

SUPPLEMENTARY DATA

Material and methods supplementary data

In vitro immunomodulation assays

For assessing immunomodulation on intestinal cells, HT-29 cells were seeded in 24-well plates (1.8×10^5 cells per well). Bacteria at a multiplicity of infection (MOI) of 40 or controls —either PBS glycerol, or butyrate at 1 mM—were added on the cells with recombinant TNF- α (Peprotech, London, UK) at a final concentration of 5 ng/ml. Each treatment was applied in 3 wells (technical triplicates). After 6 h of co-incubation, supernatants were recovered and stored at -80 °C until subsequent analysis. Interleukin-8 (IL-8) was later quantified in the supernatants using Human IL-8 ELISA MAX Standard Set (BioLegend, San Diego, CA, USA) according to the manufacturer's instructions. Each strain was tested 3 times from 3 independent cultures.

Immunomodulation on PBMCs was assessed on cells from five healthy donors (American male, Caucasian, aged <65 years, with a body mass index <30, nonsmoking, no drugs taken 15 days prior to sampling and negative for HIV and hepatitis A and B viruses). After thawing at 37 °C, cells were transferred into medium containing RPMI 1640 (Gibco) supplemented with 10% FBS, 1% L-glutamine, and 0.01% P/S. DNase (10 mg/mL) was added to this mix to avoid clumping. Cells were then centrifuged at 200xg for 15 min, counted by using trypan blue, and seeded onto 24-well plates at 1×10^6 cells/well. Bacteria at a MOI of 10 or controls —either PBS-glycerol, lipopolysaccharides (LPS) from *Escherichia coli* K-235 at 5 μ g/mL (Sigma) or a mix of phorbol 12-myristate 13-acetate (PMA) and ionomycin at 2 μ g/mL (eBioscience™ Cell Stimulation Cocktail, Thermofisher) — were added on cells and co-cultures were maintained for 24 h. Each treatment was tested in 3 wells (technical triplicates). After 24 h of co-incubation at 37°C in 10% CO₂, supernatants were recovered and stored at -80 °C until subsequent analysis. IL-10 and IL-12p70 were later quantified in supernatant by ELISA (Mabtech, Cincinnati, OH, USA) according to the manufacturer's instructions. Each strain was tested 3 times from 3 independent cultures.

In vitro permeability assay

For the assessment of the ability of strains to reinforce the intestinal barrier, transepithelial electrical resistance (TEER) was measured on Caco-2 cells, as previously described²². Briefly, Caco-2 cells were grown on Transwell® inserts (polycarbonate membrane with 0.4 μ m pore size; Costar, Corning Life Science, Kennebunk, ME, USA) for about 10 days, with regular TEER measures (REMS AutoSampler, World Precision Instruments, Sarasota, FL, USA). When measures reached 2000 Ω , bacteria at MOI of 40 or the controls (either the bacteria vehicle as negative control (PBS glycerol) or an internal positive control (butyrate at 1 mM)) were co-incubated on the apical side of the cells. Three hours later, TNF- α (100 ng/mL final concentration, Peprotech) was added to the basolateral compartment. Each treatment was tested in 2 wells (technical duplicates). TEER was measured just before co-incubation and 24 h after. A ratio measuring the evolution of TEER over 24 h with the treatment, normalized by the evolution of TEER with PBS was calculated:

$$\frac{TEER \text{ traitement } t24 / TEER \text{ traitement } t0}{TEER \text{ témoin } t24 / TEER \text{ témoin } t0}.$$

Each strain was tested 3 times from 3 independent cultures.

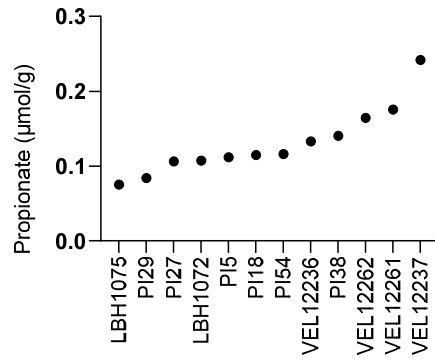


Figure S1. Propionate production in the supernatants (only the most producing strains are represented). Each strain was tested once, in technical duplicate. Quantification threshold for acetate: 0.05 mM.

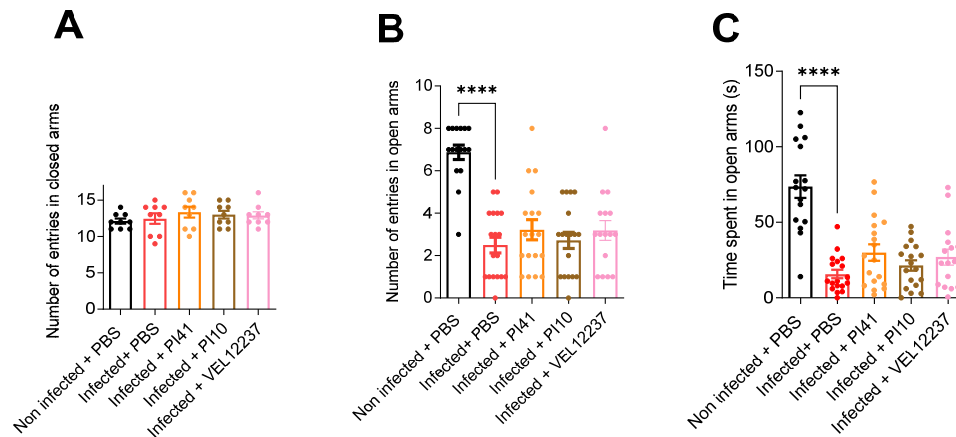


Figure S2. Effect on anxiety evaluated with Elevated Plus Maze test. A : Number of entries in the closed arms, reflecting the mobility of mice. Kruskal-Wallis non-significant. B: Number of entries in open arms as an indirect measure of anxiety. Kruskal-Wallis $p < 0.0001$. C: Time spent in open arms, as an indirect measure of anxiety. Kruskal-Wallis $p < 0.0001$. Infected + PBS $n = 17$; Infected + PI10 $n = 18$; Infected + PI41 $n = 18$; Non infected + PBS $n = 17$. Kruskal-Wallis test followed by Dunn's post-test. **** = $p \leq 0.0001$. (mean \pm SEM).