# Transcriptional regulation of organohalide pollutant utilisation in bacteria

Bruno Maucourt, Stéphane Vuilleumier, Françoise Bringel

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Bruno MAUCOURT, Stéphane VUILLEUMIER and Françoise bRINGEL*

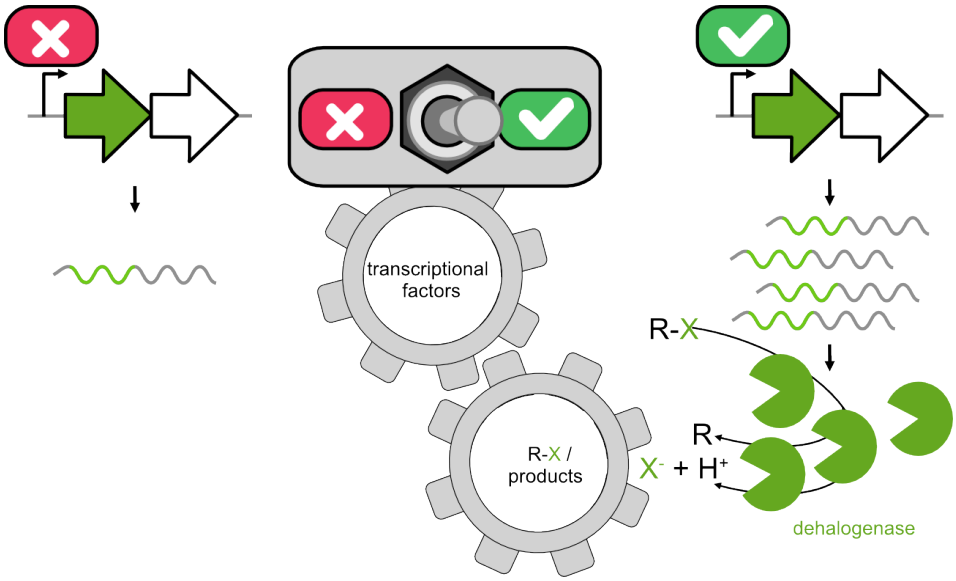
Université de Strasbourg, UMR 7156 CNRS, Génétique Moléculaire, Génomique, Microbiologie, Strasbourg, France

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

**Running title**: Regulation of organohalide pollutant degradation

**Keywords**: dehalogenation; gene expression; organohalide pollutants; regulation; transcriptional regulators; ligands

**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 (Whangsuk *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 *Dehaloccocoides mccartyi,* and of the CRP-FNR type, such as CprK in *Desulfitobacterium* 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 subsequence 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 *fcbT1T2T3* 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 e*t 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 *Methylorubrum 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 $C_1$ 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 triochloroethene 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.

**Funding.** BM was funded by a doctoral grant of the French Goverment. FB was awarded with an IdeX (Projet initiative d'excellence) grant of the University of Strasbourg.

**Conflicts of Interests**. None declared.

**Bibliography**

Abhilash PC, Jamil S, Singh N. Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics. *Biotechnol Adv* 2009;**27**:474–88.

Adrian L, Rahnenfuhrer J, Gobom J *et al.* Identification of a chlorobenzene reductive dehalogenase in *Dehalococcoides* sp. strain CBDB1. *Appl Environ Microbiol* 2007;**73**:7717–24.

Agarwal V, Miles ZD, Winter JM *et al.* Enzymatic halogenation and dehalogenation reactions: Pervasive and mechanistically diverse. *Chem Rev* 2017;**117**:5619–74.

Albers P, Weytjens B, De Mot R *et al.* Molecular processes underlying synergistic linuron mineralization in a triple-species bacterial consortium biofilm revealed by differential transcriptomics. *MicrobiologyOpen* 2018, DOI: 10.1002/mbo3.559.

Amos BK, Ritalahti KM, Cruz-Garcia C *et al.* Oxygen effect on *Dehalococcoides* viability and biomarker quantification. *Environ Sci Technol* 2008;**42**:5718–26.

Anantharaman V, Aravind L. MEDS and PocR are novel domains with a predicted role in sensing simple hydrocarbon derivatives in prokaryotic signal transduction systems. *Bioinformatics* 2005;**21**:2805–11.

Ang T-F, Maiangwa J, Salleh A *et al.* Dehalogenases: From improved performance to potential microbial dehalogenation applications. *Molecules* 2018;**23**:1100.

Atashgahi, S., Liebensteiner, M.G., Janssen, D.B., Smidt, H., Stams, A.J.M., and Sipkema, D. Microbial synthesis and transformation of inorganic and organic chlorine compounds. *Front Microbiol* 2018, DOI: 10.3389/fmicb.2018.03079.

Baker JL, Sudarsan N, Weinberg Z *et al.* Widespread genetic switches and toxicity resistance proteins for fluoride. *Science* 2012;**335**:233–5. DOI: 10.1126/science.1215063.

Beck CF, Warren RA. Divergent promoters, a common form of gene organization. *Microbiol Rev* 1988;**52**:318–26.

Becker JG. A modeling study and implications of competition between *Dehalococcoides ethenogenes* and other tetrachloroethene- respiring bacteria. *Environ Sci Technol* 2006;**40**:4473–80.

Beggah S, Vogne C, Zenaro E *et al.* Mutant HbpR transcription activator isolation for 2-chlorobiphenyl via green fluorescent protein-based flow cytometry and cell sorting. *Microb Biotechnol* 2007, DOI: 10.1111/j.1751-7915.2007.00008.x.

Belchik SM, Schaeffer SM, Hasenoehrl S *et al.* A β-barrel outer membrane protein facilitates cellular uptake of polychlorophenols in *Cupriavidus necator*. *Biodegradation* 2010;**21**:431–39.

Bernat P, Nykiel-Szymańska J, Stolarek P *et al.* 2,4-dichlorophenoxyacetic acid-induced oxidative stress: Metabolome and membrane modifications in *Umbelopsis isabellina*, a herbicide degrader. *PLoS ONE* 2018, DOI: 10.1371/journal.pone.0199677.

Bers K, Leroy B, Breugelmans P *et al.* A novel hydrolase identified by genomic-proteomic analysis of phenylurea herbicide mineralization by *Variovorax* sp. strain SRS16. *Appl Environ Microbiol* 2011;**77**:8754–64.

Bhandari V, Houry WA. Substrate interaction networks of the *Escherichia coli* chaperones: Trigger factor, DnaK and GroEL. In: Krogan, PhD NJ, Babu, PhD M (eds.). *Prokaryotic Systems Biology*. Vol 883. Cham: Springer International Publishing, 2015, 271–94.

Bibi-Triki S, Husson G, Maucourt B *et al*. N-terminome and proteogenomic analysis of the *Methylobacterium extorquens* DM4 reference strain for dichloromethane utilization. *J Proteomics* 2018;**179**:131-9.

Boopathy R. Factors limiting bioremediation technologies. *Bioresour Technol* 2000;**74**:63–67.

Bringel F, Couée I. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Front Microbiol* 2015, DOI: 10.3389/fmicb.2015.00486.

Bringel F, Besaury L, Amato P *et al*. Methylotrophs and methylotroph populations for chloromethane degradation. In: Chistoserdova L (ed). *Methylotrophs And Methylotroph Communities*, Poole: Caister academic press, 2019,149–72. DOI: 10.21775/9781912530045.08.

Cai M, Xun L. Organization and regulation of pentachlorophenol-degrading genes in *Sphingobium chlorophenolicum* ATCC 39723. *J Bacteriol* 2002;**184**:4672–80.

Cases I, de Lorenzo V. Genetically modified organisms for the environment: stories of success and failure and what we have learned from them. *Int Microbiol* 2005a;**8**:213–22.

Cases I, de Lorenzo V. Promoters in the environment: transcriptional regulation in its natural context. *Nat Rev Microbiol* 2005b;**3**:105–18.

Chae J-C, Zylstra GJ. 4-Chlorobenzoate uptake in *Comamonas* sp. strain DJ-12 Is mediated by a tripartite ATP-independent periplasmic transporter. *J Bacteriol* 2006;**188**:8407–12.

Chaignaud P, Maucourt B, Weiman M *et al.* Genomic and transcriptomic analysis of growth-supporting dehalogenation of chlorinated methanes in *Methylobacterium*. *Front Microbiol* 2017, DOI: 10.3389/fmicb.2017.01600.

Chaignaud P, Morawe M, Besaury L *et al.* Methanol consumption drives the bacterial chloromethane sink in a forest soil. *ISME J* 2018, DOI: 10.1038/s41396-018-0228-4.

Chen C, He J. Strategy for the rapid dechlorination of polychlorinated biphenyls (PCBs) by *Dehalococcoides mccartyi* strains. *Environ Sci Technol* 2018, DOI: 10.1021/acs.est.8b03198.

