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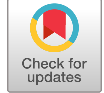

Lifestyle and Horizontal Gene Transfer-Mediated Evolution of *Mucispirillum schaedleri*, a Core Member of the Murine Gut Microbiota

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ABSTRACT *Mucispirillum schaedleri* is an abundant inhabitant of the intestinal mucus layer of rodents and other animals and has been suggested to be a pathobiont, a commensal that plays a role in disease. In order to gain insights into its lifestyle, we analyzed the genome and transcriptome of *M. schaedleri* ASF 457 and performed physiological experiments to test traits predicted by its genome. Although described as a mucus inhabitant, *M. schaedleri* has limited capacity for degrading host-derived mucosal glycans and other complex polysaccharides. Additionally, *M. schaedleri* reduces nitrate and expresses systems for scavenging oxygen and reactive oxygen species *in vivo*, which may account for its localization close to the mucosal tissue and expansion during inflammation. Also of note, *M. schaedleri* harbors a type VI secretion system and putative effector proteins and can modify gene expression in mucosal tissue, suggesting intimate interactions with its host and a possible role in inflammation. The *M. schaedleri* genome has been shaped by extensive horizontal gene transfer, primarily from intestinal *Epsilon-* and *Deltaproteobacteria*, indicating that horizontal gene transfer has played a key role in defining its niche in the gut ecosystem.

IMPORTANCE Shifts in gut microbiota composition have been associated with intestinal inflammation, but it remains unclear whether inflammation-associated bacteria are commensal or detrimental to their host. Here, we studied the lifestyle of the gut bacterium *Mucispirillum schaedleri*, which is associated with inflammation in widely used mouse models. We found that *M. schaedleri* has specialized systems to handle oxidative stress during inflammation. Additionally, it expresses secretion systems and effector proteins and can modify the mucosal gene expression of its host. This suggests that *M. schaedleri* undergoes intimate interactions with its host and may play a role in inflammation. The insights presented here aid our understanding of how commensal gut bacteria may be involved in altering susceptibility to disease.

KEYWORDS DNRA, *Deferribacteres*, gut microbiota, *Helicobacter*, fluorescence *in situ* hybridization, metatranscriptomics

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Mucispirillum schaedleri is a non-spore-forming, flagellated anaerobe with a spiral or broken-stick morphology thought to assist movement through the viscous gut mucus layer (1, 2). *M. schaedleri* has a long history of being included in defined microbial consortia for gnotobiotic laboratory animal studies (3–5) and is one of eight species in the widely used category altered Schaedler flora (ASF) (6). Members of the genus *Mucispirillum* has been detected in a variety of hosts, including pigs, goats, dogs, rats, mice, turkeys, termites, and cockroaches (7–15). *M. schaedleri* is a core member of the laboratory mouse microbiota and can colonize the intestinal tract from the stomach to the colon (16). As part of the phylum *Deferribacteres* (17) (Fig. 1), *Mucispirillum* stands out as one of the few taxa (genus classification and above) commonly found in mice but not humans (18). It has, however, occasionally been detected in humans (19), which may be due to either transient or infrequent colonization or its presence at an abundance below the detection limit of standard sequencing efforts.

Mucispirillum has been associated with both inflammatory markers and active colitis in the T-bet$^{-/-}$ Rag2$^{-/-}$ mouse model, in chemically induced colitis, and during *Citrobacter rodentium* infection (20–23). ASF mice infected with *Helicobacter bilis* exhibited an IgG response to *M. schaedleri* (24), indicating that it can become the target of a systemic immune response potentially via translocation across the intestinal mucosal barrier (25). In a study of diet-induced weight modification, *Mucispirillum* was positively correlated with serum leptin levels (26), which may be a feed-forward loop to maintain its niche, as luminal leptin induces mucin secretion (27, 28). Leptin is also thought to be released into the lumen during colitis (29), which may contribute to *Mucispirillum* expansion during intestinal inflammation. Despite its localization to the mucus layer and association with mucus production, it has not, however, been identified as a significant degrader of host-derived compounds *in vivo* (30).

Though it is a core member of the murine gut microbiota and increases during conditions of inflammatory stress, the genetic and physiological features of *M. schaedleri* remain poorly understood. In this study, we analyzed and compared the draft genomes of two recently diverged lineages of *M. schaedleri* ASF 457. We performed physiological experiments to test key features predicted by the genomes. We also identified genes expressed by *M. schaedleri in vivo* using newly generated and previously published metatranscriptomic data from gnotobiotic and conventional mice. Together, these results provide a comprehensive picture of the evolution and intestinal lifestyle of this inflammation-associated mucus-dwelling bacterium and further our understanding of its potential to be an intestinal pathobiont.

RESULTS

Genomic features. (i) Genome reconstruction and comparison. The assembled genomes of variants MCS and AYGZ have 36 and 39 contigs, respectively, and were estimated to be largely complete based on detection of a complete set of tRNAs and conserved housekeeping genes (see Table S1 in the supplemental material). The two genomes are very similar, with only a few shared genes having nonidentical sequences. The nonidentical, shared genes generally have high sequence identity (>99%) and include genes for hydrogenase 2, transposases, transporters, and multiple proteins with unknown functions, indicating that the genomes diverged little from one another and that differences consist of only a small number of single nucleotide polymorphisms.

(ii) Central metabolism. The genomes predict that *M. schaedleri* altered Schaedler flora 457 (ASF 457) harbors a complete Embden-Meyerhof-Parnas (EMP) pathway and a nonoxidative pentose phosphate pathway, as well as a complete tricarboxylic acid (TCA) cycle that features a *Helicobacter*-type succinyl-coenzyme A (CoA):acetoacetate CoA transferase (SCOT; EC 2.8.3.8) (Fig. 2). It has complete biosynthesis pathways for most amino acids, but several pathways, such as for methionine and tryptophan, are incomplete or not detected (Text S1). *M. schaedleri* may therefore be reliant on amino acid or oligopeptide transporters for growth. Ammonia can be assimilated via a glutamate dehydrogenase (GdhA, EC 1.4.1.4), and a type 3 glutamine synthetase (GlnA, EC 6.3.1.2) (31). A description of cofactor and vitamin biosynthesis pathways, storage

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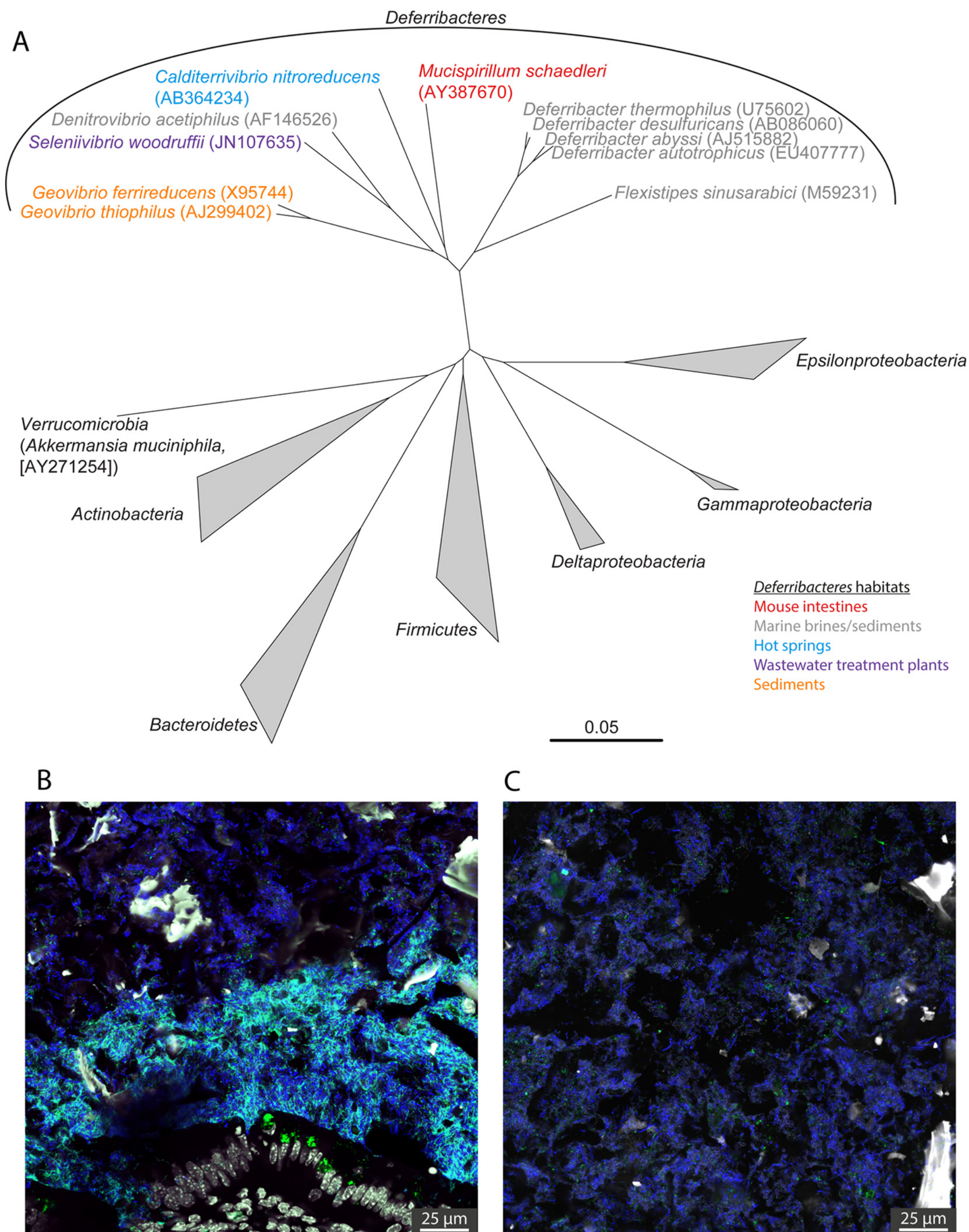

