Mechanisms of Microbial Hydrogen Disposal in the Human Colon and Implications for Health and Disease

Noriko Nakamura,[1,4] Henry C. Lin,[5] Christopher S. McSweeney,[6] Roderick I. Mackie,[1,3,4] and H. Rex Gaskins[1,2,3,4]

[1]Departments of Animal Sciences and [2]Pathobiology, [3]Division of Nutritional Sciences, [4]Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801; email: hgaskins@illinois.edu

[5]New Mexico VA Health Care System, Department of Medicine, The University of New Mexico, Albuquerque, New Mexico 87108

[6]CSIRO Livestock Industries, Queensland Bioscience Precinct, St. Lucia, Brisbane, Queensland 4067, Australia

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Abstract

In the human gastrointestinal tract, dietary components, including fiber, that reach the colon are fermented principally to short-chain fatty acids, hydrogen, and carbon dioxide. Microbial disposal of the hydrogen generated during anaerobic fermentation in the human colon is critical to optimal functioning of this ecosystem. However, our understanding of microbial hydrogenotrophy is fragmented and, at least as it occurs in the colon, is mostly theoretical in nature. Thorough investigation and integration of knowledge on the diversity of hydrogenotrophic microbes, their metabolic variation and activities as a functional group, as well as the nature of their interactions with fermentative bacteria, are necessary to understand hydrogen metabolism in the human colon. Here, we review the limited data available on the three major groups of H_2-consuming microorganisms found in the human colon [methanogens, sulfate-reducing bacteria (SRB), and acetogens] as well as evidence that end products of their metabolism have an important impact on colonic health.

METABOLIC FEATURES OF THE COLONIC ECOSYSTEM

Overview of Fermentation

Short-chain fatty acids (SCFA): primary fermentation products in the colon that include acetate, propionate and butyrate; formed primarily from resistant starch and nonstarch polysaccharides

Fermentation: anaerobic energy-yielding processes in which ATP is formed by substrate-level phosphorylation through association with redox transformations between organic compounds

Sulfate-reducing bacteria (SRB): bacteria capable of obtaining energy by reducing sulfate or other sulfur species to hydrogen sulfide

Hydrogenotroph: hydrogen-consuming microorganisms critical to disposal of hydrogen during bacterial fermentation

Methanogen: microorganisms unique to Archaea that obtain metabolic energy by methane production

Acetogen: bacteria that utilize the acetyl-CoA pathway to generate acetate for the purpose of conserving energy and assimilating CO_2 into cell carbon

In humans, dietary components that escape digestion by host enzymes in the upper intestinal tract reach the colon (**Figure 1**), where they are fermented by the cooperative metabolism of an enormous variety of bacterial species. Endogenously produced substrates, such as mucin, are also nutrient sources that support the colonic microbiota. Complex polymerized carbohydrates are degraded to smaller oligomers or monomers, which are subsequently fermented to short-chain fatty acids (SCFA), H_2, CO_2, and a variety of other products. SCFA are quantitatively the principal fermentation products and are rapidly absorbed across the intestinal epithelium. It is estimated that 95–99% of the SCFA are absorbed and utilized by the host (Macfarlane & Gibson 1997). The three major SCFA detected in the human colon are acetate, propionate, and butyrate (Cummings et al. 1987). Resistant starch (RS) and nonstarch polysaccharides (NSP) are major sources of colonic SCFA. Resistant starch is a component of dietary starch that escapes host digestion in the small intestine and reaches the colon undigested. Starch fermentation is associated with greater production of butyrate than fermentation of NSP (Cummings & Englyst 1987). Consumption of high RS diets has been suggested to be protective against large bowel disorders (Topping & Clifton 2001).

Mucins are likely to be important nutrient sources for fermentative microbes in addition to those of dietary origin. Mucins are complex glycoproteins secreted by goblet cells lining the gastrointestinal (GI) tract and constitute the mucus gel layer that serves to protect the mucosal surface as well as provide an ecological niche for intestinal microbes. Mucin oligosaccharide side chains may be sulfated or sialylated, and the extent of such modifications varies substantially in different parts of the GI tract. Colonic mucins are more highly sulfated than the small intestinal and gastric mucins (Filipe 1979). Mucins are degraded by colonic bacteria using a variety of hydrolytic enzymes (glycosidases, proteases, peptidases, and sulfatases) (Macfarlane et al. 2005). Some bacteria such as *Bacteroides fragilis* possess sulfatase activity and are able to release sulfate in a free form, which subsequently becomes available for sulfate reduction by sulfate-reducing bacteria (SRB) (Willis et al. 1996).

Many colonic fermentative bacteria possess branched fermentation pathways that allow a flexibility of metabolism in response to changes of redox balance. Different metabolic pathways result in differences in the flow of carbon and electrons, energy yield from the substrate, and the final fermentation products. The production of H_2 is an efficient mechanism of disposing of reducing power generated during bacterial metabolism of carbohydrates and protein. However, accumulation of H_2 inhibits the reoxidation of pyridine nucleotides, resulting in the production of electron sink products such as ethanol, lactate, or succinate. The symbiosis between fermentative bacteria and hydrogenotrophic microbes enables the former to shift fermentation patterns toward the production of more reduced substrates with additional ATP synthesis via substrate-level phosphorylation, as opposed to production of electron sink products. The syntrophic coupling of hydrogen producers and consumers was first recognized by Bryant et al. (1967) and was later termed interspecies hydrogen transfer (Iannotti et al. 1973) (See sidebar, Interspecies H_2 Transfer).

There are three major groups of H_2-consuming microorganisms (hydrogenotrophs) in the human colon: methanogens, SRB, and acetogens (**Figure 2**). Colonic hydrogen may also be consumed by other microbes via hydrogenation of unsaturated fatty acids (Polan et al. 1964) or reduction of fumarate (Asanuma & Hino 2000) or nitrate (Allison & Macfarlane 1988). These types of hydrogen consumption are quantitatively less important and not covered in this review. Hydrogenotrophic organisms are typically present at much lower densities than

INTERSPECIES H_2 TRANSFER

This is a mutually beneficial, unidirectional process that plays a central role in the anaerobic fermentation of organic matter in which one species degrades an organic substrate and releases reducing equivalents in form of hydrogen, which, in turn, is oxidized by the second species. Generally, the first organism profits from H_2 removal by the syntrophic partner. Hydrogen-based syntrophy was first demonstrated by M. P. Bryant and coworkers at the University of Illinois when they reported in 1967 that a culture called *Methanobacillus omelianskii*, which oxidized ethanol to acetate and used the electrons to reduce CO_2 to CH_4, actually consisted of two organisms (Bryant et al. 1967). The so-called S-organism fermented ethanol to acetate and hydrogen, and the hydrogen was used by *Methanobacterium bryantii* strain MoH, which created thermodynamically favorable conditions for growth of the S-organism. This interaction became the paradigm for other reactions involving obligate interspecies hydrogen transfer, in which growth of the H_2 producer is possible only if the H_2 partial pressure is maintained below a certain threshold by a H_2 consumer.

fermentative bacteria. However, in the absence of H_2-consuming organisms, the H_2 partial pressure rapidly reaches a level that thermodynamically restricts fermentation.

One characteristic of the colonic ecosystem is a lack of complete degradation of organic matter. In many environmental anaerobic ecosystems, fermentation products such as organic acids and alcohols are further degraded to acetate, H_2, and CO_2, which are subsequently utilized by methanogens and SRB. Thus, acetate is a key intermediate in the complete anaerobic degradation of organic matter. Acetogens may also play an important role in interspecies hydrogen transfer by functioning both as H_2 producers and consumers. On the other hand, in the colonic ecosystem the importance of acetate as a fermentation intermediate is less significant. This is because a short retention time in the colon does not accommodate slow-growing aceticlastic methanogens or H_2-producing acetate degraders (Bryant 1979, McInerney et al. 1979). As a result, most end-products produced during colonic fermentation are typically not further metabolized by resident microbes but are rather absorbed and utilized by the host. As a consequence, competition between methanogens and SRB in the colon occurs mainly for H_2 and not for acetate.

Numerous environmental parameters differ significantly between the right and the left colon, affecting fermentation patterns in each region (**Figure 1**). In particular, pH of the colonic lumen has important effects. A pH shift between 5.5 and 6.5, which corresponds to the pH gradient from the right to the left colon (Cummings et al. 1987), affects microbial species composition and fermentation patterns in the continuous culture of human fecal microbiota, with a lower pH favoring a higher proportion of Firmicutes phylum and butyrate production (Duncan et al. 2009, Walker et al. 2005). The pH gradient may affect H_2-consuming microbiota as well. Methanogenesis and sulfate reduction occur optimally at neutral or slightly alkaline pH respectively, whereas acetogenesis appears to be maximal at an acidic pH (Gibson et al. 1990). Methanogens exist in higher concentration in the left than the right colon, whereas SRB may be present throughout the colon (Macfarlane et al. 1992). Increased hydrogenotrophic activity in the left colon may also reflect a greater in situ H_2 partial pressure in this region, which results from less stirring of the colonic contents as a result of decreased liquidity (Strocchi & Levitt 1992).

Gas Composition and Volumes

Unabsorbed, fermentable dietary carbohydrate is the primary source of intestinal gas, with quantities varying according to the type and amount of fermentable carbohydrate consumed. Although

most of the gas that accumulates in the intestinal lumen is absorbed into the circulation and expelled with exhaled breath, gas that exceeds the capacity for pulmonary excretion is expelled as flatus. The volume of flatus excreted by individuals ranges from 400 ml to 1200 ml per day (Askevold 1956, Beazell & Ivy 1941, Kirk 1949). Flatus gas is composed predominately (~74%) of the intraluminally produced gases H_2, CO_2, and CH_4 together with other trace gases including volatile amines, NH_3, mercaptans, and sulfur-containing gases (Suarez et al. 1997). Two additional major flatus gases, N_2 and O_2, are considered to be derived solely from swallowed air (Levitt & Bond 1980). Marked individual differences exist in the proportional composition of major intestinal gas (Levitt & Bond 1970, Suarez et al. 1997).

CO_2 is the predominant component of flatus (Suarez et al. 1997). There are three possible sources of CO_2 in the intestinal tract: diffusion from the blood into the lumen, neutralization of acid by bicarbonate, and production by intestinal microbes (Levitt & Bond 1970). Although large quantities of CO_2 are produced in the duodenum after food consumption, most is absorbed into blood during passage through the bowel, and thus this CO_2 does not appear in flatus. Diffusion of CO_2 from blood is likely to be negligible because CO_2 concentrations in flatus are much greater than that in blood. Thus, most CO_2 in flatus originates from bacterial metabolism.

