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Physiology of the gut bacteria *Sutterella wadsworthensis*, *Sutterella parvirubra*, and
Turicimonas muris

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Zusammenfassung

Sutterellaceae, eine Familie weit verbreiteter anaerober Darmbakterien, wurde vor fast 30 Jahren erstmals identifiziert. In den letzten zehn Jahren wurden durch den Aufstieg der Metagenomik viele neue Arten entdeckt. Es wurde festgestellt, dass sie mit verschiedenen menschlichen Gesundheitszuständen wie Colitis ulcerosa, entzündlichen Darmerkrankungen, Autismus oder einem gesunden Zustand assoziiert sind. Sie bleiben jedoch wenig erforscht, da nur wenig über ihren Stoffwechsel und ihre Auswirkungen auf den Magen-Darm-Trakt bekannt ist. Eine zugrundeliegende Re-Analyse öffentlich zugänglicher metagenomischer und metatranskriptomischer Datensätze von Stuhlproben verschiedener menschlicher Kohorten, die in unserem Labor durchgeführt wurde, zeigte eine hohe Expression mikrobieller Gene für die Nutzung von Formiat und Wasserstoff als Elektronendonatoren, Fumaratreduktion über hoch exprimierte Fumaratreduktase und vermutlich Sulfatreduktion über die Octahaem-Cytochrom-c-Sulfatreduktase MccA. Ziel dieser Arbeit war es, diese physiologischen Kapazitäten, die in den Genomen von *Sutterella wadsworthensis*, *Sutterella parvirubra* und *Turicimonas muris* kodiert sind, experimentell durch eine Reihe von Reinkultur-Wachstumsexperimenten zu beweisen und das Verständnis der Rolle dieser Organismen im Darm zu vertiefen. Wir haben getestet, ob das Wachstum dieser Stämme in Gegenwart von Formiat als Elektronendonator durch die Zugabe verschiedener potenzieller Elektronenakzeptoren (Sauerstoff, Fumarat, Aspartat, Sulfit, Sulfat, Thiosulfat und Nitrat) stimuliert wird. Es wurde beobachtet, dass die Zugabe von Formiat und Fumarat in Kombination das Wachstum dieser Kulturen sowie deren Succinatproduktion stimulierte, was auf eine Fumaratreduktion hinweist. Außerdem scheint sich in Gegenwart von Sulfit und Sulfat über die Zeit vermehrt Schwefelwasserstoff im Medium anzureichern, was eher auf den Abbau von Cystein als auf die Aktivität von MccA hindeutet.

Abstract

Sutterellaceae, a family of widespread gut anaerobes, first identified almost 30 years ago, have seen many species discovered over the past decade with the rise of metagenomics. They are found to be associated with several human health conditions, such as ulcerative colitis, inflammatory bowel disease, autism or a healthy state. They remain understudied as little is known about their metabolism and effect on the gastrointestinal tract. An underlying re-analysis of publicly available metagenomic and metatranscriptomic datasets of faecal samples from different human cohorts, conducted in our lab, showed high expression of microbial genes for use of formate and hydrogen as electron donors, fumarate reduction via highly expressed fumarate reductase and presumable sulphite reduction via the octahaem cytochrome-c sulphite reductase MccA. The aim of this work was to experimentally prove these physiological capacities encoded in the *Sutterella wadsworthensis*, *Sutterella parvirubra*, and *Turicimonas muris* genomes via a series of pure culture growth experiments and further deepen the understanding of the role of these organisms in the gut. We tested if in the presence of formate as an electron donor, the growth of these strains is stimulated by the addition of various potential electron acceptors (oxygen, fumarate, aspartate, sulphite, sulphate, thiosulphate and nitrate). It was observed that the addition of formate and fumarate combined stimulated the growth of these cultures as well as their succinate production indicating fumarate reduction, and that in the presence of sulphite and sulphate, hydrogen sulphide seems to accumulate more over time in the media suggesting cysteine degradation rather than MccA's activity.

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Introduction

Sutterella spp. are a genus of Gram-negative bacteria from the phylum Pseudomonadota often associated with the gastrointestinal tract. They were first identified in 1996, with the isolation of *Sutterella wadsworthensis* from human appendiceal and peritoneal fluids from patients with a variety of gastrointestinal diseases. *S. wadsworthensis* was distinguished from *Campylobacter gracilis* due to its ability to resist human bile acid and to reduce triphenyl tetrazolium chloride only in anaerobic conditions. This new genus was classified as part of the *Alcaligenaceae* (Wexler et al., 1996). *S. wadsworthensis* remained the only bacteria known to belong to the genus *Sutterella* until 2004, when a strain later named *Sutterella stercoricanis* was isolated from the faeces of a labrador dog, presenting very similar particularities. *S. stercoricanis* also presented the highest similarity (94.7%) to the 16S rRNA gene sequence of *S. wadsworthensis* but was shown to constitute a different species (Greetham et al., 2004). In 2008, *Sutterella parvirubra* was isolated from the faeces of three healthy Japanese men, also displaying 94.3% similarity to the 16S rRNA of *S. wadsworthensis* and 94.9% similarity to *S. stercoricanis* (Sakon et al., 2008). *Parasutterella excrementihominis* was then isolated from human faeces but phylogenetically represented a new genus distinct from *Sutterella* (Nagai et al., 2009). In 2011, with the discovery of *Parasutterella secunda*, isolated from human faeces and displaying 90% sequence similarity to *P. excrementihominis* was suggested to form a new family for the members of *Sutterella* and *Parasutterella*: the *Sutterellaceae* (Morotomi et al., 2011). Many other species have since then been discovered through culturomics of stool samples and gut samples such as *Sutterella massiliensis* (which to the difference of most *Sutterellaceae* displays motility), *Sutterella timonensis* (Ndongo et al., 2017), *Sutterella seckii* (Dione et al., 2018), *Sutterella megalosphaeroides* as well as *Mesosutterella multiformis*, a new genus phylogenetically classified in between *Sutterella* and *Parasutterella* (Sakamoto et al., 2018). Another genus, *Turicimonas* was also discovered, containing so far one species, *Turicimonas muris* isolated from the murine gut, whose closest phylogenetic relative is *Parasutterella excrementihominis* (Lagkouvardos et al., 2016).

According to one study, *Sutterellaceae* members are reported to be mostly prevalent in the duodenum (about 10% but they can go up to 20%); then their concentration decreases along the gastrointestinal tract to reach about 1% in the rectum (Hiippala et al., 2018). It was later discovered that members of the *Sutterellaceae* family could also be found outside the gut. Molitoris et al shortly after the isolation of *S. wadsworthensis* reported its presence in varied severe complications such as brain or liver abscesses and bacteremia (Molitoris et al., 1997). *Sutterella* and *Parasutterella* have also been found more frequently in the urines of pregnant individuals who delivered their baby prematurely, prior to 37 weeks, possibly by contamination due to the proximity of the rectum (Ollberding et al., 2016). A new species that was also phylogenetically classified as *Sutterellaceae* was found in the female reproductive tract, with the discovery of *Dakarella massiliensis* isolated from a woman with bacterial vaginosis (Dione et al., 2017).

Since their discovery, *Sutterellaceae* have been reported to be implicated in several conditions, mostly gastro-enterical. A study from 2012 demonstrated that dogs with acute diarrhoea had a significant increase of *Sutterella* spp. in their faeces compared to healthy dogs (Suchodolski et al., 2012). A study from 2004 also discovered the presence of a *Sutterella* not yet identified (whose the closest relative is *Sutterella wadsworthensis*) present in unusual quantities in the gastrointestinal tract of a patient with one of two types of IBD – Crohn disease among other phylogenetic groups (Mangin et al., 2004). *Sutterella* spp. are also incriminated in correlating with the autism spectrum disorder where a study demonstrates their presence in increased amounts, along with *Ruminococcus torques* in gastrointestinal biopsies and faeces of children with autism (Wang et al., 2013). Increased levels of *Sutterella* spp. were also observed in patients with Down syndrome likely due to premature ageing leading to decreased quality of the gut microbiota (Biagi et al., 2014). There were also reports of bacteremia with *S. wadsworthensis* after intra-abdominal surgery (Kirk et al., 2021). Other studies claim that *Sutterella* spp., despite having pro-inflammatory properties, cannot be considered the cause of IBD. For example, in a study by Engberg et al., it was shown that *S. wadsworthensis* after isolation from the faeces was more prevalent in the healthy cohort (0.93%) than in the IBD cohort (Engberg et al., 2000). The result is however considered inconclusive by the authors due to the much smaller size of the healthy cohort (107 compared to 1483 clinical samples) (Engberg et al., 2000). A later study based on an optimised PCR assay with specific *S. wadsworthensis* primers demonstrated that this bacterium is present with insignificant differences between individuals with IBD and healthy individuals in mucosal colonic isolates samples (Mukhopadhyay et al., 2011). It was also demonstrated that the levels of *Sutterella* spp. were similar in healthy children with coeliac disease or inflammatory bowel disease (Hiippala et al., 2018). The same study shows that *S. wadsworthensis* adheres to extracellular matrix proteins and mucus indicating that at least some *Sutterella* species might be commensals in the human gut.

Despite being widely associated with the gut microbiome of mammals, little is known yet about the *Sutterellaceae* core metabolism. In the gut where the environment is on a greater length microaerophilic or anaerobic, many bacteria use various available electron acceptors through the reductases. A recent study found that *Pseudomonadota*, including *Sutterella wadsworthensis*, encode a uniquely high amount of flavin and molybdopterin reductases; *Sutterella wadsworthensis* is suspected to be able to respire electron acceptors such as DMSO, shikimate, aspartate, malate and fumarate as all these metabolites stimulated its growth rate in the incubation experiments carried out (Little et al., 2024).

Recent metagenomic and metatranscriptomic analysis of faecal samples from diverse human cohorts was reanalysed by a former member of our group Dr. Kerim Dimitri Kits, with a special focus on *Sutterella wadsworthensis*. He observed a similar trend – high expression of some reductase in *Sutterellaceae* in the gut. All metagenomes and metatranscriptomes for this preliminary study originated from two datasets: 367 paired metagenomes and metatranscriptomes from 308 adult men belonging to the Men's Lifestyle Validation Study (MLVS) (Abu-Ali et al., 2018) and 735 paired metagenomes/metatranscriptomes from a

gender-balanced cohort comprised of 117 patients on the dynamics of meta transcription in inflammatory bowel disease (IBD) (Schirmer et al., 2018). The MLVS cohort was grouped along with the non-IBD cohort for the analysis. The final dataset contained metagenomic and metatranscriptomic data from 1076 samples. This analysis showed that nitrate (NapAB), fumarate (FrdABC) and presumably sulfite reduction (MccABCD octahaem cytochrome-c sulfite reductase) enzymes are highly expressed by *S. wadsworthensis* in the gut. Also, from the higher levels of expression of the formate dehydrogenase (FrdABC) and respiratory H₂ uptake hydrogenase (HyaABC), it is suspected that formate and hydrogen are fueling several reactions as electron donors.

Even though formate was identified as a widely used electron donor by microbes a while ago (e.g. *Vibrio succinogenes* (now known as *Wolinella succinogenes*)) (Kröger, 1978), and can be synthesised through various nutrients and foods, for example in the mice plasma, 50% of formate is produced from serine (Meiser et al., 2016), but also from lactate (Pham et al., 2017), from methanol after alcohol consumption that is turned into formaldehyde and converted into formate (Dorokhov et al., 2015). A study demonstrated that formate is produced by the gut microbiota during inflammation and that it can contribute to the proliferation of *Escherichia coli* when a dysbiosis caused by inflammation induced with dextran sodium sulphate (Hughes et al., 2017). The same study also showed that the formate concentration was increased in the gut lumen of the mice treated with dextran sodium sulphate compared to mice treated with placebo. Another important electron donor present in the gastrointestinal tract is hydrogen. It acts as an electron sink and fuels reduction reactions (Gibson et al., 1993). It is used mostly by sulphate-reducing bacteria, methanogens and acetogens (Doré et al., 1995).

Based on the D. Kit's data, the level of the putative cytochrome-c sulfite reductase *mccA* transcription was the highest among other microorganisms in the gut. Therefore, we hypothesise that *S. wadsworthensis* might be able to reduce sulfite and produce hydrogen sulphide as an end product of that reaction. Hydrogen sulphide is widely present and highly relevant in the gastrointestinal tract (Suarez et al., 1997). It can affect the intestinal tract differently, however in high concentrations H₂S facilitates the invasion of bacteria into the epithelium by degrading the protective mucus layer and causing inflammation (Ijssennagger et al., 2015). Moreover, hydrogen sulphide is a cytotoxic compound which causes DNA damage and inhibits the cytochrome c oxidase and butyrate oxidation in colonic epithelial cells (Singh et al., 2015). Other studies suggest that H₂S could act as a gasotransmitter (Gadalla & Snyder, 2010). The discovery in the hippocampus of cystathionine beta-synthase responsible for the production of hydrogen sulphide suggests that it is produced endogenously in the hippocampus and could act as a neuromodulator by acting on NDMA receptors (Abe & Kimura, 1996). Although the level of H₂S in the intestine is physiologically crucial, the largest producer(s) (strains or higher taxa) of sulphide in the gut are still unknown. The biggest known producers of hydrogen sulphide in the gastrointestinal tract so far are sulphate-reducing bacteria. Multiple species of bacteria performing the sulphate reduction were discovered in faeces such as *Desulfovibrio desulfuricans* which performs

dissimilatory sulphate reduction by first reducing sulphate into sulphite (depending on ATP) as an intermediate product and then sulphite into hydrogen sulphide (Ishimoto, 1959; Peck, 1959). A similar system was also found in the widespread gut bacteria *Bilophila wadsworthia*, which is not able to perform sulphate reduction but can reduce the sulphite produced via taurine degradation into hydrogen sulphide with the action of desulfoviridin (Laue et al., 1997), a dissimilatory sulphite reductase which in *Bilophila wadsworthia* contains a domain that has high homology with DsrB and DsrD (Laue et al., 2001). Here we interest ourselves in the cytochrome-c sulphite reductases such as encoded by the *mccABCD* gene cluster (Hartshorne et al., 2006) as an alternative pathway to contribute to the hydrogen sulphide production in the gut. A multi-heme cytochrome c gene cluster was first identified in *Wolinella succinogenes*. *Wolinella succinogenes* belongs to the *Epsilonproteobacteria*, just as *Helicobacter pylori* or *Campylobacter jejuni* and has been widely studied due to their high similarities in terms of virulence genes (Vandamme et al., 1991). It was first identified as part of the *Vibrio* genus and was isolated from the rumen fluid of bovines. Presumably similar to our strain of interest, *W. succinogenes* uses formate and dihydrogen as electron donors, and fumarate, malate and nitrate as electron acceptors. It can also grow in the presence of oxygen up to 2% (Wolin et al., 1961). However the most interesting is that *W. succinogenes* was demonstrated to possess a gene cluster *mccABCD* which encodes a respiratory sulphite reductase. It provides them with the advantage of growing and gaining energy from sulphite when there are no other electron acceptors available (Kern et al., 2011). The enzyme complex *mccABCD* consist of several subunits: MccA acting as a octahaem respiratory sulphite reductase located in the periplasm enabling sulphite respiration in *W. succinogenes*, MccC and MccD are thought to be iron-sulphur proteins involved in the transport of electrons from quinone to MccA and finally MccB's structure has been described as a peptidyl-propyl cis-trans-isomerase but its function remains unclear (Hartshorne et al., 2006; Hartshorne et al., 2007; Kern et al., 2011). Five years later, SirA, another sulphite reductase, part of the MccA family was discovered in *Shewanella oneidensis* MR-1, also part of the *Pseudomonadota* phylum, such as *Sutterellaceae* but that is quite specific to the *Shewanella* genus so far and not observed in the gastro-intestinal tract (Shirodkar et al., 2011).

