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A proteomic approach to pesticide stress management in microalgae

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Abstract

Pesticides are one of the major sources of water pollution.Some pesticides are persistent organic pollutants and contribute to soil contamination. Microalgae are primary producers of ecosystem which are rich source of compounds having industrial values. Proteomic studies have highlighted the various mechanisms adapted by the microalgae in combating the pesticide stress. This review highlights the proteomics approach using Two-dimensional gel electrophoresis and MALDI-TOF for analysing the protein profile of microalgae.

Keywords: Pesticide, Microalgae, Two-dimensional gel electrophoresis, Mass Spectrometry

Introduction

Pesticides are used to control weeds, to repel or kill pests (insects, mites, nematodes etc.) in paddy fields, crops.They are used for controlling vector-borne diseases for health care of humans and animals. It includes insecticides, herbicides, fungicides, rodenticides, molluscicides etc. used to control pests (Damalas, 2009; Agrawal *et al.,* 2010; EPA 2012). Application of pesticides enhances the crop productivity, however under constant exposure to high concentration of chemicals, some pests have developed resistance to the pesticides which affects the non-target organisms such as microalgae and cyanobacteria and other aquatic organisms (Damalas, 2009). These pesticides enter the aquatic environment in different ways such as runoff, aerial drift and spill and affect non-target biota (Damala, 2009; Prado *et al.,* 2009). Pesticide drift occurs when pesticide particles suspended in the air are carried away by wind to other areas, potentially contaminating them. Pesticides heavily contribute to soil and sediment contamination (Lee *et al.,* 2011). In this way, pesticides enter into aquatic ecosystems from agricultural runoff or leaching and, as a consequence, have become some of the most frequent organic pollutants in aquatic ecosystems (Herrero *et al.,* 2009).

Pesticides are classified as (i) Organophosphates, (ii) Organochlorines, (iii) Carbamates and (iv) Pyrethroids. Organophosphate pesticides are the ester forms of phosphoric acid and are most widely used insecticide. Their main mechanism of action

is blocking the enzyme acetyl cholinesterase causing nervous and respiratory damages that result in the insect's death, but they are also hazardous to humans (Buchanan *et al.,* 2001). Some organophosphate pesticides are Chlorpyrifos, Malathion, Profenofos, Glyphosate, Quinalphos, Monocrotophos, Parathion, etc. The widespread use of these pesticides in agriculture has led to serious environmental pollution (Beard *et al.,* 2003). Organochlorine pesticides are derived from chlorinated hydrocarbonswhich alter the movement of ions across nerve membrane of the insects. They contribute in many acute nervous and chronic illnesses. Some chlorinated hydrocarbon pesticides are lipophilic i.e. they are easily accumulated and dissolve in fats and animal tissues and are not excreted, leading to adverse health effects in animals and human beings. Examples are DDT (Dichloro diphenyl trichloroethane) and PCBs (Polychlorobiphenyls) (Clary *et al.,* 2003; Rogan *et al.,* 2005).Carbamateare esters of carbamic acids. They are insecticide that work by inactivating the enzyme cholinesterase and alter the signal transduction in synapse. Example: Aldicarb, carbofuran, carbaryl, ethienocarb, fenobucarb, oxamyl and methomyl etc.Carbofuran is one of the most toxic carbamate pesticides. It is used to control insects in a wide variety of field crops, including potatoes, corn and soybeans. Pyrethroid pesticides are potent neuron poisons, endocrine disruptors and cause paralysis. Pyrethroids are synthetic version of pyrethrin a natural insecticidewhich is extracted from plant *Chrysanthemum.* They work by blocking the sodium ion channel in the insects. These are most commonly used insecticides introduced after organophosphates and organochlorine pesticides.Someexamples are- deltamethrin, cypermethrin, alpha-cypermethrin(Dorman *et al.,* 1991; Damala, 2009).

