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Effects of sodium acetate on the production of stereoisomers of lactic acid by *Lactobacillus sakei* and other lactic acid bacteria

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Lactobacillus sakei **and other lactic acid bacteria were studied on the change of the type of stereoisomers (the ratio of L-form to D-form) of lactic acid produced in the presence of sodium acetate and under other cultural conditions. Of 49 strains tested, only *L. sakei* NRIC 1071^T and *L. coryniformis* subsp. *coryniformis* NRIC 1638^T changed the type in the presence of 50 mM sodium acetate compared with the absence of sodium acetate. The type produced by *L. sakei* NRIC 1071^T was shifted 30% or more from the DL-type to the L-type in the presence of 50 mM sodium acetate. *L. sakei* NRIC 1071^T produced not only twice or more the amount of L-lactic acid but decreased the amount of D-lactic acid compared with the absence of sodium acetate. The shift of the DL-type to the L-type by *L. sakei* is due to the high production of L-lactic acid and the low production of D-lactic acid. The type of stereoisomers produced by 11 *L. sakei* strains was also shifted from the DL-type to the L-type in the presence of 50 mM sodium acetate. The shift of stereoisomers by the majority of *L. sakei* strains seems interesting from the viewpoint of the delineation of this species.**

Key Words——effects of sodium acetate; lactate racemase; lactic acid bacteria; *Lactobacillus coryniformis*; *Lactobacillus sakei*; production of lactic acid; type of stereoisomers of lactic acid

Introduction

Lactobacillus sake (sic) was first isolated from a sake starter used in sake production in Japan (Katagiri et al., 1934). Recently, the specific epithet *sake* was corrected to *sakei* according to Latin grammar (International Code of Nomenclature of Bacteria, Rule 12c, Lapage et al. 1992; Trüper and De Clari, 1997). *L. sakei* was found in meat and meat products (Hammes et al., 1990; Kandler and Weiss, 1986; Morishita and Shiromizu, 1986; Samelis et al., 1994), starter cultures for sausage fermentation, and sake starters, sauerkraut and other fermented plant materials (Kandler and Weiss, 1986; Lücke, 1996; Toyoda et al., 1979). In contrast, *L. sakei* caused spoilage in vacuum-packaged vienna sausages (Dykes and von Holy, 1994).

Lactic acid bacteria produce two stereoisomers of lactic acid, L-form and D-form. The type of stereoisomers has been shown as the ratio of L-form to D-form of lactic acid produced, and employed for the classification and identification of lactic acid bacteria (Hammes et al., 1992; Kandler and Weiss, 1986; Kitahara, 1940; Orla-Jensen, 1919, 1942). However, the type produced by *L. sakei* was reported to change with several cultural conditions (Katagiri and Kitahara, 1937), and the type shifted from DL-form to L-form in the presence of sodium acetate (Kitahara et al., 1957;

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Ôbayashi and Kitahara, 1959; Toyoda et al., 1979).

L-Form and D-form of lactic acid are principally produced by L-lactate dehydrogenase [EC 1.1.1.27, L-LDH] and D-lactate dehydrogenase [EC 1.1.1.28, D-LDH], respectively (Gravie, 1980). These enzyme reactions were clarified in case of *Lactobacillus plantarum* (Dennis and Kaplan, 1960; Hiyama et al., 1965; Mizushima et al., 1964). In contrast, *L. sakei* was reported to produce lactate racemase [EC 5.1.2.1]. L-Lactic acid once produced is transformed to D-lactic acid by lactate racemase until the reaction reaches equilibrium, and optically active lactic acid turns to inactive lactic acid in consequence (Hiyama et al., 1968; Katagiri and Kitahara, 1937; Stetter and Kandler, 1973). *L. sakei* was described as producing L-lactic acid exclusively when the formation of lactate racemase was repressed by sodium acetate (Kitahara et al., 1957; Ôbayashi and Kitahara, 1959). However, the effects of sodium acetate on the production of stereoisomers have not been clarified yet. It is of interest to note that the ratio of L-form to D-form of biological substances such as L-lactic acid and D-lactic acid are largely shifted from each other by environmental conditions.

This paper deals with the change of the type of stereoisomer of lactic acid produced by *L. sakei* and other lactic acid bacteria cultivated under various cultural conditions, and with discussion on the characteristics of *L. sakei* from the viewpoint of the type of stereoisomers, especially in the presence of sodium acetate.

Materials and Methods

Bacterial strains. Strains used in this study are listed in Tables 1 and 9.

Cultivation. Strains were cultivated in 5 ml of GYP broth. GYP medium was composed of 10 g of glucose, 10 g of yeast extract (Difco Laboratories, Detroit, MI, USA), 5 g of Polypepton (Nihon Seiyaku Ltd., Tokyo, Japan), 0.025 g of Tween 80, 5 ml of a salt solution, and 1,000 ml of distilled water, and pH was adjusted to 6.8. The salt solution contained 40 mg of $MgSO_4 \cdot 7H_2O$, 2 mg of $MnSO_4 \cdot 4H_2O$, 2 mg of $FeSO_4 \cdot 7H_2O$, and 2 mg of NaCl in 1,000 ml of distilled water. Strains were cultivated at 30°C, but *L. sakei* NRIC 1071^T and NRIC 1764, and other *L. sakei* strains at 25°C. *Lactobacillus acidophilus* NRIC 1547^T, *L. delbrueckii* subsp. *delbrueckii* NRIC 1053^T, *L. fermentum* NRIC 1752^T, *Lactococcus lactis* subsp. *lactis* NRIC 1150^T, *Streptococcus bovis* NRIC 7877^T, *Streptococcus thermophilus* NRIC 1747, and *Sporolactobacillus inulinus* NRIC 1133^T were cultivated at 37°C. The strains were mainly cultivated stationarily for two or three days, and aerobically with shaking for two or three days. Anaerobic conditions were produced by replacing air in test tubes with nitrogen gas and sealing the tubes with tight-fitting rubber stoppers.

Concentration of sodium acetate. Referring to the report of Kitahara et al. (1957), 50 mM of sodium acetate was used throughout this study, unless otherwise mentioned.

Monitoring of bacterial growth. Strains were stationarily precultured in GYP broth for two days. Cells were collected by centrifugation (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 µl of the suspension was inoculated into GYP broth and other liquid media with a pipette. Bacterial growth was monitored spectrophotometrically by reading absorbance at 660 nm with a single-beam spectrophotometer (NOVASPEC II, Amersham Pharmacia Biotech, Tokyo, Japan). If necessary, bacterial growth was monitored every 2 h.

Analysis. After bacteria were cultivated in liquid media for two or three days, cells were collected by centrifugation and discarded. The resulting supernatant was used for analysis. Glucose was determined with the Glucose CII test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The total lactic acid and stereoisomers of lactic acid were determined by the methods described by Otsuka et al. (1994) and Manome et al. (1998). Unless otherwise mentioned, the increase and decrease of lactic acid produced in the presence of sodium acetate were expressed compared with the absence of sodium acetate. Stereoisomers were classified into three types (L-, DL-, and D-) according to the definition of Otsuka et al. (1994) and Manome et al. (1998). Namely, the L-type means a content of 75% L-lactic acid or more in a total of lactic acid; the DL-type means a content of L-lactic acid from 25% to 75%; and the D-type means a content of L-lactic acid of less than 25%. Acetic acid was determined by HPLC. Analytical conditions were as follows: Instrument, Shodex OA (Showa Denko K.K., Tokyo, Japan); column, Ionpak KC-811×2, 8.0 mm ID×300 mm (Showa Denko K.K.); eluent, 3 mM $HClO_4$; flow rate, 1.0 ml/min; pressure, 42 kgf/cm^2; reagent, 1/10 ST3-R

(0.7 ml/min) (Showa Denko K.K.); column temperature, 60°C; detector, Shodex VD-1 (430 nm) (Showa Denko K.K.); and a sample volume, 20 µl.

Results

Effects of sodium acetate on the production of stereoisomers of lactic acid by a variety of lactic acid bacteria

All strains tested grew in the presence of 50 mM sodium acetate. Of 49 strains tested, 23 strains produced 1.5 times or more the amount of lactic acid in the presence of 50 mM sodium acetate than in the absence of sodium acetate, and 9 strains produced from 1.2 to 1.5 times the amount of lactic acid. The other 17 strains produced almost the same amount of lactic acid compared with the absence of sodium acetate (Table 1).

