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Microbial Metabolism of Organophosphates: Key for Developing Smart Bioremediation Process of Next Generation

Santanu Pailan, Kriti Sengupta, and Pradipta Saha

Abstract

Currently organophosphate compounds constitute one of the largest families of chemical compounds that are used for pest control, mainly for better crop yield worldwide. Due to their toxicity, persistence, and adverse effects, some organophosphates (like parathion and methyl parathion) were classified and registered as extremely hazardous by the World Health Organization (WHO) and US EPA (US Environmental Protection agency) and have been banned in many countries. Some of the hydrolysis intermediates (such as 4-nitrophenol and trichloropyridinol) of these organophosphates are more toxic and environmentally mobile (due to greater water solubility) and therefore more dangerous. However, existing reports suggest their illegal, extensive use and application without proper technical know-how (especially by illiterate farmers in underdeveloped/developing countries). Their indiscriminate and extensive application and use are responsible for possible contamination of several ecosystems and groundwater. Continuous and excessive use of organophosphates has been reported to be responsible for various ever-ending global problems such as contamination of air, water, and terrestrial ecosystems, decline in diversity of productive soil microflora, disruption of biogeochemical cycles, and death of nontarget macroscopic life forms. Organophosphates have been documented as neurotoxic and are potent inhibitors of acetylcholinesterase. They are responsible for serious adverse effect on the nervous, excretion, endocrine, reproductive, cardiovascular, and respiratory systems of target as well as nontarget organisms including humans. Moreover, these compounds are one of the major causes of accidental and suicidal deaths in rural population of the world. The situation therefore is of huge public interest, and hence, suitable cost-effective bioremediation technique

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P. K. Arora (ed.), *Microbial Technology for Health and Environment*,
Microorganisms for Sustainability 22,
https://doi.org/10.1007/978-981-15-2679-4_14

must be developed for the restoration of organophosphate-contaminated environmental niches. Bioremediation of pollutants by biological system has emerged as the most effective method for clean up the contaminated sites. In order to implement bioremediation approach, proper understanding of microbial metabolism of these organophosphates compounds is of extreme importance. Microbial metabolism of OP compounds can be carried out catabolically (with organophosphates serving either as a sole source for C, N, or P) or co-metabolically (in the presence of other compounds, mainly carbohydrates). The metabolic conversion of organophosphates to CO_2 and H_2O (i.e., complete mineralization) is carried out through three main processes such as degradation, conjugation, and rearrangements that involves reactions like oxidation, hydrolysis, and reduction, all mediated through the enzyme-mediated pathways. The main enzymes that are involved in hydrolysis are phosphotriesterases (PTE) and phosphatase. The three major types of PTE are reported so far, such as organophosphate hydrolase (OPH), methyl parathion hydrolase (MPH), and organophosphorus acid anhydrolase (OPAA) encoded by *opd*, *mpd*, and *opaA* genes, which are either located on plasmid or on chromosomal DNA. Since most of the organophosphates are less soluble to make it physiologically available for microbes, solubilization is carried out either through the secretion of organic acid or by biosurfactants by the microbial cells. This is followed by adsorption and or uptake. Most of these adsorption and uptake mechanisms remain largely unknown. However, being lipophilic and small in size, these organophosphates can be transported to the periplasmic space where the metabolic transformation starts. The metabolic transformation involves either an initial oxidation or reduction followed by hydrolysis to release the toxic functional group and phosphate group. This hydrolysis step is most critical as it reduces the toxicity of organophosphates. The metabolic transformation of the toxic functional group is most well-studied and reported in literature. This is followed by a series of reactions that involves interconversion ultimately leading to ring cleavage reaction that opens up the molecule. Further reactions then convert these intermediates into a product that can act as suitable metabolite to be entered into the TCA cycle. The end products released from the TCA cycle are CO_2 and H_2O. Most of initial reactions are mediated in the periplasmic space of the bacterial cell. The interconversion of much less toxic metabolites occurs in the cytoplasm. Although many facets of organophosphates biodegradation have been excavated, still there remain many lacunas. Understanding microbial diversity, ecological aspects, and adaptation strategies might cater better prospects to hope for smart technologies.

Keywords
Organophosphates; 4-Nitrophenol · Parathion · Methyl parathion

14.1 Introduction

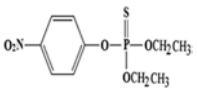
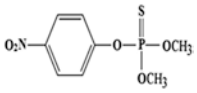
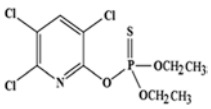
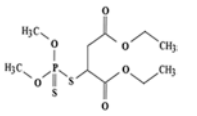
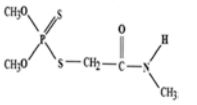
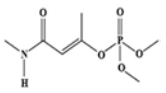
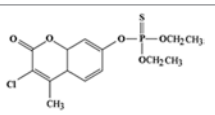
The population of human is probably going through zenith phase of development and to cater its need steady food supply for all is an absolute requirement. The latter is dependent on the continuous increase in food production. Unfortunately, nearly 15–20% (sometimes up to 33%) of the agricultural production are lost due to pest infestation (Puri et al. 2013). For tropical countries, products are damaged due to high humidity, temperature, and several conditions that provide highly favorable environment for the multiplication of insect pests (Lakshmi 1993; Abhilash and Singh 2009). Thus, to protect crops and food from insect attack, insecticides were introduced (Kannan et al. 1997). Initially, organochlorine (OC) insecticides were used; however, due to their high toxicity, long persistence in the environment, bioaccumulation, biomagnifications, and devastatingly ill ecological effects, the majority has been replaced by organophosphate insecticides (Aktar et al. 2009). Some common organophosphate insecticides used worldwide along with their chemical structure, mode of action, year of introduction, half-life, and toxicity are illustrated in Table 14.1.

14.2 Introduction of Organophosphate: Historical Perspectives and Current Scenario

The first organophosphate insecticide to be commercialized was Bladan, which contained tetraethyl pyrophosphate (TEPP) and was formulated by German chemist Gerhard Schrader in 1937 (Gallo and Lawryk 1991; Kanekar et al. 2004; Ghosh 2010). Parathion was synthesized in 1944 by same chemist-scientist (Gallo and Lawryk 1991) and was introduced in 1947; later on its methyl derivative, methyl parathion, was introduced in 1949 (Singh and Walker 2006). Chlorpyrifos was introduced in 1965 as acaricide and insecticide (Singh and Walker 2006). Due to its broad-spectrum nature, chlorpyrifos was used throughout the world to control a variety of chewing and sucking insect pests and mites on a range of economically important crops, including citrus fruit, bananas, vegetables, potatoes, coffee, cocoa, tea, cotton, wheat, and rice (Thengodkar and Sivakami 2010, Chen et al. 2012).

Currently, more than 140 organophosphates are reported to be used worldwide as insecticides, fertilizers, fungicides, weedicides, plant growth factors, and other agrochemicals for better crops yield and chemical warfare agents like soman and sarin. These organophosphates are used as a component of 100 different types of commercially available insecticides, and it has also been estimated that more than 1500 different types of organophosphates have been synthesized during the period of the last century. Presently, organophosphates represent the largest group of chemical insecticides used in plant protection throughout the world after the prohibition on use of organochlorine insecticides (Bhagobaty and Malik 2008; Ortiz-Hernandez and Sanchez-Salinas 2010).

Table 14.1 Some commonly used organophosphate compounds

Name of OP insecticides	Structure	Mode of action	Year of introduction	Half-life in soil (days)
Parathion		Insecticides	1947	30–180
Methyl parathion		Insecticides	1949	25–130
Chlorpyrifos		Acaricide/ insecticide	1965	10–120
Malathion		Insecticides	1950	1–25
Dimethoate		Insecticides	1955	2–40
Monocrotophos		Insecticides	1965	40–60
Coumaphos		Insecticides	1952	24–1400

Data taken from Singh and Walker (2006); Kanekar et al. (2004)

14.2.1 Usage of Organophosphates

Historically, organophosphates were used as chemical warfare agents such as Sarin, Soman, and VX. About 200,000 tons of these extremely toxic organophosphates chemical warfare agents were manufactured and are stored. As per Chemical Weapons Convention (CWC) of 1993, these stocks must be destroyed within 10 years of ratification by the member states (Singh and Walker 2006).

Abhilash and Singh (2009) categorically pointed out the following six sectors where organophosphates insecticides are used extensively:

1. Agriculture—for control of weeds, insects, pests, and rodents mainly
2. Public health—for control of insect (mainly mosquito and others) vectors that spread various diseases (malaria, filariasis, dengue fever, Japanese encephalitis, etc.)
3. Domestic—for controlling insects (mosquitos, louse, etc.), flies that are common in houses and gardens (insects such as spiders that affect ornamental plants), ectoparasites (scab mites, blowfly, ticks, and lice) of domestic farmhouse cattle

4. Personal—applied in clothing or body for controlling head and body lice, mites, other small insects, etc.
5. Material building—incorporated into paints, plastics, wood (furniture, etc.), and other materials as well in building foundation, to prevent insect infestation
6. Others—control of vegetation in forests and factory sites, fumigation of buildings, and ships

14.3 Toxic Organophosphates: A Global Threat of Huge Public Interest

Organophosphates act as neurotoxic agents (Shimazu et al. 2001; Ghosh et al. 2010) and are mainly potent inhibitors of acetyl cholinesterase (Tago et al. 2006. Chao et al. 2008; Ortiz-Hernandez and Sanchez-Salinas 2010). Acetylcholine is a neurotransmitter and acetylcholinesterase constitutes a key enzyme of the nervous system. Generally, after completion of nerve impulse transmission, the function of acetylcholinesterase is to hydrolyze acetylcholine (neurotransmitter) into choline and acetyl-CoA (inactive components), so that these become available for further function. Upon irreversible binding of organophosphate to acetylcholinesterase, it loses its normal hydrolysis function. This results into accumulation of acetylcholine at the junction of the synaptic cleft. Eventually, overstimulation occurs that ultimately leads to paralysis and, under extreme condition, death (Kumar et al. 2010; Theriot and Grunden 2011; Chaudhry et al. 1988; Cho et al. 2004; Bhagobaty and Malik 2008; Ortiz-Hernandez and Sanchez-Salinas 2010). The failure of nerve impulse transmission, due to the organophosphate pesticide poisoning, causes health problems such as weakness, headache, excessive sweating, salivation, nausea, vomiting, diarrhea, abdominal pain, and paralysis which can ultimately lead to death (under extreme condition) (Kanekar et al. 2004). Some other health disorders reported due to organophosphate poisoning are malfunctioning of the endocrinal, respiratory, excretory, and cardiovascular systems as well as miscarriage during pregnancy, abnormal/retarded fetus development, etc. (Kumar et al. 2010).

Approximately, two million tons of organophosphate pesticides are used per year throughout the world. The major consumers are Europe (45%) followed by the USA (24%) and the rest of the world (25%). Herbicides are the main category of pesticide used globally followed by insecticides and fungicides (Gupta 2004).

14.4 Microbial Bioremediation: Best for Effective Environmental Cleanup of Organophosphates

Although several chemical, physical, and physicochemical methods have been developed for the removal of these toxic chemicals from its contaminated sites, bioremediation is considered to be the best. It is the green process of cleaning the environment by using different biological means (i.e., with the help of plants, animals, and microorganisms). It offers a more effective, cheap, eco-friendly, and safer alternative

process toward cleaning up of toxic and hazardous contaminants/pollutants (Chen et al. 2012, 2014). Bioremediation using microorganisms has received huge attention in the last one decade. Organophosphate-hydrolyzing enzymes of bacterial origin are considered for detoxification (and bioremediation) due to broader substrate specificities and better kinetics (Dumas et al. 1989; Cheng et al. 1993).

The organophosphate-degrading microorganisms may be used for systematic investigation toward development of suitable technology for bioremediation of these toxic organophosphate agrochemicals from the contaminated agricultural fields (and other adjoining niches). This is a strong need and demand of the day toward greener and clean tomorrow.