FIG 1 Phylogeny and habitat of *Mucispirillum*. (A) Phylogenetic tree of *M. schaedleri* in relation to other cultured members of the *Deferribacteres* and abundant gut taxa based on the maximum-likelihood method using the 16S rRNA gene and 500 bootstraps. The source of isolation of the members of the *Deferribacteres* is indicated by color. GenBank accession numbers are shown in parentheses. Scale bar, 0.05 change per nucleotide position. (B and C) Fluorescence *in situ* hybridization image of the ceca of ASF[4] mice colonized with *M. schaedleri* ASF 457 (Cy3, green), showing localization proximal to the mucosa (B) and its almost complete absence in the lumen (C). All bacteria (targeted by the EUB338I-III mix, Cy5) are blue, and DAPI (4′,6-diamidino-2-phenylindole)-stained cells are shown in gray.

compounds, motility and chemotaxis genes, a clustered regularly interspaced short palindromic repeat (CRISPR) system, mobile genetic elements, and transporters can be found in Text S1.

(iii) Putative electron donors and carbon sources. The *M. schaedleri* genome features an extremely limited repertoire of polysaccharide degradation machinery, consisting of just 3 glycoside hydrolases (family 57 α-amylases) that are likely used for

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Abbrevations

Cat	Catalase
CCO	Cytochrome c oxidase
CCP	Cytochrome c peroxidase
DNRA	Dissimilatory nitrate reduction to ammonium
EMP	Embden–Meyerhof– Parnas
FR	Fumarate reductase
GCK	Glucokinase
GK	Glycerol kinase
GLDH	Glutamate dehydrogenase
Glp	Sn-glycerol-3-phosphate dehydrogenase
GP	Glycerophosphodiester phosphodiesterase
GS	Glutamine synthetase
HK	Histidine kinase
HYD	Hydrogenase
Hyb	Hydrogenase 2
MCP	Methyl-accepting chemotaxis protein
NapA	Perisplasmic nitrate reductase
NrfA	Cytochrome c nitrite reductase
PolyP	Polyphosphate
Rbr	Rubrerythrin
Rnf	Ferredoxin:NAD+ oxidoreductase
SOR	Superoxide reductase
TMAO	Trimethylamine-N-oxide reductase
TrxR	Thioredoxin reductase
TCA	Tricarboxylic acid

Amino Acid Biosynthesis

COMPLETE: Alanine, Arginine, Aspartate, Cysteine, Glutamate, Glutamine, Glycine, Homocysteine, Isoleucine, Leucine, Phenylalanine, Proline, Tyrosine, Valine

NEARLY COMPLETE: Histidine, Lysine, Ornithine

INCOMPLETE/ABSENT: Asparagine, Homoserine, Methionine, Selenocysteine, Serine, Threonine, Tryptophan

FIG 2 Selected genomic features of *M. schaedleri* ASF 457. Predicted metabolic and physiological capabilities based on genome annotations are shown.

glycogen storage (Text S1). Genes for degradation of glycerophosphodiester and glycerol, as well as for transport of glucose, dicarboxylic acids, and short-chain fatty acids, were also detected (Text S1). The genomes encode 15 proteases and 3 aminopeptidases, a subset of which (4 for AYGZ, 5 for MCS) are predicted to be secreted. Catabolic pathways for glutamine, asparagine, and cysteine were identified. We detected multiple ABC transporters for amino acids, including for leucine, isoleucine, valine, and methionine. Transporters were also present for peptides and polyamines (ABC type), oligopeptides, and a permease for oligopeptides. We detected hydrogenase 2 (EC 1.12.7.2), which is a membrane-bound Ni-Fe hydrogenase that reduces menaquinone to menaquinol in a reversible reaction (32). Although we detected a formate dehydrogenase, which allows for reversible NAD-dependent interconversion of formate and CO_2, we found neither hydrogenase 3 nor hydrogenase 4, so there is no evidence for the presence of a formate-hydrogen lyase.

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(iv) Respiration and oxidative-stress response. *M. schaedleri* has genes for dissimilatory nitrate reduction to ammonia (DNRA), with a periplasmic nitrate reductase (NapA, EC 1.7.99.4) as well as a nitrite reductase (NrfA, EC 1.7.2.2). The presence of genes for fumarate reductase suggests that fumarate can also be used as a terminal electron acceptor for anaerobic respiration. We also detected genes for the membrane-bound Rnf complex, which is proposed to couple the electron transfer from reduced ferredoxin to NAD^+ with the translocation of Na^+ ions across the cytoplasmic membrane via a Na^+-translocating ferredoxin:NAD^+ oxidoreductase and thereby generate a sodium ion gradient (33).

M. schaedleri has genes for a high-affinity cbb3-type cytochrome *c* oxidase (EC 1.9.3.1), which may be used either for protection from O_2 stress (34, 35) or for microaerobic respiration (36, 37). Several genes for detecting and defending against oxidative stress, including a superoxide reductase, catalase, cytochrome *c* peroxidase, rubrerythrin, and thioredoxin reductase, were detected. The genome also includes genes for a nitroreductase as well as other nitroreductase family proteins, which may be used for scavenging nitrogen radicals formed during nitrate and nitrite reduction. We detected genes for a putative trimethylamine-*N*-oxide reductase (EC 1.7.2.3) for the reduction of trimethylamine-*N*-oxide (TMAO) into trimethylamine (TMA), which may serve as a trophic link to methylotrophic methanogens in the gut that use methylated amino compounds like TMA in conjunction with H_2 during methanogenesis (38).

(v) Secretome and putative interaction genes. Parts of either a type II secretion system (T2SS) or a type IV pilus biogenesis machinery (T4P) were present in the genome. The T2SS is widely distributed especially among *Proteobacteria*, most of which are extracellular pathogens, and is usually encoded by at least 12 genes in a single operon (39, 40). We detected genes for only 5 proteins, the major prepilins T2SC to T2SG. Seven of the T2SS core proteins (T2SH to T2SO) (41) seem to be missing, indicating either a nonfunctioning T2SS system or, alternatively, the presence of a T4P or DNA uptake machinery (42). A gene encoding PilZ, a putative T4P assembly protein, is located in close vicinity to the T2SS/T4P genes. We also detected a PilT protein, which has been proposed to be a force-generating protein for pilus retraction (43).

We detected a putative type IVA secretion system (T4ASS), including five Tra conjugal transfer proteins (TraC, TraD, TraF, TraG, TraL), three Trb conjugal transfer proteins (TrbB, TrbD, TrbG), and a VirB complex with a type IV secretion/conjugal transfer ATPase VirB4 family protein and a VirB8 family protein belonging to the putative T4ASS (44), which may mediate horizontal gene transfer (HGT) (45) via conjugation or play a role in pathogenicity (46, 47). Virulence-associated protein D (VapD) was found located between the Tra and Trb loci of the T4ASS and several transposases, integrases, and tRNA genes. The exact biological role of VapD has not yet been established, but it is known as alpha-toxin in *Haemophilus influenzae* and as a typical prokaryotic toxin with the activity of an mRNA interferase (48). Though many pathogens carry multiple *vap* genes, *Helicobacter pylori* also carries only the *vapD* gene (48).

The type VI secretion system (T6SS) consists of 13 conserved core proteins necessary for function (49). *M. schaedleri* has bacteriophage-like components of the T6SS, including hemolysin-coregulated protein (Hcp), valine-glycine repeat protein G (VgrG, putatively), TssB/C, which complexes to form a needle sheath (50), and TssE, which is homologous to the bacteriophage baseplate protein gp25 (51). It also has membrane-associated proteins TssL, TssM, TssJ, and other T6SS proteins with unknown functions (TssA, TssF, TssG, and TssK). Additionally, homologs to other associated proteins, such as ClpV and a putative eukaryote-like phospholipase D protein, which is involved in destabilization of the host cell membrane (52), were also detected.