Pesticides applied in the field are extremely detrimental to cyanobacteria(Agrawal *et al.,* 2013).Cyanobacteria (Blue green algae) are the largestand most widely distributed photosynthetic diazotrophs contributingto the carbon and nitrogen economy of paddy field soils and aquatic environment (Agrawal *et al.,* 2013).It has been reported that the interaction between *Anabaena (*cyanobacterium*) and Azolla (*water fern*)* have great importance in paddy fields, where nitrogen is frequently a limiting nutrient (Chellappa *et al.,* 2004).Some of the prominent rice field cyanobacteria are *Anabaena, Aulosira, Cylindrospermum, Gloeotrichia, Nostoc, Rivularia*and others (Venkataraman *et al.,*1975). Greenmicroalgaeare photosynthetic eukaryoteswhose primary function is to produce starch or carbohydrates. Inspite of their simple physiology they can survive in harsh environmental conditions (Raven and Falkowski, 1999). The green microalgae and higher plants share metabolic pathways, which makes it susceptible to the action of herbicides (Lipok *et al.,* 2010; Tohge *et al.,* 2013). They are the primary producers in the aquatic environment and are rich source of compounds which have medicinal and industrial value (Borowitzka *et al.,* 2016). Pesticides which enter the aquatic environment has negative impact on these microalgae which have adverse effects on the whole aquatic ecosystem. Some microalgae are reported to accumulate and bioconcentrate, and biotransform xenobiotics. Microalgae are excellent biomonitors of pollution because of their sensitive nature to various contaminants. Studying the effects of these contaminants gives early warning of a polluting situation (Torres *et al.,* 2008). Different pollutants

induce oxidative stress in the microalgae. Some microalgae are able to tolerate such contaminants in a higher concentration. Omics approaches which involve genomics, proteomics, transcriptomics and metabolomics are applied to investigate the underlying principles of these organisms tolerating the contaminant. In this review we discuss about a proteomic approach in brief.

Proteomics Approach: Two Dimensional Gel Electrophoresis

Proteomics provides an effective way to identify and quantify proteome of an organism. It gives comprehensive insight into proteome changes in an organism upon stresses and reveals the mode of action, and identifies potential biomarkers (Tan *et al.,* 2012; Qiao *et al.,* 2012). Two dimensional gel electrophoresis coupled with mass spectrometry is an acclaimed tool for qualitative and quantitative assessment of proteomic changes (Zhang *et al.,* 2014). iTRAQ (isobaric tags for relative and absolute quantitation) is also a widely utilized quantitative proteomics approach to obtain relative quantification information of peptides in up to eight samples simultaneously.This method uses several isobaric tags to label peptides from various samples followedby liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis to performprotein identification and quantitation. Since iTRAQ can quantify proteins from up toeight samples simultaneously, it's an appropriate method to investigate altered proteinexpression level from control and treated samples and determine the effect ofexposure or treatment time on the organism (Tan *et al.,* 2012; Qiao *et al.,* 2012). Recently, alteration in the algal protein expressions induced by cypermethrin exposure has been studied by iTRAQ where photosynthetic proteins, stress responsive proteins and carbohydrate metabolism were found to alter (Gao *et al.,* 2016). However,two dimensional gel electrophoresis is more affordable and employed by many researchers than iTRAQ technique. Two dimensional gel electrophoresis coupled to mass spectrometry isan objective for the high quality separation of proteins. Proteins extracted from the control and pesticide stressed samples are subject to the 2-D gel electrophoresis.Two dimensional gel electrophoresis consist of first dimension i.e. isoelectric focussing and second dimension i.e. SDS-PAGE. In isoelectric focussing the isolated and purified protein sample is separated based on Isoelectric point in IPG strip in an isoelectric focussing chamber, and in second dimension this protein on the IPG strip is separated by SDS-PAGE where it gets separated based on their molecular mass. After this the gel is stained with Coomassie brilliant blue stain. Analysis of the gel image is carried out to check the differences in the spot intensity of both the control and treated protein samples. Following this, the differentially expressed protein spots are excised and in gel trypsin digestion is done. The digested peptides are further analysed by MALDI-TOF MS/MS. The results from the MALDI-TOF MS/MS analysis are further searched by MASCOT database software for the identification of the proteins.