All H_2 production in the human intestine appears to be of bacterial origin and depends primarily upon the fermentation of dietary substrates (Levitt & Bond 1980). It is estimated that approximately 40 g of carbohydrate per day remain unabsorbed in a typical Western diet (Levitt et al. 1987). With this amount of substrate available for bacterial fermentation, and hydrogen production occurring at a rate of 340 mL/g of carbohydrate (Wolin & Miller 1983), approximately 13,600 mL of hydrogen would be generated daily (**Figure 2**). Fermentable substances of endogenous origin also likely contribute to colonic H_2 production (Perman & Modler 1982) given that H_2 is produced consistently at low levels in the colon after prolonged fasting (Levitt & Ingelfinger 1968). The main mechanisms by which H_2 can be produced by carbohydrate-fermenting bacteria include (*a*) cleavage of pyruvate to formate and subsequent metabolism by formate hydrogen lyase, (*b*) generation from pyruvate through the activity of pyruvate:ferredoxin oxidoreductase and hydrogenase, and (*c*) formation from oxidation of pyridine and flavin nucleotides (Macfarlane & Gibson 1997). The absolute production of H_2 per gram of glucose fermented is similar among various individuals (Strocchi & Levitt 1992). A substantial amount of H_2 is consumed very rapidly in situ by colonic hydrogenotrophic microbes (Strocchi & Levitt 1992), which convert hydrogen into methane or hydrogen sulfide (H_2S), resulting in a great reduction of the total volume of gas excreted. The amount of hydrogen expelled in exhaled breath may reach zero when H_2 consumption by hydrogenotrophic microbes is nearly complete.

All intestinal CH_4 derives from microbial methanogenesis (**Figure 2**). Significant catabolism of CH_4 by intestinal microbes or host cells has not been observed. Therefore, the measurement of respiratory CH_4 excretion provides a simple means of studying the in situ metabolism of intestinal methanogens. However, the data should be interpreted with caution because the proportions of both H_2 and CH_4 excreted in breath are influenced by their production rates in the colon. At higher production rates (>500 mL/day), a greater proportion of these gases is excreted in flatus than in breath; at lower rates (<200 mL/day), the proportion excreted in breath increases (Christl et al. 1992b). Slower intestinal transit is often reported in methane-producers compared with nonproducers (El Oufir et al. 1996, Mah et al. 1977), which is consistent with slower transit time being more favorable for the growth of slow-growing methanogens. Pimentel et al. (2006) demonstrated that methane may slow small intestinal transit by augmenting nonperistaltic type of small bowel contractile activity and suggested that colonic methanogenesis may predispose to constipation, which opposes the common assumption that methane produced in the gut is inert.

Colonic bacteria also produce large quantities of sulfur-containing gases such as H_2S and methanethiol (CH_3SH), which are highly toxic and odorous. These gases are present in trace concentrations (H_2S, 1.06 μmol/l; CH_3SH, 0.21 μmol/l) in flatus (Suarez et al. 1997). Intracolonic concentrations of these gases might be substantially higher than that in flatus given that H_2S and CH_3SH rapidly permeate the colonic mucosa and are detoxified (Suarez et al. 1998). When colonic transit is accelerated by disease, these gases may escape detoxification to be passed in much greater amounts in flatus. Contrary to the competition that is thought to exist between methanogens and SRB, a negative correlation is not observed between CH_4 and H_2S concentrations, indicating a coexistence of both microbial groups in the colon (Suarez et al. 1997).

MICROBIAL MECHANISMS OF HYDROGEN DISPOSAL

Methanogenesis

Methanogenesis, in which four moles of hydrogen are converted to one mole of methane, is a property unique to Archaea and an efficient pathway for hydrogen disposal (**Figure 3**).

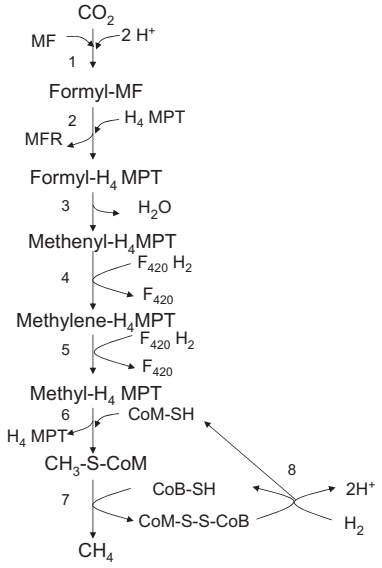

Figure 3

Methanogenic pathway from H_2 and CO_2. The reduction of CO_2 to CH_4 proceeds via sequential reduction of the C_1 group bound to coenzymes: methanofuran (MF), tetrahydromethanopterin (H_4MPT), and 2-mercaptoethanesulfonate (CoM-SH) *(reaction 1–7)*. Factor$_{420}$ (F_{420}), a fluorescent electron carrier that is specific to methanogens, is often used to detect their presence in mixed microbial suspensions. Methyl coenzyme M is a central intermediate in all methanogenic pathways. Methyl coenzyme M methylreductase (MCR; *reaction* 7) catalyzes the reduction of a methyl group bound to coenzyme-M, forming the final product methane. Reduction of the coproduct, heterodisulfide, is coupled with energy conservation (*reaction 8*). This terminal reaction of methanogenesis is shared by all types of methanogenic pathways regardless of the initial substrate. 1. formylmethanofuran dehydrogenase (FMD), 2. formyl-MFR:H_4MPT formyltransferase (FTR), 3. 5,10-methenyl-H_4MPT cyclohydrolase, 4. 5,10-methylene-H_4MPT-dehydrogenase, 5. 5,10-methylene-H_4MPT reductase, 6. 5-methyl-H_4MPT:CoM-SH methylransferase (MTR), 7. methyl coenzyme M methylreductase (MCR), 8. heterodisulfide reductase.

Significant individual differences exist in colonic methanogenesis. Methane producers harbor an average of 10^9 CFU/g of methanogens in feces, whereas apparent nonproducers harbor approximately 10^4 CFU/g (Pochart et al. 1992). For methane to be detectable in breath, a colonic methanogen density greater than 10^7–10^8 CFU/g is required (Lewis & Cochrane 2007, Pochart et al. 1992, Weaver et al. 1986).

Biochemistry of methanogenesis. Methanogens derive all or most of their metabolic energy from methanogenesis by reducing CO_2 to CH_4 using H_2 or formate as electron donors (Hedderich & Whitman 2006). Some methanogens also reduce C_1-compounds containing a methyl group carbon, such as methanol and methylated amines. Acetate is a further possible substrate for aceticlastic methanogenesis, in which the methyl carbon of acetate is reduced to methane. The reader is referred to **Figure 3** for the biochemical pathway and Hedderich & Whitman (2006) for an up-to-date review of the major physiological and metabolic characteristics of methanogens.

Phylogeny of intestinal methanogens. Methanogens are phylogenetically and physiologically distinct members of the Euryarchaeota. They are classified into five well-established orders: Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales, and Methanopyrales. Culture- and molecular-based studies to date indicate that *Methanobrevibacter smithii* is the predominant methanogen in the human colon (Abell et al. 2006, Eckburg et al. 2005, Miller & Wolin 1982, Scanlan et al. 2008, Weaver et al. 1986, Zhang et al. 2009). *Methanosphaera stadtmanae*, also a member of the order Methanobacteriales, has been isolated from the human intestinal tract at a lower abundance (Miller & Wolin 1985). These two methanogenic species have different biochemical characteristics. The genome of *M. smithii* is significantly enriched with genes involved in CO_2, H_2, and formate utilization during methanogenesis and also possesses the capacity for nonmethanogenic removal of methanol and ethanol (Samuel et al. 2007). These metabolic capabilities may allow *M. smithii* to form syntrophic relationships with a broad range of bacterial members in the human colon (Samuel et al. 2007). In contrast to *M. smithii*, *M. stadtmanae* has a more restricted energy metabolism and uses hydrogen to reduce methanol to methane (Fricke et al. 2006, Miller & Wolin 1985).

Five strains of colonic methanogen isolates are currently available in reference culture collections, and whole genome sequences are completed or in progress for these methanogens (**Table 1**). However, characterization of methanogens in the human colon remains incomplete. There are many examples where the universal archaeal 16S rDNA primers failed to amplify archaeal sequences because of too many base mismatches (Chaban et al. 2006). The most comprehensive 16S rDNA-based microbial diversity study (Eckburg et al. 2005), in which only *M. smithii* was recovered among 1524 archaeal clones sequenced, might also reflect a limitation of the 16S rDNA-based approach for Archaea. The gene encoding the subunit A of methyl coenzyme M methylreductase (MCR; *mcrA*) is highly conserved among methanogens and thus serves as an alternative marker for detection of methanogens in a wide range of anaerobic environments (Denman et al. 2007, Hales et al. 1996, Lueders et al. 2001). Recent molecular fingerprinting studies targeting 16S rDNA or *mcrA* have identified several different phylotypes closely related to *M. smithii*, *M. stadtmanae*, *M. oralis*, or *Methanosarcinales* from the human colon (Abell et al. 2006, Mihajlovski et al. 2008, Scanlan et al. 2008). Scanlan et al. (2008) reported that the *mcrA* gene sequences recovered from human feces were only distantly related to cultured methanogens, highlighting the importance of culture-independent approaches in the investigation of colonic methanogen diversity. Mihajlovski et al. (2008) studied fecal methanogen diversity by targeting the *mcrA* gene and recovered three operational taxonomic units (OTUs) attributed to *M. smithii*,

Table 1 Hydrogenotrophic microbes isolated from human feces or colon

Organism	Collection No.	Source	NCBI Taxonomy ID & Refseq No. for whole genome	References
Methanobrevibacter smithii PS	ATCC 35061 DSM 861	Sewage digester	*Taxonomy ID:* 420247 Refseq: NC_009515	Samuel et al. 2007, Smith 1966
Methanobrevibacter smithii F1	DSM 2374	Feces	*Taxonomy ID:* 521002 Refseq: NZ_ABYV00000000	Miller et al. 1982
Methanobrevibacter smithii ALI	DSM 2375	Colon	*Taxonomy ID:* 483214 Refseq: NZ_ABYW00000000	Miller & Wolin 1981, Miller et al. 1982
Methanobrevibacter smithii B181	DSM 11975	Feces	*Taxonomy ID:* 521001	Lin & Miller 1998, Miller et al. 1986
Methanosphaera stadtmanae	ATCC 43021 DSM 3091 JCM 11832	Feces	*Taxonomy ID:* 339860 Refseq: NC_007681	Fricke et al. 2006, Miller & Wolin 1985
Desulfovibrio piger	ATCC 29098 DSM 749 JCM 12224	Feces	*Taxonomy ID:* 901 Refseq: NZ_ABXU00000000	Loubinoux et al. 2002b, Moore et al. 1987
Desulfitobacterium hafniense	DSM 13498	Feces	*Taxonomy ID:* 537010	Niggemyer et al. 2001, van de Pas et al. 2001
Blautia hansenii	ATCC 27752 DSM 20583	Feces	*Taxonomy ID:* 1322 Refseq: NZ_ABYU00000000	Haack & Moore 1974, Liu et al. 2008
Blautia hydrogenotrophicus	DSM 10507	Feces	*Taxonomy ID:* 53443 Refseq: NZ_ACBZ00000000	Bernalier et al. 1996c, Liu et al. 2008
Marvinbryantia formatexigens	DSM 14469	Feces	*Taxonomy ID:* 168384 Refseq: NZ_ACCL00000000	Wolin et al. 2003, Wolin et al. 2008

M. stadtmanae, and a distant phylotype that does not cluster with any of the five methanogenic orders. These observations indicate that the use of multiple molecular targets may be important in recovering a wider range of colonic methanogens. **Table 2** summarizes primers and probes that have been used successfully as molecular markers for detection of methanogens in the human colon.

