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Transcriptional regulation of organohalide pollutant utilisation in bacteria

Bruno MAUCOURT, Stéphane VUILLEUMIER and Françoise bRINGEL*

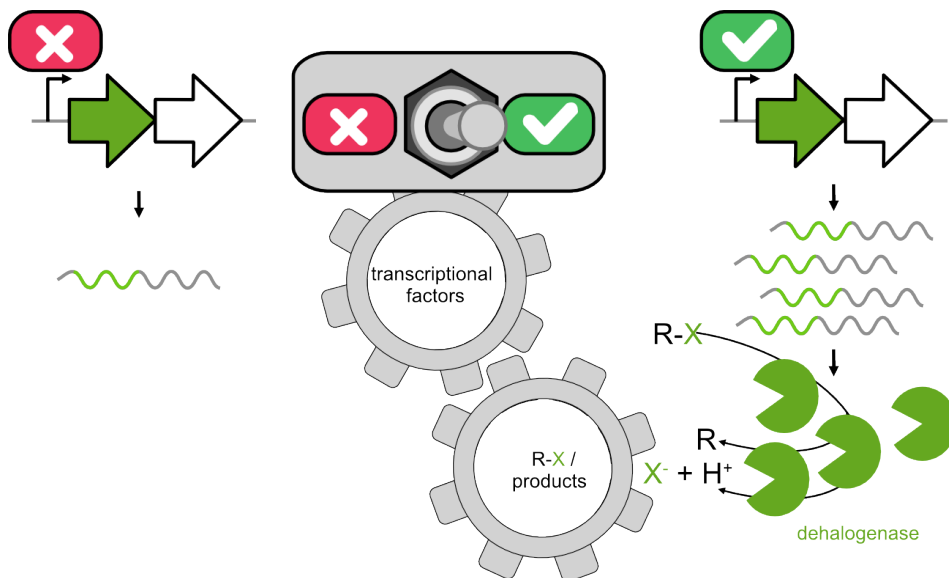
Université de Strasbourg, UMR 7156 CNRS, Génétique Moléculaire, Génomique, Microbiologie,
Strasbourg, France

* Correspondence: Françoise Bringel; Email: francoise.bringel@unistra.fr; phone: + 33 (0)3 68 85 18
15; address: 4 allée Konrad Roentgen, 67000 Strasbourg, France.

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Graphical Abstract:



Abstract

Organohalides are organic molecules formed biotically and abiotically, both naturally and through industrial production. They are usually toxic and represent a health risk for living organisms, including humans. Bacteria capable of degrading organohalides for growth express dehalogenase genes encoding enzymes that cleave carbon-halogen bonds. Such bacteria are of potential high interest for bioremediation of contaminated sites. Dehalogenase genes are often part of gene clusters that may include regulators, accessory genes and genes for transporters and other enzymes of organohalide degradation pathways. Organohalides and their degradation products affect the activity of regulatory factors, and extensive genome-wide modulation of gene expression helps dehalogenating bacteria to cope with stresses associated with dehalogenation, such as intracellular increase of halides, dehalogenase-dependent acid production, organohalide toxicity, and misrouting and bottlenecks in metabolic fluxes. This review focuses on transcriptional regulation of gene clusters for dehalogenation in bacteria, as studied in laboratory experiments and *in situ*. The diversity in gene content, organization, and regulation of such gene clusters is highlighted for representative organohalide-degrading bacteria. Selected examples illustrate a key, overlooked role of regulatory processes, often strain-specific, for efficient dehalogenation and productive growth in presence of organohalides.

Highlights

- Regulatory processes are key to bacterial dehalogenation and bioremediation of sites contaminated with organohalide pollutants
- Genes for dehalogenases, regulators, and associated proteins are often found in clusters
- Expression of dehalogenation gene clusters is mostly coordinately regulated
- Regulation of dehalogenation is often strain-specific and may also involve alternative non-halogenated growth substrates

Introduction

Organic compounds containing one or several carbon-halogen bonds are produced by both biotic and abiotic processes (Gribble, 2010; Fig. 1). They are often toxic to animals (Jayaraj, Megha and Sreedev 2016), fungi (Bernat *et al.* 2018), plants (Clausen and Trapp 2017), and micro-organisms (Tobajas *et al.* 2016). Many such compounds have been massively produced industrially, and released into the environment. They may thereby threaten human health, especially at the high concentrations found in chemical production and usage sites. Such compounds have been diversely referred to in the literature as ‘halogenated organic compounds’, ‘organohalogens’, or ‘organohalides’. In this review, we chose to use the latter term throughout, in agreement with the prevailing consensus among scientists in the field (Leys, Adrian and Smidt, 2013).

Some bacteria degrade and use organohalides for growth, as carbon or energy source, or as electron acceptors for respiration (Atashgahi *et al.* 2018). So far, research on microbial dehalogenation has

focussed on the process of enzymatic dehalogenation itself (see recent reviews of Agarwal *et al.*, 2017; Ang *et al.*, 2018; Fincker and Spormann, 2017), and essentially on bacteria, with only relatively few studies on fungi (e.g. Marco-Urrea *et al.* 2015). This review addresses a neglected aspect of dehalogenation: the regulation of dehalogenase production, mainly at the transcriptional level, and the impact of different factors and processes on organohalide degradation. To our knowledge, only the regulation of reductive dehalogenases has recently been reviewed in-depth (Maillard and Willemin 2019). Here, we will focus on acquired knowledge of regulated gene clusters for growth with organohalide pollutants released into the environment as the result of agricultural and industrial practices, and emphasize both general and substrate-specific processes involved in the detection, detoxification and degradation of these chemicals. We chose not to include pharmaceutically relevant organohalides, such as antibiotics (e.g. chloramphenicol, vancomycin) and biocides (e.g. triclosan), since reviews on such compounds have also recently become available (e.g. Novotna, Kwun and Hong 2016). Most of the examples discussed in this review will involve organochlorine pollutants, which traditionally have been better studied. However, much of this research is also relevant to homologous (notably brominated) organohalides, which are often transformed by the same systems (Chae and Zylstra 2006; Ferreira *et al.* 2009; Farhan Ul Haque *et al.* 2013).

Differences in the structural type (e.g. aliphatic or aromatic) and nature of substituents (e.g. nitrogen- or sulphur-containing) of organohalides provide them with diverse properties in terms of usage, toxicity, and stability. Carbon-halogen bonds are often poorly reactive, rendering many organohalides recalcitrant to biodegradation. Half-lives of organohalides vary extensively (e.g. 45 days for pentachlorophenol, and 15 years for DDT) (Jayaraj, Megha and Sreedev 2016). Some organohalide pesticides are still detected in the environment many years after having been banned by the Stockholm Convention on persistent organic pollutants (Wöhrnschimmel *et al.* 2016). In this context, the capacity of specific bacteria to degrade organohalides has attracted much interest, starting already over half a century ago (e.g. Kearney and Kaufman 1965; Wedemeyer 1967; Goldman *et al.* 1968). Carbon-halogen bond cleavage by environmental bacteria is catalysed by a wide diversity of enzymes (see e.g. Temme *et al.*, 2019), and may involve oxidative (e.g. 4-chlorophenylacetate 3,4-dioxygenase; EC 1.14.12.9), reductive (e.g. reductive dehalogenase; EC 1.97.1.8), hydrolytic (e.g. haloalkane dehalogenase; EC 3.8.1.5) or thiolytic (e.g. dichloromethane dehalogenase; EC 4.5.1.3) reactions (Agarwal *et al.* 2017; Fincker and Spormann 2017; Ang *et al.* 2018). Regulatory processes of dehalogenase gene expression are often an overlooked aspect of bioremediation of organohalides although dehalogenases are generally among the most abundant proteins in bacteria growing with these compounds as the sole carbon and energy source, reaching up to at least 20% of total soluble proteins (Muller *et al.* 2011; van den Wijngaard, Reuvekamp and Janssen 1991). It has been hypothesized that high cellular levels of dehalogenases may be needed for faster growth with organohalides, especially when enzymes have low turnover and low substrate affinity, or when the substrates are cytotoxic (van den Wijngaard, Reuvekamp and Janssen 1991; Gisi *et al.* 1998).

Conversely, the energy required to express dehalogenases at such high levels in the absence of pollutants would put an unnecessary burden on cellular metabolism. Thus, it makes sense that bacteria efficiently regulate the expression of dehalogenases and other genes associated with degradation of organohalides.

