Full Paper

The effect of sodium acetate on the activity of \(L\)- and \(D\)-lactate dehydrogenases in *Lactobacillus sakei* NRIC 1071\(^T\) and other lactic acid bacteria

Takao Iino,* Tai Uchimura, and Kazuo Komagata

Laboratory of General and Applied Microbiology, Department of Applied Biology and Chemistry, Faculty of Applied Bioscience, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156–8502, Japan

(Received September 19, 2002; Accepted January 28, 2003)

The effect of sodium acetate on the production of stereoisomers of lactic acid produced by *Lactobacillus sakei* NRIC 1071\(^T\) and other lactic acid bacteria was studied. *L. sakei* NRIC 1071\(^T\) started producing \(L\)-lactic acid at the early logarithmic phase and \(D\)-lactic acid at the late logarithmic phase. The activity of \(L\)-lactate dehydrogenase [EC 1.1.1.27, \(L\)-LDH] from the resting cells of *L. sakei* NRIC 1071\(^T\) appeared at the early stage of the logarithmic phase during the growth, and the activity of \(D\)-lactate dehydrogenase [EC 1.1.1.28, \(D\)-LDH] at the late stage of the logarithmic phase. The resting cells and cell-free extracts of *L. sakei* NRIC 1071\(^T\) did not produce \(D L\)-lactic acid from \(L\)- or \(D\)-lactic acid. Stained bands of \(L\)-LDH and \(D\)-LDH appeared in the cell-free extracts from the cells of *L. sakei* NRIC 1071\(^T\). Consequently, *L. sakei* conclusively produced \(L\)- and \(D\)-lactic acid by the action of \(L\)-LDH and \(D\)-LDH. This finding leads to the conclusion that lactate racemase [EC 5.1.2.1] does not exist in this strain. When the specific activity of LDHs (the total activity of \(L\)-LDH plus \(D\)-LDH) from the cells cultivated in the presence of sodium acetate is compared with that cultivated in its absence, the ratio of the activity between the cells cultivated in the former condition and those in the latter fell from 1.7 on the cell-free extracts to 1.3 on the preparation of the QAE-Toyopearl 550c chromatography. This result indicates that the amount of LDHs in the cells of *L. sakei* NRIC 1071\(^T\) cultivated in the presence of 50 mM sodium acetate was much more than that in the cells cultivated in the absence of sodium acetate. The shift of the type of stereoisomers of lactic acid from the \(D L\)-type to the \(L\)-type is discussed in the case of *L. sakei* strains.

Key Words—\(D\)-lactate dehydrogenase; \(L\)-lactate dehydrogenase; lactate racemase; *Lactobacillus sakei*; the effect of sodium acetate; type of stereoisomers of lactic acid

Introduction

*Lactobacillus sakei* was reported to shift the type of stereoisomers of lactic acid produced from the \(D L\)-type to the \(L\)-type in the presence of sodium acetate because of the repression of the formation of lactate racemase by sodium acetate (Katagiri and Kitahara, 1937; Kitahara et al., 1957). However, the substantial properties of lactate racemase have not been clarified since lactate racemase [EC 5.1.2.1] was reported about 50 years ago. Furthermore, the effect of sodium acetate on the formation of lactate racemase has not

---

* Present address and address reprint requests to: Dr. Takao Iino, Independent Administrative Institution (A Government Agency) National Institute of Technology and Evaluation NITE Biological Resource Center, 2–5–8 Kazusa-kamatari, Kisarazu, Chiba 292–0818, Japan.
E-mail: iino-takao@nite.go.jp
been clarified.

The high production of \(\alpha\)-lactic acid and the low production of \(\beta\)-lactic acid were reported to cause the shift of the \(\alpha\)-type to the \(\beta\)-type by \(L.\ sakei\) (Iino et al., 2001). In addition, this fact revealed the increase of the ratio of \(\alpha\)-lactic acid to \(\beta\)-lactic acid by the activation of \(\alpha\)-lactate dehydrogenase [EC 1.1.1.27, \(\alpha\)-LDH] and the stabilization of the activity of LDHs (the total activity of \(\alpha\)-LDH plus \(\beta\)-lactate dehydrogenase [EC 1.1.1.28, \(\beta\)-LDH]) (Iino et al., 2002). Furthermore, racemization of \(\alpha\)-lactic acid or \(\beta\)-lactic acid was not found by using the resting cells and cell-free extracts of \(L.\ sakei\) NRIC 10717 (NRIC, Culture Collection Center, Tokyo University of Agriculture, Tokyo) (Iino et al., 2002). It is of interest to note that the ratio of \(\alpha\)-form to \(\beta\)-form of biological substances, for example, \(\alpha\)-lactic acid and \(\beta\)-lactic acid, are largely shifted from one to the other by environmental conditions.

