Full Paper

***Trans* fatty acids promote the growth of some *Lactobacillus* strains**

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Five *Lactobacillus* strains (2 *L. gasseri*, 2 *L. plantarum* and 1 *L. reuteri*) were cultured in modified MRS medium containing fatty acids (FAs) instead of Tween 80 for 24 h at 37°C, to learn the effect of saturated and unsaturated FAs on the *Lactobacillus* growth. Free FAs included palmitic (16:0), palmitoleic (c9-16:1), stearic (18:0), oleic (c9-18:1), elaidic (t9-18:1), *cis*-vaccenic (c11-18:1), vaccenic (t11-18:1), linoleic (c9, c12-18:2), conjugated linoleic (c9, t11- and t10, c12-18:2), α -linolenic (c9, c12, c15-18:3), α -eleostearic (c9, t11, t13-18:3), eicosapentaenoic (20:5), and docosahexaenoic (22:6) acids. Among free FAs, oleic acid stimulated the growth of all *Lactobacillus* strains, whereas palmitoleic acid had almost no effect on the *Lactobacillus* growth. Saturated FAs such as stearic and palmitic acids inhibited or did not affect the *Lactobacillus* growth. Polyunsaturated FAs such as α -linolenic, eicosapentaenoic and docosahexaenoic acids strongly inhibited the *Lactobacillus* growth at 7.6×10^{-4} M. Octadecenoic acids such as oleic, elaidic, *cis*-vaccenic and vaccenic acids remarkably promoted the growth of *L. gasseri*, regardless of the different double bond positions and configurations. When oleic or *cis*-vaccenic acid was incubated with *L. gasseri*, the FAs were transformed to cyclopropane FAs (methyleneoctadecanoic acids) after incorporation into the cells. On the other hand, *trans* FAs such as elaidic and vaccenic acids incorporated into the cells were not converted to another FAs. Conjugated linoleic and α -eleostearic acids having a *trans* double bond promoted the *Lactobacillus* growth. The growth of *L. gasseri* was also stimulated by *trans*-rich free FAs from hydrogenated canola and fish oils. These results showed that octadecenoic acid and *trans* FAs had strong promotion activities for the *Lactobacillus* growth due to their incorporation into membrane lipids.

Key Words—elaidic acid; lactic acid bacteria; *Lactobacillus*; octadecenoic acid; *trans* fatty acid; vaccenic acid

Introduction

Some fatty acids (FAs) can inhibit or stimulate the growth of bacteria. In general, polyunsaturated FAs such as linoleic and linolenic acids have more inhibitory effects on bacteria than saturated and mono-

unsaturated FAs (Nieman, 1954; Raychowdhury et al., 1985). Gram-positive bacteria, especially, are more sensitive to polyunsaturated FAs than Gram-negative bacteria (Greenway and Dyke, 1979). We found that eicosapentaenoic and docosahexaenoic acids seriously damaged rumen bacteria such as *Butyrivibrio fibrisolvens* (Fujimoto et al., 1993). In addition, Butcher et al. (1976) reported that *Staphylococcus aureus* was more susceptible to *cis* FAs. The stereochemistry of the double bonds in FAs may play an important role in the growth of microorganisms.