Organization and regulation of gene clusters involved in metabolism of organohalides

Dehalogenase-encoding genes are often clustered together with regulator genes, other genes involved in the catabolism and transport of organohalides, and accessory genes (Fig. 2). For example, as recently reviewed specifically (Kruse, Smidt and Lechner 2016; Maillard et Willemin 2019), reductive dehalogenase (*rdh*) gene clusters are generally located immediately adjacent to transcriptional regulatory genes. Gene clusters for organohalide degradation are diverse in genetic organisation, regulatory genes, and genomic localization (e.g. chromosomal or plasmidic, Fig. 2) (Sentchilo *et al.* 2003; Tabata *et al.* 2016; Nielsen *et al.* 2017). Such oftentimes patchwork assembly can be explained in part by horizontal gene transfer events driven by selective pressure for the capacity to degrade organohalides in contaminated environments. This is confirmed by ample co-occurrence of insertion sequences (Schmid-Appert *et al.* 1997), transposases (Rhee *et al.*, 2003; Maillard *et al.*, 2005), recombinases (Muller *et al.* 2004), or CRISPR-Cas systems (Molenda *et al.* 2019) in gene clusters for dehalogenation. Frequent association of gene clusters for degradation of organohalides with mobile genetic elements, genomic islands or integrative and conjugative elements (Sentchilo *et al.* 2003; McMurdie *et al.*, 2009; Liang *et al.* 2012) has likely been instrumental for selection and distribution of the most appropriate dehalogenation pathways (Liang *et al.* 2012). Gene clusters for dehalogenation occur both in single polycistronic operons as well as multiple co-regulated operons (i.e., a regulon).

The organization of gene clusters involved in transformation of organohalides may be classified in two groups. The first comprise gene clusters with a dehalogenase gene and vary greatly in size (from 2 to 20 genes including often one or several regulatory genes). In the lindane-degrading strain *Sphingobium japonicum* UT26, as an impressive example, 16 *lin* genes associated with 6 dehalogenation reactions of the pathway are found in several gene clusters scattered across two chromosomes and one plasmid (Fig. 2A) (Tabata *et al.* 2016). This represents a challenge for robust and streamlined applications of dehalogenation metabolism for bioremediation. So far, only a few compact synthetic gene clusters for dehalogenation have been reported, e.g. for utilisation of 1,3-dichloropropene and dichloromethane (Nikel and de Lorenzo 2013; Michener *et al.* 2014a) (Fig. 2B).

In contrast, organohalide-relevant catabolic gene clusters lacking dehalogenase genes lead to the formation of halogenated metabolites that often accumulate. For example, the *bph* gene cluster affects both biphenyl and polychlorinated biphenyl degradation, e.g. in *Rhodococcus jostii* RHA1 (Takeda *et al.* 2010) (Fig. 2C). Transcription factors in such clusters contribute to regulate expression of cluster genes, and also recognize halogenated homologs of biphenyls (Takeda *et al.* 2010).

Transcription factors and their ligands in regulation of organohalide degradation

In gene clusters with dehalogenase genes, regulatory genes are commonly found in direct proximity to those of dehalogenases, and often in opposite orientation, a common characteristic of transcriptional regulators in bacteria (Beck and Warren 1988) (Fig. 2). This facilitates concerted expression (Beck and Warren 1988) and production of transcription factors nearby targeted genes, and favours efficient recognition of corresponding DNA-binding sites by preventing spatial diffusion (Weng and Xiao 2014). Transcription factors feature at least two domains: a DNA-binding domain recognizing a specific DNA sequence (often an inverted repeat located upstream or around the target promoter), and a sensor domain, often termed companion domain or effector-binding domain (Perez-Rueda *et al.* 2018). Additional domains may also be found, such as an RNA polymerase sigma 54 factor domain which favours RNA polymerase interactions for increased gene expression in the presence of pollutant (see Fig. 3A, MopR).

Transcription factors involved in organohalide detection usually carry a Helix-Turn-Helix (HTH) DNA-binding domain (Fig. 3). This HTH domain is found in about half of known bacterial transcription factors (Rivera-Gomez, Segovia and Perez-Rueda 2011), and indeed in the majority of regulators involved in biodegradation (de Lorenzo *et al.* 2010).

Sensor domains in organohalide-responsive transcription factors usually recognize the organohalide itself or one of its metabolites. Binding of small ligands induces conformational changes in transcription factors (Fig. 4), thereby affecting their DNA-binding properties (Anantharaman and Aravind 2005; Möglich, Ayers and Moffat 2009). Ligand binding and its role in regulation can be tested using electrophoretic mobility shift assays of DNA and DNase footprints in the presence or absence of regulator proteins (Whangskuk *et al.* 2010; Starr, Fruci and Poole 2012; Sallabhan *et al.* 2013; Torii *et al.* 2013; Wagner *et al.* 2013). Regulatory processes can also be investigated by comparing global gene expression in cultures in the presence or absence of organohalides, or by using gene-specific transcriptional fusion and RT-qPCR assays (Table 1). New powerful techniques continue to be developed for this purpose. For example, binding of trichloroethene by dehalogenase and a two-component system was demonstrated using unbiased, high-throughput thermal proteome profiling (Türkowsky *et al.* 2018b).

Crystal structures can suggest how ligands bind to transcription factors (Table 1). Several organohalides act as ligands binding their transcriptional regulators (Chae and Zylstra 2006; Sanchez and Gonzalez 2007; Hayes *et al.* 2014). Some transcription factors feature one cavity in the sensor domain and can bind several different organohalides (Ray *et al.*, 2016). Other factors have distinct cavities that allow binding of different molecules for regulatory fine-tuning (Fig. 4, PcpR) (Hayes *et al.* 2014). Halogen atoms themselves are not always directly involved in ligand binding (Fig. 4). In some cases, metabolites obtained after cleavage of organohalide carbon, bonds are also recognized by their transcription factors (Platero *et al.* 2012). Thus, transcription factors relevant to organohalides degradation are not restricted to organohalides themselves. Ligand specificity is determined by

properties of the identified binding sites (volume, solvent accessibility, hydrophobicity) (Liang, Woodward and Edelsbrunner 1998), so that detailed structures of transcription factors serve to predict the binding of new ligands by molecular docking, similarly to what can be done for enzymes and their substrates, including dehalogenases (Daniel *et al.* 2015; Yang and Lai, 2017). Some families of transcription factors have been associated with specific organohalides. For example, transcription factors of the LysR family (e.g. CatR, ClcR, LinR, PcpR; Fig. 2; Table 1) are mainly involved in the detection of aromatic organohalides such as chlorobenzoate or chlorophenol. Transcription factors of the NtrC family (DmpR, MopR, XylR) (Ray *et al.* 2016) are able to detect both aromatic compounds with or without halogen substituents, albeit with lesser specificity. On the other hand, transcription factors of the MarR-type, such as RdhR in *Dehalococcoides mccartyi*, and of the CRP-FNR type, such as CprK in *Desulfotobacterium* strains, are often involved in the regulation of halogenated aliphatic solvents (Pop *et al.*, 2004) (Fig. 5D). For other transcription factor families, in contrast, no clear trends have been found. For example, transcription factor ChpR, associated with chlorpyrifos detection and transformation, is the only member of the CadC family known to respond to organohalides (Whangsuk *et al.* 2010).

Regulatory mechanisms in gene clusters for dehalogenation expression

The mechanistic details of regulatory processes can be studied at many levels, but the transcriptional level has been the most investigated so far. For organohalides, most studies have focused on transcription factors activating or repressing gene expression in response to organohalides. In some cases, the interference of other non-halogenated growth substrates on dehalogenase expression has been studied. The diverse ways by which transcription factors are known to affect gene expression in the context of degradation of organohalides are reviewed in the next paragraphs.

