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Factors affecting conjugated linoleic acid production by *Lactobacillus plantarum* GSI 303

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Abstract

Conjugated linoleic acids (CLAs), an isomerization product of linoleic acid (LA), have been found to provide beneficial physiological effects for health. *Lactobacillus plantarum* GSI 303, a strain, selected for its potential in CLAs synthesis, was studied to understand the conditions that may enhance CLA production. The maximum concentration of CLA was 6.0 mg/g fat obtained from the optimum conditions for culturing in de Mann Rogosa Sharp (MRS) broth and skim milk media contain 2 mg/mL LA, initial pH 6.5, and incubation at 37°C for 24 h. By performing either in aerobic or anaerobic conditions, CLA production was not significantly different and prolonging incubation time from 24 to 48 h did not enhance CLA formation ($p>0.05$). Incubation at 37°C, CLA content was higher than at 43°C and 15°C. In addition, *L. plantarum* GSI 303 produced CLA in skim milk media higher than did *L. reuteri* ATCC 55739 which was used as a positive bacterial control for CLA production.

Introduction

Conjugated linoleic acids (CLAs, isomerization product of linoleics acid (LA; *c*9,*c*12-C18:2), have been shown to provide some beneficial physiological effects such as antioxidative, cancer inhibition, cholesterol lowering agents and growth promoting factors (Bhattacharya *et al*., 2006; Benjamin and Spener, 2009). The main biologically active isomers are *c*9,*t*11-CLA and *t*10,c12-CLA (Pariza, 2004; Collomb *et al*., 2006), with *c*9,*t*11-CLA being the predominant isomer in the diet (≈90% of dietary CLA is *c*9,*t*11-CLA and <10% is t10,c12-CLA) (Chin *et al*., 1992). Normally, CLAs are found in ruminant foodstuff such as dairy and meat products; however, the concentrations of CLA isomers are present at relatively low levels (4–6 mg/g fat). Consequently, large quantities of these foods are must be consumed in order to obtain any beneficial effects (Hwang *et al*., 2012). Typical dietary intake of CLA in humans has been determined for several different countries which range from 400-800 mg/d (Wahle *et al,* 2004). For beneficial effects on body weight or atherosclerosis in humans, daily doses of 3 to 4 g CLA are thought to be effective (Tricon *et al*., 2005; Bhattacharya *et al*., 2006). Many researchers are looking for incorporation of bacterial strains to be able to produce CLA in processed foods in order to increase CLA for the human consumption. Moreover, there are some information demonstrating several strains of lactic acid bacteria (LAB) isolated from human and animal intestines are able to convert LA to CLA (Lin *et al.,* 2005; Puniya *et al*., 2008; Zeng *et al*., 2009; Maldonado *et al*., 2011; Li *et al*., 2013). For example, *L. plantarum* has been identified as a CLA producer with hydroxy fatty acids (HFA) as intermediates (Kishino *et al*., 2002; Ogawa *et al*., 2005). The mechanism of CLA production has been proposed to be the hydration of LA to 10-hydroxy-18:1 with subsequent dehydration of this HFA to CLA via a multicomponent enzyme system (Kishino *et al*., 2011a, 2011b). The LAB strains capable of producing CLA from LA were the most inhibited strain by LA whereas a CLA mixture had no inhibitory effect on growth. Many strains were found to produce CLA in the media after cultures reached stationary phase. Together, this suggests that fatty acid isomerization has a detoxifying effect for LAB (Gorissen *et al*., 2011).

Using LAB as starter cultures, adjunct cultures

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or health-promoting ingredients in food has recently drawn much interest. This research evaluated, the potential of *L. plantarum* GSI 303 to serve as a CLA producing bacteria in foods such as fermented milk products. This strain was isolated from the small intestine of a goat and found to exhibit good probiotic properties. Therefore, CLA-enriched dairy products may not only increase the amount of CLA consumed but may also help in modifying the gut flora.