L. sakei NRIC 1071^T produced twice or more the amount of L-lactic acid and about one-third the amount of D-lactic acid in the presence of 50 mM sodium acetate as in the absence of sodium acetate. *L. coryniformis* subsp. *coryniformis* NRIC 1638^T produced about twice the amount of D-lactic acid and about one-third the amount of L-lactic acid in the presence of 50 mM sodium acetate. *L. fermentum* NRIC 1752^T produced two-thirds the amount of D-lactic acid and almost the same amount of L-lactic acid in the presence of 50 mM sodium acetate as in the absence of sodium acetate. *Pediococcus acidilactici* NRIC 0097^T produced about twice the amount of L-lactic acid and almost the same amount of D-lactic acid in the presence of 50 mM sodium acetate as in the absence of sodium acetate. *Sporolactobacillus inulinus* NRIC 1133^T produced about three times the amount of D-lactic acid in the presence of 50 mM sodium acetate. This strain barely produced L-lactic acid in the presence of 50 mM sodium acetate. *L. plantarum* NRIC 1067^T produced L- and D-lactic acid to the same extent in the presence of 50 mM sodium acetate. The remaining 44 strains produced different amounts of L- or D-lactic acid in the presence of 50 mM sodium acetate but the type of stereoisomers slightly changed.

Effects of sodium acetate on the production of stereoisomers of lactic acid during the growth

L. sakei NRIC 1071^T grew in the absence of sodium acetate, and started producing L-lactic acid at an early stage of the logarithmic phase and produced D-lactic acid at a late stage of the logarithmic phase (Fig. 1A). This strain grew well in the presence of 50 mM sodium acetate, and produced L-lactic acid in parallel with the growth but barely produced D-lactic acid. Finally, twice the L-lactic acid or more produced compared with the absence of sodium acetate (Fig. 1B). *L. sakei* NRIC 1764 grew, and produced L-lactic acid but barely produced D-lactic acid, regardless of the presence of sodium acetate (Fig. 1C, D). *L. coryniformis* subsp. *coryniformis* NRIC 1638^T grew in the absence of sodium acetate, and produced L-lactic acid and D-lactic acid concomitantly. The D-lactic acid represented two-thirds of the total lactic acid (Fig. 1E). This strain grew, and produced D-lactic acid along with the growth and a small amount of L-lactic acid in the presence of 50 mM sodium acetate (Fig. 1F). *L. curvatus* NRIC 1052^T grew, and produced L- and D-lactic acid concomitantly in the absence of sodium acetate. In contrast, this strain produced L-lactic acid earlier in the logarithmic phase than D-lactic acid in the presence of 50 mM sodium acetate (Fig. 1G, H). *L. plantarum* NRIC 1067^T grew, regardless of the presence of 50 mM sodium acetate, and produced L- and D-lactic acid concomitantly to the same extent (Fig. 1I, J).

Effects of concentrations of sodium acetate on the production of stereoisomers of lactic acid

L. sakei NRIC 1071^T grew well as the concentration of sodium acetate increased. This strain produced twice or more the amount of L-lactic acid and a smaller amount of D-lactic acid in the presence of 50 or 100 mM sodium acetate than in the absence of sodium acetate (Table 2). However, this strain produced 1.4 times or more the amount of L-lactic acid and 1.5 times or more the amount of D-lactic acid in the presence of 10 or 20 mM sodium acetate. *L. coryniformis* subsp. *coryniformis* NRIC 1638^T grew well, and produced a larger amount of D-lactic acid while decreasing the amount of L-lactic acid as the concentration of sodium acetate increased. *L. plantarum* NRIC 1067^T grew at any concentration of sodium acetate, and produced L- and D-lactic acid to the same extent.

Effects of addition of sodium acetate at different growth phases on the production of stereoisomers of lactic acid by L. sakei NRIC 1071^T

L. sakei NRIC 1071^T grew well, and produced 1.5 times or more the amount of lactic acid when 50 mM sodium acetate was added at any phase during the

Table 1. Effects of sodium acetate on the production of stereoisomers of lactic acid by a variety of lactic acid bacteria.[a]

Species	Strains[b]	Presence of NaAc[c]	Concentration of lactic acid (mM)			Ratio of stereoisomers		Type of stereoisomers	Remarks[d]
			Total	L-Form	D-Form	L (%)	D (%)		
Lactobacillus acidophilus	NRIC 1547^T	WO[e]	43.5	22.0	21.5	50.6	49.4	DL	B
		W[f]	58.0	29.8	28.2	51.4	48.6	DL	
Lactobacillus alimentarius	NRIC 1640^T	WO	41.9	36.0	5.9	86.0	14.0	L[g]	A
		W	87.7	72.6	15.1	82.8	17.2	L	
Lactobacillus brevis	NRIC 1684^T	WO	24.0	14.3	9.7	59.7	40.3	DL[g]	A
		W	48.5	30.4	18.1	62.7	37.3	DL	
Lactobacillus buchneri	NRIC 1040^T	WO	52.0	24.3	27.7	46.8	53.2	DL[g]	C
		W	50.3	26.0	24.3	51.7	48.3	DL	
Lactobacillus casei subsp. *casei*	NRIC 1042^T	WO	74.1	69.8	4.3	94.2	5.8	L[g]	B
		W	95.8	90.8	5.0	94.8	5.2	L	
Lactobacillus coryniformis subsp. *coryniformis*	NRIC 1638^T	WO	65.6	21.3	44.3	32.5	67.5	DL[g]	A
		W	99.6	8.5	91.1	8.5	91.5	D	
Lactobacillus coryniformis subsp. *torquens*	NRIC 1051^T	WO	36.5	2.8	33.7	7.8	92.2	D[g]	A
		W	60.0	3.9	56.1	6.5	93.5	D	
Lactobacillus curvatus	NRIC 1052^T	WO	73.5	37.1	36.4	50.5	49.5	DL[g]	B
		W	106.0	57.0	49.0	53.8	46.2	DL	
Lactobacillus delbrueckii subsp. *delbrueckii*	NRIC 1053^T	WO	109.7	14.0	95.7	12.8	87.2	D[g]	C
		W	107.9	13.3	94.6	12.3	87.7	D	
Lactobacillus fermentum	NRIC 1752^T	WO	52.7	28.8	23.9	54.7	45.3	DL[g]	C
		W	47.5	30.6	16.9	64.5	35.5	DL	
Lactobacillus frigidus	NRIC 1079	WO	31.3	14.1	17.2	45.1	54.9	DL[g]	B
		W	46.9	21.9	25.0	46.6	53.4	DL	
Lactobacillus fructivorans	NRIC 0224^T	WO	48.5	29.9	18.6	61.7	38.3	DL[g]	C
		W	45.5	30.0	15.5	66.0	34.0	DL	
Lactobacillus malefermentans	NRIC 1081^T	WO	34.2	19.4	14.8	56.7	43.3	DL[g]	B
		W	46.0	26.8	19.2	58.2	41.8	DL	
Lactobacillus mali	NRIC 1076^T	WO	55.5	46.5	9.0	83.8	16.2	L[g]	A
		W	95.9	82.7	13.2	86.2	13.8	L	
Lactobacillus paracasei subsp. *paracasei*	NRIC 1044	WO	112.9	56.7	56.2	50.2	49.8	DL[g]	C
		W	105.5	53.3	52.2	50.5	49.5	DL	
Lactobacillus paracasei subsp. *paracasei*	NRIC 1046	WO	103.6	97.3	6.3	93.9	6.1	L[g]	C
		W	98.8	92.8	6.0	93.9	6.1	L	
Lactobacillus parvus	NRIC 1082	WO	13.6	8.5	5.1	62.4	37.6	DL[g]	A
		W	40.7	23.3	17.4	57.2	42.8	DL	
Lactobacillus pentosus	NRIC 1069^T	WO	92.8	46.5	46.3	50.1	49.9	DL[g]	C
		W	100.8	49.9	50.9	49.5	50.5	DL	
Lactobacillus plantarum	NRIC 1067^T	WO	115.0	57.0	58.0	49.6	50.4	DL[g]	C
		W	106.3	51.3	55.0	48.3	51.7	DL	
Lactobacillus rhamnosus	NRIC 1043^T	WO	92.6	87.2	5.4	94.2	5.8	L[g]	C
		W	99.9	95.2	4.7	95.3	4.7	L	
Lactobacillus sakei	NRIC 1071^T	WO	37.6	23.8	13.8	63.4	36.6	DL[g]	A
		W	89.5	84.3	5.2	94.2	5.8	L	

Table 1. (Continued)

