Title

Propionic acid enhances the virulence of Crohn's disease-associated adherent-invasive *Escherichia coli*

Authors

Michael J. Ormsby[1], Síle A. Johnson[1], Lynsey M. Meikle[1], Robert J. Goldstone[2], Anne McIntosh[1], Hannah M. Wessel[1], Heather E. Hulme[1], Ceilidh C. McConnachie[1], James P. R. Connolly[1], Andrew J. Roe[1], Eamonn Fitzgerald, Konstantinos Gerasimidis[3], Douglas Morrison[4], David G.E. Smith[2] and Daniel M. Wall[1*]

Affiliations

[1]*Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, Sir Graeme Davies Building, University of Glasgow, Glasgow G12 8TA, United Kingdom*
[2]*Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot-Watt University, Edinburgh, EH14 4AS, United Kingdom*
[3]*Human Nutrition, School of Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, Glasgow Royal Infirmary, Glasgow G31 2ER, United Kingdom.*
[4]*Scottish Universities Environmental Research Centre, University of Glasgow, Glasgow G75 0QF, United Kingdom.*

**Corresponding author email: Donal.Wall@glasgow.ac.uk*

**Corresponding author address:*
Dr. Daniel (Dónal) M. Wall
Institute of Infection, Immunity and Inflammation
College of Medical, Veterinary and Life Sciences
Sir Graeme Davies Building
University of Glasgow
120 University Place
Glasgow G12 8TA

Abstract

Short chain fatty acids (SCFA), such as propionic acid (PA), are natural human intestinal antimicrobials and immune modulators that are also used in Western food production and agriculture. Here we examine the effect of PA on the pathogenicity of the Crohn's disease-associated microbe, adherent-invasive *Escherichia coli* (AIEC). We show that AIEC is insensitive to the antimicrobial effects of PA and adapts to utilize it for efficient growth. Repeated exposure of AIEC to PA significantly increases AIEC adherence to human intestinal epithelial cells, acid tolerance and biofilm formation. RNA-sequencing identified transcriptional changes in response to PA with upregulation of genes involved in biofilm formation, stress responses, metabolism, membrane integrity and use of alternative carbon sources. Finally, after pre-exposure to PA, AIEC demonstrated an increased ability to colonize (>5-fold) and persist (>50-fold) in an *in vivo* model where the low murine intestinal PA concentrations were increased to mimic those found in humans. Our data indicates that exposure of AIEC to PA evolves bacteria that are both resistant to this natural human intestinal antimicrobial, and increasingly virulent in its presence.

Importance

Propionic acid (PA) is a common agricultural and industrial antimicrobial in the Western world. Its use is increasing as efforts intensify to reduce reliance on antibiotics, particularly in food production. Here we show that exposure of adherent-invasive *Escherichia coli* (AIEC) to PA induced significant virulence associated phenotypic changes. AIEC exhibited greater adherence and biofilm formation *in vitro*, translating into greater colonization and persistence in an *in vivo* mouse model. RNAseq analysis identified transcriptional changes directly related to these phenotypic changes. While PA is generally regarded as safe for both human and animal use, the significance of SCFAs such as PA to maintaining human intestinal immune health means their widespread use requires further scrutiny. This will allow us to understand if, in a similar manner to antibiotics, SCFAs are facilitating horizontal transmission of microorganisms.

Introduction

The effect of increased preservative use on bacteria, particularly in agriculture and food producing animals (FPAs), has remained largely unexplored. While there is increasing evidence for antibiotic driven enhanced genome wide mutation rates [1] and horizontal transmission of bacteria from FPAs to humans, the role of alternative antimicrobials in driving a similar form of horizontal transmission of microbes has not been addressed [2–4].

One such group of alternative antimicrobials are short chain fatty acids (SCFAs). SCFAs are naturally produced within the gut as inflammatory mediators as the microbiota facilitate the breakdown of undigested carbohydrates and starches into small carbon molecules with acetic (AA), butyric (BA) and propionic acids (PA) accounting for approximately 90% of these. SCFAs are also easy to manufacture on an industrial scale and are now commonly used in agriculture, as in addition to their anti-inflammatory effects, are antimicrobial. SCFAs have exhibited particular successes in reducing pathogen numbers in poultry, with PA particularly effective in reducing *Salmonella* and *Campylobacter* carriage [5–8]. PA is also an effective antimicrobial agent used in Western food production and agriculture to reduce the need for antibiotic use amidst growing antibiotic resistance concerns [9–11]. Preservation of animal feed, grain, and food accounted for almost 80% of PA consumption across the world in 2016 with Western Europe (40% of total use), North America (30%) and Asia (23%), the main consumers (17). However, the success of SCFAs in reducing antibiotic dependence is now seeing their use spread to countries across Africa, the Middle East and Central and South America [12].

PA has attracted recent interest, as PA supplementation in murine models has been shown to reduce the severity of Crohn's disease (CD) and the associated colitis [13,14]. CD is a debilitating and incurable inflammatory disease of a multi-factorial nature that occurs in genetically susceptible individuals. The mechanisms underlying the disease are not fully understood however it is thought that defects in the immune response to the gut microbiota are a contributing factor [15–18]. Sudden changes in diet have been shown to result in rapid changes in the gut microbiota [19–21], while the inflammation associated with CD results in markedly decreased microbial diversity [22–24]. Levels of

Enterobacteriaceae in particular are higher in intestinal samples from CD patients when compared to healthy controls [25–27]. One group of *Enterobacteriaceae* that is of particular interest is the *Escherichia coli* pathotype, adherent-invasive *E. coli* (AIEC). These bacteria are overrepresented in the intestinal microbiota in the inflamed gut of CD patients being present in 51.9% of mucosal samples from CD patients compared with 16.7% in healthy controls [28]. Key features of the AIEC pathotype that distinguish them from non-invasive commensal strains include; adherence to and invasion of the intestinal epithelium, an increased ability to form biofilms, and the ability to survive and replicate within macrophages without inducing cell death [28]. While AIEC strains harbour genetic similarity, in terms of phylogenetic origin and virulence genotype, with extra-intestinal pathogenic *E. coli* (ExPEC), they have few known virulence factors [29]. This apparent lack of virulence factors, and the discovery of AIEC strains across all five major diverse phylogroups of *E. coli* mean that an overarching explanation for the origin and virulence of AIEC has remained out of reach. This is of significant interest given that PA is a natural human intestinal antimicrobial; and AIEC is both phylogenetically and genomically linked to avian pathogenic *E. coli* (APEC) species [28,30–34] which doubtless come into contact with SCFAs that are used extensively in poultry production [9,11]. Additionally, there is mounting evidence of horizontal transmission of *E. coli* with poultry considered a potential vehicle or reservoir for other human disease causing *Escherichia coli*, such as extraintestinal pathogenic *E. coli* (ExPEC) [3,35–37].

In this study, we show that exposure of AIEC to PA results in an ability to utilize PA as a carbon source whilst simultaneously enhancing the virulent adherent and invasive phenotype of AIEC. The basis of these phenotypic changes was determined by RNA sequencing and the phenotype was determined to be reversible by removal of PA, suggesting an epigenetic basis for the changes. Exposure to PA also increases the colonization and long-term persistence of LF82 within a humanized murine model where PA levels have been increased from the low levels of the mouse intestine to those closer to that seen in the human intestine. This is the first time that a direct link has been made between bacteria over-represented in CD and a common SCFA preservative. Our work indicates that further research is needed to

115 understand the consequence of the use of SCFA antimicrobials especially when, as in the case of PA, they
116 play such important roles in maintaining human intestinal health.

