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Dr. M. Zahid Rizvi

REVIEW ARTICLE

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Molecular and Analytical Advancements in Environmental Biotechnology: A Synergistic Approach

Mohd. Zahid Rizvi and Arshad H. Rizvi
Department of Botany, Shia P.G. College, Lucknow, U.P., India

ABSTRACT

Molecular techniques are important to enhance practice in various fields of biology touching basic understanding and exploring diversity and modulation of biomolecules such as nucleotides, proteins and lipids. It is really tricky and almost impossible to know about the stress response in environment. To make stress response detection easy, hybridization of nucleic acids, such as DNA and RNA, proteins, and lipid content play useful role as they deviates their fate and functions from normal to abnormal one which can be used as a biomarkers. Article insisted to give glimpse on various molecular techniques that helps to know about environmental hurdles, maintenance, and responses. Hybridization of DNA or RNA, Mass spectrometer analysis with combinations of analytical techniques, chromatography and in silico databases are discussed briefly to have an idea about the advancement and application of molecular techniques.

Keywords: Molecular techniques, Environmental biotechnology, PCR, Nucleic acid hybridization, Mass spectrometer and DGGE.

INTRODUCTION

Biotechnology is explored to make commercial and beneficial applications of it using reactions occurring in living forms(Płaza et al., 2001). Environmental biotechnology deals with the destruction, removal, and conversion of many hazardous materials in the environment to keep environment secure and safe (Ivanov and Hung, 2010). Environmental health is an additional responsibility of the growing field of biotechnology and molecular techniques are the tools by which one can solve issues of environmental biotechnology. There are several aspects to which molecular techniques are useful, like finding contamination in water and soil, characterizing the biochemical processes of livestock diversification and similarity, building biosensors based on the physiological events happening in the cell, etc. The molecular techniques deal with identification, interaction, mechanism for responses of nucleic acid, protein, and lipid composition under normal and stressed situations (Płaza et al., 2001).

Molecular and analytical techniques have been in use since many years like PCR, RT PCR, Recombinant DNA technology, DGGE etc(Płaza et al., 2001). To validate all reaction happening in healthy, stressed, or dead materials affected by compromised environmental health is the major responsibility of environmental biotechnology and molecular techniques are promising step to solve the clue. In this review, the various molecular techniques are discussed with their applications in environmental biotechnology.

MOLECULAR TECHNIQUES USED IN ENVIRONMENTAL BIOTECHNOLOGY

Molecular techniques are important to enhance practice in various field of biology touching basic understanding and exploring diversity and modulation of biomolecules such as nucleotides, proteins and lipids(Grahl-Nielsen and Barnung, 1985; Ivanov and Hung, 2010). Parameters that causes environmental stress such as a temperature, salt, chemicals have an impact on environment and that drastically affects the live stocks in the ecosystem. To make stress response detection easy, hybridization of nucleic acids, proteins, and lipid content play useful role as they deviates their fate and functions from normal to abnormal one which can be used as a biomarkers. Any changes to the environmental parameters makes changes in the rhythm of these macro molecules suggesting their exploration at vary efficient and accurate level in the future (Wu et al., 2003; Parrish, 2013; Mosier et al., 2015; Zhou et al., 2015;) Analyzing pattern of the whole genomics, proteomics, entire or sub population of different RNA, and lipid profiles are only analyzed using various molecular tools such as next generation sequencing, pyro sequencing, mass spectrometer. These techniques can be complexed with various analytical method, HPLC, GC, GC-MS (gas chromatography-mass spectrometry), GC-IRMS (gas chromatography-isotope ratio mass spectrometer) and HPLC-IRMS (high performance liquid chromatography- isotope ratio mass spectrometer) are high through put analytical method for molecules such as nucleic acids (including DNA or RNA), proteins and peptides and fatty acid diversification in the different community (Parrish, 2013). Various analytes and the advanced molecular tools to get clue about them are listed in Table 1.

Table 1. A diverse group of analytes and molecular techniques.

