



## Protective effects of *Lactococcus chungangensis* CAU 28 on alcohol-metabolizing enzyme activity in rats

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### ABSTRACT

In this study, we investigated the beneficial effects of *Lactococcus chungangensis* CAU 28, a bacterial strain of nondairy origin, on alcohol metabolism in rats treated with ethanol, focusing on alcohol elimination and prevention of damage and comparing the effects with those observed for *Lactococcus lactis* ssp. *lactis* ATCC 19435. Male Sprague-Dawley rats were orally administered 20% ethanol and 3 substrates (freeze-dried cells, cream cheese, and yogurt) containing *Lc. chungangensis* CAU 28 or *Lc. lactis* ssp. *lactis* ATCC 19435, which were provided 1 h before or 1 h after ethanol ingestion. Blood samples were collected from the tail veins of the rats at 1, 3, 6, 12, and 24 h after ingestion of ethanol, *Lc. chungangensis* CAU 28 substrate, or *Lc. lactis* ssp. *lactis* ATCC 19435 substrate. Alcohol and acetaldehyde concentrations in the *Lc. chungangensis* CAU 28 substrate-treated rats were significantly reduced in a time-dependent manner compared with those in the *Lc. lactis* ssp. *lactis* ATCC 19435 substrate-treated rats. Among the experimental groups, treatment with cream cheese before ingestion of 20% ethanol was found to be the most effective method for reducing both alcohol and acetaldehyde levels in the blood. Alanine aminotransferase and aspartate aminotransferase activities in the *Lc. chungangensis* CAU 28 substrate-treated rats were significantly lower than those in the positive controls. Moreover, in the *Lc. chungangensis* CAU 28 cream cheese-treated group, rats showed a reduction of liver enzymes by up to 60%, with good effectiveness observed for both pre- and post-ethanol ingestion. These results suggested that intake of lactic acid bacteria, particularly in *Lc. chungangensis* CAU 28-supplemented dairy products, may reduce blood alcohol and acetaldehyde concentrations, thereby mitigating acute

alcohol-induced hepatotoxicity by altering alcohol-metabolizing enzyme activities.

**Key words:** *Lactococcus*, alcohol dehydrogenase, aldehyde dehydrogenase, aspartate aminotransferase, alanine aminotransferase

### INTRODUCTION

Alcoholic beverages have been consumed by humans since the beginning of recorded history (Room et al., 2005). Fermented drinks are now prepared and consumed globally, affecting the cultural acceptance of alcohol (Room et al., 2002). However, problems associated with alcohol consumption have increased in the last 30 yr, with substantial advances in our understanding of drinking problems as well as their prevention and treatment (Room et al., 2005). After consumption of alcoholic beverages, alcohol is absorbed into the blood and diluted by the total body water. The oxidation of alcohol occurs primarily in the liver, and the major pathway for alcohol metabolism involves the production of alcohol dehydrogenase (ADH), which converts alcohol into acetaldehyde through enzymatic oxidation. Acetaldehyde is an organic chemical compound that is highly toxic to the body, even at low concentrations. However, aldehyde dehydrogenase (ALDH) rapidly oxidizes acetaldehyde to acetate (Maher, 1997; Vanni et al., 2000). Pearl (1926) reported that excessive alcohol consumption could lead to death. Moreover, alcohol consumption contributes to the incidence of several diseases, such as breast cancer, coronary heart disease, stroke, diabetes, and liver injury (Rehm et al., 1997; Smith-Warner et al., 1998; Corrao et al., 2000).

Various lactic acid bacteria (LAB) are currently being used to prevent and treat diseases. The most commonly used species include *Lactobacillus acidophilus*, *Lactobacillus bifidus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Streptococcus thermophilus*, and *Enterococcus faecium*. Lactic acid bacteria benefit the host when administered at the proper amounts (Kalliomäki et al., 2001; Brown and Valiere, 2004). Additionally, probiotics can be beneficial to human health

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by affecting gastrointestinal infections, antimicrobial activity, lactose metabolism, serum cholesterol levels, immune system function, and because of their antimutagenic, anticarcinogenic, and antidiarrheal properties (Gomes and Malcata, 1999; Agerholm-Larsen et al., 2000; Gotcheva et al., 2002; Nomoto, 2005; Imase et al., 2007; Shah, 2007).

Some oral streptococci have been shown to exhibit ADH activity and produce acetaldehyde in vitro during ethanol incubation. In particular, *Streptococcus salivarius*, *Streptococcus intermedius*, and *Streptococcus mitis* produce high amounts of acetaldehyde in response to exposure to 11 or 1,100 mM ethanol (Kurkivuori et al., 2007). *Leuconostoc cremoris* also shows NADP-dependent alcohol dehydrogenase activity for reducing acetaldehyde to ethanol (Lees and Jago, 1976). The thermophilic bacterium *Thermoanaerobium brockii* contains both NADP-linked ADH and NAD-linked ADH and has thus attracted considerable interest for industrial alcohol and enzyme production (Lamed and Zeikus, 1981). In addition, LAB of the genus *Lactobacillus*, including *Lactobacillus* GG ATCC 53103, can metabolize ethanol and acetaldehyde in vitro, as determined by gas chromatography of the bacterial suspension after incubating the culture for 1 h with 22 mM ethanol (Nosova et al., 2000).

Another LAB, *Lactococcus chungangensis* CAU 28, has been shown to express several genes that are relevant for dairy milk manufacturing, such as *S*-adenosylmethionine (Konkit et al., 2014). In addition, this strain has been shown to produce ADH and ALDH to reduce alcohol and acetaldehyde levels in vitro and in vivo (Konkit et al., 2015, 2016). A recent study showed that this strain could reduce atopic dermatitis symptoms in mice (Choi et al., 2016).

Accordingly, in this study, the ADH and ALDH activities of *Lc. chungangensis* CAU 28 were characterized and compared with those of *Lactococcus lactis* ssp. *lactis* ATCC 19435 through administration in freeze-dried cells, cream cheese, or yogurt 1 h before or after administration of ethanol. Thus, *Lc. chungangensis* CAU 28 may have applications as an effective alcohol detoxicant.

## MATERIALS AND METHODS

### Bacterial Strain Culture and Sample Preparation

*Lactococcus chungangensis* CAU 28 and *Lc. lactis* ssp. *lactis* ATCC 19435 (hereafter, *Lc. chungangensis* and *Lc. lactis*) were cultured in tryptic soy broth (Becton, Dickinson and Co., Sparks, NV) at 30°C for 24 h.

