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REVIEW ARTICLE

Lateral transfer of organophosphate degradation (*opd*) genes among soil bacteria: mode of transfer and contributions to organismal fitness

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Abstract. Genes encoding structurally independent phosphotriesterases (PTEs) are identified in soil bacteria. These *pte* genes, often identified on mobilizable and self-transmissible plasmids are organized as mobile genetic elements. Their dissemination through lateral gene transfer is evident due to the detection of identical organophosphate degradation genes among soil bacteria with little or no taxonomic relationship. Convergent evolution of PTEs provided selective advantages to the bacterial strain as they convert toxic phosphotriesters (PTs) into a source of phosphate. The residues of organophosphate (OP) compounds that accumulate in a soil are proposed to contribute to the evolution of PTEs through substrate-assisted gain-of-function. This review provides comprehensive information on lateral transfer of *pte* genes and critically examines proposed hypotheses on their evolution in the light of the short half-life of OPs in the environment. The review also proposes alternate factors that have possibly contributed to the evolution and lateral mobility of PTEs by taking into account their biology and analyses of *pte* genes in genomic and metagenomic databases.

Keywords. phosphotriesterases; *opd* gene; *mpd* gene; lateral gene transfer; molecular evolution.

Introduction

Neurotoxic organophosphates (OPs) were introduced as insecticides about 60 years ago to replace more persistent organochloride insecticides like DDT, HCH etc. Mainly due to their indiscriminate use in agriculture, the residues of OP compounds are found in various environments, including groundwater (Singh *et al.* 2014). Due to the evolution of novel degradative enzymes, certain soil bacteria use OP insecticide residues as sole source of carbon and phosphate. Initially these hydrolytic enzymes were designated as parathion hydrolases, methyl parathion hydrolases and paraoxonases etc. depending on the substrate (OP insecticide) used to assay the enzyme activity. Subsequently they were divided into three major subgroups namely organophosphate hydrolases (OPH), methyl parathion hydrolases (MPH) and organophosphate acid anhydrolases (OPAA). Based on the similarities in structure and catalytic mechanisms, the OP-degrading enzymes are assigned to one of these three subgroups. Among these OP-hydrolyzing enzymes, the physiological substrate is known only for OPAA. Dipeptides ending with a prolyl residue serve as substrates for OPAA. Therefore, the OPAA has been renamed as prolidase (Cheng *et al.* 1999). Since physiological substrate is known for OPAA, they are not taken as part of genes evolved for degradation of organophosphates.

Authentic physiological substrates are not known for either OPH or MPH. Despite structural differences, these two enzymes have identical active sites and follow similar catalytic mechanisms while hydrolyzing OPs (Afriat *et al.* 2006; Afriat-Jurnou *et al.* 2012; Parthasarathy *et al.* 2017c). They hydrolyze certain OP insecticides with rates close to their substrate limits (Chaudhry *et al.* 1988). Therefore, OPs are considered as cognate substrates for both MPH and OPH (Pandeeti *et al.* 2012; Purg *et al.* 2016; Parthasarathy *et al.* 2017a). It is also proposed that these two structurally different enzymes have converged functionally to provide selective advantage to the bacteria. They minimize toxic effects of OP residues and generate a phosphate pool to be used as a phosphate source (Dong *et al.* 2005; Afriat *et al.* 2006; Tawfik 2006; Afriat-Jurnou *et al.* 2012). This proposition gained strength due to the existence of identical mobile *pte*-elements in geographically and taxonomically well separated bacteria. However, this review critically examines this point of view in the light

of the short half-life of OP residues and proposes a possible physiological role for *pte* genes by taking into account the existing knowledge on *pte* genes and sequence information from genome and meta-genome databases.

Studies on the biodegradation of organophosphates gained momentum with the isolation of parathion-degrading *Flavobacterium* sp. from soil samples collected by the International Rice Research Institute, Manila (Sethunathan and Yoshida 1973). Since then a number of reports appeared on biodegradation of OP compounds. Most of them, either described degradation pathways or the characterization of the corresponding enzymes. Only a few studies have focussed on the genetics of OP degradation in bacteria (Serdar *et al.* 1982; Mulbry *et al.* 1987; Zhongli *et al.* 2001; Horne *et al.* 2002a, b; Siddavattam *et al.* 2003; Yang *et al.* 2003; Zhang *et al.* 2006; Pandeeti *et al.* 2011, 2012; Parthasarathy *et al.* 2017b). Unlike their eukaryotic homologues discovered accidentally in patients with injured kidneys, the bacterial *pte* genes were identified and cloned while specifically trying to understand the genetic basis of organophosphate degradation (Ali *et al.* 2012; Mulbry *et al.* 1987; Harper *et al.* 1988; McDaniel and Wild 1988; Mulbry and Karns 1989; Somara and Siddavattam 1995; Zhongli *et al.* 2001; Horne *et al.* 2002a, b). Initial studies reported the existence of identical *pte* genes on indigenous plasmids isolated from soil bacteria strains. However, the survey of genome and meta-genome sequences has revealed the existence of *pte* homologues both on plasmids and chromosomes of archaea and bacteria, including human pathogen like *Mycobacterium tuberculosis*.

The opd plasmids

The *opd* genes are found both on plasmids and on chromosomes (Harper *et al.* 1988; McDaniel and Wild 1988; Horne *et al.* 2002b; Ali *et al.* 2012; Parthasarathy *et al.* 2017b) and detailed studies have been conducted on plasmid-borne *opd* sequences cloned from *Flavobacterium* sp. ATCC 27551 and *B. diminuta*. These two soil isolates, reclassified as *Sphingobium fuliginis* ATCC 27551 (Kawahara *et al.* 2010) and *Sphyngophyxis wildii* (Parthasarathy *et al.* 2017c), respectively, were isolated on different continents. *Sphingobium fuliginis* ATCC 27551 was isolated from rice fields of the International Rice Research Institute, Manila (Sethunathan and Yoshida 1973), whereas the *Sphyngophyxis wildii* species was isolated from sewage samples collected in California, USA (Munnecke and Hsieh 1974). In both cases, the *opd* sequences were localized on large indigenous plasmids designated as pPDL2 and pCMS1. The plasmid pPDL2 isolated from *S. fuliginis* ATCC 27551 is a 40-kb plasmid and the 65-kb pCMS1 is an indigenous plasmid of *S. wildii*. Except for the region (5.1 kb) containing *opd* gene, no obvious similarity was seen between these two plasmids (Mulbry *et al.* 1987; Pandeeti *et al.* 2011, 2012; Parthasarathy *et al.* 2017c). Sequence analyses of both plasmids has provided significant insights into the lateral transfer of *opd* genes among soil bacteria.

Multiple strategies to maintain plasmidome

Mobilizable plasmids do not always encounter favourable situations to replicate in their recipient cells. If they fail to replicate they can be lost, particularly if they do not integrate into the genome. Plasmid pPDL2 follows unique strategies to maintain its genome in recipient cells. A toxin–antitoxin module ensures survival of the cells that retain the plasmid. The replication and site-specific integration modules facilitate plasmid maintenance in the host either as an episome or as a plasmid. The mobilization module consisting of *oriT* and *relA* gene enables its horizontal mobility in the presence of a genetic repertoire coding for type 4 secretion system (T4SS). However, no T4SS-coding sequences are found on pPDL2. Consistent with the sequence information, the plasmid pPDL2 derivative, pPDL2-K has shown lateral mobility only in the presence of helper plasmid (Pandeeti *et al.* 2012).