Sulfate Reduction

The SRB are a diverse group of bacteria sharing the ability to use sulfate as a terminal electron acceptor for respiration, with a concomitant production of H_2S. Four moles of hydrogen are consumed in the formation of one mole of H_2S. The electrons may also be provided from the oxidation of organic compounds, such as lactate. This process is termed dissimilatory sulfate reduction and clearly distinguished from assimilatory sulfate reduction, which is the process of generating reduced sulfur for biosynthesis of cell materials. Although assimilatory sulfate reduction is a biochemical process widespread among microbes, only restricted microbial groups are capable of dissimilatory sulfate reduction. SRB are ubiquitously present in the human intestinal mucosa (Fite et al. 2004, Kleessen et al. 2002, Zinkevich & Beech 2000), and have been enumerated from human feces in numbers ranging from 10^3 to 10^{11}/g (Gibson et al. 1988c, 1993; Pochart et al. 1992).

Hydrogen sulfide is highly toxic to colonocytes and impairs their metabolic function, especially butyrate oxidation (Roediger et al. 1993a,b). In aqueous solutions, H_2S dissociates into hydrosulfide anion (HS^-) and sulfide ion (S^{-2}) with pKa values of 7.04 and 11.96, respectively (O'Neil et al.

Table 2 Primers and probes used for the detection of hydrogenotrophs in the human colon

Target	Primer/probe[a]	Sequence (5′→3′)	Reference
Archaea 16S rDNA	Arch21F	TTC CGG TTG ATC CYG CCG GA	DeLong 1992
	300fEyAr	AGC RRG AGC CCG GAG ATG G	Kulik et al. 2001
	Arch333	TCC AGG CCC TAC GGG	Lepp et al. 2004
	344F	ACG GGG HGC AGC AGG CGC GA	Raskin et al. 1994
	Met448F	GGT GCC AGC CGC CGC	Wright & Pimm 2003
	519R	GWA TTA CCG CGG CKG CTG	Amann et al. 1995
	A571F	GCY TAA AGS RIC CGT AGC	Baker et al. 2003
	Arc767F	ATT AGA TAC CCS BGT AGT CC	Yu et al. 2005
	Arch806r	GGA CTA CVS GGG TAT CTA AT	Takai & Horikoshi 2000
	850f	GAG CAC CAC AAC GCG U	Abell et al. 2006
	Arc915F	AGG AAT TGG CGG GGG AGC AC	Yu et al. 2005
	954rEyAr	CGG CGT TGA RTC CAA TTA AAC	Kulik et al. 2001
	958R	YCC GGC GTT GAM TCC AAT T	DeLong 1992
	Met1027F	GTC AGG CAA CGA GCG AGA CC	Wright & Pimm 2003
	Arc1059R	GCC ATG CAC CWC CTC T	Yu et al. 2005
	1260rc	CTA CGC ATT CCA GCT TC	Abell et al. 2006
Methanobacteriales 16S rDNA	MBT857f	CGW AGG GAA GCT GTT AAG T	Yu et al. 2005
	MBT929F	AGC ACC ACA ACG CGT GGA	Yu et al. 2005
	MBT1196R	TAC CGT CGT CCA CTC CTT	Yu et al. 2005
Methanobrevibacter 16S rDNA	MET-105f	TGG GAA ACT GGG GAT AAT ACT G	Ufnar et al. 2006
	MET-386r	AAT GAA AAG CCA TCC CGT TAA G	Ufnar et al. 2006
mcrA	ME1	GCM ATG CAR ATH GGW ATG TC	Hales et al. 1996
	ME2	TCA TKG CRT AGT TDG GRT AGT	Hales et al. 1996
M. smithii nifH	Mnif-342f	AAC AGA AAA CCC AGT GAA GAG	Ufnar et al. 2006
	Mnif-363r	ACG TAA AGG CAC TGA AAA ACC	Ufnar et al. 2006
Desulfovibrionales 16S rDNA	DSV230	GRG YCY GCG TYY CAT TAG C	Daly et al. 2000
	DSV838	SYC CGR CAY CTA GYR TYC ATC	Daly et al. 2000
Desulfovibrio 16S rDNA	687	TAC GGA TTT CAC TCC T	Devereux et al. 1992
	DSV691-F	CCG TAG ATA TCT GGA GGA ACA TCA G	Fite et al. 2004
	DSV826-R	ACA TCT AGC ATC CAT CGT TTA CAG C	Fite et al. 2004
	DSV1292	CAA TCC GGA CTG GGA CGC	Manz et al. 1998
aprA	APS-FW	TGG CAG ATM ATG ATY MAC GG	Deplancke et al. 2000
	APS-RV	GGG CCG TAA CCG TCC TTG AA	Deplancke et al. 2000
	Forward (969–992)	CCA GGG CCT GTC CGC CAT CAA TAC	Zinkevich & Beech 2000
	Reverse (1603–1624)	CCG GGC CGT AAC CGT CCT TGA A	Zinkevich & Beech 2000
dsr	P94-F	ATC GGW ACC TGG AAG GAY GAC ATC AA	Karkhoff-Schweizer et al. 1995
	P93-R	GGG CAC ATS GTG TAG CAG TTA CCG CA	Karkhoff-Schweizer et al. 1995
dsrB	DSR1F	ACS CAC TGG AAG CAC G	Wagner et al. 1998
	DSR4R	GTG TAG CAG TTA CCG CA	Wagner et al. 1998
FTHFS	FTHFS-f	TTY ACW GGH GAY TTC CAT GC	Leaphart & Lovell 2001
	FTHFS-r	GAT TTG DGT YTT RGC CAT ACA	Leaphart & Lovell 2001

[a]Primer and probe names are listed as published.

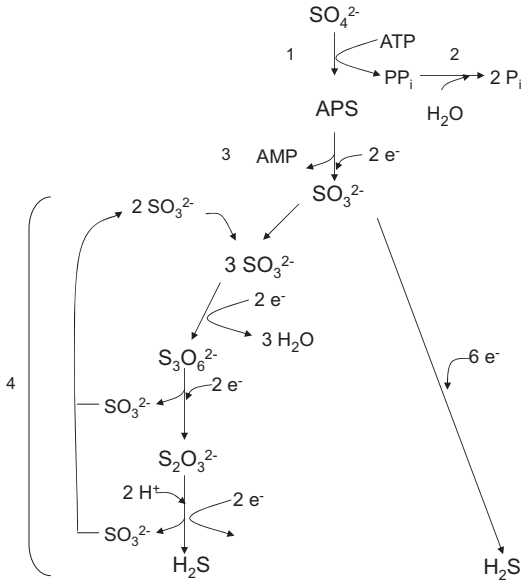

Figure 4

Dissimilatory sulfate-reducing pathway. Sulfate is an unfavorable electron acceptor that has low redox potential and cannot be directly reduced by most intracellular electron mediators. Therefore, sulfate must be activated by the formation of adenosine phosphosulfate (APS) before reduction, at the expense of ATP. The enzyme that catalyzes this reaction is ATP sulfurylase (*reaction 1*). Pyrophosphate (PPi) formed by this reaction is hydrolyzed by pyrophosphatase (*reaction 2*), which pulls the ATP sulfurylase reaction in favor of APS formation. The reduction of APS to sulfite (or the protonated form bisulfate) and AMP is the first redox reaction catalyzed by APS reductase (APR) (*reaction 3*). The following reduction of sulfite to sufide, which involves the transfer of six electrons by dissimilatory (bi)sulfite reductase (DSR) (*reaction 4*), is the central energy-generating process. The mechanism for sulfite reduction to sulfide has not yet been fully established, and two possible mechanisms are suggested; sequential three two-electron reducing steps with the formation of trithionate and thiosulfate as intermediates (*reaction 4; left pathway*), or a direct six-electron reduction of bisulfate to sulfide (*reaction 4; right pathway*). 1. APS sulfurylase, 2. pyrophosphate, 3. APS reductase (APR), 4. dissimilatory (bi)sulfite reductase (DSR).

2001). In the human colon, sulfide exists largely in the volatile, highly toxic undissociated form (H_2S), which is quickly absorbed by the mucosa or passes as flatus (Suarez et al. 1998). More than 90% of sulfate disappears during passage through the colons of subjects lacking a sulfate-reducing microbiota, indicating that a variety of colonic processes compete for sulfate (Strocchi et al. 1993).

Biochemistry of sulfate reduction. The biochemistry of dissimilatory sulfate reduction has been investigated most extensively with *Desulfovibrio* species, which is also the predominant SRB genus in the human colon (Gibson et al. 1988c, 1991, 1993; Goudar et al. 2004). **Figure 4** summarizes the biochemical pathway of dissimilatory sulfate reduction, and the reader is referred to Rabus et al. (2006) and Barton & Fauque (2009) for an up-to-date review of the major physiological and metabolic characteristics of SRB. SRB are able to utilize a wide range of substrates as electron donors, including sugars, amino acids, and one-carbon compounds such as methanol, carbon monoxide, and methanethiol (summarized by Muyzer & Stams 2008). There is also evidence that colonic SRB are capable of utilizing electron donors other than molecular hydrogen, including SCFA, succinate, and lactate (Gibson et al. 1993, Newton et al. 1998). Lactate and pyruvate-oxidizing SRB were found to be numerically predominant, with butyrate, succinate, valerate,

amino acid, and H_2/CO_2 utilizers present in lower numbers (Gibson et al. 1988c). Detection of SRB in the feces of infants less than one month of age (Fite et al. 2004, Hopkins et al. 2005) also likely reflects the fact that SRB are not entirely dependent on the H_2 produced by intestinal microbiota but can grow on other electron donors in vivo, such as lactate.