Concerning LAB species, several strains have been described that are able to produce CLA via isomerase activity. For instance, *Lactococcus lactis* subsp. *lactis*, *L. acidophilus, L. plantarum, L. reuteri, L. delbrueckii* subsp. *bulgaricus, L. paracasei, L. pentosus, L. brevis* and *L. rhamnosus* (Jiang *et al.*, 1998; Kishino *et al*., 2002; Ogawa *et al*., 2005; Gholami and Khosravi-Darani, 2014; Yang *et al*., 2014; Gorissen *et al*., 2015). There are differences among different strains and in the effects of cultured conditions affecting CLA production, including culture media, initial LA concentration, atmospheric conditions, initial pH, incubation time and temperature (Van Nieuwenhove *et al*., 2007; Zeng *et al.*, 2009; Gorissen *et al*., 2011; Yang *et al.*, 2014). Consequently, in order to apply CLA-producing bacteria effectively in commercial food products, the incubation conditions must be optimized. LAB production of CLA using oxidation and biohydrogenation is also a posibility. CLA density increases through rapid formation of the free radicals of LA followed by isomerization of the double bond and formation of conjugated double bonds. While the CLA content in the rumen fluid of ruminants is also affected by certain variables. CLA content of a dairy product is affected by the origin of milk, seasonal variation and time of ripening. General comparison of the results revealed that an increase of CLA through biotechnological methods is quite competitive and replaceable by the livestock production of CLA (Gholami and Khosravi-Darani, 2014). The production of CLA by LAB during cultivation may be affected by different factors, such as the concentration of added LA and the pH and temperature of the incubations, as well as by the cultivation medium (Gorissen *et al.*, 2015). Therefore, the purpose of this study was to optimize the production of CLA (*c*9,*t*11-CLA and *t*10,*c*12-CLA) by varying various incubation conditions of a *L. plantarum* GSI 303 and to evaluate the possibility of using this strain as an adjunct culture for production of fermented milk. This could offer novel opportunities for developing health-promoting functional dairy products with multiple benefits of CLA and probiotic activity.

Materials and Methods

Microbial strains and incubation conditions

For preparation of inoculums, *L. plantarum* GSI 303, selected from previous study, and *L. reuteri* ATCC 55739 from stock culture were transferred to 10 mL of MRS broth pH 6.5 (Difco, Becton, Dickenson and company, USA), anaerobically incubated in an anaerobic chamber (Coy Laboratory Products Inc., Michigan, USA) at 37°C for 18 h under 85% N_2, 10% H2, and 5% CO_2 without shaking. Next, 5% of the cultured cell were transferred to 100 mL of MRS broth in flask and incubated for 18 h as the starter culture for further study. Cell growth was monitored by measuring an increase in optical density at 600 nm after washing the cells twice in a saline solution (0.9% NaCl) using a Beckman DU 640 spectrophotometer (Beckman Instrument, Inc., Fullerton, Calif., USA.). Total viable cell count (TVC) was determined by plating out serial dilutions of suspension on MRS agar to measure the cell growth and calculated as log CFU/m. The pH of the cultured broth was measured by pH meter (Mettler-Toledo, LLC, OH, USA).

Effect of free LA concentration

L. plantarum GSI 303 was grown under the following conditions: 5% of 24-h-grown inoculum was prepared using 1% (w/v) Tween-80 and 0.5, 1.0, 2.0, 3.0 or 4.0 mg/ml LA in 100 ml MRS broth pH 6.5, anaerobic incubation at 37ºC and sampled for CLA content after 24 h fermentation. The media without LA addition was used as the control treatment. For initial cell culture, an optical density at 600 nm of 0.05 corresponding to a cell density of 4-5 log CFU/ml.

Effect of atmospheric incubation condition

L. plantarum GSI 303 was grown under the following conditions: 5% of a 24-h-grown inoculum was prepared using 1% (w/v) Tween-80 and 2 mg/ml of LA in MRS broth, and aerobic or anaerobic static incubation at 37°C. CLA content was determined after 24 h of incubation. The above conditions without LA addition were used as the control treatment.

