Organophosphorus-degrading bacteria: ecology and industrial applications

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Abstract | The first organophosphorus (OP) compound-degrading bacterial strain was isolated from a paddy field in the Philippines in 1973. Since then, several phylogenetically distinct bacteria that can degrade OP by co-metabolism, or use OPs as a source of carbon, phosphorus or nitrogen, have been isolated from different parts of the world. There is huge potential for industrial applications of OP-degrading bacteria. Important advances in our understanding of the microbiology, genomics and evolution of OP-degrading bacteria have been made over the past four decades, and are discussed in this Review.

Bioremediation
A biological process that uses living organisms or their products (enzymes) to convert a harmful substance to a non-toxic substance or to return the contaminated environment to its original condition.

Biosensor
A detector device made from a biological component combined with a physico–chemical detector that is used for detection of a substance or chemical.

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Synthetic organophosphorus (OP) compounds have been used worldwide as pesticides, petroleum additives and plasticizers, and as pesticides have been in use since the end of the Second World War[1]. More than 100 OP pesticides are in use worldwide, accounting for ~38% of total pesticide usage[2]. In the United States alone, approximately 50,000 tonnes of OP pesticides are used per year[3], and, as a result of excessive and continuous use, many terrestrial and aquatic ecosystems across the world are contaminated with OP compounds[4,5]. One survey found that all 20 sites sampled in Scotland and 75% of 107 sites sampled in Wales were contaminated by diazinon, an OP compound used in sheep dips[6]. Although they are biodegradable, OP compounds are highly toxic to mammals and are toxic to other non-target animals, and the toxic effects of OP compounds on invertebrates, vertebrates and wildlife are well documented[7]. Approximately 3 million poisonings and 300,000 human deaths occur per year owing to OP ingestion, and OP poisoning is a global clinical problem[8]. Poisoning occurs owing to accidental spillage, terrorist attacks, suicide attempts and occupational hazards, such as accidents that involve workers and farmers. Acute or chronic toxicity of OP compounds has also been implicated in a range of nerve and muscular disorders[7,9]. Another major source of OP compounds is the storage of extremely toxic chemical warfare agents (CWAs). It is estimated that ~30,000 tonnes of OP CWAs are stored in the United States alone and ~200,000 tonnes of OP CWAs are stored worldwide. Lists of OP compounds are available from pesticide manuals and various web sites. Some of the more widely known compounds are listed in TABLE 1.

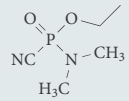
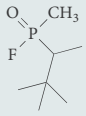
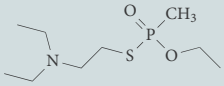
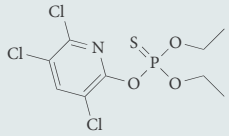
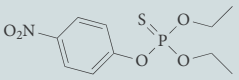
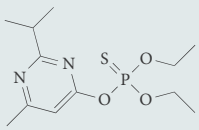
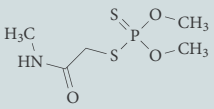
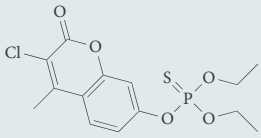
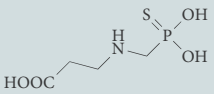
The biochemical basis, types and clinical features of OP poisoning have been previously reviewed[9,10]. All OP compounds have similar chemical structures (BOX 1) and therefore have similar mechanisms of toxicity. In brief, OP compounds inhibit acetylcholine breakdown in synapses and red blood cell membranes[11]. Acetylcholine is a vital component of the nervous system and enables the transmission of nerve impulses in the brain, skeletal and muscular systems. However, for proper functioning, and to avoid overstimulation, acetylcholine is hydrolysed to choline and acetyl-CoA by acetylcholine esterase (AChE). OP compounds inhibit the activity of AChE by covalently binding to its active site. The hydrolysis of phosphorylated AChE is extremely slow and results in the overstimulation of acetylcholine receptors in synapses of autonomic and central nervous systems and neuromuscular junctions, which in turn causes agitation, hypersalivation, confusion, convulsion, respiratory failure and ultimately death of insects and mammals[9]. However, the duration and severity of intoxication by different OP compounds varies according to both the nature of the compounds and the route of exposure[8,10] (TABLE 1).

In this Review, I summarize recent research and identify future challenges in OP research. I also describe approaches that might help us realize the potential of OP-degrading bacteria (OPDB) for bioremediation, biosensors and human therapy.

Environmental fate and microbial degradation

Despite high toxicity to mammals, including humans, OP compounds have been increasingly used because they are considered to be biodegradable. Most OP

Table 1 | **Selected organophosphorus compounds**

Compound	Use	Half-life (days)	Mammalian toxicity*
Tabun 	CWA	1.5–2.5	150–400
Sarin 	CWA	1.5–2.5	75–100
Soman 	CWA	1.5–2.5	35–50
VX 	CWA	4–42	10
Chlorpyrifos 	Insecticide	16–120	135–163
Parathion 	Insecticide	30–180	2–10
Diazinon 	Insecticide	11–21	80–300
Dimethoate 	Insecticide	2–41	160–387
Coumaphos 	Acaricide	24–1,400	16–41
Glyphosphate 	Herbicide	30–174	3,530–5,600