Chen G, Kleindienst S, Griffiths DR *et al.* Mutualistic interaction between dichloromethane- and chloromethane-degrading bacteria in an anaerobic mixed culture: Mutualistic degradation of chlorinated methanes. *Environ Microbiol* 2017, DOI: 10.1111/1462-2920.13945.

Chen K, Mu Y, Jian S *et al.* Comparative transcriptome analysis reveals the mechanism underlying 3,5-dibromo-4-hydroxybenzoate catabolism via a new oxidative decarboxylation pathway. *Appl Env Microbiol* 2018;**84**:e02467–17.

Chen K, Xu X, Zhang L, Gou Z *et al*. Comparison of four *Comamonas* catabolic plasmids reveals the evolution of pBHB to catabolize haloaromatics. *Appl Environ Microbiol* 2015:82:1401–11.

Choudhary PK, Duret A, Rohrbach-Brandt E *et al.* Diversity of cobalamin riboswitches in the corrinoid-producing organohalide respirer *Desulfitobacterium hafniense*. *J Bacteriol* 2013:**195**:5186-95.

Chow WL, Cheng D, Wang S *et al.* (2010) Identification and transcriptional analysis of trans-DCE-producing reductive dehalogenases in *Dehalococcoides* species. *ISME J* 2010:**8**:1020–30. DOI: 10.1038/ismej.2010.27.

Clausen LPW, Trapp S. Toxicity of 56 substances to trees. *Environ Sci Pollut Res* 2017;**24**:18035–47.

Collins FA, Fisher K, Payne KAP *et al.* NADPH-driven organohalide reduction by a nonrespiratory reductive dehalogenase. *Biochem* 2018:**57**:3493–502.

Cunningham JJ, Kinner NE, Lewis M. Protistan predation affects trichloroethene biodegradation in a bedrock aquifer. *Appl Environ Microbiol* 2009;**75**:7588–93.

Daniel L, Buryska T, Prokop Z *et al.* Mechanism-based discovery of novel substrates of haloalkane dehalogenases using *in silico* screening. *J Chem Inf Model* 2015;**55**:54–62.

Díaz-Marrero AR, Rovirosa J, Darias J *et al.* Plocamenols A−C, novel linear polyhalohydroxylated monoterpenes from *Plocamium cartilagineum*. *J Nat Prod* 2002;**65**:585–8.

Dvořák P, Nikel PI, Damborský J *et al.* Bioremediation 3.0 : Engineering pollutant-removing bacteria in the times of systemic biology. *Biotechnol Adv* 2017, DOI: 10.1016/j.biotechadv.2017.08.001.

Esquirol L, Peat TS, Wilding M *et al*. A novel decarboxylating amidohydrolase involved in avoiding metabolic dead ends during cyanuric acid catabolism in *Pseudomonas* sp. strain ADP. Oberer M (ed.). PLoS ONE 2018;13:e0206949.

Farhan Ul Haque M, Nadalig T, Bringel F *et al*. Fluorescence-based bacterial bioreporter for specific detection of methyl halide emissions in the environment. *Appl Environ Microbiol* 2013; **79**:6561-7.

Farhan Ul Haque M, Besaury L, Nadalig T *et al.* Correlated production and consumption of chloromethane in the *Arabidopsis thaliana* phyllosphere. *Sci Rep* 2017, DOI 10.1038/s41598-017-17421-y.

Ferreira MIM, Iida T, Hasan SA *et al.* Analysis of two gene clusters involved in the degradation of 4-fluorophenol by *Arthrobacter* sp. strain IF1. *Appl Environ Microbiol* 2009;**75**:7767–73.

Fincker M, Spormann AM. Biochemistry of catabolic reductive dehalogenation. *Annu Rev Biochem* 2017;**86**:357–86.

Futagami T, Tsuboi Y, Suyama A *et al.* Emergence of two types of nondechlorinating variants in the tetrachloroethene-halorespiring *Desulfitobacterium* sp. strain Y51. *Appl Microbiol Biotechnol* 2006 70(6):720–8. DOI:10.1007/s00253-005-0112-9

Gábor K, Hailesellasse Sene K, Smidt H *et al.* Divergent roles of CprK paralogues from *Desulfitobacterium hafniense* in activating gene expression. *Microbiol* 2008;**154**:3686–96. DOI:10.1099/mic.0.2008/021584-0

Gaillard M, Pernet N, Vogne C *et al.* Host and invader impact of transfer of the *clc* genomic island into *Pseudomonas aeruginosa* PAO1. *Proc Natl Acad Sci USA* 2008;**105**:7058–63.

Geldart K, Borrero J, Kaznessis YN. Chloride-inducible expression vector for delivery of antimicrobial peptides targeting antibiotic-resistant *Enterococcus faecium*. *Appl Env Microbiol* 2015;**81**:3889–97.

Gisi D, Willi L, Traber H *et al.* Effects of bacterial host and dichloromethane dehalogenase on the competitiveness of methylotrophic bacteria growing with dichloromethane. *Appl Environ Microbiol* 1998;**64**:1194–202.

Gisi D, Leisinger, Vuilleumier S. Enzyme-mediated dichloromethane toxicity and mutagenicity of bacterial and mammalian dichloromethane-active glutathione S-transferases. *Arch Toxicol* 1999;73:71-9.

Goldman, P., G. W. A. Milne, and D. B. Keister. Carbon halogen bond cleavage. III. Studies on bacterial halidohydrolases. *J Biol Chem* 1968;243:428-434.

Gong T, Xu X, Che Y *et al.* Combinatorial metabolic engineering of *Pseudomonas putida* KT2440 for efficient mineralization of 1,2,3-trichloropropane. *Sci Rep* 2017; DOI: 10.1038/s41598-017-07435-x.

Goris T, Schiffmann CL, Gadkari J *et al.* Proteomics of the organohalide-respiring *Epsilonproteobacterium Sulfurospirillum multivorans* adapted to tetrachloroethene and other energy substrates. *Sci Rep* 2015, DOI: 10.1038/srep13794.

Govantes F., García-González,V., Porrúa O *et al*. Regulation of the atrazine-degradative genes in *Pseudomonas* sp. strain ADP. *FEMS Microbiol Lett* 2010;**310**: 1–8.

Green PN, Ardley JK. Review of the genus *Methylobacterium* and closely related organisms: a proposal that some *Methylobacterium* species be reclassified into a new genus, *Methylorubrum* gen. nov. *Int J Syst Evol Microbiol* 2018;**68**:2727–48.

Gribble GW. *Naturally Occurring Organohalogen Compounds - A Comprehensive Update*. Wien: Springer-Verlag, 2010.

Gvakharia BO, Permina EA, Gelfand MS *et al.* Global transcriptional response of *Nitrosomonas europaea* to chloroform and chloromethane. *Appl Environ Microbiol* 2007;**73**:3440–5.

Hayes RP, Moural TW, Lewis KM *et al.* Structures of the inducer-binding domain of pentachlorophenol-degrading gene regulator PcpR from *Sphingobium chlorophenolicum*. *Int J Mol Sci* 2014;**15**:20736–52.

Heavner G, Mansfeldt C, Debs G *et al.* Biomarkers' responses to reductive dechlorination rates and oxygen stress in bioaugmentation culture KB-1TM. *Microorganisms* 2018, DOI: 10.3390/microorganisms6010013.

Heipieper, HJ, Neumann, G, Cornelissen, S *et al*. Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. *Appl Microbiol Biotechnol* 2007;**74**:961–73. DOI: 10.1007/s00253-006-0833-4.

Hermon L, Denonfoux J, Hellal J *et al.* Dichloromethane biodegradation in multi-contaminated groundwater: Insights from biomolecular and compound-specific isotope analyses. *Water Res* 2018;**142**:217–26.

Hoffmann D, Müller RH. 2,4-Dichlorophenoxyacetic acid (2,4-D) utilisation by *Delftia acidovorans* MC1 at alkaline pH and in the presence of dichlorprop is improved by introduction of the *tfdK* gene. *Biodegradation* 2006;**17**:263–73.

Hua A, Gueuné H, Cregut M *et al.* Development of a bacterial bioassay for atrazine and cyanuric acid detection. *Front Microbiol* 2015, DOI: 10.3389/fmicb.2015.00211.

Ibrahim ES, Kashef MT, Essam TM *et al.* A degradome-based polymerase chain reaction to resolve the potential of environmental samples for 2,4-dichlorophenol biodegradation. *Curr Microbiol* 2017, DOI: 10.1007/s00284-017-1327-6.

Islam MA, Waller AS, Hug LA *et al.* New insights into *Dehalococcoides mccartyi* metabolism from a reconstructed metabolic network-based systems-level analysis of *D. mccartyi* transcriptomes. *PLoS ONE* 2014, DOI: 10.1371/journal.pone.0094808.

