Contributions of the microbial hydrogen economy to colonic homeostasis

Franck Carbonero, Ann C. Benefiel and H. Rex Gaskins

Abstract | Colonic gases are among the most tangible features of digestion, yet physicians are typically unable to offer long-term relief from clinical complaints of excessive gas. Studies characterizing colonic gases have linked changes in volume or composition with bowel disorders and shown hydrogen gas (H_2), methane, hydrogen sulphide, and carbon dioxide to be by-products of the interplay between H_2-producing fermentative bacteria and H_2 consumers (reductive acetogens, methanogenic archaea and sulphate-reducing bacteria [SRB]). Clinically, H_2 and methane measured in breath can indicate lactose and glucose intolerance, small intestinal bacterial overgrowth and IBS. Methane levels are increased in patients with constipation or IBS. Hydrogen sulphide is a by-product of H_2 metabolism by SRB, which are ubiquitous in the colonic mucosa. Although higher hydrogen sulphide and SRB levels have been detected in patients with IBD, and to a lesser extent in colorectal cancer, this colonic gas might have beneficial effects. Moreover, H_2 has been shown to have antioxidant properties and, in the healthy colon, physiological H_2 concentrations might protect the mucosa from oxidative insults, whereas an impaired H_2 economy might facilitate inflammation or carcinogenesis. Therefore, standardized breath gas measurements combined with ever-improving molecular methodologies could provide novel strategies to prevent, diagnose or manage numerous colonic disorders.

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Introduction

Despite a long history of dealing with bowel gas and a basic accounting of its components and their origins, our understanding of the mechanisms by which these gases are produced and metabolized remains rudimentary. Human colonic gases are comprised of hydrogen (H_2), carbon dioxide (CO_2), methane (CH_4), nitrogen and oxygen as well as several odiferous trace gases. Nitrogen and oxygen are exclusively derived from swallowed air; on the other hand—making up ~74% of the flatus—H_2, CO_2 and CH_4 are produced solely by colonic microbes, which ferment dietary components that escape digestion by host enzymes and endogenous substrates derived from the colonic mucosa.[1] Other microbial gases are present in flatus in trace concentrations; for example, hydrogen sulphide (H_2S) at 1.06 μmol/l, methanethiol at 0.21 μmol/l and dimethyl sulphide at 0.08 μmol/l in one study.[1] Intracolonic concentrations of these trace gases might be substantially higher than detected in flatus, as H_2S and methanethiol rapidly permeate the colonic mucosa and are detoxified.[2] Marked individual differences also exist in the proportional composition of major colonic gases.[1,3]

The accumulation of gas in the colonic lumen is dependent on the interplay between various microbial metabolic pathways and host physiology. Most CO_2, and 30–40% of H_2 and CH_4, are absorbed by the colonic mucosa and recirculated in the blood, the remaining being metabolized (H_2 only) or passed as flatus.[4] H_2 metabolism—reflecting the balance between H_2-producing (hydrogenogenic) bacteria and H_2-utilizing (hydrogenotrophic) microbes—has a primary influence on the final composition of colonic gases. Here, we review the microbiology of H_2 production and utilization, and mechanisms by which these pathways influence colonic health and disease.

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Competing interests
The authors declare no competing interests.

Colonic gases

Early measurements

Early attempts to measure excretion of colonic gases focused on total volume and composition of flatus gases under baseline and varying dietary conditions (Figure 1).[5–9] Ruge is credited with the first attempts to collect colonic gases from humans in the 1800s, using a glass tube from the anus to a water displacement system beneath a specialized chair.[10,11] In 1942, Beazell and Ivey[8] measured flatus gases from healthy individuals over a 24 h period and determined that the daily excretion rate was in the range of 380–655 ml per day. Kirk demonstrated that total flatus production increased with dietary fibre consumption.[5] In later studies, Steggerda[6] collected an average of 360 ml flatus per day, of which 7.4% was CH_4 and 19.8% H_2, from individuals on a baseline diet of foods typically considered non-flatus-producing (such as boiled eggs, lean beef and apple sauce), demonstrating the microbial origin of these two gases in the colon.[6,12] By adding varying types and amounts of beans to the diet over subsequent 7-day trials—whilst

maintaining the baseline level of fat, protein, carbohydrates and calories—Steggerda also examined the effect of altering the type, but not overall amount, of dietary carbohydrate on production of flatus.[6,12] He concluded that the low-molecular-weight fraction of carbohydrates (monosaccharide, disaccharide and oligosaccharides) were responsible for an increase in overall gas excretion, up to an average of 4,224 ml per day when a commercial pork and bean diet comprised 57% of the diet.[6] Using a constant infusion technique, Levitt and Ingelfinger[13] measured the rate of H_2 and CH_4 production in healthy individuals directly from the intestinal lumen, demonstrating that H_2 was produced primarily in the large intestine of all individuals, correlated with breath excretion levels, and was almost completely dependent upon fermentation of dietary substances. Of the nine study participants, four were CH_4 producers (producing colonic CH_4 after lactose infusion at a rate of 0.5–0.6 ml/min); the remaining participants produced no detectable CH_4.[13]

Less than 20% of carbohydrate remains unabsorbed in people consuming a typical Western diet.[14] Theoretically, with this amount of substrate available for bacterial fermentation and H_2 production occurring at a rate of 340 ml/g of glucose,[15] the potential exists for >13 l of H_2 to be generated daily. However, Strocchi and Levitt[16] measured a mean absolute H_2 production rate during glucose fermentation of 80 ml/g, suggesting that fermentation by stool bacteria might involve metabolic pathways that do not liberate H_2. Hammer,[17] likewise, found H_2 production in flatus after fasting to be very low and in the range of 50–200 ml per 6 h period after ingestion of 12.5 g lactulose. Indeed, H_2 excretion varies markedly with different substrates, throughout the day and among individuals.[6,7,17,18] Although yet to be quantitatively determined, such interindividual differences probably reflect complex interactions between host physiological and microbial factors, including: quantity and type of substrate; the ability of colonic microbes to ferment carbohydrates; the abundance and location of different types of H_2-producing and H_2-consuming microbes; the efficiency of stirring of gut contents; and environmental factors such as pH and sulphate availability.[11] That methane excretion also varies markedly demonstrates the key role of H_2 microbial consumption in determining intraluminal concentrations of this gas.

Current understanding

Whole-body calorimetry demonstrated the dynamic relationship between flatus and breath gas excretion of both H_2 and CH_4.[4] Improvements in means of measuring H_2 from exhaled breath have enabled reliable, noninvasive estimates of colonic H_2 levels in a clinical setting.[19] Gas chromatography systems have now become common for detecting the somewhat low concentrations of H_2 (1–200 parts per million) in breath.[11] Calloway[20] demonstrated changes in respiratory H_2 and CH_4 levels with consumption of gas-forming food such as beans, linking early direct measurements from the lumen or in flatus with levels detectable in breath.

Key points

- The colonic gases hydrogen (H_2), carbon dioxide and methane (CH_4) are end products of microbial fermentation; their concentrations depend on the interplay between host physiology and H_2-producing (hydrogenogenic) and H_2-using (hydrogenotrophic) microbes
- Colonic H_2 production is most readily measured via excretion in breath; clinically, breath H_2 and CH_4 are commonly measured to assess lactose and glucose intolerance and small intestinal bacterial overgrowth, and increasingly IBS
- Improved understanding of microbial H_2 metabolism and its relation to expired gas concentrations will reinforce the breath gas test as a widely applicable, easy and cost-effective diagnostic or prognostic tool
- Use of breath gas tests in diagnosis could enable novel therapeutic or preventative measures for a wide array of colonic diseases
- Although emphasis has been given to the potential inflammatory or carcinogenic properties of colonic gases, emerging evidence suggests these gases might have a beneficial effect in colonic health

Calloway and Murphy[21] replicated the findings of Levitt and Ingelfinger—that a subgroup of individuals seem to produce little to no colonic CH_4—using breath testing.

Although all intestinal CH_4 derives from microbial methanogenesis, estimates of colonic production based on breath measurements should be interpreted with caution, as proportions of both H_2 and CH_4 excreted in breath are influenced by the production rates in the colon; 65% of the gas is excreted in breath at low production rates (<200 ml per day) and 25% at high rates (>500 ml per day).[4] Several studies show that breath H_2 concentrations are lower in CH_4-excretors than non-excretors.[4,22–24] The clinical value of breath testing for colonic gases was further demonstrated by Calloway *et al.*[25] and Levitt and Donaldson[26] who linked an increase in breath H_2 concentration with fermentation in the colon of malabsorbed lactose, suggesting breath H_2 levels as a measure of lactose intolerance. Metz *et al.*[27] compared intestinal lactase activity with symptoms such as abdominal pain and bloating, increases in blood glucose concentration, and breath H_2 production in a group of patients with diarrhoea and found end-expiratory breath H_2 to be as reliable as blood glucose concentration, and better than symptoms, in the diagnosis of lactase deficiency.

Easy to administer, H_2 breath tests (typically following a period of fasting and ingestion of lactulose) have become one of the primary means of detecting small intestinal bacterial overgrowth (SIBO).[28] Theoretically, an early peak in breath H_2 indicates fermentation of lactulose in the small intestine;[11] however, conditions such as rapid intestinal transit, in which lactulose would reach the colon much earlier than is typical, can confound results. In addition, breath H_2 peaks do not always correlate with results of gold-standard tests for SIBO (such as cultures from jejunal aspirate), and high H_2 concentrations can be found in apparently healthy individuals with no other indication of SIBO.[29,30] Although the value of breath testing in detecting SIBO—and even the importance of SIBO in disorders such as IBS, immunodeficiency syndromes and motility disorders—is controversial, breath testing to detect H_2 and CH_4 production in the colon remains a valuable indicator of microbial activity. Early

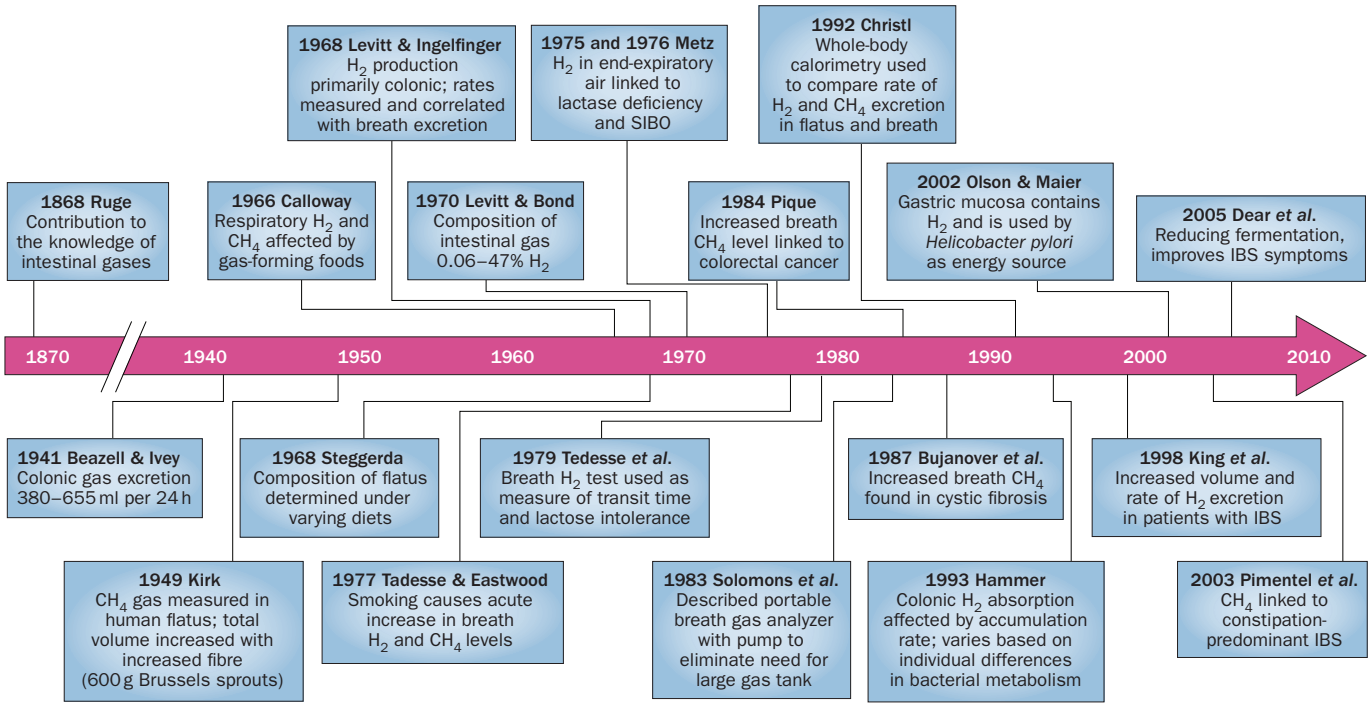

Figure 1 | Milestones in the measurement of colonic gases in breath and flatus. Colonic gases in breath and flatus have been investigated since at least the late 1800s. The clinical utility of breath testing, however, emerged much later in the mid-to-late 1970s when it was demonstrated to be an accurate measurement of disaccharide malabsorption. Since then, the sensitivity and practicality of instrumentation has improved considerably, and the measurement of H_2 and CH_4 in breath is now generally accepted by most gastroenterologists as a reliable indicator of lactose intolerance and SIBO. Its value in evaluating IBS has been controversial, but might be increased by standardization of substrate, sampling frequency and duration and routinely including CH_4 as well as H_2 testing. This noninvasive tool is fairly inexpensive, easy to use and is one of few methods that provides real-time assessment of fermentation and colonic gas physiology. The utility of breath gas analysis could be expanded substantially with greater knowledge of the microbiology of gas production relative to diet or disease states. Abbreviations: CH_4, methane; H_2, hydrogen; SIBO, small intestinal bacterial overgrowth.

