Forty Years of *Oxalobacter formigenes*, a Gutsy Oxalate-Degrading Specialist

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ABSTRACT *Oxalobacter formigenes*, a unique anaerobic bacterium that relies solely on oxalate for growth, is a key oxalate-degrading bacterium in the mammalian intestinal tract. Degradation of oxalate in the gut by *O. formigenes* plays a critical role in preventing renal toxicity in animals that feed on oxalate-rich plants. The role of *O. formigenes* in reducing the risk of calcium oxalate kidney stone disease and oxalate nephropathy in humans is less clear, in part due to difficulties in culturing this organism and the lack of studies which have utilized diets in which the oxalate content is controlled. Herein, we review the literature on the 40th anniversary of the discovery of *O. formigenes*, with a focus on its biology, its role in gut oxalate metabolism and calcium oxalate kidney stone disease, and potential areas of future research. Results from ongoing clinical trials utilizing *O. formigenes* in healthy volunteers and in patients with primary hyperoxaluria type 1 (PH1), a rare but severe form of calcium oxalate kidney stone disease, are also discussed. Information has been consolidated on *O. formigenes* strains and best practices to culture this bacterium, which should serve as a good resource for researchers.

KEYWORDS oxalate degradation, *Oxalobacter formigenes*, gut microbiome, kidney stone disease, probiotics, culture methods

DISCOVERY OF *O. FORMIGENES*

Studies from the 1940s and 1950s showed that oxalate is degraded by microbes in human feces and in the rumen, although little was known about the nature of the microorganisms responsible for this activity (1, 2). Oxalate is a compound commonly found in plants consumed by humans (e.g., rhubarb, spinach, and beets) and in a variety of forage plants (e.g., *Halogeton glomeratus*) consumed by ruminants (3). When oxalate is ingested in large quantities, renal toxicity can occur from the formation and deposition of calcium oxalate crystals in the kidney (Fig. 1A). This problem was first made relevant when sheep died after grazing in fields predominant in oxalate-rich plants (Fig. 1B) (4). Although calcium supplementation was used to reduce acute oxalate toxicity in herding animals (5), the most successful approach was via gradual acclimation to a high-oxalate intake (6). It was also reported that seasonal eating patterns affected oxalate degradation by bacteria in the rumen of these animals, and degradation was highest in seasons when oxalate-rich plants predominated the grazing crops (7). The original hypothesis was that oxalate-degrading organisms lived symbiotically on oxalate-rich plants and were ingested when the livestock would graze (7), but this was later rejected by Allison and associates, who demonstrated that selection of resident oxalate-degrading bacteria, rather than introduction of a new species, was responsible for the increase in both rates of oxalate degradation and numbers of oxalate-degrading bacteria in the rumen (8, 9). Considering the strictly anaerobic conditions
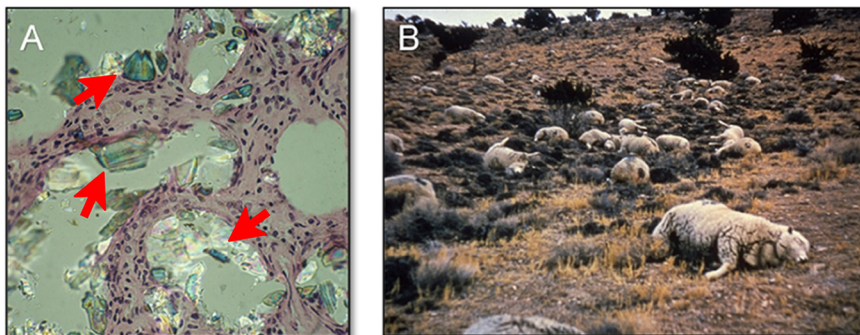
FIG 1 (A) Oxalate crystals (arrows indicate some of the crystals present) in stained thin-sections of renal tissue from oxalate-fed laboratory rats (R. C. Cutlip and S. L. Daniel, unpublished data). (B) Dead sheep (>1,200) as a result of oxalate intoxication from consuming the high oxalate-containing plant *Halogeton glomeratus* (reprinted from reference 101).

of the rumen, Allison and associates hypothesized that strict anaerobes were responsible for the majority of ruminal oxalate degradation and, in 1980, Karl Dawson isolated an anaerobic oxalate-degrading bacterium from the rumen of sheep (Fig. 2A) (10). This same bacterium was later isolated from human feces by Milton Allison and colleagues in 1985 (Fig. 2B) and given the name *Oxalobacter formigenes* (11). Importantly, this was the first discovery of a commensal obligate anaerobe in the human gut with a total dependency on oxalate. This beneficial gut bacterium is therefore considered a "specialist" when it comes to oxalate utilization, compared to "generalists," which can use a variety of substrates, including oxalate. Since its isolation, numerous studies have examined the impact of colonization by *O. formigenes* on intestinal oxalate levels, urinary oxalate excretion, and transport of oxalate in the mammalian gut (12–14).

TAXONOMY AND PHYSIOLOGY OF *O. FORMIGENES*

O. formigenes is a Gram-negative, obligately anaerobic, rod or curve-shaped, nonmotile, non-spore-forming bacterium (Fig. 3) that has been assigned to the following taxa: *Bacteria* (domain); *Proteobacteria* (phylum); *Betaproteobacteria* (class); *Burkholderiales* (order); *Oxalobacteraceae* (family); *Oxalobacter* (genus); *formigenes* (species); and OxB (type strain). Table 1 lists details on various strains of *O. formigenes* isolated to date. Comparisons of the profiles of cellular fatty acids of 17 strains of *O. formigenes*, including strains isolated from the gastrointestinal contents from humans, sheep, cattle, pigs,

FIG 2 Karl A. Dawson (A) and Milton J. Allison (B), the two microbiologists responsible for the isolation and naming of the oxalate-degrading gut bacterium *Oxalobacter formigenes*. Dr. Dawson (Vice President & Chief Scientific Officer of Alltech, now retired) is shown here speaking at the Alltech 30th Annual International Symposium in 2014. Dr. Allison is shown here in his laboratory at Iowa State University in 2018.
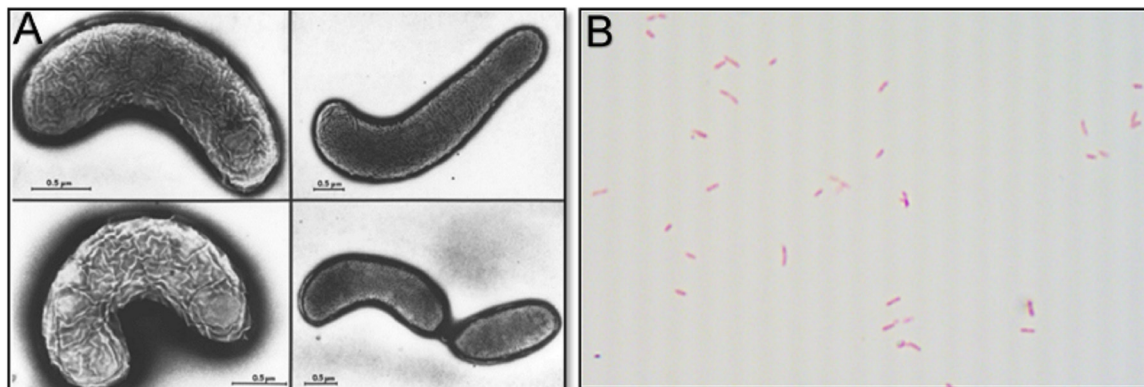
FIG 3 (A) Electron micrographs of *Oxalobacter formigenes* strain OxWR1 (reprinted from reference 102). (B) Gram stain of *Oxalobacter formigenes* strain HOxBLS (N. Pareek and S. L. Daniel, unpublished data; ×1,000 magnification) with its typical varied morphology and size.

guinea pigs, and rats (both wild and laboratory) and from freshwater lake sediments, support the concept of separating these strains into two main groups (currently designated group I and II). In group I strains, a cyclic 17-carbon fatty acid predominates, whereas in group II a cyclic 19-carbon fatty acid is dominant (11, 15). In addition, this separation of strains into two groups based on cellular fatty acids is well supported by differential profiles observed between strains based on cell proteins, serological tests, 16S rRNA sequences, and specificity of oligonucleotide probes/primers directed at the *oxc* gene (11, 15–17). To date, the only exception appears to be strain OxGP1 (isolated from the guinea pig cecum), which falls into group II based on cellular fatty acids and *oxc* gene analysis and into group I when based on rRNA sequence comparisons (Fig. 4) (16). Interestingly, another strain, HOxBLS (a group II member), falls somewhere between groups I and II in the 16S rRNA-based phylogenetic tree (Fig. 4). Overall, group II strains appear to be more diverse than group I strains, leading to the suggestion by Sidhu et al. that group II strains could be further sorted into 3 subgroups (17). What this grouping/ subgrouping of strains means relative to *O. formigenes* and its ecology, nutrition and physiology, antibiotic susceptibility, colonization of humans, and impact on urinary oxalate and kidney stone prevention (to name just a few) is presently unclear and warrants further study. It is worth noting that group II strains in general tend to be more difficult to maintain in broth cultures and do not achieve the same final optical densities as group I strains (unpublished observations). Thus, when working with group II strains, an ample supply of frozen glycerol stocks should be made available for culture recovery.