Effect of initial pH of culture broth

L. plantarum GSI 303 was grown under the following conditions: 5% of 24-h-grown inoculum was prepared in 1% (w/v) Tween-80; grown medium of 2.0 mg/ml LA in MRS broth different initial pH values (4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0); anaerobic incubation at 37°C. CLA content was determined after 24 h of incubation. The above conditions without LA addition were used as the control treatment.

Use of L. plantarum *GSI 303 for CLA production*

Potential use of *L. plantarum* GSI 303 as a milk culture was studied in two conditions, i.e., time and temperature of fermentation as following: For the study of fermentation time, a 5% 24-h-grown inoculum of *L. plantarum* GSI 303 was prepared in 1% (w/v) Tween-80 and 2.0 mg/ml of LA in MRS broth and 12% (w/v) skim milk media containing 0.25% (w/v) of yeast extract (Difco), adjusted pH at 6.5, incubated at 37°C and sampled for TVC and CLA content after incubation for every 6 h for up to 48 h. The above conditions without LA addition were used as the control treatment.

For the study of fermentation temperature, a 5% 24-h-grown inoculum of *L. plantarum* GSI 303 or *L. reuteri* ATCC 55739 were prepared under the same media conditions as above. The medium was incubated at 15, 37 and 43°C and CLA content determined after 24 h. The media without LA addition were used as the control treatments.

Analysis of microbial CLA production

A stock solution of LA was prepared by mixing 30 mg/ml in 1% (v⁄v) Tween 80 (polyoxyethylene sorbitan monooleate) (Merck, Darmstadt, Germany) to improve its solubility. The bacterial strain was cultured in MRS broth and/or skim milk media supplemented with 0.5 mg/ml of free LA (97% purity; VWR) in 1% (v⁄v) Tween 80 (Merck, Germany), and incubated at 37°C for 24 hr. The fat was extracted from the fermentation broth by adding 2 ml isopropanol (99% purity; Alkem Chemicals, Cork, Ireland),followed by Vortex mixing for 30 s. Hexane (1.5 ml; 99% purity; Lab Scan, Dublin, Ireland) was added, mixed and another 3 ml of hexane was added, mixed and centrifuged at 2500 x g for 5 min. The hexane layer containing the lipid was collected in a glass tube and the hexane was evaporated under a gentle stream of nitrogen gas (Coakley *et al.*, 2003).

To a lipid sample in a screw-capped glass test tube, 4.0 ml of anhydrous methanolic HCl was added, and the mixture was heated at 80°C for 1 h in a boiling water bath. After cooling, 2 ml of water was added, and then fatty acid methyl esters (FAMEs) were extracted with 2 ml of hexane (Ichihara and Fukubayashi, 2010). Heptadecanoic acid (C17:0; 99% pure; Sigma) and tricosanoic acid methyl ester (C23:0; 99% pure; Sigma, St. Louise, USA.) were used for internal standard. The FAMEs were analyzed by using a GC equipped with an FID and a SP2560 capillary column (100 m x 0.25 mm i.d.x 0.20 µm film; with 1 m of a 0.25 mm i.d. polar deactivated guard column; Sigma-Aldrich) with 43.5 psi He, and constant pressure. The injector and detector temperatures were set at 280°C and 250°C, respectively. The oven temperature was programed from 60°C for 1 min, increased to 170°C at a rate of 10oC/min, maintained at 170°C for 10 min, increased to 224ºC at a rate of 4°C/min, and maintained at 224°C for 25 min. CLA methyl ester was identified by comparing peak retention times of samples with those of a commercial standard containing *c*9,*t*11-CLA and *t*10,*c*12-CLA and *t*9,*t*12-CLA (Sigma-Aldrich, St. Louise, MO, USA).

Statistical analysis

Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Significant differences among treatments were tested by ANOVA followed by Duncan's test with a level of significance at $p \leq 0.05$. All experiments were performed in duplicate with three analyses.

