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In vitro interactions between *Blautia hydrogenotrophica*, *Desulfovibrio piger* and *Methanobrevibacter smithii* under hydrogenotrophic conditions

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ABSTRACT

Methanogens, reductive acetogens and sulfate-reducing bacteria play an important role in disposing of hydrogen in gut ecosystems. However, how they interact with each other remains largely unknown. This *in vitro* study cocultured *Blautia hydrogenotrophica* (reductive acetogen), *Desulfovibrio piger* (sulfate reducer) and *Methanobrevibacter smithii* (methanogen). Results revealed that these three species coexisted and did not compete for hydrogen in the early phase of incubations. Sulfate reduction was not affected by *B. hydrogenotrophica* and *M. smithii*. *D. piger* inhibited the growth of *B. hydrogenotrophica* and *M. smithii* after 10 h incubations, and the inhibition on *M. smithii* was associated with increased sulfide concentration. Remarkably, *M. smithii* growth lag phase was shortened by coculturing with *B. hydrogenotrophica* and *D. piger*. Formate was rapidly used by *M. smithii* under high acetate concentration. Overall, these findings indicated that the interactions of the hydrogenotrophic microbes are condition-dependent, suggesting their interactions may vary in gut ecosystems.

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Coculture; methanogen; hydrogen; formate; acetate; sulfide

Introduction

The gut microbiota consists of a wide variety of microbial species with the ability to ferment dietary fibers and other complex substrates that escape digestion and absorption, resulting in the production of short chain fatty acids as well as carbon dioxide and hydrogen.[1–3] The accumulation of hydrogen thermodynamically restricts further microbial fermentation and growth.[4–6] Hydrogenotrophic microbes using hydrogen as the electron donor for their anaerobic respiration play an important role in maintaining the hydrogen balance in gut ecosystems.[4,7] Moreover, hydrogenotrophic microbes have been suggested to play an important role in human health.[5,6] Methanogens are considered beneficial or harmful for human health, and the associations of methanogens with obesity, anorexia, colorectal cancer, inflammatory bowel disease, irritable bowel syndrome, diverticulosis, atherosclerosis and periodontitis were described by Chaudhary and colleagues.[8] Hydrogen sulfide has been implicated in the development of colorectal cancer.[9,10] Higher sulfate-reducing bacteria (SRB) abundance or hydrogen sulfide concentrations have been reported in ulcerative colitis patients compared to healthy individuals.[11,12]

Hydrogenotrophic microbes in humans consist of three major functional groups, namely methanogens, reductive acetogens and SRB.[6] Methanogens reduce carbon dioxide to methane using hydrogen as electron donor ($4\,H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O$).[13] Reductive acetogens use hydrogen and carbon dioxide producing acetate via the Wood-Ljungdahl metabolic pathway ($4\,H_2 + 2\,CO_2 \rightarrow CH_3COOH + 2\,H_2O$).[13] SRB reduce sulfate to hydrogen sulfide using hydrogen as electron donor ($4\,H_2 + SO_4^{2-} + 2\,H^+ \rightarrow H_2S + 4\,H_2O$).[13] The prevalence of gut methanogens varies between populations with estimates of ~ 30% prevalence in the Western world and ~ 80% in Africa with *Methanobrevibacter smithii* as the dominant methanogenic archaeal species in the human gut.[5,14] SRB that colonize the guts of ~50% of

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humans show greater taxonomic diversity than methanogens with *Desulfovibrio piger* described as the most common species.[15] Reductive acetogens are phylogenetically diverse, and *Blautia hydrogenotrophica* is the most well-known and studied reductive acetogenic species.[5] It has been estimated that one-third or one-fourth of acetate in the gut is produced via reductive acetogenesis.[16]

Competition between the three hydrogenotrophic functional groups has been considered because all of them can use hydrogen as an energy source.[4,5] Thermodynamically, sulfate reduction with hydrogen is more favorable with a Gibbs free energy change under standard conditions of −152.2 kJ mol^{-1}, compared to methanogenesis and acetogenesis with Gibbs free energy changes of −131 kJ mol^{-1} and −95 kJ mol^{-1}, respectively.[17] It has been reported that SRB and methanogens are mutually exclusive.[18] SRB were rarely detected in the gut microbiota of so-called methane excretors that are subjects with an above average methanogen abundance, while the gut microbiota of non-methane excretors harbors a higher abundance of SRB.[18] In addition, lower acetogenesis has been found in the presence of methanogens, and inhibition of methanogens concomitantly led to higher acetate production in fecal cultures.[19] However, mutual exclusivity is not always found, and several studies have reported that no significant relationship was observed between methanogens and SRB.[20,21] A recent study by Wang et al (2022) found that methanogens, reductive acetogens and SRB abundances did not show a negative correlation with each other indicating their coexistence in adult fecal samples.[22] Although these previous studies indicate that the three hydrogenotrophic functional groups may impact each other considering all of them use hydrogen as energy source, detailed insights into the interactions between them remains unknown.