On the other hand, some microorganisms demand

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FA. *Lactobacillus* strains, being lactic acid bacteria, which are common in fermented foods and mammalian gastrointestinal tracts, are well known to need oleic acid for their growth (Johnsson et al., 1995; Nikkila et al., 1995). Oleic acid is considered necessary for the formation of cell membranes. In fact, a surfactant, Tween 80 containing oleic acid is usually added to a culture medium such as MRS broth for *Lactobacillus* growth. However, the effect of saturated and unsaturated FAs other than oleic acid on the *Lactobacillus* growth is not fully understood. Partanen et al. (2001) investigated the effect of surfactants with different FA compositions on the growth of *Lactobacillus delbrueckii*. Tween 80 containing oleic acid promoted the growth of *L. delbrueckii* but not Tween 40 and 60 rich in saturated FAs (palmitic and stearic acids). They also reported that dodecanoic acid inhibited the growth of *L. delbrueckii*. Jenkins and Courtney (2003) observed that linoleic acid inhibited the *Lactobacillus* growth but not conjugated linoleic acid. Kankaanpää et al. (2004) reported that the addition of polyunsaturated FAs such as linoleic, α -linolenic, arachidonic and docosahexaenoic acids to the growth medium affected the FAs composition of total lipids in *Lactobacillus rhamnosus*, *L. casei* and *L. delbrueckii*. However, effects of position, configuration and number of double bond in the FAs on the growth of *Lactobacillus* strains were poorly understood.

In this study, we cultured 5 *Lactobacillus* strains including probiotics in the presence of different FAs, to assess the role of position, configuration and number of double bonds in the FAs on the *Lactobacillus* growth.

Materials and Methods

Materials. Palmitic acid (hexadecanoic acid, 16:0), palmitoleic acid (*cis*9-hexadecenoic acid, *c*9-16:1), stearic acid (octadecanoic acid, 18:0), oleic acid (*cis*9-octadecenoic acid, *c*9-18:1), elaidic acid (*trans*9-octadecenoic acid, *t*9-18:1), *cis*-vaccenic acid (*cis*11-octadecenoic acid, *c*11-18:1), vaccenic acid (*trans*11-octadecenoic acid, *t*11-18:1), linoleic acid (*cis*9, *cis*12-octadecadienoic acid, *c*9, *c*12-18:2), conjugated linoleic acid (*cis*9, *trans*11- and *trans*10, *cis*12-octadecadienoic acids, *c*9, *t*11- and *t*10, *c*12-18:2), and α -linolenic acid (*cis*9, *cis*12, *cis*15-octadecatrienoic acid, *c*9, *c*12, *c*15-18:3) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). α -Eleostearic acid (*cis*9, *trans*11, *trans*13-octadecatrienoic acid, *c*9, *t*11, *t*13-

18:3) was prepared from commercial tung oil by silicic acid column chromatography after hydrolysis. Eicosapentaenoic acid (*cis*5, *cis*8, *cis*11, *cis*14, *cis*17-eicosapentaenoic acid, 20:5) and docosahexaenoic acid (*cis*4, *cis*7, *cis*10, *cis*13, *cis*16, *cis*19-docosahexaenoic acid, 22:6) were obtained from Nippon Surisan Kaisha, Ltd. (Tokyo, Japan). The purity of free FAs used in this study was more than 95% except for α -eleostearic acid (80%). Conjugated linoleic acid consisted of 50% *c*9, *t*11- and 50% *t*10, *c*12-18:2. Tween 80 was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

Hydrogenated canola and fish oils were obtained from Tsukishima Food Industry Co., Ltd. (Tokyo, Japan). *Trans*-rich free FAs were prepared by the hydrolysis of hydrogenated canola and fish oils, and they contained 51% and 43% *trans* FAs, respectively.

Microorganisms and cultivation. *Lactobacillus gasseri* LA158 was isolated from the feces of a human infant in our laboratory (Graduate School of Agricultural Science, Tohoku University, Sendai, Japan). *L. gasseri* JCM1131, *L. reuteri* JCM1112 and *L. plantarum* JCM1149 were obtained from the Japan Collection of Microorganisms (Wako, Japan). *L. plantarum* IFO15891 was obtained from the Institute for Fermentation of Osaka University (Osaka, Japan).