To survive in recipient cells, the plasmid should have a broad host-range replicative origin (*oriV*). Analyses of the plasmid sequence deposited in GenBank (NC_019376.1) revealed the existence of a replicative origin typically seen in theta-replicating plasmids, with a well conserved DnaA binding box. A 776-bp long *oriV* sequence was identified immediately upstream of *repA* (17238 to 18114). The RepA protein of pPDL2 shows 99% amino acid sequence identity with the RepA of plasmid pUT1 isolated from *Sphingobium japonicum* UT26S. Likewise, considerable similarities were also found between the *oriV*s of pPDL2 and pUT1 (Pandeeti *et al.* 2012). The special arrangements of direct repeats of DnaA boxes resemble the structure of *oriV* sequences found in pUT1. In general, the replicative origins of plasmids show configuration conservation (spatial arrangement of repeats) rather than showing strict sequence conservation (Puyet *et al.* 1988). The *oriV* of plasmid pPDL2 has shown similarities in spatial arrangements of repeats and palindromic sequences with several plasmids isolated from *Sphingobium* and *Pseudomonas* strains (figure 1). The existence of such a conserved *oriV* facilitates its replication in a wide range of soil bacteria. In addition to the well conserved replication origin, a *par* locus and toxin–antitoxin modules exist among the 42 ORFs predicted in the sequence of pPDL2 to ensure its segregation and stability in recipient cells.

Integration module

In the sequence of plasmid pPDL2, two ORFs, *int1* (28584-29570c) and *int2* (31182-32028) code for phage integrase (*IntP*) family proteins forming two integration modules CIP-I (24734-28063) and CIP-II (28913-30866) along with the other ORFs (figure 2). These two proteins show

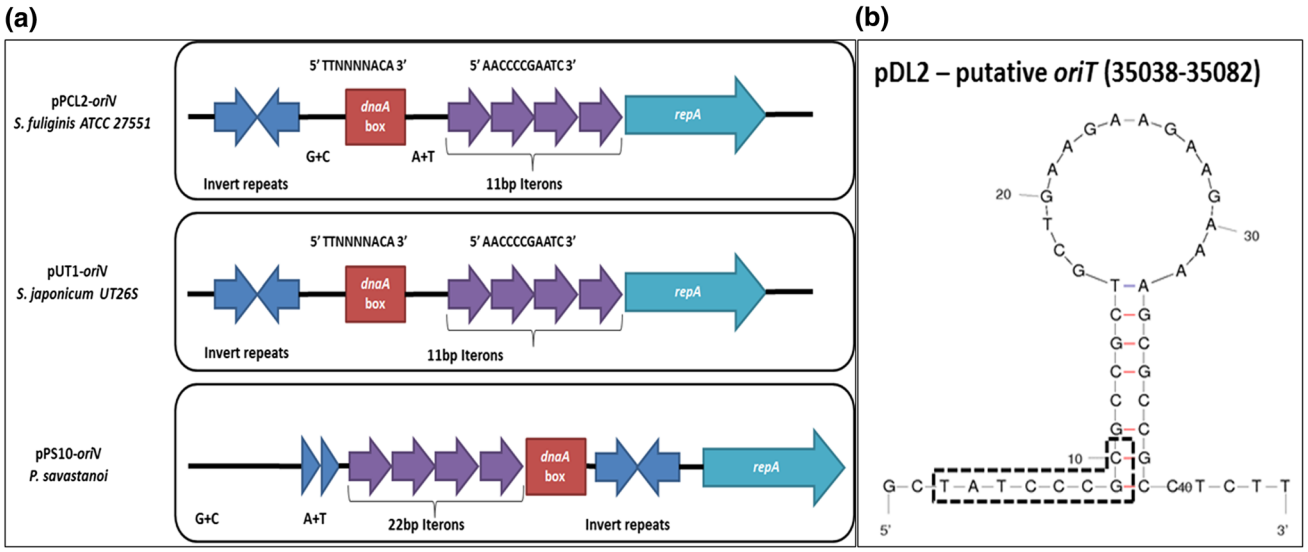

Figure 1. (a) Replicative origin (*oriV*) of plasmid pPDL2. Comparison of the particular arrangements of *dnaA* boxes and intron sequences in plasmids pUT1 and pPS10 isolated from *S. japonicum* and *P. savastanoi* is shown. (b) Secondary structure of predicted *ori*T sequence. The dotted box indicates the relaxase target sequence. The figure is reproduced from the article published in G3: Genes, Genomics and Genetics (Pandeeti *et al.* 2012).

