Utilization of Pristane by a Yeast Candida lipolytica
Fatty Acid Composition of Pristane-grown Cells

Takeshige Hagihara, Masayoshi Mishina, Atsuo Tanaka
and Saburo Fukui

Laboratory of Industrial Biochemistry, Department of Industrial Chemistry,
Faculty of Engineering, Kyoto University, Kyoto
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A hydrocarbon-utilizable yeast, Candida lipolytica NRRL Y-6795, was found to utilize pristane (2, 6, 10, 14-tetramethylpentadecane) as the sole source of carbon and energy. The maximum cell yield (about 4 mg dry cell/ml) was obtained in 6 to 7 days' cultivation on 1% (v/v) pristane. Pristanoic acid (2, 6, 10, 14-tetramethylpentadecanoic acid) was identified as an oxidation product of pristane. Analysis of cellular fatty acids of C. lipolytica grown on pristane showed that pristanoic acid was contained in neutral lipids as major component and in polar lipids as minor one. Accumulation of pristanoic acid in culture filtrate was not appreciable.

Extensive studies on microbial assimilation of hydrocarbons reveal that n-alkanes serve as suitable substrates for a variety of microorganisms. However, isoalkanes are inferior carbon sources to n-alkanes, and especially, alkanes having a branched alkyl group larger than methyl group or those having multiple methyl branches seem to be inert against microbial attack.1) Pristane (2,6,10,14-tetramethylpentadecane), which distributes widely in nature (see, ref. 2 and 3), has been used as a biological marker2) and a solvent for solid n-alkanes4) because of its biological and chemical inertness.

However, several strains of bacteria can degrade pristane. McKenna and Kallio5) found a strain of Corynebacterium to grow on pristane and proposed a pathway for pristane catabolism. Pirnik et al.5) also investigated a degradation pathway of pristane by Brevibacterium erythrogenes. Nakajima et al.5) isolated two major oxidation products from the culture of Nocardia species grown on pristane and identified them as pristanoic acid (2,6,10,14-tetramethylpentadecanoic acid) and pristanol (2,6,10,14-tetramethylpentadecan-1-ol).

Jones6) reported oxidation of pristane by a yeast Torulopsis gropengiesseri growing on glucose. Pristane was incorporated into glycolipids of the yeast in the forms of pristanol, 2,6,10,14-tetramethylpentadecane-1,15-di-ol and 15-hydroxypristanoic acid. However, there is no available information concerning growth of yeast on pristane used as the sole source of carbon and energy.

This paper deals with growth of a yeast Candida lipolytica NRRL Y-6795 on pristane and identification of pristanoic acid in the cellular lipids as well as in the culture filtrate.

MATERIALS AND METHODS

Cultivation of yeasts. The yeasts used in this study were Candida lipolytica NRRL Y-6795, C. guilliermondii IFO 0566, C. krusei IFO 0592, C. parapsilosis IFO 0583 and C. tropicalis pK 233, which were maintained on malt extract agar slants. Yeasts were cultivated aerobically for 22 hr at 30°C in 500 ml flasks containing 100 ml of a glucose medium (see, below), harvested by centrifugation, and suspended into 25 ml of sterilized saline. One ml of the cell suspension was inoculated into a 500 ml flask containing 100 ml of a glucose medium (see, below), harvested by centrifugation, and suspended into 25 ml of sterilized saline. One ml of the cell suspension was inoculated into a 500 ml flask containing 100 ml of a medium and cultivation was carried out with shaking (220 rpm) at 30°C. The composition of the medium was: NH₄H₂PO₄, 5.0 g; KH₂PO₄, 2.5 g; MgSO₄·7H₂O, 1.0 g; FeCl₃·6H₂O, 0.02 g; corn steep liquor 1.0 g; and alkane, 10 ml; or glucose, 16.5 g per
Extraction of lipids and analysis of fatty acids. Cellular lipids were extracted with a mixture of chloroform and methanol (2:1) according to the method of Folch et al. and then separated by silicic acid column chromatography. Neutral lipids were eluted with chloroform and polar lipids with methanol from the column. Each lipid was saponified in 20% methanolic KOH for 3 hr at 80°C under reflux. Fatty acids extracted and purified were converted to their methyl esters by treatment with boron trifluoride, as described previously. Culture filtrate was extracted with diethyl ether under acidic conditions and the extract was treated as described above. The methyl esters of fatty acids were analyzed with a gas chromatograph under the same conditions as described previously. Identification of fatty acid methyl esters was made by calculating the equivalent chain lengths on gas chromatogram. Authentic samples used were methyl esters of myristic, pentadecanoic, palmitic, heptadecanoic, stearic, oleic and linoleic acids. Peak area was determined by triangulation. Mass spectra were obtained with a Hitachi GC Mass Spectrometer RM-50 GC. A glass column, 1 m x 6 mm (outer diameter) was packed with 5% diethylene glycol succinate (DEGS) on Neopak 1A, 60/80 mesh (Nishio Industry Co., Japan).