To evaluate alcohol metabolism, freeze-dried cells, cream cheese, and yogurt were prepared with *Lc. chun-*

*gangensis* and *Lc. lactis*. For freeze-dried cell preparation, cells cultured in tryptic soy broth at 30°C for 24 h were harvested by centrifugation at 12,000 × *g* and 4°C for 20 min and then dried using a freeze dryer (Hanil Science Inc., Gimpo, Korea). Cream cheese was made as described previously (Konkit et al., 2015). Briefly, pasteurized milk (Pasteur Milk Co., Seoul, Korea) was heated at 68°C for 30 min, cooled, and then 5% (vol/vol) cell culture of *Lc. chungangensis* or *Lc. lactis* was added and the milk incubated at 30°C for 48 h. The milk was acidified during this period, stirred, and heated at 70°C for 5 min, and the whey was separated using a cloth bag. Curd was set, and the whey was drained by adding 0.5% salt. Yogurt was prepared according to a method described previously (Konkit et al., 2015). Briefly, pasteurized milk was heated at 93°C, stirred gently, and then cooled until it was warm to touch, around 44 to 46°C, after which the milk was occasionally stirred to prevent skin formation. A cup of the warm milk was scooped out, the inoculum strain (1% vol/vol) was added to it, and the yogurt was kept to set overnight. Finally, both cream cheese and yogurt samples were freeze-dried and stored in the dark at 4°C until further use.

### Administration of Substrate to Animals

Five-week-old male Sprague Dawley rats were purchased from Samtako (Osan, Korea). Rats were acclimated to cages at 22°C and a 12-h day/night cycle, and these conditions were maintained throughout the experiments, beginning 1 wk before the start of the experiments. The rats were divided into 8 groups (*n* = 3 for each group): (1) negative control group (water administration); (2) positive control group (20% ethanol administration); (3) group administered *Lc. chungangensis* freeze-dried cells (10<sup>9</sup> cfu/mL); (4) group administered cream cheese made with *Lc. chungangensis* (1.4 g/kg); (5) group administered yogurt made from *Lc. chungangensis* (1.4 g/kg); (6) group administered *Lc. lactis* freeze-dried cells (10<sup>9</sup> cfu/mL); (7) group administered cream cheese made from *Lc. lactis* (1.4 g/kg); and (8) group administered yogurt made from *Lc. lactis* (1.4 g/kg). All rats except those in the negative group were administered the substrate orally and groups were either given 20% ethanol first followed by substrate or substrate first and then 20% ethanol (Figure 1).

### Determination of Alcohol and Acetaldehyde Concentrations in Rat Blood

Whole-blood samples were collected from the tail veins of Sprague Dawley rats in serum separation tubes at 1, 3, 6, 12, and 24 h after ethanol and substrate

administration. The blood samples were centrifuged at  $12,000 \times g$  for 15 min and incubated at  $4^{\circ}\text{C}$  for 10 min. The supernatant (comprising blood serum) was used for analyses of alcohol and aldehyde concentrations. The reaction mixture, containing  $0.3\text{ M KH}_2\text{PO}_4$  buffer (pH 9.0),  $49\text{ mM NAD}^+$ , and serum in a final volume of  $3\text{ mL}$ , was incubated at  $20^{\circ}\text{C}$  for 5 min, and its absorbance (A1) was measured at  $340\text{ nm}$  using a spectrophotometer (Tecan, Männedorf, Switzerland). After measurement,  $50\text{ }\mu\text{L}$  of ADH for alcohol analysis or  $50\text{ }\mu\text{L}$  of ALDH for aldehyde analysis was added to the mixture, and the mixture was incubated at  $20^{\circ}\text{C}$  for 5 min. The absorbance (A2) was measured at  $340\text{ nm}$  using the Infinite 200 NanoQuant spectrophotometer (Tecan). Blood alcohol and aldehyde concentrations were calculated using the following equations:

$$\text{Alcohol concentration} = 0.7259/3.6 \times \Delta A,$$

$$\text{Aldehyde concentration} = 0.7158/3.6 \times \Delta A,$$

where  $\Delta A = \text{sample (A2 - A1)} - \text{blank (A2 - A1)}$ .

#### Determination of Aspartate Aminotransferase and Alanine Aminotransferase in Rat Blood

All rats were fasted for 12 h before blood collection. Whole-blood samples were collected from the hearts of the experimental Sprague Dawley rats and analyzed for total aspartate aminotransferase (AST) and alanine aminotransferase (ALT) at Green Cross Biotherapeutics Inc. (Yongin, South Korea).

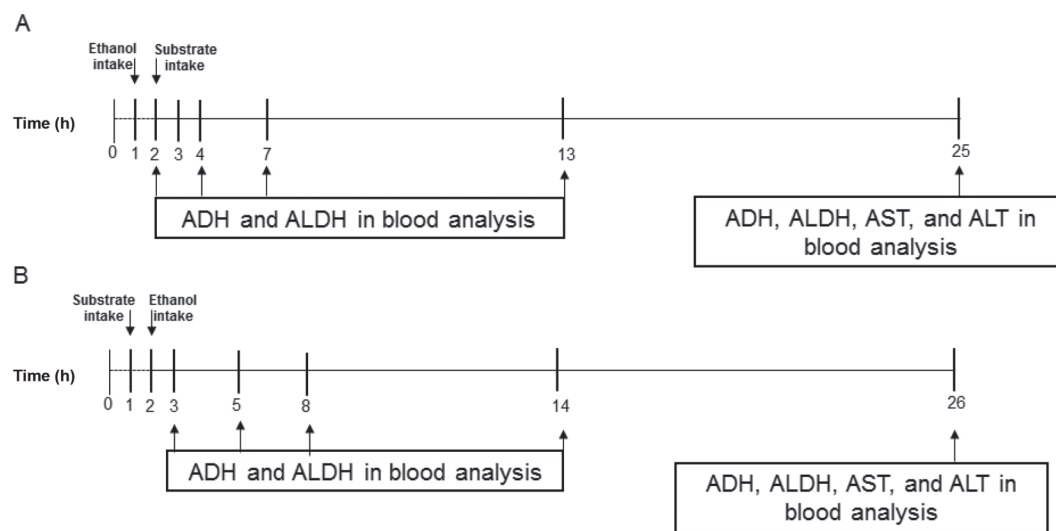
#### Statistical Analysis

All results are presented as means  $\pm$  standard deviations. One-way ANOVA with Tukey's post hoc test was applied using the predictive analytic software statistics package for Windows (SPSS Inc./IBM Corp., Armonk, NY). Differences with  $P$ -values  $< 0.05$  were considered statistically significant.

