ORIGINAL

Production of Fatty Acids from 1-Propanol in *Pseudomonas* sp

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Pseudomonas sp (ATCC 12085) grown in a medium containing 1-propanol produced pentadecanoic (C₁₅), heptadecanoic (C₁₇), heptadecenoic (C_{17:1}), and methyleneheptadecanoic acids (C_{18cyc}), a cyclopropane fatty acid. The ratio of these fatty acids except for heptadecenoic acid to all fatty acids increased with the growth of *Pseudomonas*. Maximum concentrations of these fatty acids were obtained at 0.5 % 1-propanol. The fatty acids were also produced in the presence of sodium propionate instead of 1-propanol. The production of odd-numbered fatty acids and methylene-heptadecanoic acid by *Pseudomonas* sp can be explained based on the intermediary propionyl-CoA from 1-propanol and propionic acid.

Introduction

The effect of *n*-alcohols on fatty acid composition of *E. coli*^{1)~4)}, *Bacillus*⁵⁾ and *Clostridium*⁶⁾ has been previously studied. These results showed that the concentration of individual fatty acids present was dependent on the alcohols employed, however these alcohols were not assimilated.

Pseudomonas sp (ATCC 12085) produced C₁₆, C_{16:1}, C_{18:1}, and cyclopropane acids (C_{17cyc} and C_{19cyc}) when grown in a medium containing peptone and yeast extract. C₁₆, C_{16:1}, and C_{18:1} were synthesized from acetyl-CoA (ACP) which is a primer of fatty acid synthesis and malonyl-ACP supplying C₂ unit to acyl-ACP. As the growth advanced, C_{17cyc} and C_{19cyc} were produced as the result of methyl transfer reaction of *S*-adenosyl-L-methionine onto the double bond of C_{16:1} and C_{18:1}^{7),8)}.

Key words : *Pseudomonas* sp, 1-propanol, pentadecanoic acid, heptadecanoic acid, heptadecenoic acid, methyleneheptadecanoic acid

Abbreviation : ACP, acyl carrier protein

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Aside from the fatty acids mentioned above, this strain was capable of producing C₁₅, C₁₇, C_{17:1} and C_{18cyc} in the presence of 1-propanol. This fact indicated that after oxidation 1-propanol was converted to propionyl-CoA (ACP) which was used as a primer for production of odd-numbered fatty acids (C₁₅, C₁₇, C_{17:1}). In the next step, C_{17:1} was converted to C_{18cyc} by the methyl transfer from *S*-adenosyl-L-methionine.

Fatty acid synthesis from *n*-alcohols, whose number of acyl chain is longer than 3, has not been studied extensively. Therefore production of odd-numbered and cyclopropane fatty acids from 1-propanol and propionic acids was hereby studied.

Materials and Methods

Organism and cultivation

All experiments were performed with *Pseudomonas* sp (ATCC 12085) grown at 25 °C. Polypepton which was purchased from Nihon Seiyaku (Tokyo, Japan) was used for peptone. Yeast extract was purchased from Nakarai Tesque (Kyoto, Japan). Tropic

Marin was purchased from Aquarientechnik (Wartenberg, Germany) to prepare artificial sea water. The basal medium contained 3 g peptone and 1 g yeast extract in 1 L of artificial sea water. Buffered medium was prepared by adding 200 mM HEPES in the basal medium before sterilization (pH 7.5). The medium containing 1-propanol was prepared by adding this alcohol through a membrane filter with 0.2 μ m pore size to the medium after sterilization. The medium containing sodium acetate or sodium propionate was prepared by adding each reagent to the basal medium before sterilization. *Pseudomonas* sp was grown in pre-culture flask and subsequently inoculated into 500 mL flask containing 150 mL of the medium. Cell growth was measured with turbidimeter at 650 nm with pH measurement at the same time.