RESULTS AND DISCUSSION

Growth ability of n-alkane-utilizable yeasts on pristane

Five strains of yeasts were cultivated on pristane as sole carbon source. As shown in Figure 1, C. lipolytica could utilize pristane, while C. guilliermondii, C. krusei, C. parapsilosis and C. tropicalis could not. The growth of C. lipolytica on pristane was significantly slower than that on n-alkanes. The maximum cell yield with 1% (v/v) substrate (about 4 mg dry cell/ml) was about two-thirds of that on n-alkanes. Addition of a non-ionic detergent Tween 85 to the medium slightly stimulated the growth of C. lipolytica on pristane, but the effect was not significant as the case of n-alkane utilization by this yeast. Figure 2 shows that no remarkable difference was observed in the yeast growth on pristane among three kinds of the seed cultures harvested from the glucose medium, n-hexadecane medium and 3% malt extract broth. Preculture of C. lipolytica on n-alkane seemed not to induce a pristane assimilation system. Degradation system of pristane skeleton having methyl branches might be different from that of n-alkanes.
Utilization of Pristane by *Candida lipolytica*

**Fatty acid composition of neutral and polar lipids from pristane-grown *C. lipolytica***

Figure 3 illustrates a typical gas chromatographic pattern of the fatty acid methyl esters of neutral lipids extracted from *C. lipolytica* cells grown on pristane. One major peak with a retention time of about 4 min and an equivalent chain length of 15.7 did not correspond to those of authentic samples of straight-chain fatty acid methyl esters. This compound showed the same degradation pattern on mass spectrometry as that of methyl pristanoate (methyl 2,6,10,14-tetramethylpentadecanoate) as reported by Hansen and Morrison and Pirnik et al. Its characteristic peaks were: m/e = 312 (parent ion); M–31; M–43; M–90; 101; and 88 (Fig. 4).

**TABLE I. FATTY ACID COMPOSITION OF NEUTRAL AND POLAR LIPIDS FROM *Candida lipolytica* NRRL Y–6795 GROWN ON PRISTANE**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Neutral lipids (%)</th>
<th>Polar lipids (%)</th>
<th>Neutral lipids (%)</th>
<th>Polar lipids (%)</th>
<th>Neutral lipids (%)</th>
<th>Polar lipids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.8</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4</td>
<td>ND</td>
<td>0.5</td>
<td>ND</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>1.2</td>
<td>2.5</td>
<td>0.8</td>
<td>1.9</td>
<td>0.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Pristanoic acid</td>
<td>40.6</td>
<td>6.4</td>
<td>27.0</td>
<td>6.0</td>
<td>33.3</td>
<td>5.7</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1&lt;/sub&gt;</td>
<td>10.2</td>
<td>14.0</td>
<td>12.2</td>
<td>16.1</td>
<td>10.6</td>
<td>15.1</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:1&lt;/sub&gt;</td>
<td>11.1</td>
<td>20.7</td>
<td>11.0</td>
<td>21.1</td>
<td>9.8</td>
<td>17.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>1.6</td>
<td>0.8</td>
<td>2.1</td>
<td>0.6</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:1&lt;/sub&gt;</td>
<td>5.7</td>
<td>11.9</td>
<td>7.2</td>
<td>9.6</td>
<td>4.9</td>
<td>10.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>0.4</td>
<td>0.1</td>
<td>2.1</td>
<td>0.5</td>
<td>1.8</td>
<td>0.3</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1&lt;/sub&gt;</td>
<td>13.1</td>
<td>19.5</td>
<td>22.4</td>
<td>21.6</td>
<td>20.2</td>
<td>22.6</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:2&lt;/sub&gt;</td>
<td>14.8</td>
<td>23.6</td>
<td>14.3</td>
<td>22.2</td>
<td>16.4</td>
<td>25.4</td>
</tr>
<tr>
<td>Total fatty acids (mg/g dry cell)</td>
<td>2.44</td>
<td>7.91</td>
<td>2.37</td>
<td>9.75</td>
<td>11.41</td>
<td>14.67</td>
</tr>
<tr>
<td>Growth (mg dry cell/ml)</td>
<td>1.05</td>
<td>2.23</td>
<td>3.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> ND, not detected.
these results, the compound was identified as methyl pristanoate.

Fatty acid composition of neutral and polar lipids was summarized in Table I. In this study, we did not examine occurrence of alcohols, which were the components of glycolipid of pristane-oxidizing T. gropengiesseri.\(^6\) It is striking that pristanoic acid was the most abundant fatty acid in neutral lipids of C. lipolytica independent of the growth phase. A part of this acid seems to be incorporated in triglyceride, the major component of neutral lipids of C. lipolytica.\(^12\) Accumulation of short-chain fatty acids corresponding to alkane substrates was remarkable in triglyceride of C. tropicalis cultivated on n-alkane mixture consisting of n-decane to n-tridecane.\(^13\) These non-physiological fatty acids may be stored in the form of triglyceride or other neutral lipids. In polar lipids, the content of pristanoic acid corresponded to several percents of fatty acids throughout the cultivation. It is not clear that pristanoic acid will be physiologically active as a constituent of phospholipid. At the late growth phase (148 hr cultivation), the content of pristanoic acid reached 4.6 mg/g dry cell along with the increase of fatty acid content in the cells, especially that of neutral lipids.

Another interesting feature of C. lipolytica grown on pristane is a relatively high proportion of C\(_{17}\) acids (especially C\(_{17,1}\) acid) to total fatty acids in polar lipids. Pristanoic acid is considered to be degraded in bacteria via the \(\beta\)-oxidation pathway to yield acetyl-CoA and propionyl-CoA.\(^2,5\) The latter may serve as priming substrate for the \textit{de novo} synthesis of C\(_{17}\) acids.

Pristanoic acid was also detected in the culture filtrate of C. lipolytica. However, the amount was small, less than 5 mg/liter filtrate. Several products of di-terminal oxidation and \(\beta\)-oxidation have been demonstrated in the culture filtrates of bacteria grown on pristane.\(^2,5\) Although we did not examine occurrence of such compounds in detail, 15-hydroxypristanoic acid and pristanedioic acid, at least, were not detected in the culture filtrate of pristane-grown C. lipolytica.

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\textbf{REFERENCES}