Original research

Novel *myo*-inositol to butyrate fermentation pathway in the prevalent human gut species *Dysosmobacter welbionis,* a bacterium associated with improved metabolic and liver health

Chi-Hsien Lee ,[1,2] Thi Phuong Nam Bui,[3] Camille Petitfils,[1,2] Ching Jian ,[4] Giselle C Wong,[1,2] Anthony Puel,[1,2] Tiphaine Le Roy ,[5] Samuel Bellais,[6] Bouthaina Ben Abdallah,[6] Mélanie Nehlich,[6] Thomas Leicht,[6] Manyi Jia ,[7] Lesley Hoyles,[8] Massimo Federici ,[9,10] Jose Manuel Fernández-Real ,[11,12,13] Remy Burcelin ,[14] Marc-Emmanuel Dumas ,[7,15] Nathalie M Delzenne ,[1] Thomas Clavel ,[16] Sjef Boeren,[17] Antonio Dario Troise,[18] Andrea Scaloni,[18] Giulio G Muccioli ,[19] Willem M De Vos ,[3,4,20] Matthias Van Hul ,[1,2] Patrice D Cani [1,2,7,21]

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/gutjnl-2025-336617).

For numbered affiliations see end of article.

Correspondence to
Professor Patrice D Cani; patrice.cani@uclouvain.be and Dr Matthias Van Hul; Matthias.Vanhul@uclouvain.be

C-HL, TPNB and CP contributed equally.

MVH and PDC are joint senior authors.

Received 5 August 2025
Accepted 6 December 2025

To cite: Lee C-H, Bui TPN, Petitfils C, *et al*. *Gut* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/gutjnl-2025-336617

ABSTRACT

Background *Dysosmobacter welbionis* is a recently discovered butyrate producer whose presence in stool correlates with improved metabolic health. Whether its abundance is reduced in individuals with metabolic dysfunction-associated steatotic liver disease (MASLD) remains unknown. Mechanistic insight into its butyrate production from *myo*-inositol, a dietary compound from fruits, beans, grains and nuts with metabolic benefits, is also limited.

Objective To assess population-level distribution, relative abundance and strain diversity of *D. welbionis* in humans, and to elucidate its metabolic capacity to ferment *myo*-inositol into butyrate.

Design We analysed several human cohorts for associations with liver health and evaluated *D. welbionis* J115^T supplementation in a diet-induced steatosis mouse model. An antibody-guided anaerobic cell-sorting strategy enabled isolation of distinct strains. We combined ^{13}C-labelled inositol isotopes with NMR, mass spectrometry, genomics and proteomics.

Results We found that *D. welbionis* and two related species (*D. hominis* and *D. segnis*) are prevalent gut bacteria in the human gut. *D. welbionis* abundance was reduced in MASLD across two cohorts and inversely correlated with fibrosis score in a third cohort. Treatment with *D. welbionis* J115^T improved glycaemia and hepatic steatosis in high-fat diet fed mice. We identified a non-canonical *myo*-inositol-to-butyrate fermentation pathway. 19 human strains were isolated, comparative genomics of 23 strains revealed an open pangenome (about 2100 core genes) including the full *myo*-inositol fermentation pathway.

Conclusion *D. welbionis* possesses a unique, conserved route to convert dietary *myo*-inositol into butyrate, distinguishing it from other commensals and supporting its potential as a next-generation probiotic for metabolic and liver health.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Butyrate is a short-chain fatty acid (SCFA) produced by gut bacteria that plays a crucial role in metabolic and liver health.
⇒ *myo*-Inositol is a dietary compound known to influence insulin sensitivity and gut microbial composition.
⇒ Certain gut bacteria can metabolise *myo*-inositol into SCFAs like acetate and propionate, but until now no gut species had been experimentally confirmed to convert it into butyrate.

INTRODUCTION

The human gut harbours a dense and metabolically active microbial community,[1 2] which plays a key role in host health. A key function of these microbes is the production of metabolites that influence intestinal and systemic physiology. Among these metabolites, short-chain fatty acids (SCFAs), including acetate, propionate and butyrate, are the most extensively studied. SCFAs modulate host responses by engaging G-protein coupled receptors, altering gene expression via epigenetic mechanisms, and influencing metabolic, immune and inflammatory pathways.[3 4] Butyrate is particularly notable for its role as the primary energy source for colonocytes, its anti-inflammatory properties and its overall involvement in metabolic regulation.[1 5 6]

The production of butyrate in the gut is primarily carried out by anaerobic commensals from the families *Oscillospiraceae* (synonym, *Ruminococcaceae*) and *Lachnospiraceae*, both within the phylum *Bacillota* (formerly *Firmicutes*).[7] Well-characterised butyrogenic taxa include *Roseburia intestinalis*, *Faecalibacterium prausnitzii* and *Eubacterium* spp. Other species also contribute to butyrate synthesis

WHAT THIS STUDY ADDS

⇒ This study reveals that *Dysosmobacter welbionis* is highly prevalent across diverse human populations, including children and adults, with a notable heritability pattern observed in monozygotic twins, suggesting host-genetic influences on its colonisation.

⇒ The relative abundance of *D. welbionis* is significantly reduced in patients with metabolic-associated fatty liver disease (MASLD) and is negatively associated with a fibrosis score, highlighting a potential protective role in liver health.

⇒ Through the isolation and genomic analysis of 23 human-derived *D. welbionis* strains, the study demonstrates that this inositol-to-butyrate pathway is conserved across the species, reinforcing its ecological and therapeutic relevance.

⇒ Functionally, the study identifies a previously uncharacterised metabolic pathway in *D. welbionis* that converts *myo*-inositol into butyrate.

⇒ Advanced techniques, including antibody-guided anaerobic cell-sorting strategy, ^{13}C-labelling, NMR, proteomics and genomics were used to isolate the new strains and to map the unique biochemical steps of this pathway.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ *D. welbionis* could become a next-generation probiotic candidate for preventing or managing metabolic diseases such as MASLD, obesity and type 2 diabetes.

⇒ The discovery that inositol fermentation produces butyrate may shift current paradigms in diet-microbiota-host interaction research, especially regarding inositol supplementation.

⇒ These findings highlight the need for strain-level evaluation in probiotic development, including antibiotic resistance profiling, to ensure safety and efficacy in therapeutic applications.

by fermenting dietary oligosaccharides, polysaccharides and metabolic intermediates (eg, lactate and acetate), including *Anaerobutyricum* and *Anaerostipes* spp.[8 9] We recently expanded this repertoire with the identification of *Dysosmobacter welbionis* J115^T, which stains gram-negative but encodes a monoderm (gram-positive type) cell envelope and is a member of *Oscillospiraceae*, that produces butyrate through an unusual reliance on inositol as a primary carbon source.[10]

The relative and absolute abundance of *D. welbionis* in human faeces has been reported to decline significantly in individuals with obesity and type 2 diabetes, showing inverse correlations with fasting glucose and glycated haemoglobin levels.[11] In high-fat diet-fed mice, supplementation with *D. welbionis* J115^T improved glucose tolerance more effectively than metformin and lowered fasting glycaemia through mechanisms independent of glucagon and hepatic gluconeogenic enzymes.[12] More recently, *D. welbionis* J115^T was shown to metabolise cholesterol, and its higher abundance, along with that of *Oscillibacter* spp., was associated with reduced faecal and plasma cholesterol levels, suggesting a potential role in cardiovascular and metabolic regulation.[13]

Inositol, a hexahydroxycyclohexane alcohol structurally derived from cyclohexane, belongs to the glucose family and exists in nine stereoisomeric forms, with *myo*-inositol being the most prevalent in human diet.[14 15] Although *myo*-inositol can be endogenously synthesised in humans—primarily by the kidneys—it is also obtained through dietary intake in free form or as phytic acid (inositol hexaphosphate, IP6).[16–18] Major dietary sources include fruits (especially cantaloupe and oranges), whole grains, legumes (such as beans and lentils), seeds and nuts. *Myo*-inositol is particularly abundant in foods high in fibre, where it is often present in bound form as phytates. During food processing or digestion, phytates can be hydrolysed to release free *myo*-inositol. Recent studies have implicated *myo*-inositol deficiency in the development of several metabolic disorders, including polycystic ovary syndrome, type 2 diabetes and gestational diabetes.[19–21] Supplementation with *myo*-inositol and its isomer, D-*chiro*-inositol, has been shown to improve insulin sensitivity and lower blood glucose levels.[22] *Myo*-inositol derivatives also function as a second messenger of insulin, promoting glycogen synthesis, facilitating GLUT4 translocation and enhancing glucose uptake in insulin-sensitive tissues.[22]

Beyond its role in host metabolism, *myo*-inositol and phytic acid have been shown to modulate the gut microbiota in animal studies, increasing the relative abundance of lactobacilli and enhancing the production of SCFAs. Until now, only a limited number of bacterial species have been experimentally demonstrated to metabolise *myo*-inositol, including *Aerobacter aerogenes*, *Rhizobium leguminosarum bv. viciae*, *Bacillus subtilis*, *Lacticaseibacillus casei*, *Corynebacterium glutamicum* and *Mitsuokella jalaludinii*.[23–28] However, most of these species are not resident members of the human gut microbiota. In recent years, a human gut-associated bacterium has been identified with the capacity to metabolise inositol, *Anaerostipes rhamnosivorans*, which is able to produce propionate and acetate from inositol.[29] But to date, none has been shown to convert *myo*-inositol into butyrate. A potential exception is *A. hadrus*, in which a genomic structural variant suggests a possible inositol-to-butyrate pathway, although this has not been experimentally validated.[30] It was later demonstrated that not all *A. hadrus* strains grow in *myo*-inositol and only some can ferment inositol to propionate and acetate, but not butyrate. Notably, like *D. welbionis*, *A. hadrus* has been associated with favourable host metabolic markers, including reduced body mass index, body weight and waist-to-hip ratio.[30] These findings strongly suggest a mechanistic link between inositol-to-butyrate fermentation in the gut and host metabolic regulation. However, there has been to date no detailed analysis of *D. welbionis* phylogenetic and functional diversity, limiting its potential use as a next-generation probiotic.

Given its metabolic capacity and health-associated profile, *D. welbionis* emerges as an ideal next-generation probiotic candidate. In this article, we report comprehensive genomic and functional data on newly isolated strains and provide mechanistic evidence for the conversion of *myo*-inositol into butyrate and acetate by this important human gut bacterial species.

RESULTS

D. welbionis is a dominant *Dysosmobacter* species associated with liver health

Since our proposal of *Dysosmobacter* as a novel genus in 2020,[10] three human-associated *Dysosmobacter* spp. have been isolated and described: *D. welbionis*, *D. segnis* and *D. hominis*.[31] However, their population-level distribution and relative abundance have not been explored. In the TwinsUK cohort of 977 adults, *Dysosmobacter* spp. were detected in 94% of faecal samples, while the prevalence was 93% in a healthy Chinese cohort (n=1073) spanning a wide age range[32] (figure 1a,b). *D. welbionis* was the most prevalent species, detected in 81% of the TwinsUK cohort (relative abundance (RA) 0–2.4%) and 72% of