Strains were cultured in modified MRS medium containing free FA instead of Tween 80. The modified MRS medium consisted of 2% glucose, 1% peptone, 1% beef extract, 0.5% yeast extract, 0.5% sodium acetate, 0.2% diammonium hydrogen citrate, 0.2% KH_2PO_4 , 0.058% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.028% $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, and free FAs. The modified MRS medium did not contain Tween 80 at all. Before addition of free FAs into the modified MRS medium, the medium was sterilized by autoclaving at 121°C for 15 min. Free FAs was resolved in ethanol and then was added into the medium after passing through a 0.45 μm membrane filter. The concentrations of free FAs were finally adjusted to 7.6×10^{-4} to 10^{-6} M.

To prepare the inoculum culture, the stationary phase culture of each *Lactobacillus* strain in MRS medium was diluted with fresh MRS medium omitting Tween 80 at the concentration of 1% and cultured at 37°C for 24 h. This culture was finally diluted to 1/5 with MRS medium without Tween 80. The inoculum culture (10 μl) and the modified MRS medium containing each free FA (90 μl) were mixed on the 96-well plate and aerobically incubated for 24 h (stationary

Table 1. Effects of saturated and unsaturated fatty acids on the growth of *Lactobacillus* strains.

Fatty acid ^a	Growth ^b				
	<i>L. gasseri</i> LA158	<i>L. gasseri</i> JCM1131	<i>L. reuteri</i> JCM1112	<i>L. plantarum</i> JCM1149	<i>L. plantarum</i> IFO15891
Tween 80	7.6	1.2	1.6	10.4	2.9
Palmitic (16:0)	0.6	1.0	0.4	0.9	0.8
Palmitoleic (c9-16:1)	0.6	1.1	0.9	2.2	0.7
Stearic (18:0)	<0.1	<0.1	<0.1	<0.1	0.1
Oleic (c9-18:1)	7.5	1.7	1.5	12.3	4.3
Linoleic (c9, c12-18:2)	0.0	0.7	0.5	0.7	0.9
α -Linolenic (c9, c12, c15-18:3)	<0.1	0.0	<0.1	<0.1	<0.1
Eicosapentaenoic (20:5)	<0.1	<0.1	<0.1	<0.1	<0.1
Docosahexaenoic (22:6)	0.4	0.1	0.4	0.2	0.3

^a 7.6×10^{-4} M.^b Expressed as the ratio of absorbance of cultures incubated with and without free FAs for 24 h at 37°C.

growth phase reached) at 37°C. The absorbance at 595 nm (turbidity) of cultures was measured to estimate the cell growth. The growth value was expressed as the ratio of absorbance of the culture after incubation with and without free FA for 24 h. A growth value >1.0 indicates the promotion activity of free FAs, whereas a growth value <1.0 indicates its inhibitory activity.

For lipid analyses, *L. gasseri* was aerobically cultured in 50 ml modified MRS medium containing 7.6×10^{-4} M free FAs in a 100-ml flask for 24 h at 37°C. Cells were harvested by centrifugation (3,000×g) for 10 min, and washed twice with 0.85% NaCl solution and then *n*-hexane. The washed cells were used for later analysis.

Lipid and lactic acid assays. Total lipids were extracted from cultured *L. gasseri* with a mixture of chloroform and methanol (2:1, by vol) after the addition of water according to the method of Bligh and Dyer (1959). Phospholipids were isolated from total lipids by thin-layer chromatographic method using Silica gel 60 plate (Merck, Germany) and a mixture of *n*-hexane/diethyl ether/formic acid (70:30:1, by vol) as a mobile phase. The FAs compositions of prepared phospholipids were measured by gas liquid chromatography (GLC) with a flame-ionization detector using a CP-Sil 88 fused silica capillary column (0.25 mm×50 m, Chrompack, Netherlands) after the methylation with 0.5 M sodium methylate, as described previously (Lee et al., 2002). The column temperature was programmed at 170°C for 25 min, and then heated to

225°C at 4°C/min. The injection and detector temperature was 250°C.

The concentration of lactic acid in the culture medium was determined by enzymatic assay kits (J.K. International, Tokyo, Japan).

Data describe the mean of 6 experiments.