14.5 Hunting Bacteria for Organophosphates: Key for Developing Bioremediation Process

Research works carried out over the past three decades have shown microorganisms as the major component of biological diversity on our planet earth with the representation of 10^{30} cells. These huge number of microbes are fundamental components toward the successful execution of biogeochemical cycles and all other processes that take care of the health of our planet earth (Whitman et al. 1998). Several studies has now unequivocally proven that a successful existence and survival of most of the other life forms (including macroscopic plants and animals) depends on the proper functioning and interaction of the very basic normal microbiota that varies from one living system to another (Berg et al. 2014).

Therefore, to understand the fate of organophosphate compounds in the ecosystems, its metabolic transformation must be properly investigated in the laboratory under precisely controlled conditions (Fig. 14.1). Since the diversity of bacteria is considered huge, lot being unknown and unexplored, this group is supposed to serve as the major reservoir of novel gene pool to hunt for. Since less than 1% of the total diversity is known, it is best to explore more. Bacterial systems are less complicated compared to eukaryotic ones (fungal and plants), and their genetic regulation has been well explored and better understood and thus can be better manipulated for biotechnological applications and bioremediation purposes. In general bacterial enzymes are given more importance than the same from other (plants and animals) sources due to the following reasons (Dumas et al. 1989; Cheng et al. 1993; Chen et al. 2011; Cycon' et al. 2011; Arora et al. 2012; Chen et al. 2014):

- They are generally cheaper to produce.
- Their enzyme contents are more predictable and controllable.
- Reliable supplies of raw material of constant composition are more easily arranged.
- Plant and animal tissues contain more potentially harmful materials than microbes, including phenolic compounds (from plants), endogenous enzyme inhibitors, and proteases.

Fig. 14.1 A brief overview of the current trends in the study of organophosphate (OP) metabolism (catabolic) in microorganism, from isolation and hydrolysis product identification to pathway reconstruction

- Their enzyme-based biodegradation and bioremediation are more cost-effective and eco-friendly.
- Their enzymes have broad substrate specificity.
- Their enzyme can be used easily with bead-based remediation of toxic pollutant.

Although many organophosphate hydrolytic enzymes have been reported, considering the huge estimated diversity of the microbial world, these represent only the tip of hidden, unknown iceberg. From the rich collection, such as organophosphate-degrading microbes, much has been excavated in terms of microbial metabolism, biodegradation pathways, evolution, genetic, and molecular mechanisms. Still, in order to realize the full potential of organophosphate-degrading bacteria, their applications, and development of better strategies for bioremediation of contaminated sites, more intensive research is required. This involves isolation of organophosphate-degrading microorganisms from different ecological habitat (extreme habitats), understanding the detail molecular events of degradation and signaling pathways that initiate/activate the organophosphate-degrading genes, and development of modern technologies for better field applications (Singh 2009).

14.6 Study of Microbial Metabolism of Organophosphate Compounds

In general, the study of microbial metabolism of organophosphate compounds was started by Sethunathan and Yoshida (1973), when they reported a bacterial strain *Flavobacterium* sp. ATCC 27551 (now reclassified as (*Sphingobium fuliginis*), which could degrade and utilize diazinon and parathion as the sole carbon source and degrade chlorpyrifos co-metabolically followed by *Bacillus* sp. and *Pseudomonas* sp. (Siddaramappa et al. 1973); *Xanthomonas* (Rosenberg and Alexander 1979); *Arthrobacter* sp. (Nelson 1982); and *Pseudomonas diminuta* MG (Serdar et al. 1982; Mulbry et al. 1986). Singh et al. (2004) for first time reported the degradation of chlorpyrifos as the sole carbon source by *Enterobacter asburiae* strain B-4, which was followed by *Alcaligenes faecalis* (Yang et al. 2005); *Stenotrophomonas* sp. YC-1 (Yang et al. 2006); and *Sphingomonas* sp. DSP-2 (Li et al. 2007a, b). The overall general methodologies followed toward their studies are summarized in Fig. 14.2. So far, many bacterial strains have been reported to degrade parathion, chlorpyrifos, and other organophosphate compounds either catabolically or co-metabolically. A thorough and extensive list of bacterial spp. reported to be involved in the degradation of organophosphate compounds (mainly parathion and/or chlorpyrifos) is documented in Table 14.2.

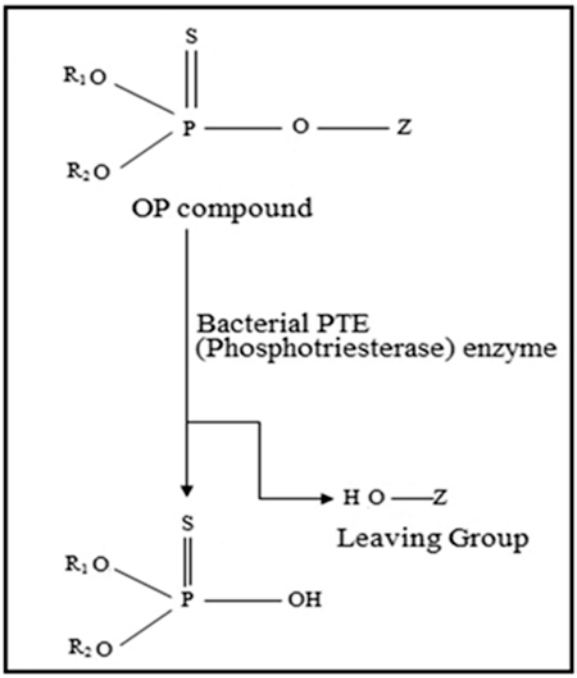


Fig. 14.2 General pathway for biodegradation of organophosphate compounds (Singh 2009)

14.7 General Trend for Organophosphate Metabolism in Microorganisms

The process of microbial metabolism of organophosphate compounds takes place through multistep pathway each being catalyzed by an enzyme. In most of the cases, the general reactions involved are hydrolysis and oxidation and rarely reduction.

All the organophosphate compounds share a similar general pattern for their degradation (Fig. 14.2). There are usually three ester bonds and breakdown of any one reduces toxicity of the compound. The most important step is the breakdown of ester bond with the main group (Z in Fig. 14.2) is bonded. This releases the group [4-NP in case of parathion and methyl parathion; 3,5,6-trichloro-2-pyridinol (TCP) in case of chlorpyrifos] to be metabolized further through enzyme catalyzed multiple steps. Finally, the ultimate end product enters into the TCA cycle for complete metabolic utilization (Singh 2009; Singh and Walker 2006).

14.8 Microbial Metabolism of Organophosphate: A Potential Source of C, P, and N for Growing Cells

Most of the studies related to understanding of microbial metabolism of organophosphate compounds started with isolation and degradation of organophosphate compounds by microorganisms. Two categories for metabolism studies have been