117 ## Results

118 ### *Short chain fatty acids as antimicrobials*

119 To examine the impact of PA on the phenotype of AIEC, LF82 was grown in minimal media
120 supplemented with PA as the sole carbon source (20 mM). PA concentration increases across the human
121 intestine from 1.5 mM in the ileum to 27 mM in the colon, with the greatest inhibitory effect on bacterial
122 colonization seen where PA is highest [5,38]. Therefore 20 mM was chosen as a physiologically relevant
123 concentration to grow LF82, as AIEC are known to colonize the ileal/colonic junction [25]. While LF82
124 was able to grow in PA, the human commensal *E. coli* strain, F-18 (38), replicated extremely poorly.
125 Following successive sub-culturing over five growth cycles in PA supplemented minimal media, a 'PA-
126 adapted' strain of LF82 was generated termed LF82-PA. LF82-PA had a significantly reduced doubling
127 time (3.98 h) in PA when compared to wild type LF82 (25.59 h; Fig. 1a). We were unable to generate a
128 corresponding PA-adapted version of F-18 due to its reduced ability to metabolize and grow in PA. The
129 increased rate of growth was specific to PA and did not impact growth of LF82-PA in nutrient rich
130 Lysogeny broth (LB; Fig. 1b).

131 ### *Propionic acid exacerbates the adherent and invasive and biofilm forming phenotypes of AIEC*

132 AIEC adherence to, and invasion of, the intestinal epithelium and their increased ability to form
133 biofilms are key features of this pathotype that distinguish them from non-invasive commensal strains
134 [28]. To better understand any effects of increased growth on PA on these attributes in LF82-PA a
135 number of virulence assays were performed. LF82-PA showed a significant increase in adherence to
136 Caco-2 human intestinal epithelial cells when compared to wild type LF82 (>7.45 fold; p-value = 0.0371;
137 Fig. 1c), whilst a trend towards increased invasion by LF82-PA was also observed but was not significant
138 (>5.26 fold; p-value = 0.3297; Fig. 1d). Biofilm formation by LF82-PA was also significantly enhanced
139 under anaerobic conditions (>1.72 fold; p-value = 0.00019; Fig. 1e). Finally, LF82-PA was more acid

tolerant than LF82 despite adaptation to PA being carried out at pH 7.4 (Fig. 1f). A reduction in cell number was seen for both strains in response to acidic conditions (pH 3) however the LF82-PA strain survived in greater numbers and for longer periods of time (At 20 min, LF82-PA recovered >30.4 fold higher than LF82 [p-value = 0.0286]; at 40 min, LF82-PA was >22.7 fold higher [p-value = 0.028].

We hypothesized that this increased virulence was likely to be mediated by changes in the composition of the bacterial membrane following growth on PA. Direct incorporation of PA into the membrane is a mechanism employed by bacteria to minimize the toxic effects of excess PA in the environment [40–42]. Gas chromatography coupled to isotope ratio mass spectrometry (GC-IRMS) using ^{13}C labelled PA (1-^{13}C sodium propionate) revealed that PA was not incorporated into odd chain long chain fatty acids (LCFAs). However, there appeared to be significant ^{13}C enrichment in 12 fatty acid methyl esters (FAMEs) that did not correspond to any of 37 FAMEs in our reference standard (see *Supplementary experimental procedures*). However we speculated that the proximity of these labelled peaks to LCFAs was due to incorporation of PA into methylated or branched chain fatty acids (BCFAs) as described previously during *Mycobacterium tuberculosis* growth on and detoxification of PA [40].

A transcriptional basis for enhanced LF82-PA virulence

Genome sequencing of three independently adapted biological replicates of LF82-PA revealed a number of single nucleotide polymorphisms (SNPs; Supplementary File S1). However, no SNPs were conserved across all strains. Detailed analysis of the genes and pathways in which the SNPs were identified did not lead to the identification of any candidate pathways that may explain the changes in virulence observed. However, we cannot exclude the possibility that different combinations of small genomic changes could result in the same outcome at the transcriptional level.

Therefore, given no mutational basis for the observed increase in virulence was detected, we employed a comparative RNA-sequencing (RNA-seq) approach to probe the global transcriptional profiles of LF82 and LF82-PA grown on PA. RNA-sequencing revealed 25 differentially expressed genes (DEGs; P-value $\leq$0.05) between LF82 and LF82-PA (Fig. 2; Supplementary Table S2); 24 were

upregulated in the LF82-PA strain and one (*mcbR;* -20.85 fold) was downregulated. Of the 25 DEGs identified by RNA-seq, 21 including *mcbR*, were validated as significantly altered by PA using qRT-PCR (Supplementary Fig. S1). Functional grouping of these 21 DEGs revealed their roles in diverse processes including biofilm formation, stress responses, metabolism, membrane integrity and transport of alternative carbon sources (Fig. 2c; Supplementary Table S2). Eight DEGs have well described roles in biofilm formation further adding to our *in vitro* findings indicating that PA was a driver of adhesion and biofilm formation (Fig. 1c & e). Upregulation of another DEG, a regulator of membrane fatty acid composition *yibT*, adds further evidence for potential detoxification of PA through membrane incorporation [40,41]. This RNA-seq analysis indicates that prior exposure of LF82 to PA can result in significant changes in transcription leading to an altered virulence phenotype.

The enhanced LF82-PA phenotype is reversible

Recently, a long-term epigenetic memory switch with a role in controlling bacterial virulence bimodality was identified in enteropathogenic *E. coli* (EPEC) [43]. This 'resettable phenotypic switch' results in populations of virulent and hypervirulent genetically identical subpopulations that are retained through generations. As the virulent phenotype persists over a number of generations, and was not explained by genetic analysis, we hypothesised that the phenotype we see may be as a result of a similar epigenetic memory switch in LF82-PA. In order to examine this hypothesis, the LF82-PA strain was repeatedly passaged through rich (LB) media with no PA selective pressure. After five successive subcultures, the strain (LF82-PA-LB) no longer exhibited an increased growth rate in PA as LF82-PA did; and its virulence phenotype with regards adherence and invasion reverted to that of the wild type LF82 strain (Fig. 3). This indicates that the changes induced by PA were epigenetic and not due to SNPs or mutations.

PA supplementation in a murine model promotes colonization and long-term persistence of AIEC

Knowledge of any role for PA in bacterial infection in the intestine is lacking as murine models are limited by distinct differences in basal levels of SCFAs between the murine and human gut

(Supplementary Fig. S2a) [38]. To address this in our *in vivo* infection model, levels of PA in the murine intestine were increased by supplementing drinking water with 20 mM PA for three days prior to infection and for the duration of the infection. Caecal SCFA levels post-PA supplementation indicated that PA levels had increased while no significant changes were seen in either AA or BA levels (Supplementary Fig. S2b; AA:PA:BA ratios without PA 84.6:7.3:8.1; with PA 78.9:12.6:8.5). PA supplementation led to significantly greater LF82-PA colonization of both the ileum (Fig. 4a, >4.96 fold; p-value = 0.044) and colon (Fig. 4b, >5.57 fold; p-value = 0.028) compared to LF82 (Fig. 4). This effect was PA specific, with no significant difference in colonization observed at either site in the absence of PA supplementation (Fig. 4e, ileum: >1.99 fold; p-value = 0.550; Fig. 4f, colon: >2.36 fold; p-value = 0.282). No changes in colonization efficiency for either strain were noted in the caecum under either condition (With PA: >0.29 fold; p-value = 0.277; Without PA: >0.14 fold; p-value = 0.052; Supplementary Fig. S3).

For analysis of long-term persistence of both LF82 and LF82-PA, mice were again provided PA supplemented (20 mM) water for three days prior to infection, and for the 21-day duration of the infection. LF82-PA was again found to persist in significantly greater numbers than LF82 in both the ileum (Fig. 4c, >54.02 fold; p-value = 0.034) and colon (Fig. 4d, >73.44 fold; p-value = 0.013). Again this increased persistence was PA dependent, as LF82-PA did not colonize either site to a greater extent in the absence of PA (Fig. 4g, ileum: >0.16 fold; p-value = 0.237; Fig. 4h, colon: >0.81 fold; p-value = 0.719). No significant difference in the persistence of either strain was observed in the caecum, irrespective of the presence of PA (Supplementary Fig. S3, >13.05 fold increase; p-value = 0.376).

