Water Air Soil Pollut (2023) 234:482
https://doi.org/10.1007/s11270-023-06495-2

# Comparative Genomics Analysis of the Aromatic and Xenobiotic Degradation Capacities and Heavy Metal Resistance in Seven Environmentally Derived Bacterial Isolates

**Alexander R. Kneubehl · Rupa Iyer**

Received: 12 April 2023 / Accepted: 30 June 2023 / Published online: 14 July 2023


**Abstract** This work is a comparative genomics investigation of the aromatic and xenobiotic compound degradation capabilities and heavy metal resistance of environmental bacterial isolates previously identified by our lab, *Achromobacter xylosoxidans* ADAF13, *Exiguobacterium* sp. KKBO11, *Ochrobactrum anthropi* FRAF13, *Pseudomonas putida* CBF10-2, *Pseudomonas stutzeri* ODKF13, *Rhizobium radiobacter* GHKF11, and *Stenotrophomonas maltophilia* CBF10-1. This work sought to assess the potential of these isolates as bioremediation tools. We found a variety of aromatic degradation pathways though none directly acts on industrial compounds such as polycyclic aromatic compounds, benzene, phthalate, or xylene. *Achromobacter xylosoxidans* ADAF13, *P. putida* CBF10-2, and *P. stutzeri* ODKF13 showed the most complete pathways for aromatic compound degradation and halobenzoate degradation. All isolates contained heavy metal resistance genes for arsenic, cadmium, copper, chromium, lead, mercury, and zinc. Arsenic resistance genes were the most common among isolates and were organized into structurally diverse *ars* operons. Collectively, our data indicated that *A. xylosoxidans* ADAF13, *P. putida* CBF10-2, and *P. stutzeri* ODKF13 are strong candidates for further enhancement and development as bioremediation tools.

**Keywords** Comparative genomics · Pesticides · Bioremediation · Aromatic compounds · Heavy metals · Microbiology

## 1 Introduction

Global industrialization has brought the world into the modern age but has resulted in the release of many pollutants into the environment (Brimblecombe, 2005; Carpenter et al., 1998; Pimentel, 2005; Power et al., 2018; Thevenon et al., 2011; Tilman, 1998). Soil is particularly at risk from contamination either by direct application of agrochemicals (e.g., pesticides) (Pimentel, 2005; Tang et al., 2021), accident chemical release via chemical spills (Shin et al., 2018; FAO and UNEP, 2021), or through atmospheric deposition of pollutants (Cai et al., 2022; Swain et al., 1992). Generally, these contaminants, which can include herbicides, fungicides, insecticides, polycyclic aromatic hydrocarbons, and heavy metals, can have long-term adverse effects on agricultural soil, resulting in decreased soil productivity (Alengebawy et al., 2021; Kaur et al., 2017; Srivastava et al., 2017)

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11270-023-06495-2.

A. R. Kneubehl
Houston, USA

R. Iyer (✉)
Research, Innovation and Economic Development, Tarleton State University, Stephenville, TX, USA
e-mail: iyer@tarleton.edu

as well as potential contamination of the food product itself (Ahmad et al., 2021; Alengebawy et al., 2021; Haddad et al., 2023; Zhang et al., 2017). Pesticides (e.g., chlorpyrifos, malathion, glyphosate) are commonly used in agricultural practices, and their benefit to the industry has been massive; however, these compounds can have adverse effects on the environment and the soil they are applied to (Fox et al., 2007; Gunstone et al., 2021; Kaur et al., 2017; Lu et al., 2020; Pimentel, 2005; Sharma et al., 2019; Tang et al., 2021; Woodcock et al., 2016). Further, pesticide poisoning in humans is a significant concern, resulting in 385 million unintentional poisonings globally, with approximately 11,000 deaths yearly (Boedeker et al., 2020).

Polycyclic aromatic carbons (PAHs) such as benzene, anthracene, and benzo[α]pyrene are pollutants of concern for public health. PAHs can be carcinogenic (Rengarajan et al., 2015) and can be absorbed into plant material, contaminating the food chain (Fan et al., 2020; Zhang et al., 2017). Soil can also be contaminated by long-range transport of PAHs through the atmosphere, which are then deposited into topsoil (Arellano et al., 2018; Gocht et al., 2007; Nam et al., 2008). Due to the high hydrophobicity of PAH compounds, they can bind to soil particles and have half-lives between days and months (Duan et al., 2015; Luo et al., 2008; Roslund et al., 2018; Yang et al., 2010).

Heavy metal contamination of soil is also a concern and can result from natural geochemical processes, direct application in the agricultural industry, or pollution (Alengebawy et al., 2021). Heavy metals such as arsenic, cadmium, chromium, lead, and mercury are toxic to humans, plants, and wildlife and can be long-lived in soil. These contaminants also threaten the food chain since plants can absorb these elements into food products, which can accumulate in humans (Haddad et al., 2023; Xiang et al., 2021; Zhang et al., 2017). Given these threats to the soil and agricultural production, methods to remove or neutralize pesticides, PAHs, and heavy metals are necessary. Bioremediation of contaminated soils by microbes is a cost-effective and efficient bioremediation method actively pursued by the field at large (Bala et al., 2022; Zhang et al., 2020).

Soil microbes, particularly rhizospheric microbes that interact closely with plant roots, are crucial to soil and plant health and productivity (Bulgarelli et al., 2013; Li et al., 2021; Mendes et al., 2011; Panke-Buisse et al., 2015). Microbes have evolved mechanisms to utilize plant aromatic compounds for tricarboxylic acid cycle intermediates. Through this evolution, these microbes can also degrade human-related aromatic compounds (e.g., pesticides and aromatic hydrocarbons) (Fuenmayor et al., 1998; Harwood & Parales, 1996; Jencova et al., 2004; Lessner et al., 2002; Mohapatra & Phale, 2021; Swetha & Phale, 2005). These same microbes also have mechanisms for resistance to heavy metals (Mathivanan et al., 2021; Pal et al., 2022). Given these characteristics, soil microbes are being pursued for deployment in bioremediation scenarios to degrade contaminants to restore healthy environments (Bala et al., 2022; Narayanan et al., 2023). There is also great potential for discovery and innovation in biotechnology to exploit the mechanisms of how these microbes degrade xenobiotic compounds and resist heavy metal exposure. By isolating and refining these systems, there could be significant benefits to agricultural production, human, and environmental disaster cleanup.

The Iyer Lab hosts an extensive library of environmentally derived bacterial isolates. Previous work investigating a particular cohort of bacterial isolates has determined that they can degrade organophosphate compounds individually (Iyer & Iken, 2013; Iyer et al., 2016, 2018) or as consortia (Islam & Iyer, 2021). This work furthers the previous studies by investigating the genomes of these isolates (*Achromobacter xylosoxidans* ADAF13 (Iyer & Damania, 2016c, d), *Exiguobacterium* sp. KKBO11 (Iyer & Damania, 2016a, b), *Ochrobactrum anthropi* FRAF13 (Iyer & Damania, 2016a, b), *Pseudomonas putida* CBF10-2 (Iyer & Damania, 2016c, d), *Pseudomonas stutzeri* ODKF13 (Iyer & Damania, 2016e), *Rhizobium radiobacter* GHKF11 (Iyer & Damania, 2016f), and *Stenotrophomonas maltophilia* CBF10-1 (Iyer & Damania, 2016g) to assess their potential degradation abilities for aromatics and xenobiotics as well as resistance to heavy metals (arsenic, cadmium, chromium, mercury, and lead) to determine the potential of these isolates in broad spectrum bioremediation.

## 2 Results and Discussion

### 2.1 Aromatic Compound and Xenobiotic Degradation

Plants often produce aromatic compounds exuded into the soil via roots and are a substantial carbon source for soil microbes capable of degrading them (Chaparro et al., 2013; Zhalnina et al., 2018). Consequently, soil microbes are often investigated for potential use in the bioremediation of PAH, pesticides, and even chemical weapons (Alves et al., 2018; Islam & Iyer, 2021; Iyer et al., 2018; Narayanan et al., 2023). To investigate the potential aromatic and xenobiotic compound degradation capacity of the isolates *A. xylosoxidans* ADAF13, *Exiguobacterium* sp. KKBO11, *O. anthropi* FRAF13, *P. putida* CBF10-2, *P. stutzeri* ODKF13, *R. radiobacter* GHKF11, and *S. maltophilia* CBF10-1, their genomes were annotated by the bacterial and viral bioinformatics resource center (BV-BRC)'s annotation service using RASTtk (Brettin et al., 2015; Olson et al., 2023). KEGG annotation and pathway mapping of the predicted proteins by RASTtk was used for aromatic and xenobiotic degradation pathway analysis (Kanehisa & Sato, 2020).

We used KEGG map01220 as a basis for a general overview of aromatic degradation pathways. An abridged map01220 showing only the pathways present among these isolates is shown in Fig. 1, where each isolate's KEGG reconstruct results are indicated. The number of map01220 KEGG

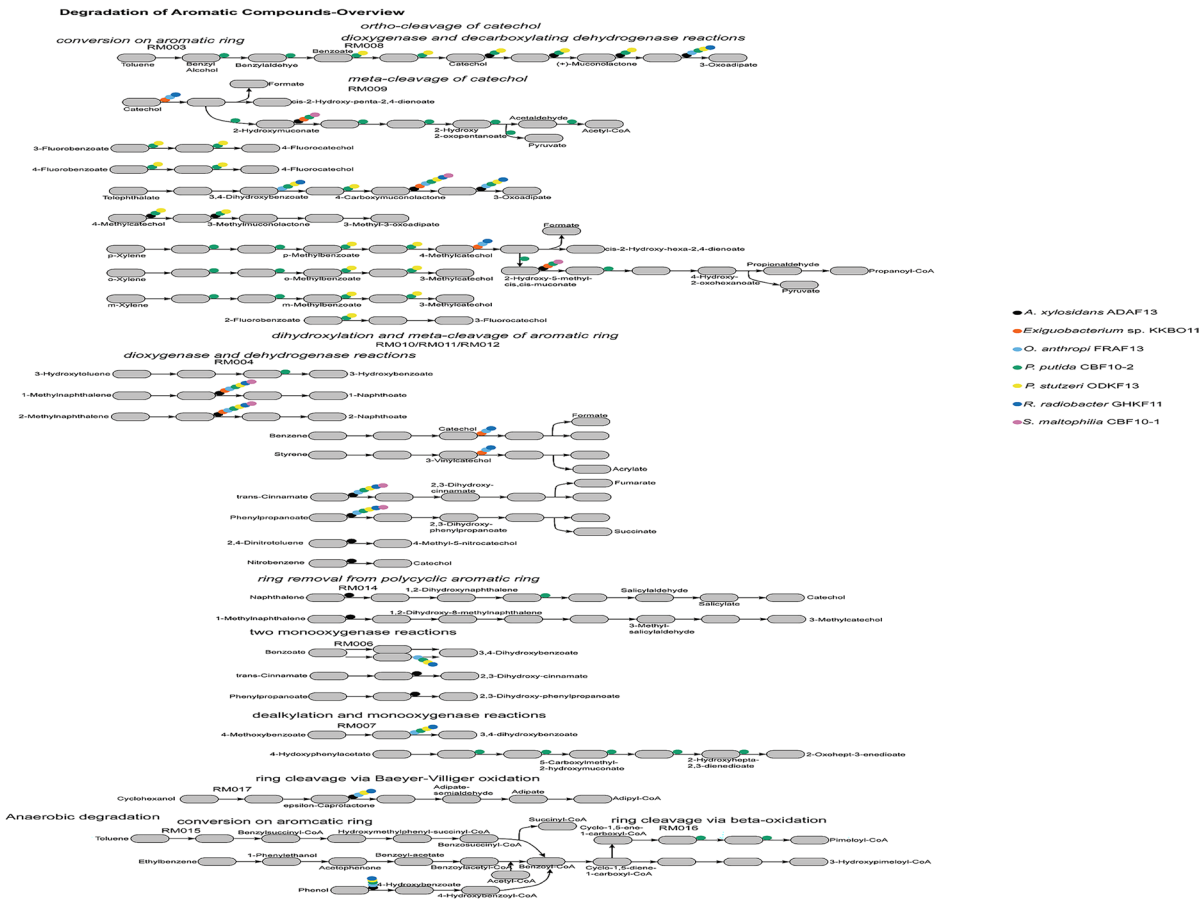


**Fig. 1** Abridged overview of aromatic degradation pathways (KEGG map01220). This is an abridged version of KEGG map01220 showing only the pathways/reactions present in this dataset. Dots at each arrow indicate the presence of genes for that particular isolate for that reaction which mediate the conversion of the upstream compound to the downstream product or vice versa in the case of reactions that can go either forward or backward. Each colored dot corresponds to a specific isolate

orthologs found in each of the isolates was enumerated in Online Resource 1. On average, there were 19 genes classified by KEGG in map01220 though these ranged widely from 45 genes in *Pseudomonas putida* CBF10-2 to only 5 genes in *Exiguobacterium* sp. KBBO11. *Pseudomonas putida* CBF10-2's 45 genes were found in 20 different pathways/reactions, whereas *Exiguobacterium* sp. KBBO11's five genes were found in 7 pathways/reactions (Fig. 1 and Online Resource 1). *Stenotrophomonas maltophilia* CBF10-1 also had few genes in KEGG map01220, with only six genes identified in 7 pathways/reactions.