Therefore, we cocultured the hydrogenotrophic microbial species *B. hydrogenotrophica*, *D. piger* and *M. smithii* with each other *in vitro* under hydrogenotrophic conditions, aiming to understand how these three species affect each other's growth and metabolic activity *in vitro*. Moreover, the impact of sulfide concentrations on these species was investigated as hydrogen sulfide produced by sulfate reduction is highly reactive and toxic to microbes.[23,24] Furthermore, we described the impact of formate and acetate concentrations produced during incubations on the growth of *M. smithii* as they could serve as substrates for methanogens.[25,26]

Results

Coculturing with B. hydrogenotrophica or D. piger shortens the lag phase of M. smithii

To study the interactions between the three hydrogenotrophic species, *B. hydrogenotrophica* DSM 10,507^T (B), *D. piger* DSM 749^T (D) and *M. smithii* DSM 11,975 (M) were cultured in monocultures, in binary cocultures and in triculture (Experiment one, Figure 1a). Hydrogen was consumed by the three hydrogenotrophic species over time in all the cultures (Figure 1b). As expected, methane was only detected in the presence of *M. smithii*. Sulfate was consumed and sulfide produced concomitantly, only in the presence of *D. piger*. Acetate production was only observed in the presence of *B. hydrogenotrophica*.

For monocultures, *D. piger* and *B. hydrogenotrophica* consumed hydrogen much faster than *M. smithii* in the early phase of incubation. *B. hydrogenotrophica* and *D. piger* grew rapidly and their 16S rRNA gene copy numbers peaked at 24 h. In contrast, *M. smithii* showed no growth in the first 24 h, but started fast growth afterward and consumed hydrogen throughout the incubation (Figure 1c).

When cocultured with *B. hydrogenotrophica* or *M. smithii*, sulfate reduction and the growth of *D. piger* were not affected. In contrast, *B. hydrogenotrophica* and *M. smithii* growth and metabolism were impacted when cocultured with other hydrogenotrophic species. The monoculture of *B. hydrogenotrophica* produced acetate throughout the 72 h incubations. However, *B. hydrogenotrophica* stopped producing acetate in the presence of *D. piger* (BD and BDM) after 10 h incubation. The 16S rRNA gene copy numbers of *B. hydrogenotrophica* stopped increasing as well. When cocultured with *M. smithii* (BM), the 16S rRNA gene copy number of *B. hydrogenotrophica* was not