studies by Christl confirmed the predictable relationship between H_2 and CH_4 production and breath excretion of these two colonic gases.[4] Likewise, breath testing remains a validated means of determining intestinal transit time[31] and distinguishing two fairly stable phenotypes, excretors versus nonexcretors of CH_4.[32]

A particularly provocative finding regarding the distinction between CH_4-excretors and non-CH_4-excretors is the variation in percentages of CH_4-excretors among different ethnic and racial populations (range 34–87%; summarized in Levitt *et al.*[33]). Black Africans tend to be highly methanogenic compared with North American white or Asian individuals (including those of Indian origin).[34–36] Moreover, the percentage of a given population that is methanogenic seems to remain stable over time.[32,33] However, distinguishing the multifactorial interactions between host ethnicity, environment and diet is difficult. Segal *et al.*[35] reported that the percentage of CH_4-producers was lower in urban (72%) than rural black Africans (84%). Moreover, O'Keefe *et al.*[37] found that the H_2 and CH_4 breath emission patterns of African Americans and white Americans—both consuming a typical Western diet—were more similar to each other than the excretion patterns of native Africans consuming a maize-based diet low in animal-based protein and high in resistant starch.

Microbial guilds in the hydrogen economy

Host colonic cells derive energy from aerobic respiration, in which nutrients are fully oxidized in mitochondria, with oxygen serving as the terminal electron acceptor. Similar to fermentation, the metabolic reactions involved in respiration are catabolic and based on redox reactions (that is, oxidation of one molecule coupled to the reduction of another). In fermentation—the anaerobic process by which most colonic microbes gain energy—nutrient substrates are incompletely oxidized and the reduced fermentation products serve as terminal electron acceptors. In such cases, the amount of energy (ATP) that can be produced depends on the difference in redox potential between the substrate and the reduced end products. Another distinction is that in fermentation the reduced pyridine (NADH) and flavin (FADH) nucleotides must be reoxidized to maintain redox balance, a reaction that is the primary source of H_2 in the colon (Figure 2).

Accordingly, the production of H_2 by hydrogenogenic microbes is crucial to the efficiency of fermentation. However, H_2 accumulation would rapidly lead to a H_2 partial pressure that would thermodynamically restrict further fermentation. Such an outcome typically is prevented by the simultaneous oxidation of H_2 by three groups of hydrogenotrophic (H_2-utilizing) microbes that conserve energy through anaerobic respiration: reductive

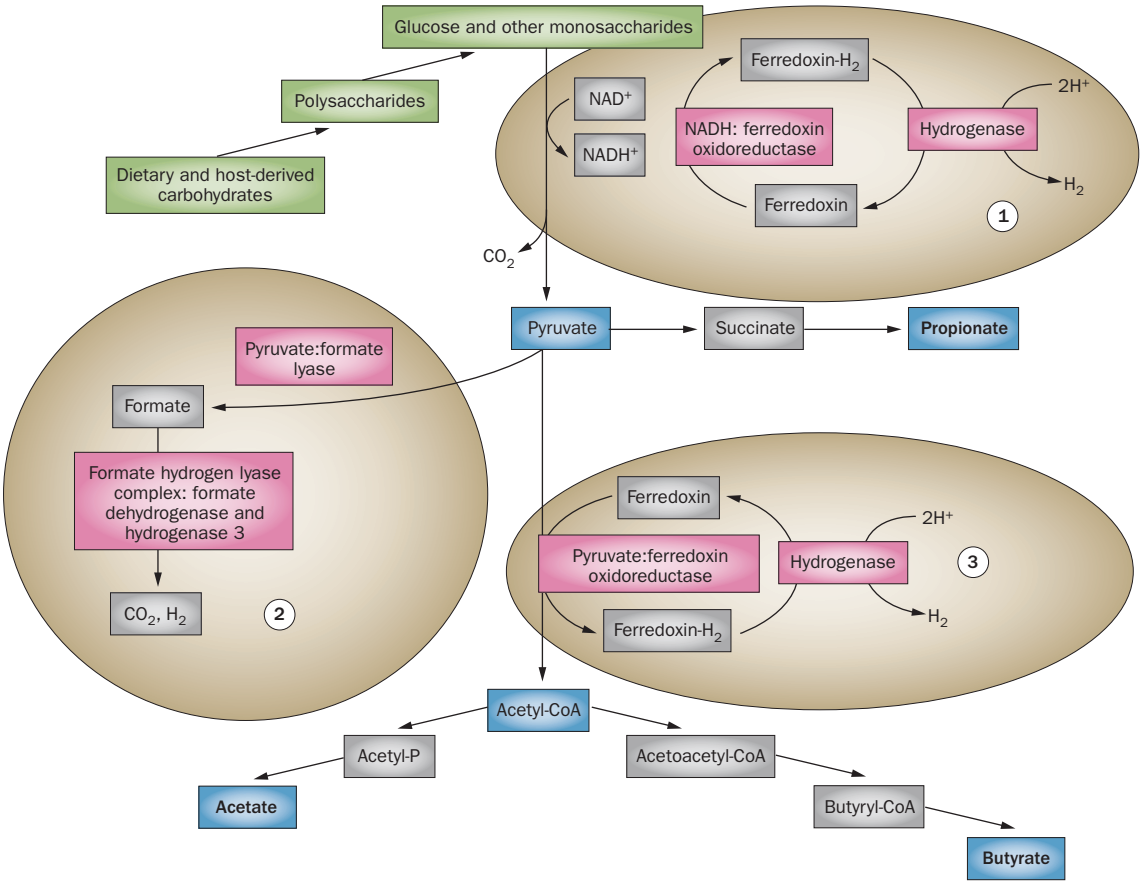

Figure 2 | Biochemical pathways of H_2 production from bacterial fermentation. The primary source of H_2 derives from (1) the reoxidation of reduced pyridine and flavin nucleotides. This pathway is inhibited by a high partial pressure of H_2, whereas the other two pathways of H_2 production are not. These two pathways are (2) the cleavage of pyruvate to formate and subsequent metabolism by formate hydrogen lyase (primarily *Clostridia*), and (3) the generation from pyruvate through the activity of pyruvate: ferredoxin oxidoreductase and hydrogenase (primarily *Enterobacteria*). These biochemical pathways were defined mainly from *in vitro* cultivation of fermentative bacteria. The availability of genomics and molecular-based methods provides opportunities for further metabolic characterization of colonic H_2 production *in situ*. Abbreviations: CO_2, carbon dioxide; H^+, hydrogen ion; H_2, hydrogen.

acetogens, methanogenic archaea and sulphate-reducing bacteria (SRB; Figure 3). Microbial hydrogenotrophy—together with excretion in flatus and breath (15–20% for each route)—results in efficient removal of H_2, and shifts fermentation to more oxidized end products, hence, increasing the energy yield of fermentative microbes.[4] Although the existence of H_2 disposal mechanisms is crucial to colonic fermentation, the extent to which the three metabolic guilds (reductive acetogens, methanogenic archaea, SRB) co-occur in the healthy human colon has been the subject of debate. Reductive acetogenesis has been presented as a facultative H_2 disposal pathway owing to sulphate reduction and methanogenesis being thermodynamically more favourable reactions for this requirement of fermentation.[38] In addition, models of competitive exclusion between methanogenic archaea and SRB in the human colon have been suggested to explain the observed disparities in detectable breath CH_4 excretion and in differential outcomes of culture-dependent approaches.[13,39–48] Culture-based and molecular-based studies have demonstrated that SRB persistently colonize the healthy human colon.[39–41,49]

Of note, the colon is composed of different sites with various physiological and chemical characteristics, which have a role in shaping microbial communities.[50] Specifically, it has been suggested that the more acidic right colon is mainly colonized by reductive acetogens, whereas methanogenic archaea would thrive better in the more neutral pH of the distal colon.[51–54] Indeed, coherent gradients of microbial abundance were demonstrated by a 2011 molecular survey,[39] but all microbial communities were ubiquitous throughout the colon. However, this observation does not preclude regional differentiation, which could also occur on a microscale, enabling acid-intolerant methanogens to grow in microniches exhibiting neutral pH, in the right colon for example.

Hydrogen gas producers

In addition to the reoxidation of reduced pyridine and flavin nucleotides, H_2 can be produced by cleavage of pyruvate to formate and subsequent metabolism by formate hydrogen lyase, or by generation from pyruvate through the activity of pyruvate:ferredoxin oxidoreductase and hydrogenase (Figure 2).[55] With H_2 production

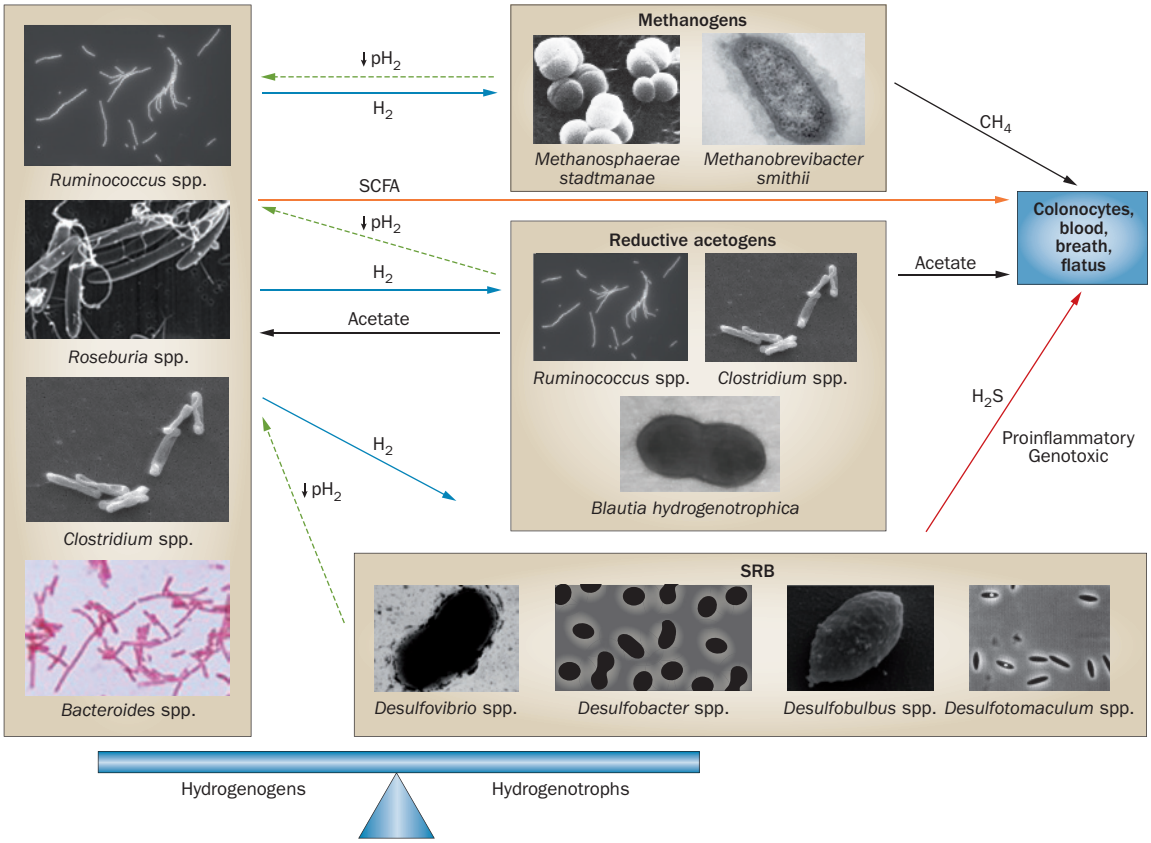

Figure 3 | H_2 gas is inherently produced during microbial fermentation in the human colon. The accumulation of H_2 leads rapidly to a pH_2 that thermodynamically restricts further fermentation. Accordingly, three groups of hydrogenotrophic (H_2-utilizing) microbes possess the crucial ability to simultaneously oxidize H_2, which lowers pH_2 enabling fermentation to proceed. Given the centrality of H_2 production in fermentative pathways, this metabolic feature is almost certainly widespread among colonic bacteria. The genera depicted are among those that have been characterized *in vitro* for H_2 production. Hydrogenotrophic microbes include methanogenic archaea, reductive acetogens and SRB. Abbreviations: CH_4, methane; H_2, hydrogen; pH_2, partial pressure of hydrogen; SCFA, short-chain fatty acids; SRB, sulphate-reducing bacteria. All images shown are microscopy images with the exception of a schematic representation of *Desulfobacter* spp. *Ruminococcus* spp. image courtesy of Harry Flint and Sylvia Duncan. *Desulfovibrio* spp. image courtesy of Grahame Bradley. *Clostridium* spp. and *Bacteroides* spp. images both courtesy of CDC. *Desulfobulbus* spp. image taken from Pagani, I. *et al. Stand. Genomic Sci.* **4**, 100–110 (2011), which is published under an open-access license by the Genomic Standards Consortium. *Desulfotomaculum* spp. and *Roseburia* image used with permission from the Society for General Microbiology © Fardeau *et al. Int. J. System. Bacteriol.* **45**, 218–221 (1995) and Duncan, S. H. *et al. Int. J. System. Evol. Microbiol.* **56**, 2437–2441 (2006), respectively. *M. stadtmaniae* and *B. hydrogenotrophica* image used with permission from Springer © Miller, T. L. & Wolin, M. J. *Arch. Microbiol.* **141**, 116–122 (1985) and Bernalier *et al. Arch. Microbiol.* **166**, 176–183 (1996), respectively. *M. smithii* image used with permission from National Academy of Sciences © Samuel, B. S. *et al. Proc. Natl Acad. Sci. USA* **104**, 10643–10648 (2007).

