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Halogenases and dehalogenases: mechanisms, engineering, and applications

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Halogenated organic compounds (HOCs) are essential building blocks in pharmaceuticals, agrochemicals, and advanced materials. However, their conventional chemical synthesis often relies on hazardous reagents and generates significant environmental waste. Harnessing nature's solutions, halogenases and dehalogenases offer selective, eco-friendly alternatives for the biosynthesis and degradation of HOCs. Halogenases, including electrophilic (*e.g.*, haloperoxidases, flavin-dependent), radical (α-ketoglutarate-dependent), and nucleophilic (*S*-adenosylmethionine (SAM)-dependent) types, facilitate precise C–X bond formation under mild conditions. Recent advances in protein engineering, such as the modification of tryptophan halogenases and fluorinases, have greatly expanded the repertoire and efficiency of biocatalytic halogenation, enabling the production of new-to-nature compounds for synthetic biology applications. In parallel, dehalogenases, ranging from reductive to hydrolytic and oxidative enzymes, play crucial roles in removing halogens from persistent pollutants, thereby supporting effective bioremediation and environmental detoxification. This review summarizes recent progress in enzyme discovery, mechanistic elucidation, protein engineering, and applied synthetic biology, with a focus on the integration of halogenases and dehalogenases into scalable platforms for both biosynthetic and remediation. Continued research aimed at improving enzyme stability, substrate scope, and operational robustness will be critical to fully realizing the industrial and environmental potential of these versatile biocatalysts.

1. Introduction

HOCs are carbon-containing molecules with halogen substituents (Cl, Br, I, and F). To date, approximately 8400 naturally occurring organohalogen compounds have been identified,[1] including well-known bioactive molecules such as the antitumor agent rebeccamycin, and the antibiotics chloramphenicol and vancomycin. The vast majority are either chlorinated

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($\sim$50%) or brominated ($\sim$45%), while iodinated ($\sim$100 compounds) and fluorinated (only $\sim$5 compounds) metabolites remain comparatively rare.[2,3] Because halogen substituents can fine-tune molecular bioactivity, bioavailability, and metabolic stability,[4,5] HOCs are widely used across industrial, agrochemical, pharmaceutical, and materials sectors (Fig. 1). Notable examples include thiamethoxam, a novel neonicotinoid that has rapidly become one of the world's best-selling insecticides;[6] Nirmatrelvir (trade name Paxlovid®), a fluorinated antiviral recently approved for the treatment of COVID-19;[7] imidacloprid, a historically important insecticide; and loratadine (Claritin®), a popular chlorinated antihistamine.[5,8] Reflecting their significance, halogenated compounds accounted for 96% of new herbicides, fungicides, insecticides, acaricides, and nematicides introduced to the market since 2010.[5] Currently, most HOCs are synthesized through chemical reactions such as halogen addition, hydrohalogenation, nucleophilic substitution, and electrophilic aromatic substitution.[5] However, these traditional halogenation processes often require toxic and hazardous reagents, harsh conditions, and environmentally damaging solvents.[9] They frequently generate complex and harmful byproducts, lack regioselectivity,[2] and are generally expensive, time-consuming, and inefficient. Moreover, the widespread use and persistence of HOCs have led to significant environmental risks due to their bioaccumulative properties and toxicity, which can adversely impact plants, animals, and humans. Given these challenges, developing eco-friendly strategies for both HOC synthesis and remediation has become an urgent priority. In this review, we summarize recent advances in halogenase and dehalogenase discovery, mechanistic understanding, protein engineering, and their growing potential for sustainable halogenation and dehalogenation in synthetic biology.

Jing Luo

Jing Luo completed her postdoctoral training at Shanghai Jiao Tong University, where her research centered on developing compact and high-efficiency, TnpB-based, STAGE genome editing tools for Streptomyces *to advance synthetic biology and strain engineering. She obtained her PhD in Toxicology and Environmental Biology from the University of Strasbourg, focusing on microbial pathway for methyl halide degradation. Her research interests include genome editing, microbial metabolic engineering, enzymatic mechanisms of halogenated compound degradation, natural product biosynthesis, and environmental biotechnology.*

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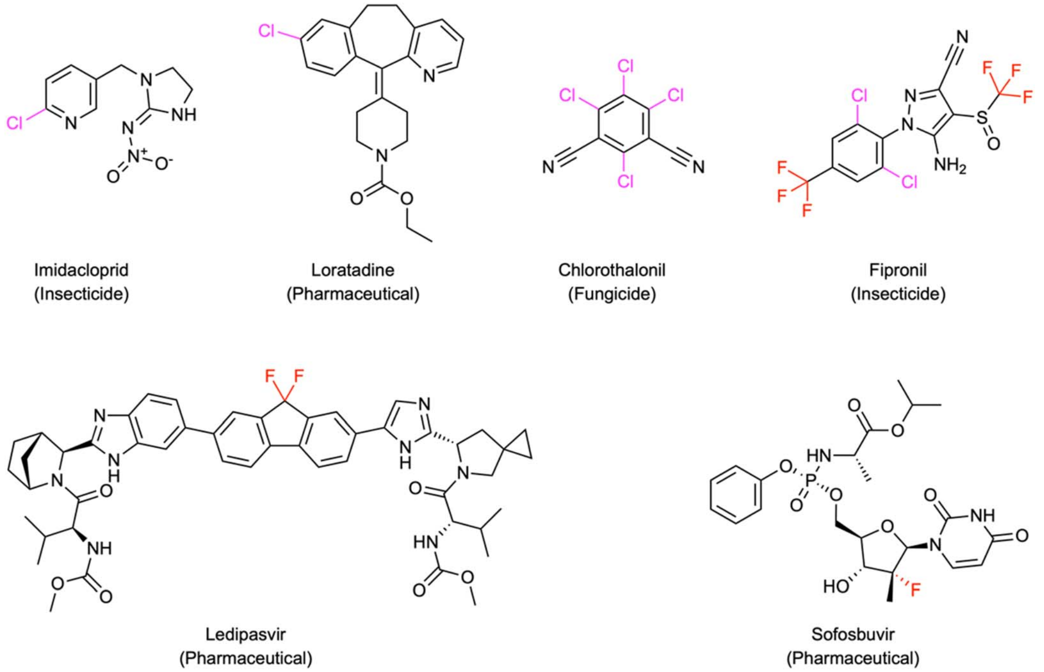

Fig. 1 Representative commercial organohalides. Structures of widely used halogenated compounds across agrochemical and pharmaceutical sectors: Imidacloprid (insecticide), Loratadine (pharmaceutical), Chlorothalonil (fungicide), Fipronil (insecticide), Ledipasvir (pharmaceutical), and Sofosbuvir (pharmaceutical). Halogen atoms are color-coded by type: chlorine (Cl) in bright magenta, fluorine (F) in red.

2. HOCs synthesis

A diverse set of enzymes mediate halogenation in nature, incorporating halogens into a wide array of molecular scaffolds.[10] The introduction of halogens often has a profound impact on biological activity of natural products, including many antifungal agents. As a result, the structural and mechanistic diversity of halogenases has been the subject of extensive study over the last few decades, halogenases are broadly categorized into three classes: electrophilic, radical, and nucleophilic types[2] (Fig. 2).

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2.1 Electrophilic halogenation

Electrophilic halogenases represent the largest group of halogenating enzymes and are responsible for generating electrophilic halogen species *via* halogen oxidation.[5] These enzymes predominantly catalyze the formation of C–Cl, C–Br, and C–I bonds through electrophilic pathways. Depending on their catalytic mechanisms and required cofactors, electrophilic halogenases can be further subdivided into two major categories.

2.1.1 Haloperoxidases. Haloperoxidases are a diverse group of oxidative enzymes that catalyze the halogenation of organic molecules using hydrogen peroxide (H_2O_2) and halide ions (Cl^-, Br^-, or I^-) to generate hypohalous acids (HOX)[5] (Fig. 2A and Table 1). As one of the earliest and most studied biological halogenating agents, haloperoxidases enabled halogen incorporation into organic scaffolds long before the discovery of flavin-dependent halogenases.[2,13] Their catalytic versatility, selectivity, and ability to function under mild aqueous conditions make them valuable for synthesizing halogenated compounds with pharmaceutical, agricultural, and industrial applications.

Haloperoxidases are generally divided into vanadium-dependent (VHPOs) and heme-dependent types, based on their cofactors and mechanisms. VHPOs utilize a vanadate cofactor to form peroxovanadate intermediates that oxidize halides into HOX for subsequent halogenation of electron-rich substrates. These enzymes are widely distributed across marine

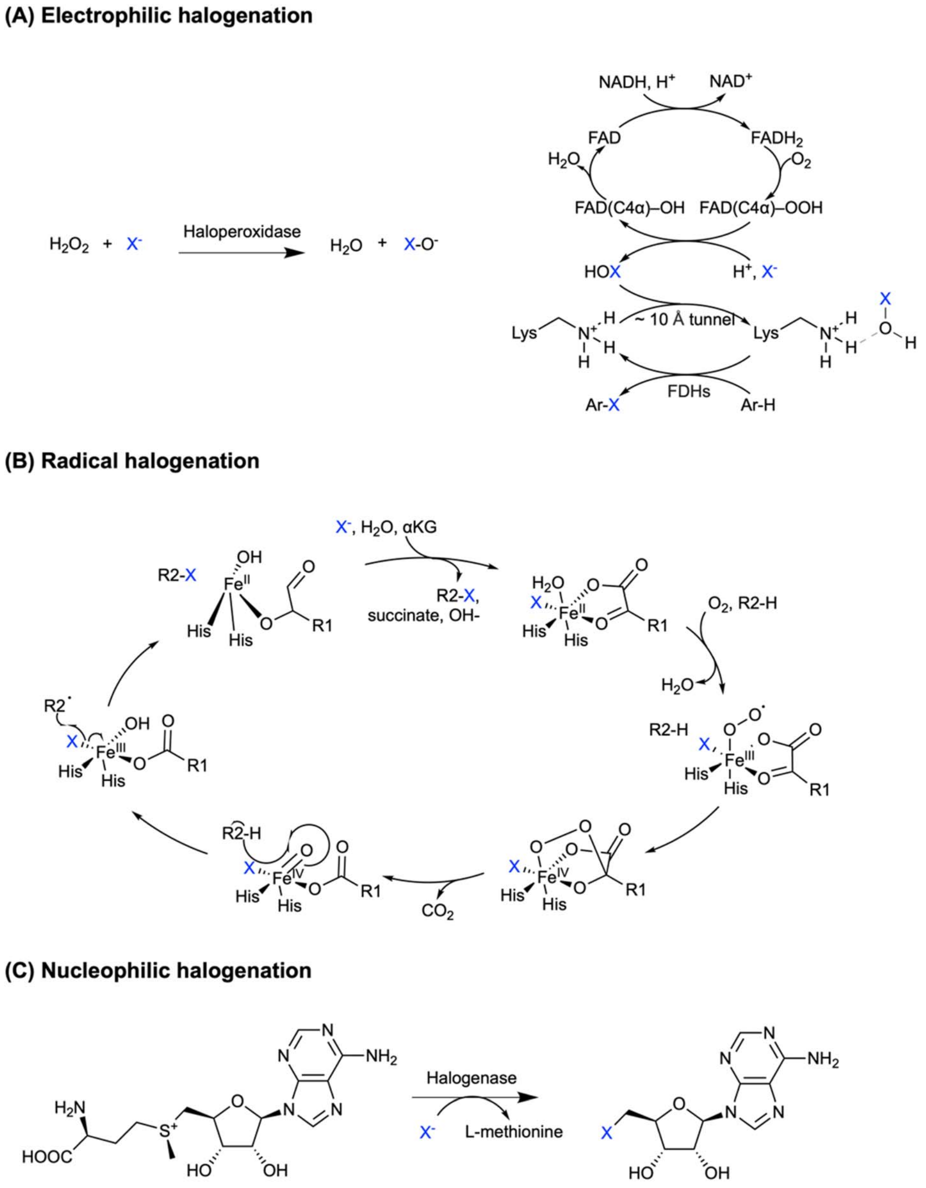

Fig. 2 Representative mechanisms of halogenation reactions classified by reaction pathway. (A) Electrophilic halogenation *via* haloperoxidases or FDHs (adapted from ref. 11), where halide ions are oxidized to electrophilic species (*e.g.*, HOX) that react with electron-rich substrates. (B) Radical halogenation by non-heme Fe(II)/αKG-dependent enzymes, involving C–H bond activation and radical recombination with halides. Adapted from ref. 12. (C) Nucleophilic halogenation by SAM-dependent halogenases, where halide ions directly displace leaving groups in nucleophilic substitution reactions.

macroalgae, bacteria, fungi, and cyanobacteria.[14–16,19,20] Structural studies, such as those on NapH1 and NapH3 from *Streptomyces*, have revealed their role in regio- and stereoselective halogenation during the biosynthesis of meroterpenoids like napyradiomycin[16] (Fig. 3A). Notably, key active-site residues and substrate-binding pockets shape both reactivity and selectivity, and post-translational modifications (for example, histidine phosphorylation in NapH3) can shift enzymatic function toward isomerization. In marine macroalgae, VHPOs are central to iodine metabolism and contribute to ecological processes such as halogenated metabolite production and embryo development. Genomic surveys in brown algae (*e.g.*, *Saccharina japonica*) have revealed massive expansion and diversification of VHPO genes, likely driven by horizontal gene transfer and adaptive evolution, reflecting their role in environmental adaptation and chemical defense.[17] Recently, haloperoxidases, particularly VHPOs, have gained significant attention for their ability to catalyze selective, late-stage halogenation of complex molecules. They have been used for regioselective bromination of indoles,[21] site-selective halogenation of flavonoids,[22] and functionalization of quorum sensing molecules such as alkyl quinolones.[23] VHPOs also enable chemoenzymatic heterocycle formation,[24] including the oxidative dimerization of thioamides to thiadiazoles, N-halogenation of benzamidines to 1,2,4-

Table 1 Representative haloperoxidases recently discovered or characterized

Enzyme	Source organism	Catalytic function/role	References
AmVHPO	Marine cyanobacterium	Halogenation of bromophycolide-related compounds	14
HwvCPO	*Hortaea werneckii* UBOCC-A-208029	Catalyzes HOX generation and bromination reactions	15
NapH1/NapH3	*Streptomyces* sp. CNQ-525	Regio- and stereoselective halogenation in napyradiomycin biosynthesis	16
SjavHPOs	*Saccharina japonica* (brown algae)	Iodine metabolism; ecological halogenated metabolite biosynthesis	17
VPO-RR	*Rhodoplanes roseus*	Halogenation	18

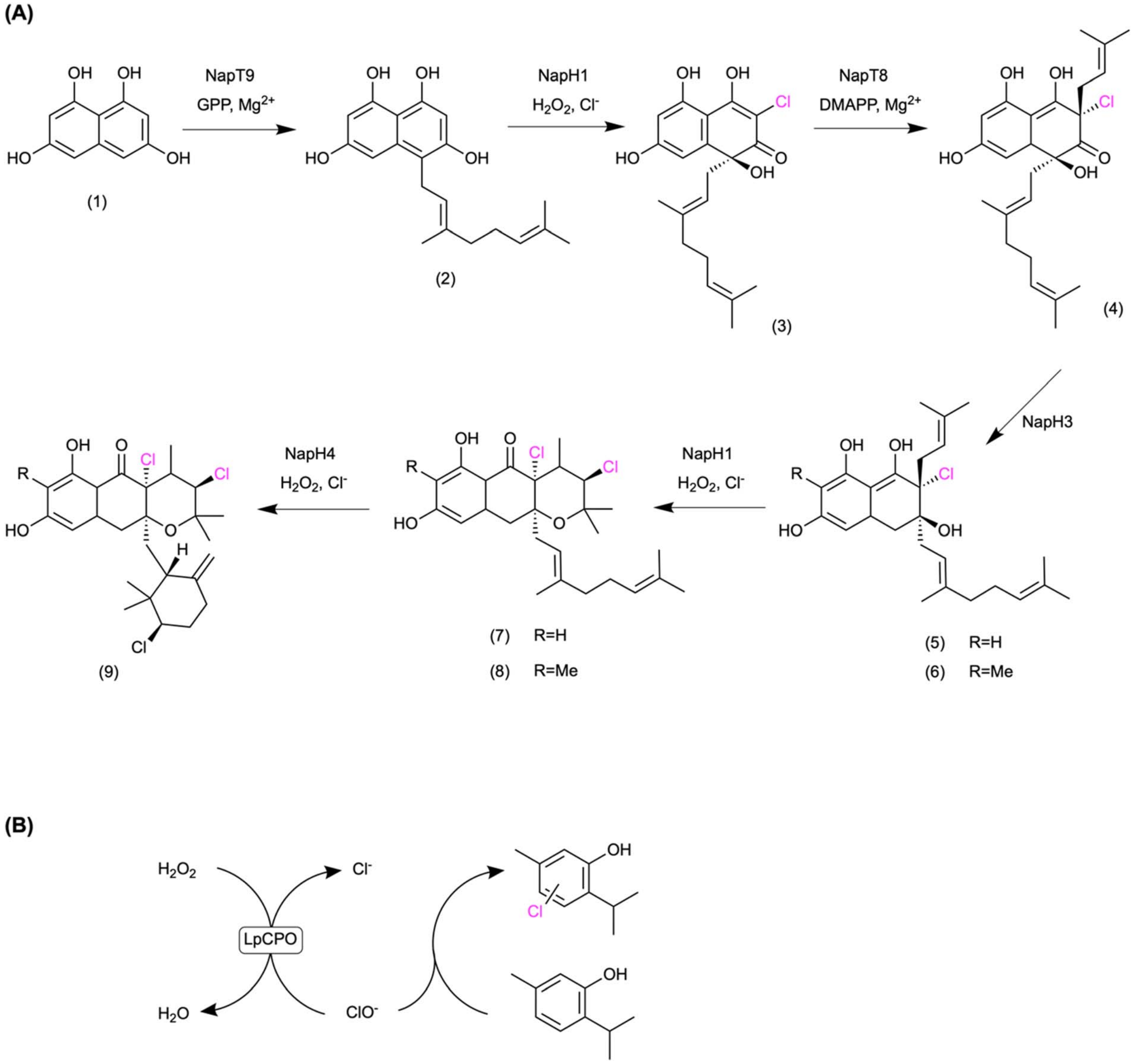

Fig. 3 Vanadium- and heme-dependent haloperoxidases as versatile biocatalysts in natural product biosynthesis and selective halogenation. (A) The biosynthesis of napyradiomycin B1, highlighting VHPO-catalyzed transformations. Starting from 1,3,6,8-tetrahydroxynaphthalene (**1**), the biosynthesis of napyradiomycin B1 (**9**) proceeds through six enzyme-catalyzed steps, four of which are mediated by vanadium-dependent haloperoxidases (VHPOs). NapH1 catalyzes two key halofunctionalization reactions: an asymmetric arene chlorination (**2** → **3**) and a regio- and stereoselective alkene halocyclization (**5** → **7** or **6** → **8**). NapH4 facilitates a chloronium-induced diastereoselective cyclization of the geranyl side chain to yield the final product. In contrast, NapH3 catalyzes a vanadium-independent α-hydroxyketone rearrangement. The pathway integrates aromatic and terpene moieties derived from THN and geranyl diphosphate (GPP), respectively, showcasing the functional versatility of VHPO enzymes in meroterpenoid biosynthesis. Adapted from ref. 16. (B) Chloroperoxidase from *Leptoxyphium fumago* (LfCPO)-mediated *in situ* generation of hypochlorous acid (HOCl) for the electrophilic chlorination of thymol. Upon reaction with H_2O_2 and Cl^-, CPO catalyzes the formation of HOCl, which selectively chlorinates the aromatic ring of thymol to yield mono- or dichlorinated products under mild aqueous conditions. Adapted from ref. 42.