Species	Strains[b]	Presence of NaAc[c]	Concentration of lactic acid (mM)			Ratio of stereoisomers		Type of stereoisomers	Remarks[d]
			Total	L-Form	D-Form	L (%)	D (%)		
Lactobacillus sakei	NRIC 1764	WO	44.6	42.4	2.2	95.1	4.9	L[g]	A
		W	96.8	95.6	1.2	98.8	1.2	L	
Lactobacillus viridescens	NRIC 1536^T	WO	17.1	3.5	13.6	20.3	79.7	D	A
		W	38.7	6.2	32.5	16.1	83.9	D	
Leuconostoc carnosum	NRIC 1772^T	WO	23.6	1.4	22.2	6.0	94.0	D[g]	A
		W	49.7	3.9	45.8	7.8	92.2	D	
Leuconostoc fallax	NRIC 0210^T	WO	42.5	1.7	40.8	3.9	96.1	D[g]	C
		W	50.1	1.5	48.6	3.0	97.0	D	
Leuconostoc mesenteroides subsp. *mesenteroides*	NRIC 1541^T	WO	42.4	1.8	40.6	4.3	95.7	D[g]	B
		W	53.1	2.0	51.1	3.8	96.2	D	
Leuconostoc mesenteroides subsp. *dextranicum*	NRIC 1539^T	WO	33.7	2.9	32.0	8.5	95.0	D[g]	B
		W	47.6	4.5	45.8	9.5	96.3	D	
Leuconostoc mesenteroides subsp. *dextranicum*	NRIC 1085	WO	36.3	1.8	34.5	5.0	95.0	D	B
		W	46.7	1.7	45.0	3.7	96.3	D	
Weissella confusa	NRIC 1544	WO	46.5	30.5	16.0	65.6	34.4	DL[g]	C
		W	45.8	32.2	13.6	70.3	29.7	DL	
Weissella halotolerans	NRIC 1627^T	WO	42.9	15.6	27.3	36.4	63.6	DL[g]	C
		W	49.1	19.1	30.0	38.8	61.2	DL	
Weissella paramesenteroides	NRIC 1542^T	WO	36.1	0.6	35.5	1.7	98.3	D	C
		W	38.1	0.6	37.5	1.7	98.3	D	
Pediococcus acidilactici	NRIC 0097^T	WO	53.7	28.5	25.2	53.1	46.9	DL[g]	A
		W	92.6	60.5	32.1	65.3	34.7	DL	
Pediococcus acidilactici	NRIC 0115	WO	51.9	30.1	21.8	58.0	42.0	DL[g]	A
		W	84.4	48.7	35.7	57.7	42.3	DL	
Pediococcus damnosus	NRIC 0214^T	WO	80.7	42.5	38.2	52.6	47.4	DL	C
		W	80.3	47.1	33.2	58.6	41.4	DL	
Pediococcus dextrinicus	NRIC 0096^T	WO	32.6	31.4	1.2	96.3	3.7	L	A
		W	61.0	59.4	1.6	97.4	2.6	L	
Pediococcus inopinatus	NRIC 1773^T	WO	58.5	29.0	29.5	49.5	50.5	DL	B
		W	82.6	41.1	41.5	49.7	50.3	DL	
Pediococcus parvulus	NRIC 0095^T	WO	81.7	37.4	44.3	45.8	54.2	DL	C
		W	80.0	31.5	48.5	39.4	60.6	DL	
Pediococcus pentosaceus	NRIC 0099^T	WO	63.0	31.8	31.2	50.4	49.6	DL	C
		W	61.9	32.2	29.7	52.0	48.0	DL	
Enterococcus durans	NRIC 0274^T	WO	34.3	33.5	0.8	97.6	2.4	L[g]	A
		W	71.8	69.7	2.1	97.1	2.9	L	
Enterococcus faecalis	NRIC 1142^T	WO	32.0	30.7	1.3	96.0	4.0	L[g]	A
		W	53.3	51.2	2.1	96.1	3.9	L	
Enterococcus faecium	NRIC 1145^T	WO	32.2	30.9	1.3	96.1	3.9	L[g]	A
		W	54.9	53.3	1.6	97.0	3.0	L	
Lactococcus lactis subsp. *lactis*	NRIC 1150	WO	33.1	32.0	1.1	96.8	3.2	L[g]	A
		W	59.1	58.5	0.6	99.0	1.0	L	

Table 1. (Continued)

Species	Strains[b]	Presence of NaAc[c]	Concentration of lactic acid (mM)			Ratio of stereoisomers		Type of stereoisomers	Remarks[d]
			Total	L-Form	D-Form	L (%)	D (%)		
Lactococcus raffinolactis	NRIC 1781^T	WO	16.1	15.9	0.2	99.0	1.0	L	A
		W	40.8	40.6	0.2	99.4	0.6	L	
Streptococcus agalactiae	NRIC 1137^T	WO	15.1	14.9	0.2	98.8	1.2	L	A
		W	32.7	32.5	0.2	99.4	0.6	L	
Streptococcus bovis	NRIC 1535	WO	23.3	22.7	0.6	97.6	2.4	L	A
		W	54.7	54.2	0.5	99.1	0.9	L	
Streptococcus equinus	NRIC 1139^T	WO	10.4	10.3	0.1	98.6	1.4	L	A
		W	39.3	39.1	0.2	99.4	0.6	L	
Streptococcus thermophilus	NRIC 1747	WO	37.5	37.0	0.5	98.6	1.4	L	C
		W	31.1	30.7	0.4	98.7	1.3	L	
Sporolactobacillus inulinus	NRIC 1133^T	WO	36.7	1.6	35.1	4.4	95.6	D[g]	A
		W	103.6	1.6	102.0	1.5	98.5	D	
Bacillus coagulans	NRIC 1005^T	WO	32.0	30.1	1.9	94.0	6.0	L[g]	A
		W	70.5	68.2	2.3	96.8	3.2	L	

[T] Type strain.

[a] After two-day cultures.

[b] NRIC, Culture Collection Center, Tokyo University of Agriculture, Tokyo.

[c] NaAc, sodium acetate.

[d] A, produced from 1.2 to 1.5 times the amount of lactic acid in the presence of 50 mM sodium acetate compared with the absence of sodium acetate; B, produced 1.5 times or more; C, produced almost the same amount of lactic acid as the absence of sodium acetate.

[e] WO, without sodium acetate.

[f] W, with 50 mM sodium acetate.

[g] Cited from *J. Gen. Appl. Microbiol.*, **44**, 371–374 (1998).

growth (Fig. 2A, B, C, D). This strain produced twice or more the amount of L-lactic acid and barely produced D-lactic acid compared with no addition of sodium acetate, regardless of growth phase.

Effects of the salt of organic acids other than sodium acetate on the production of stereoisomers of lactic acid

L. sakei NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T grew well in the presence of the salt of most organic acids (Table 3). However, *L. sakei* NRIC 1071^T and *L. coryniformis* subsp. *coryniformis* NRIC 1638^T grew poorly in the presence of 50 mM trisodium citrate (data not shown). *L. sakei* NRIC 1071^T consumed 1.3 times or more the amount of glucose in the presence of the salt of organic acids other than sodium fumarate, and produced 1.3 times or more the amount of lactic acid. In particular, this strain produced about three times the amount of lactic acid in the presence of 50 mM sodium L-malate compared with the basal medium. *L. sakei* NRIC 1071^T produced about twice the amount of L-lactic acid and almost the same amount of D-lactic acid in the presence of 20 mM trisodium citrate compared with the absence of the salt of organic acids (Table 3). *L. coryniformis* subsp. *coryniformis* NRIC 1638^T consumed 1.2 times or more the amount of glucose in the presence of the salt of organic acids other than sodium fumarate, and produced 1.3 times or more the amount of lactic acid. This strain produced twice or more the amount of lactic acid in the presence of 20 mM trisodium citrate, 50 mM sodium L-malate, or 50 mM sodium oxalacetate. *L. coryniformis* subsp. *coryniformis* NRIC 1638^T produced L- and D-lactic acid to the same extent in the presence of the salt of most organic acids. *L. plantarum* NRIC 1067^T produced 1.5 times or more the amount of lactic acid in the presence of 50 mM sodium L-malate. This strain produced L- and D-