Results and Discussion

Effect of free LA concentration

Unsaturated fatty acids, including LA, are generally toxic to a wide spectrum of bacteria (Kepler *et al*., 1970; Maczulak *et al*., 1981; Khaskheli *et al*., 2013). However, it has been reported that bioconversion of linoleic acid to CLA might be a key step for fatty acid detoxicification in bacteria (Jiang *et al*., 1998; Maia *et al.*, 2010).

The reason why LA is toxic could be explained by the presence of double bonds, which alter the shape of the molecule. Incorporation of unsaturated fatty acids in the cell membrane can disrupt the lipid bilayer structure. Another possibility is that diffusion of fatty acids across the membrane causes chemiosmotic difficulties, disturbs the membrane potential, or disconnects intramembrane pathways (Maia *et al*., 2010). Accordingly, LA concentration is one factor that could impact the production rate of CLA. Although many LAB strains are able to convert LA to CLA, the products are highly variable among species. In addition, different production levels were obtained when the substrate concentrations were supplemented differently. For example, *Lactobacillus acidophilus* F0221 produced the highest *c*9,*t*11-CLA in MRS broth supplemented with 0.5 mg/ml of LA (Li *et al*., 2013). Supplementation of 0.02% LA in the medium was most effective in promoting *c*9,*t*11-CLA production by *L. acidophilus* L1 (Alonso *et al*., 2003). *L. acidophilus* CCRC 14079 produced relatively large amounts of *c*9,*t*11-CLA after the addition of 1.0 mg/mL of LA to the medium (Lin *et al*., 1999). Additionally, Kishino *et al.* (2002) showed that in the presence of 0.06% LA, the highest

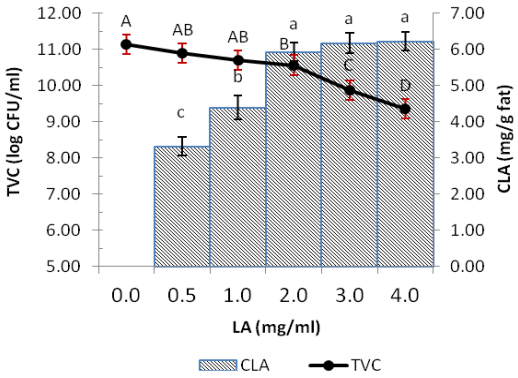

Figure 1. Effect of linoleic acid (LA) concentrations on the growth of *L. plantarum* GSI 303 and conjugated linoleic acid (CLA) production in MRS broth incubated at 37°C for 24 h in anaerobic condition. TVC with different capital letters, and CLA production with different lower-case letters are significantly different ($p < 0.05$)

CLA conversion rate (33%, including 38% *c*9,*t*11-CLA and 62% *c*9,*t*11-CLA) was obtained from *L. plantarum* AKU 1009 while the optimal *c*9,*t*11-CLA production from *L. reuteri* ATCC 55739 was obtained by supplementing 20 mg/ml of LA to the medium (Hernandez-Mendoza *et al*., 2009). In the present study, the LA concentration between 0.0-4.0 mg/ml was studied (Figure 1). The result indicated that TVC of *L. plantarum* GSI 303 and CLA production in MRS broth were dependent on the amounts of LA added. After 24 h, the initial TVC (4.98 log CFU/ml) increased to 11.13 log CFU/ml in control sample which not contain LA while TVCs were significantly lower increase in the treatments containing LA over 3.0 mg/ml (9.88 log CFU/ml) and higher LA ($p < 0.05$).

On the other hand, LA was effective in enhancing CLA production, with the maximum content of the total CLA isomers being 5.94 mg/g fat in the media when supplemented with 2.0 mg/ml of LA. There was no higher CLA contents found with an increase of LA addition although higher microbial reduction was observed with higher amounts of LA were added. Therefore, it was unnecessary to supplement more than 2.0 mg/ml LA in broth in order to obtain higher CLA production from *L. plantarum* GSI 303. From here forward, we used 2.0 mg/ml LA as the base supplementation and optimized other parameters.