oxadiazoles,[25] and even diazo compound formation from hydrazones *via* N–N bond oxidation,[26] all with high chemo-, regio-, and stereoselectivity. Protein engineering and structure-guided mutagenesis have further broadened the substrate range and selectivity of haloperoxidases. For instance, point mutations in cyanobacterial VHPOs (*e.g.*, AmVHPO R425S) have enabled switching between bromination and chlorination, with redesigned substrate-binding tunnels improving catalytic efficiency.[14] Combined computational and experimental approaches, including molecular dynamics simulations, density functional theory (DFT), and AlphaFold modeling, have provided valuable insights for rational enzyme design.

Heme-dependent haloperoxidases catalyze similar HOX-generating reactions *via* a heme-iron center[22,27] (Fig. 3B). Compared with VHPOs, they are less extensively studied, and most known examples were discovered more than two decades ago in fungi, algae, plants, mammals, and certain bacteria.[28] Among them, chloroperoxidase (CPO) from *Leptoxyphium fumago* (formerly *Caldariomyces fumago*) remains the best-characterized representative. CPO exhibits mechanistic features of both peroxidases and cytochrome P450s, proceeding through compound I intermediates.[27] Beyond halogenations, CPO catalyzes diverse oxidative reactions, including dehydrogenation, hydrogen peroxide disproportionation, and oxygen insertion,[28] enabling applications such as oxidative halocyclization of allenes and chemoenzymatic cascades with palladium catalysts.[29,30] Quantum chemical calculations and molecular dynamics simulations further indicate that substrate orientation relative to the heme plane governs enantio- and regioselectivity.[31] Mechanistic analyses show that halide oxidation follows conserved Glu/His-mediated proton transfers,[27] favoring iodination over bromination and chlorination due to redox potential differences, whereas fluorination is inaccessible under natural conditions.[28]

Despite their catalytic versatility, CPO and related enzymes are prone to peroxide-driven inactivation, mainly through heme destruction and oxidation of redox-sensitive residues.[32,33] This fragility highlights the need for stabilization strategies,

Table 2 Overview of characterized flavin-dependent halogenases (FDHs), including their source organisms, substrate classes, halogenation positions, enzymatic variants (A: acting on free substrates; B: carrier protein-tethered), and key biochemical or engineering features

Enzyme name	Source organism	Substrate	Halogenation site	Variant	References
AbeH	Soil-DNA library	L-Tryptophan	C5	A	45 and 46
AoiQ (FDH domain: AoiQ-FDH)	*Aspergillus oryzae* RIB40	1,3-Diketone substrate; aromatic scaffold	*Gem*-α,α-dichlorination of enolizable sp^3 carbon; chlorination at aromatic ring (C7/C9 positions)	A	47 and 48
AetF	*Aetokthonos hydrillicola*	L-Tryptophan (native); electron-deficient arenes, heterocycles, alkenes, alkynes (non-native)	Native: C5, C7 of tryptophan; non-native: selective halogenation of arenes, heteroarenes, alkenes, alkynes	A	49–51
Bmp5	*Pseudoalteromonas* spp.	4-Hydroxybenzoic acid	C2, C4	A	52
BorH	Uncultured soil actinomycetes	L-Tryptophan	C6	A	53 and 54
FasV	*Streptomyces* sp.	Polyketide naphthacemycin	Five consecutive chlorinations on multiple aromatic ring positions (multi-site halogenation)	A	55
GsfI	*Penicillium aethiopicum*	Dechlorogriseofulvin	C13	A	56
JamD	*Moorea producens*	Terminal alkyne-containing lipopeptides	Chlorination at terminal alkyne C–H bond	A	57
KtzQ/KtzR	*Kutzneria* sp. 744	L-Tryptophan	C7, C6	A	58
MalA	*Malbranchea aurantiaca*	Premalbrancheamide	C8, C9	A	59
PltM	*Pseudomonas fluorescens* Pf-5	Phloroglucinol	C2, C6	A	60 and 61
PrnA	*Pseudomonas fluorescens*	L-Tryptophan	C7	A	62 and 63
PrnC	*Pseudomonas fluorescens*	Monodechloroaminopyrrolnitrin	C3	A	64 and 65
PyrH	*Streptomyces rugosporus* LL-42D005	L-Tryptophan	C5	A	66 and 67
Rdc2	*Pochonia chlamydosporia*	Aromatic macrolactones	Aromatic ring	A	68
RebH	*Lechevalieria aerocolonigenes*	L-Tryptophan	C7	A	69
SatH	*Streptomyces albus*	L-Tryptophan	C6	A	70
SttH	*Streptomyces toxytricini* NRRL 15443	L-Tryptophan	C6	A	71
Tar14	*Saccharomonospora* sp. CNQ-490	L-Tryptophan	C6	A	72
Thal	*Streptomyces albogriseolus*	L-Tryptophan	C6	A	43
Th-Hal	*Streptomyces violaceusniger*	L-Tryptophan	C6	A	73
VirX1	Cyanophage	Indole derivatives (*e.g.*, L-tryptophan)	C5 or C7	A	4
Bmp2	Marine *Pseudoalteromonas*	Pyrrole	C2, C3, C4, C5	B	52
Mpy16	*Streptomyces* sp. CNQ-418	Pyrrole	C4, C5	B	74 and 75
PltA	*Pseudomonas fluorescens*	Pyrrole	C4, C5	B	3 and 76

including protein engineering, directed evolution, and computational redesign, which have already begun to improve catalytic performance and oxidative resistance.[34,35] Biomimetic catalysts, such as non-heme iron complexes and bismuth molybdate-based nanozymes, have also been developed to mimic CPO-like reactivity under mild conditions.[36] Although natural heme-HPOs have not been shown to catalyze stereoselective fluorination, advances in non-heme metalloenzymes and photoenzymatic systems demonstrate that strategies such as substrate orientation control, second-sphere redesign, and distal-site mutations could, in principle, enable such reactivity.[37–41]

While haloperoxidases remain underused in industrial biocatalysis, ongoing discovery of new haloperoxidases from extremophilic fungi, red and brown algae, and diverse bacterial continues to expand the available repertoire. The unique advantages of VHPOs for selective C–X bond formation, combined with the mechanistic versatility of heme-dependent haloperoxidases, highlight their potential. Future efforts aimed at improving stability, broadening substrate range, and integrating computational design will be crucial for translating haloperoxidases into scalable applications across biocatalysis, green chemistry, and synthetic biology.

2.1.2 Flavin dependent halogenases (FDHs). Flavin-dependent halogenases (FDHs) are a well-characterized class of electrophilic halogenases that catalyze regioselective halogenation of electron-rich substrates, particularly aromatic rings, under mild conditions (Table 2 and Fig. 4). These enzymes use reduced flavin adenine dinucleotide ($FADH_2$) and oxygen to generate a hypohalous acid (HOX)-like intermediate within a tightly controlled active site (Fig. 2A). HOX migrates through a conserved ~10 Å tunnel and selectively halogenates the target molecule, while active-site residues ensure regioselectivity and suppress undesired reactions such as hydroxylation or HOX leakage.[43] Recent genomic surveys of thousands of bacterial genomes have uncovered conserved FDH motifs (FAD-binding, halide-binding, substrate recognition) and revealed extensive misannotation in public databases.[44] These findings have provided a framework for discovering and predicting new FDH activities. Structural and mechanistic studies show FDHs use $FADH^-$-generated HOX delivered through a 10 Å tunnel, and targeted engineering (*e.g.*, Thal-V82I) has reduced HOX escape and improved both thermostability and substrate scope.[43]

FDHs are generally divided into two mechanistic variants: variant A, acts on free substrates, and variant B, requires substrates tethered to carrier proteins (ACPs). Among variant A enzymes, tryptophan halogenases (PrnA, RebH, Thal) are key examples, selectively chlorinating L-tryptophan at distinct positions to initiate the biosynthesis of natural products like pyrrolnitrin, rebeccamycin, and related alkaloids[62,69,77] (Fig. 4). Other tryptophan halogenases (*e.g.*, KtzR, SatH, Tar14, AfnX, AbeH, PyrH, and BorH) display distinct regioselectivities across different strains.[46,58] In contrast, variant B FDHs drive multistep halogenation in complex polyketide and nonribosomal peptide biosynthetic pathways, as seen in PltA, Bmp2, and Mpy16.[3,52,75] PrnC, although classified as variant A, uniquely chlorinates a pyrrolic intermediate, demonstrating FDH mechanistic diversity.[65] Recent work has significantly broadened the substrate scope of FDHs. Enzymes like RadH can halogenate quinolines, benzothiophenes, and other non-tryptophan scaffolds, as revealed by structural studies at high resolution.[78] Fungal FDHs such as Rdc2 and GsfI act on phenolic compounds, supporting biosynthesis of radicicol and griseofulvin, respectively.[56,79]

Functionally diverse FDHs have emerged from both natural evolution and protein engineering. Some (*e.g.*, Bmp5 and PltM) accept a broader range of halides (including I^-), and rare enzymes like VirX1 from cyanophages can catalyze iodination.[4] Innovations such as FDR-XanH fusions and engineered photoenzymes (*e.g.*, PyrH-W281F) have enhanced catalytic efficiency and enabled light-driven halogenation.[67,80] Crystal structures of AetF in various states further clarify the structural basis for selectivity and reactivity.[49] Continued advances in protein engineering have minimized HOX leakage, improved substrate scope, and enhanced overall enzyme performance. Examples include rational mutagenesis of tunnel residues, cofactor fusion, and photochemical regeneration systems. Current challenges include boosting turnover numbers, precisely predicting regioselectivity, and developing robust, scalable FDH platforms. Efforts in directed evolution, metagenomic mining, and computational design promise to further advance FDH applications in pharmaceutical and agrochemical synthesis.

2.2 Radical halogenation

Radical halogenases, mainly represented by non-heme Fe(II) and α-ketoglutarate (αKG)-dependent enzymes, catalyze the selective halogenation of unactivated aliphatic C–H bonds *via* a hydrogen atom transfer (HAT) mechanism (Fig. 2B). Unlike electrophilic or nucleophilic halogenases that act on activated centers, these enzymes generate carbon-centered radical through the oxidative decarboxylation of αKG, producing a high-valent Fe(IV)=O intermediate that activates the substrate C–H bond.[83–85] The resulting radical then reacts with halide (typically chloride or bromide) to form a new C–X bond, a process that directly competes with hydroxylation. The geometry of the active site and specific amino acid residues are crucial for steering the reaction toward halogenation over hydroxylation[85,86] (Table 3). Early studies of this enzyme family include SyrB2, which catalyzes γ-chlorination of L-threonine bound to the carrier protein SyrB1 during syringomycin E biosynthesis in *Pseudomonas syringae*.[87–90] Similar chemistries are observed in CytC3 from *Streptomyces* (acting on L-Aba or L-valine conjugated to CytC2)[91] (Fig. 5A) and BarB1/BarB2, which trichlorinate L-leucine in the marine cyanobacterium *Lyngbya majuscule* during barbamide biosynthesis[92] (Fig. 5B). In parallel, CurA and JamE, halogenase domains embedded within the biosynthetic machineries for curacin A and jamaicamide A, catalyze the chlorination of (*S*)-3-hydroxy-3-methylglutaryl-ACP (Fig. 5E). The resulting intermediates are subsequently dehydrated and cyclized by associated dehydratase and reductase domains, yielding cyclopropane or alkene structures within these complex natural products.[83,93–95] Other notable examples include KthP and KtzD in kutzneride pathways (Fig. 5C and D)

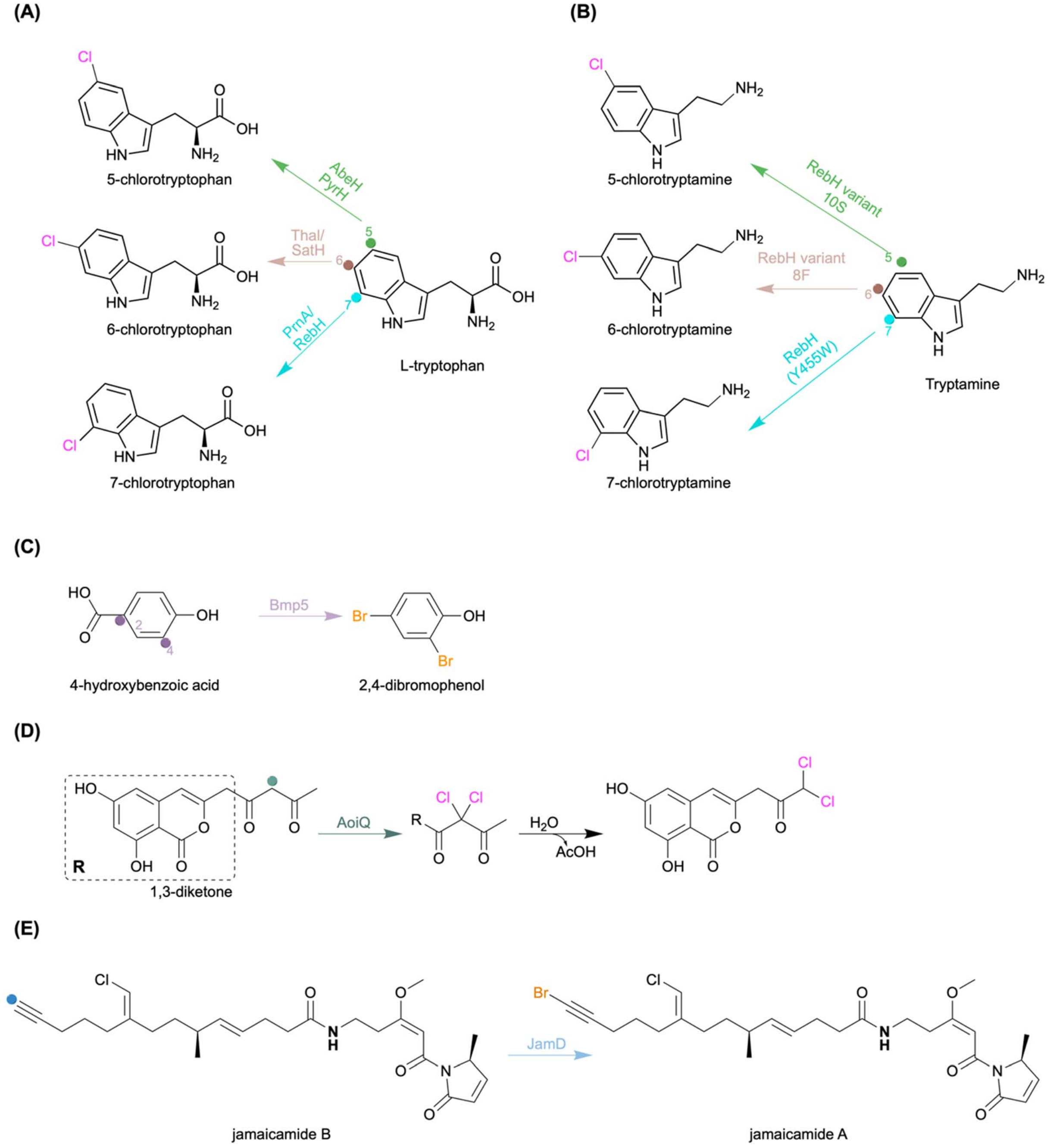

Fig. 4 The reactions catalyzed by representative flavin-dependent halogenases (FDHs). (A) FDHs catalyzing regioselective halogenation of tryptophan: C5-halogenation by PyrH (adapted from ref. 66 and 67) and AbeH (adapted from ref. 46); C6-halogenation by Thal (adapted from ref. 43) and SatH (adapted from ref. 70); and C7-halogenation by wild-type PrnA (adapted from ref. 62 and 63) and RebH (adapted from ref. 69). (B) Engineered RebH variant 10S (adapted from ref. 81), 8F (adapted from ref. 81), and Y445W (adapted from ref. 82) catalyzing at the C5, C6, C7 positions, respectively. (C) Bmp5 catalyzing halogenation of 4-hydroxybenzoic acid to yield 2,4-dibromophenol (adapted from ref. 52). (D) AoiQ-mediated *gem*-α,α-dichlorination of 1,3-diketone substrates at enolizable sp^3 carbons (adapted from ref. 48). (E) JamD catalyzing terminal alkyne halogenation in lipopeptide scaffolds (adapted from ref. 57). Halogenation positions are indicated by color-coded solid circles.