*Mammalian toxicity for all chemical warfare agents (CWAs) is expressed in terms of the lethal dose, whereas the LD_{50} value (concentration ingested (mg per kg of animal weight) at which half of the tested animals die) is provided for the pesticides. Because CWAs are highly vaporized, most deaths occur owing to inhalation or skin contact. The values provided for the CWAs indicate the lethal dose by inhalation (mg per min per m^3). All CWAs have incapacitating effects that occur in 1–10 minutes and the lethal effect occurs in 10–15 minutes.

compounds have a short half-life (TABLE 1) in the environment, as they are degraded by microorganisms. In general, OP compounds do not adversely affect bacteria, because bacteria do not possess AChE, and some microorganisms can even use OPs as an energy source[12]. There have been several reports that repeated applications of OP pesticides lead to enhanced biodegradation[13,14]. Enhanced biodegradation of OP compounds is also influenced by soil properties and the chemical structure of the OPs. Alkaline soils have been shown to be conducive to a higher degradation rate of two OP insecticides, the widely used chlorpyrifos[13,15] and fenamiphos[14]. These observations led to the hypothesis that OP-degrading genes might have evolved from genes that are required for survival in an alkaline pH[13]. OP compounds share similar chemical structures, and therefore soil that developed enhanced degradation for one OP compound also rapidly degraded other OP compounds, in a well-known phenomenon called cross-enhanced degradation[16].

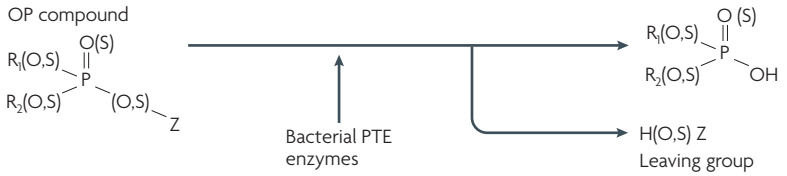
Ecological studies of OPDB have mainly been carried out on isolated bacteria. As <1% of environmental bacteria can be isolated into pure culture, it is important that culture-independent methods are used to probe the true diversity and community structure of OPDB. In the environment, multiple species of microorganisms occupy one niche. One or more populations can adapt quickly to the availability of a substrate, such as OP, and thus grow to become the dominant species, whereas other species coexist as minority members of the community. Culture-independent methods can identify dominant groups that might elude culturing methods[17]. OPDB belong to several different phylogenetic groups, so the use of universal markers (such as the 16S ribosomal RNA gene) is less informative. A better approach is to target functional genes that encode enzymes for OP degradation. Singh *et al.* unsuccessfully attempted to analyse one such functional marker (the OP hydrolase (OPH) encoding gene *opd* (organophosphorus degrading)) directly from soils using PCR, but the use of DNA hybridization produced positive results[13]. Since then, several new sequences of OP-degrading genes have been identified and submitted to public databases, allowing the design of primers with broad specificity. This, in turn, should enable the PCR amplification of these functional genes directly from the environment. The use of culture-independent methods (see BOX 2 for a summary of different approaches) should provide a platform to understand the diversity of OPDB and their ecological niche, and could help unravel the environmental factors that influence expression and regulation of OP-degrading functions. This information could be further exploited to predict the environmental fate and efficacy of OP compounds against target organisms.

OP-degrading bacteria and enzymes

In 1973, the first bacterium to degrade OP compounds was isolated from a soil sample from the Philippines and was identified as *Flavobacterium* sp. ATCC 27551 (REF. 18). Since then, several bacteria, a few fungi and cyanobacteria, have been isolated that can use OP compounds as a source of carbon, nitrogen or phosphorus. A few consortia with the capability to metabolize OP compounds have also been reported[12,14]. A list of well-known OP-degrading bacteria and their degrading gene or enzymes are provided in TABLE 2. Most microorganisms can degrade one OP or a narrow range of OP compounds[12]. For biotechnological applications, it is important that bacterial

Biodegradation
A process by which an indigenous bacterial population acquires genes that encode enzymes to allow the use of xenobiotics as an energy source.

Box 1 | **General structure of organophosphorus compounds**



All organophosphorus (OP) compounds share a similar general structure (see the figure). They are mainly esters or thiols, and R_1 and R_2 can be aryl or alkyl groups that are attached to a phosphorus atom either directly (phosphinates) or through an oxygen (phosphates) or a sulphur (phosphorothioates) atom[56]. In phosphonates, R_1 is directly bonded to the P atom and R_2 is linked to either an oxygen or a sulphur atom (phosphorothioates). In phosphoramidates, at least one of the R groups is attached to $–NH_2$. The Z group, which is released during hydrolysis (see the figure), and is thus also known as the leaving group, can be a halogen or an aliphatic, aromatic or hetrocyclic group. The double-bonded atom to phosphorous may be oxygen or sulphur (called phosphates or phosphorothioate, respectively). The structure of OP compounds and degradation products during hydrolysis by the bacterial enzymes is shown in the figure. PTE, phosphotriesterase.

degraders have activity against all or most OP compounds. A few suitable species have been isolated; for example, *Flavobacterium* sp. ATCC 27551, which was isolated by virtue of its ability to degrade diazinon metabolism, can degrade almost all known OP bonds, with a range of efficacies, by enzymatic hydrolysis[18] (BOX 1). Similarly, *Brevundimonas diminuta*, which was isolated from an enrichment culture in the United States, can cleave all known OP bonds[19]. These two microorganisms have received the most scientific attention during the past three decades. Another *Flavobacterium* sp. isolated from an Indian soil sample, as well as *Agrobacterium radiobacter* and an *Enterobacter* sp. isolated from Australian soils, can also degrade several OP compounds[13,20,21].