Ismaeil M, Yoshida N, Katayama A. Identification of multiple dehalogenase genes involved in tetrachloroethene-to-ethene dechlorination in a *Dehalococcoides*-dominated enrichment culture. *BioMed Res Int* 2017, DOI: 10.1155/2017/9191086.

Jayaraj R, Megha P, Sreedev P. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdiscip Toxicol* 2016;**9**:90–100.

Jennings LK, Chartrand MMG, Lacrampe-Couloume G *et al.* Proteomic and transcriptomic analyses reveal genes upregulated by cis-dichloroethene in *Polaromonas* sp. strain JS666. *Appl Environ Microbiol* 2009;**75**:3733–44.

Kahng H-Y, Byrne AM, Olsen RH *et al.* Characterization and role of *tbuX* in utilisation of toluene by *Ralstonia pickettii* PKO1. *J Bacteriol* 2000;**182**:1232–42.

Kavita K, de Mets F, Gottesman S New aspects of RNA-based regulation by Hfq and its partner sRNAs. *Curr Opin Microbiol.* 2018 Apr;42:53-61. doi: 10.1016/j.mib.2017.10.014.

Kayser MF, Vuilleumier S. Dehalogenation of dichloromethane by dichloromethane dehalogenase/glutathione S-transferase leads to formation of DNA adducts. *J Bacteriol* 2001;**183**:5209–12.

Kearney PC, Kaufman DD. Enzyme from soil bacterium hydrolyzes phenylcarbamate herbicides. *Science* 1965;147:740-1.

Kim SH, Harzman C, Davis JK *et al.* Genome sequence of *Desulfitobacterium hafniense* DCB-2, a Gram-positive anaerobe capable of dehalogenation and metal reduction. *BMC Microbiol* 2012, DOI: 10.1186/1471-2180-12-21.

Kranzioch I, Ganz S, Tiehm A. Chloroethene degradation and expression of *Dehalococcoides dehalogenase* genes in cultures originating from Yangtze sediments. *Environ Sci Pollut Res* 2015;**22**:3138–48.

Krasper L, Lilie H, Kublik A *et al.* The MarR-type regulator Rdh2R regulates *rdh* gene transcription in *Dehalococcoides mccartyi* strain CBDB1. *J Bacteriol* 2016;**198**:3130–41.

Kruse T, Smidt H, Lechner U. Comparative genomics and transcriptomics of organohalide-respiring bacteria and regulation of *rdh* gene transcription. In Lorenz Adrian L and Löffler F E (ed.) Organohalide-Respiring Bacteria 2016:345–76.

La Roche SD, Leisinger T. Identification of *dcmR*, the regulatory gene governing expression of dichloromethane dehalogenase in *Methylobacterium* sp. strain DM4. *J Bacteriol* 1991;**173**:6714–21.

Lang G, Ogawa N. Mutational analysis of the inducer recognition sites of the LysR-type transcriptional regulator TfdT of *Burkholderia* sp. NK8. *Appl Microbiol Biotechnol* 2009;**83**:1085–94.

Lang KS, Johnson TJ. Transcriptome modulations due to A/C2 plasmid acquisition. *Plasmid* 2015;**80**:83–9.

Lee PKH, Johnson DR, Holmes VF *et al.* Reductive dehalogenase dene expression as a biomarker for physiological activity of *Dehalococcoides* spp. *Appl Environ Microbiol* 2006;**72**:6161–8.

Lee PKH, Macbeth TW, Sorenson KS *et al.* Quantifying genes and transcripts to assess the *in situ* physiology of " *Dehalococcoides*" spp. in a trichloroethene-contaminated groundwater site. *Appl Environ Microbiol* 2008;**74**:2728–39.

Leys D, Adrian L, Smidt H. Organohalide respiration: microbes breathing chlorinated molecules. *Phil Trans R Soc B* 2013;**368**:20120316.

Li N, Tong R-L, Yao L *et al.* Identification of two glutathione-dependent 3,6-dichlorogentisate dehalogenases and their roles in the catabolism of the herbicide dicamba in *Rhizorhabdus dicambivorans* Ndbn-20. *Appl Environ Microbiol* 2018, DOI: 10.1128/AEM.00623-18.

Li N, Yao L, He Q *et al.* 3,6-Dichlorosalicylate catabolism is initiated by the DsmABC cytochrome P450 monooxygenase system in *Rhizorhabdus dicambivorans* Ndbn-20. Zhou N-Y (ed.). *Appl Environ Microbiol* 2017, DOI: 10.1128/AEM.02133-17.

Liang yi, Liu X, Singletary MA *et al.* Relationships between the abundance and expression of functional genes from vinyl chloride (VC)-degrading bacteria and geochemical parameters at VC-contaminated sites. *Environ Sci Technol* 2017, DOI: 10.1021/acs.est.7b03521.

Liang B, Jiang J, Zhang J *et al.* Horizontal transfer of dehalogenase genes involved in the catalysis of chlorinated compounds: Evidence and ecological role. *Crit Rev Microbiol* 2012;**38**:95–110.

Liang J, Woodward C, Edelsbrunner H. Anatomy of protein pockets and cavities: Measurement of binding site geometry and implications for ligand design. *Protein Sci* 1998;**7**:1884–97.

Lillis L, Clipson N, Doyle E. Quantification of catechol dioxygenase gene expression in soil during degradation of 2,4-dichlorophenol. *FEMS Microbiol Ecol* 2010;**73**:363–9.

Liu X, Germaine KJ, Ryan D *et al.* Development of a GFP-based biosensor for detecting the bioavailability and biodegradation of polychlorinated biphenyls (PCBs). *J Environ Eng Landsc Manag* 2007;**15**:261–8.

Liu X, Germaine KJ, Ryan D *et al.* Genetically modified *Pseudomonas* biosensing biodegraders to detect PCB and chlorobenzoate bioavailability and biodegradation in contaminated soils. *Bioeng Bugs* 2010;**1**:198–206.

Lopes N, Hawkins SA, Jegier P *et al.* Detection of dichloromethane with a bioluminescent (*lux*) bacterial bioreporter. *J Ind Microbiol Biotechnol* 2012;**39**:45–53.

de Lorenzo V. Recombinant bacteria for environmental release: what went wrong and what we have learnt from it. *Clin Microbiol Infect* 2009;**15**:63–5.

de Lorenzo V, Silva-Rocha R, Carbajosa G *et al.* Sensing xenobiotic compounds: lessons from bacteria that face pollutants in the environment. 2010, In Spiro S and Dixon R (ed.) Sensory mechanisms in bacteria: Molecular aspects of signal recognition.

Lovley DR. Cleaning up with genomics: applying molecular biology to bioremediation. *Nat Rev Microbiol* 2003;**1**:35–44.

Mac Nelly A, Kai M, Svatoš A *et al.* Functional heterologous production of reductive dehalogenases from *Desulfitobacterium hafniense* Strains. *Appl Environ Microbiol* 2014;**80**:4313–22.

McMurdie PJ, Behrens SF, Müller JA *et al.* Localized plasticity in the streamlined genomes of vinyl chloride respiring *Dehalococcoides*. *PLoS Genet* 2009;5:e1000714. DOI: 10.1371/journal.pgen.1000714.

Maillard J, Genevaux P, Holliger C. Redundancy and specificity of multiple trigger factor chaperones in *Desulfitobacteria*. *Microbiol* 2011;**157**:2410–21.

Maillard J, Regeard C, Holliger C. Isolation and characterization of Tn-Dha1, a transposon containing the tetrachloroethene reductive dehalogenase of *Desulfitobacterium hafniense* strain TCE1. *Environ Microbiol* 2005;**7**:107–17.

Maillard J, Willemin MS. Regulation of organohalide respiration. *Adv Microbial Physiol* Vol 74. Elsevier, 2019, 191–238.

Mao X, Stenuit B, Polasko A *et al.* Efficient metabolic exchange and electron transfer within a syntrophic trichloroethene-degrading coculture of *Dehalococcoides mccartyi* 195 and *Syntrophomonas wolfei*. Kostka JE (ed.). *Appl Environ Microbiol* 2015;**81**:2015–24.

Maphosa F, de Vos WM, Smidt H. Exploiting the ecogenomics toolbox for environmental diagnostics of organohalide-respiring bacteria. *Trends Biotechnol* 2010;**28**:308–16.

Marco-Urrea E, García-Romera I, Aranda E. Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. *Nature Biotechnol* 2015;32:620-8.

Mattes TE, Jin YO, Livermore J *et al.* Abundance and activity of vinyl chloride (VC)-oxidizing bacteria in a dilute groundwater VC plume biostimulated with oxygen and ethene. *Appl Microbiol Biotechnol* 2015;**99**:9267–76.

Matturro B, Rossetti S. GeneCARD-FISH: Detection of *tceA* and *vcrA* reductive dehalogenase genes in *Dehalococcoides mccartyi* by fluorescence *in situ* hybridization. *J Microbiol Methods* 2015;**110**:27–32.