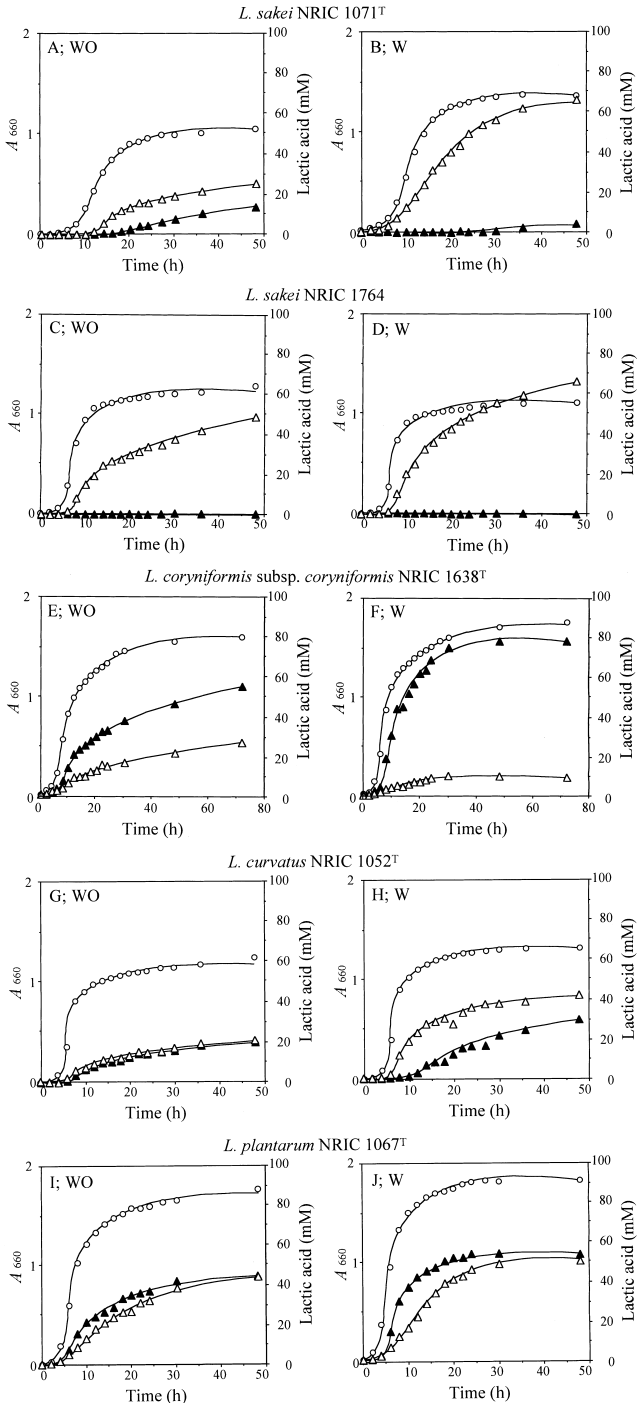

Fig. 1. Effects of sodium acetate on the production of stereoisomers of lactic acid during the growth.

Symbols: ○; Absorbance at 660 nm, △; the production of L-lactic acid, ▲; D-lactic acid, WO; GYP broth, W; GYP broth containing 50 mM sodium acetate.

lactic acid to the same extent in the presence of the salt of most organic acids. No effect of sodium chloride was found on the growth of the above three strains tested or the production of stereoisomers of lactic acid.

Effects of L-, DL-, and D-lactic acid on the production of stereoisomers of lactic acid

L. sakei NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T grew under the addition of L-, DL-, or D-lactic acid. When the amount of lactic acid added was subtracted from the total amount of lactic acid, the above three strains consumed sugar to the same extent compared with no addition of lactic acid, and produced almost the same amount of lactic acid (Table 4).

When the amount of lactic acid added was subtracted from the total amount of lactic acid determined, *L. sakei* NRIC 1071^T produced about three times the amount of D-lactic acid and barely produced L-lactic acid under the addition of L-lactic acid. Remarkably, this strain utilized L-lactic acid between the lag phase and the logarithmic phase under the addition of L-lactic acid, and increased L-lactic acid a little later (Fig. 3A, B, C). In contrast, *L. sakei* NRIC 1071^T produced about 1.5 times the amount of L-lactic acid and did not produce D-lactic acid under the addition of D-lactic acid (Table 4). Furthermore, this strain produced L-lactic acid exclusively and did not utilize D-lactic acid under the addition of D-lactic acid. *L. sakei* NRIC 1071^T produced about 1.5 times the amount of L-lactic acid and did not produce D-lactic acid with the addition of DL-lactic acid compared with no addition of DL-lactic acid. *L. coryniformis* subsp. *coryniformis* NRIC 1638^T produced about 1.5 times the amount of D-lactic acid and did not produce L-lactic acid under the addition of L-lactic acid. In contrast, this strain produced almost the same amount of L- and D-lactic acid under the addition of D- or DL-lactic acid compared with no addition of D- or DL-lactic acid (Table 4). *L. plantarum* NRIC 1067^T produced 1.5 times or more the amount of D-lactic acid and about one-fourth the amount of L-lactic acid under the addition of L-lactic acid compared with no addition of lactic acid. Conversely, *L. plantarum* NRIC 1067^T produced about twice the amount of L-lactic acid and about half the amount of D-lactic acid under the addition of D-lactic acid compared with no addition of D-lactic acid (Table 4). This strain utilized L-lactic acid between the lag phase and the logarithmic phase under the addition of L-lactic acid, and increased L-lactic acid

Table 2. Effects of the concentration of sodium acetate on the production of stereoisomers of lactic acid produced by *L. sakei* NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T.

Strains	Presence of NaAc[c]	Absorbance at 660 nm	Consumed glucose (mM)[d]	Concentration of lactic acid			Ratio of stereoisomers		Type of stereoisomers
				Total	L-Form	D-Form	L (%)	D (%)	
L. sakei NRIC 1071^T[a]	None	0.909	24.3	44.9	28.6	16.3	63.6	36.4	DL
	10 mM	1.077	31.9	64.7	39.5	25.2	61.0	39.0	DL
	20 mM	1.182	37.9	75.5	51.3	24.2	68.0	32.0	DL
	50 mM	1.313	47.7	81.4	67.4	14.0	82.8	17.2	L
	100 mM	1.388	46.7	78.3	76.8	1.5	98.1	1.9	L
L. coryniformis subsp.	None	1.325	32.1	59.9	22.8	37.1	38.1	61.9	DL
coryniformis NRIC 1638^T[b]	10 mM	1.440	40.3	72.9	16.8	56.1	23.1	76.9	D
	20 mM	1.461	44.8	76.3	13.3	63.0	17.4	82.6	D
	50 mM	1.516	53.0	84.0	10.0	74.0	11.9	88.1	D
	100 mM	1.540	52.9	87.4	8.3	79.1	9.5	90.5	D
L. plantarum NRIC 1067^T[a]	None	1.768	51.8	113.5	55.4	58.1	48.8	51.2	DL
	10 mM	1.839	52.6	102.1	45.8	54.2	46.9	53.1	DL
	20 mM	1.868	52.8	102.4	46.0	55.2	46.1	53.9	DL
	50 mM	1.842	53.1	102.4	46.0	57.3	44.0	56.0	DL
	100 mM	1.828	53.1	101.4	45.5	59.0	41.9	58.1	DL

[a] After two-day cultures.
[b] After three-day cultures.
[c] NaAc, sodium acetate.
[d] The initial concentration of glucose was 53.3 mM.

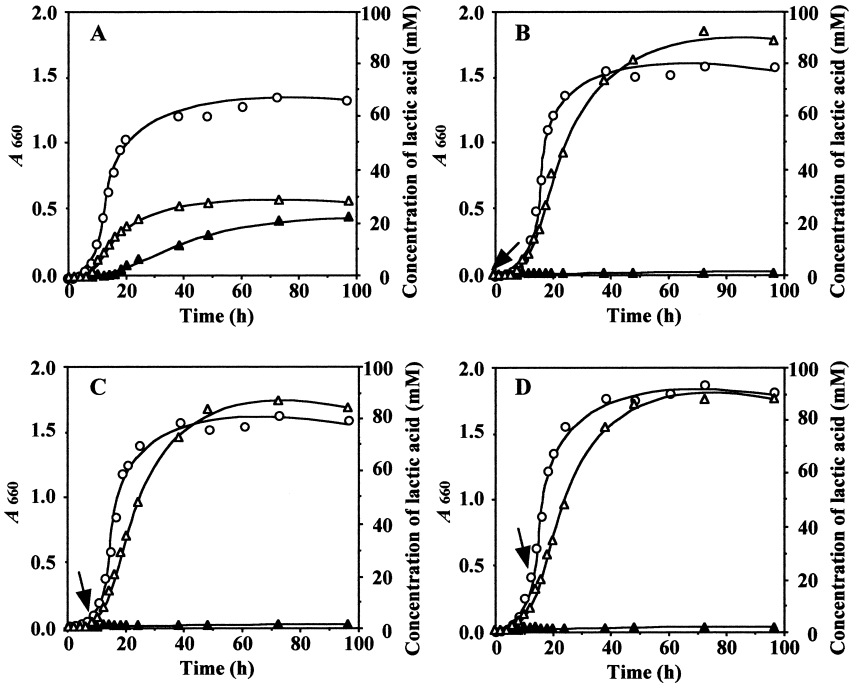

Fig. 2. Effects of addition of sodium acetate at different growth phases on the production of stereoisomers of lactic acid by *L. sakei* NRIC 1071^T.

Symbols: ○; Absorbance at 660 nm, △; L-lactic acid, ▲; D-lactic acid. A; Basal medium, B; 0 h, C; 6 h, D; 12 h.

Table 3. Effects of the salt of organic acids and sodium chloride on the production of stereoisomers of lactic acid by *L. sakei* NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T.

