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Laccase-mediated biotransformation potential for fluorinated compounds by geographically diverse human gut microbiota

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ABSTRACT The growing prevalence of synthetic organofluorine substances in agrochemicals, food packaging, and consumer products has led to increasing gastrointestinal exposure, with potential consequences for human health. Despite the extreme stability of fluorinated compounds, several microbial pathways for their transformation are known, including those involving laccases, a type of multicopper oxidase. However, the functionality of laccases in the gut microbiome, a natural contact point between food-associated chemicals and microbial biotransformation pathways, is poorly defined. Through a multi-study analysis of 1,578 human gut metagenomes spanning a global gradient from hunter-gatherer societies to industrialized urban populations, we found that laccase-coding gene homologs are widely distributed in the human gut microbiome. We identified a significant association between both the abundance and phylogenetic diversity of laccase homologs and the degree of urbanization. As human gut microbial laccase activity has not been experimentally demonstrated, eight gut metagenome-derived laccases were heterologously expressed and screened for activity with a redox mediator system. Six of the eight laccases demonstrated activity. One of these gut microbial laccases, derived from *Veillonella*, and three previously characterized laccases were then tested for their capacity to deplete 11 different food-associated chemicals. The *Veillonella* laccase depleted the agrochemicals cyflumetofen and fluazinam, as well as the industrial chemical bisphenol AF, to a lesser extent. By linking global gut metagenomes with activity assays, this work demonstrates the untapped potential of mining human gut metagenomes for laccases and other microbial enzymes that can actively modify various agricultural and industrial chemicals.

IMPORTANCE As adverse effects of fluorinated compounds on human health are emerging, the responsible enzymes from the human gut microbiome of geographically diverse human cohorts mediating interactions with fluorinated compounds in the gastrointestinal tract remain poorly characterized. In a multi-study analysis of publicly available microbiome sequencing data, we linked the abundance, diversity, and phylogeny of laccases within the gut microbiome to the degree of urbanization of human cohorts and experimentally demonstrated the ability of these laccases to deplete a range of food-associated fluorochemicals. Another significant contribution of our study is in the integration of rural catchment areas as a quantitative metric of urbanization in gut microbiome metagenomics and enzyme activity surveys.

KEYWORDS laccase, gut microbiome, human microbiome, fluorinated compounds, urbanization

Microbial biotransformation represents a critical yet insufficiently addressed factor in chemical risk assessment of fluorinated compounds, which have been linked with genotoxicity and endocrine disruption in humans (1–5). Fluorinated compounds are widely used in agriculture due to attractive properties, such as increased stability,

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uptake, and bioavailability (1, 6–9), and the share of fluorinated chemicals among newly approved agrochemicals has risen from 9% to nearly 70% in the past quarter-century (3, 6, 10). While concerns have long been raised over the impacts of fluorinated pesticides on the environment and human health, transformation products can exhibit disparate toxicities compared to their parent compounds, potentially intensifying or mitigating environmental and human health risks (1, 5, 11–15). Gut bacteria have been shown to bioaccumulate and biotransform fluorinated compounds, with potential effects on bioavailability and side effects of fluorinated drugs (16–19). However, more research is needed on different classes of enzymes capable of fluorinated compound biotransformation, specifically their distribution, evolutionary relationships, and fluorinated substrate specificity. The human gut microbiome has been studied extensively in the past decade to understand its influence on human health (20, 21), but the capacity of gut microbiota to act on fluorinated compounds and other environmental pollutants remains insufficiently explored (16, 22).

While the majority of gut microbiome samples sequenced so far originate from industrialized populations, studies have revealed that there are major differences in gut microbiome composition between industrialized and traditionalist societies (23–25). Lifestyle and urbanization play a crucial role in shaping the taxonomic structure (26) and functional gene composition of the gut microbiome, leading to differences in the resistome and metabolic capacity, e.g., carbohydrate-active enzymes (CAZymes) (27). More recent studies have also explored the microbiota of intermediate cohorts, to which a large part of the world's population belongs, i.e., neither living in highly industrialized urban centers nor living traditionalist lifestyles such as hunter-gatherers (28, 29). Our study builds on these findings by investigating a class of CAZymes relevant for biotransformation of fluorinated compounds from the human gut microbiota along an urbanization gradient (30).

Specifically, our study focuses on laccases, a class of CAZymes previously shown to biotransform xenobiotics including fluorinated compounds (31). Laccases are multicopper oxidases (MCOs) encoded in plants, archaea, fungi, and bacteria that are capable of oxidizing phenolic and other substrates with molecular oxygen as an electron acceptor (32, 33). The active site of MCOs, including laccases, consists of four copper (Cu) atoms of Type 1 (T1), Type 2 (T2), and Type 3 (T3) (34). Substrate oxidation occurs at the mononuclear T1 site, whereas reduction of oxygen occurs at the trinuclear site with two T3 Cu and one T2 Cu (35). Small-molecule redox mediators can also act as electron shuttles between the enzyme and the substrate. These mediator-assisted reactions significantly expand the oxidative capabilities of laccases, allowing them to target a broader range of substrates that are not directly accessible to the enzyme's active site. Recently, we demonstrated the capacity of a bacterial laccase-mediator system to biotransform diverse xenobiotic organic compounds, highlighting its wide substrate range (33). Laccase homologs are widespread in environmental and host-associated microbiomes, yet their role in the human gut has not been defined, despite the intestine being a natural contact point between xenobiotics and microbial biotransformation enzymes.

We hypothesized that the diversity of laccase enzymes may vary in the gut microbiota of humans living in different locations and urbanization levels because of dietary and/or environmental exposures that shape the gut microbiome. Using publicly available metagenomic shotgun sequencing data from six different studies, we investigated associations of laccase-coding gene abundance and laccase sequence diversity and phylogeny, and the degree of urbanization of sampling locations, classified according to urban-rural catchment areas (URCA) (30) as a quantitative measure of the degree of urbanization. To our knowledge, this is the first microbiome study to use the URCA classification, allowing us to establish more objective and quantitative associations between microbiome and high-resolution geographical data studies across different studies and large geographic distances (30). We investigated the distribution and taxonomic lineages of laccase homologs in this multi-study analysis to differentiate

putatively transient versus resident populations of the human gut. Using heterologous expression of diverse bacterial laccases, including eight derived from the human gut microbiome, we demonstrate that host-associated laccases depleted several fluorinated compounds, suggesting that such reactions could also occur within the gut.

RESULTS

Multi-study analysis and MAG recovery

To evaluate the global distribution of bacterial laccase homologs in the gut microbiome across diverse populations, we re-analyzed a set of six published studies from distinct geographic locations and lifestyles. These represent a gradient of urbanization following the urban-rural catchment areas (URCA) classification system, from urban centers (URCA 1–7) to peri-urban (URCA 8–13) to peri-rural (URCA 14–27) to hinterland (URCA 28-30), including hunter-gatherer communities (30). We recovered a total of 158,198 MAGs from our data set, of which 48,312 were medium quality (completeness ≥ 50% and contamination <10%) and 17,626 were high quality (completeness >90% and contamination <5%) (Fig. S1).

Laccase-coding gene abundance, as well as laccase protein sequence diversity and phylogeny, correlates with urbanization

We examined the diversity of laccase homologs across human cohorts in our multi-study analysis, with the hypothesis that laccase homolog diversity and taxonomic affiliations will vary across lifestyle gradients. We found that the abundance of contigs containing putative laccase genes in the metagenomic samples correlates negatively with the URCA index for the samples (linear regression, adjusted R^2 = 0.07, P < 0.001) (Fig. 1A). This is a more pronounced effect size than that for the housekeeping gene *rpoB* (linear regression, adjusted R^2 = 0.02, P < 0.001) (Fig. S2). Additionally, we used Faith's phylogenetic diversity (Faith's PD) as an index of laccase genetic diversity (based on sequence similarity), demonstrating that the genetic diversity of laccase homologs in each sample with full laccase sequences is also negatively correlated with the URCA index (linear regression, adjusted R^2 = 0.12, P < 0.001) (Fig. 1B). Unweighted UniFrac distance was calculated based on laccase genetic diversity and abundance in each sample to evaluate how laccase homolog composition varies across populations and URCA settings (Fig. 1C), demonstrating a significant association with the URCA index (PERMANOVA, R^2 = 0.07, P = 0.001). This further highlights that gut microbial laccases differ in protein sequence and abundance across urbanization levels.

As laccase homolog alpha diversity (represented by Faith's PD) and beta diversity (represented by UniFrac distance) correlate with URCA degree of urbanization, next, we examined the laccase protein diversity in more detail to assess whether these differences were driven by differences in taxonomic composition. Construction of a phylogenetic tree of the laccases found indicates that the mean URCA of laccase origin sites correlates with phylogenetic distance (Moran's I, I = 0.35, P = 0.001; Blomberg's K, K = 0.0014, P = 0.003), indicating that laccases from cohorts with similar levels of urbanization share similar laccase clades and taxonomic origins as well (36). Many laccases found in samples from urbanized cohorts are from contigs assigned to the genus *Akkermansia* by Kraken2, 47 of which are from the species *Akkermansia muciniphila* (37). Overall, the laccases from (peri-)rural (URCA ≥ 14) samples are from contigs assigned to more mixed taxa, with laccases found in 12 different families compared to five families in (peri-)urban samples (Fig. 2) (30).

Next, we examined the typical source location of bacterial species associated with laccase homologs in our multi-study analysis with the aim of distinguishing between putatively resident and transient members of the gut microbiota and identifying potential influences from food sources. Using the BacDive database, isolation sources of all strains associated with species with contigs coding for laccase genes were retrieved and classified as either animal, food, human gut, human non-gut, or other (38). Notably,