Sulfite reduction and taurine degradation are not the only potential sources of hydrogen sulphide in the gut. Another important source of hydrogen sulphide production in the gastro-intestinal tract as well as in other tissues is the degradation of L-cysteine. As much as we, as humans, possess genes for the degradation of L-cysteine into hydrogen sulphide as mentioned above with the discovery of cystathionine beta-synthase in the hippocampus (Abe & Kimura, 1996), the gut microbiota is also suspected to play a huge part in this. According to one study, the ability to produce hydrogen sulphide from cysteine is a common feature in the human microbiome (Braccia et al., 2021). By looking into the Unified Human Gastrointestinal Genome data for hydrogen sulphide-producing enzymes, we observe that some members of *Sutterellaceae* possess one primary cysteine-degrading gene

(*yhaM*), as well as several secondary cysteine degrading genes (*malY*, *metC*, *cysK*, *cysM* and *mccB*) (Braccia et al., 2021).

Another important type of respiration for *Sutterella wadsworthensis* is through fumarate reduction which was recently reported (Little et al., 2024). The fumarate reduction gene cluster (*frdABCD*) encodes multiple subunits such as FrdA which is a flavoprotein catalytic subunit (Weiner et al., 1979), FrdB which is also a catalytic subunit containing a three iron-sulphur sites (Morningstar et al., 1985) and FrdC and FrdD which are both transmembrane proteins used as anchors for FrdA and FrdB (Lemire et al., 1982). We hypothesise that *Sutterella wadsworthensis* also has the ability to reduce or respire fumarate, a capacity that is seen in other members of the gastrointestinal tract, such as in *Salmonella typhimurium* and *Escherichia coli*. In *E. coli*, fumarate reductase was widely described to be activated under anaerobic conditions (Lambden et al., 1976; Weiner et al., 1979; Weiner et al., 1986). In turn, *S. typhimurium* also possesses functioning fumarate reductase and transporter DcuABC, which imports malate and aspartate (subsequently turned into fumarate) inside the cell in exchange for succinate (Nguyen et al., 2020). This process fuels *S. typhimurium* metabolism and allows it to establish an early colonisation of the mouse gut. Aspartate and malate are turned into fumarate via aspartate ammonia lyase (AspA) and TCA cycle enzyme fumarase (FumABC) (Nguyen et al., 2020). A similar system was also uncovered in *Escherichia coli* K-12 where the presence of aspartate in the mice gut lumen stimulated the expression of fumarate reduction genes and promoted better colonisation of the gut lumen (Schubert et al., 2021). It therefore seems that the ability to perform fumarate reduction improves fitness to colonise the gut lumen by pathogens.

To further understand the metabolism of *Sutterellaceae*, we focused our work on the investigation of the utilisation of fumarate, as well as some other electron acceptors, namely aspartate, thiosulphate, sulphite, nitrate and sulphate, which are suspected to be utilised by *Sutterellaceae* members. We studied their impact on the growth of our strains, as well as the consequent production of succinate and malate from fumarate and the possible hydrogen sulphide production indicating activity from MccA. In order to discover more about the physiology of *Sutterellaceae* members, the conducted research was focused on a few members of this family: *Sutterella parvirubra*, *Sutterella wadsworthensis*, *Turicimonas muris* and up to a certain extent *Parasutterella excrementihominis*.

First, to test the utilisation of some chosen electron acceptors which all have a likelihood of being present in the gastrointestinal tract, such as aspartate, fumarate, sulphite and nitrate, as well as thiosulphate and sulphate. Aspartate as an essential amino acid is widely present in the gut released via protein degradation. Nitrate can be found in water (McRae, 1909), and in root vegetables like turnips or beets (Kilgore et al., 1963). Sulphite can be produced as part of the catabolism of methionine and cysteine before being oxidised into sulphate (Forsberg, 1979; Stipanuk & Ueki (2011). Sulphite can also be ingested due to its use as a preservative agent in the food industry (Chichester et al., 1972). Thiosulphate can be produced from mitochondrial sulphide oxidation (Hildebrandt et al., 2008). We also used formate as an electron donor to fuel the reactions and hydrogen which is also a known

electron donor for *S. wadsworthensis* was present in the atmosphere of the anaerobic tent in which the tests were conducted. To test for this we monitored the growth of our bacterial strains, *S. parvirubra* and *S. wadsworthensis*, in presence of all the different electron acceptors and quantified the hydrogen sulphide production over time with Cline assay (Cline, 1969).

A test to check the influence of formate and fumarate at various concentrations (5 mM and 50 mM) in presence of 1% oxygen on growth of selected *Sutterella* spp. was also conducted in a microaerophilic environment. Succinate production over time was quantified with capillary electrophoresis. A similar test was conducted prior to this research in anaerobic conditions and this new experiment in a hypoxic environment was to observe the impact of 1% oxygen on the growth of *S. parvirubra*, *S. wadsworthensis* and *T. muris*.

Finally, in order to investigate the potential of *Sutterellaceae* members possessing Dimethylsulfoxide A reductase (DmsA) (in this case, *Sutterella parvirubra* and *Turicimonas muris*) as well as Tetrathionate reductase A (TtrA) (possessed by *Parasutterella excrementihominis*), an incubation experiment was performed. This experiment was carried out in presence of various level of DMSO and serves as a preliminary study regarding the possibility of our strains to perform DMSO reduction. No incubation experiment was done to assess the ability of *Parasutterella excrementihominis* to reduce tetrathionate due to the complexity of growing this strain in liquid media.

Materials and methods

Gifu Anaerobic Medium preparation

Were dissolved for each litre of ddH₂O 59.00 grammes of GAM Broth from HIMEDIA according to the manufacturer's instructions. The pH was then adjusted to 6.0-7.0 and autoclaved (121°C, 15 minutes). Media was stored after autoclaving at +4°C. Before use, the media would be poured in Hungate tubes under the laminar flow and introduced into the tent 24 hours before inoculation to purge the dioxygen present into the media.

Anaerobic *Akkermansia* Medium media preparation

Anaerobic *Akkermansia* medium as described by Brugiroux et al., 2016 in the protocol provided by Bärbel Stecher from Max von Petterkofen Institut, was prepared for the inoculation of *Turicimonas muris*. 1 L was prepared using 18,5 g of Brain Heart Infusion (Oxoid/Fischer), 15 g of Trypticase soy broth (Oxoid/Fischer), 5 g of yeast extract (Oxoid), 2,5 g of K₂HPO₄ (Carl Roth), 1 mg of Hemin (Sigma Aldrich), 0,5 mg of D-glucose (Sigma Aldrich) were added and the bottle was filled up to 1 litre with distilled H₂O. The bottle was stirred until all the compounds dissolved. Then 1.5% of agar (Thermo Fischer Scientific) was added and the bottles were autoclaved (121°C, 15 minutes). The medium was split into several 100 mL bottles. After autoclaving, all the compounds that could not be autoclaved were added in every bottles under the laminar flow and after sterile filtration (0,22 µm): 1 mL Na₂CO₃ (5% stock solution (Carl Roth) (autoclaved)), 1 mL of L-cysteine hydrochloride (x100 stock

solution (Carl Roth)), 0,1 mL of menadione (x1000) stock solution (Sigma Aldrich), 3 mL of inactivated foetal bovine serum (x100 stock solution (Gibco)) and 1 mL of mucin from porcine stomach (2.5% in ddH₂O x100 stock solution (Sigma Aldrich) (autoclaved)). Media was stored after autoclaving at +4°C. Before use, the media would be poured in Hungate tubes under the laminar flow and introduced into the tent 24 hours before inoculation to purge the dioxygen present into the media.

Inoculation and incubation of anaerobic agar cultures

1.5% Agar (Thermo Fischer Scientific) plates were prepared with either Gifu Anaerobic Broth (GAM) (HIMEDIA) (for *S. parvirubra* and *S. wadsworthensis*) or Anaerobic *Akkermansia* Medium (for *T. muris*) the media was poured into plates after autoclaving (and adding the additional compounds needed in the case of AAM) in the laminar flow and left to dry for about 20 minutes. The dry plates were then introduced into the anaerobic vinyl glove box from CoyLabs with an atmosphere of 5% H₂, 10% CO₂ and 85% N₂ (gas level fluctuates because of catalysts which therefore lead to a H₂ concentration of between 1.6 and 2.5% inside the tent) and left inside the tent for at least 24 hours before inoculation to leave time for the plates to become anaerobic. The plates were then inoculated with bacterial stocks stored at -75°C in airtight bottles. Once thawed, between 50µL and 200µL of bacterial stock was withdrawn with a needle and a syringe inside the tent and spread over the plate with single-use plastic rattle for a duration of a few minutes (especially with higher stock volume to avoid leakage). The plates were then stored upside down in a bag at 37°C for a duration of between three to five days (usually three days, but if no huge sign of growth they would be kept a few days longer). The plates were removed from the incubator, sealed with parafilm and stored at 30°C inside the tent for up to three months.

Inoculation and incubation of anaerobic liquid cultures

Liquid inoculums were inoculated from agar cultures by withdrawing one colony from a plate with a single-use plastic loop (Thermo Fischer Scientific) and dispersing it in 10 mL liquid media (either Gifu Anaerobic Broth for *Sutterella parvirubra* and *Sutterella wadsworthensis* or Anaerobic *Akkermansia* Medium for *Turicimonas muris*). This inoculum was incubated for one or two days and used to further inoculate other liquid cultures. Anaerobic liquid cultures inoculated with 0,1mL of the inoculum culture of either *Sutterella parvirubra*, *Sutterella wadsworthensis* or *Turicimonas muris* grown in the hypoxic tent and were incubated in a vinyl glove box from CoyLabs containing 5% H₂ and 10% CO₂ and 85% N₂ at 37°C without shaking. Optical density (OD₆₀₀) was measured twice a day with Biochrom Ultraspec 10.

SL-10 micro-element solution from *Clostridium cellulovorans* medium 320 (DMSZ)

In a 1 litre bottle were dissolved 1.5 g iron(III) chloride tetrahydrate FeCl₂·4H₂O (Sigma Aldrich) in 10 mL of hydrogen chloride 25%. Then, 99.0mL H₂O were added. Were then added and dissolved 70 mg ZnCl₂ (from Carl Roth), 100 mg manganese(II) chloride

tetrahydrate $\geq 99\%$ $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (Carl Roth), 6 mg boric acid H_3BO_3 (from Carl Roth), 190.0 mg cobalt(II) chloride hexahydrate $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (Sigma Aldrich), 2 mg Copper(II) chloride dihydrate $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (Sigma Aldrich), 24 mg nickel(II) chloride hexahydrate $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (Sigma Aldrich) and 36 mg sodium molybdate dihydrate $\geq 99.5\%$ $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (Roth).

Preparation of the treatment groups for electron acceptor tests

In four hungate tubes (three for inoculation and one uninoculated as a negative control) were added by treatment group: 1) 10 mL Gifu medium; 2) 10 mL Gifu medium + SL10; 3) 10 mL Gifu medium + SL10 + sodium formate (Sigma Aldrich) 5 mM; 4) 9 mL Gifu medium + SL10 + sodium formate 5 mM + 1 mL di-sodium fumarate (Sigma Aldrich) 50 mM (Final concentration of 5 mM); 5) 9 mL Gifu medium + SL10 + sodium formate 5 mM + 1 mL L-aspartic acid sodium salt (Sigma Aldrich) 50 mM (Final concentration 5 mM); 6) 9,9 mL Gifu medium + SL10 + sodium formate 5 mM + 100 μL of sodium sulfite BioUltra anhydrous 98% (Sigma Aldrich) 50 mM (Final concentration 0,5 mM); 7) 9 mL Gifu medium + SL10 + sodium formate 5 mM + 1 mL sodium sulfite BioUltra anhydrous 50 mM (final concentration 5 mM), 8) 9,9 mL Gifu medium + SL10 + sodium formate 5 mM + 100 μL sodium thiosulfate ReagentPlus® 99% (Sigma Aldrich) 50 mM (Final concentration 0,5 mM); 9) 9 mL Gifu medium + SL10 + Formate 5 mM + 1 mL sodium thiosulphate ReagentPlus® 99% 50 mM (final concentration 5mM); 10) 9 mL Gifu medium + SL10 + sodium formate 5 mM + 1 mL sodium nitrate ACS reagent $\geq 99.0\%$ (Sigma Aldrich) 50 mM (final concentration 5 mM); 11) 9,5 mL Gifu medium + SL10 + Formate 5 mM + 500 μL sodium sulphate (Sigma Aldrich) (50 mM) (final concentration 2,5 mM).

Preparation of treatment groups for hypoxic (1% oxygen) and aerobic (21%) experiments

Four treatment groups with three replicates and one negative control were prepared for each strain. In all four hungate tubes for each treatment group following components have been added: 1) 10 mL of media (Gifu Anaerobic Broth (GAM) (HIMEDIA) for *Sutterella parvirubra* and *Sutterella wadsworthensis* and Anaerobic *Akkermansia* Medium (AAM) for *Turicimonas muris*); 2) 9,9 mL GAM or AAM + 100 μL sodium formate (Sigma Aldrich) 0,5 M (final concentration of 5 mM of sodium formate); 3) 9,8 mL GAM or AAM + 100 μL sodium formate 0,5M + 100 μL di-sodium fumarate (Sigma Aldrich) 0,5 M (final concentration of 5 mM of sodium formate and di-sodium fumarate); 4) 8 mL of GAM or AAM + 1 mL of 0,5 M of sodium formate + 1 mL of 0,5 M of di-sodium fumarate (final concentration of 50mM of sodium formate and di-sodium fumarate). For the hypoxic experiment, the tubes were left for 24 hours in the hypoxic tent before inoculation to become hypoxic. Three tubes for each treatment group were then inoculated with *Sutterella parvirubra*, *Sutterella wadsworthensis* and *Turicimonas muris* and the non-inoculated tube per treatment group acted as a negative control.