Overall, these isolates putatively lack the ability to degrade polycyclic aromatic compounds (e.g., naphthalene) or dioxygenase and dehydrogenase reactions capable of degrading compounds such as benzene, styrene, toluene, phthalate, and chloro- or fluorobenzene (Fig. 1). However, the benzoate degradation pathway was present at varying levels across the isolates, from complete in *P. putida* CBF10-2 to nearly absent in *Exiguobacterium* sp. KKBO11 and *S. maltophilia* CBF10-1. *Pseudomonas putida* CBF10-2 and *P. stutzeri* ODKF13 putatively were able to degrade 3/4-fluorobenzoate to 4-fluorocatechol. *Pseudomonas putida* CBF10-2 was the only isolate likely capable of degrading 4-methyl benzyl alcohol (a degradation product of p-xylene) to 2-hydroxy-cis-hex-2,4-dienoate as well as a nearly complete 4-hydroxyphenaylacetate catabolism pathway.

Initially, KEGG reconstruction of map01220 indicated *A. xylosoxidans* ADAF13 as the only isolate capable of degrading nitrobenzene, though further analysis revealed this to be incorrect. For the degradation of nitrobenzene to catechol to occur, four genes are required, naphthalene 1,2-dioxygenase subunit alpha (*nbzAc*), naphthalene 1,2-dioxygenase subunit beta (*nbzAd*), a naphthalene 1,2-dioxygenase ferredoxin reductase component (*nbzAa*), and a naphthalene 1,2-dioxygenase ferredoxin component (*nbzAb*) (Lessner et al., 2002). We found that only the *nbzAb* gene was present in *A. xylosoxidans* ADAF13. The genes upstream and downstream of *nbzAb* were unrelated to the *nbz* operon, suggesting that *A. xylosoxidans* ADAF13 cannot convert nitrobenzene to catechol.

Analysis of the genes surrounding *A. xylosoxidans* ADAF13's *nbzAb* gene (fig|222.333.peg.2710) suggested that this gene was part of an oxygenolytic ortho-dehalogenation operon (*ohbRABD*). The two genes upstream of the *nbzAb* gene (fig|222.333.peg.2710) were annotated by KEGG as a tRNA threonylcarbamoyladenosine dehydratase (fig|222.333.peg.2709) and not classified (fig|222.333.peg.2708). The three genes downstream of *nbzAb* were annotated by KEGG as a salicylate 5-hydroxylase small subunit (*nagH*) gene, a salicylate 5-hydroxylase large subunit (*nagG*) gene, and a LysR family transcriptional regulator, mexEF-oprN operon transcriptional activator (*mexT*) gene (fig|222.333.peg.2711, fig|222.333.peg.2712, fig|222.333.peg.2713, respectively). These four genes likely constitute an *ohb* operon, with *ohbR* being the regulatory gene (fig|222.333.peg.2713), *ohbA* encoding the small beta-ISP (terminal oxidoreductase) subunit ((fig|222.333.peg.2712), *ohbB* encoding the large alpha-ISP subunit (fig|222.333.peg.2711), and *ohbD* encoding a ferredoxin gene that enhances the activity of OhbAB (fig|222.333.peg.2710) (Tsoi et al., 1999). The *ohbC* gene in other *ohb* operons is an overlapping gene with *ohbB* and encodes an ATP-binding cassette (ABC) transporter family protein of unknown function (Tsoi et al., 1999); however, we did not find an *ohbC* gene in this operon. The putative *ohbRABD* operon was found on an ~ 480 kb contig (fig|222.333.con.0054), which did not appear to be derived from a plasmid suggesting this operon was localized on the chromosome. Neither the *ohbRABD* operon nor its constituent genes were found in the other isolates investigated in this work.

To better understand the relatedness of *A. xylosoxidans* ADAF13's OhbA and OhbB sequences to similar proteins in different bacterial species, including plasmid-borne OhbA and OhbB from a chlorobenzoate degrading *A. xylosoxidans* isolate (A8) (Jencova et al., 2004), we performed phylogenetic (Fig. 2) and BLASTp analyses (Johnson et al., 2008). Phylogenetic analysis revealed that ADAF13's OhbA and OhbB were more closely related to the functionally validated OhbA (Fig. 2A) and OhbB (Fig. 2B) from *P. aeruginosa* JB2 (Hickey & Sabat, 2001) and *Ralstonia* sp. U2 (Fuenmayor et al., 1998) to the exclusion of the plasmid-borne OhbA and OhbB reported from the *A. xylosoxidans* A8 isolate. BLASTp analysis of ADAF13's OhbA and OhbB showed that these genes were found in other *Achromobacter* species. OhbA had strong BLASTp hits in other *A. xylosoxidans* isolates, *Achromobacter mucicolens*, and *Achromobacter spanius* (query coverage = 100%,

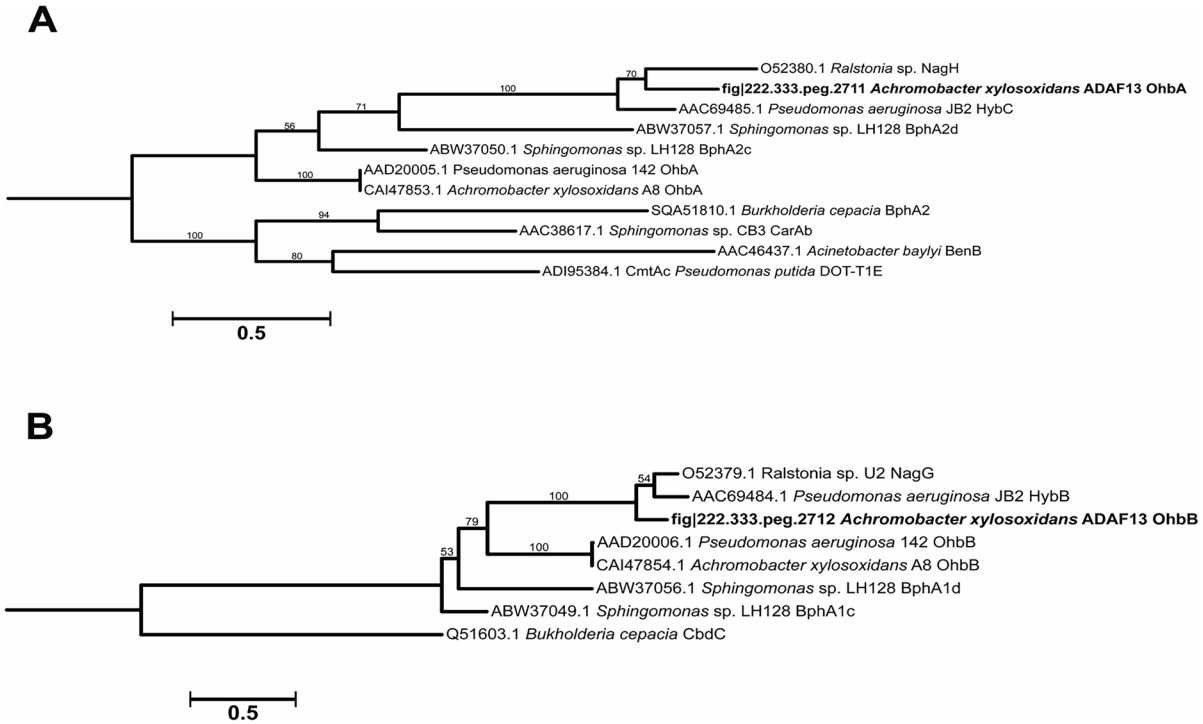


**Fig. 2** Phylogenetic analysis of *A. xylosoxidans* ADAF13 OhbA and OhbB. Maximum likelihood trees were inferred from amino acid alignments of proteins related to OhbA (**A**) and OhbB (**B**). The tip labels indicating this study's *A. xylosoxidans* ADAF13 sequences are bolded. The numbers above the branches indicate the percent branch support of 1000 ultra-fast bootstrap replicates. The trees are midpoint rooted. Scale bars indicate the number of substitutions per site

percent sequence identity ≥ 98%, *e*-value ≤ 1e-111). OhbB had BLASTp hits in other *A. xylosoxidans* isolates and in *A. mucicolens, Achromobacter animicus*, *Achromobacter insolitus*, and *Achromobacter aresnitoxydans* (query coverage = 100%, percent sequence identity ≥ 98%, *e*-value = 0.0). BLASTp analysis of OhbA and OhbB from the A8 isolate did not indicate any other *Achromobacter* spp. contained these plasmid-borne proteins. The best BLASTp hits for A8's OhbA was a sequence from Burkholderiales and *P. aeruginosa* and for OhbB Pseudomonadota. These data suggest that plasmid-borne *ohbAB* was likely a rare event for *Achromobacter* spp. Collectively, our data suggested that *A. xylosoxidans* ADAF13 cannot convert nitrotoluene to catechol, but it can mediate the dehalogenation of halobenzoates.

Further analysis of the KEGG annotations revealed genes in 20 different xenobiotic degradation pathways between the isolates. The number of genes classified into the 20 different pathways was enumerated in Online Resource 1. *Achromobacter xylosoxidans* ADAF13 contained the most pathways with gene(s) in all 20 identified pathways, while *Exiguobacterium* sp. KBBO11 had the least with genes in only 11 pathways. The number of genes in each pathway within each isolate ranged widely from zero to 62. The benzoate degradation pathway (KEGG ko00362) had the greatest number of genes classified across all isolates. *Pseudomonas putida* CBF10-2 had the highest number at 65 genes in the benzoate degradation pathway, followed closely by *A. xylosoxidans* ADAF13 (59 genes). *Stenotrophomonas maltophilia* CBF10-1 had the least number of genes in this pathway, with 12 genes. The benzoate degradation was particularly interesting to us since it is a pathway associated with the beta-ketoadipate pathway (Harwood & Parales, 1996).