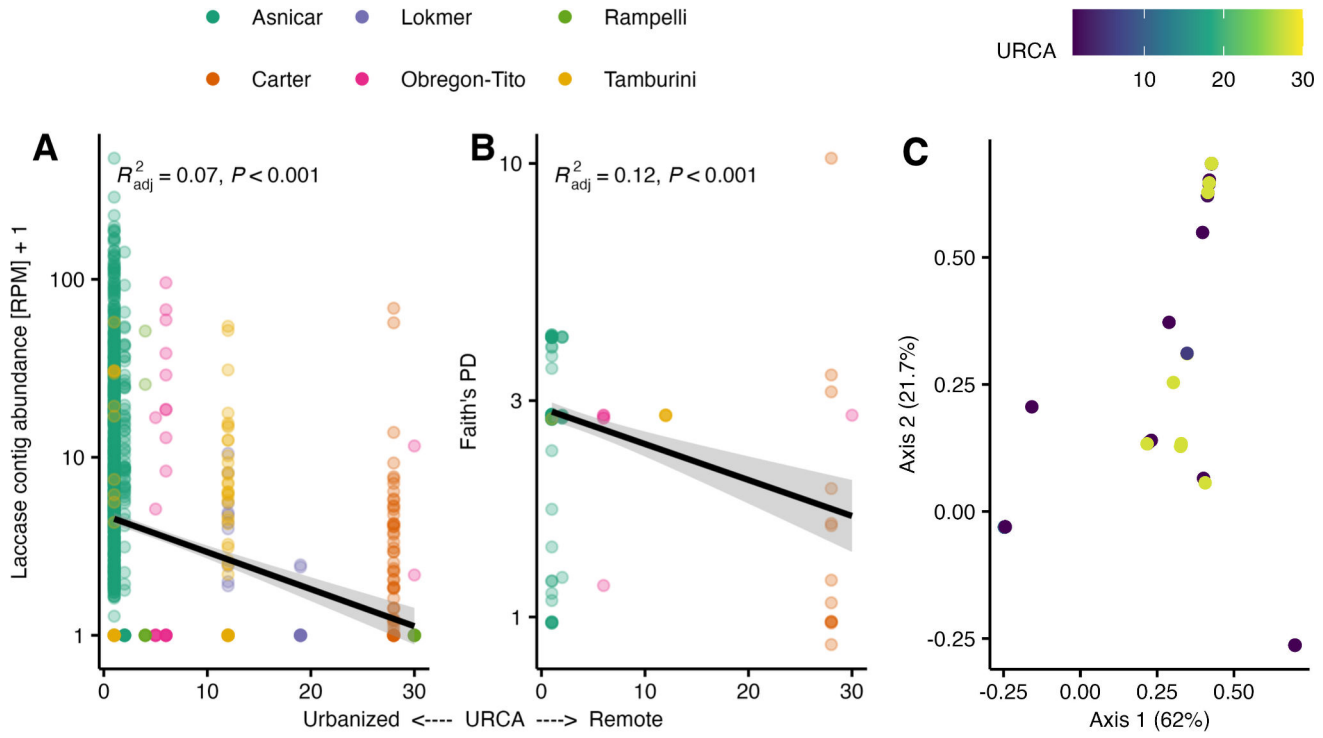


FIG 1 Laccase abundance and diversity metrics in association with URCA. (A) Per-sample abundance measured as reads per million (RPM) of contigs containing putative laccase-coding genes plotted against URCA index values. A lower URCA index value indicates a greater degree of urbanization. (B) Faith's phylogenetic diversity of laccases in samples that contain laccase-coding genes plotted as a function of the URCA index. In panels A and B, individual points are colored according to the study of origin of samples (labeled by study first author), while black lines represent linear regression lines, with pointwise 95% confidence intervals indicated by shaded areas. (C) PCoA of Unifrac distances between samples based on the abundance of contigs with laccase-coding genes and phylogeny of laccase protein sequences, indicating a significant association of laccase homolog composition and URCA index (PERMANOVA $R^2 = 0.07$; $P = 0.001$).

Akkermansia muciniphila is the only species with laccase-coding genes for which the majority of isolated strains originate from the human gut (Fig. S3). This corresponds well with the fact that most medium- or high-quality MAGs that could be recovered and assigned a species-level taxonomy and that contained laccase-coding genes were classified as *Akkermansia* at the genus level (Fig. S4). A large number of bacteria detected in the gut metagenomes (six out of 23 species containing laccase-coding genes) are primarily isolated from foods, namely *Furfurilactobacillus rossiae*, *Lactobacillus delbrueckii* subsp. *allosunkii*, *Lactococcus cremoris*, *Lentilactobacillus hilgardii*, *Limosilactobacillus pontis*, and *Weissella cibaria*, suggesting that these are more likely transient residents (Fig. S3).

Laccases from gut bacteria show activity with ABTS mediator system

As laccase activity has not been experimentally demonstrated from human gut bacteria previously, we selected several laccase homologs from our gut metagenome data set to recombinantly express and purify for activity assays with an 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) mediator system. Two laccase homologs from *Akkermansia* (Akk1 and Akk2) were selected as representatives of the *Akkermansia* laccase homologs found in the gut microbiome of urbanized cohorts, whereas a *Veillonella* laccase homolog (Vei1) was selected as an example found in the gut bacteria of less urbanized cohorts. Additionally, we selected laccase homologs from the genera *Lactiplantibacillus* (Lac) and *Kurthia* (Kur) as examples from food- and animal-associated bacteria that are transiently present in the human gut as well as other environments. Two laccases encoded in *Enterococcus* species (Ent1 and Ent2), isolated from feces, were also

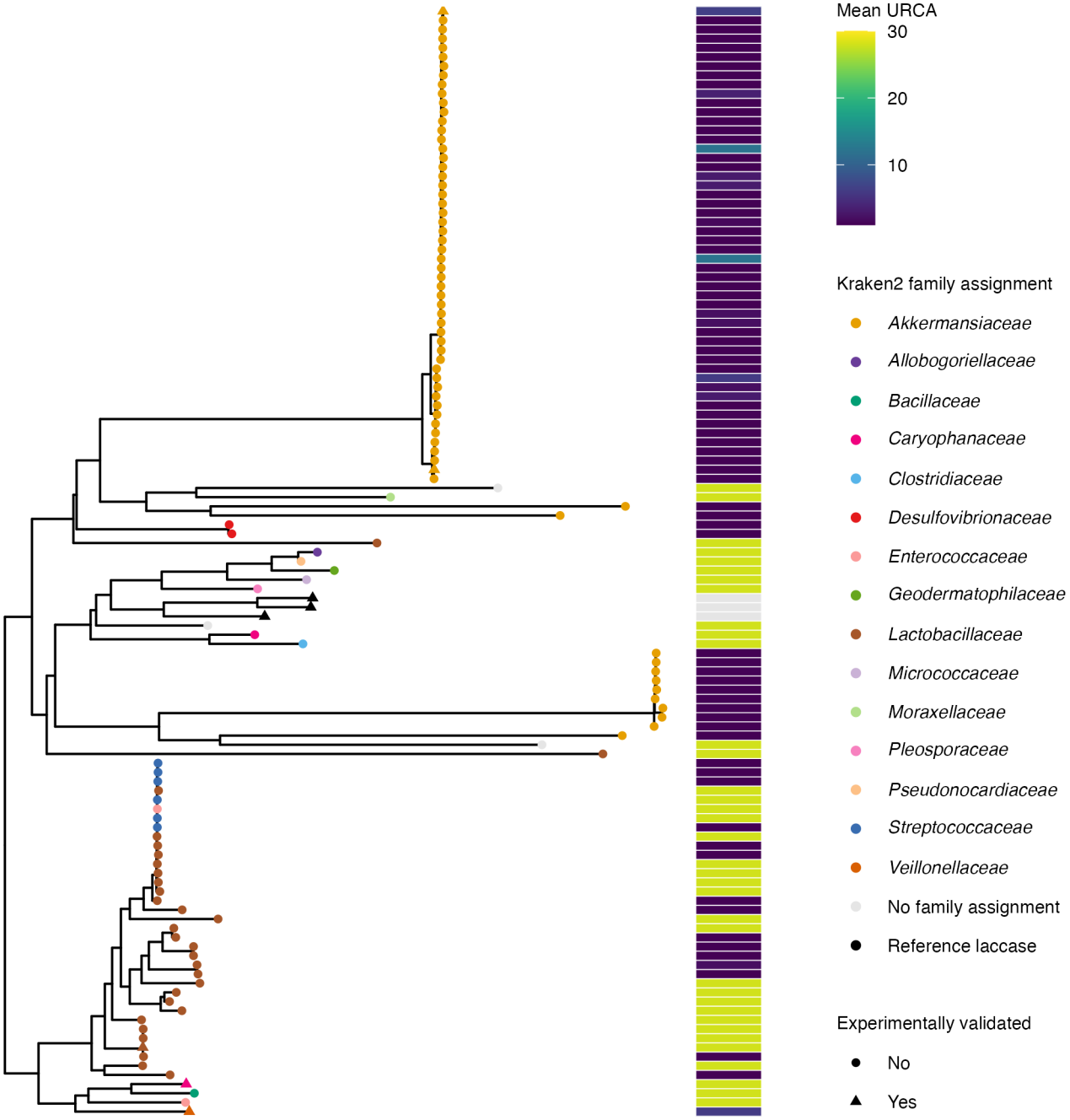


FIG 2 Phylogenetic tree of laccase amino acid sequences, with tips labeled according to family-level NCBI taxonomy classification assigned to contigs of origin using Kraken2, the mean URCA index of all samples in which the laccase sequence was detected, and experimental validation status.

included. All selected laccase homologs were expressed, purified to the greatest extent possible, and assayed.

The majority of gut microbial laccases demonstrated activity with ABTS after 1-h reactions compared to no-enzyme controls (Fig. 3A). Each triplicate reaction contained a fixed volume of eluted enzyme (5 µL) rather than a fixed enzyme concentration because the purity of laccases varied as assessed by SDS-PAGE (Fig. S5). A truncated *Veillonella* (Vei1_Δ120) showed the highest activity among gut laccases. Accordingly, SDS-PAGE revealed a clean band at the predicted 52.5 kDa size. The lowest activity was found in *Akkermansia* column eluate (Akk1), which encodes a membrane-embedded C-terminal beta-barrel as predicted by AlphaFold 3 (Fig. S6) and showed little discernible purified

Akk1, with significant contamination in the SDS-PAGE gel (39). We hypothesized that the beta-barrel could limit solubility, and thus, activity might be present in the cell extract. Indeed, cell extract from induced cultures expressing Akk1 showed significantly more activity with ABTS than uninduced samples, and SDS-PAGE shows clear induction of Akk1 (Fig. 3B). Neither the second *Akkermansia* laccase (Akk2), which naturally lacks the C-terminal beta-barrel, nor Ent2 showed activity above baseline. While we were able to demonstrate activity for a wide range of gut-metagenome-derived laccases, it is worth considering that the assay we used is sufficient to demonstrate activity for one substrate only, and not the full range of substrates that laccases can potentially oxidize (32). Finally, we assessed the activity of Vei1_Δ120 at different pH values in an ammonium acetate and MES mixed buffer system. Vei1_Δ120 exhibited a pH optimum of 5–5.5 which is consistent with the mildly acidic environment of the duodenum and caecum (Fig. 3C) (40).

Depletion of fluorinated chemicals by selected archaeal and bacterial laccases

To examine the activity of laccase-mediator systems with fluorinated chemicals, we evaluated the removal of a panel of 11 chemicals (Fig. S6) by laccases. We selected four bacterial and archaeal laccases to maximize diversity of organisms and environments: Vei1, from the gut bacterium *Veillonella*, as an example of our characterized metagenomic laccases, as well as an archaeal laccase (aMCO) and two bacterial laccases (MCO1, mMCO) (33, 41). As it has been well characterized, MCO1 was considered a positive control (33). Biotransformation experiments were conducted in a laccase-mediator system using ABTS, a laccase-only system (without ABTS), and abiotic controls to account for chemical losses in a laccase-mediator system, pure enzymatic, and non-enzymatic losses, respectively.