Ankyrin repeats (ANK) are found primarily in eukaryotic genomes, but proteins with ANK domains are also present in some symbiotic and pathogenic bacteria (53). We detected a total of 10 genes with ANK domains in ASF 457. Eleven genes were identified with tetratricopeptide repeat (TPR) domains, which are involved in virulence

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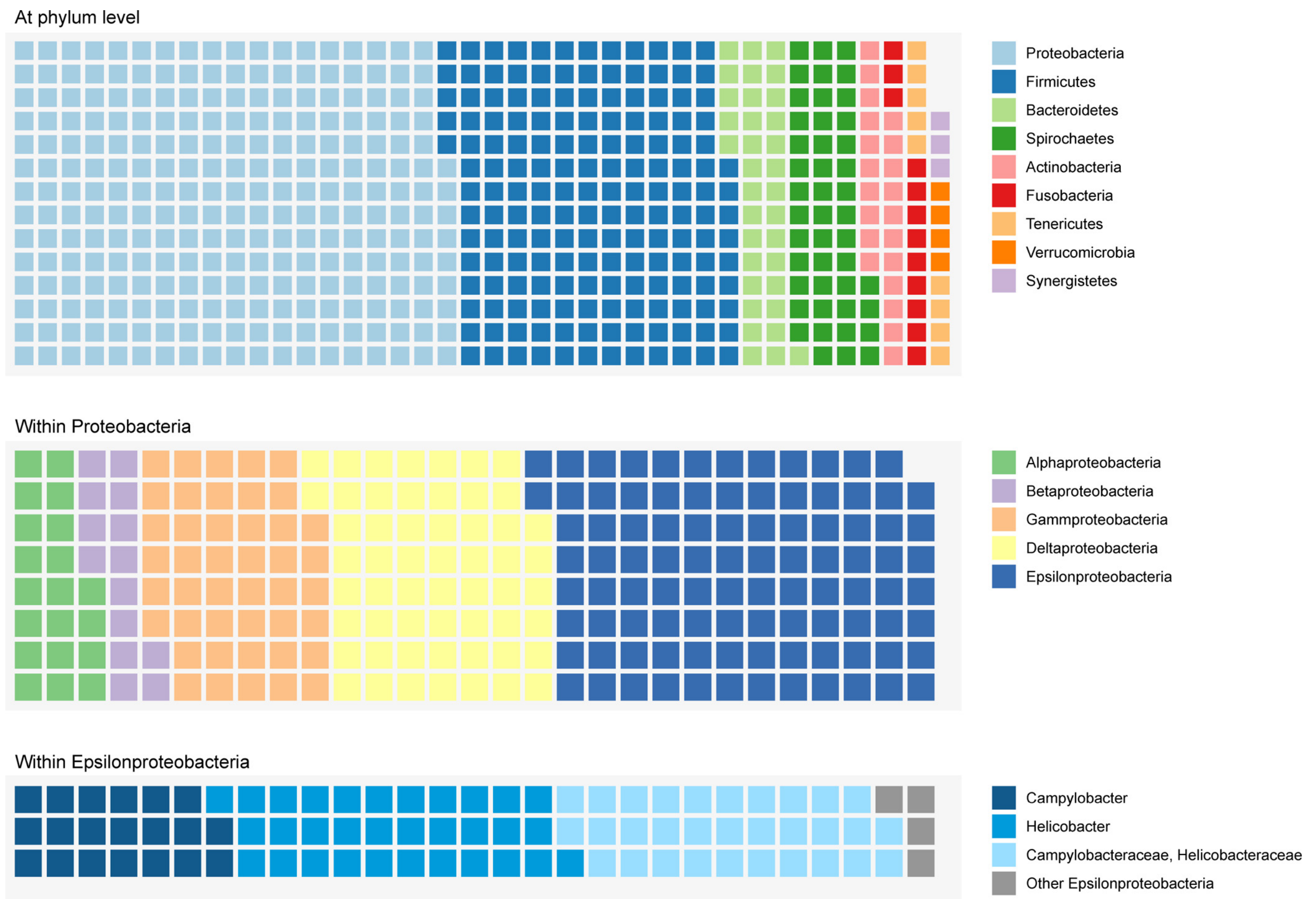

FIG 3 Putative sources of interphylum horizontal gene transfer in the *M. schaedleri* ASF 457 AYGZ genome. Each block represents a putative horizontally transferred gene. Blocks are colored by the predicted source of the gene and are shown at the phylum level; classes within the *Proteobacteria* and genera within the *Epsilonproteobacteria* are also shown. Nonhorizontally transferred genes are not shown.

in bacterial pathogens (54). In addition, we detected hemolysin-3, a putative colicin V production protein (55, 56), and seven genes encoding putative β-lactamases (EC 3.5.2.6).

Putative horizontally transferred genes. Phylogenetic trees could be calculated for most (1,599) of the genes in the AYGZ genome. More than half of the genes have putatively been horizontally transferred between *M. schaedleri* and bacteria not belonging to the phylum *Deferribacteres*. In comparison, only 7% and 4% of genes of the genomes of the abundant gut bacteria *Bacteroides thetaiotaomicron* and *Ruminococcus bromii*, respectively, were found by the same analysis to be putative interphylum transfers. According to our analysis, many putatively horizontally transferred genes originate from *Proteobacteria* ($n = 261$) and *Firmicutes* ($n = 168$) (Fig. 3). Among *Proteobacteria*, *Epsilonproteobacteria* contributed the majority of transferred genes ($n = 97$), with the largest fraction coming from *Campylobacter* and *Helicobacter* spp. Among the *Firmicutes*, *Clostridia* contributed the largest number of genes ($n = 108$). Many of the nearest neighbors within the gene trees derived from genomes classified to the genus level as *Eubacterium* and *Clostridium*.

Most of the putative horizontally transferred genes are classified as being involved in replication, recombination, and repair (cluster of orthologous groups [COG] category L), with a large fraction coming from *Firmicutes* (Fig. S1). *Firmicutes* are also by far the largest group contributing to coenzyme transport and metabolism (COG category H) and inorganic iron transport and metabolism (COG category P), whereas *Proteobacteria* appear to be an important source for genes in most of the other COG categories. In

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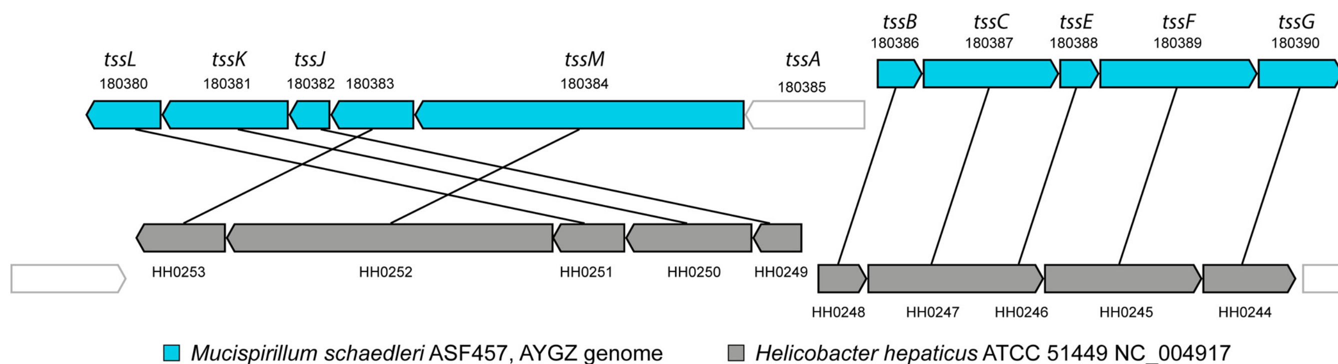

FIG 4 Synteny of type VI secretion system (T6SS) genes of *M. schaedleri* ASF 457 AYGZ and *Helicobacter hepaticus*. Gene names and loci are listed. TssB and TssC are components of the bacteriophage-like contractile sheath. TssE, TssJ, TssK, TssL, and TssM are components of the baseplate (97).

addition, *M. schaedleri* putatively acquired several genes involved in virulence, resistance, and defense, and mobile genetic elements from other bacteria (Text S1; Fig. S1). Of note, the T6SS appears to have been horizontally transferred from *Epsilonproteobacteria*. No homologs of *M. schaedleri* genes belonging to the T6SS are present in other *Deferribacteres* genomes, and nearly all genes share a node in their phylogenetic trees with just *Campylobacter* and/or *Helicobacter* (Fig. S2); in addition, T6SS genes (minimum of 30% identity, matching at least 80% of the gene) shared between *M. schaedleri* and *Helicobacter hepaticus* had high synteny, with an almost identical gene order (Fig. 4).

Physiological experiments. We tested selected features of the genome predictions in pure-culture physiological experiments with *M. schaedleri* ASF 457 MCS. Addition of nitrate significantly boosted the growth of *M. schaedleri*, and nitrate was completely reduced to ammonium, with no detectable nitrite accumulation, indicating that *M. schaedleri* is indeed capable of DNRA (Fig. 5A). To determine whether increased growth was due to nitrate reduction or to additional ammonium as a nitrogen source, *M. schaedleri* was incubated with different combinations of nitrate and/or ammonium. While ammonium alone could not elevate growth, it also did not inhibit growth in combination with nitrate (Fig. S3A). Fumarate reduction to succinate, which was also predicted by the genome, was also confirmed (Fig. S3B). Incubations with combinations