Several microbial degradation pathways for industrially produced aromatic compounds (e.g., naphthalene, aniline, toluene, p-cresol, and benzene) eventually funnel through either catechol or protocatechuate as degradation intermediates (Cao et al.,

2009; Phale et al., 2020; Pimviriyakul et al., 2020). Therefore, the catechol and protocatechuate pathways are essential for the mineralization of aromatic compounds allowing for the complete degradation and use as a carbon source by the microbe. All seven isolates being investigated had at least one gene in the benzoate degradation pathway (KEGG ko00362) (Fig. 3), which catabolizes aromatic compounds for TCA-cycle intermediates. However, the data suggested that only *P. putida* CBF10-2 was capable of catabolism from benzoate or protocatechuate to 3-oxoadipate. *Achromobacter xylosoxidans* ADAF13 contained *catABC*, *pcaDIJ*, and *fadA* genes whose proteins should be able to catabolize catechol to succinyl-CoA and acetyl-CoA via the ortho-cleavage pathway. However, it was missing the 3-oxoadipyl-CoA thiolase gene, *pcaF*. *Exiguobacterium* sp. KKBO11 had only *catE*, *praC*, and *pcaC*, suggesting it could cleave catechol via meta-cleavage but not process the 2-hydroxymuconate semialdehyde product further in this pathway. *Ochrobactrum anthropi* FRAF13 encoded *pcaCDGH*, *fadA*, and *catE*, a nearly complete protocatechuate catabolism pathway to succinyl- and acetyl-CoA products. However, KEGG did not annotate a 3-carboxy-cis, cis-muconate cycloisomerase, PcaB, which converts β-carboxymuconate to γ-carboxymuconolactone. BLASTp analysis of FRAF13's predicted proteins using an *O. anthropi* PcaB (AIK42087.1) as the query identified fig|529.295.peg.3137 as a PcaB (query coverage = 100%, percent sequence identity = 93.5%, *e*-value = 0.0). This protein was not annotated by KEGG but was classified as a 3-carboxy-cis, cis-muconate cycloisomerase by BV-BRC's RASTtk annotation. *Pseudomonas putida* CBF10-2 had the most extensive benzoate degradation gene repertoire of all the isolates. CBF10-2 had a complete set of *benABCD* genes to convert benzoate to catechol and a complete set of ortho-cleavage of catechol genes (*catABC*, *pcaDIJF*, and *fadA*). Moreover, CBF10-2 also had *pcaBCGH* genes to allow for protocatechuate catabolism and a nearly complete meta-cleavage of catechol gene complement (*dmpCH*, *praC*, *mhpDEF*). *Pseudomonas putida* CBF10-2 was missing the catechol 2,3-dioxygenase gene (*dmpB* or *catE*). Neither KEGG nor RASTtk annotated a catechol 2,3-dioxygenase gene. BLASTp analysis of CBF10-2's predicted proteins using a

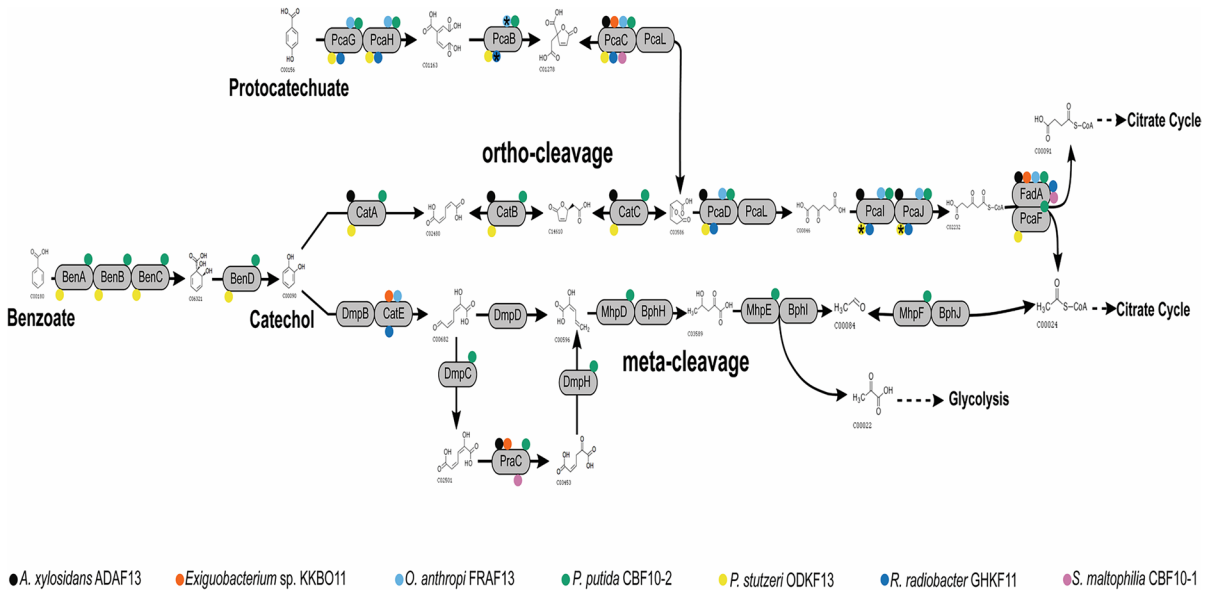


**Fig. 3** Abridged benzoate degradation pathway (KEGG map00362). This figure is an abridged KEGG map00362 that focuses on benzoate degradation via ortho- and meta-cleavage of catechol and protocatechuate degradation. Dots present for each enzyme indicate the presence of that enzyme for a particular isolate. Each colored dot corresponds to a specific isolate. Dotted arrows indicate there are other intermediate steps not listed. KEGG compound numbers are indicated for each chemical structure

*Pseudomonas* spp. Catechol 2,3-dioxygenase protein sequence (WP_011475388.1) did not return any hits. *Pseudomonas stutzeri* ODKF13 had similar capabilities as *P. putida* CBF10-2 except for the partial meta-cleavage pathway and was missing PcaI and PcaJ. ODKF13 did not contain any meta-cleavage pathway genes. Given the nearly complete benzoate catabolism pathway, we thought it was unlikely that *P. stutzeri* ODKF13 was missing PcaI and PcaJ. Therefore, we performed a BLASTp analysis of ODKF13 predicted proteins against *P. stutzeri* PcaI (VEI36278.1) and PcaJ (AEJ04444.1). BLASTp analysis yielded significant hits with fig|316.732.peg.3339 (query coverage 100%, percent sequence identity = 93.68%, *e*-value = 0.0) for PcaI and fig|316.732.peg.3340 (query coverage 100%, percent sequence identity = 100%, *e*-value = 0.0) for PcaJ. KEGG annotated these proteins as K01039 (glutaconate CoA-transferase, subunit A) and K01040 (glutaconate CoA-transferase, subunit B), respectively; however, RASTtk annotated both proteins as 3-oxoadipate CoA-transferase subunits A and B. *Rhizobium radiobacter* GHKF11 and *O. anthropi* FRAF13 were similarly equipped containing a near-complete protocatechuate catabolism pathway except for a PcaB in GHKF11. Using BLASTp, we used an *Agrobacterium tumefaciens* (synonymous with *Rhizobium radiobacter*) (Flores-Félix et al., 2020; Young et al., 2001) PcaB sequence (WP_262526641) as a query against the predicted proteins of GHKF11, which yielded a significant hit (fig|379.725.peg.2964, query coverage 99%, percent sequence identity = 78.29%, *e*-value = 0.0). Again, we found that KEGG failed to annotate PcaB, but RASTtk identified fig|379.725.peg.2964 as a 3-carboxy-cis, cis-muconate cycloisomerase. Lastly, *Stenotrophomonas maltophilia* CBF10-1 had only three genes in this pathway (*praC*, *pcaC*, and *fadA*), and none confer any ring cleavage capabilities. Overall, catechol cleavage was likely possible in all isolates except *S. maltophilia* CBF10-1 with protocatechuate cleavage in *O. anthropi* FRAF13, *P. putida* CBF10-2, *P. stutzeri* ODKF13, and *R. radiobacter* GHKF11. Further catabolism of cleavage products was possible in all cases, with the exception of *Exiguobacterium* sp. KKBO11. The fact that the isolates investigated (excluding *Exiguobacterium* sp. KKBO11 and *S. maltophilia* CBF10-1) can likely degrade catechol and protocatechuate could be exploited for genome engineering of these microbes to allow for complete degradation of industrially significant aromatic compounds.

Given the use of halogenated aromatic compounds in industrial and agricultural applications (Jeschke, 2017, 2022), their toxicity in humans (Heid et al., 2001; Vickers et al., 1985), and their longevity in the environment (Salkinoja-Salonen et al., 1995), we investigated the degradation of these compounds further (Fig. 4). Proteins within the benzoate degradation pathway also confer the ability to catabolize halobenzoates (Haddad et al., 2001; Neidle et al., 1991). With *benABCD*, *catAB*, and carboxymethylenebutenolidase genes, *P. putida* CBF10-2 and *P. stutzeri* ODKF13 likely could catabolize 3- and 4-fluorobenzoate compounds to 2-malelyacetate and hydrofluoric acid (Fig. 4A). *Achromobacter xylosoxidans* ADAF13 did not possess BenABCD but did have the OhbAB proteins, which could act on 2-fluorobenzoate after which the CatAB and carboxymethylenebutenolidase proteins could further catabolize the compound to 2-malelyacetate and hydrofluoric acid. None of these isolates had a maleylacetate reductase protein that would convert 2-malelyacetate to 3-oxoadipate for further catabolism in the benzoate degradation pathway. Furthermore, the CatAB proteins working with a carboxymethylenebutenolidase in *A. xylosoxidans* ADAF13, *P. putida* CBF10-2, and *P. stutzeri* ODKF13 likely confer the ability to catabolize 4-chlorocatechol to cis-acetylacrylate (Fig. 4B). Halogenated aromatics are long-lived in the environment, toxic, and pose a threat to contaminating soil and water sources (Goswami et al., 2022; Heid et al., 2001; Vickers et al., 1985; Xie et al., 2021; Zhang et al., 2017); therefore, the ability of these three isolates, *A. xylosoxidans* ADAF13, *P. putida* CBF10-2, and *P. stutzeri* ODKF13, to degrade halogenated aromatics should be further investigated.

Lastly, we expanded our search for aromatic compound degradation ability by investigating and assessing the putative ability of these isolates to catabolize gentisate, homogentisate, and 3,4-dihydroxyphenylacetate (homoprotocatechuate), pathways of which are found in tyrosine metabolism (KEGG map00350). Counting the genes in the subpathways of KEGG map00350 (only genes involved in the ring cleavage and catabolism of gentisate, homogentisate, and homoprotocatechuate) indicated that, on average, each isolate had approximately nine genes annotated by KEGG (Online

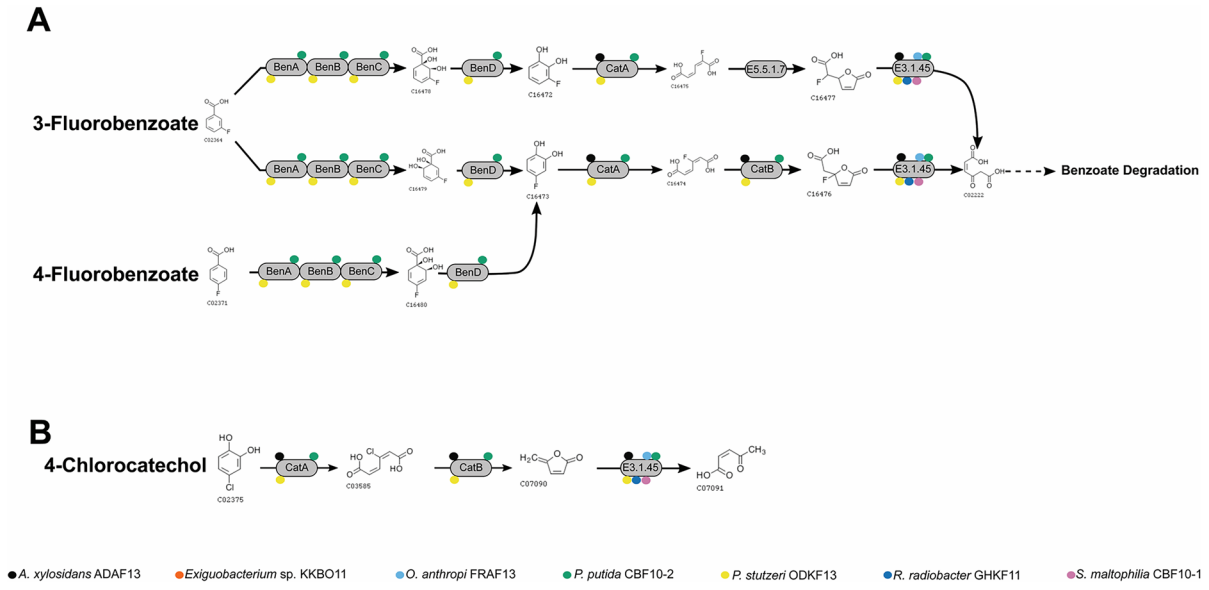


**Fig. 4** Halobenzoate degradation pathways. The fluorobenzoate pathways (**A**) are adapted from KEGG map00364. The 4-chlorocatechol degradation pathway (**B**) is adapted from KEGG map00361. Dots present for each enzyme indicate the presence of that enzyme for a particular isolate. Each colored dot corresponds to a specific isolate. Dotted arrows indicate there are other intermediate steps not listed. KEGG compound numbers are indicated for each chemical structure

