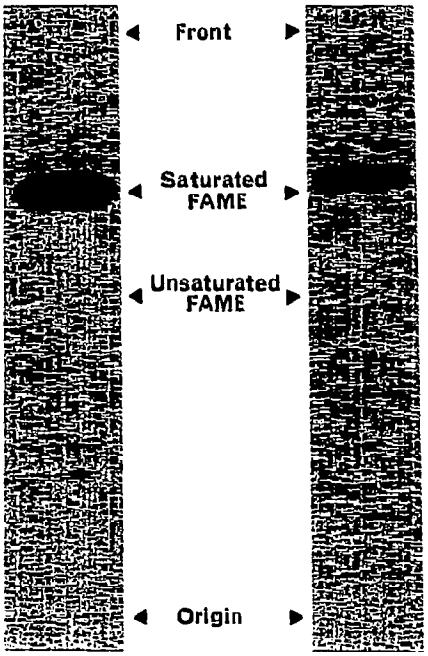

Fig. 5. TLC profiles of radiolabeled FAME fractions of total lipids prepared from cells labeled with [1-^{14}C]14:0 (A) and with [1-^{14}C]18:0 (B), in the presence of cerulenin (10 μg/ml) under aerobic conditions. A minor spot in (A) was not determined.

distribution among organisms, the anaerobic pathway and aerobic desaturation in the synthesis of unsaturated fatty acids are mutually exclusive; no organism has been found, so far, to contain the enzymes required for both the aerobic and anaerobic routes. Recently, however, it was reported [12] that an aerobic bacterium, *Pseudomonas* sp., in addition to the anaerobic pathway, has the capacity for aerobic desaturation during the synthesis of unsaturated fatty acid. In *Pseudomonas* sp., unsaturated fatty acids were synthesized in vitro by the type II FAS and the membrane fraction converted palmitoyl-CoA to its unsaturated counterpart [12]. However, it is unknown which pathway is operative in *Pseudomonas* sp. during the aerobic growth of cells [12].

It is clear that the two pathways coexist in *Vibrio* ABE-1, although the aerobic desaturation of saturated fatty acids has not been demonstrated in vitro. Furthermore, the cells of this bacterium, which contain 12:1 and 14:1, fatty acids that are produced only via the anaerobic pathway, convert 14:0 to 16:1 via 16:0 under aerobic conditions. Thus, it is concluded that the anaerobic pathway and aerobic desaturation are involved cooperatively in the synthesis of unsaturated fatty acids in *Vibrio* ABE-1. This phenomenon has not previously been reported in any other system.

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