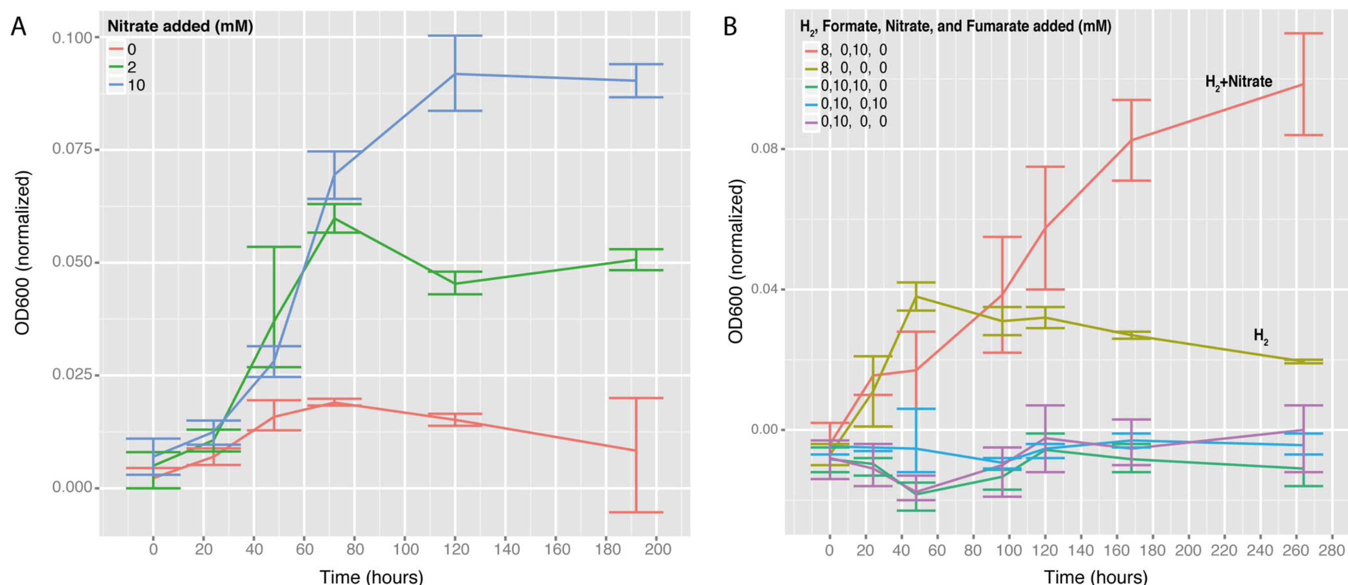

FIG 5 Physiological experiments of *M. schaedleri* ASF 457 MCS. Pure cultures were grown in AMM with various amounts of nitrate (A) or combinations of nitrate, formate, fumarate, and hydrogen (B). Growth was determined spectrophotometrically (at 600 nm) and was normalized by subtracting the background absorbance of the medium. Means and standard deviations of results from three replicate experiments are shown.

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of H_2 or formate as an electron donor and nitrate and fumarate as electron acceptors were also performed. H_2 supported growth, but no growth was detected for cultures with formate as the electron donor, even in the presence of nitrate or fumarate (Fig. 5B).

Gene expression in gnotobiotic and conventional mice. Published metatranscriptomes from the intestinal microbiota of conventional mice were screened for reports of *Mucispirillum*. Reads from metatranscriptomes containing *Mucispirillum* spp. were then mapped to the AYGZ genome, as it has slightly more genes. Putative *M. schaedleri* transcripts were detected in 48 libraries from three studies and included cecum, colon, or pooled cecum and colon contents of healthy mice (57), dextran sulfate sodium (DSS)-treated mice (58), and nonobese diabetic mice (59). Transcripts were detected at relatively low abundance in all samples (median, 515 reads; range, 4 to 11,910 reads), which is consistent with the typically low abundance of *Mucispirillum* organisms. In total, reads mapped to 851 genes (37% of predicted genes in the genome). Among the most highly expressed were genes involved in DNRA as well as cytochrome *c* biogenesis, though not the hydrogenase 2 or the T6SS gene (Table S2).

The published study with the largest number of mapped reads compared cecum and colon metatranscriptomes (59), and we attempted to determine whether there was differential gene expression in *M. schaedleri* between these two intestinal compartments. No differential gene expression was detected, though this may have been due to limited sequencing depth (median, 694 reads; range, 195 to 4,984 reads). The properties of the mucus layer are different between these two compartments (60), so to further explore whether there is differential expression of *M. schaedleri* genes in the cecum and colon, we compared levels of gene expression in gnotobiotic mice colonized with a consortium of four ASF species (ASF^4) and *M. schaedleri*. Between 1.2 and 3% of mapped reads were mapped to *M. schaedleri*, with no statistically significant difference in relative abundances between the two compartments (median, 68,800 reads; range, 38,570 to 193,800 reads). Transcripts from 2,015 genes (87% of all genes) were detected in the RNA sequencing (RNA-seq) libraries. Among the most-expressed genes included those involved in DNRA, hydrogenase 2, the oxidative-stress response (rubrerythrin and catalase), and the T6SS. Five *M. schaedleri* genes were detected as differentially expressed (all upregulated) in the colons of the gnotobiotic animals relative to in their ceca (Table S3A). All five genes have unknown functions, though three are predicted to be exported.

Mucispirillum has been reported to be elevated in abundance during intestinal inflammation (22, 23, 61), and we therefore evaluated its gene expression using a gnotobiotic DSS colitis mouse model harboring the eight strains of the ASF (ASF^8). As expected, DSS treatment induced colitis, including significant weight loss, colonic shortening, and gross lesions compared to conditions for control mice (Fig. S4). RNAs from pooled cecal and colon contents were sequenced, and between 1.3 and 4.1% of mapped reads were mapped to *M. schaedleri* (median, 81,760 reads; range, 55,300 to 286,700 reads). Transcripts from 2,036 genes (89% of all genes) were detected overall. Among the most highly expressed genes included those involved in DNRA, cytochrome *c*, hydrogenase 2, and the oxidative-stress response (rubrerythrin, catalase, and superoxide dismutase). Surprisingly, only 12 genes were differentially expressed during inflammation, 11 of which were upregulated and 1 of which was downregulated (Table S3B). Genes in the putative type IVA secretion system were upregulated, as were genes encoding an uncharacterized reductase system with homology to dimethyl sulfoxide (DMSO) reductase of *Escherichia coli* and the tetrathionate reductase and trimethylamine-*N*-oxide reductase of *Salmonella enterica* serovar Typhimurium (32 to 36% identity) (AYGZv1_260003 and AYGZv1_260004, respectively).

Mouse mucosal tissue gene expression. To test whether *M. schaedleri* affects host physiology, we compared the transcriptional profiles of the cecal mucosal tissues of ASF^4 mice with or without *M. schaedleri* ASF 457 MCS using microarray technology. Gene set enrichment analysis (GSEA) revealed that the presence of *M. schaedleri* was associated with the selective transcription of several gene sets, including those for

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upregulation of translation and respiratory electron transport, as well as chemokine receptors and chemokines (Table S4A). Gene sets associated with the complement and coagulation cascades, lipoprotein metabolism, and mitosis were downregulated (Table S4B). Upstream regulator analysis predicted several regulators that may be activated (such as NF-κB and PPAR-delta) or inhibited (such as epidermal growth factor [EGF]) by *M. schaedleri* (Table S5).

DISCUSSION

Metabolic strategies of *M. schaedleri*. *M. schaedleri* has an extremely limited repertoire of carbohydrate degradation machinery, with just 3 glycoside hydrolases (family 57 α-amylases) that are likely used for processing the storage compound glycogen (see Text S1 in the supplemental material). The absence of specialized glycan-degrading enzymes was unexpected, as *M. schaedleri* inhabits a mucus layer composed of abundant complex glycoproteins, such as mucin. In comparison, gut polysaccharide degraders, such as *Bacteroides* spp., have on average 137 glycoside hydrolases (62), and *Akkermansia muciniphila*, a dedicated intestinal mucin degrader, has 35 predicted glycoside hydrolases (63). It therefore seems that *M. schaedleri* is not a primary degrader of host-derived glycans and has limited capacities to utilize dietary polysaccharides. The genome predicts that *M. schaedleri* rather uses monosaccharides, oligopeptides, amino acids, glycerol, and short-chain fatty acids (SCFAs) as the substrates for its energy metabolism (Fig. 2). It is therefore likely a consumer of breakdown products produced by hydrolytic/fermentative microorganisms, such as *Bacteroidaceae* and *Ruminococcaceae* species (62). *M. schaedleri* also has a hydrogenase (*hyb*), and addition of H_2 in pure cultures dramatically improved its growth (Fig. 5B). As hydrogenases 3 and 4 were not found and the addition of formate did not improve growth in pure cultures (Fig. 5B), it is unlikely that *M. schaedleri* produces H_2. It is therefore probably dependent on cross-feeding of H_2 produced by other fermentative species, analogously to how Salmonella Typhimurium is dependent on microbiota-derived H_2 for establishment in the gut (64). Future colocalization and coculture studies are needed to provide insights into whether *M. schaedleri* is preferably associated with certain polysaccharide-degrading species in the mucus layer, as these species may provide it with nutrients such as monosaccharides, amino acids, and H_2.

Nitrate is an important electron acceptor in the gut, particularly during inflammation, when levels are increased due to release of nitrogen radicals from the oxidative burst (65). *M. schaedleri* can utilize nitrate as a terminal electron acceptor via dissimilatory reduction of nitrate to ammonia (DNRA) using the periplasmic enzyme NapA for conversion of nitrate to nitrite and NrfA for reduction of nitrite to ammonia (66). In addition to nitrate reduction, *M. schaedleri* has genes for a fumarate reductase that converts fumarate to succinate and encodes a C_4-dicarboxylate transport/antiport system (*dcuAB*), which is necessary for anaerobic respiration with fumarate. In pure-culture experiments, the addition of nitrate or fumarate substantially enhanced the growth rate and yield (Fig. 5), suggesting that nitrate may partially fuel the *Mucispirillum* blooms observed during inflammation (22).

Resistance to oxidative stress. The intestinal mucosa is thought to be micro-oxic, and additionally, reactive oxygen and nitrogen species are increased during inflammation (67, 68). *M. schaedleri* has several systems for scavenging oxygen and reactive oxygen species, which may explain its persistence and increased relative abundance in the inflamed gut (22). Besides encoding superoxide reductase, catalase, and cytochrome *c* oxidase, the genome encodes rubrerythrin, an oxidative-stress response protein that acts as a hydrogen peroxidase reductase (69). *M. schaedleri* therefore seems to be well adapted to the micro-oxic conditions at the mucosa and in the elevated-redox environment in the gut during inflammation.