Table 14.2 List of organophosphate-degrading microorganisms

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Flavobacterium sp. (ATCC 27551), reclassified as *Sphingobium fuliginis*	Par, Couma (Cat, C) Chlp (Co-met, C)	Paddy field water, Philippines	Sethunathan and Yoshida (1973); Kawahara et al. (2010)
Pseudomonas sp.	Par, 4-NP (Cat, C)	Parathion-amended soil	Siddaramappa et al. (1973)
4 species of *Pseudomonas* sp. (mixed culture)	Par (Co-met C)	Agri. wastes	Munnecke and Hsieh (1974)
Pseudomonas stutzeri	Par (Co-met, C)	–	Daughton and Hsieh (1977)
Pseudomonas sp.	Par (Cat, P)	Soil and sewage	Rosenberg and Alexander (1979)
Xanthomonas sp.	Par (Cat, C)	Soil and sewage	Rosenberg and Alexander (1979)
Pseudomonas diminuta MG	Par, chlp (Cat)	American isolate	Serdar et al. (1982), Mulbry et al. (1986)
Arthrobacter sp.	Par (Co-met, C)	Par-treated soil (Gilat, Israel)	Nelson (1982)
Bacillus sp.	Par (Co-met)		
Pseudomonas sp. (mixed culture)	Par, MPar (Co-met, C)	MPar-treated soil of farmland	Chaudhry et al. (1988)
Arthrobacter sp.	Chlp (Co-met)	Flooded soil treated with MPar	Misra et al. (1992)
Pseudomonas putida	MPar (Cat, C, and P)	–	Rani and Lalithakumari (1994)
Flavobacterium balustinum	MPar	Agri. soils (Anantapur, AP, India)	Somara and Siddavattam (1995)
Pseudomonas sp. A3	MPar (Cat, C, and P)	Rice field soil	Ramanathan and Lalithakumari (1996, 1999)
Micrococcus sp. (M-36 and AG-43)	Chlp (Cat)	Soil	Guha et al. (1997)
Bacillus sp.	MPar (Cat)	Cotton field soil (Guntur, AP, India)	Sreenivasulu and Aparna (2001)
Plesiomonas sp. strain M6	MPar (Co-met)	(Nanjing, Jiangsu, China)	Zhongli et al. (2001)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Burkholderia cepacia, Bacillus sp.	MPar	Agri. soil	Keprasertsupa et al. (2001)
Agrobacterium radiobacter P230	MPar, Par	Soil, domestic yard (Brisbane, Australia)	Horne et al. (2002a)
Pseudomonas putida KT2442	Par (Cat)	–	Walker and Keasling (2002)
Enterobacter, Enterobacter asburiae strain B-4 (AJ564997 and AJ564998)#	Chlp (Co-met and Cat, C)	Soils of the UK and Australia	Singh et al. (2003, 2004)
Pseudomonas pseudoalcaligenes	MPar (Co-met)	Organophosphate-treated soil	Ningfeng et al. (2004)
Pseudomonas sp. strain WBC-3	MPar, 4-NP, Mala, Fen, Diazin (Cat, C, and N)	–	Liu et al. (2005)
	Chlp, TCP (Cat, C)	Soils (che. factory)	Yang et al. (2005)
7 bacterial species (*Pseudaminobacter* sp., *Achromobacter* sp., *Brucella* sp., *Ochrobactrum* sp.) (AY627033 to AY627039)#	MPar	MPar-contam. soil	Zhang et al. (2005, 2006a, b)
Ochrobactrum sp. B2 (AY661464)#	MPar (Co-met)	MPar-polluted soil	Qiu et al. (2006)
Stenotrophomonas sp. YC-1 (DQ537219)#	Chlp (Cat, C, and P)	Sludge (WW, OP pest. manuf.)	Yang et al. (2006)
Bacillus laterosporus strain DSP	Chlp	–	Wang et al. (2006); Zhang et al. (2012a, b)
Sphingomonas sp. DSP-2 (AY994060)#	Chlp (Cat, C)	Poll. water (chlp manuf. indust., Nantong, China	Li et al. (2007b)
Klebsiella sp.	Chlp	Acti. sludge (Damascus WW Treatment Plant, Syria)	Ghanem et al. (2007)
Serratia sp. (EF070125)#	Chp (Cat, C)	Acti. sludge (Tiancheng pesti. Co., Shandong, China)	Xu et al. (2007)
Bacillus sp. DM-1 (DQ201643)#	MPar (Co-met)	Organophosphate-polluted soil	Yang et al. (2007)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Acinetobacter radioresistens USB-04	MPar, Par (Cat, C)	Sedi., WW treat., pesti., Shandong, China	Fang-Yao et al. (2007)
Burkholderia sp. JBA3	Par (Cat)	Agri. soil (Korea)	Kim et al. (2007)
Serratia sp. (AM050059)#	MPar, 4-NP (Cat, C)	Agri. soil (Anantapur, AP, India)	Pakala et al. (2007)
Delftia sp. XSP-1	MPar, chlp, Fen, Phoxim	Sludge collected from a pesti. manuf.	Shen et al. (2007)
Bacillus firmus strain BY6	Chlp (Cat, C)	Coral was collected from Teluk Awur North Java Sea, Indonesia	Sabdono (2007)
Pseudomonas stutzeri strain HS-D36	Me-Par (Cat, C)	Acti. sludge water treat. pond pesti. facto. in Hubei, China	Wang et al. (2008)
Arthrobacter sp. L1	MPar (Cat, C, and N)	Acti. sludge, enrich. tech.	Li et al. (2008a, b)
Brachybacterium sp., *Kytococcus* sp., *Brevibacterium* sp., *Chromobacterium* sp., *Oceanobacillus* sp., *Bacillus* sp. (AB449753, AB449754, AB449755, AB449757, AB449758, AB449765)	Chlp, Diazin, Ethn (Cat, C)	Coral surface (Teluk Awur, N. Java Sea, Indonesia	Sabdono and Radjasa (2008)
Paracoccus sp. strain TRP (EF070124)#	Chlp/TCP (Cat, C)	Acti. sludge (pesti. manuf., Shandong, China)	Xu et al. (2008)
Pseudomonas aeruginosa (NCIM 2074)	Chlp (Cat, C)	From NCIM, Pune, India	Fulekar and Geetha (2008)
Providencia stuartii (MTCC 8099)	Chlp (Cat, C)	Agri. soil (Chittoor, AP, India	Rani et al. (2008)
Pseudomonas sp. DSP-1 (DQ482656), DSP-3 (DQ482655), and DSP-5 (DQ115539), *Sphingomonas* sp. DSP-2 (AY994060), *Stenotrophomonas* sp. DSP-4 (DQ482654), *Bacillus* sp. DSP-6 (DQ237947), *Brevundimonas* sp. DSP-7 (DQ676936)#	Chlp (Cat, C)	Water sample of chlp indust. Pt. (Nan Tong, Jiangsu and soil agri. field Nanjing, China)	Li et al. (2008a, b)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Bacillus pumilus C2A1	Chlp (Cat, C)	Soil sample from cotton fields at NIBGE, Jhang Road, Faisalabad, Pakistan	Anwar et al. (2009)
Pseudomonas aeruginosa	Chlp and TCP (Cat, C)	Pesti.-contam. soils (Punjab, India)	Lakshmi et al. (2009)
Pseudomonas sp., Burkholderia, *Arthrobacter, Pseudomonas, Variovorax, Ensifer*	Par, Fen, 4-NP, MPar (Cat, C)	Rice field soils	Min-Kyeong et al. (2009)
P. fluorescens, Brucella melitensis, Bacillus subtilis, Bacillus cereus, Klebsiella sp., *Serratia* sp., *P. aeruginosa* (consortium)	Chlp (Cat, C)	Pesti.-contam. soils of Punjab	Lakshmi et al. (2009)
Burkholderia sp. strain KR100 (HM101281)#	Chlp-Me, TCP (Cat, C)	Korean rice paddy soil	Kim and Ahn (2009)
Bacillus sp. and *Pseudomonas* sp.	Chlp, MPar, phorate, dichlorvos	Soil sample	Madhuri and Rangaswamy (2009)
Pseudomonas aeruginosa	MPar, Mono	MTCC, Chandigarh, India	Balamurugan et al. (2010)
Stenotrophomonas sp. SMSP-1 (EU312979)#	Par, MPar, Fen, Phoxim –	Sludge of a WW of pesticide manuf.	Shen et al. (2010a, b)
Bacillus licheniformis ZHU-1 (KC197213)#	Chlp (Cat, C)	Soil sample from Wuqi Farm in Shanghai, China	Zhu et al. (2010)
Sinorhizobium sp., *Pseudoxanthomonas* sp., *Streptomyces iakyrus*, *Microbacterium takaoensis*, *Isoptericola dokdonensis* (GU902282 to GU902303)#	Par (Cat, C)	Soil sample	Fodale et al. (2010)
Spirulina platensis (cyanobacteria)	Chlp	Obtained from Indian Agricultural Research Institute, Delhi, India	Thengodkar and Sivakami (2010)
Pseudomonas sp. *(aeruginosa/putida)*	Paraoxon (Cat)	Soil samples Houston, Texas, Alvin Texas, League City, Texas Sealy, Texas Katy, Texas	Iyer et al. (2011)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
4 species of *Pseudomonas* sp., 2 species of *Agrobacterium* sp. and *Bacillus* sp. (GQ149502-GQ149508)#	Chlp (Cat, C)	Soil sample from agri. farm of Banaras Hindu University, Varanasi, India	Maya et al. (2011)
Synechocystis sp. strain PUPCCC 64 (GQ907237)#	Chlp	Rice field of the village Dera Bassi of Patiala district of Punjab state, India	Singh et al. (2011)
Pseudomonas sp. strains RCC-2, *Staphylococcus* sp. GCC-1, *Flavobacterium* sp. GCC-3, and *Streptococcus* sp. JCC-3	Chlp	Soil samples from cultivated fields of Rajkot, Gujarat, India	Kumar (2011a, b)
Acinetobacter sp., *Pseudomonas putida, Bacillus* sp., *Pseudomonas aeruginosa, Citrobacter freundii, Stenotrophomonas* sp., *Flavobacterium* sp., *Proteus vulgaris, Pseudomonas* sp., *Acinetobacter* sp., *Klebsiella* sp., *Proteus* sp., and *Pseudomonas* sp. (consortium)	Chlp, MPar (Co-Met), 4-NP	Contam. garbage dump of Moravia, Medellin	Pino et al. (2011); Pino and Peñuela (2011)
Pseudomonas stutzeri, Pseudomonas Pseudoalcaligenes, Pseudomonas maltophilia, Pseudomonas vesicularis	Chlp (Cat, C)	Pest.-contaminated soil in Egypt	Awad et al. (2011)
Agrobacterium sp. strain Yw12 (DQ468100)#	MPar (Cat, C, and P)	OP-contaminated sludge Huayang pesti. manuf., Shandong, China	Wang et al. (2012)
Enterobacter sp. strain Cons002	Par, MPar, phorate (Co-met)	Agri. soil	Concepcio'n et al. (2012)
Bacillus pumilus W1	MPar	OP-contaminated soil of Khairpur, N. Sindh, Pakistan	Ali et al. (2012)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Klebsiella sp., (NII 1118), *Pseudomonas putida* (NII 1117), *Pseudomonas stutzeri* (NII 1119), *Pseudomonas aeruginosa* (NII 1120) (consortium) (HM135446, HM135447, HM135448, HM135449)#	Chlp (Cat)	Chlp-contam. soil sample paddy field, Kancheepuram, Tamil Nadu, India	Sasikala et al. (2012)
Pseudomonas putida	Chlp (Co-Met)	Soil samples collected from different sites in and around Bangalore, India, having a history of repeated application of chlp	Vijayalakshmi and Usha (2012)
5 species of *Pseudomonas* sp. (individually)	Chlp (Cat, C, and P)	Efflu. storage pools of facto. producing pesti. and from soil moisture around them	Latifi et al. (2012)
Pseudomonas fluorescens, Bacillus subtilis, Klebsiella sp.	Chlp, Mono (Co-Met)	Pesti.-contam. soil of paddy field, Annamalai Nagar, Tamil Nadu, India	KaviKarunya and Reetha (2012)
Bacillus stearothermophilus, B. circulans, B. macerans	Chlp (Co-Met)	Soil from cabbage cultivated private agri. farm, Bangalore, India	Savitha and Raman (2012)
Bacillus cereus	Chlp,TCP (Cat N)	Soil from Jiangsu Jinghong Chemical Co., Ltd, China	Liu et al. (2012)
Four species of *Actinobacteria* (*Streptomyces* sp.) (JQ289350-JQ289353)#	Chlp (Co-Met)	Chlp-contam. agri. soil from blueberry field, Gorbea City in southern Chile	Briceño et al. (2012)
Stenotrophomonas maltophilia strain MHF ENV 20 and MHF ENV (HM625746, HQ661376)#	Chlp/TCP	Soil from banks of Surya River, Palghar (100 km away from Mumbai)	Dubey and Fulekar (2012)
Pseudomonas putida MAS-1	Chlp (Co-Met)	Indigenous agri. soil of Karachi, Pakistan	Ajaz et al. (2012)
Pseudomonas sp. WW5	Chlp (Co-Met)	–	Farhan et al. (2012)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Pseudomonas diminuta (EMP11c), *P. putida* (EMP12a), *P. aeruginosa* (EMP12b)	OP (Cat, C)	Agri. soil from Gwalior, Madhya Pradesh, India	Sharma et al. (2013)
Pseudomonas putida POXN01	MPar	Soil sample collected from rice field of Harlingen (Cameron Country, Texas)	Iyer et al. (2013)
Sphingobacterium sp. JAS3 (JQ514560)#	Chlp (Cat, C)	Soil collected from a paddy field in Vellore district, Tamil Nadu state, India	Abraham and Silambarasan (2013)
Naxibacter sp. strain CY6 (JX987079)#	Chlp, Par, MPar (Cat, C, P)	Soil samples from pesti.-contam. soil of a greenhouse	Kim et al. (2013)
Cupriavidus sp. DT-1 (JQ750642)#	Chlp,TCP (Cat, C)	Sludge collected from a chlp manuf. site in Changzhou, Jiangsu Province, China	Lu et al. (2013)
Kocuria sp.	Chlp	Agri. soil of West Godavari district of AP, India	Neti and Zakkula (2013)
Acinetobacter radioresistens, Pseudomonas frederiksbergensis, Bacillus pumilus, Serratia liquefaciens, Serratia marcescens, Burkholderia gladioli	Chlp, MPar, Diazin, Mala, Dime	Agri. soil of Beed district, Maharashtra, India	Hussaini et al. (2013)
Nocardia mediterranei	Chlp, MPar (Co-Met)	–	Sukirtha and Usharani (2013)
Pseudomonas aeruginosa, Bacillus megaterium, Staphylococcus aureus	MPar	Rhizos. soil MP-treated agri. res. farm, guava orchad. SHIATS and comm. farm, Jhunsi, Allahabad	Peter et al. (2014)
Bacillus subtilis strain C5 (JN942155)#	MPar	Marine sludge (China Bohai Sea)	Hao et al. (2014)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Pseudomonas aeruginosa, Serratia marcescens, and Klebsiella oxytoca	Chlp	Rice fields in Anaku, Omor, and Igbakwu towns in Ayamelum Local Govt. Area of Anambra State, Nigeria	Ifediegwu et al. (2015)
Bacillus cereus strain LR5 (JX966388)#	Chlp	Soil (treated with chlp) was collected from Zhejiang Academy of Agri. Sciences, Hangzhou, China	Chen et al. (2014)
Pseudomonas sp. strain YF-5 (KF584917)#	MPar, chlp (Cat, C)	Sludge (China)	Liu et al. (2014)
Pseudomonas sp. BF1–3 (KJ849233)#	Chlp	Balloon flower root	Barman et al. (2014)
Paenibacillus (*Bacillus*) *polymyxa* and *Azospirillum lipoferum*	Chlp, chlp-Me, Mala	–	Romeh and Hendawi (2014)
Stenotrophomonas sp. G1 (JN688160)#	Par, chlp, MPar, Diazin	Sludge, drain outlet (chlorpyrifos manufac. Plant, China)	Deng et al. (2015)
Achromobacter sp. C1	MPar (Cat, C)	Agri. soil, Jabalpur, India	Mishra (2015)
Mesorhizobium sp. HN3 (JN119831)#	Chlp, TCP (Cat, C)	Chlp-contam. agri. soil samples	Jabeen et al. (2015)
Cupriavidus taiwanensis (JN688161)#	Chlp	Sludge from outlet of a chlp manuf. in Jiangsu Province, China	Wang et al. (2015)
Bacillus aerius	Chlp	Soil samples from locations of the Nandimandalam village of YSR district Kadapa, AP, India	Jayasri et al. (2015)
Bacillus thuringiensis strain BRC-HZM2 (GQ140344)#	Chlp	Samples were collected from a facto (Fujian Sannong che. and pest. facto.), manuf. OP pesti., Sanming, Fujian Province, China	Wu et al. (2015)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Bacillus aryabhattai SanPs1	MPar (Cat, C)	Rhizosphere soil of paddy field. Burdwan, India	Pailan et al. (2015)
Pseudomonas sp. BUR11	MPar (Cat, C)	Rhizosphere soil of paddy field. Burdwan, WB, India	Pailan and Saha (2015)
Acinetobacter sp. MemCl4	Chlp (Cat, C)	Rhizosphere soil of paddy field. Memari, WB, India	Pailan et al. (2016)
Pseudomonas putida X3	MPar (Cat, C)	–	Zhang et al. (2016)
Pseudomonas sp. *R1, R2, and R3*	Mpar (Cat)	Agri. soil, Visakhapatnam, AP, India	Begum and Arundhati (2016)
Cupriavidus nantongensis X1	Chlp	Isolated from sludge collected at drain outlet of a chlorpyrifos manuf. plant	Fang et al. (2016)
Staphylococcus warneri (CPI2), *Pseudomonas putida* (CPI 9), *and Stenotrophomonas maltophilia* (CPI 15) (consortium)	Chlp	Soil from different agric. areas in Kerala, India	John et al. (2016)
Xanthomonas sp. 4R3-M1, Pseudomonas sp. 4H1-M3, and Rhizobium sp. 4H1-M1	Chlp (catabolically as a sole source of C and N)	Sugarcane farms in the Mackay, Burdekin, and Tully areas in Queensland, Australia	Rayu et al. (2018)
Fungi			
Penicillium waksmani	Par	Flooded sulfate soil	Rao and Sethunathan (1974)
Trichoderma harzianum, Penicillium vermiculatum, and *Mucor* sp.	Chlp	Forest sample	Jones and Hastings (1981)
Phanerochaete chrysosporium	Chlp (Cat, N)	US Dept. of agri. Forest Products Laboratory, Madison, WI	Bumpus et al. (1993)
Aspergillus terreus, Trichoderma harzianum	Chlp	A clay soil taken from the Botanical Garden of Assiut University, Assiut, Egypt	Omar (1998)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Coriolus versicolor, Hypholoma fasciculare	Chlp	–	Bending et al. (2002)
Aspergillus sp., *Trichoderma* sp.	Chlp	Soil pre-treated with chlp, China	Liu et al. (2003)
Fusarium sp. LK (WZ-I)	Chlp	–	Wang et al. (2005); Xie et al. (2010)
Verticillium sp. (DQ153250)##	Chlp (Cat, C)	Samples from farm soil, tree rhizos. soil, sedi. of a sewer, sludge, and piggery soil from Huajiachi Campus, Zhejiang University, Hangzhou, China	Yu et al. (2006)
Trichosporon sp. (EF091819)##	Chlp,TCP	Acti. sludge from Tiancheng pesti. Co., Shandong, China	Xu et al. (2007)
Verticillium sp. DSP	Chlp	Soil samples collected from farm field at Huajiachi Campus, Zhejiang University, Hangzhou, China	Fang et al. (2008)
Trichoderma viride	MPar	MTCC, Chandigarh, India	Balamurugan et al. (2010)
Aspergillus niger AN400	MPar (Co-Met, C)	–	Marinho et al. (2011)
Acremonium sp. strain GFRC-1	Chlp (Cat, C)	From agri. soils	Kulshrestha and Kumari (2011)
Cladosporium cladosporioides Hu-01	Chlp (Cat, C)	–	Chen et al. (2012)
Aspergillus terreus JAS1 (JQ361749)##	Chlp (Co-Met, C)	Paddy field chlp-contam. soil sample from Vellore, Tamil Nadu, India	Silambarasan and Abraham (2013)
Aspergillus sp. F1 (JQ898687), *Penicillium* sp. F2 and F3 (JQ898688, JQ898689), *Eurotium* sp. F4 (JQ898690), and *Emericella* sp. F5 (JQ898691)##	Chlp, TCP	Soil of Agri. farm of Banaras Hindu University, Varanasi (25° 18′ N, 83° 3′ E)	Maya et al. (2012)

