Materials and Methods

Mouse experiments

An experimental model of ALD was used to study the impact of ethanol introit on intestinal *Bt* and whether oral supplementation with *Bt* could exert beneficial effects. All experiments were performed according to ethical principles and legal laws. 8- to 10-week-old female wild type (C57BL/6) mice were fed with a Lieber-DeCarli diet containing up to 5% alcohol for 15 days (EtOH-fed) or Lieber-DeCarli diet alone (pair-fed) as shown in Figure 1A and previously described[1, 2].

In order to study the potential properties of *Bt* to protect liver against ethanol introit, the mice were treated every day (from day 1 to day 14) with *Bt* (3×10^9 bacteria/200 µl phosphate-buffered saline (PBS)) or vehicle (PBS) by oral gavage. Mice were weighed every other day and drinking amounts were monitored daily. At day 15 mice were sacrificed by being anaesthetized with xylazine (5 mg/kg) and ketamine (100 mg/kg), and blood, liver and intestinal samples were collected.

Quantification of *Bt* in mice faeces.

DNA extraction from faeces was performed by DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) according to manufacturer's protocol. Following, the amount of *Bt* DNA was quantified by qPCR with SybrGreen (Eurogentec, Köln, Germany) on MXPro3000 Cycler (Agilent Technology, Waldbronn, Germany). The primers for *Bt* DNA detection were based on 16rDNA gene sequences: forward-GGCAGCATTTCAGTTTGCTTG; reverse-GGTACATACAAAATTCCACACGT. The cycle was performed using 30 ng of faecal DNA, primers concentration at 250 nM and 60° C as annealing temperature. A standard curve was obtained by serial dilutions of bacterial DNA extracted from *Bt* colonies, and the cycle threshold of each sample was compared with the standard. The quantity of *Bt* DNA in the faeces was expressed in Log_{10} ng/g.

Cultivation of *Bt*

Bt (DSM 2079) was purchased by DSMZ (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures) and cultured on blood agar (Biomerieux, Marcy l'Etoile, France)

at 37° C under anaerobic conditions with GENbox and GENbox anaer systems (Biomeriux, Marcy l'Etoile, France).

Statistical analysis

GraphPad PRISM v6 (La Jolla,California, USA) was used for statistical analysis. Unpaired two-tailed Student's t-test and one-way analysis of variance followed by post hoc Bonferroni test were used when appropriate. Results are shown as mean ± standard error of mean (SEM). Data were considered statistically significant when $p<0.05$.

Further methods are described in Supplementary section.

Reference List

1. Grabherr F, Grander C, Adolph TE, et al. Ethanol-mediated suppression of IL-37 licenses alcoholic liver disease. Liver Int 2018;38:1095-1101.
2. Sangineto M, Grabherr F, Adolph TE, et al. Dimethyl fumarate ameliorates hepatic inflammation in alcohol related liver disease. Liver Int 2020;40:1610-1619.