Inoculation and incubation of hypoxic liquid cultures

To introduce the cultures of *Sutterella parvirubra*, *Sutterella wadsworthensis* and *Turicimonas muris*, we grew them first in the anaerobic tent (100 µL of inoculum in 10 mL media (either GAM for *Sutterella wadsworthensis* and *Sutterella parvirubra* and AAM for *Turicimonas muris*)) for three days and then introduced them in the hypoxic tent after checking the identity of the cultures by sending the PCR product (using the 16s rRNA primers 1492r and 616v) to microsynth. Tubes of fresh media (GAM for *Sutterella parvirubra* and *Sutterella wadsworthensis* and AAM for *Turicimonas muris*) were introduced a few days before and were inoculated with the anaerobic cultures (only one tube of each). Hypoxic cultures for the hypoxic incubation experiment in presence of sodium formate and di-sodium fumarate were inoculated the next day in triplicates with 0,1mL of an overnight culture of either *Sutterella parvirubra*, *Sutterella wadsworthensis* or *Turicimonas muris* grown inside of the hypoxic tent in 10 mL of fresh media and were incubated in a vinyl glove box from CoyLabs containing 1% O₂ and 99% N₂ at 37°C without shaking. Optical density (OD600) was measured twice a day using Biochrom Ultrospec 10 and sampled once a day for capillary electrophoresis. The samples were stored at -20°C.

Preparation of tubes for testing the effect of dimethylsulfoxide (DMSO) on growth

For each strain (*Sutterella parvirubra* and *Turicimonas muris*) five treatment groups were prepared. For each treatment group were prepared four Hungate tubes (three replicates and one uninoculated tube to serve as a negative control) with either: 1) 5 mL Media (Gifu Anaerobic Medium (GAM) or Anaerobic *Akkermansia* Medium (AAM)); 2) 4.950 mL Media + 50 µL sodium formate 0.5 M (final concentration of 5mM); 3) 4,900 mL Media + 50 µL sodium formate 0.5 M + 50 µL dimethylsulfoxide (DMSO) from Roth (final concentration of 5 mM Formate and 0.5 mM DMSO), 4)4.900 mL media + 50 µm sodium formate 0.5 M + 50 µm DMSO 0.5 M (final concentration 5 mM of sodium formate and 5 mM of DMSO); 5) 4,450 mL media + 50 µL sodium formate 0.5 M + 500 µL DMSO 0.5 M (final concentration 5 mM sodium formate and 50 mM DMSO).

DNA extraction of cultures for identification check of cultures

DNA extraction was performed according to the protocol from Walsh et al., 1991. 100 µL of liquid culture was sampled into an Eppendorf tube or selected colonies from plates were sampled with single-use plastic loops (Thermo Fisher Scientific) and resuspended in 100 µL of ddH₂O. The tubes were centrifuged at 13,400 rpm for three minutes, the supernatant was discarded and the cultures were resuspended in 100 µL ddH₂O with 0,005 g Chelex from Bio-Rad as described by Jung et al., 1991. The bacteria in 5% Chelex were boiled at 100°C for 100 minutes in ThermoBlock and centrifuged for two minutes. The supernatants containing the extracted DNA were then transferred into new Eppendorf tubes and stored at -20°C. The extracted DNA was then analysed with nanodrop to check for the concentration and purity of the extraction.

16S rRNA gene PCR reactions for identification check of cultures

The bacterial cultures were regularly submitted for identification checks. The MasterMix was prepared as follows: for 1 PCR reaction were mixed 5 μ L Taq Polymerase Buffer, 5 μ L 2 μ M dNTPs, 5 μ L 10 pm 616v primer, 5 μ L 10 pm 1462r primer, 0,5 μ L Bovine Seralbumine, 0,5 μ L Taq Polymerase, 28 μ L of RNase-free H₂O and 1 μ L of extracted DNA. The PCR cycle was set up as follows: 1. 95°C, 3:00; 2. 95°C, 0:30; 3. 52°C, 0:30; 4. 72°C 1:30, 5.repeat step 2 to 4 30X; 6. 72°C, 10:00; 7. 4°C, ∞ . After every 16S rRNA gene PCR, the PCR products were checked on a 1% agarose gel electrophoresis. To the gel, was added 0.8 μ L of GelRed for a small gel (0.5 g agarose in 50 mL 1X TBE) or 1.6 μ L for a big gel (1 g agarose in 100 mL 1X TBE). The gels were run for approximately 45 minutes at 100 V. They were visualised with GelDoc for the presence of the 16S rRNA gene. When a PCR product was observable, the leftover PCR reactions were sent to Microsynth for Sanger sequencing followed by a nucleotide BLAST analysis for identification check and an ApE analysis to check for the purity of the sample.

Preparation of stock solutions of bacterial strains

In 0,5 mL 60% glycerol stock was added 1 mL of overnight bacterial culture to make a 20% glycerol stock inside the anaerobic tent. The stocks were then flash-frozen in liquid N₂ for about two minutes and stored at -75°C.

Quantification of hydrogen sulphide with Cline assay (Cline, 1969)

A dilution series was prepared under anaerobic conditions of 5 mM, 2.5 mM, 1.25 mM, 0.625 mM, 0.3125 mM, 0.156 mM, 0.078 mM and 0 mM from a stock solution of 5 mM sodium sulphide Na₂S from Sigma Aldrich by always diluting by half from the most concentrated solution into MilliQ water until reaching the lowest concentration of the series to create a standard curve as a mean to determine the concentration of hydrogen sulphide in our cultures. 20 μ L of each tube of the dilution series were introduced under the anaerobic tent because of the toxic, volatile and oxidisation-prone nature of Na₂S into a fixative made from 200 μ L of MilliQ ddH₂O and 100 μ L zinc acetate (preparation of zinc acetate solution: 2.393 grammes of zinc acetate dihydrate Zn[OOCCH₃]₂·2H₂O (Sigma Aldrich), 100 mL ddH₂O, 2 mL acetic acid ROTIPURAN 100% (Carl Roth)). The tubes were then removed from the tent and under the hood were added 100 μ L ADMA solution (preparation of ADMA solution: 4 grammes of NN-dimethyl-p-phenylenediamine dihydrochloride (Thermo Fischer Scientific), 200 mL ddH₂O, 200 mL sulphuric acid H₂SO₄ (Sigma Aldrich) and then add ddH₂O to fill up to 1 L), 100 μ L Fe(III) solution (preparation of the Fe(III) solution: 2.29 g ammonium iron(III) sulphate dodecahydrate NH₄Fe(SO₄)₂·12H₂O (Sigma Aldrich), 10 mL ddH₂O, 250 μ L sulphuric acid H₂SO₄ (Sigma Aldrich), and then add water up to 250 mL) and 400 μ L MilliQ H₂O. The tubes were left to incubate for 30 minutes in the dark and then analysed with a Tecan plate reader M2000Pro with as settings five seconds shaking and measurement at 670 nm. This was done following the protocol stated by Cline in Spectrophotometric Determination of Hydrogen Sulfide in Natural Waters (Cline,

1969). The association of zinc sulphide, Fe(III) and N,N-dimethyl-p-phenylenediamine triggers a blue colouration that is visible at 670 nm. For the sample preparation, 0.1mL of culture was added to the 300mL zinc acetate fixative under the anaerobic tent. Then the protocol was followed the same way as for the standard curve up until the measurement with the Tecan plate reader.

Capillary electrophoresis analysis

500 μ L of bacterial cultures were sampled for capillary electrophoresis and frozen at -20°C . For analysis, the tubes were thawed and centrifuged for 15 minutes at 13,400 rpm. In capillary electrophoresis tubes, 1 μ L of supernatant from bacterial cultures was diluted in 99 μ L of ddH₂O. Acidic buffer pH 5.6 from Agilent Technologies was used for washing the capillary in between every 10 samples. Standards were prepared for L-aspartic acid sodium salts (Sigma Aldrich), sodium formate (Sigma Aldrich), di-sodium fumarate (Sigma Aldrich), DL malic acid disodium salt (Sigma Aldrich) and sodium succinate dibasic purum (Sigma Aldrich) at concentration 0.01, 0.05, 0.1, 0.5, 1 and 2 mM and 100 μ L ddH₂O for the negative control. Capillary electrophoresis (Agilent Technologies) was set up to take two injections from the first sample and one injection from every other sample. The concentration gradient of each metabolite was used to determine the retention time of the metabolites, and to establish a standard curve from the area under the curve data and to calculate the equation of the linear curve. For every sample then was noted the retention time and the area under the curve and the concentration of each metabolite were determined using the equation acquired from the standards for each metabolite.

Statistic tests

Statistics for growth data were performed first by determining the growth rate of cultures with R (R Core team, 2023) and R package “growthcurver” according to the basic instructions on the user’s guide provided by R (developed by Sprouffske & Wagner, 2016). The growth rate of each replicate was determined and a table was made with all the empirical areas under the curve per treatment group. A one-way ANOVA was then performed on the empirical areas under the curve table with a Bonferroni correction. In case of significant difference, a Tukey HSD *post-hoc* test was performed. Growthcurver does not always fit data properly: when no growth is observed, a fitting is not always possible, and in this case, the data was kept because nothing is observed, however sometimes, it does not fit data for which growth was observed, and in these case those values were omitted not to impact the data analysis.

Statistics for quantification of hydrogen sulphide, formate, malate and succinate were performed using one-way ANOVA ($\alpha = 0.05$) on the difference between the replicates from the last time point and the first time point of data of each quantification experiment. A Bonferroni correction was also calculated due to the high number of treatment groups. Finally, when the p-value obtained with the one-way ANOVA is lower than the p-value

calculated with the Bonferroni correction, a post-hoc Tukey HSD *post-hoc* test was performed. These tests were performed using the software RStudio.

Plots

All figures were plotted in R language (R Core team, 2023) with the software R studio using the ggplot2 package (Wickham et al., 2016). For the double-Y-axes figures, both axes were plotted separately but made to be the same size to be perfectly superimposable and the plots were merged using the GNU Image Manipulation Program. All text that was present twice (titles, number labels on the X axis and X axis title) were erased from one of the plots to remove the shadowing effect.

Gene table

Gene table was created by using KEGG and UniProt databases to retrieve protein sequences either from KEGG if the gene was confirmed present in *Sutterella wadsworthensis* or to browse UniProt in KEGG and blasting the protein FASTA sequences either from *Sutterella wadsworthensis*, *Sutterella parvirubra*, *Turicimonas muris*, *Parasutterella excrementihominis* or a related member in the *Sutterellaceae* family if none were found. If well-characterised genes could not be found, they were blasted against the *Escherichia coli* K-12 protein sequence. An exception was made for MccA which was blasted against the protein sequence of MccA from *Wolinella succinogenes*. From the blast results, only hits with e-values under 1e-30 were considered present.