McFall SM, Abraham B, Narsolis CG *et al.* A tricarboxylic acid cycle intermediate regulating transcription of a chloroaromatic biodegradative pathway: fumarate-mediated repression of the clcABD operon. *J Bacteriol* 1997;**179**:6729–35.

McFall SM, Chugani SA, Chakrabarty AM. Transcriptional activation of the catechol and chlorocatechol operons: variations on a theme. *Gene* 1998;**223**:257–67.

Men Y, Feil H, Verberkmoes NC *et al.* Sustainable syntrophic growth of *Dehalococcoides ethenogenes* strain 195 with *Desulfovibrio vulgaris* Hildenborough and *Methanobacterium congolense*: global transcriptomic and proteomic analyses. *ISME J* 2012;**6**:410–21.

Men Y, Seth EC, Yi S *et al.* Sustainable growth of *Dehalococcoides mccartyi* 195 by corrinoid salvaging and remodeling in defined lactate-fermenting consortia. *Appl Environ Microbiol* 2014;**80**:2133–41.

Men Y, Yu K, Baelum J *et al.* Metagenomic and metatranscriptomic analyses reveal the structure and dynamics of a dechlorinating community containing *Dehalococcoides mccartyi* and corrinoid-providing microorganisms under cobalamin-limited conditions. *Appl Environ Microbiol* 2017, DOI:10.1128/AEM.03508-16.

Mena-Benitez GL, Gandia-Herrero F, Graham S *et al.* Engineering a catabolic pathway in plants for the degradation of 1,2-dichloroethane. *Plant Physiol* 2008;**147**:1192–8.

Michener JK, Camargo Neves AA, Vuilleumier S *et al.* Effective use of a horizontally-transferred pathway for dichloromethane catabolism requires post-transfer refinement. *eLife* 2014a, DOI: 10.7554/eLife.04279.

Michener JK, Vuilleumier S, Bringel F *et al.* Phylogeny poorly predicts the utility of a challenging horizontally transferred gene in *Methylobacterium* strains. *J Bacteriol* 2014b;**196**:2101–7.

Michener JK, Vuilleumier S, Bringel F *et al.* Transfer of a catabolic pathway for chloromethane in *Methylobacterium* strains highlights different limitations for growth with chloromethane or with dichloromethane. *Front Microbiol* 2016, DOI: 10.3389/fmicb.2016.01116.

Miyauchi K, Lee HS, Fukuda M *et al.* Cloning and characterization of *linR*, involved in regulation of the downstream pathway for hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. *Appl Environ Microbiol* 2002;**68**:1803–7.

Möglich A, Ayers RA, Moffat K. Structure and signaling mechanism of Per-ARNT-Sim domains. *Structure* 2009;**17**:1282–94.

Mohn WW, Garmendia J, Galvao TC *et al.* Surveying biotransformations with à la carte genetic traps: translating dehydrochlorination of lindane (gamma-hexachlorocyclohexane) into *lacZ*-based phenotypes. *Environ Microbiol* 2006;**8**:546–55.

Molenda O, Tang S, Lomheim L *et al.* Extrachromosomal circular elements targeted by CRISPR-Cas in *Dehalococcoides mccartyi* are linked to mobilization of reductive dehalogenase genes. *ISME J* 2019;**13**:24–38.

Moreno-Forero S., Rojas E, Beggah S *et al.* Comparison of differential gene expression to water stress among bacteria with relevant pollutant-degradation properties. *Environ Microbiol Rep* 2016;**8**:91–102.

Morita Y, Futagami T, Goto M *et al.* Functional characterization of the trigger factor protein PceT of tetrachloroethene-dechlorinating *Desulfitobacterium hafniense* Y51. *Appl Microbiol Biotechnol* 2009;**83**:775–81.

Muller EEL, Hourcade E, Louhichi-Jelail Y *et al.* Functional genomics of dichloromethane utilisation in *Methylobacterium extorquens* DM4. *Environ Microbiol* 2011;**13**:2518–35.

Muller EEL. Determining microbial niche breadth in the environment for better ecosystem fate predictions. *mSystem* 2019;**4**:pii: e00080-19. DOI: 10.1128/mSystems.00080-19.

Muller JA, Rosner BM, von Abendroth G *et al.* Molecular identification of the catabolic vinyl chloride reductase from *Dehalococcoides* sp. strain VS and its environmental distribution. *Appl Environ Microbiol* 2004;**70**:4880–8.

Muraoka S, Okumura R, Ogawa N *et al.* Crystal structure of a full-length LysR-type transcriptional regulator, CbnR: unusual combination of two subunit forms and molecular bases for causing and changing DNA bend. *J Mol Biol* 2003;**328**:555–66.

Murínová S, Dercová K. Response Mechanisms of Bacterial Degraders to Environmental Contaminants on the Level of Cell Walls and Cytoplasmic Membrane. *Int J Microbiol* 2014, DOI: 10.1155/2014/873081.

Nielsen TK, Rasmussen M, Demanèche S *et al.* Evolution of *Sphingomonad* gene clusters related to pesticide catabolism revealed by genome sequence and mobilomics of *Sphingobium herbicidovorans* MH. *Genome Biol Evol* 2017;**9**:2477–90.

Nikel PI, de Lorenzo V. Engineering an anaerobic metabolic regime in *Pseudomonas putida* KT2440 for the anoxic biodegradation of 1,3-dichloroprop-1-ene. *Metab Eng* 2013;**15**:98–112.

Nirmalakhandan N, Xu S, Trevizo C *et al.* Additivity in microbial toxicity of nonuniform mixtures of organic chemicals. *Ecotoxicol Environ Saf* 1997;**37**:97–102.

Norman A, Hansen LH, Sørensen SJ. A flow cytometry-optimized assay using an SOS–green fluorescent protein (SOS–GFP) whole-cell biosensor for the detection of genotoxins in complex environments. *Mutat Res Toxicol Environ Mutagen* 2006;**603**:164–72.

Novotna GB, Kwun MJ, Hong H-J. *In vivo* characterization of the activation and interaction of the VanR-VanS two-component regulatory system controlling glycopeptide antibiotic resistance in two related *Streptomyces* species. *Antimicrob Agents Chemother* 2016;**60**:1627–37.

Ohtsubo Y, Goto H, Nagata Y *et al.* Identification of a response regulator gene for catabolite control from a PCB-degrading *beta-proteobacteria, Acidovorax* sp. KKS102. *Mol Microbiol* 2006;**60**:1563–75.

Olaniran AO, Balgobind A, Pillay B. Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *Int J Mol Sci* 2013;**14**:10197–228.

Parnell JJ, Park J, Denef V *et al.* Coping with polychlorinated biphenyl (PCB) toxicity: Physiological and genome-wide responses of *Burkholderia xenovorans* LB400 to PCB-mediated stress. *Appl Env Microbiol* 2006;**72**:6607–14.

Paulin MM, Nicolaisen MH, Sorensen J. Abundance and expression of enantioselective *rdpA* and *sdpA* dioxygenase genes during degradation of the racemic herbicide (R,S)-2-(2,4-dichlorophenoxy)propionate in soil. *Appl Environ Microbiol* 2010;**76**:2873–83.

Pavlova M, Klvana M, Prokop Z *et al.* Redesigning dehalogenase access tunnels as a strategy for degrading an anthropogenic substrate. *Nat Chem Biol* 2009;**5**:727–33.

Peng X, Yamamoto S, Vertès AA *et al.* Global transcriptome analysis of the tetrachloroethene-dechlorinating bacterium *Desulfitobacterium hafniense* Y51 in the presence of various electron donors and terminal electron acceptors. *J Ind Microbiol Biotechnol* 2012;**39**:255–68.

Perez JC, Groisman EA. Evolution of transcriptional regulatory circuits in bacteria. *Cell* 2009;**138**:233–44.

Perez-Rueda E, Hernandez-Guerrero R, Martinez-Nuñez MA *et al.* Abundance, diversity and domain architecture variability in prokaryotic DNA-binding transcription factors. *PLoS ONE* 2018, DOI: 10.1371/journal.pone.019533.

Platero AI, Garcia-Jaramillo M, Santero E *et al.* Transcriptional organization and regulatory elements of a *Pseudomonas* sp. strain ADP operon encoding a LysR-type regulator and a putative solute transport system. *J Bacteriol* 2012;**194**:6560–73.

Poelarends GJ, Kulakov LA, Larkin MJ *et al.* Roles of horizontal gene transfer and gene integration in evolution of 1, 3-dichloropropene-and 1, 2-dibromoethane-degradative pathways. *J Bacteriol* 2000;**182**:2191–9.

Pop SM, Kolarik RJ, Ragsdale SW. Regulation of anaerobic dehalorespiration by the transcriptional activator CprK. *J Biol Chem*. 2004;**279**:49910-8.