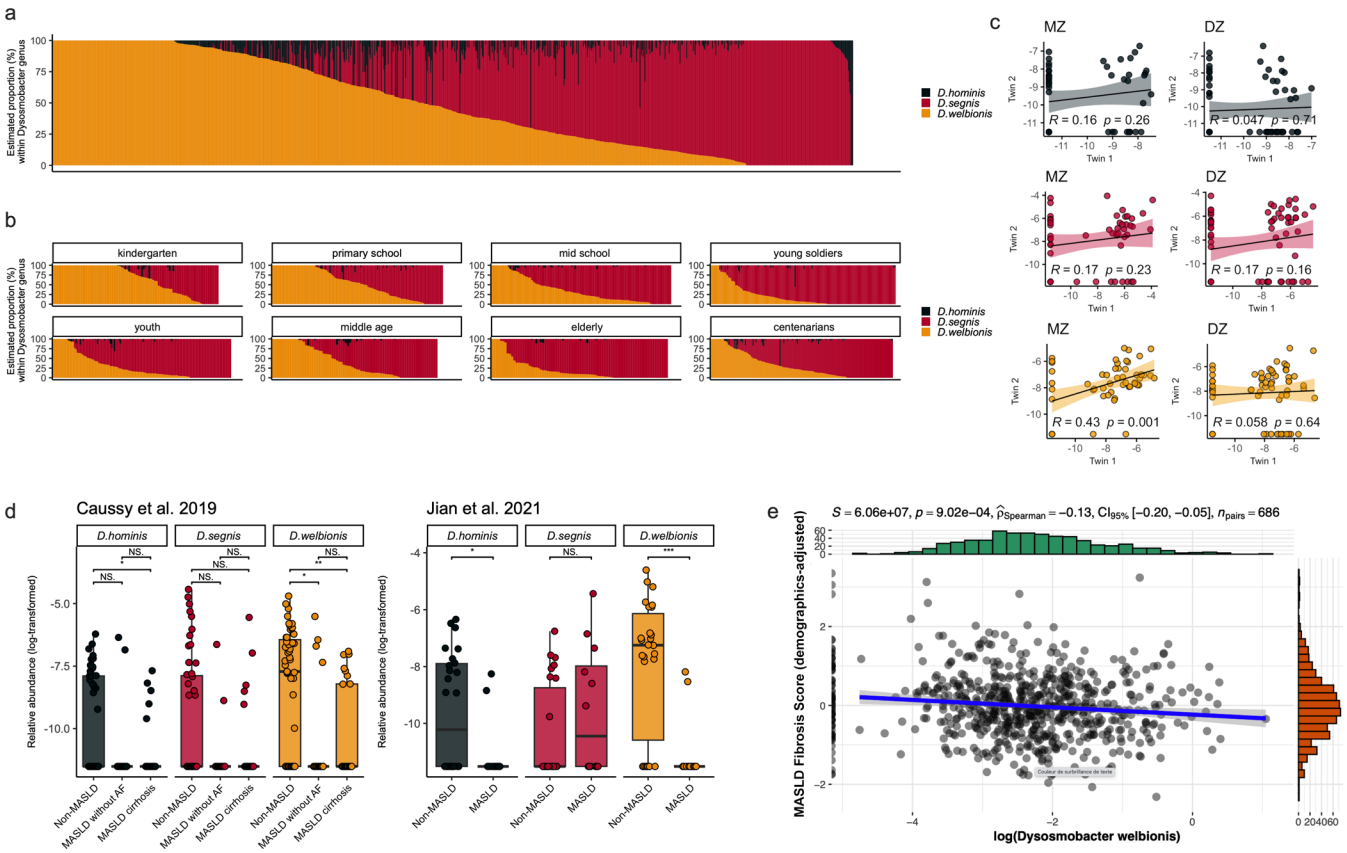

Figure 1 Abundance and distribution of *Dysosmobacter* species across multiple human cohorts. (a) *Dysosmobacter* spp. abundance in the TwinsUK cohort of 977 adults, *Dysosmobacter* spp. were detected in 94% of faecal samples, while the prevalence was 93% in a healthy Chinese cohort (n=1073) spanning a wide age range. (b) In a large cross-sectional cohort of healthy Chinese individuals spanning ages 3–100+, *Dysosmobacter* spp. were less abundant in children, with *D. welbionis* being the most dominant species across age groups. (c) *Dysosmobacter* spp. abundances in 53 healthy MZ and 66 DZ twin pairs from the TwinsUK cohort. The relative abundance of *D. welbionis* was significantly more correlated between MZ twins (R=0.43, p=0.001) than DZ twins (R=0.058, p=0.64). (d) Abundance was significantly lower in subjects with MASLD or MASH compared with controls. Among identified species, *D. welbionis* and *D. hominis* showed the most pronounced reductions in patients with MASLD/MASH, while *D. segnis* remained low across groups. (e) Spearman's correlation between *D. welbionis* faecal abundance and the MASLD fibrosis score in a large cohort of Italian and Spanish individuals (n=686) with MASLD/steatosis. The data were adjusted for demographics (country, sex and age). DZ, healthy dizygotic; MASLD, metabolic-associated fatty liver disease; MASH, metabolic-associated steatohepatitis; MZ, monozygotic.

the Chinese cohort (RA 0–1.7%), in line with our previous findings from the American Gut Project and the Flemish Gut Flora Project.[11] *D. segnis* showed similar prevalence (64% in TwinsUK, 78% in the Chinese cohort; RA up to 2.5%), whereas *D. hominis* was less common (45% and 19%, respectively). Notably, *D. welbionis* was the dominant species in children aged 3–6 years (kindergarten; figure 1b). To assess host genetic influences, we analysed *Dysosmobacter* spp. relative abundances in 53 healthy monozygotic (MZ) and 66 healthy dizygotic (DZ) twin pairs from the TwinsUK cohort. A correlation in relative abundances of *D. welbionis* was detected in MZ (R=0.43, p=0.001) but not DZ twins (R=0.058, p=0.64), suggesting its heritability (figure 1c).

Given recent evidence that *D. welbionis* J115^T attenuates liver weight gain in high-fat diet-fed mice,[12] we analysed three well-characterised gut microbiota datasets from patients with metabolic-associated fatty liver disease (MASLD): one from the USA (non-MASLD n=51; MASLD without fibrosis n=17; MASLD with cirrhosis n=25),[33] one from Finland (non-MASLD n=26; MASLD n=12)[34] and one with individuals from Spain and Italy (n=686). In the first two cohorts, the relative abundance of *D. welbionis*, and to a lesser extent *D. hominis*, was significantly reduced in MASLD cases (p<0.05; figure 1d). Although we did not find a correlation between the relative abundance of *D. welbionis* and MASLD in the third study, *D. welbionis* abundance was inversely correlated with MASLD fibrosis score (figure 1e). These inverse correlations imply that higher abundance of this bacterium may be linked to improved liver health, without being directly linked to the total liver fat content. Altogether, these findings reinforce the dominance of *D. welbionis* within the genus, and its potential role in maintaining liver health across diverse human populations.

To experimentally assess whether *D. welbionis* can mitigate hepatic steatosis development, we explored additional hepatic health parameters in previously published mice cohorts[12 35] with an addition of two unpublished cohorts with similar experimental design. In these four independent studies comparing animals fed a normal diet (ND) or a high-fat diet (HFD) for 8–10 weeks, as previously observed, J115^T supplementation (HFD + J115^T) limited the HFD-induced hyperglycaemia and prevented the increase in liver weight associated with HFD (figure 2a,b). Histological quantification of hepatic fat droplets revealed that J115^T supplementation had limited the development of steatosis observed in the HFD group (figure 2c,d). Plasma transaminase measurements revealed no significant differences in

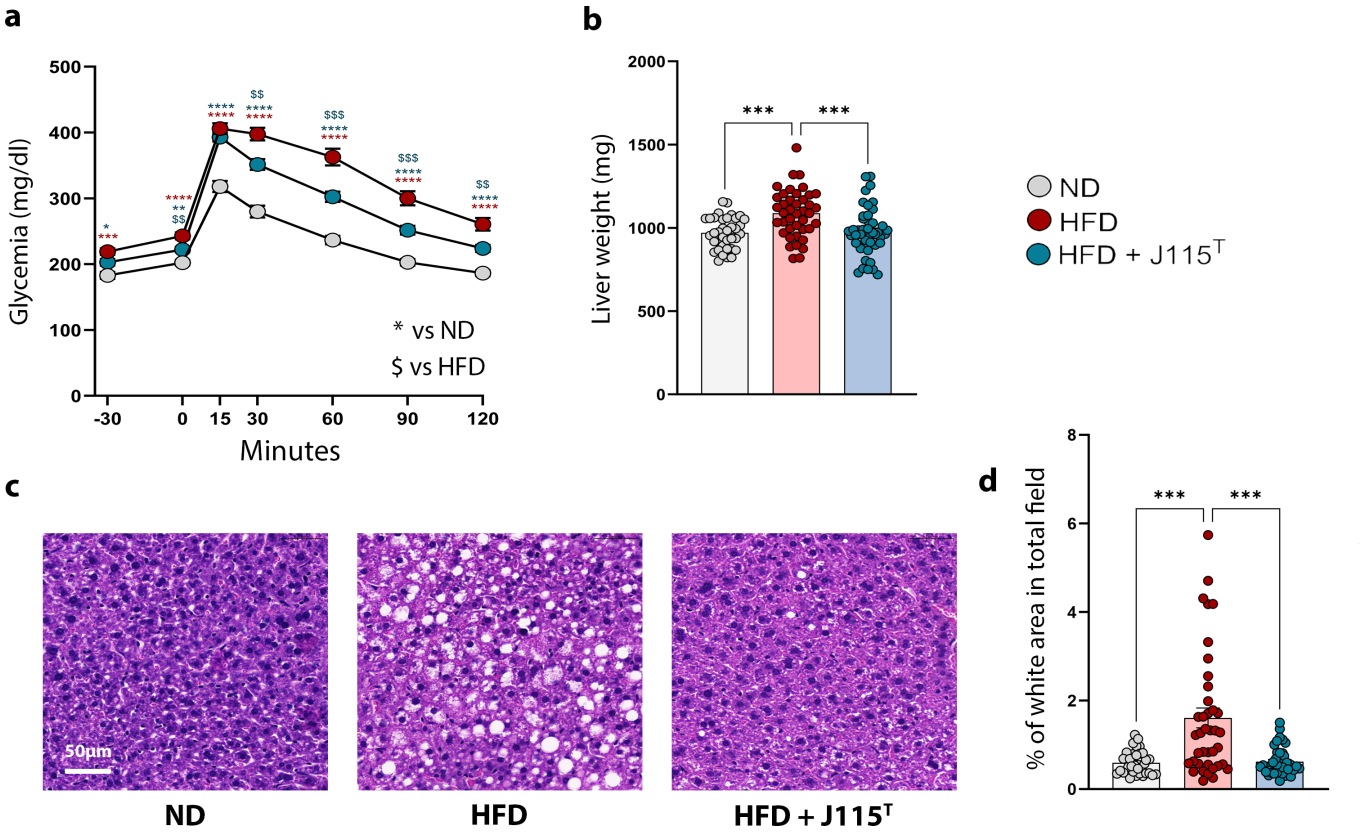

Figure 2 *D. welbionis* J115^T improves glycaemia, hepatic weight and steatosis in a mouse model of diet-induced obesity and diabetes. (a) Plasma glucose profile during a 2-hour OGTT in mice fed an ND or a HFD, treated with either a daily oral gavage of vehicle or live *D. welbionis* J115^T (HFD + J115^T) (>1×10^9 CFUs) for 8 weeks. (b) Liver weights at the end of the protocol. (c) Representative images of liver slices for each group. (d) Percentage of white (unstained/empty zone) per field, representing hepatic lipid droplets. Data represent the mean of 10 fields per sample. n=25–44/group. Data are represented as the mean±SEM. (a) Was analysed using a repeated measures two-way ANOVA with a Geisser-Greenhouse correction and a Tukey's multiple comparisons test. (b) Was analysed using a one-way ANOVA followed by a Tukey's multiple comparisons test. (d) Was analysed using a Kruskal-Wallis test followed by a Dunn's multiple comparisons test. *p<0.05, **p<0.01, ***p<0.001. ANOVA, analysis of variance; CFU, colony forming units; *D. welbionis, Dysosmobacter welbionis;* HFD, high-fat diet; ND, normal diet; OGTT, oral glucose tolerance test.

aspartate aminotransferase (AST) between groups, while alanine aminotransferase (ALT) levels were significantly elevated in both HFD and HFD+J115^T compared with ND, with no significant difference observed between HFD and HFD+J115^T (online supplemental figure S1a and b). Collectively, these data indicate that *D. welbionis*, at least partially, protects against diet-induced hepatic steatosis, supporting its positive association with liver health observed in human cohorts.

Inositol fermentation by *D. welbionis* J115^T

Given the beneficial effects of *D. welbionis* on host metabolism and liver health, we next sought to investigate the bacterial metabolic capacities that may support its activity in the gut. *D. welbionis* J115^T can produce butyrate from *myo*-inositol, a feature not observed in other members of the *Oscillospiraceae* family.[10] Reduced microbial-derived butyrate has been linked to metabolic diseases, including MASLD,[36] highlighting the relevance of investigating corresponding metabolic pathways in *D. welbionis*.