Strains	Salt of organic acid (50 mM)	Absorbance at 660 nm	Consumed glucose (mM)[c]	Concentration of lactic acid (mM)			Ratio of stereoisomers		Type of stereoisomers
				Total	L-Form	D-Form	L (%)	D (%)	
L. sakei NRIC 1071^{T,a}	Basal medium	0.924	23.7	41.8	26.0	15.8	62.1	37.9	DL
	Sodium acetate trihydrate	1.312	43.6	78.5	74.5	4.0	94.9	5.1	L
	Sodium pyruvate	1.411	46.9	86.7	52.7	34.0	60.8	39.2	DL
	Trisodium citrate[d]	1.103	41.6	65.9	50.8	15.1	77.1	22.9	L
	Sodium α-ketoglutarate	1.229	39.4	72.1	38.5	33.6	53.4	46.6	DL
	Sodium succinate	1.085	38.2	66.5	34.8	31.7	52.4	47.6	DL
	Sodium fumarate	0.975	25.6	51.0	27.3	23.7	53.6	46.4	DL
	Sodium L(−)-malate	1.516	42.5	120.7	63.7	57.0	52.8	47.2	DL
	Sodium oxalacetate	1.615	50.1	99.4	60.0	39.4	60.3	39.7	DL
	Sodium gluconate	1.019	31.1	55.4	30.1	25.3	54.4	45.6	DL
	Sodium chloride	0.772	22.8	39.9	22.2	17.7	55.7	44.3	DL
L. coryniformis subsp. *coryniformis* NRIC 1638^{T,b}	Basal medium	1.362	29.2	49.5	19.2	30.2	38.9	61.1	DL
	Sodium acetate trihydrate	1.525	53.9	91.2	15.7	75.6	17.2	82.8	D
	Sodium pyruvate	1.549	53.8	96.5	44.5	51.9	46.2	53.8	DL
	Trisodium citrate[d]	1.457	47.9	132.5	64.2	68.3	48.4	51.6	DL
	Sodium α-ketoglutarate	1.506	51.0	90.6	35.1	55.5	38.7	61.3	DL
	Sodium succinate	1.386	47.7	80.2	36.7	43.4	45.8	54.2	DL
	Sodium fumarate	1.250	31.2	77.3	36.0	41.3	46.6	53.4	DL
	Sodium L(−)-malate	1.554	53.9	134.6	67.4	67.2	50.1	49.9	DL
	Sodium oxalacetate	1.632	53.2	103.2	57.9	45.3	56.1	43.9	DL
	Sodium gluconate	1.363	35.4	63.0	23.5	39.5	37.4	62.6	DL
	Sodium chloride	1.247	31.5	55.7	20.0	35.7	35.9	64.1	DL
L. plantarum NRIC 1067^{T,a}	Basal medium	1.765	50.5	89.4	44.2	45.2	49.5	50.5	DL
	Sodium acetate trihydrate	1.836	53.3	101.9	46.9	55.0	46.0	54.0	DL
	Sodium pyruvate	1.776	52.7	90.4	39.7	50.7	43.9	56.1	DL
	Trisodium citrate	1.601	54.0	96.1	47.4	48.7	49.3	50.7	DL
	Sodium α-ketoglutarate	1.842	51.1	85.3	36.1	49.2	42.4	57.6	DL
	Sodium succinate	1.703	53.2	99.8	49.9	49.9	50.0	50.0	DL
	Sodium fumarate	1.809	53.4	106.4	54.0	52.4	50.7	49.3	DL
	Sodium L(−)-malate	1.814	53.4	144.8	70.9	73.9	49.0	51.0	DL
	Sodium oxalacetate	1.642	53.3	107.1	47.6	59.5	44.4	55.6	DL
	Sodium gluconate	1.830	52.5	115.1	57.4	57.7	49.9	50.1	DL
	Sodium chloride	1.702	48.7	89.0	44.7	44.3	50.2	49.8	DL

[a] After two-day cultures.
[b] After three-day cultures.
[c] The initial concentration of glucose was 53.3 mM.
[d] 20 mM.

a little later. (Fig. 3D, E, F). *L. plantarum* NRIC 1067^T produced L- and D-lactic acid to the same extent under the addition of DL-lactic acid.

Effects of buffers on the production of stereoisomers of lactic acid

L. sakei NRIC 1071^T grew in the presence of Na_2HPO_4-NaH_2PO_4 buffer, K_2HPO_4-KH_2PO_4 buffer, and KH_2PO_4-NaOH buffer, and grew particularly well in the presence of an acetic acid-sodium acetate buffer

Table 4. Effects of the salt of lactic acid on the production of stereoisomers of lactic acid by *L. sakei* NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T.

Strains	Salt of organic acid (50 mM)	Absorbance at 660 nm	Consumed glucose (mM)[c]	Concentration of lactic acid (mM)[d]			Ratio of stereoisomers		Type of stereoisomers
				Total	L-Form	D-Form	L (%)	D (%)	
L. sakei NRIC 1071^{T,a}	Basal medium	0.924	23.7	41.8	26.0	15.8	62.1	37.9	DL
	Lithium L(+)-lactic acid	0.964	23.4	88.9	44.9	44.0	0.0	100.0	D
	Lithium D(−)-lactic acid	0.978	21.1	81.8	39.0	42.8	100.0	0.0	L
	Lithium DL-lactic acid	0.985	21.4	84.6	61.5	23.1	100.0	0.0	L
L. coryniformis subsp. *coryniformis* NRIC 1638^{T,b}	Basal medium	1.362	29.2	49.5	19.2	30.2	38.9	61.1	DL
	Lithium L(+)-lactic acid	1.281	27.6	95.6	47.6	48.0	0.0	100.0	D
	Lithium D(−)-lactic acid	1.320	29.6	94.7	20.3	74.4	40.5	59.5	DL
	Lithium DL-lactic acid	1.278	28.2	94.3	39.5	54.9	33.9	66.1	DL
L. plantarum NRIC 1067^{T,a}	Basal medium	1.765	50.5	89.4	44.2	45.2	49.5	50.5	DL
	Lithium L(+)-lactic acid	1.724	49.2	149.4	74.5	74.9	14.0	86.0	D
	Lithium D(−)-lactic acid	1.756	49.8	140.7	67.2	73.5	76.2	23.8	L
	Lithium DL-lactic acid	1.776	49.7	144.8	71.6	73.2	49.1	50.9	DL

[a] After two-day cultures.
[b] After three-day cultures.
[c] The initial concentration of glucose was 53.3 mM.
[d] The amount of lactic acid added was subtracted from a total amount of lactic acid.

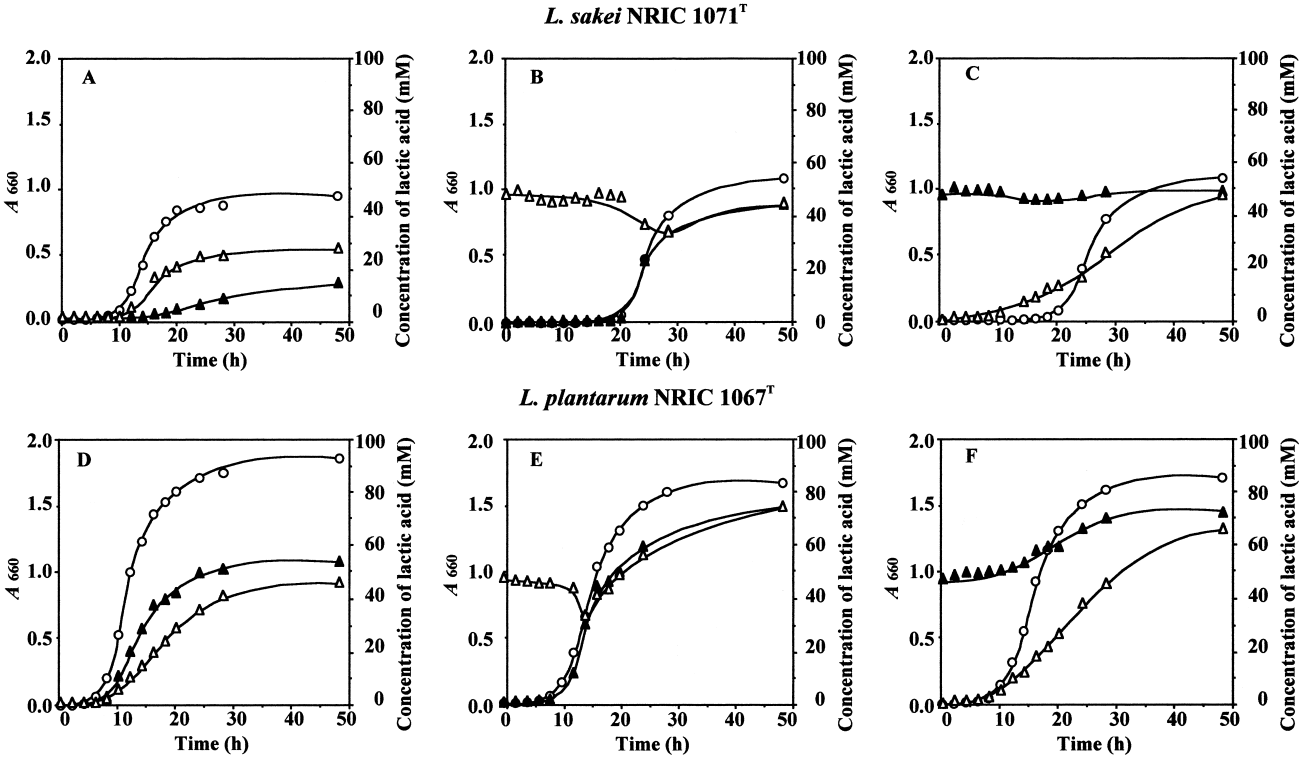

Fig. 3. Effects of L- and D-lactic acid on the production of stereoisomers of lactic acid by *L. sakei* NRIC 1071^T and *L. plantarum* NRIC 1067^T.

