Acetogenic Bacteria Mainly Contribute to the Disposal of Hydrogen in the Colon of Healthy Japanese

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Hydrogen-utilizing bacteria in the feces of Japanese individuals were analyzed with the specific polymerase chain reaction targeting the functional genes. The formyltetrahydrofolate synthetase gene derived from acetogenic bacteria was predominantly detected in all subjects. We consider that reductive acetogenesis might be an important H₂ disposal pathway in healthy Japanese.

Key words: acetogenic bacteria; methanogenic archaea; sulfate-reducing bacteria; hydrogen; colonic fermentation

Intestinal bacteria ferment undigested food materials and endogenous substances, such as mucus. Short-chain fatty acids, carbon dioxide, and hydrogen are produced during this fermentation. In the process of anaerobic fermentation, hydrogen must be removed to allow the reoxidation of electron carriers that are essential to the fermentation process (24). Hydrogen-utilizing bacteria, such as sulfate-reducing bacteria, methanogenic archaea, and acetogenic bacteria, contribute to the disposal of hydrogen in the mammalian large intestine (2, 8). Sulfate-reducing bacteria are obligatory anaerobic bacteria that utilize hydrogen and sulfate as an electron donor and an electron acceptor, respectively (7). The major end product of sulfate reduction is sulfide. Sulfide inhibits butyrate oxidation in colonic epithelial cells (17). Because butyrate plays a physiological role in colonocytes, such as the upregulation of ion transports, stimulation of mucin synthesis, increase of colonic blood flow, and stimulation of growth on colonic epithelial cells (4, 21), it has been suggested that sulfide produced by sulfate-reducing bacteria is involved in chronic intestinal diseases, such as ulcerative colitis (20). Sulfate-reducing bacteria found in the human colon belong to the genera *Desulfovibrio*, *Desulfobacter*, *Desulfomonas*, *Desulfobulbs*, and *Desulfotomaculum* (10). Methanogenic archaea utilize H₂ to reduce CO₂ and produce methane. The predominant methanogenic archaea in the human large intestine is *Methanobrevibacter smithii* (16). Acetogenic bacteria utilize H₂ to reduce CO₂ and form acetate (14). The predominant acetogenic bacteria in the human intestinal

tract are *Ruminococcus productus* (18). These hydrogen-utilizing microbes compete for H₂ in an anaerobic fermentation system. In about 30–50% of the people from European countries, methanogenesis has been shown to be an important H₂-disposal pathway (10). In 85–90% of the people from African countries, methane production was the main method of H₂ disposal (8, 9). In non-methane producers, H₂ was consumed with sulfate reduction or reductive acetogenesis (2, 3, 10, 19). The predominant H₂ disposal bacteria in the human colon seem to differ across ethnic groups (9, 22), and the hydrogen-utilizing microbes in Japanese have not yet been investigated. In this study, we analyzed sulfate-reducing bacteria, methanogenic archaea, and acetogenic bacteria in the feces of Japanese individuals with the specific polymerase chain reaction (PCR) targeting the functional genes, adenosine-5'-phosphosulfate (APS) reductase (5), methyl-coenzyme M reductase (*mcrA*) (23), and formyltetrahydrofolate synthetase (FTHFS) (13).

A total of 27 fecal samples from healthy volunteers (22 males and 5 females) aged between 21 and 24 years old were analyzed. Bacterial DNA was extracted from 0.1 g of feces as described by Godon et al. (11). Partial APS reductase, *mcrA*, and the FTHFS gene were amplified with PCR using specific primers (Table 1). The PCR conditions were as follows: 10 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 1.5 mmol/l MgCl₂, 160 μmol/l of each deoxynucleoside triphosphate (dNTP), 400 μmol/l of each primer, 1 U *rTaq* polymerase (TOYOBO, Tokyo, Japan), and 1 μl of extracted bacterial DNA in a total volume of 25 μl. The PCR for APS and *mcrA* was performed as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing for 30 sec and elongation at 72°C for 30

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Table 1. Specific primers used in this study