Secretome and possible interactions with the host. Protein secretion is used by bacterial pathogens as well as symbionts for mediating interactions with their hosts. We detected a eukaryote-like phospholipase D protein, a member of the type VI lipase effector superfamily that targets bacterial and eukaryotic membranes (52). It is possible

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that *M. schaedleri* uses its T6SS to antagonize other bacteria or for promoting the establishment of a mutualistic or pathogenic relationship with its host (70). The T6SS of *M. schaedleri* has probably been laterally transferred from either *Helicobacter* or *Campylobacter*, and the gene order is the same as in *H. hepaticus*, a spiral-shaped pathogen that also inhabits the murine intestinal mucus layer and plays an important role in the development of severe inflammatory bowel disease (71). Interestingly, the presence of the T6SS in *H. hepaticus* limits intestinal inflammation (72). It has yet to be shown whether the presence of *M. schaedleri* affects inflammation status or disease susceptibility. Microarray data, however, suggest that the presence of *M. schaedleri* does modify host mucosal tissue gene expression, and it appears to have proinflammatory properties (Tables S4 and S5). *M. schaedleri* also has several putative effector proteins with eukaryote-like domains, namely, ANK repeats and TPR-containing proteins, that can be used for interactions with the host and may also play a role during inflammation (73). Future studies are needed to establish whether *M. schaedleri* can act as a pathobiont, a member of the microbiota present in healthy hosts but able to alter susceptibility to inflammatory bowel disease or enteric infection (74), or is rather a commensal that benefits from the altered gut environment during inflammation.

Putatively horizontally transferred genes. Horizontal gene transfer (HGT) is a major source of phenotypic innovation and a way to facilitate niche adaptation. The amount of newly acquired genes in a bacterial genome is on average less than 15% (75, 76), though interphylum HGT is thought to occur more frequently in anaerobic bacteria (77). Microbiota perturbations and intestinal inflammation can, however, boost the frequency of HGT (78). More than half of the genes in the *M. schaedleri* genome were putative interphylum-transferred genes, which is much greater than the percentage of other abundant gut bacteria transferred. Many of these genes were not related to metabolic capacity but rather to features that may enhance survival and competitive growth in a selective mammalian gut environment. In particular, these genes are involved in interactions with other bacteria or the host (e.g., T6SS), resistance and defense (e.g., CRISPR, drug resistance), and mobile genetic elements. Horizontally transferred pathways like the T6SS and glycerol-3-phosphate utilization might be especially important for facilitating survival and establishment in the mammalian intestinal tract. Interestingly, several genes involved in chemotaxis, motility, and conjugation (those for T4P, T4ASS, and Tra conjugal transfer proteins) were putatively acquired via HGT. *Proteobacteria*, one of the core phyla in the mammalian gut, were the largest phylogenetic group contributing to the gene pool of *M. schaedleri*, and this was dominated by genes shared with the epsilonproteobacterial families *Helicobacteraceae* and *Campylobacteraceae*, which include inflammation-inducing enteric pathogens. Many of these genes are involved in pathogenicity and/or host interaction, which suggests that HGT contributes significantly to the putative pathobiont lifestyle of *M. schaedleri*.

Conclusions and outlook. Comprehensive study of *M. schaedleri* revealed that this mucus-associated bacterium is adapted to the high-redox environment of the mucus layer and is well equipped to handle oxidative bursts that occur during inflammation. In stark contrast to characterized mucus degraders, *M. schaedleri* has virtually no capacity to degrade complex polysaccharides. It therefore likely specializes in the utilization of small molecules. An exceptionally large number of genes were putatively horizontally transferred from other gut bacteria and particularly from members of the *Proteobacteria*, which are generally facultative anaerobes, inhabit the same gut microenvironment, and can tolerate high-reduction-potential conditions. This genome evolution led to the acquisition of a range of molecular mechanisms and effector proteins for interactions with the host. These genomic features, as well as the ability of *M. schaedleri* to modulate gene expression of immune-related genes, suggest that *M. schaedleri* may indeed be a pathobiont for certain diseases. Our analyses did not suggest any explanation for why *M. schaedleri* would not survive in the human gut, and it may be that its niche is already occupied by *Epsilonproteobacteria* such as *Helicobacter*

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spp., which are slightly better adapted to the human gut due to long-term coevolution (79).

MATERIALS AND METHODS

DNA sequencing and assembly. *Mucispirillum schaedleri* ASF 457 variant MCS was provided to Bärbel Stecher by Charles River Laboratories, Inc. (Wilmington, MA, USA). Nucleic acids were extracted using a phenol-chloroform-based extraction method (80). DNAs were sequenced using the Illumina HiSeq2000 with 3-kb mate pair libraries and MinION technology (Oxford Nanopore Technologies, Oxford, United Kingdom). Illumina data were quality filtered with prinseq-lite (81), and MinION data were filtered with poretools (82) and prinseq-lite. *De novo* genome assembly was performed using SPAdes (83).

Genome annotation. The MCS genome and the genome of *M. schaedleri* ASF 457 variant AYGZ (84) were annotated with the MicroScope Microbial Genome Annotation and Analysis Platform (85). Metabolic pathways were reconstructed using the MicroCyc and the KEGG (86) classification schemes within MicroScope. Further details about genome analysis are provided in Text S1.

Physiological studies. Unless otherwise stated, *M. schaedleri* ASF 457 MCS was cultured under anaerobic conditions under an N_2 and 8% H_2 atmosphere at 37°C without shaking using anaerobic *Mucispirillum* medium (AMM), which is based on Trypticase soy agar and contains (per liter) 18 g brain heart broth (Merck), 15 g tryptone soy broth (Oxoid), 5 g yeast extract (Bacto yeast extract), 2.5 g K_2HPO_4 (Carl Roth), 1 mg hemin (Sigma), 0.5 mg vitamin K_1 (Carl Roth), 0.4 g Na_2CO_3 (Carl Roth), 3% fetal calf serum (Sigma), 0.5 mg L-cysteine hydrochloride (Sigma), and 0.5 mg alpha-(D+)-glucose monohydrate (Carl Roth) (87). *M. schaedleri* ASF 457 MCS was analyzed for growth in AMM with or without the presence of the following compounds: hydrogen (8%), formate (0.5, 2.5, 10, or 50 mM), nitrate (2 or 10 mM), and fumarate (10 or 50 mM). Growth was quantified by optical density measured at 600 nm (OD_{600}) (M107 high-specification visible spectrophotometer; Spectronic Camspec Ltd., Leeds, United Kingdom). OD_{600} values were normalized by subtracting the background absorbance values of abiotic-medium controls.

Animal experiments. In order to evaluate the gene expression of *M. schaedleri* MCS in the cecum and colon, 4- to 6-week-old C57BL/6 mice harboring a reduced altered Schaedler flora (ASF 356, ASF 361, SB2 [a reisolate of ASF 502], and ASF 519 [ASF^4]; $n = 3$) were inoculated with *M. schaedleri* MCS and housed under gnotobiotic conditions in gnotocages. Ten days after inoculation, mice were euthanized and cecum and colon contents were collected separately and immediately frozen in liquid nitrogen for subsequent RNA-seq analysis. Animal experiments were approved by the Regierung von Oberbayern, Germany, and the local ethics committee.

To evaluate the gene expression of *M. schaedleri* during acute intestinal inflammation, age- and sex-matched 8-week-old C57BL/6 mice harboring the 8 taxa of the altered Schaedler flora (17) were maintained under gnotobiotic conditions and treated with 3% dextran sodium sulfate (DSS; molecular weights [MW], 36,000 to 50,000; MP Biomedicals, Solon, OH, USA) in the drinking water for 5 days ($n = 8$) and then given regular drinking water for another 3 days. Control animals ($n = 8$) were given drinking water without DSS for the entire study. Three days after DSS treatment, all animals were euthanized; cecal contents were collected and immediately frozen in liquid nitrogen for RNA-seq analysis. To assess disease severity, colon lengths and scores (0 to 5) were recorded at necropsy based on the presence (+1) or absence (0) of enlarged cecal tonsils, cecal atrophy, intestinal emptying, mucoid contents, and blood (modified from reference 88). All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Nebraska—Lincoln.

RNA-seq and metatranscriptomic analysis. Nucleic acids were extracted from collected samples, and DNase was digested twice and checked to be DNA free using PCR. rRNA was removed using the Ribo-Zero bacterial kit (Illumina, San Diego, CA) and evaluated using an RNA HighSens kit (Experion, Hercules, CA). RNA was prepared for multiplexed Illumina RNA-seq (NEBNext Ultra RNA library prep kit for Illumina with NEBNext multiplex oligonucleotides; New England Biolabs, Ipswich, MA) and sequenced on the HiSeqV4 SR100 platform (Campus Science Support Facilities GmbH, Vienna, Austria). Sequence data are available at the European Nucleotide Archive (ENA) under BioProject no. PRJEB13534. Published metatranscriptomic data sets from mice with detectable levels of *Mucispirillum* were downloaded from the National Center for Biotechnology Information Short Read Archive database and quality filtered using Trimmomatic (89). Reads were mapped to the *M. schaedleri* genome using BWA (90) and analyzed with HTSeq (91).