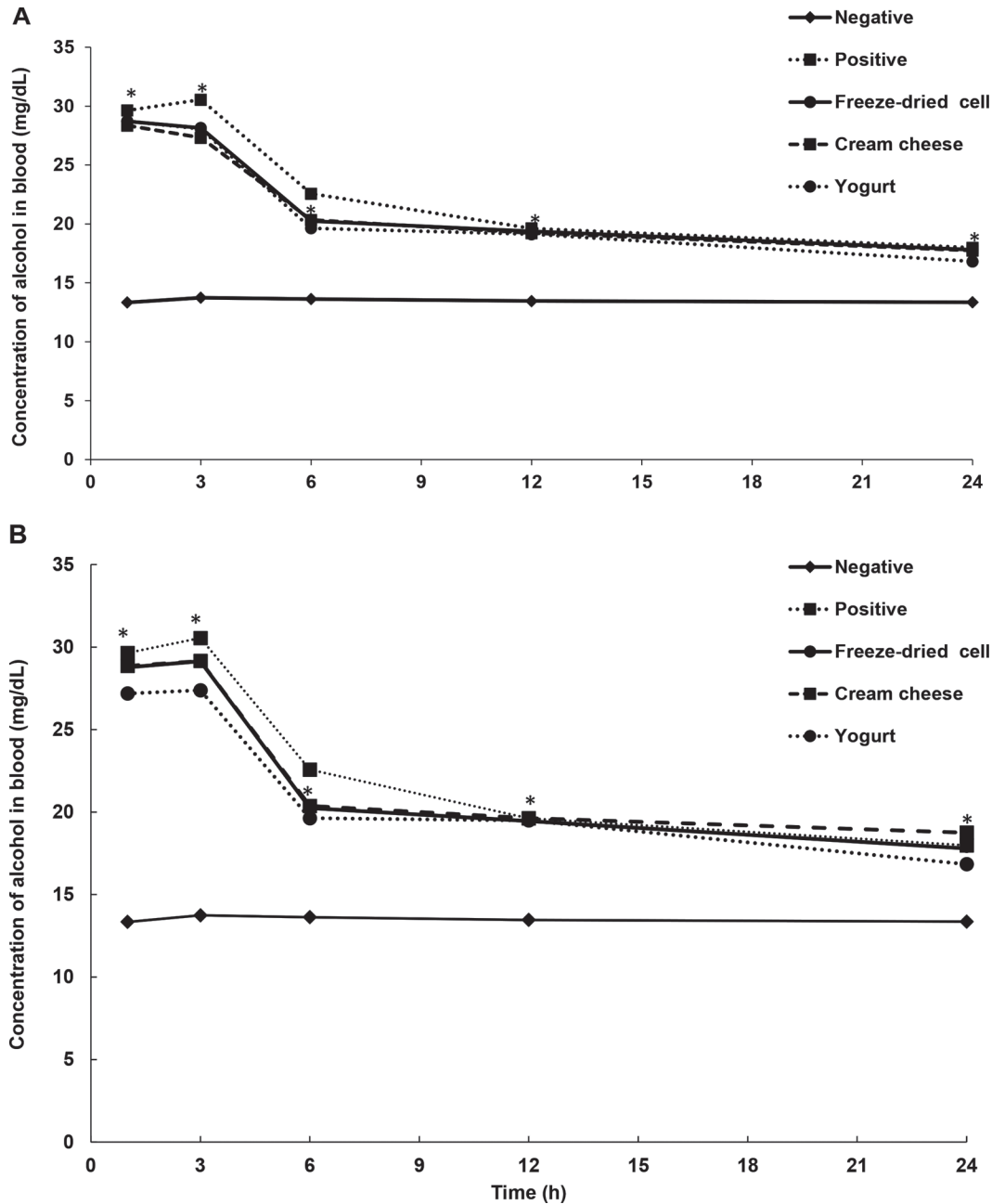
## RESULTS

#### ADH Activity in Rats Treated with 20% Ethanol First and then Administered Substrate

In vivo studies were conducted to measure alcohol concentrations in rat sera after administration of 20% ethanol, followed by administration of *Lc. chungangensis* or *Lc. lactis* substrates after 1 h. From 1 to 3 h, the blood alcohol concentration of the positive group was significantly increased ( $29.64\text{--}30.55\text{ mg/dL}$ ;  $P = 0.01$ ) compared with those of the treated sample groups (Figure 2A and 2B). At 6, 12, and 24 h, we observed differences in blood alcohol levels between treated groups administered freeze-dried cells ( $P = 0.01$ ), cream cheese ( $P = 0.03$ ), and yogurt ( $P = 0.01$ ) and compared with the positive control group ( $P = 0.01$ ). The blood alcohol concentrations in the rats treated with freeze-dried cells, cream cheese, and yogurt were  $20.25$ ,  $20.34$ , and  $19.64\text{ mg/dL}$ , respectively, for *Lc. chungangensis* and  $22.57$ ,  $21.45$ , and  $20.79\text{ mg/dL}$ , respectively, for *Lc. lactis*, with a positive control of  $22.61\text{ mg/dL}$  at 6 h;  $19.37$ ,  $19.23$ , and  $19.13\text{ mg/dL}$ , re-



**Figure 1.** Schematic of the experiment schedule. (A) Treatment of rats with 20% ethanol first and then with *Lactococcus chungangensis* CAU 28 and *Lactococcus lactis* ssp. *lactis* ATCC 19435 in substrates (freeze-dried cells, cream cheese, and yogurt). (B) Administration of *Lc. chungangensis* CAU 28 and *Lc. lactis* ssp. *lactis* ATCC 19435 substrates first and then 20% ethanol. ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.



**Figure 2.** Alcohol dehydrogenase concentrations in rat blood after treatment with 20% ethanol first and then substrates. (A) *Lactococcus chungangensis* CAU 28 in 1 of 3 substrates: freeze-dried cells, cream cheese, or yogurt; (B) *Lactococcus lactis* ssp. *lactis* ATCC 19435 in substrates. Negative = water only, Positive = 20% ethanol only. Results are expressed as means  $\pm$  SD of 3 independent experiments; the 3 values of alcohol dehydrogenase are very close, so error bars are not clearly visible. \* $P < 0.05$ .

spectively, for *Lc. chungangensis* and 21.68, 20.67, and 20.79 mg/dL, respectively, for *Lc. lactis*, with a positive control of 22.07 mg/dL at 12 h; and 17.81, 17.74, and 16.82 mg/dL, respectively, for *Lc. chungangensis* and 20.67, 19.75, and 17.83 mg/dL, respectively, for *Lc. lactis*, with a positive control of 19.33 mg/dL at 24 h. Interestingly, at 24 h, the blood alcohol concentrations in rats from the yogurt group of *Lc. chungangensis* were

lower than those of the other treated groups and the positive control ( $P = 0.03$ ).