Discussion

Dietary additives, and a mock Western diet, have been demonstrated to contribute to increased colonization of AIEC in murine models [20,21]. Additionally, recent evidence has implicated the food additive trehalose as a contributory factor in the emergence and hypervirulence of two epidemic lineages of *Clostridium difficile* [44]. Here we add to these findings demonstrating that a dietary and common-use

antimicrobial can significantly increase the virulence of AIEC. The use of PA as an antimicrobial stems from its inhibitory effects on bacterial colonization of the host, being both toxic and acting as a signal for virulence gene repression [5–7]. Given its potent antimicrobial effect, AIEC adapting to and growing on PA was unexpected (Fig. 1a). PA is of limited nutritional value to most prokaryotes with sustained growth leading to the production of toxic by-products [45]. However, a small number of bacteria are capable of growing on PA and it is recognized as being a major virulence determinant for *Mycobacteria* spp. which can degrade [46] or incorporate PA into membrane surface lipids [40–42]. Here, we saw similar changes in lipid content post-growth on PA with appearance of novel, and what we speculated to be branch chain, fatty acid methyl esters (FAMEs). Adaptation to PA also manifested in increases in; adherence to a human colonic intestinal epithelial cell line, biofilm formation and colonization and persistence in the murine intestine. Importantly, after exposure to PA LF82-PA exhibited increased resistance to low pH meaning these bacteria are likely to be increasingly resistant to stomach acids and therefore potentially better able to reach and colonize the human intestine.

Mucosa-associated AIEC have been predominantly recovered from both the ileum [30,47,48] and the colon [25,48–50] of CD patients. In the present study, both LF82 and LF82-PA colonized the ileum, caecum and colon of male C57BL/6 mice but with no significant difference in colonization levels between LF82 and LF82-PA in a standard mouse model. However, given PA levels are significantly lower in the gastrointestinal tract of mice compared to humans (31; Fig. S2a), we employed PA supplementation to raise PA levels in our *in vivo* mouse model closer to those observed in humans. These mice were significantly better colonized by LF82-PA in both the ileum and colon (Fig. 4). This suggests that the prior exposure to PA renders LF82-PA better equipped to colonize an intestine, such as that in humans, with higher PA levels than those in a normal mouse. The increased intestinal PA also resulted in greater persistence of LF82-PA in both the ileum and the colon. Again this persistence was LF82-PA specific with the LF82 strain exhibiting a trend towards reduced persistence in the presence of increased PA, as previously described for *Campylobacter* and *Salmonella* [5–8].

Our findings raise a number of important issues. Firstly, present mouse models, given their distinct differences in SCFA levels to those of humans, are likely even poorer models for human intestinal disease than is already known [51]. Secondly, PA reduces colonization and persistence of a wild type AIEC strain as expected, but repeated exposure to PA creates a readily adapted strain of AIEC (LF82-PA) that easily colonizes and persists in the mouse where higher PA levels are representative of the human gut. Given the wide use of PA environmentally and agriculturally it is not inconceivable that bacteria such as AIEC come into contact with such concentrations of PA with concentrations in animal water, feed and silage reported to be 20 mM and higher [5,7,8,52]. Such exposure would likely make the PA concentrations in the human intestine, which increase from 1.5 mM in the ileum to 27 mM in the colon, easily tolerable to a strain such as the one we indicated here can be adapted [5,38].

To elucidate a mechanism behind the increased LF82-PA virulence, genomic and transcriptomic approaches were undertaken. While no reproducible differences were observed between LF82 and three independent replicates of LF82-PA at a sequence level, it cannot be ruled out that diverse combinations of SNPs may converge on the same phenotype. RNA-sequencing did, however, identify significant transcriptional differences between the strains, with 21 DEGs identified and validated (Fig. 2 and supplementary Fig. S1). These included DEGs with roles in biofilm formation, stress responses and metabolism that explained the mechanisms behind LF82-PA enhanced virulence. Biofilm gene regulation was most dramatically affected with increased expression for a number of known factors involved while the biofilm repressor, *mcbR*, was decreased greater than 20-fold [53]. Stress and metabolism genes were also significantly altered with the glyoxylate shunt repressor activated and increased shuttling of 3-carbon PA through the 2-methylcitrate cycle, as previously reported [54]. A number of DEGs of unknown function were also identified, requiring further characterization. Interestingly, these results indicate that *E. coli* behaves in stark contrast to *Salmonella* during intestinal infection. While it is reported that *Salmonella* actively colonize the small intestine [55,56], where PA concentrations are relatively low [38], the high PA concentration of the caecum and colon have been shown to represses *Salmonella* pathogenicity island 1 (SPI1) which is essential for colonization [5,57,58]. In contrast to these findings,

we show that AIEC are not only able to tolerate these higher PA concentrations, but actively use PA as a stimulus for colonization with genes for such highly upregulated in the presence of PA. However, the LF82-PA phenotype is reversible (Fig. 3), which ultimately suggests that the adaptation to PA is most likely the result of an epigenetic trait in a similar manner to the epigenetic memory switch seen in EPEC [43]. This ability to rapidly adapt to the surrounding environment makes AIEC more likely to cross from the environment, poultry or FPAs to humans, colonize and persist in the human intestine.

In conclusion, the data presented in this study demonstrate that increased exposure to PA results in a more virulent AIEC strain that is readily adapted to utilize abundant SCFAs such as in the human gastrointestinal tract. This exposure of AIEC to PA confers no competitive advantage on the bacteria in a mouse model, unless PA levels are raised in the murine intestine to bring them closer to human intestinal PA levels. With the AIEC pathotype closely related to APEC and other ExPEC strains, there are suggestions that AIEC and closely related ExPEC may be environmentally- or avian-derived [28,30–34]. Given the now widespread use of PA as an antimicrobial and the increasing evidence that *E. coli* are crossing host species boundaries with antimicrobial use a major driver, any role of alternative antimicrobials in facilitating AIEC spread must be examined [2–4]. The potential for AIEC crossing from the environment, poultry or FPAs to humans is a greater risk in our opinion as PA is not only an antimicrobial but also a crucial antibacterial component of the human intestine as well as playing critical roles in the immune response to infection. The significance of SCFAs to human intestinal immune health therefore warrants increased scrutiny of their industrial and agricultural use as any effects of their widespread use on bacterial dissemination need to be better understood.

Materials and Methods

Bacterial strains and growth conditions. Pathogenic AIEC strain LF82 and intestinal commensal *E. coli* strain F-18 were used in this study and were cultivated on Lysogeny broth or agar. M9 minimal medium supplemented with 20 mM PA (M9-PA [20% M9 salts (32g $Na_2H_2PO_42H_2O$ (Merck), 12.5 g NaCl (Merck), 2.5 g NH_4Cl (Fisher scientific), 7.5g KH_2PO_4 (Merck) and 400 ml H_2O], 0.1% Trace metal

291 solution, 0.2 mM $MgSO_4$ [VWR chemicals], 0.02 mM $CaCl_2$ [Fisher scientific], 1 mM Thiamine, 0.01 %
292 5 g/L $FeCl_3$, 0.01 % 6.5 g/L EDTA, 0.1% taurocholic acid, 20 mM Sodium propionate and dH_2O) was
293 used for growth. Strains were grown in 100 ml of M9-PA at 37°C at 180 rpm, unless stated. Bacterial
294 growth was measured at optical density 600nm (OD_{600nm}). To obtain adapted cells, upon reaching
295 stationary phase, cultures were back-diluted into fresh M9-PA. Strains for infection were back-diluted
296 after overnight growth into 10 ml cultures of RPMI-1640 (Sigma) supplemented with 3% foetal calf
297 serum (FCS) and L-glutamine. These were then grown at 37°C in a shaking incubator at 180 rpm to an
298 OD_{600nm} of 0.6 before further dilution to give a final multiplicity of infection (MOI) of 100. Real-time
299 PCR was conducted using bacteria grown in No-Carbon-E (NCE) media [59]. Twenty millimolar sodium
300 propionate (Sigma), 1,2-propanediol (Fisher Scientific) or D-glucose (Sigma) were added with 200 nM
301 cyano-cobalamin (Sigma) to act as an electron acceptor [60]. Cultures were grown overnight in LB,
302 washed three times in NCE media with no carbon source added, and inoculated 1:100 into 10 ml NCE
303 media containing each respective carbon source. Cultures were grown until mid-log phase (OD_{600} of 0.6)
304 and used for RNA-extraction.