In the laccase-mediator system, eight out of 11 tested chemicals showed no notable depletion by any of the tested laccases, while three showed significant depletion by at least one laccase. Two compounds, namely cyflumetofen and fluazinam, showed significant removal across all laccases compared to abiotic controls, indicating that laccases possess the capacity to mediate the transformation of these fluorinated compounds (Fig. 4). Among the enzymes tested, MCO1 exhibited the broadest activity spectrum. In addition to cyflumetofen and fluazinam, this enzyme achieved greater than 40% removal of bisphenol AF. While the other three laccases also showed measurable removal of bisphenol AF, their activities were lower and did not consistently exceed abiotic controls to the same extent as the positive control enzyme. These results suggest that although laccases share a conserved oxidative capability, they differ in their capacity to transform specific fluorinated substrates. Such differences likely reflect variations in active-site structure or redox potential among enzymes from different ecological origins.

We also tested the ability of laccases to deplete the same fluorinated chemicals in the absence of the ABTS mediator. Fluazinam removal was measured for aMCO (51%), mMCO (57%), and, to a lesser extent, Vei1 (23%). Limited removal of cyflumetofen (<30%) was also measured for the same three enzymes. It is worth noting that in all cases, the measured removal was below that measured for the same enzyme/chemical combination within the ABTS mediator system.

DISCUSSION

Fluorinated compounds have become widespread in food-contact applications in recent years, especially as agrochemicals and in food packaging materials (3, 42). In humans, the gut microbiome represents a major site for the biotransformation of xenobiotic compounds, including fluorinated agrochemicals (43–45). In this study, we characterized diverse archaeal and bacterial laccases, including from the human gut microbiota of geographically diverse cohorts (31). We mapped the distribution of laccases in gut microbiota and experimentally validated their activity in mediator systems and their role in fluorinated chemical removal.

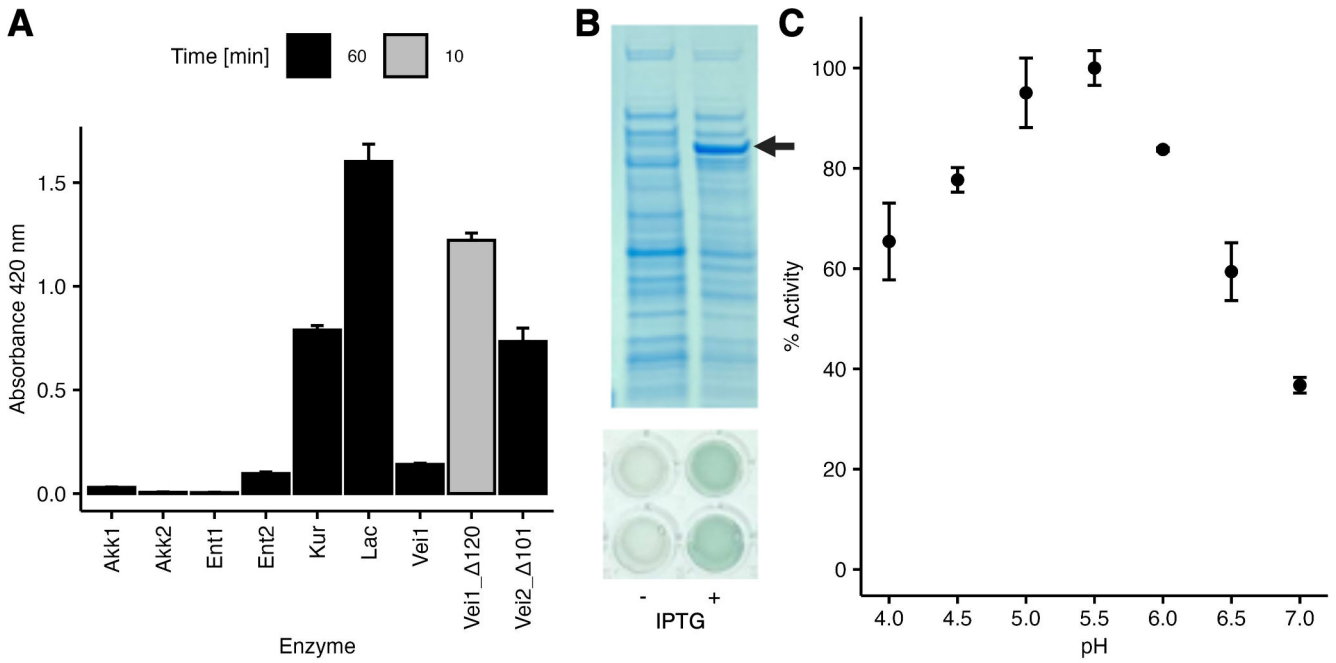


FIG 3 Verification of ABTS activity in predicted gut microbiome laccases. (A) Activity from 5 µL of purified laccases after 1 h for all enzymes except Vei1_Δ120 (10 min). Activity assays were performed in triplicate. Activity for Ent1 and Akk1 is below 0.01 Abs/hr and is not considered significant. (B) Duplicate activity after 2 h from 5 µL of cell extract of uninduced and induced Akk1 (indicated by arrow) expressed in *E. coli*. (C) pH activity profile of Vei1_Δ120 with ABTS. Activity assays were performed in triplicate.

Our results show that the gut microbiota of different human cohorts differs in terms of laccase abundance and diversity, implying that urbanization and lifestyle factors may influence fluorinated xenobiotic metabolism in the human gut. In line with studies showing that host lifestyles shape gut microbiome composition, we found that urbanization was associated with increased abundance of genes coding for laccases, as well as increased Faith's phylogenetic diversity (used here as a metric of laccase homolog sequence diversity, not community diversity) (46). In other words, both laccase abundance and genetic diversity varied across an urbanization gradient, suggesting that urbanization-associated factors may influence the spectrum of laccase activities within the human body, with wide-ranging effects, as these encompass a range of enzymatic targets not limited to the fluorinated compounds tested in this study. A plausible explanation is that exposure to larger concentrations of xenobiotics (including increased dietary diversity) in urbanized populations leads to selection of a diverse array of bacteria capable of biotransforming these compounds (45, 47–49). Laccases can also oxidize plant structural components, such as lignin, flavonoids, or tannins, which are more abundant in non-industrialized diets (50–52). Thus, the trends observed in this study suggest that the effects of diverse dietary and/or xenobiotic exposures (and potentially other environmental exposures) in urban settings outweigh the effects of increased plant components on laccase abundance in human gut microbiota, even if it is not possible to link laccase abundance to exposure to one specific compound or group of compounds.

We also found a significant association between laccase phylogeny, as well as UniFrac distances of laccases between samples with the URCA index. This suggests that the functionality (e.g., substrate range and kinetic parameters) of gut-microbiota-derived laccases may differ between more and less urbanized cohorts, corresponding with established differences between other classes of enzymes in the gut microbiota of humans with different lifestyles, for example, CAZymes (27). Although phylogenetic diversity was highest in urbanized populations, there was a notable diversity of laccases from less urbanized cohorts as a whole, including higher representation of unique

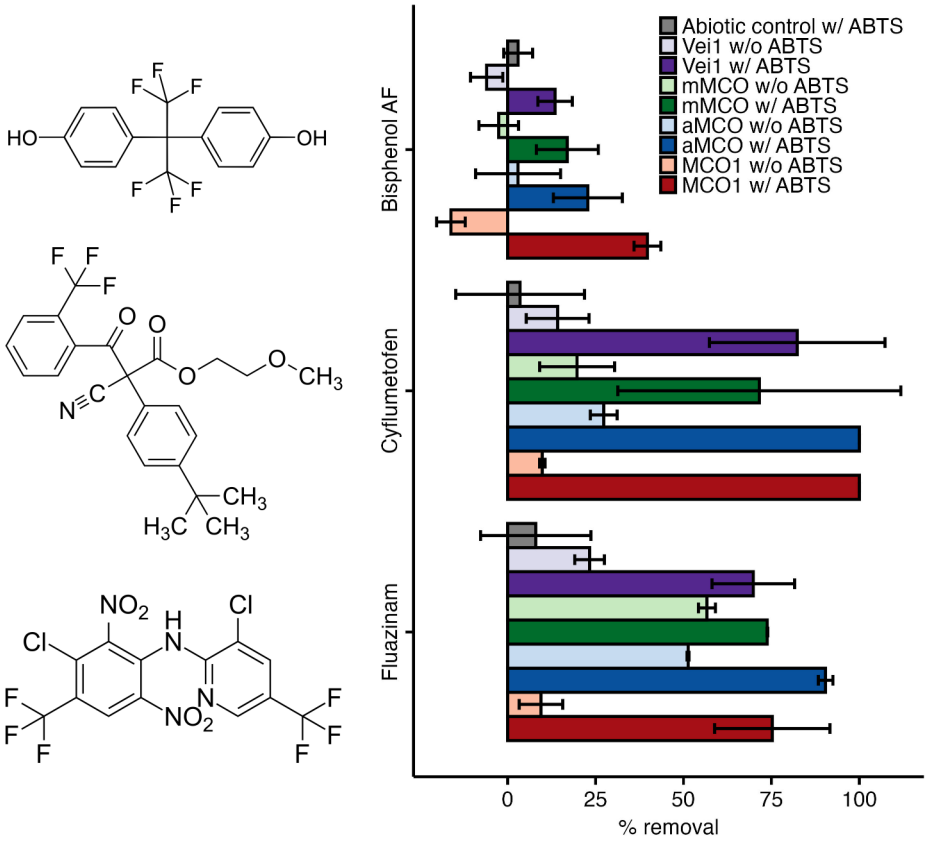


FIG 4 Removal of selected fluorinated chemicals by four representative laccase-mediator systems with the laccases MCO1, aMCO, mMCO1, and Vei1.

laccase clades. Consistent with this finding, urbanized microbiomes are particularly enriched in laccase homologs originating from a single bacterial genus, *Akkermansia*. Many laccases were from the species *Akkermansia muciniphila*, which has been identified as being strongly associated with an industrialized microbiome (26). Conversely, the laccases from the gut microbiota of less industrialized cohorts originated from more varied taxa, including some associated with fermented foods, which aligns with previous studies indicating higher alpha diversity in the gut microbiota of rural cohorts (53).

Laccases from both human-gut-specialists (*Akkermansia*, *Enterococcus*, and *Veillonella*) and animal- and food-associated genera (*Kurthia* and *Lactiplantibacillus*) demonstrated activity as part of laccase-mediator systems, representing the first report of laccase activity from these bacterial genera to the best of our knowledge. This is especially significant given that it represents the presence of oxygen-dependent enzymes in these mostly anoxic environments (32, 54). Localized pockets of oxygen have been shown to exist within the human gut, which would provide an environment where enzymes such as laccases could function (55, 56). Moreover, the apparent K_m for O_2 for fungal laccases is in the range of 20–50 µM, indicating a high affinity for oxygen (57). Testing the affinity of laccases of gut microbial origin would further shed light on their potential to scavenge oxygen in oxygen-depleted environments. In addition to gut- and food-associated bacteria, we also describe the activity of archaeal laccases, where previous studies have focused on fungal and plant laccases (50, 58). In short, our study significantly expands the range of organisms with characterized laccases, highlighting the potential for functional enzyme discovery from human and food microbiomes.