and CmaB in coronatine biosynthesis, where halogenation steps enable further cyclopropane or alkene formation within complex natural product scaffolds.[83,96–101] Recent discoveries have revealed radical halogenases that do not require carriers. For example, WelO5, involved in welwitindolinone biosynthesis, catalyzes stereoselective chlorination of complex indole alkaloids and can also use bromide as a halogen source[102,103] (Fig. 5F). AmbO5, a close homolog, demonstrates even broader substrate scope, efficiently halogenating multiple hapalindole derivatives at specific positions.[104] These enzymes utilize solvent-exposed active sites and on C-terminal domains to recognize diverse substrates, as shown by domain-swapping

Table 3 Representative radical halogenases involved in selective halogenation of unactivated aliphatic C–H bonds in natural product biosynthesis

Enzyme	Source organism	Reaction/substrate	Halogenation site	Carrier protein required	References
CmaB	*P. syringae*	L-Allo-isoleucine tethered to CmaD	γ-C	Yes	101
BarB1/BarB2	*Lyngbya majuscule*	L-Leucine tethered to BarA	C5 methyl group (trichlorination)	Yes	92
CurA/JamE (Hal domain)	*Lyngbya majuscule*	3-Hydroxy-3-methylglutaryl-ACP	γ-C	Yes	83 and 93–95
CytC3	*Streptomyces* sp.	L-Aba or L-valine tethered to CytC2	γ-C (methyl group)	Yes	91
HtcB	*Lyngbya majuscule*	Fatty acyl chain tethered to internal ACP domain	Aliphatic C–H	Yes	83, 96 and 97
KthP	Kutzneride-producing actinomycete	Piperazyl moiety tethered to KtzC	C5 (piperazate ring)	Yes	83 and 98
KtzD	Kutzneride-producing actinomycete	Isoleucyl residue	γ-C (alkyl)	Yes	99 and 100
SyrB2	*P. syringae*	L-Threonine tethered to SyrB1	γ-C (methyl group)	Yes	87–90
AdeV	*Actinomadura* sp. ATCC 39365	dAMP, ddAMP, or dIMP	C2′	No	106
AmbO5	*Fischerella ambigua*	Free hapalindole-type alkaloids	C13	No	104
BesD	Heterologously expressed	Small organic molecules	Aliphatic C	No	85
McDAH	*Menispermum canadense* (Menispermaceae plants)	Meroterpenoid/polycyclic	C10, C12	No	107
SaDAH	*Sinomenium acutum* (Menispermaceae plants)	Indole-terpenoid-like	C11	No	107
WelO5	*Hapalosiphon welwitschii*	Free indole alkaloids	Bridgehead aliphatic C (*e.g.*, C13)	No	102 and 103
WelO5*	*Hapalosiphon welwitschii*	Macrocyclic indole alkaloids	Aliphatic C	No	105
Wi-WelO15	*Westiella intricata* HT-29-1	Non-natural hapalindole analogs (with ketone instead of isonitrile)	Unactivated C(sp^3)–H bond	No	108

experiments.[83,104] Mechanistic studies have shown that protein structure is key to selectivity in radical halogenation. For instance, in BesD, QM/MM simulations indicate that equatorial Fe(III)–OH intermediates formed after HAT lower steric hindrance and favor halide rebound, stabilized by a conserved asparagine.[85] In WelO5, Ser189 is crucial for orienting the Fe(IV)=O intermediate to suppress undesired hydroxylation and promote halogenation selectivity.[102]

The synthetic potential of radical halogenases has inspired active protein engineering. Directed evolution, structure-guided mutagenesis, and machine learning-aided design have generated variants with expanded or altered halogenation profiles, including WelO5* mutants with improved activity on bulky substrates[84,104,105] (Fig. 5B). Beyond iron enzymes, copper-dependent radical halogenases such as ApnU, capable of iterative halogenation and thiocyanation, are expanding the catalytic landscape of this family.[111] Despite rapid advances, challenges remain in the discovery of new enzymes, fine control of reactivity, and expansion of substrate flexibility. Future priorities include large-scale genome mining for novel candidates,[83] detailed mechanistic studies to refine halogenation selectivity,[85] and the development of high-throughput engineering platforms.[84] Together these efforts are expected to further expand the use of radical halogenases for selective C–H functionalization in the synthesis of complex pharmaceuticals and other valuable compounds.[84,105]

2.3 Nucleophilic halogenation

Compared to other types of halogenases, nucleophilic halogenases represent a rare and mechanistically distinct category, uniquely catalyzing halogenation *via* nucleophilic attack of halide ions (X^-) on SAM (Fig. 2C). The electrophilic sulfonium center of SAM allows substitution at the 5′-carbon, enabling halide transfer reactions under mild conditions.[2,112] All known nucleophilic halogenases are SAM-dependent. The best-characterized example is the fluorinase FlA from *Streptomyces cattleya*, which catalyzes the formation of a C–F bond, a chemically challenging transformation, during the biosynthesis of fluoroacetate and 4-fluoro-L-threonine. FlA converts SAM and fluoride ion into 5′-fluoro-5′-deoxyadenosine (5′-FDA) and L-methionine.[113,114] A homologous enzyme, SalL, also from *S. cattleya*, produces 5′-chloro-5′-deoxyadenosine (5′-ClDA) from SAM and chloride, and can also accept bromide and iodide, but not fluoride[115] (Fig. 6), indicating both the specificity and limitations of this enzyme family. In addition, SAM-dependent halide methyltransferases such as AtHOL1 (from *Arabidopsis thaliana*) and Pthtmt (from *Phaeodactylum tricornutum*) generate volatile methyl halides (*e.g.*, CH_3Cl, CH_3Br, CH_3I) using SAM as a methyl donor.[116,117] Despite their unique mechanistic and synthetic promise, only 11 SAM-dependent fluorinases and 3 chlorinases have been identified to date, suggesting that this enzyme class remains largely unexplored.[118] Recent protein engineering has shown that SAM-dependent enzymes can be repurposed for halogenation: for example, a study on hydroxide

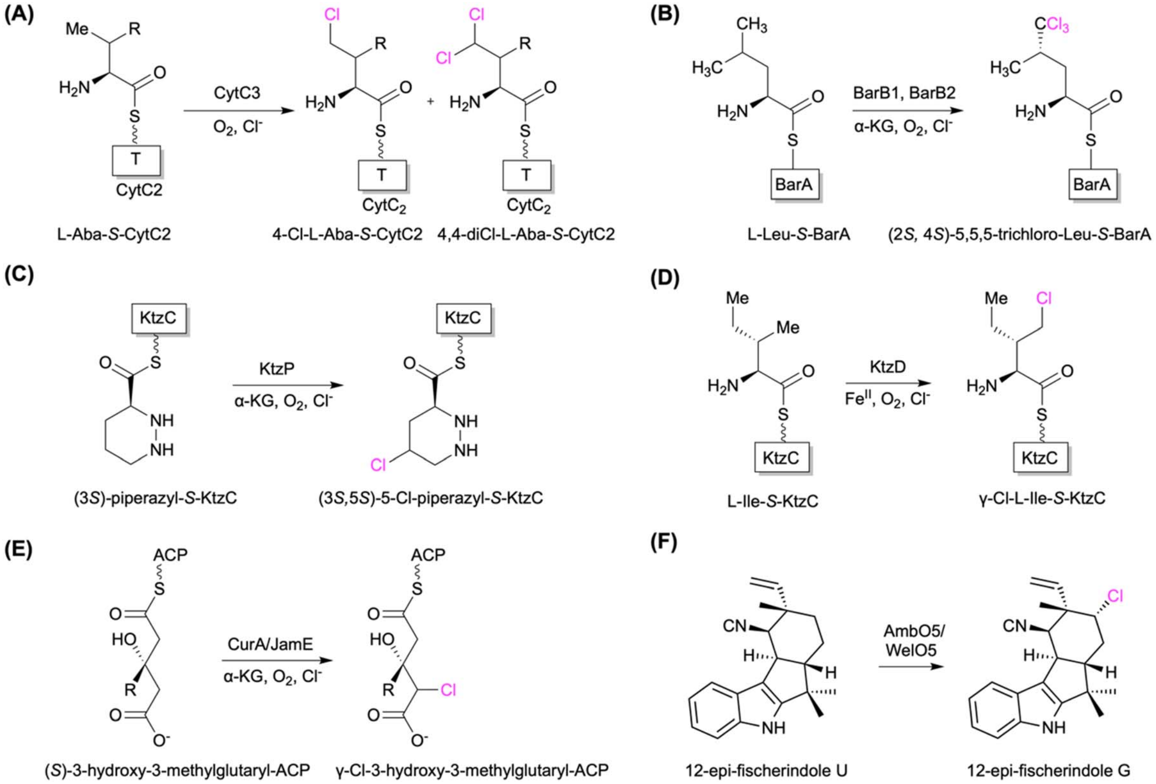

Fig. 5 Representative reactions catalyzed by radical halogenases. (A) CytC3-mediated chlorination of L-Aba-*S*-CytC2 to yield mono- and di-chlorinated L-Aba (adapted from ref. 91 and 109). (B) BarB1/BarB2-catalyzed trichlorination of L-Leu-*S*-BarA (adapted from ref. 92). (C) KtzP catalyzed chlorination of a piperazyl moiety tethered to a carrier protein during kutzneride 2 biosynthesis (adapted from ref. 98). (D) KtzD mediated chlorination of KtzC-tethered L-Ile (adapted from ref. 99 and 100). (E) CurA or JamE-catalyzed chlorination of (*S*)-3-hydroxy-3-methylglutaryl-ACP (adapted from ref. 83). (F) AmbO5/WelO5-catalyzed late-stage chlorination of the indole alkaloid 12-*epi*-fischerindole U to yield 12-*epi*-fischerindole G (adapted from ref. 102 and 110). Halogenation sites are indicated in magenta, and carrier protein domains are shown in boxes.

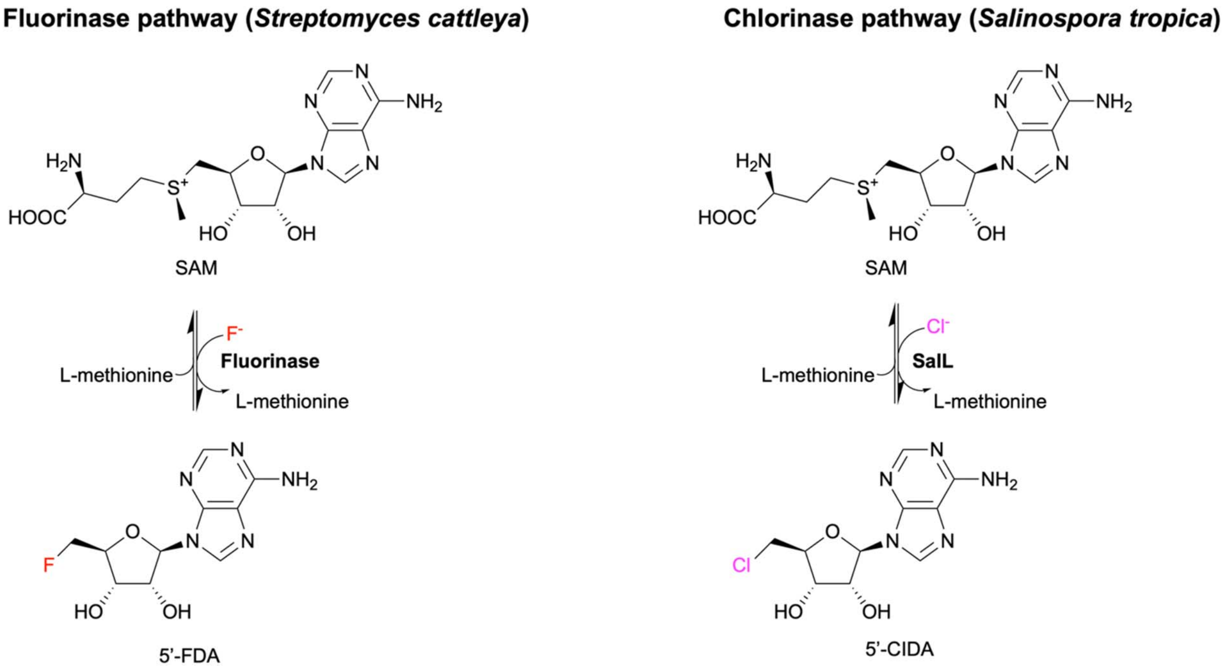

Fig. 6 Representative reaction catalyzed by nucleophilic halogenases. Fluorinase and SalL identified from *Streptomyces cattleya* and *Salinispora tropica*, respectively, catalyze the formation of a C–F or C–Cl bond *via* nucleophilic attack of a halide ion (fluoride or chloride) on the 5′-carbon of *S*-adenosyl-L-methionine (SAM), with concomitant displacement of L-methionine. These enzymes are involved in the biosynthesis of fluoroacetate by *S. cattleya* and the chlorinated unit of salinosporamide A by *S. tropica*. Adapted from ref. 113, 115 and 119.

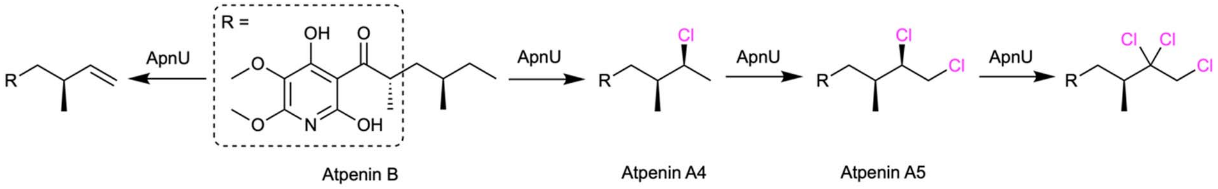

Fig. 7 Halogenation of Atpenin B catalyzed by the copper-dependent halogenase ApnU. Adapted from ref. 111.

adenosyltransferase (HATase) from *Thermotoga maritima* demonstrated that, although the native enzyme uses water as a nucleophile, it can be rationally redesigned to accept halide ions.[118] Through specific mutations (*e.g.*, W8L and V71T), HATase variants capable of using Cl^-, Br^-, and I^- as substrates were generated. Notably, the M4 variant retained thermostability and outperformed SalL in iodination at 80 °C. QM/MM simulations confirmed that these mutations optimized halide binding geometry, improving nucleophilic attack on SAM.

2.4 Emerging and noncanonical halogenases

In addition to classical flavin-dependent, radical, and haloperoxidase-type halogenases, recent research has identified a growing number of noncanonical halogenating enzymes with unique structures and mechanisms. A notable example is ApnU, a copper-dependent halogenase discovered in actinobacteria. ApnU catalyzes iterative halogenation and thiocyanation during the biosynthesis of secondary metabolites containing alkyl thiocyanates and polyhalogenated motifs[111] (Fig. 7). Unlike haloperoxidases or radical halogenases, ApnU does not require hydrogen peroxide, does not generate hypohalous acids (HOX), and does not rely on a high-valent iron–oxo intermediates. Instead, it uses a mononuclear copper center, though the detailed mechanism remains to be fully elucidated. ApnU is remarkable for its substrate flexibility, catalyzing C–H halogenation at multiple aliphatic positions and enabling enzymatic installation of thiocyanate (–SCN) groups, a transformation rarely seen in halogen biochemistry. Bioinformatic analyses indicate that ApnU homologs are widespread across actinobacteria and other soil-dwelling microbes, hinting at a broader, yet untapped, diversity of non-heme, non-iron halogenating enzymes in nature. From a synthetic perspective, ApnU and its relatives could expand biocatalytic strategies for C–H activation under mild, redox-neutral conditions.