being integral to microbial fermentation, a broad assemblage of hydrogenogens must exist in the human colon—probably most abundant in the right colon, the colonic region with the greatest extent of microbial fermentation.[50] Few studies have focused on the phylogenetic diversity of these microbes and the metabolic niches they occupy. Among abundant bacterial genera detected in the colon, several strains of *Roseburia* spp.[56,57] and *Ruminococcus* spp.[44,45] produce substantial concentrations of H_2 *in vitro*. Other prominent colonic bacteria known to produce H_2 include *Anaerostipes caccae*,[46] *Clostridium* spp.[47,48] *Eubacterium rectale*,[58] *Bacteroides* spp.[43,59] and *Victivallis vadensis*.[60] Conversely, *Akkermansia muciniphila*[61] and *Faecalibacterium prausnitzii*[62] were clearly shown to produce no detectable levels of H_2. Thus, it seems that colonic H_2 is produced mainly by members of the Firmicutes and much less by members of the Bacteroidetes.

Commonly, H_2 production has been characterized by breath testing; however, data indicating the extent to which such tests accurately reflect the abundance or activity of hydrogenogens in luminal or mucosal microbiota are scarce. A proof-of-principle description of a protocol for selective enrichment of hydrogenogens offers promise of successful culture-based approaches for application to the colonic environment.[63] A more readily available approach would be the direct characterization and quantification of microbial H_2 production by targeting the hydrogenase enzymes involved in H_2 production (Figure 2). Functional gene approaches are particularly

useful for measuring the DNA-based abundance or the RNA-based activity of phylogenetically diverse metabolic groups such as fermentative hydrogenogens. Of the two main types of hydrogenases, [Fe-Fe]-hydrogenases are involved primarily in H_2 production and can be used as molecular targets.[64] The utility of this approach was demonstrated for the characterization of H_2 producers in an acidic fen[65] and earthworm gut,[66] and thus offers promise for unravelling greater detail on the extent to which the abundance and activities of hydrogenogens vary among individuals and in response to diet or disease.

Reductive acetogens

The reductive acetogens are a group of obligately anaerobic bacteria that utilize the acetyl-CoA (Wood–Ljungdhal) pathway to synthesize acetate from CO_2 and H_2.[67] Colonic acetogenesis was first demonstrated using slurries from human stool,[68] and later cultivation-based studies estimated that the number of acetogens ranged from 1×10^2–1×10^8 colony-forming units (CFUs) per g wet human stool.[69,70] Acetogens isolated from human stool were related to the genera *Ruminococcus*, *Clostridium* or *Streptococcus*.[70–72] The gene sequences of formyl tetrahydrofolate synthetase (*fhs*) and acetyl-CoA synthase (*acs*) are highly conserved among acetogens and thus serve as useful functional gene targets.[48,73,74] Analysis of *fhs* sequences amplified from human stool identified *Blautia producta* (formerly *Ruminococcus productus*) as the predominant acetogen, and detected several *fhs* sequences that had not been identified previously.[75] Both *fhs* and *acs* were persistently detected in colonic biopsies from 25 healthy individuals, with values ranging from 1.8×10^3 to 8.8×10^6 and from 9.8×10^3 to 3.8×10^7 gene copy numbers per g of tissue, respectively.[39] It should be noted, however, that the presence of these functional genes does not necessarily correlate with reductive acetogenesis.

Acetogenesis has been postulated to be a less important hydrogenotrophic pathway in the colon than methanogenesis and sulphate reduction[4] because both the conversion of H_2 and CO_2 into CH_4 and sulphate reduction are thermodynamically more favourable than reductive acetogenesis.[38,76] Nevertheless, a metagenomic study of the *in vivo* metabolic potential of human gut acetogens concluded that acetogenesis was the most prevalent pathway of H_2 disposal in the human colon.[77] This model is supported by a previous study that used radioisotope analysis to quantify the fraction of acetate derived from the reduction of CO_2 by H_2 in stool suspensions from two individuals.[78] However, the model conflicts with the theoretical consideration of the thermodynamics of H_2 utilization among hydrogenotrophs as well as experimental evidence of the relative prevalence of methanogenic archaea and SRB in the human colon. Without question, polyphyletic reductive acetogens are more metabolically versatile than methanogenic archaea or SRB, and defining the nature of their interactions with these two groups of hydrogenotrophic microbes and the extent to which they contribute to H_2 disposal in CH_4-excretors versus nonexcretors needs to be resolved.

Methanogenic archaea

Colonic methanogenic archaea derive all (or most) of their metabolic energy from methanogenesis by reducing CO_2 or methanol to CH_4 using H_2 or formate as electron donors.[79] Substantial interindividual differences exist in colonic methanogenesis. A threshold value of 1×10^8 methanogens per g of stool for CH_4 to be detected in breath was defined by Miller and Wolin[80] and confirmed in a subsequent study in which breath CH_4 excretors harboured an average of 1×10^9 CFU per g of methanogens in stool, whilst nonexcretors harboured ~1×10^4 CFU per g.[49] Chassard *et al.*[81] demonstrated that the structure of the cellulose-degrading bacterial community in individuals differs according to their CH_4 status. The dominant cellulose degraders isolated from non-CH_4-excreting individuals belonged mainly to Bacteroidetes; CH_4-excreting individuals harboured predominantly Firmicutes. Methanogens were also observed to co-occur predominantly with members of the Clostridiales order (Firmicutes) in individuals harbouring abundant methanogens.[82] This same study confirmed that host genotype influences methanogen carriage, as the concordance rate for carriage of methanogens was markedly greater in monozygotic adult twin pairs than in dizygotic twins. However, carriage of methanogens between mother and daughter was discordant.[82] Thus, host genotype and various environmental factors are among the potential determinants of persistent colonization by methanogens.

The two methanogenic species isolated from the human colon, *Methanobrevibacter smithii* and *Methanosphaera stadtmanae*, have different biochemical characteristics. *M. smithii* converts CO_2 and H_2 to CH_4, but *M. stadtmanae* uses H_2 to reduce methanol to CH_4.[83,84] To date, studies (using both culture-based and molecular-based approaches) indicate that *M. smithii* is the predominant methanogen in the human colon;[85–90] *M. stadtmanae* has been isolated from the human intestinal tract at a lower abundance.[83] Several different phylotypes closely related to *M. smithii*, *M. stadtmanae*, *M. oralis* or *Methanosarcinales* have been identified using molecular fingerprinting studies targeting 16S ribosomal DNA and functional gene coenzyme M reductase (*mcrA*).[89–91] The *mcrA* gene was persistently detected with values ranging from 3.0×10^2 to 4.5×10^9 in colonic biopsy samples from 25 healthy individuals.[39] These data further confirm initial observations that breath CH_4 concentration reflects the relative abundance or activity of colonic methanogens, and not the presence or absence of this hydrogenotrophic group.

Sulphate-reducing bacteria

SRB are a diverse group sharing the ability to use sulphate as a terminal electron acceptor for respiration, with the concomitant production of H_2S. Colonic SRB generally use H_2 as their electron donor, but electrons can also be provided from the oxidation of organic compounds, such as lactate.[92] SRB are ubiquitously present in the human intestinal mucosa[40,41,93] and have been enumerated from human stool within the range 1×10^3–1×10^{11}

Table 1 | Studies on the link between IBS and colonic H_2

Study	No. of participants	Major findings
Levitt & Donaldson (1970)[26]	55	Abnormal breath H_2 and CH_4 in carbohydrate malabsorption
Metz *et al.* (1975)[194]	24	IBS linked with lactose intolerance
Metz *et al.* (1976)[195]	17	IBS symptoms linked to SIBO
Rhodes *et al.* (1979)[28]	64	Lactulose breath testing for SIBO
El Oufir *et al.* (1996)[196]	8	Altering intestinal transit times leads to a change in bacterial abundance and activity
King *et al.* (1998)[113]	12	Increased volume and rate of H_2 excretion in patients with IBS
Pimentel *et al.* (2000)[116]	202	Reduction of SIBO with antibiotics reduces IBS symptoms
Pimentel *et al.* (2003)[153]	551	CH_4 linked to constipation-predominant IBS
Pimentel *et al.* (2004)[197]	18	Patients with methanogenic IBS have reduced postprandial serotonin levels relative to patients with hydrogenogenic IBS
Dear *et al.* (2005)[198]	12	Reduction of fermentation with metronidazole or exclusion diet improves IBS symptoms

Abbreviations: CH_4, methane; H_2, hydrogen; SIBO, small intestinal bacterial overgrowth.

bacteria per g.[34,49,94] *In vitro*, colonic SRB are metabolically flexible and can oxidize a variety of short-chain fatty acids.[94,95] Detection of SRB in the stool of infants <1 month indicates that these bacteria are facultative H_2 utilizers.[40,96] A range of nutritionally and physiologically distinct SRB has been detected in human stool.[34,94,97,98] The genes encoding adenosine 5'-phosphosulfate reductase (*apsr1*) and dissimilatory sulphite reductase (*dsrAB*), which are enzymes in the sulphate reduction pathway, are also useful molecular markers owing to their highly conserved nature and congruence with the evolutionary history of SRB.[99–102] However, few studies have been published that have examined the diversity or abundance of human colonic SRB using molecular-based techniques. The functional gene *dsrAB* was persistently detected with values ranging from 1.8×10^2–1.4×10^9 copy numbers in colonic biopsy samples from 25 healthy individuals.[39] Furthermore, in the same set of biopsy samples, four different SRB genera identified previously by culturing[34] were consistently detected with relevant 16S rRNA gene probes.[39]

Colonic gases and human disease

Links with IBS

IBS is categorized as a functional intestinal disorder and afflicts ~14% of the US population;[103] it is the first-listed diagnosis in >1.6 million individuals according to the National Hospital Ambulatory Medical Care Survey for 2004–2005.[104] However, based on findings from a 2005 survey of 5,009 individuals, Hungin *et al.*[103] estimated that >76% of IBS sufferers may go undiagnosed. IBS subtypes include constipation-predominant IBS (IBS-C), diarrhoea-predominant, and mixed or unspecified.[105,106] Primary symptoms—in addition to diarrhoea or constipation in 30–40% of patients—include pain, bloating and/or abdominal distension, flatulence and belching.[107,108] Despite its prevalence and severity, no confirmatory diagnostic test exists for IBS.