The spectrum of carbon sources that support the growth of *D. welbionis* J115^T was subjected to 190 assays of carbon sources using Biolog phenotypes microarray plates. Only three carbon substrates consistently stimulated the growth by more than 50%: *myo*-inositol (a carbocyclic sugar alcohol), uridine and inosine (both nucleosides) (figure 3a and online supplemental table S1 for the list of negative compounds). The strong growth response to *myo*-inositol and uridine highlighted its potential as a key metabolic substrate for *D. welbionis* J115^T. To confirm these results and determine the optimal concentration, we grew *D. welbionis* J115^T on medium containing increasing concentrations, between 0% and 2.5% (weight/vol), of *myo*-inositol or uridine for 72 hours. A maximal increase (threefold) of biomass production was obtained with 0.75% (weight/vol) of *myo*-inositol and 2.5% of uridine, confirming their growth-promoting effects (figure 3b). *Myo*-inositol utilisation, which reached 75% after 52 hours, was associated with a pH decrease from 8.25 to 5.70 while no pH change was observed for uridine (figure 3b). *D. welbionis* J115^T was able to ferment *myo*-inositol to butyrate and acetate after 48 hours (figure 3c), as well as isovalerate and isobutyrate in small quantities, suggesting branched-chain amino acid fermentation capability (online supplemental figure S1). We also observed that the production of butyrate and acetate was dependent on *myo*-inositol addition, suggesting a robust capability of *D. welbionis* J115^T to ferment inositol (online supplemental figure S2). Maximal production of butyrate was obtained with a medium supplementation of 0.75% *myo*-inositol (online supplemental figure S2a). Consistent

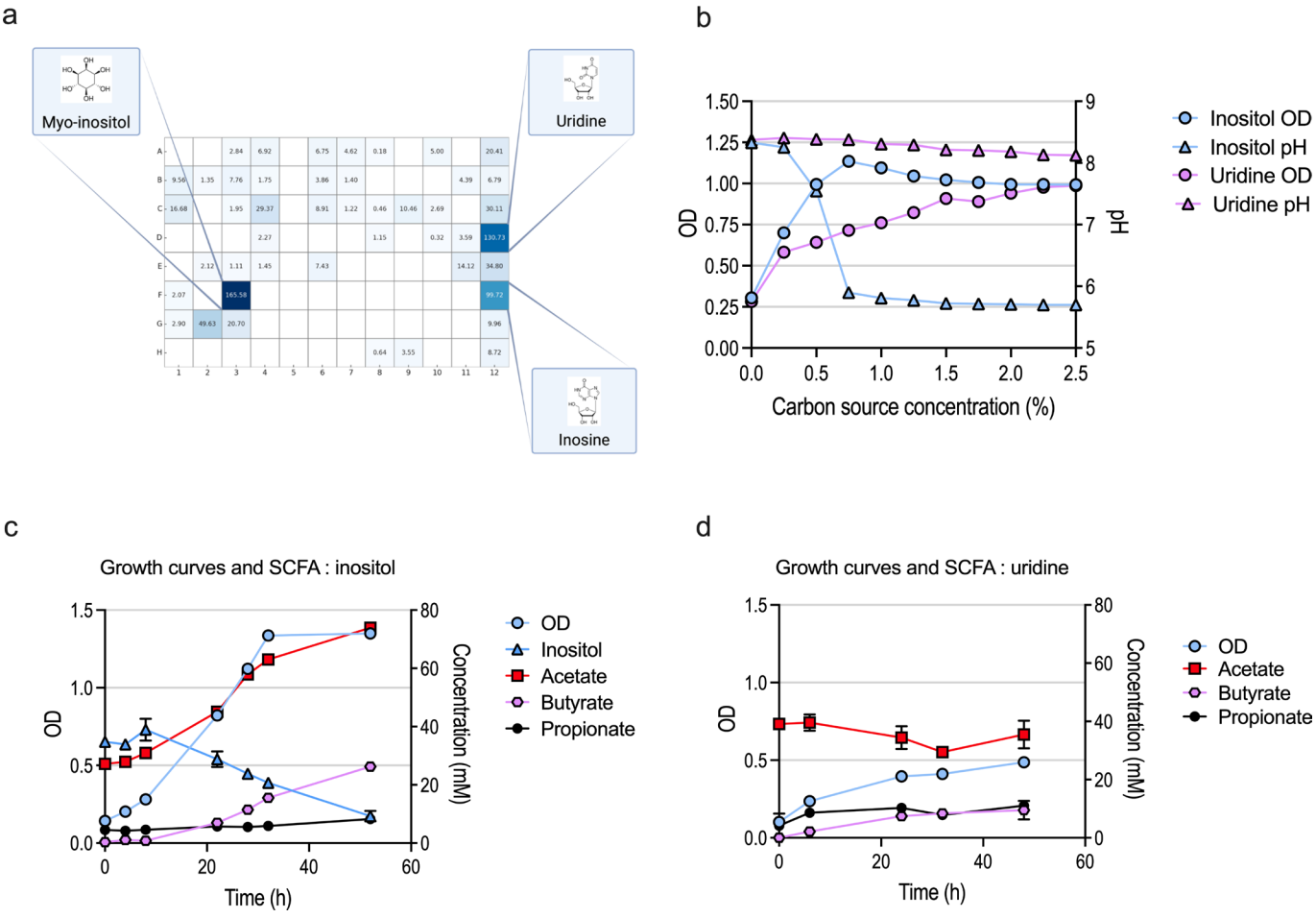

Figure 3 *Myo*-inositol and uridine utilisation by *D. welbionis* J115^T and their impact on biomass, pH and SCFA production. (a) Percentage of biomass (evaluated by OD_{680nm}) increase in comparison to the negative control for positive substrates in the 190 substrates utilisation assays for carbon sources (PM1 and PM2 Biolog plates). (b) Biomass (left Y axis) and pH (right Y axis) modification of *D. welbionis* J115^T after 72 hours of growth in modified YCFA supplemented with 0 to 2.5% (weight/vol) of *myo*-inositol or uridine. (c) Biomass (left Y axis) and SCFAs (right Y axis) produced by *D. welbionis* J115^T after several hours of growth in modified YCFA medium supplemented with *myo*-inositol. (d) Biomass (left Y axis) and SCFAs (right Y axis) produced by *D. welbionis* J115^T after several hours of growth in modified YCFA medium supplemented with uridine. *D. welbionis, Dysosmobacter welbionis;* OD, optical density; OD, optical density; SCFAs, short-chain fatty acids.

with no change in pH, providing uridine led to a limited growth and no production of SCFAs (figure 3b and d, online supplemental figure S2a).

Fermentation of *myo*-inositol by *D. welbionis* J115^T is essential to produce energy

Using a multichannel isothermal microcalorimeter, we monitored bacterial growth by measuring metabolic heat production to assess real-time energy release during active metabolism.[37 38] Among all concentrations tested, *myo*-inositol 0.75% led to the highest energy production (figure 4a). To evaluate the importance of each individual carbon source originally used to isolate strain J115^T, we removed one substrate per condition and monitored metabolic activity. Notably, the total heat production was analysed to assess metabolic activity profiles. All tested conditions supported similarly elevated metabolic rates in the presence of *myo*-inositol. However, the removal of *myo*-inositol led to a decrease in total heat production, suggesting reduced metabolic activity of the bacteria under this condition and that *myo*-inositol plays a key role in supporting the growth and energy metabolism (figure 4b). These findings reinforce the strong metabolic response elicited by *myo*-inositol and confirm the maximum growth observed at 0.75% (figure 3). These results suggest that *myo*-inositol supports robust bacterial metabolism, in contrast to all other tested sugars.

Elucidation of *myo*-inositol fermentation pathway using ^{13}C-NMR and mass spectrometry

To further investigate the *myo*-inositol pathway at the molecular level, we grew *D. welbionis* J115^T in YCFA medium containing either [$^{13}C_6$]*myo*-inositol, [4-^{13}C]*myo*-inositol or [4,5-$^{13}C_2$]*myo*-inositol as the carbon source.[29] Supernatants were collected after 48 hours and analysed by using NMR. High-resolution ^{13}C-NMR spectra showed a complete conversion of [$^{13}C_6$]*myo*-inositol to [1-^{13}C]acetate, [1-^{13}C]butyrate, [2-^{13}C]acetate, [2-^{13}C]butyrate, [3-^{13}C]butyrate, [4-^{13}C]butyrate and [^{13}C]CO_2 (figure 4a). To investigate the biochemical pathway of [4-^{13}C]*myo*-inositol or [4,5$^{13}C_2$]*myo*-inositol fermentation, we detected the following products: [1-^{13}C]butyrate, [1-^{13}C]acetate, [^{13}C]CO_2, [2-^{13}C]acetate, [2-^{13}C]butyrate, [3-^{13}C]butyrate and [4-^{13}C]butyrate (figure 5a). These results confirmed that inositol was stoichiometrically converted into butyrate and acetate. To further dissect the biochemical pathway, *D. welbionis* J115^T was grown in [4-^{13}C]*myo*-inositol

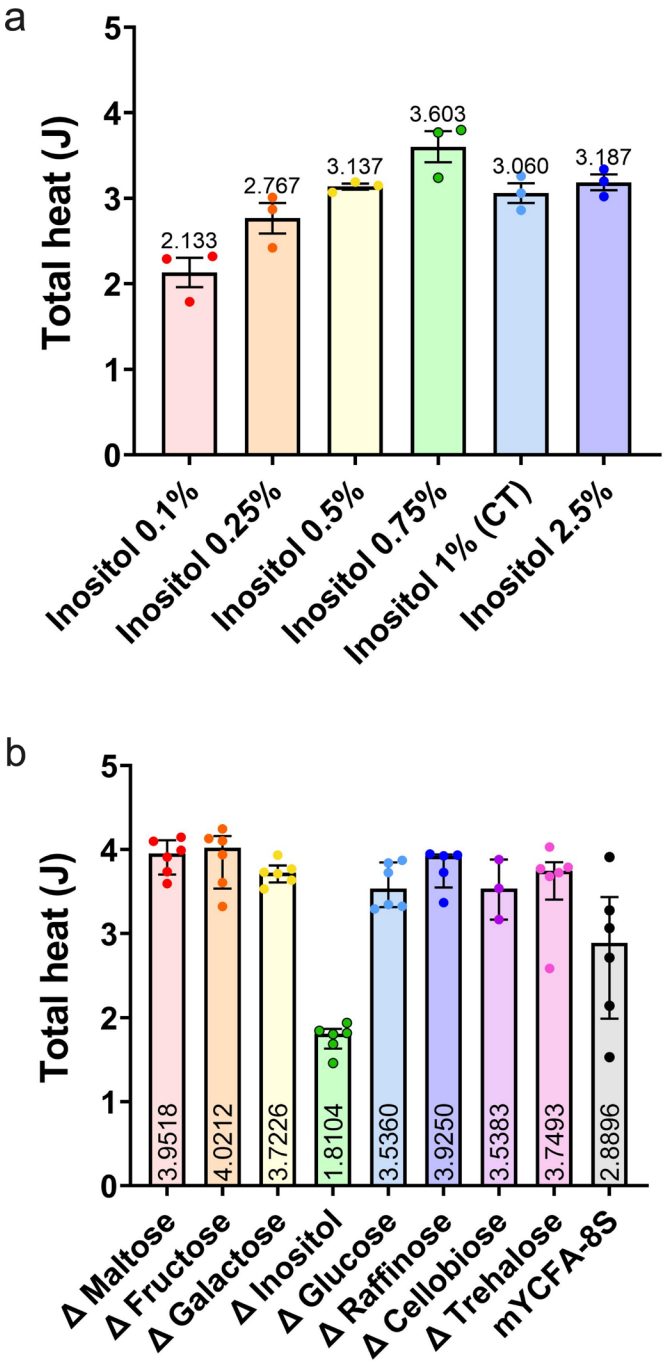

Figure 4 *Myo*-inositol stimulates energy production in *D. welbionis* J115^T as measured by isothermal microcalorimetry. (a) Total heat released by *D. welbionis* J115^T after 72 hours of incubation in modified YCFA medium supplemented with increasing concentrations of *myo*-inositol (0.1%–2.5%, w/v). The highest metabolic activity was observed at 0.75%, indicating an optimal concentration for energy production. CT represents the control group, which was cultured with 1% myo-inositol, the concentration routinely used for growing *D. welbionis* J115^T. (b) Comparison of total heat production in cultures supplemented with various carbon sources, including monosaccharides and disaccharides. Absence of *myo*-inositol triggered significantly lower energy output than all other tested sugars, highlighting its preferential use and importance in supporting robust metabolism. mYCFA-8s is a modified YCFA medium supplemented with eight different sugar sources (g/L) with maltose, fructose, galactose, *myo*-inositol, glucose, raffinose, cellobiose, trehalose. *D. welbionis, Dysosmobacter welbionis.*

or [4,5-$^{13}C_2$]*myo*-inositol. Subsequent NMR analysis revealed the formation of [2-^{13}C]acetate, [4-^{13}C]butyrate or [1,2-^{13}C] acetate, [3,4-^{13}C]butyrate, respectively (figure 5a). This result suggests that the cleavage of 5-dehydro-2-deoxy-D-gluconate 6-phosphate occurs between the C3-C4 bond to form [2-^{13}C]3-oxopropionate or [1,2-^{13}C]3-oxopropionate and non-labelled glycerone phosphate from [^{13}C]inositols (figure 5b). In addition, the dehydration of *scyllo*-inosose was across the C4–C5 bond rather than C1–C6 bond. The produced 3-oxopropionate was subsequently converted into [2-^{13}C]acetate or [1,2-^{13}C]acetate. Production of [4-^{13}C]butyrate and [3,4-^{13}C]butyrate indicates condensation of 2 labelled acetyl-Coenzyme A (acetyl-CoA) later in the fermentative pathway. The proposed inositol utilisation route was further supported by detection of four intermediates by liquid chromatography high-resolution mass spectrometry (LC-MS/MS) including 3,5/4-trihydroxycyclohexan-1,2-dione, *scyllo*-inosose, 5-dehydro-2-deoxy-D-gluconate and 5-dehydro-2-deoxy-D-gluconate 6-phosphate in cell lysates (online supplemental figure S3 and online supplemental table S2). We also observed that the first three intermediates were present at the beginning of the growth and subsequently used and their levels remained low in the bacterial cells; whereas 5-dehydro-2-deoxy-D-gluconate 6-phosphate was only accumulated in small quantities after 2 hours and fully converted after 24 hours. These combined results confirm the precise molecular pathway by which *D. welbionis* J115^T ferments *myo*-inositol into acetate and butyrate, providing clear biochemical markers and reaction intermediates for further metabolic studies.