Phosphotriesterases (PTEs) are a group of enzymes that can degrade OP compounds, and are found in microorganisms, animals and plants. There are three different types of well-characterized bacterial PTEs.

Organophosphorus hydrolase (OPH). The OPHs purified from *B. diminuta* and *Flavobacterium* spp. have identical, or very similar, amino-acid sequences[19,20,22]. A variant of OPH called OPDA (OP-degrading enzyme) has been purified from *A. radiobacter*[21]. OPHs are members of the amidohydrolase superfamily and share the same $(\alpha–\beta)_8$ barrel structural folds and an active site with two transition metal ions, such as zinc, iron, manganese or cobalt. Whereas the protein-scaffold and key active-site features of all amidohydrolases are conserved, the substrate specificity and reaction mechanisms of different family members vary owing to differences in the sequence and length of the β- or α-loops that comprise the substrate-binding loops (in particular loops one, seven and eight)[23]. OPH is a dimer of two identical subunits that contain 336 amino-acid residues and have a molecular mass of ~72 kDa[23]. It has a broad substrate specificity and can hydrolyse P–O, P–F and P–S bonds, but with different efficiencies[12]. OPDA has 90% amino-acid homology, and shares a similar secondary structure, with OPH[21,24,25]. The most important difference between OPH and OPDA is the presence of 20 amino-acid residues at the carboxyl terminus[25].

OPH is thought to have evolved during the past 70 years, as OPs were not present in the environment until after the Second World War. The course of PTE evolution remains unresolved, as it is difficult to understand the speed at which this enzyme has evolved. Recent studies have provided evidence that OPH and OPDA may have evolved from other existing enzymes. The emergence of a completely novel enzyme through multiple amino-acids substitutions is unlikely, because the frequency of point mutations is low (1 in 10^6 per gene per generation) and the likelihood of a specific mutation occurring in a gene is accordingly low (10^9 per gene per generation)[26,27]. Therefore, evolutionary processes occur gradually and are only maintained if they improve fitness. Afriat *et al.*[28] proposed that the ability of a progenitor enzyme to promiscuously catalyse low levels of the evolving activity could facilitate the divergence of a new function by providing an immediate selective advantage. To prove the direct role of promiscuity in the divergence of natural enzymes, they positively tested the activity of OPH and lactonase against both OP compounds and lactones. They also tested some putative OPHs (genes obtained from whole genome sequences from several bacteria that encode an unknown function but share significant similarity with OPH sequences) for both lactonase and OP-degrading activities[28]. Three genes had high lactonase and low promiscuous OPH activities, and were therefore named PTE-like lactonases (PLLs). These enzymes were active against *N*-acyl homoserine lactone (AHL), a bacterial signal molecule; the chemical structure, ecological role and lactonase-dependent degradation mechanism of AHLs are summarized in BOX 3. This observation was further strengthened by the recent purification and characterization of the SsoPox protein from the hyperthermophilic archaeon *Sulfolobus solfataricus* MT4 (REFS 29,30). SsoPox only has 30% sequence identity to other known OPHs. Despite this, the amino acids that coordinate the binuclear metal centres of different OPHs are conserved. SsoPox degrades OP compounds at a similar k_m (Michaelis constant), but lower k_{cat} (catalytic constant), value[29], and can degrade several lactones at a 20-fold higher rate than lactonase AHL1 (REF. 28). It has been proposed that, given the roles of AHL, PLLs have probably existed for many millions of years, and therefore it has been argued that PTEs could have evolved from a PLL family with the promiscuous OP-degrading activity of PLLs, thereby acting as an essential starting point of divergence[28,29]. In this respect, SsoPox resembles a generalist intermediate[31] from which OPH might have evolved, specifically by insertion of the $(\alpha–\beta)_8$ barrel into loop seven[28].

The availability of numerous bacterial genome sequences provides an opportunity to search for similar sequences. Analysis of whole genome sequences reveals that several bacteria have similar PTE gene

Box 2 | **Approaches to study unculturable OPDB in natural environments**

- The diversity and community structure of organophosphorus (OP)-degrading bacteria (OPDB) can be studied using direct PCR amplification combined with other DNA-fingerprinting methods, such as terminal restriction fragment length polymorphism (T-RFLP) or denaturing gradient gel electrophoresis (DGGE)[62]. This approach could be combined with real-time PCR to quantify the number of particular functional genes in an OPDB community.
- Radiolabelled OP compounds are widely used to determine their environmental fate and persistence. In combination with fluorescence *in situ* hybridization (FISH) and radioactive substrates, the activity of the degrading population can be visualized in a technique known as microautoradiography–FISH[63]. This technique has been successfully used to identify the activity of 'unculturable' bacteria from other substrates[64]. However, for safety reasons, the use of radioisotopes for field experiments is problematic.
- Stable isotope probing has been used to identify unculturable microorganisms that degrade natural and man-made compounds[65,66] and could be applied to OPDB.
- Microarray, transcriptomic and proteomic approaches could be used to probe the ecology and activity of OPDB in natural environments; for example, to analyse environmental samples.