Puglisi E, Cahill MJ, Lessard PA *et al.* Transcriptional response of *Rhodococcus aetherivorans* I24 to polychlorinated biphenyl-contaminated sediments. *Microb Ecol* 2010;**60**:505–15.

Ravikumar S, Baylon MG, Park SJ *et al.* Engineered microbial biosensors based on bacterial two-component systems as synthetic biotechnology platforms in bioremediation and biorefinery. *Microb Cell Factories* 2017, DOI: 10.1186/s12934-017-0675-z.

Ray S, Gunzburg MJ, Wilce M *et al.* Structural basis of selective aromatic pollutant sensing by the effector binding domain of MopR, an NtrC family transcriptional regulator. *ACS Chem Biol* 2016;**11**:2357–65.

Rhee S-K, Fennell DE, Häggblom MM *et al.* Detection by PCR of reductive dehalogenase motifs in a sulfidogenic 2-bromophenol-degrading consortium enriched from estuarine sediment. *FEMS Microbiology Ecology* 2003;**43**:317–24.

Rivera-Gomez N, Segovia L, Perez-Rueda E. Diversity and distribution of transcription factors: their partner domains play an important role in regulatory plasticity in bacteria. *Microbiology* 2011;**157**:2308–18.

Roselli S, Nadalig T, Vuilleumier S *et al.* The 380 kb pCMU01 plasmid encodes chloromethane utilisation genes and redundant genes for vitamin B12- and tetrahydrofolate-dependent chloromethane metabolism in *Methylobacterium extorquens* CM4: A proteomic and bioinformatics study. *PLoS ONE* 2013, DOI: 10.1371/journal.pone.0056598.

Rupakula A, Lu Y, Kruse T *et al.* Functional genomics of corrinoid starvation in the organohalide-respiring bacterium *Dehalobacter restrictus* strain PER-K23. *Front Microbiol* 2015;**5**. DOI: 10.3389/fmicb.2014.00751.

Sallabhan R, Kerdwong J, Dubbs JM *et al.* The *hdhA* gene encodes a haloacid dehalogenase that is regulated by the LysR-type regulator, HdhR, in *Sinorhizobium meliloti*. *Mol Biotechnol* 2013;**54**:148–57.

San Millan A, Toll-Riera M, Qi Q *et al.* Interactions between horizontally acquired genes create a fitness cost in *Pseudomonas aeruginosa*. *Nat Commun* 2015, DOI: 10.1038/ncomms7845 |www.nature.com/naturecommunications.

Sanchez MA, Gonzalez B. Genetic characterization of 2,4,6-trichlorophenol degradation in *Cupriavidus necator* JMP134. *Appl Environ Microbiol* 2007;**73**:2769–76.

Scheublin TR, Deusch S, Moreno-Forero SK *et al.* Transcriptional profiling of Gram-positive *Arthrobacter* in the phyllosphere: induction of pollutant degradation genes by natural plant phenolic compounds. *Environ Microbiol* 2014;**16**:2212–25.

Schirmer F, Ehrt S, Hillen W. Expression, inducer spectrum, domain structure, and function of MopR, the regulator of phenol degradation in *Acinetobacter calcoaceticus* NCIB8250. *J Bacteriol* 1997;**179**:1329–36.

Schmid-Appert M, Zoller K, Traber H *et al.* Association of newly discovered IS elements with the dichloromethane utilisation genes of methylotrophic bacteria. *Microbiol Read Engl* 1997;**143**:2557–67.

Selifonova OV, Eaton RW. Use of an *ipb-lux* fusion to study regulation of the isopropylbenzene catabolism operon of *Pseudomonas putida* RE204 and to detect hydrophobic pollutants in the environment. *Appl Environ Microbiol* 1996;**62**:778–83.

Sentchilo V, Ravatn R, Werlen C *et al.* Unusual integrase gene expression on the *clc* genomic island in *Pseudomonas* sp. Strain B13. *J Bacteriol* 2003;**185**:4530–38.

Smidt H, van Leest M, van der Oost J *et al.* Transcriptional regulation of the *cpr* gene cluster in ortho-chlorophenol-respiring *Desulfitobacterium dehalogenans*. *J Bacteriol* 2000;**182**:5683–91.

Speed MC, Burkhart BW, Picking JW *et al.* An archaeal fluoride-responsive riboswitch provides an inducible expression system for hyperthermophiles. *Appl Env Microbiol* 2018, DOI: 10.1128/AEM.02306-17.

Starr LM, Fruci M, Poole K. Pentachlorophenol induction of the *Pseudomonas aeruginosa mexAB-oprM* efflux operon: involvement of repressors NalC and MexR and the antirepressor ArmR. *PLoS ONE* 2012, DOI: 10.1371/journal.pone.0032684.

Stierand K, Rarey M. Drawing the PDB: Protein−ligand complexes in two dimensions. *ACS Med Chem Lett* 2010;**1**:540–5.

Su X, Tsang JSH. Existence of a robust haloacid transport system in a *Burkholderia* species bacterium. *Biochim Biophys Acta BBA - Biomembr* 2013;**1828**:187–92.

Tabata M, Ohhata S, Nikawadori Y *et al.* Comparison of the complete genome sequences of four γ-hexachlorocyclohexane-degrading bacterial strains: insights into the evolution of bacteria able to degrade a recalcitrant man-made pesticide. *DNA Res* 2016;**23**:581–99.

Takeda H, Shimodaira J, Yukawa K *et al.* Dual two-component regulatory systems are Involved in aromatic compound degradation in a polychlorinated-biphenyl degrader, *Rhodococcus jostii* RHA1. *J Bacteriol* 2010;**192**:4741–51.

Tas N, van Eekert MHA, Wagner A *et al.* Role of "*Dehalococcoides*" spp. in the aaerobic transformation of hexachlorobenzene in European rivers. *Appl Environ Microbiol* 2011;**77**:4437–45.

Temme HR, Carlson A, Novak PJ. Presence, diversity, and enrichment of respiratory reductive dehalogenase and non-respiratory hydrolytic and oxidative dehalogenase genes in terrestrial environments. *Front Microbiol* 2019;**10**:1258. DOI: 10.3389/fmicb.2019.01258.

Tittlemier SA, Blank DH, Gribble GW *et al.* Structure elucidation of four possible biogenic organohalogens using isotope exchange mass spectrometry. *Chemosphere* 2002;**46**:511–7.

Tobajas M, Verdugo V, Polo AM *et al.* Assessment of toxicity and biodegradability on activated sludge of priority and emerging pollutants. *Environ Technol* 2016;**37**:713–21.

Torii H, Machida A, Hara H *et al.* The regulatory mechanism of 2,4,6-trichlorophenol catabolic operon expression by HadR in *Ralstonia pickettii* DTP0602. *Microbiology* 2013;**159**:665–77.

Trefault N, Guzmán L, Pérez H *et al.* Involvement of several transcriptional regulators in the differential expression of *tfd* genes in *Cupriavidus necator* JMP134. *Int Microbiol* 2009;**12**:97. DOI: 10.2436/20.1501.01.86.

Tsai W-T. Fate of chloromethanes in the atmospheric environment: Implications for human health, ozone formation and depletion, and global warming impacts. *Toxics* 2017, DOI: 10.3390/toxics5040023.

T'Syen J, Tassoni R, Hansen L *et al.* Identification of the amidase BbdA that initiates biodegradation of the groundwater micropollutant 2,6-dichlorobenzamide (BAM) in *Aminobacter* sp. MSH1. *Environ Sci Technol* 2015;**49**:11703–13.

Türkowsky D, Esken J, Goris T *et al.* A retentive memory of tetrachloroethene respiration in *Sulfurospirillum halorespirans* - involved proteins and a possible link to acetylation of a two-component regulatory system. *J Proteomics* 2018a, DOI: 10.1016/j.jprot.2018.03.030.

Türkowsky D, Lohmann P, Mühlenbrink M *et al.* Thermal proteome profiling allows quantitative assessment of interactions between tetrachloroethene reductive dehalogenase and trichloroethene. *J Proteomics* 2018b, DOI: 10.1016/j.jprot.2018.05.018.

Villacieros M, Whelan C, Mackova M *et al.* Polychlorinated biphenyl rhizoremediation by *Pseudomonas fluorescens* F113 derivatives, using a *Sinorhizobium meliloti nod* system to drive *bph* gene expression. *Appl Environ Microbiol* 2005;**71**:2687–2694.

Wagner A, Segler L, Kleinsteuber S *et al.* Regulation of reductive dehalogenase gene transcription in *Dehalococcoides mccartyi*. *Philos Trans R Soc B Biol Sci* 2013, DOI: 10.1098/rstb.2012.0317.

Waller AS, Hug LA, Mo K *et al.* Transcriptional analysis of a *Dehalococcoides*-containing microbial consortium reveals prophage activation. *Appl Environ Microbiol* 2012;**78**:1178–86.

