Xylitol inhibits carcinogenic acetaldehyde production by Candida species

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Acetaldehyde is a highly toxic and mutagenic product of alcohol fermentation and metabolism which has been classified as a Class I carcinogen for humans by the International Agency for Research on Cancer of the World Health Organisation (WHO). Many Candida species representing oral microbiota have been shown to be capable of marked acetaldehyde production. The aim of our study was to examine the effects of various sugar alcohols and sugars on microbial acetaldehyde production. The study hypothesis was that xylitol could reduce the amount of acetaldehyde produced by Candida. Laboratory and clinical isolates of seven Candida species were selected for the study. The isolates were incubated in 12 mM ethanol and 110 mM glucose, fructose or xylitol at 37°C for 30 min and the formed acetaldehyde was measured by gas chromatography. Xylitol significantly (p < 0.0001) reduced the amount of acetaldehyde produced from ethanol by 84%. In the absence of xylitol, the mean acetaldehyde production in ethanol incubation was 220.5 μM and in ethanol–xylitol incubation 32.8 μM. This was found to be mediated by inhibition of the alcohol dehydrogenase enzyme activity. Coincubation with glucose reduced the amount of produced acetaldehyde by 23% and coincubation with fructose by 29%. At concentrations that are representative of those found in the oral cavity during the intake of proprietary xylitol products, xylitol was found to reduce the production of carcinogenic acetaldehyde from ethanol by Candida below the mutagenic level of 40–100 μM.

Key words: acetaldehyde, xylitol, Candida, cancer

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of the solution at 492 nm was adjusted spectrophotometrically to 0.4 (Multiscan RC, Labsystems, Helsinki, Finland) corresponding 1 × 10^7 colony forming units per millilitre by quantitative microbial culture. Aliquots of 350 µl of this yeast suspension were transferred into gas chromatograph vials. Thereafter, 50 µl of PBS buffer containing ethanol (final concentration 12 mM) and 50 µl PBS buffer containing glucose, fructose or xylitol (final concentration 110 mM) was added and the vial was immediately sealed. The ability of microbes to produce acetaldehyde from ethanol, glucose, fructose or xylitol alone was analyzed by adding 50 µl of one of these and 50 µl of PBS buffer to the yeast aliquots. The samples were thereafter incubated for 30 min at 37°C. The reaction was stopped by injecting 50 µl of 6 M perchloric acid (PCA) through the rubber septum of the vial. Three parallel samples were processed and the experiment was repeated. The means were calculated and used for the statistical analysis. To measure the baseline and artefactual acetaldehyde, 50 µl of PCA was immediately added to control vials and the suspension was equally incubated for 30 min at 37°C. The formed acetaldehyde was measured by gas chromatography (Perkin Elmer Headspace sampler HS 40XL, Perkin Elmer Autosystem Gas Chromatograph equipped with Ionization Detector FID, USA) and subtracted from the initial values as reported earlier.14

**ADH analyses**

ADH activity was analyzed for the highest (Candida glabrata CCUG 32725), the lowest (Candida krusei ATCC 6258) and an average (Candida albicans ATCC 90029) acetaldehyde producer. The ADH activity was measured using fluorescence analysis with cofactor nicotinamide adenine dinucleotide (NAD) as described earlier.6 For the analyses, the yeast cells were first grown as described above and then lysed by glass bead vortexing in the presence of a protease inhibitor cocktail (SIGMA, P 8340, Missouri, USA). Five 1 min vortexing cycles and glass beads of 1.0 mm diameter were used. The samples were cooled on ice before each cycle. Cell lysates were centrifuged for 5 min at 2,900g (Hettich EBA 20, Germany), the supernatants were collected and further centrifuged at 139,700g for 65 min at +4°C (Beckman Optima LE-80k Ultracentrifuge, USA). This supernatant was collected and used for the analyses. Cytosolic ADH activity was determined by measuring the fluorescence (ex 340 nm, em 440 nm) after addition of ethanol or ethanol and xylitol (final concentration 100 mM) and NAD (final concentration 2.5 mM) at 37°C in 0.1 M glycine buffer (pH 9.6). Ethanol concentrations 0.68 to 2174 mM were used. ADH activity was determined by using Tecan SAFIRE monochromator-based microplate detection system and Magellan Software V6.05 (Tecan Trading AG, Switzerland). The Lineweaver–Burk plot was used to determine the enzyme activities.