sequences to *opd* (and *opd*A), including the pathogens *Mycobacterium tuberculosis* (locus tag YP 001286177) and *Mycobacterium bovis* (locus tag YP 976375), the radioresistant *Deinococcus radiodurans* (locus tag AAF 10507), the anaerobic bacterium *Desulfatibacillum alkenivorans* (locus tag ZP 02130319), the facultatively aerobic, thermophilic bacterium *Geobacillus* sp. (locus tag EDV 77399) that was found in a deep soil reservoir, the thermophilic bacterium *Thermoanaerobacter* sp. X514 (locus tag NC 010320), *Escherichia coli* (*yhfV*) and several other groups of phylogenetically and physiologically distinct bacteria, as well as some Archaea. Bioinformatics was used to identify a PTE activity in the halophilic archaeal species *Sulfolobus acidocaldarius*[32]. The putative homologue of SsoPox, which was designated SAC12140, was then cloned to evaluate the activity of translated proteins against OP compounds[33]. The SAC12140 protein shares 76% sequence identity with SsoPox and 34% sequence identity with OPH and is active against all tested OP compounds. As the number of whole-genome sequences for bacteria is growing exponentially, a similar approach for the identification and expression of various PTE and PLL genes from bacteria will further our knowledge of the mechanism of PTE evolution.

Methyl parathion hydrolase (MPH). MPH is present in several phylogenetically unrelated bacteria, and is active against several OP compounds, but has a narrower substrate range than OPH. The crystal structure of the MPH (which is a member of the β-lactamase superfamily) from *Pseudomonas* sp. WBC-3 has been solved[34]. MPH is a dimer in which each subunit has a mixed-hybrid, binuclear zinc centre. MPH is not homologous to any other PTEs, however, even though several PTEs can degrade methyl parathion[34]. Several *mpd* (methyl parathion degrading) genes have been cloned recently, and phylogenetic analysis confirmed that *mpd* genes have evolved separately from *opd* genes (FIG. 1). Unlike *opd* genes, all known *mpd* genes have been isolated from one country (China), suggesting that the environment has an influence on *mpd* evolution. Whole genome-sequence analysis also suggests that *mpd* and β-lactamase gene homologues are present in other bacteria, such as *Methylibium petroleiphilum* (locus tag NC 008825), *Azoarcus* sp. (locus tag AM 406670), *Leptothrix cholodnii* (locus tag CP 00001013), *Chromobacterium violaceum* (locus tag AE O16825) and *Sinorhizobium meliloti* 1021 (locus tag AE 006469). Interestingly, an AHL lactonase from *Bacillus thuringiensis* also belongs to the β-lactamase superfamily. AHL lactonase has some promiscuous PTE activities, so it is possible that OPH and MPH have evolved from different lactonase enzymes[28]. Further insight into the progenitor of MPH can be obtained by evaluating β-lactamase activity against OP compounds and the level of MPH activity against lactones and lactamase substrate.

Organophosphorus acid anhydrolase (OPAA). Another OP-degrading enzyme that has received considerable attention is OPAA (encoded by *opaA* (organophosphorus acid anhydrolase)), which was isolated from *Alteromonas undina* and *Alteromonas haloplanktis*[35,36]. This enzyme belongs to the dipeptidase family and does not share enzyme or gene-sequence similarity either with OPH or MPH (FIG. 1). This suggests that the OP-degrading function of OPAA might have evolved from different progenitors.

Because of differences in the chemical structure of lactones and OP, and in the scaffold and catalytic mechanism of lactonase and bacterial PTEs, it was proposed that a promiscuous phosphotriesterase activity of lactonases emerged from the overlap between the transition states of lactones and OP hydrolysis[28]. Although the catalytic mechanism of microbial PTE activity, including transition states, is still controversial, the fact that other hydrolases with similar transition states, such as esterase and prolidase (of which OPAA is a member), also have promiscuous phosphotriesterase activities supports this hypothesis[29]. Recent studies have unravelled some evolutionary aspects of microbial PTEs by assigning a natural substrate (AHL) for their progenitors. However, this may be just the 'tip of the iceberg' and many more progenitors for other PTEs will emerge in the future. Several hundreds of bacteria with OP-degrading capability have been isolated, but the genes and enzymes that are involved in degradation are known only for a few isolates. By systematically purifying enzymes from all known OPDB and examining their enzymatic activity, three-dimensional structure, substrate specificity, catalytic mechanisms and encoding genes, we will further advance our knowledge of microbial PTE evolution.

