Evaluation of the role of *Lactobacillus casei* on alcohol metabolism and liver functions of rats

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Abstract

Alcohol consumption causes numerous consequences on the health of the human body. Heavy drinking on a daily base has caused liver diseases. Furthermore, some products such as acetaldehyde produced from alcohol metabolism are more toxic than alcohol itself. This study was carried out to evaluate the role of *Lactobacillus casei* on alcohol metabolism, especially, the removal of the toxic effect of alcohol. The maximum alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activities from *L. casei* were observed at 4 hr of culture. *L. casei* was confirmed to produce the ADH and ALDH by the SDS-PAGE. From *in vivo* test using SD rats with 22% alcoholic drink, blood alcohol concentration (BAC), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) of the rats feeding the medium containing *L. casei* were lower than those of the rats feeding the medium containing an alcoholic drink only. This demonstrates that the ADH and ALDH produced by *L. casei* have virtual functions to detoxicate the alcohol *in vivo* and the fermentation broth of *L. casei* can be used as an alcohol detoxification drink.

INTRODUCTION

The major pathway for alcohol metabolism involves the production of alcohol dehydrogenase (ADH). This enzyme converts alcohol to acetaldehyde through a chemical oxidation process. Acetaldehyde is highly toxic to the body even in low concentration. However, aldehyde dehydrogenase (ALDH) rapidly oxidizes acetaldehyde to acetate. The acetate oxidizes into carbon dioxide and water principally in the extra hepatic muscle tissues. Heavy drinking has been associated with liver diseases. Furthermore, some products generated during alcohol metabolism are more toxic than alcohol itself. The liver is

particularly susceptible to alcohol-related injuries because it is the organ that metabolizes alcohol. Lactic acid bacterium(LAB) inhibit the proliferation and the activities of putrefactive and pathogenic bacteria in several ways. This study was carried out to evaluate the role of *L. casei* on alcohol metabolism to decrease the toxic effect of alcohol.

MATERIALS AND METHODS

The ten species of LAB were cultured in the MRS broth medium containing the various concentrations of 0, 10, 15, 20 and 25% alcohol at 37℃. For the measurement of the activities of ADH and ALDH, the products of LAB, the cultured cells were sonicated in a ice bath; supernatants were then centrifuged to obtain cytosols. ADH and ALDH activities were determined by a spectrophotometer (λ =340nm). ADH and ALDH produced by *L. casei* were analyzed by the SDS-PAGE. For the determination of ADH and ALDH activities from *L. casei* on *in vivo* alcohol metabolism, SD rats (8 week old) were fed with culture broth of *L. casei* including 22% alcoholic drink. The blood samples were centrifuged at 3,000 rpm for 10 min to obtain the serum for the measurement of BAC using an alcohol kit (Sigma Chemical, Missouri, USA). For the measurements of *in vivo* GOT and GPT activities, the SD rats were divided into three groups and fed different supplements: feeding water to the group 1, *L. casei* containing 22% alcohol to the group 2 and only 22% alcohol to the group 3. The GOT and GPT of serums were obtained by the same method as the BAC assay. The GOT and GPT were measured at the time of just before and 2 hr after oral administration of the supplements.

RESULTS AND DISCUSSIONS

One species of 10 lactic acid bacteria(LABs) tested in this study, *L. casei* had higher cell mass than those of other LABs at various alcohol concentrations as shown in Fig. 1. The LAB species, *L. casei* had alcohol dehydrogenase (ADH) and aldehyde dehydrogenase(ALDH) activities as shown in Fig. 2. The maximum enzyme activities of ADH and ALDH in *L. casei* were shown in the early log phase (4 hr) as 2.5 unit/mg protein of ADH and 0.5 unit/mg protein of ALDH as shown in Fig. 3. However, the activities of the enzymes decreased rapidly to 0.25 unit/mg protein of ADH and 0 unit/mg protein of ALDH in the stationary phase. The activities of ADH and ALDH in the culture broth containing 10% alcohol were the highest as compared to the controls

as shown in Fig. 4, although the cell mass was lowest in the culture broth. It was considered that the enzyme production was increased to eliminate the added alcohol instead of cell growth.

The production of ADH and ALDH from *L. casei* was confirmed by the SDS-PAGE. The molecular weight of ADH in *L. casei* was about 40 kDa similar to that of the standard ADH of Baker's yeast. The molecular weight of the ALDH in *L. casei* was the same size to that the standard ALDH (Bakers yeast) in 55 kDa and 97 kDa.

