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ORIGINAL ARTICLE

Phylogenetic distribution of three pathways for propionate production within the human gut microbiota

This article has been corrected since Advance Online Publication and a corrigendum is also printed in this issue

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Propionate is produced in the human large intestine by microbial fermentation and may help maintain human health. We have examined the distribution of three different pathways used by bacteria for propionate formation using genomic and metagenomic analysis of the human gut microbiota and by designing degenerate primer sets for the detection of diagnostic genes for these pathways. Degenerate primers for the acrylate pathway (detecting the *lcdA* gene, encoding lactoyl-CoA dehydratase) together with metagenomic mining revealed that this pathway is restricted to only a few human colonic species within the Lachnospiraceae and Negativicutes. The operation of this pathway for lactate utilisation in *Coprococcus catus* (Lachnospiraceae) was confirmed using stable isotope labelling. The propanediol pathway that processes deoxy sugars such as fucose and rhamnose was more abundant within the Lachnospiraceae (based on the *pduP* gene, which encodes propionaldehyde dehydrogenase), occurring in relatives of *Ruminococcus obeum* and in *Roseburia inulinivorans*. The dominant source of propionate from hexose sugars, however, was concluded to be the succinate pathway, as indicated by the widespread distribution of the *mmdA* gene that encodes methylmalonyl-CoA decarboxylase in the Bacteroidetes and in many Negativicutes. In general, the capacity to produce propionate or butyrate from hexose sugars resided in different species, although two species of Lachnospiraceae (*C. catus* and *R. inulinivorans*) are now known to be able to switch from butyrate to propionate production on different substrates. A better understanding of the microbial ecology of short-chain fatty acid formation may allow modulation of propionate formation by the human gut microbiota.

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Introduction

The human large intestine is inhabited by a diverse microbial community that influences host health through a number of mechanisms, including the production of metabolites, a barrier function against pathogens, and interactions with the host's immune system and physiology (Flint *et al.*, 2012b). The breakdown of non-digestible carbohydrates originating from the diet leads to the formation of fermentation acids, mainly the short-chain fatty acids (SCFAs) acetate, propionate and butyrate. SCFAs are absorbed by the host and used as an energy source but also have a variety of distinct physiological effects. Butyrate, in particular, is believed to counteract colorectal cancer and inflammation (Hamer *et al.*, 2008; Berni Canani *et al.*, 2012). Propionate also has potential health-promoting effects that include anti-lipogenic, cholesterol-lowering, anti-inflammatory and anti-carcinogenic action (Hosseini *et al.*, 2011; Vinolo *et al.*, 2011). Furthermore, the potential role of propionate in enhancing satiety (Arora *et al.*, 2011) is of increasing interest given the rising incidence of obesity across the world. Recent proteomic work suggests that some of the effects of propionate at the cellular level

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differ from the action of butyrate (Kilner *et al.*, 2012). The recently deorphanised SCFA receptors FFA2 and FFA3 are likely to mediate some of the actions of propionate; however, the exact mechanisms remain to be established (Ulven, 2012). Enhancing propionate production in the colon through dietary intervention with non-digestible carbohydrates is an attractive approach for increasing satiety and maintaining health; however, in-depth knowledge of propionate producers within the gut microbiota is required for the development of dietary strategies.

In order to understand and manipulate SCFA formation by the human colonic microbiota, we need to know which phylogenetic groups, substrates and pathways have major roles in the formation of each acid. Relevant information is now available for butyrate formation (Louis and Flint, 2009, Louis *et al.*, 2010) but is currently lacking for propionate. Three different biochemical pathways for propionate production are known to be present in the microbiota (Figure 1). Bacteroidetes utilise the succinate pathway via methylmalonyl-CoA (Macy and Probst, 1979), which is also present in several Firmicutes bacteria belonging to the recently proposed new class of Negativicutes (formerly classed as *Veillonellaceae* or Clostridial cluster IX (Marchandin *et al.*, 2010)). Bacteroidetes mainly utilise polysaccharides and peptides for growth (Macy and Probst, 1979, Flint *et al.*, 2012a), whereas in Firmicutes propionate formation has been reported from organic acids as well (Seeliger *et al.*, 2002; Watanabe *et al.*, 2012). *Veillonella parvula* gains additional energy from succinate in the presence of lactate as the main growth substrate (Janssen, 1992), whereas *Phascolarctobacterium succinatutens,* isolated from human faeces, can grow on succinate alone (Watanabe *et al.*, 2012). *Selenomonas ruminantium* strains isolated from the rumen produce lactate, acetate and propionate from carbohydrates, and some are also able to utilise lactate for growth (Bryant, 1956; Gilmour *et al.*, 1994). This class of bacteria remains understudied in the human gut and it remains to be established which genera dominate in this ecosystem. The acrylate pathway for propionate formation has been characterised in detail in the soil bacterium *Clostridium propionicum* (Hetzel *et al.*, 2003), and the corresponding genes have recently been described (Kandasamy *et al.*, 2013). This pathway is also present in the rumen bacterium *Megasphaera elsdenii* within the Negativicutes (Hino and Kuroda, 1993), which produces butyrate during growth on glucose, but propionate during growth on lactate (Hino and Kuroda, 1993). It is possible to distinguish the succinate pathway from the acrylate pathway by incubation with stable isotope-labelled substrates (Bourriaud *et al.*, 2005; Morrison *et al.*, 2006). Finally, several different bacteria are known to produce 1,2-propanediol from deoxy sugars such as fucose and rhamnose, or via different pathways from dihydroxyacetonephosphate or lactate (Saxena *et al.*, 2010). In some bacteria, including *Salmonella enterica* serovar Typhimurium, 1,2-propanediol can be further metabolised to propionate or propanol (Bobik *et al.*, 1999). Propionate formation from

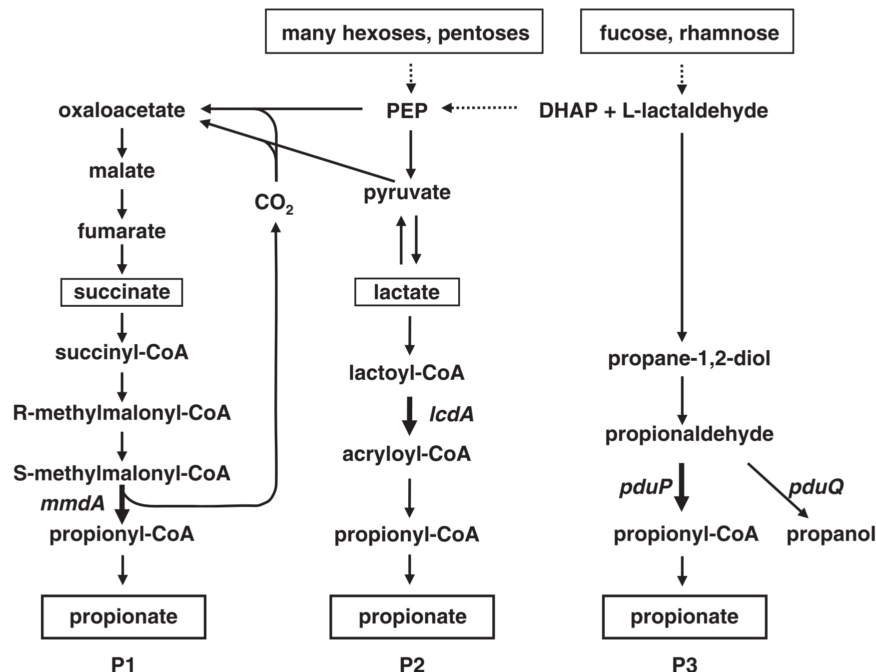

Figure 1 Known pathways for propionate formation in human gut bacteria. (P1), Succinate pathway; (P2), acrylate pathway; (P3), propanediol pathway. Substrates utilised are shown in boxes. Genes targeted as molecular markers for the specific pathways are indicated. DHAP, dihydroxyacetonephosphate; PEP, phosphoenolpyruvate.

fucose via propanediol has also been described in the human gut anaerobe *Roseburia inulinivorans*, which produces butyrate rather than propionate when grown on glucose (Scott *et al.*, 2006).

Our primary aim here was to explore the distribution of the three known pathways for propionate production within the human microbiota with genomic and metagenomic approaches. In addition, we demonstrate the operation of the acrylate pathway for propionate production for the first time in an isolated human colonic bacterium by means of stable isotope labelling.