Extraction of lipids

Cells were collected after centrifugation and washed with a 0.8 % NaCl solution and suspended in approximately 0.5 mL NaCl solution (0.8 %). Two milliliters of a CHCl_3 -MeOH solution (1:2 vol/vol) was added and stirred using a vortex, where 6 mL CHCl_3 and 2 mL saturated NaCl solution was added. After stirring the mixture, the CHCl_3 layer was separated. Any water present was removed by addition of sodium sulfate, whose mixture was then filtered and concentrated for fatty acid analysis.

Fatty acid analysis

Fatty acid methyl ester was prepared by the following procedure: a 0.2 mL lipid extract in CHCl_3 was saponified with 0.5 mL of 0.5 N NaOH-methanol solution at 80°C for 5 min and cooled in ice. The solution was then esterified with BF_3 in MeOH at 80°C for 5 min. The fatty acid methyl esters were extracted by adding 0.7 mL hexane and a 1 mL saturated NaCl solution. The hexane layer was then examined by GC and GC-MS. Shimadzu GC 8A equipped with CPS-2 bonded capillary column was used for GC, while HP 5971 A and HP 5890 for GC-MS. Individual fatty acid methyl esters of the lipid extract were identified by their retention time and mass spectral fragmentation

pattern. With the equipment used, $\text{C}_{18:1}$ ($n-7$) and $\text{C}_{18\text{cyc}}$ ($n-6$) were not separated. To obtain the ratio of $\text{C}_{18:1}$ ($n-7$) and $\text{C}_{18\text{cyc}}$ ($n-6$), mild hydrogenation was done (5 % Pd/C, for 5~10 min at room temperature). After filtration and removal of the solvent used (diethyl ether) under nitrogen stream, the product was diluted with a small amount of hexane, and was injected into GC. After the procedure, $\text{C}_{18:1}$ was converted to C_{18} with $\text{C}_{18\text{cyc}}$ intact. Subsequently we could obtain the ratio of $\text{C}_{18:1}$ ($n-7$) and $\text{C}_{18\text{cyc}}$ ($n-6$). Positions of the double bond and cyclopropane ring were determined by picoliny method^{9),10)}.

Results

Fig.-1 shows the difference in the fatty acid composition of lipid extract from the whole cell grown in basal medium and in 1-propanol-supplemented buffered medium. C_{15} , C_{17} , $\text{C}_{17:1}$, and $\text{C}_{18\text{cyc}}$ were produced only in the latter medium.

The growth at different 1-propanol concentrations from 0 % to 3 % are shown in Fig.-2. There was no apparent effect on the

Cell was grown in the basal medium (a) and the buffered medium containing 0.5% 1-propanol (b).

1, hexadecanoic acid, C_{16} ; 2, 9-hexadecenoic acid, $\text{C}_{16:1}$ ($n-7$); 3, 9, 10-methylenehexadecanoic acid (8-(2-hexyl-1-cyclopropyl) octanoic acid), $\text{C}_{17\text{cyc}}$ ($n-7$); 4, 11-octadecenoic acid, $\text{C}_{18:1}$ ($n-7$); 5, 11, 12-methyleneoctadecanoic acid (10-(2-hexyl-1-cyclopropyl) decanoic acid), $\text{C}_{19\text{cyc}}$ ($n-7$); 6, pentadecanoic acid, C_{15} ; 7, heptadecanoic acid, C_{17} ; 8, heptadecenoic acid, $\text{C}_{17:1}$; 9, 9, 10-methyleneheptadecanoic acid (8-(2-heptyl-1-cyclopropyl) octanoic acid), $\text{C}_{18\text{cyc}}$ ($n-8$); 10, 11, 12-methyleneheptadecanoic acid (10-(2-pentyl-1-cyclopropyl) decanoic acid), $\text{C}_{18\text{cyc}}$ ($n-6$). The peak 3, 5, 9, and 10 are cyclopropane acids. The peak 10 contained both $\text{C}_{18:1}$ ($n-7$) and $\text{C}_{18\text{cyc}}$ ($n-6$) because of their inseparable nature.