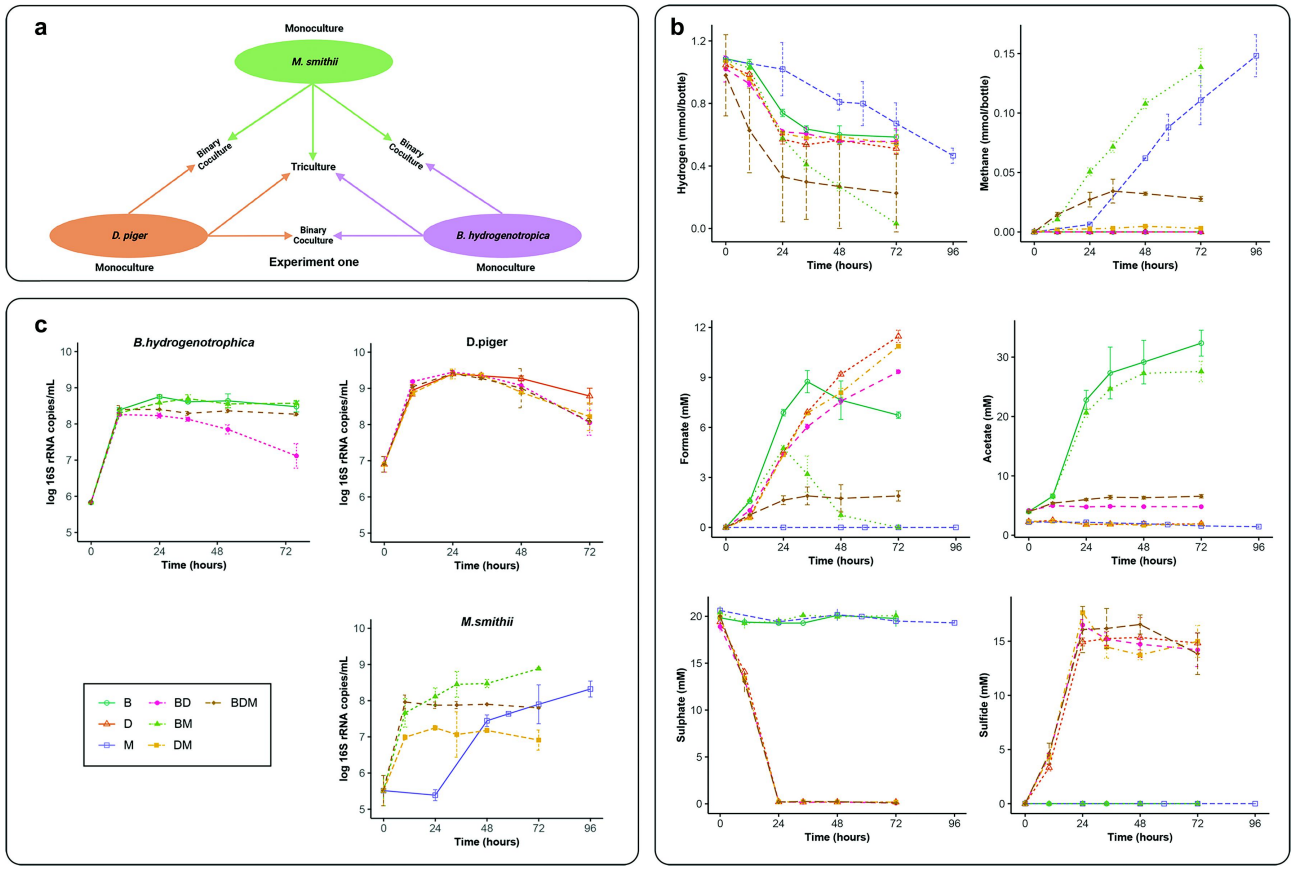

Figure 1. Interactions between hydrogenotrophic species. (a) schematic overview of Experiment one. *B. hydrogenotrophica*, *D. piger* and *M. smithii* were cultured in monoculture, or together in binary cocultures and a triculture. (b) hydrogen consumption and metabolite production of *B. hydrogenotrophica*, *D. piger* and *M. smithii* under hydrogenotrophic conditions. (c) log 16S rRNA gene copies per mL of *B. hydrogenotrophica*, *D. piger* and *M. smithii* in monocultures, binary cocultures and a triculture. Data are shown as average ± standard deviation ($n = 2$). B: *B. hydrogenotrophica* monoculture; D: *D. piger* monoculture; M: *M. smithii* monoculture; BD: *B. hydrogenotrophica* and *D. piger* binary coculture; BM: *B. hydrogenotrophica* and *M. smithii* binary coculture; DM: *D. piger* and *M. smithii* binary coculture; BDM: *B. hydrogenotrophica*, *D. piger* and *M. smithii* triculture.

affected compared to its monoculture. However, the BM coculture ended up with a lower acetate concentration at the end of the incubation compared to the monoculture of *B. hydrogenotrophica*. Remarkably, in BM and DM cocultures, *M. smithii* had a much shorter lag phase compared to its monoculture: < 10 h versus > 24 h. Afterward, *M. smithii* stopped growing when cocultured with *D. piger* (DM and BDM). However, it continued to grow in the coculture with *B. hydrogenotrophica* (BM) with a similar trend as in its monoculture. Consistently, a small amount of methane (0.0018 ± 0.0007 mmol/bottle) was observed in DM in the first 10 h without a further increase afterward, and in the BDM culture methane was only produced in the first 34 h. In contrast, the BM coculture showed a faster methane production in the first 10 h of incubation compared to the monoculture of *M. smithii*. After 10 h, methane continued to be rapidly produced in the BM coculture with a similar trend as the monoculture of *M. smithii* (Figures 1b,c).

Formate was only detected in cultures with *B. hydrogenotrophica* or *D. piger*. For monoculture of *B. hydrogenotrophica*, formate was produced fast and peaked at 34 h, and started to decrease afterward. Interestingly, in the BM coculture, the highest formate concentration was observed at 24 h, after which it started to decrease until depletion at 72 h. In contrast, the formate in the BD and DM cocultures followed a similar trend as the monoculture of *D. piger*. The BDM triculture had a fast formate production in the first 24 h, after which its concentration remained stable over time (Figure 1b).

High sulfide concentrations inhibit the growth of M. smithii

When cocultured with *D. piger*, methane production by *M. smithii* and acetate production by *B. hydrogenotrophica* were inhibited in the late phase of incubations. We speculated this could be caused by the increased sulfide concentration produced by *D. piger* during incubations as hydrogen sulfide is highly reactive and toxic to microbes.[27] To confirm the effect of sulfide on the growth of the hydrogenotrophic species, monocultures of the three species were performed with different sulfide concentrations ranging from 1 to 10 mM (Experiment two, Figure 2a). We observed that the growth of *D. piger* and *B. hydrogenotrophica* was not impacted by sulfide concentrations. Interestingly, low concentration sulfide (1 mM) had no effect on the growth of *M. smithii*, whereas 3 mM, 6 mM and 10 mM sulfide inhibited the *M. smithii* growth. These findings suggest that produced sulfide in the DM and BDM cocultures over time inhibited the growth of *M. smithii* (Figure 2b).

High acetate concentrations contribute to the usage of formate by M. smithii

The lag phase of *M. smithii* was shortened in the presence of *B. hydrogenotrophica* or *D. piger* (Figure 1c). We determined whether the production of acetate and formate could explain this phenomenon by growing *M. smithii* in media supplemented different concentrations of acetate and formate in hydrogenotrophic and non-hydrogenotrophic conditions (Experiment three, Figure 3a). We observed that both hydrogen and formate were consumed, concomitantly with an increase of methane and culture density (Figure 3b). Compared to HANF-H_2 (20 mM acetate, 0 mM formate, 1.7 atm H_2-CO_2 in the headspace), LANF-H_2 (2 mM acetate, 0 mM formate, 1.7 atm H_2-CO_2 in the headspace) showed higher methane production after 92 h indicating that a lower acetate concentration (2 mM) was more favorable for hydrogenotrophic methanogenesis than the higher one (20 mM). Using formate as an electron donor, HAF-N_2 (20 mM acetate, 15 mM formate, 1.7 atm N_2-CO_2 in the headspace) showed increased formate consumption with increased methane production compared to LAF-N_2 (2 mM acetate, 15 mM formate, 1.7 atm N_2-CO_2 in the headspace) after 92 h incubations, indicating that a higher