This paper deals with the effect of sodium acetate on the activity of \(\alpha\)-LDH and \(\beta\)-LDH caused by resting cells of \(L.\ sakei\) NRIC 10717, racemization by resting cells of \(L.\ sakei\) strains, and the enzyme activity of LDHs isolated from cell-free extracts of the cells from \(L.\ sakei\) NRIC 10717 cultivated in the absence of sodium acetate and in the presence of 50 mM sodium acetate. Furthermore, discussion is concerned with the participation of \(\beta\)-LDH in the production of \(\beta\)-lactic acid by \(L.\ sakei\) NRIC 10717 and the conclusion that lactate racemase does not exist in this strain.

Materials and Methods

**Bacterial strains.** \(L.\ sakei\) NRIC 10717, NRIC 1764, IFO 12456, No. 16, No. 27, and No. 30 were used in this study. \(L.\ sakei\) No. 16, No. 27, and No. 30 were isolated from sake starters in this laboratory. \(Lactobacillus curvatus\) NRIC 10527, \(Lactobacillus paracasei\) subsp. \(paracasei\) NRIC 1044, and \(Lactobacillus plantarum\) NRIC 10677 were used as references.

**Cultivation.** Strains were cultivated in 5 ml of GYP broth as described in previous papers (Iino et al., 2001, 2002). \(L.\ sakei\) NRIC 10717 and \(L.\ sakei\) strains were stationarily cultivated at 25°C for two days. \(L.\ curvatus\) NRIC 10527, \(L.\ paracasei\) subsp. \(paracasei\) NRIC 1044, and \(L.\ plantarum\) NRIC 10677 were stationarily cultivated at 30°C for two days. These strains were stationarily precultured in GYP broth for two days. Cells were collected by centrifugation at 3,500 rpm for 15 min at room temperature, and washed twice with sterile saline. The washed cells were resuspended in sterile saline, and 50 \(\mu\)l of the suspension was inoculated into GYP broth and other liquid media with a pipette.

**Preparation of resting cells.** Resting cells were prepared by the method described in a previous paper (Iino et al., 2002).

**Assay of the activity of \(\alpha\)-LDH and \(\beta\)-LDH in the resting cells.** The activity of \(\alpha\)-LDH and \(\beta\)-LDH was respectively assayed by 2,6-dichlorophenol indophenol (DCIP) reduction. The resting cells were suspended in 1 ml of Tris-HCl buffer (pH 7.5), and used for the assay of the activity of \(\alpha\)-LDH and \(\beta\)-LDH. A reaction mixture containing 100 \(\mu\)l of the suspension of the resting cells and 800 \(\mu\)l of 50 mM Tris-HCl buffer (pH 7.5) containing 0.5 mM DCIP was preincubated at 25, 30, or 35°C. A reaction was started by adding 100 \(\mu\)l of 500 mM lithium \(\alpha\)-lactate or 500 mM lithium \(\beta\)-lactate, and the decrease in absorbance was monitored at 600 nm. The activity of \(\alpha\)-LDH and \(\beta\)-LDH in the resting cells was assayed under aerobic and anaerobic conditions. The anaerobic conditions were produced by replacing atmosphere in a sealed cell with nitrogen gas.

**Production of \(\alpha\)-lactic acid from \(\alpha\)-lactic acid by the resting cells.** The production of \(\alpha\)-lactic acid from \(\alpha\)-lactic acid was determined by the method described in a previous paper (Iino et al., 2002).

**Preparation of cell-free extracts.** Cell-free extracts were prepared by the method described in a previous paper (Iino et al., 2002).