Autotrophy: the ability of an organism to utilize inorganic compounds for generating energy

Heterotrophy: the ability of an organism to use organic substrates for generating energy

Phylogeny of intestinal sulfate reducers. The SRB are composed of numerous genera and species widely differing in their growth rates and physiological activities, including the ability to reduce sulfate (Rabus et al. 2006). Based on comparative analysis of 16S rRNA gene sequences, the known SRB can be grouped into seven phylogenetic lineages, five within the domain Bacteria and two within the Archaea. Most of the SRB belong to the division of Deltaproteobacteria with more than 25 genera, followed by the Gram-positive SRB within the class Clostridia (*Desulfotomaculum*, *Desulfosporosinus*, and *Desulfosporomusa* genera).

A range of nutritionally and physiologically distinct SRB has been detected in human feces (Gibson et al. 1988c, 1991, 1993; Willis et al. 1997). Despite the detection of diverse SRB from the human colon, there are only two colonic isolates currently available in reference culture collections (**Table 1**). In a culture-based study by Gibson et al. (1993), the principal SRB were lactate- and hydrogen-utilizing *Desulfovibrio* spp. (64–81%), acetate-utilizing *Desulfobacter* spp. (9–16%), propionate- and hydrogen-utilizing *Desulfobulbus* spp. (5–8%), lactate-utilizing *Desulfomonas* spp [reclassified with genus *Desulfovibrio* (Loubinoux et al. 2002b)] (3–10%), and acetate- and butyrate-utilizing *Desulfotomaculum* spp. (2%). However, these observations are based on cultivation, which underestimates true bacterial diversity. Recently molecular-based techniques have been applied successfully to describing SRB diversity in various environments. Because the sulfate-reducing trait is relatively restricted to a coherent assemblage within the division of Deltaproteobacteria (Devereux et al. 1989), 16S rDNA-based approaches are reasonable for characterizing natural populations in a mesophilic environment, despite the phylogenetic diversity of SRB. Genes encoding two of the enzymes in the sulfate reduction pathway, APS reductase (APR) and dissimilatory (bi)sulfite reductase (DSR), are often also useful molecular markers because of their highly conserved nature and congruence with the evolutionary history of SRB (Klein et al. 2001, Meyer & Kuever 2007, Wagner et al. 1998, Zverlov et al. 2005). **Table 2** summarizes primers and probes that have been used successfully as molecular markers for detection of SRB in the colon. However, relatively few studies have examined the diversity of human colonic SRB using molecular-based techniques.

Reductive Acetogenesis

The acetogens are a group of obligately anaerobic bacteria that utilize the acetyl-CoA (Wood-Ljungdhal) pathway to synthesize acetyl-CoA from CO_2 while conserving energy and assimilating CO_2 into cell carbon (Drake 1994). Cultivation-based studies have estimated that the number of acetogens ranges from 10^2 to 10^8 CFU/g human feces (Bernalier et al. 1996a, Doré et al. 1995). Acetogenesis is thought to be a relatively less important hydrogenotrophic pathway in the colon, compared with methanogenesis and sulfate reduction (Christl et al. 1992b). This is because the oxidation of H_2 by methanogenesis or sulfate reduction is thermodynamically more favorable than reductive acetogenesis (Cord-Ruwisch et al. 1988, Thauer et al. 1977).

Biochemistry of acetogenesis. Acetogens utilize the acetyl-CoA pathway to grow autotrophically on H_2 and CO_2 or heterotrophically on a variety of organic compounds. The reader is referred to **Figure 5** for the autotrophic biochemical pathway of acetogenesis and Drake et al. (2006, 2008) for recent reviews of the major physiological and metabolic characteristics of

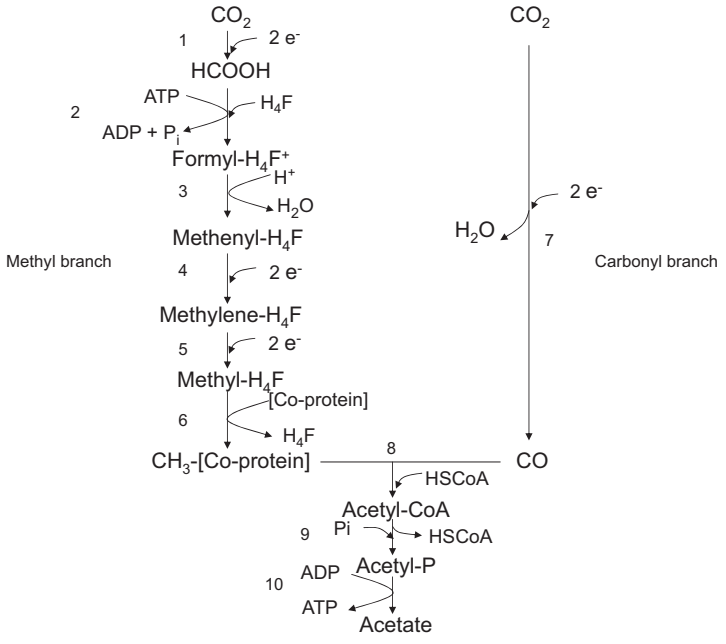

Figure 5

Acetyl-CoA (Wood-Ljungdahl) pathway. This pathway contains two branches: the methyl branch and the carbonyl branch. The methyl group of acetate is formed via tetrahydrofolate (H_4folate)-bound C_1 intermediates (*reaction 1–6*), and the carboxyl group of acetate is formed from CO_2 via reduction to CO (*reaction 7*). The methyl branch is widely distributed in nature, whereas the carbonyl branch, which has novel biochemical properties, is unique to acetogens, methanogens, and SRB. The two branches merge at the synthesis of acetyl-CoA catalyzed by the enzyme acetyl-CoA synthase (*reaction 8*). Energy conservation may occur both by substrate-level phosphorylation during reductive synthesis of acetate (*reaction 10*) and by chemiosmiotic processes. However, during autotrophic growth with H_2 and CO_2, energy conservation is dependent solely on the chemiosmotic process because the ATP produced by substrate-level phosphorylation is consumed during the activation of formate (*reaction 2*). 1. formate dehydrogenase, 2. formyl-H_4folate synthetase, 3. methyl-H_4folate cyclohydrolase, 4. methenyl-H_4folate cyclohydrolase and methylene-H_4folate dehydrogenase complex, 5. methylene-H_4folate reductase, 6. methyltransferase, 7, 8. carbon monoxide dehydrogenase/acetyl CoA synthase (CODH/ACS), 9. phosphotransacetylase, 10. acetate kinase.

acetogens. During the autotrophic growth of acetogens, two moles of CO_2 are reduced by four moles of H_2 to produce one mole of acetate. In heterotrophic growth, one mole of hexose is converted to three moles of acetate, which is formed in a ratio of 2:1 from the oxidation of pyruvate and from the reduction of CO_2, respectively (Drake et al. 2006). It is assumed that both autotrophic and heterotrophic acetogenesis occur simultaneously in the intestinal ecosystem. An early report of the isolation of acetogens from human feces identified three Gram-positive bacteria that produced acetate from CO_2 but varied in their ability to grow on a range of substrates (Wolin & Miller 1993). These isolates either did not grow or grew poorly in vitro with H_2 and CO_2 alone. However, two of them cometabolized the gases rapidly when they were grown with glucose. These data indicate that reductive acetogenesis in the colon likely depends primarily on the organic substrate and that mixotrophic growth enhances the H_2-consuming capacity of colonic acetogens.

Phylogeny of intestinal acetogens. Acetogens are among the most metabolically versatile anaerobes and are phylogenetically diverse. To date, more than 100 acetogenic species representing 22

genera have been isolated, most belonging to the genera *Acetobacterium* and *Clostridium* (Drake et al. 2008).

Because of their metabolic versatility, there is no single selective agent or substrate that can be employed to enumerate and isolate acetogens. These characteristics have made it difficult to culture and identify the broad diversity of acetogens in the human gut. Using conventional culturing techniques, H_2/CO_2-utilizing acetogens isolated from human feces were related to genera *Ruminococcus*, *Clostridium*, or *Streptococcus* (Bernalier et al. 1996b,c; Doré et al. 1995). Some acetogenic species of *Ruminococcus* and *Clostridium* genera were recently reclassified within the genus *Blautia* (Liu et al. 2008). These isolates exhibited nutritional versatility, possessing the ability to ferment a large variety of organic substrates, a strategy that would produce greater energy for growth in the colon. Human colonic isolates of acetogens that are available currently in reference culture collections are included in **Table 1**.

As a result of the polyphyletic distribution of acetogens, their identification via 16S rDNA-based molecular approaches is also problematic. Recently, functional genes in the reductive acetogenesis pathway have been targeted for use in molecular ecology approaches to facilitate studies of acetogen diversity (Lovell & Leaphart 2005). In particular, the gene sequence of formyl tetrahydrofolate synthetase (FTHFS; *fhs*), which catalyzes the ATP-dependent activation of formate, is highly conserved among acetogens and thus serves as a useful molecular target (Lovell & Hui 1991). Although FTHFS is present in nonacetogens, phylogenetic analysis of the *fhs* sequences amplified from a range of anaerobic environments formed distinctive clusters represented by known acetogens, SRB, and other FTHFS-containing nonacetogens (Lovell & Leaphart 2005). Analysis of *fhs* sequences amplified from human feces has identified *Blautia producta* (formerly *Ruminococcus productus*) as the predominant acetogen and detected several FTHFS gene sequences that had not been identified previously (Ohashi et al. 2007). Acetogens were also found in periodontal lesions in the human oral cavity based on the phylogenetic placement of recovered *fhs* sequences (Vianna et al. 2008). The FTHFS gene has proven to be a useful marker for acetogens; however, the presence of the gene in bacteria that do not possess the acetyl-CoA pathway means that assignment of sequences to acetogenic groups needs to be carefully assessed. Consequently, the use of broad *fhs* primer sets for estimating abundance of acetogens is unreliable. Recently, a FTHFS gene-based, real-time quantitative PCR assay was developed that has higher specificity to the phylogenetic cluster that is affiliated with known reductive acetogens (Xu et al. 2009). Furthermore, other researchers have addressed this problem by recovering sequence information from acetogenic isolates and environmental samples for genes specific to the carbonyl branch of the acetyl-CoA pathway (e.g., acetyl-CoA synthase and carbon monoxide dehydrogenase), which should aid in the development of molecular probes and primers for acetogens (Denman et al. 2009). **Table 2** includes the FTHFS primer pair that has been used to detect acetogens in human feces (Ohashi et al. 2007).