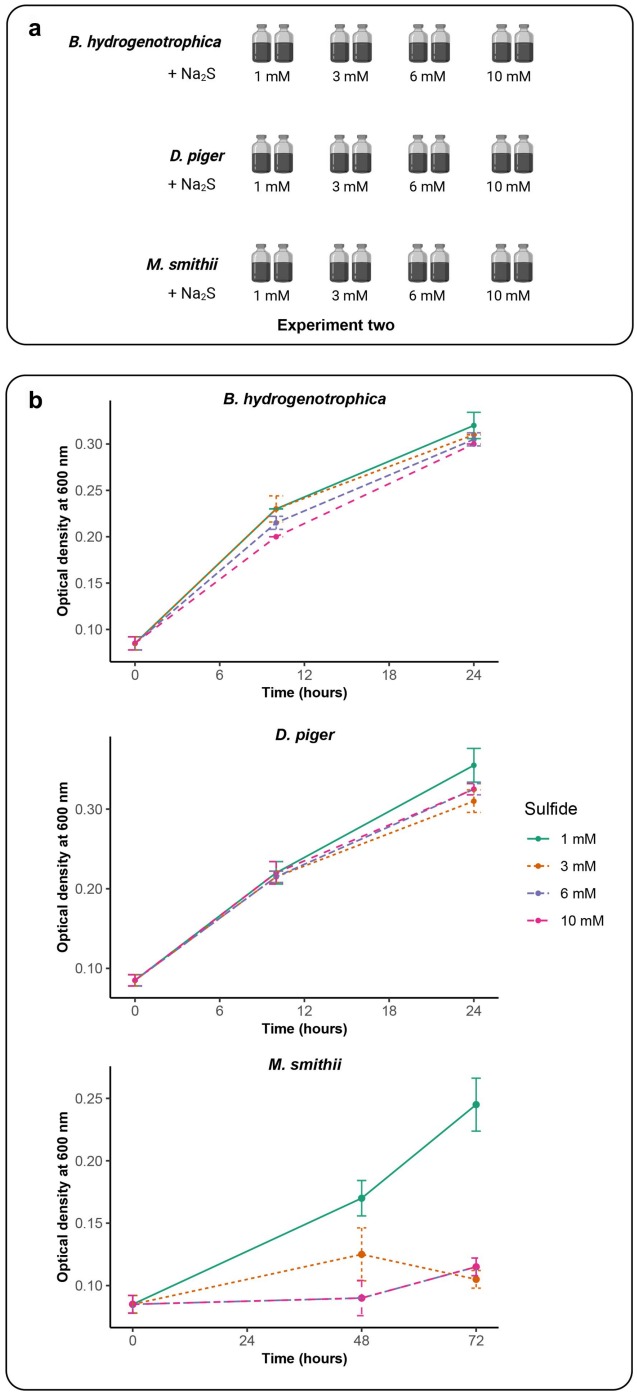

Figure 2. Impact of sulfide concentrations on the growth of *B. hydrogenotrophica*, *D. piger* and *M. smithii*. (a) schematic overview of Experiment two (b) the optimal density of *B. hydrogenotrophica*, *D. piger* and *M. smithii* under different sulfide concentrations during incubations. Data are shown as average ± standard deviation ($n = 2$).