Biomolecule	Molecular tools and techniques used	References
Nucleotide	PCR, FISH, 16s RNA probing, 18s RNA probing.	Antón et al., 1999; MacNaughton et al., 1999; Eickhorst and Tippkötter, 2008;Franke-Whittle et al., 2008; Decelle et al., 2014; Zhou et al., 2015
Protein	1D & 2D gel electrophoresis, 2D-DGGE, Mass spectrometer (MS), GC-MS, Tandem mass tag (TMT) and isobaric TMT.	Askari et al., 2006; Wang et al., 2014; Kim et al., 2015; Mosier et al., 2015
Lipid (phospholipid and fatty acid)	Gas chromatography, HPLC, HPLC-IRMS, GC-IRMS, Chromared thin layer chromatography, and Capillary gas chromatography	Di Pasqua et al., 2006; Zhang and Rock 2008; Parrish, 2013

The community of the organism that shows variation in these molecules as a consequence of the environmental stress (Hou et al., 2016). Analysis has to be we documented in case of its retrieval in the future and made a databases which is only possible with the help of collaborating molecular markers detected by molecular tools with the bio informatics like marine genomics database. Molecular tools and the combinations of various fields in biology helps to build better environment with accurate detection of stress and responses (Kim et al., 2015).

MOLECULAR TECHNIQUES THAT SOLVES NUCLEIC ACID PUZZLES

Not all diversity of microorganism present are identifiable (Amann et al., 1995; Zhou et al., 2015). Nucleic acids like DNA and RNA present are the most important molecules to detect surreptitious events in environment. Method that employs 5s, 16s and 23s RNA detection from mixed community based on gel electrophoresis helps primary screening for microorganisms limiting use of this technique with smaller RNA polymer such as 5s as it may not possess diversity and accuracy in separation on gel electrophoresis (Amann et al., 1995). In case of protist, diversity in 18s sequence was used to identify (Decelle et al., 2014).PCR of a specific DNA sequence acting as gene is an old method to identify the phylogenetic closeness is of great use (Amann et al., 1995; Zhou et al. , 2015). Next generation sequencing is the most advanced technique complementing many of the PCR limitations (Zhou et al., 2015). PCR amplification of the unique genes and expression profile provides an idea to the meta-genomics and functionality(Amann et al., 1995; Zhou et al. , 2015).

FISH-fluorescence *in situ* hybridization where probes bind to either DNA or RNA and helps in detection of unique community or community with altered nucleotide make up is one of the useful method (Lenaerts et al., 2007). EUB 338 and NON338 are an example of probes against 16s RNA (Eickhorst and Tippkötter, 2008). Prokaryotes residing in high salt habitats are studied using probes against 16s RNA (Antón et al., 1999). MacNaughton et al. (1999), have reported that microbes surviving at decontaminating sites were checked against 16s RNA DGGE profile which indicates the importance of nucleic acid to resolve ambiguity amongst microbe community. Figure 1 represents nucleotide probing for DNA and c-DNA along with its application in identification of microbial community. Different types of nucleic acid arrays help in identification of the microbial community like PhyloChip has an array that helps identifying various taxa of community of gut flora (Paliy et al., 2009).COMPOChip helps identifying compost degrading microbial community involving probe labelled with Cy5 against 16S rRNA(Franke-Whittle et al., 2008), and SRP PhyloChip helps identifying sulphur reducing microbial community (Zhou et al., 2015). Microbial source tracking (MST) array helps identifying fecal contamination microbial community (Li et al., 2016), amoA (ammonia monooxygenase) array helps identifying community capable of oxidizing ammonia (Abell et al., 2012). Techniques that uses such kind of array has prior information about nucleotide sequences and so called is closed format (Zhou et al., 2015).

LIPID CONTENT REVEALS ENVIRONMENTAL STRESS

As all boundaries of the cell in prokaryotes or eukaryotes are made up of a phospholipid layers, one can use the stress responsiveness or sensitivity of phospholipid and fatty acid composition of cellular membranes to decipher environmental stress like temperature, pollution etc(Grahl-Nielsen and Barnung, 1985).