Evidence for a non-canonical *myo*-inositol to butyrate pathway in *D. welbionis* by proteogenomics and comparative genomics

The genome of *D. welbionis* J115^T comprised 3 576 546 bp with a guanine (G) and cytosine (C) bases (GC) content of 58.9% and a total of 3510 protein-coding sequences (CDSs) (online supplemental table S3).

To reconstruct the entire inositol fermentative pathway, a proteogenomic approach was used. We have reconstructed the entire pathway from *myo*-inositol to butyrate based on the use of stable isotopes [^{13}C] *myo*-inositol (figure 5b) and have identified gene candidates involved in the pathway based on proteogenomic analysis (figure 5c). This includes a *myo*-inositol utilisation gene cluster and enzymes encoded by this gene cluster that were highly abundant with a twofold to threefold induction in cells grown with inositol. The conversion of 2-deoxy-5-keto-D-gluconate 6-phosphate into 3-oxopropionate is canonically done by 5-dehydro-2-deoxyphosphogluconate aldolase (*iolJ*). However, although the *iolJ* gene is absent in the genome of *D. welbionis* J115^T, we identified two genes by the annotation pipeline as class II fructose-bisphosphate aldolases (online supplemental table S4 and see fold changes in online supplemental table S5). Interestingly, both enzymes are associated with the TIGR01859 conserved domain, which has been linked to 5-dehydro-2-deoxy-D-gluconate-6-phosphate aldolase activity. This raises the possibility that one of these two aldolases may catalyse the cleavage of 2-deoxy-5-keto-D-gluconate-6-phosphate into 3-oxopropionate. This strongly suggests that *D. welbionis* likely uses a non-canonical set of enzymes to produce butyrate using *myo*-inositol. Indeed, some class II fructose-bisphosphate aldolases, although specialised for fructose-1,6-bisphosphate, can sometimes exhibit secondary aldolase activity on modified aldonic substrates (like 2-deoxy-5-keto-D-gluconate-6P). Therefore, it is possible that *D. welbionis* has recruited another aldolase enzyme (not

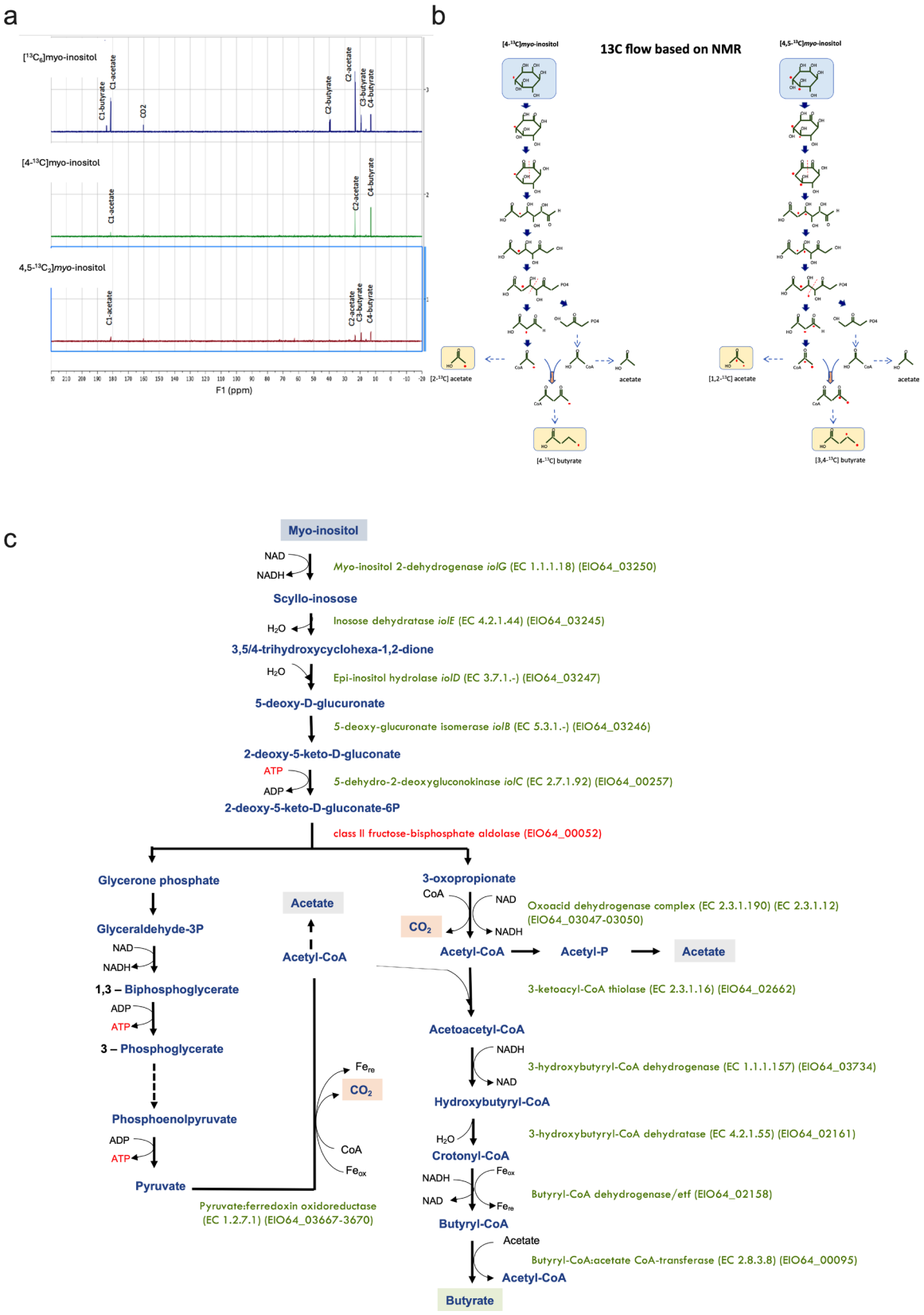

Figure 5 Reconstruction of the *myo*-inositol-to-butyrate fermentation pathway in *D. welbionis* J115^T through proteogenomic and isotopic analyses. (a) High-resolution ^{13}C-NMR spectra showing [$^{13}C_6$]*myo*-inositol, [4-^{13}C]*myo*-inositol or [451$^3C_2$]*myo*-inositol fermentation products that are [1-^{13}C]butyrate, [1-^{13}C]acetate, [^{13}C]CO_2, [2-^{13}C]acetate, [2-^{13}C]butyrate, [3-^{13}C]butyrate, [4-^{13}C]butyrate. (b) Detection of ^{13}C-labelled fermentation intermediates and products using ^{13}C-NMR supports the presence of a functional pathway from *myo*-inositol to butyrate with anticipated scheme of ^{13}C flow. (c) Reconstruction of the entire *myo*-inositol degradation pathway to butyrate production based on metabolomic analyses using ^{13}C NMR spectra and high-resolution LC-MS/MS data, genomic and proteomic analysis. See fold changes in the online supplemental table S5. CoA, Coenzyme A; *D. welbionis, Dysosmobacter welbionis;* LC-MS/MS, liquid chromatography high-resolution mass spectrometry.

annotated as *iolJ*) to carry out this step in *myo*-inositol catabolism. Along the same line, we propose that the conversion of 3-oxopropionate into acetyl-CoA may be realised by an oxoacid dehydrogenase complex (EIO64_03 047–03050) (EC 2.3.1.190) (EC 2.3.1.12). We found that the complex was highly abundant and 1.1–1.8 times induced in inositol-grown cells compared with uridine-grown cells (online supplemental table S4 and online supplemental table S5). This oxoacid dehydrogenase has been shown to convert 3-oxopropionate to acetyl-CoA and CO_2.[39] Produced acetyl-CoA is postulated to condense with another acetyl-CoA to form acetoacetyl-CoA which would enter the acetyl-CoA pathway for butyrate formation.[40] This proposed route is in line with the detection of various intermediates by LC-MS/MS (figure 5b).

Isolation of novel *D. welbionis* strains using an anaerobic cell sorting method

Building on our findings, we next aimed to isolate novel *D. welbionis* strains from the human gut to better capture the species' diversity and functional potential. This allowed us to examine whether the identified non-canonical *myo*-inositol-butyrate pathway is strain-specific. Expanding the strain repertoire also enabled the assessment of genomic features such as antimicrobial resistance genes, which is important for therapeutic use. Establishing a strain bank thus provides a basis for identifying safe and metabolically competent candidates for future probiotic applications.

We employed the species-specific isolation and cultivation technology developed by Bellais *et al*[41] that enables the targeted recovery of gut commensal bacteria via anaerobic flow cytometry (figure 6a). First, polyclonal antibodies against *D. welbionis* were generated by injecting rabbits with the heat-inactivated type strain J115^T. These antibodies were then tested against a pure culture of the same strain to test sensitivity while *Dysosmobacter* sp SEL_291 and *Escherichia coli* ATCC 35218 were used as control bacteria to test specificity. Antibodies raised against *D. welbionis* J115^T detected >90% of target bacteria, whereas only 3% and 0.7% of *Dysosmobacter* sp SEL_291 and *E. coli* were detected by the antibodies, respectively (figure 6b). We then tested whether *D. welbionis*-specific antibodies could detect the J115^T strain at varying concentrations within a complex bacterial mixture. To validate this, we used a control stool sample quantified by flow cytometry, in combination with stool samples spiked with 0.1%, 0.3%, 1% or 3% of *D. welbionis* J115^T. First, the viability of the spiked stool samples was comparable to that of the non-spiked controls. As shown in online supplemental