Symbols: ○; Absorbance at 660 nm, △; L-lactic acid, ▲; D-lactic acid. A and D; GYP broth, B and E; GYP broth containing 50 mM L-lactic acid, C and F; GYP broth containing 50 mM D-lactic acid.

Table 5. Effects of buffers on the production of stereoisomers of lactic acid by *L. sakei* NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T.

Strains	Buffer solutions	Absorbance at 660 nm	Final pH	Concentration of lactic acid (mM)			Ratio of stereoisomers		Type of stereoisomers
				Total	L-Form	D-Form	L (%)	D (%)	
L. sakei NRIC 1071^{T,a}	Basal medium	0.924	3.80	41.8	26.0	15.8	62.1	37.9	DL
	Na_2HPO_4-NaH_2PO_4 buffer	0.941	4.08	73.3	37.8	35.5	51.5	48.5	DL
	K_2HPO_4-KH_2PO_4 buffer	0.944	4.06	67.5	34.7	32.8	51.4	48.6	DL
	KH_2PO_4-NaOH buffer	0.987	3.89	60.7	31.2	29.5	51.4	48.6	DL
	Acetic acid-sodium acetate buffer	1.298	4.05	82.7	75.8	6.9	91.6	8.4	L
	Citric acid-sodium citrate buffer[c]	1.101	4.14	62.2	42.2	20.0	67.9	32.1	DL
L. coryniformis subsp. *coryniformis* NRIC 1638^{T,b}	Basal medium	1.298	3.61	51.1	19.7	31.4	38.6	61.4	DL
	Na_2HPO_4-NaH_2PO_4 buffer	1.449	3.94	90.3	43.9	46.4	48.6	51.4	DL
	K_2HPO_4-KH_2PO_4 buffer	1.448	3.90	89.1	43.2	45.9	48.5	51.5	DL
	KH_2PO_4-NaOH buffer	1.525	3.72	79.8	35.6	44.2	44.6	55.4	DL
	Acetic acid-sodium acetate buffer	1.530	4.00	89.8	14.2	75.6	15.8	84.2	D
	Citric acid-sodium citrate buffer[c]	1.472	3.99	77.5	39.7	37.8	51.2	48.8	DL
L. plantarum NRIC 1067^{T,a}	Basal medium	1.731	3.37	93.2	45.7	47.5	49.1	50.9	DL
	Na_2HPO_4-NaH_2PO_4 buffer	1.697	3.89	91.2	43.9	47.3	48.1	51.9	DL
	K_2HPO_4-KH_2PO_4 buffer	1.690	3.87	90.5	44.4	46.1	49.0	51.0	DL
	KH_2PO_4-NaOH buffer	1.730	3.66	91.1	44.5	46.6	48.9	51.1	DL
	Acetic acid-sodium acetate buffer	1.850	3.98	96.9	44.6	52.3	46.0	54.0	DL
	Citric acid-sodium citrate buffer	1.476	4.75	92.0	45.5	46.5	49.5	50.5	DL

[a] After two-day cultures.
[b] After three-day cultures.
[c] 20 mM.

and a citric acid-sodium citrate buffer. *L. coryniformis* subsp. *coryniformis* NRIC 1638^T and *L. plantarum* NRIC 1067^T grew well in the presence of all of the buffers (Table 5).

L. sakei NRIC 1071^T produced 1.5 times or more the amount of lactic acid in the presence of any of the buffers compared with a basal medium. This strain produced L- and D-lactic acid to the same extent in the presence of a buffer other than the acetic acid-sodium acetate buffer. *L. sakei* NRIC 1071^T produced about three times the amount of L-lactic acid and about half the amount of D-lactic acid in the presence of the acetic acid-sodium acetate buffer compared with a basal medium (Table 5). *L. coryniformis* subsp. *coryniformis* NRIC 1638^T produced 1.5 times or more the amount of lactic acid in the presence of any of the buffers. This strain produced L- and D-lactic acid to the same extent in the presence of a buffer other than the acetic acid-sodium acetate buffer (Table 5). *L. plantarum* NRIC 1067^T scarcely changed the production of stereoisomers in the presence of any of the buffers (Table 5).

Effects of the source of sugars on the production of stereoisomers of lactic acid

L. sakei NRIC 1071^T grew at the expense of L-arabinose, D-fructose, D-glucose, maltose, D-mannose, melibiose, D-ribose, sucrose, or D-trehalose as a sole source of sugar, and poorly at the expense of D-galactose or lactose. *L. sakei* NRIC 1071^T produced about two-thirds the amount of lactic acid and produced acetic acid concomitantly from L-arabinose or D-ribose compared with D-glucose. Furthermore, this strain produced almost the same amount of L-lactic acid and half or less the amount of D-lactic acid at the expense of L-arabinose and D-ribose. Moreover, *L. sakei* NRIC 1071^T produced 1.4 times or more the amount of L-lactic acid and one-fourth the amount of D-lactic acid at the expense of D-trehalose. The production of L- and D-lactic acid by this strain was barely changed when sugars other than L-arabinose, D-ribose, or trehalose were used (Table 6).

Table 6. Effects of sugars on the production of stereoisomers of lactic acid by *L. sakei* NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T.

Strains	Sugars	Absorbance at 660 nm	Concentration of lactic acid (mM)			Ratio of stereoisomers		Type of stereoisomers	Acetic acid (mM)
			Total	L-Form	D-Form	L (%)	D (%)		
L. sakei NRIC 1071^{T,a}	L-Arabinose	1.233	37.7	29.9	7.8	79.3	20.7	L	37.9
	D-Fructose	1.065	59.6	37.5	22.1	62.9	37.1	DL	0.0
	D-Glucose	1.101	55.4	34.9	20.5	63.0	37.0	DL	0.0
	Maltose	1.409	63.5	35.3	28.2	55.6	44.4	DL	0.0
	D-Mannose	1.293	59.4	35.3	24.1	59.4	40.6	DL	0.0
	Melibiose	1.072	47.9	28.7	19.2	59.9	40.1	DL	0.0
	D-Ribose	1.252	34.7	30.9	3.9	88.8	11.2	L	45.3
	Sucrose	1.279	60.3	36.7	23.6	60.9	39.1	DL	3.7
	D-Trehalose	1.355	54.4	48.9	5.5	89.9	10.1	L	0.0
L. coryniformis subsp.	D-Fructose	1.607	126.4	34.5	91.9	27.3	72.7	DL	0.0
coryniformis NRIC 1638^{T,b}	D-Galactose	1.516	96.7	29.5	67.2	30.5	69.5	DL	0.0
	D-Glucose	1.597	110.1	34.6	75.5	31.4	68.6	DL	0.0
	Lactose	1.658	84.6	35.3	49.3	41.7	58.3	DL	0.0
	Maltose	1.678	105.1	37.8	67.3	36.0	64.0	DL	4.1
	D-Mannose	1.645	116.7	31.5	85.2	27.0	73.0	DL	0.0
	Sucrose	1.554	98.9	28.9	69.9	29.3	70.7	DL	0.0
	D-Trehalose	1.914	140.4	64.0	76.4	45.6	54.4	DL	0.0
L. plantarum NRIC 1067^{T,a}	L-Arabinose	1.757	61.2	25.5	35.7	41.7	58.3	DL	62.3
	D-Fructose	1.910	139.3	60.8	78.5	43.6	56.4	DL	0.0
	D-Galactose	1.982	132.0	62.3	69.7	47.2	52.8	DL	0.0
	D-Glucose	2.018	130.6	60.6	69.9	46.4	53.6	DL	0.0
	Lactose	1.840	121.0	53.8	67.2	44.5	55.5	DL	0.0
	Maltose	2.077	137.0	64.5	72.5	47.1	52.9	DL	0.0
	D-Mannose	2.034	132.8	62.0	70.8	46.7	53.3	DL	0.0
	Melibiose	1.967	139.6	66.0	73.6	47.3	52.7	DL	0.0
	D-Ribose	1.524	69.3	29.0	40.3	41.9	58.1	DL	68.3
	Sucrose	2.069	161.2	76.9	84.3	47.7	52.3	DL	0.0
	D-Trehalose	2.010	137.7	64.9	72.9	47.1	52.9	DL	0.0

[a] After two-day cultures.
[b] After three-day cultures.