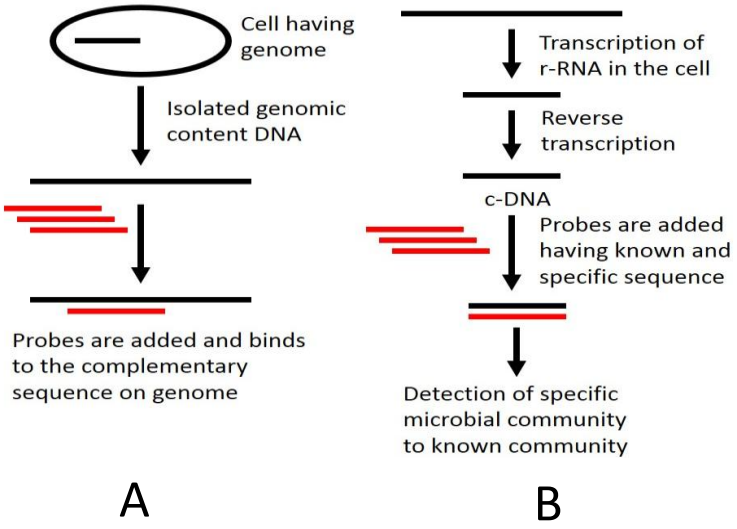

Figure 1: Nucleotide probing and application in identification of microbial community. (A) Probes are shown for DNA (Left), (B) Probes for c-DNA reverse transcribed from r-RNA (Right).

As lipids are found at surface of the cell, it helps in adsorption and absorption of chemical pollutant in the marine environment making analysis of lipid one of the crucial tools to check status of environmental stress over the life forms. Lipid composition of the living community helps as a biomarkers to stress because of the diverse groups of lipids are present in the nature (Parrish, 2013). Analysis of PLFA (phospholipid and fatty acid) gives an idea about microbial community (Willers et al., 2015). Heat stress, salt stress etc. regulate the signaling mediated by the cell as membrane lipids are a good sources of signal molecules such as phoshoinositides, phospholipids, free fatty acids etc(Hou et al., 2016). Bacteria changes their lipid content in response to the stress to survive against diverse environmental condition (Di Pasqua et al., 2006; Zhang and Rock 2008). Figure 2 depicts membrane bilayer having phospholipid contentand changes observed when it is exposed to environmental stress conditions. To measure the lipid quantity and quality, various analytical techniques are used that comprises gravimetric, colorimetric, chromatography. Gravimetric analysis is not an accurate method as it counts non lipid compounds also which may interfere with the analysis.

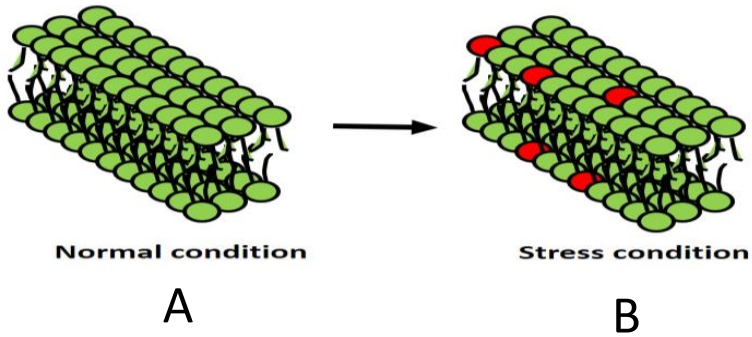

Figure 2: Membrane bilayer having phospholipid content. (A) Normal condition (B) Abnormal condition upon exposure to environmental stress conditions (Some proteins become red coloured).

Chromatography is the better option for analysis of the lipid content in the samples. Types of chromatography and combinations with other techniques emerged out with the need of accuracy and specificity required over time like chromarod thin layer chromatography, capillary gas chromatography, GC-MS, GC-IRMS and HPLC-IRMS (Parrish, 2013).

ANALYSIS OF PROTEOMICS OPENS WAY TO UNDERSTAND ENVIRONMENTAL STRESS

Environmental stress directly affects cell and organisms in their protein profile. A small cell carries out various metabolic events with the help of protein macromolecule. Environmental stress such as higher temperature, presence of pollutant, higher salt concentration etc. changes protein pattern indicating compensating mechanism against stress.