3. HOCs degradation

The extensive use of HOCs in agriculture and industry has resulted in their global distribution across air, soils, water, and even human tissues. Such pervasive presence poses significant environmental and health risks. For example, some halogenated volatile organic compounds (VOCs) contribute to global warming and ozone depletion,[120] while others are associated with diseases ranging from oral infections to reproductive toxicity and organ damage.[121] Fortunately, many bacteria can degrade HOCs by utilizing them as sole carbon and energy sources, playing a crucial role in natural attenuation of these contaminants. These microbes underpin bioremediation strategies that are safe, cost-effective, and environmentally friendly. Dehalogenases produced by HOC-degrading microorganisms mediate diverse dehalogenation reactions, including reductive, oxidative, hydrolytic, and dehydrohalogenation pathways (Fig. 8), enabling the breakdown and detoxification of a broad spectrum of halogenated pollutants.[121]

3.1 Reductive dehalogenation

Organohalide-respiring bacteria (OHRB) are anaerobes that conserve energy by coupling the reductive dehalogenation of organohalides to grow, using these compounds as terminal electron acceptors.[123,124] This metabolism underpins cost-effective and environmentally friendly strategies for the removal of highly halogenated pollutants, including polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), chloroethenes, chlorophenols, and chlorobenzenes, especially in anoxic environments.[125] Since the discovery of *Desulfomonile tiedjei* DCB-1 in 1984,[126] OHRB have been identified across diverse Gram-positive and Gram-negative phyla. Although many reductive dehalogenases (RDases) are now known, their biochemical characterization remains challenging due to the slow growth of native OHRB, difficulties with heterologous expression, and poor solubility of recombinant forms. Tetrachloroethene (PCE) and trichloroethene (TCE) are among the most abundant anthropogenic organohalides, historically used as dry cleaning solvents and metal degreasing agents.[124] Early studies focused on *Sulfurospirillum multivorans*, which dichlorinates PCE to *cis*-dichloroethene (*cis*-DCE).[123] More recently, research has expanded our understanding of complete dechlorination pathways. For example, *Dehalococcoides mccartyi* strains FL2 and 195 utilize can convert vinyl chloride (VC) to ethene in the presence of vitamin B_{12}, with this activity linked to expression of the TceA RDase.[127] The recently isolated strain NIT01 encodes at least 19 *rdhA* genes, expressing several novel RDases and achieving quantitative TCE-to-ethene conversion even at high substrate loads[128] (Fig. 9 and Table 4). Other organisms, such as *Candidatus Dehalogenimonas etheniformans* strain GP, can also fully dechlorinate VC.[129] Innovative tools are improving our ability to track these processes. Metaproteomic RDase quantification and the Dehalochip microarray[130] have enhanced *in situ* detection of dechlorination activity. Meanwhile, density functional theory (DFT) calculations suggest a proton-coupled two-electron transfer (PC-TET) mechanism mediated by cob(I)alamin and a B_{12}-coordinated tyrosine, which helps predict aromatic-halide reactivity.[131] Bioelectrochemical cultivation of *D. mccartyi* strain CBDB1 using

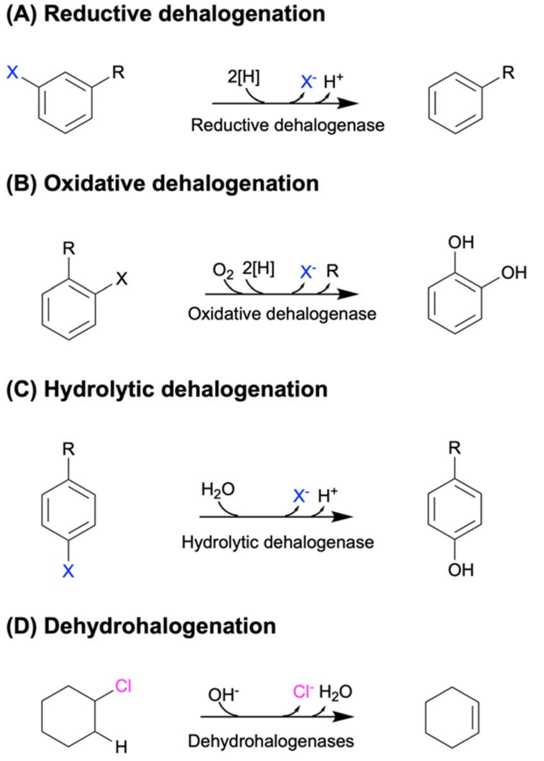

Fig. 8 Representative dehalogenation mechanisms. (A) Reductive dehalogenation: removal of a halogen atom (X) *via* electron transfer, typically catalyzed by reductive dehalogenases under anaerobic conditions, adapted from ref. 122. (B) Oxidative dehalogenation: incorporation of oxygen into the substrate, catalyzed by oxygenases, leading to hydroxylated products and halide release, adapted from ref. 122. (C) Hydrolytic dehalogenation: replacement of a halogen by a hydroxyl group through nucleophilic attack by water, often catalyzed by haloalkane or haloacid dehalogenases, adapted from ref. 122. (D) Dehydrohalogenation: base-catalyzed elimination of HX, resulting in the formation of a double bond. X represents a halogen atom.

cobalt chelates as electron shuttles offers a halogen-free route to sustain organohalide respiration.[132] In *Firmicutes*, multi-omics analysis of the *pceABCT* operon in *Dehalobacter restrictus* and *Desulfitobacterium hafniense* indicates that a functional $PceA_2B$ complex forms with little or no PceC, challenging previous models of RDase membrane assembly.[133] Comparative genomics of a newly enriched *Dehalobacter* consortium from North China Plain sediments has revealed versatile dechlorination of both aliphatic and aromatic halides, supported by syntrophic partners supplying corrinoids and hydrogen.[134]

Polybrominated diphenyl ethers (PBDEs), widely used as flame retardants since the 1960s, have become pervasive environmental pollutants, with bioaccumulation observed across many ecosystems, including human tissues.[142,159] Although regulatory bans implemented in the early 2000s have reduced new PBDE inputs, their environmental persistence remains a major remediation challenge. Several bacterial genera, including *Dehalococcoides*, *Dehalogenimonas*, *Dehalobacter*, *Desulfitobacterium*, *Sulfurospirillum*, *Acetobacterium* and *Geobacter*, have demonstrated debromination potential.[160,161] However, most isolates exhibit limited activity, and can only partially debrominate highly brominated congeners, resulting in intermediates that are more bioavailable and, in some cases, more toxic.[159,162–165] A major breakthrough was the identification of *Dehalococcoides mccartyi* strain GY50, which is capable of fully debrominate tetra- and penta-BDEs to diphenyl ether within 12 days, using hydrogen as the electron donor.[142] The genes *pbrA1*, *pbrA2*, and *pbrA3* encode the associated RDases, which have been proposed as biomarkers for *in situ* PBDE debromination.[142] Further studies have uncovered strains such as CG1, CG4, and 11a5, which harbor novel RDases (*PbrA4*, *TceA*, *11a5_e001*) and can also achieve complete PBDE debromination.[147] These discoveries demonstrate the widespread PBDE-degrading potential of *Dehalococcoides* populations. Some strains, like *D. mccartyi* MB and TZ50, display broad substrate specificity, catalyzing the debromination of penta-BDEs, the dichlorination of PCBs and PCE, and sequential debromination/dechlorination through multifunctional RDases.[144,166]

Polychlorinated biphenyls (PCBs), once widely used as industrial dielectric and coolant fluids, persist as organic pollutants (POPs) of global concern due to their high chemical stability, toxicity, and bioaccumulation.[166–168] Despite regulatory bans, PCBs continue to contaminate ecosystems, including

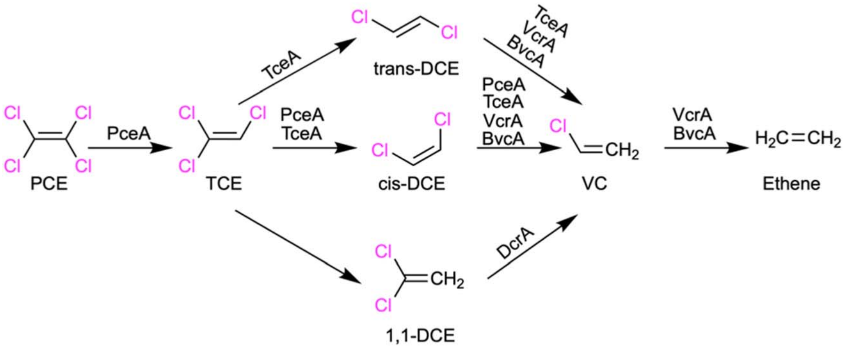

Fig. 9 Genes and enzymes involved in the reductive dechlorination pathways of chlorinated ethenes. Pathways depict the sequential transformation of tetrachloroethene (PCE) through trichloroethene (TCE), dichloroethene (DCE, including *cis*-, *trans*-, and 1,1-DCE isomers), and vinyl chloride (VC) to ethene. Key enzymes catalyzing each step are indicated: PceA, TceA, VcrA, BvcA, and DcrA. Abbreviations: PCE, tetrachloroethene; TCE, trichloroethene; DCE, dichloroethene; VC, vinyl chloride.

Table 4 Representative organohalide-respiring bacteria and biochemically characterized native reductive dehalogenases

Source organism	Reductive dehalogenase	Catalyzed reaction/major substrate(s)	PDB	References
Dehalobacter sp. strain UNSWDHB	TmrA	CF → DCM	—	135
Dehalobacter-containing mixed cultures	CfrA	CF,1,1,1-TCE	—	136
Dehalobacter-containing mixed cultures ACT-3	DcrA	1,1-DCE	—	136
Dehalococcoides ethenogenes 195	TceA	TCE → ethene	—	137 and 138
Dehalococcoides mccartyi BAV1	BvcA	All dichloroethane isomers and 1,2-DCE → ethene	—	139 and 140
Dehalococcoides mccartyi CBDB1	—	1,2,3-TCB → 1,3-DCB	—	141
Dehalococcoides mccartyi GY50	PbrA1, PbrA2, PbrA3	Tetra- and penta-BDEs → diphenyl ether	—	142
Dehalococcoides mccartyi MB	MbrA	Penta-BDEs → di- to tetra-BDEs; PCE, PCB	—	143
Dehalococcoides mccartyi TZ50	PteA$_{TZ50}$, TceA $_{TZ50}$	Penta- and tetra-BDEs → di-BDE; PCE → TCE; TCE → VC/ethene	—	144
Dehalococcoides mccartyi TZ50	BdeA $_{TZ50}$	di-BDE → diphenyl ether	—	144
Dehalococcoides mccartyi VS	VcrA	VC → ethene; 1,2-DCE → ethene	—	145 and 146
Microcosms derived from E-waste-contaminated sites; *Dehalococcoides mccartyi* CG1, CG4, and 11a5	PbrA4, TceA, 11a5_e001	Penta-BDE mixture → diphenyl ether	—	147
Dehalogenimonas-containing culture	—	1,2-DCP → propene	—	148
Desulfitobacterium dehalogenans	CprA	*Ortho*-chlorophenols	—	149
Mixed culture enriched from groundwater/ *Desulfitobacterium dichloroeliminans* strain DCA1	DcaA	1,2-DCA → ethene	—	150
Desulfitobacterium hafniense PCP-1	CrdA	2,4,6-Trichlorophenol	—	151
Geobacter thiogenes K	—	Trichloroacetate → dichloroacetate	—	152
Geobacter thiogenes SZ	—	PCE → *cis*-1,2-DCE	—	153
Sulfurospirillum multivorans	PceA	PCE → TCE → *cis*-1,2-DCE	4UQU, 4UR2, 4UR0, 4UR1;[154] 5MAA, 5MA1, 5M8X, 5M8Y, 5M8Z, 5MA0, 5M90, 5M91, 5M92;[155] 5OBI[156]	157 and 158

marine food webs where they impair apex predators such as *Orcinus orca*.[125,168,169] Reductive dichlorination by OHRB, particularly *Dehalococcoides mccartyi*, is now a key bioremediation approach. Early studies showed that *Dehalococcoides mccartyi* 195 could reductively dechlorinate both chlorinated ethenes and certain PCB congeners.[170–173] Subsequently work with other strains, including CBDB1, CG1–CG5, JNA, and MB, has extended the substrate range to higher chlorinated PCBs *via* strain-specific RDases such as MbrA.[125,166,174–178] Microcosm experiments have shown that native *Dehalococcoides* populations can remove up to 70% of selected tetra- and penta-chlorinated PCBs under anaerobic incubations, even without added electron donors.[179] The identification and enrichment of RDase genes such as *rd14*, *pcbA5*, and *rd4*/*rd8* homologs highlight their use as biomarkers for *in situ* PCB dechlorination monitoring.[179] Additional studies revealed that *Dehalococcoides*- and *Dehalogenimonas*-dominated consortia can carry out *para*- and *meta*-dechlorination in PCB180,[180] mediated by distinct RDase clusters. The development of high-throughput *in vitro* dechlorination platforms such as HINVARD has enabled the systematic screening of PCB congeners and microbial consortia, uncovering how both congener structure and bacterial strain determine dechlorination specificity and efficiency.[181]

Recent studies have greatly broadened the known range of OHRB, RDases and degradable substrates, extending far beyond classical chloroethene dechlorination. Diverse microorganisms, such as *Desulfoluna spongiiphila*,[182] *Pseudomonas* sp. CP-1,[183] *Dehalogenimonas*,[148,184,185] and deep-sea *Peptococcaceae*-affiliated strains,[186] have been implicated in reductive dehalogenation processes. These discoveries reveal that the phylogenetic diversity of OHRB is much greater than previously recognized. Beyond expanding microbial diversity, new work continues to identify additional organohalide substrates. Polyfluorinated compounds,[187] polybrominated flame retardants (*e.g.*, TBBPA[188]), chlorinated aromatic fungicides (*e.g.*, chlorothalonil derivatives[184]), and trihalopropanes,[185] are now recognized as targets for microbial dehalogenation. In parallel, researchers are uncovering the importance of non-canonical habitats,[189]

Table 5 Representative oxidative dehalogenase involved in halogenated compound degradation

Enzyme class	Oxidative dehalogenase	Microbial source	Typical substrates	Catalytic mechanism	References
Monooxygenase	Cytochrome P450 enzyme CYP199A4	*Rhodopseudomonas palustris* HaA2	Halogenated benzoic acids (*e.g.* 4-methoxybenzoic acid)	Aromatic hydroxylation	202
Monooxygenase	Toluene *ortho*-monooxygenase (TOM)	*Burkholderia cepacian* G4	TCE, all three dichloroethylenes, and vinyl chloride	Aromatic hydroxylation	203
Monooxygenase	Toluene-*o*-xylene-monooxygenase (ToMO)	*P. stutzeri* OX1	Chlorophenols	Aromatic hydroxylation	204
Monooxygenase	HadA	*Ralstonia pickettii* DTP0602	Halogenated phenols	$FADH_2$-dependent hydroxylation	205
Dioxygenase	Biphenyl dioxygenase (BphA)	*P. pseudoalcaligenes* KF707; *Burkholderia* sp. LB400	PCBs	Aromatic ring dioxygenation	206 and 207
Dioxygenase	Chlorobenzene dioxygenase (TcbA)	*Pseudomonas* sp. strain P51	Chlorobenzenes	Aromatic ring hydroxylation	208
Dioxygenase	Toluene dioxygenase (TodC)	*P. putida* F1	Trichloroethylene	Aromatic ring hydroxylation	209
Peroxidase	Lignin peroxidase (Lip)	*Phanerochaete chrysosporium*	2,4-Dichlorophenol 2,4,5-Trichlorophenol	Aromatic ring oxidation	210 and 211
Peroxidase	Manganese peroxidase (MnP)	*Phanerochaete chrysosporium*	2,4-Dichlorophenol 2,4,5-Trichlorophenol	Mn^{3+}-mediated one-electron oxidation	210 and 211
Multicopper oxidase	Laccase	*Trametes versicolor*	Chlorophenols	A single-electron oxidation	212

auxiliary physicochemical processes,[190–193] and stimulatory amendments[190,194] in supporting or accelerating dehalogenation. For example, landfill leachates[195] harbor previously uncharacterized *Dehalococcoidia* and facultative OHRB, with their dechlorination activities shaped by complex syntrophic interactions among sulfate reducers, fermenters, and methanogens. The dominance of *Methanosarcina versus* OHRB in these systems often follows acetate-competition thresholds and reflects energy optimization principles. In iron-rich river sediments, synergism between Fe(III) reduction and 2,4,6-trichlorophenol dechlorination is observed, with nano-hematite boosting electron-transfer gene expression and more than doubling dechlorination rates.[190] Similarly, waste-activated sludge amendments can provide redox mediators, electron donors, and vitamins, substantially accelerating PCE and PCB dechlorination across different soils.[194]

In addition to biological processes, several abiotic or hybrid remediation technologies have gained traction. UV/sulfite advanced-reduction systems generate hydrated electrons that efficiently dechlorinate recalcitrant aromatic AOX (*e.g.*, 2,5-dichloronitrobenzene) in saline wastewaters.[191] Pd-nanoparticle membrane reactors can achieve >99% conversion of herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) to less-toxic products under continuous-flow conditions.[192] Environmental factors also play a role: for instance, microplastics tend to enhance *Dehalococcoides*-mediated dechlorination, while 80 nm nanoplastics can inhibit it. Community resilience is often supported by non-dehalogenating taxa.[193] Heavy metals like Cu^{2+} may suppress RDase expression and metabolism in some strains (*e.g.*, *Pseudomonas* CP-1 (ref. 196)), yet facultative pathways can still support efficient dechlorination under fluctuating redox conditions.[183] Alongside ecological and technological progress, the systematic discovery of new RDases continues. Recent B_{12}-dependent screens have identified enzymes capable of both PCE dichlorination and regioselectively deiodination of 2,4,6-triiodophenol. A deiodinase from *Clostridioides difficile* able to reduce L-halotyrosines hints at host-associated reservoirs of halogen-transforming activity.[197] Isotope probing studies help distinguish between concerted dihalo-elimination and S_N2-type mechanisms,[189] deepening our understanding of non-canonical dehalogenation. Together, these advances provide new opportunities, from stimulatory amendments and redox-active materials to high-specificity enzymes, for tackling chemically recalcitrant and mixed-pollutant environments.