Previous studies have reported the upregulation of various stress related proteins in microalgae such as oxidoreductase, photosynthesis related proteins, transporter proteins, carbohydrate metabolism, stress proteins and others to tolerate the stress (Gau *et al.,* 2016; Ismaiel *et al.,* 2018).Some cyanobacteria are reported to mineralize organophosphorus

compounds and use them as nutrient source. Phosphate specific transporters (Pst) and alkaline phosphatases were identified by proteomic approach to play an important role in utilization of these compounds as phosphate source (Tiwari *et al.,* 2014). Similarly, in another study *Nostoc muscorum* was found to be more tolerant to different concentrations of malathion than *Anabaena oryzae* and *Spirulina platensis* due to the presence of the enzymes which can hydrolyse and utilize this organophosphorus compound as nutrient source (Ibrahim *et al.,* 2014).Genes encoding a number of organic pollutant degrading enzymes are also reported. The gene cbaA that encodes enzyme 3-chlorobenzoate-3, 4-dioxygenase is upregulated by 1.26 to 8.9- fold during PCB (Polychlorobiphenyl) degradation by *Anabaena* PD-1. These proteins were identified using Two dimensional gel electrophoresis (2-DE) coupled with matrix-assisted laser desorption/ ionization time of flight mass spectrometry (MALDI-TOF MS). These findings reveal the resistance and adaptation of cyanobacterium to the presence of PCBs (Zhang *et al.,* 2014). Some cyanobacteria and green algae are reported to degrade pesticides into less toxic metabolites. As example, *Spirulina platensis* is found capable of degrading chlorpyrifos to its less toxic primary metabolite 3,5,6-trichloro-2-pyridinol (TCP) in laboratory cultures by HPLC analysis (Thengodkar *et al.,* 2010).Green algae such as *Scenedesmus* sp. MM1, *Scenedesmus* sp. MM2, *Chlamydomonas* sp*., Chlorella* sp*., Stichoccus* sp*.,* and five cyanobacteria *i.e. Nostoc* sp. MM1*, Nostoc* sp.MM2*, Nostoc* sp.MM3*, Nostoc muscorum* and *Anabaena* sp. were reported to biodegrade organophosphorus pesticide fenamiphos intofenamiphos sulfoxide (FSO), which is further hydrolyzed to fenamiphos sulfoxide phenol (FSOP) analysed by using HPLC (Caceres *et al.,* 2008). *Synechocystis* sp. strain PUPCCC 64 can tolerate chlorpyrifos pesticide up to 15 mg/L. GC-MS analysis showed this organism can degrade chlorpyrifos into less toxic products as 3,5,6-trichloro-2-pyridinol (TCP) (Singh *et al.,* 2011). Studying these organisms by proteomic approach will further reveal the proteins involved in the various degrading pathways. Specific gene characterized fromtolerant species of cyanobacteria was further explored for its potential by cloning in other bacteria. As reported, in *Anabaena* sp. PCC7120 phytochelatin synthase (pcs) is involved in the synthesis of phytochelatins (PCs) which plays role in heavy metal detoxification. The effect of an extensively used rice field herbicide butachlor was also studied on three *Anabaena* species e.g. *Anabaena* sp. PCC 7120, *Anabaena doliolum* and *Anabaena* L31. 75 differentially expressed proteins from each *Anabaena* sp. were reported which are related to photosynthesis, carbon, nitrogen and protein metabolism, redox homeostasis, and signal transduction. Early accumulated proteins involved in photosynthesis (atpA, atpB), carbon metabolism (glpx, fba and prk), protein folding (groEL, PPIase), regulation (orrA) and late accumulated proteins are involved in *Anabaena* L31 and in *Anabaena* sp. PCC 7120 to tolerate prolonged exposure to butachlor (Agrawal *et al.,* 2014).

Recent study also reported the tolerance strategy of cyanobacterium *Fischerella* sp. under methyl parathion (MP) stress which is investigatedthrough proteomics analysis using 2-DE technique coupled with MALDI- TOF MS/MS. This cyanobacterium treated with MP for 2 and 8 days showed differential expressions of proteinsrelated to

photosynthesis, energy and protein metabolism, redox homeostasis, signal transduction and cellular defence compared to the control (Tiwari *et al.,* 2018).

Conclusion

Proteomics approach has highlighted the comprehensive view of various mechanisms adapted by the microalgae in combating the pesticide stress. These microalgae further are a rich source of various compounds which have industrial value. So studying the microalgae in their proteome level will helpto identify stress responsive proteins which can further be characterisedas potential biomarker for the future investigation of pesticide exposed plants and microalgae.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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