Mouse microarray analysis. C57BL/6 mice harboring a reduced altered Schaedler Flora (ASF^4; $n = 6$) or ASF^4 mice colonized with *M. schaedleri* MCS for 10 days ($n = 6$) were sacrificed. The cecum was washed in phosphate-buffered saline (PBS) to remove contents and stored in RNAlater (Qiagen). RNA was purified from cecal tissue samples using TRIzol (Life Technologies, Inc., Carlsbad, CA, USA) followed by RNeasy Microkit columns (Qiagen, Venlo, the Netherlands). RNA quality was assessed on the Agilent 2100 bioanalyzer (Agilent Technologies, Amsterdam, the Netherlands). RNA was labeled using an Affymetrix WT plus reagent kit and hybridized to GeneChip Mouse Gene 1.1 ST arrays (Affymetrix, Santa Clara, CA). Sample labeling, hybridization to chips, and image scanning were performed according to the manufacturer's instructions. Microarray analysis was performed using the MADMAX pipeline (92). Quality control was performed, and all arrays met our criteria. A custom annotation that combines all individual probes for a gene (93) was used. Expression values were calculated and normalized using the robust multichip average (RMA) method (94), and significant differences were assessed using the paired intensity-based moderated T statistic (IBMT) (95). Pathway analysis was performed by gene set enrichment analysis (96); upstream regulator analysis (Ingenuity) was also performed.

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Accession number(s). Genome and RNA-seq data are available at the European Nucleotide Archive (ENA) under BioProject no. PRJEB13534, and microarray data are available at the NCBI GEO repository under accession no. GSE83625.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mSystems.00171-16.

TEXT S1, PDF file, 0.6 MB.
FIG S1, PDF file, 0.3 MB.
FIG S2, PDF file, 0.4 MB.
FIG S3, PDF file, 0.4 MB.
FIG S4, PDF file, 0.2 MB.
TABLE S1, PDF file, 0.3 MB.
TABLE S2, XLSX file, 0.4 MB.
TABLE S3, PDF file, 0.4 MB.
TABLE S4, PDF file, 0.3 MB.
TABLE S5, PDF file, 0.3 MB.

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REFERENCES

1. Robertson BR, O'Rourke JL, Neilan BA, Vandamme P, On SLW, Fox JG, Lee A. 2005. *Mucispirillum schaedleri* gen. nov., sp. nov., a spiral-shaped bacterium colonizing the mucus layer of the gastrointestinal tract of laboratory rodents. Int J Syst Evol Microbiol 55:1199–1204. https://doi.org/10.1099/ijs.0.63472-0.
2. Young KD. 2006. The selective value of bacterial shape. Microbiol Mol Biol Rev 70:660–703. https://doi.org/10.1128/MMBR.00001-06.
3. Savage DC, Dubos R, Schaedler RW. 1968. The gastrointestinal epithelium and its autochthonous bacterial flora. J Exp Med 127:67–76. https://doi.org/10.1084/jem.127.1.67.
4. Dubos R, Schaedler RW, Costello R, Hoet P. 1965. Indigenous, normal, and autochthonous flora of the gastrointestinal tract. J Exp Med 122:67–76. https://doi.org/10.1084/jem.122.1.67.
5. Schaedler RW, Dubos R, Costello R. 1965. The development of the bacterial flora in the gastrointestinal tract of mice. J Exp Med 122:59–66. https://doi.org/10.1084/jem.122.1.59.
6. Orcutt RP, Giani FJ, Judge RJ. 1987. Development of an 'altered Schaedler flora' for NCI gnotobiotic rodents. Microecol Ther 17:59.
7. Scupham AJ, Patton TG, Bent E, Bayles DO. 2008. Comparison of the cecal microbiota of domestic and wild turkeys. Microb Ecol 56:322–331. https://doi.org/10.1007/s00248-007-9349-4.
8. Li RW, Wu S, Li W, Navarro K, Couch RD, Hill D, Urban JF. 2012. Alterations in the porcine colon microbiota induced by the gastrointestinal nematode Trichuris suis. Infect Immun 80:2150–2157. https://doi.org/10.1128/IAI.00141-12.
9. Hildebrand F, Nguyen TL, Brinkman B, Yunta RG, Cauwe B, Vandenabeele P, Liston A, Raes J. 2013. Inflammation-associated enterotypes, host genotype, cage and inter-individual effects drive gut microbiota variation in common laboratory mice. Genome Biol 14:R4. https://doi.org/10.1186/gb-2013-14-1-r4.
10. Roesch LFW, Lorca GL, Casella G, Giongo A, Naranjo A, Pionzio AM, Li N, Mai V, Wasserfall CH, Schatz D, Atkinson MA, Neu J, Triplett EW. 2009. Culture-independent identification of gut bacteria correlated with the onset of diabetes in a rat model. ISME J 3:536–548. https://doi.org/10.1038/ismej.2009.5.
11. Schauer C, Thompson CL, Brune A. 2012. The bacterial community in the gut of the cockroach Shelfordella lateralis reflects the close evolutionary relatedness of cockroaches and termites. Appl Environ Microbiol 78:2758–2767. https://doi.org/10.1128/AEM.07788-11.
12. Carrasco P, Pérez-Cobas AE, van de Pol C, Baixeras J, Moya A, Latorre A. 2014. Succession of the gut microbiota in the cockroach Blattella germanica. Int Microbiol 17:99–109. https://doi.org/10.2436/20.1501.01.212.