Regarding synthetic organofluorine compounds, we found that the gut *Veillonella* laccase, as well as three environmental laccases, were active in the removal of fluorinated compounds (especially within an ABTS mediator system). Future studies could

investigate whether other gut microbial laccases, including those we characterized, possess the same capabilities, as Vei1 cannot be considered representative of all gut microbial laccases. Notably, only three of the fluorinated compounds we tested, namely cyflumetofen, fluazinam, and bisphenol AF, were depleted, whereas the remaining compounds were largely stable under the same conditions. These compounds are all aromatics with electron-withdrawing substituents including $-CF_3$ groups; however, this common backbone does not readily distinguish them from the other fluorinated agrochemical and industrial chemicals that were not depleted. Laccases preferentially oxidize substrates that can form stabilized radical intermediates, such as phenolic or aromatic amines, and are therefore more susceptible to single-electron transfer followed by coupling, rearrangement, or fragmentation reactions (58–62). In contrast, many fluorinated xenobiotics are dominated by electron-poor, highly substituted $-CF_3$ motifs and lack readily oxidizable functional groups, which can make them poor laccase substrates even in laccase-mediator systems (63–66).

Knowledge of biotransformations for these compounds is key for understanding potential adverse environmental or health effects associated with these compounds. Cyflumetofen, fluazinam, and bisphenol AF have been linked with various negative effects in humans and/or other animals, for example, cytotoxicity or endocrine disruption (67–70). Gut biotransformations have previously been shown to alter the effects of fluorinated chemotherapeutic drugs through either deactivation or enhancement of cytotoxic effects, indicating that the potential for microbial biotransformations to increase toxicity should be taken into account when evaluating the safety of food-associated fluorinated compounds as well (19, 71, 72). However, an accurate toxicological assessment would need to consider gut conditions which differ considerably from our experimental conditions.

While we did show limited depletion of some fluorinated compounds without the ABTS mediator system, the most notable depletion occurred under conditions with ABTS, a compound which does not occur naturally. However, previous studies have proven the existence of naturally occurring lignin-derived phenolic compounds that can act as laccase mediators (73). Laccases from the gut microbiota of animals with lignin-rich diets, namely pandas and termites, have been previously characterized, suggesting that gut-based laccase-mediator systems could exist in nature (74, 75). Future studies will test the gut laccases we identified with naturally occurring phenolic mediators to further characterize their substrate range. Metabolomics approaches could be used to test whether these mediators are present in the human gut, which would provide further evidence for laccase activity during intestinal transit.

In addition to our findings on laccases and their removal of fluorinated compounds, our study demonstrated a cross-cohort, multi-study approach to working with publicly available metagenomes by linking sequencing data to geographic metadata such as the URCA index (30). This approach allowed us to detect trends across cohorts from different studies and locations with high geographic resolution, whereas previous studies on urbanization and the gut microbiome have been limited to a single country (76) classification systems to distinguish study populations (e.g., binary or trinary classification of urban and non-urban cohorts) (25, 27). Future studies could expand on this approach to investigate other questions on environmental or host-associated microbiota, as well as integrating other relevant data sets such as chemical exposure data.

In conclusion, we demonstrate that phylogenetically disparate laccases are widespread in diverse human cohorts and capable of depleting various food-associated fluorinated compounds. This lends deeper insights into how human lifestyle factors relate to the prevalence of pathways for metabolism of xenobiotic fluorinated compounds. Together, these findings expand knowledge of the substrate range of laccases to include fluorinated compounds of public health relevance, link patterns of laccase abundance and diversity to human geographical factors, and highlight potential approaches for further studies with publicly available microbiome sequencing data.

These outcomes point to the possibility for microbial biotransformation of fluorinated food contact chemicals, with potential health risks that should be taken seriously in chemical safety assessments. Further research to assess *in vivo* activity and consequences is warranted, as is further investigation of a larger range of enzyme classes capable of fluorinated compound biotransformation.

MATERIALS AND METHODS

Study selection and metadata curation

Six studies (Table 1) on the healthy human gut microbiome with publicly available metagenomic sequencing data were selected for inclusion based on the availability of latitude/longitude metadata for the samples. The studies were intended to cover cohorts with a range of different lifestyles, including industrialized, subsistence farming, and hunter-gatherer. Using the geographic metadata from the NCBI sequencing reads archive (SRA), each sample's collection location was classified in terms of urbanization on a scale of 1 (most urbanized) to 30 (least urbanized) using the urban-rural catchment areas (URCA) approach (30, 77). Only metagenomes that could be successfully downloaded using q2-fondue were retained (78). In the Carter et al. (2023) (26) study, in some cases, multiple metagenomes originated from the same individual. For statistical tests where independent observations were necessary, the first collected sample from an individual was used.

Bioinformatics

Metagenome data analysis was performed using MOSHPIT (81). Sequences and metadata, including location, were downloaded from the NCBI sequencing read archive (SRA) using q2-fondue (78). Raw sequences were trimmed to remove adapters and filtered with a phred score cutoff of 30 using fastp (82). Human-associated reads were removed by mapping to the GRCh38 reference human pangenome using Bowtie2 (83). While this method may not completely exclude all human sequences, it was sufficient in the context of our study focused on laccase homologs (84). Reads were assembled into contigs with MEGAHIT using the meta-sensitive preset (85). Contigs were annotated functionally against the eggNOG database using DIAMOND (86, 87). Contigs that included at least one open reading frame (ORF) with the eggNOG annotations "Cu-oxidase, Cu-oxidase_2, Cu-oxidase_3" for PFAMs and either "PFAM multicopper oxidase type" or "Multicopper oxidase" for description were considered to contain laccase homologs (88). Contig abundances were calculated according to the reads per million (RPM) method following read mapping using Bowtie2 (83, 89). Contigs were classified taxonomically using Kraken2 (37). Strain isolation sources were retrieved for contigs that could be classified at the species level using BacDive and categorized as "Animal," "Food," "Human gut," "Human other," or "Other" using a custom Gem created using the Google Gemini large language model (38, 90, 91). Laccase amino acid sequences

TABLE 1 Studies with human gut microbiome metagenomic shotgun sequencing data used for our analysis, with sample countries of origin and total number of samples indicated for each study

Study (reference)	Country	No. of samples (no. of individuals)
Obregon-Tito et al. (2015) (25)	Peru	17 (17)
Obregon-Tito et al. (2015) (25)	USA	18 (18)
Rampelli et al. (2015) (27)	Italy	10 (10)
Rampelli et al. (2015) (27)	Tanzania	27 (27)
Lokmer et al. (2019) (79)	Cameroon	57 (57)
Asnicar et al. (2021) (80)	UK	997 (997)
Asnicar et al. (2021) (80)	USA	97 (97)
Tamburini et al. (2023) (28)	South Africa	169 (169)
Carter et al. (2023) (26)	Tanzania	186 (74)

coded by open reading frames (ORFs) with laccase annotations from eggNOG were predicted from contigs using Prodigal (92). Protein sequences were filtered such that only sequences with a length greater than 150 amino acids containing a start and stop codon as well as at least one copper binding motif (HXHG, HXH, HXXHXH, or HCHXXXHXXXXM/L/F) (93). Protein sequences were aligned using Clustal Omega (94). A phylogenetic tree of protein sequences was generated using IQ-TREE with ModelFinderPlus and bootstrapping with 100 iterations (95). Phylogeny-aware distance metrics, namely Faith's phylogenetic diversity and unweighted UniFrac, were calculated using the piccante R package (96–99). Associations between phylogeny and URCA classification were computed using Moran's I and Blomberg's K using the phylosignal R package (36, 99, 100). Contigs were binned into metagenome-assembled genomes (MAGs) with MetaBAT2 (101). MAG quality was assessed using BUSCO; medium- and high-quality MAGs with completeness >50% and contamination <10% were retained (102, 103). Taxonomy of contigs binned to medium- and high-quality MAGs was predicted using Kraken2, with MAGs being assigned the lowest taxonomic rank to which over 50% of the contigs were assigned (37). A comprehensive overview of the bioinformatics pipeline for sequence processing can be found in Fig. 5A.

Statistics and plotting

Statistics and plotting were performed using R (version 4.5.1) within the R Studio integrated development environment (IDE) using the packages ape (version 5.8-1), ggnewscale (version 0.5.2), ggpmisc (version 0.6.3), ggpubr (version 0.6.1), ggtree (version 3.16.3), janitor (version 2.2.1), pacman (version 0.5.1), phylobase (0.8.12), phyloseq (version 1.52.0), RColorBrewer (version 1.1-3), readxl (version 1.4.5), rentrez (version 1.2.4), scales (version 1.4.0), tidyverse (version 2.0.0), treeio (version 1.32.0), ursa (version 3.11.5), and xml2 (version 1.5.0) (99, 104–122).

Laccase selection and synthesis

All synthesized genes and plasmids used in this study can be found in Table S1. Codon-optimized gene sequences are also available to be downloaded from https://github.com/MSM-group/gut-laccases/tree/main/sequences. Several ORFs representing laccase homologs from contigs assigned to the genera *Akkermansia* (Akk1 and Akk2), *Kurthia* (Kur), *Lactiplantibacillus* (Lac), and *Veillonella* (Vei1) were selected for synthesis as representative laccase homologs from our gut metagenomes. The full-length Vei1 sequence was predicted by TMHMM-2.0 and AlphaFold to contain an N-terminal disordered region (39, 123). Thus, a truncated version of the original *Veillonella* (Vei1_Δ120) was obtained. We searched for additional putative laccases associated with the gut microbiome using NCBI Blast (124). We identified a second *Veillonella* sequence with 66% amino acid sequence identity to Vei1 that also contained a predicted disordered region. Thus, a second truncated *Veillonella* (Vei2_Δ101) was procured. Two laccases encoded in *Enterococcus* species isolated from feces were selected (Ent1 and Ent2). Both source organisms are available from the American Type Culture Collection (ATCC), ATCC 51266 and ATCC 6056, respectively. Finally, three additional non-gut reference bacterial and archaeal laccases (MCO1, aMCO, and mMCO) for comparison were selected, synthesized, and cloned as described in Yu et al. (33, 41). Genes ordered from Integrated DNA Technologies (IDT) were assembled in-house via Gibson Assembly into plasmids linearized at the cut-sites specified in Table S1. Final constructs were verified by Sanger sequencing. Additional cloned genes were provided directly in the plasmids specified in Table S1 by GenScript and the Joint Genome Institute.