Materials and methods

Bacterial isolation and cultivation

Strain GD/7 was isolated on yeast extract-casitone-fatty acid (YCFA) medium (Lopez-Siles *et al.*, 2012) containing 25 mM DL lactate from a faecal sample of a 42-year-old man consuming an omnivorous diet as described previously (Louis *et al.*, 2004). The 16S rRNA sequence of GD/7 was determined (accession number EU266552) as described before (Louis *et al.*, 2004) and blastn analysis revealed that it was 97% identical to *Coprococcus catus* VPI-C6-61. A fluorescent *in situ* hybridisation (FISH) probe was designed for *C. catus* and was used to determine the abundance of this species in human volunteers (for details see Supplementary Information S1). All other bacterial strains used were described before (Dabek *et al.*, 2008) or obtained from the German Collection of Microorganisms and Cell Cultures, the American Type Culture Collection, the National Collection of Industrial and Marine Bacteria (as indicated by DSM, ATCC and NCIMB numbers) or from the Rowett Institute strain collection (*V. parvula* L59). Bacteria were grown anaerobically in M2GSC (Miyazaki *et al.*, 1997) or YCFAGSC medium (Lopez-Siles *et al.*, 2012) apart from *Akkermansia muciniphila* DSM 22959 (M2GSC + 0.2% procine mucin, Sigma-Aldrich, Gillingham, UK) and *V. parvula* L59 (M2GSC medium with maltose replacing starch + 1% lactate). Growth experiments on specific substrates were performed in triplicate on basal YCFA medium supplemented with substrates as detailed in the results (at 0.5% substrate, unless specified otherwise). Growth experiments were performed by inoculating 0.1 ml of overnight culture into 7.5 ml of anaerobic medium prepared in Hungate tubes (in a 100% CO_2 atmosphere) followed by incubation for 24 h at 37 °C (Lopez-Siles *et al.*, 2012). The SCFA and lactate content of samples was determined by gas chromatography analysis following conversion to t-butylmethylsilyl derivatives (Richardson *et al.*, 1989).

Determination of concentrations and ^{13}C enrichments in propionate

Duplicate culture supernatant samples from YCFA and 25 mM L-lactate with additional universally ^{13}C-labelled L-lactate at 10 molar % excess were prepared for estimation of concentrations by isotope dilution and for determination of enrichments of SCFA and lactate by gas chromatography coupled with mass spectrometry. They were measured by analysis of the *tert*-butyldimethylsilyl derivatives. Procedures were as described previously (Belenguer *et al.*, 2007). The mass spectrometer was operated under electron impact ionisation conditions. For the concentration determinations, appropriate corrections were applied for the enrichments in the sample. Lactate flows to propionate, either through acrylate or through succinate, were obtained from the changes in labelled propionate (M + 2 and M + 3), assuming that the succinate route gives half M + 2 and M + 3.

Degenerate primer design, PCR and clone library analysis

Degenerate primers (Table 1) specific for genes *lcdA* (lactoyl-CoA dehydratase subunit alpha), *pduP* (CoA-dependent propionaldehyde dehydrogenase), *pduQ* (propanol dehydrogenase; for further details see Supplementary Information) and *mmdA* (methylmalonyl-CoA decarboxylase α-subunit of Negativicutes, equivalent to propionyl-CoA carboxylase β-subunit of Bacteroidetes, for details see Results) were designed by visual inspection of protein sequence alignments of genes detected by blastp analysis (Altschul *et al.*, 1990) against the non-redundant database at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and using the CoGe database (http://genomevolution.org/CoGe/) (Lyons and Freeling, 2008). Query sequences: LcdA, *C. propionicum* AEM62994; PduP, *R. inulinivorans* ABC25528; PduQ, *R. inulinivorans* ABC25529; and MmdA, *Bacteroides thetaiotaomicron* NP_810363. Degenerate primer design was carried out as described previously (Louis *et al.*, 2004). Primer specificity was validated with genomic DNA prepared with a DNeasy Blood and Tissue Kit (Qiagen, Manchester, UK) from pure cultures of 21 bacterial strains (Supplementary Table S1). To confirm specificity of the primers, they were used to amplify the respective genes from faecal DNA from a healthy human volunteer. Fresh faeces was homogenised for 3 × 1 min in a stomacher and 0.2 g was used to extract genomic DNA using the FastDNA spin kit for soil (MP Biomedicals, Cambridge, UK). PCRs contained 10 ng template DNA, 3 mM $MgCl_2$, 10 mM dNTPs, 100 nM of each primer (Table 1) and 1 U Taq polymerase (Bioline, London, UK) per 50 μl reaction, and were performed at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, primer-specific annealing temperature (Table 1) for 30 s, 72 °C for 40 s (1 min for *lcdA*, 2 min for 16S rRNA gene) and a final extension at 72 °C for 5 min (10 min for 16S rRNA gene). Clone libraries specific for Bacteroidetes and Negativicutes were constructed by amplification of faecal DNA with primers targeting the 16S

Table 1 Primers used in this study

Target gene	*Primer name*	*Primer sequence*	*Amplicon size (bp)*	*Ann. temp.*[a]	*Reference*
Functional genes					
lcdA	lcdAF	5′-CTGGTGTGCTGGWSIGCIWSIGTIGCNCC-3′	771	58	This study
	lcdAR	5′-CAGATAGGTCCAIAYIGCDATNCCYTCCCA-3′			This study
pduP	pduPF1	5′-GTGGATGARACIGGIATGGGNAAYGTNGG-3′	1088	57	This study
	pduPF2[b]	5′-TGGCTGGAACATGGIAAYMRICAYWSIGCICA-3′			This study
	pduPR	5′-CAATAGCCYTCICCICCRAAICCIADNGC-3′			This study
pduQ	pduQF	5′-GATCTGAAYAARMARAAYRTIGGIGGIGC-3′	584	57	This study
	pduQR1[b]	5′-CGGATCATCTTTRAAIATCATRCAISWNAC-3′			This study
	pduQR2	5′-ATGCGGGTTAATNKKIGCRTCICCICCRTTDAT-3′			This study
mmdA	mmdAF	5′-AATGACTCGGGIGGIGCIMGNATHCARGA-3′	874	56	This study
	mmdAR	5′-GATTGTTACYTTIGGIACNGTNGCYTC-3′			This study
16S rRNA gene					
Universal	7-f	5′-AGAGTTTGATYMTGGCTCAG-3′	~1495	52	Satokari *et al.* (2001)
	1510-r	5′-ACGGCTACCTTGTTACGACTT-3′			Satokari *et al.* (2001), modified
Bacteroidetes	Bac303F	5′-GAAGGTCCCCCACATTG-3′	610	60	Bartosch *et al.* (2004)
	BacPre-rev	5′-CTTTGAGTTTCACCGTTGCCGG-3′			Wood *et al.* (1998), modified
Negativicutes	IX552F[c]	5′-GTTGTCCGGAATYATTGGGC-3′	321	63	This study
	IX854R2A[d]	5′-ATTGCGTTAACTCCGGCACA-3′			Daly and Shirazi-Beechey (2003); modified
	IX854R2G[d]	5′-ATTGCGTTAACTCCGGCACG-3′			Daly and Shirazi-Beechey (2003); modified

[a]ann. temp.: annealing temperature.
[b]Primers have not been used here for clone library analysis but can be used to amplify across *pduP* and *pduQ*.
[c]Primer is not entirely specific for Negativicutes and will work specifically only in conjunction with reverse primers.
[d]Both primers are used together at equimolar concentrations.

rRNA gene (Table 1). For primer design of Negativicutes primers see Supplementary Information. The PCR amplification protocol was the same as described above. PCR products were cloned and sequenced as described before (Louis *et al.*, 2010). Sequence accession numbers were KF385507 (*lcdA* of *Clostridium lactatifermentans* DSM 14214) and KF385508-KF385859 (faecal clones).