Optimal growth of *O. formigenes* in culture occurs under anaerobic conditions at a pH between 6 and 7 and in a CO_2-bicarbonate buffered undefined medium containing minerals, oxalate, acetate, and a small amount of yeast extract (Table 2). Yeast extract, though not required, does appear to improve the growth of some strains of *O. formigenes*, especially upon initial isolation from the gut. However, regardless of the presence or absence of yeast extract, a low concentration (0.5 to 2 mM) of acetate is added since *O. formigenes* assimilates small amounts of carbon from acetate, as well as from carbon dioxide, for the synthesis of cell biomass (Fig. 5). Acetate alone does not support the growth of this organism; in fact, none of the more than 60 different compounds tested to date have been found to be growth supportive for *O. formigenes* (11, 18, 19). Even enriched complex media (e.g., anaerobic peptone yeast glucose or brain heart infusion) fail to support the growth of *O. formigenes* and are therefore often inoculated to help ascertain the purity of *O. formigenes* cultures (18).

One of the hallmark features of *O. formigenes* is its absolute requirement for oxalate as both an energy-yielding substrate and a major source of carbon for growth (Fig. 5) (20–22). Given the highly oxidized state of oxalate, the energy yield is low, but sufficient to support growth. The products from oxalate metabolism are carbon dioxide

TABLE 1 Group classification and strains of *Oxalobacter formigenes*

Group	Strain (source)[a]	Selected background information	Reference(s)
I	OxB (sheep rumen)	Type strain (ATCC 35274); 16S rRNA and *oxc* and *frc* genes sequenced; cellular fatty acids measured; antibiotic susceptibility tested; rat colonization studied; bile salts tolerance tested	(10, 11, 16, 19, 52, 102, 109–111)
	OxWR1 (wild rat cecum)	Rat/mouse colonization and enteric oxalate excretion studied; antibiotic susceptibility tested; cellular fatty acids measured; 16S rRNA sequenced	(16, 18, 53, 58, 71, 102)
	OxDB12 (human feces)	Cellular fatty acids measured; 16S rRNA sequenced	(16)
	OxCC13 (human feces)	Genome, 16S rRNA, and *oxc* gene sequenced; antibiotic susceptibility tested; proteomics studied; mouse colonization examined; cellular fatty acids measured; strain used in ongoing trial (NCT03752684; clinicaltrials.gov) with healthy volunteers	(13, 16, 29, 31, 33, 48, 52, 55, 57, 64)
	HOxHM18 (human feces)	16S rRNA and *oxc* gene sequenced; cellular fatty acids measured	(16, 52)
	HOxRA (human feces)	*oxc* gene sequenced	(52)
	HOxUK90 (human feces)	*oxc* gene sequenced	(52)
	HC-1 (human feces)	Genome, 16S rRNA, and *oxc* gene sequenced; human colonization studied; mouse colonization and enteric oxalate excretion studied; mouse colonic metabolome examined; cellular fatty acids measured; antibiotic susceptibility tested; air, pH, bile salts tolerance tested; used in ongoing PH1 clinical trials (Oxthera.com)	(11, 16, 32, 64, 94, 97, 100, 112)
	HC-3 (human feces)	Cellular fatty acids measured	(11, 16)
	SOx4 (lake sediment)	ATCC 43053; cellular fatty acids measured; 16S rRNA sequenced	(16, 113)
	SOx6 (lake sediment)	Cellular fatty acids measured	(16, 113)
	POxC (swine feces)	Cellular fatty acids measured; 16S rRNA sequenced	(11, 16)
	HOxSLD-1 (human feces)	Analyzed by pulsed-field gel electrophoresis (PFGE)	(114)
	HOxSLD-2 (human feces)	Analyzed by PFGE	(114)
	OxMC386-1 (mouse feces)	Analyzed by PFGE	(114)
	OxMC386-2 (mouse feces)	Analyzed by PFGE	(114)
	OxMC405 (mouse feces)	Analyzed by PFGE	(114)
	SSYG-15 (human feces)	CCTCC M201618; genome sequenced; group classification is tentative; strain appears to be very similar to OxCC13 (group I) with a 99.6% identity based on 16S rRNA sequencing	(30)
II	OxK (human feces)	16S rRNA and *oxc* gene sequenced; antibiotic susceptibility tested; rat colonization studied; cellular fatty acids measured	(11, 16, 52, 64)
	BA1 (human feces)	16S rRNA and *oxc* gene sequenced; cellular fatty acids measured	(16, 52)
	BA2 (human feces)	DSM 4420; cellular fatty acids measured; bile salts tolerance tested	(11, 109)
	BA4 (human feces)	16S rRNA sequenced	(16)
	BA6 (human feces)	Cellular fatty acids measured	(16)
	HOxRW (human feces)	16S rRNA and *oxc* gene sequenced; cellular fatty acids measured; bile salts tolerance tested	(16, 109)
	HOxBLS (human feces)	Genome and 16S rRNA sequenced; cellular fatty acids measured	(13, 16, 17, 52)
	HOxUK5 (human feces)	*oxc* gene sequenced	(52)
	HOxUK88 (human feces)	*oxc* gene sequenced	(52)
	HOxHS (human feces)	*oxc* gene sequenced	(52)
	OxCR6 (lab rat cecum)	Cellular fatty acids measured; rat colonization and oxalate balance studied; 16S rRNA sequenced	(16, 18, 102, 115)
	OxGP1 (guinea pig cecum)	Cellular fatty acids measured; rat colonization studied; bile salts tolerance tested; 16S rRNA sequenced	(16, 53, 102, 109)
	Va3 (human feces)	Antibiotic susceptibility tested; air, pH, and bile salts tolerance tested	(64, 97)
	89-112 (unknown)	Cellular fatty acids measured	(16)
	HOxNP-1 (human feces)	Analyzed by PFGE	(114)
	HOxNP-2 (human feces)	Analyzed by PFGE	(114)
	HOxNP-3 (human feces)	Analyzed by PFGE	(114)

[a]The following strains of *Oxalobacter formigenes* are commercially available: OxB (ATCC 35274) and Sox4 (ATCC 43053) from the American Type Culture Collection (ATCC; https://www.atcc.org/); BA2 (DSM 4420) from DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen (https://www.dsmz.de/); OxCC13 from BEI Resources (https://www.beiresources.org/); and SSYG-15 (CCTCC M201618) from the China Center for Type Culture Collection (CCTCC; http://cctcc.whu.edu.cn/).

and formate, with approximately 1 mol of each produced per mol of oxalate metabolized. Energy conservation is centered on the development of a proton-motive force through the electrogenic exchange of oxalate (in) and formate (out) across the cell membrane, together with the consumption of a proton inside the cell when the CoA-ester of oxalate is decarboxylated by oxalyl-CoA decarboxylase (23, 24). Proton consumption ultimately results in the alkalization of the external environment.
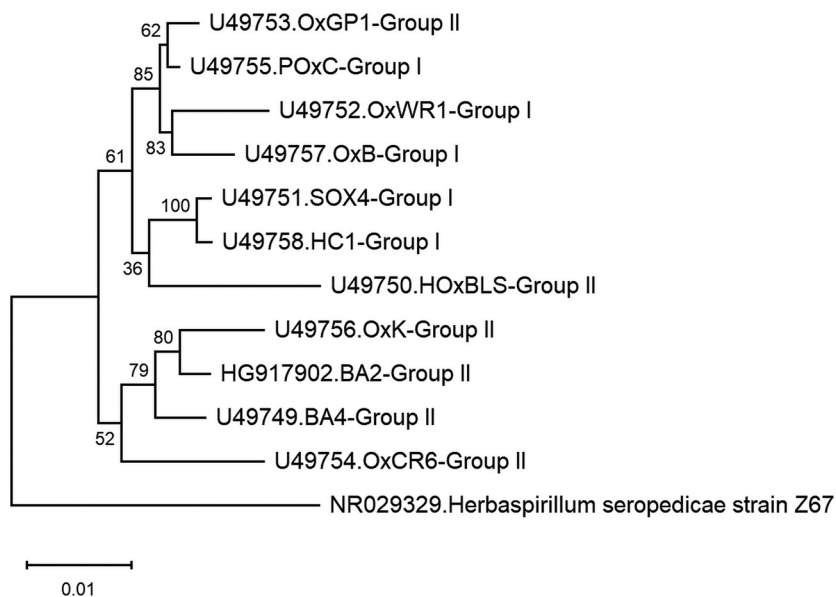
FIG 4 Phylogenetic analysis of *Oxalobacter formigenes* group I and group II strains and *Herbaspirillum seropedicae* strain Z67 (outgroup and a member of the *Betaproteobacteria* class) based on 16S rRNA sequences obtained from the National Center for Biotechnology Information (NCBI). GenBank accession numbers are shown with the strain names. Evolutionary history and distances were computed using the neighbor-joining method (103) and maximum composite likelihood method, respectively (104). Bootstrap values, expressed as percentages of 1,000 replicates, are shown next to each internal node in the tree (105). The scale bar represents an estimate of the numbers of substitutions per base. Evolutionary analyses were conducted in Molecular Evolutionary Genetics Analysis (MEGA) (106).