Effect of atmospheric incubation conditions

To investigate the effect of atmospheric conditions on CLA production, *L. plantarum* GSI 303 was incubated under both aerobic and anaerobic conditions (Table 1). After 24 h incubation, the results showed that there were not significantly different in *c*9,*t*11-CLA isomer and total CLA production although the concentrations of *t*10,*c*12-CLA isomer were higher in aerobic condition than in anaerobic condition ($p < 0.05$). This was in agreement with previous reports that showed *L. reuteri* ATCC 55739 and *Butyrivibrio fibrisolvens* A38 produced more *c*9,*t*11-CLA under aerobic versus anaerobic conditions (Wallace *et al*., 2007; Hernandez-Mendoza *et al*., 2009). In contrast, the CLA production of *B. breve* LMC 520 produced more *c*9,*t*11-CLA concentration under anaerobic conditions (Park *et al*., 2009). In addition, the TVC in both conditions was not different which indicated that the microbial growth and yield of total CLA produced by *L. plantarum* GSI 303 were not dependent on atmospheric incubation conditions. Consequently, CLA can be produced and accumulate under either aerobic or anaerobic conditions.

Table 1. Conjugated linoleic acid (CLA) production in MRS broth supplemented with 2.0 mg/ml LA by *L. plantarum* GSI 303 at 37°C for 24 h in aerobic and anaerobic conditions

Condition	CLA (mg/g fat)			TVC
	*c*9,*t*11-CLA	*t*10,*c*12-CLA	Total CLA	(log CFU/ml)
Aerobe	3.65±0.24 [a]	2.20±0.05 [a]	5.85±0.29 [a]	10.50±0.30 [a]
Anaerobe	3.73±0.18 [a]	1.83±0.12 [b]	5.57±0.28 [a]	10.75±0.23 [a]

Note: Values in the same column with different superscript letters are significantly different ($p < 0.05$).

Regarding biohydrogenation of lactic acid bacteria, many researchers have demonstrated that bacterial growth and complete biohydrogenation of unsaturated fatty acids require anaerobic conditions, yet isomerization, the first step in the biohydrogenation process, is not inhibited by aerobiosis (Kim, 2003; Li *et al*., 2013) and anaerobic conditions are not required for isomerisation (Gorissen *et al*., 2015). The restriction of oxygen did not increase the CLA yield, but instead it favored the formation of trans,trans isomers (Macouzet *et al*., 2009).

Effect of initial pH of culture broth

L. plantarum GSI 303 was incubated in MRS broth supplemented with 2.0 mg/ml of LA at different initial pH levels and CLA production was analyzed after 24 h of incubation. The results demonstrated that production of the *c*9,*t*11-CLA isomer decreased as a result of low initial pH while production of the *t*10,*c*12-CLA was not affected (Figure 2). The maximum content of the total CLA isomers (*c*9,*t*11-CLA and *t*10,*c*12-CLA) in media in which the

Table 2. CLA production by *L. plantarum* GSI 303 and *L. reuteri* ATCC 55739 at 15 37 and 43°C for 24 h in MRS broth and skim milk media supplemented with 2.0 mg/ml LA (initial pH 6.5)

Sample	CLA (mg/g fat)		
	15°C	37°C	43°C
MRS_GSI 303	1.00±0.04 ^{Cb}	6.36±0.29 ^{Ab}	5.50±0.23 ^{Ba}
MRS_*L.reuteri*	1.58±0.14 ^{Ca}	7.68±0.30 ^{Aa}	5.48±0.14 ^{Ba}
Skim milk_GSI 303	0.30±0.06 ^{Bc}	5.31±0.38 ^{Ac}	5.00±0.26 ^{Ab}
Skim milk_*L.reuteri*	0.92±0.04 ^{Bb}	3.29±0.14 ^{Ad}	3.28±0.04 ^{Ac}