Modulation of dehalogenase expression by transcription factors

Expression of dehalogenases usually increases in the presence of organohalides. Activation of dehalogenase expression can involve binding of transcription factors to DNA, or alternatively their release from DNA, as well as their interaction with other proteins. For example, transcription of the reductive dehalogenase gene *cprA* is increased when CprK binds to chlorophenol (Maillard and Willemin 2019). Conversely, other transcription factors bind to operator sequences to block access to RNA polymerase in the absence of their ligands, thereby repressing transcription of downstream genes. This is the case for DcmR (La Roche and Leisinger 1991), HdhR (Sallabhan *et al.* 2013) and RdhR1625 (Krasper *et al.* 2016), identified as repressors of the expression of genes involved in the degradation of dichloromethane, chloroacetic acid and trichlorobenzene, respectively (Fig. 2). No ligand has yet been demonstrated experimentally for these factors. Interestingly, some regulators can switch between repressing and activating gene expression. In chlorobenzoate degradation, for example, the transcriptional regulator ClcR represses transcription of the chlorocatechol-1,2-dioxygenase gene *ClcA* by promoting DNA bending. Upon binding of 2-chloro-cis,cis-muconate,

ClcR switches from repressor to activator (Fig. 5), by interacting directly with the alpha subunit of the RNA polymerase to increase transcription activation (McFall, Chugani and Chakrabarty 1998).

Regulation of dehalogenase expression by multiple transcriptional regulators

Several studies have investigated the effects of multiple copies of gene clusters within a genome on regulation of organohalide degradation. For example, duplication of the *bphST* genes in *Rhodococcus jostii* RHA1 (Fig. 2C) and subsequent mutational drift and selection resulted in a *bphST* gene variant more responsive to chlorinated biphenyls, with unchanged DNA-binding sites but different ligand-sensing specificities (Takeda *et al.* 2010). Duplicated *bphST* copies are highly similar (92 and 97% protein identity between BphS1/BphS2 sensor histidine kinases and BphT1/BphT2 response regulators, respectively). BphS1T1 and BphS2T2 both detect chlorobenzene and 1,2-dichlorobenzene, but only BphS1T1 recognizes biphenyl (Takeda *et al.* 2010). Similarly, *Pseudomonas putida* RB1 carries two orthologous gene clusters involved in benzoate metabolism: *catR-catBCA* and *clcR-clcABD* of which only the latter is specific for chlorinated compounds (McFall, Chugani and Chakrabarty 1998). Transcriptional regulator CatR activates both *cat* and *clc* operons in presence of *cis,cis*-muconate, whereas ClcR only activates expression of the *clc* operon in presence of 2-chloro-*cis,cis*-muconate despite being able to bind the *cat* operon promoter. Thus, duplication of transcription factor genes can lead to ligand binding specificities (Perez and Groisman 2009).

Repression of dehalogenation metabolism by non-halogenated compounds

Catabolic repression is often observed in bacteria able to utilise other carbon sources and energy sources besides organohalides for growth (Govantes *et al.* 2010; Weir *et al.* 2006). For example, the tricarboxylic acid cycle intermediate fumarate competes with 2-chloro-*cis,cis*-muconate for binding to the transcription factor ClcR, inhibiting *clcABC* gene activation for chlorobenzoate utilisation by ClcR (McFall *et al.* 1997) (Fig. 5B). Intermediates of the tricarboxylic acid cycle and sugars repress *bph* gene expression and thereby PCB degradation in *Acidovorax* sp. KKS102 (Ohtsubo *et al.* 2006). This system involves a two-component regulator-sensor (*bphPQ*) located outside the *bph* gene cluster. BphQ recognizes and binds to the pE promoter upstream of the *bph* gene cluster when carbon sources other than PCBs are present (Ohtsubo *et al.* 2006). Catabolic repression of organohalide degradation is not limited to carbon when an organohalide can also serve as a source of nitrogen for bacterial growth. For example, repression of expression of genes for atrazine catabolism and inhibition of dehalogenation is observed in the presence of an alternative preferential nitrogen source (Platero *et al.* 2012). In this specific example, the gene cluster for dehalogenation *atzRSTUVW* includes a fixation site for transcription factor NtrC, in addition to a nucleoid protein binding site (integration host factor; IHF) (Platero *et al.* 2012) (Fig. 5), and NtrC represses transcription of *atz* dehalogenase genes in the presence of ammonium, nitrate or urea. Similarly, for some organohalides used as both carbon and sulphur source for growth, as in the case of degradation of the pesticide endosulfan by *Arthrobacter* sp. KW (Weir *et al.* 2006), the presence of other sulphur sources inhibits endosulfan degradation by an unknown mechanism.

Uptake of pollutants

In many cases, transport of organohalides into bacterial cells will also affect dehalogenase expression. In *Comamonas* sp. DJ-12 for example, the genes *fbT1T2T3* located in the gene cluster for 4-chlorobenzoate degradation encode a transporter essential for 4-chlorobenzoate import which is induced by 4-chlorobenzoate, as the enzymes involved in its degradation (Chae and Zylstra 2006). Transporters involved in organohalide uptake are often encoded by genes found nearby dehalogenase genes (Fig. 2), and can be located at the outer membrane (e.g. beta-barrel transporter (Belchik *et al.* 2010)) or at the inner membrane (e.g. ABC-type, TRAP-type or major facilitator-type superfamily transporters; Platero *et al.* 2012; Chae and Zylstra 2006; Hoffmann and Müller 2006; Su and Tsang 2013).

Post-transcriptional regulatory mechanisms

Known post-transcriptional regulatory mechanisms for organohalide gene expression include aspects of both protein expression and dehalogenase activity. Translation can be modulated by formation of stem-loop structures at the level of mRNA leader sequences (riboswitches, metabolite-binding mRNA structures) (Speed *et al.* 2018), or by interactions with proteins such as Hfq and small RNAs (Kavita, de Mets and Gottesman 2018). Some riboswitches specifically bind fluoride halide ions, and to trigger the translation of halide transporters (Speed *et al.* 2018), as well as of enzymes known to be inhibited by fluoride and proteins of unknown function (Baker *et al.*, 2012). Intriguingly, the dichloromethane dehalogenating strain *Methylobacterium extorquens* DM4 (formerly *Methylobacterium extorquens* (Green and Ardley 2018)), contains ten homologs of this “fluoride” riboswitch (Baker *et al.*, 2012). All these riboswitch copies are located in a genomic island involved in dehalogenation (Bringel and Vuilleumier, unpublished data). This suggests a potential link of halide-specific riboswitches with regulatory processes of organohalide degradation.

Dehalogenases and their associated transcription factors also represent key targets for post-translational protein modifications to modulate both dehalogenase activity and associated regulatory processes. For instance, acetylation of a two-component system regulates dehalogenase gene *pceA* transcription in *Sulfurospirillum halorespirans* (Fig. 5C). In the presence of tetrachloroethene (PCE), acetylation of the DNA-binding response regulator (SHALO_1502) induces conformational changes of the protein that mimics phosphorylation by a PCE-responsive histidine kinase (SHALO_1503) (Türkowsky *et al.* 2018a). Acetylation was shown to induce *pceAB* transcription (although less strongly than by phosphorylation), so that *pceAB* remains transcribed and activated even in absence of PCE. Besides acetylation, other protein modifications such as phosphorylation and glycosylation have been found. However, post-transcriptional regulatory mechanisms have not yet been much investigated in the context of degradation of organohalides. This clearly represents an attractive area for future study.

Global responses in gene expression upon growth with organohalides

Bacteria that grow with organohalides are exposed to different stresses that may trigger changes in gene expression extending beyond the genes associated with dehalogenation *per se*. Also and as already mentioned, dehalogenase genes are often located on mobile genetic elements (Liang *et al.* 2012) which often have wide-ranging effects on the expression of genes not necessarily linked to the functions encoded by the element (Lang and Johnson 2015; San Millan *et al.* 2015). In *Pseudomonas aeruginosa* PAO1 for example, acquisition of the genomic island ICE_{clc} for chlorobenzoate degradation, changes patterns of gene expression outside ICE_{clc} itself even in the absence of chlorobenzoate (Gaillard *et al.* 2008).

Large-scale gene expression changes during growth with chlorinated compounds have been observed in other studies (Table 2), notably by cDNA micro-array and RNA-seq approaches. Frequently, hundreds of transcripts change expression levels between organohalide and non-halogenated substrates. Similarly large changes were also observed in proteomic studies (Zhang *et al.* 2014; Goris *et al.* 2015; Bibi-Triki *et al.* 2018; Türkowsky *et al.* 2018a, 2018b). Such investigations at the genome-scale will clearly continue to provide much-needed valuable detailed information on the global effects of dehalogenation metabolism on bacterial physiology in the future.