305 ***Cell Culture and Maintenance.*** The Caco-2 human intestinal epithelial cell (IEC) line obtained from the
306 American Type Culture Collection (ATCC) was maintained in Dulbecco's Modified Eagle Medium
307 (DMEM) medium (Sigma) supplemented with 10% FCS, L-glutamine and penicillin/streptomycin
308 (Sigma). Cells were maintained at 37°C and 5% CO_2 with regular media changes.

309 ***Biofilm assays.*** Crystal violet static biofilm assays were performed as described previously [61].
310 Anaerobic culture conditions were achieved using a microaerophilic cabinet. PA was added to a final
311 concentration of 20 mM.

312 ***Adherence and invasion assays.*** Caco-2 IECs were washed once before infection and bacterial
313 suspensions were added at an MOI of 100. The infection was allowed to proceed for 2 h at 37°C in 5%
314 CO_2 atmosphere. Non-adhered bacteria were washed away and the infected cells were lysed with 1%

315 Triton X-100 for 5 min. Bacteria were serially diluted in Luria Bertani (LB) broth and spread onto LB
316 agar plates. Total bacteria were enumerated by counting colony forming units (CFUs) after overnight
317 incubation at 37ºC. To determine bacterial invasion, cells were infected for 6 h, extracellular bacteria
318 were then washed away and 50 µg/ml gentamycin sulphate was added for 1 h to kill any remaining cell-
319 associated bacteria before triton X-100 treatment.

320 ***Acid survival assays.*** Cultures of bacteria were grown overnight at 37°C in LB. The pH of these cultures
321 was lowered to pH 3 using 1 M HCl. Samples were taken every 20 min for 1 h and serially diluted in LB.
322 Dilutions were plated in triplicate onto LB agar and incubated overnight at 37°C. Colonies were counted
323 to determine the number of surviving cells.

324 ***Total RNA extraction and mRNA enrichment.*** Bacterial cultures were grown as above and mixed with
325 two volumes of RNAprotect reagent (Qiagen, Valencia, CA, USA), before incubating for 5 min at room
326 temperature. Total RNA was extracted, genomic DNA removed and samples enriched for mRNA as
327 described previously by Connolly *et al.* (2016). Samples for RNA-sequencing (RNA-seq) analysis were
328 QC tested for integrity and rRNA depletion using an Agilent Bioanalyzer 2100 (University of Glasgow,
329 Polyomics Facility).

330 ***Genomic analysis and SNP identification.*** A bacterial lawn generated from single overnight colonies of
331 LF82 and three independent cultures of LF82-PA were resuspended in a microbank bead tube, inverted
332 four times and incubated at room temperature for 2 min. The cryopreservative was removed and the
333 samples sent to MicrobesNG (Birmingham University, UK) for sequencing. Genomic DNA was extracted
334 using a Illumina Nextera XT DNA sample kit as per manufacturer's protocol (Illumina, San Diego, USA).
335 Samples were sequenced on the Illumina MiSeq using a 2x250 paired-end protocol, De novo assembled
336 using SPAdes version 3.5, aligned to the reference genome using BWA-MEM 0.7.5. Variants were called
337 using samtools 1.2 and VarScan 2.3.9 and annotated using snpEFF 4.2. Subsequent genomic analysis was
338 performed using a combination of MAUVE, CLC genomics (Version 7.0.1), ExPASY and EMBOSS

Needle. The sequence reads in this paper have been deposited in the European Nucleotide Archive under study (upload in progress).

RNA-seq transcriptome generation and data analysis. cDNA synthesis and sequencing was performed at the University of Glasgow Polyomics Facility, essentially as described by Connolly *et al.* (2016). Briefly, sequencing was preformed using an Illumina NextSeq 500 platform obtaining 75 bp single end reads. Samples were prepared and sequenced in triplicate. Raw reads were QC checked using FastQC (Babraham Bioinformatics, Cambridge, UK) and trimmed accordingly using CLC Genomics Workbench (CLC Bio, Aarhus, Denmark). Trimmed reads were mapped to the LF82 reference genome (NCBI accession number: CU651637) allowing for 3 mismatches per read. Analysis of differential expression was performed using the Empirical analysis of DGE tool, which implements the EdgeR Bioconductor tool [63]. Differentially expressed genes were identified by absolute fold change (cutoffs $\log_2$) and a P-value of ≤ 0.05. Volcano plots were generated in CLC Genomics Workbench. The sequence reads in this paper have been deposited in the European Nucleotide Archive under study (upload in progress).

Quantitative real-Time PCR (qRT-PCR). cDNA was generated from total RNA using an Affinity Script cDNA Synthesis Kit (Agilent) following the manufacturer's instructions. Levels of transcription were analyzed by qRT-PCR using PerfeCTa® SYBR® Green FastMix® (Quanta Biosciences). Individual reactions were performed in triplicate within each of three biological replicates. The 16S rRNA and *rpoS* genes were used to normalize the results. RT-PCR reactions were carried out using the ECO Real-Time PCR System (Illumina, San Diego, CA, USA) according to the manufacturer's specifications and the data were analyzed according to the $2^{-\Delta\Delta CT}$ method [64]. All primers used are listed in Supplementary Table S1.

Construction of p16S*lux*. LF82 and LF82-PA *lux* integrated strains containing the erythromycin cassette were generated using the protocol of Riedel *et al.* [65]. The bioluminescent properties of these strains allowed visualization of the establishment of infection but despite bacteria being recovered it was

noted that bioluminescent signal was lost. However, upon plating the murine microbiome onto LB agar containing ampicillin (100 µg/ml), it was observed that several members of the microbiota also harboured ampicillin resistance. LB supplemented with erythromycin (500 µg/ml) did not support the growth of any microbiota species; therefore utilizing the erythromycin cassette inserted as part of the *lux* integration allowed for the selection of LF82 and LF82-PA, and was used in subsequent animal experiments.

Animal experiments. All animal procedures were approved by an internal University of Glasgow ethics committee and were carried out in accordance with the relevant guidelines and regulations as outlined by the U.K. Home Office (PPL 70/8584). Male C57BL/6 mice aged between eight and ten weeks were obtained from The Jackson Laboratory (Envigo). Twenty millimolar sodium propionate was administered to C57BL/6 mice in drinking water three days prior to infection. Control mice were given only sterile water. Twenty-four hours prior to infection, mice were treated with an oral dose of 20 mg streptomycin before oral infection with 0.1 ml PBS (mock-infected) or with approx. 1 x 10^9 colony forming units (CFU) of LF82 (*lux*) or LF82-PA (*lux*). Mice were euthanized 3 days after infection for colonization experiments and 21 days after infection for persistence experiments, with caecal contents collected for SCFA analysis. Ileal, caecal and colonic tissue were weighed and homogenized for enumeration of bacterial numbers. Bacterial numbers were determined by plating tenfold serial dilutions onto LB agar containing the appropriate antibiotics. After 24 h of incubation at 37 °C, colonies were counted and expressed as CFU per gram of tissue.