(continued)

Table 14.2 (continued)

Name of strain	Organophosphate compound (Cat/Co-Met utilization as C/P source)	Isolation (from) site	References
Trichoderma harzianum, *Rhizopus nodosus*	Chlp, Ethn (Cat, C)	Chlp- and Ethn-contam. soil	Harish et al. (2013)
Fusarium sp. CR10 (JX915255); *Fusarium oxysporum* CR9 (JX915246); *Fusarium* sp. GR4 and CR13 (JX915247); *Gibberella moniliformis* CR11, GR1, GR3, and CR4 (JX915252, JX915251, and JX915250); *Dipodascaceae* sp. GR2 and CR12 (JX915245); *Chaetomium globosum* CR1 and CR14 (JX915254)##	Chlp	Soil (treated with chlp) was collected from Zhejiang Academy of Agri. Sciences, Hangzhou, China	Chen et al. (2014)
Isaria farinosa	Chlp	Chlp-contam. soil samples from Idukki, Kerala, India	Karolin et al. (2015)
Penicillium citrinum, *Fusarium proliferatum*	MPar	Isolated from the ascidian *Didemnum ligulum*	Rodrigues et al. (2016)

Abbreviation: *Acti* activated, *agri* agriculture, *Che* chemical, *Cat* catabolic, *C* carbon, *chlp* chlorpyrifos, *Couma* coumaphos, *Co-met* co-metabolic, *contam* contaminated, *Diazin* diazinon, *Efflu* effluent, *Ethn* ethion, *facto* factory, *Fen* fenitrothion, *Mpar* methyl parathion, *Par* parathion, *pesti* pesticides, *Mala* malathion, *manuf* manufacturer, *Mono* monocrotophos, *N* nitrogen, *P* phosphorus, *poll* polluted, *res* research, *rhizos* rhizosphere, *sedi* sediment, *WW* wastewater, # 16S rRNA gene sequence, ## 18S rRNA gene sequence

addressed in literature. This includes the following: the first includes the catabolic utilization/biodegradation studies, where, organophosphate compound has been used as the sole source of C, and the second includes co-metabolic utilization/biodegradation studies, where another C compound (along with organophosphate compound) has been used as sources of C for growth (Singh 2009). The metabolic conversion of organophosphate compounds has been proposed to occur through pathways, each having multiple steps. In this chapter, parathion has been considered as a representative compound.

Till date, three different pathways for metabolic conversion of parathion have been reported as shown in Fig. 14.3 (Singh and Walker 2006). The first pathway involves an initial oxidative step to generate paraoxon which is hydrolyzed to generate 4-NP and diethyl thiophosphoric acid (DETP). For the second pathway, the first step is hydrolysis, leading to the formation of 4-NP and DETP. While the third pathway is reductive one facilitated under anaerobic condition [although some oxygen-insensitive reductase from *Bacillus* (Yang et al. 2007) and *Anabaena* sp. PCC7120 (Barton et al. 2004) has been reported]. The reactions involve reduction

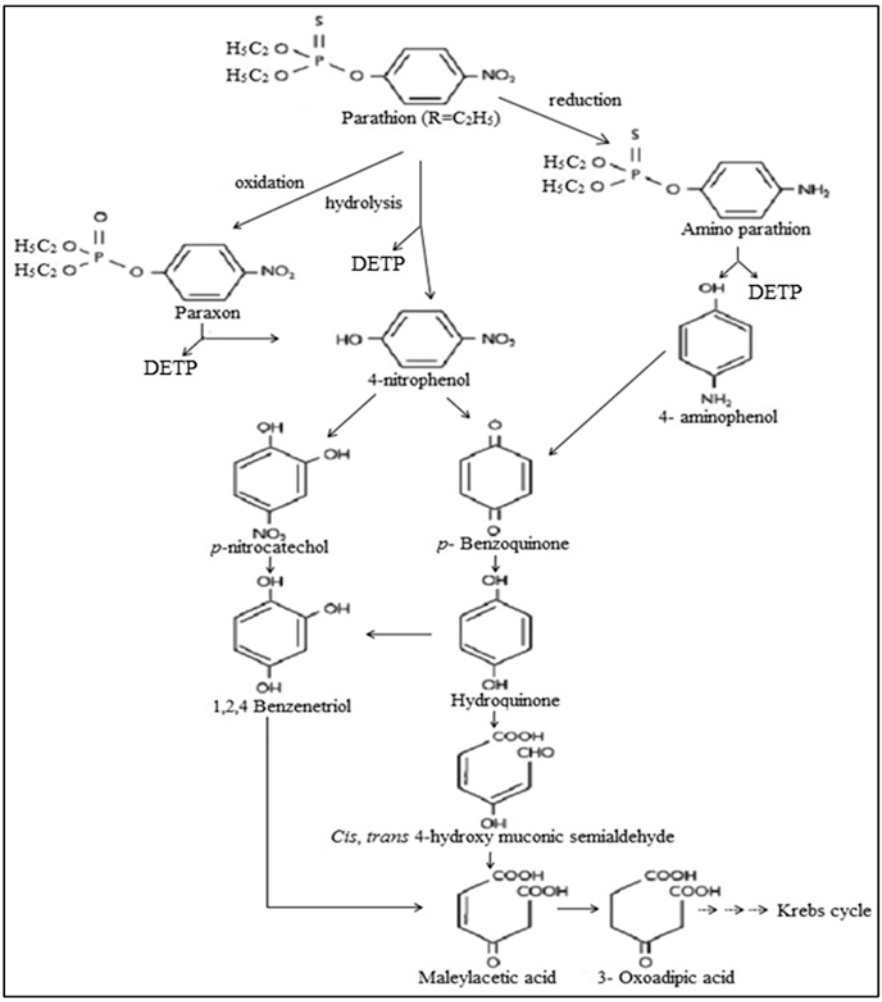


Fig. 14.3 Pathway of parathion biodegradation (Singh and Walker 2006)

of nitro group to amine (leading to formation of 4-aminoparathion), which up on further hydrolysis releases 4-aminophenol (4-AP) and DETP. In most of the literatures, the metabolisms of the main functional leaving groups are discussed. The fate of DETP, being common to all, is not followed.

It is clear from available literature that the second pathway (the hydrolysis one) is the most widely reported one. The 4-NP that is generated is reported to be utilized via two pathways: one that operates through formation of 4-NC and BT is more prevalent among Gram-positive bacteria [*Bacillus sphaericus* JS905 (Kadiyala and Spain 1998) and *Rhodococcus opacus* SAO101 (Kitagawa et al. 2004)], while the second that operates through formation of PBQ and HQ is more common among the Gram negatives [*Moraxella* sp. (Spain and Gibson 1991) and *Pseudomonas* sp.

strain WBC-3 (Zhang et al. 2009)]. However, in *Pseudomonas* sp. 1–7, both the pathways have been reported to be operative (Zhang et al. 2012b).

Very few reports on the degradation of parathion to paraoxon before hydrolysis of phosphotriester bond (i.e., the first pathway) were reported, except that from a mixed bacterial culture (Mastumura and Boush 1968; Tomlin 2000).

The third pathway has mainly been reported from a mixed bacterial consortium (by Munnecke and Hsieh 1976) under anaerobic environment. This pathway was also reported from aerobically growing *Bacillus* sp. (Sharmila et al. 1989 and Yang et al. 2007) and *Anabaena* sp. PCC7120 (Barton et al. 2004). The presence of possible involvement of oxygen-insensitive reductases is suggested for conversion in the aerobic bacteria (Barton et al. 2004). Very recently, Pailan and Saha (2015) reported evidence of two possible pathways (first, through 4-NP formation and, second, through 4-aminoparathion and 4-aminophenol) operative in *Pseudomonas* sp. strain BUR11. Through analytical techniques and growth-dependent experimental evidences, they reported on this aspect.