Competitive and Mutualistic Interactions Among Hydrogenotrophic Microbes

In the human colon, direct competition among methanogens, SRB, and acetogens may occur for the common substrate H_2. The interactions among microbes competing for the same growth-limiting substrate are often explained by both kinetic and thermodynamic models. A Monod-type growth kinetics model is used frequently in modeling microbial growth under substrate-limiting conditions. It has been reported that SRB in general have more favorable kinetic growth parameters for H_2 than do methanogens (Kristjansson et al. 1982, Lovley et al. 1982, Robinson & Tiedje 1984, Stams et al. 2005), which likely accounts for the dominance of SRB in sulfate-rich, H_2-limiting environments. The thermodynamic model is based on the free-energy change

(available energy) associated with the chemical reaction at standard conditions and equimolar substrate concentrations. A greater Gibbs free-energy change is associated with the reduction of sulfate by hydrogen than with the reduction of CO_2 by hydrogen to methane or to acetate (Thauer et al. 1977), which would theoretically allow higher growth yield of SRB per mole of hydrogen.

The mechanism whereby one organism outcompetes others can also be explained by a minimum threshold model. Specifically, organisms with higher substrate affinity and growth yield outcompete others by maintaining the substrate concentration below the minimum concentration necessary for other organisms to conserve energy (Lovley et al. 1982). The threshold concentrations of H_2 are reported to be 10–20 ppm for SRB, 30–100 ppm for methanogens, and 400–950 ppm for acetogens (Cord-Ruwisch et al. 1988). Environmental H_2 concentrations are primarily dependent upon the predominant microbial hydrogenotrophic process, and the environmental H_2 concentration associated with sulfate reduction is lower than that with methanogenesis (Lovley & Goodwin 1988).

Although these theoretical explanations seem to agree with environmental observations, they are based on the assumption of an idealized environment in which the competing species are homogeneously distributed in space without significant variations in growth conditions. Chemostats represent such an environment and are used commonly as model systems to study microbial competition under nutrient limitation. However, most natural microbial ecosystems, including the human colon, are not idealized environments, and as opposed to the theoretical competitive order, coexistence of competing microbes is commonly observed. The colon is a unique microbial ecosystem characterized by a pulsed supply of nutrients, host secretions, fluctuations in pH, and a complex anatomical structure, all of which provide a variety of different ecological niches for resident microbes. Colonic microbial populations are spatially organized (Palestrant et al. 2004, Swidsinski et al. 2005) and considered to reside within specific microhabitats (Lee 1984) that likely serve as niches for distinct microbial groups based on different physical characteristics and substrate availability. Such environmental heterogeneity may enable distinct types of hydrogenotrophic reactions to proceed simultaneously in the human colon. It thus appears impossible to fully explain the outcome of the competition among hydrogenotrophs in the human colon by idealized theory or in vitro chemostat systems.

Although it commonly has been assumed that methanogenesis and sulfate reduction are mutually exclusive processes, there is evidence that SRB and methanogens can coexist in the human and nonhuman primate colon (Bernalier et al. 1996a, Doré et al. 1995, Gibson et al. 1993, Nakamura et al. 2009, Pitcher et al. 2000, Pochart et al. 1992). A recent molecular-based study also concludes that the competition of methanogens and SRB does not necessarily lead to the predominance of one group in the human fecal microbiota (Stewart et al. 2006). Strocchi et al. (1994a) showed that fluctuations in the methanogenic or sulfate-reducing status within individuals occur over time without serious illnesses or major dietary alterations, supporting the coexistence of two hydrogenotrophic populations in an individual over time.

Contradictory observations have also been reported regarding competitive interactions among human colonic hydrogenotrophs. It has been reported that addition of sulfate and sulfated mucopolysaccharides to mixed fecal slurries containing metabolically active SRB results in the stimulation of sulfide production and inhibition of methanogenesis (Gibson et al. 1988a,b). In addition, dietary sulfate supplementation results in increased activity of SRB and inhibition or reduction of methanogenesis in the feces (Christl et al. 1992a, Lewis & Cochrane 2007). These observations support the common assumption that sulfate availability is the key determinant of which of the two hydrogenotrophic pathways predominate in a given environment. On the other hand, Strocchi et al. (1991, 1994a,b) demonstrated repeatedly that human fecal methanogens consume hydrogen more rapidly and efficiently than SRB even in the presence of sulfate and suggest that

the presence or absence of methanogens determines which of the hydrogenotrophic pathways takes place in the human colon. Their observations contradict the previously reported kinetic and thermodynamic values for sulfate reduction and methanogenesis as well. Indeed, they have observed very low hydrogen concentrations in the in vitro culture of methanogenic fecal homogenates, which was only approximately 1/180 of that of the sulfate-reducing homogenate and 1/60 of the half saturation constant (K_s) reported for sediment methanogens (Strocchi et al. 1994b). An interesting observation has been reported in the competitive order of methanogens and acetogens in the wood-feeding termite gut (Breznak & Blum 1991). In this system, an acetogen *Sporomusa termitida* outcompetes methanogens although the H_2 threshold value of this organism is much higher than that of methanogens. It is concluded that the ability of *S. termitida* to perform mixotrophy allows conservation of more energy per unit time and per mole H_2 consumed, and this may ultimately contribute to the ability of acetogens to outcompete methanogens for H_2. Their observation clearly indicates that factors other than H_2 threshold can determine the competitive order among hydrogenotrophs. In this regard, more detailed study of in situ metabolic activities of human colonic hydrogenotrophs is required to fully understand the nature of relationships among them.

There is limited information regarding the roles of acetogens and their competitiveness with other hydrogenotrophs in the human colon. Although acetogens are poor hydrogen utilizers, the ability to grow mixotrophically may enhance the competitiveness of acetogens for H_2. In addition, almost all known acetogens can utilize alternative terminal electron acceptors, such as nitrate, in addition to the acetyl-CoA pathway, which may also significantly improve H_2-consuming capacity of acetogens (Drake et al. 2006). In the ruminal ecosystem, it appears that autotrophic acetogenesis could only become a significant metabolic pathway if methanogenesis were inhibited, which would allow the partial pressure of hydrogen to increase to levels above the required threshold for reductive acetogenesis (Nollet et al. 1997). However, a study with pigs indicated that acetogenic activity occurs in the colon even in the presence of methanogenesis (De Graeve et al. 1990). In the human colon, acetogenesis was a major hydrogenotrophic pathway only in nonmethanogenic individuals (Bernalier et al. 1996a, Lajoie et al. 1988, Wolin & Miller 1983). Correspondingly, the number of acetogens was significantly higher in nonmethanogenic than methanogenic individuals (Bernalier et al. 1996a, Doré et al. 1995), consistent with a competitive relationship between these two groups of hydrogenotrophs. In contrast, competition for H_2 was not observed between colonic acetogens and SRB (Bernalier et al. 1996a). Overall, the extent of the metabolic contribution of reductive acetogenesis to the growth of other hydrogenotrophs in the human colon is not fully understood at present.

In a recent review, Muyzer & Stams (2008) pointed out that the occurrence of high numbers of SRB does not necessarily reflect the occurrence of sulfate reduction and that, in many recent publications, this link is made too easily. Their statement seems applicable to studies of colonic SRB, as they are often explained solely in terms of their H_2-consuming sulfate-reducing activity, and their metabolic versatility often seems to be overlooked. In this regard, the presence of large populations of SRB in a sulfate-depleted, methanogenic reactor (Raskin et al. 1996) demonstrates the ability of SRB to grow with various environmental conditions. It is also known that H_2-producing, fermentatively-growing SRB can have a syntrophic relationship with methanogens via interspecies H_2 transfer (Bryant et al. 1977), although the extent to which this type of relationship occurs in the human colon is not clear. In fact, few studies have examined in detail metabolic pathways underlying potential competition or coexistence of SRB and methanogens in the human colon. Functional gene-based molecular approaches may provide some insight into the metabolic activities of SRB. Transcript levels of *dsr* were related to the cell-specific sulfate reduction rate, thus providing information on the metabolic state of SRB (Neretin et al. 2003, Villanueva et al. 2008).

Dar et al. (2007) examined SRB diversity in sulfidogenic wastewater treatment reactors by both DNA- and RNA-based DGGE for 16S rRNA and *dsrB* genes, and observed marked differences between the SRB populations that were present and those that were metabolically active.

Physiological differences among strains of SRB in the human colon may also partially explain discrepancies reported in the competition between SRB and methanogens. For example, Pitcher et al. (2000) reported that the growth characteristics of human-derived SRB strains can be divided into rapid- and slow-growing phenotypes, which show high and low sulfate-reducing activity, respectively. Competition for sulfate between different types of SRB may also explain partially the competitive interaction of hydrogenotrophic microbes in sulfate-limited environments. Clearly, careful integration of molecular techniques with biochemical analyses will be required to gain insights into metabolic activities and population dynamics of SRB in the human colon.

HOST INFLUENCES ON AND RESPONSES TO MICROBIAL HYDROGENOTROPHY

Effects of Diet on Microbial Hydrogenotrophy

Dietary intake and composition affect the supply of substrates to the colonic microbiota both quantitatively and qualitatively and thus influence colonic microbial metabolism and competition. In regards to dissimilatory sulfate reduction, food sources of inorganic sulfate include commercial breads, dried fruits and vegetables, nuts, fermented beverages, and brassica vegetables (Florin et al. 1993). Diets supplemented with inorganic sulfate stimulate H_2S production within the colon (Christl et al. 1992a, Lewis & Cochrane 2007). Hydrogen sulfide can also be generated through bacterial fermentation of cysteine through cysteine desulfhydration. Although predominant groups of intestinal microbes including *Bacteroides*, *Clostridia*, and *Fusobacteria* spp. possess this potential, there is little to no information in the literature regarding the extent to which this pathway is utilized. Of note, concentrations of free sulfur amino acids in colonic mucosa are relatively low (Ahlman et al. 1993). In vitro incubation studies using human feces also indicate that the organic sulfur-containing compounds including cysteine, taurocholic acid, and mucin provide a more readily utilizable source of sulfides than inorganic sulfate (Florin 1991, Levine et al. 1998). Magee et al. (2000) demonstrated that dietary protein, especially meat, was an important substrate for sulfide production in the colon with sulfur amino acids rather than inorganic sulfate being the major sulfur substrate. Together, these observations indicate that microbial sulfur amino acid degradation likely plays an important role in colonic sulfidogenesis, in addition to dissimilatory sulfate reduction by SRB. Consequently, fecal sulfide concentration, which is often used as an indicator of in situ SRB activities, may not serve as a reliable marker for this purpose.