**Purification of LDHs from the cells of \(L.\ sakei\) NRIC 10717.** The cell-free extracts were treated with a 10% streptomycin sulfate solution to remove nucleoproteins. The resulting mixture was shaken several times, and centrifuged at 15,000 rpm for 30 min. The supernatant was applied to a DEAE Sephadex (Amersham Biosciences, Piscataway, USA) anion-exchange column (7 x 60 mm) equilibrated with 50 mM Tris-HCl buffer (pH 7.5). The column was washed with 2 ml of 50 mM Tris-HCl buffer (pH 7.5) and 2 ml of the same buffer containing 100 mM NaCl. Proteins were eluted with 2 ml of the same buffer containing 200 mM NaCl. The elute was filled up to 20 ml with 50 mM Tris-HCl buffer (pH 7.5), and applied to a QAE-Toyopearl 550c (Tosoh, Tokyo, Japan) anion-exchange column (7 x 60 mm) equilibrated with 50 mM Tris-HCl buffer (pH 7.5). The column was washed with 2 ml of 50 mM Tris-HCl buffer (pH 7.5) and 2 ml of the same buffer containing 100 mM NaCl. Proteins were eluted with 2 ml of 50 mM
Tris-HCl buffer (pH 7.5) containing 200 mM NaCl.

Assay of enzymes. The total activity of \( \text{L-LDH} \) plus \( \text{D-LDH} \) of the cell-free extracts was expressed as LDHs, and assayed by NADH oxidation as described in a previous paper (Lino et al., 2002). The activity of \( \text{L-LDH} \) and \( \text{D-LDH} \) was determined by using two parameters such as the activity of LDHs and the ratio of \( \text{L-lactic} \) acid to \( \text{D-lactic} \) acid produced (Lino et al., 2002).

Active staining of \( \text{L-LDH} \) and \( \text{D-LDH} \) in the cell-free extracts. Referring to the method of Laemmli (1970), 10% separating gel and 4.5% stacking gel were prepared from a stock solution containing 29.2% acrylamide and 0.8% \( \text{N,N'-methylene-bis-acrylamide} \). The separating gel was composed of 0.325 m Tris-HCl buffer (pH 8.8), and was polymerized chemically by the addition of 0.05% \( \text{N,N',N',N'-tetramethylenediamine (TEMED)} \) and 0.03% ammonium persulphate. The stacking gel was composed of 0.125 m Tris-HCl buffer (pH 6.8), and was polymerized chemically in the same way as the separating gel. An amount of enzyme corresponding to 1 mg protein was used for each run. Proteins were concentrated at 10 mA, and electrophoresis was carried out at 15 mA by using an electrode buffer containing 0.3% Tris(hydroxymethyl) aminomethane and 1.44% glycine. Biochemical forward and reverse reactions were performed because the activity of \( \text{D-LDH} \) from \( \text{L. sakei NRIC 1071} \) was difficult to detect. The electrophoresed gel was incubated in either of the following two mixtures (Hensel et al., 1977) to detect the activity of \( \text{L-LDH} \) and \( \text{D-LDH} \) from pyruvic acid to lactic acid (biochemically forward reaction) or the activity from lactic acid to pyruvic acid (reverse reaction).

The reaction from pyruvic acid to \( \text{L-} \) or \( \text{D-lactic} \) acid was determined as follows: The electrophoresed gel was incubated in reaction mixture A, containing 50 mg of sodium pyruvate, 25 mg of NADH, 3.75 mg of fructose-1,6-bisphosphate (FBP), and 2.0 mg of MnSO₄ per 10 ml of 100 mM Tris-HCl buffer (pH 7.5). After incubation of reaction mixture A for 60 min, the electrophoresed gel was rinsed with distilled water, and transferred to reaction mixture B, containing 18 mg of nitroblue-tetrazolium (NBT) and 3 mg of phenazinemethosulfate (PMS) per 10 ml of 100 mM Tris-HCl (pH 8.5). After a few minutes, the area occupied by \( \text{L-LDH} \) and \( \text{D-LDH} \) retained unstained, and the other area was stained blue. When the staining was completed, the stained gel was transferred to 7% acetic acid solution, and stored in a preservation solution containing 6.7% methanol and 10% acetic acid.