14.9 Overall Process of Organophosphate Metabolism

Several enzymes are reported to participate in the process of metabolism of organophosphate compounds. These can be broadly categorized into two major groups, namely, phase I and phase II enzymes. Phase I enzymes participate in reactions that makes the molecule much more polar, water-soluble, and amenable for enzymes of phase II to act. It may also be pointed that microbes can solubilize organophosphate by organic acid secretion and also by biosurfactants (Monteiro et al. 2007). In general, increase in solubility reduces half-life of the compounds rapidly. The major processes involved in metabolism are biodegradation, conjugation, and rearrangements. These include many chemical reaction types such as oxidation, reduction, dealkylation, ring cleavage, oxygenase, and peroxidize mechanisms.

Interaction of toxic organophosphate compound with microorganisms can proceed through three processes:

1. Transformation reaction leading to detoxification of parent organophosphate compound
2. Direct degradation and mineralization through catabolic pathway
3. Maintenance of cellular homeostasis

These three processes can occur together or in isolation depending up on what kind of genetic information the organism is equipped with.

Most of the literature has worked up on the second issue (Singh and Walker 2006; Pailan and Saha 2015). While, Longkumar et al. (2014) showed existence glutathione *S*-transferase mediated detoxification system in *Acinetobacter baumannii* strain DS002. The enzyme was reported to be involved in a dealkylation reaction that eventually reduced the toxicity of parent methyl parathion. There is a huge lacuna as far

as the third issue is concerned. This issue is particularly true for those strain that do not have the capacity to degrade organophosphates but can tolerate them.

14.10 Quantitative Study of Microbial Metabolism

Most of the studies in literature have addressed the quantitative aspect of metabolism study by any one of the following two ways (Peter et al. 2014; Pailan and Saha 2015; Fang et al. 2016):

1. By monitoring gradual decrease in the amount of parent organophosphate compound in the growth medium (due to microbial metabolism) with respect to time
2. By monitoring gradual increase in the amount of hydrolytic intermediates followed by their subsequent decrease, indicating their utilization and metabolic conversion

As case study, for example, for metabolic study of parathion, the decrease in the residual amount of parathion in microbial culture inoculated test growth flask can be compared with blank (i.e., where no microbial inoculants are added) with respect to time as shown in Fig. 14.4a.

Another way of monitoring the metabolism is by quantifying the amounts of major hydrolytic intermediates produced as a result of the degradation of parent compound. As evident from Fig. 14.4b, by studying the fate of four major hydrolysis intermediates of parent organophosphate compound, parathion, one can conclude that the bacterial culture in the question can metabolically utilize parent organophosphate compound with concomitant formation of the first intermediate (4-nitrophenol, which accumulates in culture medium initially) followed by its gradual utilization (as its amount decreases) and then by formation of other intermediates (*p*-benzoquinone, hydroquinone, and benzenetriol). The temporal trend of the graph indicated the utilization of all the intermediates (as they decrease gradually).

Quantification of organophosphate compounds and its other hydrolytic intermediates can be carried out by HPLC technique. As evident from Fig. 14.5, compounds can be identified by comparing retention time of the test samples to that for authentic standards (from a well-known source like Sigma Aldrich). For quantification, specific peak area as well as height of the analytes from the test sample (extracted at different time intervals) is compared to that of the standard (for which standard curves are generated).

14.11 Identifying the Intermediate Compounds Produced Due to Metabolic Breakdown of Organophosphate

The reliable techniques to detect and identify different hydrolytic intermedites of organophosphate compound (e.g. parathion) are TLC, GC screening, GC-MS and LC-MS/MS followed by NIST (National Institute of Standard Technology) library

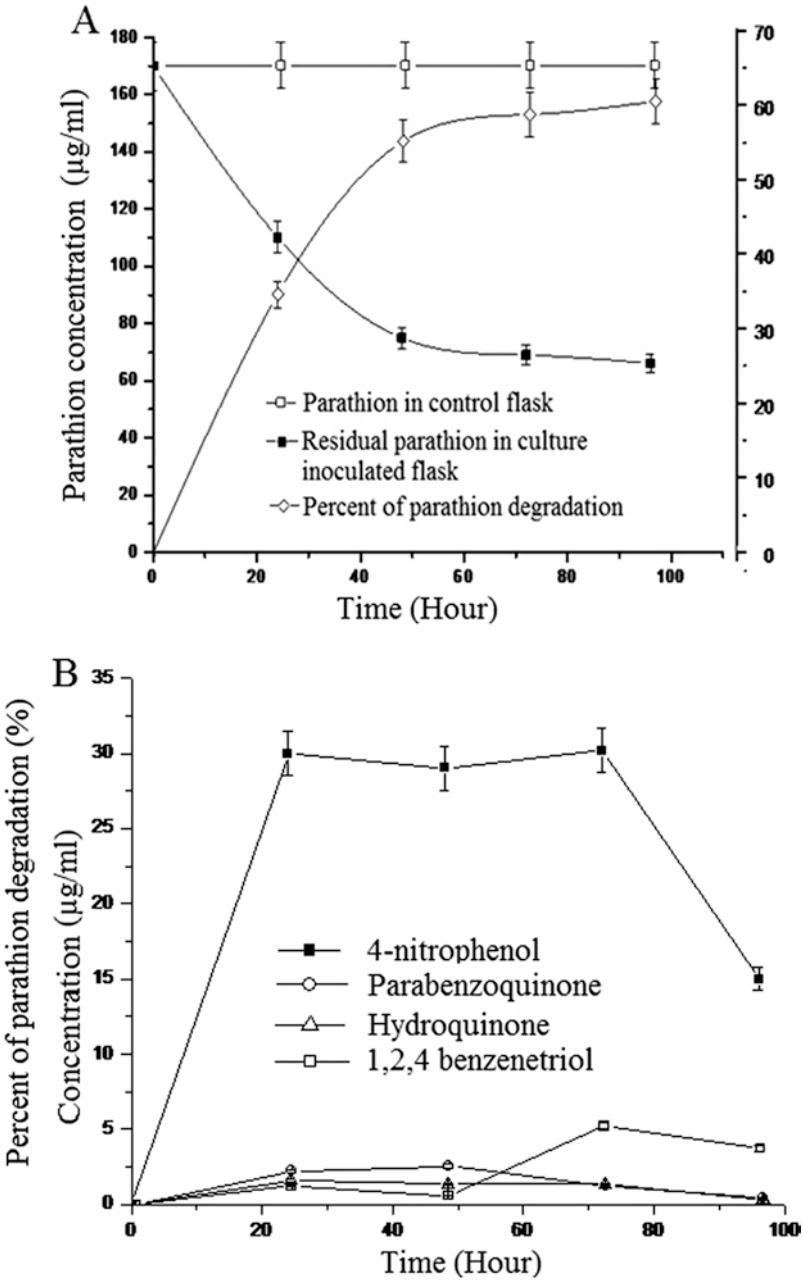


Fig. 14.4 Parathion degradation profile of BUR11. (**a**) Parathion degradation profile by the strain BUR11 and (**b**) fate of intermediates during parathion degradation by the strain BUR11 (Pailan and Saha 2015)

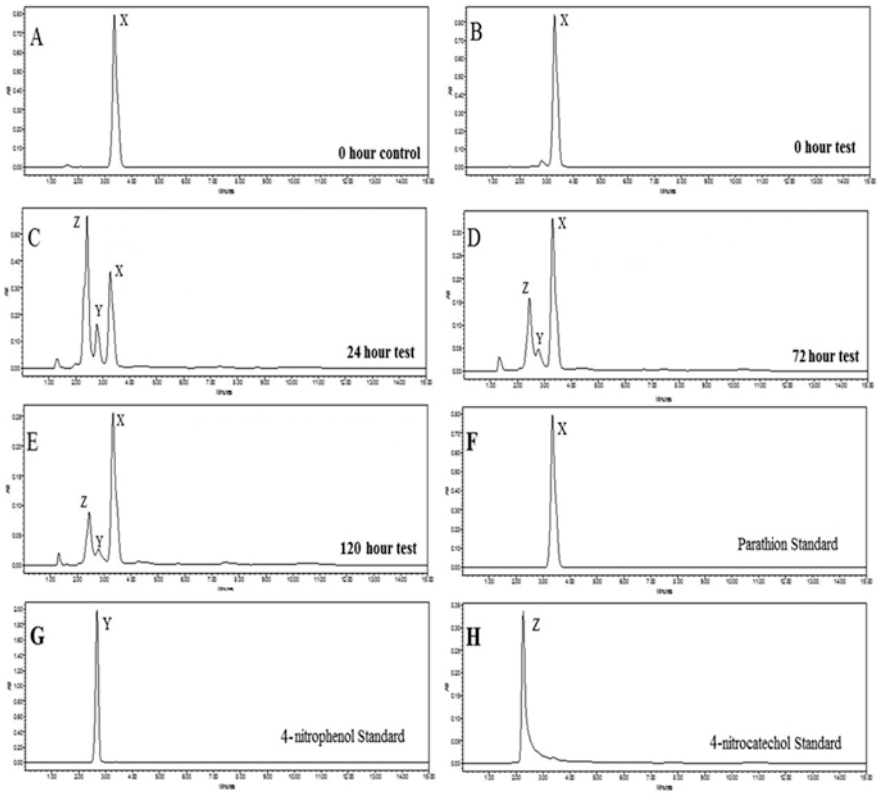


Fig. 14.5 Parathion degradation by a bacterial strain. The elution profile of each sample is shown as individual chromatograms. 0 h control sample (**a**), 0 h test sample (**b**), 24 h test sample (**c**), 72 h test sample (**d**), 120 h test sample (**e**), and elution profiles of standards (**f**, **g**, **h**). X-, Y-, and Z-labeled peak denotes parathion, 4-NP, and 4-NC, respectively

search. For the preliminary identification of hydrolytic intermediates during organophosphate (e.g., parathion) degradation, TLC is performed. Compounds were identified (Fig. 14.6) by comparing R_f value of the test samples to that for authentic standards (from Sigma Aldrich).

Through GC screening and library match, also the preliminary idea of hydrolytic intermediates can be obtained. However, for confirmed results, separation by GC-MS and/or LC-MS/MS techniques followed by the identification of intermediate compounds by comparing their mass spectrum profiles to that of the NIST library are universally accepted (Fig. 14.7)

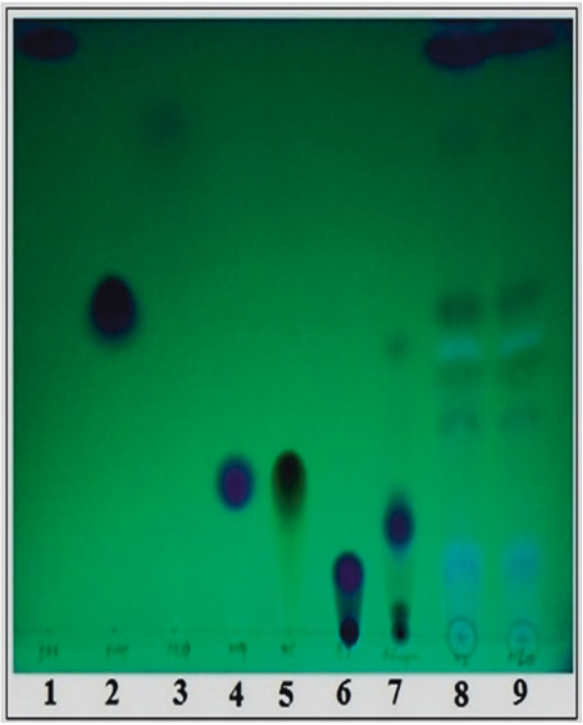


Fig. 14.6 Identification of metabolites of parathion degradation by TLC. Authentic standards **1**, parathion; **2**, 4-NP; **3**, PBQ; **4**, HQ; **5**, 4-NC; **6**, BT; **7**, 4-AP. And **8** and **9** correspond to 72 and 120 h extract of parathion-grown culture, indicating the detection of 4-NP, PBQ, HQ, and BT during the course of degradation (Pailan and Saha, 2015)

14.12 Factors That Affect Organophosphate Degradation

Several factors have been reported to affect the process of organophosphate degradation (both in soil and in laboratory batch cultures). These are as follows:

14.12.1 Substrate Concentration

Biodegradation of a particular pollutant depends upon the concentration of pollutant occurring in the contaminated site. Usually, a concentration which is too high may be toxic for the microbes, while lower concentration may not be sufficient to induce the microbial enzyme system involved in the degradation process (Block et al. 1993; Morra 1996). It has been reported that with the increasing concentration of organophosphate pesticides, there is a decrease in the microbial population (Shan et al. 2006). A dosage of 4 l/hac of chlorpyrifos was recorded to be inhibitory to the total soil microbial population (Pandey and Singh 2004). The average half-life of chlorpyrifos was reported to be increased with the increasing chlorpyrifos concentration of the soil (Hua et al. 2009).