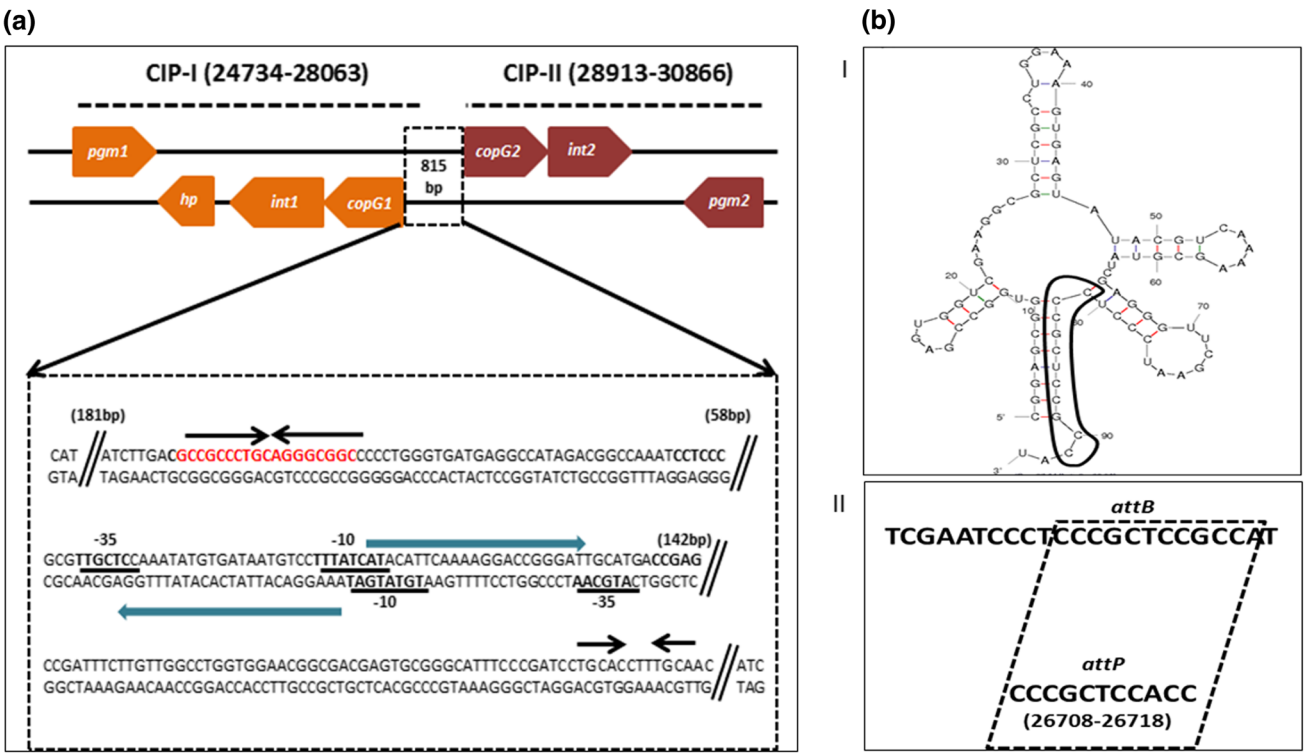

Figure 2. (a) Organization of plasmid pPDL2-borne *xerD* sequences in *Sphingobium fuliginis* ATCC 27551. Panel b-I shows the existence of the *attB* site at the 3′ end of the tRNA coding gene. Comparison of *attB* and *attP* sequences is shown in panel b-II. The figure is reproduced from the article published in G3: Genes, Genomics and Genetics (Pandeeti *et al.* 2012).

high sequence similarity to the site-specific recombinase XerD (integrase/recombinase) of *Pseudomonas syringae* pv. tomato str. DC3000. Phage integrases are recombinases that mediate unidirectional site-specific recombination between two recognition sequences namely *attP* (the phage attachment site) and *attB* (the bacterial attachment site). The phage integrases are tyrosine recombinases that are characterized by the presence of a signature sequence H-X-X-R in their C-terminal domain. The integrase of pPDL2 aligns well with the integrases present in other bacteria

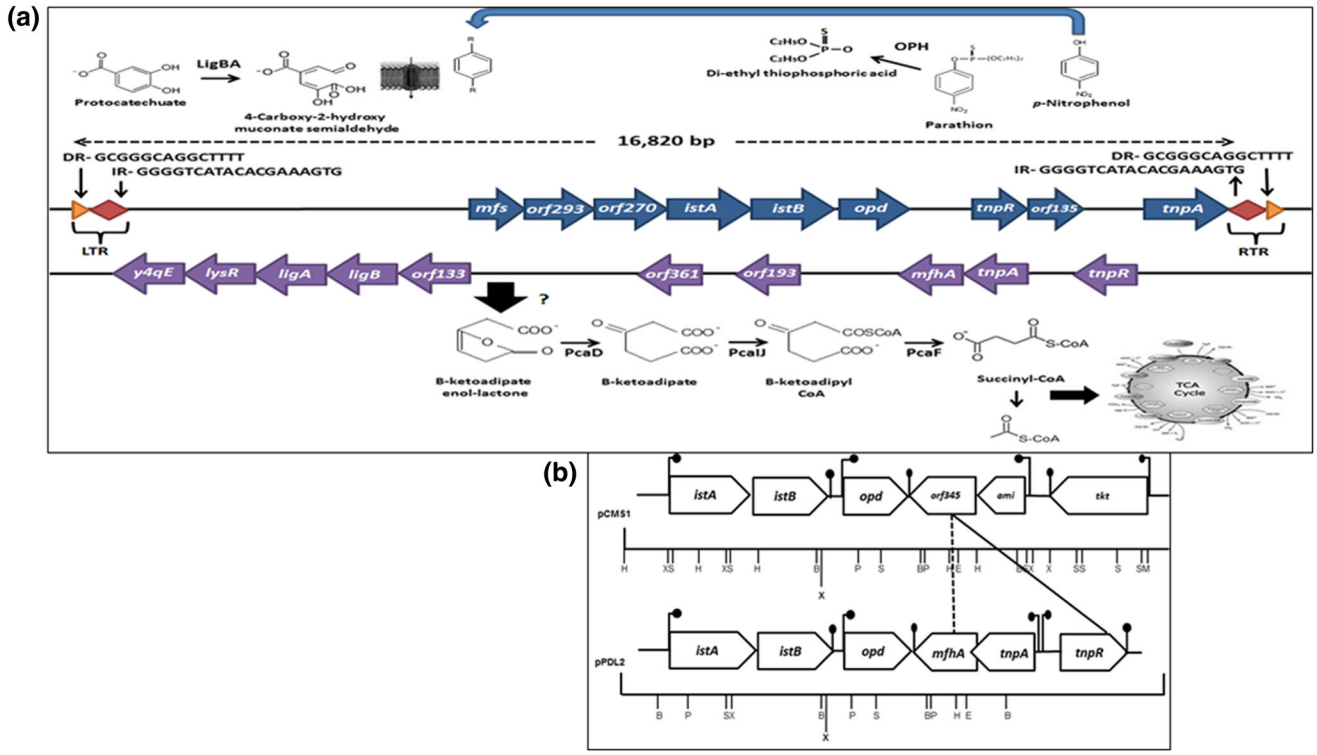

Figure 3. (a) Genetic organization of the plasmid pPDL2-borne *opd* element in *Sphingobium fuliginis* ATCC 27551. Panel b indicates the existence of an identical *opd* region found between plasmids pPDL2 and pCMS1. Creation of *orf306* due to insertion of Tn*3* in plasmid pPDL2 is shown with vertical lines. The figure is reproduced from the article published in G3: Genes, Genomics and Genetics (Pandeeti *et al.* 2012).

suggesting the possibility of site-specific integration of pPDL2 in the recipient chromosome in case if it fails to replicate in the recipient bacterium. The *attP* attachment site (figure 2b, I&II), shows sequence identity to the 3′ end of the seryl-tRNA gene of *S. japonicum*, and is found on the sequence of pPDL2 (Pandeeti *et al.* 2012). The plasmid pPDL2 has also been shown to integrate site-specifically at an artificially generated *attB* site on a compatible plasmid (Pandeeti *et al.* 2012). Considering its ability to site-specifically integrate and its lateral mobility, the plasmid pPDL2 is designated as an integrative mobilizable element.