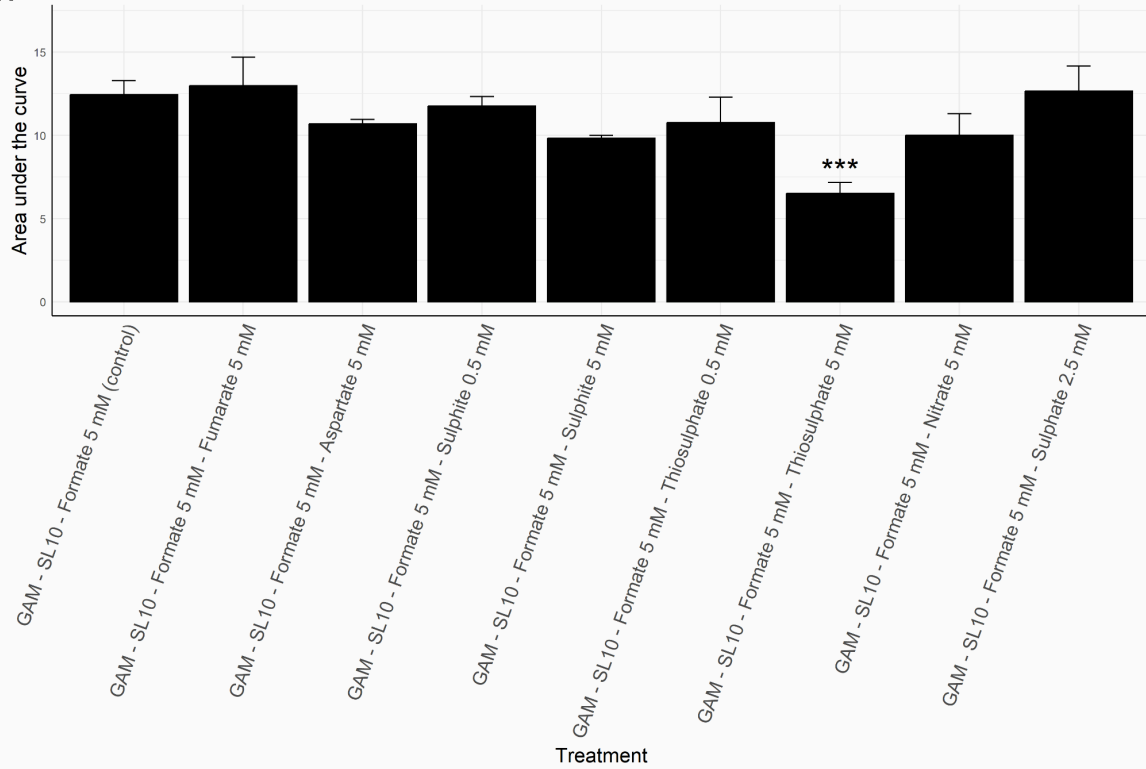
Results

Electron acceptor tests

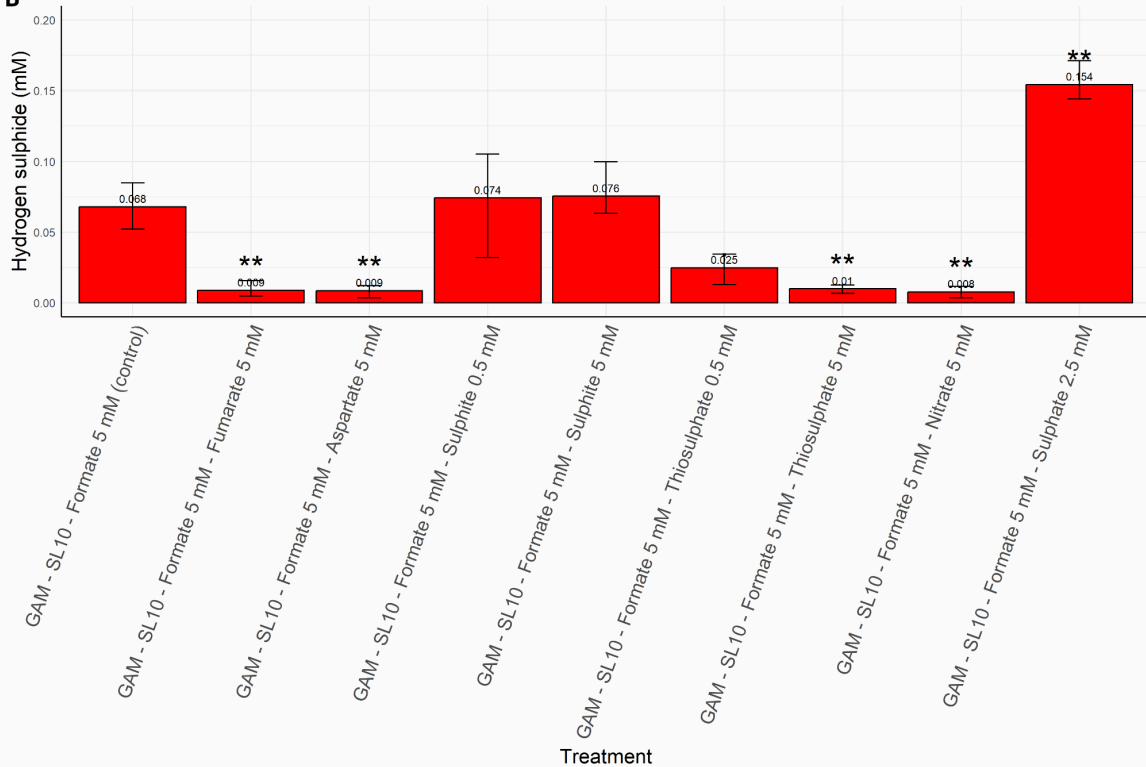
We set up two tests featuring electron acceptor that can be utilised by both *S. parvirubra* and *S. wadsworthensis* according to the genome analysis. All featured various electron acceptors got the potential to be present in the gut, namely: fumarate (5 mM), aspartate (5 mM), sulphite (0.5 and 5 mM), thiosulphate (0.5 and 5 mM), nitrate (5 mM) and sulphate (2.5 mM). In addition to 5 mM formate and the chosen electron acceptors, SL10 micro-element solution was added to potentially stimulate the activity of copper-dependent cytochrome-c sulfite reductase MccA (Hermann et al., 2015). We monitored the growth over time as well as quantified the hydrogen sulphide levels in the media.

<i>Sutterella wadsworthensis</i>

A Growth



B Hydrogen sulphide production over time



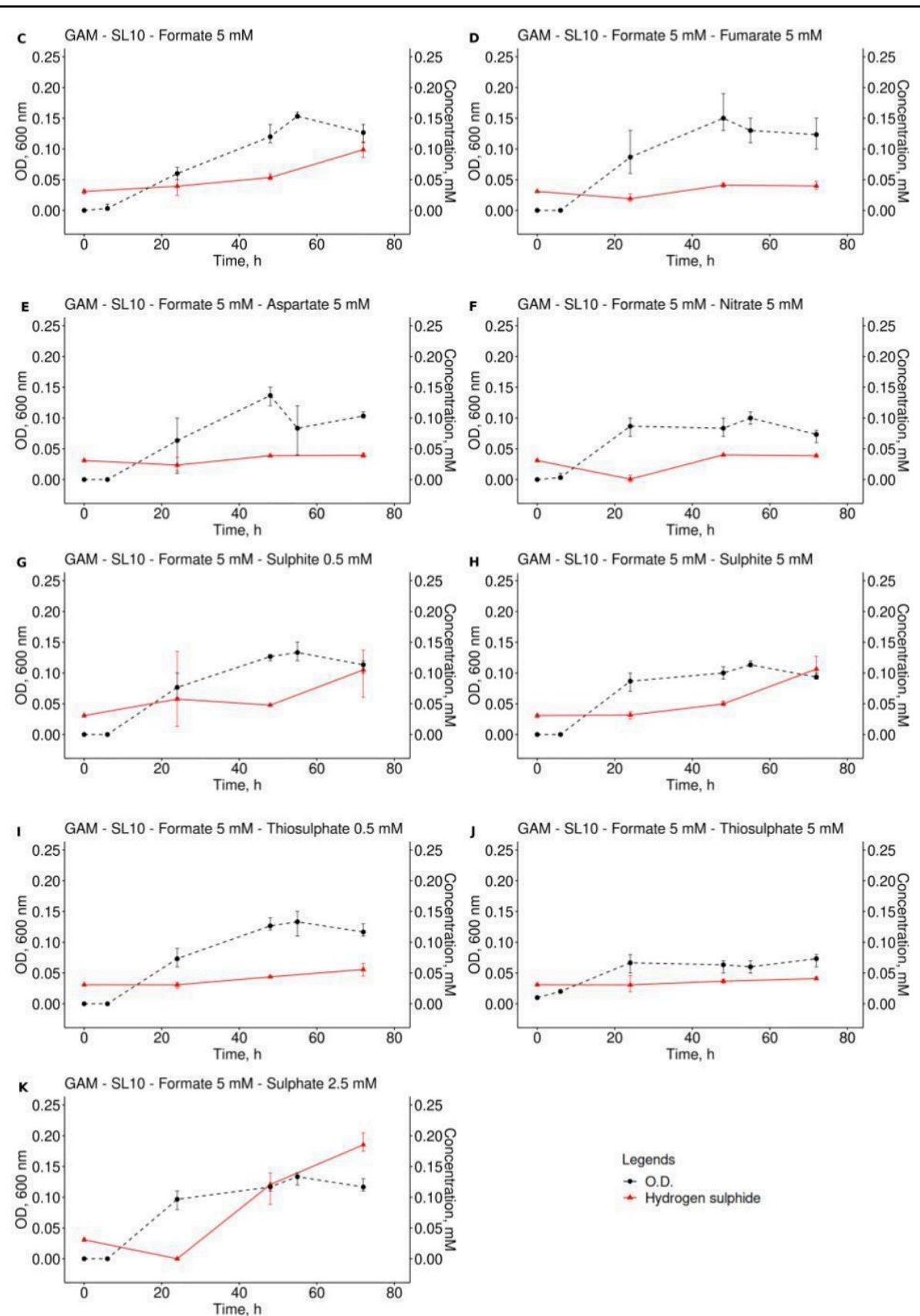


Figure 1 | *Sutterella wadsworthensis* - Growth and hydrogen sulphide concentration over time in presence of various electron acceptors. From left to

right and up to down: **A:** Growth represented by the empirical area under the curve using treatment group GAM - SL10 - Formate 5 mM as a control (Significance code: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1); **B:** Hydrogen sulphide production over time represented by the difference of hydrogen sulphide quantity of the last timepoint and of the first timepoint using GAM - SL10 - Formate 5 mM as a control (Significance code: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1). Average hydrogen sulphide production per treatment group is shown above its respective bar; **C:** GAM - SL10 - Formate 5 mM; **D:** GAM - SL10 - Formate 5 mM - Fumarate 5 mM; **E:** GAM - SL10 - Formate 5 mM - Aspartate 5 mM; **F:** GAM - SL10 - Formate 5 mM - Sulfite 0.5 mM; **G:** GAM - SL10 - Formate 5 mM - Sulfite 5 mM; **H:** GAM - SL10 - Formate 5 mM - Thiosulfate 0.5 mM; **I:** GAM - SL10 - Formate 5 mM - Thiosulfate 5 mM; **J:** GAM - SL10 - Formate 5 mM - Nitrate 5 mM; **K:** GAM - SL10 - Formate 5 mM - Sulphate 2.5 mM. On each graph, in black, dotted is represented the growth and in red is represented the production of hydrogen sulphide.

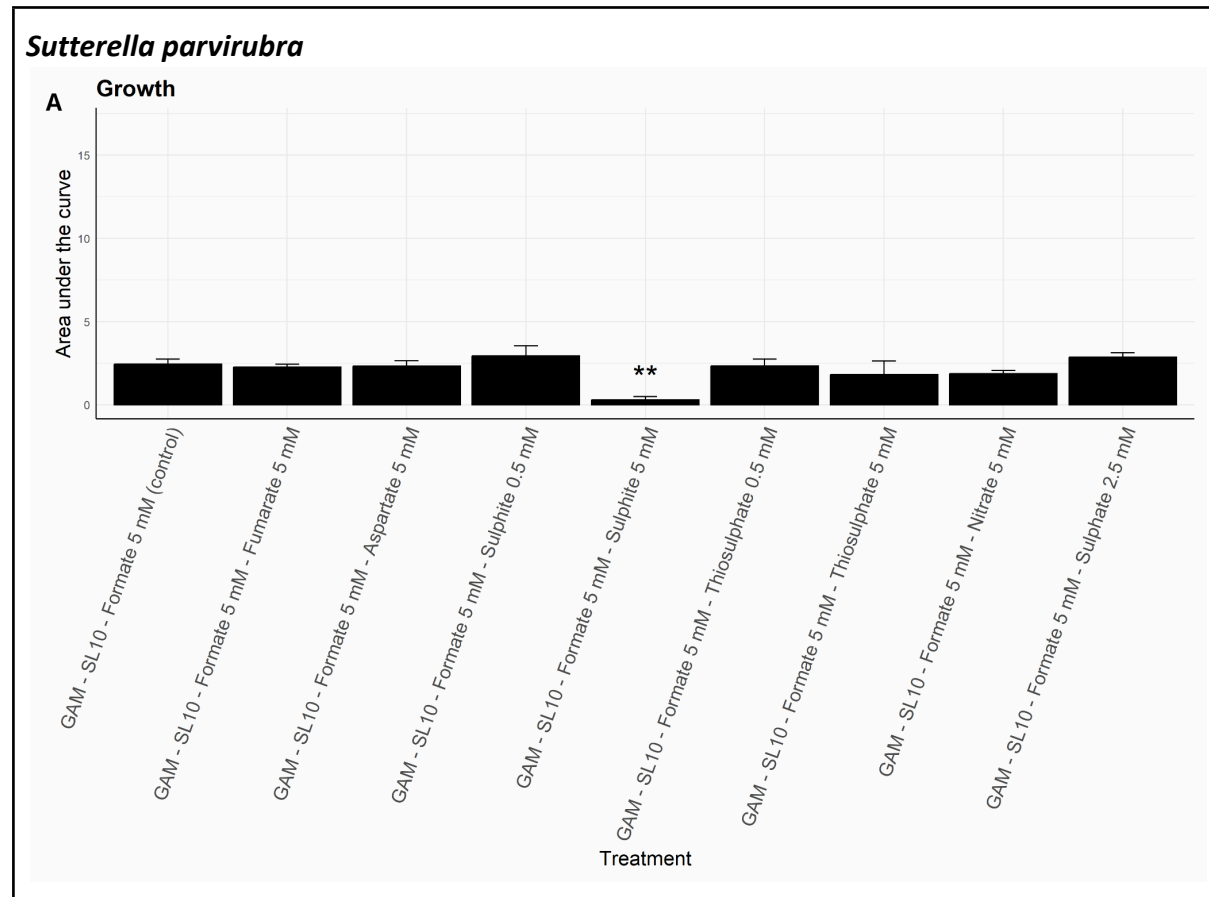
In terms of growth, the comparison was made by calculating the area under the curve with the R package “growthcurver” and comparing the area under the curve of the replicates with a one-way ANOVA and a Tukey Honestly Significant Difference test. Here, the growth analysis for *S. wadsworthensis* shows that treatment groups supplemented in 5 mM thiosulphate has a significantly lower area under the curve than the control containing formate only (Fig. 1A & 1J). Treatment groups formate 5 mM (Fig. 1C), in formate 5 mM and fumarate 5 mM (Fig. 1D), in sulphite 0,5 mM (Fig. 1G) and in sulphate 2.5 mM (Fig. 1K) have a statistically higher area under the curve than the GAM-only (Supplementary materials) treatment group.

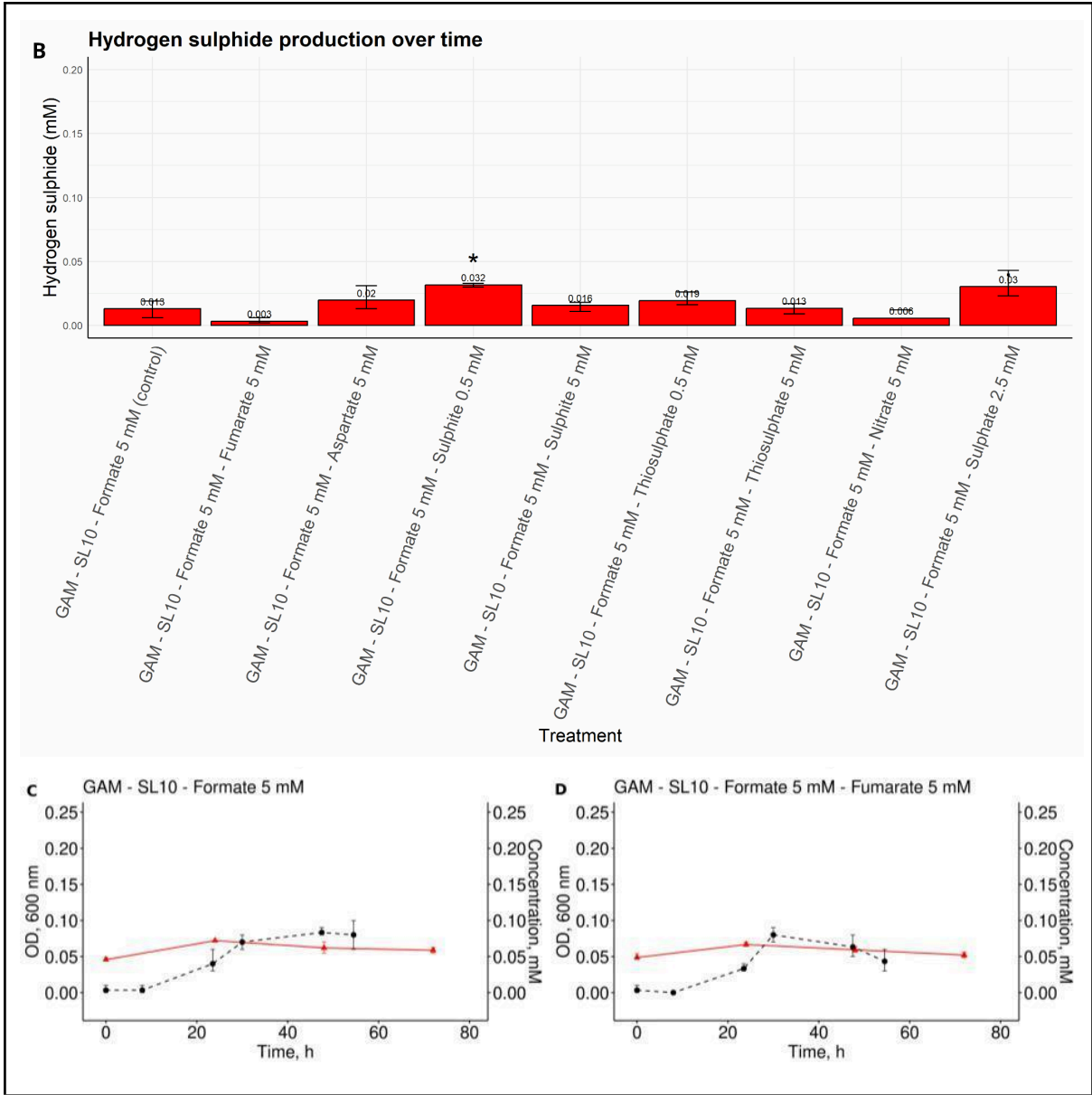
In order to determine the difference between treatment groups, we compared absolute production over time by deducting the concentrations of the very first measurement at T_0 , to the very last concentration point as our measurements show empirical production over time and due to the standard curve, none of our calculated hydrogen sulphide curves start around 0. Therefore the values that will be referred to here are the actual production of hydrogen sulphide over time and not the raw final concentration (Fig. 1B).