Indeed, the global bacterial response to organohalides and their toxicity usually involves several still incompletely characterised aspects of the general stress response, including transcription factors that respond to halogenated pollutants and their degradation products (Bringel *et al.* 2019; Heipieper *et al.* 2007). For instance, some organohalides act as solvents and disrupt membrane fluidity and integrity, potentially affecting the proton-motive force (Murínová and Dercová, 2014). In addition, besides the inherent toxicity of organohalides, the process of degradation itself may be a major source of organohalide toxicity, with degradation products being more of a problem than organohalides themselves. For example, glutathione S-transferase driven degradation of dichloromethane yields S-chloromethylglutathione, a reactive metabolite which leads to the formation of alkylated DNA adducts (Kayser and Vuilleumier 2001), with associated mutagenic effects (Gisi *et al.* 1999). Similarly, pentachlorophenol degradation leads to the formation of highly toxic hydroxyl radicals by reaction of the degradation pathway intermediate tetrachlorobenzoquinone with hydrogen peroxide (Zhu *et al.* 2007). Higher expression of enzymes of oxidative stress such as catalases or peroxidases is indeed often observed during degradation of organohalides by oxygenases (Jennings *et al.* 2009; Puglisi *et al.* 2010). Finally, halide production from dehalogenation is another source of stress associated with degradation of organohalides. In dichloromethane dehalogenation, for example, high transcription of the ClcA chloride/proton antiporter gene was shown to be of advantage in laboratory experimental evolution studies of bacterial dichloromethane utilisation (Michener *et al.* 2014a, 2014b). This highlighted the importance of gene expression re-tuning in the host for efficient growth with an organohalide following acquisition of the corresponding gene cluster.

Taken together, the toxic effects associated with organohalides and their degradation contribute to explaining the differential expression of a large variety of stress response genes in the context of organohalide degradation (e.g. Gvakharia *et al.* 2007; Jennings *et al.* 2009; Puglisi *et al.* 2010; Islam *et al.* 2014). This was shown to include common chaperones DnaK, GroES and GroEL, known to target a wide range of proteins (Bhandari and Houry 2015), further emphasizing the major effects of dehalogenation on global gene expression. Moreover, some chaperones encoded within dehalogenase operons are only expressed in the context of dehalogenation metabolism, suggesting a specific role of some chaperones in the folding of structurally complex dehalogenases such as reductive dehalogenases (Morita *et al.*, 2009; Maillard *et al.*, 2011; Mac Nelly *et al.*, 2014).

Aspects of metabolic fluxes and energy balance clearly also impact global gene expression in organohalide metabolism. For example, the energy retrieved from organohalide degradation may sometimes be insufficient to match the energy needs of dehalogenase synthesis and adaptation to toxic organohalides (Cases and de Lorenzo 2005a). This provides a rationale for the often-observed adjustments in central metabolism and corresponding expression levels of genes for enzymes and also transporters of growth-supporting organohalides involved in energy metabolism (Jennings *et al.* 2009; Zhang *et al.* 2014). Similarly, modulation of the level of expression of genes involved in cofactor and redox balance has been observed such as the glutathione reductase gene found downstream of glutathione S-transferase dehalogenase gene *dsmH2* involved in the degradation of the broad-spectrum herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid) (Li *et al.* 2018), or the FAD reductase gene *tcpX* in 2,4,6-trichlorophenol degradation (Sanchez and Gonzalez 2007). Moreover, requirements for increased cellular pools of essential dehalogenase cofactors can also trigger higher expression of gene sets involved in cofactor biosynthesis. Reductive dehalogenation, for example, usually involves corrinoid-dependent dehalogenases, with concomitant increased expression of genes for cobalamin biosynthesis upon growth with organohalides (Kruse, Smidt and Lechner 2016), such as in *Desulfitobacterium hafniense* Y51 growing with tetrachloroethene (Peng *et al.* 2012), or of genes regulated by riboswitches that detect cobalamin (Choudhary *et al.*, 2013; Rupakula *et al.*, 2015). Another example of dehalogenation-dependent regulation of cobalamin-associated genes was demonstrated in *M. extorquens* CM4 growing with chloromethane: an additional set of such genes is co-localized with chloromethane dehalogenase on a plasmid, and its expression is specifically regulated in a chloromethane-dependent way, unlike that of the homologous chromosomal gene set (Roselli *et al.* 2013; Chaignaud *et al.* 2017).

Moving out of the laboratory: Assessing regulation of dehalogenation expression in the environment

Most transcriptomics studies have been conducted under reproducible, low complexity conditions, such as with pure cultures of reference dehalogenating strains growing with different substrates under controlled nutrients, temperature, pH, and oxygen conditions. In real-world polluted environments,

however, physical, chemical and biological parameters are constantly changing, making it likely that complex adaptive processes, prominently also at the transcriptional level, are associated with organohalide degradation and bacterial growth in the environment (Cases and de Lorenzo 2005b).

In this context, it is striking to note that many pollutant-degrading bacteria that show promising results when cultivated in the laboratory have been ineffective in *in situ* studies aiming at bioremediation (Boopathy 2000; Lovley 2003; de Lorenzo 2009). This is also in part because organohalides are usually found together with other contaminants in the environment, resulting in additive or synergistic toxic effects (Nirmalakhandan *et al.* 1997), as notably reported for fungicides (White, Potter and Culbreath 2010) and heavy metals (Olaniran, Balgobind and Pillay 2013). Indeed, the presence of several unrelated pollutants has been shown to affect transcriptional regulation, for example when transcription factors recognize several molecules with antagonistic (e.g. inducing and inhibitory) effects (Selifonova and Eaton 1996).

Other differences between laboratory and *in situ* experiments include predation of organohalide-degrading strains by other microorganisms (Cunningham, Kinner and Lewis 2009), competition for growth substrates (Becker 2006), and water stress encountered during the passage of cells grown as liquid cultures under laboratory conditions to *in situ* generally rather drier environments (Moreno-Forero *et al.* 2016). Conversely, organohalide-degrading bacteria may benefit from living with other microorganisms as aggregates (Mao *et al.* 2015) and as biofilms (Yoshida *et al.* 2009) in the environment, e.g. also by sharing and exchanging metabolites and cofactors, with underlying regulatory processes differing from those observed in laboratory experiments (Mao *et al.* 2015; Chen *et al.* 2017) (Table 3). Indeed, many organohalide-respiring bacteria strongly depend on other bacteria for corrinoid production (Fincker and Spormann 2017).

The relative scarcity of *in situ* transcriptomic studies specific of dehalogenation metabolism reflects this complexity, and the difficulty of correlating observed changes in gene expression with dehalogenation metabolism. In this context, laboratory microcosms using environmental samples of water, sediment and soil have increasingly been developed to study bacterial dehalogenation. Such microcosms provide conditions mimicking *in situ* environmental conditions, while allowing for controlled growth conditions and monitoring (Amos *et al.* 2008; Chaignaud *et al.* 2018; Lillis, Clipson and Doyle 2010; Xiu *et al.* 2010; Men *et al.* 2017). For example, pollutant degradation could be quantitatively correlated with transcription of dehalogenase gene *cbdbA* in water microcosms exposed to hexachlorobenzene at several temperatures (Tas *et al.* 2011). Microcosms also enabled investigations of bacterial dehalogenation in the context of microbial interactions with other types of organisms such as plants (Liu *et al.* 2007; Scheublin *et al.* 2014; Chaignaud *et al.* 2018; Wang *et al.* 2018). Such studies are likely to continue to develop in the future, and to allow improved assessment of environmentally relevant processes, such as the fate of plant-emitted chlorinated C₁ volatile organic compounds and their consumption by dehalogenating methylotrophs (reviewed in Bringel and Couée 2015, Bringel *et al.*, 2019).