3.2 Oxidative dehalogenase

Oxidative dehalogenases catalyze the cleavage of carbon-halogen bonds *via* oxygen-dependent reactions and are emerging as key players in the detoxification of persistent pollutants, including polychlorinated biphenyls (PCBs), halophenols, and brominated flame retardants.[198–201] These enzymes, mainly mono- or dioxygenases, typically function under aerobic conditions and display broad substrate ranges spanning haloaromatic and haloaliphatic compounds (Table 5).

A major challenge in this field has been the limited cultivability of efficient degraders, many of which enter a viable but non-culturable (VBNC) state under stress. Recent studies have demonstrated that resuscitating VBNC bacteria can unlock new oxidative dehalogenation activities. For example, strains like *Streptococcus* SPC0 and *Bacillus* LS1 have been revived and shown to degrade various PCB congeners, using *bph*-encoded biphenyl dioxygenases and funnelling intermediates through protocatechuate and β-ketoadipate pathways[198,213] (Fig. 10).

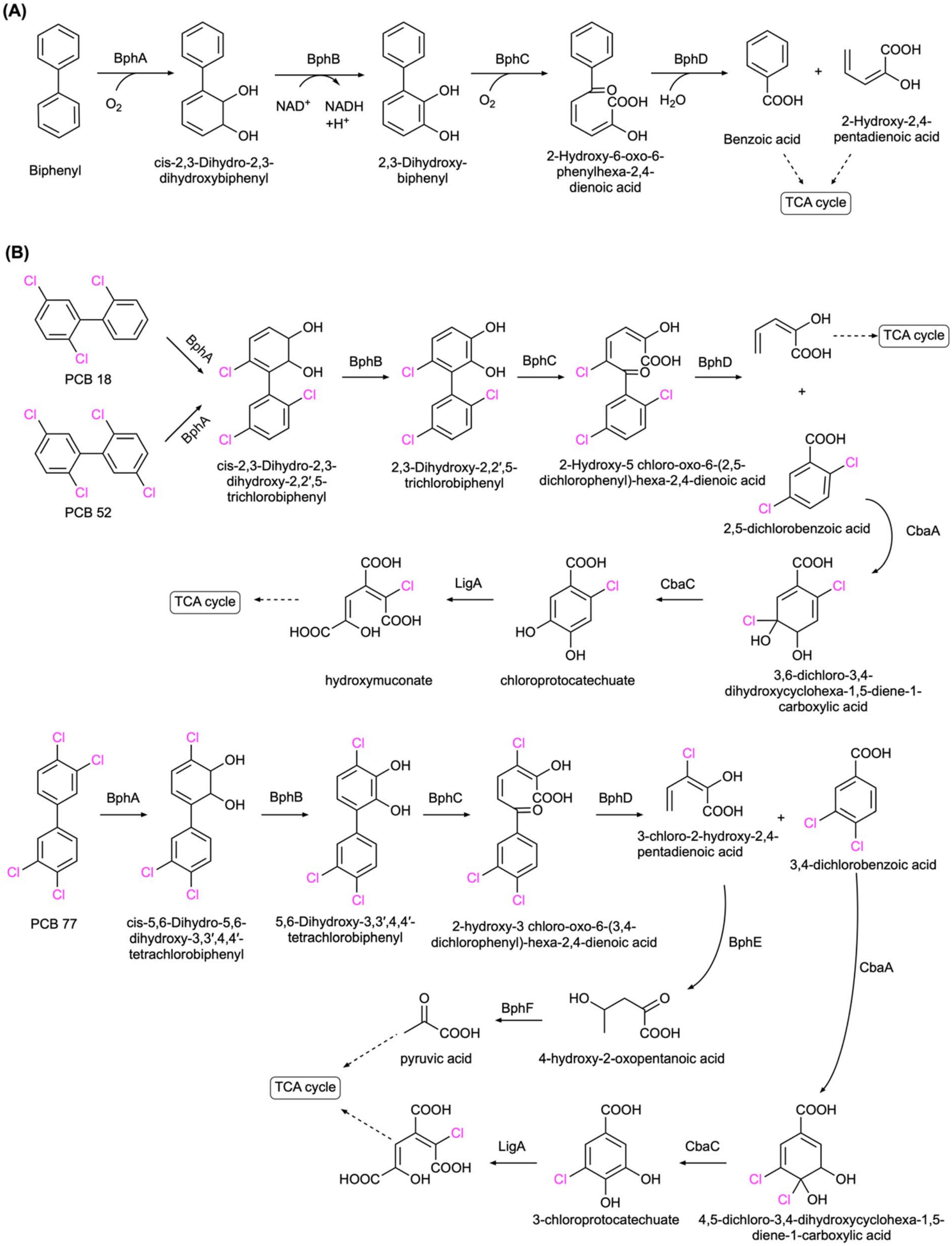

Fig. 10 Aerobic degradation pathways of biphenyl and polychlorinated biphenyls (PCBs). (A) Reaction catalyzed by the biphenyl dioxygenase (*bph*) gene cluster. Adapted from ref. 219. (B) Proposed aerobic degradation pathway for PCBs 18, 52 and 77 by the strain *Streptococcus* sp. SPC0, including subsequent catabolic steps leading to TCA cycle intermediates. Adapted from ref. 198. Abbreviations: BphA/B/C/D/E/F, biphenyl/PCB catabolic enzymes; LigA/C, ring-cleavage dioxygenases; CbaA/C, chlorobenzoate pathway enzymes; TCA, tricarboxylic acid cycle.

Engineered and artificial oxidative dehalogenases have also advanced rapidly. Miniaturized heme-based enzyme such as Fe(III)-MC6*a outperform natural horseradish peroxidase in H_2O_2-mediated oxidation of 2,4,6-trichlorophenol (TCP), relying on high-valent oxoferryl intermediates similar to peroxidase compound.[199] In nature, monooxygenases like TcpA from *Cupriavidus nantongensis* X1T, and two-component FAD-dependent monooxygenases such as HnpAB from *Cupriavidus* sp. CNP-8, mediate oxidative dehalogenation of di- and tri-bromophenols.[200,214] Structural analysis revealed that TcpA's substrate specificity is shaped by its binding pocket,[214] while HnpAB performs sequential oxidative and hydrolytic debromination, generating ring-cleavage intermediates that are further degraded by HnpC.[200] Such findings illustrate the functional diversity and the molecular specialization of oxidative dehalogenases, especially in species like *Cupriavidus* that encode unique hnpABC gene clusters.[200] In addition, biomimetic non-heme iron complexes featuring iron(IV)–oxo species have been shown to oxidatively dehalogenate mono- to tri-halophenols.[201] These model systems rely on hydrogen atom abstraction and second-sphere substrate interactions, offering valuable insights for designing synthetic catalysts for environmental use. Oxidative dehalogenases also play an essential role in integrated bioremediation strategies. For example, sequential bi-oelectrochemical systems (BESs)[215] combine reductive dechlorination of highly chlorinated solvents (*e.g.*, PCE to VC) with oxidative dehalogenation of less chlorinated byproducts (*e.g.*, VC), the latter step mediated by aerobic mono- and di-oxygenases.[216] Biomarkers such as *etnC* and *etnE*, encoding alkene monooxygenase and epoxyalkane coenzyme M transferase respectively, have been identified as key contributors in these pathways.[217] Recent studies on CYP199A4, a cytochrome P450 monooxygenase, reveal fine-tuned substrate specificity for halogenated benzoic acids.[218] While *para*-halobenzoates are only weakly oxidized, halomethyl-substituted benzoates undergo efficient benzylic hydroxylation, demonstrating that the position of the halogen atom can significantly influence enzyme reactivity and substrate binding. Altogether, oxidative dehalogenases are becoming increasingly important for tackling persistent halogenated pollutants.

3.3 Hydrolytic dehalogenases

Hydrolytic dehalogenation involves the substitution of a halogen atom with a hydroxyl group derived from water.[220] The enzymes that catalyze this process, hydrolytic dehalogenases, are typically cytosolic proteins that require no cofactors, use water as the only co-substrate, and are generally tolerant of water-miscible organic solvents. These properties make them excellent candidates for biodegradation catalysts in the bioremediation of halogenated compounds.[220,221] Hydrolytic dehalogenases are usually classified into groups such as haloacid dehalogenases, haloalkane dehalogenases, and fluoroacetate dehalogenases.[220] These enzymes primarily act on aliphatic halogenated organic compounds, catalyzing the removal of halogens through a straightforward hydrolytic mechanism.

3.3.1 Haloalkane dehalogenases. Haloalkane dehalogenases (HLDs), together with 2-haloacid dehalogenases, form a well-characterized group of hydrolytic dehalogenases within the α/β-hydrolase fold superfamily. Structurally, these enzymes share a conserved core of seven parallel and one antiparallel β-sheet flanked by α-helices.[222] HLDs catalyze the hydrolytic cleavage of carbon-halogen bonds in HOCs, producing alcohols, halide ions, and protons.[223] The catalytic mechanism involves

Table 6 Major haloalkane dehalogenases

Representative enzymes	Microbial source	Typical substrates	References
DadB	*Alcanivorax dieselolei* strain B-5	1,2-Dichloroethane, 1,2-dichloropropane and 1,2,3-trichloropropane	231
DbeA	*Bradyrhizobium elkanii* USDA94	1,3-Diiodopropane	232
DbjA	*Bradyrhizobium diazoefficiens* USDA110 (formerly *Bradyrhizobium japonicum* USDA110)	1-Chloro-2-methylpropane, 3-chloro-2-methylpropene	233
DccA	*Caulobacter crescentus*	Long brominated and chlorinated alkanes	234
DhaA	*Rhodococcus rhodochrous* NCIMB 13064	1-Haloalkanes	226 and 227
DhlA	*Xanthobacter autotrophicus* GJ10	1,2-DCA, 1,2-dibromoethane	224 and 225
DhmA	*Mycobacterium avium* subsp. *avium* N85	1,2-Dichloroethane	235
DmbA	*Mycobacterium tuberculosis* H37Rv	1,3-Propanediol, 1,2-dichloroethane	236
DmlA	*Mesorhizobium loti* MAFF303099	1-Chloro-2-methylpropane, 3-chloro-2-methylpropene	233
DmmA (formerly CurN)	Marine microbial consortium	1,3-Dibromopropane	237
DmrA	*Mycobacterium rhodesiae* JS60	4-Bromobutyronitrile	238
DmrB	*Mycobacterium rhodesiae* JS60	1-Bromohexane	238
DppA	*Plesiocystis pacifica* SIR-1	1-Bromobutane	239
DsaA	*Saccharomonospora azurea*	Long (greater than 3 carbons) brominated substrates	234
HanR	*Rhodobacteraceae* bacterium	1,6-Dichlorohexane, 1-bromooctane, 1,3-dibromopropane and 1-bromohexane	240
LinB	*Sphingobium japonicum* UT26 (formerly *Sphingomonas paucimobilis* UT26)	1-Chlorohexane, chlorocyclohexane and bromocyclohexane	241

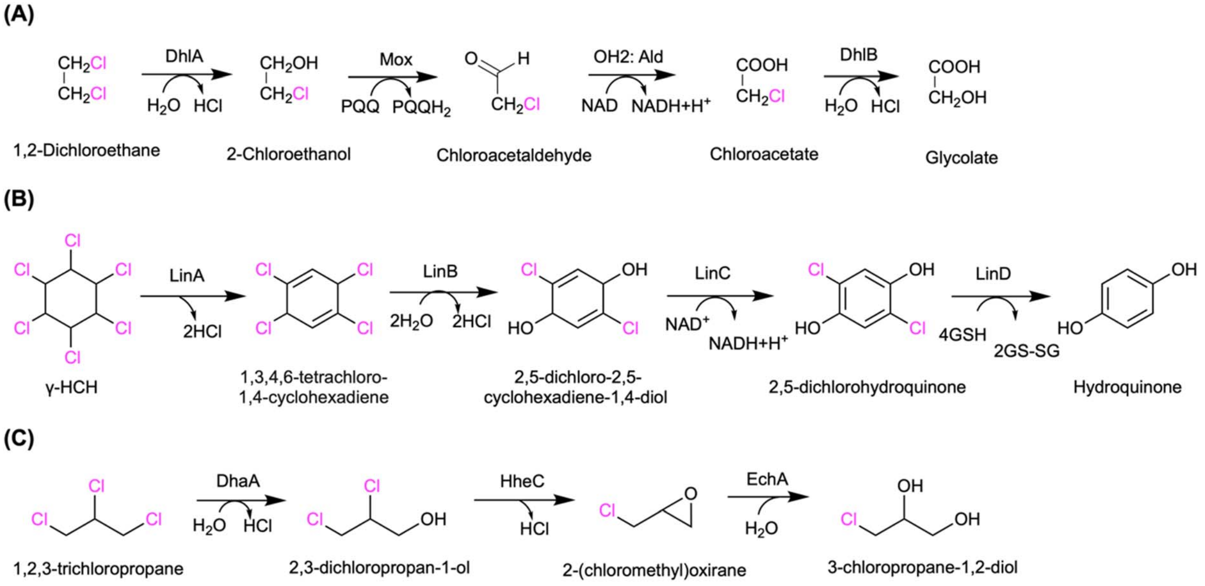

Fig. 11 Representative haloalkane dehalogenases involved dehalogenation. (A) Degradation of 1,2-dichloroethane by *Xanthobacter autotrophicus* GJ110. Adapted from ref. 242. (B) Proposed γ-hexachlorocyclohexane degradation pathway in *Sphingomonas paucimobilis* UT26. Adapted from ref. 243 and 244. (C) Proposed degradation pathway of TCP for *Agrobacterium radiobacter* AD1. Adapted from ref. 245.

S_N2 nucleophilic substitution and transient ester intermediate, which is then hydrolyzed to regenerate the active site.[222]

Over the past five years, HLDs have emerged as a data-driven platform for green chemistry and environmental biotechnology (Table 6). Three key HLDs, DhlA, DhaA, and LinB, have been instrumental in understanding the substrate specificity, regulation, and catalytic breadth of the family (Fig. 11). DhlA, discovered in *Xanthobacter autotrophicus* GJ10, initiates 1,2-dichloroethane (1,2-DCA) degradation and acts on a range of chlorinated, brominated, and iodinated substrates.[224,225] DhaA, first identified in *Rhodococcus rhodochrous* NCIMB 13064, starts the breakdown of 1-haloalkanes, with its gene widespread among haloalkane-degrading strains.[226,227] LinB, known for degradation γ-hexachlorocyclohexane (γ-HCH), displays broad substrate specificity toward monochloroalkanes, dichloroalkanes, bromoalkanes, and select alcohols.[228–230]

Advances in genome mining and high-throughput screening, such as EnzymeMiner,[246] have doubled the number of validated HLDs. Machine learning approaches, including variational-autoencoder design, have produced multi-site variants with up to 3.5-fold increased activity and improved stability.[247] Structural studies now span diverse architectures, from Asp–His–Asp-triad (S)-selective dimer DmmarA[248] and psychrophilic tetramer DpaA[249] to a 20-mer archaeal ring, DhmeA.[250] Kinetic isotope effect (KIE) and MD studies reveal that minor substrate modifications can invert the rate-limiting step, challenging the idea of a universal catalytic model.[251] Recent studies have also expanded our understanding of HLDs in complex environmental and engineered systems. For example, bacterial consortia from mangrove sediments dominated by *Alcanivorax* can transform up to 97.7% of persistent organic pollutants (such as HBCDs), with metagenomic and transcriptomic data pinpointing two inducible HLDs, DadAH and DadBH, as key players.[252,253] In addition, *Microbacterium* J1-1 and a range of wastewater microbial genomes have been found to encode more than 100 distinct HLDs,[254] illustrating the genetic diversity and metabolic potential for hydrolytic dehalogenation in various environments. These dehalogenation steps often integrate with downstream oxidative and metabolic pathways, underscoring the metabolic cooperation typical of natural communities. Beyond marine habitats, HLDs genes are found in oligotrophic subsurface environments such as aquifers and iron mines. For instance, *Marinobacter subterrain* JG233 from the Soudan Underground Iron Mine uses chloroacetate hydrolysis as a carbon source, supported by isotope labelling and Raman spectroscopy evidence.[255] Notably *Pseudomonas* sp. 273 demonstrates the functional integration of oxidative and hydrolytic pathways, coupling AlkB monooxygenases with (*S*)-2-haloacid dehalogenase for the mineralization of terminally fluorinated alkanes. This process releases inorganic fluoride and fluorinated fatty acids, with transcriptomic data revealing coordinated upregulation of relevant genes.[256] Such findings highlight the key role of HLDs in supporting microbial survival and adaptation under nutrient-limited or contaminated conditions. Mechanistic insights from computational studies, combining KIEs, halogen binding isotope effects (BIEs), and free energy simulations, demonstrate that even subtle changes in substrate structure can alter the catalytic rate-limiting step in enzymes like LinB and DhaA,[251] indicating the mechanistic complexity across this enzyme family.