Results

Effect of saturated and unsaturated FA

Five *Lactobacillus* strains were cultured in the modified MRS medium containing free FA (7.6×10^{-4} M corresponding to the concentration of Tween 80 in the conventional MRS medium) instead of Tween 80 for 24 h at 37°C. Palmitic and stearic acids were used as saturated FAs, and oleic, linoleic, α -linolenic, eicosapentaenoic and docosahexaenoic acids were used as unsaturated FAs. Table 1 shows the *Lactobacillus* growth estimated by the turbidity (OD₅₉₅) of the culture after incubation with each free FAs. In the absence of Tween 80 or free FAs (control), all *Lactobacillus* strains slightly grew and they showed low absorbance below 0.3. When oleic acid was added to the culture medium, the absorbance was higher than the control for all *Lactobacillus* strains. Their growth value was more than 1.5. *L. gasseri* LA158, *L. plantarum* JCM1149 and *L. plantarum* IFO15891 showed the especially high growth values of 7.5, 12.3 and 4.3, respectively. Palmitoleic acid, a monounsaturated FA, did not affect the *Lactobacillus* growth except for that of *L. plantarum* JCM1149, because the growth value was lower than

Table 2. Effects of octadecenoic acids and conjugated fatty acids on the growth of *L. gasseri* LA158.

Fatty acid	Growth		
	7.6×10^{-6} M	7.6×10^{-5} M	7.6×10^{-4} M
Tween 80	4.0	7.0	8.4
Oleic (c9-18:1)	2.6	7.6	9.3
Elaidic (t9-18:1)	3.5	8.2	8.1
cis-Vaccenic (c11-18:1)	2.9	7.1	9.3
Vaccenic (t11-18:1)	2.6	6.3	7.6
Linoleic (c9, c12-18:2)	2.1	6.2	<0.1
Conjugated linoleic (c9, t11- and t10, c12-18:2)	3.4	7.9	8.6
α -Linolenic (c9, c12, c15-18:3)	2.6	4.2	0.5
α -Eleostearic (c9, t11, t13-18:3)	2.2	6.9	7.7

1.0 of the control. On the other hand, *Lactobacillus* strains hardly grew in the presence of stearic acid, and they showed very low growth values (<0.1). *Lactobacillus* strains cultured with palmitic acid showed almost the same absorbance as the control except for *L. reuteri* JCM1112, and their growth value was about 1.0. When polyunsaturated FAs such as eicosapentaenoic and docosahexaenoic acids were added to the culture medium at 7.6×10^{-4} M, the absorbance of the culture was very low after cultivation for 24 h. The low growth value (<0.4) showed that polyunsaturated FAs might have inhibitory activity for the *Lactobacillus* growth at the concentration of 7.6×10^{-4} M. From these results, it was shown that oleic acid, being an octadecenoic acid, could promote the *Lactobacillus* growth, but not other unsaturated and saturated FAs.

Effect of octadecenoic acids

The effect of the double bond position and configuration of octadecenoic acids on the *Lactobacillus* growth was investigated using *L. gasseri* LA158 as a typical probiotic *Lactobacilli* species which is generally distributed in human gastrointestinal tracts. *L. gasseri* LA158 was cultured for 24 h at 37°C after supplementation with elaidic, vaccenic and cis-vaccenic acids, being octadecenoic acids, as well as oleic acid. In the absence of free FAs, *L. gasseri* LA158 usually showed low absorbance below 0.3 after cultivation for 24 h at 37°C. However, *L. gasseri* LA158 grown with octadecenoic acids showed high absorbance, and the growth value was more than 2.6, as shown in Table 2. The growth value was higher with the concentration of octadecenoic acids. All octadecenoic acids remarkably

promoted the growth of *L. gasseri* LA158, regardless of the different double bond positions and configurations. A similar tendency was obtained for 4 *Lactobacillus* strains other than *L. gasseri* LA158 (data not shown). Octadecenoic acids proved to have strong growth-promotion activity for some *Lactobacillus* strains.