It is observed that the treatment group containing 5 mM of formate and fumarate (Fig. 1B & C), formate 5 mM and aspartate 5 mM (Fig. 1E), formate 5 mM and thiosulphate 0.5mM (Fig. 1I) and 5 mM (Fig. 1J) as well as in both formate 5 mM and nitrate 5 mM (Fig. 1F) show lower production of H_2S than control group supplemented in formate only. The two treatment groups supplemented in either 0.5 mM or 5 mM sulphite (Fig. 1G & H) display equivalent hydrogen sulphide production over time reaching respectively overtime productions of 0.074 mM and 0.076 mM following the trend of the control group (Fig. 1B & C), but also significantly higher production compared to additional treatment groups containing either GAM or GAM with added SL10 (Supplementary materials Fig. 1), or treatment groups with the added electron acceptors fumarate 5 mM (Fig. 1D), aspartate 5

mM (Fig. 1E), thiosulphate 0.5 mM (Fig. 1I), thiosulphate 5 mM (Fig. 1J) as well as nitrate 5mM (Fig. 1F) (Supplementary materials, Table 2).

Interestingly, the treatment group containing both formate 5 mM and sulphate 2.5 mM (Fig. 1K) as an electron acceptor showed significantly the highest hydrogen sulphide production compared to all other treatment groups, reaching an average end production of 0.154 mM (Supplementary materials, Table 2).





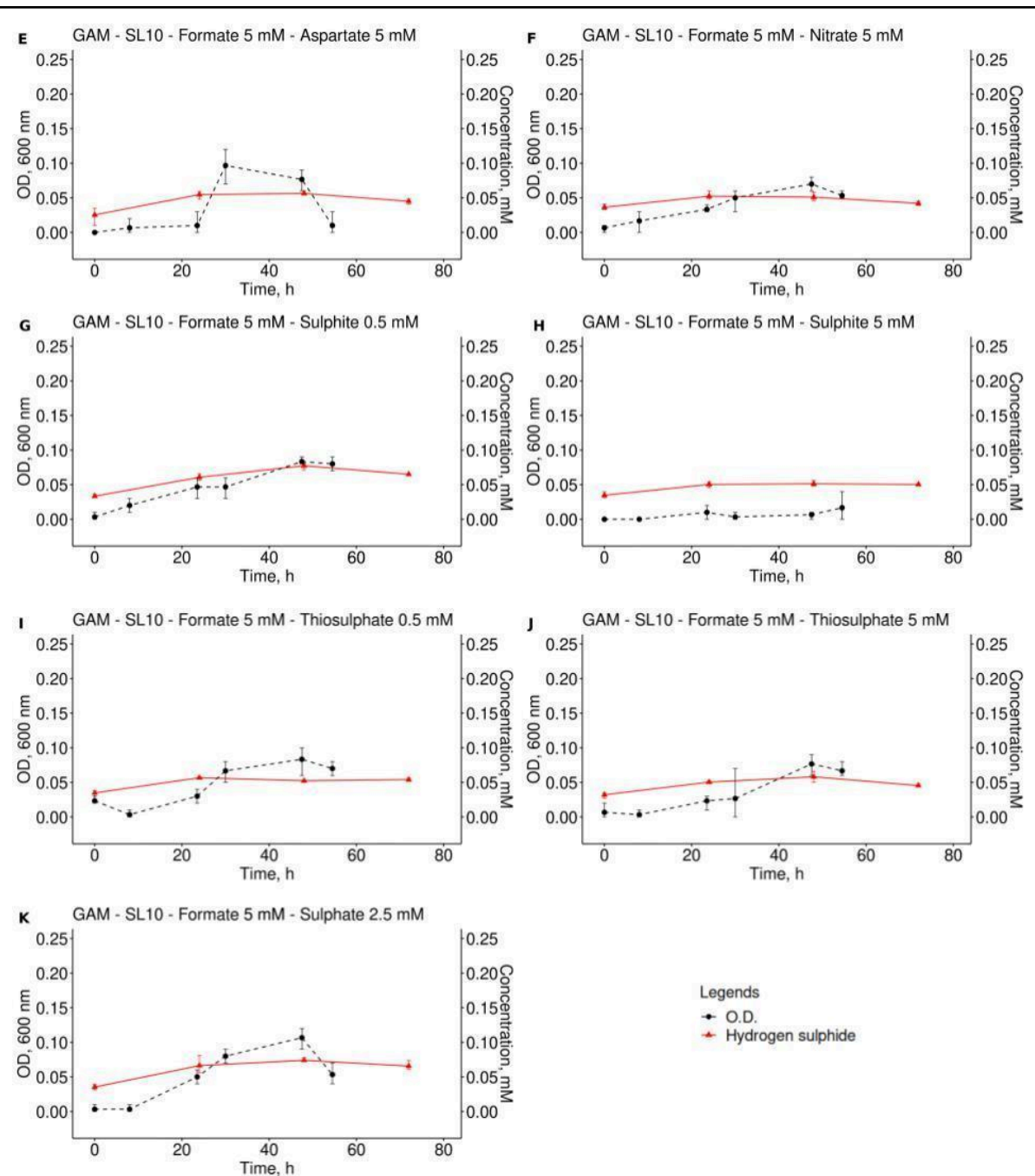


Figure 2 | *Sutterella parvirubra* - Growth and hydrogen sulphide concentration over time in presence of various electron acceptors. From left to right and up to down: **A:** Growth represented by the empirical area under the curve using treatment group GAM - SL10 - Formate 5 mM as a control (Significance code: 0 **** 0.001 *** 0.01 ** 0.05 ' 0.1 ' ' 1); **B:** Hydrogen sulphide production over time represented by the difference of hydrogen sulphide quantity of the last timepoint and of the first timepoint using GAM - SL10 - Formate 5 mM as a control (Significance code: 0 **** 0.001 *** 0.01 ** 0.05 ' 0.1 ' ' 1). Average hydrogen sulphide production per treatment group is shown above its respective bar.; **C:** GAM - SL10 - Formate 5 mM; **D:** GAM - SL10 - Formate 5 mM - Fumarate 5 mM; **E:** GAM - SL10 - Formate 5 mM -

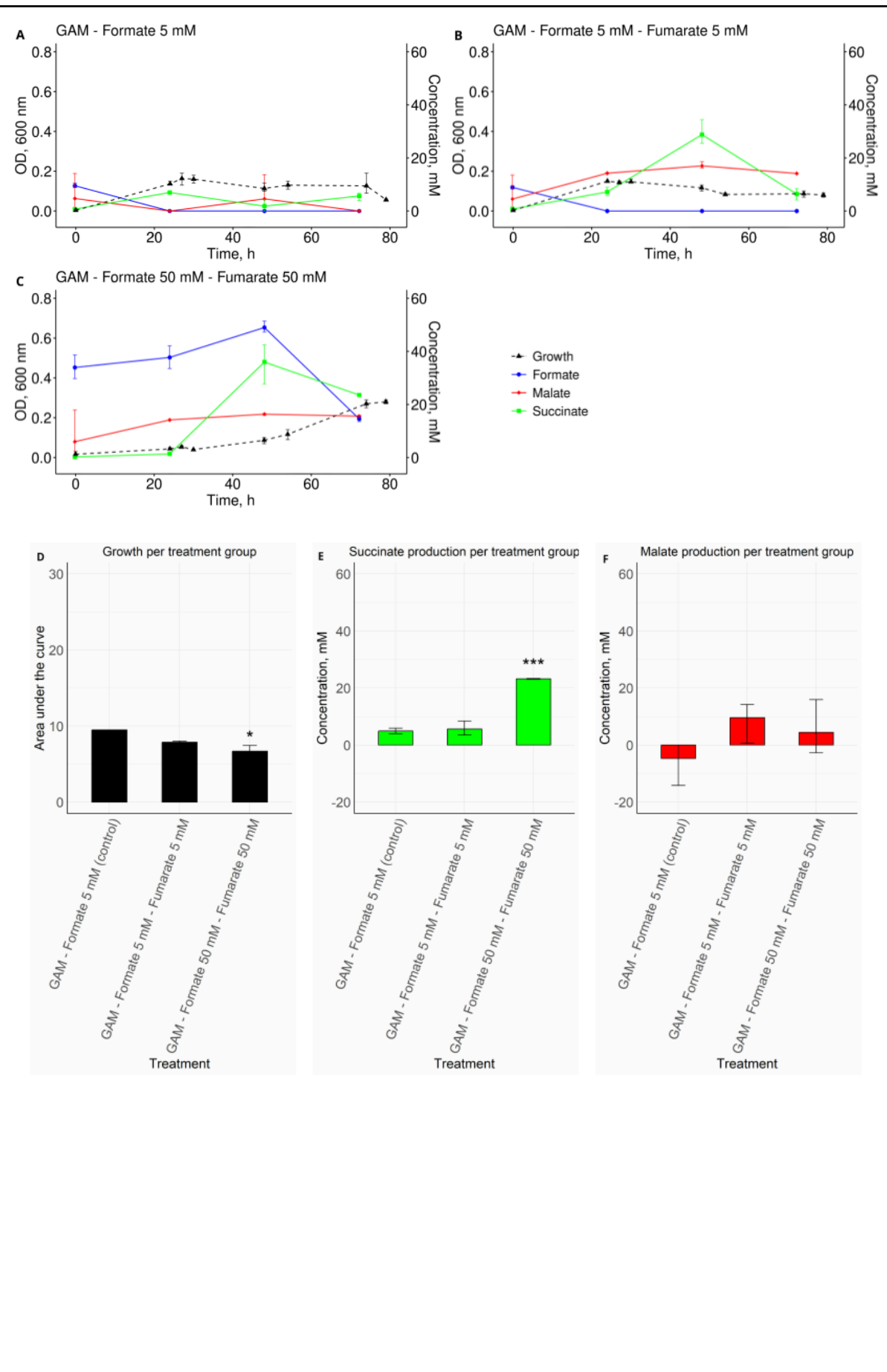
Aspartate 5 mM; **F**: GAM - SL10 - Formate 5 mM - Sulfite 0.5 mM; **G**: GAM - SL10 - Formate 5 mM - Sulfite 5 mM; **H**: GAM - SL10 - Formate 5 mM - Thiosulfate 0.5 mM; **I**: GAM - SL10 - Formate 5 mM - Thiosulfate 5 mM; **J**: GAM - SL10 - Formate 5 mM - Nitrate 5 mM; **K**: GAM - SL10 - Formate 5 mM - Sulphate 2.5 mM. On each graph, in black, dotted is represented the growth and in red is represented the production of hydrogen sulphide.

In the case of *S. parvirubra*, the empirical area under the curve analysis is showing less striking results. All growths here represented by the empirical area under the curve show equivalent growth to the control except for the treatment group supplemented in 5 mM of sulphite (Fig. 2A & 2H) with a significantly lower growth which can be a sign of the growth inhibition by a higher sulphite concentration. Only the treatment group containing both formate 5 mM and sulphite 0,5 mM (Fig. 2G) and both formate 5 mM and sulphate 2,5 mM (Fig. 2K) are showing a marginally significantly bigger area under the curve than the additional groups GAM and GAM with added SL10 (Supplementary materials, Table 1) (but show no statistical difference with the control treatment group supplemented in formate 5 mM).