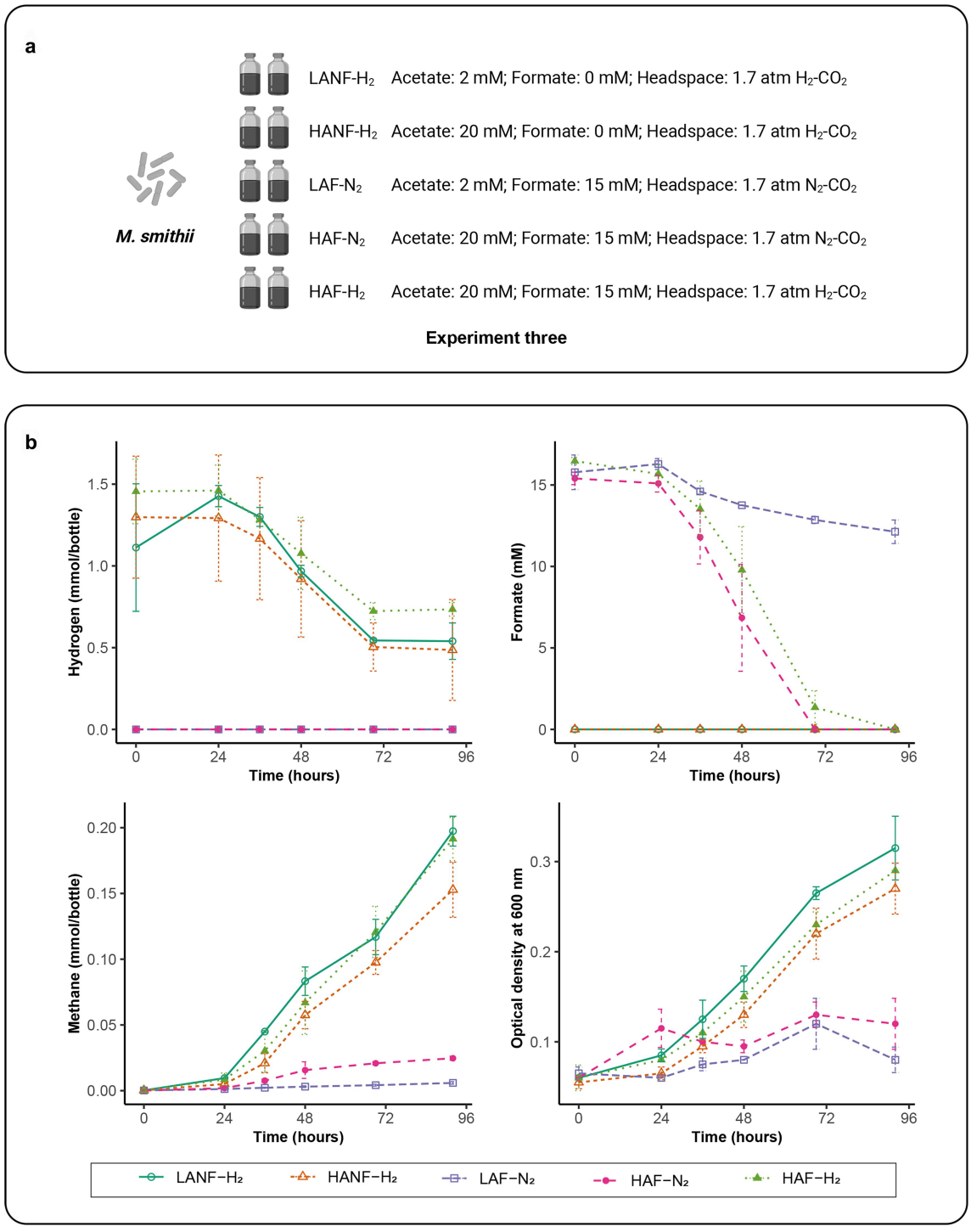

Figure 3. Impact of acetate and formate concentrations on the growth of *M. smithii*. (a) schematic overview of Experiment three. (b) hydrogen and formate consumption, methane production and microbial density of *M. smithii* during incubations. Data are shown as average ± standard deviation ($n = 2$). LANF-H_2: 2 mM acetate, 0 mM formate, H_2-CO_2 headspace; HANF-H_2: 20 mM acetate, 0 mM formate, H_2-CO_2 headspace; LAF-N_2: 2 mM acetate, 15 mM formate, N_2-CO_2 headspace; HAF-N_2: 20 mM acetate, 15 mM formate, N_2-CO_2 headspace; HAF-H_2: 20 mM acetate, 15 mM formate, H_2-CO_2 headspace.

acetate concentration (20 mM) was more favorable for formate-dependent methanogenesis. Moreover, compared to HANF-H_2, HAF-H_2 (20 mM acetate, 15 mM formate, 1.7 atm H_2-CO_2 in the headspace) showed more methane produced with concomitant depletion of formate after 92 h incubation. Although we observed differences in metabolic activities when the

concentrations of formate and acetate were modified, a shortened lag phase of *M. smithii* was not observed, suggesting that a yet unidentified mechanism in the cocultures is responsible for this.

Discussion

In this study, *B. hydrogenotrophica*, *D. piger* and *M. smithii* were cocultured to investigate their interactions under hydrogenotrophic conditions. The main findings are summarized in Figure 4 and indicated that the three hydrogenotrophic species coexisted and did not compete for hydrogen in the early phase of incubations. Coculturing with *B. hydrogenotrophica* and *D. piger* shortened the lag phase of *M. smithii*, concomitantly resulting in faster methane production. However, the presence of *D. piger* inhibited the growth of *B. hydrogenotrophica* and *M. smithii* and their metabolite production in the late phase of incubations. In addition, we found that high sulfide concentrations inhibited methanogenesis. A higher acetate concentration stimulated the usage of formate by *M. smithii*.

It has been considered that the three hydrogenotrophic functional groups may compete because all of them use hydrogen as an energy source in the human gut.[4,5] However, our results by coculturing the three hydrogenotrophic species clearly showed that the interactions between the three hydrogenotrophic functional groups are condition dependent. Accordingly, considering the complexity of the human gut ecosystem including its nutrient supply, the variable environmental conditions throughout the gut, as well as the metabolic flexibility of some hydrogenotrophic microbes, the interactions between hydrogenotrophic microbes in the gut are potentially complex and certainly environment-dependent,[5,28,29] which might explain the inconsistent findings between studies.