Metagenomic mining methods

The deduced protein sequences of *mmdA* from *V. parvula* (accession number ZP_06259922), *lcdA* from *C. propionicum* (AEM62994) and *pduP* and *pduQ* from *R. inulinivorans* (ABC25528 and ABC25529) were blasted (tblastn) against the metagenomic data set comprising 124 European individuals (Qin *et al.*, 2010) on the in-house Rowett/BioSS Beowulf cluster as described before (McIntosh *et al.*, 2012). Matches with at least 50% sequence identity for LcdA and 55% for PduP and PduQ were selected on the basis of the blastp results in the non-redundant (nr) database indicating which identity level is likely to represent a true hit ($>$60% for LcdA, as blastp results with query sequence AEM62994 had at least 60% identity for strains known to carry this pathway, followed by a sharp drop in sequence identity to $\leqslant$43% for other matches; $>$65% for PduP and PduQ, as blastp results with query sequences ABC25528/9 had at least 73% identity for strains known to carry this pathway, followed by a sharp drop in sequence identity to $\leqslant$58%; for PduP (but not PduQ) two matches of 63% identity were also present in *Thermoanaerobacterium* spp.). Maximum likelihood phylogenetic trees (poisson model, 100 times bootstrap) were constructed using Mega5 (Tamura *et al.*, 2011). Sequences with at least 95% identity were grouped into phylotypes. Individual bacterial genomes were obtained from multiple sources and included 20 strains of human colonic Firmicutes bacteria (listed in Table 2) isolated at the Rowett Institute that were sequenced at the Sanger Institute as part of the MetaHit EU project (http://www.sanger.ac.uk/pathogens/metahit/).

Results

Succinate pathway

The succinate pathway of propionate formation has mainly been studied in bacteria belonging to the class Negativicutes. In *V. parvula*, the decarboxylation of methylmalonyl-CoA to propionyl-CoA is coupled with sodium transport across the membrane, which facilitates ATP generation via a sodium-translocating ATPase (Dimroth and von Ballmoos, 2008), and the respective genes for the sodium pump decarboxylase have been characterised (*mmdA-E*, (Huder and Dimroth, 1993)). The other pathway genes are encoded upstream of the *mmd* gene cluster, with the same gene configuration also being present in the human colonic anaerobes *Dialister succinatiphilus* YIT 11850 and *P. succinatutens* YIT 12067 (Supplementary Table S2). Genes with high similarity to *V. parvula* ATCC 17745

Table 2 Occurrence of genes diagnostic for different pathways of propionate and butyrate production in the genomes of dominant species of human colonic anaerobes

Phylum, family and species	*Origin*	*% Protein sequence identity*[a]				
		Fermentation pathway[b] *and diagnostic gene*[c]				
		P1 MmdA[d]	*P2 LcdA*	*P3 PduP*	*B1 Buk*	*B2 BCoAT*
Actinobacteria						
Bifidobacterium adolescentis DSM 20083	Adult intestine	48	No	No	No	No
Bacteroidetes						
Bacteroides thetaiotaomicron VPI-5482, DSM 2079[T]	Human faeces	60	No	No	49	No
Bacteroides fragilis YCH46	Clinical	62	No	No	52	No
Bacteroides vulgatus DSM 1447[T]	Human faeces	60	No	No	50	No
Firmicutes-Lachnospiraceae						
Roseburia intestinalis M50/1	Human faeces	35	No	No	No	94
Eubacterium rectale A1-86	Human faeces	38	No	No	No	81
Roseburia inulinivorans A2-194	Human faeces	38	No	100	No	87
Unknown sp SR1/5	Human faeces	44	No	78	No	No
Ruminococcus obeum A2-162	Human faeces	30	No	77	No	No
Ruminococcus gnavus ATCC 29149	Human faeces	39	No	78	No	No
Ruminococcus torques L2-14	Human faeces	40	No	74	No	No
Unknown sp SM4/1	Human faeces	No	No	No	No	82
Clostridium sp. M62/1	Human faeces	36	39	No	No	76
Clostridiales bacterium SS3/4	Human faeces	38	37	No	No	73
Eubacterium hallii L2-7	Human faeces	38	No	53	No	77
Anaerostipes hadrus SSC/2	Human faeces	No	No	No	No	74
Coprococcus catus GD/7	Human faeces	39	67	No	No	75
Coprococcus eutactus L2-50	Human faeces	40	No	No	100	51
Coprococcus eutactus ART55/1	Human faeces	42	No	No	92	49
Coprococcus comes ATCC 27758	Human faeces	39	No	No	76	No
Clostridium symbiosum WAL-14163	Human faeces	54	37	33	No	76
Clostridium asparagiforme DSM 15981	Human faeces	40	36	43	No	No
Clostridium sp. MSTE9 (cluster XIVb)	Oral	54	78	53	No	41
Firmicutes-Ruminococcaceae						
Faecalibacterium prausnitzii A2-165	Human faeces	No	No	No	No	74
Faecalibacterium prausnitzii S3L/3	Human faeces	No	No	No	No	73
Faecalibacterium prausnitzii L2-6	Human faeces	No	No	No	No	74
Ruminococcus bromii L2-63	Human faeces	34	No	32	No	No
Eubacterium siraeum 70/3	Human faeces	35	No	34	No	No
Eubacterium siraeum V10Sc8a	Human faeces	35	No	34	No	No
Firmicutes–Negativicutes						
Veillonella parvula DSM 2008	Intestinal tract	99	No	No	No	No
Dialister succinatiphilus YIT 11850, DSM 21274[T]	Human faeces	77	No	No	No	No
Phascolarctobacterium succinatutens YIT 12067	Human faeces	81	No	No	No	No
Selenomonas ruminantium subsp. *lactilytica* TAM 6421	Bovine rumen	81	No	No	No	No
Megasphaera elsdenii LC1 DSM 20460[T]	Sheep rumen	74	62	No	No	55
Firmicutes–Peptostreptococcaceae						
Clostridium difficile 630	Clinical	No	43	32	62	39
Firmicutes–Clostridiaceae						
Clostridium botulinum A2 Kyoto	Infant botulism	No	42	35	65	No
Clostridium botulinum C Eklund		No	75	No	No	No
Clostridium novyi NT	Gas gangrene	No	76	31	No	No
Clostridium beijerickii NCIMB 8052	Corn meal	No	No	51	67	41
Verrucomicrobia						
Akkermansia muciniphila DSM 22959[T]	Human faeces	52	No	No	No	No

[a]On the basis of blastp and tblastn analysis of genomes in the NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and CoGe (http://genomevolution.org/CoGe/) databases. Per cent protein identity is given for all matches >30% with >80% query coverage. Shading from light grey to black: 40–49%, 50–59%, 60–69%, 70–100%.
[b]Propionate pathways: P1, succinate pathway; P2, acrylate pathway; P3, propanediol pathway (see Figure 1). Butyrate pathways: B1, butyrate kinase pathway (as Bacteroides do not produce butyrate, Buk matches below 60% in the Bacteroidetes genomes are likely to have another function); B2, butyryl-CoA:acetate CoA-transferase pathway (Louis and Flint, 2009).
[c]Genes used for blastp analyses are MmdA, ZP_06259922; LcdA, AEM62994; PduP, ABC25528; Buk, AAR19758; BCoAT, AAX19660.
[d]Genes with high sequence similarity to MmdA may also be involved in other pathways as detailed in the main text.

mmdA were also found in the genomes of the rumen species *M. elsdenii* DSM 20460 and *S. ruminantium* TAM 6421, although most of the remaining pathway genes were lacking in *M. elsdenii* (Supplementary Table S2).

Bacteroidetes strains also carry genes with similarity to *mmdA* (Table 2), and this gene was therefore chosen as a molecular target for this pathway, as it exhibited the highest level of sequence identity between Negativicutes and Bacteroidetes (Supplementary Table S2). Degenerate primers were designed and validated against pure bacterial DNA of 21 strains, which indicated that they worked specifically for bacteria carrying the *mmdA* gene, most of which are known to use the succinate pathway (Supplementary Table S1). A clone library was constructed from human faecal DNA to confirm specificity of the primers, and clone libraries of 16S rRNA genes specific for both phylogenetic groups of bacteria carrying this pathway were generated in parallel. For the functional gene primers, 64 of 65 analysed clone sequences belonged to Bacteroidetes, with the final sequence having 72% identity to *Clostridium hathewayi* DSM 13479 ZP_6112601 (the *C. hathewayi* gene exhibits 67% protein sequence identity to *V. parvula* MmdA; however, only one further gene (*mmdB*, 60% protein identity) with similarity to the *V. parvula* gene cluster ZP_06259927-18 was found by blastp analysis in this bacterium). The clone distribution between different species agreed well with a Bacteroidetes-specific clone library based on the 16S rRNA gene (Supplementary Table S3), whereas a 16S rRNA gene clone library targeted specifically against Negativicutes detected mainly *Phascolarctobacterium faecium* (32 clones; also 1 clone closely related to *Veillonella dispar*, out of 44 clones analysed, Supplementary Table S3). *P. faecium* is reported to convert succinate to propionate (Del Dot *et al.*, 1993).