Most, if not all, of the strains listed in Table 1 were isolated using modifications of the anaerobic roll tube technique developed by Hungate (25–27) and culture media (Table 2) developed by Dawson and others in the 1980s following the isolation of *O. formigenes* strain OxB from the sheep rumen (10). One such novel approach is the use

TABLE 2 Anaerobic oxalate broth for the enrichment and routine cultivation of *Oxalobacter formigenes*

Component[a] and preparation[b]	Amt per liter (final concn)
Deionized water	1,000 ml
Potassium oxalate monohydrate	3.7 g (20 mM)
Yeast extract	1.0 g (0.1%)
Mineral solution[c]	50.0 ml
Sodium acetate·trihydrate (0.4 M)	5.0 ml (2 mM)
Trace metal solution[d]	2.0 ml
Resazurin (0.1%)	1.0 ml

[a]Add components in the order indicated above into an Erlenmeyer flask; if a defined medium is desired, the yeast extract can be left out. For safety, the flask should be twice the volume of the amount of medium being prepared. The total volume is greater than 1 liter in order to account for the water lost during boiling.

[b]Adjust pH to 7, add sodium bicarbonate (7.5 g per liter), and bring medium to a boil on a hotplate while bubbling with CO_2. After the resazurin (O/R indicator) in the medium has turned from blue to pink ($\sim$5 to 10 min of boiling), carefully remove flask from heat using autoclave gloves and continue to bubble with CO_2 and cool medium to room temperature in an ice bath. Add the reducing agent cysteine·HCl·H_2O (0.5 g per liter), mix, and flush headspace with CO_2 until medium is reduced (colorless). Dispense 10-ml aliquots into culture tubes (18 by 150 mm; series 2048 [Bellco Glass]; $\sim$27.2-ml stoppered volume) which are being flushed with a gentle stream of CO_2. Flush headspace with CO_2 for 5 s and then stopper with gray butyl rubber-stopper and seal with an aluminum-crimp seal. While tube is being sealed, gassing cannula should be placed in the next empty tube for flushing prior to adding medium. Repeat process tube by tube until the entire medium has been dispensed. Autoclave at 121°C for 15 min and fast exhaust. After autoclaving, pH of the medium is 6.6 to 6.8.

[c]Mineral solution contains (g per liter): KCl, 20; NH_4Cl, 10; KH_2PO_4, 10; and $MgSO_4·7H_2O$, 1 (dissolve one at a time and store solution at 4°C).

[d]Trace metal solution contains (g per liter): trisodium nitrilotriacetate, 1.500; $MnSO_4·H_2O$, 0.500; $FeSO_4·7H_2O$, 0.100; $CO(NO_3)_2·6H_2O$, 0.100; $ZnCl_2$, 0.100; $NiCl_2·6H_2O$, 0.050; H_2SeO_3, 0.050; $CuSO_4·5H_2O$, 0.010; $AlK(SO_4)_2·12H_2O$, 0.010; H_3BO_3, 0.010; $Na_2MoO_4·2H_2O$, 0.010; and $Na_2WO_4·2H_2O$, 0.010 (dissolve one at a time and store solution at 4°C).
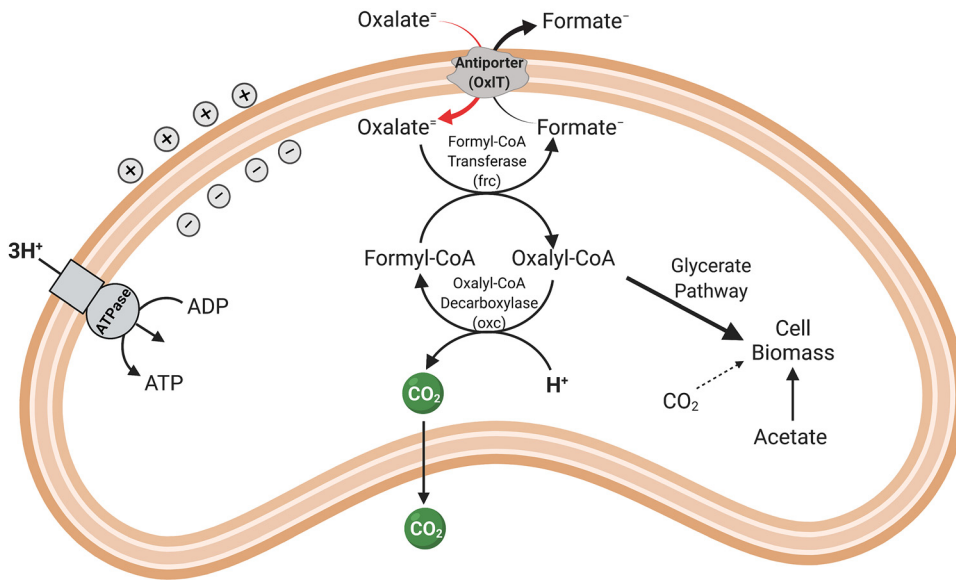
FIG 5 Schematic of oxalate metabolism by *Oxalobacter formigenes*.

of anaerobic roll tubes containing calcium oxalate, where colonies of anaerobic oxalate-degrading bacteria (e.g., *O. formigenes*) can be detected based on the formation of clear zones around colonies (Fig. 6; see Table 3 for preparation of anaerobic calcium oxalate roll tubes and plates). This approach has been successfully used over the years for the isolation (with or without prior enrichment), enumeration, and characterization of *O. formigenes* strains from gut and environmental samples (16, 28).
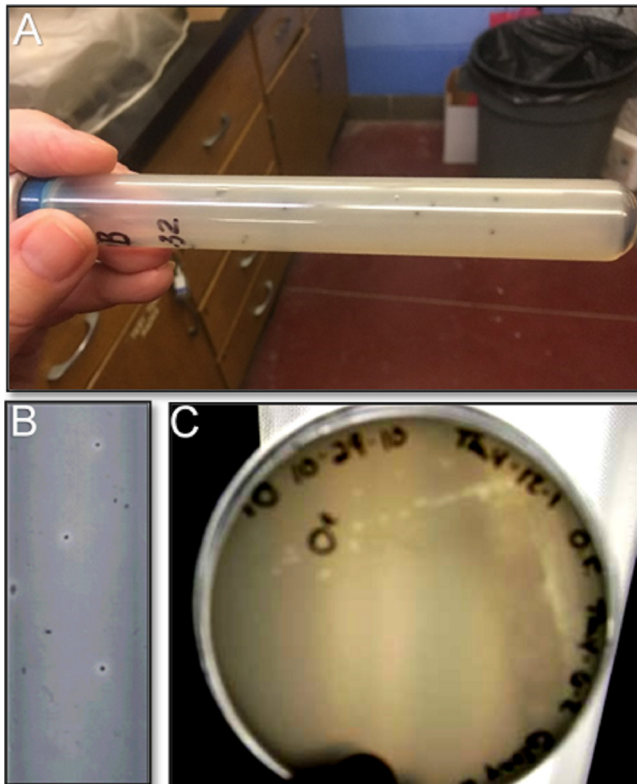
FIG 6 Clear zones formed around colonies by *Oxalobacter formigenes* after incubation in anaerobic roll tubes (A and B) and on anaerobic plates (C) containing calcium oxalate. See Table 3 for the preparation of anaerobic calcium oxalate agar in tubes and plates.

TABLE 3 Anaerobic calcium oxalate agar for the enumeration and isolation of *Oxalobacter formigenes*

Component[a] and preparation of roll tubes[b] and plates[c]	Amt per liter (final concn)
Deionized water	750 ml
Potassium oxalate monohydrate	3.7 g (20 mM)
Yeast extract	1.0 g (0.1%)
Mineral solution (see Table 2)	50.0 ml
Sodium acetate·trihydrate (0.4 M)	5.0 ml (2 mM)
Trace metal solution (see Table 2)	2.0 ml
1% $CaCl_2$·$2H_2O$ (147.0 g/mol; 68 mM) solution	200.00 ml (14 mM)
Cysteine·HCl·H_2O (175.61 g/mol)	0.5 g (3.4 mM)
Sodium bicarbonate (84.01 g/mol)	7.5 g (89.3 mM)
Agar	17.5 g (1.5%)
Resazurin (0.1%)	1.0 ml

[a]Add all of the above components in the order indicated into a 2-liter Erlenmeyer flask with a stirring bar; for safety, the flask should be twice the volume of the amount of medium being prepared.