Resource 2). *Achromobacter xylosoxidans* ADAF13 had the most genes at 20 with *Exiguobacterium* sp. KBBO11 with the least two genes (Fig. 5). Gentisate is an intermediate degradation product of industrial aromatic compounds such as naphthalene and the pesticide carbaryl (Mohapatra & Phale, 2021; Swetha & Phale, 2005; Zhu et al., 2019). *Achromobacter xylosoxidans* ADAF13 was the only isolate with a gentisate 1,2-dioxygenase, which mediates ring cleavage, and had two different copies of this gene (figl222.333.peg.2644 and figl222.333.peg.4034). BLASTp analysis of the proteins indicated they are quite different in amino acid sequence (query coverage = 95%, *e*-value 9e-91, percent identity = 41.91%). Another bacterium, *Rhizorhabdus dicambivorans* Ndbn-20, was recently reported with two different gentisate 1,2-dioxygenases involved in degrading the herbicide dicamba (Li et al., 2020). One of these dioxygenases could cleave a 3-chlorogentisate, while the other could not. Further investigation is warranted into the function of the two gentisate 1,2-dioxygenases in *A. xylosoxidans* ADAF13 and their role in the potential degradation of halogenated gentisates. *Achromobacter xylosoxidans* ADAF13 was also the only isolate to have *nagLK*, whose proteins further catabolize gentisate to pyruvate and fumarate. Homogentisate degradation is involved in the catabolism of phenylalanine, tyrosine, and 3-hydrophenylacetate (Arias-Barrau et al., 2004) but may also be a downstream degradation intermediate of styrene via side-chain oxygenation (Baggi et al., 1983). Styrene is reasonably anticipated to be a human carcinogen and toxin, and bioremediation could be beneficial for environmental cleanup (ATSDR, 2010; Migliore et al., 2006; NTP, 2021). Homogentisate catabolism to fumarate and acetoacetate was likely in *A. xylosoxidans* ADAF13, *P. putida* CBF10-2, *P. stutzeri* ODKF13, and *S. maltophilia* CBF10-1. Two gene copies of homoprotocatechuate 2,3-dioxygenase were found in *P. putida* CBF10-2 and were not found in any other isolate. The homoprotocatechuate degradation pathway is also associated with aromatic amino acid catabolism (Cooper & Skinner, 1980; Díaz et al., 2001; Roper et al., 1993); however, to our knowledge, this pathway does not play a direct role in aromatic or xenobiotic compound degradation. Moreover, *P. putida* CBF10-2 was the only isolate likely to catabolize homoprotocatechuate to succinate. An expanded search into tyrosine metabolism

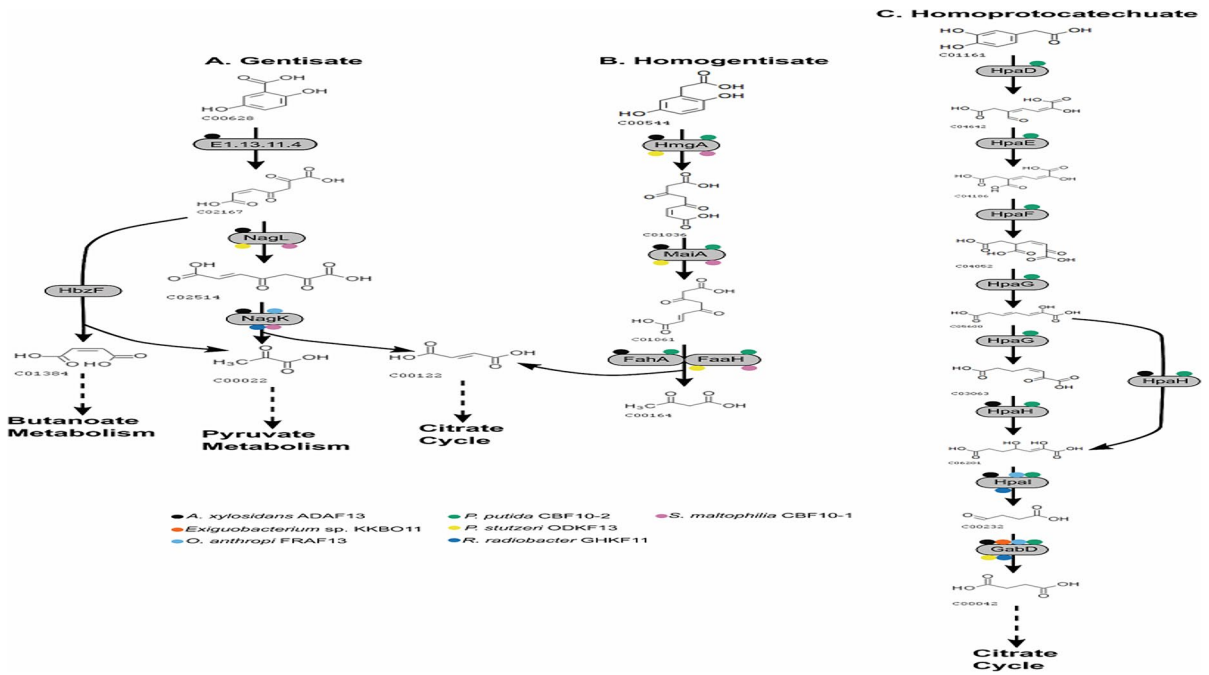


**Fig. 5** Gentisate, homogentisate, and homoprotocatechuate degradation pathways. This is adapted from KEGG map00350. The gentisate (**A**), homogentisate (**B**), and homoprotocatechuate (**C**) degradation pathways are shown. Dots present for each enzyme indicate the presence of that enzyme for a particular isolate. Each colored dot corresponds to a specific isolate. Dotted arrows indicate there are other intermediate steps not listed. KEGG compound numbers are indicated for each chemical structure

sub-pathways suggested additional aromatic ring cleavage capabilities in *A. xylosoxidans* ADAF13, *P. putida* CBF10-2, *P. stutzeri* ODKF13, and *S. maltophilia* CBF10-1.

## 2.2 Heavy Metal Resistance

We investigated the proteins associated with arsenic, cadmium, chromium, lead, and mercury resistance in our data set. Heavy metal contamination in soil and other environments is common worldwide (Xiang et al., 2021; Xiao et al., 2020; Zhou et al., 2020); therefore, any potential bioremediation strains should either be able to resist these hostile conditions or help remediate these environments. These metals are present as a result of both natural geochemical processes and as human-derived pollutants (Ahmad et al., 2021; Briffa et al., 2020; Tchounwou et al., 2012). Microbes have developed numerous systems to resist the presence of heavy metals found in soil (Gonzalez Henao & Ghneim-Herrera, 2021; Mathivanan et al., 2021; Nies, 2003; Pal et al., 2022). We used a combined approach with the annotations from RASTtk and the BacMet v2.0 Predicted database (Pal et al., 2014). The numbers of metal resistance proteins are graphed in Fig. 6. RASTtk found far more metal resistance proteins (273/295) than were identified using the BacMet v2.0 Predicted database (39/295). Both strategies overlapped on only 17 proteins. Arsenic resistance-related proteins were the most prevalent, with, on average, approximately 15 proteins per isolate. Many of these were ArsR proteins, the regulatory protein for *ars* operons (Ji & Silver, 1992; Wu & Rosen, 1991). On average, seven arsenic resistance proteins are predicted to be ArsR family transcriptional regulators. These regulators were not always associated with *ars* operons. The ArsR family of transcriptional regulators are metalloregulatory repressors that repress operons encoding genes that increase intracellular concentrations of heavy metals (e.g., arsenic import proteins) (Wu & Rosen, 1991). ArsR family regulators also control operons related to other heavy metals (e.g., zinc, nickel, cobalt, and cadmium) (Cavet et al., 2002; Yoon et al., 1991). Lead

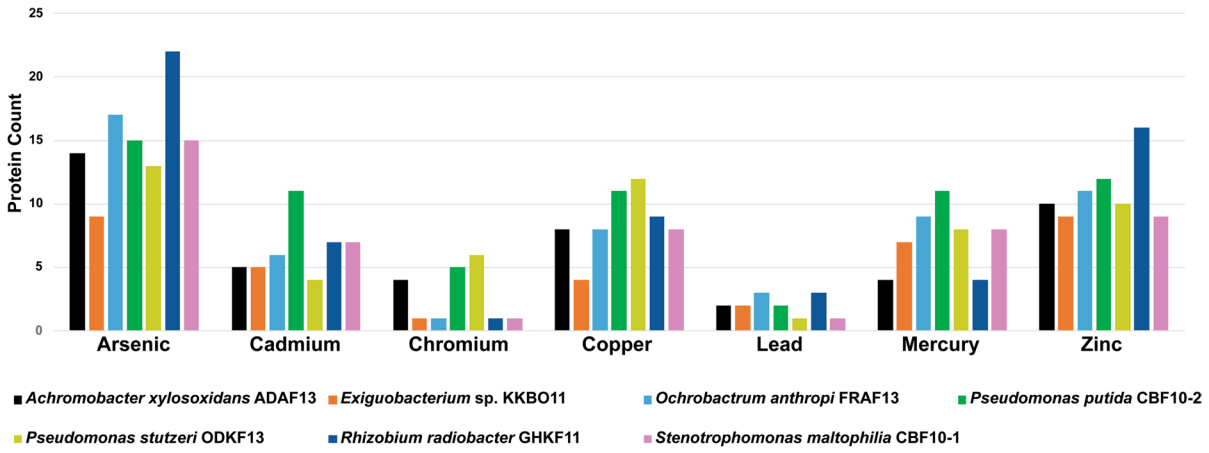


**Fig. 6** Heavy metal resistance protein counts. Heavy metal resistance proteins were enumerated for each isolate. These proteins were plotted as histograms. The proteins classified into each resistance type are found in the Zenodo repository in a Microsoft Excel workbook "Heavy_Metal_Resistance_Proteins"

resistance-related proteins were the least prevalent, with only about two lead resistance-related proteins present per isolate, with none of these being found in a *pbr* operon. We also found the presence of proteins conferring resistance to multiple heavy metals. These proteins included CzcD and FieF/YiiP, which confer resistance to cobalt, zinc, and cadmium (Anton et al., 1999; Wei & Fu, 2005), and proteins annotated as heavy metal translocating ATPases likely conferring lead, cadmium, zinc, mercury, and copper resistance.