Effect of conjugated FAs

The effect of conjugated FAs on the *Lactobacillus* growth was also investigated using *L. gasseri* LA158. Conjugated linoleic and α -eleostearic acids, which are positional and stereochemical isomers of linoleic and α -linolenic acids, respectively, have a *trans* double bond. When *L. gasseri* LA158 was incubated with conjugated FA for 24 h at 37°C, a high growth value (>2.2) was observed, as shown in Table 2. The growth value was higher with the concentration of conjugated linoleic and α -eleostearic acids. These observations were also obtained for 4 *Lactobacillus* strains other than *L. gasseri* LA158 (data not shown). Conjugated linoleic and α -eleostearic acids having *trans* double bonds were able to promote the *Lactobacillus* growth at all concentrations. On the other hand, *L. gasseri* LA158 grown with linoleic or α -linolenic acid showed a low growth value (<0.5) at 7.6×10^{-4} M. However, it showed a high growth value (2.1–6.2) at a concentration below 7.6×10^{-5} M. These observations suggested that only linoleic and α -linolenic acids with *cis* double bonds had the ability to promote the *Lactobacillus* growth at a low concentration, but not at a high concentration. Probably, oxidation products formed from linoleic and α -linolenic acids during incubation show a

toxicity to inhibit the *Lactobacillus* growth at a high concentration.

Effect of *trans* fatty acids

To confirm the promotion activity of *trans* FAs for *Lactobacillus* growth, *trans*-rich free FAs prepared from hydrogenated canola and fish oils were added to the culture medium, and then *L. gasseri* LA158 was cultured for 24 h at 37°C. *L. gasseri* LA158 grown with free FAs from hydrogenated canola and fish oils showed high absorbance, and the growth value was more than 1.6. The growth value was higher with increased free FAs from hydrogenated canola and fish oils, as shown in Table 3. Both *trans*-rich free FAs from hydrogenated canola and fish oils stimulated the growth of *L. gasseri* LA 158 at all concentrations. Simi-

lar observations were also obtained for other *Lactobacillus* strains (data not shown). These results showed that *trans*-rich free FAs had an ability to promote the *Lactobacillus* growth, regardless of the different positions of double bonds and carbon chain lengths.

Lipid content and FA compositions of *L. gasseri*

Table 4 shows the lipid content and FA composition of membrane lipids in *L. gasseri* LA158 grown with oleic, elaidic, *cis*-vaccenic or vaccenic acid as the octadecenoic acid. All octadecenoic acids increased the lipid content in cells of *L. gasseri* LA158. In particular, *trans* FAs such as elaidic and vaccenic acids significantly increased the lipid content to ca. 17% or more in *L. gasseri* LA158. It was suggested that the supplemented octadecenoic acids were specifically incorporated into the cells of *L. gasseri*.

To discover the metabolism of octadecenoic acid added to the culture medium, we investigated the FA composition of membrane lipids in *L. gasseri* LA158. When adding oleic, elaidic, *cis*-vaccenic or vaccenic acid to the culture medium, the corresponding octadecenoic acid was found in membrane lipids in *L. gasseri* LA158. *L. gasseri* LA158 incorporated the supplemented octadecenoic acid as a major FAs into membrane lipids, because considerable amounts (>60%)

Table 3. Effects of free fatty acids from hydrogenated oils on the growth of *L. gasseri* LA158.

Fatty acid	Growth		
	7.6×10^{-6} M	7.6×10^{-5} M	7.6×10^{-4} M
Tween 80	1.6	5.9	6.8
Hydrogenated canola oil	1.6	3.1	7.7
Hydrogenated fish oil	1.8	2.2	8.4

Table 4. Lipid content and fatty acid composition of membrane lipids in *L. gasseri* LA158 grown with octadecenoic acids.

