

Gift of Microbial Life: Engineering Earth's Habitability

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Our galaxy has more than 100 billion stars and about 300 planetary systems which are similar to the solar system. The recent measurements from NASA's Kepler space observatory suggest that about two billion planets may have liquid water on their surfaces, like Earth, and orbit around their parent stars [1]. These planets may support life as Earth does. Life appeared about 3.8 billion year ago, which was evident from the stratigraphic records in Earth. Early earth primarily consisted of archaea and bacteria, the unique domains of life. These microscopic organisms harvest energy through varied chemical reactions and continue to be the fundamental ecological circuitry of life [2]. Since the Precambrian earth, these organisms play vital roles in the chemical cycles. As of now, half of the biomass in Earth belongs to these microorganisms [3]. Complex life forms appeared only about 540 million years ago, i.e. during the Cambrian Explosion. But, many biological species have become extinct, with the rate of extinction being 0.1 to 1 per million species-years. The diverse life-forms had also endured ice ages, frequent climate change events and even mass extinctions. The notable among them are archaea, eubacteria and eukaryotic algae, which are contributing immensely to make Earth habitable.

Humans have come lately, in the geologic time scale of Earth. The modern human species have evolved about 200,000 years ago in Africa, arrived in India about 70,000 years ago and in Australia about 51,000 years ago, settled in Europe about 40,000 years ago and migrated to North America around 10,000 years ago. Human population reached the first billion in 1804 and it took 123 years to add the next billion. But it took only 12 years from five billion in 1987 to become six billion. The world's population is 7.147 billion as of now. Both the ever-increasing human populations and their activities have impacted the habitability of Earth. Nearly 50 percent of the Earth's natural habitats have been altered for human agricultural and industrial activities. The mass extinctions of mammoths and other large mammals had occurred due to hunting by humans. Earlier predictions on the limitations of Earth to feed people was proved wrong with new innovative technologies for growing higher yields of food, fuel and fibre. Both the demands of industrialization and human lifestyle rely heavily on the energy sources. In addition, the human population pressure on land, water and energy sources have led to the generation of contaminants and waste and consequently pollution. Many forms of pollution now affect the environment at the local, global and even planetary levels.

The modern humans are exposed to chemical substances, both natural and manmade, from the cradle to the grave. Certain manmade chemical substances threaten human life or have harmful effects on respiratory, reproductive or nervous systems of other complex life-forms too. These chemical substances are synthesized for their use in public health, agriculture, industries and during wars. One among them is the class of organophosphorus (OP) compounds which include insecticides, herbicides, antihelminthics, ophthalmic agents, plasticizers, petroleum additives, and nerve gases [4]. The first description of the synthesis of an OP compound (tetraethyl pyrophosphate) was made by Philip de Clermout in 1854. Organophosphates as insecticides became popular after the World War II though some of them were developed as chemical warfare agents (e.g., soman, sarin, and tabun) before. Even now, the OP compounds are stored as chemical warfare agents worldwide (about

30,000 tonnes in the United States and 200,000 tonnes in the rest of the world) [5]. Synthetic OP compounds, which are more than 100 now, account for 35% of global pesticide usage. Every year, about 3 million is poisoned and 300,000 humans die due to poisoning by OPs and no such systematic records on the poisoning and death are available for other life-forms.

The OP compounds inhibit acetylcholine esterase of the central and peripheral nervous system of vertebrates; serine hydroxyl moiety is phosphorylated and hydrolysis of acetylcholine is prevented. The accumulation of acetylcholine at the nerve synapses disrupts the propagation of nerve impulses [6]. Interestingly, there is no adverse effect of OPs on bacteria since they do not possess acetylcholine esterase. The first bacterium capable of degrading OP compound was isolated from a paddy field in the Philippines and identified as *Flavobacterium* sp. ATCC 27551 in 1973 [7]. Several bacteria are now known to use OPs as a source of carbon, phosphorus or nitrogen or by co-metabolism [5]. Notable among them are *Brevundimonas diminuta* and *Alteromonas haloplanktis* from the United States, *Pseudomonas* sp. WBC-3 from China, and *Agrobacterium radiobacter* and *Enterobacter* sp. from Australia. Besides microorganisms, animals and plants also possess phosphotriesterases (PTE) capable of degrading OPs. The molecular studies on Organophosphorus Hydrolase (OPH), one of the bacterial phosphotriesterases, suggest that this enzyme has evolved recently, only during the past 70 years [5,7]. Methyl parathion hydrolase and organophosphorus acid anhydrolase are other two phosphotriesterases reported in several OP-degrading bacteria [8]. Instead of OPH, *Agrobacterium radiobacter* has an enzyme variant, known as OP-degrading enzyme (OPDA).