Laccase expression and purification

A full overview of experimental methods can be found in Fig. 5B. All genes contained an N-terminal His-tag, and plasmids were transformed into *Escherichia coli* BL21(DE3) for heterologous expression. Genes were ligated into plasmids by Gibson assembly

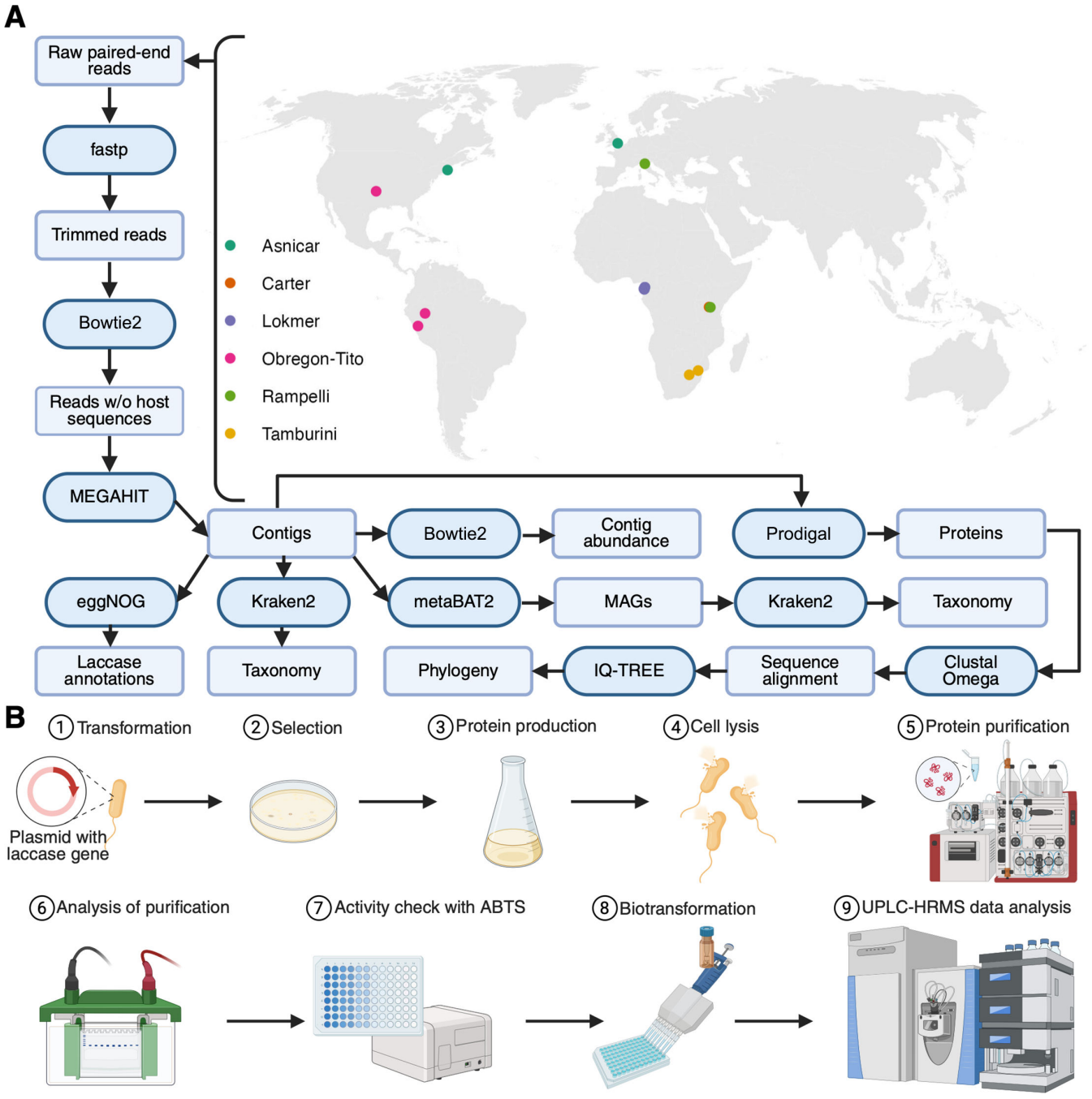


FIG 5 Overview of methods. (A) Map of sample origin and overview of bioinformatics pipeline used for sample processing. Points on the map are labeled according to the study of origin, as shown in Table 1. (B) Experimental methods used to assess enzyme activity.

following the use of restriction enzyme cut sites for plasmid linearization. An overview of cloning vectors and corresponding antibiotics can be found in Table S1. Single colonies were picked and inoculated into 5 mL of terrific broth containing antibiotic selection and cultured overnight at 37°C with shaking. For protein expression, a 400 µL aliquot of the overnight culture was inoculated into 400 mL of terrific broth supplemented with the appropriate antibiotic and incubated at 37°C with shaking at 200 rpm until the optical density at 600 nm (OD_{600}) reached approximately 0.5–0.6. The protein expression was induced by the addition of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) into the medium. To promote functional multicopper oxidase formation, $CuCl_2$ was added

simultaneously with IPTG at a final concentration of 0.25 mM. Cultures were then shifted to 18°C and shaken for 4 h, followed by overnight incubation at 18°C without shaking to establish microaerobic conditions conducive to active enzyme production.

After centrifugation, cell pellets were resuspended in 50 mL of lysis buffer containing 50 mM Tris-HCl (pH 7.5), 300 mM NaCl, 10–50% glycerol (we initially purified with 50% glycerol based on our previously published methods but found throughout the course of the study that acceptable results were achievable down to 10% glycerol), 5 mM imidazole, and 1 mM phenylmethylsulfonyl fluoride (PMSF) (41, 125). Cell breakage was carried out using a high-pressure homogenizer (Avestin Emulsiflex C3) for approximately 5 min, or until the cell extract became visibly less viscous and non-sticky, with a pressure range of 1,200–1,500 bar considered acceptable. A sharp drop in pressure during operation typically indicated complete cell disruption. Vei1_Δ120 lysis buffer lacked PMSF, and cell extract was created using three separate one-minute passes through a sonicator at 50% power (Qsonica Q125).

Cell extract was clarified by centrifugation before loading onto a 1.0 mL Histrap FF column (Cytiva) using an ÄKTA FPLC system (Cytiva). The column was washed with 50 mM Tris-HCl (pH 7.5), 300 mM NaCl, 10–50% glycerol, and 5 mM imidazole, and the protein was eluted using a linear imidazole gradient (20–500 mM imidazole in wash buffer). Eluted fractions exhibiting protein peaks were pooled and further processed by HiTrap desalting columns (Cytiva) with Sephadex G-25 resin to exchange into the final storage buffer (TNG buffer, 10 mM Tris-HCl, pH 7.5; 30 mM NaCl; 10% vol/vol glycerol) to remove residual imidazole. Peak fractionation based on A280 UV absorbance, with confirmation based on A610 UV absorbance, using the UNICORN 7 control software was performed. The purified laccase was aliquoted and stored at –80°C until use. Concentrations of purified laccase were determined by Bradford assay (Bio-Rad) or Pierce assay (Thermo Scientific).

Colorimetric activity screening of selected laccases using ABTS assay in 96-well plates

Laccase activity was determined colorimetrically using the chemical mediator 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a chromogenic substrate, following a previously described protocol (33). Briefly, enzyme activity was assessed in clear, flat-bottom 96-well plates. Standard 200 µL reactions contained 100 mM ammonium acetate buffer (pH 5.50), 500 µM ABTS solution, and 5 µL of purified laccase preparation. The oxidation of ABTS to its radical cation was monitored spectrophotometrically at 420 nm using a microplate reader at 30°C, with absorbance recorded every 2 min for up to 24 h. The molar extinction coefficient (ε) of the ABTS radical at 420 nm was taken as 36,000 M^{-1} cm^{-1}, and the optical path length for a 200 µL reaction volume was approximated as 0.56 cm.

pH purification profile

The truncated *Veillonella* laccase (Vei1_Δ120) was used to generate a pH activity profile using the described ABTS assay. A stock buffer containing both 111 mM ammonium acetate and 111 mM MES was created to buffer between pH 4.0 and 7.0, and aliquots were adjusted with HCl to create solutions at 0.50 pH unit increments. Reactions (200 µL) were run in 96-well plates containing final concentrations of 4.0 mM ABTS and 200 mM of the appropriate buffer. Assays were initiated with 5 µg of truncated *Veillonella* laccase and were monitored at 420 nm for 10 minutes. Slopes for no laccase control reactions were subtracted from initial reaction slopes.

Fluorinated chemical selection

A set of fluorinated contaminants (Fig. S7) was selected based on usage data in the fluorinated pesticide PFAS (per- and polyfluoroalkyl substances) Data Hub and/or the USGS National Water-Quality Assessment Project (NAWQA) pesticide database, as well

as their commercial availability and the availability of established detection methods by ultra-high-performance liquid chromatography coupled to high-resolution tandem mass spectrometry (UHPLC-HRMS/MS) (126, 127).

High-throughput biotransformation experiments of fluorinated chemicals in 96-well plates

A mixture of selected fluorinated chemicals was used for enzymatic biotransformation screening. Reactions were performed in 96-well plates following our previously established workflow with slight modifications (33, 125). Briefly, individual fluorinated compounds were prepared as stock solutions (5–10 mM) in ethanol, depending on compound solubility, and stored at −20°C until use. Prior to experiments, stock solutions were equilibrated to room temperature and sonicated for 10 min to ensure complete dissolution. Stocks were then diluted in ethanol and mixed to generate a working mixture containing each compound at 200 µM. For the screening experiments, 25 µL of the working mixture was first added to each well of a 96-well plate. The solvent was fully evaporated at room temperature. Subsequently, 125 µL of the buffer medium was added to each well, and the plate was mixed by pipetting up and down for approximately 1 min, followed by a 15-min incubation at room temperature to facilitate re-dissolution of compounds. An additional 100 µL of medium was then added, followed by a further 10-min incubation to facilitate complete re-dissolution of the compounds. Depending on the experimental condition, enzyme, mediator (ABTS), or nuclease-free water was subsequently added to reach a final reaction volume of 250 µL, corresponding to an intended concentration of ~20 µM per compound. Prior to taking the first time point samples, wells were visually inspected for precipitation, and no visible precipitation was observed under these conditions. For the biological (enzyme-active) control, each well contained purified laccase enzyme, approximately 250 µM ABTS as the mediator, and the fluorinated chemical mixture in 100 mM ammonium acetate buffer (~pH 5.0). In the mediator-free enzyme control, the same components were included except for ABTS. For the abiotic buffer control, the fluorinated compounds and 250 µM ABTS were added without enzyme. Plates were incubated in a dark incubator, shaking at 120 rpm, at 30°C for up to 48 h, with samples collected at 0, 24, and 48 h to measure the parent compound removal by ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS/MS). Enzyme stocks used in this study differed in concentration and activity with ABTS (MCO1: 0.583 mg/mL; Vei1: 0.591 mg/mL; aMCO: 0.462 mg/mL; mMCO: 0.436 mg/mL). Approximately 3 µL of MCO1 and 20 µL of VEI, aMCO, and mMCO were added per well to achieve observable ABTS oxidation activity under the assay conditions. This approach was adopted because the primary objective of this study was to assess qualitative biotransformation potential across a broad set of compounds for different laccases, rather than to perform quantitative comparisons of enzymatic activity or normalize enzyme loading by concentration or activity. Accordingly, enzyme amounts were adjusted to ensure measurable reactivity within the experimental time frame rather than standardized across enzymes.