Wang X, Teng Y, Tu C *et al.* Coupling between nitrogen fixation and tetrachlorobiphenyl dechlorination in a rhizobium–legume symbiosis. *Environ Sci Technol* 2018;**52**:2217–24.

Ward WO, Swartz CD, Porwollik S *et al.* Toxicogenomic analysis incorporating operon-transcriptional coupling and toxicant concentration-expression response: analysis of MX-treated *Salmonella*. *BMC Bioinformatics* 2007;**8**:378. DOI: 10.1186/1471-2105-8-378.

Wedemeyer G. Dechlorination of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane by Aerobacter aerogenes. I. Metabolic products. *Appl Microbiol* 1967;15:569-74.

Weir KM, Sutherland TD, Horne I *et al.* A single monooxygenase, Ese, is involved in the metabolism of the organochlorides endosulfan and endosulfate in an *Arthrobacter* sp. *Appl Environ Microbiol* 2006;**72**:3524–30.

Weng X, Xiao J. Spatial organization of transcription in bacterial cells. *Trends Genet TIG* 2014;**30**:287–97.

Werner JJ, Ptak AC, Rahm BG *et al.* Absolute quantification of *Dehalococcoides* proteins: enzyme bioindicators of chlorinated ethene dehalorespiration. *Environ Microbiol* 2009;**11**:2687–97.

Whangsuk W, Dubbs JM, Sallabhan R *et al.* ChpR Is a chlorpyrifos-responsive transcription regulator in *Sinorhizobium meliloti*. *J Mol Microbiol Biotechnol* 2010;**18**:141–7.

Whangsuk W, Thiengmag S, Dubbs J *et al.* Specific detection of the pesticide chlorpyrifos by a sensitive genetic-based whole cell biosensor. *Anal Biochem* 2016;**493**:11–3.

White PM, Potter TL, Culbreath AK. Fungicide dissipation and impact on metolachlor aerobic soil degradation and soil microbial dynamics. *Sci Total Environ* 2010;**408**:1393–402.

van den Wijngaard AJ, van der Kamp KW, van der Ploeg J *et al.* Degradation of 1,2-dichloroethane by *Ancylobacter aquaticus* and other facultative methylotrophs. *Appl Environ Microbiol* 1992;**58**:976–83.

van den Wijngaard AJ, Reuvekamp PT, Janssen DB. Purification and characterization of haloalcohol dehalogenase from *Arthrobacter* sp. strain AD2. *J Bacteriol* 1991;**173**:124–9.

Wise AA, Kuske CR. Generation of novel bacterial regulatory proteins that detect priority pollutant phenols. *Appl Environ Microbiol* 2000;**66**:163–9.

Wöhrnschimmel H, Scheringer M, Bogdal C *et al.* Ten years after entry into force of the Stockholm convention: What do air monitoring data tell about its effectiveness? *Environ Pollut Barking Essex 1987* 2016;**217**:149–58.

Xiu Z, Gregory KB, Lowry GV *et al.* Effect of bare and coated nanoscale zerovalent iron on *tceA* and *vcrA* gene expression in *Dehalococcoides* spp. *Environ Sci Technol* 2010;**44**:7647–51.

Yang W, Lai L. Computational design of ligand-binding proteins. *Curr Opin Struct Biol* 2017;**45**:67–73. DOI: 10.1016/j.sbi.2016.11.021.

Yoshida S, Ogawa N, Fujii T *et al.* Enhanced biofilm formation and 3-chlorobenzoate degrading activity by the bacterial consortium of *Burkholderia* sp. NK8 and *Pseudomonas aeruginosa* PAO1. *J Appl Microbiol* 2009;**106**:790–800.

Zhang H, Jiang X, Xiao W *et al.* Proteomic strategy for the analysis of the polychlorobiphenyl-degrading cyanobacterium *Anabaena* PD-1 exposed to aroclor 1254. *PLoS ONE* 2014, DOI: 10.1371/journal.pone.0091162.

Zhang H, Wan H, Song L *et al.* Development of an autofluorescent *Pseudomonas nitroreducens* with dehydrochlorinase activity for efficient mineralization of γ-hexachlorocyclohexane (γ-HCH). *J Biotechnol* 2010;**146**:114–9.

Zhu BZ, Kalyanaraman B, Jiang GB. Molecular mechanism for metal-independent production of hydroxyl radicals by hydrogen peroxide and halogenated quinones. *Proc Natl Acad Sci USA* 2007; 104:17575–8.

Table 1. Transcription factors involved in detection of organohalides

| Transcription factor | Degradation pathway | Ligand | Target DNA sequence | Crystallographic structure | Organism | Reference |
|---|---|---|---|---|---|---|
| Directly organochlorine pollutants recognized as ligands | | | | | | |
| BphS1, BphR1 | polychlorinated biphenyls | biphenyl; dichlorobenzene [a] | TTCCGTAGTTTTCCCGGATGTTCG | No | *Rhodococcus jostii* RHA1 | (Takeda *et al.* 2010) |
| HadR | 2,4,6-trichlorophenol | 2,4,6-trichlorophenol | ATGCCGCTGAGGAAT | No | *Cupriavidus pickettii* DTP0602 | (Torii *et al*. 2013) |
| MopR | 3-chlorophenol | 3-chlorophenol [b] | TTCATCAAATAATGGA-8nt-ATGCTGATTCATCAA | Yes | *Acinetobacter calcoaceticus* NCIB8250 | (Ray *et al*. 2016) |
| PcpR | pentachlorophenol; 2,4,6-trichlorophenol | pentachlorophenol [b]; 2,4,6-trichlorophenol [b] | ATTC-7nt-GAAT | Yes | *Sphingobium chlorophenolicum L-1* | (Hayes *et al*. 2014) |
| Degradation products recognized as ligands | | | | | | |
| | | | | | | |
| CbnR | 3-chlorobenzoate | cis,cis-muconate | unknown | Yes | *Ralstonia eutropa* NH9 | (Muraoka *et al*. 2003) |
| CprK1 | chlorophenols | orthochlorophenolacetic acid [b] | TTTA-8nt-CTAA-20nt-TATAAT | Yes | *D. hafniense* DCB-2 | (Gábor *et al*. 2008) |
| LinR | lindane | 2,5-dichlorohydroquinone; chlorohydroquinone | ATTCACAATCTGAAT | No | *Sphingomonas paucimobilis* UT26 | (Miyauch*i et a*l. 2002) |
| Rdh2R | trichlorobenzene | 2,3-dichlorophenol [b]; 2,4-dichlorophenol [b]; 3,4-dichlorophenol [b] | TAGTATACGAT-2nt-TAGTATACGATTA | No | *Dehalococcoides mccartyi* CBDB1 | (Krasper *et al.* 2016) |
| Unknown ligands | | | | | | |
| CbrC | trichlorobenzene | unknown | unknown | No | *Dehalococcoides mccartyi* CBDB1 | (Adrian *et al*. 2007) |
| DcmR | dichloromethane | unknown | unknown | No | *Methylorubrum extorquens* 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 |
|---|---|---|---|---|---|---|---|
| *Arthrobacter chlorophenolicus* A6 | 4-chlorophenol | tryptone soy agar | agar plate | 569/4 255 | 580/4 255 | micro-array | (Scheublin *et al.* 2014) |
| | | N.A. | microcosm with plant (phyllosphere low humidity) | 390/4 255 | 412/4 255 | | |
| | | N.A. | microcosm with plant (phyllosphere high humidity) | 430/4 255 | 474/4 255 | | |
| *Burkholderia xenovorans* LB400 | aroclor 1242 | biphenyl | liquid culture | 28/8 249 | 19/8 429 | RNA-seq | (Parnell *et al.* 2006) |
| *Dehalococcoides* spp. KB1™ | vinyl chloride | methanol | microcosm | 452/19 200 [b] | 95/19 200 [b] | micro-array | (Waller *et al*. 2012) |
| *Desulfitobacterium hafniense* DCB-2 | 3-chloro-4-hydroxybenzoate<br>3,5-dichlorophenol<br>ortho-bromophenol | uranyl acetate, sodium selenate, ferric citrate | liquid culture | N.A. [c] | N.A. | micro-array | (Kim *et al*. 2012) |
| *Desulfitobacterium hafniense* Y51 | trichloroethene | MMYPF-medium (pyruvate and fumarate) | liquid culture | 24/5 060 | N.A./5 060 | micro-array | (Peng *et al*. 2012) |
| *Methylorubrum extorquens* CM4 | chloromethane | methanol | liquid culture | 137/6 262 | 13/6 262 | RNA-seq | (Chaignaud *et al.* 2017) |
| *Methylorubrum extorquens* DM4 | dichloromethane | methanol | liquid culture | 69 /5 701 | 121/5 701 | RNA-seq | (Chaignaud *et al*. 2017) |
| *Nitrosomonas europaea* | chloromethane | mineral medium | liquid culture | 67 /2 460 | 148/2 460 | micro-array | (Gvakharia *et al*. 2007) |
| | chloroform | mineral medium | liquid culture | 175/2 460 | 501/2 460 | | |
| *Pigmentiphaga* sp. H8 | 3,5-dibromo-4-hydroxybenzoate | non induced culture | liquid culture | 98/5 678 | 31/5 678 | RNA-seq | (Chen *et al*. 2018) |
| *Polaromonas* sp. JS666 | cDCE | glycolate | liquid culture | 140/5 569 | 450/5 569 | micro-array, proteomics | (Jennings *et al*. 2009) |
| *Rhodococcus aetherivorans* I24 | aroclor 1254 | glucose | liquid culture | 129/3 524 | 87/3 524 | micro-array | (Puglisi *et al.* 2010) |
| | | | microcosm (contaminated Rotterdam harbour sediments) | 207/3 524 | 176/3 524 | | |
| | | | | 44/3 524 | 67/3 524 | | |
| *Salmonella typhimurium* TA100 | MX chlorinated furanone | dimethyl sulfoxide | liquid culture | 169/4 253 [b] | | micro-array | (Ward *et al*. 2007) |