SRB have the greatest affinity for hydrogen and dissimilatory sulfate reduction is thermodynamically more favorable than methanogenesis and reductive acetogenesis.[17,30] It has been suggested this advantage is negated when sulfate is depleted

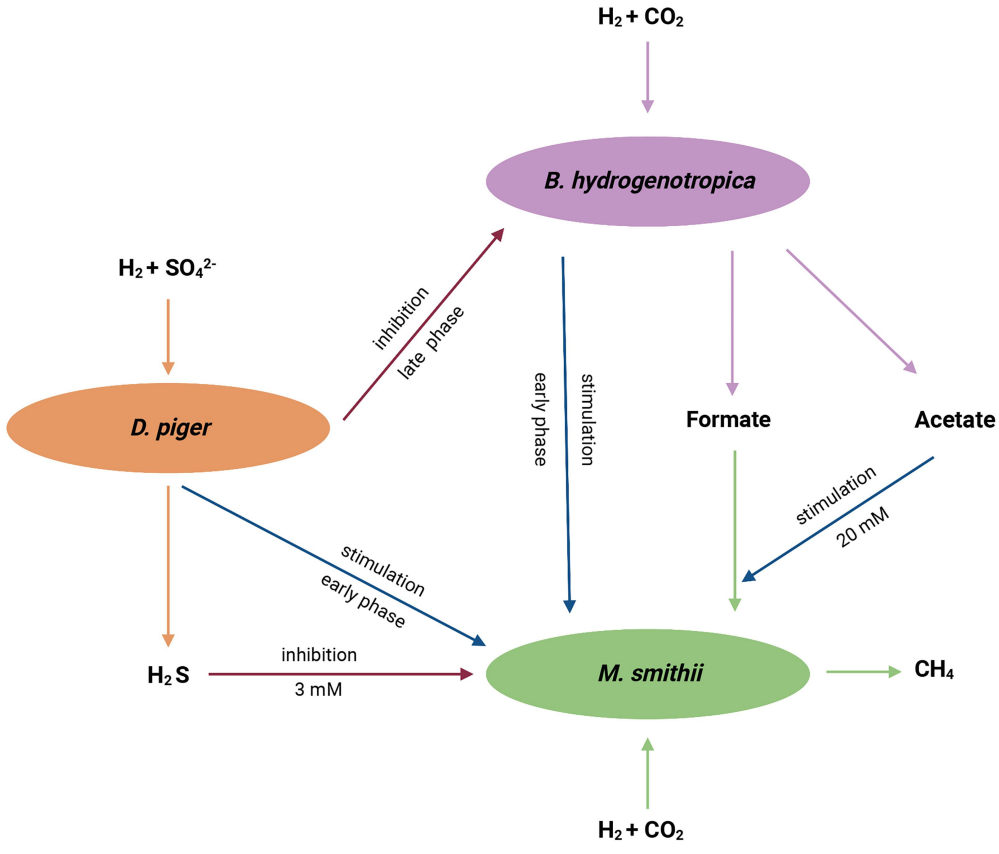

Figure 4. Summary of the interactions between *B. hydrogenotrophica*, *D. piger* and *M. smithii* under hydrogenotrophic conditions.

in the gut. Consistently, in our study, we observed that 20 mM of sulfate was quickly depleted by *D. piger* within 24 h, which limits the growth of *D. piger* under hydrogenotrophic conditions. Hydrogen sulfide is highly reactive and toxic to microbes since it can diffuse across the cell membrane and is involved in protein denaturation and enzyme inhibition.[23,24] Moreover, this toxicity has been suggested to be associated with hydrogen sulfide concentrations.[24] We indeed confirmed that 3 mM sulfide inhibited the growth of *M. smithii*. However, the growth of *D. piger* and *B. hydrogenotrophica* was not affected by high sulfide concentrations, indicating that the toxicity of sulfide is species dependent. Sulfide concentrations vary between individuals. It has been reported that the mean total sulfide content in wet feces was 0.66 mmol/kg.[31] However, a study in which individuals consumed a high-meat diet (600 g/day) rich in sulfur-containing amino acids, showed a much higher fecal sulfide content reaching levels of 3.38 mmol/kg.[32] This suggests that interactions between SRB and *M. smithii* may vary and are partially determined by the daily diet consumed as multiple sources of sulfur are present in the gut, including organic components from consumed plant-based diets as well as host-secreted components, such as mucus. SRB and methanogens have been reported to be coexisted or mutually exclusive[18,20,21] and these inconsistent observations could be associated with the luminal sulfide concentrations, which should be further confirmed to illustrate the importance of hydrogen sulfide in the hydrogen metabolism in gut ecosystems.