SCFA analysis by gas chromatography. Faecal contents of murine caeca were isolated from PBS treated mice three dpi. The concentrations of acetate, propionate and butyrate per gram of dry weight were measured by gas chromatography as previously described [66] and expressed as a ratio for comparison to the known human acetate:propionate:butyrate SCFA ratio [38]. The effect of PA supplementation in drinking water on PA levels was measured by extraction of caecal contents from PA treated and untreated mice and the levels of SCFAs again calculated per gram of dry weight.

Statistical analysis. Values are represented as means and standard deviation. All statistical tests were performed with GraphPad Prism software, version 7.0c. All replicates in this study were biological; that is, repeat experiments were performed with freshly grown bacterial cultures, immortalized cells and additional mice, as appropriate. Technical replicates of individual biological replicates were also conducted, and averaged. Significance was determined using t-tests (multiple and individual as indicated in the figure legends) and one-way ANOVA corrected for multiple comparisons with a Tukey post hoc test (as indicated in the figure legends). RT-PCR data was log-transformed before statistical analysis. Values were considered statistically significant when P values were $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

References and Notes

1. Long H, Miller SF, Strauss C, Zhao C, Cheng L, Ye Z, et al. Antibiotic treatment enhances the genome-wide mutation rate of target cells. Proc Natl Acad Sci. 2016; E2498–E2505. doi:10.1073/pnas.1601208113

2. Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, et al. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from pork farms and visiting veterinary students. PLoS One. 2013;8: 1–10. doi:10.1371/journal.pone.0053738

3. Lazarus B, Paterson DL, Mollinger JL, Rogers BA. Do human extraintestinal *Escherichia coli* infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. Clin Infect Dis. 2015;60: 439–452. doi:10.1093/cid/ciu785

4. Norizuki C, Wachino J-I, Suzuki M, Kawamura K, Nagano N, Kimura K, et al. Specific blaCTX-M-8/IncI1 plasmid transfer among genetically diverse *Escherichia coli* isolates between humans and chickens. Antimicrob Agents Chemother. 2017;61: 1–7. doi:10.1128/AAC.00663-17

5. Hung CC, Garner CD, Slauch JM, Dwyer ZW, Lawhon SD, Frye JG, et al. The intestinal fatty acid propionate inhibits *Salmonella* invasion through the post-translational control of *HilD*. Mol Microbiol. 2013;87: 1045–1060. doi:10.1111/mmi.12149

6. Iba AM, Berchieri A. Studies on the use of a formic acid-propionic acid mixture (Bio-add) to

control experimental *Salmonella* infection in broiler chickens. Avian Pathol. 1995;24: 303–311. doi:10.1080/03079459508419071

7. Hinton M, Linton AH. Control of *Salmonella* infections in broiler chickens by the acid treatment of their feed. Vet Rec. 1988;123: 416–421.

8. González-Fandos E, Maya N, Pérez-Arnedo I. Effect of propionic acid on *Campylobacter jejuni* attached to chicken skin during refrigerated storage. Int Microbiol. 2015;18: 171–175. doi:10.2436/20.1501.01.247

9. Haque MN, Chowdhury R, Islam KMS, Akbar MA. Propionic acid is an alternative to antibiotics in poultry diet. Bangladesh J Anim Sci. 2012;38: 115–122. doi:10.3329/bjas.v38i1-2.9920

10. Defoirdt T, Boon N, Sorgeloos P, Verstraete W, Bossier P. Short-chain fatty acids and poly- β -hydroxyalkanoates: (New) Biocontrol agents for a sustainable animal production. Biotechnol Adv. Elsevier Inc.; 2009;27: 680–685. doi:10.1016/j.biotechadv.2009.04.026

11. Khan SH, Iqbal J. Recent advances in the role of organic acids in poultry nutrition. J Appl Anim Res. 2016;44: 359–369. doi:10.1080/09712119.2015.1079527

12. Bizzari SN, Blagoev M. Propionic acid. In: Chemical economics handbook: IHS chemical. 2013.

13. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science (80-). 2013;341: 569–573. doi:10.1126/science.1241165

14. Tedelind S, Westberg F, Kjerrulf M, Vidal A. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. World J Gastroenterol. Baishideng Publishing Group Inc; 2007;13: 2826–32. doi:10.3748/WJG.V13.I20.2826

15. Imhann F, Vich Vila A, Bonder MJ, Fu J, Gevers D, Visschedijk MC, et al. Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. Gut. 2016;0: 1–12. doi:10.1136/gutjnl-2016-312135

16. Molodecky NA, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Challenges associated with

identifying the environmental determinants of the inflammatory bowel diseases. Inflamm Bowel Dis. 2011;17: 1792–1799. doi:10.1002/ibd.21511

17. Keestra-Gounder AM, Byndloss MX, Seyffert N, Young BM, Chávez-Arroyo A, Tsai AY, et al. NOD1 and NOD2 signalling links ER stress with inflammation. Nature. Nature Publishing Group; 2016;532: 1–15. doi:10.1038/nature17631

18. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. Nature Publishing Group; 2012;491: 119–24. doi:10.1038/nature11582

19. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med. 2009;1: 1–19. doi:10.1126/scitranslmed.3000322.The

20. Agus A, Denizot J, Thévenot J, Martinez-Medina M, Massier S, Sauvanet P, et al. Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive *E. coli* infection and intestinal inflammation. Sci Rep. Nature Publishing Group; 2016;6: 19032. doi:10.1038/srep19032

21. Martinez-Medina M, Denizot J, Dreux N, Robin F, Billard E, Bonnet R, et al. Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. Gut. BMJ Publishing Group; 2014;63: 116–24. doi:10.1136/gutjnl-2012-304119

22. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis as a prerequisite for IBD. Gut. 2004;53: 1057. Available: http://www.ncbi.nlm.nih.gov/pubmed/15194668

23. Gophna U, Sommerfeld K, Gophna S, Doolittle WF, Veldhuyzen Van Zanten SJO. Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. J Clin Microbiol. 2006;44: 4136–4141. doi:10.1128/JCM.01004-06

24. Frank DN, Robertson CE, Hamm CM, Kpadeh Z, Zhang T, Chen H, et al. Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel

diseases. Inflamm Bowel Dis. 2011;17: 179–184. doi:10.1002/ibd.21339

25. Willing B, Halfbarson J, Dicksved J, Rosenquist M, Jarnerot G, Engstran L, et al. Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. Inflamm Bowel Dis. 2009;15: 653–660.

26. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward D V, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 2012;13: R79. doi:10.1186/gb-2012-13-9-r79

27. Honneffer JB, Minamoto Y, Suchodolski JS. Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs. World J Gastroenterol. 2014;20: 16489–16497. doi:10.3748/wjg.v20.i44.16489

28. Martinez-Medina M, Aldeguer X, Lopez-Siles M, González-Huix F, López-Oliu C, Dahbi G, et al. Molecular diversity of *Escherichia coli* in the human gut: New ecological evidence supporting the role of adherent-invasive *E. coli* (AIEC) in Crohn's disease. Inflamm Bowel Dis. 2009;15: 872–882. doi:10.1002/ibd.20860

29. Palmela C, Chevarin C, Xu Z, Torres J, Sevrin G, Hirten R, et al. Adherent-invasive *Escherichia coli* in inflammatory bowel disease. Gut. 2018;67: 574–587. doi:10.1136/gutjnl-2017-314903

30. Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, et al. High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. Gastroenterology. 2004;127: 412–421. doi:10.1053/j.gastro.2004.04.061

31. Baumgart M, Dogan B, Rishniw M, Weitzman G, Bosworth B, Yantiss R, et al. Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of *Clostridiales* in Crohn's disease involving the ileum. ISME J. 2007;1: 403–418. doi:10.1038/ismej.2007.52

32. Boudeau Ã, Barnich N, Intestinale Â, De L, Pharmacie Â De. Type 1 pili-mediated adherence of *Escherichia coli* strain LF82 isolated from Crohn's disease is involved in bacterial invasion of intestinal epithelial cells. Mol Microbiol. 2001;39: 1272–1284.