[a] Number 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 | *Variovorax* sp WDL1 [b] | 1 372 | | RNA-seq | (Albers *et al.* 2018) |
| | | *Comamonas testosteroni* WDL7 | 169 | | | |
| | | *Hyphomicrobium sulfonivorans* WDL | N.A. [c] | | | |
| trichloroethene | liquid culture | *Dehalococcoides mccartyi* 195 [b] | 102 | | micro-array | (Men *et al*. 2012) |
| | | *Desulfovibrio vulgaris* Hildenborough | N.A. | N.A. | | |
| | | *Methanobacterium congolense* | N.A. | N.A. | | |
| trichloroethene | liquid culture with vitamin $B_{12}$ | *Dehalococcoides mccartyi* 195 [b] | 28 | 18 | micro-array | (Men *et al.* 2014) |
| | | *Desulfovibrio vulgaris* Hildenborough | N.A. | N.A. | | |
| | | *Pelosinus fermentans* R7 | N.A. | N.A. | | |
| trichloroethene | liquid culture without vitamin $B_{12}$ | *Dehalococcoides mccartyi* 195 [b] | 30 | 44 | micro-array | (Men *et al*. 2014) |
| | | *Desulfovibrio vulgaris* Hildenborough | N.A. | N.A. | | |
| | | *Pelosinus fermentans* R7 | N.A. | N.A. | | |
| trichloroethene | microcosm (contaminated groundwater with or without cobalamin) | 7 bin genomes | 550 | | RNA-seq, micro-array | (Men *et al.* 2017) |
| trichloroethene | liquid culture | *Dehalococcoides mccartyi* 195 [b] *Syntrophomonas wolfei* | 18 | 196 | micro-array | (Mao *et al.* 2015) |

[a] Number of genes regulated; [b] Dehalogenating partner; [c] N.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.

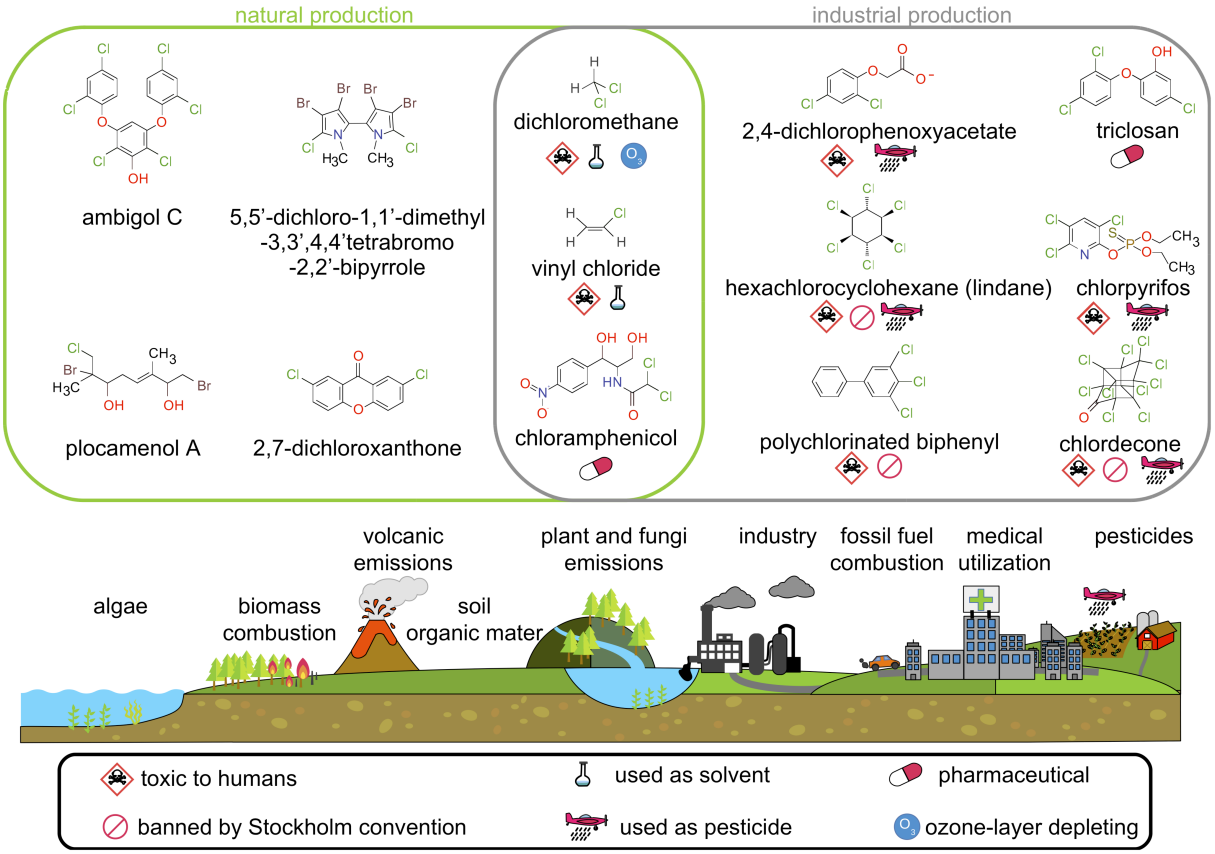


**Figure 2. Gene clusters for organohalide degradation.** (**A**) Gene clusters for dehalogenation in representative bacterial degraders. (**B**) Synthetic gene clusters. (**C**) Organohalide-relevant catabolic gene clusters lacking dehalogenase genes. References are for gene clusters *atz* (Esquirol 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, Ehrt 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).