The role of gas byproducts of microbial fermentation has been implicated in IBS in general, and IBS-C in particular (Table 1).[109,110] Malabsorption of carbohydrates from the intestine and SIBO are commonly, but inconsistently, associated with IBS, and their relevance to the cause of the disorder has been controversial.[111] In particular, CH_4 has been linked to decreased colonic transit time in patients with IBS-C; meanwhile H_2 accumulation, possibly due to a failure of colonic microbes to dispose of H_2 produced via fermentation, has been postulated to account for the bloating and pain that often distinguish IBS-C from chronic constipation.[110,112] Using whole-body calorimetry, King *et al.*[113] found that patients with IBS excreted substantially more H_2 overall than healthy individuals. Although H_2 plus CH_4 excretion was slightly higher in those with IBS than healthy controls, the difference in volume was not statistically significant; however, the rate of excretion of both gases was 400% higher in patients with IBS than controls. Dietary changes that improved IBS symptoms decreased gas excretion levels, particularly H_2.[113]

Because bloating and abdominal pain are suggested to result from carbohydrate malabsorption and subsequent bacterial fermentation in the colon, clinicians and scientists have sought to correlate elevated breath levels of H_2 produced by colonic bacteria during fermentation with IBS.[114,115] Several studies have measured increases in breath H_2 concentration in patients with IBS,[116] as well as premature peaks in breath H_2, implicating SIBO as a cause of abdominal pain and bloating. However, evidence exists that early H_2 peaks associated with IBS reflect differences in small-bowel transit time rather than SIBO.[117] Quigley[118] suggests that IBS owing to SIBO might present a distinct category of this disorder. Nonetheless, reliable data verifying a link between colonic microbiota, H_2 metabolism and IBS are sparse.[119]

Finally, a correlation was observed between high breath CH_4 levels and the occurrence of motility disorders.[120,121] As demonstrated in human and mammalian model systems, high levels of CH_4 are correlated with decreased intestinal motility;[121] however, it has not been confirmed that this increase in breath gases is associated

with increased abundance of colonic methanogens. For example, increased CH_4 production could result from increased bacterial H_2 production. Global and deep microbial analysis of stool samples from patients with IBS demonstrated the presence of methanogenic archaea in a higher percentage of those with IBS-C than in healthy controls or patients with diarrhoea-predominant or alternating IBS. However, among those patients harbouring methanogens, their abundance was fourfold greater in healthy individuals than in either the IBS group as a whole or those with IBS-C.[122] In addition, patients with IBS who excreted CH_4 produced lower levels of serotonin in response to glucose than those excreting primarily H_2.[123] Serotonin in IBS has been linked to abnormal gut motility as well as visceral hypersensitivity; however, the potential success of treatments capitalizing on this relationship has been hindered by serious adverse effects.[124–127] This lack of knowledge on the relationship between measures of microbial abundance and activities relative to breath gas concentrations highlights the need for more systematic investigation of host–microbe aspects of H_2 metabolism.

H_2S has been shown to modulate peripheral nociceptive (pain-related) signals.[128] As abdominal pain is a primary symptom of IBS, these data implicate a potential, direct role for colonic H_2S. Indeed, a pro-nociceptive effect has been suggested from mouse model studies that could support this hypothesis.[129,130] Nevertheless, antinociceptive effects have also been reported,[131] and, thus, the importance of the role of H_2S in abdominal pain in IBS remains controversial.

Links with IBD

A role for bacterial-generated H_2S in IBD aetiopathogenesis has support from both clinical and experimental studies (Table 2). H_2S is highly toxic to colonocytes and impairs their metabolic function, especially butyrate oxidation.[132,133] In aqueous solutions, H_2S dissociates into hydrosulphide anion (pKa 7.04) and sulphide ion (pKa 11.96).[134] Generally, sulphide exists in the human colon in the volatile, highly toxic undissociated form (H_2S), which is quickly absorbed by the mucosa or passed as flatus.[2] The majority of sulphate (>90%) disappears during passage through the colon of individuals lacking SRB; thus, a number of colonic processes, in addition to sulphate reduction by resident bacteria, must compete for sulphate.[135]

Anionic sulphide concentrations were elevated in the colon of patients with ulcerative colitis.[136] These patients also ingested more protein, and thereby more sulphur amino acids, than healthy control individuals.[137] Removing foods rich in sulphur amino acids (milk, eggs and cheese) has proven therapeutic benefits in those with ulcerative colitis.[138] However, other studies examining SRB-related variables have not confirmed a possible link with IBD. These conflicting observations might reflect the common use of 5-aminosalicylic acid (5-ASA), which has a proven therapeutic value for the treatment of ulcerative colitis[136] but also diminishes H_2S production by colonic bacteria.[139,140] Indeed, no difference in stool sulphide concentrations was observed between patients with ulcerative colitis who were treated with 5-ASA and noncolitic individuals,[141] whereas stool sulphide concentrations were markedly higher, by comparison, in patients with ulcerative colitis who were not administered 5-ASA.[139] Aminoglycoside antibiotics also inhibit SRB growth and are of therapeutic benefit in active ulcerative colitis.[136] Further supporting a role for H_2S in ulcerative colitis is the observation that SRB were found in surgically constructed ileoanal pouches of patients with ulcerative colitis but not in pouches of patients with familial adenomatous polyposis.[141] Moreover, H_2S production in ulcerative colitis pouches was 10 times greater than that in familial adenomatous polyposis pouches.[142] In addition, the severity of pouchitis is positively correlated with stool concentrations of H_2S,[143] possibly reflecting a pathogenic role for this gas.

Early studies used culture-based approaches to examine colonic SRB in patients with IBD (Table 2). The numbers of SRB and rate of sulphidogenesis were greater in those with ulcerative colitis than in healthy controls.[144,145] In another *in vitro* study, production of H_2S from stool of patients with ulcerative colitis was 3–4 times greater than that from controls.[146] Molecular-based techniques have been used to evaluate the prevalence and diversity of SRB species (Table 2). For example, *Desulfovibrio piger* was more abundant in stool of patients with IBD than in healthy individuals or in patients with other gastrointestinal symptoms.[147] However, Fite and coworkers[40] reported that patients with active ulcerative colitis did not harbour more *Desulfovibrio* spp. than healthy controls in either stool or rectal mucosal samples (as measured by quantitative PCR). Thus, either increased sulphidogenic activities or reduced sulphide detoxification in the colonic epithelium might explain the increased H_2S concentrations in these patients and their potential inflammatory impact.

Indirect support for a role of H_2S and SRB in the aetiology of ulcerative colitis (but not Crohn's disease) is the observed increased activity of mucin sulphatase, an enzyme that cleaves sulphate groups from mucosal sulphomucins.[148] Additionally, in most patients, fluctuations in stool sulphatase activities correspond to clinical disease activity.[148] Therefore, a model in which increased sulphatase activity results in increased availability of endogenous sulphate for H_2S production by SRB might contribute to perpetuation of the disease. This model is supported by the finding of an association between the abundance of sulphomucins and the quantity of several SRB genera in the colonic mucosa of healthy individuals.[149]

A few, but consistent, reports indicate that the prevalence of the methanogenic phenotype is markedly lower in patients with Crohn's disease or ulcerative colitis than in healthy individuals. However, these intriguing findings have received limited attention, and it is not known whether the potentially reduced prevalence of CH_4 excretion in IBD is a cause or consequence of, for example, reduced transit time or pH. Breath CH_4 was detected in 44% of healthy white individuals but absent in patients

Table 2 | Studies on the link between IBD, SRB and H_2S

Study	No. of participants	Major findings
Gibson *et al.* (1991)[97]	30	Faecal slurries from patients with ulcerative colitis produce markedly higher rates of H_2S than healthy controls
Roediger *et al.* (1993)[132]	31	H_2S impairs butyrate acquisition by colonocytes
Christl *et al.* (1996)[167]	10	NaHS induces mucosal hyperproliferation
Levine *et al.* (1996)[199]	100	No difference in SRB stool carriage between populations at high risk of IBD (Ashkenazi Jews) and control lower-risk populations
Roediger *et al.* (1997)[136]	NA*	Therapeutic value of 5-ASA for ulcerative colitis might result from SRB inhibition
Pitcher *et al.* (2000)[139]	49	5-ASA inhibits H_2S production from SRB and might confound correlation between ulcerative colitis and SRB
Zinkevich *et al.* (2000)[41]	74	SRB cultivated successfully from 92% of patients with ulcerative colitis and only 52% of noncolitic ones. SRB-specific PCR successful from all biopsy samples, indicating differences in abundance
Duffy *et al.* (2002)[200]	25	SRB colonize pouches formed for ulcerative colitis, but not for familial adenomatous polyposis
Kleessen *et al.* (2002)[93]	38	Biopsy samples from patients with ulcerative colitis always positive for SRB detection by FISH; only some patients with Crohn's disease had positive samples
Loubinoux *et al.* (2002)[147]	151	Higher prevalence of cultivable SRB (68%) and *Desulfovibrio piger* DNA (55%) in stool from patients with IBD compared with healthy individuals or patients with other digestive diseases (24–37% for SRB and 12–25% for *D. piger*, respectively)
Bullock *et al.* (2004)[201]	12	No difference in SRB abundance detected between patients with active ulcerative colitis and those in remission
Ohge *et al.* (2005)[143]	50	Stool H_2S markedly higher in patients with ulcerative colitis who have a recent history of pouchitis than patients with ulcerative colitis who have an older or no history of pouchitis
Smith *et al.* (2005)[202]	14	SRB only colonizes ulcerative colitis pouches, but not adenomatous polyp pouches
Bambury *et al.* (2008)[203]	21	Ulcerative colitis pouches characterized by high sulphomucin expression that correlates with high SRB colonization
Coffey *et al.* (2009)[204]	NA*	Model for ulcerative colitis pouchitis suggested in which an increase in sulphomucin production enables SRB development and H_2S-induced inflammation
Lim *et al.* (2009)[205]	20	Two *Desulfosporosinus* sequences and other uncultivated Proteobacteria (potential SRB) detected only in inflamed colonic pouches
Rowan *et al.* (2010)[206]	39	*Desulfovibrio* absolute and relative abundance increased in patients with acute and chronic ulcerative colitis compared with healthy controls
Verma *et al.* (2010)[207]	149	SRB (and *Methanobrevibacter smithii*) genes more abundant in patients with ulcerative colitis and those with Crohn's disease compared with healthy controls
Strauss *et al.* (2011)[208]	56	H_2S-producer *Fusobacterium nucleatum* isolated more often from IBD biopsy samples than from healthy controls. *F. nucleatum* strains originating from inflamed biopsy tissue were more invasive in a Caco-2 invasion assay than strains isolated from healthy tissue

*Review article. Abbreviations: 5-ASA, 5-aminosalicylic acid; FISH, fluorescence *in situ* hybridization; H_2S, hydrogen sulphide; NA, not applicable; NaHS, sodium hydrogen sulphide; SRB, sulphate-reducing bacteria.

with Crohn's ileitis.[22,150] McKay *et al.*[151] reported a 13% prevalence of CH_4 excretion in patients with Crohn's disease and 15% in those with ulcerative colitis compared with 54% in healthy controls. Peled and coworkers[152] found that among healthy individuals, 50% produced CH_4, whereas breath CH_4 was detected in only 6.1% of patients with Crohn's disease and 31.4% of patients with ulcerative colitis. A 2003 study[153] compared the excretion of either H_2 or CH_4 alone to combined excretion of these two gases following a lactulose breath test. The predominant gas excreted by patients with IBD was H_2 alone (76 of 78 individuals with Crohn's disease or ulcerative colitis). By contrast, breath CH_4 was detected as the predominant gas in only two of 78 individuals with IBD in this study.[153] To date, only a single report, using a molecular-based approach, has compared the incidence and density of colonic methanogens in healthy individuals versus patients with IBD. Targeting the *mcrA* gene, Scanlan *et al.*[90] reported that although the abundance of methanogens was reduced in both IBD groups relative to healthy controls, statistical significance was observed only for those with ulcerative colitis.