[b]Anaerobic roll tubes (Fig. 6): Stir to mix and bring the medium to a boil on a hotplate while bubbling with CO_2. Continue to boil medium until agar has melted. Once agar has melted, continue to bubble medium with CO_2 and carefully remove flask from heat using autoclave gloves to transfer flask to insulated bucket which contains hot water ($\sim$50°C). The hot water bucket should be on a small stirring plate so the medium can be stirred while being dispensed. It is important to keep the calcium oxalate suspended while dispensing. Stir and continue to bubble medium with CO_2 and allow medium to cool down for $\sim$5 min. Dispense 7 ml into an 18 × 150-mm crimp-seal culture tube which is being flushed with CO_2 following the procedure described in Table 2. Autoclave at 121°C for 15 min and fast exhaust. After autoclaving, medium pH is 6.6 to 6.8. If the medium is used that day, place tubes in a water bath ($\sim$48°C) to temper. Once tempered, add sample (inoculum) to tubes via a sterile needle and syringe; after inoculation, gently mix inoculum into medium by inverting tube several times and then place tube on its side on ice (contained in an ice bucket with no water added) and quickly spin tube so agar solidifies on the inside wall of the tube. If agar medium is not used that day, store medium at room temperature and reheat to melt agar when needed. Once the inoculated tubes have been placed in the incubator, condensation (water) will accumulate inside at the bottom of the tube. Keep inoculated roll tubes upright to prevent water at the bottom from contaminating colonies.

[c]Anaerobic plates (Fig. 6): Use the same components as described above but with the following modifications: 500 ml of medium is prepared in a 1-liter flask; sodium bicarbonate and CO_2 are omitted in the preparation; pH of the medium is adjusted to 6.6 to 6.8 before autoclaving; after autoclaving, tempered agar medium ($\sim$20 ml; detection of clear zones is easier if plates contain a thin layer of agar medium) is poured into sterile petri plates on the lab bench and, after agar has solidified, plates are transferred to an anaerobic chamber (90% N_2/5% CO_2/5% H_2) and allowed to degas overnight before being used (e.g., streaked) and incubated in the chamber.

The unique features of this bacterium, which can differ among strains, may influence its ability to be formulated into a probiotic preparation. A recent study examining some common processes and conditions associated with manufacturing of probiotic strains highlighted the resilience of the group I *O. formigenes* strain OxCC13 to lyophilization and storage in yogurt (29). Perhaps humans may be able to be colonized with favorable strains in a lyophilized form or mixed in yogurt.

GENOMICS, PROTEOMICS, AND METABOLOMICS OF *O. FORMIGENES*

The availability of the genomes (13, 30–32) and proteome (33) of *O. formigenes* has provided an opportunity to increase our understanding of the important biological properties of this beneficial gut bacterium, including identification of important proteins and pathways that promote colonization resilience, aerotolerance, and enteric secretion of host-derived oxalate. A review of the genomic sequences of two strains of *O. formigenes* identified some interesting differences that may suggest that group I and group II strains utilize different pathways to survive and flourish within the intestine (13).

General features of the genomes of *O. formigenes* are shown in Table 4. Currently, there are only 4 cultured strains of *O. formigenes* (group I strains HC-1 and OxCC13, the putative group I strain SYGSS-15, and a group II strain HOxBLS) with publicly available, sequenced genomes (13, 30–32). In comparison, there are more than 50 and 500 sequenced genomes from cultivated strains of the resident gut anaerobe *Phocaeicola vulgatus* (*Bacteroides vulgatus*) and gastric pathogen *Helicobacter pylori*, respectively. Despite this limited data set, it is clear that the genomes of *O. formigenes* HC-1 and HOxBLS (Table 4), as well as those reported for OxCC13 (31) and SYGSS-15 (30), are small, with a size of $\sim$2.5 Mb. In comparison, the genome of *Escherichia coli* Nissle

TABLE 4 Genomic features of strains of *Oxalobacter formigenes*[a]

Genomic feature	*Oxalobacter formigenes* HC-1	*Oxalobacter formigenes* HOxBLS	*Escherichia coli* Nissle 1917
Oxalobacter formigenes group designation[b]	I	II	Not applicable
Genome size (Mb)	2.46887	2.48830	5.44120
Total gene count	2,275	2,292	5,303
Protein-coding genes	2,205	2,126	4,694
GC%	49.6	52.7	50.6
CRISPR count	4	6	0
Phage protein-producing sequences (ORFs)[c]	154 (154)	184 (212)	59 (161)

[a]Data and resources from the Joint Genome Institute (JGI)/Integrated Microbial Genomes and Microbiomes (IMG/M) (107), the National Center for Biotechnology Information (NCBI) websites and PHASTER (116) websites were utilized for the preparation of this data set. *Escherichia coli* Nissle 1917 is included for comparative purposes (34).

[b]Group designations are from Jensen and Allison (15).

[c]ORF, open reading frame. The value represents the number of proteins with matches in the phage and bacterial protein databases in regions scored as "intact" by PHASTER scoring criteria for level of completeness. *O. formigenes* HC-1, *O. formigenes* HOxBLS, and *E. coli* Nissle 1917 contained 3 (out of 3), 3 (out of 4), and 1 (out of 5) intact prophage regions, respectively. The parenthetical values include matches in the phage and bacterial protein databases in all prophage regions scored as intact, incomplete, and questionable.

1917, a well-studied, Gram-negative, probiotic gut bacterium (sold commercially as Mutaflor), is ~5.4 Mb (34, 35). Prokaryotic genome sizes range from 0.5 to well over 10 Mb, indicating that the *O. formigenes* genome is more toward the lower (smaller) end of the genome size scale. Gao et al. recently reported that the combination of CRISPR systems and viruses (prophages) associated with the genomes of prokaryotes appears to impede genome expansion and thus over time contributes to a smaller genome size (36). These may be some of the driving forces behind the small genome size of *O. formigenes*, given the CRISPR counts and the large volume of phage-coding elements observed in the genome of this organism (Table 4).

Additional features of the genomes of *O. formigenes* are illustrated in Fig. 7. The spectrum of genes (e.g., Pfam, COG, KEGG Orthology, TIGRfam, enzymes, KEGG pathway, MetaCyc pathway, transmembrane proteins, signal peptide proteins, and IMG pathway) is fairly similar between *O. formigenes* strains HC-1 and HOxBLS. Some notable exceptions are with HOxBLS (a group II strain) in the category of protein-coding genes with function prediction, where gene numbers (%) are reduced, or in the category of protein-coding genes in biosynthetic clusters, where genes are completely absent. The latter continues to build on the potential reasons for the decreased robustness of group II strains relative to growth *in vitro*. Interestingly, in the category of fused protein-coding genes, *O. formigenes* HC-1 and HOxBLS gene numbers (%) are actually higher (7.7 and 10.2%, respectively) than that of *E. coli* Nissle 1917 (3.9%) (Fig. 7). Gene fusion (also referred to as "composite genes") is one of the mechanisms for the creation of new genes in organisms, including prokaryotes, which contain on average 14.5% composite genes (37, 38). How gene fusion, as well as CRISPR systems and phage-coding elements, have collectively helped to shape the genome and thus evolutionary history of *O. formigenes*, is waiting to be elucidated.