Nevertheless, direct future development of *in situ* studies of polluted sites will remain indispensable to better understand and improve bioremediation processes. In the few available *in situ* studies on organohalide degradation focussing on gene regulation, differential detection of mRNA of gene biomarkers by RT-qPCR remains by far the preferred approach until now (Lee *et al.* 2008; Kranzioch, Ganz and Tiehm 2015; Mattes *et al.* 2015; Ismaeil, Yoshida and Katayama 2017; Liang *et al.* 2017). The mainly used biomarkers are of course dehalogenases (Werner *et al.* 2009; Kranzioch, Ganz and Tiehm 2015; Matturro and Rossetti 2015; Ibrahim *et al.* 2017; Liang *et al.* 2017; Hermon *et al.* 2018), although other genes, for example related to stress or cofactor biosynthesis, may also prove useful for this purpose. To our knowledge, however, regulator genes have not yet been used, likely because of the generally low level of expression of such genes compared to usually highly expressed dehalogenases. The presence and relative abundance of gene transcripts can usually be correlated with biodegradation (for an example see Chow *et al.* 2010), and also uncover specific actively dehalogenating populations during bioremediation (Maphosa *et al.* 2010). In tetrachloroethene and trichloroethene bioremediation for example, key dehalogenase genes *bvcA* and *vcrA* were measured at both DNA and RNA levels, and found in 99% and 58% of 95 samples from 6 contaminated environments, respectively (Liang *et al.*, 2017). The added value of RNA detection compared to DNA is that it may help to distinguish dehalogenase gene copies expressed by living cells from those in extracellular DNA or dead cells. As a caveat, however, RNA transcript abundance is not always strictly correlated with biodegradation (Lee *et al.* 2006), also because some dehalogenase genes are constitutively expressed (Paulin, Nicolaisen and Sorensen 2010; Peng *et al.* 2012; T'Syen *et al.* 2015). At this stage, therefore, interpretation of DNA, mRNA and protein biomarkers in environmental samples still requires some caution, in the context of organohalide degradation studies (Heavner *et al.*, 2018), as well as in the general context of microbial ecology (Muller 2019).

Applications of molecular tools based on regulatory processes

In addressing the challenge of worldwide environmental pollution and by organohalides particularly, we still mainly proceed today by tinkering, and trial-and-error. In the future, bioremediation strategies will clearly benefit from bacterial strains with optimised *in situ* fitness, and we have reviewed here how this may also depend on regulatory processes. Many more studies investigating and improving these processes at the molecular level are still needed to develop the most effective bioremediation processes possible. Clearly, the use of genetically engineered strains continues to be unacceptable to the public in many parts of the world, including for *in situ* bioremediation. Nevertheless, ongoing progress in our understanding of the molecular processes associated with degradation of organohalides, in the context of the rapid development of synthetic biology approaches that also include the design of efficient and specific regulatory circuits, is fuelling advances in the field. For example, targeted engineering of transcription factors can improve and expand recognition ability for specific organohalides of interest (Wise and Kuske 2000; Mohn *et al.* 2006; Beggah *et al.* 2007; Lang

and Ogawa 2009), in analogy to what has been done extensively to improve activity of dehalogenases and modify their substrate range (Pavlova *et al.* 2009; Dvořák *et al.* 2017; Ang *et al.* 2018). Synthetic biology approaches combined with metabolic engineering (Dvořák *et al.* 2017) may also help design and assemble new and specifically regulated optimised dehalogenation pathways, by applying previously streamlined and optimised synthetic gene clusters (Fig. 2B).

Such synthetic gene clusters for dehalogenation hold the promise of conferring improved fitness to bacteria, for use in bioremediation (Zhang *et al.* 2010) or in phytoremediation (Mena-Benitez *et al.* 2008; Abhilash, Jamil and Singh 2009). They can be driven by either constitutive or regulated heterologous promoters (e.g. *lac*, *tac*, *nod*) placed upstream of dehalogenase genes to optimize dehalogenase gene expression. Some exploratory studies along these lines have already been reported. For example, bacterial *bph* genes for degradation of polychlorinated biphenyls were expressed from the *nod* promoter induced by plant-produced flavonoids to enhance biodegradation capacity of *Pseudomonas* strains in the rhizosphere (Villacieros *et al.* 2005). Conversely, detrimental growth behaviour may be avoided by deletion of corresponding regulatory genes. For example, growth of *P. putida* KT2440 with glycerol was prevented, and 1,2,3-trichloropropane utilisation favoured, following deletion of the transcription factor gene *glpR* (Gong *et al.* 2017).

Better expression of genes involved in the adaptive response to specific organohalides may also improve bacterial growth driven by organohalide degradation, as exemplified above for the chloride/proton antiporter *clcA* gene (Michener *et al.* 2014a). In this case, a synthetic construct containing the DCM dehalogenase gene together with *clcA* under the control of an experimentally evolved *clcA*-specific promoter was designed to confer improved growth with DCM to appropriate bacterial hosts (Fig. 2B, Michener *et al.* 2014b). The results of this study indeed suggest that efficient halide extrusion may represent a major, although often overlooked, adaptive advantage for growth with organohalide pollutants.

Finally, regulatory processes can also be exploited not only to improve degradation of organohalides, but also to monitor and predict bioremediation potential, as well as to evaluate their bioavailability and quantify them in natural environments (Lopes *et al.* 2012; Hua *et al.* 2015; Whangsuk *et al.* 2016; Farhan Ul Haque *et al.* 2017). Bioreporter strains expressing a fluorescent protein as a function of organohalide identity and concentration have been developed (e.g. (Whangsuk *et al.* 2016; Zhang *et al.* 2010)). This approach was successfully applied in several cases, e.g. to demonstrate the expression of polychlorinated biphenyls and chloromethane dehalogenase genes in the rhizosphere and the phyllosphere, respectively (Liu *et al.* 2010; Farhan Ul Haque *et al.* 2017). In the environment, monitoring of bioreporter activity is often constrained by the limitations of spectrophotometric or fluorimetric approaches, arising e.g. from natural autofluorescence of humic acids. Use of bioreporters coupled with fluorescence-activated cell sorting (FACS) may help to overcome these problems (Norman, Hansen and Sørensen 2006; Beggah *et al.* 2007).

Bioreporters for organohalide quantification have so far mainly made use of transcription factors that sense cytoplasmic processes. In the future, membrane-based two-component systems may offer the advantage of directly sensing pollutants in the environment without potential biases associated with pollutant uptake (Ravikumar *et al.* 2017). Similarly, use of halide-inducible promoters (Geldart, Borrero and Kaznessis 2015) and halide-specific riboswitches (Speed *et al.* 2018) could also be envisaged, to detect the occurrence of dehalogenation, and to drive dehalogenation-dependent gene expression. Insights along these lines will certainly also emerge from further fundamental explorations on the regulation of organohalide degradation pathways, and will likely provide new, improved tools to monitor bioremediation of organohalides, and help define improved criteria for optimal dehalogenating strain efficiency in bioremediation.

To conclude, many aspects of the regulation of organohalide degradation pathways are still largely unexplored, in contrast to extensive available knowledge on genes and enzymes for degradation of organohalides. It is our contention that regulatory aspects may represent a key, often potentially limiting step for effective biodegradation of organohalides, in particular in natural environments. We hope that this review, together with the opportunities offered by increasingly robust omics approaches available today, will encourage to revisit this specific aspect of bacterial dehalogenation metabolism after some 40 years of intensive research focus on dehalogenases.

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Table 1. Transcription factors involved in detection of organohalides

Transcription factor	Degradation pathway	Ligand	Target DNA sequence	Crystallographic structure	Organism	Reference
<u>Directly organochlorine pollutants recognized as ligands</u>						
BphS1, BphR1	polychlorinated biphenyls	biphenyl; dichlorobenzene ^a	TTCCGTAGTTTTCCCGGATGTTTCG	No	<i>Rhodococcus jostii</i> RHA1	(Takeda <i>et al.</i> 2010)
HadR	2,4,6-trichlorophenol	2,4,6-trichlorophenol	ATGCCGCTGAGGAAT	No	<i>Cupriavidus pickettii</i> DTP0602	(Torii <i>et al.</i> 2013)
MopR	3-chlorophenol	3-chlorophenol ^b	TTCATCAAATAATGGA-8nt- ATGCTGATTCATCAA	Yes	<i>Acinetobacter calcoaceticus</i> NCIB8250	(Ray <i>et al.</i> 2016)
PcpR	pentachlorophenol; 2,4,6-trichlorophenol	pentachlorophenol ^b ; 2,4,6-trichlorophenol ^b	ATTC-7nt-GAAT	Yes	<i>Sphingobium chlorophenolicum</i> L-1	(Hayes <i>et al.</i> 2014)
<u>Degradation products recognized as ligands</u>						
CbnR	3-chlorobenzoate	cis,cis-muconate	unknown	Yes	<i>Ralstonia eutropa</i> NH9	(Muraoka <i>et al.</i> 2003)
CprK1	chlorophenols	ortho-chlorophenolacetic acid _b	TTTA-8nt-CTAA-20nt-TATAAT	Yes	<i>D. hafniense</i> DCB-2	(Gábor <i>et al.</i> 2008)
LinR	lindane	2,5-dichlorohydroquinone; chlorohydroquinone	ATTCACAATCTGAAT	No	<i>Sphingomonas paucimobilis</i> UT26	(Miyachi <i>et al.</i> 2002)
Rdh2R	trichlorobenzene	2,3-dichlorophenol ^b ; 2,4-dichlorophenol ^b ; 3,4-dichlorophenol ^b	TAGTATACGAT-2nt-TAGTATACGATTA	No	<i>Dehalococcoides mccartyi</i> CBDB1	(Krasper <i>et al.</i> 2016)
<u>Unknown ligands</u>						
CbrC	trichlorobenzene	unknown	unknown	No	<i>Dehalococcoides mccartyi</i> CBDB1	(Adrian <i>et al.</i> 2007)
DcmR	dichloromethane	unknown	unknown	No	<i>Methylorubrum extorquens</i> DM4	(La Roche and Leisinger 1991)