Links with colorectal cancer

Multiple lines of evidence for a possible association of both methanogens and SRB with sporadic colorectal cancer (CRC) have been reported (Table 3). In the 1970s and 1980s, numerous studies reported a higher prevalence of methane CH_4 excretion among patients with CRC compared with healthy individuals and, in some cases, patients with other gastrointestinal disease.[154–159] However, subsequent studies did not find major differences in CH_4 status between patients with CRC and healthy individuals,[160,161] and the use of the breath test was apparently abandoned as a possible CRC diagnostic tool. It was suggested that observations of higher breath CH_4 levels in patients with CRC might have resulted from reduced transit time owing to at least partial obstruction by tumour tissue.[154] Karlin *et al.*[155] found that, among a group of 55 patients with unresected CRC

Table 3 | Studies on the link between CRC, SRB and H_2S

Study	No. of study participants/ patient samples	Major findings
Kanazawa *et al.* (1996)[171]	27	Higher H_2S concentration in high-risk patients compared with controls
Deplancke *et al.* (2003)[165]	5	H_2S induces cell-cycle entry in rat intestinal epithelial cells
Attene-Ramos *et al.* (2006)[168]	NA*	Physiological H_2S concentrations genotoxic to mammalian cells
Ramasamy *et al.* (2006)[172]	NA	Levels of sulphide-detoxifying enzymes in the human colon decreased in patients with cancer
Attene-Ramos *et al.* (2007)[169]	NA*	H_2S induces direct radical-associated DNA damage
Balamurugan *et al.* (2008)[173]	46	No major differences in abundance of *Desulfovibrio* between stool from patients with CRC and healthy individuals
Scanlan *et al.* (2009)[209]	90	No major differences in abundance of *Desulfovibrio* between stool from patients with CRC and healthy individuals
Attene-Ramos *et al.* (2010)[170]	NA*	Physiological H_2S concentrations genotoxic to colonic epithelial cells
Cai *et al.* (2010)[164]	NA*	H_2S induces human colon cancer cell proliferation
Castellarin *et al.* (2011)[176]	99	H_2S-producer *Fusobacterium nucleatum* DNA sequences over-represented in CRC tumour tissues
Kostic *et al.* (2011)[175]	95	H_2S-producer *Fusobacterium nucleatum* sequences over-represented in microbial metagenomes from CRC tumour tissues
Marchesi *et al.* (2011)[174]	6	H_2S-producer *Fusobacterium nucleatum* sequences more abundant in 16S pyrosequencing from CRC tumour tissues than in healthy tissue

**In vitro* studies. See references for details. Abbreviations: CRC, colorectal cancer; H_2S, hydrogen sulphide; NA, not applicable; SRB, sulphate-reducing bacteria.

in varying regions of the colon and rectum, no difference was observed in the frequency or amount of CH_4 excretion for the groups; however, of those with more distal cancer (descending or sigmoid), the likelihood of excreting breath CH_4 was twice as high as for patients with proximal colon or rectal cancer. Holma *et al.*[162] reported an extensive assessment of methanogenic status in CRC. Again, breath and stool CH_4 levels were similar in healthy individuals and those with CRC. However, patients with right-sided CRC exhibited lower methanogenesis, lower stool pH and increased abdominal discomfort compared with patients with left-sided CRC.[162]

H_2S can damage the intestinal epithelium leading to chronic inflammation,[132,136,163] as well as perturbing the balance between cellular proliferation and apoptosis.[164–167] At concentrations similar to those found in the human and mouse intestine, the H_2S donor sodium hydrosulphide produced genomic DNA damage in Chinese hamster ovary and human HT29-Cl.16E colonic epithelial cells when DNA repair was inhibited.[168] Sodium hydrosulphide also induced DNA damage in the absence of cellular metabolism, and this damage was at least in part produced by free radicals.[169] A subsequent study confirmed the genotoxic properties of sodium hydrosulphide in nontransformed human intestinal epithelial cells with intact DNA repair pathways, and demonstrated that H_2S modulates the expression of genes involved in cell-cycle progression and triggers both inflammatory and DNA repair responses.[170]

Kanazawa[171] measured higher stool H_2S levels in individuals with high risk of CRC than in healthy controls. Ramasamy[172] found that thiosulphate sulphotransferase, an enzyme purported to be involved in H_2S detoxification, was present in lower abundance in biopsy tissue from patients with CRC (and ulcerative colitis). In a limited study targeting only a few microbial taxa in stool from patients with CRC and healthy individuals, major differences were not observed for *Desulfovibrio* abundance.[173] In 2011, Marchesi *et al.*[174] described a potential CRC tumour-associated microbiome, based on high-throughput sequencing. Intriguingly, the highest H_2 producers belong to genera over-represented in colonic tumour tissue (*Eubacterium* and *Roseburia*). This study, as well as two subsequent reports, also consistently detected an enrichment of *Fusobacterium* spp. in colonic tumour tissue, a genus known to produce H_2S via degradation of sulphur-containing amino acids.[174–176] Thus, it is possible that the observed association of increased H_2S with CRC derives from cysteine fermentation rather than sulphate respiration.

Endogenously produced H_2S has been demonstrated from a wide range of tissues, including the gastrointestinal tract,[177] and this molecule is receiving a lot of attention as a possible intracellular 'gaseous transmitter'.[178] Numerous examples exist whereby the exogenous administration of H_2S donors (such as sodium hydrosulphide) exerts anti-inflammatory effects in a wide range of *in vitro* and *in vivo* settings.[179] However, at present, a large discrepancy remains between the concentrations of H_2S in tissue versus those needed for alteration of tissue function *in vitro*.[180] One hypothesis that has not been tested is a direct modulation of the gut microbiota by H_2S rather than physiological effects. H_2S is known to be toxic to microbes in general and also to help maintain anaerobic status.[181,182] Thus, it is possible that the suggested beneficial effects of H_2S might actually reflect their shaping of a more beneficial microbiome, possibly by inhibiting the growth of pathogenic facultative anaerobes.

What is clear is that SRB are tightly associated with the colonic mucosa and presumably their presence provides a source of H_2S that directly influences the colonic epithelium. Hence, numerous host and microbial components might fit multifactorial models that could explain gene–environment interactions that predispose to sporadic CRC.

Links with obesity

Obesity has been hypothesized to correlate with elevated levels of colonic CH_4 and H_2.[86] This hypothesis is based on the assumption that increased methanogenesis would improve fermentation efficiency, resulting in increased production of short-chain fatty acids, which potentially promotes adipogenesis by the host. The idea was first suggested by observations that methanogens were more abundant in homozygous *ob*/*ob* mice than in their non-obese heterozygotic littermates and wild-type controls.[183] Subsequently, an intriguing study detected markedly higher numbers of methanogenic archaea in obese individuals than in normal-weight individuals or patients after gastric bypass.[86] Alternatively, four reports demonstrate a reduced number of CH_4 excretors among obese individuals compared with lean individuals,[184] a lower level of *M. smithii* in obese individuals,[185,186] and greater abundance of methanogens in those with anorexia compared with obese and lean individuals.[187] Clearly, much additional work is needed to determine the extent to which colonic H_2 metabolism might influence the development of obesity. Determining the relationship between colonic microbial populations and breath gas measurements in obese individuals, populations at risk of obesity, and in response to diet would help to clarify this matter.

Hydrogen as a therapeutic gas

Perhaps the most exciting link between H_2 and human disease is emerging evidence that this microbial-derived gas has potent antioxidative, antiapoptotic and anti-inflammatory activities in a wide range of disease models.[188] The breakthrough in this area of research was the report that molecular H_2 selectively reduced the levels of hydroxyl radicals *in vitro* and that H_2 molecules also exerted therapeutic antioxidant activities in a rat model of middle cerebral artery occlusion.[189] H_2 selectively reduces hydroxyl radicals and peroxynitrite, which are strong oxidants that react indiscriminately with nucleic acids, lipids and proteins resulting in DNA fragmentation, lipid peroxidation and protein inactivation.

Accumulating evidence suggests that H_2 can protect various cells, tissues and organs against oxidative injury.[190,191] Of specific interest to the field of gastroenterology is the demonstration that H_2-enriched water reduced colitis (induced by dextran sodium sulphate) in a rat model.[192] Evidence that H_2-infused water and H_2 gas exerted beneficial effects in animal models of, or patients suffering from, obesity, diabetes mellitus and metabolic syndrome expands the potential clinical relevance of the H_2 economy.[193] Thus, it would seem that the H_2 endogenously produced by the resident microbiota might exert a similar protective antioxidant role in the healthy colon, and that decreases in the net production of H_2 might increase the risk of inflammatory or metabolic diseases. It is intriguing to consider whether manipulation of microbial H_2 metabolism, either through enhanced production or diminished utilization, might provide a novel means of regulating colonic homeostasis. At present, technological limitations preclude interrogation of microbial H_2 metabolism at temporal and spatial scales relevant to the colonic mucosal ecosystem.

Conclusions

Despite long-standing evidence demonstrating the importance of microbial H_2 metabolism, minimal efforts have been exerted to manipulate relevant host–microbe interactions to affect colonic health. The few studies that demonstrate the ease with which colonic gases can be regulated by dietary substrate offer promise for relatively innocuous strategies to alter the balance between hydrogenogenic and hydrogenotrophic microbes, and thereby broaden preventative and therapeutic options for managing multiple colonic and possibly metabolic disorders. However, for this promise to be fulfilled, additional research will be required to better understand the microbial and molecular bases of colonic H_2 production and utilization as well as the effectiveness of the H_2 breath test as a reliable readout of microbial metabolic activities. Such findings are expected to reveal explanations for the marked variation among individuals in both host and microbial aspects of H_2 metabolism, thus enabling these parameters to be utilized for novel biomarkers of health and disease.

Review criteria

This Review is based upon data from systematic reviews, review papers and individual studies known to the authors. Other relevant studies were identified by MEDLINE and ISI Web of Knowledge searches of English-language papers published up to November 2011 using the search terms: "acetogen", "breath test", "colorectal cancer", "Crohn's disease", "hydrogen", "hydrogen sulfide", "hydrogenotroph", "inflammatory bowel disorder", "irritable bowel syndrome", "methanogen", "methane", "obesity", "sulfate-reducing bacteria", "ulcerative colitis", and any relevant combination of terms. When appropriate, the reference lists of key papers were checked to identify additional articles of interest.

1. Suarez, F., Furne, J., Springfield, J. & Levitt, M. Insights into human colonic physiology obtained from the study of flatus composition. *Am. J. Physiol.* **272**, G1028–G1033 (1997).
2. Suarez, F., Furne, J., Springfield, J. & Levitt, M. Production and elimination of sulfur-containing gases in the rat colon. *Am. J. Physiol.* **274**, G727–G733 (1998).
3. Levitt, M. D. & Bond, J. H. Jr. Volume, composition, and source of intestinal gas. *Gastroenterology* **59**, 921–929 (1970).
4. Christl, S. U., Murgatroyd, P. R., Gibson, G. R. & Cummings, J. H. Production, metabolism, and excretion of hydrogen in the large intestine. *Gastroenterology* **102**, 1269–1277 (1992).
5. Kirk, E. The quantity and composition of human colonic flatus. *Gastroenterology* **12**, 782–794 (1949).
6. Steggerda, F. R. Gastrointestinal gas following food consumption. *Ann. NY Acad. Sci.* **150**, 57–66 (1968).
7. Tomlin, J., Lowis, C. & Read, N. W. Investigation of normal flatus production in