L. coryniformis subsp. *coryniformis* NRIC 1638^T grew at the expense of D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, sucrose, or D-trehalose as a sole source of sugar, but did not grow on L-arabinose, melibiose, or D-ribose as a sole source of sugar. The production of L- and D-lactic acid by this strain was barely changed at the expense of any sugar.

L. plantarum NRIC 1067^T grew on all of the sugars tested. This strain produced about half the amount of lactic acid and produced acetic acid concomitantly from L-arabinose or D-ribose compared with D-glucose. The production of L- and D-lactic acid by this strain was barely changed when sugars other than L-arabinose or D-ribose were used.

Effects of aerobic, stationary, and anaerobic conditions on the production of stereoisomers of lactic acid

L. sakei NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T grew with shaking, stationarily and under anaerobic conditions. These strains decreased the production of lactic acid and produced acetic acid concomitantly in aerobic cultures compared with stationary cultures (Table 7). *L. sakei* NRIC 1071^T produced about three-fourths the amount of L-lactic acid and almost the

Table 7. Effects of cultural conditions on the production of stereoisomers of lactic acid by *L. sakei* NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T.

Strains	Cultural condition	Absorbance at 660 nm	Consumed glucose (mM)[c]	Concentration of lactic acid (mM)			Ratio of stereoisomers		Type of stereoisomers	Acetic acid (mM)
				Total	L-Form	D-Form	L (%)	D (%)		
L. sakei NRIC 1071^T[a]	Shaking	0.901	19.5	27.3	13.4	13.9	49.2	50.8	DL	13.8
	Stationary	0.834	20.2	35.0	18.9	16.0	54.2	45.8	DL	0
	Anaerobic	1.005	28.2	41.5	33.9	7.5	81.8	18.2	L	0
L. coryniformis subsp.	Shaking	1.350	25.3	39.6	16.7	22.8	42.3	57.7	DL	7.7
coryniformis	Stationary	1.553	32.4	51.5	18.0	33.5	35.0	65.0	DL	0
NRIC 1638^T[b]	Anaerobic	1.694	36.0	56.5	17.7	38.8	31.4	68.6	DL	0
L. plantarum NRIC 1067^T[a]	Shaking	1.840	50.1	65.4	28.3	37.1	43.2	56.8	DL	35.2
	Stationary	1.660	49.1	88.7	44.3	44.5	49.9	50.1	DL	0
	Anaerobic	1.769	52.2	87.5	43.2	44.4	49.3	50.7	DL	0

[a] After two-day cultures.

[b] After three-day cultures.

[c] The initial concentration of glucose was 54.8 mM.

same amount of D-lactic acid in shaking cultures compared with stationary cultures, while this strain produced about twice the amount of L-lactic acid and half the amount of D-lactic acid under anaerobic cultures. *L. coryniformis* subsp. *coryniformis* NRIC 1638^T and *L. plantarum* NRIC 1067^T produced L- and D-lactic acid to the same extent under any of above cultural conditions (Table 7).

Effects of cultural temperatures on the production of stereoisomers of lactic acid

L. sakei NRIC 1071^T grew poorly at 5°C and 37°C, and produced about one-third and two-thirds the amount of lactic acid, respectively, compared with that at 25°C. This strain did not grow at 45°C. The production of D-lactic acid was decreased to the one-fourth the amount or less at 30°C and 37°C (Table 8). *L. coryniformis* subsp. *coryniformis* NRIC 1638^T grew poorly at 5°C compared with its growth at 30°C, and produced one-fourth the amount of lactic acid. This strain did not grow at 45°C (Table 8). *L. plantarum* NRIC 1067^T grew poorly at 5°C and 45°C compared with its growth at 30°C, and produced one-fifth and about three-fifths the amount of lactic acid, respectively (Table 8).

Effects of initial pHs on the production of stereoisomers of lactic acid

L. sakei NRIC 1071^T did not grow at pH 4.5, and *L. coryniformis* subsp. *coryniformis* NRIC 1638^T did not grow at pH 3.5. *L. plantarum* NRIC 1067^T grew well at all of the pHs tested (data not shown). These three strains scarcely changed the production of stereoisomers of lactic acid at any of the initial pHs tested.

Effects of sodium acetate on the production of stereoisomers of lactic acid by L. sakei strains

Effects of sodium acetate on the production of stereoisomers were investigated for 11 *L. sakei* strains because among the 49 strains tested *L. sakei* NRIC 1071^T clearly changed the type of stereoisomers in the presence of 50 mM sodium acetate. All strains produced twice or more the amount of L-lactic acid and about one-tenth the amount of D-lactic acid in the presence of 50 mM sodium acetate compared with the absence of sodium acetate (Table 9).

Discussion

A variety of lactic acid bacteria tested grew well in the presence of 50 mM sodium acetate, and the two-thirds of the strains produced much larger amounts of lactic acid compared with the absence of sodium acetate. Sodium acetate will play not only the role of a buffer but also other roles in the production of lactic acid because *L. sakei* NRIC 1071^T produced twice or more the amount of lactic acid in the presence of the acetic acid-sodium acetate buffer and about 1.6 times

Table 8. Effects of cultural temperatures on the production of stereoisomers of lactic acid by *L. sakei* NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T.

Strains	Temperature (°C)	Absorbance at 660 nm	Consumed glucose (mM)[f]	Concentration of lactic acid (mM)			Ratio of stereoisomers		Type of stereoisomers
				Total	L-Form	D-Form	L (%)	D (%)	
L. sakei NRIC 1071^T	5[a]	0.417	6.9	13.7	7.0	6.7	51.0	49.0	DL
	15[b]	0.881	26.7	37.0	19.5	17.5	52.7	47.3	DL
	25[c]	0.907	25.9	35.0	20.5	14.5	58.5	41.5	DL
	30[c]	0.879	33.1	38.8	35.2	3.6	90.7	9.3	L
	37[c]	0.524	16.4	27.9	26.5	1.4	94.9	5.1	L
L. coryniformis subsp. *coryniformis* NRIC 1638^T	5[a]	0.815	10.5	17.6	2.2	15.4	12.6	87.4	D
	15[d]	1.531	41.7	55.2	9.5	45.7	17.3	82.7	D
	25[e]	1.589	48.6	65.5	18.9	46.6	28.9	71.1	DL
	30[e]	1.563	51.7	70.9	22.3	48.6	31.5	68.5	DL
	37[e]	1.403	29.5	56.3	20.3	36.0	36.0	64.0	DL
L. plantarum NRIC 1067^T	5[a]	0.638	3.8	15.0	6.1	8.9	40.4	59.6	DL
	15[b]	1.624	40.5	57.7	24.0	33.7	56.4	43.6	DL
	25[c]	1.666	42.3	58.8	27.1	31.7	46.0	54.0	DL
	30[c]	1.623	50.4	75.2	37.2	38.0	49.4	50.6	DL
	37[c]	1.543	50.7	75.3	40.2	35.1	53.3	46.7	DL
	45[c]	0.919	29.5	44.0	28.5	15.5	64.8	35.2	DL

[a] After ten-day cultures.
[b] After five-day cultures.
[c] After two-day cultures.
[d] After six-day cultures.
[e] After three-day cultures.
[f] The initial concentration of glucose was 50.3 mM.

the amount of lactic acid in phosphate buffers. Mechanisms have not been clarified yet on the high production of lactic acid by lactic acid bacteria in the presence of sodium acetate. However, the following might be possible explanations: sodium acetate deals with the activation of LDHs, the production of LDHs, and/or the strengthening of the glycolytic pathway or the pentose cycle that will result in the high production of pyruvic acid. However, details are still obscure, and further studies are needed.