The reaction from \( \text{L-} \) or \( \text{D-lactic} \) acid to pyruvic acid was determined as follows: The electrophoresed gel was incubated in a reaction mixture containing 50 mg of lithium \( \text{L-lactate} \) or lithium \( \text{D-lactate} \), 15 mg of NAD, 15 mg of FBP, 30 mg of MnSO₄, 10 mg of NBT, and 1.5 mg of PMS. After incubation, the area occupied by the \( \text{L-LDH} \) or \( \text{D-LDH} \) was stained blue. When the staining was completed, the stained gel was transferred to 7% acetic acid solution, and stored in a preservation solution containing 6.7% methanol and 10% acetic acid.

\( \text{L-LDH} \) preparation from rabbit muscle (Rosche Diagnostics, Basel, Switzerland) and \( \text{D-LDH} \) preparation from \( \text{Lactobacillus leichmanni} \) (Rosche Diagnostics) were used as references. Proteins were determined by the dye-binding method (Bradford, 1976) by using bovine serum albumin as the standard.

Results
Activity of \( \text{L-LDH} \) and \( \text{D-LDH} \) and production of \( \text{L-form} \) and \( \text{D-form} \) of lactic acid during the growth of \( \text{L. sakei NRIC 1071} \)

\( \text{L. sakei NRIC 1071} \) started growing logarithmically after about 8 h, and reached the stationary phase after 20–24 h (Fig. 1). The resting cells of \( \text{L. sakei NRIC 1071} \) obtained from the cultures showed the activity of \( \text{L-LDH} \) 4 h after inoculation, and the activity of \( \text{D-LDH} \) after 16 h. In addition, \( \text{L. sakei NRIC 1071} \) started

![Graph](image-url)

Fig. 1. The activity of \( \text{L-LDH} \) and \( \text{D-LDH} \), and the production of \( \text{L-form} \) and \( \text{D-form} \) of lactic acid during the growth of \( \text{L. sakei NRIC 1071} \).

Symbols: ● growth; ▲ activity of \( \text{L-LDH} \) (mU/mg); ▲ activity of \( \text{D-LDH} \) (mU/mg); □ production of \( \text{L-lactic} \) acid (mm); ■ production of \( \text{D-lactic} \) acid (mm).
producing L-lactic acid at the early logarithmic phase, and produced D-lactic acid at the late logarithmic phase.

Activity of L-LDH and D-LDH at various temperatures by the resting cells of L. sakei NRIC 1071\textsuperscript{T} and L. plantarum NRIC 1067\textsuperscript{T}

The activity of L-LDH was detected at 25°C against the resting cells of L. sakei NRIC 1071\textsuperscript{T} cultivated in the absence of sodium acetate in the reaction mixture (Fig. 2). Moreover, the activity was detected at 30 and 35°C to the same extent. In contrast, the activity of D-LDH was detected at 25°C on the cells of L. sakei NRIC 1071\textsuperscript{T} in the reaction mixture. However, the activity was scarcely detected at 30°C, and not at 35°C.

The activity of L-LDH was detected at any of the temperatures tested against the resting cells of L. plantarum NRIC 1067\textsuperscript{T} cultivated in the absence of sodium acetate tested in the reaction mixture (Fig. 2). However, L-LDH was less active at 25°C than at 30 and 35°C. The activity of D-LDH was detected at any of the temperatures tested against the cells of L. plantarum NRIC 1067\textsuperscript{T}.

Activity of L-LDH and D-LDH under aerobic and anaerobic conditions against the resting cells of L. sakei NRIC 1071\textsuperscript{T} and L. plantarum NRIC 1067\textsuperscript{T}

The activity of L-LDH and D-LDH was detected under aerobic and anaerobic conditions on the resting cells of L. sakei NRIC 1071\textsuperscript{T} cultivated in the absence of sodium acetate (Fig. 3). The activity of D-LDH was less active under the aerobic conditions than under anaerobic conditions. In contrast, mostly the same activity of L-LDH and D-LDH was detected under aerobic and anaerobic conditions against the resting cells of L. plantarum NRIC 1067\textsuperscript{T} cultivated in the absence of sodium acetate (Fig. 3).

Production of D-lactic acid from L- or D-lactic acid by the resting cells of L. sakei strains and other lactic acid bacteria

The resting cells of six L. sakei strains and L. curvatus NRIC 1052\textsuperscript{T} cultivated in the absence of sodium acetate scarcely produced D-lactic acid from L-lactic acid or D-lactic acid (Fig. 4). The resting cells of L. paracasei subsp. paracasei NRIC 1044 and L. plantarum NRIC 1067\textsuperscript{T} cultivated in the absence of sodium...
Lactic acid produced by *Lactobacillus sakei*

**Fig. 4.** The production of D-lactic acid from L- or D-lactic acid by the resting cells of *L. sakei* strains and other lactic acid bacteria.