In natural ecosystems, HLDs also contribute to the metabolism of chlorinated natural organic matter (Cl-NOM), facilitating contaminant breakdown and influencing microbial community structures.[257] Engineering innovations mirror this functional breadth. Multi-point mutations have stabilized DhaA variants;[258] ionic-liquid-soaked crystals have clarified DhaA80

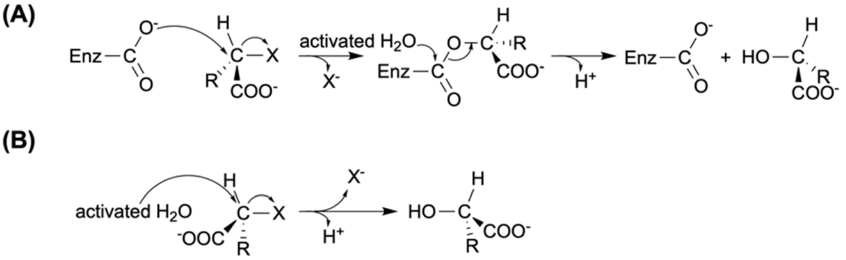

Fig. 12 Reaction mechanisms of haloacid dehalogenases. (A) L-DEX: catalysis is initiated by nucleophilic attack from an acidic residue in the active site to form a covalent ester intermediate. (B) DL-DEX: an activated water molecule directly attacks the C2 carbon of the substrate, bypassing the formation of an ester intermediate. Adapted from ref. 265.

rigidity;[259] LinB displayed on *Bacillus subtilis* spores can detoxify sulfur-mustard simulants;[260] and Halo-Tag fusions have enabled efficient receptor chromatography without prior purification.[261] These advances, building on foundational studies of DhlA, DhaA and LinB, reveal HLDs as structurally diverse and mechanistically adaptable biocatalysts. Their expanding functional repertoire positions them as valuable tools for next-generation applications in synthetic biology, bioremediation, and analytical chemistry.

3.3.2 Haloacid dehalogenases. Haloacid dehalogenases (HADs) are a family of hydrolase enzymes that catalyze the hydrolytic cleavage of carbon-halogen bonds in halogenated aliphatic acids, especially 2-haloalkanoic acids, yielding corresponding 2-hydroxy acids.[262,263] This transformation is central to microbial degradation of many xenobiotic compounds. Based on substrate stereospecificity and product configuration, 2-haloacid dehalogenases are classified into four main types: D-2-haloacid dehalogenases (D-DEX), L-2-haloacid dehalogenases (L-DEX), configuration-inverting DL-2-haloacid dehalogenases (DL-DEXi), and configuration-retaining DL-2-haloacid dehalogenases (DL-DEXr)[264] (Fig. 12). Phylogenetically, D-DEX and DL-DEXs (group I) are distinct from L-DEXs (group II) due to sequence divergence.[265] L-DEXs (EC 3.9.1.2) are the most extensively studied HADs. These enzymes are widely distributed in nature and specifically catalyze the dehalogenation of L-2-haloalkanoic acids to produce D-2-hydroxyalkanoic acids. Structurally, L-DEXs adopt an α/β-type fold with a Rossmann-like core and a distinct subdomain, where the active site lies between the two.[266] Model enzymes such as L-DEX YL from *Pseudomonas* sp. YL, DhlB from *Xanthobacter autotrophicus* GJ10, and DehIVa from *Burkholderia cepacian* MBA4 have revealed a two-step catalytic mechanism: nucleophilic attack by a conserved aspartate generates an ester intermediate and releases a halide ion, followed by hydrolysis of the intermediate by an activated water molecule.[267] Most L-DEXs act efficiently on chlorinated, brominated, and iodinated substrates but show little defluorination capacity. Only a handful (such as Bpro0530, Bpro4516, RHA1_ro00230, and Adeh3811) can defluorinate haloacids,[268] a property associated with a more compact active sites, as shown by crystallographic studies.[267] Substrate specificity is also variable:[266] for example, L-DEX YL prefers L-2-chloropropionic acid, while the L-DEX from *Bacillus* strain I37C prefers chloroacetic acid.[269] D-DEXs catalyze the conversion of D-2-haloalkanoic acids to L-2-hydroxyalkanoic acids and are comparatively rare, with a few examples identified in *Rhizobium* sp. RC1, *Agrobacterium* sp. NHG3, *Pseudomonas putida* AJ1, and *Pseudomonas* sp. ZJU26. These enzymes tend to act on short-chain (C2–C4) chlorinated and brominated acids, and their all-α homotetrameric structures are distinct from the α/β fold of L-DEXs.[265] DL-DEXs act on both enantiomers of 2-haloalkanoic acids. DL-DEXi enzymes invert the substrate's configuration, while DL-DEXr enzymes retain it. Well-characterized DL-DEXi enzymes, such as DehI, DehE, DL-DEX 113, and DhlIV, prefer L-isomers and use a single-displacement mechanism. The only reported DL-DEXr enzyme comes from *Pseudomonas putida* PP3 and is proposed to act *via* a double inversion, though experimental validation is still needed. Genomic analyses reveal a broad distribution of HADs in various bacteria. For example, *Bacillus megaterium* strains BHS1 and WSH-002 encode multiple HAD genes and associated regulators (dehR) and transporters (dehP), suggesting that horizontal gene transfer plays a role in HAD diversification.[270] Novel HADs have also been identified in *Staphylococcus*,[271] *Bacillus thuringiensis*,[272] and halotolerant strains from extreme environments,[273] demonstrating adaptability to diverse ecological niches.

HADs are important for microbial bioremediation. For instance, *Paracoccus denitrificans* detoxifies monoiodoacetic acid (MIAA), a disinfection byproduct, *via* hydrolytic dehalogenation to support denitrification under oxidative stress.[274] HADs also appear in plants metabolism, rice encodes 37 HAD genes, with several induced by the herbicide oxadiazon, highlighting their potential in phytoremediation.[275] Crystallographic and molecular modeling studies provide insights for rational enzyme design.[267,276] Molecular dynamics and substrate docking have revealed key residues and structural features that determine substrate preference and catalytic efficiency (*e.g.*, Tyr12, Lys46, Asp182 in DehHsAAD6 from *Halomonas smyrnensis*).[276] The compactness of the active site is critical for defluorination activity, while regulatory operons (such as dehR/dehP) may be harnessed for improved gene expression and substrate uptake.[270] Haloacid dehalogenases thus represent a structurally and mechanistically diverse enzyme family with wide ecological significance. Advances in structural biology, genome mining, AI-assisted protein engineering, and synthetic biology continue to expand their potential in bioremediation, green chemistry, and agricultural biotechnology.

3.3.3 Fluoroacetate dehalogenases. Fluoroacetate dehalogenases (FAcDs) are specialized hydrolases that catalyze the cleavage of the carbon–fluorine (C–F) bond in fluoroacetate, one of the most chemically stable bonds. First identified in the 1960s for their ability to hydrolyze fluoroacetate to glycolate, FAcDs have become model systems for studying enzymatic defluorination. With growing environmental and health concerns over per- and polyfluorinated substances (PFAS), these enzymes are now seen as promising tools for bioremediation and green chemistry. Structurally, FAcD adopts an α/β-hydrolase fold with an α/β/α core and a cap domain.[277] Catalysis proceeds *via* a classical two-step mechanism involving a conserved catalytic triad (Asp110–His280–Asp134); substrate binding is stabilized by Arg111 and Arg114, while the leaving group is stabilized by His155, Trp156, and Tyr219.[277] Microsecond-scale molecular dynamics simulations and ultrahigh-resolution crystallography have revealed "half-of-the-site reactivity", only one subunit of the FAcD homodimer is active at a time. Allosteric control across the dimer interface is mediated by a water network, with conformational changes triggered by substrate binding in the non-catalytic subunit.[278,279] Although FAcDs are optimized for fluoroacetate, several homologs exhibit broader substrate tolerance.[280] Enzymes such as RPA1163, DAR3835, and NOS0089 can defluorinate defluorination and trifluoroacetate to glyoxylate *via* sequential C–F cleavage,[280] and can also act on bulkier α-fluorinated acids and aryl-substituted fluorocarboxylates. Comparative kinetic and computational studies show that while defluorination is generally more favorable than dechlorination or debromination, the latter can be enhanced *via* active site engineering.[277,281,282] Mechanistic insights from crystallography and MD simulations highlight dynamic allosteric regulation and catalytic asymmetry within these enzymes.[279] A wide variety of bacteria carry FAcD-like enzymes with defluorinating activity. Genera such as *Pseudomonas mosselii*, *Delftia acidovorans*, *Rhodococcus* sp., *Caballeronia* sp., and *Acidimicrobium* sp. are known to degrade PFAS or fluoroacetate derivatives.[283–287] Genome analyses have identified a diverse set of dehalogenases and accessory genes, including haloacid dehalogenases (*e.g.*, dehH1), fluoroacetate dehalogenases (*e.g.*, DeHa4), fluoride transporters (crcB), and novel enzymes from the MhPC superfamilies.[283,286,288] In some anaerobic bacteria such as *Cloacibacillus porcorum* and *Pyramidobacter piscolens*, unique operons linked to glycine reductase complexes support fluoroacetate metabolism in environments like the rumen microbiota.[289,290] Recent studies also suggest that non-cytochrome P450 enzymes in human liver microsomes may contribute to xenobiotic fluorine removal.[291]

FAcDs and related enzymes are now being explored as biocatalysts for the remediation of persistent fluorinated pollutants. For example, mixed consortia of *Pseudomonas* and *Acidimicrobium* can degrade long-chain perfluorocarboxylic acids (*e.g.*, PFOA) in soil and water, releasing fluoride and shortening perfluorinated chains.[284,287,288] In some cases, microbial electrolysis cells have been used to support PFAS degradation by autotrophic species such as *Acidimicrobium* sp. A6, overcoming Fe(III)-dependency.[287] Heterologous expression in *E. coli* expressing dehalogenases from *D. acidovorans* has yielded biocatalysts active against mono- and difluoroacetate. The continued discovery and engineering of new enzymes with expanded activity toward diverse PFAS structures, including unsaturated or sulfonated forms, promises to broaden the available biodegradation toolkit.

Other haloaromatic dehalogenases, such as 4-chlorobenzoyl-CoA dehalogenases and tetrachlorohydroquinone dehalogenases, are also important for haloaromatic degradation. 4-Chlorobenzoate (4CBA) is a key intermediate in the breakdown of many HOCs, including PCBs.[292] Several 4CBA-degrading bacteria have been isolated, including *Pseudomonas* sp. CBS3, *Comamonas* sp. DJ-12, *Arthrobacter* spp.[292,293] The hydrolytic dehalogenation of 4CBA is CoA-dependent,with the relevant genes located in the fcd gene cluster. This cluster encodes the dehalogenases (fcbA, fcbB, fcbC) and transporters (fcdT1T2T3) for 4CBA uptake, and transcription is regulated by a TetR-type repressor responsive to 4-chlorobenzoyl-CoA.[293,294]

3.3.4 Dehydrohalogenases. Although halogenated organic compounds are degraded through hydrolytic or reductive pathways, enzymes capable of dehydrohalogenation reactions are comparatively rare. These enzymes eliminate hydrogen halide (HX) from halogenated substrates, typically generating alkenes or epoxides. The best-characterized members of this group are halohydrin dehalogenases (HHDHs), which convert vicinal halohydrins to epoxides. Another important example is LinA, a dehydrochlorinase that participates in γ-hexachlorocyclohexane degradation by catalyzing successive eliminations of HCl to form unsaturated chlorocyclohexenes.[295] HHDHs were first described in 1968, when Castro and Bartnicki discovered their ability to convert 2,3-dibromo-1-propanol to epibromohydrin.[296] Belonging to the short-chain dehydrogenase/reductase (SDR) superfamily.[297], HHDHs catalyze both reversible dehalogenation of halohydrins to epoxides and irreversible nucleophile-mediated ring-opening of epoxides.[298] In the latter, these enzymes accommodate a wide variety of small anionic nucleophiles (N_3^-, CN^-, NCO^-, SCN^-, NO_2^-, $HCOO^-$), producing structurally diverse β-substituted alcohols with high chemo-and stereoselectivity[299] (Fig. 13). With the growth of sequence databases, over 70 HHDHs have been identified and divided phylogenetically into subtypes A–G, with D-type being the largest group.[300,301]

Recent efforts have focused on G-type HHDHs (HheG, HheG2, HheG3),[299] whose dynamic active sites and flexible loop regions confer remarkable tolerance toward bulky, cyclic, and internal epoxides.[304] Molecular dynamics simulations and tunnel analyses have helped explain their substrate promiscuity, revealing transient access channels and flexible N-terminal segments that enable broad substrate ranges. Protein-engineering has leveraged these mechanistic insights to yield practical catalysts. Rational and semi-rational redesign of enzymes like *Ic*HheG and *Ab*HheG has enabled precise control of regio- and enantioselectivity in the synthesis of optically pure oxazolidinones from styrene oxides[305,306] (Fig. 14). Loop engineering in HheG led to the M45F variant, which shows a tenfold activity increase and up to 96% enantiomeric excess in

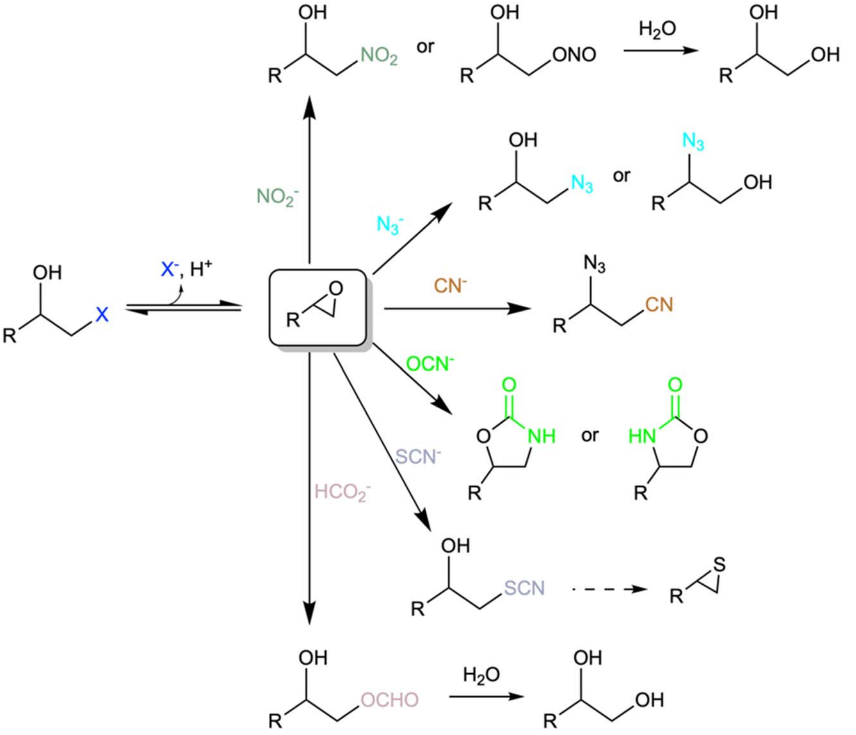

Fig. 13 HHDH-catalyzed dehalogenation of vicinal halohydrins and nucleophilic ring-opening of epoxides with selected anionic nucleophiles. Pathways illustrate the hydrolytic dehalogenation of halohydrins (left) and the transformation of epoxides *via* nucleophilic attack by N_3^-, CN^-, OCN^-, SCN^-, HCO_2^-, NO_2^-, or H_2O, yielding structurally diverse β-substituted alcohols. Adapted from ref. 302 and 303.