The Clusters of Orthologous Genes (COGs) is a versatile tool that has been used over the years for the annotation and comparative analysis of sequenced genomes from *Bacteria* and *Archaea* (39, 40). COGs are composed of families of orthologous protein-coding genes and can be assigned to one or more of the 26 functional categories. This allows for detailed comparisons of organisms relative to their metabolism, cellular processing and signaling, and information storage and processing, as well as to poorly characterized proteins. Small differences (0.5 to 1.0%) in gene distribution can be seen between *O. formigenes* HC-1 and HOxBLS among the following COG functional categories: J, translation, ribosomal structure, and biogenesis; P, inorganic ion transport and metabolism; L, replication, recombination, and repair; C, energy production and
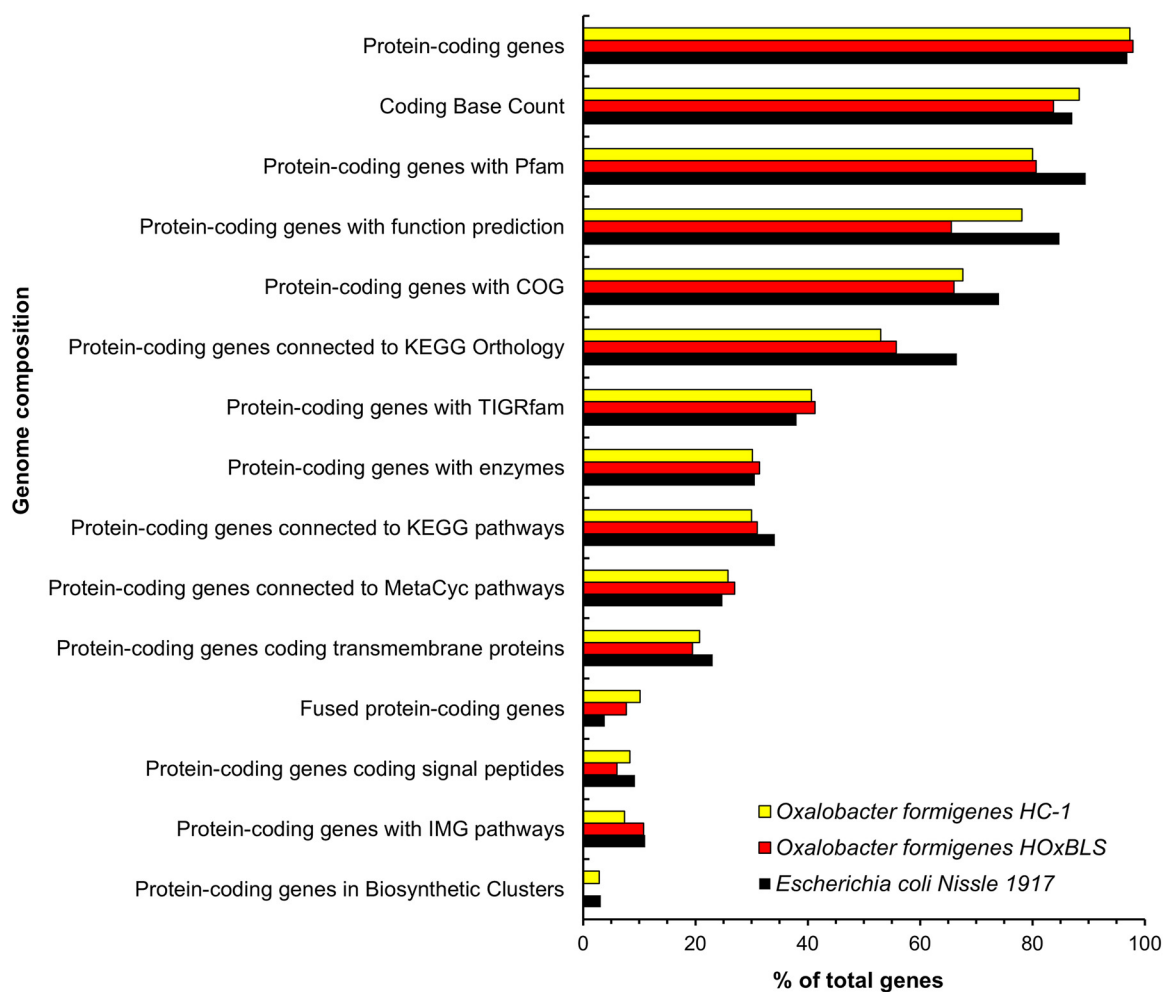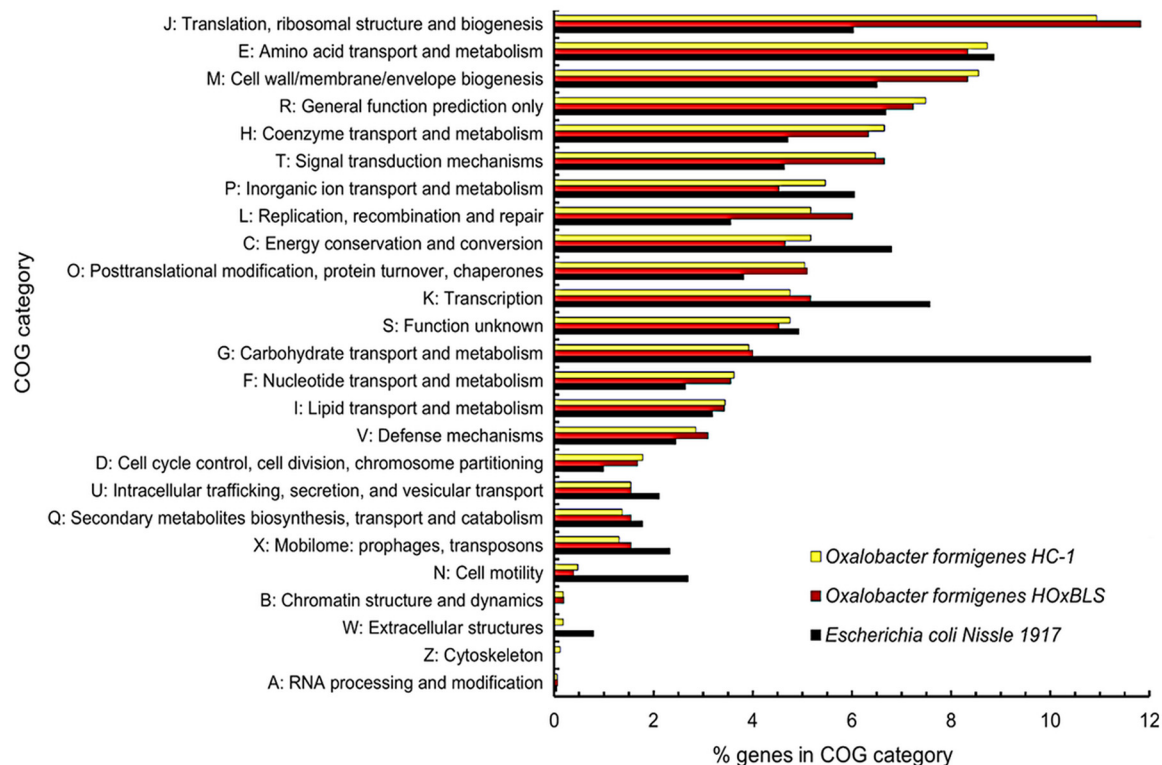
FIG 7 Genome features and composition for strains of *Oxalobacter formigenes*. Data and resources from the Joint Genome Institute (JGI)/Integrated Microbial Genomes (IMG) (107) and the National Center for Biotechnology Information (NCBI) websites were utilized for the preparation of this data set. *Escherichia coli* Nissle 1917 (34) is included for comparative purposes.

conversion; and X, mobilome: prophages, transposons (Fig. 8). An example of the differences between strains is that 118 COGs are shared between group I strains HC-1 and OxCC13, while these same 118 COGs are absent in the group II strain HOxBLS. Likewise, there are 70 COGs unique to HOxBLS. Larger differences (1 to 3%) can be seen in genes assigned to COG categories between *O. formigenes* strains and *E. coli* Nissle 1917, with the largest difference in the category "carbohydrate transport and metabolism" (category G). In *E. coli* Nissle 1917, ~11% of the protein-coding genes with COGs are associated with this functional category compared to the ~4% in *O. formigenes* strains (Fig. 8). This seems reasonable given the nature of *O. formigenes* as an oxalate-metabolizing specialist that is unable to utilize other substrates, including carbohydrates, as a source of energy.

Mass spectrometry-based shotgun proteomics identified 1,822 proteins of the 1,867 unique protein-coding genes in the group I *O. formigenes* strain OxCC13 (33). From the protein data sets presented, it is clear this organism contains a repertoire of metabolic pathways that mediate adaptation with nutrient shifts and environmental stress. For example, the proteomic analysis showed superoxide dismutase increases in stationary phase relative to log phase, suggesting *O. formigenes* has the ability to persist outside the anaerobic environment of the large intestine.

More recent investigations have focused on whether *O. formigenes* expresses features in its metabolome that are unique and/or undetectable in other oxalate-degrading bacteria

FIG 8 Distribution and abundance of genes in Clusters of Orthologous Genes (COG) categories for *Oxalobacter formigenes* HCl-1, *Oxalobacter formigenes* HOxBLS, and *Escherichia coli* Nissle 1917. *Escherichia coli* Nissle 1917 (34) is included for comparative purposes. Data and resources from the Joint Genome Institute (JGI)/Integrated Microbial Genomes (IMG) website (107) were used in the preparation of this data set. For *O. formigenes* HC-1, *O. formigenes* HOxBLS, and *E. coli* Nissle 1917, there are 746, 737, and 1291 genes not in COGs, respectively.

(41, 42). These intriguing studies have revealed a set of 12 exclusive features in a lysate preparation of *O. formigenes* strain HC-1 compared with lysate preparations of *Lactobacillus acidophilus*, *Lactobacillus gasseri*, and *Bifidobacterium animalis* (41). With respect to differences in the metabolomes and lipidomes of *O. formigenes* strains HC-1 and OxWR, there are notable and significant differences in the relative expression of individual metabolic products, although the metabolomes and lipidomes are found to be largely conserved between the two strains (42). Future investigations are needed to "expand the defined metabolome and lipidome of *Oxalobacter* spp." (42). Such studies may, for example, lead to the identification of specific molecules that can enhance enteric oxalate secretion and colonization resilience.