Synergistic metabolic relationships have been proposed between *M. smithii* and hydrogen-producers.[29,33] *Bacteroides thetaiotaomicron* is a well-known saccharolytic bacterium, and its fermentation resulting in production of hydrogen, formate and acetate supports the growth of *M.smithii*.[29,34] In turn, the presence of *M. smithii* increases the metabolic efficiency of *Bacteroides thetaiotaomicron*.[29] Interestingly, cocolonization of *M. smithii* and *Bacteroides thetaiotaomicron* in a humanized gnotobiotic mouse model showed an increased population size of both in cecum and distal colon.[35] In addition, coculturing *M. smithii* and the species of the bacterial family *Christensenellaceae* indicated a syntrophic relationship via interspecies hydrogen transfer, resulting in higher acetate but lower butyrate production compared to monocultures of *Christensenellaceae* strains.[33] Although *B. hydrogenotrophica* and *D. piger* are hydrogenotrophic microbes, they are not obligatorily dependent on hydrogen and could even potentially have similar syntrophic interactions as described above to *B. thetaiotaomicron* and strains of *Christensenellaceae*. *B. hydrogenotrophica* can use glucose and fructose to grow and produce acetate and formate.[36,37] Besides hydrogen consumption *D. piger* is also able to perform fermentation of pyruvate resulting in the production of hydrogen, formate and acetate.[38] Both formate and acetate can favor the growth of *M. smithii* as indicated in a study published recently,[26] suggesting possible syntrophic relationships between the *M. smithii* and the *B. hydrogenotrophica* and *D. piger*. Interestingly, we found that the lag phase of *M. smithii* was consistently shortened by both *B. hydrogenotrophica* and *D. piger*. However, this was not due to the increased formate and acetate concentrations. The exact mechanism of this stimulation effect by *B. hydrogenotrophica* and *D. piger* remains unknown and needs further studies. Moreover, we found that formate was rapidly converted to methane only under high acetate concentration (20 mM). Therefore using formate as an energy source with high acetate concentration can be an alternative of hydrogen and carbon dioxide for growing *M. smithii*.[26]

In this study, we cocultured the three hydrogenotrophic functional groups to give insights into their interactions, which could improve our understanding in their interactions in the human gut. However, our study, like any *in vitro* study, cannot completely mimic the complexity of the gut environment *in vivo* and further research is needed to evaluate our findings *in vivo*. Unfortunately, given the multiple comparative cultivations in our study, we could only obtain duplicate samples that are not sufficient to support sound statistical assessment of potential differences between groups. Nevertheless, although having at least triplicate measurements would be necessary to allow for statistical analyses, our data generally showed that the duplicate measurements followed the expected trend with acceptable hydrogen balances (Supplementary Table S1).

In conclusion, this *in vitro* study gives a detailed overview of interactions between the three hydrogenotrophic species under hydrogenotrophic

conditions. Results revealed that the interactions and metabolisms of these hydrogenotrophic functional groups are condition-dependent *in vitro*. Their relationships are complex and may vary throughout the gut considering the variable environmental conditions *in vivo*, and thus not easy to extrapolate to the *in vivo* situations in the gut. Our study thus may explain why inconsistent observations are reported about the coexistence of hydrogenotrophs in the gut.

Materials and methods

Study set up

To study the interactions between the three hydrogenotrophic species, *Blautia hydrogenotrophica* DSM 10,507^T (B), *Desulfovibrio piger* DSM 749^T (D) and *Methanobrevibacter smithii* DSM 11,975 (M) were cultured in duplicate, in monoculture, in binary cocultures: *B. hydrogenotrophica* and *D. piger* (BD), *B. hydrogenotrophica* and *M. smithii* (BM), *D. piger* and *M. smithii* (DM), and all of them together (BDM) (Figure 1a, Experiment one) using a basal medium (Supplementary Table S2) as previously described[39] with some modifications that included the addition of 2 mM sodium acetate, 20 mM sodium sulfate, 1 g/L yeast extract (OXOID) and 1 g/L tryptone (OXOID). Incubation was performed in 30 mL serum bottles containing 10 mL medium. The bottle headspace consisted of a mixture of H_2 and CO_2 (80:20, v/v; 1.7 atm). Individual precultures of the three species were prepared prior to the inoculations with the same medium. After inoculations of each species, the optical density (OD) at 600 nm with a spectrophotometer (600 DiluPhotometer, Implen GmbH, Munich, Germany) was measured when their growth was in the mid-log phase. Subsequently, precultures were diluted with the same medium and 0.5 mL precultures (OD = 0.2) were used to inoculate monocultures, binary cocultures and tricultures for each species. Cultures were incubated at 37°C with 150 rpm. All samples were taken at 0 h, 10 h, 24 h, 34 h, 48 h and 72 h except for the monoculture of *M. smithii* for which samples were taken at 0 h, 24 h, 48 h, 58 h, 72 h and 96 h due to its slower growth compared to other cultures.

To study the effect of sulfide concentrations on the growth of *B. hydrogenotrophica*, *D. piger* and *M. smithii*, the same medium as described above with different sulfide concentrations (1 mM, 3 mM, 6 mM and 10 mM) modified by adding sodium sulfide were used to incubate these three species (Figure 2a, Experiment two). The incubation condition and inoculation were the same as described above. For *B. hydrogenotrophica* and *D. piger*, samples were taken at 0 h, 10 h, 24 h. For *M. smithii*, samples were taken at 0 h, 48 h, 72 h due to its slower growth compared to other cultures.

To study the effect of formate and acetate concentrations on the growth of *M. smithii*, the same medium as mentioned in Experiment one was used with the following modifications (Figure 3a, Experiment three): no modification (2 mM acetate, 0 mM formate, 1.7 atm H_2-CO_2 in the headspace; LANF-H_2); addition of 20 mM acetate without modification of the headspace (HANF-H_2); addition of 15 mM formate with the headspace flushed with a mixture of N_2-CO_2 (80:20, v/v; 1.7 atm; LAF-N_2,); addition of 20 mM acetate and 15 mM formate with the headspace flushed with a mixture of N_2-CO_2 (80:20, v/v; 1.7 atm; HAF-N_2); addition of 20 mM acetate and 15 mM formate without modification of the headspace (HAF-H_2). For Experiment three, samples were taken at 0 h, 24 h, 36 h, 48 h, 69 h and 92 h for all incubations.