#### **ADH Activity in Rats Administered Substrate First and then Treated with 20% Ethanol**

Alcohol concentrations in rat serum were measured after administration of *Lc. chungangensis* and *Lc. lactis*

substrates, followed by treatment with 20% ethanol after 1 h. At 1 h after administration of 20% ethanol, the blood alcohol concentration of the positive group was significantly increased (29.54 mg/dL;  $P = 0.01$ ) compared with those of the other treated groups (Figure 3A and 3B). Interestingly, rats administered cream cheese with *Lc. chungangensis* or *Lc. lactis* had the lowest blood alcohol concentrations (23.05 and 25.2 mg/dL, respectively;  $P = 0.01$ ) among all groups. From 3 to 6 h, rats in groups administered freeze-dried cells, cream cheese, and yogurt had lower blood alcohol concentrations than those in the positive group. At 12 h, rats in the group treated with *Lc. chungangensis* and *Lc. lactis* cream cheese had the lowest blood alcohol concentrations of 16.26 and 19.35 mg/dL, respectively ( $P = 0.01$ ), compared with those of the other treated groups. In addition, rats in groups administered freeze-dried cells and yogurt also had low blood alcohol concentrations of 17.73 and 17.74 mg/dL, respectively, in *Lc. chungangensis*, and 21.37 and 20.83 mg/dL, respectively, in *Lc. lactis*; however, there was a significant difference between groups. At 24 h, we observed differences in blood alcohol levels between treated groups administered freeze-dried cells ( $P = 0.01$ ), cream cheese ( $P = 0.01$ ), and yogurt ( $P = 0.01$ ), and the positive control group ( $P = 0.01$ ). Moreover, the blood alcohol concentrations of the *Lc. chungangensis* substrate groups were 16.30, 16.14, and 17.48 mg/dL, respectively, and those in the *Lc. lactis* substrate groups were 20.09, 18.19, and 19.58 mg/dL, respectively. Notably, at 24 h, the blood alcohol concentration in rats administered cream cheese from *Lc. chungangensis* was the lowest among all treated groups, *Lc. lactis*-treated groups, and the positive control ( $P = 0.01$ ).

#### **ALDH Activity in Rats Treated with 20% Ethanol First and then Administered Substrate**

From 1 to 3 h, blood acetaldehyde concentrations in the positive group were significantly increased (27.94–29.22 mg/dL;  $P = 0.01$ ) compared with those of the other treated groups (Figure 4A and 4B). At 6 h, we observed differences in blood acetaldehyde levels between treated groups administered freeze-dried cells ( $P = 0.01$ ), cream cheese ( $P = 0.03$ ), and yogurt ( $P = 0.01$ ) and the positive control group ( $P = 0.01$ ). Furthermore, blood acetaldehyde concentrations in the *Lc. chungangensis* substrate groups were 20.34, 18.58, and 17.93 mg/dL, respectively, and those in the *Lc. lactis* substrate groups were 21.72, 19.65, and 19.40 mg/dL, respectively. Even at 12 and 24 h, there were differences in blood acetaldehyde concentrations between rats in treated groups administered freeze-dried cells ( $P = 0.01$ ), cream cheese ( $P = 0.03$ ), and yogurt ( $P = 0.01$ )

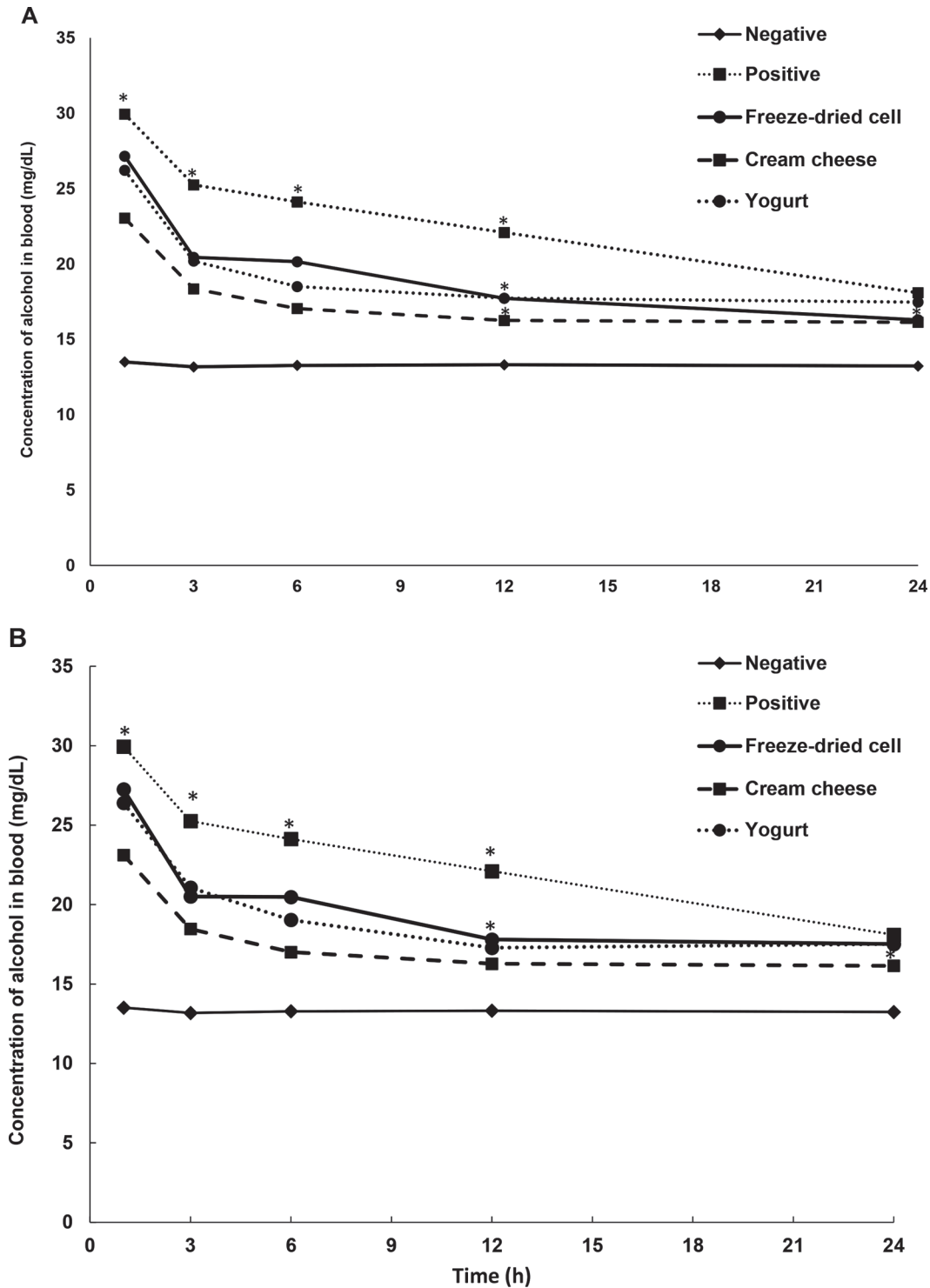
and in the positive control group ( $P = 0.01$ ). The blood acetaldehyde concentrations of the groups treated with *Lc. chungangensis* at 24 h were 17.47, 16.84, and 16.67 mg/dL, respectively, whereas those of the *Lc. lactis* substrate groups were 17.90, 17.74, and 17.66 mg/dL, respectively. In addition, from 1 to 24 h, the blood acetaldehyde concentrations in rats fed yogurt made with *Lc. chungangensis* were significantly reduced compared with those of the other treated groups, the *Lc. lactis*-treated groups, and the positive control ( $P = 0.03$ ).