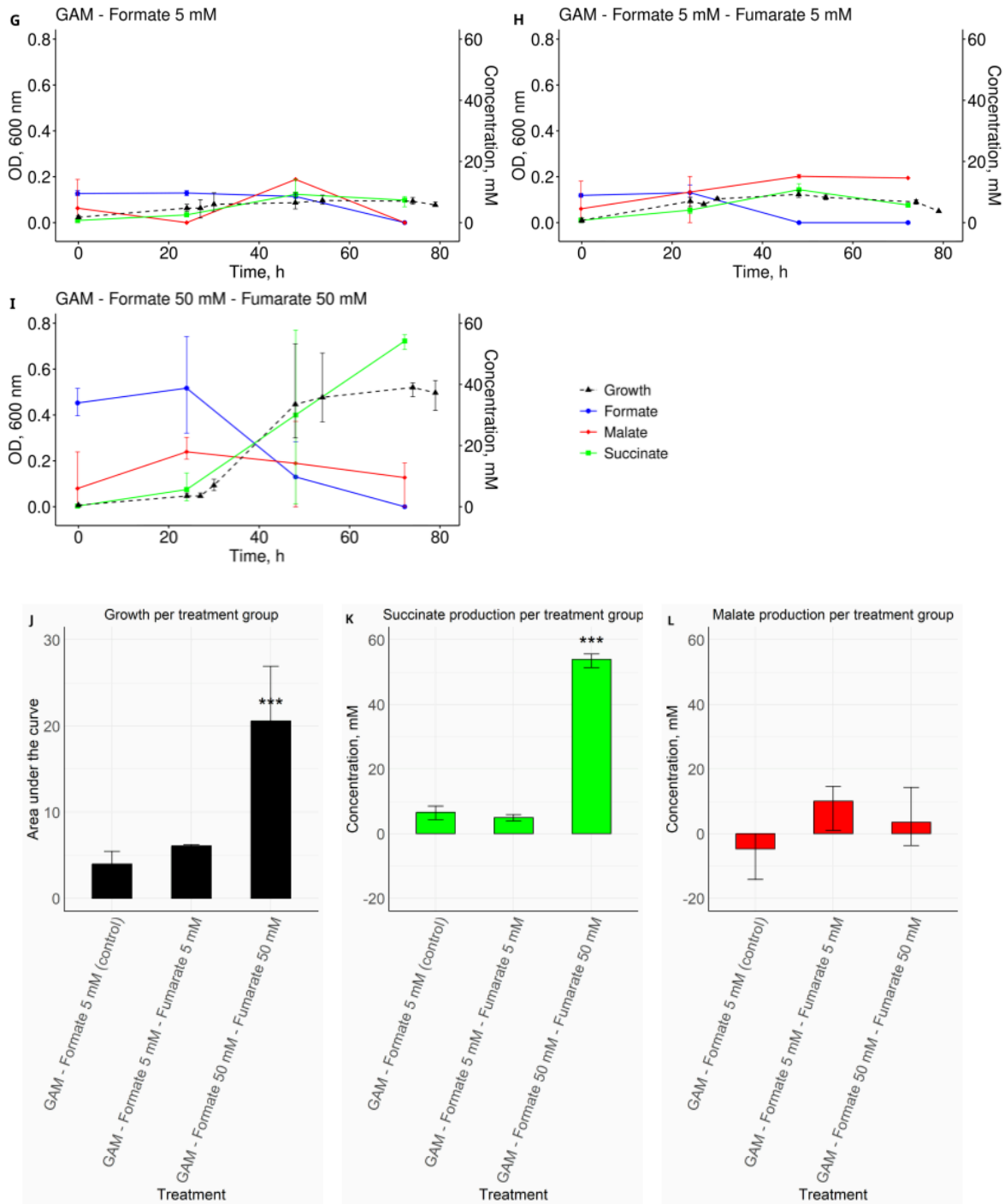
For *S. parvirubra*, some similar trends as *S. wadsworthensis* are observed for the treatment groups supplemented in both formate 5 mM and sulfite 0.5 mM (Fig. 2G) and in both formate 5 mM and sulphate 2.5 mM (Fig. 2K) in respect to hydrogen sulphide production. Over time 0.032 mM and 0.031 mM of H₂S respectively have been produced. These two groups show marginally higher production of hydrogen sulphide when comparing the difference between concentrations of the first timepoint and the last timepoint compared to control treatment supplemented in formate 5 mM. No other differences are observed with the control (Fig. 2B).

Hypoxic incubation experiment

Sutterella parvirubra



Sutterella wadsworthensis



Turicimonas muris

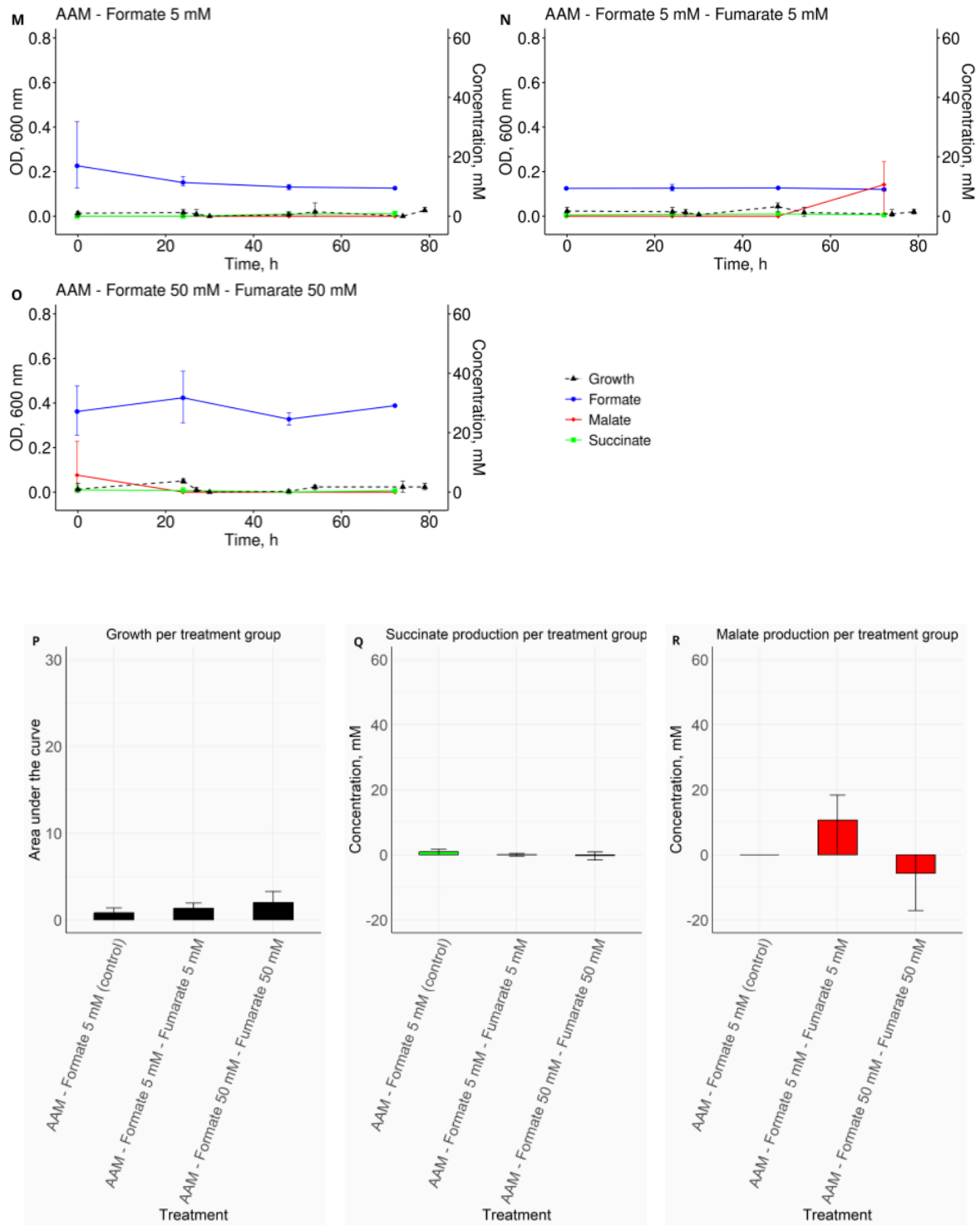


Figure 3 | Concentration of Succinate, Malate and Formate over time for *Sutterella parvirubra*, *Sutterella wadsworthensis* and *Turicimonas muris* determined via capillary electrophoresis. **A, B, C: Optical density (600 nm) of *Sutterella parvirubra* in parallel to the concentration of succinate, formate and malate over time (in black dotted line: growth; in blue: formate, in red: malate, in green: succinate); **D:** Comparison of empirical area under the curve per treatment**

for the growth of *Sutterella parvirubra* showing statistical analysis using “GAM - Formate 5 mM” as a control group (Significance code: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1); **E** Comparison of succinate production (difference between the concentrations of the last timepoint and of the first timepoint) per treatment group for *Sutterella parvirubra* showing statistical analysis using “GAM - Formate 5 mM” as a control group (Significance code: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1); **F**: Comparison of malate production (difference between the concentration of the last timepoint and of the first timepoint) per treatment group for *Sutterella parvirubra* showing statistical analysis using “GAM - Formate 5 mM” as a control group (Significance code: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1); **G, H, I**: Optical density (600 nm) of *Sutterella wadsworthensis* in parallel to the concentration of succinate, formate and malate over time (in black dotted line: growth; in blue: formate, in red: malate, in green: succinate); **J**: Comparison of empirical area under the curve per treatment for the growth of *Sutterella wadsworthensis* showing statistical analysis using “GAM - Formate 5 mM” as a control group (Significance code: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1); **K**: Comparison of succinate production (difference between the concentrations of the last timepoint and of the first) per treatment group for *Sutterella wadsworthensis* showing statistical analysis using “GAM - Formate 5 mM” as a control group (Significance code: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1); **L**: Comparison of malate production (difference between the concentration at the first timepoint and at the last) per treatment group for *Sutterella wadsworthensis* showing statistical analysis using “GAM - Formate 5 mM” as a control group (Significance code: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1); **M, N, O**: Optical density (600 nm) of *Turicimonas muris* in parallel to the concentration of succinate, formate and malate over time (in black dotted line: growth; in blue: formate, in red: malate, in green: succinate); **P**: : Comparison of empirical area under the curve per treatment for the growth of *Turicimonas muris* showing statistical analysis using “GAM - Formate 5 mM” as a control group (Significance code: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1); **Q**: Comparison of succinate production (difference between the concentrations of the last timepoint and of the first) per treatment group for *Turicimonas muris* showing statistical analysis using “GAM - Formate 5 mM” as a control group (Significance code: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1); **R**: Comparison of malate production (difference between the concentrations of the last timepoint and of the first) per treatment group for *Turicimonas muris* showing statistical analysis using “GAM - Formate 5 mM” as a control group (Significance code: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1).

As several studies demonstrated the microaerophilic properties of *Sutterella parvirubra*, *Sutterella wadsworthensis* and *Turicimonas muris*, we designed an experiment to monitor their growth in an environment with 1% dioxygen, as well as their ability to use formate and fumarate if dioxygen is present in the environment. We therefore implemented

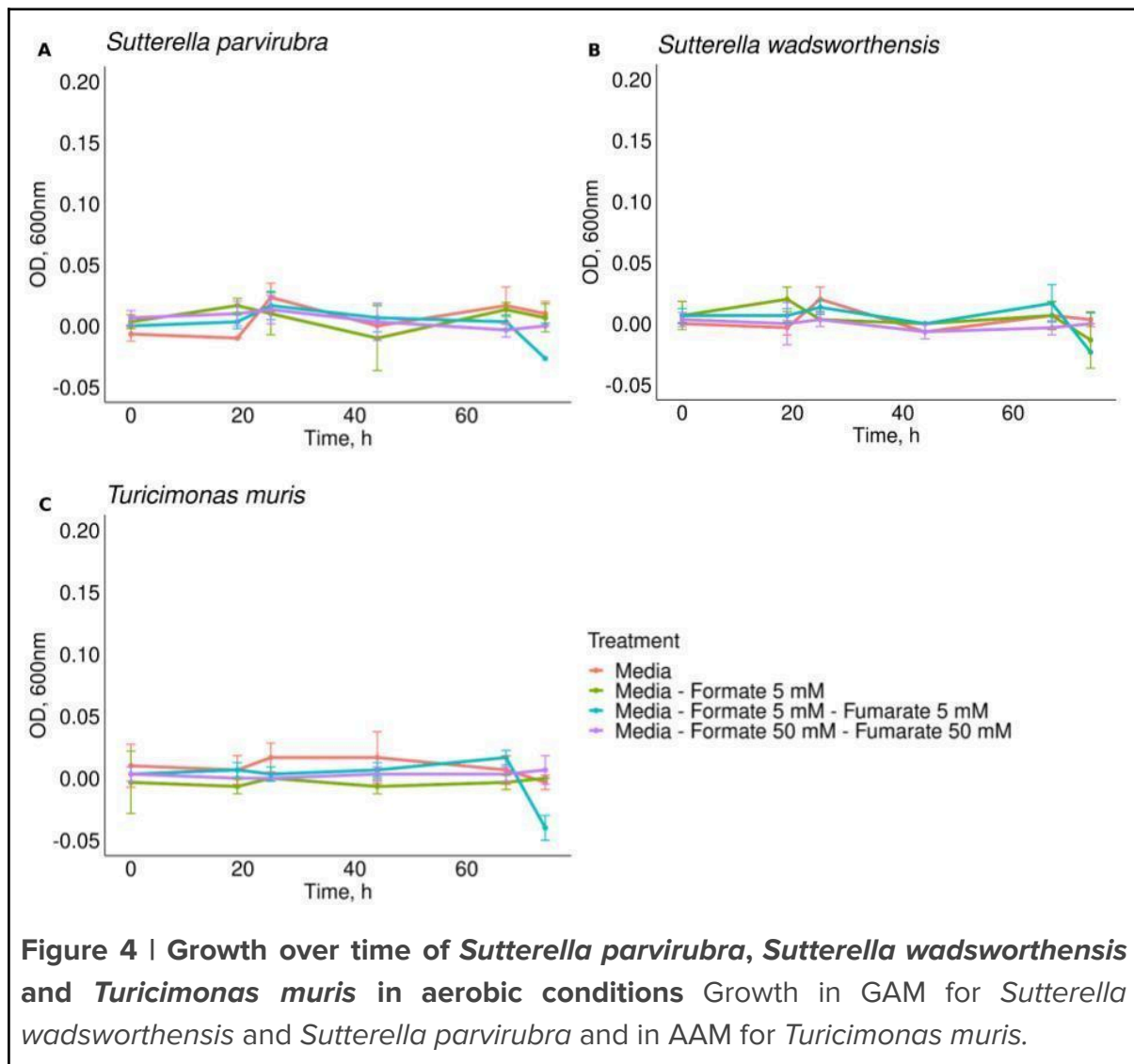
several treatment groups (using as basis for media GAM for *Sutterella* species and AAM for *T. muris*): media with 5mM formate (which acts as a control), media with 5mM formate and 5mM fumarate and media with 50mM formate and 50mM fumarate, and measured the growth over the course of a few days. Every 24 hours, all tubes except the controls, were sampled for later analysis with capillary electrophoresis.

Almost no growth was observed with *Turicimonas muris* in hypoxic conditions. For both *Sutterella wadsworthensis* and *Sutterella parvirubra*, we observe contradictory results in terms of growth. In the case of *Sutterella wadsworthensis*, treatment group containing 50 mM of formate and fumarate show significantly higher growth (Fig. 3I & J) while for *Sutterella parvirubra*, it is significantly lower (Fig. 3C & D). The growth for both *S. parvirubra* and *S. wadsworthensis* is however significantly higher as soon as any type of formate or fumarate is added to the media (Supplementary materials, Table 3)).

For both *S. parvirubra* and *S. wadsworthensis*, we observe a decrease of formate over time, in most cases with formate being completely utilised by the last timepoint (Fig. 3A, B, C, H, I, J). We also observe the production of succinate over time for both *S. parvirubra* and *S. wadsworthensis* for these same groups. Production of succinate is significantly higher compared to all treatment groups for *S. wadsworthensis* for treatment supplemented in formate 50 mM fumarate 50 mM (Fig. 3I & K), reaching at the last timepoint an average production of 54 mM/ μ L which is an in equimolar concentrations of the fumarate added. In the case of *S. parvirubra*, succinate production was also significantly higher for the treatment enriched in formate 50 mM and fumarate 50 mM (Fig. 3C & 3E) compared to all other groups, reaching a final concentration of 23,56 mM, around half of the concentration of the fumarate initially added. None of these trends were observed for *T. muris* most likely due to the lack of growth for this strain.