The *Tnopd* element

As stated in earlier sections, the DNA region containing the *opd* gene is highly conserved in plasmids pPDL2 and pCMS1. Sequence analyses of the conserved *opd* region has revealed a transposon-like organization on plasmid pPDL2 of *Flavobacterium* sp ATCC 27551 (Siddavattam *et al.* 2003). The presence of an insertion sequence (IS) element (IS*21* class) and a transposon (Tn*3*) upstream and downstream of the *opd* gene, respectively, resembled that of a typical complex catabolic transposons reported in a number of bacterial species involved in the degradation of recalcitrant aromatic compounds (Tan 1999; Nojiri *et al.* 2004). However, in a standard transposition assay performed in *E. coli*, this conserved region alone failed to show transposition (Siddavattam *et al.* 2003). Moreover, this conserved region did not show the existence of inverted and direct repeat sequences typically seen in complex transposons (Siddavattam *et al.* 2003). Nevertheless, such sequences were identified in flanking mobile elements Tn*3* and *y4qE* (figure 3a). Transposition was only observed when the plasmid pPDL2 derivative pPDL2-K was used as donor plasmid while performing the transposition assay. The left and right border sequences of the *opd* cluster at the target sequence revealed the repeat sequences indicated flanking the mobile elements Tn*3* and *y4qE*, suggesting that the *opd* region found between these two mobile elements is an active transposon (figure 3a). In addition to the *opd* gene and *orf306*, the *opd* element contains genes that code for an outer membrane transporter (MFS permease), and protocatechuate dioxygenase, which is required for degradation of organophosphate insecticides and the degradation products like catechols and substituted catechols generated during biodegradation of OP insecticides (figure 3a). The mobilizable nature of plasmid pPDL2 and transposability of the *opd* gene cluster, if seen together with the existence of identical *opd* genes among taxonomically distant bacterial strains provides strong evidence to

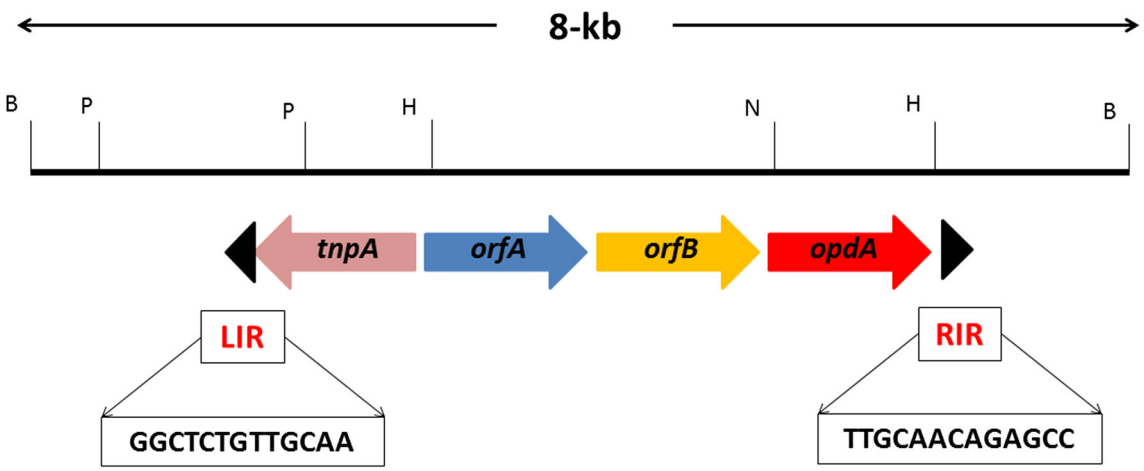

Figure 4. Organization of *opdA* element in *Agrobacterium radiobacter* P230: The *opdA* gene is shown in red colour arrow. The ORFs, *orfA* and *orfB* flanking the *opdA* gene are shown with blue and yellow colour arrows, respectively. The *orfA* codes for a truncated transposase and *orfB* codes for an ATP-binding protein. The transposase gene *tnpA* is coloured in pink. Small black arrows represent left (LIR) and right (RIR) inverted repeats.

suggest the existence of horizontal mobility of *opd* genes among soil bacteria.

pCMS1 is a self-transmissible plasmid

The complete genome sequence for *S. wildii* is available. It contains a chromosome and a 65, 908-bp plasmid designated as pCMS1 (Parthasarathy *et al.* 2017c). The annotated sequence of plasmid pCMS1 is available in the genome database (NZ_CP026382.1). It is a self-transmissible plasmid and contains all necessary genes for coding functional T4SS. In a typical conjugation experiment, the pCMS1-selectable derivative pCMS1::*tet*, constructed by replacing the *opd* gene with *opd::tet*, is horizontally transferred into *P. putida* (Pandeeti *et al.* 2011). In addition to the *opd* gene, the genes involved in degradation of aromatic compounds, especially those encoding lower pathway enzymes are identified on plasmid pCMS1. Nevertheless, the *opd* gene does not show a typical transposon-like organization on plasmid pCMS1 (Pandeeti *et al.* 2011). The conserved *opd* regions found in plasmids pCMS1 and pPDL2 show sequence identity only in the upstream region of the *opd* gene (figure 3b). There is a marked difference in sequence in the regions downstream of the *opd* gene. In contrast to pPDL2, pCMS1 does not contain Tn*3* downstream of the *opd* gene. Instead, it contains a larger ORF, *orf345*, that codes for a carboxyesterase. Interestingly, the sequence of *orf306*, later identified as *mfhA* (Khajamohiddin *et al.* 2006) on pPDL2 is identical to the 3′ end of the *orf345* sequence. Insertion of the Tn*3* sequence in the coding region of *orf345* created a new ORF, called *orf306* in plasmid pPDL2. Our unpublished results indicate that *orf306* is an independent transcriptional unit. It appears to be transcribed from the distally located promoter element generated due to insertion of Tn*3* in the coding region of *orf345*. The newly created *orf306* codes for an active esterase/lipase and hydrolyzes meta-fission products generated from aromatic compounds (Khajamohiddin *et al.* 2006). Further, the lipase activity of Orf306 caused accumulation of propionate in *E. coli* (Chakka *et al.* 2015). The propionate-dependent metabolic diversion in *E. coli* enabled the cells to survive on less preferred carbon compounds like *p*-nitrophenol, which are generated during biodegradation of OP insecticides such as parathion and methyl parathion (Chakka *et al.* 2015). Since the *orf306* is part of the plasmid pPDL2-borne *opd* element, its existence probably stimulates the innate ability of the recipient strain, and promotes, to mineralization of OP insecticide residues completely, which have accumulated in the soil.

The *TnopdA* element

The *opd* gene homologues are also found in a number of soil bacteria (Iyer *et al.* 2013). The most notable among them is *opdA*, which has been isolated from *Agrobacterium radiobacter* P230 (Horne *et al.* 2002b). The chromosomally located *opdA* gene is organized as a transposable element (figure 4). In addition to *opdA*, the Tn*opdA* element contains three ORFs and inverted repeats typically seen in transposon Tn*610* of *Mycobacterium fortuitum* (Horne *et al.* 2003). The transposase (TnpA) of Tn*opdA* is identical to the TnpA sequence of Tn*610*. The two ORFs found between *tnpA* and *opdA* are predicted to code for a truncated transposase (*orfA*) and an ATP-binding protein (*orfB*). The event of transposition was successfully demonstrated in *E. coli*, demonstrating the ability of horizontal mobility of *opdA* among soil bacteria (Horne *et al.* 2003)

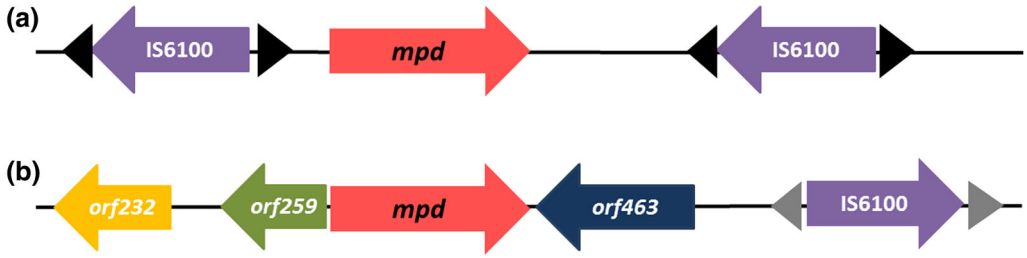

Figure 5. Genetic organization of *mpd* elements: The *mpd* gene is shown in red colour arrow. The IS elements flanking the *mpd* gene of *Pseudomonas* sp. strain WBC-3 are shown using purple colour arrows (panel a). Genetic organization of the 4.7-kb *mpd* element identified in seven bacterial strains is shown in panel b. The *mpd* gene is shown with red colour arrow. The *orf463*, *orf232* and *orf259*, which code for sigma factor 70, permease and an energy-transducing component, ExeB, are shown with blue, yellow and green colour arrows, respectively.

