Colonic mucosa-associated Diffusely-Adherent *afaC+ Escherichia coli* expressing *lpfA* and *pks* are increased in inflammatory bowel disease and colon cancer. Maelle Prorok-Hamon, Melissa K. Friswell, Abdullah Alswied, Fei Song, Carol L. Roberts, Paul Flanagan, Paul Knight, Caroline Codling, Julian R. Marchesi, Craig Winstanley, Neil Hall, Jonathan M. Rhodes and Barry J. Campbell.

Supplementary file S1

Table S1(A) - Oligonucleotide primers used for RT-PCR and amplicon sizes

Gene	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Reference or *Genbank Accession Nº.*
afaC	fp: CGGCTTTTCTGCTGAACTGGCAGGC rp: CCGTCAGCCCCCACGGCAGACC	672	25
dsbA	fp: CTGCCGGAAGGCGTGAAC rp: GCTGTTCCACGCCGCGTC	237	18
fimA	fp: GCTGAATGATTGCGATACCA rp: AGCACCGGTTGCAAAATAAC	255	16
fimC	fp: AAAAACGTCAATGTAAGGAAATCG rp: GCACCATAATCATTTATTGTTCGG	682	***FJ866045***
fimH	fp: TGCAGAACGGATAAGCCGTGG rp: GCAGTCACCTGCCCTCCGGTA	508	5
fliC	fp: ATGGCACAAGTCATTAAT rp: TTAACCCTGCAGTAGAGA	Full gene 1497bp K12	a
htrA	fp: TTCCAGCAGTTCTTCGGTGA rp: ATCAGTTCGCCGTTCAGGTT	530	***M36536***
$lpfA_{Shigella}$	fp: AGGCGGTGCATTCACTCTGGCATCT rp: CCGCGTCGATAGCGGTATAGGCAGA	448	***NC_004337***
$lpfA_{LF82}$	fp: CTGGAAAACTGCGATATCTCC rp: GGCCTTCTTTCAGACGGTA	199	19
ompC	fp: GCGCCGACATCAACGTATTT rp: GCCAACAAAGCGCAGAACTT	141	b
papC	fp: GACGGCTGTACTGCAGGGTGTGGCG rp: ATATCCTTTCTGCAGGGATGCAATA	328	5
pks	fp: AGCCGTATCCTGCTCAAAAC rp: TCGGTATGTCCGGTTAAAGC	1413	22

[a] Subramanian S *et al.* (2008). Characterization of epithelial IL-8 response to inflammatory bowel disease mucosal *E. coli* and its inhibition by mesalamine. Inflamm. Bowel Dis. 14: 162–175

[b] Balaji B *et al.* (2005). Timing of induction of osmotically controlled genes in *Salmonella enterica* Serovar Typhimurium, determined with quantitative real-time reverse transcription-PCR. Appl. Environ. Microbiol. 71: 8273-83

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Methods S1(A): PCR amplification conditions

E. coli, grown overnight on LB agar, were re-suspended in 100µL sterile water, boiled for 5 min and centrifuged (13,000 *g*, 10 min). Aliquots of supernatant (2µL) were used as amplification template in 50µL reactions containing 45µl 1.1X ReddyMix™ PCR Mastermix (Thermofisher; Loughborough, UK) with 300nM of forward and reverse.

All amplifications were performed in a thermal cycler (Hybaid Omnigene; Middlesex, UK) and carried out using conditions described within references listed in Table S1 or as follows:

afaC - amplification of was carried out over 25 cycles (94°C for 1 min, 65°C for 1 min and 72°C for 2 min).

htrA - amplification was carried out over 35 cycles (95°C for 1 min, 55°C for 1 min, and 72°C for 2 min).

dsbA - additional $MgCl_2$ was added to a final concentration of 3.4 mM.

$lpfA_{Shigella}$ - amplification over 35 cycles (94°C for 1 min, 60°C for 1 min, and 72°C for 1 min).

All PCR-generated cDNA products were applied to 2% (wt/vol) agarose gel containing 1:10K Gelred nucleic acid gel stain and electrophoresed for ~1 h at 100V.

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Table S1(B): Oligonucleotide primers and probes used for qPCR

Gene	Human Universal Probe Library Set (Roche)	Oligonucleotide sequence (5'-3')
beta-Actin	#64	fp: CCAACCGCGAGAAGATGA rp: CCAGAGGCGTACAGGGATAG
VEGF-A	#29	fp: CCTTGCTGCTCTACCTCCAC rp: CCACTTCGTGATGATTCTGC