Acrylate pathway

Analysis of the acrylate pathway genes revealed the lactoyl-CoA dehydratase sequence to be most suitable as a marker gene for this pathway (Supplementary Table S4). The protein sequences of genes *lcdA*, *lcdB* and *lcdC* from the soil bacterium *C. propionicum* (accession numbers AEM62993-5), encoding the α- and β-subunits of the enzyme as well as the activator (Kandasamy *et al.*, 2013), were blasted against non-redundant protein sequences at NCBI, and phylogenetic trees were generated from the blast results (data not shown). Genes belonging to a limited number of bacteria (*C. propionicum*, *C. botulinum* type C and D, *C. novyi*, *Megasphaera* spp., *C. catus*, *Desulfosporosinus* spp., *Peptinophilus indolicus* and *Fusobacterium* sp.) formed a separate cluster for all three genes, which was most clearly delineated from other sequences for LcdA. The presence of *C. botulinum* type C and D but not other *C. botulinum* types in this cluster is in line with those strains (together with *C. novyi*) being able to convert lactate to propionate, whereas other *C. botulinum* types cannot utilise lactate (Rainey *et al.*, 2009). Furthermore, this cluster contained *M. elsdenii*, known to carry the pathway (Hino and Kuroda, 1993), and we therefore postulated that it represents true lactoyl-CoA dehydratase genes. Analysis of genome arrangements of *lcdABC* was in agreement with this, as all the bacteria within this cluster contained propionate CoA-transferase two genes upstream, whereas other bacteria carrying genes of lower identity to *lcdABC* did not (Supplementary Table S4).

Interestingly, the gut bacterium *C. catus* GD/7 (Lachnospiraceae) also contained two homologues of the lactoyl-CoA dehydratase genes, one of which had a similar genome arrangement to that of *C. propionicum* and *M. elsdenii* (Supplementary Table S4). *C. catus* has been reported to produce butyrate and propionate, whereas *Coprococcus eutactus* and *Coprococcus comes* produce butyrate together with formate or lactate, respectively, as main fermentation products (Ezaki, 2009a). We tested different *Coprococcus* strains for their ability to utilise lactate for growth. *C. eutactus* ATCC 27759 and *C. comes* ATCC 27758 could not grow on lactate, but *C. catus* GD/7 was able to grow with 25 mM lactate on YCFA medium containing 30 mM acetate. Propionate became the main product and substantial lactate utilisation also occurred in the presence of 10 mM fructose, whereas growth on fructose in the absence of lactate led to the production of butyrate and net consumption of acetate (Figure 2). In order to determine the pathway used for lactate utilisation, 25 mM DL lactate with additional universally ^{13}C-labelled lactate at 10 molar % excess was added to the batch culture medium. All three labelled carbons are expected to appear in propionate when the acrylate pathway is used to convert lactate to propionate, whereas 50% of the propionate formed should contain only two labelled carbons when the succinate pathway is used (Gottschalk, 1979; Counotte *et al.*, 1981). On this basis, it was estimated

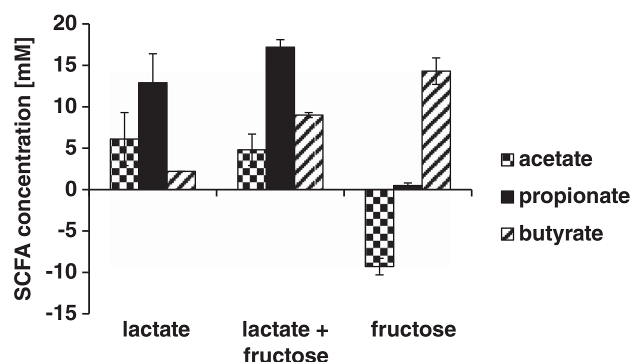

Figure 2 Fermentation acid profiles for *C. catus* GD/7 grown (24 h) on basal YCFA medium with 30 mM acetate, supplemented with either lactate (25 mM), fructose (10 mM) or both substrates (average and s.d. of triplicate experiments).

that 94–96% of the ^{13}C from lactate flowed to propionate via the acrylate pathway, regardless of whether growth was with lactate alone or with lactate and fructose. Probe Ccat62, specific for *C. catus*, was designed and employed to determine the abundance of the *C. catus* group in faecal samples from 10 healthy volunteers (5 females and 5 males) between 24 and 60 years of age. *C. catus* was detected in samples from 9 out of 10 volunteers at mean populations of 2.02×10^7 cells g^{-1}, averaging 0.035% of total bacteria detected with the broad Eub338 probe.

To establish how widespread the acrylate pathway is within the human gut microbiota, the human metagenomic data set from Qin *et al*. (2010), comprising data from 124 individuals (85 healthy, overweight and obese subjects and 39 IBD patients), was searched to identify genes with similarity to the *lcdA* gene from *C. propionicum*. Thirty-four hits with over 50% identity were found, with most of them falling within a cluster related to *C. propionicum*. Other sequences were closely related to *M. elsdenii* or *C. catus*, and one sequence (scaffold12607 3 V1 CD15) was not closely related to reference sequences (Figure 3, Supplementary Table S5). Thus, it appears that this pathway for propionate formation is present in a very restricted number of human gut bacteria. Degenerate primers designed against *lcdA* were employed to investigate whether they could specifically amplify the gene from a human faecal sample. Clone library analysis of 78 clones revealed that the primers showed limited cross-specificity to other genes (9 clones carried genes not closely related to *lcdA*). Three types of novel *lcdA* genes clustering with the oral isolate *Clostridium* sp. MSTE9gi and *C. propionicum* were found

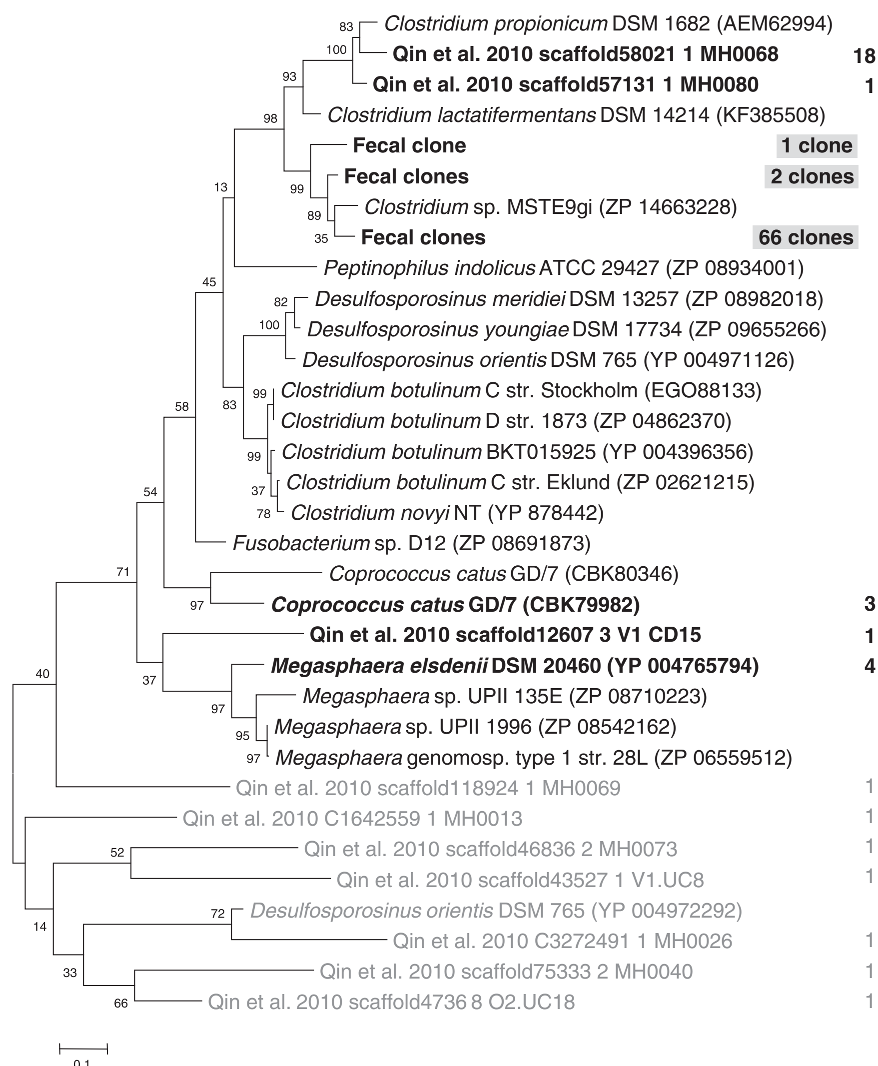

Figure 3 Phylogenetic tree of deduced protein sequence of lactoyl-CoA dehydratase gene *lcdA*. Database matches to *C. propionicum* (AEM62994) of at least 60% as well as the top hit below this cutoff (*Desulfosporosinus orientis* YP_004972292, 41%) are shown. The *lcdA* gene fragment of *C. lactatifermentans* DSM 14214 was sequenced in this study. The number of hits within the metagenomic data set of Qin *et al*. (2010) with at least 50% identity is indicated to the right (sequences with at least 95% identity were grouped; for a list of all hits see Supplementary Table S5). Sequences from clone library analysis of a human faecal sample ($\geqslant$95% identity grouped) are shaded. Grey tree branches indicate genes with lower identity to *C. propionicum* assumed not to be *bona fide lcdA* genes.