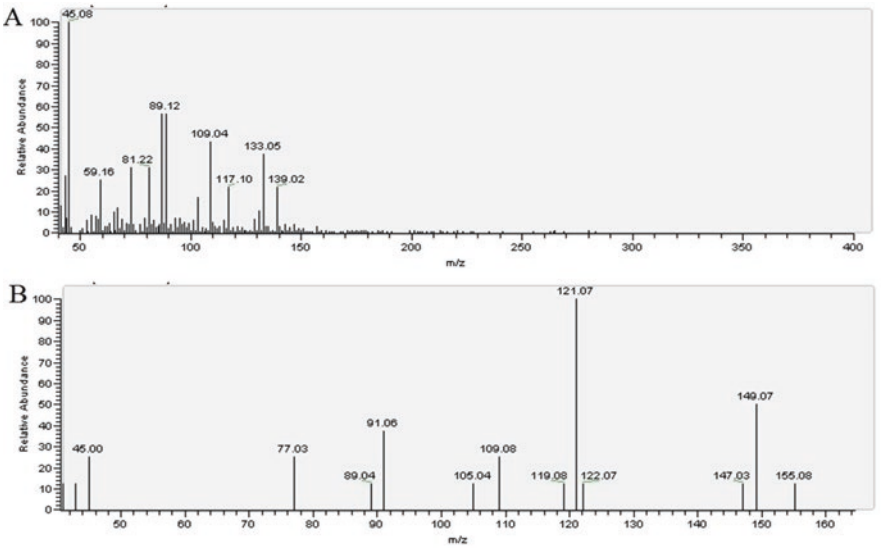


Fig. 14.7 GC-MS spectra obtained from the bacterial culture extract of parathion-grown broth culture. (**a**) 4-NP and (**b**) 4-NC were found as major compounds as hydrolysis products). The compounds were identified and confirmed from the NIST library

14.12.2 pH

It is one of the most important factors for the degradation of organophosphate compounds in soil and other habitats. Majority of the organophosphate pesticides are subject to base catalyzed hydrolysis at higher alkaline pH, around 8 (Greenhalgh et al. 1980). The degradation of chlorpyrifos was reported to be slow in acid soil (pH 4.7) and high in alkaline soil (pH 7.7–8.4), by Singh et al. 2003. Biodegradation of chlorpyrifos by *Bacillus laterosporus* DSP was reported to be enhanced by increasing the pH from 7 to 9 (Wang et al. 2006; Zhang et al. 2012b). A study of the effect of pH on biodegradation of malathion and dimethoate by *Pseudomonas frederiksbergensis* indicated decrease in half-life (almost by twofold) with increasing pH from neutral to pH 8 (Al-Qurainy and Abdel-Megeed 2009). For fungal culture, *Fusarium* sp. LK, biodegradation of chlorpyrifos was reported in the range of pH 6.5–9 (Wang et al. 2005).

14.12.3 Inoculum Size

The population of microorganisms involved in degradation is also reported to be an important factor. Inoculum size ranging from 10^6 to 10^8 cells/g of soil was suggested to be sufficient for bioremediation of pesticides from their contaminated sites (Comeau et al. 1993). Biodegradation of fenamiphos and chlorpyrifos was reported to be influenced by inoculum size, while no degradation of chlorpyrifos by *Enterobacter* sp. was recorded below an inoculum concentration of 103 cells/g of

soil. When soil was supplied with less than 105 cells/g of soil, no biodegradation of fenamiphos was recorded (Singh and Walker 2006).

14.12.4 Bioavailability/Solubility

For proper biodegradation, it is very essential that the pollutant be available/made available to the degrading microorganism(s). In general, many organophosphate compounds have less water solubility, and this factor has been reported to be responsible for its decreased degradation (Alexander 1999). Many hydrophobic organophosphate pesticides become entrapped in the nanopores of the organic matter of the soil and thus are not available for biodegradation at all. Addition of suitable material that solubilizes the pollutant or selection of biosurfactant-producing microorganisms has been reported to make these hydrophobic molecules available for biodegradation. The biosurfactants desorb the hydrophobic chemicals so as to make them available for degradation (Aronstein et al. 1991; Brown and Jaffe 2006; Zhu and Zhou 2008).

Biosurfactants are anionic or neutral (some are cationic) rhamnolipids, glycolipids, lipopeptides, phospholipids, fatty acids, particulate compounds, etc. which are of microbial origin and are used for solubilization of hydrophobic pollutants, with the aim of making it bioavailable and more suitable for biodegradation (Monteiro et al. 2007).

14.13 Chemotaxis and Metabolism of Organophosphate Insecticides

The movement of bacteria either toward or away from a chemical gradient is called bacterial chemotaxis. Chemotaxis is a natural phenomenon and is reported from diverse groups of bacteria. A chemical compound that affects the bacterium's movement is called the chemoeffector (stimulant). Chemicals that attract bacteria are called chemoattractants, and chemicals that repel them are called chemorepellents. Chemotaxis can be classified into two types, namely, metabolism dependent and metabolism independent (Pandey and Jain 2002; Baker et al. 2005). Till date several assays have been developed to check the chemotactic activity of a bacterium. These are swarm plate assay, drop plate assay, agarose-plug assay, etc. (Bhushan et al. 2000; Samanta et al. 2000; Pandey et al. 2002; Bhushan et al. 2004). As far as chemotaxis to pesticides/insecticides are concerned, survey of literature revealed reports pertaining only to two bacteria, namely, *Pseudomonas* sp. strain ADP (Liu and Parales 2009) and *Ralstonia eutropha* JMP134 (Hawkins and Harwood 2002). Both of them are reported to exhibit chemotaxis-mediated biodegradation of atrazine and 2,4-dichlorophenoxyacetate herbicides, respectively. There is hardly any literature on chemotaxis of bacteria toward organophosphate compounds except by *Pseudomonas* sp. strain WBC-3 (Zhang et al. 2008) and by *Pseudomonas putida* DLL-1. However, the latter publication is only available in Chinese language, and its English version is currently not available (Wen et al. 2007).

Recently, *Pseudomonas* sp. strain BUR11 was reported to exhibit positive chemotaxis toward two OP compounds, namely, parathion and chlorpyrifos (as well as their degraded intermediate products 4-NP, 4-AP, and TCP). Through a series of plate-based qualitative assays (drop plate & swarm plate) and quantitative assay, the chemotactic response was confirmed for the strain BUR11 (Figs. 14.8 and 14.9). However, the authors could not conclude whether this chemotactic response was metabolism dependent or independent. The study concluded on the importance of genetic analyses for better understanding of this chemotactic process; nevertheless, this was one of the unique confirmed reports of chemotactic response of bacterium toward organophosphate compounds in recent times (Pailan and Saha 2015).

14.14 Discovery of Organophosphate-Degrading Enzyme

Organophosphate-degrading enzyme was first described by Mazur in 1946 when he discovered the hydrolysis of diisopropyl fluorophosphate (DFP) by enzymes found in rabbit and human tissue extracts (Mazur 1946). For the first time, DFPase and sarinase enzymes were found to degrade organophosphate compounds. Later, the DFPase activities of several bacterial isolates for organophosphate degradation were described by Attaway et al. (1987). In 1992, the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology listed them in the category of phosphoric triester hydrolases. These enzymes were further categorized into two subgroups based on their substrate specificities. The first subgroup is the organophosphorus hydrolases (also referred to as paraoxonase and phosphotriesterase; PTE) that prefer the substrates paraoxon and P-esters, which have P–O and P–S bond. The second subgroup is diisopropyl fluorophosphates (also including

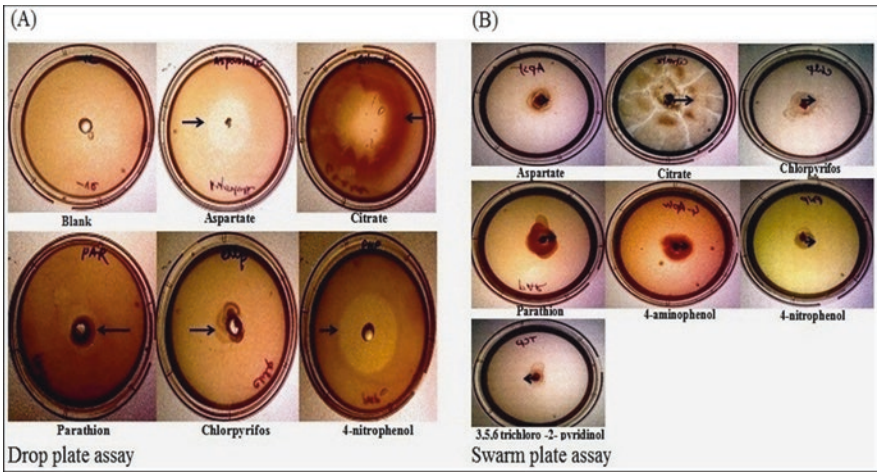


Fig. 14.8 (**a**) Drop plate assay and (**b**) swarm plate assay. Qualitative chemotactic response of BUR11 toward parathion, chlorpyrifos, 4-NP, 4-AP, and TCP (Pailan and Saha 2015)

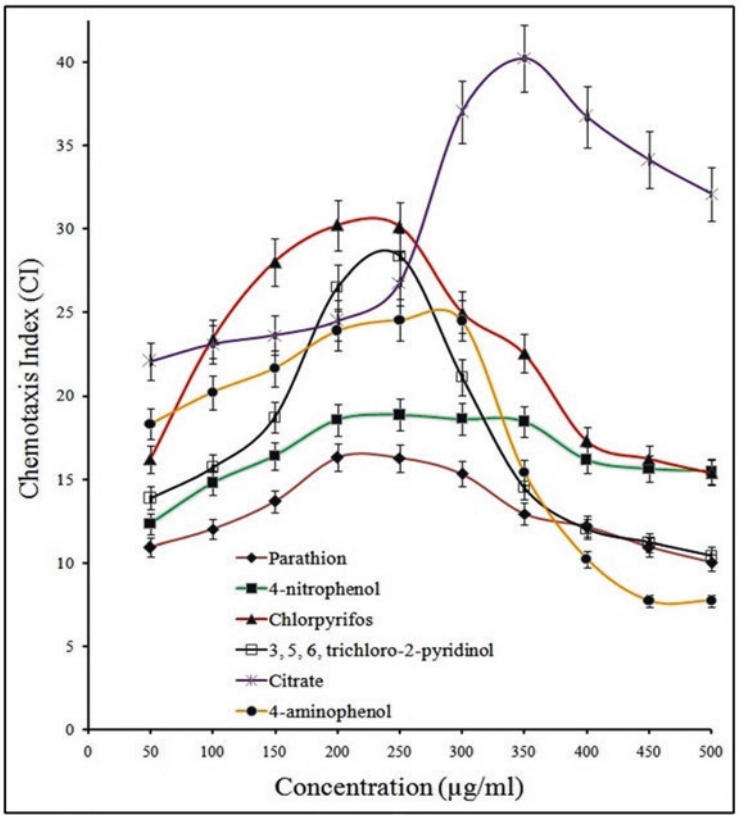


Fig. 14.9 Quantitative capillary assay. Quantitation of the chemotactic response and determination of the optimal response concentration for BUR11 chemotaxis toward different test compounds using capillary assays (Pailan and Saha 2015)

organophosphorus acid anhydrolase, *OPAA*), which are most active against organophosphate compounds with P–F or P–CN bonds (Cheng and DeFrank 2000).