	Octadecenoic acid ^a				
	Tween 80	Oleic (c9-18:1)	Elaidic (t9-18:1)	<i>cis</i> -Vaccenic (c11-18:1)	Vaccenic (t11-18:1)
Lipid content, %	6.3	13.4	16.6	8.9	17.7
Fatty acid, %					
Myristic (14:0)	3.2±0.4	0.4±0.0	0.6±0.0	0.2±0.1	0.9±0.0
Palmitic (16:0)	44.0±0.1	2.7±0.1	11.5±0.6	1.2±0.1	16.0±2.0
Palmitoleic (16:1)	1.3±0.1	0.9±0.0	0.9±0.0	0.6±0.1	1.0±0.0
Stearic (18:0)	5.0±0.1	0.9±0.1	2.1±0.1	4.9±4.3	4.1±0.8
Oleic (c9-18:1)	4.1±0.1	64.8±0.8	1.4±0.1	N.D.	2.4±0.1
Elaidic (t9-18:1)	N.D. ^c	N.D.	71.4±1.4	N.D.	N.D.
<i>cis</i> -Vaccenic (c11-18:1)	4.1±1.4	N.D.	1.4±0.2	71.5±1.9	2.1±0.2
Vaccenic (t11-18:1)	4.6±1.9	5.1±0.0	N.D.	N.D.	63.4±4.8
Cyclopropane FA ^b	32.6±0.1	22.5±2.2	7.2±0.6	21.5±2.2	7.4±1.7
Others	1.2±0.0	2.7±1.4	3.5±0.1	0.1±0.1	2.8±0.1

^a 7.6×10^{-4} M.

^b 9, 10- and 11, 12-methyleneoctadecanoic acids.

^c Not detected.

of octadecenoic acids were found in *L. gasseri* (Table 4). On the other hand, palmitic acid and cyclopropane FAs (9,10-methyleneoctadecanoic acid) were found as major FAs in membrane lipids of *L. gasseri* LA158 grown with Tween 80. *L. gasseri* could metabolize oleic acid contained in Tween 80 to 9, 10-methyleneoctadecanoic acid. Moreover, *L. gasseri* LA158 grown with oleic or *cis*-vaccenic acid contained a high level (ca. 22%) of 9, 10- or 11, 12-methyleneoctadecanoic acid in membrane lipids, while those grown with elaidic or vaccenic acids did not. These observations demonstrated that oleic and *cis*-vaccenic acids incorporated in the cell of *L. gasseri* were partially converted to 9, 10- and 11, 12-methyleneoctadecanoic acids as cyclopropane FAs, respectively, but not *trans* FAs such as elaidic and vaccenic acids. Thus, the FAs composition of membrane lipids in *L. gasseri* LA158 was remarkably different according to the varieties of supplemented octadecenoic acids.

Lactic acid production of *L. gasseri*

We also investigated the lactic acid production of *L. gasseri* LA158, to learn the effect of octadecenoic acids on fermentation by *Lactobacillus*. After the cultivation of *L. gasseri* LA158 with oleic, elaidic, *cis*-vaccenic or vaccenic acid for 24 h at 37°C, the concentration of lactic acid in the culture medium was in the range of 9.6 to 10.0 g/L. The concentrations of D- and L-lactic acid were 2.6–2.9 and 6.8–7.2 g/L, respectively, and they were almost same in all samples. The position and configuration of the double bond on the supplemented octadecenoic acids did not affect the production of lactic acid by *L. gasseri*. This result showed that octadecenoic acids did not inhibit the fermentation by *L. gasseri* at all, although they varied the FAs composition of the membrane lipid.

Discussion

We added saturated and unsaturated FAs to the culture medium, and then cultured 5 *Lactobacillus* strains for 24 h at 37°C, to learn the effect of FAs on *Lactobacillus* growth. Among FAs, octadecenoic acids such as oleic acid could stimulate the growth in *Lactobacillus* strains tested. It was very interesting that octadecenoic acids showed strong growth-promotion activity regardless of the different double bond positions and configurations. We also detected the supplemented octadecenoic acids in membrane lipids of *Lactobacil-*

lus grown with octadecenoic acids. These observations suggested that supplemented octadecenoic acids were incorporated into membrane lipids and could act as the structural FAs of the cell membrane.