**Statistical analysis**

SPSS ver. 16.0 (SPSS, Chicago, IL, USA) was used for the statistical analyses. The results are expressed as means (±SEM)

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**Table 1. Candida isolates used in the study**

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate/identification no.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. glabrata</td>
<td>CCUG 32725</td>
<td>Blood</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>ATCC 22019</td>
<td>Stool</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>ATCC 750</td>
<td>Sputum</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>UK NEQAS 2/07</td>
<td>Pharynx</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>ATCC 90029</td>
<td>Blood</td>
</tr>
<tr>
<td>C. albicans</td>
<td>ATCC 90029</td>
<td>Blood</td>
</tr>
</tbody>
</table>

Abbreviations: ATCC: American Type Culture Collection; CCUG: Culture Collection of University of Gothenburg; UK NEQAS: United Kingdom National External Quality Assessment Service.
Large variation was seen in acetaldehyde production between the Candida species whereas within-species variation was less striking (Supporting Information Fig. 2). C. glabrata isolates were found to be the highest producers and C. krusei isolates the lowest producers of acetaldehyde under all conditions. In ethanol incubation, the C. glabrata isolates produced mean 366.1 ± 10.1 μM of acetaldehyde whereas the C. krusei isolates produced mean 53.7 ± 9.1 μM. The differences between the species within each experimental condition were statistically significant (p < 0.0001). C. glabrata isolates differed from the other Candida species by showing higher acetaldehyde production in ethanol–glucose and ethanol–fructose coincubation than in ethanol alone. In all Candida isolates acetaldehyde production in ethanol–glucose and in ethanol–fructose incubations showed a significant positive correlation \[ r_S = 0.94 \text{(0.81–0.98)}, \quad p < 0.0001 \]. No correlation was found in acetaldehyde production between ethanol–xylitol incubation and ethanol–glucose or in ethanol–fructose incubation. C. albicans and C. glabrata isolates showed high ADH activities \( (V_{\text{max}} = 3.40 \text{ s}^{-1}, K_m = 0.16 \text{ mM} \) and \( V_{\text{max}} = 4.47 \text{ s}^{-1}, K_m = 2.83 \text{ mM}, \) respectively) whereas the ADH activity of the C. krusei isolate was low \( (V_{\text{max}} = 1.66 \text{ s}^{-1}, K_m = 0.13 \text{ mM}) \). Xylitol reduced the ADH activity of the C. glabrata isolate by 61% and of the C. albicans isolate by 100% when coincubated with 110 mM ethanol for 10 min (Supporting Information Fig. 3). When coincubated with 11 mM ethanol, the reduction was 66% in C. glabrata isolate and 100% in C. albicans isolate. In C. krusei no effect of xylitol on ADH activity could be detected.

**Discussion**

Xylitol was found to significantly inhibit candidal acetaldehyde production from ethanol. It reduced the production below the mutagenic acetaldehyde level of 40–100 μM for all Candida species tested. In the absence of xylitol, the mean acetaldehyde production in ethanol incubation was high (220.5 μM). Xylitol has previously been shown to inhibit the metabolism of sugars by acidogenic oral bacteria and thus prevent tooth decay. It is therefore incorporated in chewing gums and tablets as well as in health care products such as dentifrice and oral rinses. Five to six grams and three daily exposures are required for this clinical effect. A xylitol concentration of 65 mM has been shown to have antimicrobial activity against otopathogenic bacteria. In our study, the final concentration of 110 mM xylitol was used. This equals to 17 mg/mL readily available in the oral cavity during intake of xylitol products. Concentrations higher than 30 mg/mL can be detected in saliva during chewing xylitol-sweetened chewing gum and the levels have been found to remain significantly elevated up to 30 min.

Xylitol strongly inhibited candidal ADH activity. It is possible that xylitol directly inhibits the ADH via blockage of the substrate binding site. XDH belongs to the ADH enzyme family and the NAD-binding part of XDH resembles that of the liver ADH and other enzymes within the family.
Xylitol metabolism may also compete for the nicotinamide adenine dinucleotide (NADH) coenzyme leading to downregulation of ADH. Product inhibition and other regulatory circuits may also counter-regulate the ADH activity. The nature of the inhibition of ADH enzyme by xylitol remains to be established in future studies. Fructose and glucose reduced acetaldehyde production in ethanol incubation to lesser extent. This is likely to be due to catabolite repression and preference of sugars as carbon and energy source over ethanol. C. glabrata isolates were found to differ from the other Candida species by producing increased amounts of acetaldehyde when incubated with ethanol and fructose or glucose. This is not completely surprising as carbohydrate metabolism of C. glabrata differs in many ways from that of other Candida species and it is more adapted to fermentative anaerobic growth.21,22

In conclusion, our in vitro study shows that xylitol in concentrations that are comparable to those in vivo during intake of xylitol products reduce remarkably the production of carcinogenic acetaldehyde from ethanol by Candida representing normal oral flora. This appears to be caused by the xylitol-induced inhibition of the microbial ADH enzyme. Further studies are warranted to find out the potency of xylitol to control microbial acetaldehyde production in vivo after an alcohol challenge.

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References