Effects of diet on the methanogenic microbiota are not clear. Whereas breath H_2 concentration increases after ingestion of nonabsorbable carbohydrates, breath CH_4 concentration does not increase appreciably after ingestion of various slowly fermented compounds (Levitt et al. 2006). This may be explained by the physical separation of the primary site of carbohydrate fermentation and CH_4 production or may be due to the slow transit time for dietary fibers to reach the left colon where methanogenesis primarily takes place. The lack of correlation between methane production and diet may also indicate that intestinal methane production relies more on endogenous sources such as mucins (Haines et al. 1984, Perman & Modler 1982) rather than dietary substrates.

It is largely accepted that diet affects the development of microbiota during infancy (Fanaro et al. 2003). In regards to hydrogenotrophic microbes, gas release by infant feces is strongly influenced by the infant's diet. Breastfeeding is associated with production of high CH_4 and low CH_3SH and H_2S, whereas soy-based formula is associated with high production of CH_4 and H_2S (Jiang et al.

2001). A study with rats indicated that environmental effects on colonic methanogenesis were most evident during the weaning period (Florin et al. 2000). These observations indicate the possibility that dietary exposure during infancy may play a role in the development of hydrogenotrophic microbiota that persists into adulthood.

Inflammatory bowel disease (IBD): gene-environment disorder characterized by chronic inflammation of intestinal mucosa, restricted to the colon (ulcerative colitis) or involving any segment of the gastrointestinal tract (Crohn's disease)

Effects of Ethnicity on Microbial Hydrogenotrophy

Host genetic determinants likely shape the microbial composition of the intestinal microbiota. For example, a significant impact of host genotype on the microbiota has been demonstrated by comparison of individuals and inbred mouse strains with varying degrees of genetic relatedness (Stewart et al. 2005, Toivanen et al. 2001, Vaahtovuo et al. 2001, Van de Merwe et al. 1983, Zoetendal et al. 2001). Genetic effects on colonization of the hydrogenotrophic microbiota are not clear. Hackstein & Van Alen (1996) investigated methane emissions by 253 vertebrate species and showed that the methanogenic trait is shared by related species and higher taxa, irrespective of differing feeding habits. Based on this observation, they suggested that methane production is a primitive shared character (pleisomorphic) and that loss of that competence appears to be a synapomorphic (shared-derived) character. Their screening of methane emissions from five Dutch and German families also indicated that the methanogenetic trait is controlled by genetic factors, which segregate as an autosomal, dominant character (Hackstein et al. 1995). Similar potential genetic effects were also shown in a study by Bond et al. (1971), in which high concordance for methane production was observed between siblings and between parents and their children but not in spouses. However, they also observed an unusually high incidence of methane producers among institutionalized children living together in closed units for a long period of time and concluded that, although genetic effects cannot be denied, early environmental influences are important in the establishment of methanogenetic microbiota (Bond et al. 1971). Florin et al. (2000) also evaluated genetic and environmental effects on the methanogenic trait in humans and rats and concluded that shared and unique environmental factors were the main determinants.

It is well established that the percentage of methane producers varies significantly in humans of different ethnic groups, ranging from 34% to 87% (summarized in Levitt et al. 2006). In addition, the methanogenic phenotype appears to be remarkably stable within a population over time (Levitt et al. 2006). It is commonly found that black Africans are highly methanogenic compared with Caucasians (Gibson et al. 1988c, Segal et al. 1988). However, it is difficult to sort out the multifactorial interactions among host ethnicity, environment, and diet. Segal et al. (1988) reported that the percentage of methane producers was lower in urban black Africans (72%) compared with the rural population (84%). O'Keefe et al. (2007) found that the hydrogen and methane breath emission patterns of African Americans are more similar to those of Caucasian Americans than to native Africans. In this case, the two American groups were consuming typical Western diets, whereas native Africans consumed a maize-based diet low in animal-based protein and high in RS. The need to understand how genetic background and diet interact to influence the composition and metabolic activity of hydrogenotrophic microbes is further justified by evidence that Africans consuming a diet high in RS exhibit a significantly reduced risk for developing chronic intestinal inflammatory disorders as well as sporadic colorectal cancer (O'Keefe et al. 2007).

Inflammatory Bowel Disease

Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of inflammatory bowel disease (IBD) afflicting 0.1–0.5% of individuals in Western countries (Hanauer 2006, Podolsky

2002). The hallmark of IBD is poorly-controlled chronic inflammation of the intestinal mucosa, which can affect all parts of the gastrointestinal tract. Recent studies provide strong evidence that IBD results from multifactorial interactions among genetic and environmental factors that lead to a dysregulation of the innate immune response to the intestinal microbiota in genetically predisposed individuals (Podolsky 2002). Substantial evidence also exists for a potential pathogenic role of H_2S in IBD, particularly in ulcerative colitis. (Pitcher & Cummings 1996).

Patients with UC ingest more protein, and thereby sulfur amino acids, than control subjects (Tragnone et al. 1995). Removing foods rich in sulfur amino acids (e.g., meat, eggs, dairy) has therapeutic benefits in UC (Truelove 1961). It has also been reported that the numbers of SRB and rate of sulfidogenesis were greater in UC patients than control cases (Gibson et al. 1991, Pitcher et al. 2000). Another study using PCR techniques to evaluate the presence of different SRB species found that the prevalence of *D. piger* was significantly higher in IBD patients as compared with healthy individuals or with patients with other gastrointestinal symptoms (Loubinoux et al. 2002a). In a study of patients with UC, production of H_2S from feces was 3–4 times greater than from feces of controls (Levine et al. 1998). However, this difference in H_2S production was apparently not due to colonization by a greater number of SRB, as patients with active UC did not harbor more SRB than healthy controls in either stool or rectal mucosal samples as measured by qPCR (Fite et al. 2004). Based on their results, Fite and coworkers proposed that if SRB were involved in the pathogenesis of UC, there would have to be a defect in tissue detoxification of H_2S. The rate-limiting step in sulfide detoxification is oxidation by a sulfide oxidase to thiosulfate. Rhodanese then converts this thiosulfate to thiocyanate, but this reaction does not increase the rate of sulfide detoxification (Wilson et al. 2008). Identification of the specific sulfide oxidase involved in colonic sulfide detoxification would be a useful endeavor, as would a determination of the extent of individual variation in the activity of this enzyme(s). Indeed, impairment of mucosal function and exposure of the colonic epithelium to H_2S concentrations that exceed the detoxification capacity of the mucosa may predispose to UC (Levitt et al. 1999).

Conflicting results regarding the density of SRB populations may reflect the confounding effect of a common treatment regimen for patients with UC. Specifically, 5-aminosalicylic acid (5-ASA), an anti-inflammatory medication commonly prescribed for UC, also inhibits SRB growth and production of H_2S (Edmond et al. 2003, Pitcher et al. 2000). Thus, although no difference in stool sulfide concentrations was found between patients with UC and noncolitic controls when the use of salicylates in colitic patients was not accounted for (Moore et al. 1998), fecal sulfide concentrations were significantly higher in patients with UC who were not administered 5-ASA (Pitcher et al. 2000).

Increased activity of mucin sulfatase, an enzyme that frees sulfate groups from sulfomucins (Tsai et al. 1992), was observed in patients with active UC but not Crohn's disease (Tsai et al. 1995). In most patients, fluctuations in fecal sulfatase activities corresponded to clinical disease activity. Thus, it was suggested that the increased fecal sulfatase activity contributed to perpetuation of the disease. Enhanced sulfatase activity in individuals genetically predisposed to a high SRB carriage rate would represent a particularly threatening scenario because of the increased availability of endogenous sulfate for SRB sulfide production. Similarly, diets high in exogenous sources of sulfate would likely represent the greatest risk for those genetically predisposed to high SRB carriage rate.

Further supporting a role for H_2S in UC is the observation that SRB were found in surgically constructed ileo-anal pouches of UC patients but not in pouches of patients with familial adenomatous polyposis (FAP) and that H_2S production in UC pouches was 10 times greater than that in FAP pouches (Duffy et al. 2002). In addition, the severity of pouchitis is correlated with fecal concentrations of H_2S (Ohge et al. 2005) possibly reflecting a pathogenic role for this gas. Coffey

et al. (2009) proposed that colonic metaplasia may follow the surgical creation of a pouch in UC patients, leading to increased production of sulfomucin which, in turn, supports colonization by SRB. The adverse consequence of such colonization is greater exposure to H_2S, potentially at proinflammatory concentrations (Coffey et al. 2009).

In addition to an exogenous source resulting from bacterial sulfate reduction, H_2S is also endogenously produced in various tissues by cystathionine β-synthase (CBS) or cystathionine gamma lyase (CSE) (Abe & Kimura 1996, Kery et al. 1994, Meier et al. 2001, Wang 2002). The contributions of endogenously produced H_2S as a gaseous signaling molecule have generated a great deal of interest lately as a possible mediator of various physiological functions particularly in neuronal and cardiovascular tissues (Cleare et al. 1999, Kimura & Kimura 2004, Scott & Dinan 1999, Wang 2002, Zhao et al. 2001, 2003). However, a recent report provided convincing evidence that whole tissue and blood concentrations of free H_2S are orders of magnitude less than conventionally accepted values (Furne et al. 2008). Endogenous H_2S in the colon of the mouse and rat appears to be produced primarily by constitutively expressed CBS in the muscularis mucosae, submucosa, and cells of the lamina propria rather than by the epithelium (Martin et al. 2009, Wallace et al. 2009). Intriguingly, a recent report demonstrated that inhibition of H_2S synthesis in healthy rats resulted in mucosal injury and inflammation in the small intestine and colon, whereas intracolonic administration of H_2S significantly reduced the severity of trinitrobenzene sulfonic acid–induced colitis (Wallace et al. 2009). These data indicate that the outright assumption that colonic H_2S is deleterious may be flawed and justify additional study of both bacterial and endogenous sources of H_2S in the human colon.