^a Other ligands are described in Takeda *et al.* 2010; ^b ligand co-crystallized with transcription factor

Table 2. Differentially expressed genes in pure cultures of bacteria growing with organohalides

Bacteria	Organohalide substrate	Reference substrate	Culture condition	Up-regulated ^a	Down-regulated ^a	Method	Reference
		tryptone soy agar	agar plate	569/4 255	580/4 255		
<i>Arthrobacter chlorophenolicus</i> A6	4-chlorophenol	N.A.	microcosm with plant (phyllosphere low humidity)	390/4 255	412/4 255	micro-array	(Scheublin <i>et al.</i> 2014)
		N.A.	microcosm with plant (phyllosphere high humidity)	430/4 255	474/4 255		
<i>Burkholderia xenovorans</i> LB400	aroclor 1242	biphenyl	liquid culture	28/8 249	19/8 429	RNA-seq	(Parnell <i>et al.</i> 2006)
<i>Dehalococcoides</i> spp. KB1 TM	vinyl chloride	methanol	microcosm	452/19 200 ^b	95/19 200 ^b	micro-array	(Waller <i>et al.</i> 2012)
<i>Desulfitobacterium hafniense</i> DCB-2	3-chloro-4-hydroxybenzoate 3,5-dichlorophenol ortho-bromophenol	uranyl acetate, sodium selenate, ferric citrate	liquid culture	N.A. ^c	N.A.	micro-array	(Kim <i>et al.</i> 2012)
<i>Desulfitobacterium hafniense</i> Y51	trichloroethene	MMYPF-medium (pyruvate and fumarate)	liquid culture	24/5 060	N.A./5 060	micro-array	(Peng <i>et al.</i> 2012)
<i>Methyloburbrum extorquens</i> CM4	chloromethane	methanol	liquid culture	137/6 262	13/6 262	RNA-seq	(Chaignaud <i>et al.</i> 2017)
<i>Methyloburbrum extorquens</i> DM4	dichloromethane	methanol	liquid culture	69 /5 701	121/5 701	RNA-seq	(Chaignaud <i>et al.</i> 2017)
<i>Nitrosomonas europaea</i>	chloromethane	mineral medium	liquid culture	67 /2 460	148/2 460	micro-array	(Gvakharia <i>et al.</i> 2007)
	chloroform	mineral medium	liquid culture	175/2 460	501/2 460		
<i>Pigmentiphaga</i> sp. H8	3,5-dibromo-4-hydroxybenzoate	non induced culture	liquid culture	98/5 678	31/5 678	RNA-seq	(Chen <i>et al.</i> 2018)
<i>Polaromonas</i> sp. JS666	cDCE	glycolate	liquid culture	140/5 569	450/5 569	micro-array, proteomics	(Jennings <i>et al.</i> 2009)
			liquid culture	129/3 524	87/3 524		
<i>Rhodococcus aetherivorans</i> I24	aroclor 1254	glucose	microcosm (contaminated Rotterdam harbour sediments)	207/3 524	176/3 524	micro-array	(Puglisi <i>et al.</i> 2010)
				44/3 524	67/3 524		
<i>Salmonella typhimurium</i> TA100	MX chlorinated furanone	dimethyl sulfoxide	liquid culture		169/4 253 ^b	micro-array	(Ward <i>et al.</i> 2007)

^aNumber of up- or down-regulated genes / total number of genes in genome. When available, total number of regulated genes / total number of genes in genome are shown in the centre of the columns; ^b Number of spots on micro-array with differential hybridization (not number of genes); ^c N.A., Not Applicable, no numbers provided.

Table 3. Differentially regulated genes in bacterial consortia growing with organohalides

Organohalide	Culture condition	Consortia partners	Partner regulated gene number ^a		Method	Reference
			Up-regulated	Down-regulated		
linuron, 3,4-dichloroaniline	liquid culture	<i>Variovorax</i> sp WDL1 ^b	1 372		RNA-seq	(Albers <i>et al.</i> 2018)
		<i>Comamonas testosteroni</i> WDL7	169			
		<i>Hyphomicrobium sulfonivorans</i> WDL	N.A. ^c			
trichloroethene	liquid culture	<i>Dehalococcoides mccartyi</i> 195 ^b	102		micro-array	(Men <i>et al.</i> 2012)
		<i>Desulfovibrio vulgaris</i> Hildenborough	N.A.	N.A.		
		<i>Methanobacterium congolense</i>	N.A.	N.A.		
trichloroethene	liquid culture with vitamin B ₁₂	<i>Dehalococcoides mccartyi</i> 195 ^b	28	18	micro-array	(Men <i>et al.</i> 2014)
		<i>Desulfovibrio vulgaris</i> Hildenborough	N.A.	N.A.		
		<i>Pelosinus fermentans</i> R7	N.A.	N.A.		
trichloroethene	liquid culture without vitamin B ₁₂	<i>Dehalococcoides mccartyi</i> 195 ^b	30	44	micro-array	(Men <i>et al.</i> 2014)
		<i>Desulfovibrio vulgaris</i> Hildenborough	N.A.	N.A.		
		<i>Pelosinus fermentans</i> R7	N.A.	N.A.		
trichloroethene	microcosm (contaminated groundwater with or without cobalamin)	7 bin genomes	550		RNA-seq, micro-array	(Men <i>et al.</i> 2017)
trichloroethene	liquid culture	<i>Dehalococcoides mccartyi</i> 195 ^b <i>Syntrophomonas wolfei</i>	18	196	micro-array	(Mao <i>et al.</i> 2015)

^aNumber of genes regulated; ^bDehalogenating partner; ^cN.A., Not Applicable, no numbers provided.

Figure 1. Natural and industrial sources of organohalides. Some organohalides are found in the environment as a result of natural abiotic production, biomass combustion and volcano emissions (Gribble 2010). Biological production involves mainly macro-algae, microorganisms and plants (Diaz-Marrero et al. 2002; Tittlemier et al. 2002; Atashgahi et al. 2018). Some organohalides have been massively produced synthetically, for agriculture (Jayaraj, Megha and Sreedev 2016) and for industrial use as refrigerants, solvents (Tsai 2017), electrical insulators and in plastic production.