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13. Otani S, Mikaelyan A, Nobre T, Hansen LH, Koné NA, Sørensen SJ, Aanen DK, Boomsma JJ, Brune A, Poulsen M. 2014. Identifying the core microbial community in the gut of fungus-growing termites. Mol Ecol 23:4631–4644. https://doi.org/10.1111/mec.12874.
14. Liu J, Xu T, Zhu W, Mao S. 2014. High-grain feeding alters caecal bacterial microbiota composition and fermentation and results in caecal mucosal injury in goats. Br J Nutr 112:416–427. https://doi.org/10.1017/S0007114514000993.
15. Suchodolski JS, Markel ME, Garcia-Mazcorro JF, Unterer S, Heilmann RM, Dowd SE, Kachroo P, Ivanov I, Minamoto Y, Dillman EM, Steiner JM, Cook AK, Toresson L. 2012. The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. PLoS One 7:e51907. https://doi.org/10.1371/journal.pone.0051907.
16. Sarma-Rupavtarm RB, Ge Z, Schauer DB, Fox JG, Polz MF. 2004. Spatial distribution and stability of the eight microbial species of the altered schaedler flora in the mouse gastrointestinal tract. Appl Environ Microbiol 70:2791–2800. https://doi.org/10.1128/AEM.70.5.2791-2800.2004.
17. Dewhirst FE, Chien CC, Paster BJ, Ericson RL, Orcutt RP, Schauer DB, Fox JG. 1999. Phylogeny of the defined murine microbiota: altered Schaedler flora. Appl Environ Microbiol 65:3287–3292.
18. Krych L, Hansen CHF, Hansen AK, van den Berg FWJ, Nielsen DS. 2013. Quantitatively different, yet qualitatively alike: a meta-analysis of the mouse core gut microbiome with a view towards the human gut microbiome. PLoS One 8:e62578. https://doi.org/10.1371/journal.pone.0062578.
19. Harrell L, Wang Y, Antonopoulos D, Young V, Lichtenstein L, Huang Y, Hanauer S, Chang E. 2012. Standard colonic lavage alters the natural state of mucosal-associated microbiota in the human colon. PLoS One 7:e32545. https://doi.org/10.1371/journal.pone.0032545.
20. El Aidy S, Derrien M, Aardema R, Hooiveld G, Richards SE, Dane A, Dekker J, Vreeken R, Levenez F, Doré J, Zoetendal EG, Van Baarlen P, Kleerebezem M. 2014. Transient inflammatory-like state and microbial dysbiosis are pivotal in establishment of mucosal homeostasis during colonisation of germ-free mice. Beneficial Microbes 5:67–77. https://doi.org/10.3920/BM2013.0018.
21. Rooks MG, Veiga P, Wardwell-Scott LH, Tickle T, Segata N, Michaud M, Gallini CA, Beal C, van Hylckama-Vlieg JE, Ballal SA, Morgan XC, Glickman JN, Gevers D, Huttenhower C, Garrett WS. 2014. Gut microbiome composition and function in experimental colitis during active disease and treatment-induced remission. ISME J 8:1403–1417. https://doi.org/10.1038/ismej.2014.3.
22. Berry D, Schwab C, Milinovich G, Reichert J, Ben Mahfoudh K, Decker T, Engel M, Hai B, Hainzl E, Heider S, Kenner L, Müller M, Rauch I, Strobl B, Wagner M, Schleper C, Urich T, Loy A. 2012. Phylotype-level 16S rRNA analysis reveals new bacterial indicators of health state in acute murine colitis. ISME J 6:2091–2106. https://doi.org/10.1038/ismej.2012.39.
23. Hoffmann C, Hill DA, Minkah N, Kirn T, Troy A, Artis D, Bushman F. 2009. Community-wide response of the gut microbiota to enteropathogenic *Citrobacter rodentium* infection revealed by deep sequencing. Infect Immun 77:4668–4678. https://doi.org/10.1128/IAI.00493-09.
24. Jergens AE, Wilson-Welder JH, Dorn A, Henderson A, Liu Z, Evans RB, Hostetter J, Wannemuehler MJ. 2007. Helicobacter bilis triggers persistent immune reactivity to antigens derived from the commensal bacteria in gnotobiotic C3H/HeN mice. Gut 56:934–940. https://doi.org/10.1136/gut.2006.099242.
25. Fox JG. 2007. Helicobacter bilis: bacterial provocateur orchestrates host immune responses to commensal flora in a model of inflammatory bowel disease. Gut 56:898–900. https://doi.org/10.1136/gut.2006.115428.
26. Ravussin Y, Koren O, Spor A, LeDuc C, Gutman R, Stombaugh J, Knight R, Ley RE, Leibel RL. 2012. Responses of gut microbiota to diet composition and weight loss in lean and obese mice. Obesity (Silver Spring) 20:738–747. https://doi.org/10.1038/oby.2011.111.
27. El Homsi M, Ducroc R, Claustre J, Jourdan G, Gertler A, Estienne M, Bado A, Scoazec JY, Plaisancié P. 2007. Leptin modulates the expression of secreted and membrane-associated mucins in colonic epithelial cells by targeting PKC, PI3K, and MAPK pathways. Am J Physiol Gastrointest Liver Physiol 293:G365–G373. https://doi.org/10.1152/ajpgi.00091.2007.
28. Plaisancié P, Ducroc R, El Homsi M, Tsocas A, Guilmeau S, Zoghbi S, Thibaudeau O, Bado A. 2006. Luminal leptin activates mucin-secreting goblet cells in the large bowel. Am J Physiol Gastrointest Liver Physiol 290:G805–G812. https://doi.org/10.1152/ajpgi.00433.2005.
29. Sitaraman S, Liu X, Charrier L, Gu LH, Ziegler TR, Gewirtz A, Merlin D. 2004. Colonic leptin: source of a novel proinflammatory cytokine involved in IBD. FASEB J 18:696–698. https://doi.org/10.1096/fj.03-0422fje.
30. Berry D, Stecher B, Schintlmeister A, Reichert J, Brugiroux S, Wild B, Wanek W, Richter A, Rauch I, Decker T, Loy A, Wagner M. 2013. Host-compound foraging by intestinal microbiota revealed by single-cell stable isotope probing. Proc Natl Acad Sci USA 110:4720–4725. https://doi.org/10.1073/pnas.1219247110.
31. Pengpeng W, Tan Z. 2013. Ammonia assimilation in rumen bacteria: a review. Anim Biotechnol 24:107–128. https://doi.org/10.1080/10495398.2012.756402.
32. Pinske C, Jaroschinsky M, Linek S, Kelly CL, Sargent F, Sawers RG. 2015. Physiology and bioenergetics of [NiFe]-hydrogenase 2-catalyzed H2-consuming and H2-producing reactions in Escherichia coli. J Bacteriol 197:296–306. https://doi.org/10.1128/JB.02335-14.
33. Biegel E, Schmidt S, González JM, Müller V. 2011. Biochemistry, evolution and physiological function of the Rnf complex, a novel ion-motive electron transport complex in prokaryotes. Cell Mol Life Sci 68:613–634. https://doi.org/10.1007/s00018-010-0555-8.
34. Pitcher RS, Watmough NJ. 2004. The bacterial cytochrome cbb3 oxidases. Biochim Biophys Acta 1655:388–399. https://doi.org/10.1016/j.bbabio.2003.09.017.
35. Bertini I, Cavallaro G, Rosato A. 2006. Cytochrome c: occurrence and functions. Chem Rev 106:90–115. https://doi.org/10.1021/cr050241v.
36. Colburn-Clifford J, Allen C. 2010. A cbb(3)-type cytochrome C oxidase contributes to Ralstonia solanacearum R3bv2 growth in microaerobic environments and to bacterial wilt disease development in tomato. Mol Plant Microbe Interact 23:1042–1052. https://doi.org/10.1094/MPMI-23-8-1042.
37. Preisig O, Anthamatten D, Hennecke H. 1993. Genes for a microaerobically induced oxidase complex in Bradyrhizobium japonicum are essential for a nitrogen-fixing endosymbiosis. Proc Natl Acad Sci USA 90:3309–3313. https://doi.org/10.1073/pnas.90.8.3309.
38. Gaci N, Borrel G, Tottey W, O'Toole PW, Brugère JF. 2014. Archaea and the human gut: new beginning of an old story. World J Gastroenterol 20:16062–16078. https://doi.org/10.3748/wjg.v20.i43.16062.
39. Korotkov KV, Sandkvist M, Hol WGJ. 2012. The type II secretion system: biogenesis, molecular architecture and mechanism. Nat Rev Microbiol 10:336–351. https://doi.org/10.1038/nrmicro2762.
40. Sandkvist M. 2001. Type II secretion and pathogenesis. Infect Immun 69:3523–3535. https://doi.org/10.1128/IAI.69.6.3523-3535.2001.
41. Cianciotto NP. 2005. Type II secretion: a protein secretion system for all seasons. Trends Microbiol 13:581–588. https://doi.org/10.1016/j.tim.2005.09.005.
42. Peabody CR, Chung YJ, Yen MR, Vidal-Ingigliardi D, Pugsley AP, Saier MH, Jr. 2003. Type II protein secretion and its relationship to bacterial type IV pili and archaeal flagella. Microbiology 149:3051–3072. https://doi.org/10.1099/mic.0.26364-0.
43. Merz AJ, So M, Sheetz MP. 2000. Pilus retraction powers bacterial twitching motility. Nature 407:98–102. https://doi.org/10.1038/35024105.
44. Christie PJ, Atmakuri K, Krishnamoorthy V, Jakubowski S, Cascales E. 2005. Biogenesis, architecture, and function of bacterial type IV secretion systems. Annu Rev Microbiol 59:451–485. https://doi.org/10.1146/annurev.micro.58.030603.123630.
45. Glöckner G, Albert-Weissenberger C, Weinmann E, Jacobi S, Schunder E, Steinert M, Hacker J, Heuner K. 2008. Identification and characterization of a new conjugation/type IVA secretion system (trb/tra) of Legionella pneumophila Corby localized on two mobile genomic islands. Int J Med Microbiol 298:411–428. https://doi.org/10.1016/j.ijmm.2007.07.012.
46. O'Callaghan D, Cazevieille C, Allardet-Servent A, Boschiroli ML, Bourg G, Foulongne V, Frutos P, Kulakov Y, Ramuz M. 1999. A homologue of the Agrobacterium tumefaciens VirB and Bordetella pertussis Ptl type IV secretion systems is essential for intracellular survival of Brucella suis. Mol Microbiol 33:1210–1220. https://doi.org/10.1046/j.1365-2958.1999.01569.x.
47. Cascales E, Christie PJ. 2003. The versatile bacterial type IV secretion systems. Nat Rev Microbiol 1:137–149. https://doi.org/10.1038/nrmicro753.
48. Kwon AR, Kim JH, Park SJ, Lee KY, Min YH, Im H, Lee I, Lee KY, Lee BJ. 2012. Structural and biochemical characterization of HP0315 from Helicobacter pylori as a VapD protein with an endoribonuclease activity. Nucleic Acids Res 40:4216–4228. https://doi.org/10.1093/nar/gkr1305.
49. Boyer F, Fichant G, Berthod J, Vandenbrouck Y, Attree I. 2009. Dissecting the bacterial type VI secretion system by a genome wide in silico analysis: what can be learned from available microbial genomic re-