There are few but consistent reports that the prevalence of the methanogenic phenotype is significantly lower in patients with CD or UC than in healthy control subjects. However, this intriguing finding has received limited attention. There are minimal microbiological data, and it is not known if the reduced prevalence of methane excretion in IBD reflects a predisposing host trait or simply a pathophysiological correlate of disease. It was reported that breath methane was detected in 44% of healthy Caucasian subjects (Bjorneklett & Jenssen 1982), but absent in a cohort of 28 patients with Crohn's ileitis (Bjorneklett et al. 1983). McKay et al. (1985) reported significantly lower prevalence of methane excretion in patients with CD and UC (13% and 15%, respectively) compared with healthy controls (54%). Peled et al. (1987) studied breath methane and found that among controls, 50% produced methane. In contrast, breath methane was detected in only 6.1% of patients with CD, whereas 31.4% of UC patients excreted this microbial gas. A more recent study (Pimentel et al. 2003b) compared the excretion of either hydrogen or methane alone to combined excretion of these two gases following a lactulose breath test. The predominant gas excreted by patients with IBD was hydrogen alone (47 of 49 subjects with CD and all 29 subjects with UC) (Pimentel et al. 2003b). In fact, breath methane was detected as the predominant gas in only 2 of the 78 subjects with IBD in this study. To date, there is only a single report on the use of a molecular-based approach to compare the incidence and density of colonic methanogens in healthy controls versus IBD patients. Targeting the *mcrA* gene, Scanlan et al. (2008) reported that although methanogen incidence was numerically reduced in both IBD groups relative to healthy controls, statistical significance was observed only for subjects with UC. Numerous metabolic and physiologic processes, of both microbial and host origin, including blood flow, tissue oxygen tension, and epithelial integrity, are involved in the complex pathway from the production of methane to its excretion in breath. Thus, a multitude of mechanisms may account for the consistent observation of reduced breath methane in IBD patients.

Collectively, available data illuminate the significant amount of work required to determine if a better understanding of the methanogenic niche in the human colon might offer novel prophylactic or therapeutic options for IBD.

Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by chronic abdominal pain, bloating, and abnormal bowel habits (Mayer 2008). Diarrhea or constipation may predominate, or they may alternate; these are classified as IBS-D, IBS-C, or IBS-A, respectively. The exact cause of IBS is unknown. Current explanations for IBS (Mayer & Collins 2002) neither account for postprandial bloating, a symptom that affects 92% of IBS patients (Chami et al. 1991), nor explain the physical evidence of increased abdominal girth and greater small intestinal gas after meals in these patients (Koide et al. 2000, Lea et al. 2003). Additionally, the breath of IBS patients has greater hydrogen and methane excretion than controls (King et al. 1998). Given that these gases are strictly microbial in origin (King et al. 1998), any explanation of IBS must account for abnormal microbial fermentation.

Irritable bowel syndrome (IBS): idiopathic functional bowel disorder characterized by chronic abdominal pain, bloating, and abnormal bowel habits

Colorectal cancer: third most common type and second leading cause of cancer deaths in the Western world; classified as sporadic, hereditary, or IBD-associated

A recent study of IBS-C patients observed decreased fecal populations of lactic acid bacteria, lactate utilizers, and butyrate-producing *Roseburia* species, and increased populations of *Enterobacteriaceae* and SRB relative to control subjects (Chassard et al. 2009). These results suggest that abnormal fermentation may take place in the gut of IBS subjects, possibly leading to production of deleterious metabolites such as H_2S that could contribute to the digestive symptoms. Consistent with this possibility, it was demonstrated recently that exogenous H_2S (NaHS) inhibits in vitro motor patterns in the human, rat, and mouse colon and jejunum, mainly through an action on multiple potassium channels (Gallego et al. 2008).

Along with H_2S, a pathogenic role for methane in constipation is also emerging. Although breath excretion of methane was absent in children without altered bowel habits, 86.3% of children with constipation and fecal soiling exhibited methane excretion (Soares et al. 2002). During a randomized, controlled trial of the effect of antibiotics on symptoms of IBS, the type of breath gas was observed to correlate with bowel symptoms (Pimentel et al. 2003a). Although methane excretion was not observed in patients with IBS-D, 12 out of 31 patients with IBS-C excreted methane. Correspondingly, IBS patients with methane excretion were found to have a reduced plasma concentration of serotonin, the key mediator of the peristaltic reflex (Pimentel et al. 2004). Colonic transit of radio-opaque markers was also observed to be abnormally slow in children with chronic constipation who excreted breath methane (Soares et al. 2005). The relationship between methane and constipation can be explained on the basis of the biologic effect of this gas. Methane slows intestinal transit by converting the pattern of motility from peristaltic to nonperistaltic (Pimentel et al. 2006). Finally, the presence of methane in the breath can be used as a biomarker for IBS-C given that the detection of this gas has a sensitivity of 91.7% and a specificity of 81.3% for this diagnosis (Hwang et al. 2009).

COLORECTAL CANCER

Colorectal cancer is the third most frequent cancer worldwide, producing greater than 940,000 new cases and being responsible for approximately 492,000 deaths annually (Weitz et al. 2005). Genetic and environmental factors play a significant role in the development of colorectal cancer (de la Chapelle 2004, Kinzler & Vogelstein 1996, Potter 1999, Rhodes & Campbell 2002). Although etiologically divided into sporadic (90% of cases), hereditary (5–10%), and IBD-associated (2%), all colorectal cancers show multistep development with several mutations (de la Chapelle 2004, Kinzler & Vogelstein 1996, Rhodes & Campbell 2002). Doll & Peto (1981) estimated that more than 90% of gastrointestinal cancers are determined by environmental factors such as diet. It has been suggested that environmental cancer risk is determined by the interaction between diet and colonic microbial metabolism (O'Keefe et al. 2007). Particularly, there is strong epidemiologic and

experimental evidence showing that diets with high animal fat and protein (meat) are associated with increased risk of colorectal cancer (Norat et al. 2002, Sandhu et al. 2001, Willett et al. 1990). As discussed earlier, meat provides high dietary sulfur, which can promote bacterial production of H_2S in the colon.

Kanazawa et al. (1996) demonstrated that H_2S concentrations were significantly greater in male patients who had previously undergone surgery for sigmoid colon cancer and who later developed new epithelial neoplasia of the colon, compared with males of similar age with a healthy colon. The ability of the colon to detoxify H_2S is also reduced in patients with colon cancer (Ramasamy et al. 2006). The association of H_2S with colon cancer is further supported by the finding that H_2S induces colonic mucosal hyperproliferation with this effect reversed by butyrate (Christl et al. 1996). This effect of H_2S may be mediated by mitogen-activated protein kinase (MAPK)–mediated proliferation (Deplancke & Gaskins 2003). Hydrogen sulfide is also a potent genotoxin that induces direct free radical–associated DNA damage (Attene-Ramos et al. 2006, 2007). Colon cancer in UC and, perhaps, sporadic colon cancer, might reflect genomic instability resulting from exposure to H_2S (Attene-Ramos et al. 2007). Given that the number of SRB was reported to be either not different (Balamurugan et al. 2008) or reduced in colorectal cancer patients when compared with healthy controls (Scanlan et al. 2009), impaired detoxification of H_2S may be critical to the role of this compound in colon cancer.

Regarding an association between colorectal cancer and methanogenesis, findings remain controversial. Haines et al. (1977) reported a high proportion (80%) of methane producers among colon cancer patients compared with control subjects (40%). However, rural black Africans have a low risk for colorectal cancer even though rural Africans are significantly more methanogenic than Western populations (Segal et al. 1988). Pique et al. (1984) reported a high percentage of methane producers among patients with unresected colon cancer (86–92%), which decreased to normal levels (43–47%) after resection. This observation indicates a possibility that the presence of the colonic tumor increases the incidence of methane production. Recently, a negative correlation was found between mean fecal butyrate concentration and methanogen abundance, which may indicate indirect association of methanogens with colorectal cancer or other gastrointestinal disorders (Abell et al. 2006). However, much additional data are needed before causal relationships between hydrogenotrophic microbes and colorectal cancer can be confirmed.

Obesity

Recent findings with animal models and human studies support the emerging concept that the composition and metabolic functions of intestinal microbiota also exert considerable influence on host energy balance. Two recent reviews (DiBaise et al. 2008, Tilg et al. 2009) summarize pathways involving both microbial and host mechanisms that possibly link the microbiota to host energy metabolism. Most pertinent to the present report is evidence for involvement of hydrogenotrophic microbes in energy homeostasis of the host. Overall, the findings confirm the importance of interspecies hydrogen transfer for fermentation efficiency. Although this is a well-documented phenomenon, recent empirical evidence from human and animal model studies revealed the relevance of this key concept in microbial ecology to mammalian energy homeostasis. A study by Samuel & Gordon (2006) demonstrated that cocolonization of germ-free mice with *Bacteroides thetaiotaomicron*, a prominent hydrolyzer of nondigestible polysaccharides in the human colon (Xu et al. 2003), and *M. smithii* not only increased the efficiency of energy extraction from dietary polysaccharides but also changed the specificity of polysaccharide fermentation by *B. thetaiotaomicron*, leading to a significant increase in adiposity in *B. thetaiotaomicron/M. smithii* biassociated mice (Samuel & Gordon 2006). Comparisons were made in gnotobiotic mice harboring *D. piger* as the possible

hydrogenotrophic partner; however, neither *B. thetaiotaomicron* fermentation characteristics nor host markers of lipid metabolism responded in a similar manner in *B. thetaiotaomicron/D. piger* biassociated mice. Flux studies enabling a comparison of the extent of hydrogen utilization by *M. smithii* versus *D. piger* were not performed, and thus, conclusions regarding the relative contribution of these two hydrogenotrophs as syntrophic partners of *B. thetaiotaomicron* are preliminary. Nevertheless, these data were the first to reveal the likely importance of interspecies hydrogen transfer in colonic fermentation to host energy balance. Further, metagenomic studies of the gut microbiota of genetically obese mice and their lean littermates demonstrated that the former possess a greater abundance of Archaea, contain an enhanced representation of genes involved in polysaccharide degradation, and exhibit a greater capacity to promote adiposity when transplanted into germfree recipients (Turnbaugh et al. 2006). A greater abundance of archaeal sequences was also observed in obese human subjects in an intriguing study that used high-throughput sequencing technology and qPCR to compare stool microbial communities of three individuals each in normal-weight, morbidly obese, and postgastric-bypass surgery groups (Zhang et al. 2009). Numbers of the H_2-producing family Prevotellaceae were highly enriched in the obese individuals. Using real-time PCR and targeting the order Methanobacteriales, significantly higher numbers of H_2-utilizing methanogenic Archaea were also detected in obese subjects relative to normal-weight or postgastric-bypass subjects. These investigators also hypothesized that interspecies H_2 transfer between bacterial and archaeal species is an important mechanism for increasing uptake of SCFA from the human colon in obese persons. In contrast to the more recent findings, an earlier study by Haines et al. (1984) reported an inverse association between breath methane concentrations and obesity as measured by skinfold thickness. However, this area of study has just begun and definitive prospective studies are needed to determine, in this case, whether specific hydrogenotrophic signatures predispose to or result from obesity.