Fig.-1 Gas chromatogram of fatty acid methyl esters derived from the whole cell lipid

Fig.-2 Cell growth in various 1-propanol concentration.

growth rate below 0.5 % concentration. At concentrations above 1 %, the growth initiation was delayed and the lower growth rate was indicated. Final cell mass took maximum value at 0.5 and 1.0 % concentrations.

Fatty acid composition of lipid extract harvested at the end of culture in Fig.-2 is shown in Fig.-3. Ratio of C_{15} and C_{18cyc} to all the fatty acids was maximum at 0.5 % 1-propanol. The maximum for C_{17} was at both 0.5 and 1.0 % concentrations. Decreases of the ratio of C_{15} , C_{17} , C_{18cyc} were exhibited at 1-propanol concentrations above 1 %. Reversely, ratios of C_{16} and C_{19cyc} became the minimum at 0.5 % 1-propanol. C_{17cyc} was not so changed. Only small amounts of $C_{16:1}$ and $C_{18:1}$ were detected because these were easily converted to C_{17cyc} and C_{19cyc} .

Relationship of fatty acid composition and growth phase is shown in Fig.-4. The ratio of fatty acids produced from 1-propanol was small at the early stage and increased as the growth progressed. Quantities of C_{16} , $C_{16:1}$, and $C_{18:1}$ decreased with increase of the growth while the ratio of C_{19cyc} was unchanged.

Fig.-5 shows the production of C_{15} , C_{17} , and C_{18cyc} when sodium propionate was added instead of 1-propanol. Concentrations of C_{15} , C_{17} , C_{18cyc} ($n-8$), and C_{18cyc} ($n-6$) in 100 mM, sodium propionate were 16.5, 23.2, 8.1, and 5.7 %, respectively. These values were almost the same to those obtained with

Cell was harvested at the end of culture shown in Fig. - 2.

(a) Fatty acids formed from 1-propanol; C_{15} (○), C_{17} (△), $C_{17:1}$ (▲), C_{18cyc} ($n-8$) (●), C_{18cyc} ($n-6$) (□)

(b) Fatty acids other than the ones shown in (a); C_{16} (○), $C_{16:1}$ ($n-7$) (▲), C_{17cyc} ($n-7$) (●), $C_{18:1}$ ($n-7$) (△), C_{19cyc} ($n-7$) (□)

Fig.-3 Fatty acid composition at various 1-propanol concentration.

0.5 % 1-propanol.

Comparison of the growth in sodium acetate-, 1-propanol-, and sodium propionate-supplemented medium is shown in Fig.-6. In 0.5 % 1-propanol (approximately 66 mM) supplemented medium, growth rate was similar to that of the basal medium, and final cell mass was larger than the one obtained in the basal medium. In sodium propionate-supplemented medium, the growth initiation was delayed and the growth rate was lower than the one in basal medium, however final cell mass was large-

Fig.-4 The fatty acid composition change according to the cell growth.

er. In sodium acetate-supplemented medium, the growth rate and final cell mass was higher and larger than the ones in basal medium.^{11),14),15).}

Discussion

The ability of *Pseudomonas* sp (ATCC 12085) to produce C_{15} , C_{17} , $C_{17:1}$, and C_{18cyc} in the presence of 1-propanol is hereby presented. This finding indicates that the fatty acid synthetase (FAS) system of this strain can utilize propionyl-CoA (ACP) as well as acetyl-CoA (ACP) for primer. The FAS system of the other bacteria was reported to be able to utilize various acyl-CoAs as a primer *in vitro*^{11),14),15).} It is thought that C_{18cyc} ($n-6$) and C_{18cyc} ($n-8$) are formed from $C_{17:1}$ ($n-6$) and $C_{17:1}$ ($n-8$), respectively with *S*-adenosyl-L-methionine. Therefore, the FAS system of this strain can form a double bond at the position of $n-6$ and $n-8$

Cell was grown in the basal medium containing sodium propionate.
 Symbols of fatty acids are the same as the ones shown in Fig. - 3 (a).