33. Martinez-medina M, Mora A, Blanco M, Lo C, Alonso MP, Darfeuille-michaud A, et al. Similarity and divergence among adherent-invasive *Escherichia coli* and extraintestinal pathogenic *E. coli* strains. J Clin Microbiol. 2009;47: 3968–3979. doi:10.1128/JCM.01484-09

34. Sepehri S, Kotlowski R, Bernstein CN, Krause DO. Phylogenetic analysis of inflammatory bowel disease associated *Escherichia coli* and the fimH virulence determinant. Inflamm Bowel Dis. 2009;15: 1737–1745.

35. Bergeron CR, Prussing C, Boerlin P, Daignault D, Dutil L, Reid-Smith RJ, et al. Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. Emerg Infect Dis. 2012;18: 415–421. doi:10.3201/eid1803.111099

36. Ewers C, Antão EM, Diehl I, Philipp HC, Wieler LH. Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic *Escherichia coli* strains with zoonotic potential. Appl Environ Microbiol. 2009;75: 184–192. doi:10.1128/AEM.01324-08

37. Nash JHE, Villegas A, Kropinski AM, Aguilar-valenzuela R, Konczy P, Mascarenhas M, et al. Genome sequence of adherent-invasive *Escherichia coli* and comparative genomic analysis with other *E. coli* pathotypes. BMC Genomics. 2010;11: 1–15.

38. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut. 1987;28: 1221–1227. doi:10.1136/gut.28.10.1221

39. Ormsby MJ, Johnson SA, Wall DM. Draft genome sequence of the commensal *Escherichia coli* strain F-18. Genome Announc. 2016;4: 1–2. doi:10.1128/genomeA.01416-16.Copyright

40. Lee W, VanderVen BC, Fahey RJ, Russel DG. Intracellular *Mycobacterium tuberculosis* exploits host-derived fatty acids to limit metabolic stress. J Biol Chem. 2013;288: 6788–6800.

41. Si HM, Zhang F, Wu AN, Han RZ, Xu GC, Ni Y. DNA microarray of global transcription factor mutant reveals membrane-related proteins involved in n-butanol tolerance in *Escherichia coli*. Biotechnol Biofuels. 2016;9: 1–14. doi:10.1186/s13068-016-0527-9

42. Jain M, Petzold CJ, Schelle MW, Leavell MD, Mougous JD, Bertozzi CR, et al. Lipidomics

reveals control of *Mycobacterium tuberculosis* virulence lipids via metabolic coupling. Proc Natl Acad Sci U S A. 2007;104: 5133–5138. doi:10.1073/pnas.0610634104

43. Ronin I, Katsowich N, Rosenshine I, Balaban NQ. A long-term epigenetic memory switch controls bacterial virulence bimodality. Elife. 2017;6: 1–26. doi:10.7554/eLife.19599

44. Collins J, Robinson C, Danhof H, Knetsch CW, van Leeuwen HC, Lawley TD, et al. Dietary trehalose enhances virulence of epidemic *Clostridium difficile*. Nature. Nature Publishing Group; 2018;0. doi:10.1038/nature25178

45. Horswill AR, Dudding AR, Escalante-Semerena JC. Studies of propionate toxicity in *Salmonella enterica* identify 2-Methylcitrate as a a potent inhibitor of cell growth. J Biol Chem. 2001;276: 19094–19101. doi:10.1074/jbc.M100244200

46. Upton AM, McKinney JD. Role of the methylcitrate cycle in propionate metabolism and detoxification in *Mycobacterium smegmatis*. Microbiology. 2007;153: 3973–3982. doi:10.1099/mic.0.2007/011726-0

47. Darfeuille-Michaud A, Neut C, Barnich N, Lederman E, Di Martino P, Desereumaux P, et al. Presence of adherent *Escherichia coli* strains in ileal mucosa of patients with Crohn's disease. Gastroenterology. 1998;115: 1405–1413.

48. Martinez-Medina M, Aldeguer X, Gonzalez-Huix F, Acero D, Garcia-Gil LJ. Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis. Inflamm Bowel Dis. 2006;12: 1136–1145.

49. Martin HM, Campbell BJ, Hart CA, Mpofu C, Nayar M, Singh R, et al. Enhanced *Escherichia coli* adherence and invasion in Crohn's disease and colon cancer. Gastroenterology. 2004;127: 80–93. doi:10.1053/j.gastro.2004.03.054

50. Mylonaki M, Rayment N, Rampton D, Hudspith B, Brostoff J. Molecular characterization of rectal mucosa-associated bacterial flora in inflammatory bowel disease. Inflamm Bowel Dis. 2005;11: 481–487.

543 51. Thompson JL, Hinton M. Antibacterial activity of formic and propionic acids in the diet of hens
544 on *Salmonellas* in the crop. Br Poult Sci. 1997;38: 59–65. doi:10.1080/00071669708417941
545 52. European Food Safety Authority (EFSA). Scientific Opinion on the safety and efficacy of
546 propionic acid, sodium propionate, calcium propionate and ammonium propionate for all animal
547 species. EFSA J. 2011;9: 1–22. doi:10.2903/j.efsa.2011.2446.
548 53. Zhang XS, Garcia-Contreras R, Wood TK. *Escherichia coli* transcription factor YncC (McbR)
549 regulates colanic acid and biofilm formation by repressing expression of periplasmic protein YbiM
550 (McbA). ISME J. 2008;2: 615–631. doi:10.1038/ismej.2008.24
551 54. Dolan SK, Wijaya A, Geddis SM, Spring DR, Silva-Rocha R, Welch M. Loving the poison: the
552 methylcitrate cycle and bacterial pathogenesis. doi:10.1099/mic.0.000604
553 55. Carter PB, Collins FM. The route of enteric infection in normal mice. J Exp Med. 1974;139:
554 1189–203. Available: http://www.ncbi.nlm.nih.gov/pubmed/4596512
555 56. Jones BD, Ghori N, Falkow S. Salmonella typhimurium initiates murine infection by penetrating
556 and destroying the specialized epithelial M cells of the Peyer's patches. J Exp Med. 1994;180: 15–
557 23. Available: http://www.ncbi.nlm.nih.gov/pubmed/8006579
558 57. Voravuthikunchai SP, Lee A. Cecectomy causes long-term reduction of colonization resistance in
559 the mouse gastrointestinal tract. Infect Immun. 1987;55: 995–9. Available:
560 http://www.ncbi.nlm.nih.gov/pubmed/3549568
561 58. Garner CD, Antonopoulos DA, Wagner B, Duhamel GE, Keresztes I, Ross DA, et al. Perturbation
562 of the Small Intestine Microbial Ecology by Streptomycin Alters Pathology in a Salmonella
563 enterica Serovar Typhimurium Murine Model of Infection. Infect Immun. 2009;77: 2691–2702.
564 doi:10.1128/IAI.01570-08
565 59. Davis R, Botstein D, Roth J. A manual for genetic engineering. Advanced Bacterial Genetics. New
566 York: Cold Spring Harbor; 1980.
567 60. Price-carter M, Tingey J, Bobik TA, Roth JR. The alternative electron acceptor tetrathionate
568 supports B12-dependent anaerobic growth of *Salmonella enterica* serovar Typhimurium on

ethanolamine or 1,2-Propanediol. J Bacteriol. 2001;183: 2463–2475. doi:10.1128/JB.183.8.2463

61. O'Toole GA. Microtiter dish biofilm formation assay. J Vis Exp. 2011; 3–5. doi:10.3791/2437

62. Connolly JPR, Gabrielsen M, Goldstone RJ, Grinter R, Wang D, Cogdell RJ, et al. A highly conserved bacterial D-Serine uptake system links host metabolism and virulence. PLoS Pathog. 2016;12. doi:10.1371/journal.ppat.1005359