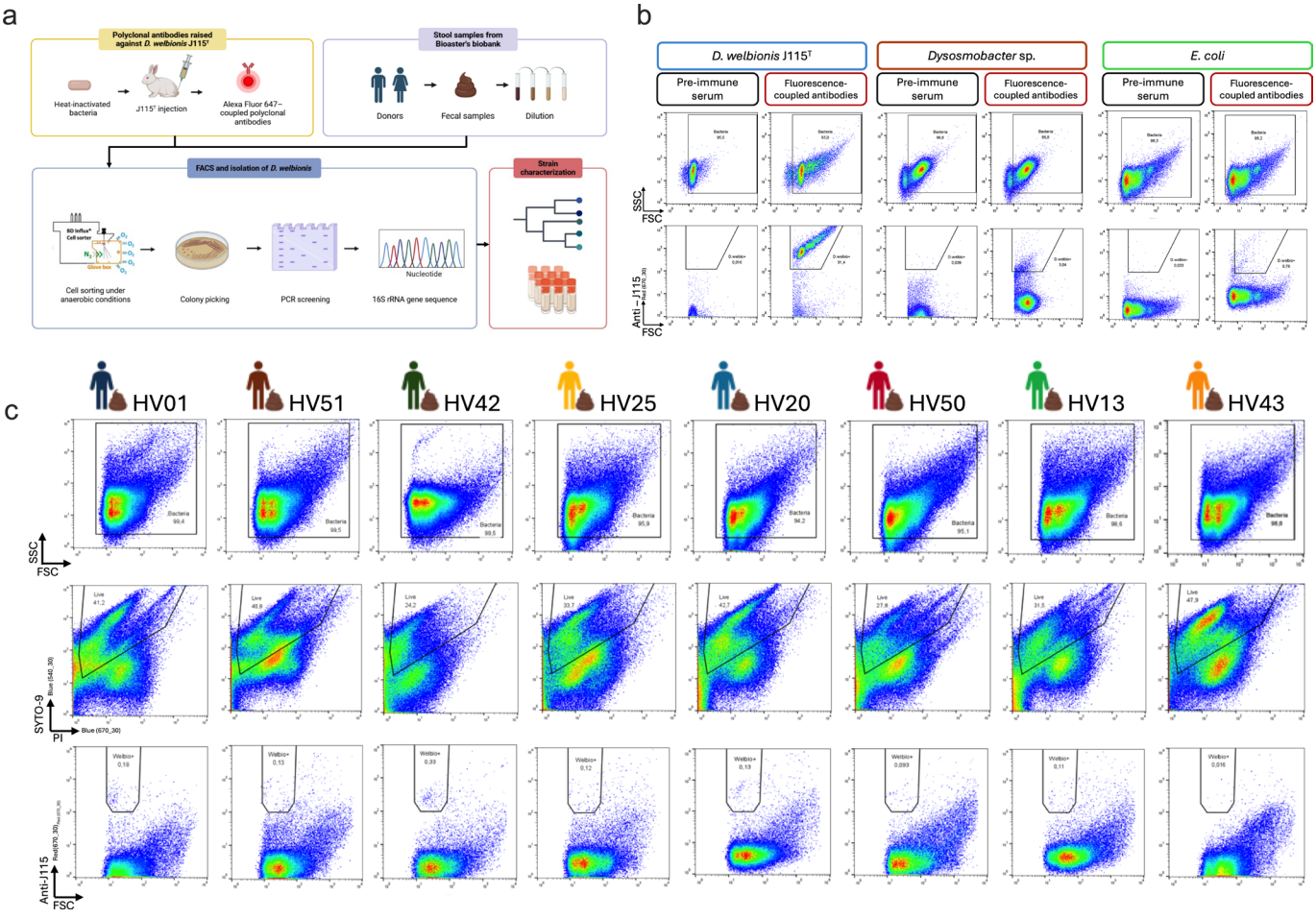

Figure 6 Species-specific isolation of *D. welbionis* strains using anaerobic flow cytometry and antibody-based sorting. (a) Schematic overview of the anaerobic cell sorting method developed for targeted isolation of *D. welbionis* from faecal samples, based on the strategy by Bellais *et al*.[41] (b) Polyclonal antibodies raised against heat-inactivated *D. welbionis* J115^T showed >90% specificity toward the target strain in pure culture, while exhibiting minimal cross-reactivity with a related *Dysosmobacter* sp. (3%) and *E. coli* (0.7%). (c) Cell sorting and culture from faecal samples of healthy donors led to the successful isolation of 19 novel *D. welbionis* strains from eight individuals. *D. welbionis, Dysosmobacter welbionis; E. coli, Escherichia coli.*

figure S4, *D. welbionis* spiking was detectable in all four spiked samples. Even at a low abundance of 0.1%, a distinct population could be observed, with 0.098% detected in the sample. At higher concentrations, from 0.3% to 3%, the *D. welbionis* population became clearly visible and denser. In conclusion, this spiking experiment validated the specificity of our anti-*D. welbionis* antibody in a complex mixture of bacteria, demonstrating its ability to detect as little as 0.1% of the total bacterial population.

Using quantitative PCR analysis on available stool samples, we identified 16 samples with the highest relative abundance of *D. welbionis*; these samples were then selected for cell sorting. Out of the 16 stools tested, we successfully isolated 19 new isolates of *D. welbionis* from 8 healthy volunteers. Using faecal samples collected from these healthy volunteers, we found that live bacteria (live: SYTO nine-positive and propidium iodide (PI)-negative) ranged from 24.2% to 47.9% (figure 6c). Similarly to what was observed in online supplemental figure S4, *D. welbionis* bacteria accounted for 0.016%–0.33% of the events for eight volunteers. All colonies isolated were then tested by PCR with *Dysosmobacter*-specific primers and samples for which the 16S rRNA gene could be amplified were sent for Sanger sequencing. The obtained sequences had similarities ranging from 99.78% to 100% between themselves.

Pangenomic analysis and phylogenetic differences among all the *D. welbionis* isolates

In addition to the 19 strains isolated using the anaerobic cell sorting method and the type strain J115^T, we isolated 1 specific strain via conventional approaches that we named *D. welbionis* Ino, and obtained *D. welbionis* CLA-AA-H189 and *D. welbionis* CLA-JM-H43 from the Human intestinal Bacteria Collection.[42] Comparative genomic analysis of these 23 *Dysosmobacter* genomes and nine additional genomes from phylogenetically related species showed a variable range of CDSs (figure 7a). The results of paired average nucleotide identity (ANI) analysis showed intraspecies differences within the different isolates (including the type strain J115^T) but all ANI were above 98% of similarities (an ANI threshold of 95% has been commonly used for species demarcation[43]) confirming that all of the strains isolated belong to *D. welbionis* (figure 7b). To determine the core genes and strain-specific genes within the species, genomes of 23 strains were selected for pan-genome analysis. Additionally, two more strains, *Oscillibacter acetigenes* H4-59 and *Oscillibacter* sp. PEA192, were included based on their high ANI with all other *D. welbionis* strains. Among the 25 genomes, we obtained a total of 8141 unique protein-coding genes in the pan-genome, corresponding to more than twofold the average total genes of the 25 genomes. These also included the 2136 core genes present in all strains, 174–457 persistent genes, 75–419 shell genes and 148–880 cloud genes (figure 7c). The gene accumulation curve showed a rapid stabilisation of the core genome after the inclusion of only a few strains, indicating a set of genes conserved across all isolates, while the pan-genome showed an increasing trend, suggesting the presence of a large and diverse shell and cloud gene pool (figure 7d). This is characteristic of an open pangenomic structure, indicating extensive genomic variability and a high frequency of strain-specific genes. At the species level, the genome sizes are 3 594 757±12 628 bp, GC content is 59.05%±0.02% and there are 3469.7±14.9 coding sequences. We constructed a phylogenetic tree of the strains based on concatenated core genes (figure 7e). To gain further insight into the functional diversity of the *D. welbionis* pan-genome, we categorised the persistent, shell and cloud genes according to Clusters of Orthologous Groups functions (figure 7f). Persistent genes, present in almost all strains, were predominantly involved in essential metabolic and cellular functions, including transcription (category K), energy production and conversion (C), translation and ribosomal biogenesis (J) and amino acid metabolism (E), highlighting a conserved genetic backbone central to core bacterial physiology. Shell genes (ie, shared by several but not all strains) were more evenly distributed across functional categories, with notable enrichment in cell wall/membrane biogenesis (M) and signal transduction mechanisms (T), suggesting roles in strain-specific adaptation. Cloud genes (ie, unique to only a few strains) showed a broader functional range, including a substantial fraction of poorly characterised or unknown function genes (categories R and S), as well as defence mechanisms (V). These findings indicate that while the core genome supports the fundamental biology of *D. welbionis*, the accessory genome likely contributes to ecological flexibility and strain-specific traits, such as environmental sensing or niche specialisation.

Genomic analyses revealed the presence of a *myo*-inositol pathway to butyrate in all the *D. welbionis* strains

To determine whether the newly isolated strains carry the same metabolic pathways as the type strain and specifically the pathway related to the production of butyrate from *myo*-inositol, we compared the genome of each strain. As depicted in figure 8a, we found that all strains harboured the same genes involved in the *myo*-inositol to butyrate pathway, with some variations in gene copy numbers. In addition, all strains were able to grow on *myo*-inositol as sole carbon source, with the exception of *D. welbionis* W36, which showed no growth, and *D. welbionis* CLA-AA-H189, which exhibited reduced growth capacity (online supplemental figure S5a and b).

We have previously identified a tetracycline-resistant gene (*tetW*) as antimicrobial resistance (AMR) gene in the genome of *D. welbionis* J115^T. To further characterise the resistome of the *D. welbionis* strains, we analysed AMR using both Comprehensive Antibiotic Resistance Database (CARD) (via RGI) and AMRFinderPlus. The CARD-based analysis predicted the presence of multiple AMR genes associated with tetracyclines (eg, *tet(W)*), aminoglycosides (eg, *aad(6)*, *SAT-4*, *APH(3')-IIIa*) and macrolides (*ErmB*). Notably, genes from various vancomycin resistance clusters (eg, *vanA*, *vanG*, *vanX*) were consistently detected across strains with 'strict' hits (figure 8b). Concordantly, AMRFinderPlus identified several of the same AMRs with high confidence, including *tet(W)*, *erm(B)* and *APH(3')-IIIa*, confirmed via BLASTP or EXACT alignments. Strain W27 stood out for carrying a broader range of AMR genes, including *erm(G)*, *aadE*, *ant(6)-Ia* as well as a PARTIALX hit for *erm(G)*, suggesting a potentially more complex resistance profile. The detection of *vanT*, *vanG* and *vanR* genes across nearly all strains likely reflected non-functional genetic fragments rather than active glycopeptide resistance, consistent with the CARD findings. Interestingly, *D. welbionis* CLA-AA-H189 and *D. welbionis* W28 did not encode any resistance genes other than the vancomycin clusters identified using CARD and confirmed with AMRFinderPlus (figure 8c). However, genomic analyses did not identify canonical genes involved in lipopolysaccharide biosynthesis, outer-membrane assembly or porins typically found in diderm gram-negative bacteria, while genes involved in teichoic acid biosynthesis were present.

Together, these results underscore the value of integrating multiple annotation tools to robustly assess resistome content,