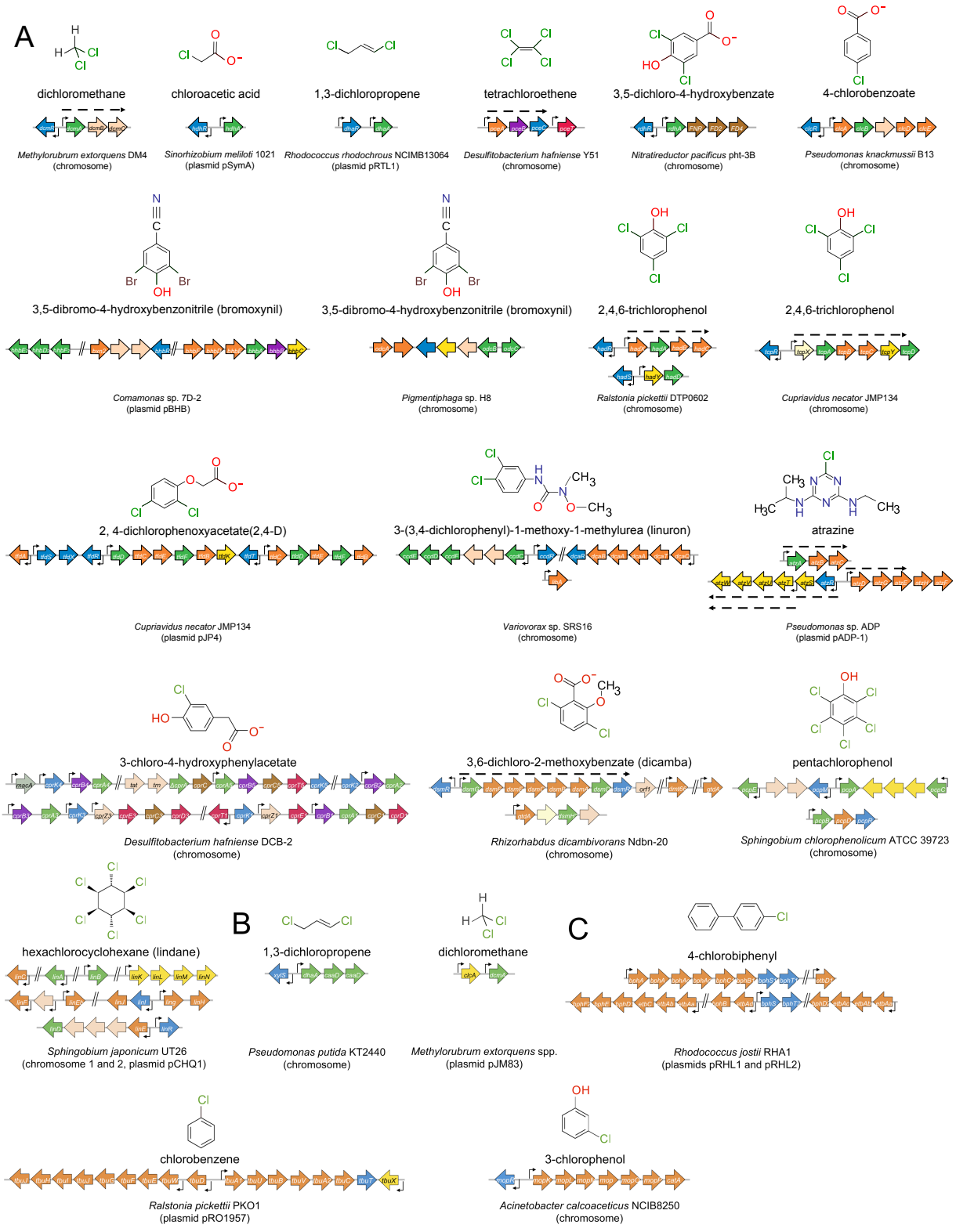


**Figure 3. Functional domains of selected transcriptional factors associated with dehalogenation**. (**A**) Transcription factors; (**B**) Proteins of two component systems. The HTH (helix-turn-helix) DNA binding domain (blue) present in almost all transcription factors is fused to organohalide-specific 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).

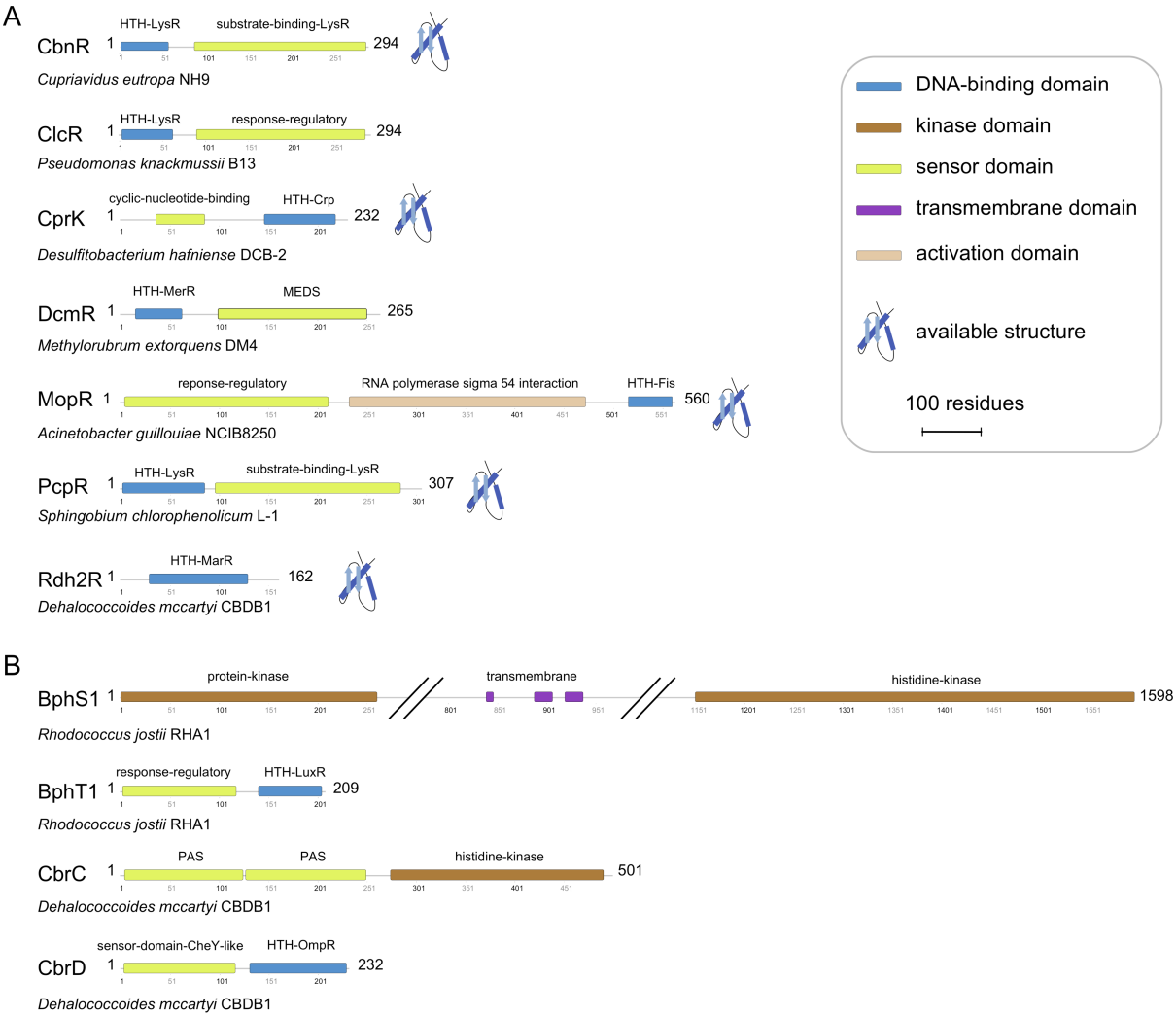


**Figure 4. Interactions between organohalides and transcription factors**. Black and green dashed lines represent hydrogen bounds and hydrophobic interactions, respectively. Orange dashed lines indicate interactions between aromatic rings. 2D representations generated by PoseView (Stierand and Rarey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

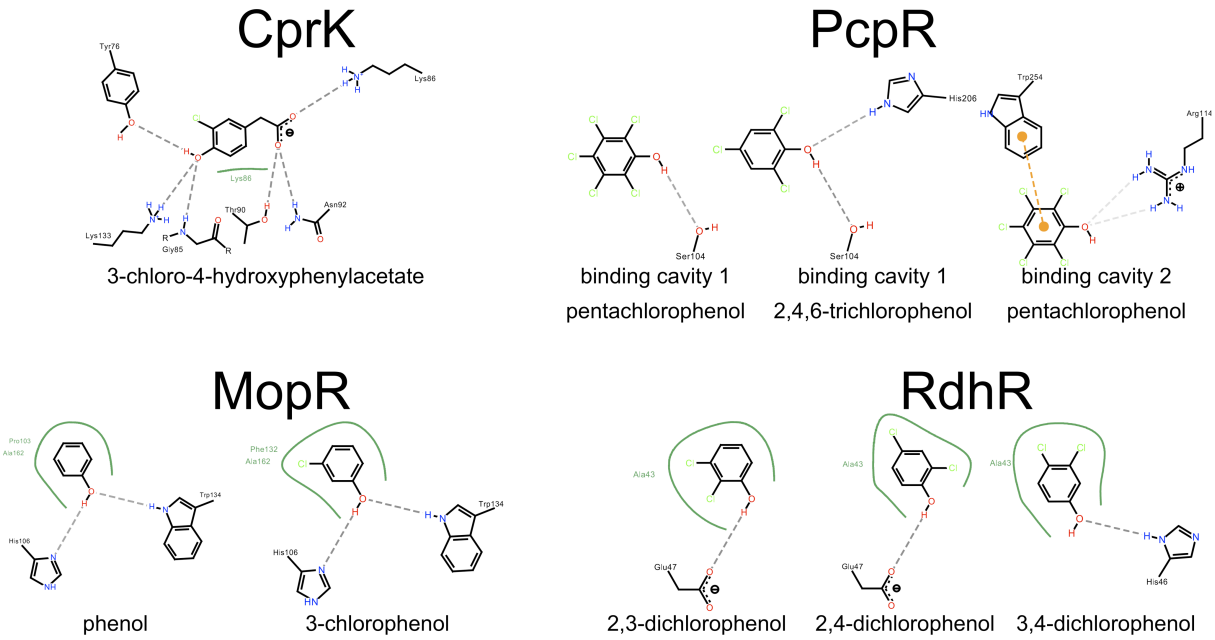


**Figure 5. Examples of transcriptional regulation in organohalide degradation in Gram-negative and Gram-positive bacteria**. (**A**) Atrazine utilization in *Pseudomonas* sp. ADP. Binding of cyanuric acid to AtzR activates expression of *atzDEF*. Expression of *atzR* and downstream genes is activated 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 Desulfitobacterium 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.