The genome sequences of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Deinococcus radiodurans*, *Desulfatibacillum alkenivorans*, *Geobacillus* sp., *Thermoanaerobacter* sp. X514, *Escherichia coli* (*yhfV*) and *Sulfolobus acidocaldarius* suggest that they have PTE gene sequences similar to the OP hydrolase (OPH) encoding *opd* (organophosphorus degrading) gene [5]. *Pseudomonas* sp. WBC-3 was found to have methyl parathion hydrolase. Interestingly, the genome sequences of *Methylobium petroleiphilum*, *Azoarcus* sp., *Leptothrix cholodnii*, *Chromobacterium violaceum* and *Sinorhizobium meliloti* 1021 possess the *mpd* (methyl parathion degrading) gene homologues. *Alteromonas undina* and *Alteromonas haloplanktis* have organophosphorus acid anhydrolase, encoded by *opaA* (organophosphorus acid anhydrolase). There are many microorganisms capable of degrading OPs, yet to be isolated and identified and their

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enzymes characterized. The distribution of genes of organophosphorus hydrolase among bacteria suggests the horizontal gene transfer involving mobile genetic elements or transposons. Interestingly, the OP-degrading operons are similar to the antibiotic-resistance operons [5]. The evolutionary and genetic-transfer mechanisms and diverse metabolic potential yet again indicate the tenacity and gift of microbial life in Earth.

OPs belong to the toxicity class I (highly toxic) or class II (moderately toxic) of the US-EPA classification. Although less persistent than other classes of chemical compounds, high levels of toxicity and widespread usage of OPs poses greater risks to humans and environment. The current approaches of their determination in the environment require extraction, pre-concentration and analysis using various chromatographic techniques. High costs and skill requirement make these analyses possible only in the sophisticated laboratories. Sensors with enzymes or microorganisms can make the on-line and on-site measurements possible with low cost. The OP-degrading microorganisms are made use of as biosensors for detecting and determining the concentrations of these chemical substances in the environment. The bacteria-based sensors are coupled to the electrochemical transducers. To overcome the limitations of diffusional constraints of cell wall, *Moraxella* sp., *Pseudomonas putida* or *Escherichia coli* are genetically engineered with surface-expressed OPH [9-11]. The recombinant p-nitrophenol degrading/oxidizing bacteria endowed with OPH activity is also employed in sensors based on Clark dissolved oxygen electrode, in order to measure oxygen consumption and then to correlate with the concentration of OP. Hybrid biosensor is available for direct determination of OP, with OPH enzyme for hydrolysis initially and the subsequent oxidation by *Arthrobacter* sp. JS443 for oxidation to carbon dioxide. Integration of immobilized *Arthrobacter globiformis* and free acetyl cholinesterase with a Clark type oxygen probe transducer led to the development of a biosensor with a response time of 200 s and a limit of detection level to 1 nmol dm⁻³. This biosensor is being used to determine chlorofos even in the contaminated milk [12].

The biosensors with enzymes as recognition elements for OPs are generally based on potentiometric approach to measure local pH change and amperometric approach for electroactive enzyme product measurements. Faster response and higher sensitivity and accuracy make the amperometric sensors preferred to the potentiometric sensors. The combination of these methods has given a better biosensor. The ability of OPs to inhibit acyl cholinesterases and phosphatases has been applied in the electrochemical sensors. In the first generation amperometric sensors, the sensor response, depending on the substrate concentration and the enzyme activity, is derived from the current of H₂O₂ oxidation or O₂ reduction. Since there are interferences by glutathione, ascorbates and urates, and the fluctuations of oxygen concentration alters the output signal, the synthetic substrates such as thiocholine and indoxylacetate esters in the second generation sensors. The organophosphorus hydrolase based electrochemical sensors make the direct analysis of paraoxon, parathion, coumaphos, diazinon, dursban, methyl parathion, sarin, soman, tabun and many other OPs possible. Further efforts to genetically engineer the biological recognition elements with improved selectivity and modify nanomaterial transducers for sensitive monitoring at low electrode potential can aid in the detection and determination of OPs in the environment.