Tn*mpd elements*

The methyl parathion hydrolase (MPH) encoding *mpd* genes reported to date have been isolated from agricultural soils or activated sludge collected from pesticide manufacturing plants in China. The first *mpd* gene was cloned from *Plesiomonas* sp. strain M6 (Zhongli *et al.* 2001). This chromosomally located *mpd* gene surprisingly showed no homology to any of the known OP-degrading genes. Following this discovery, a number of other *mpd* sequences were also cloned from different soil bacteria (Liu *et al.* 2005; Zhang *et al.* 2006; Iyer 2016). Isolation of a plasmid-borne *mpd* gene from *Pseudomonas* sp. strain WBC-3 provided sufficient indication for lateral gene transfer of *mpd*. In this soil isolate, an indigenous plasmid, pZWL0, contained both a *mpd* gene and genes responsible for degradation of *p*-nitrophenol (Liu *et al.* 2005). Interestingly, the plasmid-borne *mpd* gene has shown genetic organization typically seen in class I transposons (Wei *et al.* 2009). The IS elements, IS*6100* are found flanking the *mpd* gene (figure 5a). The mobile nature of the *mpd* element has also been demonstrated in a typical transposition assay performed in *Pseudomonas* sp. strain WBC-3 (Wei *et al.* 2009). The lateral transfer of *mpd* genes became evident with the discovery of a highly conserved *mpd* element in seven bacterial strains (Zhang *et al.* 2006). In all of them, a 4.7 kb region containing a *mpd* gene is highly conserved. Five ORFs were conserved in this region. One of them is similar to the TnpA-coding sequence of an IS element, IS*6100*. A perfect 14 bp inverted repeat also exists in its flanking sequences. The second ORF, designated as *orf463*, found immediately upstream of the IS element, codes for a protein that shows considerable similarity to a housekeeping sigma factor. The other ORFs, *orf232* and *orf259*, found upstream of the *mpd* sequence code for a permease and an energy-transducing component ExeB, respectively. Both ORFs are involved in the membrane transport process (figure 5b). The significance of their roles as part of the *mpd* element is currently unclear and are speculated to have a role in processing and membrane-targeting of MPH, which has a 35 amino acid-long signal peptide (Zhang *et al.* 2006). Although the functions of MPH-associated proteins are speculative, the existence of identical *mph* elements in seven different bacterial strains having weak taxonomic relationship to each other, strongly supports horizontal mobility of the *mph* genes among disparate soil microbes.

Unanswered questions

Evolution of novel genes and their lateral transfer play a very crucial role in adaptation of bacterial cells to various stress conditions. Enzyme promiscuity also plays a critical role in the evolution of new catalytic functions (Toscano *et al.* 2007; Khersonsky and Tawfik 2010; Purg *et al.* 2016). An ancillary activity can be changed into main activity of an enzyme in presence of substrate analogues due to few mutations. For example, the caboxylesterase (CE) of *Mesorhizobium loti* also shows promiscuous phosphodiesterase (PDE), phosphotriesterase (PTE) and lactonase activities. A single mutation (E183K) turns CE to PDE and substitution of E183 to glutamine abolishes all the activities (Mandrich and Manco 2009). The organisms retain such conversions if gained activity contributes to organismal fitness (Russell *et al.* 2011). The phenomenon described as 'substrate-assisted gain-of-function', the promiscuous enzymes can attain high catalytic activity towards substrates that were hitherto unknown to the native environment, in particular xenobiotics (Mandrich and Manco 2009; Russell *et al.* 2011; Davidi *et al.* 2018). A number of studies are available on evolution of PTEs, and combined structural and functional analyses have clearly established an evolutionary link between quorum-quenching lactonases and organophosphate hydrolases (OPH) (Afriat-Jurnou *et al.* 2012; Elias and Tawfik 2012; Bergonzi *et al.* 2018; Rhoads *et al.* 2018). Further, there also exists a strong structural similarity between β-lactamase and methyl parathion hydrolase (Dong *et al.* 2005). As described earlier in this review, the organization of these newly evolved genes (*opd* and *mpd*), as mobile elements and their localization on self-transmissible plasmids contribute to the spread of these genes among soil bacteria.