(Figure 3). *C. lactatifermentans* is a chicken strain phylogenetically closely related to *C. propionicum* that utilises lactate and produces propionate (Rainey *et al.*, 2009). In order to investigate whether one of the unknown sequences represents *C. lactatifermentans*, we cloned and sequenced the *lcdA* gene fragment from *C. lactatifermentans* DSM 14214. The sequence clustered with *C. propionicum*-related sequences but did not correspond to any of the unknown sequences (Figure 3).

Propanediol pathway

The propanediol pathway has been detected in the phylogenetically distant bacteria *Salmonella enterica* serovar Typhimurium (Bobik *et al.*, 1999) and *R. inulinivorans* (Scott *et al.*, 2006), suggesting that it might be present in other gut bacteria. The gene *pduP*, encoding a CoA-dependent propionaldehyde dehydrogenase that converts propionaldehyde to propionyl-CoA, was investigated for its suitability as a marker for this pathway. Blastp matches of *R. inulinivorans* PduP (ABC25528) of over 70% identity were found for several strains, most of which belonged to human gut *Lachnospiraceae* bacteria related to *R. obeum* (Figure 4). These bacteria are not reported to produce propionate (*R. obeum* produces acetate, *R. torques* produces lactate and acetate and *R. gnavus* produces acetate and formate on PYG medium, Ezaki, 2009b). However, propionate production via this pathway is dependent on the carbohydrate available for growth in other bacteria, with fucose and rhamnose reported as being propionigenic (Scott *et al.*, 2006; Saxena *et al.*, 2010). We therefore tested growth and fermentation product formation of *R. obeum* A2-162

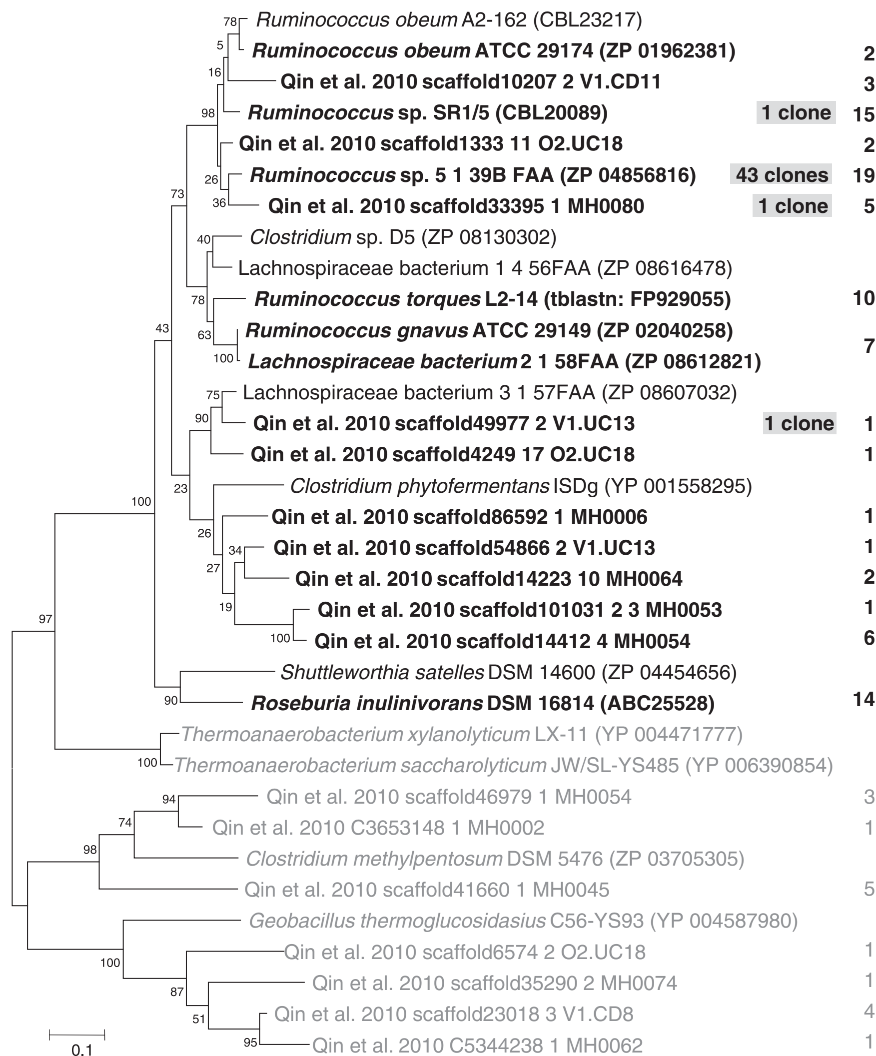

Figure 4 Phylogenetic tree of deduced protein sequence of CoA-dependent propionaldehyde dehydrogenase *pduP*. Database matches to *R. inulinivorans* (ABC25528) of at least 60% as well as the top hits below this cutoff (*Clostridium methylpentosum* ZP_03705305, 57%; *Geobacillus thermoglucosidasius* YP_004587980, 54%) are shown. The number of hits within the metagenomic data set of Qin *et al.* (2010) with at least 55% identity is indicated to the right (sequences with at least 95% identity were grouped; for a list of all hits see Supplementary Table S6). Numbers of sequences from clone library analysis of a human faecal sample (⩾95% identity grouped) are shaded. Grey tree branches indicate gene with lower identity to *R. inulinivorans* assumed not to be *bona fide pduP* genes.

in the presence of those sugars compared with glucose (OD_{650} at 24 h 0.38 ± 0.02 on rhamnose and 0.96 ± 0.06 on fucose vs 1.08 ± 0.04 on glucose). Fermentation acids produced on glucose were mainly acetate, formate and lactate (20.9 ± 4.8, 17.2 ± 1.3 and 4.2 ± 0.1 mM), with little propionate (0.2 ± 0.1 mM) detected. On fucose, *R. obeum* A2-162 revealed a clear switch to propionate (19.9 ± 0.8 mM) along with other fermentation products (26.5 ± 1.0 mM acetate, 11.4 ± 1.0 mM formate and 3.7 ± 0.1 mM lactate). On rhamnose, *R. obeum* formed 10.6 ± 1.4 mM propionate in addition to other fermentation products (18.8 ± 2.9 mM acetate, 8.1 ± 0.8 mM formate and 2.0 ± 0.1 mM lactate), thus providing strong evidence that the propanediol pathway is indeed functional in this bacterium.

The Qin *et al*. (2010) metagenomic database was searched for genes similar to PduP from *R. inulinivorans* (ABC25528) to investigate how widespread this pathway is within the gut microbial community. In total, 106 hits with at least 55% identity were found. Ninety metagenomic hits had over 70% identity to *R. inulinivorans* PduP, with 67 displaying over 95% identity to known bacteria (Figure 4, Supplementary Table S6). Twenty-three sequences distributed across 10 phylotypes did not correspond to known bacteria and thus represent novel strains carrying this pathway. The sequences with over 70% identity to the known PduP gene of *R. inulinivorans* formed a tight phylogenetic cluster (Figure 4), indicating that those genes represent *bona fide pduP* genes.

To enable a more targeted analysis of the pathway, degenerate primers were designed against *pduP* and used to amplify the gene from a human faecal sample. Forty-six clones were analysed, 43 of which were closely related to the sequence of *Ruminococcus* sp. 5_1_39B FAA, with three singletons closely related to *Ruminococcus* sp. SR1/5 and two sequences from the Qin *et al*. data set, respectively (Figure 4). As it was difficult to find highly specific sequence regions for degenerate primer design for this gene, primers were also designed for adjacent gene *pduQ* (encoding propanol dehydrogenase) to establish whether this gene could be used as an alternative marker gene. The Qin *et al*. (2010) data set was mined and a clone library was prepared from the same faecal sample as used for *pduP*. Comparison of the metagenomic hits revealed good agreement for both genes (Supplementary Figure S1, Supplementary Table S7). PduQ clone libraries were again dominated by *Ruminococcus* sp. 5_1_39B FAA, but 23 hits were also found for a metagenomic sequence only once detected in the *pduP* clone library (Supplementary Figure S1).