#### **ALDH in Rats Administered Substrate First and then Treated with 20% Ethanol**

After 1 h, blood acetaldehyde concentrations in rats in the positive group were significantly increased (32.69 mg/dL;  $P = 0.01$ ) compared with those in rats from other treated groups (Figure 5A and 5B). At 3, 6, 12, and 24 h, we observed differences in blood alcohol levels between treated groups administered freeze-dried cells ( $P = 0.01$ ), cream cheese, ( $P = 0.01$ ), and yogurt ( $P = 0.01$ ) and the positive control group ( $P = 0.01$ ). The blood acetaldehyde concentrations in the *Lc. chungangensis* substrate groups were 19.25 to 19.64, 15.91 to 17.23, and 17.33 to 18.53 mg/dL, respectively, and those in the *Lc. lactis* substrate groups were 22.96 to 21.29, 18.9 to 17.2, and 19.57 to 18.41 mg/dL, respectively, at 3 to 6 h. The blood acetaldehyde concentrations were 16.72, 15.36, and 15.43 mg/dL, respectively, in the *Lc. chungangensis* substrate groups and 17.18, 16.76, and 16.92 mg/dL, respectively, in the *Lc. lactis* substrate groups at 24 h. Additionally, from 1 to 24 h, the blood acetaldehyde concentrations in rats administered cream cheese from *Lc. chungangensis* were the lowest among all treated groups, the *Lc. lactis*-treated groups, and the positive control ( $P = 0.01$ ).

#### **Blood AST and ALT Levels in Rats Treated with 20% Ethanol and Administered Substrates**

Aspartate aminotransferase and ALT are enzymes used as blood biomarkers to assess alcohol abuse and liver injury. In this experiment, we found that blood AST levels in rats from the positive group were highest (67 U/L following treatment with 20% alcohol first, and 82.67 U/L following administration of substrates first;  $P = 0.01$ ) among all groups (Figure 6A and 7A). Interestingly, rats in groups administered cream cheese and yogurt with *Lc. chungangensis* had significantly lower blood AST levels compared with those in the *Lc. lactis* groups (35 and 40.67 U/L, respectively, for *Lc. chungangensis*, and 47.30 and 48.30 U/L, respectively, for *Lc. lactis* when rats were treated with ethanol first; 42 and 43 U/L, respectively, for *Lc. chungangensis*, and



**Figure 3.** Alcohol dehydrogenase concentrations in rat blood after administration of substrates first and then 20% ethanol. (A) *Lactococcus chungangensis* CAU 28 in 1 of 3 substrates: freeze-dried cells, cream cheese, or yogurt; (B) *Lactococcus lactis* ssp. *lactis* ATCC 19435 substrates. Negative = water only, Positive = 20% ethanol only. Results are expressed as means  $\pm$  SD from 3 independent experiments; the 3 values of alcohol dehydrogenase are very close, so error bars are not clearly visible. \* $P < 0.05$ .

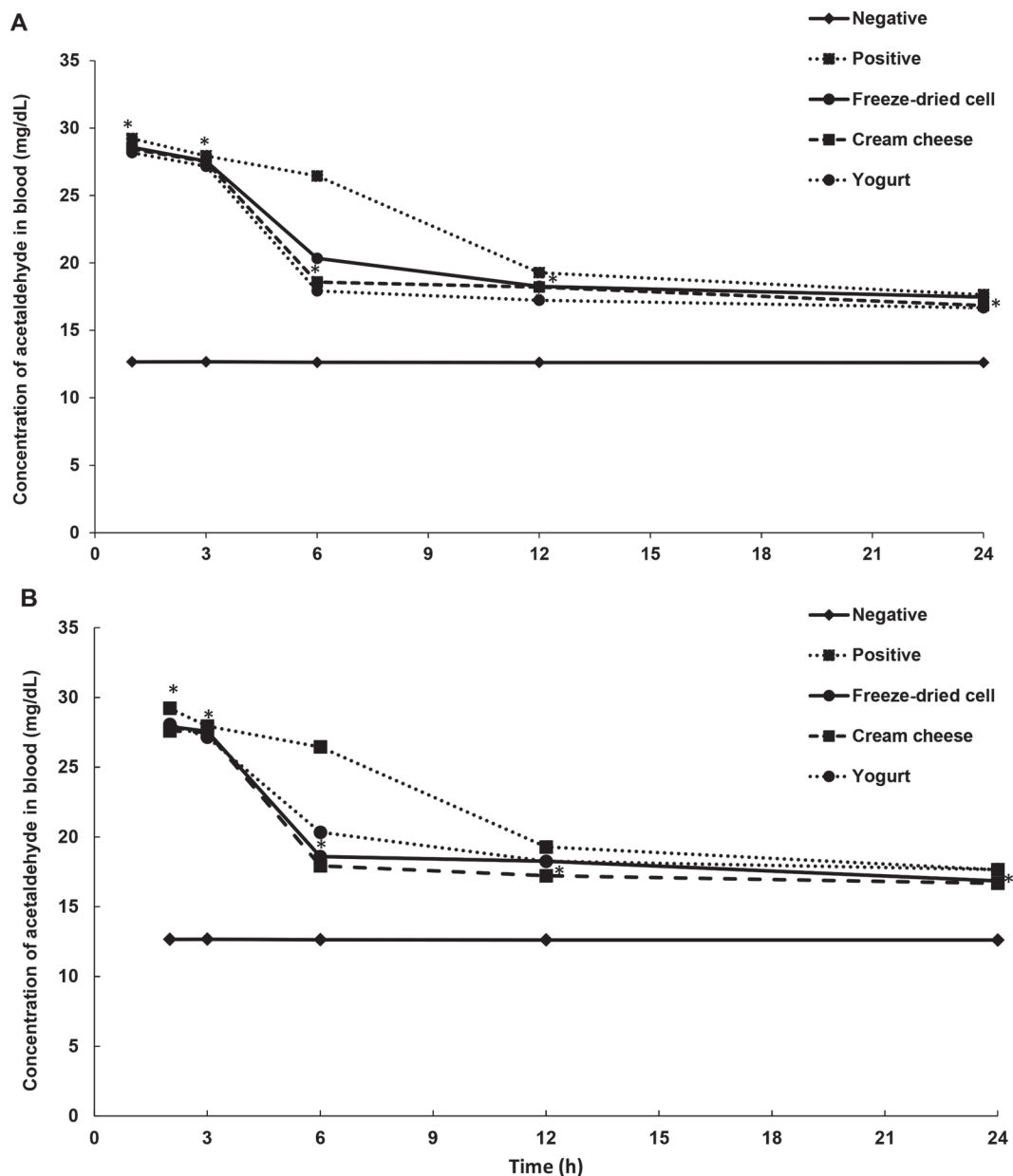
41.30 and 44.30 U/L, respectively, for *Lc. lactis* when the substrates were administered first;  $P = 0.01$ ).