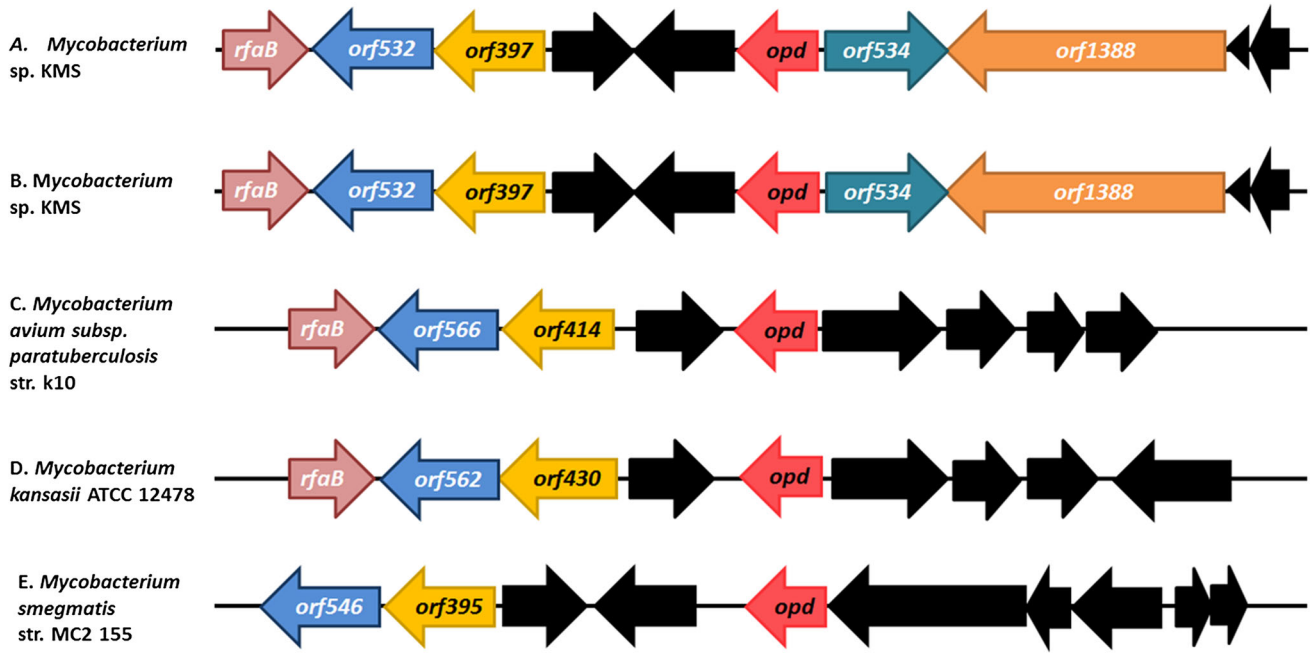

Figure 6. Organization of *opd* genes in meta-genome: (SEED, http://pubseed.theseed.org). The *opd*-linked genes encoding membrane transport proteins are shown with coloured arrows**.** The *orf1388* codes for a probable conserved transmembrane protein and *orf534* codes for a MFS permease. The ORFs *orf397*, *orf414*, *orf430*, *orf395*, *orf532*, *orf566*, *orf562* and *orf546*- code for a possible membrane proteins and *rfaB*- codes for glycosyltransferase. The black arrows represent nonmembrane proteins.

The accumulated experimental evidence obtained on the recent and rapid evolution of PTEs is indisputable. The primary question remaining, however, concerns the persistence of OP residues in agricultural soils and their contribution to the evolution of PTEs. Due to their short half-life, the recalcitrant organochlorides like HCH, DDT etc., are replaced with OPs to minimize the damage to the environment. Their half-life in most of the environments is less than 15 days. The impact of OP residues with such a short half-life to the evolution of PTEs is thus questionable. If OPs are physiological substrates, why are OPH homologues found in pathogenic bacteria like *M. tuberculosis*? These are pathogens that rarely come in contact with OPs and are known for both their genome reduction and microbial minimalism (Moran 2002). If the primary role of PTEs is indeed to metabolize OP residues, the *opd* homologues should have been lost during the adaptation of these bacteria to their pathogenic life style. Therefore, their presence and maintenance strongly suggests an unknown physiological function for PTEs. Structural insights of PTEs support their evolution through substrate-assisted gain-of-function and the lateral mobility of the corresponding genes among soil bacteria strongly suggests the existence of selection pressure necessary for retention and dispersion of the evolved genes. What is this selection pressure? If promiscuity and primary activities of PTEs are taken into consideration then they are likely to be either phosphotriesterases or lactonases. Identification of such substrates and establishing their physiological role in bacteria and archaea should be a key future goal of research in this area.

Future perspectives

Most of genomes and meta-genomes have *pte*-like genes. Interestingly, the genes linked to *opd* and *mpd* sequences are to a large extent conserved. As stated already in this review, no structural homology exists between *opd* and *mpd* genes. Their progenitors and evolutionary paths are also quite different (Parthasarathy *et al.* 2017a). Interestingly, despite following independent evolutionary paths, the genes linked to these structurally distinct *pte* genes are conserved (figures 6&7). Frequently, genes encoding MFS-permeases and proteins associated with the ABC transporter family are also linked to *pte* genes. These are interesting observations and they correlate well with the recent reports on OPH biology (Parthasarathy *et al.* 2016). OPH has recently been shown to be a periplasmically located lipoprotein, which is anchored to the periplasmic face of the inner membrane via a diacyl glycerol moiety, and which has also been shown to exist in a 293-kDa multiprotein complex (Parthasarathy *et al.* 2016). Membrane transport proteins such as outer membrane permeases, components of ABC transporters, are potential interaction partners of OPH. There are also striking similarities between OPH-interacting partners and the proteins coded by *opd*/*mph* linked genes (figure 6&7). What is the

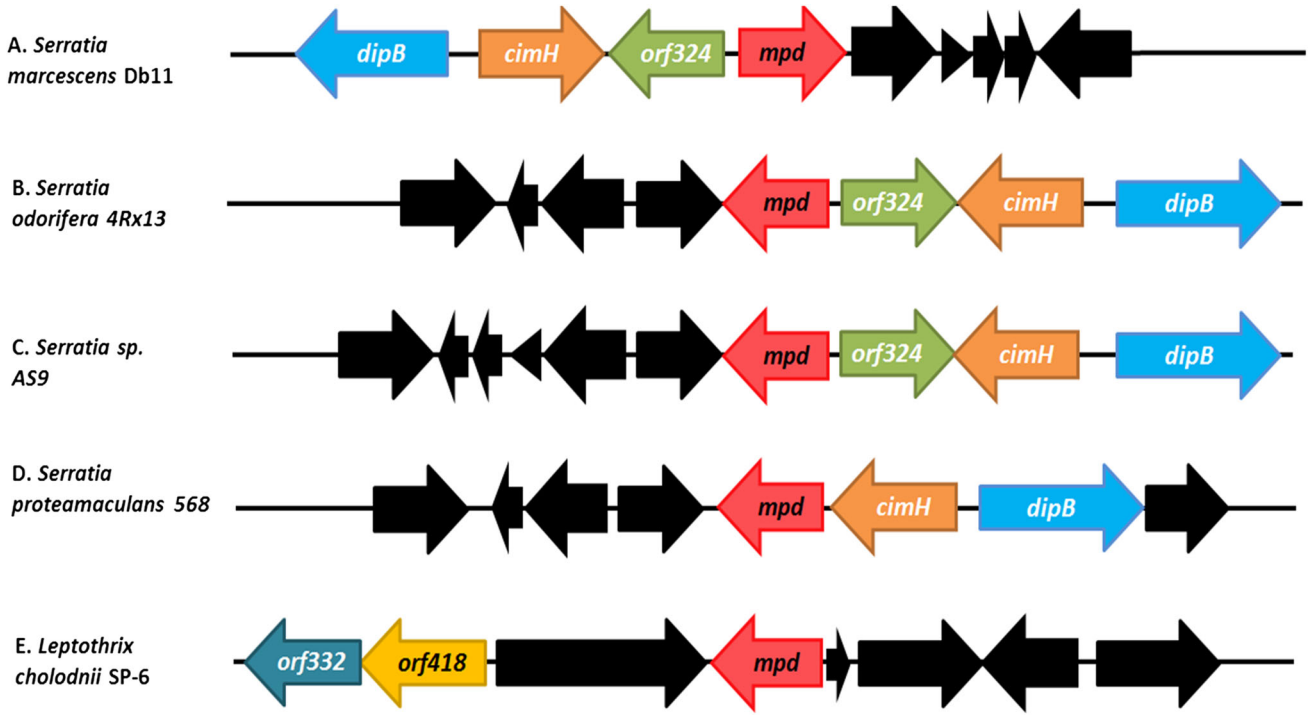

Figure 7. Organization of *mpd* genes in meta-genome: The *mpd*-linked genes coding for membrane transport proteins are shown using coloured arrows. The *orf324* codes for a possible conserved transmembrane protein; *cimH*- codes for L-malate or citrate/H+ symporter CimH; *dipB*- codes for a sensor kinase, DpiB; *orf332*- codes for a nitrate ABC transporter-permease protein; *orf418*- codes for a nitrate ABC transporter-nitrate-binding protein. The black arrows represent nonmembrane proteins.