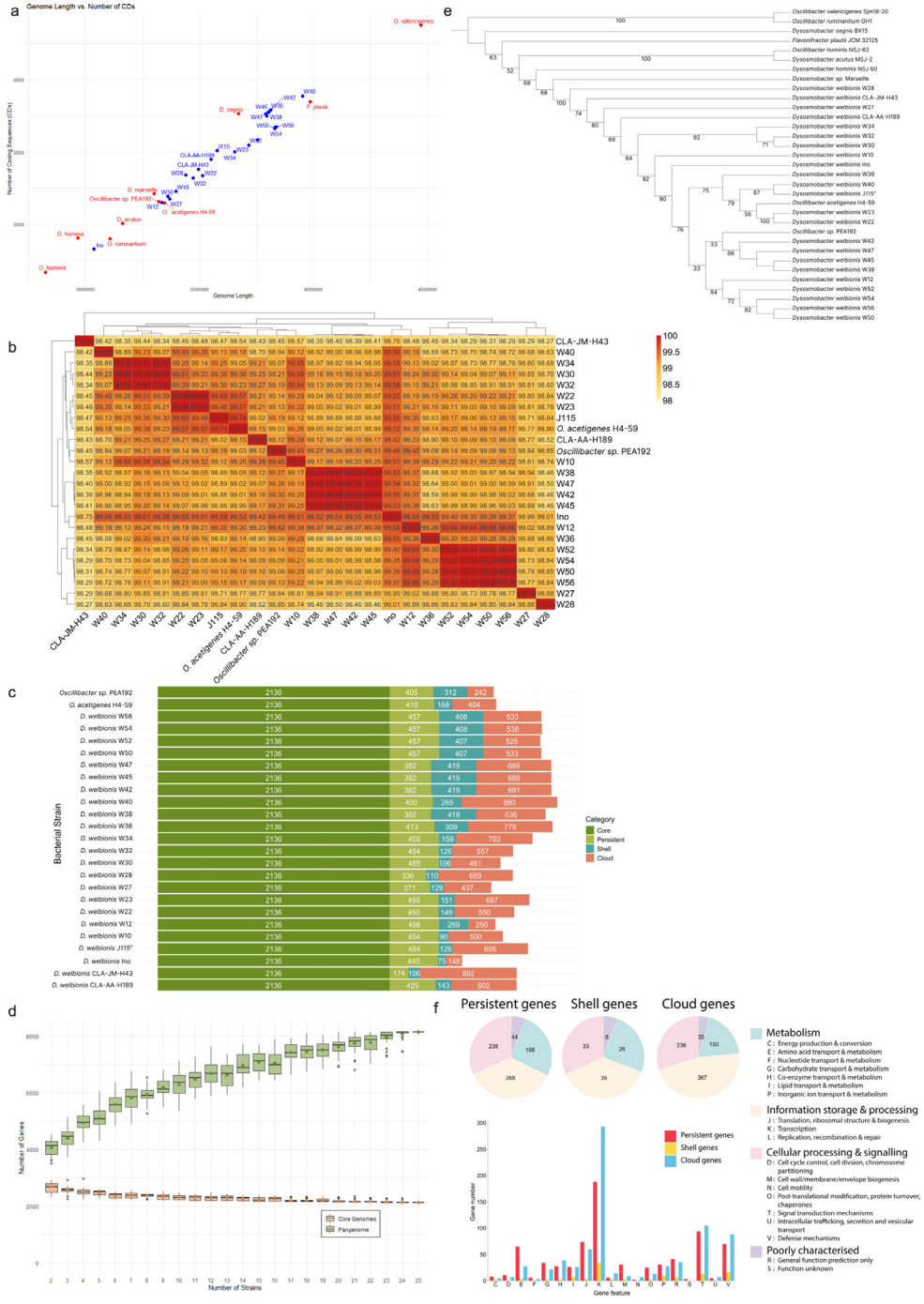

Figure 7 Comparative genomic and phylogenetic analyses of *D. welbionis* strains. (a) CDS counts across the 23 *D. welbionis* isolates (including the type strain J115^{T} and three newly named strains: Ino, H43 and CLA-AA-H189) and related *Dysosmobacter* genomes, showing intra-species genomic variability. (b) ANI matrix revealing >98% genomic similarity across all *D. welbionis* isolates, confirming species-level relatedness despite strain-level diversity. (c) Gene accumulation curves indicating a continuously expanding pangenome and a decreasing core genome size as additional strains are added. (d) Classification of gene families into core (shared by all), shell (moderately conserved) and cloud (strain-specific) genes, totalling over 8000 genes across the species. (e) Phylogenetic tree constructed from concatenated core gene alignments illustrating the evolutionary relationships among *D. welbionis* strains and their genomic clustering. (f) Pangenome analysis of 23 *D. welbionis* genomes revealed the distribution of gene functions across three categories: persistent, shell and cloud genes. The pie charts (top) illustrate the proportion of genes assigned to each functional class (COG categories) within these groups. Most persistent genes were associated with essential cellular functions such as metabolism (notably amino acid, carbohydrate and energy metabolism), transcription (category K) and translation (category J). In contrast, shell and cloud genes showed higher representation in mobile or adaptive functions, including cell wall/membrane biogenesis (M), signal transduction (T) and defence mechanisms (V). The bar graph (bottom) presents the number of genes per COG category within each pangenome subset, highlighting that core genome functions are enriched in information processing and metabolism, whereas accessory genes contribute to genomic flexibility and environmental adaptation. Cloud genes notably include a large number of unknown or poorly characterised genes (categories R and S), underscoring the unexplored functional diversity among *D. welbionis* strains. ANI, average nucleotide identity; CDS, coding sequence; COG, Clusters of Orthologous Groups; *D. welbionis, Dysosmobacter welbionis.*

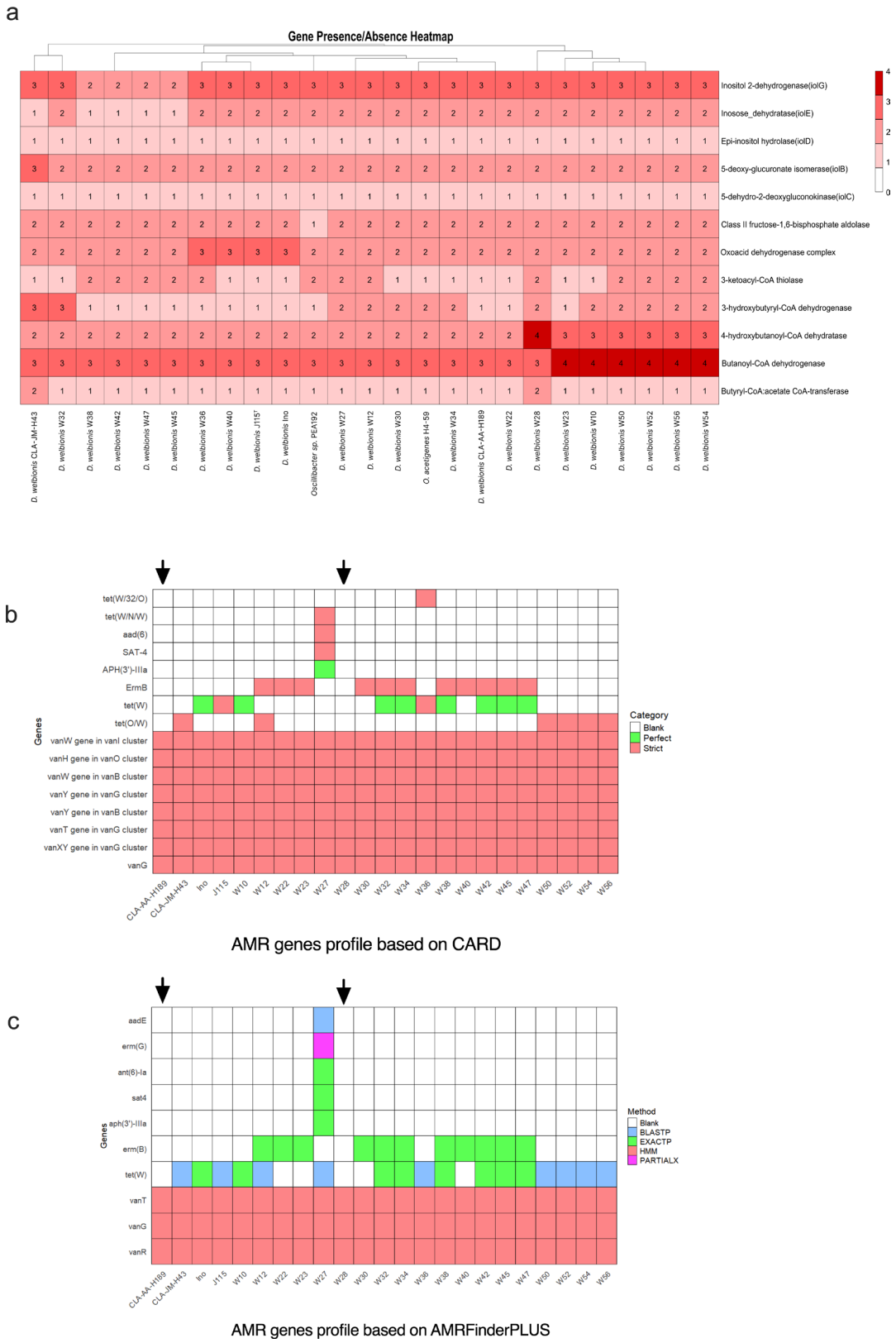

Figure 8 Gene copy variation in enzymes involved in the reconstructed *myo*-inositol to butyrate and AMR gene profiles. (a) Gene presence/absence and copy number heatmap of enzymes involved in the reconstructed *myo*-inositol to butyrate pathway across *D. welbionis* strains. Missing canonical enzymes (eg, IolJ and malonate-semialdehyde dehydrogenase) suggest the use of non-canonical enzymatic machinery. (b) AMR gene detection across strains based on CARD (Comprehensive Antibiotic Resistance Database). Several strains, including J115^{T}, harbour resistance genes against tetracyclines (*tet(W)* variants), macrolides (*ermB*) and aminoglycosides, while vancomycin resistance cluster genes (eg, *vanG*, *vanX*, *vanT*) are widely present with 'strict' confidence scores. (c) AMR gene prediction using AMRFinderPlus reveals concordant profiles, with key resistance determinants (*tet(W)*, *erm(B)*, *APH(3')-IIIa*) detected via high-confidence BLASTP or EXACT matches. Arrows depict strains without AMR except for vancomycin-resistance-associated (*van*) genes. AMR, antimicrobial resistance; CoA, Coenzyme A; *D. welbionis*, *Dysosmobacter welbionis*.

while highlighting the need for cautious interpretation of vancomycin resistance genes.

Material and methods

See online supplemental material and methods.

DISCUSSION

In this study, we discovered that *D. welbionis* is a dominant *Dysosmobacter* species and potentially associated with liver health in humans. Indeed, in two cohorts, its faecal abundance was negatively associated with MASLD, and in a third one, it was negatively associated with the MASLD fibrosis score. We then explored the impact of *D. welbionis* J115^T supplementation on steatosis in a murine model, where it mitigated hepatic steatosis in HFD-fed mice as evidenced by significantly lower liver weight and reduced lipid droplet accumulation. While we observe an increase in circulating ALT in both HFD groups, the extent of hepatocellular injury in this model appears modest. Another limitation comes from the duration of the protocol. 10–12 weeks of HFD has been described as sufficient to reach the beginning of steatosis but not hepatic inflammation, circulating liver enzyme alteration or fibrosis.[44] In addition, our *in vivo* data were obtained in a classical HFD-induced obesity model, which reliably induces steatosis and metabolic dysfunction but only partially mimics the spectrum of human MASLD. More aggressive dietary models, such as choline-deficient L-amino acid-defined (CDAA) or CDAA-HFDs, better recapitulate steatohepatitis and fibrogenesis and would provide important complementary information on the impact of *D. welbionis* on metabolic-associated steatohepatitis (MASH) and fibrosis progression.[45 46] These models, however, are also associated with pronounced weight loss and metabolic features that differ from human disease. Future work will therefore need to evaluate *D. welbionis* across multiple MASLD/MASH models, including choline-deficient diets and fibrosis-oriented settings, to fully characterise its effects on the different stages of liver disease. Therefore, our preclinical results support the idea that *D. welbionis* is associated with a better overall hepatic health in MASLD model and will require further analysis to fully delineate its putative role on MASLD and/or fibrosis in other animal models.

Beyond butyrate production, *D. welbionis* J115^T influences host metabolism through a broader network of metabolites and lipid mediators. Our previous metabolomic and lipidomic analyses identified several bioactive lipids specifically produced by (or strongly increased) following *D. welbionis* treatment.[35] Among these lipids, we can mention 9,10-diHOME, 12,13-diHOME, 9-oxoODE, 13-oxoODE, 12-HETE, 14,15-EET and 15d-PGJ$_2$. Several of these compounds act as PPARγ agonists, while others have anti-inflammatory or pro-resolving functions through yet-uncharacterised targets.[47] Notably, we previously demonstrated that *D. welbionis* J115^T exerts potent anti-inflammatory effects in the dextran sodium sulfate (DSS) induced colitis model, where it reduced disease severity and cytokine expression,[35] reinforcing the notion that this bacterium can modulate mucosal immunity and promote inflammatory resolution. The bacterium also increased C18-3OH both *in vitro* and *in vivo*, illustrating its ability to modulate hydroxylated fatty acids with anti-inflammatory properties.[35 48] Altogether, these observations support a model in which *D. welbionis* contributes to hepatic homeostasis not only through butyrate production or effects on adiposity, but also by increasing insulin sensitivity and orchestrating systemic immunometabolic signals likely including bioactive lipids, pro-resolving mediators, and pathways enhancing gut barrier function as previously observed in other studies.[11 12 35 49]

Then, we provide evidence for a previously uncharacterised metabolic pathway in *D. welbionis* J115^T for the conversion of *myo*-inositol into butyrate and acetate. Using ^{13}C-labelled substrates, NMR spectroscopy, mass spectrometry, integrative genomic and proteomic analyses, we have elucidated this fermentation pathway and identified a nearly complete gene pathway in this bacterium. To determine whether this trait is conserved across the species, we employed an antibody-guided, anaerobic cell-sorting approach to isolate 19 novel strains from healthy human stool samples. We sequenced their genomes, analysed intraspecies diversity, confirmed the presence of genes involved in the *myo*-inositol to butyrate conversion, and characterised their antimicrobial resistance profiles. These findings collectively position *D. welbionis* as a metabolically specialised member of the gut microbiota with unique potential for therapeutic applications.

The ability to use *myo*-inositol, a sugar alcohol abundant in fruits, grains, nuts and human milk, sets *D. welbionis* apart from other *Oscillospiraceae*. While a few microbes are known to ferment inositol, butyrate production from this substrate had not yet been experimentally validated in any gut commensal until now. The capacity of *D. welbionis* to grow robustly on *myo*-inositol and generate significant amounts of butyrate suggests an ecological niche based on exploiting less competitive carbon sources. Moreover, heat production assays confirmed that inositol supports higher metabolic activity than glucose, highlighting its centrality in the organism's energy metabolism.

This niche specialisation may also explain the widespread prevalence of *D. welbionis* and its dominance during childhood. The latter is particularly intriguing given the relatively high inositol content in human milk and dairy products commonly consumed during early life.[50 51] The significant correlation of *D. welbionis* abundance between monozygotic but not dizygotic twins suggests a heritable component to its colonisation, arguably a rare trait among gut microbes, as only 3–13% exhibit non-zero heritability in humans.[52 53] It is plausible that host genetic variation in inositol transport (eg, *SMIT2*) modulates colonic inositol availability and shapes microbial colonisation success.[54]

Our previous work has linked *D. welbionis* to glucose metabolism in both preclinical and clinical settings.[11 12 35] In our analyses, *D. welbionis* abundance was associated with MASLD in two cohorts, whereas this association was not observed in a third cohort (FLORINASH),[55] nor in our cohorts FOOD4GUT[12] and Microbes4U.[56] However, in the FLORINASH cohort, *D. welbionis* rather showed an association with the fibrosis score. Taken together, these findings suggest that *D. welbionis* may be involved in liver health more broadly, although a direct correlation with hepatic fat content cannot be clearly established at this stage. Additional evidence comes from a recent study on intrahepatic metastatic hepatocellular carcinoma, which reported depletion of *D. welbionis* in patients, and its restoration in a humanised mouse model following healthy donor faecal microbiota transplantation (FMT): the FMT treatment ultimately suppressed tumour progression.[57] Together, these findings point to a broader role for *D. welbionis* in maintaining metabolic and hepatic homeostasis, possibly mediated through its SCFA output and immunomodulatory potential.