Furthermore, blood ALT levels in rats from the positive group were 282.33 U/L following treatment with 20% alcohol first and were 310 U/L following administration of *Lc. chungangensis* and *Lc. lactis* first ( $P = 0.01$ ; Figure 6B and 7B). Cream cheese and yogurt from *Lc. chungangensis* were found to reduce blood ALT levels in rats treated with 20% alcohol first or when administered substrates first, and ALT levels

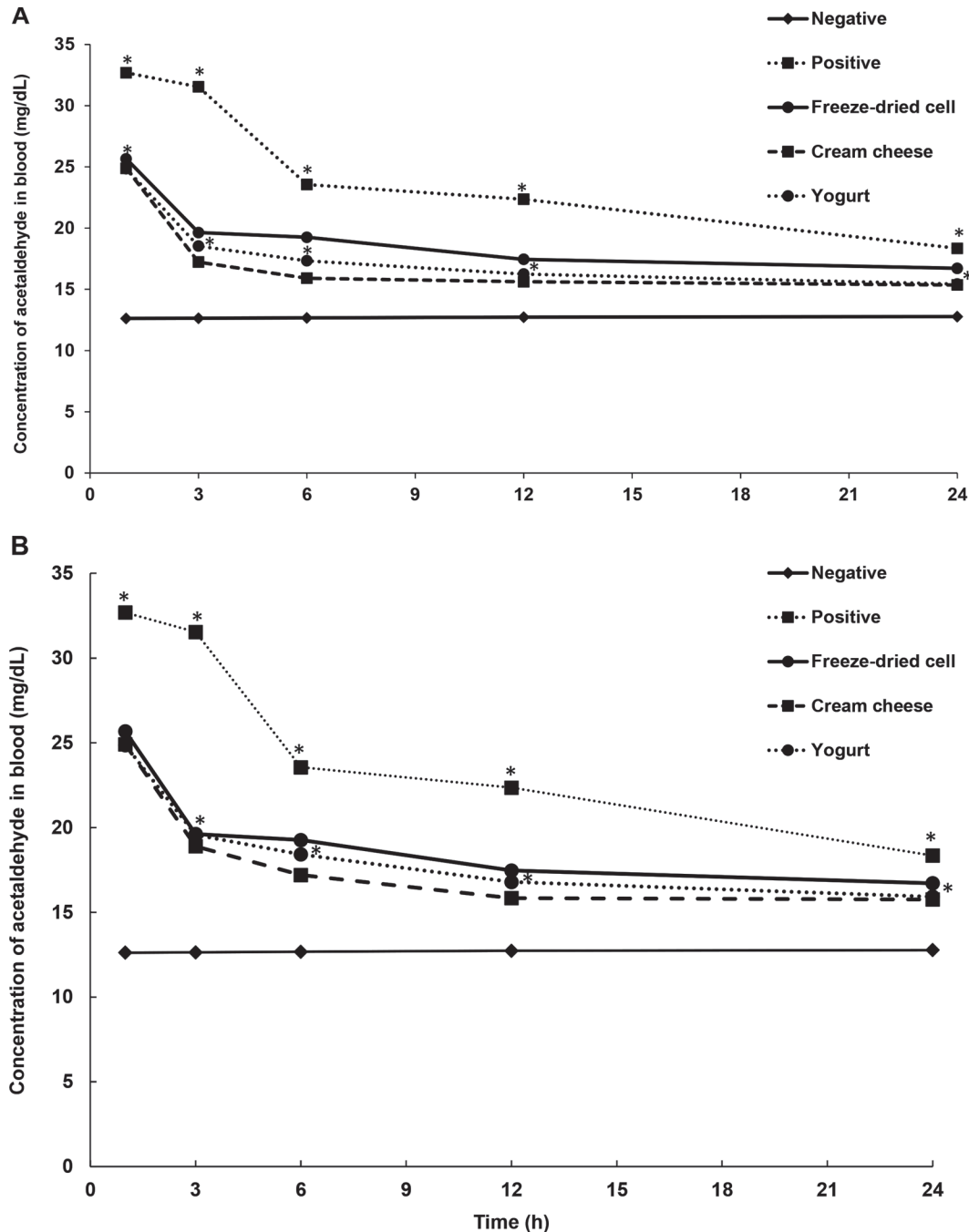
were significantly lower compared with those of positive and freeze-dried cell groups ( $P = 0.01$ ).