healthy volunteers. *Gut* **32**, 665–669 (1991).
8. Beazell, J. M. & Ivey, A. C. The quantity of colonic flatus excreted by the "normal" individual. *Am. J. Dig. Dis.* **8**, 128–129 (1941).
9. Askevold, F. Investigations on the influence of diet on the quantity and composition of intestinal gas in humans. *Scand. J. Clin. Lab. Invest.* **8**, 87–94 (1956).
10. Ruge, E. Beitrag sur kennuness der darmgase [German]. *Sitsber. Kaiserlicken Akad.* **44**, 739 (1861).
11. Levitt, M. D., Gibson, G. R. & Christl, S. U. in *Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology* (eds Gibson, G. R. & Macfarlane, G. T.) 131–154 (CRC Press, Boca Raton, 1975).
12. Steggerda, F. R. & Dimmick, J. F. Effects of bean diets on concentration of carbon dioxide in flatus. *Am. J. Clin. Nutr.* **19**, 120–124 (1966).
13. Levitt, M. D. & Ingelfinger, F. J. Hydrogen and methane production in man. *Ann. NY Acad. Sci.* **150**, 75–81 (1968).
14. Levitt, M. D., Hirsh, P., Fetzer, C. A., Sheahan, M. & Levine, A. S. H_2 excretion after ingestion of complex carbohydrates. *Gastroenterology* **92**, 383–389 (1987).
15. Wolin, M. J. & Miller, T. L. in *Human Intestinal Microflora in Health and Disease* (ed. Hentges, D. J.) 147–165 (Academic Press, New York, 1983).
16. Strocchi, A. & Levitt, M. D. Factors affecting hydrogen production and consumption by human fecal flora. The critical roles of hydrogen tension and methanogenesis. *J. Clin. Invest.* **89**, 1304–1311 (1992).
17. Hammer, H. F. Colonic hydrogen absorption: quantification of its effect on hydrogen accumulation caused by bacterial fermentation of carbohydrates. *Gut* **34**, 818–822 (1993).
18. Le Marchand, L., Wilkens, L. R., Harwood, P. & Cooney, R. V. Use of breath hydrogen and methane as markers of colonic fermentation in epidemiologic studies: circadian patterns of excretion. *Environ. Health Perspect.* **98**, 199–202 (1992).
19. Strocchi, A., Ellis, C. & Levitt, M. D. Reproducibility of measurements of trace gas concentrations in expired air. *Gastroenterology* **101**, 175–179 (1991).
20. Calloway, D. H. Respiratory hydrogen and methane as affected by consumption of gas-forming foods. *Gastroenterology* **51**, 383–389 (1966).
21. Calloway, D. H. & Murphy, E. L. The use of expired air to measure intestinal gas formation. *Ann. NY Acad. Sci.* **150**, 82–95 (1968).
22. Bjorneklett, A. & Jenssen, E. Relationships between hydrogen (H_2) and methane (CH_4) production in man. *Scand. J. Gastroenterol.* **17**, 985–992 (1982).
23. Cloarec, D. *et al.* Breath hydrogen response to lactulose in healthy subjects: relationship to methane producing status. *Gut* **31**, 300–304 (1990).
24. Vernia, P., Camillo, M. D., Marinaro, V. & Caprilli, R. Effect of predominant methanogenic flora on the outcome of lactose breath test in irritable bowel syndrome patients. *Eur. J. Clin. Nutr.* **57**, 1116–1119 (2003).
25. Calloway, D. H., Murphy, E. L. & Bauer, D. Determination of lactose intolerance by breath analysis. *Am. J. Dig. Dis.* **14**, 811–815 (1969).
26. Levitt, M. D. & Donaldson, R. M. Use of respiratory hydrogen (H_2) excretion to detect carbohydrate malabsorption. *J. Lab. Clin. Med.* **75**, 937–945 (1970).
27. Metz, G., Jenkins, D. J., Peters, T. J., Newman, A. & Blendis, L. M. Breath hydrogen as a diagnostic method for hypolactasia. *Lancet* **1**, 1155–1157 (1975).
28. Rhodes, J. M., Middleton, P. & Jewell, D. P. The lactulose hydrogen breath test as a diagnostic test for small-bowel bacterial overgrowth. *Scand. J. Gastroenterol.* **14**, 333–336 (1979).
29. Khoshini, R., Dai, S. C., Lezcano, S. & Pimentel, M. A systematic review of diagnostic tests for small intestinal bacterial overgrowth. *Dig. Dis. Sci.* **53**, 1443–1454 (2008).
30. Bures, J. *et al.* Small intestinal bacterial overgrowth syndrome. *World J. Gastroenterol.* **16**, 2978–2990 (2010).
31. Caride, V. J. *et al.* Scintigraphic determination of small intestinal transit time: comparison with the hydrogen breath technique. *Gastroenterology* **86**, 714–720 (1984).
32. Bond, J. H., Engel, R. R. & Levitt, M. D. Factors influencing pulmonary methane excretion in man—indirect method of studying *in situ* metabolism of methane-producing colonic bacteria. *J. Exp. Med.* **133**, 572–588 (1971).
33. Levitt, M. D., Furne, J. K., Kuskowski, M. & Ruddy, J. Stability of human methanogenic flora over 35 years and a review of insights obtained from breath methane measurements. *Clin. Gastroenterol. Hepatol.* **4**, 123–129 (2006).
34. Gibson, G. R., Macfarlane, G. T. & Cummings, J. H. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. *J. Appl. Bacteriol.* **65**, 103–111 (1988).
35. Segal, I., Walker, A. R., Lord, S. & Cummings, J. H. Breath methane and large bowel cancer risk in contrasting African populations. *Gut* **29**, 608–613 (1988).
36. Wilkens, L. R., Le Marchand, L., Harwood, P. & Cooney, R. V. Use of breath hydrogen and methane as markers of colonic fermentation in epidemiological studies: variability in excretion. *Cancer Epidemiol. Biomarkers Prev.* **3**, 149–153 (1994).
37. O'Keefe, S. J. *et al.* Why do African Americans get more colon cancer than Native Africans? *J. Nutr.* **137**, 175S–182S (2007).
38. Thauer, R. K., Jungermann, K. & Decker, K. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* **41**, 100–180 (1977).
39. Nava, G. M., Carbonero, F., Croix, J. A., Greenberg, E. & Gaskins, H. R. Abundance and diversity of mucosa-associated hydrogenotrophic microbes in the healthy human colon. *ISME J.* **6**, 57–70 (2011).
40. Fite, A. *et al.* Identification and quantitation of mucosal and faecal desulfovibrios using real time polymerase chain reaction. *Gut* **53**, 523–529 (2004).
41. Zinkevich, V. V. & Beech, I. B. Screening of sulfate-reducing bacteria in colonoscopy samples from healthy and colitic human gut mucosa. *FEMS Microbiol. Ecol.* **34**, 147–155 (2000).
42. Chassard, C. *et al.* Assessment of metabolic diversity within the intestinal microbiota from healthy humans using combined molecular and cultural approaches. *FEMS Microbiol. Ecol.* **66**, 496–504 (2008).
43. Harding, G. K., Sutter, V. L., Finegold, S. M. & Bricknell, K. S. Characterization of *Bacteroides melaninogenicus*. *J. Clin. Microbiol.* **4**, 354–359 (1976).
44. Simmering, R. *et al. Ruminococcus luti* sp. nov., isolated from a human faecal sample. *Syst. Appl. Microbiol.* **25**, 189–193 (2002).
45. Miller, T. L. & Wolin, M. J. Formation of hydrogen and formate by *Ruminococcus albus*. *J. Bacteriol.* **116**, 836–846 (1973).
46. Schwiertz, A. *et al. Anaerostipes caccae* gen. nov., sp. nov., a new saccharolytic, acetate-utilising, butyrate-producing bacterium from human faeces. *Syst. Appl. Microbiol.* **25**, 46–51 (2002).
47. Steer, T., Collins, M. D., Gibson, G. R., Hippe, H. & Lawson, P. A. *Clostridium hathewayi* sp. nov., from human faeces. *Syst. Appl. Microbiol.* **24**, 353–357 (2001).
48. Kamlage, B., Gruhl, B. & Blaut, M. Isolation and characterization of two new homoacetogenic hydrogen-utilizing bacteria from the human intestinal tract that are closely related to *Clostridium coccoides*. *Appl. Environ. Microbiol.* **63**, 1732–1738 (1997).
49. Pochart, P., Dore, J., Lemann, F., Goderel, I. & Rambaud, J. C. Interrelations between populations of methanogenic archaea and sulfate-reducing bacteria in the human colon. *FEMS Microbiol. Lett.* **77**, 225–228 (1992).
50. Macfarlane, G. T., Gibson, G. R. & Cummings, J. H. Comparison of fermentation reactions in different regions of the human colon. *J. Appl. Bacteriol.* **72**, 57–64 (1992).
51. Pochart, P. *et al.* Pyxigraphic sampling to enumerate methanogens and anaerobes in the right colon of healthy humans. *Gastroenterology* **105**, 1281–1285 (1993).
52. Flourie, B. *et al.* Site and substrates for methane production in human colon. *Am. J. Physiol.* **260**, G752–G757 (1991).
53. Gibson, G. R. *et al.* Alternative pathways for hydrogen disposal during fermentation in the human colon. *Gut* **31**, 679–683 (1990).
54. Marteau, P. *et al.* Comparative study of bacterial groups within the human cecal and fecal microbiota. *Appl. Environ. Microbiol.* **67**, 4939–4942 (2001).
55. Macfarlane, G. T. & Gibson, G. R. in *Gastrointestinal Microbiology* (eds Mackie, R. I. & White, B. A.) 269–318 (Chapman and Hall, New York, 1997).
56. Duncan, S. H., Hold, G. L., Barcenilla, A., Stewart, C. S. & Flint, H. J. *Roseburia intestinalis* sp nov., a novel saccharolytic, butyrate-producing bacterium from human faeces. *Int. J. Sys. Evol. Microbiol.* **52**, 1615–1620 (2002).
57. Duncan, S. H. *et al.* Proposal of *Roseburia faecis* sp. nov., *Roseburia hominis* sp. nov. and *Roseburia inulinivorans* sp. nov., based on isolates from human faeces. *Int. J. Syst. Evol. Microbiol.* **56**, 2437–2441 (2006).
58. Duncan, S. H. & Flint, H. J. Proposal of a neotype strain (A1–86) for *Eubacterium rectale*. Request for an opinion. *Int. J. Syst. Evol. Microbiol.* **58**, 1735–1736 (2008).
59. Chassard, C., Delmas, E., Lawson, P. A. & Bernalier-Donadille, A. *Bacteroides xylanisolvens* sp. nov., a xylan-degrading bacterium isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* **58**, 1008–1013 (2008).
60. Zoetendal, E. G., Plugge, C. M., Akkermans, A. D. & de Vos, W. M. *Victivallis vadensis* gen. nov., sp. nov., a sugar-fermenting anaerobe from human faeces. *Int. J. Syst. Evol. Microbiol.* **53**, 211–215 (2003).
61. Derrien, M., Vaughan, E. E., Plugge, C. M. & de Vos, W. M. *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* **54**, 1469–1476 (2004).
62. Duncan, S. H., Hold, G. L., Harmsen, H. J., Stewart, C. S. & Flint, H. J. Growth requirements and fermentation products of *Fusobacterium prausnitzii*, and a proposal to reclassify it as