We focused on better characterizing the arsenic resistance genes given that arsenic is a non-essential metalloid, nearly ubiquitous in soil (Masuda, 2018; Reimann et al., 2009), toxic to humans (Golub et al., 1998; Islam et al., 2015), and can contaminate the food chain through absorption into agricultural products (Ciminelli et al., 2017; Cubadda et al., 2010; Huq et al., 2006; Santra et al., 2013). Two different arsenic resistance operons were identified, the *ars* and *pst* operons. The *ars* operon is the most well-defined arsenic resistance system in bacteria (Ben Fekih et al., 2018). Each isolate had a single *ars* operon (Fig. 7), and the operons varied in complexity. *Exiguobacterium* sp. KKBO11 had the simplest *ars* operon with two genes, *arsR* coding for the transcriptional regulator and *arsB* coding for an arsenite (As[III]) permease (Ji & Silver, 1992; San Francisco et al., 1989; Wu & Rosen, 1991). *Pseudomonas stutzeri* ODKF13 had the most sophisticated *ars* operon. This operon consisted of *arsRHJ* with an *arsJ*-associated *gapdh* gene whose proteins collectively confer resistance to arsenate (As[V]) as well as organoarsenicals (Chen et al., 2015, 2016). The ODKF13 *ars* operon was also interrupted with two non-*ars* genes, a predicted tyrosine phosphatase, and a small multidrug resistance family-3 protein. Interestingly, upstream of the *arsR* regulator in this operon were an arsenite permease (*acr3*) (Sato & Kobayashi, 1998) and a putative monooxygenase with unknown function to arsenic resistance (*arsO*) (Wang et al., 2006). The *pst* operon is a common phosphate transport system in bacteria, which transports arsenate into the cell for detoxification and export through other mechanisms (e.g., Ars proteins) as arsenite (Rosenberg et al., 1977). All isolates had this phosphate transport system in a single *pstSCAB* operon; however, *Pseudomonas putida* CBF10-2 had 2 of these operons. Taken together, our data suggested that these isolates have diverse mechanisms for resisting arsenic in its different oxidation states (arsenite and arsenate) and, in the case of *P. stutzeri* ODKF13, potentially even organic arsenicals.

Significantly, Ars proteins do not degrade or remove arsenic in any of its forms from the soil (Yan et al., 2019). However, arsenic-resistant bacteria can either be engineered to sequester arsenic for removal from the environment through phytochelatins or metallothioneins that bind arsenate or arsenic (Li et al., 2015; Ma et al., 2011; Ruiz et al., 2011; Sauge-Merle et al., 2003). Heavy metal-resistant bacteria can also be used in phytoextraction (absorption of arsenic

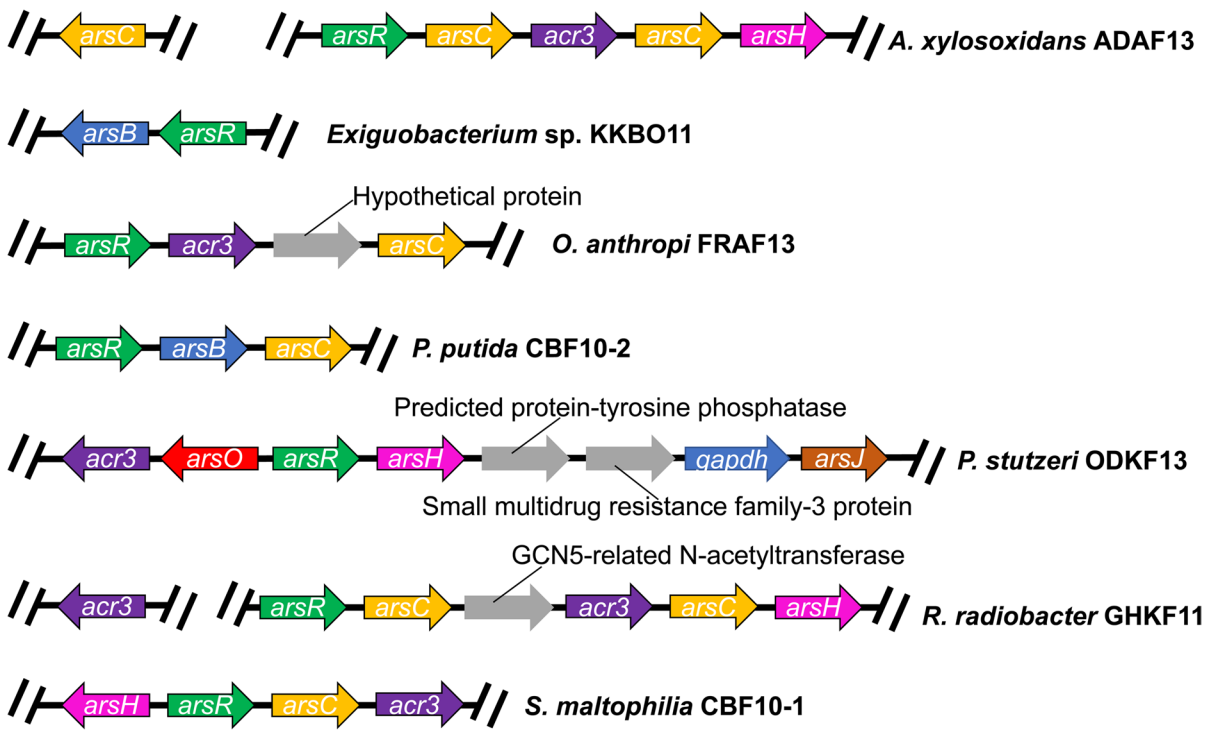


**Fig. 7** Arsenic resistance operons. Arsenic operons were drawn for each isolate. Each type of arsenic resistance gene is a similarly colored arrow between isolates. Genes without indication of conferring arsenic resistance are gray arrows with the gene annotation indicated. Double slashes indicate breaks in DNA between one group of genes and another

into plants for later removal), where these bacteria can make the phytoextraction process more efficient (Lampis et al., 2015; Mesa et al., 2017). Given the content of their *ars* operons and overall composition of meta resistance-related proteins, *A. xylosoxidans* ADAF13, *P. stutzeri* ODKF13, and *R. radiobacter* GHKF11 are likely good candidates to use in heavy metal-contaminated environments.

## 3 Limitations and Conclusions

This study investigated the genomes of *Achromobacter xylosoxidans* ADAF13, *Exiguobacterium* sp. KKBO11, *Ochrobactrum anthropi* FRAF13, *Pseudomonas putida* CBF10-2, *Pseudomonas stutzeri* ODKF13, *Rhizobium radiobacter* GHKF11, and *Stenotrophomonas maltophilia* CBF10-1 for the presence of genes and pathways that would indicate the isolates' potential as bioremediation tools. These isolates were previously shown to have low-level organophosphate degradation capabilities against either paraoxon, ethyl paraoxon, methyl parathion, or chlorpyrifos (Islam & Iyer, 2021; Iyer & Iken, 2013; Iyer et al., 2016, 2018). This study took a broad comparative genomics approach to investigate the aromatic and xenobiotic compound degradation pathways while assessing potential resistance to certain heavy metals.

This analysis was exclusively based on predictions from comparative genomics analysis and was not without limitations. The genomes analyzed were sequenced with short-read technology only and thus are not entirely assembled or "closed." This may have a bearing on accurate gene counts in instances of tandem repeats and in determining whether genes are chromosomal or plasmid-borne accurately. Future work using long-read platforms such as Oxford Nanopore Technologies or Pacific Biosciences can correct this. Functional annotation based on prediction software is also inherently limited based on the underlying data used to assign the annotations (Lobb et al., 2020; Salzberg, 2019). Non-model bacteria are at a disadvantage with these approaches since genes that

are either specific to particular genera or significantly diverged in sequence may require manual annotation (Lobb et al., 2020). Indeed, KEGG and BV-BRC functional annotation of genes in these genomes only accounted for approximately 60% of genes in each genome. Future work should empirically investigate these isolates' degradation and resistance capacities to help validate the findings from this work. Those investigations will offer exciting opportunities to discover new genes and pathways relevant to bioremediation.

Collectively, these isolates contained a variety of pathways for the degradation of aromatic and xenobiotic compounds as well as genes that likely confer resistance to a number of heavy metals. Based on the number and variety of intact pathways in this analysis, *A. xylosoxidans* ADAF13, *P. putida* CBF10-2, and *P. stutzeri* ODKF13 are the best candidates for further development through genome engineering or use in consortia for bioremediation purposes.

## 4 Methods

### 4.1 Genomes

The genomes used for analysis in this study were downloaded as FASTA files from GenBank: *A. xylosoxidans* ADAF13 LSMI00000000, *Exiguobacterium* sp. KKBO11 LUCU00000000, *O. anthropi* FRAF13 LSVB00000000, *P. putida* CBF10-2 LUCV00000000, *P. stutzeri* ODKF13 LSVE00000000, *R. radiobacter* GHKF11 LVFG00000000, and *S. maltophilia* CBF10-1 LTAC00000000.

### 4.2 Sequence Analysis

#### *4.2.1 Genome Annotation*

Genome contigs were annotated using the RASTtk tool as a part of BV-BRC's "Annotation" service (Brettin et al., 2015; Olson et al., 2023). The "Bacteria" annotation recipe and the appropriate taxonomic information for each respective genome were selected. The annotation outputs for each genome can be found in the Zenodo repository (see data availability for the DOI) in a folder for each genome (e.g., A.xylosoxidans_ADAF13_RASTtk_Annotation).

#### *4.2.2 KEGG Annotation and Pathway Reconstruction*

The predicted protein sequences for each genome were annotated using KEGG's KofamKOALA tool (ver. 2023–01-01, release 105.0) (Aramaki et al., 2020). The KEGG mapper input file generated for each genome was used with the KEGG Mapper Reconstruct tool (Kanehisa & Sato, 2020). The KEGG mapper input file was also used to enumerate the amount of KEGG genes across different pathways via Microsoft Excel based on the occurrence of specific KEGG orthology (ko) identifiers using the COUNTIF function. KEGG mapper input text files for each genome and the Microsoft Excel workbook with KEGG gene counts can be found in the Zenodo repository in the folders "KEGG_Annotation" and "KEGG_Gene_Counts," respectively. The pathway figures were generated using Inkscape (Inkscape, 2022).

#### *4.2.3 OhbAB Phylogenetic Analysis*

Phylogenetic analysis of OhbAB proteins in *A. xylosoxidans* ADAF13 was carried out using a subset of protein sequences used by Tsoi et al. (Tsoi et al., 1999) and the OhbAB proteins found in the *A. xylosoxidans* A8 isolate (Jencova et al., 2004). The amino acid sequences of the selected proteins were aligned using MAFFT (v7.490) (Katoh & Standley, 2013) using the –auto setting for the OhbA and OhbB alignments. Maximum likelihood phylogenetic trees were inferred using IQ-TREE2 using the -B 1000, -m MFP, and -T AUTO options (Hoang et al., 2018; Kalyaanamoorthy et al., 2017; Minh et al., 2020). The tree files were visualized using iTOL (v6.7.1) (Letunic & Bork, 2021), and the trees were annotated in Inkscape. The amino acid alignments and newick tree files can be found in the Zenodo repository in the "OhbAB_phylogenies" folder.