Sampling and analytical methods

For sampling, 0.2 mL gas samples were taken using a sterile 1 mL syringe from the headspace of the serum bottle and were analyzed immediately by gas chromatography (GC). 1 mL culture medium was taken at each sampling timepoint and subsequently centrifuged at 4°C at maximum speed (21130 × g) for 10 min to separate the microbial biomass and supernatant. Afterwards, 200 µL supernatant was mixed with 50 µL of $ZnCl_2$ solution ($ZnCl_2$: 50 g/L; 0.2 mL/L acetic acid) to react and precipitate the sulfide for the subsequent analysis. The remaining supernatant and pellet were stored at −20 °C for further analysis.

Hydrogen and methane amount were detected using a Compact GC 4.0 (Global Analyser Solutions, Breda, the Netherlands) equipped

with a molsieve 5A column, operated at 100°C coupled to a Carboxen 1010 pre-column. Detection was done via a Thermal Conductivity Detector. Argon was used as carrier gas with a flow rate of 5 mL/min and pressure of 325 kPa. The software Chromeleon (Version 7.2, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used for data processing with a standard curve to quantify hydrogen and methane concentrations.

Formate and acetate in the supernatant were detected via High-Performance Liquid Chromatography equipped with a Shodex SH1821 column (Showa Denko K.K., Tokyo, Japan) operated at 45°C. Four hundred microliters of supernatant were added to 600 µL of a DMSO solution at 10 mM in 0.1 N H_2SO_4. DMSO was used as an internal standard. Elution was performed with 0.01 N H_2SO_4 at a flow rate of 1 mL/min. Detection was done via a refractive index detector. The software Chromeleon (Version 7.2, Thermo Fisher Scientific) was used for data processing with a standard curve to quantify formate and acetate concentrations.

Sulfate was detected via Ion Chromatography (IC, ICS-2100, Thermo Fisher Scientific) using a Dionex IonPac AS16 column, operated at 30°C. Thirty microliters of supernatant were added to 970 µL of an internal standard solution (0.5 mM sodium iodide prepared in ultra-pure water). Ultra-pure water was used as the eluent with a flow rate of 0.1 mL/min. Detection was done via an electrochemical IC detector. The software Chromeleon (Version 7.2, Thermo Fisher Scientific) was used for data processing with a standard curve to quantify sulfate concentrations.

Fifty microliters of the sample and $ZnCl_2$ mixture were used to determine hydrogen sulfide concentrations. Hydrogen sulfide was quantified via the methylene-blue method as described previously.[40]

Quantitative polymerase chain reaction (qPCR) analysis

Microbial count for each species was determined using qPCR for Experiment one. DNA extraction was performed via the repeated beat-beating method as described previously[41] with a small modification. Briefly, 300 µL of Stool Transport and Recovery (STAR) buffer (Roche Diagnostics, United States) was mixed with each pellet for the first beat-beating, and 200 µL of the STAR buffer was added for the second bead-beating. DNA concentrations were determined with a Qubit Fluorometer (Life Technologies, Darmstadt, Germany) in combination with the dsDNA BR Assay kit (Invitrogen, Carlsbad, CA, USA), and subsequently was adjusted to 1 ng/µL with nuclease-free water for qPCR.

The three microbial species were quantified using their 16S rRNA gene fragments. A standard template for each hydrogenotrophic species was generated using purified PCR products. The 16S rRNA gene PCR products for *B. hydrogenotrophica* and *D. piger* were obtained using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492 R (5'-GGTTACCTTGTTACGACTT-3') with the DNA extracted from *B. hydrogenotrophica* DSM 10,507 and *D. piger* DSM 749 as template, respectively.[42] The 16S rRNA gene PCR product for *M. smithii* was obtained using the primers A109F (5'- ACKGCTCAGTAACACGT –3') and Arch1492R (5'- GGCTACCTTGTTACGACTT –3') with the DNA extracted from *M. smithii* DSM 11,975 as template.[43,44] All PCR products were purified using the GeneJET PCR Purification Kit (Thermo Fisher Scientific) and quantified using a Qubit Fluorometer (Life Technologies) in combination with the dsDNA BR Assay kit (Invitrogen). A dilution corresponding to 10^{10} copies/µL was prepared for each standard. Then all standards were serially ten-fold diluted with nuclease-free water and dilution from 10^9 copies/µL to 10^2 copies/µL was used for the standard curve. All qPCRs were carried out in triplicate with an iCycler iQ real-time detection system (Bio-Rad Laboratories BV). Each reaction mixture with a total volume of 10 µL contained 5 µL 2× iQ SYBR green (Bio-Rad Laboratories B.V.), 2 µL of DNA template (samples at 1 ng/µL or standards), 300 nM forward (1 µL) and reverse (1 µL) primers, and nuclease-free water (1 µL). Primers and qPCR conditions for each species are listed in Supplementary Table S3.

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Disclosure statement

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Data availability statement

All data generated or analyzed during this study are included in this report and supplementary tables .

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