Genetic organization of OP-degrading activity

Despite 100% similarity in amino-acid and DNA sequences, *opd* genes isolated from *B. diminuta* and *Flavobacterium* sp. ATCC 27551 are located on

Table 2 | **Organophosphorus-degrading bacteria**

Organism	Degrading enzyme	Enzyme structure	Encoding genes	Country of origin
Flavobacterium sp. ATCC 27551	OPH	Dimer	*opd*	Philippines
Pseudomonas diminuta	OPH	Dimer	*opd*	United States
Agrobacterium radiobacter	OPDA	Dimer	*opdA*	Australia
Alteromonas haloplanktis	OPAA	Monomer	*opa*	United States
Pseudomonas sp. WBC-3	MPH	Dimer	*mpd*	China

MPH, methyl parathion hydrolase; OP, organophosphorus; OPAA, OP acid anhydrolase; OPDA, OP-degrading enzyme; OPH; OP hydrolase.

non-homologous plasmids[19,37]. This observation suggested a role for horizontal gene transfer (HGT) in *opd* gene distribution. HGT might be aided by mobile genetic elements (MGEs) or transposons[37]. Several bacteria with almost identical *opd* sequences have been obtained from various parts of the world[12,20], and homologous *opd* genes have also been found in many bacterial genome sequences, which supports the idea that this gene has been disseminated on a transposon. From the complete sequence of a region of plasmid pPDL2 from *Flavobacterium* sp. ATCC 27551, the *opd* gene was found to be flanked by an upstream insertion sequence (IS) that encoded a complete *istAB* operon and a downstream Tn3-like element (*tnpA* and *tnpR*) that encoded a transposase and a resolvase[20] (FIG. 2). The *opdA* operon of *A. radiobacter* has a similar organization even though the *opd* gene is located on the chromosome. The *opdA* gene of *A. radiobacter* is flanked upstream by a *tnpA* gene and downstream by ISs that are homologous to the Tn*610* transposon from *Mycobacterium fortuitum*[24]. Zhang *et al.* found that the *mpd* gene clusters from seven different bacteria were conserved[38]. Further analysis suggested that this cluster contained five ORFs and was associated with IS*6100*. The cluster contains *mpd,* a *tnp* and three other ORFs with high similarity to the permease components of ATP-binding cassette-type transport systems (FIG. 2).

The organization of the *opd* and *mpd* operons is similar to that of other xenobiotic-degrading and antibiotic-resistance operons, in that all these operons are associated with an MGE. This might indicate that genes for xenobiotic degradation arose through similar evolutionary and genetic-transfer mechanisms. Such genes can be made mobile through linkage to IS elements and transposons. This configuration allows large arrays of genes for xenobiotic degradation to be transferred together in a single recombination event[39]. The role of HGT and MGEs in the evolution of xenobiotic degradation cannot be overstated. HGT provides bacteria with access to vast genetic resources, which can be used in a specific niche[40]. Despite their important role in bacterial evolution, genome sequencing and analysis of MGEs (plasmid and bacteriophage) has not been a priority, except for pathogenic bacteria. Given the role of HGT and MGEs in environmental processes and bacterial evolution, many more MGEs need to be sequenced[40].

Xenobiotic
A chemical that is usually man-made and is not found naturally in the environment.

Bioreactor
A device or system that supports a biologically active environment.

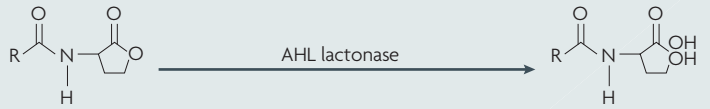
Industrial applications

Bioremediation. Excessive use, accidental spillage and the production of large volumes of waste result in pesticide pollution of the environment. For example, it is estimated that the United States alone generates more than 400,000 litres of coumaphos waste every year through the cattle-tick eradication programme[41]. Similarly, several countries from the European Union produce millions of litres of diazinon as sheep-dip waste. Bioremediation provides a cheap and environmentally friendly way to remove these toxic elements from the environment. There has been some success in the use of bacteria and bacterial enzymes for bioremediation of OP compounds. For example, Mulbry *et al.* developed a filter bioreactor with a consortium of microorganisms that could degrade 15,000 litres of coumaphos in a single batch[41]. Two such units have been operational in the United States since 1996. However, for wider application, bioremediation technology will benefit from further investigation of the induction and regulation of degrading activities of these bacteria under natural conditions, genetic and physiological relationships among cooperative bacteria and competitive advantage against other bacteria. For comparatively cheap and more-effective bioremediation, a concerted approach is essential to examine how the degrading abilities of bacteria can be maintained *in situ*, how degradation can be modelled and how to predict whether and when intervention is required.

The use of live cells in bioremediation has inherent practical difficulties (for example, nutritional requirements, availability of fresh inocula and oxygen demand), and as a result the use of purified enzymes has increased[42]. A company in Australia (Landguard; see Further information) now sells carrier-based OPH enzyme for removal of OP from sheep-dip waste before it can be applied to soil. Gene and protein engineering have been used to increase the catalytic activity and efficiency of microbial PTEs, using site-directed mutagenesis to eliminate preferential selection of particular enantiomers for PTEs[23]. Other approaches, such as DNA shuffling, have been successfully used to increase the efficiency of degradation of a poor substrate[12,25]. Single microorganisms that can completely mineralize OP compounds have been constructed by transferring one plasmid with OP-degrading activity and another plasmid with metabolite degradation[43]. OP-degrading genes have also been cloned in combination with other pesticide-degrading genes, such that two pesticides were eliminated simultaneously using a single bacterial strain[44]. This technique has been further exploited to engineer plants that harbour genes which can degrade two pesticides[45]. However, this approach can prove problematic, as regulatory guidelines in several countries preclude the use of engineered organisms for bioremediation in natural environments.