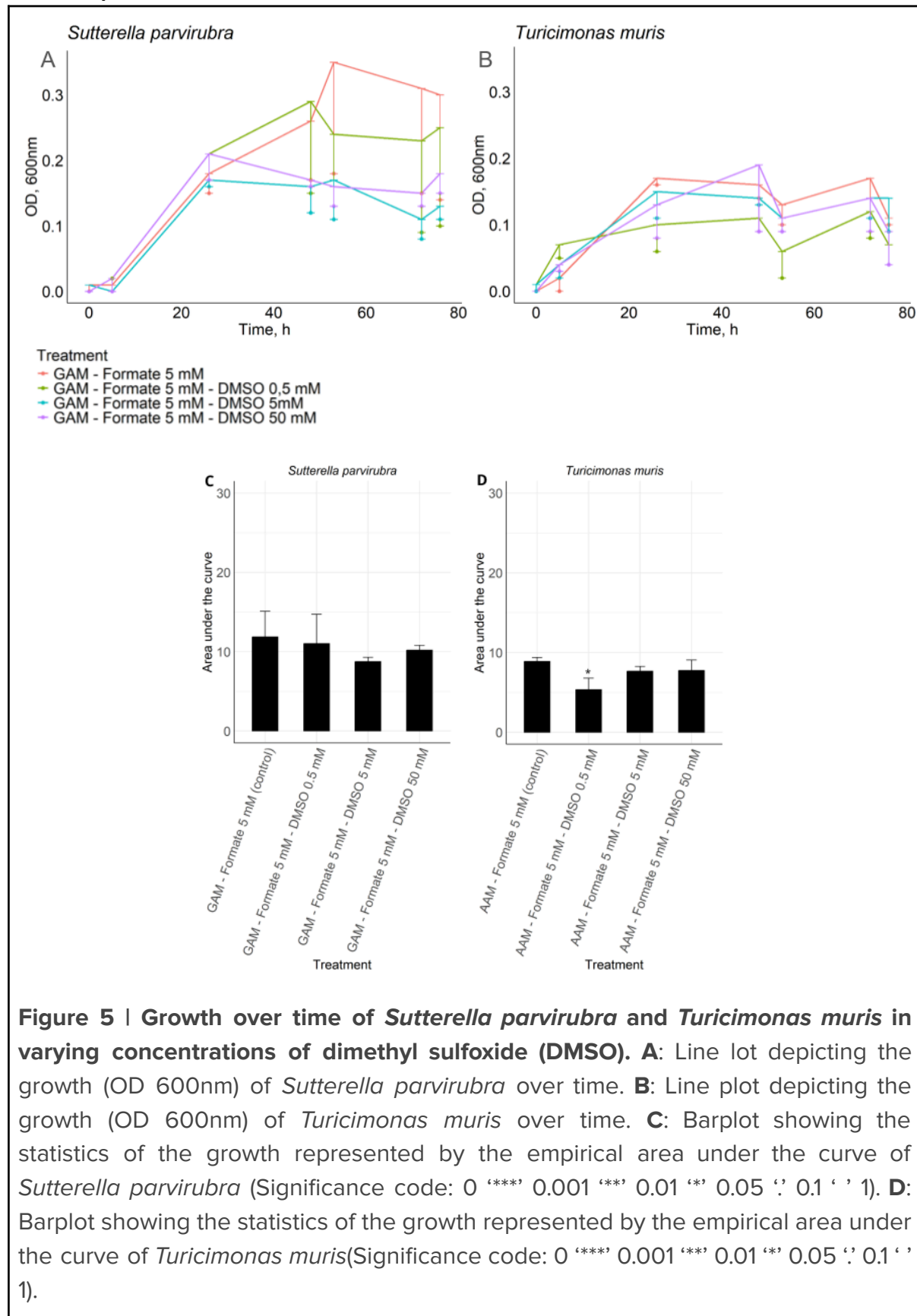
Malate peaks were also observed to fluctuate over time on capillary electrophoresis. For *S. wadsworthensis*, some malate production was observed in all treatment groups and reached concentrations between 13.5 to 27.9 mM. For *S. parvirubra*, a similar trend was observed where malate appeared at some time points before being used up. For *Sutterella parvirubra*, malate was produced one timepoint for one replicate of the control treatment group with only formate 5 mM (Fig. 3G). For both treatment groups either supplemented in formate 5 mM and fumarate 5 mM (Fig. 3H) or in formate 50 mM and fumarate 50 mM (Fig. 3H), malate seemed to be produced consistently ranging from 14 mM to 18.5 mM. Malate statistical tests however, revealed no differences in malate production between treatment groups for any of the strains due to the fluctuating nature of the observed signals (Fig. 3F, L, & R).

Aerobic experiment



The same test as the hypoxic incubation experiment was conducted in aerobic conditions at 37°C with shaking. The growth for each species and each treatment group was comprised within an optical density range going from -0.04 to 0.02 indicating no growth was observed (Fig. 4), therefore no statistical analysis was run on these data. With no log phase visible it is more complicated to fit the data with the growthcurver R package and therefore to obtain reliable analysis. From these observations, it was decided not to carry on with the capillary electrophoresis analysis.

DMSO Experiment



A team research project focuses partly on bacterial strains possessing tetrathionate reductase and DMSO reductase including *Sutterella wadsworthensis*, *Sutterella parvirubra*, *Turicimonas muris* and *Parasutterella excrementihominis*. Therefore, an incubation experiment was performed, as a preliminary test for his research, to explore the possible impact of the presence of DMSO and tetrathionate on the growth of our strains. In the genome, we found that both *Sutterella parvirubra* and *Turicimonas muris* possessed a DMSO reductase, as well as a tetrathionate reductase was present in *Parasutterella excrementihominis*.

S. parvirubra and *T. muris* were incubated for four days in an anaerobic vinyl glove box at 37°C in either media alone for control (AAM for *T. muris* and GAM for *S. parvirubra*), media and 5 mM of formate, media with 5 mM of formate and 0.5 mM of DMSO, media with 5 mM of formate and 5 mM of DMSO, media with 5 mM of formate and 50 mM of DMSO.

The growth analysis was made by comparing the empirical area under the curve of all replicates of the treatment groups. No difference in terms of growth were observed between the control group and the other treatment groups for *Sutterella parvirubra*. For *Turicimonas muris* we observe however a slightly lower growth for the treatment group supplemented with 0.5 mM of DMSO compared to the control.

Tetrathionate reductase was found to be present in *Parasutterella excrementihominis*, however, we did not perform this specific incubation test yet due to the multiple failed attempts of growing this strain in liquid media.

Discussion

Electron acceptor test

From the preliminary re-analysis of data performed in our lab directed by Kits D., unpublished, to the very recent discoveries from Little et al., 2024, *Sutterellaceae*, most specifically *Sutterella wadsworthensis*, seem to be equipped with the ability to utilise several electron acceptors, such as fumarate, aspartate, sulphite, thiosulphate, sulphate, nitrate and DMSO among others. Whether these processes are reduction reactions or actually respiration is still subject to debate. Furthermore, the re-analysis of data made in our lab suggested the presence of the octahaeme cytochrome-c sulphite reductase gene cluster MccABCD in the genome of *Sutterella wadsworthensis*, as can be found in *Wolinella succinogenes* as described by Kern et al., 2011. To further our understanding of these processes, an experiment was designed in which the growth of several *Sutterellaceae* strains, namely *Sutterella wadsworthensis*, *Sutterella parvirubra*, and *Turicimonas muris* was monitored over the course of a few days in presence of either 5 mM of fumarate, 5 mM of aspartate, 5 mM of nitrate, 2.5 mM of sulphate, 0.5 mM of sulphite, 5 mM of sulphite, 0.5 mM of thiosulphate or 5 mM of thiosulphate. Besides, all the different treatment groups

were sampled every day for Cline assay in order to quantify the amount of hydrogen sulphide produced.

This experiment with *Sutterella wadsworthensis* demonstrated that growth is stimulated as soon as 5 mM of formate were added (Supplementary materials, Table 1). In *S. parvirubra*, this trend is not observed. Furthermore, no group displayed higher growth than the control group containing only formate for either *S. wadsworthensis* or *S. parvirubra*.

Formate being an important electron donor in anaerobic conditions contributes to the reduction of the electron acceptors present in the media, whether added or pre-existing. According to the gene search performed in this work (Table 4), our strains possess formate dehydrogenase FdhABC. Gifu Anaerobic Broth is a very rich chemically undefined media that contains pre-existing electron acceptors, and the sole addition of formate could contribute to the reduction of these pre-existing electron acceptors. This is one possible explanation for the growth stimulation in presence of formate observed in *Sutterella wadsworthensis*. For *Sutterella parvirubra*, this growth boost was not observed, which might be due to the lower optical densities measured throughout this experiment. A strong growth depends on many factors with complicated strains like ours, such as strength and freshness of stocks, freshness of plates from which the inoculum was made, whether it is shaken or vortexed, or taken out of the incubator for too long, and some of these could have impacted the experiment with *Sutterella parvirubra*.

The hydrogen sulphide levels were quantified with Cline assay on every day of this experiment, to evaluate the potential activity of cytochrome c sulphite reductase MccA. The results partly concord between *S. wadsworthensis* and *S. parvirubra*. We observed for both these species a higher hydrogen sulphide production over time in treatment groups 2.5 mM of sulphate compared to our control (although very marginal for *S. parvirubra*). A higher production is also observed in *Sutterella parvirubra* for the treatment group supplemented with 0.5 mM of sulphite.

Firstly, none of the curves for the hydrogen sulphide quantification are starting at 0 mM. This is due to the protocol for Cline assay that was used throughout these experiments. During the establishment of the sodium sulphide standard, the protocol called for pipetting up and down in order to mix the contents of the tubes for the dilution series before withdrawing the contents of half the tube and transfer it to the next tube, which might have enabled the dissociation of some of the hydrogen sulphide before its fixation in zinc acetate. The obtained data points lead to an irregular standard curve instead of forming a nice line leading to a linear and an equation that calculated measured absorbance around 0 at around 0.05 mM and is no indicator that there is already hydrogen sulphide in the media.

Secondly, for all the treatment group displaying some level of hydrogen sulphide, whether significant or not, the production is empirical and indicates an accumulation over time. Both temperature and pH have an influence on the dissociation of hydrogen sulphide from water and the media we used was set at pH 6.0 before the experiment and increased to pH 6.5 by the end of the experiment, which according to one study can lead to a

dissociation of up to 30% at this pH and 35°C (Yongsiri et al., 2015). This trend of empirical production makes production via MccA unlikely.

It is also to be noted that it is not because production of hydrogen sulphide is observed for some treatment groups, or that electron acceptors are being reduced, that proper respiration is taking place.

The most likely hypothesis is the catabolism of cysteine present either in the bacterial material being recycled or in the GAM broth containing numerous protein sources (peptone, yeast extract, liver extract) as well as L-Cysteine hydrochloride. Cysteine can be turned into hydrogen sulphide via different pathways, but the most likely in this case would be *via* O-acetylserine sulfhydrylase A (CysK) which turns reversibly L-cysteine and acetate into hydrogen sulphide and O³-acetyl-L-serine (Hicks et al., 2022), and is present in our strains (except *Sutterella parvirubra*) according to the gene table (Table 4) as well as to the KEGG database. Some genes leading to hydrogen sulphide production from cysteine degradation are suspected to be present in *Sutterellaceae* bacteria as described by Braccia et al., 2021 like *yhaM* (encoding cysteine desulfidase which turns L-cysteine into ammonia, hydrogen sulphide and pyruvate (Braccia et al., 2021; Nonaka & Takumi, 2017; Tchong et al., 2005), *cysK* (as described above) and *cysM* (encoding O-acetylserine sulfhydrylase B and can produce thiosulphate and hydrogen sulphide from cysteine). This seems even more likely as for all treatment groups showing hydrogen sulphide production, hydrogen sulphide keeps increasing while bacteria are already entering their death phase as it can be observed with the decreasing growth suggesting that hydrogen sulphide production might be the result of the recycling of dead bacteria.

Hydrogen sulphide production remains, however, higher in *Sutterella wadsworthensis* growing with 2.5 mM supplementation of sulphate, compared to the formate group or the sulphite groups, for an equivalent growth, when according to the KEGG database, no gene is reported present in our strain synthesising cysteine from sulphate. Cysteine can be produced indirectly from sulphate and sulphite through the pathway of assimilatory sulphate reduction (Kredich, 2008). However, as our strains do not possess the required genes to do so, it is unclear whether some unrelated enzymes contribute to the production of cysteine by acting on sulphite and sulphate however the exact processes remain so far unclear.

To sum up, these electron acceptor tests are overall inconclusive, and although we do observe hydrogen sulphide being produced for some treatment groups, the quantity as well as the production pattern do not indicate MccA activity but rather cysteine degradation. These tests would gain being redesigned using a protocol yielding more precise results and enabling less evaporation of hydrogen sulphide in the atmosphere. It would also be interesting for the treatment groups that showed higher hydrogen sulphide production over time, to add some treatment groups containing a higher quantity of sulphite or sulphate to see whether higher quantities lead to higher hydrogen sulphide production.

Some *Sutterellaceae* strains are also known to possess some microaerophilic properties, *Sutterella wadsworthensis* is reported to grow in up to 6% dioxygen while no

data is reported on that aspect regarding *Sutterella parvirubra* and *Turicimonas muris* in the literature. Therefore, we were interested in evaluating how dioxygen influences *S. wadsworthensis*, *S. parvirubra* and *T. muris*, and whether its influence as an electron acceptor would impact the fumarate reduction reaction taking place as part of the TCA cycle as we observed in our lab in anaerobic conditions. an experiment was carried out in hypoxic conditions (1% O₂). The idea that oxygen could be used as an electron acceptor instead of fumarate would negatively impact the production of succinate resulting of fumarate reduction.

The best growth was achieved in treatment groups supplemented with formate 50 mM and fumarate 50 mM for *Sutterella wadsworthensis*, which suggests that higher growth is correlated with higher formate and fumarate concentrations. However, we cannot claim which component, whether formate or fumarate is responsible for this stimulated growth. The conducted experiments performed at lower concentrations implied that formate stimulated the growth on its own, as observed with the electron acceptor test with *Sutterella wadsworthensis*. If such tests were to be repeated, a treatment group with 50 mM of formate without fumarate added to the media should be added to observe whether clear differences in terms of growth are observed with the treatment group supplemented with both 50 mM of formate and 50 mM of fumarate are observed to better understand the impact of fumarate on growth as well as the impact the pre-existing electron acceptors in the media.