Our comprehensive characterisation of newly isolated *D. welbionis* strains offers significant insights into the

metabolic versatility, ecological relevance and potential functional roles of this emerging gut commensal. One of the most striking findings is the capacity of this bacterium to ferment *myo*-inositol into butyrate. Unlike other members of the *Oscillospiraceae* family,[10] *D. welbionis* J115^T uniquely uses *myo*-inositol as a primary carbon source, setting it apart both phenotypically and phylogenetically.[10] This capability likely provides a competitive ecological advantage in the gut environment, where niche-specific substrates such as inositol may be differentially available. As mentioned earlier, a limited number of bacterial species have demonstrated the capacity to metabolise *myo*-inositol.[23 25 26 29] While *myo*-inositol catabolism into propionate has been demonstrated in *Anaerostipes* genus *such as A. hadrus* and *A. rhamnosivorans*, our data provide the first direct evidence of a functional pathway converting *myo*-inositol into butyrate in a prevalent and health-associated commensal. This shifts the current paradigm: inositol, traditionally considered a micronutrient or signalling molecule, may also serve as a metabolic bridge between host diet and microbial SCFA output with systemic effects. To date, *D. welbionis* is the only reported gut bacterium that converts inositol to butyrate.

Mechanistically, our isotopic labelling experiments confirmed butyrate and acetate as the main fermentation products of inositol metabolism. However, genomic and proteomic data revealed the absence of the canonical enzymes, 5-dehydro-2-deoxyphosphogluconate aldolase (*iolJ*), suggesting that *D. welbionis* employs a non-canonical enzymatic route. We propose that an alternative class II fructose-bisphosphate aldolase (FBA)-type or related enzyme could fulfil the role of *iolJ*, consistent with prior reports of functional plasticity within this enzyme family. In addition, based on proteome and genome, we detected an oxoacid dehydrogenase complex. These adaptations highlight metabolic flexibility and warrant further enzymatic and structural studies to confirm the functional roles of these candidate proteins.

The pan-genomic analysis showed that the inositol-butyrate pathway is highly conserved across all isolates, pointing to its role as a core species trait. Furthermore, comparative genomic analysis revealed a pan-genome with significant diversity among strains, yet a conserved core of >2100 genes, including the complete *myo*-inositol pathway. This suggests both genetic stability of essential functions and the potential for functional divergence. Notably, variation in the number of gene copies related to the inositol pathway suggests possible strain-specific regulatory mechanisms or adaptations to different gut environments. Although all *D. welbionis* strains possess the genetically predicted capacity to ferment *myo*-inositol, two strains (W36 and CLA-AA-H189) exhibited limited to no growth under the tested conditions. This discrepancy suggests that factors beyond the mere presence of metabolic genes may influence their ability to use *myo*-inositol. The absence of the I-TBP transporter (inositol transport system sugar-binding protein), the Rnf complex, or Fdox/red could explain the lack of inositol effect on growth, despite the presence of the fermentation pathway in the genome. However, all the strains possess the I-TBP transporter and Fdox/red. Regarding the Rnf complex, most strains have two copies, one containing subunits C, D and G and another with subunits A, B, C, D, E and G. Interestingly, strain CLA-AA-H189 has only one copy and lacks the version with subunits C, D and G. These findings suggest that the absence of a complete Rnf complex in CLA-AA-H189 might be a factor worth investigating further in relation to inositol utilisation. Alternatively, these strains may require co-metabolism with another carbon source to activate or sustain *myo*-inositol utilisation. In this context, the experimental conditions, using *myo*-inositol as the only carbon source in a minimal medium, are likely far from the complex nutrient environment encountered in the gut, where a mix of host-derived and dietary sugars is present. It is therefore plausible that W36 and CLA-AA-H189 depend on additional signals or substrates to fully activate the pathway, highlighting the importance of studying bacterial metabolism under conditions that better mimic the physiological milieu.

Finally, we explored the AMR profiles of the strains using CARD and AMRFinderPlus. Several strains, including J115^T, possessed resistance genes to tetracyclines and macrolides, whereas the widespread detection of vancomycin resistance cluster genes raises questions about their functionality. Rather, they may represent horizontally acquired remnants or genomic artefacts, and their phenotypic relevance remains to be determined.[58 59]

Interestingly, *D. welbionis* J115^T was predicted to encode a *tet(W)* antibiotic resistance gene in the genome, a key obstacle to its translational potential in humans. Indeed, raising concerns regarding safety and regulatory bodies may hinder its qualification for direct human use as novel food, 'bug as drug' or probiotic. Given that we isolated two strains without predicted AMR genes, these two strains could be excellent candidates for future next-generation beneficial microbes.

A limitation of our work is that, although we provide detailed biochemical, proteogenomic and comparative genomic evidence for a conserved *myo*-inositol to butyrate pathway in *D. welbionis*, we do not formally demonstrate that this pathway is required for the metabolic or hepatic benefits associated with this species. Proving necessity would require targeted disruption of key inositol utilisation genes or transporters and subsequent testing of such mutants in relevant *in vivo* models. In addition, our previous work has shown that *D. welbionis* can modulate specific bioactive lipids linked to glucose, lipid and energy metabolism, indicating that multiple microbial and host pathways are likely to contribute to its beneficial effects.[35] Future studies combining genetic tools and integrative host-microbe phenotyping will therefore be needed to dissect the relative contribution of *myo*-inositol-derived butyrate versus other mechanisms

In summary, *D. welbionis* is a prevalent gut symbiont that possesses a conserved, non-canonical pathway for converting *myo*-inositol into butyrate. This unique metabolic trait, along with its association with metabolic and liver health and our successful isolation of strains lacking antibiotic resistance genes, underscore its potential as a next-generation probiotic. Further studies are warranted to elucidate the enzymatic basis of this pathway and its interactions with the host.

Author affiliations
[1]Louvain Drug Research Institute (LDRI), Metabolism and Nutrition research group (MNUT), UCLouvain, Université catholique de Louvain, Brussels, Belgium
[2]Walloon Excellence in LifeSciences and BIOtechnology (WELBIO) department, WELResearch Institute, Wavre, Belgium
[3]Departments of Experimental Vascular Medicine, Amsterdam University Medical Centres (AMC), Amsterdam, Netherlands

[4]Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland
[5]Nutrition and obesities : systemic approaches, Nutriomics, Sorbonne Université, Inserm, Paris, France
[6]BIOASTER, Lyon, France
[7]Section of Biomolecular Medicine, Division of Systems Medicine, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK
[8]Department of Biosciences, School of Science & Technology, Nottingham Trent University, Nottingham, UK
[9]IRCCS Neuromed, Pozzilli, Italy
[10]Department of Systems Medicine, Center for Atherosclerosis, University of Rome Tor Vergata, Rome, Italy
[11]Department of Medical Sciences, School of Medicine, University of Girona, Girona, Spain
[12]CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain
[13]Department of Diabetes, Endocrinology and Nutrition, Dr. Josep Trueta University Hospital, Girona, Spain
[14]UMR 1297 INSERM, Team INCOMM, Rangueil Hospital, Toulouse, France
[15]European Genomic Institute for Diabetes, U1283 INSERM, UMR8199 CNRS, Institut Pasteur de Lille, Lille University Hospital, University of Lille, Lille, France
[16]Functional Microbiome Research Group, Institute of Medical Microbiology, University Hospital of RWTH Aachen, Aachen, Germany
[17]Biochemistry, Wageningen University, Wageningen, Netherlands
[18]Proteomics, Metabolomics & Mass Spectrometry Laboratory, ISPAAM, National Research Council, Portici, Italy
[19]Bioanalysis and Pharmacology of Bioactive Lipids Research Group (BPBL), Louvain Drug Research Institute (LDRI), UCLouvain, Université catholique de Louvain, Brussels, Belgium
[20]Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands
[21]Institute of Experimental and Clinical Research (IREC), UCLouvain, Université catholique de Louvain, Brussels, Belgium

Social media Matthias Van Hul, LinkedIn @matthias-van-hul-997a02a; Patrice D Cani, LinkedIn @patricecani

Acknowledgements We thank Bouazza Es Saadi, Marine Oliver, Luc Gesché, Florent Maréchal, Elise Schepens and Rose-Marie Goebbels for their technical help. We thank members of the Functional Microbiome Research Group (University Hospital of RWTH Aachen, Germany), specifically Afrizal Afrizal and Johannes Masson for isolating strain CLA-AAH189 and CLA-JM-H43, respectively, Selina Nüchtern for strain curation and shipment, and Charlie Pauvert for assembling their draft genomes and for reviewing the manuscript. We thank the MetaToul-Lipidomique (I2MC, Inserm, Toulouse, France), MetaboHUB-ANR-11-INBS-0010" for the liver analysis.

Contributors PDC conceptualised the study. TPNB, PDC, CPe and MVH designed the research. CHL, TPNB, CPe, AP, TLR and GCW performed experiments. TPNB conducted 13C myo-inositol analyses for 13C-labelled compounds and NMR experiments. TPNB, SB, ADT and AS performed proteomics and LC-MS/MS analysis. SB, BB, MN and LT generated the antibodies and isolated strains. TC provided essential material and curated data. CHL cultured all the strains and performed growth experiments from novel strains. CHL prepared all the samples for genomic analysis. CHL, CPe, GCW, MVH and PDC contributed to metagenomes analysis. MJ, LH, MF-JM, FR, RB and M-ED contributed to the FLORINASH cohort. CJ performed computational analyses for microbial composition in the human cohort study and drafted the related figure. GGM measured SCFA in culture media. TPNB, CHL, CPe, GCW, PDC, MVH, CJ and WMDV analysed the data. CHL, CPe, TLR, TPNB, GCW, PDC, MVH, SB, BB, MN and LT prepared figures. PDC drafted the first manuscript. All authors commented on and approved the final manuscript. PDC (Cani PD) is the guarantor.

Funding PDC is honorary research director at FRS-FNRS (Fonds de la Recherche Scientifique) and is recipient of grants from FRFS-WELBIO: WELBIO-CR-2022A-02, FNRS T.0030.21, 25 FNRS T.0032.25, FNRS J.0027.22 EOS: program no. 40007505). WMdV and his team were supported by the SIAM Gravitation Grant 024.002.002 of the Netherlands Organization for Scientific Research and the Advanced Research Grant Microbes Inside of the European Research Council. TC received funding from the German Research Foundation (DFG), project no. 445552570, no. 395357507 – SFB1371 and no. 403224013 – SFB1382.

Competing interests PDC is an editor of the journal. WMdV and PDC are inventors on patent applications dealing with the use of specific bacteria and components in the treatment of different diseases. PDC was co-founder of Enterosys. All the other authors declare that they have no competing interests. TL, SB, BbA and MN were employees at Bioaster.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval To evaluate the *D. welbionis* abundance from the human stools of BIOASTER's biobank, the ethical committee authorisation approved the Dossier 21.00672.000002 - ID RCB N° 2021-A01392-39). CENTRE HOSPITALIER LA CHARTREUSE1 BOULEVARD CHANOINE KIR – BP 2331421033 DIJON CEDEX. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. All data relevant to the study are included in the article or uploaded as supplementary information. All elements necessary to allow interpretation and replication of results, including full datasets, are available public, open access repositories and in the Supplementary Information. The source data of the cohorts used in figure 1a,b are available in the BioProject repository under the accession number PRJNA385551 (dataset) from Bian et al.[32] The source data of the cohorts used in figure 1c are available in the BioProject repository under the accession number PRJEB6702 (dataset) from Goodrich et al.[52] and PRJEB6705 (dataset) from Goodrich et al.[52] The source data used in figure 1d are available in the European Bioinformatics Institute (EMBL-EBI) repository, under the accession number PRJEB35994 (dataset) from Jian et al.[34] and PRJEB28350 (dataset) from Caussy et al.[33] Raw metagenomic sequence data used in figure 1e are available in the EMBL-EBI repository under the study accession number ERP133867 (dataset). Proteomics dataset NB27-31 (Proteomic analysis of Dysosmobacter welbionis strains grown on myo-inositol and uridine) has been uploaded to Pride with the accession number PXD064506 (dataset) generated for this study from Lee et al. All the genomes of new D. welbionis isolates in the present manuscript have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession PRJEB90152 (dataset) generated for the present study.

