Note

Effect of Acetic Acid Bacterium on Ethanol Oxidation in Vivo

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We investigated the possible effects of acetic acid bacterium on ethanol oxidation in vivo by monitoring the blood ethanol level after injecting 5% ethanol with (treated group) or without (control group) a freeze-dried bacterial cell suspension directly into the stomach of anesthetized rats. Paired comparison t-tests of the results indicate that the blood ethanol concentration of the rats in the treated group was significantly ($p < 0.07$) lower than that in the control group.

When measured 10 min after administering ethanol into the gullet, the concentration in the stomach of the rats that received acetic acid bacterium simultaneously with ethanol was significantly ($p < 0.10$) lower than that of the rats that received ethanol alone.

We consider that freeze-dried cells of acetic acid bacterium oxidized ethanol in the stomach and could be effective for reducing the blood ethanol level after drinking.

Key words: acetic acid bacterium; ethanol oxidation; ethanol–oxidizing enzyme activity

Ethanol, when consumed as an alcoholic beverage, is absorbed via the digestive tract into the blood and then oxidized to acetaldehyde mainly in the liver. Aldehyde dehydrogenase and the microsomal ethanol–oxidation system in the liver are responsible for this metabolism. While the acetaldehyde thus produced is being further oxidized to acetic acid in the liver by aldehyde dehydrogenase, the elevated level of acetaldehyde in the blood causes such symptoms as an increased heart rate, headache, sleepiness, nausea, acute alcoholism, hangover, and in severe cases, hepatitis. To reduce these symptoms associated with drinking, a substance that can reduce the ethanol concentration in the stomach before it is absorbed into the blood may be useful.

It has been reported that membrane-bound alcohol dehydrogenase and membrane-bound aldehyde dehydrogenase are the most important enzymes involved in ethanol oxidation among acetic acid bacterium, and that both enzymes do not require NAD or NADP as an electron acceptor. These enzymes, when assayed with the ferricyanide reduction system, show their optimum activity at pH 4, but appear to be almost in active at pH 3 and below.

In this study, we found that a cell suspension of acetic acid bacterium retained ethanol-oxidizing activity at a pH value as low as 3 and less, when the enzyme activity was measured by using oxygen consumption as the indicator. We have postulate that the acetic acid bacterium, if taken orally along with an alcoholic beverage, may be able to suppress the increase in ethanol level in the blood and alleviate the intoxicating effect of drinking by oxidizing the ethanol to acetaldehyde and ultimately to acetic acid in the stomach. We, therefore, investigated the possible effect of acetic acid bacterium on ethanol oxidation in vivo with rats.

The bacterial strain used was Acetobacter aceti IFO 3284, freeze-dried cells of this bacterium being prepared as previously described. The ethanol-oxidizing activity was measured at 25°C by monitoring the decrease in dissolved oxygen concentration with an oxygen analyzer (Horiba Works, Kyoto, Japan). The initial oxygen concentration in the reaction mixture at 25°C was assumed to be 258 μM, and one unit of enzyme activity is defined as the activity that caused the consumption of 1 μmol of dissolved oxygen per min under the assay conditions.

The first series of experiments was intended to see whether the ethanol oxidative enzyme could retain its activity under the low pH conditions normally found in the stomach. The results are shown in Table I. Although the optimal pH for the activity of the ethanol-oxidizing enzyme was found to be about 4–5, about 30% of the maximum activity remained at a pH value as low as 2.5.

In our second set of in vitro experiments, 25 mg of the bacterial cells was suspended in 2 ml of distilled water in a 50-ml glass centrifuge tube with a screw cap, and to the tube was added 2 ml of 10% ethanol. The reaction mixture was incubated for 2, 5, and 10 min at 37°C, and then rapidly heated at 80°C for 15 min to stop the enzyme reaction. The mixture was then cooled and centrifuged at 3000 rpm for 5 min at room temperature. The concentration of ethanol in the supernatant was determined with an ethanol F-kit (Boehringer Mannheim) commonly used for food.

Table I. Effect of Low pH on the Ethanol-Oxidizing Enzyme Activity of Acetic Acid Bacterial Cells

<table>
<thead>
<tr>
<th>pH</th>
<th>1.5</th>
<th>2.0</th>
<th>2.5</th>
<th>3.0</th>
<th>4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activity (units/ml)</td>
<td>10.8</td>
<td>21.5</td>
<td>495.1</td>
<td>1033.2</td>
<td>1501.4</td>
</tr>
</tbody>
</table>

![Fig. 1. Time-Course of Ethanol Oxidation by Freeze-dried Cells of Acetic Acid Bacterium in Vitro.](image-url)

In a 50-mi glass centrifuge tube, 2 ml of distilled water containing 25 mg of the bacterial cells and 2 ml of a 10% ethanol solution were combined and incubated for 2, 5, and 10 min at 37°C. After inactivating the enzymatic activity by heating the reaction mixture at 90°C for 15 min, the bacterial cells were removed by centrifugation, and the ethanol concentration in the supernatant was determined with an ethanol F-kit ($n=2$).
Fig. 2. Effect of Freeze-dried Cells of the Acetic Acid Bacterium on Ethanol Oxidation in Vivo.