UHPLC-HRMS/MS analysis

The fluorinated chemicals were quantified using UHPLC-HRMS/MS (Q Exactive, Thermo Fisher Scientific). The analytical procedure was adapted from previously established protocols with minor adjustments, and full instrumental parameters have been described previously (41, 125). Briefly, for UHPLC analysis, 25 µL samples were loaded onto an ACQUITY Premier BEH C18 Column (particle size 1.7 µm, 100 × 2.1 mm, Waters) and eluted with nano-pure water (A) and methanol (B) (both amended with 0.1% formic acid) at a flow rate of 300 µL/min, with a gradient as follows: 95% A: 0-1.5 min, 95% – 5% A: 1-7.5 min, 5% A: 7.5-9.5 min, and 95% A: 9.5-11.5 min. For HRMS, mass spectra were acquired in full-scan mode at a resolution of 70,000 at m/z 200 and a scan range of m/z 100–1000 in positive/negative switching mode with electrospray

ionization (ESI). Matrix-matched calibration was used to account for matrix-induced ion suppression or enhancement effects during electrospray ionization. Xcalibur 4.0 (Thermo Fisher Scientific) was used for data acquisition and analysis. Chemical structures were drawn, visualized, and annotated using ChemDraw Professional 20.0 and MarvinSketch (v19.20.0, ChemAxon, http://www.chemaxon.com). To assess compound removal, the incubation time at which each compound reached its maximum reduction across various control treatments was determined. Removal efficiency was calculated as follows: removal (%) = $[(C_0 - C_{final})/C_0] \times 100\%$, where C_0 represents the initial concentration and C_{final} denotes the concentration after incubation. Biological activity was considered significant when two criteria were met: (i) enzymatic treatment resulted in greater than 40% removal, and (ii) removal in biological control was significantly ($P < 0.05$) higher than that in the abiotic controls.

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Milo R. Schärer, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review and editing | Yaochun Yu, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review and editing | Aaron Grawe, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review and editing | James K. Christenson, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review and editing | Serina L. Robinson, Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing | Nicholas A. Bokulich, Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

Sequencing data are reused from NCBI SRA (https://www.ncbi.nlm.nih.gov/sra) and are associated with the following BioProject accessions: PRJNA268964, PRJNA278393, PRJEB27005, PRJEB39223, PRJNA678454, and PRJEB49206 (25–28, 79, 80). Codes used to generate figures, as well as codon-optimized gene sequences, are available under https://github.com/MSM-group/gut-laccases.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental figures and tables (mBio01206-26-S0001.docx). Figures S1-S7 and Table S1.

REFERENCES

1. Jagani R, Patel H, Chovatiya J, Andra SS. 2025. The rise and risks of fluorinated pesticides: a call for comprehensive research to address environmental and health concerns. J Agric Food Chem 73:2217–2220. https://doi.org/10.1021/acs.jafc.4c12827
2. Alexandrino DAM, Mucha AP, Almeida CMR, Carvalho MF. 2022. Atlas of the microbial degradation of fluorinated pesticides. Crit Rev Biotechnol 42:991–1009. https://doi.org/10.1080/07388551.2021.1977234
3. Alexandrino DAM, Almeida CMR, Mucha AP, Carvalho MF. 2022. Revisiting pesticide pollution: the case of fluorinated pesticides. Environ Pollut 292:118315. https://doi.org/10.1016/j.envpol.2021.118315
4. Barrón Cuenca J, Tirado N, Barral J, Ali I, Levi M, Stenius U, Berglund M, Dreij K. 2019. Increased levels of genotoxic damage in a bolivian agricultural population exposed to mixtures of pesticides. Sci Total Environ 695:133942. https://doi.org/10.1016/j.scitotenv.2019.133942
5. Liu Y, Wang F, Li L, Fan B, Kong Z, Tan J, Li M. 2025. The potential endocrine-disrupting of fluorinated pesticides and molecular mechanism of EDPs in cell models. Ecotoxicol Environ Saf 289:117615. https://doi.org/10.1016/j.ecoenv.2024.117615
6. Ogawa Y, Tokunaga E, Kobayashi O, Hirai K, Shibata N. 2020. Current contributions of organofluorine compounds to the agrochemical industry. iScience 23:101467. https://doi.org/10.1016/j.isci.2020.101467
7. O'Hagan D. 2008. Understanding organofluorine chemistry. an introduction to the C-F bond. Chem Soc Rev 37:308–319. https://doi.org/10.1039/b711844a
8. Jeschke P. 2004. The unique role of fluorine in the design of active ingredients for modern crop protection. Chembiochem 5:571–589. https://doi.org/10.1002/cbic.200300833
9. Jeschke Peter. 2010. The unique role of halogen substituents in the design of modern agrochemicals. Pest Manag Sci 66:10–27. https://doi.org/10.1002/ps.1829
10. Key BD, Howell RD, Criddle CS. 1997. Fluorinated organics in the biosphere. Environ Sci Technol 31:2445–2454. https://doi.org/10.1021/es961007c
11. Martinello M, Manzinello C, Borin A, Avram LE, Dainese N, Giuliato I, Gallina A, Mutinelli F. 2019. A survey from 2015 to 2019 to investigate the occurrence of pesticide residues in dead honeybees and other matrices related to honeybee mortality incidents in Italy. Diversity 12:15. https://doi.org/10.3390/d12010015
12. Wu W, Kong D, Zhang W, Bu Y, Li J, Shan Z. 2020. Acute toxicity of fluazinam to aquatic organisms and its bioaccumulation in brachydanio rerio. Environ Sci Pollut Res 27:35000–35007. https://doi.org/10.1007/s11356-020-09779-w
13. Yang J, Sui J, Wang L, Yang J, Chen S, Cui X, Yang Y, Zhang W. 2025. Microbial degradation of chlorothalonil and its metabolite 4-OH-CTN: potential candidates and metabolic pathways. J Environ Chem Eng 13:119031. https://doi.org/10.1016/j.jece.2025.119031
14. Carvalho G, Marques R, Lopes AR, Faria C, Noronha JP, Oehmen A, Nunes OC, Reis MAM. 2010. Biological treatment of propanil and 3,4-dichloroaniline: kinetic and microbiological characterisation. Water Res 44:4980–4991. https://doi.org/10.1016/j.watres.2010.08.006
15. Boxall ABA, Sinclair CJ, Fenner K, Kolpin D, Maund SJ. 2004. When synthetic chemicals degrade in the environment. Environ Sci Technol 38:368A–375A. https://doi.org/10.1021/es040624v
16. Lindell AE, Grießhammer A, Michaelis L, Papagiannidis D, Ochner H, Kamrad S, Guan R, Blasche S, Ventimiglia LN, Ramachandran B, Ozgur H, Zelezniak A, Beristain-Covarrubias N, Yam-Puc JC, Roux I, Barron LP, Richardson AK, Martin MG, Benes V, Morone N, Thaventhiran JED, Bharat TAM, Savitski MM, Maier L, Patil KR. 2025. Human gut bacteria bioaccumulate per- and polyfluoroalkyl substances. Nat Microbiol 10:1630–1647. https://doi.org/10.1038/s41564-025-02032-5
17. Roux I, Lindell AE, Grießhammer A, Smith T, Krishna S, Guan R, Rad D, Faria L, Blasche S, Patil KR, Kleinstreuer NC, Maier L, Kamrad S, Patil KR. 2025. Industrial and agricultural chemicals exhibit antimicrobial activity against human gut bacteria *in vitro*. Nat Microbiol 10:3107–3121. https://doi.org/10.1038/s41564-025-02182-6
18. Peskett ST, Rand AA. 2022. The human fecal microbiome contributes to the biotransformation of the PFAS surfactant 8:2 monosubstituted