physiological relevance for the link between *pte* genes and genes coding for membrane transporters? For example, does OPH have a role in membrane transport? Since *opd* null mutants failed to grow in a medium having OPs as sole phosphate source, can we implicate OPH in phosphate acquisition? Phosphate is a macro-nutrient and it is used only if available in inorganic form. However, because most of the soil phosphate sources are in organic form and provide the major phosphate source available to soil bacteria, it is conceivable that PTEs in association with phosphodiesterases (PDEs) can generate an inorganic phosphate pool from these compounds in periplasmic space. The role of MFS permeases in transport of nutrients from the outer membrane to the periplasmic space is a well-established phenomenon (Pao *et al.* 1998). Thus, the MFS-permease together with ABC transporters has the potential to play an important role in the acquisition of phosphate from OPs.

The OPs need not always have to be insecticides or nerve agents. They also include a number of natural phosphate-triesters (PT) that exist in soils. All of them have the potential to serve as a phosphate source if mineralized in the presence of appropriate PTEs and PDEs. A recent study on the functional meta-genomics of these systems suggested the existence of novel PTEs that cannot be detected through homology search (Colin *et al.* 2015). Structural diversity of PTEs and their broad substrate range strongly suggest a role in the generation of inorganic phosphate from complex natural and man-made PTs. Due to the fact that phosphate is a major nutrient, its limitation can create a strong selection pressure for the evolution of novel PTEs from a variety of progenitors that show promiscuous activity towards a different PTs. The hypothesis implicating PTEs in phosphate acquisition appears to be promising, as an adequate supply of inorganic phosphate is essential to support microbial life in the soil.

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References

Ali M., Naqvi T. A., Kanwal M., Rasheed F., Hameed A. and Ahmed S. 2012 Detection of the organophosphate degrading gene opdA in the newly isolated bacterial strain *Bacillus pumilus* W1. *Ann. Microbiol.* **62**, 233–269.

Afriat-Jurnou L., Jackson C. J. and Tawfik D. S. 2012 Reconstructing a missing link in the evolution of a recently diverged phosphotriesterase by active-site loop remodeling. *Biochemistry* **51**, 6047–6055.

Afriat L., Roodveldt C., Manco G. and Tawfik D. S. 2006 The latent promiscuity of newly identified microbial lactonases is linked to a recently diverged phosphotriesterase. *Biochemistry* **45**, 13677–13686.

Bergonzi C., Schwab M., Naik T., Daude D., Chabriere E. and Elias M. 2018 Structural and biochemical characterization of AaL, a quorum quenching lactonase with unusual kinetic properties. *Sci. Rep*. **8**, 11262.

Chakka D., Gudla R., Madikonda A. K., Pandeeti E. V., Parthasarathy S., Nandavaram A. *et al*. 2015 The organophosphate degradation (opd) Island-borne esterase-induced metabolic diversion in *Escherichia coli* and its influence on p-Nitrophenol degradation. *J. Biol. Chem*. **290**, 29920–29930.

Chaudhry G. R., Ali A. N. and Wheeler W. B. 1988 Isolation of a methyl parathion-degrading *Pseudomonas* sp. that possesses DNA homologous to the *opd* gene from a *Flavobacterium* sp. *Appl. Environ. Microbiol*. textbf 54, 288–293.

Cheng T. C, DeFrank J. J. and Rastogi V. K. 1999 *Alteromonas* prolidase for organophosphorus G-agent decontamination. *Chem. Biol. Interact.* **119-120**, 455–462.

Colin P. Y., Kintses B., Gielen F., Miton C. M., Fischer G., Mohamed M. F. *et al*. 2015 Ultrahigh-throughput discovery of promiscuous enzymes by picodroplet functional metagenomics. *Nat. Commun.* **6**, 10008.

Davidi D., Longo L. M., Jablonska J., Milo R. and Tawfik D. S. 2018 A Bird's-Eye view of enzyme evolution: chemical, physicochemical, and physiological considerations. *Chem. Rev*. **118**, 8786–8797.

Dong Y. J., Bartlam M., Sun L., Zhou Y. F., Zhang Z. P., Zhang C. G. *et al*. 2005 Crystal structure of methyl parathion hydrolase from *Pseudomonas* sp. WBC-3. *J. Mol. Biol*. **353**, 655–663.

Elias M. and Tawfik D. S. 2012 Divergence and convergence in enzyme evolution: parallel evolution of paraoxonases from quorum-quenching lactonases. *J. Biol Chem*. **287**, 11–20.

Harper L. L., McDaniel C. S., Miller C. E. and Wild J. R. 1988 Dissimilar plasmids isolated from *Pseudomonas diminuta* MG and a *Flavobacterium* sp. (ATCC 27551) contain identical opd genes. *Appl. Environ. Microbiol*. **54**, 2586–2589.

Horne I., Harcourt R. L., Sutherland T. D., Russell R. J. and Oakeshott J. G. 2002a Isolation of a *Pseudomonas monteilli* strain with a novel phosphotriesterase. *FEMS Microbiol. Lett*. **206**, 51–55.

Horne I., Sutherland T. D., Harcourt R. L., Russell R. J. and Oakeshott J. G. 2002b Identification of an opd (organophosphate degradation) gene in an *Agrobacterium* isolate. *Appl. Environ. Microbiol*. **68**, 3371–3376

Horne I., Qiu X., Russell R. J. and Oakeshott J. G. 2003 The phosphotriesterase gene opdA in *Agrobacterium radiobacter* P230 is transposable. *FEMS Microbiol. Lett*. **222**, 1–8.

Iyer R., Iken B. and Damania A. 2013 A comparison of organophosphate degradation genes and bioremediation applications. *Environ. Microbiol. Rep.* **5**, 787–798.

Kawahara K, Tanaka A, Yoon J. and Yokota A. 2010 Reclassification of a parathione-degrading *Flavobacterium* sp. ATCC 27551 as *Sphingobium fuliginis*. *J. Gen. Appl. Microbiol.* **56**, 249–255.

Khajamohiddin S., Babu P. S., Chakka D., Merrick M., Bhaduri A, Sowdhamini R. *et al*. 2006 A novel meta-cleavage product hydrolase from *Flavobacterium* sp. ATCC27551. *Biochem. Biophys. Res. Commun*. **351**, 675–681.

Khersonsky O. and Tawfik D. S. 2010 Enzyme promiscuity: a mechanistic and evolutionary perspective. *Ann. Rev. Biochem*. **79**, 471–505.

Liu H., Zhang J. J., Wang S. J., Zhang X. E. and Zhou N. Y. 2005 Plasmid-borne catabolism of methyl parathion and *p*-nitrophenol in *Pseudomonas* sp. strain WBC-3. *Biochem. Biophy. Res. Commun*. **334**, 1107–1114.