#### *4.2.4 Heavy Metal Resistance Protein Analysis*

Heavy metal resistance protein analysis was conducted using a combination of the BacMet Predicted database (v2.0, March112018) (Pal et al., 2014) and RASTtk annotations. The BacMet Predicted database was downloaded as a FASTA file of amino acid sequences and clustered using CD-HIT (v4.8.1) (Fu

et al., 2012) for sequences that were 100% identical (cd-hit -c 1 -i bacmet2_predicted_database.fasta -o bacmet_predicted_clustered_c1.fasta). The bacmet2_predicted_clustered_c1.fasta file was used to generate a protein database for BLAST using BLAST v2.12.0+(Camacho et al., 2009) (makeblastdb -dbtype prot -title bacmet2_predicted_c1 -in bacmet2_predicted_clustered_c1.fasta). The predicted proteins for each genome were concatenated into a single FASTA file (total_protein.fasta) and used as the BLASTp query against the bacmet2_predicted_c1 database (blastp -query total_protein.fasta -db bacmet2_predicted_clustered_c1.fasta -out total_protein_bacmet_predicted_c1.tsv -outfmt 6 -num_threads 4). We filtered the resulting BLASTp hit file for those hits with a sequence identity hit ≥ 85%, and in the case of identical hits for the same query sequence, we selected the hit with the highest bit score. This filtering was done using the following awk script: awk -F '\t' '{if ($3 > = 85 && (!($1 in max) || $3 > max[$1])) {row[$1] = $0; max[$1] = $3} else if ($3 > = 85 && $1 in row && $3 = = max[$1]) {row[$1] = row[$1]"\n"$0}} END {for (i in row) {print row[i]}}' total_protein_bacmet_predicted_c1.tsv > filt_85_total_protein_bacmet_predicted_c1.tsv.

Only BLASTp hits in filt_85_total_protein_bacmet_predicted.tsv with an *e*-value less than 1e-4 were reported. The files for each step of the BacMet2 BLASTp analysis can be found in the Zenodo repository under the folder "BacMet2_Analysis."

The RASTtk annotations were also analyzed to identify heavy metal resistance proteins. The GFF file for each genome generated by RASTtk was used to find proteins associated with metal resistance. The GFF files were searched for specific terms for each metal/metalloid using Microsoft Excel. For arsenic resistance proteins, the terms "arsenic," "arsenate," "arsenite," "as," "ars," and "pst" were used. For cadmium resistance proteins, "cadmium," "cad," "cz," and "cd" were used. For chromium resistance proteins, the terms "chromium," "chromate," "cr," and "chr" were used. For copper resistance proteins, the terms "copper" and "cu" were used. For lead resistance proteins, the terms "lead," "pb," and "pbr" were used. For mercury resistance proteins, the terms "mercury," "hg," and "mer" were used. For zinc resistance proteins, the terms "zinc" and "zn" were used. Spurious hits that were not related to resistance proteins were removed. Proteins related to heavy metal transport/translocation and annotated with a given protein name found in the BacMet2 Predicted database were retained. The list of these proteins can be found in the Zenodo repository in a Microsoft Excel workbook "Heavy_Metal_Resistance_Proteins." These proteins were counted for each genome and plotted as histograms using Microsoft Excel. The arsenic resistance operon figure was generated using Microsoft PowerPoint.

**Acknowledgements** The authors would like to the thank Brian N. Iken for his help drafting the manuscript.

**Author Contribution** RI was the principal investigator of this research, acquired the funding, submitted compiled sequence data to NCBI for annotation, and supplied the sequence data for analysis. ARK carried out the annotation and comparative genomics analyses and wrote the manuscript. Both authors read and approved the final text.

**Funding** This work was supported by the National Institute of Standards and Technology (NIST) (G110008 58106) and by the National Science Foundation (NSF) (award no. 1726968).

**Data and Code Availability** This work made use of genome sequences already available on NCBI (see text for GenBank accessions). Data that would be necessary to replicate this study (annotations and intermediate files generated by this study) are available in the Zenodo repository under https://doi.org/10.5281/zenodo.7804129. The code used in this study is indicated in the "Methods" section.

**Declarations**

**Conflict of Interest** ARK was employed as an independent contractor to conduct the analysis and write the manuscript. RI declares no financial interests.

## References

Agency for Toxic Substances and Disease Registry (ATSDR). (2010). *Toxicological profile for Styrene*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Ahmad, W., Alharthy, R. D., Zubair, M., Ahmed, M., Hameed, A., & Rafique, S. (2021). Toxic and heavy metals contamination assessment in soil and water to evaluate human health risk. *Scientific Reports, 11*(1), 17006.

Alengebawy, A., Abdelkhalek, S. T., Qureshi, S. R., & Wang, M.-Q. (2021). Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. *Toxics, 9*(3), 42.

Alves, N. J., Moore, M., Johnson, B. J., Dean, S. N., Turner, K. B., Medintz, I. L., & Walper, S. A. (2018).

Environmental decontamination of a chemical warfare simulant utilizing a membrane vesicle-encapsulated phosphotriesterase. *ACS Applied Materials & Interfaces, 10*(18), 15712–15719.

Anton, A., Große, C., Reißmann, J., Pribyl, T., & Nies, D. H. (1999). CzcD is a heavy metal ion transporter involved in regulation of heavy metal resistance in Ralstonia sp. strain CH34. *Journal of Bacteriology, 181*(22), 6876–6881.

Aramaki, T., Blanc-Mathieu, R., Endo, H., Ohkubo, K., Kanehisa, M., Goto, S., & Ogata, H. (2020). KofamKOALA: KEGG Ortholog assignment based on profile HMM and adaptive score threshold. *Bioinformatics, 36*(7), 2251–2252.

Arellano, L., Fernández, P., van Drooge, B. L., Rose, N. L., Nickus, U., Thies, H., . . . Grimalt, J. O. (2018). Drivers of atmospheric deposition of polycyclic aromatic hydrocarbons at European high-altitude sites. *Atmospheric Chemistry and Physics*, *18*(21), 16081–16097.

Arias-Barrau, E., Olivera, E. R., Luengo, J. M., Fernández, C., Galán, B., García, J. L., . . . Minambres, B. (2004). The homogentisate pathway: A central catabolic pathway involved in the degradation of L-phenylalanine, L-tyrosine, and 3-hydroxyphenylacetate in Pseudomonas putida. *Journal of Bacteriology, 186*(15), 5062–5077.

Baggi, G., Boga, M. M., Catelani, D., Galli, E., & Treccani, V. (1983). Styrene catabolism by a strain of Pseudomonas fluorescens. *Systematic and Applied Microbiology, 4*(1), 141–147.

Bala, S., Garg, D., Thirumalesh, B. V., Sharma, M., Sridhar, K., Inbaraj, B. S., & Tripathi, M. (2022). Recent strategies for bioremediation of emerging pollutants: A review for a green and sustainable environment. *Toxics, 10*(8), 484.

Ben Fekih, I., Zhang, C., Li, Y. P., Zhao, Y., Alwathnani, H. A., Saquib, Q., . . . Cervantes, C. (2018). Distribution of arsenic resistance genes in prokaryotes. *Frontiers in Microbiology*, *9*, 2473.

Boedeker, W., Watts, M., Clausing, P., & Marquez, E. (2020). The global distribution of acute unintentional pesticide poisoning: Estimations based on a systematic review. *BMC Public Health, 20*(1), 1–19.

Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., . . . Pusch, G. D. (2015). RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific Reports*, *5*(1), 8365.

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon, 6*(9), e04691.

Brimblecombe, P. (2005). The globalization of local air pollution. *Globalizations, 2*(3), 429–441.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology, 64*, 807–838.

Cai, A., Zhang, H., Zhao, Y., Wang, X., Wang, L., & Zhao, H. (2022). Quantitative source apportionment of heavy metals in atmospheric deposition of a typical heavily polluted city in Northern China: Comparison of PMF and UNMIX. *Frontiers in Environmental Science, 10*, 2356.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics, 10*, 421. https://doi.org/10.1186/1471-2105-10-421

Cao, B., Nagarajan, K., & Loh, K.-C. (2009). Biodegradation of aromatic compounds: Current status and opportunities for biomolecular approaches. *Applied Microbiology and Biotechnology, 85*, 207–228.

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications, 8*(3), 559–568.

Cavet, J. S., Meng, W., Pennella, M. A., Appelhoff, R. J., Giedroc, D. P., & Robinson, N. J. (2002). A nickel-cobalt-sensing ArsR-SmtB family repressor: Contributions of cytosol and effector binding sites to metal selectivity. *Journal of Biological Chemistry, 277*(41), 38441–38448.

Chaparro, J. M., Badri, D. V., Bakker, M. G., Sugiyama, A., Manter, D. K., & Vivanco, J. M. (2013). Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS ONE, 8*(2), e55731.

Chen, J., Bhattacharjee, H., & Rosen, B. P. (2015). ArsH is an organoarsenical oxidase that confers resistance to trivalent forms of the herbicide monosodium methylarsenate and the poultry growth promoter roxarsone. *Molecular Microbiology, 96*(5), 1042–1052.

Chen, J., Yoshinaga, M., Garbinski, L. D., & Rosen, B. P. (2016). Synergistic interaction of glyceraldehydes-3-phosphate dehydrogenase and ArsJ, a novel organoarsenical efflux permease, confers arsenate resistance. *Molecular Microbiology, 100*(6), 945–953.

Ciminelli, V. S., Gasparon, M., Ng, J. C., Silva, G. C., & Caldeira, C. L. (2017). Dietary arsenic exposure in Brazil: The contribution of rice and beans. *Chemosphere, 168*, 996–1003.

Cooper, R. A., & Skinner, M. A. (1980). Catabolism of 3-and 4-hydroxyphenylacetate by the 3, 4-dihydroxyphenylacetate pathway in Escherichia coli. *Journal of Bacteriology, 143*(1), 302–306.

Cubadda, F., Ciardullo, S., D'Amato, M., Raggi, A., Aureli, F., & Carcea, M. (2010). Arsenic contamination of the environment−food chain: A survey on wheat as a test plant to investigate phytoavailable arsenic in Italian agricultural soils and as a source of inorganic arsenic in the diet. *Journal of Agricultural and Food Chemistry, 58*(18), 10176–10183.

Díaz, E., Ferrández, A., Prieto, M. A. A., & García, J. L. (2001). Biodegradation of aromatic compounds by Escherichia coli. *Microbiology and Molecular Biology Reviews, 65*(4), 523–569.

Duan, L., Naidu, R., Liu, Y., Palanisami, T., Dong, Z., Mallavarapu, M., & Semple, K. T. (2015). Effect of ageing on benzo[a]pyrene extractability in contrasting soils. *Journal of Hazardous Materials, 296*, 175–184.

Fan, Y., Chen, S.-J., Li, Q.-Q., Zeng, Y., Yan, X., & Mai, B.-X. (2020). Uptake of halogenated organic compounds (HOCs) into peanut and corn during the whole life cycle grown in an agricultural field. *Environmental Pollution, 263*, 114400.

FAO and UNEP. (2021). *Global assessment of soil pollution: Summary for policymakers*. Rome: FAO and UNEP. http://www.fao.org/documents/card/en/c/cb4827en/

Flores-Félix, J. D., Menéndez, E., Peix, A., García-Fraile, P., & Velázquez, E. (2020). History and current taxonomic status of genus Agrobacterium. *Systematic and Applied Microbiology, 43*(1), 126046.

Fox, J. E., Gulledge, J., Engelhaupt, E., Burow, M. E., & McLachlan, J. A. (2007). Pesticides reduce symbiotic efficiency of nitrogen-fixing rhizobia and host plants. *Proceedings of the National Academy of Sciences, 104*(24), 10282–10287.

Fu, L., Niu, B., Zhu, Z., Wu, S., & Li, W. (2012). CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics, 28*(23), 3150–3152. https://doi.org/10.1093/bioinformatics/bts565

Fuenmayor, S. L., Wild, M., Boyes, A. L., & Williams, P. A. (1998). A gene cluster encoding steps in conversion of naphthalene to gentisate in Pseudomonas sp. strain U2. *Journal of Bacteriology, 180*(9), 2522–2530.

Gocht, T., Klemm, O., & Grathwohl, P. (2007). Long-term atmospheric bulk deposition of polycyclic aromatic hydrocarbons (PAHs) in rural areas of Southern Germany. *Atmospheric Environment, 41*(6), 1315–1327.