Symbols: hatched bars, L-lactic acid (mm); open bars, D-lactic acid (mm).

acetate produced D-lactic acid (ca. 1:1) from L-lactic acid or D-lactic acid (Fig. 4). The resting cells of the above-mentioned *L. sakei*, *L. curvatus*, *L. paracasei*, and *L. plantarum* strains cultivated in the presence of 50 mM sodium acetate showed the same production pattern as those cultivated in the absence of sodium acetate (data not shown).

**Comparison of LDHs isolated from the cell-free extracts of the cells from *L. sakei* NRIC 1071^T cultivated in the absence of sodium acetate and in the presence of 50 mM sodium acetate**

LDHs were isolated from the cell-free extracts of the cells from *L. sakei* NRIC 1071^T cultivated in the absence of sodium acetate, and purified about 6.3 times. LDHs were isolated from the cell-free extracts of the cells from *L. sakei* NRIC 1071^T cultivated in the presence of 50 mM sodium acetate, and purified about 4.6 times (Table 1). The ratio of the activity of L-LDH to D-LDH could not be determined for the preparations by DEAE Sephadex chromatography and QAE-Toyopearl 550c chromatography because sufficient amounts of LDHs were not recovered for the determination of the activity of L-LDH and D-LDH.

The specific activity of LDHs in the cell-free extracts of the cells from *L. sakei* NRIC 1071^T cultivated in the
Table 1. Purification of LDHs from the cell-free extracts of the cells of *L. sakei* NRIC 1071T cultivated in the absence of sodium acetate and in the presence of 50 mM sodium acetate.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Step</th>
<th>Total activity (mU)</th>
<th>Total protein (mg)</th>
<th>Specific activity (mU/mg)</th>
<th>Fold</th>
<th>Recovery (%)</th>
<th>Ratio</th>
<th>Activity (mU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GYP</td>
<td>Cell-free extracts</td>
<td>6,099.9</td>
<td>8.407</td>
<td>725.6</td>
<td>1.00</td>
<td>100.0</td>
<td>58.5</td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td>Streptomycin sulfate treatment</td>
<td>5,842.7</td>
<td>4.623</td>
<td>1,263.8</td>
<td>1.74</td>
<td>95.8</td>
<td>61.0</td>
<td>39.0</td>
</tr>
<tr>
<td></td>
<td>DEAE Sepharcel chromatography</td>
<td>3,501.4</td>
<td>1.258</td>
<td>2,782.5</td>
<td>3.63</td>
<td>57.4</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>QAE-Toyopearl 550c chromatography</td>
<td>189.1</td>
<td>0.041</td>
<td>4,578.7</td>
<td>6.31</td>
<td>3.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>GYP-Ac</td>
<td>Cell-free extracts</td>
<td>15,613.6</td>
<td>12.546</td>
<td>1,244.5</td>
<td>1.00</td>
<td>100.0</td>
<td>96.6</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Streptomycin sulfate treatment</td>
<td>14,963.8</td>
<td>8.236</td>
<td>1,816.9</td>
<td>1.46</td>
<td>95.8</td>
<td>90.8</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>DEAE Sepharcel chromatography</td>
<td>10,508.0</td>
<td>2.847</td>
<td>3,691.2</td>
<td>2.97</td>
<td>67.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>QAE-Toyopearl 550c chromatography</td>
<td>593.3</td>
<td>0.104</td>
<td>5,732.5</td>
<td>4.61</td>
<td>3.8</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND, Not detected.

Fig. 5. The active staining of α-LDH and β-LDH in the cell-free extracts of *L. sakei* NRIC 1071T and *L. plantarum* NRIC 1067T. Symbols: P, a reaction from pyruvic acid to α- and β-lactic acid; L, a reaction from β-lactic acid to pyruvic acid; D, a reaction from α-lactic acid to pyruvic acid.