The capillary electrophoresis trends for this experiment are very similar to past observations in similar experiments set up in anaerobic conditions conducted in our lab. Formate concentration decreases over time, while succinate is being produced at the approximate concentration in which formate and fumarate were present. This implies formate is being consumed, while the succinate production suggests the reduction of fumarate into succinate. The gene search conducted by blasting related gene sequences against the *Sutterellaceae* taxa (Table 4) indicates our strains all possess fumarate reductase, which converts fumarate into succinate, hinting that the added fumarate could be used up and explaining the production of succinate. Fumarate, however, cannot be quantified with capillary electrophoresis, and this can therefore not be confirmed.

Some malate production is observed over time for treatment groups containing fumarate for *Sutterella parvirubra* and for all treatment groups in the case of *Sutterella wadsworthensis*. This malate is also possibly synthesised as part of the TCA cycle as malate dehydrogenase and fumarate hydratase have been found in the genomes of all tested species (Table 4). The fluctuating nature of malate production as well as the unreliability of capillary electrophoresis, however, did not enable us to get any results with statistical tests. These results suggest therefore that fumarate was most likely still being chosen as an electron acceptor despite the presence of oxygen in the environment and that TCA cycle was still taking place as it would in anaerobic conditions, leading to succinate production as well as malate synthesis.

Some concerns can be voiced about this experiment. First issue was about whether this experiment truly was conducted in hypoxic conditions. The hypoxic glove box does not offer the possibility to shake the tubes in the incubator due to size restrictions, and we can wonder whether the oxygen from the tent atmosphere did indeed reach the depths of the media in the tubes as our strains are non-motile (Table 3).

Second concern relates to capillary electrophoresis. It is not the most reliable tool, sometimes giving out very different results for a same sample on different days. A recurring issue concerned measurements of different formate concentrations than the initially added concentration (such as formate concentrations measured between 8 and 11 mM for tubes containing 5 mM or between 19 and 39 mM for tubes containing 50 mM).

Due to the concerns raised, this test would benefit from being redesigned and would provide more insights into the fumarate reduction process by adding a treatment group supplemented with 50 mM of formate (without fumarate) to compare with the treatment group supplemented with both 50 mM formate and 50 mM fumarate and understand whether the presence of fumarate in the media is the factor leading to a higher growth and the higher succinate production, as no significant difference in succinate production was found for the cultures growing in media supplemented with 5 mM of formate and in media supplemented in both 5 mM of formate and 5 mM of fumarate.

Furthermore, the question of dioxygen reaching the depths of the tubes where our strains were most likely located challenges the idea of the test being carried out in true hypoxic conditions. Therefore, if such a test would be reproduced, the hypoxic glove box should be equipped with a shaker in order to stimulate oxygen penetration in the media. Another option would be to use rubber stoppers and reinject every day the desired gas mix in the tubes all the while regularly checking the gas contents of tubes with gas chromatography. This option would be compatible with shaking and also with keeping the tubes in a regular 37°C chamber, which would add more convenience for measurements and sampling.

Following the hypoxic incubation experiment, an incubation experiment was set up in aerobic conditions to observe whether O₂ is effectively toxic in *Sutterellaceae* or whether some growth could be observed as well as fumarate reduction.

For this experiment where the bacteria were kept at 21% oxygen, no growth was observed, proving that *S. parvirubra*, *S. wadsworthensis* and *T. muris* do not grow in an environment that is too rich in oxygen. We therefore did not carry out the following capillary electrophoresis analysis of samples that were taken during this experiment.

It should however be reminded that the conditions of this experiment were different than all the experiments in tents, including shaking. Although the most likely explanation for the lack of growth was hindered by ambient dioxygen, it was observed so far with the *Sutterellaceae* strains we experimented with that they are particularly sensitive to change. The growth seems impaired when they are taken out for measurements outside of the incubator in the anaerobic tent, they are also sensitive to harsh motion, we can therefore

wonder whether the shaking to which the bacterial strains were submitted was not too strong resulting in the early death of the bacteria.

Following the reduction trend, a research study made in our lab by a collaborator suggests that both *Sutterella parvirubra* and *Turicimonas muris* possess dimethylsulfoxide reductase DmsA but its presence was not found in *Sutterella wadsworthensis* at the time. This latter claim was challenged by the gene search performed in Table 4 as well as the recent study from Little et al., 2024, which reports the presence of a dimethyl sulfoxide (DMSO) respiratory-like system in *Sutterella wadsworthensis*. The research performed in our lab also suggests the presence of tetrathionate reductase TtrA in the strain *Parasutterella excrementihominis*. Therefore, this hypothesis was tested by performing a preliminary incubation experiment featuring *Sutterella parvirubra* and *Turicimonas muris* growing in varying levels of DMSO. *Parasutterella excrementihominis*, however, was not grown in presence of tetrathionate due to the difficulty of culturing it in liquid media.

In this work, no difference was observed, for either strain, between the control group containing formate only, and all the other groups containing various concentrations of DMSO. The media used here being very rich, one possibility could be that reduction activities went towards the pre-existing electron acceptors in the media rather than DMSO. We therefore cannot conclude on any DMSO reduction activity performed by the dimethyl sulfoxide A (DmsA).

These results are contrasting with the recent findings from Little et al., 2024. First, and as already mentioned, they, contrarily to us, found that *Sutterella wadsworthensis* also possessed a respiratory-like system for DMSO reduction and performed an incubation experiment with *S. wadsworthensis* using 50% brain-heart infusion as a medium in presence of 10 mM formate and 10 mM DMSO. The medium used by Little et al., 2024 is different (50% BHI instead of GAM), the concentration of formate is also different (10mM instead of 5 mM) and this experiment was conducted in a plate reader which might have given them more precise results. However, some points could be discussed. The maximum optical density obtained in this study with no addition was 0.025 reached after 24 hours. In this same study, the optical density of the treatment group containing formate and DMSO stagnated during 24 and 48 hours at an optical density of 0.04 and then gradually increased to reach a maximum optical density of approximately 0.06. These optical densities are overall lower than the optical density measured in our lab without formate. Furthermore, no reduced product nor partial product was detected after the incubation experiment in this study.

In order to observe DMSO reduction activity, this test could be redesigned in a less rich media such as brain-heart infusion to check whether the richness of GAM influences the ability of *Sutterella parvirubra* and *Turicimonas muris* to reduce DMSO and reproduce the observations of Little et al., 2024. Another approach could be to monitor the fluctuation of the DMSO concentration throughout the experiment with for example high performance liquid chromatography.

Table 3 | Characteristics of aforementioned *Sutterellaceae* strains

Collection of characteristics from publications.

*: Observed in our lab.

CHARACTERISTIC	<i>Sutterella wadsworthensis</i> (WAL 9799) [71]	<i>Sutterella parvirubra</i> (YIT 11816) [54]	<i>Parasutterella excrementihominis</i> (Strain YIT 11859^T) [46]	<i>Turicimonas muris</i> (strain YL45) [33]
Size (µm)	0.5-1 x 1-3	0.4-1 x 0.4-2	0.4-1.1 - 0.9-2.6	1-2µm length
Shape	Straight rods	Cocoid/Coccobacilli	Coccobacilli	Straight rods
Motility	N/A	No	No	N/A
Gram	Negative	Negative	Negative	Negative
Spore-forming	N/A	No	No	N/A
Oxidase	No	Negative	No	N/A
Catalase		Negative	No	No
Nitrate-reduction	Yes	No	No	N/A
Urease reduction	No	N/A	No	No
Indoxyl acetate reduction	No			N/A
Assacharolytic	Yes	Yes	Yes	N/A
Resistance to bile	Yes	N/A	Yes	N/A
G+C Content	N/A	64.4%	49.8%	44.0%
Growth in anaerobic environment	GAM Brucella agar + 0.3% Formate and 0.3% Fumarate (With and without 5% laked sheep blood)	GAM PYG broth	Peptone yeast extract broth Anaerobe basal agar (pH6.0)	AAM

Growth in 1% oxygen	Agar plates: No (GAM)* Liquid culture: Yes (GAM)*	Agar plates: No (GAM)* Liquid culture: Yes (GAM)*	Not Tested	Agar plates: Yes (AAM heavily contaminated) * Liquid culture: No
Growth in 2% oxygen	GAM Brucella agar + 0.3% Formate and 0.3% Fumarate (With and without 5% laked sheep blood)	N/A	No	N/A
Growth in 6% oxygen	GAM Brucella agar + 0.3% Formate and 0.3% Fumarate (With and without 5% laked sheep blood)	N/A	No	N/A

TABLE 4 | Presence of genes of interest for the physiology and sulphur metabolism in Sutterellaceae

Gene	<i>Sutterella wadsworthensis</i> (WAL 9799) Genome accession number: HM037997	<i>Sutterella parvirubra</i> (YIT 11816) Genome accession number: AB300989	<i>Parasutterella excrementihominis</i> (Strain YIT 11859^T) Genome accession number: AB370250	<i>Turicimonas muris</i> (strain YL45) Genome accession number: KR364792
TCA Cycle				
TCA Cycle				
Aconitate hydratase A	+	-	-	-

(AcnA)	Gene locus tag: I6J16_06910 (from KEGG)			
Isocitrate deshydrogenase	-	-	-	-
2-oxoglutarate dehydrogenase E1 component (SucA)	-	-	+	+
			Gene locus tag: WP_300876567 (from BLASTp)	Gene locus tag: WP_308513893.1 (from BLASTp)
2-oxoglutarate dehydrogenase E2 component	-	-	-	-
succinate---Co A ligase (GDP-forming) (SucC)	-	-	+	+
			Gene locus tag: SucC (F3QLL7) (from Uniprot)	Gene locus tag: SucC (A0A227KI) (from Uniprot)
succinate---Co A ligase (ADP-forming) subunit alpha (SucD)	-	-	+	+
			Gene locus tag: SucD (F3QLL6) (from Uniprot)	Gene locus tag: SucD (A0A227KJ08) (from Uniprot)
succinyl-CoA: acetate CoA-transferase (AarC)	-	-	-	-
Fumarate reductase flavoprotein subunit	+	+	+	+
	Gene locus tag: I6J16_00910 (from KEGG)	Gene locus tag: MDY5202054.1 (from BLASTp)	Gene locus tag: WP_217984062.1 (from BLASTp)	Gene locus tag: WP_270208846.1 (from BLASTp)
Fumarate reductase iron sulphur subunit	+	+	+	+
	Gene locus tag: I6J16_00915 (from KEGG)	Gene locus tag: WP_008543928.1 (from BLASTp)	Gene locus tag: WP_278748110.1 (from BLASTp)	Gene locus tag: WP_304419150.1 (from BLASTp)

Fumarate reductase subunit C	+	unclear	unclear	unclear
	Gene locus tag: I6J16_11855 (from KEGG)			
Fumarate hydratase class II	+	unclear	+	+
	Gene locus tag: I6J16_07350 (from KEGG)		Gene locus tag: HMPREF9439_01476 (from Uniprot)	Gene locus tag: fumC (A0A227KKC0) (from Uniprot)
Malate dehydrogenase	+	+	+	+
	Gene locus tag: I6J16_01470 (from KEGG)	Gene locus tag: mdh (H3KEG6) (from Uniprot)	Gene locus tag: mdh (A0A6I3RZP9) (from Uniprot)	Gene locus tag: mdh (A0A227KS24) (from Uniprot)
Citrate (Si)-synthase	-	-	-	-
ATP citrate synthase	-	-	-	-
Citrate (Re)-synthase	-	-	-	-
Putative map of <i>Sutterella wadsworthensis</i> metabolism				
C4 dicarboxylate transporter (DcuA)	+	+	+	+
	Gene locus tag: HMPREF1476_01565 (strain HGA0223) (from Uniprot)	Gene locus tag: HMPREF9440_01923 (from Uniprot)	Gene locus tag: GMD42_13360 (from Uniprot)	Gene locus tag: ADH67_11070 (from Uniprot)
Aspartate ammonia lyase	+	+	+	+
	Gene locus tag: I6J16_00920 (from KEGG)	Gene locus tag: HMPREF9440_02498 (from Uniprot)	Gene locus tag: GMD42_08960 (from Uniprot)	Gene locus tag: ADH67_11075 (from Uniprot)
Nitrate reductase (cytochrome) (NapA)	+	+	+	+
	Gene locus tag: I6J16_10195 (from KEGG)	Gene locus tag: NapA (H3KE43) (from Uniprot)	Gene locus tag: NapA (A0A6I3SC05) (from Uniprot)	Gene locus tag: NapA (A0A227KPV0) (from Uniprot)

Formate dehydrogenase	+	+	+	+
	Gene locus tag: HMPREF1476_01288 (strain HGA0223) (from Uniprot)	Gene locus tag: HMPREF9440_02414 (alpha subunit) (from Uniprot)	Gene locus tag: GMD42_09850 (from Uniprot)	Gene locus tag: ADH67_11730 (from Uniprot)
Cytochrome c sulphite reductase MccA	+	+	+	+
	Gene locus tag: WP_005434753.1 (Unspecific BLAST result: Cytochrome c3 family) (from BLASTp)	Gene locus tag: MCI7710020.1 (Unspecific BLAST result: cytochrome C) (from BLASTp)	Gene locus tag: WP_373216893.1 (Unspecific BLAST result: multi-heme c-type cytochrome) (from BLASTp)	Gene locus tag: WP_283590638.1 (Unspecific BLAST result: multi-heme c-type cytochrome) (from BLASTp)
Cysteine metabolism				
Cysteine Synthase A (CysK)	+	-	+	+
	Gene locus tag: I6J16_10830 (from KEGG)		Gene locus tag: A0A6I3S743 (CysK) (from Uniprot)	Gene locus tag: ADH67_11415 (from Uniprot)
Cysteine Synthase B (CysM)	+	unclear	unclear	unclear
	Gene locus tag: WP_016474243.1 (from BLASTp)			
Thiosulphate reduction				
Thiosulfate reductase / polysulfide reductase chain A	+	+	+	+
	Gene locus tag: I6J16_05350 (from KEGG)	Gene locus tag: MDY5201591.1 (from BLASTp)	Gene locus tag: WP_008863757.1 (from BLASTp)	Gene locus tag: WP_277040750.1 (from BLASTp)
DMSO and Tetrathionate reduction				
Tetrathionate reductase A	-	-	+	-
			Gene locus tag: WP_337582520.1 (molybdopterin dependent oxidoreductase) (from BLASTp)	
Dimethyl sulphoxide reductase A	+	+	+	+
	Gene locus tag: WP_005437105.1	Gene locus tag: WP_245523567.1		Gene locus tag: WP_066592158.1

	(from BLASTp)	(from BLASTp)	Gene locus tag: HMPREF9439_01 496 (from Uniprot)	(from BLASTp)
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