ORCID iDs
Chi-Hsien Lee https://orcid.org/0000-0002-5043-5403
Ching Jian https://orcid.org/0000-0003-0577-8834
Tiphaine Le Roy https://orcid.org/0000-0002-0874-1490
Manyi Jia https://orcid.org/0000-0001-9467-1494
Massimo Federici https://orcid.org/0000-0003-4989-5194
Jose Manuel Fernández-Real https://orcid.org/0000-0002-7442-9323
Remy Burcelin https://orcid.org/0000-0002-7942-8346
Marc-Emmanuel Dumas https://orcid.org/0000-0001-9523-7024
Nathalie M Delzenne https://orcid.org/0000-0002-8271-3893
Thomas Clavel https://orcid.org/0000-0002-7229-5595
Giulio G Muccioli https://orcid.org/0000-0002-1600-9259
Willem M De Vos https://orcid.org/0000-0002-0273-3166
Matthias Van Hul https://orcid.org/0000-0002-5503-107X
Patrice D Cani https://orcid.org/0000-0003-2040-2448

REFERENCES

1 Van Hul M, Cani PD, Petitfils C, *et al*. What defines a healthy gut microbiome? *Gut* 2024;73:1893–908.
2 Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* 2016;14:e1002533.
3 Van Hul M, Neyrinck AM, Everard A, *et al*. Role of the intestinal microbiota in contributing to weight disorders and associated comorbidities. *Clin Microbiol Rev* 2024;37:e0004523.
4 Nobels A, van Marcke C, Jordan BF, *et al*. The gut microbiome and cancer: from tumorigenesis to therapy. *Nat Metab* 2025;7:895–917.
5 Mann ER, Lam YK, Uhlig HH. Short-chain fatty acids: linking diet, the microbiome and immunity. *Nat Rev Immunol* 2024;24:577–95.
6 Blaak EE, Canfora EE, Theis S, *et al*. Short chain fatty acids in human gut and metabolic health. *Benef Microbes* 2020;11:411–55.
7 Huffnagle GB, Noverr M. *GI Microbiota and Regulation of the Immune System*. Springer Science & Business Media, 2008.

8 Amiri P, Hosseini SA, Ghaffari S, *et al*. Role of Butyrate, a Gut Microbiota Derived Metabolite, in Cardiovascular Diseases: A comprehensive narrative review. *Front Pharmacol* 2021;12:837509.
9 Kullberg RFJ, Wikki I, Haak BW, *et al*. Association between butyrate-producing gut bacteria and the risk of infectious disease hospitalisation: results from two observational, population-based microbiome studies. *Lancet Microbe* 2024;5:100864.
10 Le Roy T, Van der Smissen P, Paquot A, *et al*. *Dysosmobacter welbionis* gen. nov., sp. nov., isolated from human faeces and emended description of the genus *Oscillibacter*. *Int J Syst Evol Microbiol* 2020;70:4851–8.
11 Le Roy T, Moens de Hase E, Van Hul M, *et al*. *Dysosmobacter welbionis* is a newly isolated human commensal bacterium preventing diet-induced obesity and metabolic disorders in mice. *Gut* 2022;71:534–43.
12 Moens de Hase E, Neyrinck AM, Rodriguez J, *et al*. Impact of metformin and Dysosmobacter welbionis on diet-induced obesity and diabetes: from clinical observation to preclinical intervention. *Diabetologia* 2024;67:333–45.
13 Li C, Stražar M, Mohamed AMT, *et al*. Gut microbiome and metabolome profiling in Framingham heart study reveals cholesterol-metabolizing bacteria. *Cell* 2024;187:1834–52.
14 Holub BJ, Holub BJ. Metabolism and function of myo-inositol and inositol phospholipids. *Annu Rev Nutr* 1986;6:563–97.
15 Siddiqui N, Singh V, Deshmukh MM, *et al*. Structures, stability and hydrogen bonding in inositol conformers. *Phys Chem Chem Phys* 2015;17:18514–23.
16 Clements RS, Darnell B. Myo-inositol content of common foods: development of a high-myo-inositol diet. *Am J Clin Nutr* 1980;33:1954–67.
17 NR Reddy, SK Sathe, DK Salunkhe. Phytates in legumes and cereals - PubMed. *Adv Food Res* 1982;28.
18 Schlemmer U, Frølich W, Prieto RM, *et al*. Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. *Molecular Nutrition Food Res* 2009;53.
19 P Minthami Sharon, P Mellonie, A Manivannan, *et al*. The Effectiveness of Myo-Inositol in Women With Polycystic Ovary Syndrome: A Prospective Clinical Study. *Cureus* 2024;16.
20 Pintaudi B, Di Vieste G, Bonomo M. The Effectiveness of Myo-Inositol and D-Chiro Inositol Treatment in Type 2 Diabetes. *Int J Endocrinol* 2016;2016:9132052.
21 D'Anna R, Scilipoti A, Giordano D, *et al*. myo-Inositol supplementation and onset of gestational diabetes mellitus in pregnant women with a family history of type 2 diabetes: a prospective, randomized, placebo-controlled study. *Diabetes Care* 2013;36:854–7.
22 Bevilacqua A, Bizzarri M. Inositols in Insulin Signaling and Glucose Metabolism. *Int J Endocrinol* 2018;2018:1968450.
23 Berman T, Magasanik B. The pathway of myo-inositol degradation in Aerobacter aerogenes. Ring scission. *J Biol Chem* 1966;241:807–13.
24 Poole PS, Blyth A, Reid CJ, *et al*. myo-Inositol catabolism and catabolite regulation in Rhizobium leguminosarum bv. viciae. *Microbiology (Reading, Engl)* 1994;140:2787–95.
25 Yoshida K, Yamaguchi M, Morinaga T, *et al*. myo-Inositol Catabolism in Bacillus subtilis. *Journal of Biological Chemistry* 2008;283:10415–24.
26 Yebra MJ, Zúñiga M, Beaufils S, *et al*. Identification of a gene cluster enabling Lactobacillus casei BL23 to utilize myo-inositol. *Appl Environ Microbiol* 2007;73:3850–8.
27 Krings E, Krumbach K, Bathe B, *et al*. Characterization of myo-inositol utilization by Corynebacterium glutamicum: the stimulon, identification of transporters, and influence on L-lysine formation. *J Bacteriol* 2006;188:8054–61.
28 De Vos WM, Nguyen Trung M, Davids M, *et al*. Phytate metabolism is mediated by microbial cross-feeding in the gut microbiota. *Nat Microbiol* 2024;9:1812–27.
29 Bui TPN, Mannerås-Holm L, Puschmann R, *et al*. Conversion of dietary inositol into propionate and acetate by commensal Anaerostipes associates with host health. *Nat Commun* 2021;12:4798.
30 Zeevi D, Korem T, Godneva A, *et al*. Structural variation in the gut microbiome associates with host health. *Nature New Biol* 2019;568:43–8.
31 Liu C, Du M-X, Abuduaini R, *et al*. Enlightening the taxonomy darkness of human gut microbiomes with a cultured biobank. *Microbiome* 2021;9:119.
32 Bian G, Gloor GB, Gong A, *et al*. The Gut Microbiota of Healthy Aged Chinese Is Similar to That of the Healthy Young. *mSphere* 2017;2.
33 Caussy C, Tripathi A, Humphrey G, *et al*. A gut microbiome signature for cirrhosis due to nonalcoholic fatty liver disease. *Nat Commun* 2019;10.
34 Jian C, Luukkonen P, Sädevirta S, *et al*. Impact of short-term overfeeding of saturated or unsaturated fat or sugars on the gut microbiota in relation to liver fat in obese and overweight adults. *Clin Nutr* 2021;40:207–16.
35 Moens de Hase E, Petitfils C, Alhouayek M, *et al*. Dysosmobacter welbionis effects on glucose, lipid, and energy metabolism are associated with specific bioactive lipids. *J Lipid Res* 2023;64:100437.
36 Pant K, Venugopal SK, Lorenzo Pisarello MJ, *et al*. The Role of Gut Microbiome-Derived Short-Chain Fatty Acid Butyrate in Hepatobiliary Diseases. *Am J Pathol* 2023;193:1455–67.
37 Cichos KH, Spitler CA, Quade JH, *et al*. Isothermal Microcalorimetry Improves the Time to Diagnosis of Fracture-related Infection Compared With Conventional Tissue Cultures. *Clin Orthop Relat Res* 2022;480:1463–73.
38 Cichos KH, Ruark RJ, Ghanem ES. Isothermal microcalorimetry improves accuracy and time to bacterial detection of periprosthetic joint infection after total joint arthroplasty. *J Clin Microbiol* 2023;61:e0089323.
39 YAMADA EW, JAKOBY WB. Aldehyde oxidation. V. Direct conversion of malonic semialdehyde to acetyl-coenzyme A. *J Biol Chem* 1960;235:589–94.
40 Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS MicrobiolLett* 2009;294:1–8.
41 Bellais S, Nehlich M, Ania M, *et al*. Species-targeted sorting and cultivation of commensal bacteria from the gut microbiome using flow cytometry under anaerobic conditions. *Microbiome* 2022;10:24.
42 Hitch TCA, Masson JM, Pauvert C, *et al*. HiBC: a publicly available collection of bacterial strains isolated from the human gut. *Nat Commun* 2025;16:4203.
43 Jain C, Rodriguez-R LM, Phillippy AM, *et al*. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 2018;9:5114.
44 Flessa C-M, Nasiri-Ansari N, Kyrou I, *et al*. Genetic and Diet-Induced Animal Models for Non-Alcoholic Fatty Liver Disease (NAFLD) Research. *Int J Mol Sci* 2022;23:15791.
45 Afonso MB, Rodrigues PM, Mateus-Pinheiro M, *et al*. RIPK3 acts as a lipid metabolism regulator contributing to inflammation and carcinogenesis in non-alcoholic fatty liver disease. *Gut* 2021;70:2359–72.
46 Miura A, Ikeda S, Kono Y, *et al*. Metabolic and Epigenetic Abnormalities Cause Hepatic Fibrogenesis in Metabolic Dysfunction-Associated Steatohepatitis Model Mice. *J Biol Chem* 2025;2025:110959.
47 Altmann R, Hausmann M, Spöttl T, *et al*. 13-Oxo-ODE is an endogenous ligand for PPARgamma in human colonic epithelial cells. *Biochem Pharmacol* 2007;74:612–22.
48 Pujo J, Petitfils C, Le Faouder P, *et al*. Bacteria-derived long chain fatty acid exhibits anti-inflammatory properties in colitis. *Gut* 2021;70:1088–97.
49 Wong GC, Delzenne NM, Van Hul M, *et al*. Dysosmobacter welbionis J115T reduces stress-like phenotype in high-fat diet-induced obese female mice. *Benef Microbes* 2025;2025:1–12.
50 Paquette AF, Carbone BE, Vogel S, *et al*. The human milk component *myo*-inositol promotes neuronal connectivity. *Proc Natl Acad Sci U S A* 2023;120:e2221413120.
51 Byrne ME, O'Mahony JA, O'Callaghan TF. Compositional and Functional Considerations for Bovine-, Caprine- and Plant-Based Infant Formulas. *Dairy* 2021;2:695–715.
52 Goodrich JK, Waters JL, Poole AC, *et al*. Human genetics shape the gut microbiome. *Cell* 2014;159:789–99.
53 Grieneisen L, Dasari M, Gould TJ, *et al*. Gut microbiome heritability is nearly universal but environmentally contingent. *Science* 2021;373:181–6.
54 Weston E, Pangilinan F, Eaton S, *et al*. Investigating Genetic Determinants of Plasma Inositol Status in Adult Humans. *J Nutr* 2022;152:2333–42.
55 Hoyles L, Fernández-Real J-M, Federici M, *et al*. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat Med* 2018;24:1070–80.
56 Depommier C, Everard A, Druart C, *et al*. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat Med* 2019;25:1096–103.
57 Deng Z, Mei S, Ouyang Z, *et al*. Dysregulation of gut microbiota stimulates NETs-driven HCC intrahepatic metastasis: therapeutic implications of healthy faecal microbiota transplantation. *Gut Microbes* 2025;17:2476561.
58 Delcour AH. Outer membrane permeability and antibiotic resistance. *Biochim Biophys Acta* 2009;1794:808–16.
59 Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. *Microbiol Spectr* 2016;4.