The presence of 50 mM sodium acetate was compared with that cultivated in the absence of sodium acetate. The specific activity of LDHs in the cell-free extracts of the cells from *L. sakei* NRIC 1071T cultivated in the presence of 50 mM sodium acetate was about 1.7 times higher compared with that cultivated in the absence of sodium acetate (Table 1). At the step of the QAE-Toyopearl 550c chromatography, the specific activity of LDHs in the cells of *L. sakei* NRIC 1071T cultivated in the presence of 50 mM sodium acetate was about 1.3 times higher compared with that cultivated in the absence of sodium acetate.

Active staining of α-LDH and β-LDH in the cell-free extracts of *L. sakei* NRIC 1071T and *L. plantarum* NRIC 1067T

Two protein bands with the electrophoretic mobility of 0.46 and 0.67 faintly appeared on the cell-free extracts of the cells from *L. sakei* NRIC 1071T in the reaction from pyruvic acid to α- and β-lactic acid (Fig. 5). A protein band with the electrophoretic mobility of 0.48 appeared on the cell-free extracts of the cells from *L. sakei* NRIC 1071T in the reaction from α-lactic acid to pyruvic acid (Fig. 5). Moreover, a protein band with the electrophoretic mobility of 0.68 faintly appeared on the cell-free extracts of the cells from this strain in the reaction from β-lactic acid to pyruvic acid.

Two protein bands with the electrophoretic mobility of 0.50 and 0.65 appeared on the cell-free extracts...
from the cells of *L. plantarum* NRIC 1067\textsuperscript{T} in the reaction from pyruvic acid to \(	ext{l}\)- or \(	ext{o}\)-lactic acid (Fig. 5). Moreover, protein bands with the electrophoretic mobility of 0.51 and 0.65 appeared on the cell-free extracts of the cells from this strain in the reaction from \(	ext{l}\)-lactic acid or \(	ext{o}\)-lactic acid to pyruvic acid, respectively.

Three protein bands appeared on the \(	ext{l}\)-LDH preparation from rabbit muscle, and a protein band with the electrophoretic mobility of 0.66 did on the \(	ext{o}\)-LDH preparation from *L. leichmannii* (Data not shown).

**Discussion**

The activity of \(	ext{l}\)-LDH from the resting cells of *L. sakei* NRIC 1071\textsuperscript{T} appeared at the early stage of the logarithmic phase during the growth at 25\textdegree C, and the activity of \(	ext{o}\)-LDH at the late stage of the logarithmic phase. \(	ext{l}\)-Lactic acid was produced a little later after the onset of the activity of \(	ext{l}\)-LDH. \(	ext{o}\)-Lactic acid was produced later than \(	ext{l}\)-lactic acid, and the amount of \(	ext{o}\)-lactic acid was much less than that of \(	ext{l}\)-lactic acid. This finding shows the participation of \(	ext{o}\)-LDH in the production of \(	ext{o}\)-lactic acid although the activity of \(	ext{o}\)-LDH was weak. In addition, the activity of \(	ext{o}\)-LDH was found on the resting cells of *L. sakei* NRIC 1071\textsuperscript{T} incubated at 25\textdegree C in the reaction mixture, but not at 30 and 35\textdegree C. This will explain that when *L. sakei* NRIC 1071\textsuperscript{T} was cultivated at 30\textdegree C, the type of stereoisomers shifted from the \(	ext{dl}\)-type to the \(	ext{l}\)-type (Iino et al., 2001). Additionally, all of resting cells of *L. sakei* strains tested produced scarcely any \(	ext{dl}\)-lactic acid from \(	ext{l}\)- or \(	ext{o}\)-lactic acid.

Consequently, lactate racemase was not suggested to participate in the production of \(	ext{o}\)-lactic acid by *L. sakei* strains. *L. sakei* IFO 12456 is the same strain that was used for the study of lactate racemase (Kitahara et al., 1957), but this strain did not produce \(	ext{dl}\)-lactic acid from \(	ext{l}\)-lactic acid or \(	ext{o}\)-lactic acid in this study. *L. curvatus* was also reported to produce lactate racemase (Kandler and Weiss, 1986; Stetter and Kandler, 1973), but the resting cells of *L. curvatus* NRIC 1052\textsuperscript{T} barely produced \(	ext{dl}\)-lactic acid. In contrast, the resting cells of *L. paracasei* subsp. *paracasei* NRIC 1044 and *L. plantarum* NRIC 1067\textsuperscript{T} produced \(	ext{dl}\)-lactic acid. However, it is still obscure whether lactate racemase participates in the production of \(	ext{dl}\)-lactic acid or whether \(	ext{l}\)-LDH and \(	ext{o}\)-LDH do. Further studies are needed for these strains.