initial pH 6.5 was 6.46 mg/g fat, greater amounts of *c*9,*t*11-CLA was produced when the initial pH was in the range of 6.5-7.0. Although total CLA concentration was not different in a lower pH media but CLA production was slightly reduced. This was in agreement with previous report that the amount of CLA produced by *L. plantarum* was maximal at pH 5.5 (Khaskheli *et al*., 2013). The pH above 5 during lactic acid fermentation improved CLA synthesis by Lactococcus lactis (Kim and Liu, 2002). In addition, the pH affected the growth rate and the fatty acid profile (Nikkila *et al*., 1996). Difference of CLA production at different pH levels may be related to the activity of LA isomerase (Kepler and Tove, 1967; Choi *et al*., 2005; Gorissen *et al*., 2011; Wang *et al*., 2011; Yang *et al*., 2014). From these results, it was postulated that activity of *L. plantarum* GSI 303 for CLA production may have been more active at neutral pH than lower pH.

Effect of incubation time

Changes in pH, TVC and CLA produced by *L. plantarum* GSI 303 at 37°C for 48 h in MRS broth and skim milk media supplemented with 2.0 mg/ml of LA are shown in Figure 3. Comparison among incubation times, pH decreased while TVC increased and were rather constant after 24 h of incubation. Total CLA concentration increased and slightly decreased after 30 h incubation although the maximum content of total CLA between 24 to 36 h incubation was not different. Otherwise, the CLA concentration was slightly reduced after incubation longer than 42 h. This suggested that an incubation time longer than 24 h was unnecessary for promoting CLA produced by *L. plantarum* GSI 303. In addition, CLA was formed after the cultures reached stationary phase (Alonso *et al.*, 2003). *L. plantarum* GSI 303 reached a stationary phase after 18 h. Therefore, 24

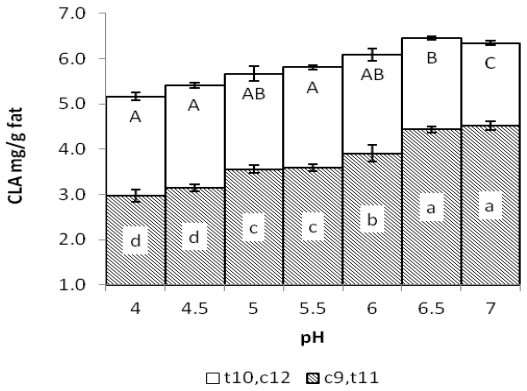

Figure 2. Effect of initial pH on *c*9,*t*11-CLA and *t*10,*c*12-CLA production by *L. plantarum* GSI 303 at 37°C for 24 h in MRS broth supplemented with 2.0 mg/ml LA. CLA production with different capital letters, and different lower-case letters are significantly ($p < 0.05$) different

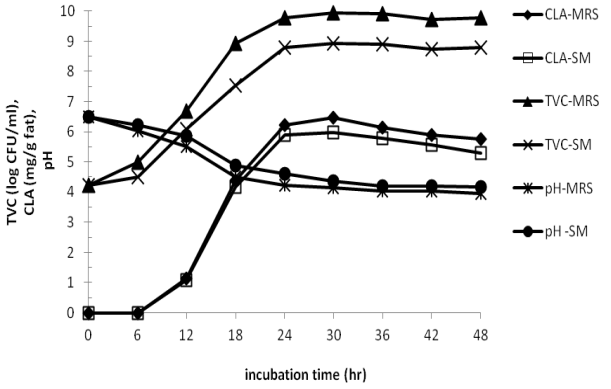

Figure 3. Change of pH, TVC and CLA production by *L. plantarum* GSI 303 at 37°C for 48 h in MRS broth and skim milk media (SM) supplemented with 2.0 mg/ml LA initial pH 6.5

h incubation was obviously appropriate for CLA productivity of this strain. According to the report of Gorissen *et al.* (2015), prolonged incubation or higher LA concentrations do not result in higher CLA formation by *Bu. fibrisolvens* A38, indicating that the LA isomerase does not recycle as a common enzyme to catalyze more substrate.