Theoretically, xenobiotic-degrading enzymes should be a powerful tool in the bioremediation industry, but in practice their use is limited because

Box 3 | **Acyl homoserine lactones: role in the environment and degradation**



Acyl homoserine lactones (AHLs) (see the figure) are a discrete group of biologically active metabolites that are produced by several members of the Proteobacteria. Different bacterial species produce different AHLs, but all AHLs share identical homoserine moieties[67].

AHLs are used for cell-to-cell communication and enable bacteria to display group behaviour in a phenomenon known as quorum sensing. When an efficient concentration of an AHL is reached, the AHL binds to a regulatory protein to control gene expression for numerous physiological activities[67,68].

AHL lactonase (see the figure) hydrolyses the homoserine lactone ring of AHL molecules and has been identified from a range of bacterial genera, including *Bacillus*, *Agrobacterium*, *Arthrobacter*, *Klebsiella*, *Comamonas* and *Rhodococcus*. The production of AHL lactonase might be a useful strategy by which certain bacteria can gain a competitive advantage by modulating the activities of AHL-producing species in the local environment. Some bacterial species (for example, *Rhodococcus erythropolis*) even use AHLs as sole sources of carbon and energy[68].

of their short shelf life, mainly owing to changes in three-dimensional structure, the activity of environmental proteases and the binding and trapping of enzymes to other substrates in the soil, such as clay and organic matter. Continuous requirements for fresh batch enzymes also make this approach expensive and labour intensive because of difficulties in purifying the degradation products of the enzymes. Enzymes can theoretically be reused for several catalysis cycles[46], as their immobilization provides extended activity. Recent advances in nanotechnology offer a range of nanostructures for enzyme immobilization[47]. Nanoparticles provide a larger surface area for enzyme attachment and shorter diffusion pathways for substrates[48]. Consequently, several nanoparticles have been evaluated as enzyme carriers[46]. The usefulness of this approach has been established by showing that an esterase enzyme–nanofibre composite could be stable and functional in a substrate reaction in repeated-batch and continuous long-term operation modes. In a 100-day experiment, this composite lost little activity even on a shaking incubator[46]. OP bioremediation technology could benefit from a similar approach, especially for large-scale, controlled waste treatment, such as cattle dip and sheep dip, manufacturing wastes and CWA destruction. Several high-efficacy OPH derivatives were obtained by using DNA- and protein-engineering approaches, and some were several-fold more efficient compared with native OPH[23]. However, these enzymes are produced in genetically modified organisms, and government regulations do not permit the release of genetically modified organisms into the environment. Nevertheless, purified enzymes encapsulated in a carrier should be able to overcome this limitation. Reuse of enzyme–nanoparticle matrices will also make this approach cheap and easy.

Nanoparticle
A small particle (one or more dimensions of ~100 nM or less) that behaves as a whole unit; for example, in terms of transportation.

Other biotechnological applications. OPH, OPAA and MPH have been successfully used to develop and evaluate biosensors for OP contamination. Two different approaches have been employed for OPH biosensors: a potentiometric approach to measure local pH change and an amperometric measurement of electroactive enzyme products[49,50]. Both of these methods have been combined to produce an improved biosensor tool[51]. Nanotechnology has increased the efficacy of OP biosensors by immobilizing OPH on a carbon nanotube[52]. An *mpd* gene for an expression vector has been used successfully as a selective marker[53]. Most of the expression vectors currently used for transgenic research and commercial purposes contain antibiotic resistance genes as selective markers. The commercialization of genetically modified organisms has been slow owing to their potential to spread antibiotic resistance genes among natural bacterial populations and their harmful effects on naturally beneficial bacteria. Expression vectors that contain *opd* or *mpd* as the selection marker offer a solution to both limitations. The positive colonies for vector acquisition can be identified by a yellow circle around colonies on culture plates (parathion or methyl parathion produce *p*-nitrophenol as a result of hydrolysis, which leads to the yellow colour). This approach has been extended to transgenic plant research, for which *opd* has been successfully used as a new 'scoreable' and selective marker system. Parathion has been used for colorimetric identification of insert-positive clones, whereas OP herbicide (haloxon and bensulide) was used as a positive selection marker for germinating seeds, which would die in the presence of herbicides without the *opd* gene[54]. Further industrial application of OP genes and enzymes includes drug-sensitivity trials and clinical diagnostics.

Medical applications

Various biotechnological efforts have been made to formulate therapies for OP poisoning. OP poisoning, caused either unintentionally or in suicide attempts, has resulted in hundreds of thousands of deaths, and exposure to several millions of individuals, every year[8]. Furthermore, the ease of synthesis and availability of OP compounds makes them suitable for terrorist purposes. Indeed, the civilian populations of Matsumoto and Tokyo, Japan, were subjected to terrorist attacks with an OP compound, which resulted in several deaths in 1994 and 1995. Clinical management for OP poisoning includes the use of atropine and oximes[10,55,56], which are generally inefficient (fatality rate of up to 40%[57]) and ineffective in preventing post-poisoning neurocognitive dysfunctions[10]. It is therefore imperative to develop an efficient therapy for patients with severe OP exposure. A limited number of PTEs (mainly enzymes of bacterial and human origins) could be used to treat OP poisoning. The dermal and intravenous administration of bacterial PTEs (obtained from *B. diminuta* and *A. radiobacter*) to experimental animals has been shown to confer prophylactic and therapeutic protection against OP poisonings[8,55]. Human butyrylcholinesterase and paraoxonase have also been used with limited success