Stained bands of \(	ext{l}\)-LDH and \(	ext{o}\)-LDH appeared on the cell-free extracts from the cells of *L. sakei* NRIC 1071\textsuperscript{T}. The electrophoretic mobility of the two bands was similar to that of two active bands from the cell-free extracts of *L. plantarum* NRIC 1067\textsuperscript{T}, and the bands respectively corresponded to \(	ext{l}\)-LDH and \(	ext{o}\)-LDH. The presence of \(	ext{l}\)-LDH and \(	ext{o}\)-LDH was described in *L. sakei* (Kandler and Weiss, 1986). In this study also, \(	ext{l}\)-LDH and \(	ext{o}\)-LDH were found in the cells of *L. sakei* NRIC 1071\textsuperscript{T}, while the activity of lactate racemase was not confirmed. Consequently, *L. sakei* strains conclusively produce \(	ext{l}\)- and \(	ext{o}\)-lactic acid by \(	ext{l}\)-LDH and \(	ext{o}\)-LDH, but not by lactate racemase.

The specific activity of LDHs in the cell-free extracts from *L. sakei* NRIC 1071\textsuperscript{T} cultivated in the presence of 50 mM sodium acetate was 1.7 times higher than that cultivated in the absence of sodium acetate. However, the ratio of the specific activity of LDHs from the cells cultivated in the presence of sodium acetate and its absence was reduced to 1.3 on the preparations of the QAE-Toyopearl 550c chromatography. This finding shows that the amount of LDHs in the cells of *L. sakei* NRIC 1071\textsuperscript{T} cultivated in the presence of 50 mM sodium acetate was much more than that in the cells cultivated in the absence of sodium acetate. Consequently, sodium acetate would play a role in the increase of the production of LDHs.

*L. sakei* has been suggested to produce lactate racemase, and described as producing \(	ext{l}\)-lactic acid exclusively when the formation of lactate racemase was repressed by sodium acetate (Hiyama et al., 1968; Kitahara et al., 1957). Recently, lactate racemase was supposed to participate in the production of \(	ext{o}\)-lactic acid by *L. sakei* because a *L. sakei* mutant that was disrupted *ldhL* gene encoded \(	ext{l}\)-LDH produced neither \(	ext{l}\)-lactic acid nor \(	ext{o}\)-lactic acid (Champomier-Verges et al., 2001; Malleret et al., 1998). However, the participation of lactate racemase in the production of \(	ext{o}\)-lactic acid by *L. sakei* has not been clarified, and detailed studies of lactate racemase have not been reported until now. Lactate racemase was named a putative enzyme involved in racemization of lactic acid (Katagiri and Kitahara, 1937; Kitahara et al., 1957). In this paper and a previous paper (Iino et al., 2002), it was mentioned that not only the resting cells of *L. sakei* strains but also the cell-free extracts of *L. sakei* strains produced scarcely any \(	ext{dl}\)-lactic acid from \(	ext{l}\)- or \(	ext{o}\)-lactic acid. Consequently, this leads to the conclusion that lactate racemase does not exist in *L. sakei* strains, and the shift of the type of stereoisomers of
lactic acid produced by *L. sakei* cannot be ascribed to the repression of the formation of lactate racemase by sodium acetate.

The activity of γ-LDH in the cell-free extracts of the cells from *L. sakei* NRIC 1071<sup>T</sup> cultivated in the presence of sodium acetate increased three times or more compared with the activity of the cells cultivated in the absence of sodium acetate (Iino et al., 2002). In contrast, the activity of α-LDH from *L. sakei* NRIC 1071<sup>T</sup> did not show a significant difference between the cells cultivated in the absence of sodium acetate and those in the presence of 50 mM sodium acetate (Iino et al., 2002). Therefore, the increase of the amount of LDHs by sodium acetate explains the increase of the amount of γ-LDH. The good growth of *L. sakei* strains by sodium acetate and the increase of the production of γ-LDH resulted in the shift of the type of stereoisomers of lactic acid produced from the αL-type to the γ-type by the strains.

References