The OP-degrading bacteria and their enzymes have an immense potential in the bioremediation efforts. In the United States, the consortium of microorganisms that is used to develop a filter bioreactor

degrades 15,000 litres of coumaphos in a single batch, from waste emanated from the cattle-tick eradication programme [13]. The Australian 'Landguard enzyme technology' applies the carrier-based OPH enzyme for treating the sheep-dip waste. Catalytic activity and efficiency of microbial OP degrading enzymes can be improved by gene and protein engineering. Transgenic plants with *opd* [14] may be used extensively for degrading OPs in the future. Nevertheless, extensive production, excessive use and frequent spillage of OPs will defy the metabolic capabilities of these microorganisms and continue to pose high risks to all other life-forms. The future of Earth is always moulded by human activities carried out today. The challenge to human ingenuity now is to recognise the balance between the social and economic benefits of manmade chemical substances or energy intensive technologies and the risks associated with them. Persistent pollution and unexpected influences of chemical substances including OPs and the engineered technologies on the life-forms in general and on microbial life in particular justify the human efforts to search for new habitable planets.

References

- Petigura EA, Howard AW, Marcy GW (2013) Prevalence of Earth-size planets orbiting Sun-like stars. *Proc Natl Acad Sci U S A* 110: 19273-19278.
- Knoll A (2003) Life on a young planet: The first three billion years of evolution on Earth. Princeton University Press, New Jersey, USA.
- Curtis TP, Sloan WT, Scannell JW (2002) Estimating prokaryotic diversity and its limits. *Proc Natl Acad Sci U S A* 99: 10494-10499.
- Edmundson RS (1988) Dictionary of organophosphorus compounds. Chapman & Hall, London, UK.
- Singh BK (2009) Organophosphorus-degrading bacteria: ecology and industrial applications. *Nat Rev Microbiol* 7: 156-164.
- Matsumura F (1980) Toxicology of insecticides. Plenum Press, New York, USA.
- Sethunathan N, Yoshida T (1973) A Flavobacterium sp. that degrades diazinon and parathion. *Can J Microbiol* 19: 873-875.
- Yang H, Carr PD, McLoughlin SY, Liu JW, Horne I, et al. (2003) Evolution of an organophosphate-degrading enzyme: a comparison of natural and directed evolution. *Protein Eng* 16: 135-145.
- Mulchandani A, Chen W, Mulchandani P, Wang J, Rogers KR (2001) Biosensors for direct determination of organophosphate pesticides. *Biosens Bioelectron* 16: 225-230.
- Mulchandani P, Chen W, Mulchandani A (2006) Microbial biosensor for direct determination of nitrophenyl-substituted organophosphate nerve agents using genetically engineered *Moraxella* sp. *Anal Chim Acta* 568: 217-221.
- Lei Y, Mulchandani P, Chen W, Mulchandani A (2006) Biosensor for direct determination of fenitrothion and EPN using recombinant *Pseudomonas putida* JS444 with surface expressed organophosphorus hydrolase. 1. Modified Clark oxygen electrode. *Sensors* 6: 466-472.
- Stoytcheva M, Zlatev R, Velkova Z, Valdez B, Ovalle M, Petkov L (2009) Hybrid electrochemical biosensor for organophosphorus pesticides quantification. *Electrochim Acta* 54: 1721-1727.
- Mulbry W, Ahrens E, Karns J (1998) Use of a field-scale biofilter for the degradation of the organophosphate insecticide coumaphos in cattle dip wastes. *Pestic Sci* 52: 268-274.
- Pinkerton TS, Howard JA, Wild JR (2008) Genetically engineered resistance to organophosphate herbicides provides a new scoreable and selectable marker system for transgenic plants. *Mol Breed* 21: 27-36.