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sources? BMC Genomics 10:104. https://doi.org/10.1186/1471-2164-10-104.
50. Cascales E, Cambillau C. 2012. Structural biology of type VI secretion systems. Philos Trans R Soc Lond B Biol Sci 367:1102–1111. https://doi.org/10.1098/rstb.2011.0209.
51. Basler M, Pilhofer M, Henderson GP, Jensen GJ, Mekalanos JJ. 2012. Type VI secretion requires a dynamic contractile phage tail-like structure. Nature 483:182–186. https://doi.org/10.1038/nature10846.
52. Russell AB, LeRoux M, Hathazi K, Agnello DM, Ishikawa T, Wiggins PA, Wai SN, Mougous JD. 2013. Diverse type VI secretion phospholipases are functionally plastic antibacterial effectors. Nature 496:508–512. https://doi.org/10.1038/nature12074.
53. Al-Khodor S, Price CT, Kalia A, Abu Kwaik Y. 2010. Functional diversity of ankyrin repeats in microbial proteins. Trends Microbiol 18:132–139. https://doi.org/10.1016/j.tim.2009.11.004.
54. Cerveny L, Straskova A, Dankova V, Hartlova A, Ceckova M, Staud F, Stulik J. 2013. Tetratricopeptide repeat motifs in the world of bacterial pathogens: role in virulence mechanisms. Infect Immun 81:629–635. https://doi.org/10.1128/IAI.01035-12.
55. Smith HW, Huggins MB. 1976. Further observations on the association of the colicine V plasmid of Escherichia coli with pathogenicity and with survival in the alimentary tract. J Gen Microbiol 92:335–350. https://doi.org/10.1099/00221287-92-2-335.
56. Smith HW. 1974. A search for transmissible pathogenic characters in invasive strains of Escherichia coli: the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated, or identical, with colicine V. J Gen Microbiol 83:95–111. https://doi.org/10.1099/00221287-83-1-95.
57. Schwab C, Tveit AT, Schleper C, Urich T. 2014. Gene expression of lactobacilli in murine forestomach biofilms. Microb Biotechnol 7:347–359. https://doi.org/10.1111/1751-7915.12126.
58. Schwab C, Berry D, Rauch I, Rennisch I, Ramesmayer J, Hainzl E, Heider S, Decker T, Kenner L, Müller M, Strobl B, Wagner M, Schleper C, Loy A, Urich T. 2014. Longitudinal study of murine microbiota activity and interactions with the host during acute inflammation and recovery. ISME J 8:1101–1114. https://doi.org/10.1038/ismej.2013.223.
59. Xiong X, Frank DN, Robertson CE, Hung SS, Markle J, Canty AJ, McCoy KD, Macpherson AJ, Poussier P, Danska JS, Parkinson J. 2012. Generation and analysis of a mouse intestinal metatranscriptome through Illumina based RNA-sequencing. PLoS One 7:e36009. https://doi.org/10.1371/journal.pone.0036009.
60. De Weirdt R, Van de Wiele T. 2015. Micromanagement in the gut: microenvironmental factors govern colon mucosal biofilm structure and functionality. NPJ Biofilms Microbiomes 1:15026.
61. Berry D, Kuzyk O, Rauch I, Heider S, Schwab C, Hainzl E, Decker T, Müller M, Strobl B, Schleper C, Urich T, Wagner M, Kenner L, Loy A. 2015. Intestinal microbiota signatures associated with inflammation history in mice experiencing recurring colitis. Front Microbiol 6:1408. https://doi.org/10.3389/fmicb.2015.01408.
62. El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B. 2013. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. Nat Rev Microbiol 11:497–504. https://doi.org/10.1038/nrmicro3050.
63. van Passel MWJ, Kant R, Zoetendal EG, Plugge CM, Derrien M, Malfatti SA, Chain PSG, Woyke T, Palva A, de Vos WM, Smidt H. 2011. The genome of *Akkermansia muciniphila*, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. PLoS One 6:e16876. https://doi.org/10.1371/journal.pone.0016876.
64. Maier L, Vyas R, Cordova CD, Lindsay H, Schmidt TSB, Brugiroux S, Periaswamy B, Bauer R, Sturm A, Schreiber F, von Mering C, Robinson MD, Stecher B, Hardt WD. 2013. Microbiota-derived hydrogen fuels Salmonella typhimurium invasion of the gut ecosystem. Cell Host Microbe 14:641–651. https://doi.org/10.1016/j.chom.2013.11.002.
65. Winter SE, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, Laughlin RC, Gomez G, Wu J, Lawhon SD, Popova IE, Parikh SJ, Adams LG, Tsolis RM, Stewart VJ, Bäumler AJ. 2013. Host-derived nitrate boosts growth of E. coli in the inflamed gut. Science 339:708–711. https://doi.org/10.1126/science.1232467.
66. Tiso M, Schechter AN. 2015. Nitrate reduction to nitrite, nitric oxide and ammonia by gut bacteria under physiological conditions. PLoS One 10:e0119712. https://doi.org/10.1371/journal.pone.0119712.
67. Keshavarzian A, Sedghi S, Kanofsky J, List T, Robinson C, Ibrahim C, Winship D. 1992. Excessive production of reactive oxygen metabolites by inflamed colon: analysis by chemiluminescence probe. Gastroenterology 103:177–185. https://doi.org/10.1016/0016-5085(92)91111-G.
68. Winter SE, Lopez CA, Bäumler AJ. 2013. The dynamics of gut-associated microbial communities during inflammation. EMBO Rep 14:319–327. https://doi.org/10.1038/embor.2013.27.
69. Mishra S, Imlay JA. 2013. An anaerobic bacterium, Bacteroides thetaiotaomicron, uses a consortium of enzymes to scavenge hydrogen peroxide. Mol Microbiol 90:1356–1371. https://doi.org/10.1111/mmi.12438.
70. Russell AB, Peterson SB, Mougous JD. 2014. Type VI secretion system effectors: poisons with a purpose. Nat Rev Microbiol 12:137–148. https://doi.org/10.1038/nrmicro3185.
71. Cahill RJ, Foltz CJ, Fox JG, Dangler CA, Powrie F, Schauer DB. 1997. Inflammatory bowel disease: an immunity-mediated condition triggered by bacterial infection with Helicobacter hepaticus. Infect Immun 65:3126–3131.
72. Chow J, Mazmanian SK. 2010. A pathobiont of the microbiota balances host colonization and intestinal inflammation. Cell Host Microbe 7:265–276. https://doi.org/10.1016/j.chom.2010.03.004.
73. Pilar AVC, Reid-Yu SA, Cooper CA, Mulder DT, Coombes BK. 2012. GogB is an anti-inflammatory effector that limits tissue damage during Salmonella infection through interaction with human FBXO22 and Skp1. PLoS Pathog 8:e1002773. https://doi.org/10.1371/journal.ppat.1002773.
74. Chow J, Tang H, Mazmanian SK. 2011. Pathobionts of the gastrointestinal microbiota and inflammatory disease. Curr Opin Immunol 23:473–480. https://doi.org/10.1016/j.coi.2011.07.010.
75. Koonin EV, Makarova KS, Aravind L. 2001. Horizontal gene transfer in prokaryotes: quantification and classification. Annu Rev Microbiol 55:709–742. https://doi.org/10.1146/annurev.micro.55.1.709.
76. Ochman H, Lawrence JG, Groisman EA. 2000. Lateral gene transfer and the nature of bacterial innovation. Nature 405:299–304. https://doi.org/10.1038/35012500.
77. Caro-Quintero A, Konstantinidis KT. 2015. Inter-phylum HGT has shaped the metabolism of many mesophilic and anaerobic bacteria. ISME J 9:958–967. https://doi.org/10.1038/ismej.2014.193.
78. Stecher B, Maier L, Hardt WD. 2013. 'Blooming' in the gut: how dysbiosis might contribute to pathogen evolution. Nat Rev Microbiol 11:277–284. https://doi.org/10.1038/nrmicro2989.
79. Moodley Y, Linz B, Bond RP, Nieuwoudt M, Soodyall H, Schlebusch CM, Bernhöft S, Hale J, Suerbaum S, Mugisha L, van der Merwe SW, Achtman M. 2012. Age of the association between Helicobacter pylori and man. PLoS Pathog 8:e1002693. https://doi.org/10.1371/journal.ppat.1002693.
80. Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ. 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol 66:5488–5491. https://doi.org/10.1128/AEM.66.12.5488-5491.2000.
81. Schmieder R, Edwards R. 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864. https://doi.org/10.1093/bioinformatics/btr026.
82. Loman NJ, Quinlan AR. 2014. Poretools: a toolkit for analyzing nanopore sequence data. Bioinformatics 30:3399–3401. https://doi.org/10.1093/bioinformatics/btu555.
83. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
84. Wannemuehler MJ, Overstreet A-M, Ward DV, Phillips GJ. 2014. Draft genome sequences of the altered schaedler flora, a defined bacterial community from gnotobiotic mice. Genome Announc 2:e00287-14. https://doi.org/10.1128/genomeA.00287-14.
85. Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, Lajus A, Le Fèvre F, Longin C, Mornico D, Roche D, Rouy Z, Salvignol G, Scarpelli C, Thil Smith AA, Weiman M, Médigue C. 2013. MicroScope—an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. Nucleic Acids Res 41:D636–D647. https://doi.org/10.1093/nar/gks1194.
86. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. 1999. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 27:29–34. https://doi.org/10.1093/nar/27.1.29.
87. Aranki A, Syed SA, Kenney EB, Freter R. 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl Environ Microbiol 17:568–576.

Downloaded from https://journals.asm.org/journal/msystems on 16 November 2021 by 129.93.161.224.

88. Liu Z, Ramer-Tait AE, Henderson AL, Demirkale CY, Nettleton D, Wang C, Hostetter JM, Jergens AE, Wannemuehler MJ. 2011. Helicobacter bilis colonization enhances susceptibility to typhlocolitis following an inflammatory trigger. Dig Dis Sci 56:2838–2848. https://doi.org/10.1007/s10620-011-1701-3.
89. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
90. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324.
91. Anders S, Pyl PT, Huber W. 2015. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31:166–169. https://doi.org/10.1093/bioinformatics/btu638.
92. Lin K, Kools H, de Groot PJ, Gavai AK, Basnet RK, Cheng F, Wu J, Wang X, Lommen A, Hooiveld GJEJ, Bonnema G, Visser RGF, Muller MR, Leunissen JAM. 2011. MADMAX—management and analysis database for multiple ~omics experiments. J Integr Bioinform 8:160. https://doi.org/10.2390/biecoll-jib-2011-160.
93. Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, Bunney WE, Myers RM, Speed TP, Akil H, Watson SJ, Meng F. 2005. Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. Nucleic Acids Res 33:e175. https://doi.org/10.1093/nar/gni179.
94. Bolstad BM, Irizarry RA, Astrand M, Speed TP. 2003. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19:185–193. https://doi.org/10.1093/bioinformatics/19.2.185.
95. Sartor MA, Tomlinson CR, Wesselkamper SC, Sivaganesan S, Leikauf GD, Medvedovic M. 2006. Intensity-based hierarchical Bayes method improves testing for differentially expressed genes in microarray experiments. BMC Bioinformatics 7:538. https://doi.org/10.1186/1471-2105-7-538.
96. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102:15545–15550. https://doi.org/10.1073/pnas.0506580102.
97. Silverman JM, Brunet YR, Cascales E, Mougous JD. 2012. Structure and regulation of the type VI secretion system. Annu Rev Microbiol 66:453–472. https://doi.org/10.1146/annurev-micro-121809-151619.

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