Faecalibacterium prausnitzii gen. nov., comb. nov. *Int. J. Syst. Evol. Microbiol.* **52**, 2141–2146 (2002).
63. Tolvanen, K. E., Mangayil, R. K., Karp, M. T. & Santala, V. P. Simple enrichment system for hydrogen producers. *Appl. Environ. Microbiol.* **77**, 4246–4248 (2011).
64. Cammack, R. Hydrogenase sophistication. *Nature* **397**, 214–215 (1999).
65. Schmidt, O., Drake, H. L. & Horn, M. A. Hitherto unknown [Fe-Fe]-hydrogenase gene diversity in anaerobes and anoxic enrichments from a moderately acidic fen. *Appl. Environ. Microbiol.* **76**, 2027–2031 (2010).
66. Schmidt, O. *et al.* Novel [NiFe]- and [FeFe]-hydrogenase gene transcripts indicative of active facultative aerobes and obligate anaerobes in earthworm gut contents. *Appl. Environ. Microbiol.* **77**, 5842–5850 (2011).
67. Drake, H. L., Gossner, A. S. & Daniel, S. L. Old acetogens, new light. *Ann. NY Acad. Sci.* **1125**, 100–128 (2008).
68. Lajoie, S. F., Bank, S., Miller, T. L. & Wolin, M. J. Acetate production from hydrogen and [13C] carbon dioxide by the microflora of human feces. *Appl. Environ. Microbiol.* **54**, 2723–2727 (1988).
69. Bernalier, A. *et al.* Acetogenesis from H_2 and CO_2 by methane- and non-methane-producing human colonic bacterial communities. *FEMS Microbiol. Ecol.* **19**, 193–202 (1996).
70. Dore, J. *et al.* Enumeration of H_2-utilizing methanogenic archaea, acetogenic and sulfate-reducing bacteria from human feces. *FEMS Microbiol. Ecol.* **17**, 279–284 (1995).
71. Bernalier, A., Rochet, V., Leclerc, M., Dore, J. & Pochart, P. Diversity of H_2/CO_2-utilizing acetogenic bacteria from feces of non-methane-producing humans. *Curr. Microbiol.* **33**, 94–99 (1996).
72. Bernalier, A., Willems, A., Leclerc, M., Rochet, V. & Collins, M. D. *Ruminococcus hydrogenotrophicus* sp. nov., a new H_2/CO_2-utilizing acetogenic bacterium isolated from human feces. *Arch. Microbiol.* **166**, 176–183 (1996).
73. Lovell, C. R. & Leaphart, A. B. Community-level analysis: key genes of CO_2-reductive acetogenesis. *Methods Enzymol.* **397**, 454–469 (2005).
74. Wolin, M. J. & Miller, T. L. Bacterial strains from human feces that reduce CO_2 to acetic acid. *Appl. Environ. Microbiol.* **59**, 3551–3556 (1993).
75. Ohashi, Y., Igarashi, T., Kumazawa, F. & Fujisawa, T. Analysis of acetogenic bacteria in human feces with formyltetrahydrofolate synthetase sequences. *Biosci. Microflora* **26**, 37–40 (2007).
76. Cord-Ruwisch, R., Seitz, H. & Conrad, R. The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. *Arch. Microbiol.* **149**, 350–357 (1988).
77. Rey, F. E. *et al.* Dissecting the *in vivo* metabolic potential of two human gut acetogens. *J. Biol. Chem.* **285**, 22082–22090 (2010).
78. Miller, T. L. & Wolin, M. J. Pathways of acetate, propionate, and butyrate formation by the human fecal microbial flora. *Appl. Environ. Microbiol.* **62**, 1589–1592 (1996).
79. Hedderich, R. & Whitman, W. B. in *The Prokaryotes* (ed. Dworkin, M.) 1050–1079 (Springer, New York, 2006).
80. Miller, T. L. & Wolin, M. J. Methanogens in human and animal intestinal tracts. *Syst. Appl. Microbiol.* **7**, 223–229 (1986).
81. Chassard, C., Delmas, E., Robert, C. & Bernalier-Donadille, A. The cellulose-degrading microbial community of the human gut varies according to the presence or absence of methanogens. *FEMS Microbiol. Ecol.* **74**, 205–213 (2010).
82. Hansen, E. E. *et al.* Pan-genome of the dominant human gut-associated archaeon, *Methanobrevibacter smithii*, studied in twins. *Proc. Natl Acad. Sci. USA* **108**, 4599–4606 (2011).
83. Miller, T. L. & Wolin, M. J. *Methanosphaera stadtmaniae* gen. nov., sp. nov.: a species that forms methane by reducing methanol with hydrogen. *Arch. Microbiol.* **141**, 116–122 (1985).
84. Fricke, W. F. *et al.* The genome sequence of *Methanosphaera stadtmanae* reveals why this human intestinal archaeon is restricted to methanol and H_2 for methane formation and ATP synthesis. *J. Bacteriol.* **188**, 642–658 (2006).
85. Eckburg, P. B. *et al.* Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638 (2005).
86. Zhang, H. *et al.* Human gut microbiota in obesity and after gastric bypass. *Proc. Natl Acad. Sci USA* **106**, 2365–2370 (2009).
87. Weaver, G. A., Krause, J. A., Miller, T. L. & Wolin, M. J. Incidence of methanogenic bacteria in a sigmoidoscopy population: an association of methanogenic bacteria and diverticulosis. *Gut* **27**, 698–704 (1986).
88. Miller, T. L. & Wolin, M. J. Enumeration of *Methanobrevibacter smithii* from human feces. *Arch. Microbiol.* **45**, 317 (1982).
89. Abell, G. C. J., Conlon, M. A. & McOrist, A. L. Methanogenic archaea in adult human faecal samples are inversely related to butyrate concentration. *Microb. Ecol. Health Dis.* **18**, 154–160 (2006).
90. Scanlan, P. D., Shanahan, F. & Marchesi, J. R. Human methanogen diversity and incidence in healthy and diseased colonic groups using *mcrA* gene analysis. *BMC Microbiol.* **8**, 79 (2008).
91. Mihajlovski, A., Alric, M. & Brugere, J. F. A putative new order of methanogenic Archaea inhabiting the human gut, as revealed by molecular analyses of the *mcrA* gene. *Res. Microbiol.* **159**, 516–521 (2008).
92. Marquet, P., Duncan, S. H., Chassard, C., Bernalier-Donadille, A. & Flint, H. J. Lactate has the potential to promote hydrogen sulphide formation in the human colon. *FEMS Microbiol. Lett.* **299**, 128–134 (2009).
93. Kleessen, B., Kroesen, A. J., Buhr, H. J. & Blaut, M. Mucosal and invading bacteria in patients with inflammatory bowel disease compared with controls. *Scand. J. Gastroenterol.* **37**, 1034–1041 (2002).
94. Gibson, G. R., Macfarlane, S. & Macfarlane, G. T. Metabolic interactions involving sulphate-reducing and methanogenic bacteria in the human large intestine. *FEMS Microbiol. Ecol.* **12**, 117–125 (1993).
95. Newton, D. F., Cummings, J. H., Macfarlane, S. & Macfarlane, G. T. Growth of a human intestinal *Desulfovibrio desulfuricans* in continuous cultures containing defined populations of saccharolytic and amino acid fermenting bacteria. *J. Appl. Microbiol.* **85**, 372–380 (1998).
96. Hopkins, M. J., Macfarlane, G. T., Furrie, E., Fite, A. & Macfarlane, S. Characterisation of intestinal bacteria in infant stools using real-time PCR and northern hybridisation analyses. *FEMS Microbiol. Ecol.* **54**, 77–85 (2005).
97. Gibson, G. R., Cummings, J. H. & Macfarlane, G. T. Growth and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. *FEMS Microbiol. Lett.* **86**, 103–111 (1991).
98. Willis, C. L., Cummings, J. H., Neale, G. & Gibson, G. R. Nutritional aspects of dissimilatory sulfate reduction in the human large intestine. *Curr. Microbiol.* **35**, 294–298 (1997).
99. Zverlov, V. *et al.* Lateral gene transfer of dissimilatory (bi)sulfite reductase revisited. *J. Bacteriol.* **187**, 2203–2208 (2005).
100. Klein, M. *et al.* Multiple lateral transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-reducing prokaryotes. *J. Bacteriol.* **183**, 6028–6035 (2001).
101. Wagner, M., Roger, A. J., Flax, J. L., Brusseau, G. A. & Stahl, D. A. Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J. Bacteriol.* **180**, 2975–2982 (1998).
102. Meyer, B. & Kuever, J. Phylogeny of the alpha and beta subunits of the dissimilatory adenosine-5'-phosphosulfate (APS) reductase from sulfate-reducing prokaryotes—origin and evolution of the dissimilatory sulfate-reduction pathway. *Microbiology* **153**, 2026–2044 (2007).
103. Hungin, A. P., Chang, L., Locke, G. R., Dennis, E. H. & Barghout, V. Irritable bowel syndrome in the United States: prevalence, symptom patterns and impact. *Aliment. Pharmacol. Ther.* **21**, 1365–1375 (2005).
104. Everhart, J. E. in *The Burden of Digestive Diseases in the United States* (ed. Everhart, J. E.) 77–87 (US Government Printing Office. US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Washington, DC, 2008).
105. Drossman, D. A. *et al.* A prospective assessment of bowel habit in irritable bowel syndrome in women: defining an alternator. *Gastroenterology* **128**, 580–589 (2005).
106. Drossman, D. A. *et al.* International survey of patients with IBS: symptom features and their severity, health status, treatments, and risk taking to achieve clinical benefit. *J. Clin. Gastroenterol.* **43**, 541–550 (2009).
107. Hasler, W. L. Irritable bowel syndrome and bloating. *Best Pract. Res. Clin. Gastroenterol.* **21**, 689–707 (2007).
108. Serra, J., Azpiroz, F. & Malagelada, J. R. Impaired transit and tolerance of intestinal gas in the irritable bowel syndrome. *Gut* **48**, 14–19 (2001).
109. Salonen, A., de Vos, W. M. & Palva, A. Gastrointestinal microbiota in irritable bowel syndrome: present state and perspectives. *Microbiology* **156**, 3205–3215 (2010).
110. Kunkel, D. *et al.* Methane on breath testing is associated with constipation: a systematic review and meta-analysis. *Dig. Dis. Sci.* **56**, 1612–1618 (2011).
111. Spiegel, B. M. R. Questioning the bacterial overgrowth hypothesis of irritable bowel syndrome: An epidemiologic and evolutionary perspective. *Clin. Gastroenterol. Hepatol.* **9**, 461–469 (2011).
112. Chatterjee, S., Park, S., Low, K., Kong, Y. & Pimentel, M. The degree of breath methane production in IBS correlates with the severity of constipation. *Am. J. Gastroenterol.* **102**, 837–841 (2007).
113. King, T. S., Elia, M. & Hunter, J. O. Abnormal colonic fermentation in irritable bowel syndrome. *Lancet* **352**, 1187–1189 (1998).
114. Simren, M. & Stotzer, P. O. Use and abuse of hydrogen breath tests. *Gut* **55**, 297–303 (2006).
115. Pimentel, M., Chow, E. J. & Lin, H. C. Comparison of peak breath hydrogen production in patients with irritable bowel syndrome, chronic fatigue syndrome and fibromyalgia. *Gastroenterology* **118**, A413 (2000).

116. Pimentel, M., Chow, E. J. & Lin, H. C. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am. J. Gastroenterol.* **95**, 3503–3506 (2000).
117. Yu, D., Cheeseman, F. & Vanner, S. Combined oro-caecal scintigraphy and lactulose hydrogen breath testing demonstrate that breath testing detects oro-caecal transit, not small intestinal bacterial overgrowth in patients with IBS. *Gut* **60**, 334–340 (2011).
118. Quigley, E. M. Germs, gas and the gut; the evolving role of the enteric flora in IBS. *Am. J. Gastroenterol.* **101**, 334–335 (2006).
119. Quigley, E. M. M. The enteric microbiota in the pathogenesis and management of constipation. *Best Pract. Res. Clin. Gastroenterol.* **25**, 119–126 (2011).
120. Pimentel, M., Park, S., Kong, Y., Low, K. & Chatterjee, S. Methane gas is associated with constipation predominant symptoms in IBS: Results from a double-blind controlled study. *Gastroenterology* **130**, A514 (2006).
121. Pimentel, M. *et al.* Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. *Am. J. Physiol. Gastrointest. Liver Physiol.* **290**, G1089–G1095 (2006).
122. Rajilic-Stojanovic, M. *et al.* Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* **141**, 1792–1801 (2011).
123. Quigley, E. M. & Quera, R. Small intestinal bacterial overgrowth: roles of antibiotics, prebiotics, and probiotics. *Gastroenterology* **130**, S78–S90 (2006).
124. Evans, B. W., Clark, W. K., Moore, D. J. & Whorwell, P. J. Tegaserod for the treatment of irritable bowel syndrome and chronic constipation. *Cochrane Database Syst. Rev.* Issue 4. Art. No.: CD003960 (2007).
125. Kim, D. Y. & Camilleri, M. Serotonin: a mediator of the brain-gut connection. *Am. J. Gastroenterol.* **95**, 2698–2709 (2000).
126. De Ponti, F. & Tonini, M. Irritable bowel syndrome: new agents targeting serotonin receptor subtypes. *Drugs* **61**, 317–332 (2001).
127. Ford, A. C. *et al.* Efficacy of 5-HT3 antagonists and 5-HT4 agonists in irritable bowel syndrome: systematic review and meta-analysis. *Am. J. Gastroenterol.* **104**, 1831–1843 (2009).
128. Kawabata, A. *et al.* Hydrogen sulfide as a novel nociceptive messenger. *Pain* **132**, 74–81 (2007).
129. Matsunami, M. *et al.* Luminal hydrogen sulfide plays a pronociceptive role in mouse colon. *Gut* **58**, 751–761 (2009).
130. Schemann, M. & Grundy, D. Role of hydrogen sulfide in visceral nociception. *Gut* **58**, 744–747 (2009).
131. Distrutti, E. *et al.* A nitro-arginine derivative of trimebutine (NO2-Arg-Trim) attenuates pain induced by colorectal distension in conscious rats. *Pharmacol. Res.* **59**, 319–329 (2009).
132. Roediger, W. E., Duncan, A., Kapaniris, O. & Millard, S. Reducing sulfur compounds of the colon impair colonocyte nutrition: Implications for ulcerative colitis. *Gastroenterology* **104**, 802–809 (1993).
133. Roediger, W. E., Duncan, A., Kapaniris, O. & Millard, S. Sulphide impairment of substrate oxidation in rat colonocytes: a biochemical basis for ulcerative colitis? *Clin. Sci. (Lond.)* **85**, 623–627 (1993).
134. O'Neil, M. J. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals (Merck & Co, Whitehouse Station, NJ, 2001).
135. Strocchi, A., Ellis, C. J. & Levitt, M. D. Use of metabolic inhibitors to study H_2 consumption by human feces: evidence for a pathway other than methanogenesis and sulfate reduction. *J. Lab. Clin. Med.* **121**, 320–327 (1993).
136. Roediger, W. E., Moore, J. & Babidge, W. Colonic sulfide in pathogenesis and treatment of ulcerative colitis. *Dig. Dis. Sci.* **42**, 1571–1579 (1997).
137. Tragnone, A. *et al.* Dietary habits as risk factors for inflammatory bowel disease. *Eur. J. Gastroenterol. Hepatol.* **7**, 47–51 (1995).
138. Truelove, S. C. Ulcerative colitis provoked by milk. *Br. Med. J.* **1**, 154–160 (1961).
139. Pitcher, M. C., Beatty, E. R. & Cummings, J. H. The contribution of sulphate reducing bacteria and 5-aminosalicylic acid to faecal sulphide in patients with ulcerative colitis. *Gut* **46**, 64–72 (2000).
140. Edmond, L. M., Hopkins, M. J., Magee, E. A. & Cummings, J. H. The effect of 5-aminosalicylic acid-containing drugs on sulfide production by sulfate-reducing and amino acid-fermenting bacteria. *Inflamm. Bowel Dis.* **9**, 10–17 (2003).
141. Moore, J., Babidge, W., Millard, S. & Roediger, W. Colonic luminal hydrogen sulfide is not elevated in ulcerative colitis. *Dig. Dis. Sci.* **43**, 162–165 (1998).
142. Duffy, M. *et al.* Sulfate-reducing bacteria colonize pouches formed for ulcerative colitis but not for familial adenomatous polyposis. *Dis. Colon Rectum* **45**, 384–388 (2002).
143. Ohge, H. *et al.* Association between fecal hydrogen sulfide production and pouchitis. *Dis. Colon Rectum* **48**, 469–475 (2005).
144. Gibson, G. R., Cummings, J. H. & Macfarlane, G. T. Growth and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. *FEMS Microbiol. Lett.* **86**, 103–111 (1991).
145. Pitcher, M. C. L., Beatty, E. R., Gibson, G. R. & Cummings, J. H. Incidence and activities of sulphate-reducing bacteria in patients with ulcerative colitis. *Gut* **36**, A63 (1995).
146. Levine, J., Ellis, C. J., Furne, J. K., Springfield, J. & Levitt, M. D. Fecal hydrogen sulfide production in ulcerative colitis. *Am. J. Gastroenterol.* **93**, 83–87 (1998).
147. Loubinoux, J., Bronowicki, J. P., Pereira, I. A., Mougenel, J. L. & Faou, A. E. Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. *FEMS Microbiol. Ecol.* **40**, 107–112 (2002).
148. Tsai, H. H., Sunderland, D., Gibson, G. R., Hart, C. A. & Rhodes, J. M. A novel mucin sulphatase from human faeces: its identification, purification and characterization. *Clin. Sci. (Lond.)* **82**, 447–454 (1992).
149. Croix, J. A. *et al.* On the relationship between sialomucin and sulfomucin expression and hydrogenotrophic microbes in the human colonic mucosa. *PLoS ONE* **6**, e24447 (2011).
150. Bjorneklett, A., Fausa, O. & Midtvedt, T. Bacterial overgrowth in jejunal and ileal disease. *Scand. J. Gastroenterol.* **18**, 289–298 (1983).
151. McKay, L. F., Eastwood, M. A. & Brydon, W. G. Methane excretion in man—a study of breath, flatus, and faeces. *Gut* **26**, 69–74 (1985).
152. Peled, Y., Weinberg, D., Hallak, A. & Gilat, T. Factors affecting methane production in humans. Gastrointestinal diseases and alterations of colonic flora. *Dig. Dis. Sci.* **32**, 267–271 (1987).
153. Pimentel, M. *et al.* Methane production during lactulose breath test is associated with gastrointestinal disease presentation. *Dig. Dis. Sci.* **48**, 86–92 (2003).
154. Perman, J. A. Methane and colorectal cancer. *Gastroenterology* **87**, 728–730 (1984).
155. Karlin, D. A., Jones, R. D., Stroehlein, J. R., Mastromarino, A. J. & Potter, G. D. Breath methane excretion in patients with unresected colorectal cancer. *J. Natl Cancer Inst.* **69**, 573–576 (1982).
156. Haines, A., Metz, G., Dilawari, J., Blendis, L. & Wiggins, H. Breath-methane in patients with cancer of the large bowel. *Lancet* **2**, 481–483 (1977).
157. Pique, J. M., Pallares, M., Cuso, E., Vilar-Bonet, J. & Gassull, M. A. Methane production and colon cancer. *Gastroenterology* **87**, 601–605 (1984).
158. Karlin, D. A., Mastromarino, A. J., Jones, R. D., Stroehlein, J. R. & Lorentz, O. Fecal skatole and indole and breath methane and hydrogen in patients with large bowel polyps or cancer. *J. Cancer Res. Clin. Oncol.* **109**, 135–141 (1985).
159. Peled, Y. Methane production and colon cancer. *Gastroenterology* **88**, 1294 (1985).
160. Kashtan, H., Rabau, M., Peled, Y., Milstein, A. & Wiznitzer, T. Methane production in patients with colorectal carcinoma. *Isr. J. Med. Sci.* **25**, 614–616 (1989).
161. Sivertsen, S. M., Bjorneklett, A., Gullestad, H. P. & Nygaard, K. Breath methane and colorectal-cancer. *Scand. J. Gastroenterol.* **27**, 25–28 (1992).
162. Holma, R. *et al.* Colonic methanogenesis *in vivo* and *in vitro* and fecal pH after resection of colorectal cancer and in healthy intact colon. *Int. J. Colorectal Dis.* **27**, 171–178 (2011).
163. Babidge, W., Millard, S. & Roediger, W. Sulfides impair short chain fatty acid beta-oxidation at acyl-CoA dehydrogenase level in colonocytes: implications for ulcerative colitis. *Mol. Cell. Biochem.* **181**, 117–124 (1998).
164. Cai, W. J., Wang, M. J., Ju, L. H., Wang, C. & Zhu, Y. C. Hydrogen sulfide induces human colon cancer cell proliferation: role of Akt, ERK and p21. *Cell. Biol. Int.* **34**, 565–572 (2010).
165. Deplancke, B. & Gaskins, H. R. Hydrogen sulfide induces serum-independent cell cycle entry in nontransformed rat intestinal epithelial cells. *FASEB J.* **17**, 1310–1312 (2003).
166. Leschelle, X. *et al.* Adaptative metabolic response of human colonic epithelial cells to the adverse effects of the luminal compound sulfide. *Biochim. Biophys. Acta* **1725**, 201–212 (2005).
167. Christl, S. U., Eisner, H. D., Dusel, G., Kasper, H. & Scheppach, W. Antagonistic effects of sulfide and butyrate on proliferation of colonic mucosa: a potential role for these agents in the pathogenesis of ulcerative colitis. *Dig. Dis. Sci.* **41**, 2477–2481 (1996).
168. Attene-Ramos, M. S., Wagner, E. D., Plewa, M. J. & Gaskins, H. R. Evidence that hydrogen sulfide is a genotoxic agent. *Mol. Cancer Res.* **4**, 9–14 (2006).
169. Attene-Ramos, M. S., Wagner, E. D., Gaskins, H. R. & Plewa, M. J. Hydrogen sulfide induces direct radical-associated DNA damage. *Mol. Cancer Res.* **5**, 455–459 (2007).
170. Attene-Ramos, M. S. *et al.* DNA damage and toxicogenomic analyses of hydrogen sulfide in human intestinal epithelial FHs 74 Int cells. *Environ. Mol. Mutagen.* **51**, 304–314 (2010).
171. Kanazawa, K. *et al.* Factors influencing the development of sigmoid colon cancer—bacteriologic and biochemical studies. *Cancer* **77**, 1701–1706 (1996).
172. Ramasamy, S., Singh, S., Taniere, P., Langman, M. J. & Eggo, M. C. Sulfide-detoxifying enzymes in the human colon are decreased in cancer and upregulated in differentiation. *Am. J.*