It has been reported that the s-adenosylmethionine (SAM) synthetase, Glutathione transferase, cysteine synthase, GST-tau and tyrosine specific protein phosphatase were elevated in response to arsenate (Ahsan et al., 2008). Environmental stress affects flowering in rice plants which is result of changes in proteomes detected by combination of conventional 1D, LC MS/MS to advanced bioinformatics tools (Kim et al., 2015). Protein analytical techniques such as tandem mass tag also helped to identify relative abundance of several proteins with respect to their synthesis indicating environmental stress not only affects proteomes but also alters protein distribution and abundance. Tandem mass tag and isobaric tandem mass tags (TMT/iTMT) possess an advantage as it produces reproducible results and more accuracy in terms of identification of closely related community of organism in which explores isotope labelling of peptides and comparison of candidate sample to known samples (Mosier et al., 2015). Most conventional method 2DE (2D gel electrophoresis) recognizes differential expression of proteins in the sample and gives an idea about stress regulated expression of unique genes and proteins as it was shown that 2D analysis in leaves against salt stress which was further coupled to MALDI-TOF analysis (Wang et al., 2014). Higher salinity stress results in changes in several protein profile which was analyzed using LC-MS/MS approach (Askari et al., 2006). Salt stress also hampers with expression of proteins involved in oxidative stress, metabolism, transport etc in canola leaf which was studied by 2DE and MS methods of proteomics (Bandehagh et al., 2011).

Proteomics deals with the changes in the post translational modifications in response to the environmental stress.

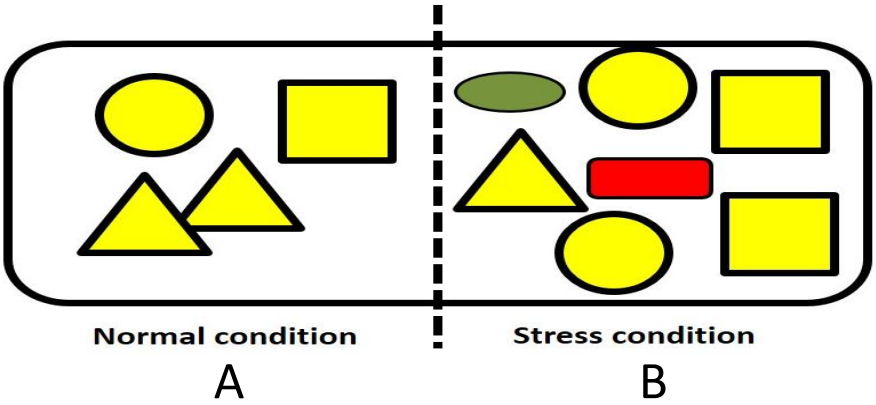

Figure 3: Stress in environment changes protein quality and quantity. (A) Dark yellow colourshows normal quality of protein (B) Change in quality and qunatitiy of protein in environmental stress condition.

Many proteins are found in membrane having two domains hydrophobic and hydrophilic and the domains that lie either of the side of membrane face hydrophilic environments and undergoes interaction with different molecules. Proteins are modified post translationally to propagate the signal against stress and can be detected by LC/MS not involving gel based method (Wu and Yates, 2003). Multi-dimensional protein identification technology discriminates soluble and membrane protein discrimination and helps to identify post translation modifications on soluble part of protein with the help of high pH and protection from protease without denaturing membrane and making it solubilized (Wu et al., 2003). Thus molecular techniques associated with proteins and proteomics helps one to understand the environmental stress. Figure 3 expalinschanges in qualitya and quantity in stress in environment conditions

CONCLUSION

Environmental stress is concerned greatly to the health of the many biological sectors. To detect and to overcome the challenges in environmental stress mediated disturbances, molecular tools are the advantageous approach over many conventional methods regarding its unique and specific nature of detection and analysis. Parameters that causes environmental stress such as a temperature, salt, chemicals have an impact on environment and that drastically affects the live stocks in the ecosystem. To detect the one out of many parameters that causes the disturbance is a tricky task as many parameters affects specific community or communities of the organisms. The community of the organism affected will show a clue in terms of altered physiology and may show hint in terms of unique expression pattern of macro molecules such as DNA in terms of set of genes(Askari et al., 2006) and lipid (Di Pasqua et al., 2006) content. Any changes to the environmental parameters makes changes in the rhythm of these macro molecules suggesting their exploration at vary efficient and accurate level in the future.

As in this article, the discussed methods target DNA, RNA, Proteins and Lipid contents, there are many more fields are to be developed in the future based on the various unique chemicals and macro molecules synthesized against environmental stress in specific community of the organisms.

Many of the method requires time and in some the accuracy of an analysis is compromised that would be more targeted to make them less time consumable and more easy to operate like many biosensors are developed.

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Corresponding author: Dr. Mohd. Zahid Rizvi, Associate Professor of Botany, Department of Botany, Shia P.G. College, Lucknow, U.P., India
Email: zahid682001@gmail.com