Two ml of distilled water containing 25 mg of bacterial cells and 2 ml of a 10% ethanol solution were mixed and immediately injected directly into the stomach of Wistar rats under anesthesia. The concentration of ethanol in blood samples withdrawn from the main vein at 5-min intervals was determined with an ethanol F-kit after deproteinization. ■, control; □, treated.

* Significantly different (p<0.07, n=4).

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Fluid weight in the stomach (g)</th>
<th>Ethanol concentration in the stomach (g/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>0 min</td>
<td>1.24±0.26</td>
<td>36.1±2.5</td>
</tr>
<tr>
<td>10 min</td>
<td>1.19±0.29</td>
<td>29.7±8.6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.9±6.9*</td>
</tr>
</tbody>
</table>

* Probably a significant difference between the control and treated groups at 10 min (p<0.10).

analysis. Figure 1 shows the time-course of ethanol oxidation by the bacterial cells during the short incubation at 37°C. The ethanol concentration after 5 min was about 77% of the initial value, and decreased further to about 73% after 10 min of incubation. These results led us to expect that the bacterial cells, when taken with ethanol, would significantly reduce the ethanol concentration in the stomach during a relatively short period of time.

With these results in mind, we proceeded to the animal test with rats. After a 16-hour fasting, male Wistar rats weighing 300-350 g (8-10 weeks old) were each anesthesized and the abdomen opened. The rats were kept under anesthesia throughout the duration of the experiments by an anesthetic injection at 1-hour intervals. One hour after the laparotomy, a cannular tube was inserted into the main vein. Distilled water (2 ml) containing 25 mg of freeze-dried cells of the acetic acid bacterium and 2 ml of a 10% ethanol solution were measured into two separate test tubes. The contents were then combined and mixed well for a few seconds, and immediately injected into the stomach of each test animal with a hypodermic syringe. Approximately 150 μl of a blood sample was withdrawn from the vein at 5-min intervals via the cannular tube. The blood samples were then deproteinized with perchloric acid using a centrifuge tube containing 800 μl of 0.33 ml/liter perchloric acid, into which 100 μl of the blood sample was added while mixing. The mixture was then centrifuged at 3000 rpm for 10 min at room temperature to obtain a clear supernatant. The ethanol concentration in the supernatant was determined with an ethanol F-kit as already described. In the control experiment, 2 ml of distilled water was used in place of the cell suspension. Each experiment was conducted on two groups of rats in pairs (a treatment and a control), and the experiment was repeated four times. A measured amount of the bacterial cell suspension and an ethanol solution at a final concentration of 5% was injected simultaneously into the stomach of each rat, and the concentration of ethanol in the blood samples was determined at intervals to see if the bacterial cells could effectively reduce the ethanol concentration in the blood. As shown in Fig. 2, the paired comparison t-tests indicate that the ethanol concentration in the blood of the treated rats was lower than that of the control rats (probably a significant difference, p<0.07).

We next investigated the reason why the decrease in ethanol concentration in the blood of the treated rats was more rapid than that of the control rats. Male Wistar rats weighing 300-350 g were used after a 16-hour fasting. Two ml of distilled water containing 25 mg of freeze-dried cells and 2 ml of a 10% ethanol solution were combined and immediately injected into the gullet of the rats with a hypodermic syringe. After 10 min, the rats were killed by decapitation, and the stomach was removed. The entire fluid content of the stomach was weighed, and the ethanol concentration was determined with an ethanol F-kit. For the control rats, 2 ml of distilled water was used in place of the cell suspension. As can be seen from Table II, the mean ethanol concentration of the treated group was significantly (p<0.10) lower than that of the control group, whereas no significant difference in the weight of the fluid content was observed between the two groups. These data indicate that freeze-dried cells of an acetic acid bacterium could effectively oxidize ethanol injected into the stomach. In conclusion, freeze-dried cells of the acetic acid bacterium taken together with an alcoholic beverage may help reduce the intoxicating effects associated with heavy drinking by oxidizing ethanol an decreasing its absorption in the stomach.

References