63. Robinson MD, McCarthy DJ, Smyth GK. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2009;26: 139–140. doi:10.1093/bioinformatics/btp616

64. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. 2001;25: 402–408. doi:10.1006/meth.2001.1262

65. Riedel CU, Casey PG, Mulcahy H, O'Gara F, Gahan CGM, Hill C. Construction of p16Slux, a novel vector for improved bioluminescent labeling of Gram-negative bacteria. Appl Environ Microbiol. 2007;73: 7092–7095. doi:10.1128/AEM.01394-07

66. Laurentin A, Edwards CA. Differential fermentation of glucose-based carbohydrates in vitro by human faecal bacteria: A study of pyrodextrinised starches from different sources. Eur J Nutr. 2004;43: 183–189. doi:10.1007/s00394-004-0457-3

Acknowledgments

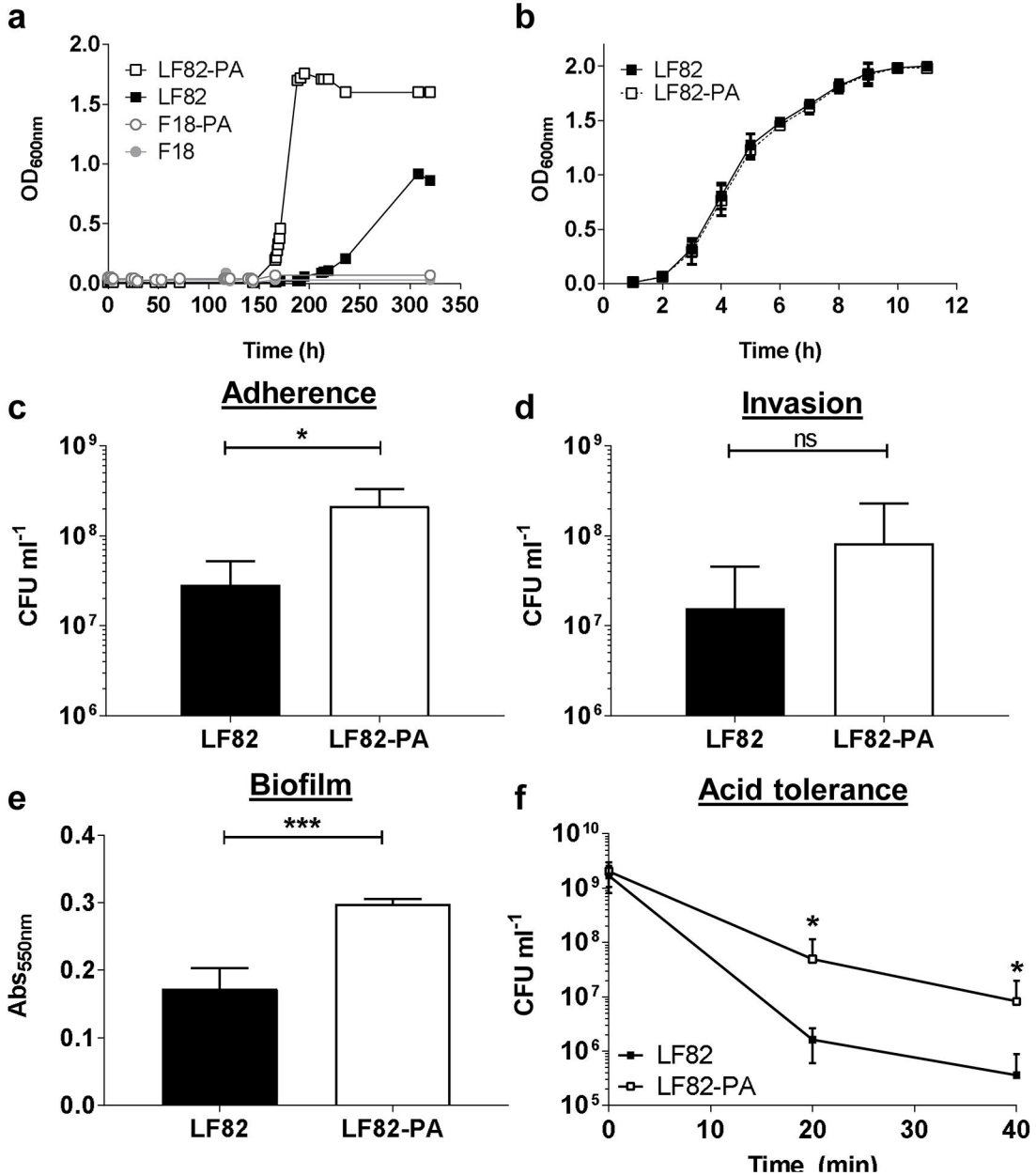
General: The LF82 strain was a kind gift from Professor Daniel Walker, University of Glasgow.

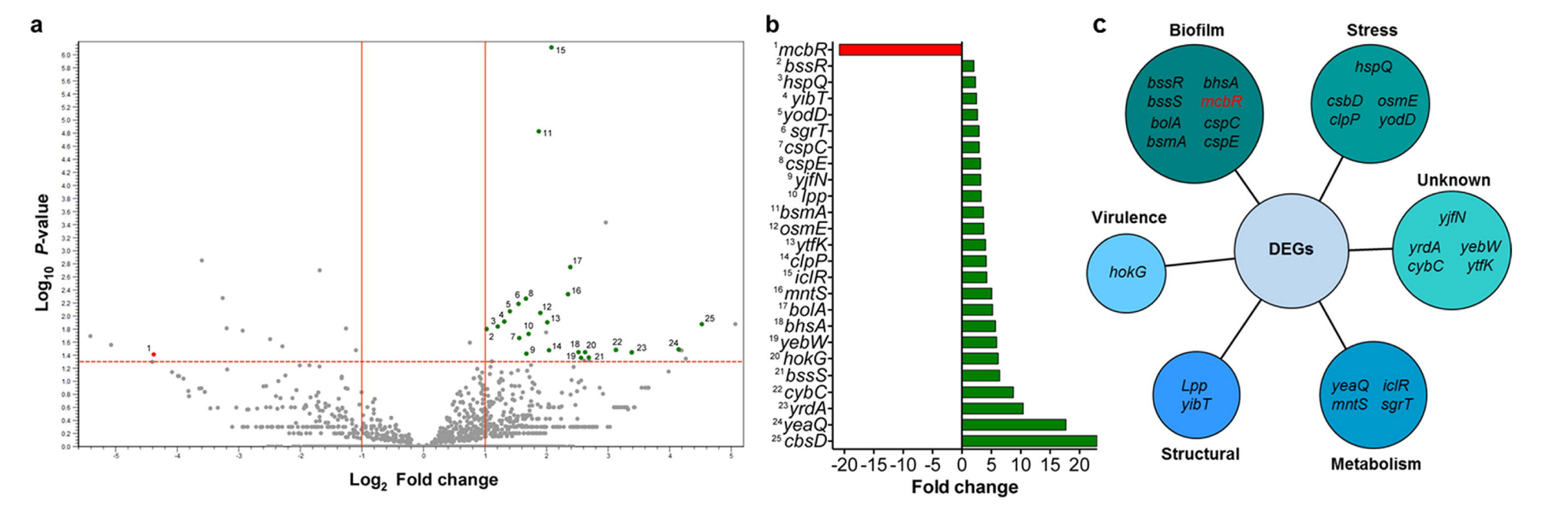
Funding: This work was funded by Biotechnology and Biological Sciences Research Council grants BB/K008005/1 & BB/P003281/1 to DMW and by the Wellcome Trust through a Wellcome Trust PhD studentship to SAJ (102460/Z/13/Z).