Fig.-5 Fatty acid concentration formed from propionic acid at various sodium propionate concentration.

Cell was grown in the basal medium (○) containing 100 mM sodium acetate (△), 50 mM sodium propionate (■), or 100 mM sodium propionate (▲), and in the buffered medium containing 0.5 % 1-propanol (□).

Fig.-6 Comparison of the growth in sodium acetate-, 1-propanol-, and sodium propionate-supplemented media.

by the β , γ -dehydration of 3-hydroxynonanoyl-ACP and 3-hydroxyundecanoyl-ACP in the process of fatty acid synthesis if a double bond is formed *via* the anaerobic pathway. However $C_{17:1}$ ($n-6$) and $C_{17:1}$ ($n-8$) could not be properly identified. One $C_{17:1}$ peak (peak no. 8) in Fig.-1 was so small that its double bond position could not be determined. The presence of another $C_{17:1}$ peak could not be ascertained because its peak may have overlapped with that of C_{17cyc} beside probably being present in small amount.

In the absence of 1-propanol this strain did not produce odd numbered-fatty acids, showing that propionyl-CoA could not be

synthesized from the materials present in the basal medium. On the other hand, in the presence of 1-propanol this strain oxidized 1-propanol to relieve its effect and the product was further converted to propionyl-CoA which is the primer for producing odd numbered fatty acid, and subsequently the cells utilized propionyl-CoA for the fatty acid synthesis. Moreover, enhancement of final cell mass in the presence of 1-propanol indicated that the supply of a primer for the fatty acid synthesis was one of the limiting step in the basal medium. A small amount of odd-numbered fatty acids and $C_{18\text{cyc}}$ derived from 1-propanol were present in the early growth phase, which was probably related to the alcohol dehydrogenase activity which increased accordingly with growth (unpublished data).

In the presence of sodium propionate, odd numbered-fatty acid and $C_{18\text{cyc}}$ were also produced. In the experiment with *E. coli*¹²⁾, C_{15} and C_{17} were also produced in the presence of propionic acid. In the present paper, the growth rate in sodium propionate-supplemented medium was lower than that in 1-propanol (Fig.-6). This is explained as follows. The conversion of propionic acid to propionyl-CoA consumed ATP and does not produce NADH. On the other hand, dehydrogenation of 1-propanol produces NADH. Assimilation of propionic acid is, therefore, less advantageous in the aspect of the energy production. The reason for the better growth in sodium acetate-supplemented medium is that acetic acid can enter into TCA cycle via acetyl-CoA and is used for production of ATP and other organic compounds as well as the fatty acid synthesis from acetyl-CoA.

We know only one report with *Streptococcus salivarius*¹³⁾ on the fatty acid synthesis from 1-alcohols with more than 3 carbon chain. There is quite a number of bacteria that can assimilate such alcohols as well as methanol and ethanol. A part of these bacteria possibly produce fatty acids from *n*-alcohol.

(Received March 13, 1992)

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Pseudomonas sp における 1-プロパノールからの脂肪酸の生産

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Pseudomonas sp (ATCC 12085) は、培地に 1-プロパノールを添加したときに、新たに C_{15} , C_{17} , $C_{17:1}$, $C_{18\text{cyc}}$ の脂肪酸を合成した。全脂肪酸に対する C_{15} , C_{17} , $C_{18\text{cyc}}$ の比率は増殖とともに増大し、1-プロパノール濃度が 0.5 % の時に最も高かった。1-プロパノールの代わりにプロピオン酸ナトリウムを加えたときも、これらの脂肪酸は同じように合成された。これらの実験結果は、*Pseudomonas* sp が、1-プロパノールに由来するプロピオニル-CoA (ACP) をプライマーとして、 C_{15} , C_{17} , $C_{17:1}$, $C_{18\text{cyc}}$ の脂肪酸を合成することを示している。

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