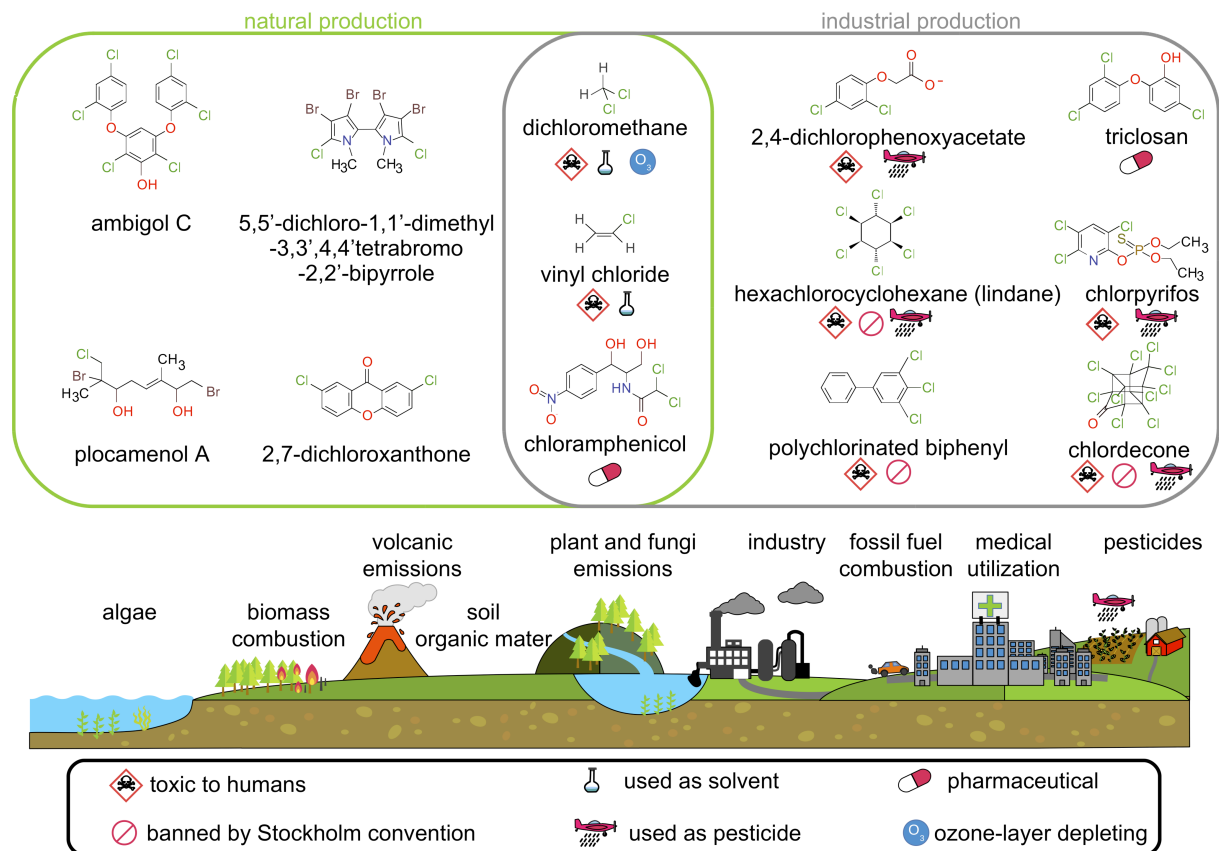


Figure 2. Gene clusters for organohalide degradation. (A) Gene clusters for dehalogenation in representative bacterial degraders. (B) Synthetic gene clusters. (C) Organohalide-relevant catabolic gene clusters lacking dehalogenase genes. References are for gene clusters *atz* (Esquirol et al. 2018), *bhb* (Chen et al. 2015), *bph* (Takeda et al. 2010), *ccd* (Bers et al. 2011), *clc* (Coco et al. 1993; Miyazaki et al. 2015), *clcA-dcmA* (Michener et al. 2014a), *cpr* (Smidt et al. 2000), *dcm* (La Roche and Leisinger 1991), *dha* (Poelarends et al. 2000), *dsm* (Li et al. 2018b), *had* (Torii et al. 2013), *hdh* (Sallabhan et al. 2013), *lin* (Tabata et al. 2016), *mop* (Schirmer, Ehrh and Hillen 1997), *odc* (Chen et al. 2018), *pce* (Futagami et al. 2006), *pcp* (Cai and Xun 2002), *rdh* (Collins et al. 2018), *tbu* (Kahng et al. 2000), *tcp* (Sanchez and Gonzalez 2007) and *tfd* (Trefault et al. 2009).

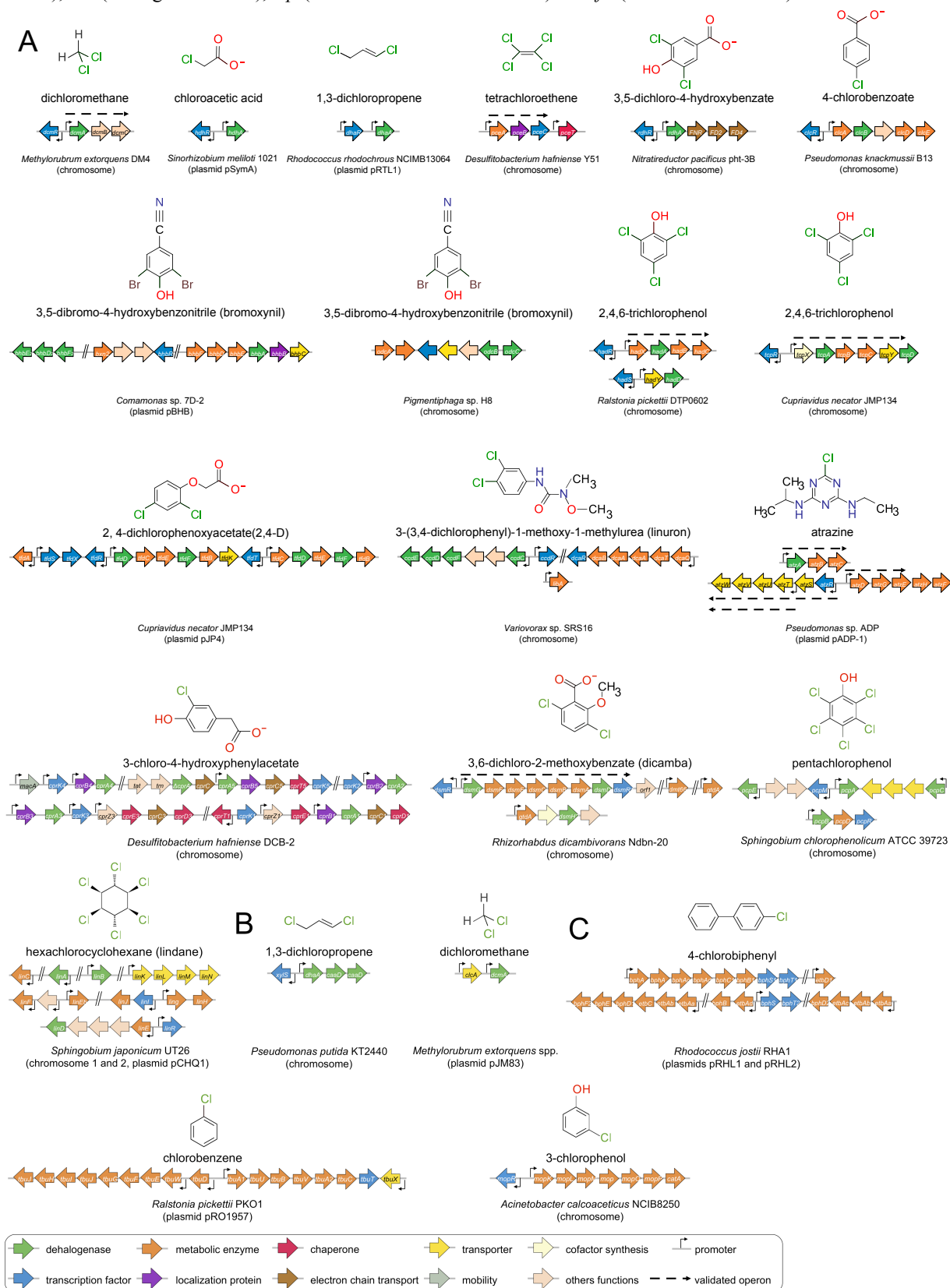


Figure 3. Functional domains of selected transcriptional factors associated with dehalogenation. (A) Transcription factors; **(B)** Proteins of two component systems. The HTH (helix-turn-helix) DNA binding domain (blue) present in almost all transcription factors is fused to organohalide-specific sensor domains (green). Abbreviations: MEDS (MEthanogen/methylotroph DcmR Sensory; (Anantharaman and Aravind 2005)) and PAS (Per sensor domain, Arnt, Sin; (Möglich, Ayers and Moffat 2009)). Accession numbers for BspS1 (WP 011598994.1), BphT1 (WP 011598993.1), CnbR (WP 011255153.1), CbrC (CAI82343.1), CbrD (CAI82344.1), ClcR (WP 012248462), CprK (ACL18797.1), DcmR (AAB68953.1), PcpR (U12290.2), (MopR (WP 004721355), and RdhR (WP 011309983).