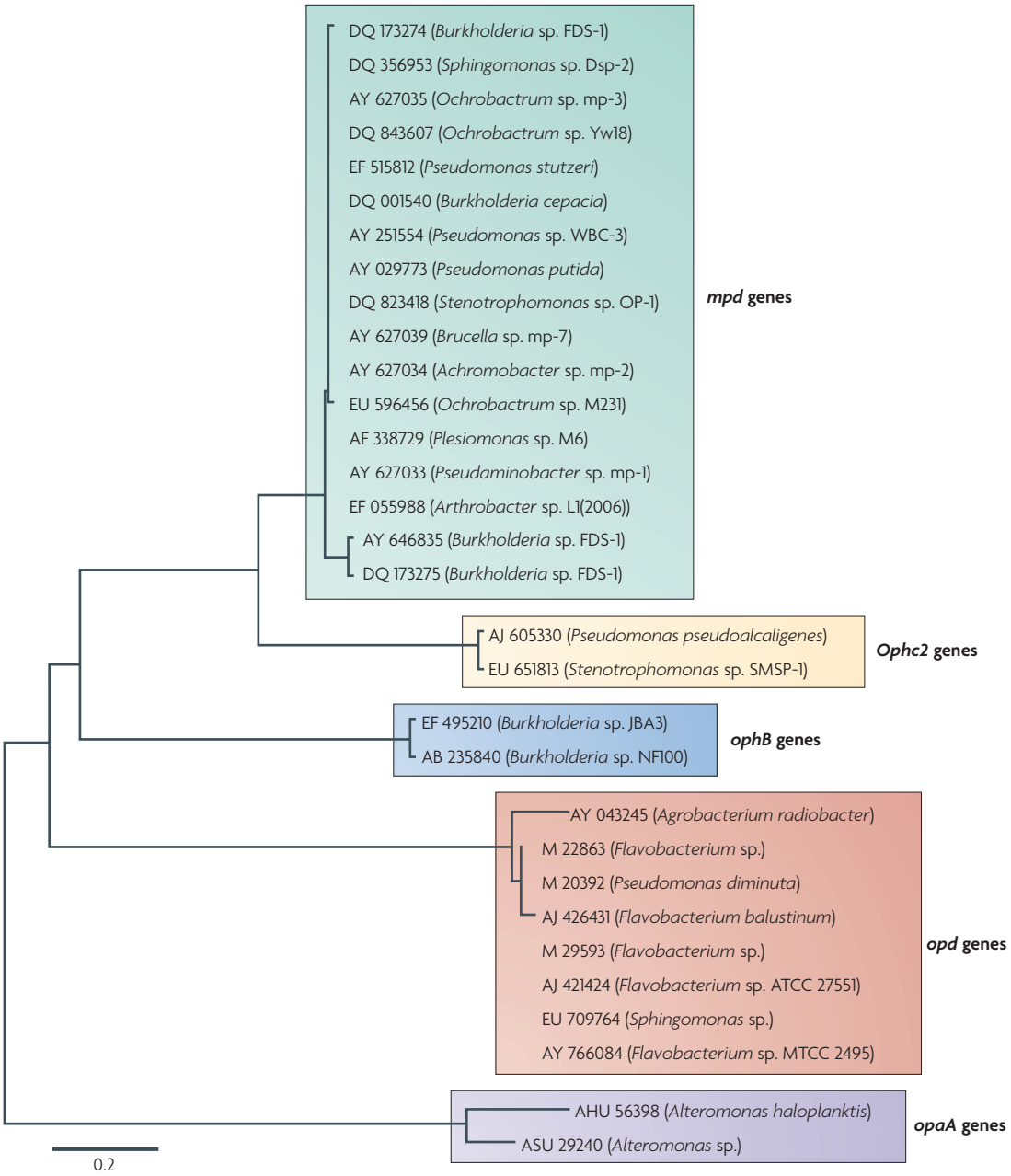


Figure 1 | **Phylogenetic tree constructed from organophosphorus-degrading gene sequences.** The tree was compiled using the sequences that were available in the NCBI database. Distinct groups that correspond to different genes, such as the *mpd* (methyl parathion degrading), *opd* (organophosphorus degrading) and *opaA* (organophosphorus acid anhydrolase) genes, are easily delineated (shown in different colours). The tree was constructed using a neighbour-joining method and Mega4 software. All major nodes are supported by a bootstrap value of >50%. The scale bar represents the number of expected changes per site. The locus tags are provided for each gene followed by the species name in brackets.

against OP poisoning *in vitro*. However, compared with human paraoxonase, bacterial PTEs have a wider substrate range, higher efficacies and minimum post-reaction re-inhibition[8]. Bacterial PTEs are particularly good enzymes for clinical therapy; for example, OPH and OPDA can hydrolyse their substrates near to their diffusion limit. However, for successful use of bacterial enzymes in human treatment, two problems must be overcome: immunological reactions occur owing to the injection of exogenous protein and blood proteases are inactivated. To address these problems, OP-degrading enzymes need to be formulated in a carrier that provides a protective environment for the enzyme while remaining permeable to OPs and their hydrolysis product and, for intravenous treatment, avoiding immunological reactions[55]. Nanotechnology has been used to show that encapsulation of bacterial PTE in liposomes enhanced PTE activity and circumvented immune defences. However, encapsulated PTE has a half-life of 45 hours in mice, which might not be long enough for complete