Discussion

We have employed a combination of genomic analysis and microbial physiology to investigate pathways of microbial propionate formation in the human gut microbiota. An overview of the pathway distribution in dominant human gut bacteria is given in Figure 5. The succinate pathway appears to be the most abundant route for propionate formation, as it is present in the abundant phylum Bacteroidetes as well as in several Negativicutes bacteria. Furthermore, tblastn analysis of the metagenomic data set of Qin *et al*. (2010) revealed 478 matches of at least 60% to the *V. parvula* MmdA gene (ZP_06259922, data not shown). This number likely underestimates the number of true hits, as the level of sequence identity between the Negativicutes and Bacteroidetes genes is around 60% (Table 2). Most of the pathway reactions are shared with central carbon metabolism pathways (conversions within the citrate cycle, amino acid degradation and so on) but the decarboxylation of methylmalonyl-CoA to propionyl-CoA appears to be specific to the succinate pathway and it was hoped that the corresponding gene could be taken as diagnostic for this pathway. Sequences amplified with the degenerate *mmdA* primers were almost exclusively related to Bacteroidetes and no *mmdA* genes were recovered from Negativicutes despite evidence that the genes were amplified well from purified DNA of representative Negativicutes (Supplementary Table S1). This is presumably due to the fact that Bacteroidetes are more abundant than Negativicutes in the human gut microbiota and also carry several copies of the gene (see below). Transcarboxylases, decarboxylases and carboxylases form part of a biotin-enzyme family (Lombard and Moreira, 2011) and the methylmalonyl-CoA decarboxylase alpha subunit (*mmdA*) from *V. parvula* shares similarity with the 12S subunit of methylmalonyl-CoA transcarboxylase of the dairy strain *Propionibacterium freudenreichii* (Kumar Bhat and Berger, 2007) and with the β-subunit of propionyl-CoA carboxylases. Many strains of *Bacteroides* carry several genes closely related to MmdA (for example, both *B. thetaiotaomicron* VPI-5482 and *B. fragilis* YCH46 have three genes with 58–62% identity to MmdA from *V. parvula*, Supplementary Table S2). Although there is no evidence for propionate carboxylation to methylmalonyl-CoA in Bacteroidetes, we did detect biotin carboxylase (BC) domains, which are considered to be present only in carboxylases, next to two *Bacteroides mmdA* genes (*B. thetaiotaomicron* VPI_5482 NP_810362 and NP_810828 and *B. fragilis* YCH46 YP_098894 and YP_100806). This is in contrast to an earlier report that concluded that BC domains were not associated with propionyl-CoA carboxylase genes in Bacteroidetes genomes (Lombard and Moreira, 2011). The presence of multiple *mmdA* gene copies, some of which might not encode true decarboxylases, therefore precluded assessment of the succinate pathway for propionate formation by degenerate PCR targeting the *mmdA* gene. As the phylogenetic distribution of this pathway is fairly

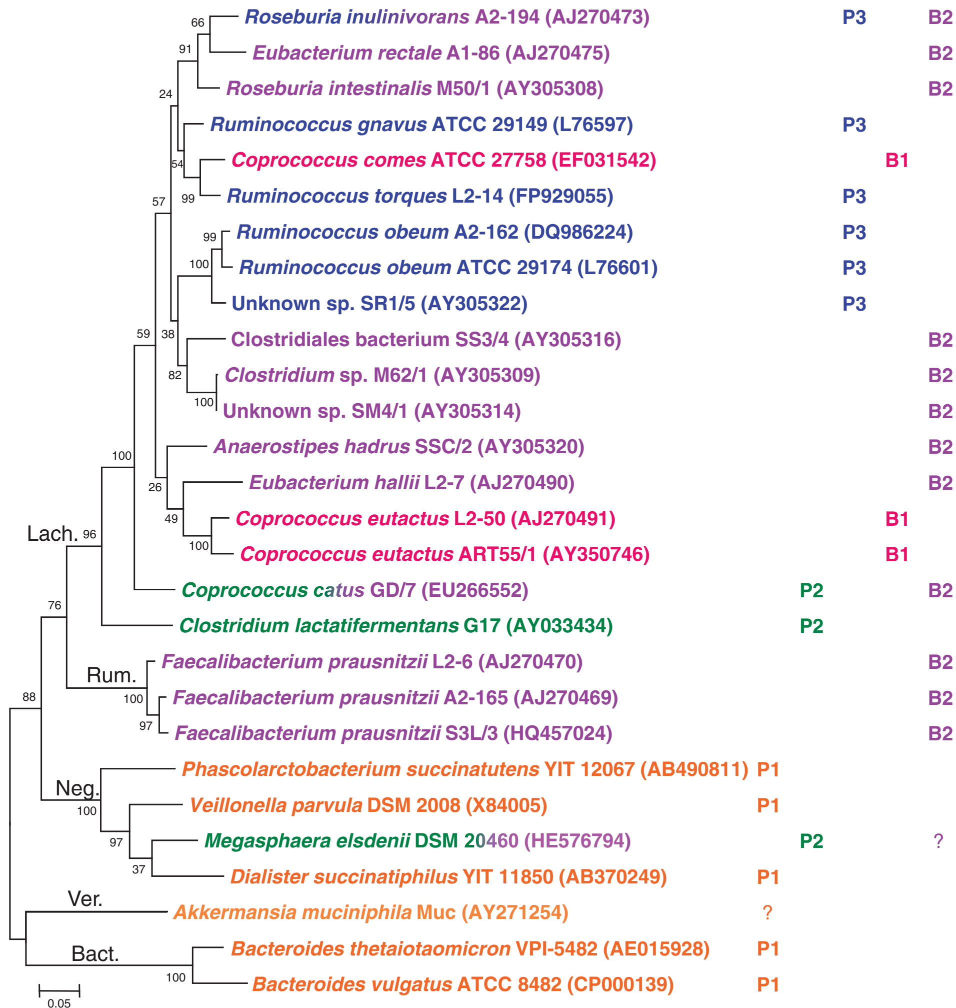

Figure 5 Distribution of different pathways for propionate and butyrate formation in dominant human gut bacteria, based on genome searches of corresponding genes (Table 2; note that no sequence information is currently available for *C. lactatifermentans*), metagenomic mining (Figures 3 and 4) and validation of degenerate primers (Supplementary Table S1). *C. lactatifermentans* and *M elsdenii* originate from animal hosts (chicken and sheep, respectively). They are included as closely related bacteria are carriers of the acrylate pathway based on human metagenome mining (Figure 3). 16S rRNA sequences were obtained from the Ribosomal Database Project (Cole *et al.*, 2009). Phylogenetic assignment—Lach.: Lachnospiraceae, Rum.: Ruminococcaceae, Neg.: Negativicutes, Ver.: Verrucomicrobia, Bact.: Bacteroidetes. P1–P3, B1–B2: Propionate and butyrate pathways as per Table 2. Question marks indicate the presence of genes with ⩾50% sequence identity (Table 2). As *M. elsdenii* is known to produce butyrate and *A. munciniphila* propionate, this has been taken into consideration for the assignment of pathways.

coherent, targeting of Bacteroidetes and Negativicutes 16S rRNA genes offers a more satisfactory strategy for quantification of bacteria carrying this important pathway. Although *M. elsdenii* has an *mmdA* homologue, it lacks other pathway genes, suggesting that the *mmdA*-related gene product performs another function in this species. *M. elsdenii* is, however, able to produce propionate via the acrylate pathway.