14.14.1 Mechanism of Enzymatic Degradation of Insecticides

In case of insecticide degradation, three main enzymes are involved under two metabolism systems. The first metabolism system includes enzymes like hydrolases, esterases, and the mixed function oxidases (MFO), and the second system includes the glutathione *S*-transferases (GST) system (Li et al. 2007a). Several enzymes that catalyze metabolic reactions including hydrolysis, oxidation, addition of an oxygen to a double bond, oxidation of an amino group (NH_2) to a nitro group, addition of a hydroxyl group to a benzene ring, dehalogenation, reduction of a nitro group (NO_2) to an amino group, replacement of a sulfur with an oxygen, metabolism of side chains, and ring cleavage are required to degrade toxic insecticide into nontoxic intermediates (Ramakrishnan et al. 2011).

In most of the microorganisms, insecticides can be metabolized by a three-phase process. In phase I metabolism, the initial properties of parent compounds are transformed through oxidation, reduction, and hydrolysis to produce a more water-soluble and usually a less toxic product than parent. The second phase (phase II) involves conjugation of a pesticide or insecticide metabolite to a sugar or amino acid, which increases the water solubility and reduces toxicity, compared to the parent pesticide/insoluble metabolite. The third phase (phase III) involves conversion of phase II metabolites into secondary conjugates, which are also nontoxic. To carry out these processes, microorganisms like fungi and bacteria produce several intracellular or extra cellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc. to accomplish the complete mineralization of toxic insecticides (Van Eerd et al. 2003).

14.14.2 Enzymes and Gene(s) Involved in Organophosphate Compounds Degradation

The organophosphate compounds are tri-esters of phosphates and their derivatives. Therefore, the most common enzyme that might be involved in their degradation is the esterase. Esterases are also categorized as hydrolases [enzyme that hydrolyzes a broad range of aliphatic, aromatic esters and organophosphates, Park and Kamble (2001)]. Various types of hydrolases involved in the degradation of organophosphate insecticides are as follows:

14.14.2.1 Phosphotriesterase (PTE)

Till date, the most well-addressed and discussed organophosphate-degrading enzyme is phosphotriesterases (PTE; Theriot and Grunden 2011). It is a metalloenzyme that hydrolyzes a variety of toxic organophosphate compounds (mainly those that act as nerve agents). PTE was first isolated from *Pseudomonas diminuta* MG (Serdar et al. 1982) and *Flavobacterium* sp. (Sethunathan and Yoshida 1973). This enzyme shows a highly catalytic activity toward various organophosphate insecticides. The PTE was further subcategorized into three groups on the basis of insecticide it acted upon (i.e., based on substrate). These are:

A. Organophosphorus hydrolase (OPH)
B. Methyl parathion hydrolase (MPH)
C. Organophosphorus acid anhydrolase (*OPAA*)

These three are encoded by *opd*, *mpd*, and *opaA* genes, respectively.

A. Organophosphorus Hydrolase (OPH)
   Many of the enzymes known to hydrolyze organophosphorus esters are referred as organophosphorus hydrolase [OPH; EC 3.1.8.1]. OPH is the most widely studied bacterial enzyme in OP degradation, exhibiting high catalytic activity and wide range of organophosphate substrate specificity (oxon and thion) (Yang et al. 2006; Ortiz-Hernandez and Sanchez-Salinas 2010). It is a zinc-containing

homodimeric membrane protein reported from *Flavobacterium* sp. strain ATCC 27551 and *Pseudomonas diminuta* MG (Sethunathan and Yoshida 1973; Serdar et al. 1982). It can hydrolyze organophosphate compounds at a rate approaching the diffusion limits (Horne et al. 2002a). The gene (*opd*) coding for OPH enzymes has been reported to be plasmid borne. The first *opd* gene (within a 66kb plasmid, pCMS1) was reported from *Pseudomonas diminuta* (Sethunathan and Yoshida 1973; Serdar et al. 1982; Mulbry et al. 1986; Singh and Walker 2006). Similar *opd* gene has been identified from various *Pseudomonas* strain by using Southern hybridization analysis (Chaudhry et al. 1988). *Flavobacterium* sp. strain ATCC 27551 and *Pseudomonas diminuta* MG contain identical *opd* genes as well as the OPHs purified from these have identical or very similar in amino acid sequences (Serdar et al. 1982; Mulbry and Karns 1989; Siddavattam et al. 2003), but it is not clear how this has occurred as the genes are on very different plasmids (Harper et al. 1988). Omburo et al. 1992 isolated an *opd* gene encoding a 40 kDa homodimer parathion hydrolase, containing divalent zinc ions as a cofactor. Horne et al. (2002a) suggested that PTE is a 384-amino-acid protein with a molecular mass of approximately 35 kDa when it is cleaved from its signal peptide. The two native Zn^{2+} ions of this enzyme can be substituted with either Co^{2+}, Ni^{2+}, Cd^{2+}, or Mn^{2+} with/without the restoration of catalytic activity. Recent findings have shown that two metal atoms are closely associated and the water molecule that attacks the phosphoryl center is bound directly to the binuclear metal center (Benning et al. 1995; Vanhooke et al. 1996).

B. Methyl Parathion Hydrolase (MPH)

Singh (2009) reported that MPH is present in several phylogenetically unrelated bacteria and is active against several organophosphate 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 by Dong et al. (2005). MPH is a dimer in which each subunit has a mixed-hybrid, binuclear zinc center. MPH is not similar to any other PTEs, even though several PTEs can degrade methyl parathion. The MPH is coded by *mpd* gene. Molecular studies and phylogenetic analyses confirmed that *mpd* genes have evolved separately from *opd* genes. Unlike *opd* genes, most of the known *mpd* genes have been isolated from one country (China), indicating that the environment has an influence on *mpd* evolution (Singh 2009). 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 (*N*-acyl homoserine lactone) 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 (Afriat et al. 2006).

C. Organophosphorus Acid Anhydrolase (OPAA)

Another organophosphate-degrading enzyme that has received considerable attention is *OPAA* [encoded by *opaA* (organophosphorus acid anhydrolase) gene], first isolated from halophilic species *Alteromonas undina* and *Alteromonas haloplanktis* (Cheng et al. 1993, 1999). This enzyme belongs to the dipeptidase family and does not share enzyme or gene-sequence homology either with OPH or MPH. This indicates that the organophosphate-degrading function of *OPAA* might have evolved from different progenitors (Singh 2009). *OPAA*s from the species of *Alteromonas* sp. JD6.5, *Alteromonas undina*, and *Alteromonas haloplanktis* are structurally and functionally similar to each other. They share a molecular weight between 50 and 60 kDa, having an optimum pH from 7.5 to 8.5 and temperature optima ranging from 40 °C to 55 °C, and require Mn^{2+} for their maximum catalytic activity (Cheng et al. 1997). *OPAA*s are highly active and more specific to OP nerve agents than OPHs. The amino acid sequence of *OPPA* of *Alteromonas* sp. JD 6.5 shares 49% and 31% similarity with dipeptidase or prolidase and aminopeptidase of *E. coli* (Theriot and Grunden 2011).

Since the property of organophosphate degradation is gene mediated, the same can be used to develop novel strains for in situ application purpose by genetic engineering process. In most of the cases, the genes are defined to be located either in plasmids or in chromosomes (Concepcio'n et al. 2012). In this way, many authors reported organophosphate degradation property using recombinant bacterial strains (Yang et al. 2005; Xu et al. 2007). Very recently, Farivar et al. (2017) reported construction of a recombinant organophosphate-degrading *Pseudomonas plecoglossicida* strains with *opd* gene from *Flavobacterium* sp. ATCC 27551 using the pUC57 plasmid.

A thorough list of organophosphate-degrading enzymes, genes, and source microorganisms from which the enzymes were isolated so far is summarized in Table 14.3.

14.14.2.2 Other Enzymes Involved in Insecticide Degradation

Survey of literature suggested some other enzymes having organophosphate-degrading activities. These are as follows:

14.14.2.2.1 Oxidoreductase

Oxidoreductases are a broad group of enzymes that carry out transfer of electrons from one molecule (the reductant or electron donor) to another (the oxidant or electron acceptor). Many of these enzymes require additional cofactors, to act as either electron donors, electron acceptors, or both. These enzymes have applications in bioremediation. There are the enzymes that catalyze an oxidation/reduction reaction by including the molecular oxygen (O_2) as electron acceptor. In these reactions, oxygen is reduced into water (H_2O) or hydrogen peroxide (H_2O_2). The oxidases are a subclass of the oxidoreductases. These enzymes not only catalyze oxidation reduction reaction of toxic compounds but also catalyze the oxidation reaction of various pesticides, insecticides, as well as herbicides (Scott et al. 2008).

Table 14.3 List of organophosphate-degrading enzymes, genes, and their source microorganisms

Organisms	Encoding genes (accession no.)	Degrading enzyme	References
Pseudomonas diminuta	*opd* (M29593)	OPH	Serdar et al. (1982)
Flavobacterium sp.	*opd* (M22863)	OPH	Harper et al. (1988)
Pseudomonas diminuta MG,	*opd* (M20392)	Phosphodiesterase	McDaniel et al. (1988)
Flavobacterium sp. strain ATCC 27551	*opd* (M29593)	Parathion hydrolase gene	Mulbry and Karns (1989)
Flavobacterium sp. ATCC27551	*opd* (AJ421424) (M20392)	OPH	Mulbry and Karns (1989)
Escherichia coli, Bacillus cereus	ND	Phosphonatase	Chen et al. (1990)
Nocardia sp.	*adpB*	ADPase	Mulbry (1992)
Mycobacterium sp. or *Nocardia* sp. strain B-1	*opaA* (AAA25371)	–	Mulbry (1992)
Pseudomonas spp.	*glpA and B*	C-P lyase	Penaloza-Vazquez et al. (1995)
Burkholderia caryophylli	*pehA*	PEH	Dotson et al. (1996)
Alteromonas sp. JD6.5	*opaA*	OPAA	Cheng et al. (1996)
Alteromonas undina Alteromonas haloplanktis ATCC 23821	*opaA* (U29240) Prolidase gene (U56398)	OPAA-2 OPAA	Cheng et al. (1996, 1997)
Nocardioides sp. strain C190	*trzN*	*s*-triazine hydrolase	Mulbry et al. (2002)
Burkholderia sp. strain NF100	*opd/mpd*	Fenitrothion-hydrolyzing enzyme	Hayatsu et al. (2000)
Plesiomonas sp. M6	*mpd* (AF338729)	MPH	Zhongli et al. (2001)
Moraxella sp.	*oph*	OPH	Shimazu et al. (2001)
Agrobacterium radiobacter	*opdA* (AY043245)	OPDA	Horne et al. (2002a)
Pseudomonas monteilii	*hocA*	HOCA (hydrolysis of coroxon)	Horne et al. (2002b)
Flavobacterium balustinum	*opd* (AJ426431)	Parathion hydrolase	Siddavattam et al. (2003)
Flavobacterium sp. ATCC 27551	*opd* (AJ421424)	–	Siddavattam et al. (2003)
Delftia acidovorans	*pdeA gene* (AF548455)	Phosphodiesterase (PdeA)	Tehara and Keasling (2003)
Escherichia coli	*pepA*	AMPP (aminopeptidase P)	Jao et al. (2004)

(continued)

Table 14.3 (continued)