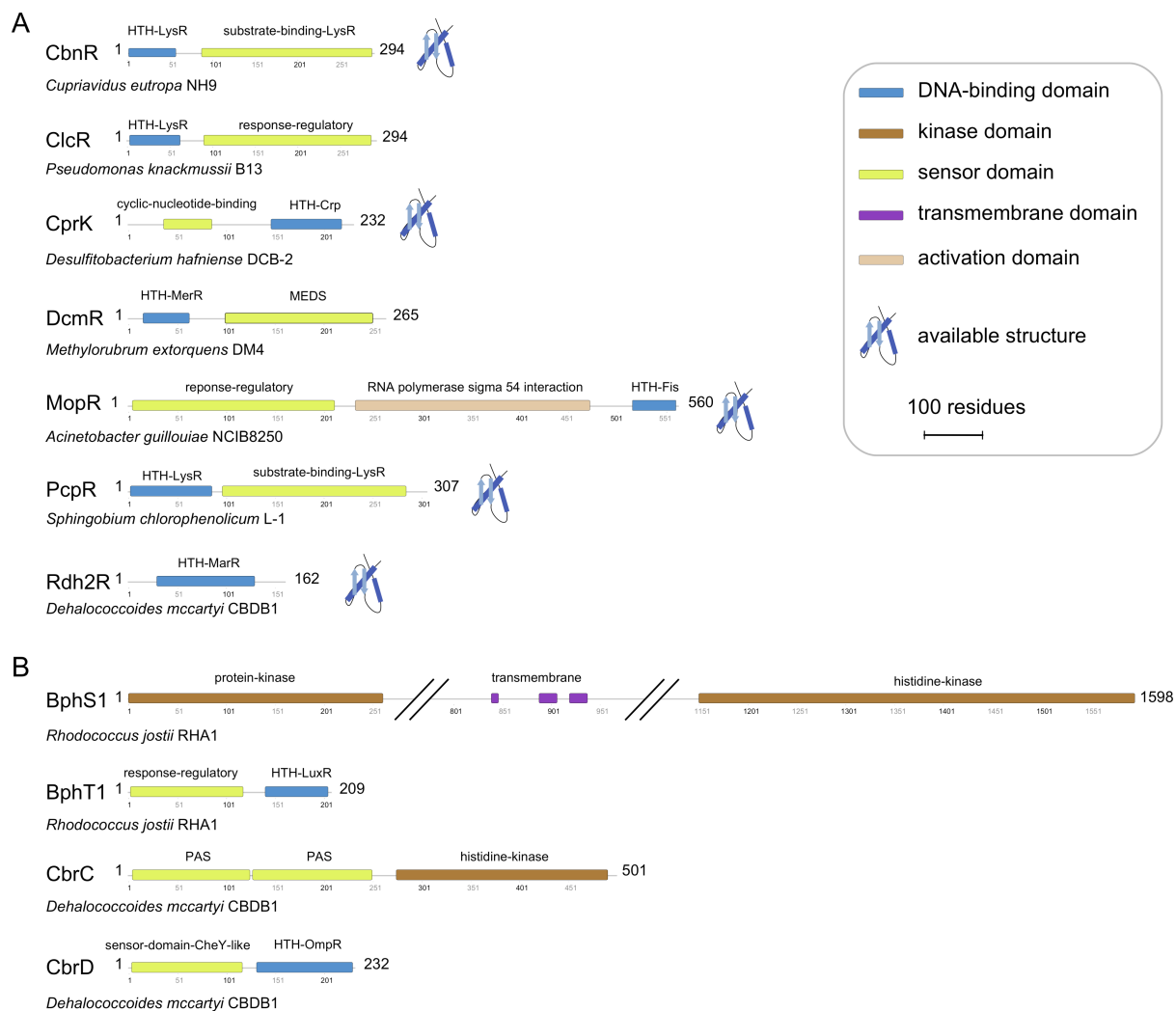


Figure 4. Interactions between organohalides and transcription factors. Black and green dashed lines represent hydrogen bonds and hydrophobic interactions, respectively. Orange dashed lines indicate interactions between aromatic rings. 2D representations generated by PoseView (Stierand and Rarey 2010).

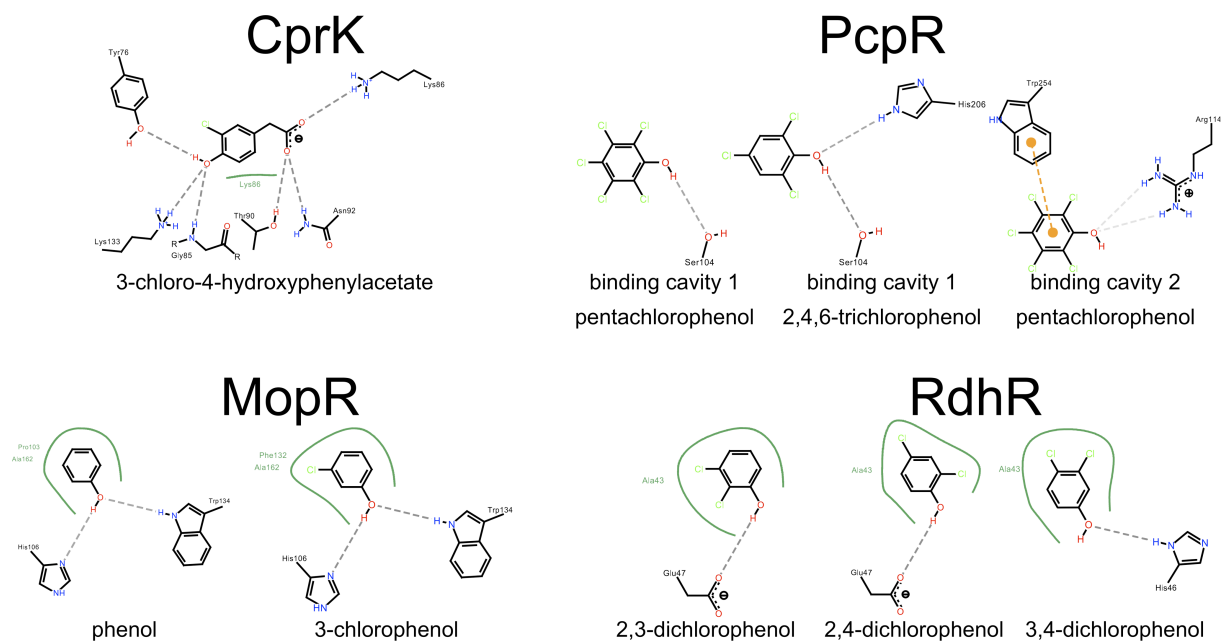


Figure 5. Examples of transcriptional regulation in organohalide degradation in Gram-negative and Gram-positive bacteria. (A) Atrazine utilization in *Pseudomonas* sp. ADP. Binding of cyanuric acid to AtzR activates expression of *atzDEF*. Expression of *atzR* and downstream genes is activated or repressed at low or high nitrogen concentration, respectively (Platero et al. 2012). (B) Chlorobenzoate utilization in *Pseudomonas putida* PRS2000. Expression is regulated by two homologous transcription regulators CatR and ClcR (McFall et al. 1997; McFall, Chugani and Chakrabarty 1998). (C) PCE utilization in *Sulfurospirillum halorespirans*. A two-component system activates expression of *pceAB* when PCE is detected. It has been proposed that a yet unknown signal subsequently activates an acetyltransferase that post-translationally modifies this two-component system (Türkowsky et al. 2018a). (D) 2,4,6-trichlorophenol utilization by *Ralstonia pickettii* DTP0602. Two regulons *hadRXABC* and *hadSYD*, separated by 146 kb in the genome sequence (Torii et al. 2013), are differentially regulated by processes involving the two transcription factors HadR and HadS. HadR binds to 2,4,6-trichlorophenol for induced expression of *hadYABC*. HadS recognizes an intermediate metabolite produced in 2,4,6-trichlorophenol degradation, probably 2-chloromaleylacetate, to alleviate repression of *hadYD* (Torii et al. 2013). (E) Dehalogenation of chlorophenols by *Desulfotobacterium hafniense* DCB-2. The reductive dehalogenase *cprA* gene is regulated by five *cprK* paralogs (Gabor et al. 2008). CprK1 and CprK2 recognize ortho-substituted phenols such as 3-chloro-4-hydroxyphenylacetate, while CprK4 recognizes meta-substituted phenols, such as 3,5-dichlorophenol. CprK activates expression of gene *cprA* when ligands are detected. CprK folding is inhibited by oxygen, which prevents disulfide bond formation.