polyfluoroalkyl phosphate ester. Environ Sci Process Impacts 24:1758–1768. https://doi.org/10.1039/d2em00225f
19. Spanogiannopoulos P, Kyaw TS, Guthrie BGH, Bradley PH, Lee JV, Melamed J, Malig YNA, Lam KN, Gempis D, Sandy M, Kidder W, Van Blarigan EL, Atreya CE, Venook A, Gerona RR, Goga A, Pollard KS, Turnbaugh PJ. 2022. Host and gut bacteria share metabolic pathways for anti-cancer drug metabolism. Nat Microbiol 7:1605–1620. https://doi.org/10.1038/s41564-022-01226-5
20. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. 2007. The human microbiome project. Nature 449:804–810. https://doi.org/10.1038/nature06244
21. McDonald D, Hyde E, Debelius JW, Morton JT, Gonzalez A, Ackermann G, Aksenov AA, Behsaz B, Brennan C, Chen Y, et al. 2018. American gut: an open platform for citizen science microbiome research. mSystems 3:e00031-18. https://doi.org/10.1128/mSystems.00031-18
22. Peskett ST, Grégoire DS, Rand AA. 2026. Human intestinal microbiota composition shapes model polyfluoroalkyl substance biotransformation. Chembiochem 27:e202500905. https://doi.org/10.1002/cbic.202500905
23. Schnorr SL, Candela M, Rampelli S, Centanni M, Consolandi C, Basaglia G, Turroni S, Biagi E, Peano C, Severgnini M, Fiori J, Gotti R, De Bellis G, Luiselli D, Brigidi P, Mabulla A, Marlowe F, Henry AG, Crittenden AN. 2014. Gut microbiome of the Hadza hunter-gatherers. Nat Commun 5:3654. https://doi.org/10.1038/ncomms4654
24. Abdill RJ, Adamowicz EM, Blekhman R. 2022. Public human microbiome data are dominated by highly developed countries. PLoS Biol 20:e3001536. https://doi.org/10.1371/journal.pbio.3001536
25. Obregon-Tito AJ, Tito RY, Metcalf J, Sankaranarayanan K, Clemente JC, Ursell LK, Zech Xu Z, Van Treuren W, Knight R, Gaffney PM, Spicer P, Lawson P, Marin-Reyes L, Trujillo-Villarroel O, Foster M, Guija-Poma E, Troncoso-Corzo L, Warinner C, Ozga AT, Lewis CM. 2015. Subsistence strategies in traditional societies distinguish gut microbiomes. Nat Commun 6:6505. https://doi.org/10.1038/ncomms7505
26. Carter MM, Olm MR, Merrill BD, Dahan D, Tripathi S, Spencer SP, Yu FB, Jain S, Neff N, Jha AR, Sonnenburg ED, Sonnenburg JL. 2023. Ultra-deep sequencing of hadza hunter-gatherers recovers vanishing gut microbes. Cell 186:3111–3124. https://doi.org/10.1016/j.cell.2023.05.046
27. Rampelli S, Schnorr SL, Consolandi C, Turroni S, Severgnini M, Peano C, Brigidi P, Crittenden AN, Henry AG, Candela M. 2015. Metagenome sequencing of the Hadza hunter-gatherer gut microbiota. Curr Biol 25:1682–1693. https://doi.org/10.1016/j.cub.2015.04.055
28. Tamburini FB, Maghini D, Oduaran OH, Brewster R, Hulley MR, Sahibdeen V, Norris SA, Tollman S, Kahn K, Wagner RG, Wade AN, Wafawanaka F, Gómez-Olivé FX, Twine R, Lombard Z, Hazelhurst S, Bhatt AS. 2022. Short- and long-read metagenomics of urban and rural south African gut microbiomes reveal a transitional composition and undescribed taxa. Nat Commun 13:926. https://doi.org/10.1038/s41467-021-27917-x
29. Maghini DG, Oduaran OH, Olubayo LAI, Cook JA, Smyth N, Mathema T, Belger CW, Agongo G, Boua PR, Choma SSR, et al. 2025. Expanding the human gut microbiome atlas of Africa. Nature 638:718–728. https://doi.org/10.1038/s41586-024-08485-8
30. Cattaneo A, Nelson A, McMenomy T. 2021. Global mapping of urban-rural catchment areas reveals unequal access to services. Proc Natl Acad Sci USA 118:e2011990118. https://doi.org/10.1073/pnas.2011990118
31. Mekureyaw MF, Junker AL, Bai L, Zhang Y, Wei Z, Guo Z. 2025. Laccase based per- and polyfluoroalkyl substances degradation: status and future perspectives. Water Res 271:122888. https://doi.org/10.1016/j.watres.2024.122888
32. Claus H. 2004. Laccases: structure, reactions, distribution. Micron 35:93–96. https://doi.org/10.1016/j.micron.2003.10.029
33. Yu Y, Zhang K, Steiner V-M, Poltorak V, Probst SI, Robinson SL, Hutter J, Satoh H, Fenner K. 2026. An enzyme-level benchmark based on environmental bacterial laccases for predicting contaminant fate in water. bioRxiv. https://doi.org/10.64898/2026.01.27.701970
34. Ali M, Bhardwaj P, Ishqi HM, Shahid M, Islam A. 2023. Laccase engineering: redox potential is not the only activity-determining feature in the metalloproteins. Molecules 28:6209. https://doi.org/10.3390/molecules28176209
35. Jones SM, Solomon EI. 2015. Electron transfer and reaction mechanism of laccases. Cell Mol Life Sci 72:869–883. https://doi.org/10.1007/s00018-014-1826-6
36. Münkemüller T, Lavergne S, Bzeznik B, Dray S, Jombart T, Schiffers K, Thuiller W. 2012. How to measure and test phylogenetic signal. Methods Ecol Evol 3:743–756. https://doi.org/10.1111/j.2041-210X.2012.00196.x
37. Wood DE, Lu J, Langmead B. 2019. Improved metagenomic analysis with Kraken 2. Genome Biol 20:257. https://doi.org/10.1186/s13059-019-1891-0
38. Schober I, Koblitz J, Sardà Carbasse J, Ebeling C, Schmidt ML, Podstawka A, Gupta R, Ilangovan V, Chamanara J, Overmann J, Reimer LC. 2025. BacDive in 2025: the core database for prokaryotic strain data. Nucleic Acids Res 53:D748–D756. https://doi.org/10.1093/nar/gkae959
39. Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard AJ, Bambrick J, et al. 2024. Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature 630:493–500. https://doi.org/10.1038/s41586-024-07487-w
40. Vandamme T. 2002. The use of polysaccharides to target drugs to the colon. Carbohydr Polym 48:219–231. https://doi.org/10.1016/S0144-8617(01)00263-6
41. Athanasakoglou A, Fenner K. 2022. Toward characterizing the genetic basis of trace organic contaminant biotransformation in activated sludge: the role of multicopper oxidases as a case study. Environ Sci Technol 56:313–324. https://doi.org/10.1021/acs.est.1c05803
42. Jeschke P. 2024. Recent developments in fluorine-containing pesticides. Pest Manag Sci 80:3065–3087. https://doi.org/10.1002/ps.7921
43. Collins SL, Patterson AD. 2020. The gut microbiome: an orchestrator of xenobiotic metabolism. Acta Pharm Sin B 10:19–32. https://doi.org/10.1016/j.apsb.2019.12.001
44. Culp EJ, Nelson NT, Verdegaal AA, Goodman AL. 2024. Microbial transformation of dietary xenobiotics shapes gut microbiome composition. Cell 187:6327–6345. https://doi.org/10.1016/j.cell.2024.08.038
45. De Filippis F, Valentino V, Sequino G, Borriello G, Riccardi MG, Pierri B, Cerino P, Pizzolante A, Pasolli E, Esposito M, Limone A, Ercolini D. 2024. Exposure to environmental pollutants selects for xenobiotic-degrading functions in the human gut microbiome. Nat Commun 15:4482. https://doi.org/10.1038/s41467-024-48739-7
46. Vangay P, Johnson AJ, Ward TL, Al-Ghalith GA, Shields-Cutler RR, Hillmann BM, Lucas SK, Beura LK, Thompson EA, Till LM, Batres R, Paw B, Pergament SL, Saenyakul P, Xiong M, Kim AD, Kim G, Masopust D, Martens EC, Angkurawaranon C, McGready R, Kashyap PC, Culhane-Pera KA, Knights D. 2018. U.S. immigration westernizes the human gut microbiome. Cell 175:962–972. https://doi.org/10.1016/j.cell.2018.10.029
47. Wang X, Chen L, Ren X, Kang S, Zhao L, Zhang H, Li X, Chen Z. 2024. Fate characteristics and risk quantification of cyflumetofen from tomato cultivation to processing based on large–scale applications. J Hazard Mater 465:133496. https://doi.org/10.1016/j.jhazmat.2024.133496
48. Popkin BM. 1999. Urbanization, lifestyle changes and the nutrition transition. World Dev 27:1905–1916. https://doi.org/10.1016/S0305-750X(99)00094-7
49. Ruel MT. 2003. Operationalizing dietary diversity: a review of measurement issues and research priorities. J Nutr 133:3911S–3926S. https://doi.org/10.1093/jn/133.11.3911S
50. Cohen R, Persky L, Hadar Y. 2002. Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. Appl Microbiol Biotechnol 58:582–594. https://doi.org/10.1007/s00253-002-0930-y
51. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA 107:14691–14696. https://doi.org/10.1073/pnas.1005963107
52. Crittenden AN, Schnorr SL. 2017. Current views on hunter - gatherer nutrition and the evolution of the human diet. Am J Phys Anthropol 162:84–109. https://doi.org/10.1002/ajpa.23148
53. Martínez I, Stegen JC, Maldonado-Gómez MX, Eren AM, Siba PM, Greenhill AR, Walter J. 2015. The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes. Cell Rep 11:527–538. https://doi.org/10.1016/j.celrep.2015.03.049
54. Wang C, Cancino A, Baste J, Marten D, Joshi AA, Nasreen A, Bhushan A. 2022. Causative role of anoxic environment in bacterial regulation of