Organisms	Encoding genes (accession no.)	Degrading enzyme	References
Pseudomonas pseudoalcaligenes	*ophc2* (AJ605330)	OPHC2	Ningfeng et al. (2004)
Pseudomonas sp. WBC-3	*mpd* (AY251554)	MPH	Liu et al. (2005)
Brucella melitensis mp-7 (AY331581)	*mpd* (AY627039)	MPH	Zhang et al. (2005, 2006a, b)
Achromobacter xylosoxidans mp-2 (AY331576)	*mpd* (AY627034)	MPH	Zhang et al. (2005, 2006a, b)
Pseudaminobacter sp. mp-1 (AY331575) strain no. AF072542	*mpd* (AY627033)	MPH	Zhang et al. (2005, 2006a, b)
Pseudaminobacter salicylatoxidans (AY331575), strain no AF072542	*mpd* (AY627033)	MPH	Zhang et al. (2005)
Ochrobactrum tritici mp-3, mp-4, mp-5, mp-6 (AY331577, AY331578, AY331579, AY331580), strain no. AF508089	*mpd* (AY627035, AY627036, AY627037, AY627038)	MPH	Zhang et al. (2005)
Burkholderia sp. FDS-1 (AY550913)	*mpd2/opd* (DQ173274, AY646835)	MPH	Zhang et al. (2006a, b)
Stenotrophomonas sp. strain YC-1 (DQ537219)	*mpd* (DQ677027)	MPH	Yang et al. (2006)
Burkholderia sp. NF100	*fedA, fedB*	Fenitrothion hydrolase gene (OPH)	Tago et al. (2006)
Flavobacterium sp. MTCC 2495	*mpd* (AY766084)	OPH	Kumar et al. (2006)
Pseudomonas putida DLL-1	*mpd*	MPH	Liu et al. (2005)
Pseudomonas pseudoalcaligenes	*ophc2*	OPH	Chu et al. (2006)
Sphingomonas sp. DSP-2 (AY994060)	*mpd* (DQ356953)	MPH	Li et al. (2007a, b)
Sphingomonas sp. CDS-1	*mpd*	MPH	Jiang et al. (2007)
Burkholderia sp. JBA3	*ophB* (EF495210)	OPH	Taesung et al. (2007)
Arthrobacter sp. L1	*mpd* (EF055988)	MPH	Li et al. (2008a, b)
Pseudomonas sp. (DSP-1, DSP-3), *Sphingomonas* sp. DSP-2, *Stenotrophomonas* sp. DSP-4	*mpd*	MPH	Li et al. (2008a, b)
Pseudomonas stutzeri strain HS-D36	*mpd*	MPH	Wang et al. (2008)

(continued)

Table 14.3 (continued)

Organisms	Encoding genes (accession no.)	Degrading enzyme	References
Pseudomonas stutzeri strain HS-D36	*mpd* (EF515812)	MPH	Guo et al. (2009)
Ochrobactrum sp. Yw18	*mpd* (DQ843607)	MPH	Singh (2009)
Ochrobactrum sp. M231	*mpd* (EU596456)		Tian et al. (2010)
Stenotrophomonas sp. SMSP-1 (EU312979)	*ophc2* (EU651813)	OPHC2	Shen et al. (2010a, b)
Lactobacillus brevis (WCP902)	*opd* B	–	Islam et al. (2010)
Pseudomonas sp.	Carboxyl esterase gene	Carboxyl esterase	Goda et al. (2010)
Sphingomonas sp. JK1	*opd* (EU709764)	OPH	Kumar and D'Souza (2010)
Burkholderia cepacia	*mpd B* (DQ001540)	MPH	Ekkhunnatham et al. (2012)
Bacillus pumilus W1	*opd A*	OPH	Ali et al. (2012)
Stenotrophomonas maltophilia MHF ENV20	*mpd*	OPH	Dubey and Fulekar (2012)
Kocuria sp.	*opd*	OPH	Neti and Zakkula (2013)
Pseudomonas sp. strain YF-5	*mpd*	MPH	Liu et al. (2014)
Sphingomonas sp. strain TDK1 and *Sphingobium* sp. strain TCM1		Haloalkylphosphorus hydrolases (TDK-HAD, TCM -HAD)	Abe et al. (2014)
Pseudomonas sp. BF1-3 (KJ849233)	*ophB*	OphB	Barman et al. (2014)
Acinetobacter sp.	*AbOPH* gene	OPH	Chen et al. (2015)
Sphingomonas sp. DC-6	*dmhA*	Amidohydrolase (DmhA)	Chen et al. (2016)
Reports from fungi			
Pleurotus ostreatus Chaetomium thermophilum		Laccase	Amitai et al. (1998)
Aspergillus niger	*opd*	A-OPH	Liu et al. (2001)
Penicillium lilacinum	*opd*	OPH	Liu et al. (2004)
Cladosporium cladosporioides Hu-01	–	CHP (chlorpyrifos hydrolase)	Gao et al. (2012)

14.14.2.2.2 Mixed Function Oxidase (MFO)

In the reaction catalyzed by the MFO (EC 1.14.14.1), an atom of one molecule of oxygen is incorporated into the substrate, while the other is reduced to water. For this reason, the MFO requires nicotinamide-adenine dinucleotide phosphate (NADPH) and O_2 for its operation. It is an enzyme system comprising of two enzymes, cytochrome P450 and NADPH-cytochrome P450 reductase; both are membrane

proteins. They are also known as cytochrome P-450-dependent monooxygenase or P450 system. The genes encoding the different isozymes comprise a superfamily of over 200 genes grouped into 36 families based on their sequence similarity. Cytochrome P450 enzymes are active in the metabolism of a wide variety of xenobiotics (Khaled et al. 2012). The cytochrome P450 family is a large, well-characterized group of monooxygenase enzymes that have long been recognized for their potential in many industrial processes, particularly due to their ability to oxidize or hydroxylate substrates in an enantiospecific manner using molecular oxygen (Urlacher et al. 2004). Many cytochrome P450 enzymes have a broad substrate range and have been shown to catalyze biochemically recalcitrant reactions such as the oxidation or hydroxylation of nonactivated carbon atoms. These properties are ideal for the remediation of environmentally persistent pesticide residues. Over 200 subfamilies of P450 enzymes have been found across various prokaryotes and eukaryotes. MFOs metabolize a wide range of compounds such as OPs, carbamates, pyrethroids, DDT, inhibitors of the chitin synthesis, juvenile hormone mimics, etc. (Alzahrani 2009).

14.14.2.2.3 Glutathione *S*-Transferase (GST)

The GSTs (EC 2.5.1.18) are a group of enzymes that catalyze the conjugation of hydrophobic components with reduced glutathione. In this reaction, the thiol group of glutathione reacts with an electrophilic place in the target compound to form a conjugate which can be metabolized or excreted. GSTs are involved in many cellular physiological activities such as detoxification of endogenous and xenobiotic compounds, intracellular transport, biosynthesis of hormones, and protection against oxidative stress (Sheehan et al. 2001; Hayes et al. 2005; Oakley 2005). Broadly, GSTs are divided into four major families: (a) cytosolic GSTs, (b) mitochondrial GSTs, (c) microsomal GSTs, and (d) bacterial fosfomycin resistance proteins (Armstrong 1997; Hayes et al. 2005). A very recent report by Longkumar et al. (2014) revealed that GST was involved in dealkylation of methyl parathion (OP compound) by a bacterial strain *Acinetobacter baumannii* DS002. Unlike in other organophosphate-degrading bacterial strains, in the genome of *Acinetobacter baumannii* DS002, there is no conserved gene encoding an organophosphate-degrading enzyme. The absence of such *opd* gene and the induction of a GST-like protein in the presence of organophosphate insecticides suggested the existence of a novel organophosphate-degrading pathway in *Acinetobacter baumannii* DS002. Longkumar and his colleagues also discovered the existence of multiple *gst* genes in *Acinetobacter baumannii* DS002 and observed the expression of these *gst* genes and involvement of resulting GST enzyme in dealkylation of methyl parathion that eventually reduces toxicity of the parent compound (Longkumar et al. 2014).

14.15 Role of OPH in Phosphate Acquisition from Organophosphate Compounds

Among the PTEs, OPH (a metalloenzyme requiring Zn as cofactor) is the most well-studied and characterized enzyme as far as structural and catalytic properties are concerned (Omburo et al. 1992; Kuo and Raushel 1994). It is best studied from *Brevundimonas diminuta* (recently reclassified as *Sphingopyxis wildii*) (Parthasarathy et al. 2017a). It located in the periplasmic space as multi-protein complexes, and it interacts with several systems like phosphate-specific transport (Pst) system, ABC transporters, and efflux pump AcrZ/TolC. It is reported to anchor to periplasmic face of the inner membrane through a diacylglycerol linked to the invariant cysteine residue. This enzyme also contains a signal peptide with a conserved cysteine residue at the junction of its cleavage site. The signal peptide contains a characteristic Tat motif which is common for proteins that are translocated across the inner membrane in a prefolded state (Parthasarathy et al. 2016). Based on bioinformatic analyses, the c-terminal of OPH has been predicted to be in the cytoplasmic side (Parthasarathy et al. 2017a, b). Apart from triesterase activity, this enzyme has also been shown to possess lactonase activity. Due to that, OPH has been hypothesized to have evolved from lactonases (whose function s for quorum quenching) for the uptake of phosphate from the surrounding environment (Afriat-Jurnou et al. 2012).

It seems PTEs located in the periplasmic space converts organic organophosphate (that enters into the periplasmic space through after crossing the outer membrane) into phosphodiesters which ultimately gets converted into inorganic phosphate by the combined action of phosphodiesterase and phosphatase. OPH has been postulated to be involved in phosphate acquisition from organophosphate compounds through its interaction with components of the outer membrane (such as ABC-type transporters, TolC, etc.) known to be involved in phosphate transport in bacterial cells (Parthasarathy et al. 2016). Although these studies provide some idea toward the utilization of phosphotriesterases as the sole source of phosphate (at least in *Sphingopyxis wildii*), there is a huge lacuna as far as the transport processes operate in this organism.

14.16 Concluding Remarks

In spite of advances in cultivation methods, the total number of culturable microbes recoverable from any environmental niche is very low compared to what exists naturally. The wealth of information, regarding organophosphate metabolism that we have gained from existing diversity, is only the tip of the iceberg as we are far from knowing the exact boundaries of microbial diversity on earth. Moreover, a lot more studies has to be carried out with anaerobic microbes and their metabolic studies with respect to organophosphate compounds. Compared to aerobic metabolism, during anaerobic process more substrates are needed to be metabolized to provide equitable amount of ATP, and prospect of cleaning xenobiotic substrate is more through anaerobic degradation than aerobic degradation. Thus, more systematic

studies for exploration of organophosphate biodegradation by anaerobic microorganisms should be made. This will not only increase our bio-resource in terms of novel microbes, gene, and enzyme pool for biotechnological aspect of the environmental cleanup process but also may lead to complete understanding of the overall degradation process and their links with other ecological processes on our planet earth. Microbial metabolism of organophosphate compounds in the environment is a complex, less understood process that depends upon the community diversity of the microflora residing in the habitat, energy, and nutrient flow as well as stress response metabolism of microbes. Unfortunately due to lack of our understanding toward holistic system wide understanding of complex interaction between degrading microbes, their genes, enzymes and multivariate environmental factors along with the complex microbial community (de Lorenzo 2008). Very recently, in order to understand the relationships in holistic manner, metagenomic approach was undertaken and it has shown promising results (Jeffries et al. 2018). The results of such approach highlighted the value holistic system-wide metagenomic approaches as a tool to predict microbial degradation in the context of the ecology of contaminated habitats. As pointed earlier, understanding the adaptation strategies taken by a microorganism to tolerate organophosphate toxicity and maintain cellular homeostasis will help us to understand the metabolism in a better way. Another huge lacuna is the process of signaling which facilitates the degradation process. Future studies will incorporate similar approaches to enrich our understanding the relationship.

Acknowledgments Authors are grateful to SERB, New Delhi, for providing fund to carry out work on organophosphate degradation and to the University of Burdwan, Burdwan, West Bengal.

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