To evaluate the *in vivo* effect of *L. casei* on alcohol metabolism, SD rats were fed with the culture broth containing *L. casei* (0.75 g/l) and 22% alcoholic drink. At 2 hr after intake, the BAC of the rats fed with 22% alcoholic drink with and without *L. casei* were 0.044% and 0.072% as shown in Fig. 5. The measured karmen unit of GOT and GPT before feeding of the supplement to rats were 24.4 and 32.0 of group 1 (water), 21.6 and 27.7 of group 2 (*L. casei*+Alc) and 20.5 and 32.6 karmen unit of group 3 (Alc only) as shown in Fig. 6A. Practically, the GOT and GPT values of the rat group 2 fed with *L. casei* with alcohol showed a similar result to that of group 1 as a control as shown in Fig. 6B. This demonstrates that the ADH and ALDH produced by *L. casei* provided virtual functions to detoxicate the alcohol *in vivo* and *L. casei* can be used as an alcohol detoxification drink.

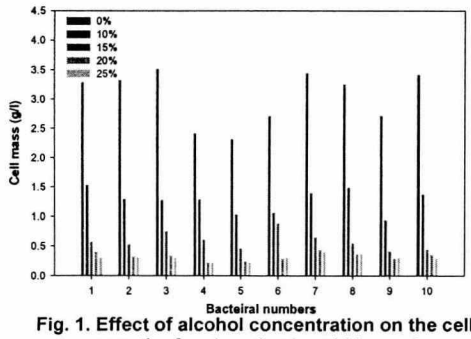

Fig. 1. Effect of alcohol concentration on the cell growth of various lactic acid bacteria.

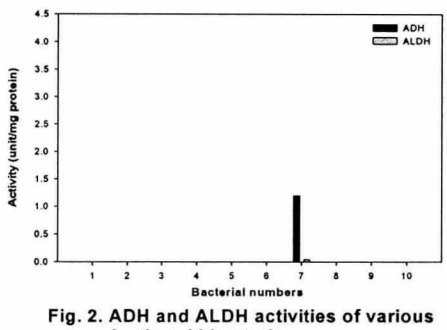

Fig. 2. ADH and ALDH activities of various lactic acid bacteria.

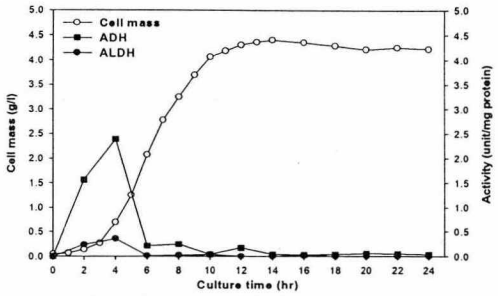

Fig. 3. Growth curve and ADH and ALDH activities of *L. casei*.

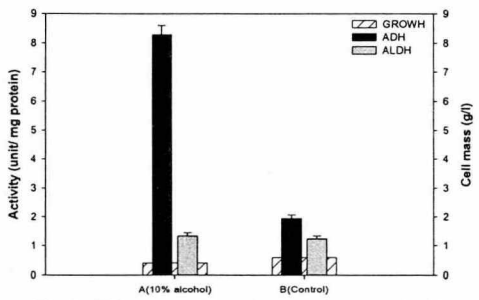

Fig. 4. ADH and ALDH activities of various lactic acid bacteria.

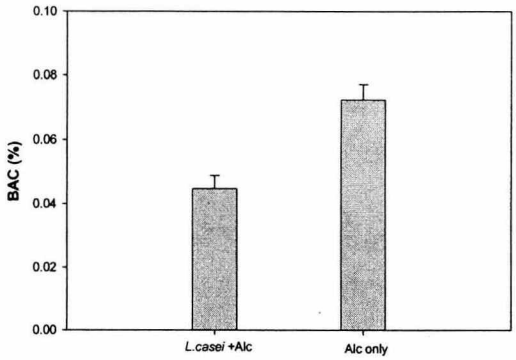

Fig. 5. Effect of L. casei addition on BAC after oral administration.

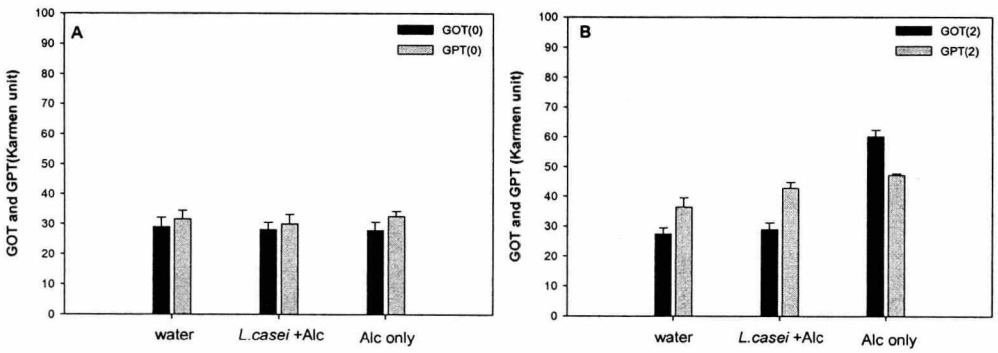

Fig 6. GOT and GPT activities *in vivo test* with SD rats fed with *L. casei* and/or 22% alcohol drink.

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