human intestinal function. Cel Mol Bioeng 15:493–504. https://doi.org/10.1007/s12195-022-00735-x
55. Donaldson GP, Lee SM, Mazmanian SK. 2016. Gut biogeography of the bacterial microbiota. Nat Rev Microbiol 14:20–32. https://doi.org/10.1038/nrmicro3552
56. Singhal R, Shah YM. 2020. Oxygen battle in the gut: hypoxia and hypoxia-inducible factors in metabolic and inflammatory responses in the intestine. J Biol Chem 295:10493–10505. https://doi.org/10.1074/jbc.REV120.011188
57. Xu F. 2001. Dioxygen reactivity of laccase. Appl Biochem Biotechnol 95:125–133. https://doi.org/10.1385/abab:95:2:125.
58. Giardina P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S, Sannia G. 2010. Laccases: a never-ending story. Cell Mol Life Sci 67:369–385. https://doi.org/10.1007/s00018-009-0169-1
59. Janusz G, Pawlik A, Świderska-Burek U, Polak J, Sulej J, Jarosz-Wilkołazka A, Paszczyński A. 2020. Laccase properties, physiological functions, and evolution. IJMS 21:966. https://doi.org/10.3390/ijms21030966
60. Cañas AI, Camarero S. 2010. Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes. Biotechnol Adv 28:694–705. https://doi.org/10.1016/j.biotechadv.2010.05.002
61. Polak J, Jarosz-Wilkolazka A. 2012. Structure/redox potential relationship of simple organic compounds as potential precursors of dyes for laccase-mediated transformation. Biotechnol Prog 28:93–102. https://doi.org/10.1002/btpr.713
62. Zille A, Munteanu F-D, Gübitz GM, Cavaco-Paulo A. 2005. Laccase kinetics of degradation and coupling reactions. J Mol Catal B Enzym 33:23–28. https://doi.org/10.1016/j.molcatb.2005.01.005
63. Luo Qi, Lu J, Zhang H, Wang Z, Feng M, Chiang S-YD, Woodward D, Huang Q. 2015. Laccase-catalyzed degradation of perfluorooctanoic acid. Environ Sci Technol Lett 2:198–203. https://doi.org/10.1021/acs.estlett.5b00119
64. Luo Q, Yan X, Lu J, Huang Q. 2018. Perfluorooctanesulfonate degrades in a laccase-mediator system. Environ Sci Technol 52:10617–10626. https://doi.org/10.1021/acs.est.8b00839
65. Marciesky M, Aga DS, Bradley IM, Aich N, Ng C. 2023. Mechanisms and opportunities for rational in silico design of enzymes to degrade per- and polyfluoroalkyl substances (PFAS). J Chem Inf Model 63:7299–7319. https://doi.org/10.1021/acs.jcim.3c01303
66. Nasrollahpour S, Purewal S, Das RK, Brar SK. 2026. Biodegradation of per- and polyfluoroalkyl substances: mechanisms, challenges, and emerging strategies for sustainable remediation. Environ Sci: Water Res Technol 12:397–420. https://doi.org/10.1039/D5EW00888C
67. Zhang Y, Kong Z, Gregoire N, Li L, Yang L, Zhao M, Jin N, Wang F, Fan B, Francis F, Li M. 2023. Enantioselective activity and toxicity of chiral acaricide cyflumetofen toward target and non-target organisms. Chemosphere 325:138431. https://doi.org/10.1016/j.chemosphere.2023.138431
68. Sun D, Pang J, Zhou Z, Jiao B. 2016. Enantioselective environmental behavior and cytotoxicity of chiral acaricide cyflumetofen. Chemosphere 161:167–173. https://doi.org/10.1016/j.chemosphere.2016.06.087
69. Li N, Xia Y, Li Y, Jia Q, Qiu J, Xu Y, Wang Z, Liu Z, Qian Y. 2023. Untargeted screening, quantitative analysis, and toxicity estimation of degradation products of fluazinam in vegetables. Microchemical Journal 190:108584. https://doi.org/10.1016/j.microc.2023.108584
70. Escrivá L, Hanberg A, Zilliacus J, Beronius A, Karolinska Institutet, Institute of Environmental Medicine, Sweden. 2019. Assessment of the endocrine disrupting properties of bisphenol AF according to the EU criteria and ECHA/EFSA guidance. EFSA J 17:e170914. https://doi.org/10.2903/j.efsa.2019.e170914
71. Geller LT, Barzily-Rokni M, Danino T, Jonas OH, Shental N, Nejman D, Gavert N, Zwang Y, Cooper ZA, Shee K, et al. 2017. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 357:1156–1160. https://doi.org/10.1126/science.aah5043
72. Lehouritis P, Cummins J, Stanton M, Murphy CT, McCarthy FO, Reid G, Urbaniak C, Byrne WL, Tangney M. 2015. Local bacteria affect the efficacy of chemotherapeutic drugs. Sci Rep 5:14554. https://doi.org/10.1038/srep14554
73. Camarero S, Ibarra D, Martínez MJ, Martínez AT. 2005. Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. Appl Environ Microbiol 71:1775–1784. https://doi.org/10.1128/AEM.71.4.1775-1784.2005
74. Xiao Y, Li J, Wu P, Ning N, Li J, Shen Y, Huang Q, Ni J. 2021. An alkaline thermostable laccase from termite gut associated strain of *Bacillus stratosphericus*. Int J Biol Macromol 179:270–278. https://doi.org/10.1016/j.ijbiomac.2021.02.205
75. Fang W, Fang Z, Zhou P, Chang F, Hong Y, Zhang X, Peng H, Xiao Y. 2012. Evidence for lignin oxidation by the giant panda fecal microbiome. PLoS One 7:e50312. https://doi.org/10.1371/journal.pone.0050312
76. Vinogradova E, Mukhanbetzhanov N, Nurgaziyev M, Jarmukhanov Z, Aipova R, Sailybayeva A, Bekbossynova M, Kozhakhmetov S, Kushugulova A. 2024. Impact of urbanization on gut microbiome mosaics across geographic and dietary contexts. mSystems 9:e00585–24. https://doi.org/10.1128/msystems.00585-24
77. Kodama Y, Shumway M, Leinonen R, International Nucleotide Sequence Database Collaboration. 2012. The sequence read archive: explosive growth of sequencing data. Nucleic Acids Res 40:D54–6. https://doi.org/10.1093/nar/gkr854
78. Ziemski M, Adamov A, Kim L, Flörl L, Bokulich NA. 2022. Reproducible acquisition, management and meta-analysis of nucleotide sequence (meta)data using q2-fondue. Bioinformatics 38:5081–5091. https://doi.org/10.1093/bioinformatics/btac639
79. Lokmer A, Cian A, Froment A, Gantois N, Viscogliosi E, Chabé M, Ségurel L. 2019. Use of shotgun metagenomics for the identification of protozoa in the gut microbiota of healthy individuals from worldwide populations with various industrialization levels. PLoS One 14:e0211139. https://doi.org/10.1371/journal.pone.0211139
80. Asnicar F, Berry SE, Valdes AM, Nguyen LH, Piccinno G, Drew DA, Leeming E, Gibson R, Le Roy C, Khatib HA, et al. 2021. Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. Nat Med 27:321–332. https://doi.org/10.1038/s41591-020-01183-8
81. Ziemski M, Gehret L, Simard A, Dau SC, Risch V, Grabocka D, Matzoros C, Wood C, Cabrera PM, Hernández-Velázquez R, Herman C, Evans K, Robeson MS, Bolyen E, Caporaso JG, Bokulich NA. 2025. MOSHPIT: accessible, reproducible metagenome data science on the QIIME 2 framework. bioRxiv:2025.01.27.635007. https://doi.org/10.1101/2025.01.27.635007
82. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560
83. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923
84. Tomofuji Y, Sonehara K, Kishikawa T, Maeda Y, Ogawa K, Kawabata S, Nii T, Okuno T, Oguro-Igashira E, Kinoshita M, et al. 2023. Reconstruction of the personal information from human genome reads in gut metagenome sequencing data. Nat Microbiol 8:1079–1094. https://doi.org/10.1038/s41564-023-01381-3
85. Li D, Liu C-M, Luo R, Sadakane K, Lam T-W. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct *de Bruijn* graph . Bioinformatics 31:1674–1676. https://doi.org/10.1093/bioinformatics/btv033
86. Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H, Mende DR, Letunic I, Rattei T, Jensen LJ, von Mering C, Bork P. 2019. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res 47:D309–D314. https://doi.org/10.1093/nar/gky1085
87. Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using DIAMOND. Nat Methods 12:59–60. https://doi.org/10.1038/nmeth.3176
88. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, Tosatto SCE, Paladin L, Raj S, Richardson LJ, Finn RD, Bateman A. 2021. Pfam: the protein families database in 2021. Nucleic Acids Res 49:D412–D419. https://doi.org/10.1093/nar/gkaa913
89. Zhao Y, Li M-C, Konaté MM, Chen L, Das B, Karlovich C, Williams PM, Evrard YA, Doroshow JH, McShane LM. 2021. TPM, FPKM, or normalized counts? a comparative study of quantification measures for the analysis of RNA-seq data from the NCI patient-derived models repository. J Transl Med 19:269. https://doi.org/10.1186/s12967-021-02936-w
90. Goeker M. 2022. BacDive: API client for BacDive. Available from: https://R-Forge.R-project.org/projects/opm
91. Google Gemini. Available from: https://gemini.google.com. Retrieved 13 Jan 2026. Accessed January 13, 2026

92. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. https://doi.org/10.1186/1471-2105-11-119
93. Reiss R, Ihssen J, Richter M, Eichhorn E, Schilling B, Thöny-Meyer L. 2013. Laccase versus laccase-like multi-copper oxidase: a comparative study of similar enzymes with diverse substrate spectra. PLoS One 8:e65633. https://doi.org/10.1371/journal.pone.0065633
94. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539. https://doi.org/10.1038/msb.2011.75
95. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300
96. Faith DP. 1992. Conservation evaluation and phylogenetic diversity. Biol Conserv 61:1–10. https://doi.org/10.1016/0006-3207(92)91201-3
97. Lozupone C, Knight R. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71:8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005
98. Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464. https://doi.org/10.1093/bioinformatics/btq166
99. R Core Team. 2025. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
100. Keck F, Rimet F, Bouchez A, Franc A. 2016. Phylosignal: an R package to measure, test, and explore the phylogenetic signal. Ecol Evol 6:2774–2780. https://doi.org/10.1002/ece3.2051
101. Kang DD, Li F, Kirton E, Thomas A, Egan R, An H, Wang Z. 2019. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. PeerJ 7:e7359. https://doi.org/10.7717/peerj.7359
102. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210–3212. https://doi.org/10.1093/bioinformatics/btv351
103. Bowers RM, Kyrpides NC, Stepanauskas R, Harmon-Smith M, Doud D, Reddy TBK, Schulz F, Jarett J, Rivers AR, Eloe-Fadrosh EA, et al. 2017. Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol 35:725–731. https://doi.org/10.1038/nbt.3893
104. Posit team. 2025. RStudio: integrated development environment for R (2025.9.2.418). Posit Software, PBC, Boston, MA, USA.
105. Paradis E, Schliep K. 2019. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–528. https://doi.org/10.1093/bioinformatics/bty633
106. Campitelli E. 2025. Ggnewscale: multiple fill and colour scales in "ggplot2"
107. Aphalo PJ, Slowikowski K, Mouksassi S. 2026. Ggpmisc: miscellaneous extensions to "ggplot2"
108. Kassambara A. 2025. Ggpubr: "ggplot2" Based publication ready plots
109. Yu G, Smith DK, Zhu H, Guan Y, Lam T-Y. 2017. Ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol 8:28–36. https://doi.org/10.1111/2041-210X.12628
110. Yu G. 2022. Data integration, manipulation and visualization of phylogenetic trees. Chapman and Hall/CRC, New York.
111. Firke S, Denney B, Haid C, Knight R, Grosser M, Zadra J. 2024. Janitor: simple tools for examining and cleaning dirty data
112. Rinker T, Kurkiewicz D, Hughitt K, Wang A, Aden-Buie G, Wang A, Burk L. 2019. Pacman: package management tool
113. Bolker B, ButlerM, CowanP, VienneD de, EddelbuettelD, HolderM, JombartT, Kembel S, Michonneau F, Orme D, O'Meara B, Paradis E, Regetz J, Zwickl D. 2024. Phylobase: base package for phylogenetic structures and comparative data
114. McMurdie PJ, Holmes S. 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217. https://doi.org/10.1371/journal.pone.0061217
115. Neuwirth E. 2022. RColorBrewer: ColorBrewer palettes. https://CRAN.R-project.org/package=RColorBrewer.
116. Wickham H, Bryan J. 2025. Readxl: read excel files. Available from: https://CRAN.R-project.org/package=readxl
117. Winter DJ. 2017. Rentrez: an R package for the NCBI eUtils API. R J 9:520. https://doi.org/10.32614/RJ-2017-058
118. Wickham H, Pedersen TL, Seidel D. 2025. Scales: scale functions for visualization. https://CRAN.R-project.org/package=scales.
119. Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H. 2019. Welcome to the tidyverse. JOSS 4:1686. https://doi.org/10.21105/joss.01686
120. Wang L-G, Lam TT-Y, Xu S, Dai Z, Zhou L, Feng T, Guo P, Dunn CW, Jones BR, Bradley T, Zhu H, Guan Y, Jiang Y, Yu G. 2020. Treeio: an R package for phylogenetic tree input and output with richly annotated and associated data. Mol Biol Evol 37:599–603. https://doi.org/10.1093/molbev/msz240
121. Platonov N. 2025. Ursa: non-interactive spatial tools for raster processing and visualization. https://CRAN.R-project.org/package=ursa.
122. Wickham H, Hester J, Ooms J. 2025. Xml2: parse XML. Available from: https://CRAN.R-project.org/package=xml2
123. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden markov model: application to complete genomes. J Mol Biol 305:567–580. https://doi.org/10.1006/jmbi.2000.4315
124. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2
125. Yu Y, Trottmann NF, Schärer MR, Fenner K, Robinson SL. 2024. Substrate promiscuity of xenobiotic-transforming hydrolases from stream biofilms impacted by treated wastewater. Water Res 256:121593. https://doi.org/10.1016/j.watres.2024.121593
126. Cordner A, Brown P, Cousins IT, Scheringer M, Martinon L, Dagorn G, Aubert R, Hosea L, Salvidge R, Felke C, Tausche N, Drepper D, Liva G, Tudela A, Delgado A, Salvatore D, Pilz S, Horel S. 2024. PFAS contamination in Europe: generating knowledge and mapping known and likely contamination with "Expert-Reviewed" journalism. Environ Sci Technol 58:6616–6627. https://doi.org/10.1021/acs.est.3c09746
127. Rosen MR, Lapham WW. 2008. Introduction to the U.S. geological survey national water - quality assessment (NAWQA) of ground - water quality trends and comparison to other national programs. J of Env Quality 37. https://doi.org/10.2134/jeq2008.0049