The acrylate pathway for propionate formation was found to have a very limited distribution within human gut bacteria, and the genera shown to carry it in this study belong to different Firmicutes families. The most abundant lactoyl-CoA dehydratase sequences were most closely related to the Lachnospiraceae (Clostridial cluster XIVb) bacterium *C. propionicum*. Only three further sequence types were found, related to *C. catus* (Lachnospiraceae), *M. elsdenii* (Negativicutes), or not related to a cultured isolate. The abundance of these bacteria appears to be quite low, based on both the limited number of metagenomic matches found and the enumeration of *C. catus* by FISH analysis. Although we have demonstrated the operation of the acrylate pathway here for the first time in a human colonic isolate, *C. catus* GD/7, this pathway seems unlikely to have a dominant role in propionate formation in the human gut. The propanediol pathway, however, appears to be more widespread within the gut community and is present in some relatively abundant bacteria. The phylogenetic analysis indicates that most, if not all, bacteria carrying

this pathway belong to the Lachnospiraceae related to *Ruminococcus* spp. (several of the misclassified ruminococci within the Lachnospiraceae are being reclassified to the genus *Blautia*, Liu *et al.*, 2008) and *R. inulinivorans*. This pathway is likely to have an important role in propionate fermentation from deoxy sugars. One of these, fucose, is a major component of host-derived glycans, suggesting that this pathway could be indicative of the ability to utilise host-derived substrates. *A. muciniphila* is a mucin-degrading bacterium originating from the human gut that produces propionate (Derrien *et al.*, 2004). Its genome contains a gene with low-level (58%) protein sequence identity to PduQ (Supplementary Figure S1); however, the closest match to PduP only has 24% protein sequence identity with a coverage of 56% (data not shown); thus, it appears that this bacterium does not harbour the propanediol pathway. No matches to LcdA were found either, but a gene with 52% protein sequence identity to MmdA is present (Table 2). The *mmdA* degenerate primer binding sites in this gene are only partially conserved (data not shown) and it remains to be established whether *A. muciniphila* uses this pathway for propionate generation. Rhamnose is another deoxy sugar that may lead to propionate formation via the propanediol pathway, and increased serum propionate levels in response to rhamnose ingestion have been reported in humans (Vogt *et al.*, 2004). In addition to the type of carbohydrate available to the microbiota, other factors also have to be considered with regard to propionate production in the gut. Thus, pH is an important determinant in the competition between Bacteroidetes and Firmicutes (Walker *et al.*, 2005; Duncan *et al.*, 2009), and the level of propionate production in Bacteroidetes is dependent on carbon dioxide levels (Macfarlane and Gibson, 1997).

The *lcdA* and *pduP* gene degenerate primers designed here for the acrylate and propanediol pathways should prove valuable in future for probing the phylogenetic diversity of propionate producers in different individuals—for example, by using signature-tagged versions of the primers in conjunction with pyrosequencing. It should be recognised, however, that the primers may not amplify genes from the different phylogenetic groups in a precisely quantitative manner, as sequence variations can be expected to lead to some variation in the efficiency of amplification. Thus, it has to be decided on a case-by-case basis, depending on the respective gene sequences, the phylogenetic distribution and how well the bacteria carrying the pathway are characterised, whether targeting the functional gene or specific phylogenetic groups via a phylogenetic marker gene (such as the 16S rRNA gene) is the better option for quantification of functional groups.

It is unusual for isolated anaerobic bacteria to produce both propionate and butyrate as major products from the fermentation of sugars (Chen *et al.*, 1977). Table 2 and Figure 5 confirm that most human colonic bacteria that possess diagnostic genes for butyrate synthesis (either butyrate kinase or butyryl-CoA:acetate CoA-transferase) lack genes concerned with propionate formation, and vice versa. Thus, these two products represent alternative 'hydrogen sinks' whose pathways have apparently evolved in different phylogenetic groups of anaerobic bacteria. Thus, there appears to be no evidence at present for the succinate pathway in the Lachnospiraceae or Actinobacteria, or for butyrate synthetic pathways in Bacteroidetes or Actinobacteria. Two exceptions were, however, found that are of some interest. *C. catus* was shown here to be able to utilise lactate via the acrylate pathway to produce propionate, although forming butyrate via the butyryl-CoA:acetate CoA-transferase route when grown on fructose. Meanwhile, *R. inulinivorans* was shown previously to utilise the propanediol pathway for propionate formation from fucose, although producing butyrate from glucose. Growth on fucose resulted in a dramatic change in the pattern of gene expression (Scott *et al.*, 2006). In *Salmonella*, acquisition of the propanediol pathway is estimated to require many genes, encoding not only the pathway enzymes themselves but also the associated proteins that are involved in polyhedral body formation and synthesis of required cofactors (Bobik *et al.*, 1999). Acquisition of additional fermentation pathways, presumably by horizontal gene transfer, may have arisen from selection for an expansion in the ability to utilise alternative substrates in the highly competitive environment of the colon.

In conclusion, the targeted analysis of three known biochemical pathways for propionate formation provides valuable information on their likely importance within the human colonic microbiota that will, in conjunction with improved knowledge of the underlying physiology of the respective bacteria and further studies on the level of gene expression, help to design strategies to boost the formation of this important metabolite in the human gut.

Conflict of Interest

The authors declare no conflict of interest.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. (1990). Basic local alignment search tool. *J Mol Biol* **215**: 403–410.

Arora T, Sharma R, Frost G. (2011). Propionate. Anti-obesity and satiety enhancing factor? *Appetite* **56**: 511–515.

Bartosch S, Fite A, Macfarlane GT, McMurdo MET. (2004). Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl Environ Microbiol* **70**: 3575–3581.

Belenguer A, Duncan SH, Holtrop G, Anderson SE, Lobley GE, Flint HJ. (2007). Impact of pH on lactate formation and utilization by human fecal microbial communities. *Appl Environ Microbiol* **73**: 6526–6533.

Berni Canani R, Di Costanzo M, Leone L. (2012). The epigenetic effects of butyrate: potential therapeutic implications for clinical practice. *Clin Epigenetics* **4**: 4.

Bobik TA, Havemann GD, Busch RJ, Williams DS, Aldrich HC. (1999). The propanediol utilization (*pdu*) operon of *Salmonella enterica* serovar Typhimurium LT2 includes genes necessary for formation of polyhedral organelles involved in coenzyme B12-dependent 1,2-propanediol degradation. *J Bacteriol* **181**: 5967–5975.

Bourriaud C, Robins RJ, Martin L, Kozlowski F, Tenailleau E, Cherbut C *et al.* (2005). Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. *J Appl Microbiol* **99**: 201–212.

Bryant MP. (1956). The characteristics of strains of *Selenomonas* isolated from bovine rumen contents. *J Bacteriol* **72**: 162–167.

Chen J, Cummins CS, Johnson JL, Smibert RM, Smith LD, Wilkins TD. (1977). *Anaerobe Laboratory Manual*. V.P.I. Anaerobe Laboratory Virginia Polytechnic Institute and State University: Blacksburg, Virginia, USA.

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ *et al.* (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* **37**: D141–D145.

Counotte GHM, Prins RA, RHAM Janssen, De Bie MJA. (1981). Role of *Megasphaera elsdenii* in the fermentation of DL-[2-13C]lactate in the rumen of dairy cattle. *Appl Environ Microbiol* **42**: 649–655.

Dabek M, McCrae SI, Stevens VJ, Duncan SH, Louis P. (2008). Distribution of β-glucosidase and β-glucuronidase activity and of β-glucuronidase gene *gus* in human colonic bacteria. *FEMS Microbiol Ecol* **66**: 487–495.

Daly K, Shirazi-Beechey SP. (2003). Design and evaluation of group-specific oligonucleotide probes for quantitative analysis of intestinal ecosystems: their application to assessment of equine colonic microflora. *FEMS Microbiol Ecol* **44**: 243–252.

Del Dot T, Osawa R, Stackebrandt E. (1993). *Phascolarctobacterium faecium* gen. nov, spec. nov., a novel taxon of the *Sporomusa* group of bacteria. *Syst Appl Microbiol* **16**: 380–384.

Derrien M, Vaughan EE, Plugge CM, de Vos WM. (2004). *Akkermansia municiphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol* **54**: 1469–1476.

Dimroth P, von Ballmoos C. (2008). ATP synthesis by decarboxylation phosphorylation. *Results Probl Cell Differ* **45**: 153–184.

Duncan SH, Louis P, Thomson JM, Flint HJ. (2009). The role of pH in determining the species composition of the human colonic microbiota. *Environ Microbiol* **11**: 2112–2122.

Ezaki T. (2009a). Genus VII. *Coprococcus* Holdemann and Moore 1974, 260[AL]. In: *Bergey's Manual of Systematic Bacteriology, Volume Three the Firmicutes*. Springer: Dordrecht, Heidelberg, London, New York, pp 940–941.

Ezaki T. (2009b). Genus I. *Ruminococcus* Sijpesteijn 1948, 152[AL]. In: *Bergey's Manual of Systematic Bacteriology, Volume Three the Firmicutes*. Springer, pp 1016–1018.

Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. (2012a). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* **3**: 289–306.

Flint HJ, Scott KP, Louis P, Duncan SH. (2012b). The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* **9**: 577–589.

Gilmour M, Flint HJ, Mitchell WJ. (1994). Multiple lactate dehydrogenase activities of the rumen bacterium *Selenomonas ruminantium*. *Microbiology* **140**: 2077–2084.

Gottschalk G. (1979). *Bacterial Metabolism*. Springer Verlag: New York, Heidelberg, Berlin.

Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer R-. (2008). Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther* **27**: 104–119.

Hetzel M, Brock M, Selmer T, Pierik AJ, Golding BT, Buckel W. (2003). Acryloyl-CoA reductase from *Clostridium propionicum*: an enzyme complex of propionyl-CoA dehydrogenase and electron-transferring flavoprotein. *Eur J Biochem* **270**: 902–910.

Hino T, Kuroda S. (1993). Presence of lactate dehydrogenase and lactate racemase in *Megasphaera elsdenii* grown on glucose or lactate. *Appl Environ Microbiol* **59**: 255–259.

Hosseini E, Grootaert C, Verstraete W, Van de Wiele T. (2011). Propionate as a health-promoting microbial metabolite in the human gut. *Nutr Rev* **69**: 245–258.

Huder JB, Dimroth P. (1993). Sequence of the sodium ion pump methylmalonyl-CoA decarboxylase from *Veillonella parvula*. *J Biol Chem* **268**: 24564–24571.

Janssen PH. (1992). Growth yield increase and ATP formation linked to succinate decarboxylation in *Veillonella parvula*. *Arch Microbiol* **157**: 442–445.

Kandasamy V, Vaidyanathan H, Djurdjevic I, Jayamani E, Ramachandran KB, Buckel W *et al.* (2013). Engineering *Escherichia coli* with acrylate pathway genes for propionic acid synthesis and its impact on mixed-acid fermentation. *Appl Microbiol Biotechnol* **97**: 1191–1200.

Kilner J, Waby JS, Chowdry J, Khan AQ, Noirel J, Wright PC *et al.* (2012). A proteomic analysis of differential cellular responses to the short-chain fatty acids butyrate, valerate and propionate in colon epithelial cancer cells. *Mol BioSyst* **8**: 1146–1156.

Kumar Bhat R, Berger S. (2007). New and easy strategy for cloning, expression, purification, and characterization of the 5S subunit of transcarboxylase from *Propionibacterium f. shermanii*. *Prep Biochem Biotechnol* **37**: 13–26.

Liu C, Finegold SM, Song Y, Lawson PA. (2008). Reclassification of *Clostridium coccoides*, *Ruminococcus hansenii*, *Ruminococcus hydrogenotrophicus*,

Ruminococcus luti, Ruminococcus productus and Ruminococcus schinkii as Blautia coccoides gen. nov., comb. nov., Blautia hansenii comb. nov., Blautia hydrogenotrophica comb. nov., Blautia luti comb. nov., Blautia producta comb. nov., Blautia schinkii comb. nov. and description of Blautia wexlerae. *Int J Syst Evol Microbiol* **58**: 1896–1902.

Lombard J, Moreira D. (2011). Early evolution of the biotin-dependent carboxylase family. *BMC Evol Biol* **11**: 232.

Lopez-Siles M, Khan TM, Duncan SH, Harmsen HJM, Garcia-Gil LJ, Flint HJ. (2012). Cultured representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii* can utilize pectin, uronic acids, and host-derived substrates for growth. *Appl Environ Microbiol* **78**: 420–428.

Louis P, Duncan SH, McCrae SI, Millar J, Jackson MS, Flint HJ. (2004). Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. *J Bacteriol* **186**: 2099–2106.

Louis P, Flint HJ. (2009). Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* **294**: 1–8.

Louis P, Young P, Holtrop G, Flint HJ. (2010). Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environ Microbiol* **12**: 304–314.

Lyons E, Freeling M. (2008). How to usefully compare homologous plant genes and chromosomes as DNA sequences. *Plant J* **53**: 661–673.

Macfarlane GT, Gibson GR. (1997). Carbohydrate fermentation, energy transduction and gas metabolism in the human large intestine. In: *Gastrointestinal Microbiology* Vol. I. Chapman and Hall: London, pp 269–318.

Macy JM, Probst I. (1979). The biology of gastrointestinal bacteroides. *Annu Rev Microbiol* **33**: 561–594.

Marchandin H, Teyssier C, Campos J, Jean-Pierre H, Roger F, Gay B *et al.* (2010). *Negativicoccus succinicivorans* gen. nov., sp. nov., isolated from human clinical samples, emended description of the family *Veillonellaceae* and description of *Negativicutes* classis nov., *Selenomonadales* ord. nov. and *Acidaminococcaceae* fam. nov. in the bacterial phylum *Firmicutes*. *Int J Syst Evol Microbiol* **60**: 1271–1279.

McIntosh FM, Maison N, Holtrop G, Young P, Stevens VJ, Ince J *et al.* (2012). Phylogenetic distribution of genes encoding β-glucuronidase activity in human colonic bacteria and the impact of diet on faecal glycosidase activities. *Environ Microbiol* **14**: 1876–1887.

Miyazaki K, Martin JC, Marinsek-Logar R, Flint HJ. (1997). Degradation and utilization of xylans by the rumen anaerobe *Prevotella bryantii* (formerly *P. ruminicola* subsp. *brevis*) B(1)4. *Anaerobe* **3**: 373–381.

Morrison DJ, Mackay WG, Edwards CA, Preston T, Dodson B, Weaver LT. (2006). Butyrate production from oligofructose fermentation by the human faecal flora: what is the contribution of extracellular acetate and lactate? *Br J Nutr* **96**: 570–577.

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C *et al.* (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**: 59–65.

Rainey FA, Hollen BJ, Small A. (2009). Genus I. *Clostridium* Prazmowski 1880, 23[AL]. In: *Bergey's Manual of Systematic Bacteriology, Volume Three the Firmicutes*. Springer: Dordrecht, Heidelberg, London, New York, pp 738–828.

Richardson AJ, Calder AG, Stewart CS, Smith A. (1989). Simultaneous determination of volatile and non-volatile acidic fermentation products of anaerobes by capillary gas chromatography. *Lett Appl Microbiol* **9**: 5–8.

Satokari RM, Vaughan EE, Akkermans ADL, Saarela M, de Vos WM. (2001). Bifidobacterial diversity in human feces detected by genus-specific PCR and denaturing gradient gel electrophoresis. *Appl Environ Microbiol* **67**: 504–513.

Saxena RK, Anand P, Saran S, Isar J, Agarwal L. (2010). Microbial production and applications of 1,2-propanediol. *Indian J Microbiol* **50**: 2–11.

Scott KP, Martin JC, Campbell G, Mayer C-, Flint HJ. (2006). Whole-genome transcription profiling reveals genes up-regulated by growth on fucose in the human gut bacterium '*Roseburia inulinivorans*'. *J Bacteriol* **188**: 4340–4349.

Seeliger S, Janssen PH, Schink B. (2002). Energetics and kinetics of lactate fermentation to acetate and propionate via methylmalonyl-CoA or acrylyl-CoA. *FEMS Microbiol Lett* **211**: 65–70.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**: 2731–2739.

Ulven T. (2012). Short-chain free fatty acid receptors FFA2/GPR43 and FFA3/GPR41 as new potential therapeutic targets. *Front Endocrinol (Lausanne)* **3**: 111.

Vinolo MAR, Rodrigues HG, Nachbar RT, Curi R. (2011). Regulation of inflammation by short chain fatty acids. *Nutrients* **3**: 858–876.

Vogt JA, Pencharz PB, Wolever TMS. (2004). L-rhamnose increases serum propionate in humans. *Am J Clin Nutr* **80**: 89–94.

Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, Flint HJ. (2005). pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol* **71**: 3692–3700.

Watanabe Y, Nagai F, Morotomi M. (2012). Characterization of *Phascolarctobacterium succinatutens* sp. nov., an asaccharolytic, succinate-utilizing bacterium isolated from human feces. *Appl Environ Microbiol* **78**: 511–518.

Wood J, Scott KP, Avguštin G, Newbold CJ, Flint HJ. (1998). Estimation of the relative abundance of different *Bacteroides* and *Prevotella* ribotypes in gut samples by restriction enzyme profiling of PCR-amplified 16S rRNA gene sequences. *Appl Environ Microbiol* **64**: 3683–3689.

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