## DISCUSSION

Our previous studies have demonstrated that treatment with *Lc. chungangensis* CAU 28 cell culture and cream cheese prepared using this microorganism could reduce both alcohol and acetaldehyde levels in mouse blood (Konkit et al., 2015, 2016). Alcohol dependence



**Figure 4.** Acetaldehyde dehydrogenase concentrations in rat blood after treatment with 20% ethanol first and then substrates. (A) *Lactococcus chungangensis* CAU 28 in 1 of 3 substrates: freeze-dried cells, cream cheese, or yogurt; (B) *Lactococcus lactis* ssp. *lactis* ATCC 19435 substrates. Negative = water only, Positive = 20% ethanol only. Results are expressed as means  $\pm$  SD from 3 independent experiments; the 3 values of alcohol dehydrogenase are very close, so error bars are not clearly visible. \* $P < 0.05$ .



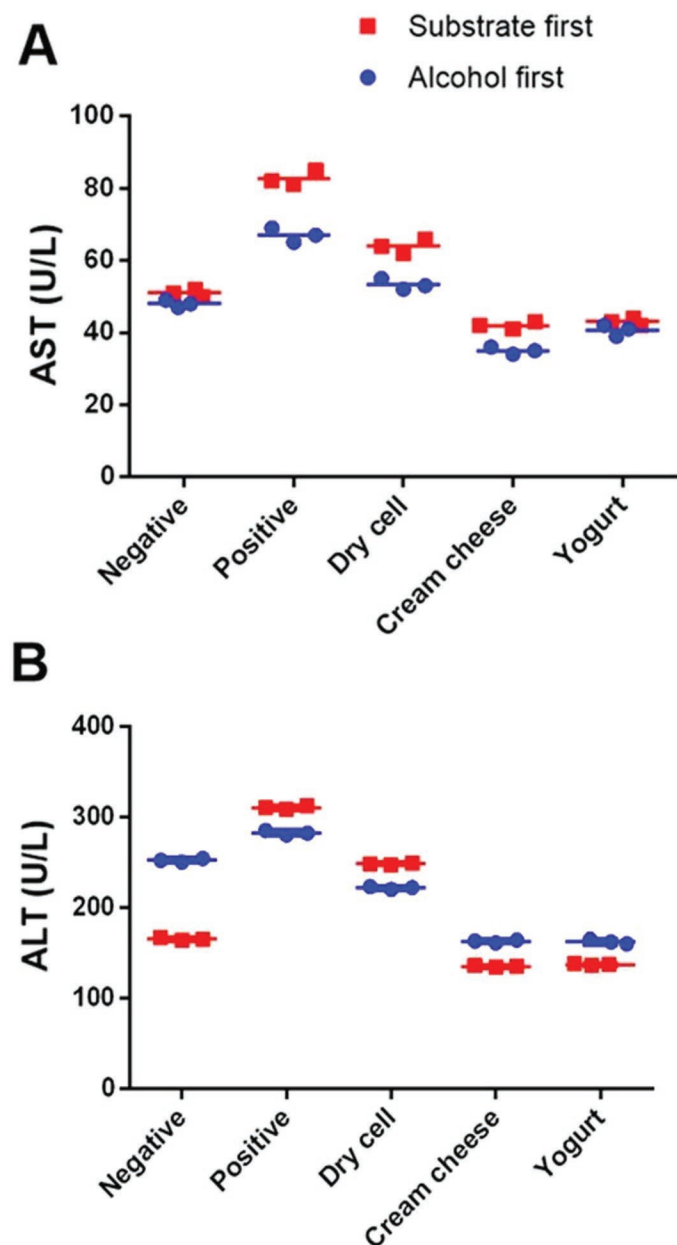
**Figure 5.** Acetaldehyde dehydrogenase concentrations in rat blood after administration of substrates first and then 20% ethanol. (A) *Lactococcus chungangensis* CAU 28 in 1 of 3 substrates: freeze-dried cells, cream cheese, or yogurt; (B) *Lactococcus lactis* ssp. *lactis* ATCC 19435 substrates. Negative = water only, Positive = 20% ethanol only. Results are expressed as means  $\pm$  SD from 3 independent experiments; the 3 values of alcohol dehydrogenase are very close, so error bars are not clearly visible. \* $P < 0.05$ .

and alcohol abuse are the primary causes of end-stage liver disease and long-term morbidity and mortality (Sehrawat and Sultana, 2006). According to studies, alcoholic liver disease (ALD) has increased in several countries showing increased alcoholic beverage consumption (Takada et al., 1995; Rehm et al., 2003).

Alcoholic liver disease is a chronic disease related to social behavior and genetic polymorphisms in various enzymes and metabolic pathways that balance ethanol metabolism (Segawa et al., 2008). It accounts for 40% of deaths due to cirrhosis and 28% of all liver disease-related deaths in the United States (Voigt,



2005). Alcohol abuse not only plays a role in ALD and hepatocellular carcinoma but also affects the brain and cardiovascular system (Setshedi et al., 2010). The most broadly recognized forms of ALD are alcoholic fatty liver disease (steatosis), acute alcoholic hepatitis, and alcoholic cirrhosis (Walsh and Alexander, 2000).