Golub, M. S., Macintosh, M. S., & Baumrind, N. (1998). Developmental and reproductive toxicity of inorganic arsenic: Animal studies and human concerns. *Journal of Toxicology and Environmental Health, Part B Critical Reviews, 1*(3), 199–237.

González Henao, S., & Ghneim-Herrera, T. (2021). Heavy metals in soils and the remediation potential of bacteria associated with the plant microbiome. *Frontiers in Environmental Science, 9*, 604216. https://doi.org/10.3389/fenvs.2021.604216

Goswami, P., Wickrama-Arachchige, A.U.-K., Yamada, M., Ohura, T., & Guruge, K. S. (2022). Presence of halogenated polycyclic aromatic hydrocarbons in milk powder and the consequence to human health. *Toxics, 10*(10), 621.

Gunstone, T., Cornelisse, T., Klein, K., Dubey, A., & Donley, N. (2021). Pesticides and soil invertebrates: A hazard assessment. *Frontiers in Environmental Science, 9*, 643847. https://doi.org/10.3389/fenvs.2021.643847

Haddad, S., Eby, D. M., & Neidle, E. L. (2001). Cloning and expression of the benzoate dioxygenase genes from Rhodococcus sp. strain 19070. *Applied and Environmental Microbiology, 67*(6), 2507–2514.

Haddad, M., Nassar, D., & Shtaya, M. (2023). Heavy metals accumulation in soil and uptake by barley (Hordeum vulgare) irrigated with contaminated water. *Scientific Reports, 13*(1), 4121. https://doi.org/10.1038/s41598-022-18014-0

Harwood, C. S., & Parales, R. E. (1996). The β-ketoadipate pathway and the biology of self-identity. *Annual Review of Microbiology, 50*(1), 553–590.

Heid, S. E., Walker, M. K., & Swanson, H. I. (2001). Correlation of cardiotoxicity mediated by halogenated aromatic hydrocarbons to aryl hydrocarbon receptor activation. *Toxicological Sciences, 61*(1), 187–196.

Hickey, W., & Sabat, G. (2001). Integration of matrix-assisted laser desorption ionization–time of flight mass spectrometry and molecular cloning for the identification and functional characterization of mobile ortho-halobenzoate oxygenase genes in Pseudomonas aeruginosa strain JB2. *Applied and Environment Microbiology, 67*(12), 5648–5655.

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution, 35*(2), 518–522. https://doi.org/10.1093/molbev/msx281

Huq, S. I., Joardar, J., Parvin, S., Correll, R., & Naidu, R. (2006). Arsenic contamination in food-chain: Transfer of arsenic into food materials through groundwater irrigation. *Journal of Health, Population, and Nutrition, 24*(3), 305.

*Inkscape*. (2022). https://inkscape.org

Islam, K., Wang, Q. Q., & Naranmandura, H. (2015). Molecular mechanisms of arsenic toxicity. In J. C. Fishbein & J. M. Heilman (Eds.), *Advances in Molecular Toxicology* (Vol. 9, pp. 77–107). Elsevier.

Islam, N., & Iyer, R. (2021). Functional analysis of chlorpyrifos biodegradation in agricultural soils augmented with a three-strain bacterial consortium. *Water, Air, & Soil Pollution, 232*, 1–11.

Iyer, R., & Damania, A. (2016a). Draft genome sequence of Exiguobacterium sp. KKBO11, isolated downstream of a wastewater treatment plant in Houston, Texas. *Genome Announcements, 4*(4), e00681-00616.

Iyer, R., & Damania, A. (2016c). Draft genome sequence of Pseudomonas putida CBF10-2, a soil isolate with bioremediation potential in agricultural and industrial environmental settings. *Genome Announcements, 4*(4), e00670-e616.

Iyer, R., & Damania, A. (2016d). Draft genome sequence of the broad-spectrum xenobiotic degrader Achromobacter xylosoxidans ADAF13. *Genome Announcements, 4*(2), e00203-00216.

Iyer, R., & Damania, A. (2016e). Draft genome sequence of Pseudomonas stutzeri ODKF13, isolated from farmland soil in Alvin, Texas. *Genome Announcements, 4*(2), e00293-e216.

Iyer, R., & Damania, A. (2016f). Draft genome sequence of Rhizobium sp. GHKF11, isolated from farmland soil in pecan grove, Texas. *Genome Announcements, 4*(4), e00682-00616.

Iyer, R., & Damania, A. (2016g). Draft genome sequence of Stenotrophomonas maltophilia CBF10-1, an organophosphate-degrading bacterium isolated from ranch soil in Fairchilds, Texas. *Genome Announcements, 4*(3), e00378-e316.

Iyer, R., Iken, B., & Leon, A. (2016). Characterization and comparison of putative Stenotrophomonas maltophilia methyl parathion hydrolases. *Bioremediation Journal, 20*(1), 71–79.

Iyer, R., Iken, B., Damania, A., & Krieger, J. (2018). Whole genome analysis of six organophosphate-degrading rhizobacteria reveals putative agrochemical degradation enzymes with broad substrate specificity. *Environmental Science and Pollution Research International, 25*(14), 13660–13675. https://doi.org/10.1007/s11356-018-1435-2

Iyer, R., & Damania, A. (2016b). Draft genome sequence of organophosphate-degrading Ochrobactrum anthropi FRAF13. *Genome Announcements, 4*(2). https://doi.org/10.1128/genomeA.00295-16

Iyer, R., & Iken, B. (2013). Identification of water-borne bacterial isolates for potential remediation of organophosphate contamination. *Advances in Biological Chemistry, 3*(1), 146–152.

Jencova, V., Strnad, H., Chodora, Z., Ulbrich, P., Hickey, W., & Paces, V. (2004). Chlorocatechol catabolic enzymes from Achromobacter xylosoxidans A8. *International Biodeterioration & Biodegradation, 54*(2–3), 175–181.

Jeschke, P. (2017). Latest generation of halogen-containing pesticides. *Pest Management Science, 73*(6), 1053–1066.

Jeschke, P. (2022). Manufacturing approaches of new halogenated agrochemicals. *European Journal of Organic Chemistry, 2022*(12), e202101513.

Ji, G., & Silver, S. (1992). Regulation and expression of the arsenic resistance operon from Staphylococcus aureus plasmid pI258. *Journal of Bacteriology, 174*(11), 3684–3694.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., & Madden, T. L. (2008). NCBI BLAST: A better web interface. *Nucleic Acids Research, 36*(Web Server issue), W5-9. https://doi.org/10.1093/nar/gkn201

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods, 14*(6), 587–589. https://doi.org/10.1038/nmeth.4285

Kanehisa, M., & Sato, Y. (2020). KEGG mapper for inferring cellular functions from protein sequences. *Protein Science, 29*(1), 28–35.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution, 30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Kaur, S., Kumar, V., Chawla, M., Cavallo, L., Poater, A., & Upadhyay, N. (2017). Pesticides curbing soil fertility: Effect of complexation of free metal ions. *Frontiers in Chemistry, 5*, 43.

Lampis, S., Santi, C., Ciurli, A., Andreolli, M., & Vallini, G. (2015). Promotion of arsenic phytoextraction efficiency in the fern Pteris vittata by the inoculation of As-resistant bacteria: A soil bioremediation perspective. *Frontiers in Plant Science, 6*, 80.

Lessner, D. J., Johnson, G. R., Parales, R. E., Spain, J. C., & Gibson, D. T. (2002). Molecular characterization and substrate specificity of nitrobenzene dioxygenase from Comamonas sp. strain JS765. *Applied and Environmental Microbiology, 68*(2), 634–641.

Letunic, I., & Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Research, 49*(W1), W293–W296.

Li, H., Cong, Y., Lin, J., & Chang, Y. (2015). Enhanced tolerance and accumulation of heavy metal ions by engineered Escherichia coli expressing Pyrus calleryana phytochelatin synthase. *Journal of Basic Microbiology, 55*(3), 398–405.

Li, J., Wang, C., Liang, W., & Liu, S. (2021). Rhizosphere microbiome: The emerging barrier in plant-pathogen interactions. *Frontiers in Microbiology, 12*, 772420.

Li, N., Peng, Q., Yao, L., He, Q., Qiu, J., Cao, H., . . . Hui, F. (2020). Roles of the gentisate 1,2-dioxygenases DsmD and GtdA in the catabolism of the herbicide dicamba in Rhizorhabdus dicambivorans Ndbn-20. *Journal of Agricultural and Food Chemistry*, *68*(35), 9287–9298.

Lobb, B., Tremblay, B. J.-M., Moreno-Hagelsieb, G., & Doxey, A. C. (2020). An assessment of genome annotation coverage across the bacterial tree of life. *Microbial Genomics, 6*(3), e000341.

Lu, C., Yang, Z., Liu, J., Liao, Q., Ling, W., Waigi, M. G., & Odinga, E. S. (2020). Chlorpyrifos inhibits nitrogen fixation in rice-vegetated soil containing Pseudomonas stutzeri A1501. *Chemosphere, 256*, 127098.

Luo, L., Zhang, S., & Ma, Y. (2008). Evaluation of impacts of soil fractions on phenanthrene sorption. *Chemosphere, 72*(6), 891–896.

Ma, Y., Lin, J., Zhang, C., Ren, Y., & Lin, J. (2011). Cd (II) and As (III) bioaccumulation by recombinant Escherichia coli expressing oligomeric human metallothioneins. *Journal of Hazardous Materials, 185*(2–3), 1605–1608.

Masuda, H. (2018). Arsenic cycling in the Earth's crust and hydrosphere: Interaction between naturally occurring arsenic and human activities. *Progress in Earth and Planetary Science, 5*(1), 1–11.

Mathivanan, K., Chandirika, J. U., Vinothkanna, A., Yin, H., Liu, X., & Meng, D. (2021). Bacterial adaptive strategies to cope with metal toxicity in the contaminated environment–A review. *Ecotoxicology and Environmental Safety, 226*, 112863.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H. M., . . . Raaijmakers, J. M. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science*, *332*(6033), 1097–1100. https://doi.org/10.1126/science.1203980

Mesa, V., Navazas, A., González-Gil, R., González, A., Weyens, N., Lauga, B., . . . Peláez, A. I. (2017). Use of endophytic and rhizosphere bacteria to improve phytoremediation of arsenic-contaminated industrial soils by autochthonous Betula celtiberica. *Applied and Environmental Microbiology*, *83*(8), e03411–03416.

Migliore, L., Naccarati, A., Coppedè, F., Bergamaschi, E., De Palma, G., Voho, A., . . . Norppa, H. (2006). Cytogenetic biomarkers, urinary metabolites and metabolic gene polymorphisms in workers exposed to styrene. *Pharmacogenetics and Genomics*, *16*(2), 87–99.

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution, 37*(5), 1530–1534. https://doi.org/10.1093/molbev/msaa015

Mohapatra, B., & Phale, P. S. (2021). Microbial degradation of naphthalene and substituted naphthalenes: Metabolic diversity and genomic insight for bioremediation. *Frontiers in Bioengineering and Biotechnology, 9*, 602445.

Nam, J. J., Thomas, G. O., Jaward, F. M., Steinnes, E., Gustafsson, O., & Jones, K. C. (2008). PAHs in background soils from Western Europe: Influence of atmospheric

deposition and soil organic matter. *Chemosphere, 70*(9), 1596–1602.

Narayanan, M., Ali, S. S., & El-Sheekh, M. (2023). A comprehensive review on the potential of microbial enzymes in multipollutant bioremediation: Mechanisms, challenges, and future prospects. *Journal of Environmental Management, 334*, 117532.

Neidle, E. L., Hartnett, C., Ornston, L. N., Bairoch, A., Rekik, M., & Harayama, S. (1991). Nucleotide sequences of the Acinetobacter calcoaceticus benABC genes for benzoate 1, 2-dioxygenase reveal evolutionary relationships among multicomponent oxygenases. *Journal of Bacteriology, 173*(17), 5385–5395.