Effect of temperature on CLA production at different mediums

The effect of temperatures (15, 37 or 43°C) on CLA production in MRS broth and skim milk media supplemented with 2.0 mg/ml of LA is shown in table 2. In MRS broth, a maximum concentration of CLA was 6.36 mg/g fat which gained more from cultured broth incubated at 37°C rather than at 43°C and 15°C. In skim milk media, the maximum concentration of CLA was 5.31 mg/g fat which also gained more from cultured broth incubated at 37°C rather than at 15°C, but not different from 43°C (5.00 mg/g fat). This

result was in accordance with Gorissen *et al.* (2011) and Soto (2013). The lower levels of CLA at 15°C indicated that not only lower temperatures would allow a small amount of growth of *L. plantarum* GSI 303 but also it showed the ability to produce a low amount of CLA.

Although CLA concentration in skim milk media was lower than in MRS broth, it was interesting that *L. plantarum* GSI 303 grew and produced CLA in skim milk media indicating that the possibility of this bacterial strain to be used for adjunct cultures in fermented milk products. This was probably due to the neutralization of the inhibitory effect of fatty acids by milk protein (Boyaval *et al*., 1995) and protection of CLA oxidation by the milk protein, especially sodium caseinate and low molecular weight whey proteins in the skim milk (Shantha and Decker, 1993). The capacity of lactic acid cultures to produce CLA in LA-added skim milk after incubation was further confirmed by linoleic isomerase studies (Lin *et al*., 1999; Lin *et al.*, 2002; Gorissen *et al.*, 2011; Kishino *et al*., 2011). *L. plantarum* GSI 303 was capable of producing CLA in milk, corresponding with an explanation why some fermented dairy products had higher levels of CLA than unfermented milk as previously described (Jiang *et al.*, 1998; Kim and Liu, 2002; Lin, 2003; Prandini *et al.*, 2007).

CLA production in skim milk media and MRS broth could be enhanced by *L. reuteri* ATCC 55739, a certain type of lactic acid bacteria which has linoleic acid isomerase activity (Hernandez-Mendoza *et al*., 2009; Abd El-Salam *et al*., 2010). At the same culture condition, *L. reuteri* ATCC 55739 produced CLA (7.68±0.30 mg/g fat higher than *L. plantarum* GSI 303 (6.36±0.29 mg/g fat) in MRS broth. On the other hand, it was interesting that *L. plantarum* GSI 303 produced higher CLA (5.31±0.38 mg/g fat) than did *L. reuteri* ATCC 55739 (3.19±0.14 mg/g fat) in skim milk media. However, the optimum production of CLA by *L. reuteri* ATCC 55739 exhibited variations with regard to cultured conditions, including culture media and the fermentation conditions used. For example, CLA were produced 0.45 mg/ml in MRS broth pH 9.5, supplemented with 0.9 mg/ml LA at 37°C (Lee *et al*., 2003), 0.11 mg/ml in MRS broth pH 6.5, supplemented with 20 mg/ml LA at 10°C (Hernandez-Mendoza *et al*., 2009) and 65.5 µg/ml in MRS broth pH 7.0, supplemented with 150 µg/ml LA at 37°C at (Roman-Nunez *et al*., 2007). In this study, the use of *L. plantarum* GSI 303 exhibits more interesting since not only having probiotic properties but also ability to producing CLA represents one of the technology options for manufacturing new dairy functional food.

Conclusions

The optimum culturing of *L. plantarum* GSI 303 for CLA production in MRS broth and skim milk media contained 2.0 mg/mL LA, initial pH 6.5 and incubation at 37°C for 24 h. Aerobic and anaerobic incubation conditions were not significantly different in CLA production. Culturing in MRS broth or skim milk media at 37°C resulted in higher CLA content than that at 43°C and 15°C. In skim milk media, *L. plantarum* GSI 303 produced more CLA than *L. reuteri* ATCC 55739, a positive control CLA-producing bacteria. Therefore, this bacterial strain could be used as an adjunct culture to increase the CLA concentration in fermented dairy products.

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