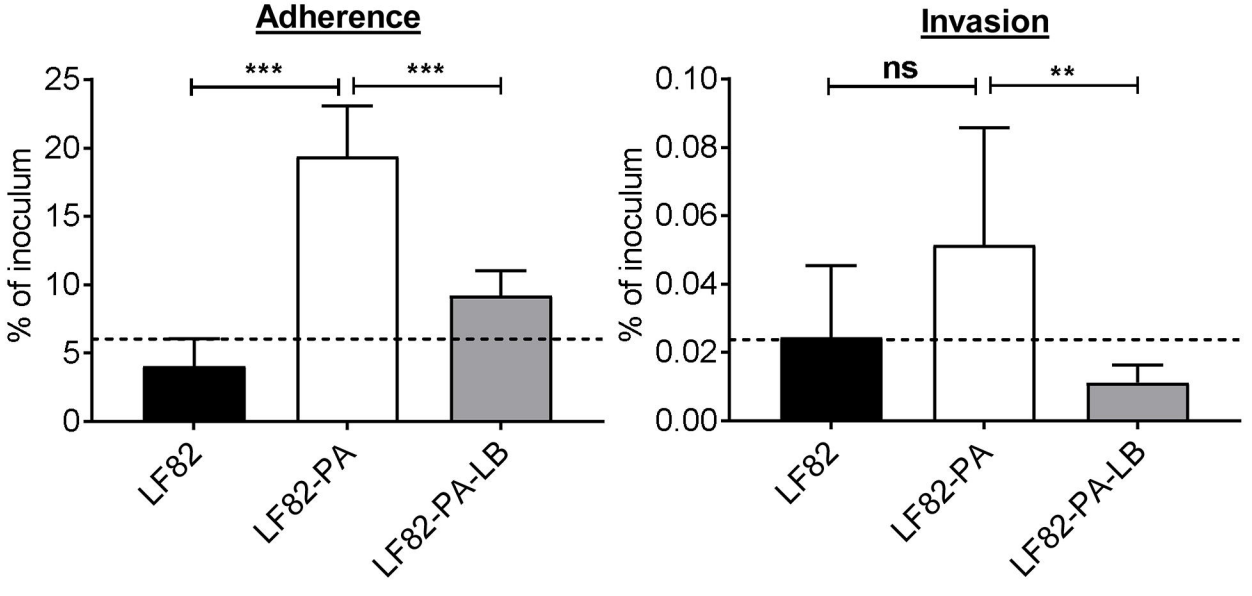
Author contributions: MJO designed and performed the experiments, analysed the data and prepared the manuscript. SAJ assisted in experimental design, animal experiments and RT-PCR. LMM, RJG and DGES assisted in experimental design. AM provided technical assistance throughout the experiments. JPRC and AJR advised on and assisted in RNA-seq analysis. CCM generated the primary LF82-PA

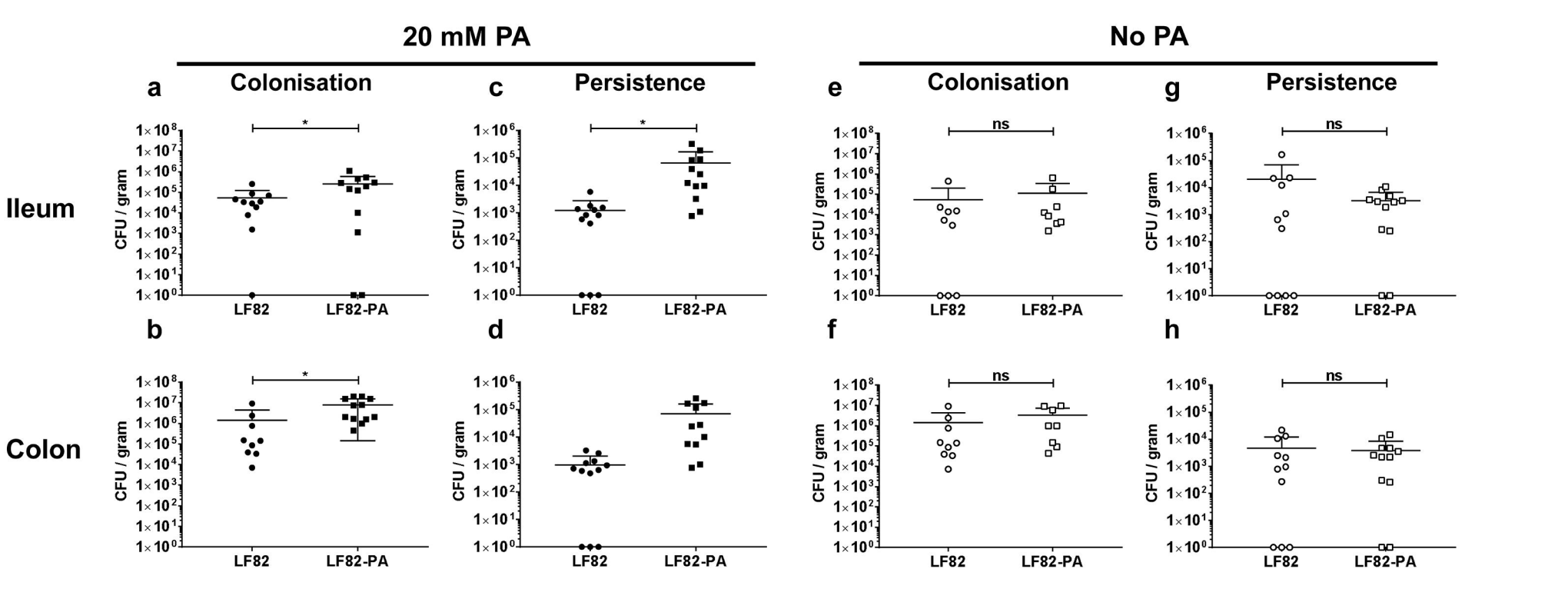
595 strain. HEH and HMW assisted in animal experiments. EF was involved in developing the initial concept
596 for the study. KG advised on and conducted SCFA experiments. DM conducted GC-IRMS experiments.
597 DMW was awarded the funding, developed the initial concept, designed and performed experiments and
598 prepared the manuscript. All authors contributed to editing the manuscript for publication.

599 **Competing interests:** The authors declare that there are no competing interests.


a
LF82-PA
LF82
F18-PA
F18
OD600nm
Time (h)
b
LF82
LF82-PA
OD600nm
Time (h)
c
Adherence
*
CFU ml-1
LF82
LF82-PA
d
Invasion
ns
CFU ml-1
LF82
LF82-PA
e
Biofilm
***
Abs550nm
LF82
LF82-PA
f
Acid tolerance
CFU ml-1
*
*
LF82
LF82-PA
Time (min)


a
Log10 P-value
Log2 Fold change
b
1 mcbR
2 bssR
3 hspQ
4 yibT
5 yodD
6 sgrT
7 cspC
8 cspE
9 yjfN
10 lpp
11 bsmA
12 osmE
13 ytfK
14 clpP
15 iclR
16 mntS
17 bolA
18 bhsA
19 yebW
20 hokG
21 bssS
22 cybC
23 yrdA
24 yeaQ
25 cbsD
-20 -15 -10 -5 0 5 10 15 20
Fold change
c
Biofilm
bssR bhsA
bssS mcbR
bolA cspC
bsmA cspE
Stress
hspQ
csbD osmE
clpP yodD
Unknown
yjfN
yrdA yebW
cybC ytfK
Virulence
hokG
DEGs
Lpp
yibT
Structural
yeaQ iclR
mntS sgrT
Metabolism


Adherence
***
***
25
20
15
10
5
0
% of inoculum
LF82
LF82-PA
LF82-PA-LB
Invasion
ns
**
0.10
0.08
0.06
0.04
0.02
0.00
% of inoculum
LF82
LF82-PA
LF82-PA-LB


20 mM PA
No PA
Ileum
Colon
a
Colonisation
c
Persistence
e
Colonisation
g
Persistence
b
d
f
h
CFU / gram
LF82
LF82-PA
*
ns