Nies, D. H. (2003). Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiology Reviews, 27*(2–3), 313–339.

NTP. (2021). *Report on Carcinogens, Fifteenth Edition: Styrene*. Atlanta, GA.

Olson, R. D., Assaf, R., Brettin, T., Conrad, N., Cucinell, C., Davis, J. J., . . . Stevens, R. L. (2023). Introducing the bacterial and viral bioinformatics resource center (BV-BRC): A resource combining PATRIC, IRD and ViPR. *Nucleic Acids Research*, *51*(D1), D678-d689. https://doi.org/10.1093/nar/gkac1003

Pal, C., Bengtsson-Palme, J., Rensing, C., Kristiansson, E., & Larsson, D. J. (2014). BacMet: Antibacterial biocide and metal resistance genes database. *Nucleic Acids Research, 42*(D1), D737–D743.

Pal, A., Bhattacharjee, S., Saha, J., Sarkar, M., & Mandal, P. (2022). Bacterial survival strategies and responses under heavy metal stress: A comprehensive overview. *Critical Reviews in Microbiology, 48*(3), 327–355.

Panke-Buisse, K., Poole, A. C., Goodrich, J. K., Ley, R. E., & Kao-Kniffin, J. (2015). Selection on soil microbiomes reveals reproducible impacts on plant function. *The ISME Journal, 9*(4), 980–989. https://doi.org/10.1038/ismej.2014.196

Phale, P. S., Malhotra, H., & Shah, B. A. (2020). Degradation strategies and associated regulatory mechanisms/features for aromatic compound metabolism in bacteria. *Advances in Applied Microbiology, 112*, 1–65.

Pimentel, D. (2005). Environmental and economic costs of the application of pesticides primarily in the United States. *Environment, Development and Sustainability, 7*, 229–252.

Pimviriyakul, P., Wongnate, T., Tinikul, R., & Chaiyen, P. (2020). Microbial degradation of halogenated aromatics: Molecular mechanisms and enzymatic reactions. *Microbial Biotechnology, 13*(1), 67–86.

Power, A. L., Tennant, R. K., Jones, R. T., Tang, Y., Du, J., Worsley, A. T., & Love, J. (2018). Monitoring impacts of urbanisation and industrialisation on air quality in the Anthropocene using urban pond sediments. *Frontiers in Earth Science, 6*, 131.

Reimann, C., Matschullat, J., Birke, M., & Salminen, R. (2009). Arsenic distribution in the environment: The effects of scale. *Applied Geochemistry, 24*(7), 1147–1167.

Rengarajan, T., Rajendran, P., Nandakumar, N., Lokeshkumar, B., Rajendran, P., & Nishigaki, I. (2015). Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific Journal of Tropical Biomedicine, 5*(3), 182–189.

Roper, D., Fawcett, T., & Cooper, R. (1993). The Escherichia coli C homoprotocatechuate degradative operon: Hpc gene order, direction of transcription and control of expression. *Molecular and General Genetics MGG, 237*, 241–250.

Rosenberg, H., Gerdes, R., & Chegwidden, K. (1977). Two systems for the uptake of phosphate in Escherichia coli. *Journal of Bacteriology, 131*(2), 505–511.

Roslund, M. I., Grönroos, M., Rantalainen, A.-L., Jumpponen, A., Romantschuk, M., Parajuli, A., . . . Sinkkonen, A. (2018). Half-lives of PAHs and temporal microbiota changes in commonly used urban landscaping materials. *PeerJ, 6*, e4508.

Ruiz, O. N., Alvarez, D., Gonzalez-Ruiz, G., & Torres, C. (2011). Characterization of mercury bioremediation by transgenic bacteria expressing metallothionein and polyphosphate kinase. *BMC Biotechnology, 11*(1), 1–8.

Salkinoja-Salonen, M., Uotila, J., Jokela, J., Laine, M., & Saski, E. (1995). Organic halogens in the environment: Studies of environmental biodegradability and human exposure. *Environmental Health Perspectives, 103*(suppl 5), 63–69.

Salzberg, S. L. (2019). Next-generation genome annotation: We still struggle to get it right. *Genome Biology, 20*(1), 1–3.

San Francisco, M. J., Tisa, L. S., & Rosen, B. P. (1989). Identification of the membrane component of the anion pump encoded by the arsenical resistance operon of R-factor R773. *Molecular Microbiology, 3*(1), 15–21.

Santra, S. C., Samal, A. C., Bhattacharya, P., Banerjee, S., Biswas, A., & Majumdar, J. (2013). Arsenic in foodchain and community health risk: A study in Gangetic West Bengal. *Procedia Environmental Sciences, 18*, 2–13.

Sato, T., & Kobayashi, Y. (1998). The ars operon in the skin element of Bacillus subtilis confers resistance to arsenate and arsenite. *Journal of Bacteriology, 180*(7), 1655–1661.

Sauge-Merle, S., Cuiné, S., Carrier, P., Lecomte-Pradines, C., Luu, D.-T., & Peltier, G. (2003). Enhanced toxic metal accumulation in engineered bacterial cells expressing Arabidopsis thaliana phytochelatin synthase. *Applied and Environment Microbiology, 69*(1), 490–494.

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., . . . Parihar, R. D. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, *1*, 1–16.

Shin, D., Kim, Y., & Moon, H. S. (2018). Fate and toxicity of spilled chemicals in groundwater and soil environment I: Strong acids. *Environmental Health and Toxicology, 33*(4), e2018019.

Srivastava, V., Sarkar, A., Singh, S., Singh, P., De Araujo, A. S., & Singh, R. P. (2017). Agroecological responses of heavy metal pollution with special emphasis on soil health and plant performances. *Frontiers in Environmental Science, 5*, 64.

Swain, E. B., Engstrom, D. R., Brigham, M. E., Henning, T. A., & Brezonik, P. L. (1992). Increasing rates of atmospheric mercury deposition in midcontinental North America. *Science, 257*(5071), 784–787.

Swetha, V. P., & Phale, P. S. (2005). Metabolism of carbaryl via 1, 2-dihydroxynaphthalene by soil isolates Pseudomonas sp. strains C4, C5, and C6. *Applied and Environmental Microbiology, 71*(10), 5951–5956.

Tang, F. H. M., Lenzen, M., McBratney, A., & Maggi, F. (2021). Risk of pesticide pollution at the global scale. *Nature Geoscience, 14*(4), 206–210. https://doi.org/10.1038/s41561-021-00712-5

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. In A. Luch (Ed.), *Molecular, clinical and environmental toxicology: volume 3: Environmental toxicology* (1st ed., pp. 133–164). Basel: Springer.

Thevenon, F., Graham, N. D., Chiaradia, M., Arpagaus, P., Wildi, W., & Poté, J. (2011). Local to regional scale industrial heavy metal pollution recorded in sediments of large freshwater lakes in central Europe (lakes Geneva and Lucerne) over the last centuries. *Science of the Total Environment, 412*, 239–247.

Tilman, D. (1998). The greening of the green revolution. *Nature, 396*(6708), 211–212.

Tsoi, T. V., Plotnikova, E. G., Cole, J. R., Guerin, W. F., Bagdasarian, M., & Tiedje, J. M. (1999). Cloning, expression, and nucleotide sequence of the Pseudomonas aeruginosa 142 ohb genes coding for oxygenolytic ortho dehalogenation of halobenzoates. *Applied and Environment Microbiology, 65*(5), 2151–2162.

Vickers, A., Sloop, T. C., & Lucier, G. W. (1985). Mechanism of action of toxic halogenated aromatics. *Environmental Health Perspectives, 59*, 121–128.

Wang, L., Chen, S., Xiao, X., Huang, X., You, D., Zhou, X., & Deng, Z. (2006). arsRBOCT arsenic resistance system encoded by linear plasmid pHZ227 in Streptomyces sp. strain FR-008. *Applied and Environmental Microbiology, 72*(5), 3738–3742.

Wei, Y., & Fu, D. (2005). Selective metal binding to a membrane-embedded aspartate in the Escherichia coli metal transporter YiiP (FieF). *Journal of Biological Chemistry, 280*(40), 33716–33724. https://doi.org/10.1074/jbc.M506107200

Woodcock, B. A., Isaac, N. J. B., Bullock, J. M., Roy, D. B., Garthwaite, D. G., Crowe, A., & Pywell, R. F. (2016). Impacts of neonicotinoid use on long-term population changes in wild bees in England. *Nature Communications, 7*(1), 12459. https://doi.org/10.1038/ncomms12459

Wu, J., & Rosen, B. (1991). The ArsR protein is a trans-acting regulatory protein. *Molecular Microbiology, 5*(6), 1331–1336.

Xiang, M., Li, Y., Yang, J., Lei, K., Li, Y., Li, F., . . . Cao, Y. (2021). Heavy metal contamination risk assessment and correlation analysis of heavy metal contents in soil and crops. *Environmental Pollution*, *278*, 116911.

Xiao, X., Zhang, J., Wang, H., Han, X., Ma, J., Ma, Y., & Luan, H. (2020). Distribution and health risk assessment of potentially toxic elements in soils around coal industrial areas: A global meta-analysis. *Science of the Total Environment, 713*, 135292.

Xie, J., Tao, L., Wu, Q., Lei, S., & Lin, T. (2021). Environmental profile, distributions and potential sources of halogenated polycyclic aromatic hydrocarbons. *Journal of Hazardous Materials, 419*, 126164.

Yan, G., Chen, X., Du, S., Deng, Z., Wang, L., & Chen, S. (2019). Genetic mechanisms of arsenic detoxification and metabolism in bacteria. *Current Genetics, 65*, 329–338.

Yang, Y., Tao, S., Zhang, N., Zhang, D., & Li, X. (2010). The effect of soil organic matter on fate of polycyclic aromatic hydrocarbons in soil: A microcosm study. *Environmental Pollution, 158*(5), 1768–1774.

Yoon, K., Misra, T., & Silver, S. (1991). Regulation of the cadA cadmium resistance determinant of Staphylococcus aureus plasmid pI258. *Journal of Bacteriology, 173*(23), 7643–7649.

Young, J., Kuykendall, L., Martinez-Romero, E., Kerr, A., & Sawada, H. (2001). A revision of Rhizobium Frank 1889, with an emended description of the genus, and the inclusion of all species of Agrobacterium Conn 1942 and Allorhizobium undicola de Lajudie et al. 1998 as new combinations: Rhizobium radiobacter, R. rhizogenes, R. rubi, R. undicola and R. vitis. *International Journal of Systematic and Evolutionary Microbiology, 51*(1), 89–103.

Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., Da Rocha, U. N., Shi, S., . . . Bowen, B. P. (2018). Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology*, *3*(4), 470–480.

Zhang, C., Wu, D., & Ren, H. (2020). Bioremediation of oil contaminated soil using agricultural wastes via microbial consortium. *Scientific Reports, 10*(1), 9188. https://doi.org/10.1038/s41598-020-66169-5

Zhang, S., Yao, H., Lu, Y., Yu, X., Wang, J., Sun, S., . . . Zhang, D. (2017). Uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by maize from soil irrigated with wastewater. *Scientific Reports*, *7*(1), 12165.

Zhou, Q., Yang, N., Li, Y., Ren, B., Ding, X., Bian, H., & Yao, X. (2020). Total concentrations and sources of heavy metal pollution in global river and lake water bodies from 1972 to 2017. *Global Ecology and Conservation, 22*, e00925.

Zhu, S., Wang, H., Jiang, W., Yang, Z., Zhou, Y., He, J., . . . Hong, Q. (2019). Genome analysis of carbaryl-degrading strain Pseudomonas putida XWY-1. *Current Microbiology, 76*, 927–929.