In this study, we found for the first time that *trans* FAs were able to stimulate *Lactobacillus* growth. Polyunsaturated FAs such as α -linolenic, eicosapentaenoic and docosahexaenoic acids inhibited the growth of *Lactobacillus* strains, but conjugated FAs having *trans* double bonds promoted the *Lactobacillus* growth. *Trans*-rich free FAs from hydrogenated canola and fish oils could also stimulate the *Lactobacillus* growth. Hydrogenated canola oil contained about 75% octadecenoic acids, while hydrogenated fish oil mainly consisted of monounsaturated FAs with 18 to 22-carbon chain lengths (42%) and palmitic acid (23%). The growth-promotion activity of free FAs from hydrogenated canola and fish oils might be due to *trans* FAs as well as octadecenoic acid.

As for the reason why *trans* FAs could promote the *Lactobacillus* growth, we discuss the melting point (mp) of FAs, because the mp of FAs in membrane lipid may be related in the fluidity and permeability of cell

Table 5. The melting point (mp) of fatty acids (Japan Oil Chem. Soc., 1990).

Fatty acid	mp, °C
Saturated fatty acids	
Palmitic (16:0)	63
Stearic (18:0)	70
Monounsaturated fatty acids	
Palmitoleic (c9-16:1)	5
Oleic (c9-18:1)	10.5
<i>cis</i> -Vaccenic (c11-18:1)	13
Elaidic (t9-18:1)	45
Vaccenic (t11-18:1)	44
Polyunsaturated fatty acids	
Linoleic (c9, c12-18:2)	−5
α -Linolenic (c9, c12, c15-18:3)	−11
Eicosapentaenoic (20:5)	−44
Docosahexaenoic (22:6)	−54
Conjugated fatty acids	
Conjugated linoleic (c9, t11-18:2)	22
α -Eleostearic (c9, t11, t13-18:3)	49
Cyclopropane fatty acid	
9,10-Methyleneoctadecanoic	29
Hydrogenated oil-free fatty acids	
Hydrogenated canola oil	35
Hydrogenated fish oil	39

membrane. Table 5 shows the mp of individual FAs (Japan Oil Chemist's Society, 1990). The mp of *trans* FAs such as elaidic, vaccenic, conjugated linoleic, and α -eleostearic acids was at the range of 20 and 50°C. Oleic and *cis*-vaccenic acids were converted into the corresponding cyclopropane FAs with mp at 29°C in the cell membrane, although their mp were about 10°C. Saturated FAs that inhibited or did not affect the *Lactobacillus* growth had mp at over 60°C. On the other hand, polyunsaturated FAs showing the inhibitory activity had very low mp (<5°C). From these results, FAs having mp at the range of 20 and 50°C may be suitable as structural FAs in cell membranes of *Lactobacillus* strains. Indeed, the mp of free FAs from hydrogenated canola and fish oils that showed the growth-promotion activity was 35 and 39°C, respectively.

Trans FAs are contained in dairy products such as milk, cheese and butter, and in hydrogenated vegetable and fish oils used as margarine and shortening. It is well known that dietary *trans* FAs increases serum cholesterol and decreases HDL-cholesterol levels in human (Zock and Katan, 1997; Zock and Mensink, 1996). Thus, *trans* FAs is regarded as a risk factor for coronary heart disease, and its excessive intake is considered unfavorable for human health. In this study, we found the growth-promotion activity for *Lactobacillus* as a nutritional function in *trans* FAs. Our data suggest that dietary *trans* FAs may be powerful prebiotics to activate *Lactobacillus* strains, which are frequently used as probiotics.

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