ECOLOGY OF *OXALOBACTER FORMIGENES* IN THE HUMAN GUT

Prevalence and abundance of *O. formigenes* colonization in humans has been investigated by several studies. However, lack of standardized methods for *O. formigenes* detection (culture-based versus molecular) and the low abundance of the organism has made the accurate assessment of colonization challenging. *O. formigenes* colonization frequency varies across different populations. A study in North India showed that *O. formigenes* colonization is present in 65% of healthy adults (43). A study of healthy adults in Seoul, South Korea showed the presence of the *oxc* gene (a marker of *O. formigenes*) in 79% of individuals (197 of 233) (44). In healthy Japanese participants, 80% of 54 male and 62% of 34 female subjects showed colonization with *O. formigenes* (45). In two large cohort studies of healthy American adults, ~30% were colonized with *O. formigenes* (46, 47). This is considerably lower than other countries. PeBenito et al. studied *O. formigenes* colonization across 3 different populations and found that *O. formigenes* colonization was much lower in the United States cohort compared to the Hadza people in Tanzania or the Amerindians from Venezuela (48). Clemente et al. studied *O. formigenes* colonization in members of a Yanomami village,

which has no contact with western medicine or culture, and also found a much higher *O. formigenes* colonization prevalence compared to that in the United States (49). Clemente et al. further stratified antibiotic use and found that westernization significantly affects *O. formigenes* colonization independently of antibiotic exposure. These data suggest that our modern lifestyles contribute to the disappearance of beneficial microbiota (50). In fact, in fecal samples where *O. formigenes* is detected, gut microbiota are more diverse and the global microbial network displays more connectivity and resilience to simulated disturbance. This suggests that the prevalence and abundance of *O. formigenes* is an indicator of the ecological state of the gut microbiome (51).

An analysis of sequencing data (whole shotgun sequencing and 16S rRNA) from the human microbiome project (HMP) and American gut project (AGP) indicated that more than one *O. formigenes* strain may colonize the human gut, with group I strains being more prevalent than group II (46, 51). Both these analyses also showed that relative abundance of *O. formigenes* varied 3 logs, with a mean of 2.9×10^4, highlighting that *O. formigenes* numbers in stool may sometimes be at or below the limit of detection when using molecular methods, including PCR.

FACTORS AFFECTING *OXALOBACTER* COLONIZATION OF THE ALIMENTARY TRACT

Children do not become colonized with *O. formigenes* "until they begin crawling around in the environment" (52). Controlled studies in rodents have also confirmed *O. formigenes* horizontal transmission (53–55). Cornelius and Peck conducted a very elegant study in rats showing that colonization of all offspring did not occur until 7 days post weaning and, importantly, colonization was derived from foster mothers, not birth mothers, after the weanlings were switched from their colonized birth mothers to foster mothers each carrying a different *O. formigenes* strain (53). In our laboratories, we also addressed this question in adult conventional mice and gnotobiotic mice. When equal numbers of conventional *O. formigenes* strain OxCC13-colonized mice and noncolonized mice are cohoused, the noncolonized mice become colonized with *O. formigenes*, confirming horizontal transmission. The introduction of a single *O. formigenes* strain OxCC13 mono-colonized gnotobiotic mouse into a cage of four noncolonized, germfree mice also leads to all germfree mice becoming colonized with *O. formigenes* (55).

One of the major gaps in our knowledge regarding *O. formigenes* is the paucity of information regarding the factors that influence *O. formigenes* colonization. Controlled diet studies in humans (56), mice (57), and rats (58) have demonstrated the profound impact of dietary oxalate and calcium on *O. formigenes* numbers. There is also evidence that luminal fat content in rats (59) inhibits the ability of *O. formigenes* to reduce urinary oxalate excretion; however, it is not clear if this is a contributing factor in humans. Jiang and colleagues determined *O. formigenes* numbers in the stool of healthy human subjects and found stool bacterial numbers increased 12-fold as dietary oxalate increased 15-fold (Fig. 9) (56). Of note, a 5-fold increase in dietary calcium intake, which will limit the bioavailability of oxalate due to the high affinity of calcium for oxalate, decreased stool *O. formigenes* numbers approximately 5-fold. The dependency for oxalate and the inverse relationship between dietary calcium and *O. formigenes* numbers may lead to a loss of colonization in kidney stone formers, as these patients are encouraged to maintain an adequate calcium and low-oxalate intake. This potential dynamic warrants further investigation.

Two large cohort studies with humans have investigated *O. formigenes*-host relationships. Kelly and colleagues' analysis of 94 *O. formigenes*-colonized subjects and 146 noncolonized healthy individuals did not show any impact of sex, race, education, region of U.S. residence, body mass index, history of urinary tract infection, family history of renal stones, and diuretic use on frequency of *O. formigenes* colonization (47). In a multivariate analysis of more than 4,900 samples in the AGP database, the relative abundance of *O. formigenes* was associated with increased age, female sex, Caucasian ethnicity, non-USA residence, normal BMI, higher educational attainment, and absence of antibiotics exposure within a year (51). Interestingly, among the 197 subjects that
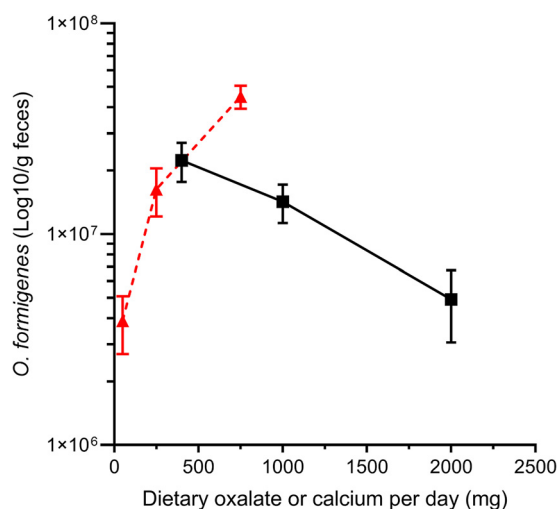
FIG 9 Amount of *O. formigenes* in the feces of human subjects relative to changes in dietary oxalate (red triangles) or dietary calcium (black squares). Daily calcium intake was 1,000 mg on the varied oxalate dietary phase and daily oxalate was 250 mg on the varied calcium dietary phase. Real-time quantitative PCR (qPCR) was used to quantitate *O. formigenes* numbers, and 5.5×10^4 CFU/ng DNA was used to convert qPCR data to number of *O. formigenes* per g feces (108). Error bars represent SEM. (Adapted from reference 56 with permission from Wolters Kluwer Health.)

completed a food frequency questionnaire, *O. formigenes* relative abundance was not significantly associated with dietary oxalate intake, but inversely associated with dietary calcium intake and the mole ratio of dietary oxalate to dietary calcium intake (51). In this regard, dietary calcium intake in a number of Eastern countries, including India, China, South Korea, and Japan, is much lower than that in the United States. (60). In light of the various findings of these studies, further investigations are needed on how culture, demographics, underlying medical conditions, access to western medicine, and diet impacts *O. formigenes* frequency rates in different populations.

Oral antibiotic use and antibiotic exposure at a young age have been associated with an increased risk for stone formation in humans (61–63) and decreased prevalence of *O. formigenes* colonization (47). Human strains of *O. formigenes* are sensitive to common antibiotics (64). Sidhu et al. suggested that the low frequency of *O. formigenes* colonization in cystic fibrosis patients is due to widespread antibiotic use (65). Sidhu et al. also reported a clear association with antibiotic therapy and lack of *O. formigenes* colonization in calcium-oxalate stone formers (66). Siener et al. assessed the relationship of *O. formigenes* colonization and urinary oxalate excretion in female calcium-oxalate stone formers treated with antibiotics for recurrent urinary tract infections (UTIs) and found significantly higher urine oxalate excretion in this cohort compared to women without recurrent UTI's (67). The same association was observed by Kharlamb et al. when looking at individuals receiving triple antibiotic therapy for *H. pylori* infection (68). The loss of oxalate-degrading bacteria, such as *O. formigenes*, following antibiotic use has been suggested to contribute to increased kidney stone risk due to augmented urinary oxalate excretion (65).

Whether *O. formigenes* colonization is influenced by other microbes is unclear, and has only been addressed in one study utilizing gnotobiotic mouse models of *O. formigenes* colonization (55). This study showed that introduction of a defined mixture of seven non-oxalate-degrading bacteria (altered Schaedler flora) into mice mono-colonized with *O. formigenes* strain OxCC13 had no impact on *O. formigenes* intestinal numbers or the capacity of *O. formigenes* to degrade intestinal oxalate. Future investigations in gnotobiotic mice should address the impact of other oxalate degraders on intestinal *O. formigenes* numbers and oxalate homeostasis. Future studies may also consider using gut chemostat models (69), since these *in vitro* approaches can directly evaluate the dynamics of a microbial community in a host-free environment. In fact, it was a methanogenic, oxalate-degrading enrichment culture established in a chemostat with sheep

rumen contents that yielded the first anaerobic oxalate-degrading bacterium, *O. formigenes* strain OxB (10, 19, 70).

Over the course of our own studies, we have observed there are other unknown factors that affect *O. formigenes* colonization in mice, in addition to the duration of the colonization. For example, we previously reported that the AGT (<u>a</u>lanine-<u>g</u>lyoxylate amino<u>t</u>ransferase) knockout mouse can be colonized with *O. formigenes* strain OxWR, but begins to lose colonization rapidly after about 2 weeks and by 7 weeks all AGT knockout mice have lost colonization (71). Recently, we also reported that downregulated in adenoma (DRA; Slc26a3) knockout mice are easily colonized with *O. formigenes* strain OxWR, but lose colonization in a similar manner to the AGT knockout model (72). Interestingly, a double knockout of DRA/AGT is resistant to *O. formigenes* strain OxWR colonization (72). It is also notable that all C57BL/6 control cohorts orally gavaged with either *O. formigenes* strain OxWR, OxCC13, or HC-1 sustain colonization for months on regular chow (54, 57). One study in C57BL/6 control mice has also shown that colonization with *O. formigenes* strain OxCC13 is resistant to short-term dietary oxalate deprivation (57). Presumably, the luminal environment of some mouse strains and perhaps some humans are not receptive, or only transiently receptive, to *O. formigenes* colonization. Examining differences in enteric oxalate secretion, intestinal chemical composition, and gut microbiomes between host species may shed further light on factors that influence *O. formigenes* colonization.