Target	Primer	Sequence (5'–3')	Reference
APS	APS-f	TGGCAGATMATGATYMACGGG	5
	APS-r	GGGCCGTAAACCGTCCTTGAA	
<i>mcrA</i>	ML-f	GGTGGTGTMMGGATTCACACARTAYGCWACAGC	23
	ML-r	TTCATTGCRTAGTTWGGRTAGTT	
FTHFS	FTHFS-f	TTYACWGGHGAYTTCCATGC	13
	FTHFS-r	GATTTGDGTYTTRGCCATACA	

sec, and final elongation at 72°C for 5 min. The annealing temperatures for APS and *mcrA* were 60°C and 55°C, respectively. For the detection of FTHFS, the touchdown thermal cycling PCR was performed according to Ohashi et al. (18). The touchdown thermal cycling protocol used initial denaturation at 94°C for 3 min, followed by 9 cycles of denaturation at 94°C for 30 sec, annealing at 63°C for 30 sec (decreased by 1°C per cycle to 55°C), and elongation at 72°C for 75 sec. After the touchdown portion of the protocol was completed, 20 additional cycles under an annealing temperature of 55°C were performed, and this was followed by a final elongation step consisting of 72°C for 5 min. Aliquots of the PCR amplicons (10 µl) were analyzed by electrophoresis on 1.5% (w/v) agarose gels and visualized after staining with ethidium bromide.

The results of detection by specific PCR are shown in Table 2. In the PCR-based analysis of fecal samples, the sensitivity of PCR is around 10⁵ to 10⁶ cells/g of feces (15). Therefore, the number of hydrogen disposable bacteria for which the target gene was not detected by specific-PCR in this study might be lower than 10⁶ cells/g feces. *mcrA* was detected from only one female subject. The other 11 subjects might be non-methane producers because the numbers of methanogens in methane producers are over 10⁷ to 10¹⁰ /g feces (1, 6). The ratio (4%) of healthy Japanese individuals who had large populations of methanogenic archaea in their intestines was much lower than that of people from both European (30–50%) (10) and African (85–90%) (9, 10) countries. Methanogenesis does not appear to be the major H₂ disposal pathway in the healthy Japanese. It has been suggested that a genetic factor in the host could have an effect on the colonization of methanogenic archaea (12). The possibility of the presence of a receptor for methanogenic archaea has also been suggested (12). Most Japanese might not have the receptor for methanogenic archaea in their gastrointestinal tract.

In non-methane producers, sulfate-reducing bacteria or homo-acetogenic bacteria contribute to H₂ disposal (1,

Table 2. Detection of hydrogen-utilizing microbes with specific PCR targeting the functional genes in the feces of Japanese individuals

Target	Numbers of positive subjects	
	Male	Female
APS	0/22	0/5
<i>mcrA</i>	0/22	1/5
FTHFS	22/22	5/5

6, 10). However, the APS gene was not detected in any subject. This suggests that sulfate-reducing bacteria might not be significant H₂-consuming bacteria among healthy Japanese. On the other hand, the FTHFS gene was detected in all subjects in this study. Therefore, reductive acetogenesis was considered to be an important H₂ disposal pathway in healthy Japanese. For sulfate-reducing bacteria, sufficient sulfate is required for the predominant utilization of H₂ (10). The amount of mucins secreted by the host and their degree of sulfation affect the growth of sulfate-reducing bacteria because sulfate is released by the degradation of colonic mucins by clostridia and the bacteria of the *Bacteroides fragilis* group (10). The dietary sulfate level is also likely to affect sulfate-reducing activity, and dietary components are likely to play an important role in the colonization and activity of sulfate-reducing bacteria. The luminal pH may also be an important factor for the H₂ disposal pathway in the large intestine (8). An alkaline pH is optimal for sulfate-reducing bacteria, whereas a neutral pH is optimal for methanogenic archaea. Acetogenesis is promoted by acidic conditions. Although the factors controlling H₂-consuming bacteria in the large intestine of Japanese are poorly understood, the luminal conditions in the large intestine of healthy Japanese might not suit the growth of sulfate-reducing bacteria. Consequently, reductive acetogenic bacteria may predominantly consume H₂ in the large intestine of healthy Japanese. This might be preferable to the host because additional energy is available from fermentation in the large intestine.

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