Physiol. Gastrointest. Liver Physiol. **291**, G288–G296 (2006).
173. Balamurugan, R., Rajendiran, E., George, S., Samuel, G. V. & Ramakrishna, B. S. Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, *Desulfovibrio* and *Enterococcus faecalis* in the feces of patients with colorectal cancer. *J. Gastroenterol. Hepatol.* **23**, 1298–1303 (2008).
174. Marchesi, J. R. *et al.* Towards the human colorectal cancer microbiome. *PLoS ONE* **6** e20447 (2011).
175. Kostic, A. D. *et al.* Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. *Genome Res.* **22**, 292–298 (2011).
176. Castellarin, M. *et al. Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. *Genome Res.* **22**, 299–306 (2011).
177. Fiorucci, S., Distrutti, E., Cirino, G. & Wallace, J. L. The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. *Gastroenterology* **131**, 259–271 (2006).
178. Wang, R. Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? *FASEB J.* **16**, 1792–1798 (2002).
179. Wallace, J. L., Ferraz, J. & Muscara, M. Hydrogen sulfide: an endogenous mediator of resolution of inflammation and injury. *Antioxid. Redox Signal* http://dx.doi.org/10.1089/ars.2011.4351.
180. Furne, J., Saeed, A. & Levitt, M. D. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **295**, R1479–R1485 (2008).
181. Brock, T. D. & Od'ea, K. Amorphous ferrous sulfide as a reducing agent for culture of anaerobes. *Appl. Environ. Microbiol.* **33**, 254–256 (1977).
182. Hungate, R. E. in *Methods in Microbiology* (eds Norris, J. R. & Ribbons, D. W.) 117–132 (Academic Press, London, 1969).
183. Turnbaugh, P. J. *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031 (2006).
184. Haines, A. P., Imeson, J. D. & Wiggins, H. S. Relation of breath methane with obesity and other factors. *Int. J. Obes.* **8**, 675–680 (1984).
185. Million, M. *et al.* Obesity-associated gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. *Int. J. Obes. (Lond.)* http://dx.doi.org/10.1038/ijo.2011.153.
186. Schwiertz, A. *et al.* Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)* **18**, 190–195 (2010).
187. Armougom, F., Henry, M., Vialettes, B., Raccah, D. & Raoult, D. Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and methanogens in anorexic patients. *PLoS ONE* **4**, e7125 (2009).
188. Zheng, X. F., Sun, X. J. & Xia, Z. F. Hydrogen resuscitation, a new cytoprotective approach. *Clin. Exp. Pharmacol. Physiol.* **38**, 155–163 (2011).
189. Ohsawa, I. *et al.* Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat. Med.* **13**, 688–694 (2007).
190. Hong, Y., Chen, S. & Zhang, J. M. Hydrogen as a selective antioxidant: a review of clinical and experimental studies. *J. Int. Med. Res.* **38**, 1893–1903 (2010).
191. Huang, C. S., Kawamura, T., Toyoda, Y. & Nakao, A. Recent advances in hydrogen research as a therapeutic medical gas. *Free Rad. Res.* **44**, 971–982 (2010).
192. Kajiya, M., Silva, M. J. B., Sato, K., Ouhara, K. & Kawai, T. Hydrogen mediates suppression of colon inflammation induced by dextran sodium sulfate. *Biochem. Biophys. Res. Commun.* **386**, 11–15 (2009).
193. Nakao, A., Toyoda, Y., Sharma, P., Evans, M. & Guthrie, N. Effectiveness of hydrogen rich water on antioxidant status of subjects with potential metabolic syndrome—an open label pilot study. *J. Clin. Biochem. Nutr.* **46**, 140–149 (2010).
194. Metz, G. L., Blendis, L. M. & Jenkins, J. A. Proceedings: alveolar H_2 in the diagnosis of carbohydrate malabsorption. *Gut* **16**, 398 (1975).
195. Metz, G., Gassull, M. A., Drasar, B. S., Jenkins, D. J. & Blendis, L. M. Breath-hydrogen test for small-intestinal bacterial colonisation. *Lancet* **1**, 668–669 (1976).
196. El Oufir, L. *et al.* Relations between transit time, fermentation products, and hydrogen consuming flora in healthy humans. *Gut* **38**, 870–877 (1996).
197. Pimentel, M., Kong, Y. & Park, S. IBS subjects with methane on lactulose breath test have lower postprandial serotonin levels than subjects with hydrogen. *Dig. Dis. Sci.* **49**, 84–87 (2004).
198. Dear, K. L., Elia, M. & Hunter, J. O. Do interventions which reduce colonic bacterial fermentation improve symptoms of irritable bowel syndrome? *Dig. Dis. Sci.* **50**, 758–766 (2005).
199. Levine, J., Furne, J. K. & Levitt, M. D. Ashkenazi Jews, sulfur gases, and ulcerative colitis. *J. Clin. Gastroenterol.* **22**, 288–291 (1996).
200. Duffy, M. *et al.* Sulfate-reducing bacteria colonize pouches formed for ulcerative colitis but not for familial adenomatous polyposis. *Dis. Colon Rectum* **45**, 384–388 (2002).
201. Bullock, N. R., Booth, J. C. & Gibson, G. R. Comparative composition of bacteria in the human intestinal microflora during remission and active ulcerative colitis. *Curr. Issues Intest. Microbiol.* **5**, 59–64 (2004).
202. Smith, F. M. *et al.* A characterization of anaerobic colonization and associated mucosal adaptations in the undiseased illeal pouch. *Colorectal Dis.* **7**, 563–570 (2005).
203. Bambury, N., Coffey, J. C., Burke, J., Redmond, H. P. & Kirwan, W. O. Sulphomucin expression in ileal pouches: emerging differences between ulcerative colitis and familial adenomatous polyposis pouches. *Dis. Colon Rectum* **51**, 561–567 (2008).
204. Coffey, J. C. *et al.* Pathogenesis of and unifying hypothesis for idiopathic pouchitis. *Am. J. Gastroenterol.* **104**, 1013–1023 (2009).
205. Lim, M. *et al.* An assessment of bacterial dysbiosis in pouchitis using terminal restriction fragment length polymorphisms of 16S ribosomal DNA from pouch effluent microbiota. *Dis. Colon Rectum* **52**, 1492–1500 (2009).
206. Rowan, F. *et al. Desulfovibrio* bacterial species are increased in ulcerative colitis. *Dis. Colon Rectum* **53**, 1530–1536 (2010).
207. Verma, R., Verma, A. K., Ahuja, V. & Paul, J. Real-time analysis of mucosal flora in patients with inflammatory bowel disease in India. *J. Clin. Microbiol.* **48**, 4279–4282 (2010).
208. Strauss, J. *et al.* Invasive potential of gut mucosa-derived *Fusobacterium nucleatum* positively correlates with IBD status of the host. *Inflamm. Bowel Dis.* **17**, 1971–1978 (2011).
209. Scanlan, P. D., Shanahan, F. & Marchesi, J. R. Culture-independent analysis of desulfovibrios in the human distal colon of healthy, colorectal cancer and polypectomized individuals. *FEMS Microbiol. Ecol.* **69**, 213–221 (2009).

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Author contributions
All authors contributed equally to all aspects of the manuscript.