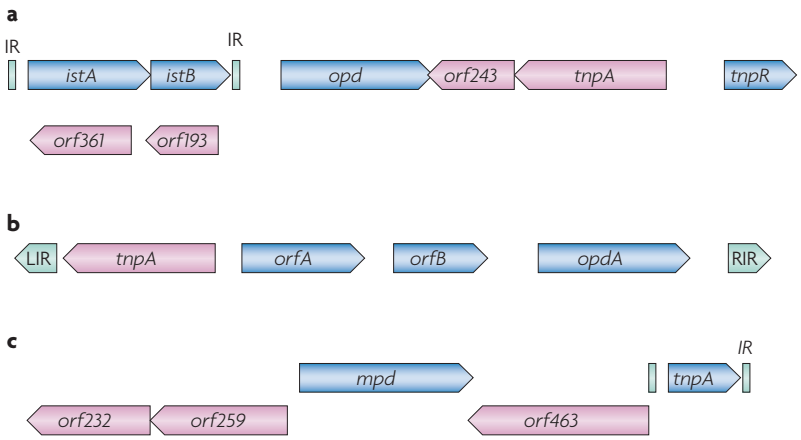


Figure 2 | **Genomic organizations of three known organophosphorus-degrading genes.** The shapes indicate different gene locations and the direction of transcription. **a** | Genomic structure of the *opd* (organophosphorus degrading) gene from the *Flavobacterium* sp. genome, which includes a complete *istAB* operon, the *tnp*A and *tnp*R genes and *orf243*, a gene that encodes for metabolite utilization. Two *orf*s on a complementary strand encode for a protein of unknown function[20]. **b** | Genomic structure of the *opdA* gene from the *Agrobacterium radiobacter* genome, which includes *tnp*A and inverted repeats (IRs) (left inverted repeats (LIRs) and right inverted repeats (RIRs)), *opdA* and two *orf*s of unknown function[24]. **c** | Genomic structure of the *mpd* gene cluster from the *Ochrobactrum* sp. genome, which includes IRs, *tnp*A and three *orf*s of unknown function[38].

Quantum dot
A type of nanoparticle that can be used for optical, electrical, biological and medical purposes.

detoxification[58]. Using more-efficient nanoparticles, such as niosomes (which have a half-life that is several times longer than liposomes), silica nanoparticles and quantum dots, might overcome this problem. These materials are less toxic and are removed from the body during the excretion of urine, which will be essential for any successful use of nanoparticles in human therapy[59,60].

Conclusions

Our understanding of OP degradation has greatly advanced in recent years. We now have a better understanding of the microbiological, biochemical, evolutionary and genetic basis of OP degradation, and this information is being exploited for several industrial applications. However, to realize the full potential of OPDB, we need to use emerging technologies. From the perspective of environmental fate and microbiology, it is important to examine OP metabolism in groundwater and extreme environments, such as hyperacidic, hypersaline and hyperthermophilic conditions, and isolate OPDB from these environments to establish a culture collection for potential bioremedial use. PLL sequences have been detected in the genomes of bacteria isolated from all these environments, and therefore isolation of OPDB from such conditions is a realistic expectation.

Repeated application of OP to the same soil results in rapid biodegradation of OP by soil bacteria[12]. Given that ~99% of soil bacteria cannot be cultured using current microbiological techniques, it can be assumed that several different types of OP-degrading bacteria and enzymes exist in the polluted environment that can only be characterized by culture-independent methods, such as metagenomics. Indeed, several industrially important enzymes have been isolated from such 'unculturable' soil bacteria using metagenomics[61], although this approach has not yet been attempted for OP-degrading enzymes. The success of metagenomic screening for OP-degrading enzymes can be enhanced by combining it with functional screening and stable isotope probing to selectively enrich DNA from the degrading community that uses OP and its metabolites. This approach is also necessary to understand the genetic basis of OP degradation in unculturable bacteria (such as flanking, promoter and regulatory genes), which is currently known only for three isolated bacteria. Such information can also be exploited later by genetic or protein engineering for better efficiency and increased production of the enzymes.

OPDB and their enzymes could form the basis of multiple biotechnological applications across several disciplines. However, to convert this promise to practice, further research is necessary. The use of modern technologies, such as metagenomics and nanotechnology, in association with more conventional biochemical and molecular analyses can help to achieve this goal and will further our understanding of the biology that underpins OP degradation in relevant environmental contexts.

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DATABASES
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Agrobacterium radiobacter | *Bacillus thuringiensis* | *Chromobacterium violaceum* | *Deinococcus radiodurans* | *Desulfatibacillum alkenivorans* | *Escherichia coli* | *Leptothrix cholodnii* | *Methylibium petroleiphilum* | *Mycobacterium bovis* | *Mycobacterium tuberculosis* | *Alteromonas haloplanktis* | *Sinorhizobium meliloti* 1021 | *Sulfolobus acidocaldarius*

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