O. FORMIGENES IMPACT ON INTESTINAL OXALATE DEGRADATION, URINARY OXALATE EXCRETION, AND RISK OF CALCIUM OXALATE STONE DISEASE

Since its discovery in 1980, the potential of *O. formigenes* as a treatment for those afflicted with calcium oxalate kidney stone disease has been under consideration. Increased urinary oxalate excretion is a recognized risk factor for the development of such stones (73). High dietary oxalate absorption leads to increased oxalate delivery to the kidney and thus an increased risk of calcium oxalate kidney stone formation. Since the mammalian body does not innately possess enzymes capable of metabolizing oxalate, the oxalate-degrading gut microbiota may influence urinary oxalate excretion. Early case-control studies with small numbers of human subjects suggested colonization with *O. formigenes* may be protective against stone disease, as measurements of urinary oxalate excretion are lower in *O. formigenes*-colonized compared to noncolonized individuals (62, 74, 75). However, there was large variability in oxalate excretion and a lack of control of dietary oxalate and calcium during urine collections. Another study showed 24-h urinary oxalate excretion and plasma oxalate were significantly lower in *O. formigenes*-colonized kidney stone patients compared to *O. formigenes*-negative patients consuming a standardized diet (76). Colonization was also found to be significantly inversely associated with the number of stone episodes (76). In 1995, Han et al. were the first to show an inverse relationship between *O. formigenes* colonization and stone formation (77). Kaufman et al. later showed in a larger cohort of patients that *O. formigenes* colonization was associated with a 70% decreased risk of recurrent kidney stone formation (78). This inverse relationship has been confirmed in many subsequent studies (62, 75, 76, 79, 80). Despite the abundance of studies showing an inverse relationship between *O. formigenes* colonization and stone disease, colonization with *O. formigenes* only demonstrated lower 24-h urinary oxalate excretion in 55% of studies, suggesting other factors may be contributing (81). These results may be partially explained by the controlled dietary study by Jiang and colleagues, where they showed healthy individuals (humans) naturally colonized with *O. formigenes* excreted less 24-h urinary oxalate compared with noncolonized individuals only when ingesting a diet moderately high in oxalate and low in calcium (Fig. 10) (56).

While there are other intestinal microorganisms that can degrade oxalate ("generalists"), including some strains of *Bifidobacterium* and *Lactobacillus* (82), studies in mice suggest that *O. formigenes* is superior by comparison in terms of reducing urinary oxalate excretion. For example, while *O. formigenes* strain HC-1 colonization led to a 90% reduction in urinary
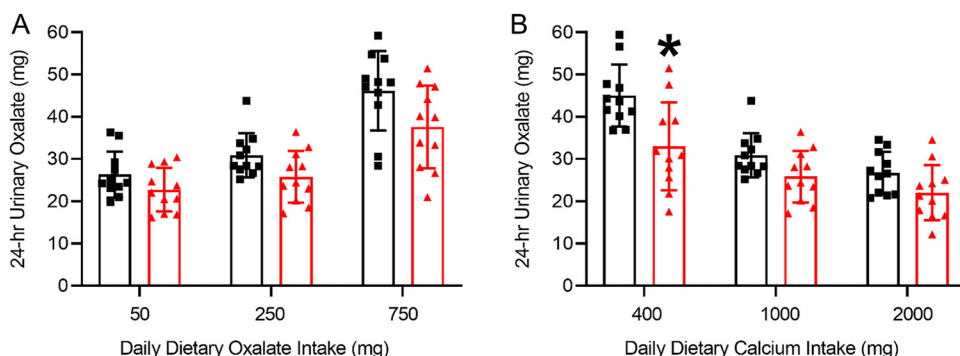
FIG 10 (A and B) 24-h urinary oxalate excretion of healthy subjects not colonized (black squares) and colonized with *O. formigenes* (red triangles) on nutrient-controlled diets varying in oxalate (A) and calcium (B). Daily calcium intake was 1,000 mg on the diets varying in oxalate and daily oxalate intake was 250 mg on the diets varying in calcium. Individuals colonized with *O. formigenes* excreted significantly less urinary oxalate than noncolonized individuals on the 250 mg oxalate/400 mg calcium diet; indicated by *, $P = 0.026$. Error bars represent SD. (Adapted from reference 56 with permission from Wolters Kluwer Health.)

oxalate excretion by conventional (C57BL/6) mice (71), colonization with *Bifidobacterium animalis* resulted in a 44% reduction (83). Similarly, reductions of 34% and 32% were observed in urinary oxalate following colonization of mice with *Lactobacillus acidophilus* and *Lactobacillus gasseri*, respectively (54). This superior effect of *O. formigenes* colonization on urinary oxalate excretion may be due to the dual actions of inducing enteric oxalate excretion coupled with highly efficient luminal oxalate degradation.

Advanced "omic" studies over the last decade have allowed a more in-depth examination of the intestinal microbiome in healthy individuals and kidney stone formers (81, 84–91), and have demonstrated that (i) *O. formigenes* is at the center of a multispecies bacterial network contributing to gastrointestinal dietary oxalate degradation, and (ii) fecal samples from stone formers have a significantly lower bacterial representation of genes involved in oxalate degradation, which may contribute to increased urinary oxalate in this cohort. In a multiomics data (>3,000 samples from >1,000 subjects) analysis of the human microbiota, Nazzal et al. (84) showed that among the diverse bacteria capable of oxalate degradation, *O. formigenes* dominates this function transcriptionally in healthy adults. Further, they demonstrated that patients with inflammatory bowel disease (IBD) had elevated enteric oxalate level and low transcription of oxalate degradation genes. Interestingly, their data indicated that the absence of *O. formigenes* colonization or colonization below the level of detection is responsible for the reduction in oxalate degradation function.

Enumeration of *O. formigenes* in human stool from healthy non-kidney-stone-forming individuals indicates that *O. formigenes* represents a tiny fraction of the total intestinal microbiota (56). Many such low-abundance bacteria are thought to survive in the intestines by occupying specific nutrient niches where competition for their food source is limited (92). Indeed, both *in vitro* culture studies (93) and a human study (56) show that *O. formigenes* utilizes oxalate more efficiently than other intestinal bacteria. Jiang and colleagues (56) also showed that the oxalate-degrading capacity of the microbiome of noncolonized individuals is negligible at low oxalate intake, but increases with adaptation to ingestion of higher levels of dietary oxalate; the oxalate recovered in stool with daily dietary oxalate intake of 250 mg and 750 mg was ~80% and ~60%, respectively (Fig. 11).

Numerous reports in rodents have documented the beneficial effects of intestinal *O. formigenes* in reducing urinary excretion of oxalate. Initially, this was assumed to be only due to *O. formigenes* degrading dietary sources of oxalate, thereby limiting its absorption. However, later studies revealed that intestinal *O. formigenes* has a unique dual action of interacting with the host intestinal epithelia by promoting enteric oxalate elimination from the systemic circulation, which correlates with the significant decreases in renal excretion (58, 59, 71, 72, 94). These observations in rats and mice led to the proposal that *O. formigenes* (strains OXWR and HC-1) may produce a secretagogue, a substance possibly proteinaceous in nature (95), which mediates alterations in transepithelial oxalate transport across
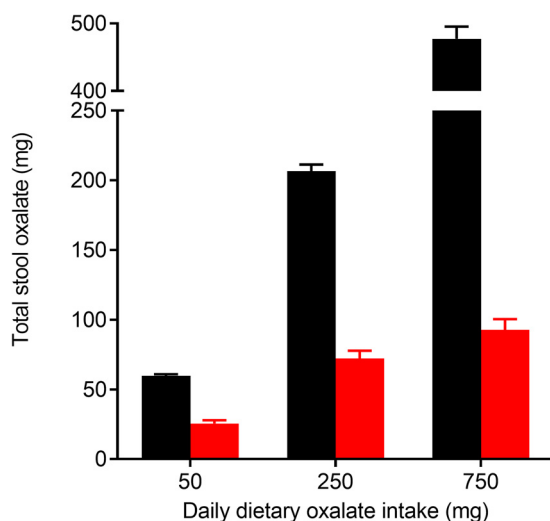
FIG 11 Stool oxalate of *O. formigenes* colonized (black bars) and noncolonized healthy subjects (red bars) on nutrient-controlled diets varying in oxalate. Daily calcium intake was 1,000 mg. Error bars represent SEM. (Adapted from reference 56 with permission from Wolters Kluwer Health.)

colonized intestinal tissues (58). In addition, this mechanism for the transport of the essential substrate (oxalate) for *O. formigenes* from blood-to-gut may account for the survival of *O. formigenes* even when the supply of dietary oxalate is negligible (57).