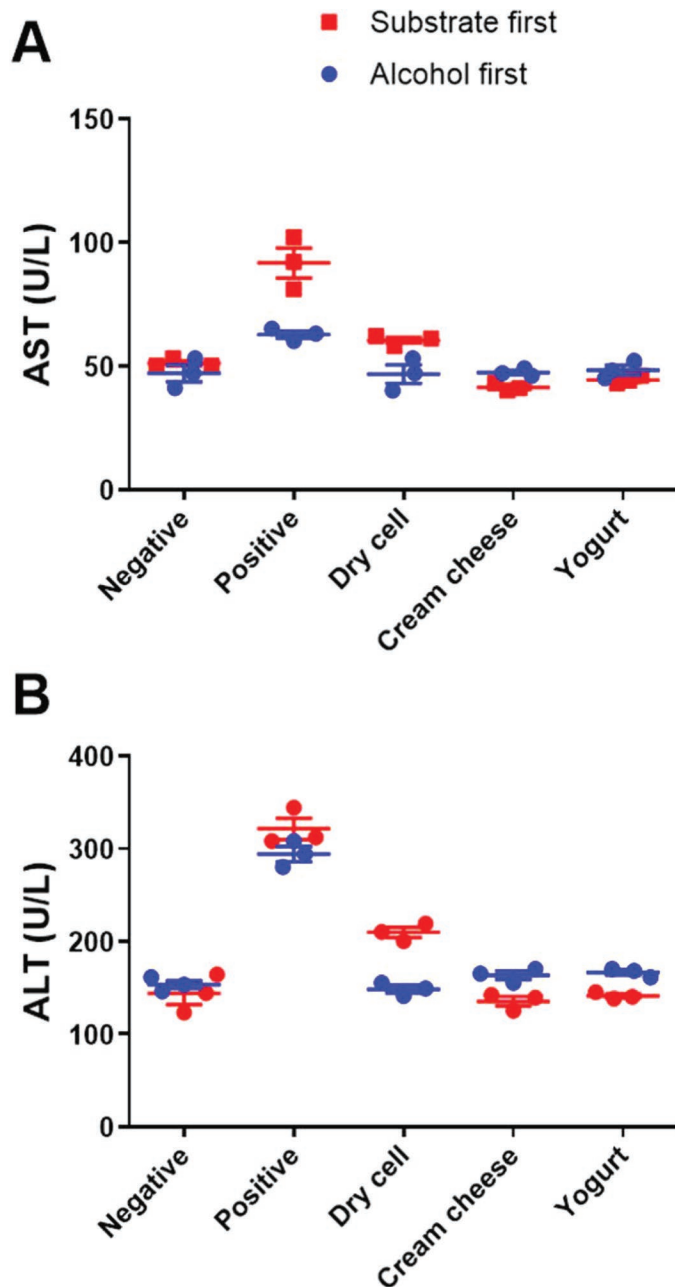


**Figure 6.** (A) Aspartate aminotransferase (AST), and (B) alanine aminotransferase (ALT) levels (in triplicate) in rat blood after treatment with 20% ethanol first and then *Lactococcus chungangensis* CAU 28 in 1 of 3 substrates: freeze-dried cells, cream cheese, or yogurt; or after administration of *Lc. chungangensis* CAU 28 in substrates first and then 20% ethanol. Negative = water only, Positive = 20% ethanol only. Data are shown as the mean  $\pm$  SEM. Color version available online.

The gut microflora plays an important role in the pathogenesis of ALD. Nanji et al. (1994) reported that feeding live *L. rhamnosus* GG reduces endotoxemia and alcohol-induced liver injury in rats continuously infused with a diet of ethanol-containing liquid through an implanted gastric tube. Probiotics are microorganisms that usually have a human or animal origin (Schrezenmeier and de Vrese, 2001). Lactic acid bacteria with probiotic properties are generally enteric flora and play an important role in the human gastrointestinal tract ecosystem. Probiotic activities can have nutritional, physiological, and antimicrobial effects. Moreover, LAB are also potential adjuvants, and their oral administration activates both mucosal and systemic immune responses (Gerritse et al., 1990). *Lactococcus* species are LAB that can be developed into probiotics because of their benefits to the food industry. Additionally, *Lactococcus* species have been given “generally recognized as safe” status, similar to *Lactobacillus* species (Kimoto et al., 1999).

In this study, consumption of *Lc. chungangensis* CAU 28 substrates, such as freeze-dried cells, cream cheese, and yogurt, was found to significantly improve serum biochemical indices and influence alcohol-metabolizing enzymes in the liver, when administered before or after ethanol. Notably, blood alcohol and acetaldehyde levels were lower when rats were treated first with 20% ethanol and then with yogurt made from *Lc. chungangensis* than when rats were administered other *Lc. chungangensis* substrates or *Lc. lactis* substrates or were not administered LAB (positive control). Moreover, when rats were administered substrate first and then 20% ethanol, blood alcohol and acetaldehyde concentrations in rats were significantly decreased, particularly in the cream cheese group. When comparing the 2 experimental groups; that is, treatment with ethanol first and administration of substrates first, we found that blood alcohol and acetaldehyde levels in rats administered substrates first followed by 20% ethanol were lowest among the freeze-dried cell, cream cheese, and yogurt groups. These findings implied that administration of *Lc. chungangensis* substrates before alcohol consumption would be better for reducing blood alcohol and acetaldehyde levels.

Rogers et al. (1987) reported that food slows the absorption of alcohol and reduces peak blood alcohol level and the area under the curve. Furthermore, carbohydrate meals may suppress the circulating and hepatic free fatty acid levels, thereby increasing alcohol metabolism (Thurman et al., 1972). The sugar in the product (fructose) increases alcohol metabolism by providing a substrate to help convert NADH to NAD<sup>+</sup> and to enhance mitochondrial oxygen uptake (Wissel, 1987; Passananti et al., 1990; Ramchandani et al., 2001). *Lac-*



**Figure 7.** (A) Aspartate aminotransferase (AST), and (B) alanine aminotransferase (ALT) levels (in triplicate) in rat blood after treatment with 20% ethanol first and then *Lactococcus lactis* ssp. *lactis* ATCC 19435 in 1 of 3 substrates: freeze-dried cells, cream cheese, or yogurt; or after administration of *Lc. lactis* ssp. *lactis* ATCC in substrates first and then 20% ethanol. Negative = water only, Positive = 20% ethanol only. Data are shown as the mean  $\pm$  SEM. Color version available online.

*tococcus chungangensis* CAU 28 produces both ADH and ALDH not only intracellularly but also in cream cheese and yogurt. Both of these enzymes have been effectively utilized for the regeneration of NADPH in multiple enzyme systems and have been used to reduce

blood alcohol and acetaldehyde levels in rats. Many facultative anaerobic and aerobic gastrointestinal bacteria have exhibited high ADH (Jokelainen et al., 1996; Nosova et al., 1997) and catalase (Tillonen et al., 1998) activities and are therefore capable of producing acetaldehyde from ethanol in vitro under aerobic and microaerobic conditions (Salaspuro et al., 1999). Alcohol-treated rats (positive group) showed high activity of the toxicity markers AST and ALT, indicating increases in membrane permeability, cellular damage, and necrosis of hepatocytes (Baldi et al., 1993; Tahir and Sultana, 2011).

Acute alcohol intake markedly increased serum AST and ALT levels. However, administration of *Lc. chungangensis* substrates decreased serum AST and ALT levels in rats. Comparison of the 2 experiments—with substrate provided before or after administration of 20% ethanol—showed that ingestion of cream cheese both before and after treatment with 20% ethanol was optimal for reducing ADH and ALDH levels in the blood. These results suggested that *Lc. chungangensis* CAU 28 substrates could be used as functional foods to reduce hepatotoxicity by decreasing the levels of alcohol and acetaldehyde in the blood and levels of AST and ALT in serum. Our results support the protective effects of dairy products from *Lc. chungangensis* CAU 28 against alcohol-induced liver diseases, suggesting that these products could be used as nutraceuticals for the prevention and control of alcohol-induced damage.

## ACKNOWLEDGMENTS

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