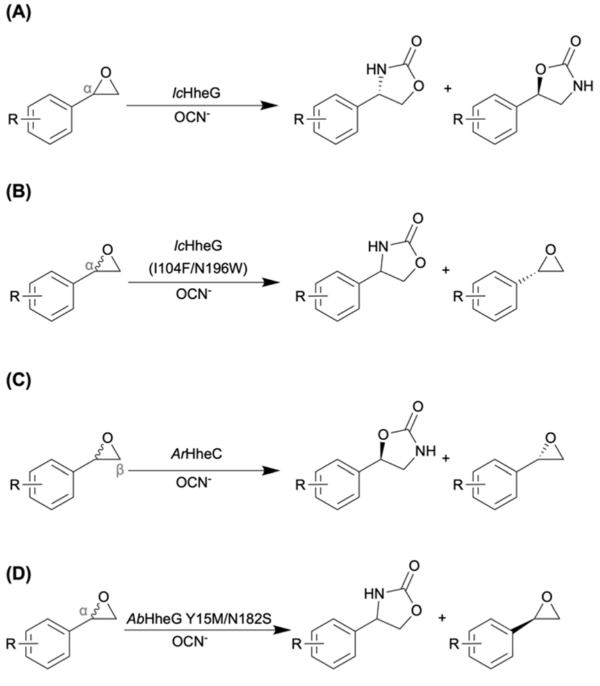

Fig. 14 Catalytic transformation of styrene oxides into 4- or 5-phenyl-2-oxazolidinones mediated by representative HHDHs and engineered variants. (A) *lc*HheG, adapted from ref. 311. (B) *lc*HheG (I104F/N196W), adapted from ref. 312. (C) *Ar*HheC, adapted from ref. 313. (D) *Ab*HheG (Y15M/N182S), adapted from ref. 312.

cyclohexene-oxide azidolysis. Targeted mutations in HheC have produced stereodivergent pathways to both enantiomers of chiral oxetanes and β-hydroxy nitriles.[306–310] Notably, even canonical "motif 1" residues have been repurposed to modulate activity, stability, and solubility.[309]

Reaction engineering has also played a role. Fed-batch, repetitive-batch, and rotating-bed operation can mitigate substrate inhibition and enzyme deactivation, leading to improved space-time yields for fluorinated β-hydroxy nitriles, azido alcohols, and spiro-epoxyoxindoles.[309,314,315] Model-guided simulations now assist in reactor design and substrate dosing.[309,314] To enhance stability and reuse, various immobilization methods have been developed, including cross-linked enzyme crystals (CLECs) and MnO_2 nanosheets.[316,317] For example, HheG D114C CLECs retain catalytic activity for months at room temperature, with high productivity in packed-bed reactors.[314] Immobilized HHDHs have also been used in colorimetric halocarbon sensors and for the bioremediation of chlorinated pollutants, highlighting their value for both synthesis and environmental cleanup.[317,318] Structural advances have further expanded our understanding. The first crystal structure of a D-type enzyme (HheD2) revealed key hydrogen-bond networks and surface helices linked thermostability,[300] while previously underexplored B-type enzymes showed unexpectedly high enantioselectivity, widening the family's landscape.[306] High-throughput screening methods, including pH-sensitive dyes and fluorescence assays, have accelerated the directed evolution and metagenomic discovery of new HHDHs,

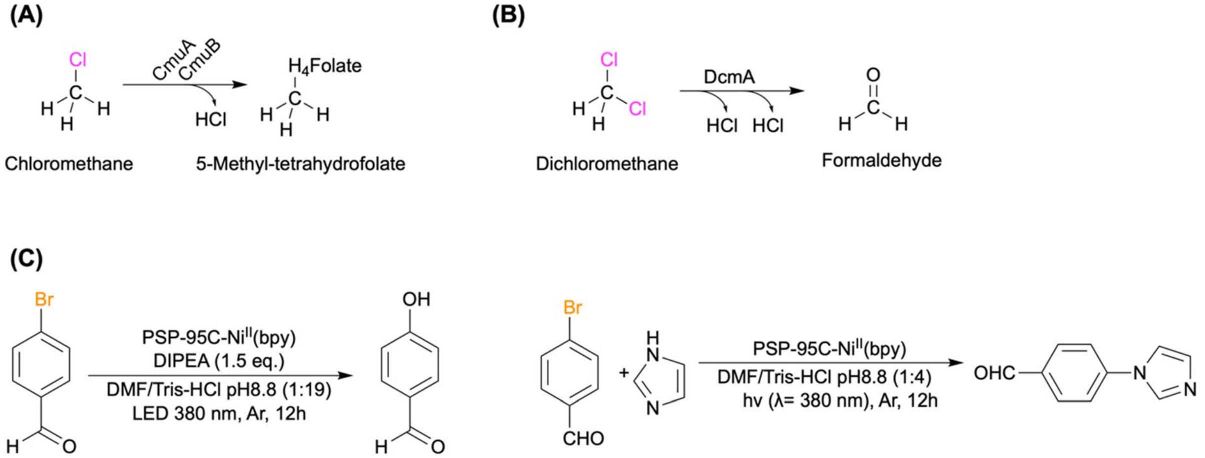

Fig. 15 Representative alternative strategies for dehalogenation. (A) Corrinoid-dependent methyl transfer catalyzed by chloromethane dehalogenases (CmuA, CmuB), adapted from ref. 319. (B) Glutathione-mediated conjugation of dichloromethane by DCM dehalogenases (DcmA), adapted from ref. 320–322. (C) Artificial dehalogenase photosensitizer-metalloenzyme PSP-95C-Nill (bpy) catalyzing the dehalogenation of *para*-bromobenzaldehyde to phenol in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in DMF/Tris–HCl buffer (pH 8.8, 1 : 19) under 380 nm irradiation for 12 h. In the absence of DIPEA, C–N cross-coupling occurred between the aryl bromide and imidazole, adapted from ref. 324.

especially from marine and extremophilic sources.[298,318] These ongoing efforts are steadily expanding the practical utility of HHDHs for both synthetic chemistry and environmental applications.

3.4 Other dehalogenases

Beyond the well-studied reductive, oxidative, hydrolytic and dehydrohalogenation mechanisms, several alternative enzymatic strategies have been discovered for the breakdown of halogenated compounds. For example, chloromethane dehalogenase (CmuA/CmuB) from *Methylobacterium extorquens* CM4 uses a methyltransferase-dependent pathway, where a corrinoid cofactor enables halide displacement *via* methyl transfer[319] (Fig. 15A). Glutathione *S*-transferase-like DCM dehalogenases (*e.g.*, DcmA) from *Methylobacterium rhodesianum* utilize glutathione as a nucleophile to catalyze conjugation and cleavage of dichloromethane, forming *S*-chloromethylglutathione intermediates[320–322] (Fig. 15B). Psychrophilic bacteria such as *Psychrobacter* TaeBurcu001 have been shown to degrade 2,2-dichloropropionic acid (Dalapon) at low temperatures through (*S*)-2-haloacid dehalogenase homologs, expanding the ecological scope of dehalogenases into polar environments.[323] In addition, artificial dehalogenases have been engineered to tackle challenging bond transformations. For instance, natural 4-chlorobenzoyl-CoA dehalogenase from *Pseudomonas* sp. strain CBS-3 has been modified with an unnatural photosensitizer and a Ni^{II} complex, enabling the conversion of diverse aryl halides to phenols as well as the formation of C–N bonds[324] (Fig. 15C). The application of genome-resolved discovery and protein-engineering continues to expand the diversity and capabilities of dehalogenases, opening up new options for the bioremediation of stubborn pollutants such as PFAS, halophenols, chlorinated solvents, and aromatic herbicides.

4. Halogenation and dehalogenation applied in synthetic biology

As more halogenases and dehalogenases are identified and structurally characterized, their applications in synthetic biology are rapidly expanding. While many known halogenases have yet to be fully explored, several have already been harnessed for the engineered biosynthesis of halogenated compounds. These examples illustrate the growing potential for using biocatalytic halogenation and dehalogenation in the production of novel chemicals, pharmaceuticals, and advanced materials.

4.1 Halogenases in synthetic biology applications

With the rapid discovery and structural elucidation of new halogenases, especially flavin-dependent halogenases (FDHs), synthetic biology has gained a powerful set of tools for regioselective halogenation under mild, environmentally friendly conditions. When expressed in microbial or plant hosts, halogenases enable the biosynthesis of structurally diverse, often bioactive halogenated compounds that are otherwise difficult to produce by traditional chemical means. While many halogenases remain undercharacterized, an increasing number have now been successfully integrated into engineered biosynthetic pathways, highlighting their promise for sustainable biomanufacturing (Table 7). Among these, tryptophan halogenases stand out as the most extensively studied and widely applied. These FDHs enable site-specific halogenation of the indole ring in tryptophan, unlocking molecular modifications that are challenging with standard chemistry. For instance, expression of RebH, Thal, and PyrH in *S. albus* led to the biosynthesis of over 30 halogenated indolocarbazole derivatives[325] (Fig. 16A). In *Catharanthus roseus*, the introduction of halogenase machinery enabled the generation of novel

Table 7 The representative examples of halogenases used in synthetic biology

Halogenase	Origin	Engineered host	Function/role	Representative product(s)	References
*D*nHal	*Dirinaria* sp.	*Pichia pastoris*	Halogenation of tryptophan and methyl haematommate	Chlorinated fungal compounds	346
FlA	*Streptomyces cattleya*	*Salinispora tropica*	C–F bond formation from fluoride ion	Fluorosalinosporamide, 5′-FDA	339
		Pseudomona putida		5′-FDA, fluorosugars	334
		E. coli		5′-FDA	340
Fre-L3-SttH (SttH and Fre fusion enzyme)	SttH from *Streptomyces toxytricini*	*E. coli*	6-Halogenation of indole for dye biosynthesis	6-Chloro-/6-bromoindigo, Tyrian purple	344
PrnA	*Pseudomonas fluorescens* BL91	*Streptomyces coeruleorubidus*	*In situ* chlorination of the uridyl peptide antibiotic pacidamycin	Pacidamycin analogs	65 and 328
		Streptomyces coelicolor M1154	Production of 7-Br-tryptophan	Br-pacidamycin D	329
	Serratia plymuthica G3/*Pseudomonas fluorescens*/*Serratia grimesii*	*Corynebacterium glutamicum*	7-Halogenation of L-tryptophan	Aminopyrrolnitrin (APRN)	332
	Pseudomonas fluorescens	*E. coli*	7-Halo-tryptophan	26 halogenated indole derivatives	333
PyrH	*Streptomyces rugosporus* NRRL 21084	*Streptomyces albus*	Complementary to RebH in indolocarbazole diversification	Halogenated indolocarbazole derivatives	325
		Catharanthus roseus (plant)	In planta chlorination of tryptamines	Chlorinated monoterpene indole alkaloids	326
		E. coli	5-Halo-tryptophan	26 halogenated indole derivatives	333
RebH	*Lechevalieria aerocolonigenes* ATCC 39243	*Catharanthus roseus* (plant)	Chlorination of tryptamines in planta	Chlorinated monoterpene indole alkaloids	326
		E. coli	7-Halo-tryptophan	26 halogenated indole derivatives	333
		S. albus	Halogenation of indole ring in indolocarbazole pathway	Over 30 halogenated indolocarbazole derivatives	325
RadH$^{\mathrm{D465E/T501S}}$	*Chaetomium chiversii*	*E. coli*	Regioselective halogenation of aromatic precursors	Halogenated coumarin derivatives	327
SpFDH-GDH-FR[a]	SpFDH from *Streptomyces pratensis*; GDH from *Priestia megaterium*; FR from *E. coli*	*E. coli*	6-Halo-tryptophan	Improved halogenation yield with cofactor self-sufficiency	330
SttH	*Streptomyces toxytricini*	*E. coli*	6-Halo-tryptophan	26 halogenated indole derivatives	333
SttH-nig02	*Pseudoalteromonas nigrifaciens*	*E. coli*	Regioselective halogenation of indole	Red and blue halogenated indigo derivatives	345
Thal	*Streptomyces albogriseolus*	*Streptomyces albus*	Complementary to RebH in indolocarbazole diversification	Halogenated indolocarbazole derivatives	325
		E. coli	6-Halo-tryptophan	26 halogenated indole derivatives	333
Th-Hal	*Streptomyces violaceusniger*	*E. coli*	6-Halo-tryptophan	26 halogenated indole derivatives	333
XszenFHal (XsHal)	*Xenorhabdus szentirmaii*	*E. coli*	5-Halo-tryptophan	26 halogenated indole derivatives	333

[a] Glucose dehydrogenase (GDH), and flavin reductase (FR).

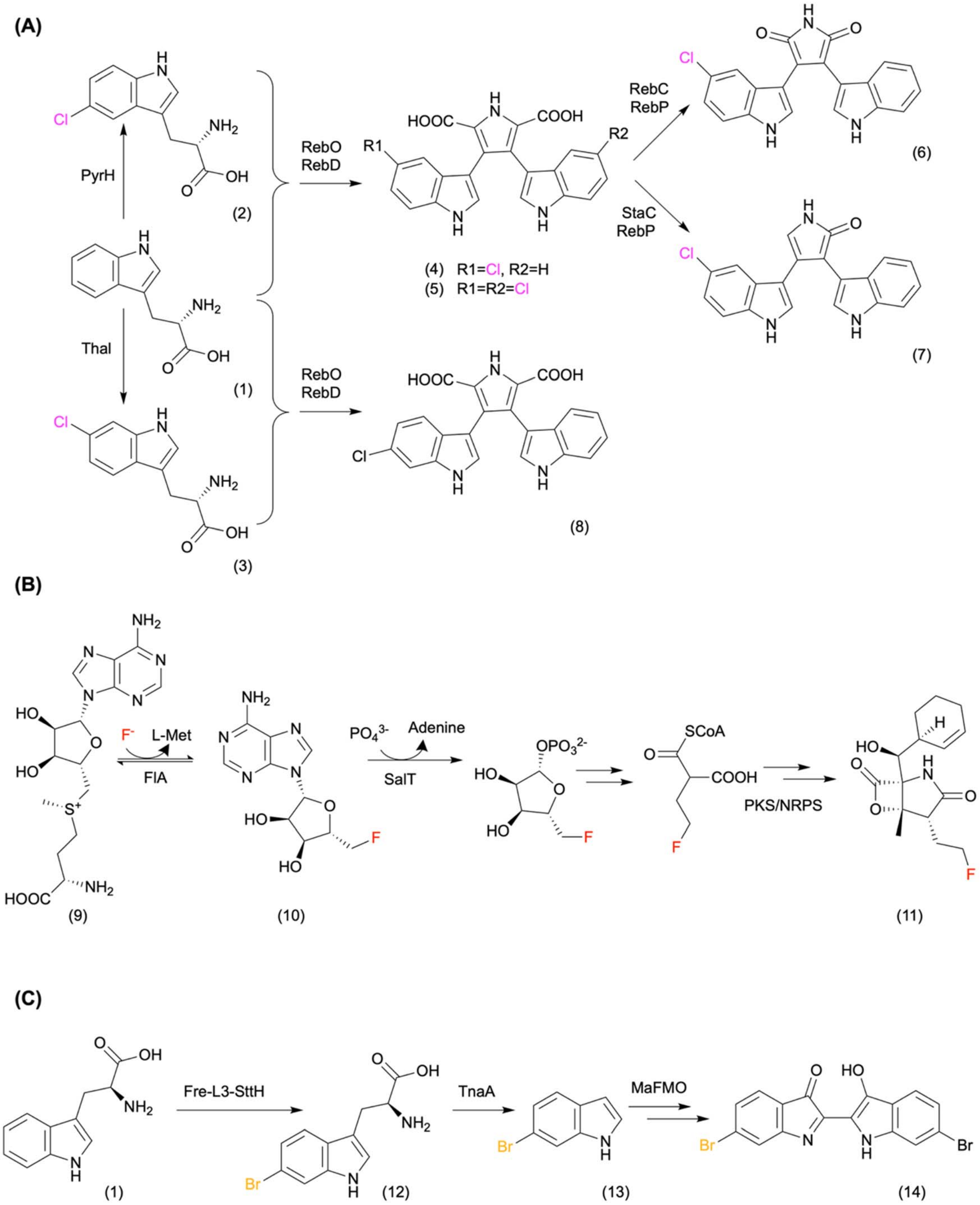

Fig. 16 Representative applications of halogenases in synthetic biology. (A) Modular biosynthetic pathways for generating halogenated bisindole compounds in *S. albus via* heterologous coexpression of tryptophan halogenases (PyrH, Thal) and rebeccamycin pathway enzymes. The system yields regioselectively chlorinated tryptophan derivatives, 5-chloro-tryptophan (**2**), 6-chloro-tryptophan (**3**), and their further conversion to halogenated bisindole intermediates, including 9-chloro-chromopyrrolic acid (CPA, (**4**)), 9,9′-dichloro-CPA (**5**), 10-chloro-CPA (**8**), and advanced products such as 3-chloro-arcyriaflavin A (**6**) and 3-chloro-staurosporine aglycone (**7**). Adapted from ref. 325. (B) FlA-mediated biosynthesis of 5′-fluoro-5′-deoxyadenosine (5′-FDA, (**10**)) and fluorosalinosporamide (**11**) in engineered *Salinispora tropica*. The fluorinase FlA catalyzes the transformation of SAM (**9**) and fluoride ion into 5′-FDA, which is incorporated into the PKS/NRPS pathway to yield fluorosalinosporamide. Adapted from ref. 339. (C) Microbial biosynthesis of Tyrian purple (6,6′-dibromoindigo, (**14**)) in engineered *E. coli* strains co-expressing tryptophan 6-halogenase SttH, tryptophanase TnaA, and flavin-containing monooxygenase MaFMO. The pathway enables the conversion of L-tryptophan (**1**) to 6-bromo-tryptophan (**12**), 6-bromoindole (**13**), and finally to the purple dye 6,6′-dibromoindigo (**14**). Adapted from ref. 344.