A recent *in vitro* study indicated that cell-free supernatant fluid from *O. formigenes* strain OxB cultures stimulates oxalate secretion in human intestinal Caco-2-BBE cells (95); however, these findings were not replicated by a different group (14) and warrant further investigation. Arvans et al. (95) also showed that rectal administration of cell-free supernatant fluid from *O. formigenes* strain OxB cultures resulted in a reduction in urinary oxalate excretion in a mouse model of PH1; however, a direct measurement of enteric oxalate secretion utilizing oxalate isotopes administered intravenously and then measured in the gut is still needed to quantify the extent of this pathway. Of interest was the filing of a patent by OxThera Pharmaceuticals in 2015 that covers the invention of the isolation and administration of secretagogues derived from *O. formigenes* that may enhance oxalate secretion into the intestinal lumen (96). Although the work described in this patent did not identify any compelling secretagogue candidates, the identification of a bioactive factor or factors secreted by *O. formigenes* that induces oxalate secretion may be an effective therapy to reduce the oxalate burden in patients with PH1.

COLONIZATION OF NONCOLONIZED INDIVIDUALS WITH *O. FORMIGENES*

The ability to colonize individuals lacking *O. formigenes* has previously been addressed by a study in which two healthy adult humans not colonized with *O. formigenes* became colonized following the ingestion of a high-oxalate meal containing $\sim 10^8$ cultured live *O. formigenes* strain HC-1 cells (97), and subsequently remained colonized for 9 months. Although this study had the subjects consume a high-oxalate meal prior to successful colonization with *O. formigenes*, a study in mice has shown that colonization may not require "priming" with an oxalate-supplemented diet (54). Thus, future human studies should explore whether an oxalate-rich diet is required for successful colonization following oral ingestion of *O. formigenes*. Other studies that have examined colonization following ingestion of *O. formigenes* have not been successful. A clinical trial conducted by the company OxThera, where *O. formigenes* strain HC-1 was provided either in lyophilized form inside an enteric coated capsule or as a frozen paste to patients with PH1, resulted in only a minority of the patients remaining colonized posttreatment (98, 99). More recent clinical studies also did not show a significant reduction in urinary oxalate excretion compared to controls (99, 100). Reasons for the poor frequency of human colonization in these studies are presently unknown but could include: (i) problems associated with the formulation of

the inoculum (strain HC-1) that was supplied or how the inoculum was administered to the patients; (ii) resistance to colonization by undefined host factors in patients with PH1; or (iii) unknown gut microbiome-associated factors in these patients. However, it should be noted that one human study (97) involving noncolonized healthy adults demonstrated long-term colonization and a significant reduction in urinary oxalate excreted in the 6 h following ingestion of a large biomass of *O. formigenes* strain HC-1 (10^8 viable cells) with an oral sodium oxalate load (2 mmol/70 kg body weight); urinary oxalate excretion pre-*O. formigenes* ingestion was 3.0 ± 0.6 mg/h ($n = 4$) compared to 1.9 ± 0.1 mg/h with *O. formigenes* ingestion. Thus, it remains possible that colonization by more resilient *O. formigenes* of noncolonized stone formers may be an effective way to help minimize stone risk in calcium oxalate stone formers.

An ongoing clinical trial (NCT03752684; clinicaltrials.gov) with healthy human volunteers conducted at the University of Alabama at Birmingham is testing whether individuals not colonized with *O. formigenes* can be colonized with live cultures of *O. formigenes* strain OxCC13. Changes in urinary oxalate excretion on fixed oxalate diets before and after *O. formigenes* colonization is also being evaluated. The trial has demonstrated that healthy individuals can be readily colonized following ingestion of a single dose of ~10^{10} live cultured *O. formigenes* cells, with only one of the 15 participants losing colonization 6 months after ingestion of *O. formigenes*.

SUMMARY

Much still remains to be learned about how *O. formigenes* establishes and maintains gut colonization. Unraveling these mechanisms may be especially important with respect to the colonization of noncolonized stone formers. Further studies on the factors involved in colonization resilience and enteric secretion of host-derived oxalate are warranted. Furthermore, in light of failed clinical trials with *O. formigenes*, more comprehensive examinations are clearly needed for (i) the best practices for optimum delivery of this strict anaerobe either as a pure culture, as a member of a synthetic, oxalate-degrading microbial consortium, or as part of a fecal microbial transplant from an *O. formigenes*-colonized donor; (ii) interactions between oxalate-degrading and non-oxalate-degrading bacteria in the gut; and (iii) how these bacteria collectively impact urinary oxalate excretion and stone risk.

ACKNOWLEDGMENTS

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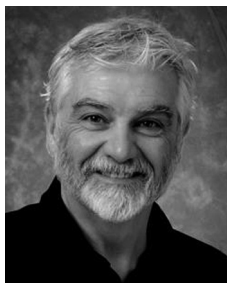
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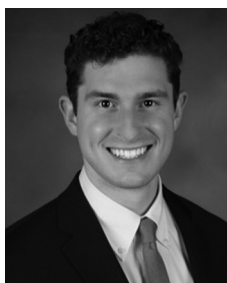
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Kyle Wood is a fellowship-trained endourologist and Assistant Professor at the University of Alabama at Birmingham (UAB). Dr. Wood was an AUA research scholar and now is an NIH-funded K08 grant scholar conducting kidney stone research with a special interest in oxalate metabolism and kidney stone disease. Dr. Wood graduated from Brown University with honors, attended medical school at the University of Massachusetts, did his residency at Wake Forest University, and his fellowship at UAB.
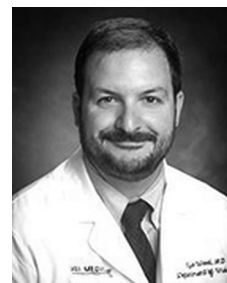
Dean G. Assimos, MD, is Professor and Chair of the University of Alabama at Birmingham (UAB) Department of Urology. His career focus has been the management of patients afflicted with kidney stones. His research has helped define the impact of dietary oxalate on urinary oxalate excretion, mechanisms of endogenous oxalate synthesis, gastrointestinal and renal oxalate handling, and the influence of the fecal microbiome in the development of kidney stones. He is currently conducting research on the relationships of obesity and calcium oxalate kidney stone formation and is the PI of two currently funded NIH grants pertaining to this subject. Dr. Assimos is the former Chair of the American Urological Association kidney stone guidelines committee.
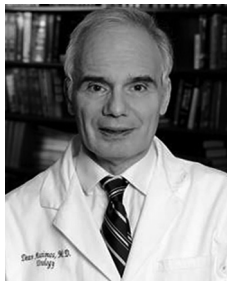
Ross Holmes is a Professor in the Department of Urology, University of Alabama at Birmingham. He received his PhD from the Australian National University and was previously on the faculty at Wake Forest University for 25 years. His research interest centers on identifying factors influencing dietary oxalate absorption and their influence on urinary oxalate excretion and calcium oxalate kidney stone formation. *O. formigenes* is a key component in this process.

Lama Nazzal is an Assistant Professor in the Department of Nephrology at New York University (NYU). She received her MD from the American University of Beirut and was trained in nephrology at New York University. She is a translational researcher focusing on the effect of the gut microbiome on kidney diseases and particularly nephrolithiasis. She uses next-generation sequencing methods to understand microbial oxalate metabolism in the human gut in health and disease conditions. She was the recipient of the Rare Kidney Stone Consortium pilot and training grant and was the first recipient of the Oxalosis and Hyperoxaluria Foundation-American Society of Nephrology career development award.

Marguerite Hatch received her undergraduate and graduate education in Dublin, Ireland, where her Ph.D., entitled *The Pathophysiology of Calcium Oxalate Kidney Stone Disease*, was awarded by Trinity College Dublin. She did two post-doctoral fellowships at the University of Arizona and at the Harvard School of Medicine in the Department of Physiology and Biophysics. Her first faculty appointment was at Louisiana State University and she then worked at SUNY-Downstate in NYC, University of California at Irvine, and Northwestern University in Chicago. Currently she is a tenured Professor at the University of Florida. She has been working in the field of kidney stone disease for 45 years, with a start in tackling oxalate methodology of biological fluids and then, for the last few decades, with a concentration on intestinal oxalate transport and the role of *Oxalobacter* in reducing urinary excretion of this stone-forming compound.

John Knight received his PhD in Physiology at the University of Newcastle-upon-Tyne, United Kingdom. He was an Assistant Professor in the Department of Urology at Wake Forest University Health Sciences, and currently is an Associate Professor in the Department of Urology at the University of Alabama at Birmingham. His research over the last 17 years has focused on investigating novel strategies to reduce the risk of calcium oxalate kidney stone disease. A large component of his current research is to increase our understanding of endogenous oxalate synthesis and potential approaches to reduce this metabolism, and to examine whether colonization of the gut with the specialist oxalate-degrading bacterium *Oxalobacter formigenes* results in lower urinary oxalate excretion.