Of 49 strains of lactic acid bacteria tested, only *L. sakei* NRIC 1071^T and *L. coryniformis* subsp. *coryniformis* NRIC 1638^T changed the type of stereoisomers of lactic acid in the presence of 50 mM sodium acetate. It is of interest to note that the production of L-lactic acid by *L. sakei* NRIC 1071^T was enhanced twice or more in the presence of 50 mM sodium acetate and the production of D-lactic acid was repressed. As a result, the type produced by *L. sakei* NRIC 1071^T was shifted from the DL-type to the L-type in the presence of 50 mM sodium acetate. In contrast, the type of stereoisomers produced by this strain retained the DL-type in the presence of 10 or 20 mM sodium acetate. Effects of sodium acetate may depend on its concentration. The type produced by *L. sakei* NRIC 1071^T was shifted from the DL-type to the L-type in the presence of 20 mM trisodium citrate, but the type was not changed remarkably in the presence of 20 mM trisodium citrate compared with the presence of 50 mM sodium acetate. The type produced by *L. coryniformis* subsp. *coryniformis* NRIC 1638^T was shifted from the DL-type to the D-type in the presence of 50 mM sodium acetate. In contrast with *L. sakei* NRIC 1071^T, it is worthy to mention that the production of D-lactic acid by this strain was enhanced in the presence of 50 mM sodium acetate and the production of L-lactic acid was repressed. *L. sakei* NRIC 1071^T, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T, and *L. plantarum* NRIC 1067^T produced much more the amount of lactic acid in the presence of L-malate compared with the amount

Table 9. Effects of sodium acetate on the production of stereoisomers of lactic acid by *Lactobacillus sakei* strains.[a]

Species	Strains[b]	Presence of NaAc[c]	Concentration of lactic acid (mM)			Ratio of stereoisomers		Type of stereoisomers
			Total	L-Form	D-Form	L (%)	D (%)	
Lactobacillus sakei	No. 14	WO[d]	58.7	29.9	28.8	51.0	49.0	DL
		W[e]	79.4	77.3	2.1	97.3	2.7	L
Lactobacillus sakei	No. 16	WO	55.7	27.3	28.4	49.0	51.0	DL
		W	77.4	75.3	2.1	97.3	2.7	L
Lactobacillus sakei	No. 17	WO	46.1	21.7	24.4	47.0	53.0	DL
		W	76.1	73.4	2.7	96.4	3.6	L
Lactobacillus sakei	No. 18	WO	57.7	30.2	27.5	52.3	47.7	DL
		W	79.4	76.4	3.0	96.2	3.8	L
Lactobacillus sakei	No. 19	WO	51.9	24.0	27.9	46.3	53.7	DL
		W	76.9	74.4	2.5	96.7	3.3	L
Lactobacillus sakei	No. 21	WO	44.7	27.7	17.0	61.9	38.1	DL
		W	77.3	76.5	0.8	99.0	1.0	L
Lactobacillus sakei	No. 22	WO	54.2	27.5	26.7	50.8	49.2	DL
		W	78.9	77.8	1.1	98.6	1.4	L
Lactobacillus sakei	No. 23	WO	47.1	27.9	19.2	59.2	40.8	DL
		W	74.3	73.6	0.7	99.1	0.9	L
Lactobacillus sakei	No. 27	WO	45.0	28.8	16.2	63.9	36.1	DL
		W	77.7	77.4	0.3	99.6	0.4	L
Lactobacillus sakei	No. 29	WO	49.4	26.3	23.1	53.2	46.8	DL
		W	76.2	72.5	3.7	95.2	4.8	L

[a] After two-day cultures.
[b] NRIC, Culture Collection Center, Tokyo University of Agriculture, Tokyo.
[c] NaAc, sodium acetate.
[d] WO, without sodium acetate.
[e] W, with 50 mM sodium acetate.

of lactic acid based on D-glucose consumed. L-Malate would be utilized to produce lactic acid by malolactic fermentation (Doelle, 1975). However, the type of stereoisomers produced by these strains was not changed in the presence of 50 mM sodium L-malate. The type of stereoisomers of lactic acid produced by the rest of 47 strains was not changed in the presence of 50 mM sodium acetate.

L. sakei NRIC 1071^T produced L-lactic acid at an early stage of the logarithmic phase in the absence of sodium acetate and D-lactic acid at a late stage of the logarithmic phase (Fig. 1A). Therefore, the type of stereoisomers was gradually shifted from the L-type to the DL-type. *L. sakei* NRIC 1071^T produced L-lactic acid exclusively and barely produced D-lactic acid during the growth in the presence of 50 mM sodium acetate (Fig. 1B). Thus, the type was keeping the L-type during the growth. On the other hand, *L. coryniformis* subsp. *coryniformis* NRIC 1638^T produced L- and D-lactic acid concomitantly in the absence of sodium acetate (Fig. 1E). As a result, the type of stereoisomers was keeping the DL-type during the growth. *L. coryniformis* subsp. *coryniformis* NRIC 1638^T increased the amount of D-lactic acid and decreased the amount of L-lactic acid during the growth in the presence of 50 mM sodium acetate (Fig. 1F). Consequently, the type was keeping the D-type during the growth in the presence of 50 mM sodium acetate. However, the type produced by this strain did not markedly change as *L. sakei* NRIC 1071^T did. *L. sakei* NRIC 1764 produced exclusively L-lactic acid and barely any D-lactic acid, regardless the presence of sodium acetate (Fig. 1C, D). Thus, the type of stereoisomers kept to the L-type during the growth. *L. plantarum* NRIC 1067^T and *L. curvatus* NRIC 1052^T produced L- and D-lactic acid concomitantly in the absence of sodium acetate (Fig.

1G, I). The type of stereoisomers produced by these strains kept to the DL-type during the growth in the presence of 50 mM sodium acetate (Fig. 1H, J). *L. sakei* and *L. curvatus* are phenotypically and phylogenetically related to each other (Collins et al., 1991; Kandler and Weiss, 1986). In addition, *L. curvatus* was reported to transform L-lactic acid to D-lactic acid by lactate racemase as *L. sakei* did (Stetter and Kandler, 1973).

The production of stereoisomers of lactic acid was significant when L-, DL-, or D-lactic acid was added to culture media. When the amount of lactic acid added was subtracted from a total amount of lactic acid (both added and produced), the type of stereoisomers produced by *L. sakei* NRIC 1071^T had remarkably shifted from the DL-type to the D-type under the addition of 50 mM L-lactic acid. In contrast, the type produced by this strain was shifted from the DL-type to the L-type under the addition of DL- or D-lactic acid. It is of interest to note that when L-lactic acid was added to the medium, its enantiomer, D-lactic acid, was produced. Conversely, when D-lactic acid was added, its enantiomer, L-lactic acid, was produced. As a result, the type of stereoisomers of lactic acid in the culture media remained the DL-type. Similar results were found in *L. coryniformis* subsp. *coryniformis* NRIC 1638^T and *L. plantarum* NRIC 1067^T. Furthermore, when L-lactic acid was added to culture media of *L. sakei* NRIC 1071^T, L-lactic acid (added and produced) decreased once at a middle logarithmic phase and increased at a late logarithmic phase. In contrast, when D-lactic acid was added to a culture medium, *L. sakei* NRIC 1071^T did not show the decrease of D-lactic acid. These facts were found in *L. plantarum* NRIC 1067^T. This finding will be ascribed to the reaction of lactic acid bacteria to stereoisomers of lactic acid, L- and D-lactic acid.

When L-arabinose or D-ribose was used as a sole source of sugar, the type of stereoisomers of lactic acid produced by *L. sakei* NRIC 1071^T was shifted from the DL-type to the L-type. This result agreed with the findings of previous studies (Katagiri and Kitahara, 1937; Kitahara et al., 1957; Ôbayashi and Kitahara, 1959). This made sense because of the production of acetic acid by facultative heterofermentatives (Kandler and Weiss, 1986), and the acetic acid produced resulted in the shift of the type of stereoisomers.

The type of stereoisomers of lactic acid produced by *L. sakei* NRIC 1071^T was considerably changed under several cultural conditions. However, the effects of sodium acetate were most striking. The shift of the DL-type to the L-type by *L. sakei* is due to the high production of L-lactic acid and the low production of D-lactic acid. Meanwhile, the lactate racemase of *L. sakei* was reported to produce D-lactic acid from L-lactic acid, and the production of this enzyme was repressed by sodium acetate (Katagiri and Kitahara, 1937; Kitahara et al., 1957; Ôbayashi and Kitahara, 1959). However, this would not explain the mechanism of enhancing the production of L-lactic acid because lactate racemase is concerned in the production of D-lactic acid but not the high production of L-lactic acid.

The type of stereoisomers of lactic acid produced by *L. sakei* NRIC 1071^T was changed by several cultural conditions. Moreover, the type produced by 11 other *L. sakei* strains, except for *L. sakei* NRIC 1764, was also shifted from the DL-type to the L-type in the presence of 50 mM sodium acetate. The type produced by *L. sakei* NRIC 1764, which was previously named *L. bavaricus* (Torriani et al., 1996), was the L-type, regardless of the presence of sodium acetate. *L. sakei* NRIC 1071^T and NRIC 1764 were genetically identical on the basis of DNA-DNA similarity (Kagaermeir-Callaway and Lauer, 1995). Consequently, *L. sakei* strains were separated into two groups: DL-former such as *L. sakei* NRIC 1071^T and other *L. sakei* strains, and L-former such as *L. sakei* NRIC 1764. The shift of stereoisomers by the majority of *L. sakei* strains seems interesting from the viewpoint of the delineation of this species. The type of stereoisomers has been considered to be species-specific and useful for identification of lactic acid bacteria. However, some exceptions may exist as shown in case of *L. sakei.* When some lactic acid bacteria share major phenotypic characteristics with one another and show different types of stereoisomers, data of DNA-DNA similarity are useful for correct identification.

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