CONCLUSIONS

Difficulties associated with accessing the unperturbed environment of the human colon have limited investigation of the in situ metabolism of colonic microbes. Hence, the spatial and temporal scales at which microbial hydrogen metabolism occurs have not been adequately studied in the context of the anatomical and physiological constraints imposed by the host. Similarly, at this point essentially nothing is known regarding the extent to which the hydrogenotrophic microbiota varies in composition or metabolic specificity among individuals. Measurements of ecological parameters at physiological scales, requiring greater knowledge of transport processes and local rates of transformations are needed. Broad interdisciplinary efforts employing the full range of "omic"-based approaches and novel chemical imaging technologies will be required to gain a reasonable understanding of how population dynamics and metabolic activities of hydrogenotrophs impact health and disease in the human colon.

SUMMARY POINTS

1. The human colon is a unique microbial ecosystem characterized by a complex anatomical and cellular structure, a pulsed supply of nutrients, a variety of host secretions, and pH gradients, which together provide diverse microbial niches.
2. The relative rapid flow rate and host absorption preclude complete degradation of organic substrates in the human colon, as opposed to that which occurs in most environmental anaerobic ecosystems.

3. Hydrogen gas produced by anaerobic fermentation in the human colon is either excreted or used in situ by three major groups of H_2-consuming microbes; methanogens, sulfate-reducing bacteria, and acetogens.
4. Many colonic hydrogenotrophs are metabolically versatile and able to utilize other terminal electron-accepting processes. The extent to which these microbes are active as hydrogen consumers in the human colon has not been adequately examined.
5. Both in vitro incubation experiments and in vivo observations indicate that competitive relationships among colonic hydrogenotrophs may not be explained solely by theoretical models estimated by thermodynamics and growth kinetics.
6. Recent molecular-based studies demonstrate greater diversity of colonic hydrogenotrophs than previously expected by culture-based studies, further indicating that their characterization remains incomplete.
7. The colonic mucosa appears to be persistently colonized by SRB, and H_2S has been linked to the pathophysiology of some chronic colonic disorders; however, emerging evidence on endogenously produced H_2S indicates that resolving the role(s) of H_2S in colonic health and disease will require significant effort.
8. Substantial evidence links host genetic background to the methanogenic phenotype, although the extent to which genetics versus diet impacts the hydrogenotrophic microbiota in general remains an open question. Resolving this issue will likely provide important insight into gene-environment disorders ranging from obesity to colorectal cancer.

FUTURE ISSUES

1. Isolation of colonic hydrogenotrophic strains and subsequent analyses by in vitro incubation and whole genome sequencing would begin to fill the substantial gap in understanding microbial hydrogenotrophy in the human colon.
2. Functional gene-based analyses are needed to detect a broader range of microbes that have hydrogenotrophic potential and to elucidate their metabolic activities in situ.
3. Metabolic flux studies, for example with stable isotopes, would provide quantitative data for modeling and thus predicting how endogenous or exogenous substrates modulate colonic hydrogen metabolism.
4. Additional in vitro culture studies will enable further definition of specific environmental conditions that affect metabolic activities of hydrogenotrophic microbes and the competitive or mutualistic interactions among them.
5. Comparative animal model studies could enhance knowledge of how anatomical, biochemical, and physiological constraints imposed by the host influence hydrogenotrophic activities in situ.
6. Emerging metagenomic data from colonic environments should provide further insight into metabolic interactions among fermentative bacteria and hydrogenotrophic microbes.

7. Novel biosensor and chemical imaging technologies are needed to measure substrates and end products in situ including those occurring in the gaseous phase of the colonic environment.

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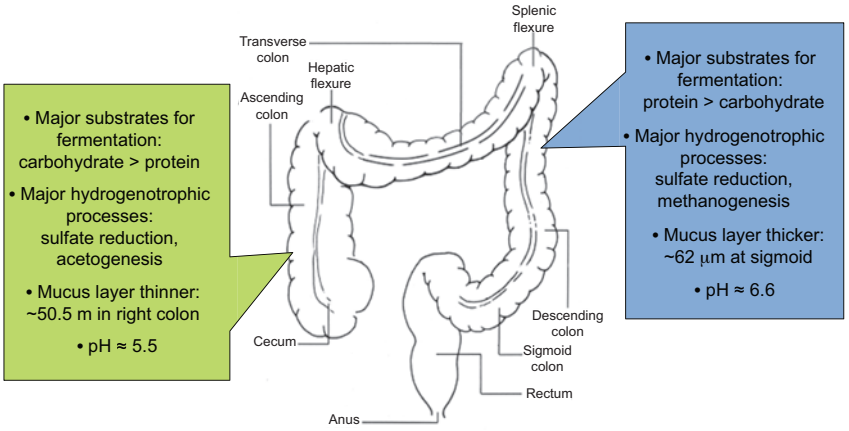

Figure 1

Differential characteristics of right and left colon. The adult human colon is approximately 150 cm in length and consists of ascending or right colon, transverse colon, descending or left colon, sigmoid colon, and rectum with a volume of approximately 0.5 liters. Microbes are the major component of colonic material with more than 10^{10} bacterial cells/ml of contents (Savage 1977). Normal colonic transit time ranges from 20 h to 56 h (Southwell et al. 2009). The mucus layer becomes thicker from ascending colon to sigmoid (Matsuo et al. 1997, Pullan et al. 1994). Mucins are likely to be important sources of carbohydrate in the distal colon, where the supply of fermentable dietary carbohydrate is usually limiting. The luminal pH of the colon becomes less acidic from ascending to descending colon, consistent with a higher proximal rate of carbohydrate fermentation (Cummings et al. 1987, Macfarlane et al. 1992). On the other hand, ammonia, the branched-chain fatty acids, phenol, p-cresol, and various phenolic acids occur predominantly in the left colon, indicating that protein breakdown and amino acid fermentation are quantitatively more dominant processes in this region (Macfarlane et al. 1992). Differences in fermentative substrates and the pH gradient from right to left colon likely impact the hydrogenotrophic processes that occur in each region. Source of colon diagram: **http://cisnet.cancer.gov/projections/colorectal/screening.php**.

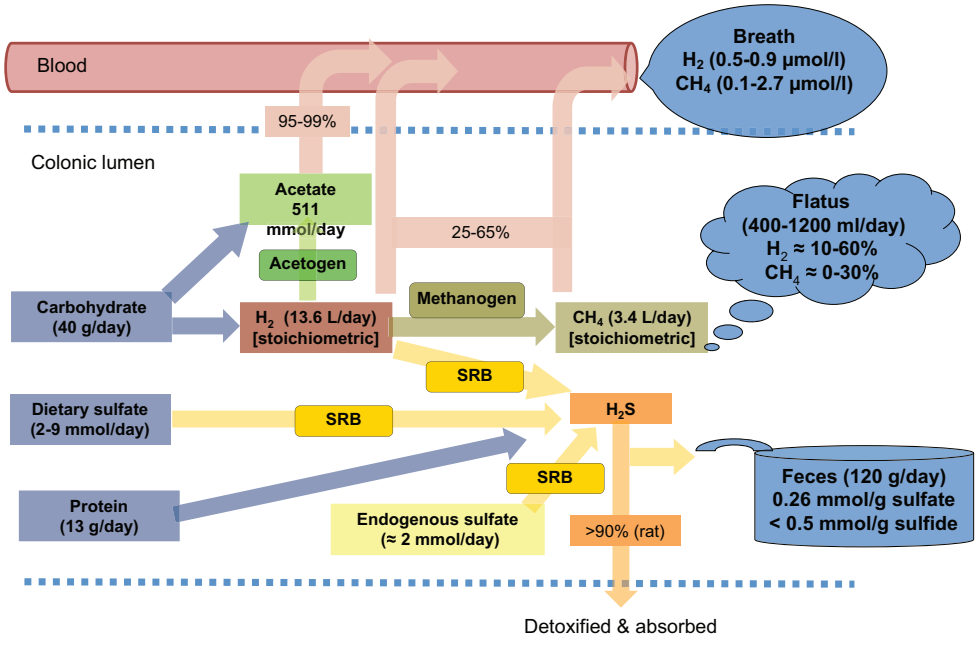

Figure 2

Anatomic schematic of the human colon illustrating fermentative and hydrogenotrophic processes central to this microbial ecosystem. Products of fermentative or hydrogenotrophic processes are often further metabolized by microbes or absorbed by the host, and only proportional amounts of these products are excreted in feces, flatus, or breath (*pink*). Large amounts of gas produced during fermentation are utilized by hydrogenotrophs, greatly reducing the volume of luminal gas. Methane is produced exclusively by methanogens primarily via CO_2 reduction with H_2 (*brown*). Colonic sulfide (*dark orange*) is produced by both SRB via sulfate reduction (*yellow*) and other resident microbes via protein fermentation (*blue*). Both dietary and endogenous sulfate is available for SRB in the colon. Approximately 1.5–2.6 mmol/day of sulfate is produced endogenously in the large intestine (Florin et al. 1991, Willis et al. 1996), and this physiological concentration of sulfate may be sufficient to support SRB growth (Willis et al. 1996). Reductive acetogenesis may occur as well (*green*). However, it is difficult to estimate the extent to which this contributes to the colonic acetate pool. CH_4, methane; H_2, hydrogen; H_2S, hydrogen sulfide; SRB, sulfate-reducing bacteria. Numerical values were taken from (Beazell & Ivy 1941, Florin et al. 1991, Glober et al. 1977, Macfarlane & Gibson 1997, Suarez et al. 1997, Tadesse et al. 1980, Wolin 1981, Wrong 1988).

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Errata

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