Mandrich L. and Manco G. 2009 Evolution in the amidohydrolase superfamily: substrate-assisted gain of function in the E183K mutant of a phosphotriesterase-like metal-carboxylesterase. *Biochemistry* **48**, 5602–5612.

McDaniel C. S. and Wild J. R. 1988 Detection of organophosphorus pesticide detoxifying bacterial colonies, using UV-photography of parathion-impregnated filters. *Arch. Environ. Contamin. Toxicol.* **17**, 189–194.

Moran N. A. 2002 Microbial minimalism: genome reduction in bacterial pathogens. *Cell.* **108**, 583–586.

Mulbry W. W., Kearney P. C., Nelson J. O. and Karns J. S. 1987 Physical comparison of parathion hydrolase plasmids from *Pseudomonas diminuta* and *Flavobacterium* sp. *Plasmid.* **18**, 173–177.

Mulbry W. W. and Karns J. S. 1989 Purification and characterization of three parathion hydrolases from gram-negative bacterial strains. *Appl. Environ. Microbiol*. **55**, 289–293.

Munnecke D. M. and Hsieh D. P. 1974 Microbial decontamination of parathion and *p*-nitrophenol in aqueous media. *Appl. Microbiol.* **28**, 212–217.

Nojiri H., Shintani M. and Omori T. 2004 Divergence of mobile genetic elements involved in the distribution of xenobiotic-catabolic capacity. *Appl. Microbiol. Biotechnol*. **64,** 154–174.

Pandeeti E. V., Chakka D., Pandey J. P. and Siddavattam D. 2011 Indigenous organophosphate-degrading (opd) plasmid pCMS1 of *Brevundimonas diminuta* is self-transmissible and plays a key role in horizontal mobility of the opd gene. *Plasmid* **65**, 226–231.

Pandeeti E. V., Longkumer T., Chakka D., Muthyala V. R., Parthasarathy S., Madugundu A. K. *et al*. 2012 Multiple mechanisms contribute to lateral transfer of an organophosphate degradation (opd) island in *Sphingobium fuliginis* ATCC 27551. *G3 (Bethesda)* **2**, 1541–1554.

Pao S. S., Paulsen I. T. and Saier M. H., Jr. 1998 Major facilitator superfamily. *Microbiol. Mol. Biol. Rev*. **62**, 1–34.

Parthasarathy S., Parapatla H., Nandavaram A., Palmer T. and Siddavattam D. 2016 Organophosphate hydrolase is a lipoprotein and interacts with Pi-specific transport system to facilitate growth of *Brevundimonas diminuta* using OP insecticide as source of phosphate. *J. Biol. Chem*. **291**, 7774–7785.

Parthasarathy S., Gudla R. and Siddavattam D. 2017a Evolution of phosphotriesterases (PTEs): how bacteria can acquire new degradative functions. *Proc. Indian Natn. Sci. Acad*. **83**, 865-875.

Parthasarathy S., Parapatla H. and Siddavattam D. 2017b Topological analysis of the lipoprotein organophosphate hydrolase from *Sphingopyxis wildii* reveals a periplasmic localisation. *FEMS Microbiol. Lett.* **364**.

Parthasarathy S., Azam S., Lakshman Sagar A., Narasimha Rao V, Gudla R., Parapatla H. *et al*. 2017c Genome-guided insights reveal organophosphate-degrading *Brevundimonas diminuta* as *Sphingopyxis wildii* and define its versatile metabolic capabilities and environmental adaptations. *Genome Biol. Evol*. **9**, 77–81.

Purg M., Pabis A., Baier F., Tokuriki N., Jackson C and Kamerlin S. C. 2016 Probing the mechanisms for the selectivity and promiscuity of methyl parathion hydrolase. *Philos. Trans. A Math. Phys. Eng. Sci.* **374**.

Puyet A., del Solar G. H. and Espinosa M. 1988 Identification of the origin and direction of replication of the broad-host-range plasmid pLS1. *Nucleic Acids Res*. **16**, 115–133.

Rhoads M. K, Hauk P., Gupta V., Bookstaver M. L., Stephens K, Payne G. F. 2018 Modification and assembly of a versatile lactonase for bacterial quorum quenching. *Molecules* **23**, 341.

Russell R. J., Scott C., Jackson C. J., Pandey R., Pandey G., Taylor M. C. *et al* 2011 The evolution of new enzyme function: lessons from xenobiotic metabolizing bacteria versus insecticide-resistant insects. *Evol. Appl*. **4**, 225–248.

Serdar C. M., Gibson D. T., Munnecke D. M. and Lancaster J. H. 1982 Plasmid involvement in parathion hydrolysis by *Pseudomonas diminuta*. *Appl. Environ. Microbiol.* **44**, 246–249.

Sethunathan N. and Yoshida T. 1973 A *Flavobacterium* sp. that degrades diazinon and parathion. *Canadian J. Microbiol*. **19**, 873–875.

Siddavattam D., Khajamohiddin S., Manavathi B., Pakala S. B. and Merrick M. 2003 Transposon-like organization of the plasmid-borne organophosphate degradation (opd) gene cluster found in *Flavobacterium* sp. *Appl. Environ. Microbiol*. **69**, 2533–2539.

Singh B., Kaur J. and Singh K. 2014 Microbial degradation of an organophosphate pesticide, malathion. *Critical Rev. Microbiol*. **40**, 146–154.

Somara S. and Siddavattam D. 1995 Plasmid mediated organophosphate pesticide degradation by *Flavobacterium balustinum*. *Biochem. Mol. Biol. Int*. **36**, 627–631.

Tan H. M. 1999 Bacterial catabolic transposons. *Appl. Microbiol. Biotechnol*. **51**, 1–12.

Tawfik D. S. 2006 Biochemistry. Loop grafting and the origins of enzyme species. *Science* **311,** 475–476.

Toscano M. D., Woycechowsky K. J. and Hilvert D. 2007 Minimalist active-site redesign: teaching old enzymes new tricks. *Angewandte Chemie*. **46**, 3212–3236.

Wei M., Zhang J. J., Liu H., Wang S. J., Fu H. and Zhou N. Y. 2009 A transposable class I composite transposon carrying mph (methyl parathion hydrolase) from *Pseudomonas* sp. strain WBC-3. *FEMS Microbiol. Lett*. **292**, 85–91.

Yang H., Carr P. D., McLoughlin S. Y., Liu J. W., Horne I., Qiu X *et al*. 2003 Evolution of an organophosphate-degrading enzyme: a comparison of natural and directed evolution. *Protein Eng.* **16**, 135–145.

Zhang R., Cui Z., Zhang X., Jiang J., Gu J. D. and Li S. 2006 Cloning of the organophosphorus pesticide hydrolase gene clusters of seven degradative bacteria isolated from a methyl parathion contaminated site and evidence of their horizontal gene transfer. *Biodegradation* **17**, 465–472.

Zhongli C., Shunpeng L. and Guoping F. 2001 Isolation of methyl parathion-degrading strain M6 and cloning of the methyl parathion hydrolase gene. *Appl. Environ. Microbiol.* **67**, 4922–4925.

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