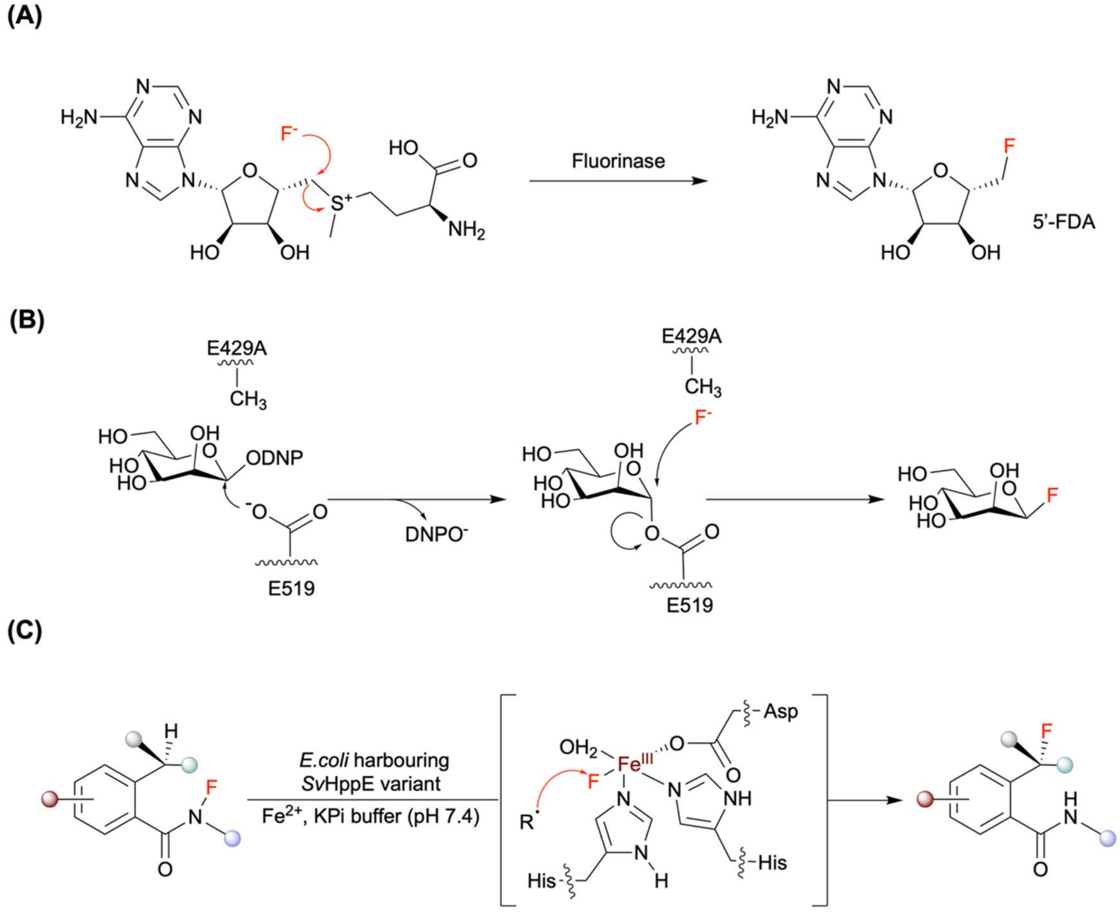

Fig. 17 Representative enzymatic pathways and intermediates for carbon–fluorine (C–F) bond formation. (A) Fluorinase employs a hydrogen-bonding network to desolvate fluoride and align SAM for nucleophilic substitution by fluoride.[119,347] Adapted from ref. 342. (B) Retaining glycosidase *Cellulomonas fimi* β-mannosidase (Man2A) mutant (E429A) catalyzing the formation of carbon–fluorine bonds through nucleophilic fluoride attack. Adapted from ref. 348. (C) Repurposed (*S*)-2-hydroxypropylphosphonate epoxidase from *Streptomyces viridochromogenes* (*Sv*HppE) catalyzing the C–F bond formation *via* an abiological, metal-mediated radical fluorination pathway in potassium phosphate (KPi) buffer. Adapted from ref. 342. Red, purple, gray, and green spheres indicate substituents.

chlorinated alkaloids.[326] Protein engineering and directed evolution have further expanded halogenases utility. High-activity RadH mutants have enabled *E. coli* to produce non-natural halogenated coumarins directly from simple precursors, bypassing the need for hazardous halogenating agents.[327] Host engineering has also proven valuable: introducing the tryptophan halogenase prnA into *S. coeruleorubidus* allowed *in situ* chlorination of the antibiotic pacidamycin, while transfer of the biosynthetic cluster into bromide-tolerant *S. coelicolor* enabled efficient bromopacidamycin production.[328,329] One challenge for FDH applications at scale is the need for redox cofactors. Multifunctional fusion proteins have been developed to enable *in situ* cofactor regeneration, as seen in tri-enzyme systems that achieve high-yield tryptophan halogenation in continuous-flow bioreactors.[330] At the same time, optimization of microbial hosts, such as engineered *Saccharomyces cerevisiae* and *Corynebacterium glutamicum*, has enabled the production of mono- and di-halogenated tryptophan derivatives and downstream products.[331,332] Modular and co-culture strategies have also broadened product diversity. A plug-and-play *E. coli* platform produced multiple halogenated tryptophan analogs from glucose, which were further diversified by downstream enzymes to yield a wide array of novel halogenated compounds.[333] The frontier of enzymatic fluorination is evolving rapidly. While classical biocatalytic C–F bond formation relies on nucleophilic fluorinases, recent work has engineered microbial hosts to overcome fluoride toxicity and boost precursor availability[334–341] (Fig. 16B and 17). Even more recently, radical-based enzymatic fluorination has been achieved by repurposing natural enzymes, such as the transformation of SvHppE from an epoxidase into an efficient radical fluorination catalyst *via* directed evolution[342] (Fig. 17C). Cooperative photoenzymatic catalysis has further expanded the synthetic possibilities, enabling the formation of stereoselective fluorinated ketones and cyclic products through enzyme–photocatalyst cascades.[343] Beyond pharmaceuticals, halogenases have found applications in sustainable dye production, such as the biosynthesis of Tyrian purple (6,6′-dibromoindigo) in *E. coli*[344] (Fig. 16C). Pigment properties can be tuned by regioselective halogenation with enzymes from marine bacteria.[345] Recent discoveries also point to a broader evolutionary and taxonomic diversity. Flavin-dependent halogenases from lichenized fungi, such as DnHal

Table 8 The representative examples of dehalogenases and dehalogenase used in synthetic biology

Dehalogenase	Origin	Engineered host	Function/role	Representative application/ product	References
DhaA	*Rhodococcus rhodochrous* NCIMB 13064	*Pseudomonas putida* KT2440 *E. coli* BL21 (DE3)	Hydrolytic dehalogenation of haloalkanes	Mineralization of 1,2,3-trichloropropane (TCP)	355 372
Hakp1	*Klebsiella pneumoniae* ITB1	*E. coli* BL21 (DE3)	Widely used as building blocks in the production of carboxymethyl cellulose (CMC), 2,4-dichlorophenoxyacetic acid (2,4-D), and 2-methyl-4-chlorophenoxyacetic acid (MCPA) herbicides	Monochloroacetic acid degradation	362
HHDHs	HheC from *Agrobacterium radiobacter* AD1; HheD15 from *Candidatus Competibacter denitrificans* Run_A_D11	*E. coli* BL21 (DE3)	Epoxide synthesis from β-haloalcohols	Biocatalytic production of chiral oxiranes/ oxazolidinones in engineered pathways	356
NpRdhA	*Nitratireductor pacificus*	*E. coli* BL21 (DE3)	Reductive dehalogenation of iodinated phenols and alkanes	Heterologous expression of cobalamin dependent class-III enzymes	357 and 373
TmrA	*Dehalobacter* sp. UNSWDHB	*E. coli* BL21 (DE3)	Chloroalkanes dehalogenation	Present a relatively straightforward *E. coli* expression system that has performed well for a variety of *Dehalobacter* spp. RDases	357

from *Dirinaria* sp., have shown activity toward tryptophan and other substrates, and can be heterologously produced in *Pichia pastoris*.[346] With new discoveries and engineering strategies emerging rapidly, the toolbox for biocatalytic halogenation in synthetic biology will only continue to grow.

4.2 Dehalogenases in synthetic biology applications

Dehalogenases have become important tools in synthetic biology, supporting both environmental and industrial applications by enabling the degradation, transformation, and valorization of halogenated compounds. Their roles now span bioremediation, biocatalysis, and the engineering of microbial consortia for synthetic metabolic pathways. A major advance has been the development of synthetic biodegradation pathways targeting stubborn pollutants that resist natural microbial attack (Table 8). For example, efficient aerobic degradation of 1,2,3-trichloropropane (TCP) required not only directed evolution of the haloalkane dehalogenase DhaA, but also careful integration of improved enzyme variants (such as DhaA31, DhaM2, and DhaA90R) into industrially relevant hosts like *Pseudomonas putida* KT2440.[245,349–354] Additional genetic modifications like removing repressors, improving oxygen uptake, and suppressing flagellar synthesis, produced robust TCP-degrading strains that have demonstrated high efficiency in bioreactor settings[245,349–353,355] (Fig. 18A). HHDHs have also demonstrated useful in asymmetric synthesis. Engineered *E. coli* expressing HHDHs can convert β-haloalcohols into optically pure epoxides and oxazolidinones in one step, providing valuable routes for pharmaceutical manufacturing with high yields and enantioselectivity[356] (Fig. 18B). RDases remain technically challenging due to their cobamide and iron-sulfur requirements, but breakthroughs in heterologous expression, such as co-expressing the *btu* operon in *E. coli* and optimizing refolding protocols, have expanded their applicability. Functional RDases, including TmrA and NpRdhA, can now be used for the detoxification of a wide range of pollutants, such as chloroform and iodinated phenolics.[357–359] Recent advances have even produced oxygen-tolerant and NADPH-dependent RDases, broadening the range of potential applications to more industrial and aerobic settings.[359–361] For hydrolytic dehalogenation, haloacid dehalogenases are being used to degrade compounds like monochloroacetic acid (MCA). Optimizing their recombinant expression, for example, through response surface methodology in *E. coli*, has yielded efficient biocatalysts for environmental remediation.[362] Mechanistic studies, such as site-directed mutagenesis of *Pseudomonas aeruginosa* homologs, have further clarified the molecular basis of activity and substrate specificity.[362,363] Significant progress has also come from protein engineering. Rational design and AI-guided methods have improved catalytic efficiency at key steps (*e.g.*, S_N2 transition state stabilization in haloalkane dehalogenases).[364] Surface charge engineering and polymer conjugation have increased tolerance to heat, low pH, salt, and organic solvents.[223,365–368] Advanced immobilization technologies,

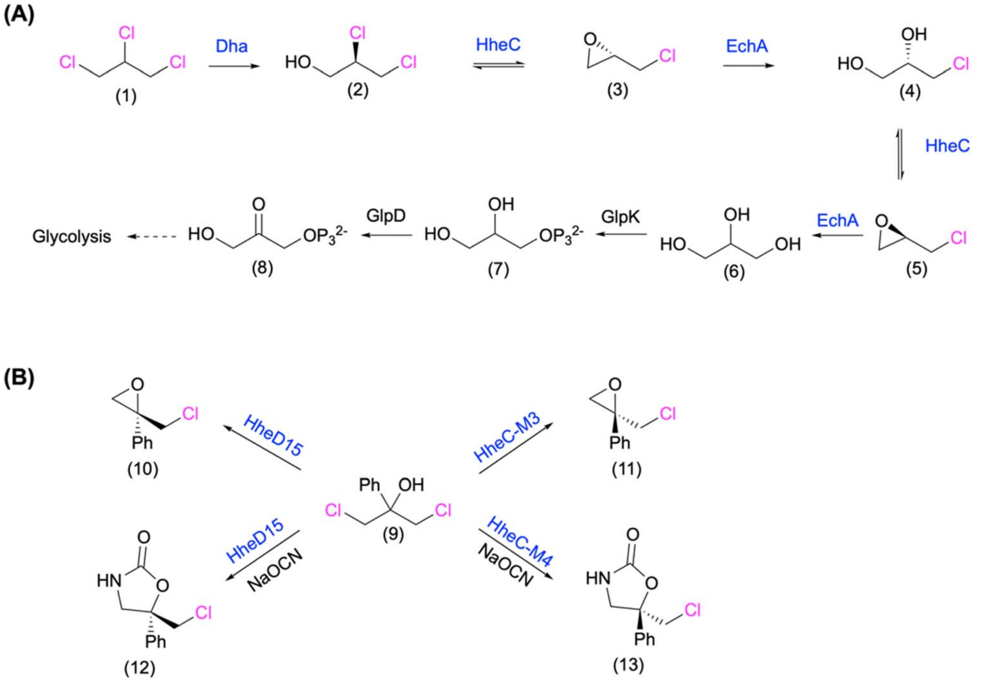

Fig. 18 Representative application of dehalogenases in synthetic biology. (A) Construction of a synthetic metabolic pathway for aerobic mineralization of 1,2,3-trichloropropane (TCP, (**1**)) in engineered *Pseudomonas putida* KT2440. The pathway incorporates three heterologously expressed dehalogenases (highlighted in blue): DhaA, a haloalkane dehalogenase from *Rhodococcus rhodochrous* NCIMB 13064; HheC, a halohydrin dehalogenase; and EchA, an epoxide hydrolase, both from *Agrobacterium radiobacter* AD1. These enzymes sequentially convert TCP through (*R*,*S*)-2,3-dichloropropane-1-ol (**2**), (*R*,*S*)-epichlorohydrin (**3**), (*R*,*S*)-3-chloropropane-1,2-diol (**4**), (*R*,*S*)-glycidol (**5**), and glycerol (**6**), which then enters central metabolism as glycerol-3-phosphate (**7**) and dihydroxyacetone phosphate (**8**). Adapted from ref. 355. (B) Biosynthesis of chiral epoxides and oxazolidinone by engineered *E. coli* expressing dehalogenases (HheD15, HheC-M3, or HheC-M4). Halohydrin dehalogenases are used to achieve stereoselective transformations from 1,3-dichloro-2-phenylpropan-2-ol (**9**) to (*R*)-chiral epoxides (**10**), (*S*)-chiral epoxides (**11**), (*R*)-chiral oxazolidinone (**12**), and (*S*)-chiral oxazolidinone (**13**). This approach enables efficient production of enantiopure intermediates for pharmaceuticals and fine chemicals. Halogen atoms are color-coded: chlorine (Cl, bright magenta), fluorine (F, red), bromine (Br, orange). Adapted from ref. 356.

including metal–organic frameworks (MOFs) and hybrid nanocomposites, further improve enzyme stability and recyclability.[369,370] The use of computational tools has accelerated these advances. AlphaFold, for example, now enables accurate modelling of dehalogenase structures in various ligand-bound states, directly informing the engineering of active sites and substrate channels.[371] Empirical valence bond and metadynamics simulations have helped clarify catalytic mechanisms and energy barriers.[364] These advances have enabled dehalogenases to move from the laboratory into real-world applications in environmental remediation and synthetic biology, laying a solid foundation for the development of more efficient and sustainable biotransformation systems.

5. Perspectives

HOCs will continue to drive innovation across pharmaceuticals, agricultural and industrial chemicals, and advanced materials. Yet, the persistent reliance on traditional chemical methods for halogenation and dehalogenation is increasingly misaligned with the global imperative for greener, more resource-efficient processes. In this context, enzyme-catalyzed halogenation and dehalogenation have emerged as powerful alternatives, offered superior site-selectivity, milder reaction conditions, and enhanced environmental compatibility. However, the field remains in a relatively early stage of development. A major bottleneck is the limited diversity of known halogenases and dehalogenases, particularly those capable of acting on challenging bonds such as C–F or C–I. Natural enzymes often exhibt narrow substrate specificity, low stability under industrial conditions, and poor compatibility with existing biocatalytic workflows due to low activity or difficulties in heterologous expression. Meanwhile, bioremediation of persistent organohalogens, especially “forever chemicals” like PFAS, often requires complex, multi-enzyme systems and remains a major technical challenge. Looking ahead, there are compelling opportunities for advancement. One of the most promising direction lies in the discovery of novel enzymes from extreme and previously untapped environments. Deep-sea, hadal zone, polar, and contaminated sites have already yielded unique microbial lineages with unexpected halogen metabolism. For instance, projects such as the Mariana Trench metagenomic surveys[374] have started to uncover halogenases and dehalogenases that defy existing mechanistic frameworks and naturally tolerate extreme pressure, salinity, or toxic substrates, traits highly desirable for industrial and environmental applications. At the same time, breakthroughs in AI-driven protein engineering, deep learning-based structure prediction tools like

AlphaFold 3,[375] and *de novo* enzyme design platforms like RFdiffusion[376] are rapidly transforming the way we discover and refine biocatalysts. These advances enable not only accelerated gene prospecting but also the custom design of enzymes for functions and environments that have no precedent in nature. Directed evolution and machine-learning-guided mutagenesis[377] are allowing fine-tuning of selectivity and stability, while synthetic biology platforms facilitate the integration of these parts into robust, modular pathways. The next decade will likely see the emergence of fully engineered "designer" halogenases and dehalogenases, some inspired by nature, others developed entirely *in silico*, that bridge the gap between laboratory innovation and real-world application. Integration with process automation, continuous manufacturing, and green chemistry principles will be critical for translating these advances into scalable solutions. Achieving these ambitions will require more than technical innovation. It will demand strong cross-disciplinary collaboration spanning synthetic biology, computational science, environmental engineering, and public policy. Equally important will be open data sharing and new partnership models between academia and industry. Ultimately, success in this field will not only enable the precise construction and deconstruction of halogenated molecules but also usher in a new era of chemical synthesis—one defined by sustainability, selectivity, and molecular innovation.

6. Author contributions

Yaojun Tong and Sang Yup Lee conceived and supervised the manuscript. Jing Luo, Na Li, Jia Wang, and Yaojie Gao, Hongzhi Tang, Linquan Bai, Sang Yup Lee, and Yaojun Tong wrote the manuscript. All authors proofread the entire manuscript and provided suggestions for improvement on all sections.

7. Conflicts of interest

There are no conflicts to declare.

8. Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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