Retrospective Review of Microbial Ecological Processes to Understand Environmental Biotechnology.

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Abstract— We—Waheed Ahmad, Andleeb Farooq, Tazeen, Maham Irfan and Nawal Naveed Abbasi— have made an attempt to explain the Retrospective review of microbial ecological processes to understand environmental biotechnology. The fields of environmental biotechnology and microbial ecology are two blossoming fields that have greatly benefited from the advancements in biology, engineering, computing and materials. Although both of the fields are traditionally varied, but the future of both the disciplines are linked to one another. Both the fields, together, provide and promise so much to help society, face and eradicate an environmental problems and challenges, sustainability, human health and security. Moreover, we have also talked about the microbial ecological processes to better understand environmental biotechnology, potential applications of these processes towards our own environment and the future perspective that where this technology is accelerating and heading towards, and what more methods and processes will be witnessed in near future to successfully eradicate and degrade the pollutants and contaminants from the environment through the interaction between microbial communities and their environment for a better, secure and sustainable ecosystem.

Index Terms— Microbial Ecology, Environmental Biotechnology, DNA Microarray, Phylo Chip, Metagenomics, Metatranscriptomics, ARDRA, Microbial fuel Cells, Bioremediation, BIOLOG.

1 Introduction

The field of Microbial ecology has remarkably developed under the past few years to help understand the microbes' relation with itself and the interaction with the environment for the ultimate betterment of an ecosystem. There have been many revolutionary changes that have taken place. The core objective of microbial ecology is to study and understand the communities of various microbes that are self-sustained and organized and how these microbes interact with Intra-communities of microbes, inter-communities of microbes and with their environment.

The microbial ecology as a field tries to answer various questions which are important and need answers. These are; Firstly, what sort of microbes or microorganisms exist within the microbial community? This phylogenetic makeup of the microbe that is number and its identity is referred to as **Community Structure**. Secondly, what sort of capabilities and strengths of microbes within the communities are present that initiate and carry out different reactions for the transformation of environment? These capabilities that exist within the microbes for carrying out reactions within the microbial community is called **Phenotypic potential of the microbial community.** In addition, a question that what reactions and transformations are performed by these microbial communities that get the work done? This realization and remembrance of the phenotypic potential of the microbial communities are referred as **Function of community or community's function.** Lastly, what are the intra-relations and inter-relations of microbes within the community and amongst the community and also their relation to the surrounding environment? This relationship generally includes the spatial location, organization and what sort of materials these microbes tend to exchange with others; to understand all these 4 basic questions that are raised in the field of microbial ecology is the ultimate goal of this prestigious field. The explanation and answers to these questions afore-mentioned might change over time and also in response to the environment. So, Resilience and Stability is generally the response of microbial communities towards the change in environment—anthropogenic or natural.

The field of microbial ecology started way back in 1940s and 1950s, however, great advancements and developments were made in the year 1960-1970s. The field of microbial ecology hugely struggled in early days when there was no availability of tools that can answer the four fundamental questions. Further, the miniaturized size and variant shapes of microbes made its identification very difficult for microbial ecologists due to the limited and insufficient amount of tools at that time. The process of selective culturing was a great success story at the start which was both applicable in practice as well as in concepts. The process relied on the metabolic function but it gave biased results and at times faced a lot of failures. The hustle for the molecular biological tools then started in 1985 which completely changed the prospect and concept of microbial ecology. The microbial ecological methods and techniques started using the 16s rRNA for prokaryotes, hybridization of nucleotides, polymerase change reaction, fluorescence in situ hybridization, and many more that is going to be discussed further in our assignment. These microbial ecological methods are the main driving factors for development and flourishment of the field.

The field of Environmental biotechnology is defined by the International Society for Environmental Biotechnology as "the development, use, and regulation of biological systems for remediation of contaminated environments and for environmental friendly processes that is green manufacturing technologies and sustainable development". The field of environmental biotechnology utilizes biological agents such as microbes and their communities to provide ultimate societal services. The most notable of the services generally include the removal of contaminants and pollutants from wastewater, sediments, soil, sludge, water and also recover products, nutrients, metals and others that could be helpful for the human activities, and dispose and degrade all those materials, contaminants and pollutants that may prove to be harmful and detrimental for human utilization. These services we have talked about are extremely essential for the essence and survival of human beings, sustainability of environment and security of all forms of life on ecosystem. If managed properly, these microbial communities can provide us economically, reliably, continuously and without imposing any sort of pollutants and hazards to human and environment.

Environmental biotechnology is relatively centuries old field but had been named differently in various eras. The environmental biotechnology was previously known as biological processes, bioenvironmental systems, bioprocess engineering and biological treatment whereas the new name, environmental biotechnology, has greatly benefited from the modern day methods and techniques of molecular biology along with the advancements in integrated science and technology. The environment biotechnology is based on the scientific foundation of microbial ecology because this field ultimately utilizes the microbes and their communities for the welfare of society—Humans and its environment. Environmental biotechnology through the use of microbial communities have indeed addressed the real world problem and is hugely working and combating for their real solutions which can be ultimately beneficial to the human beings and the environment surrounded by them. Thus, we need to scrutinize, observe and understand the microbial communities and their interaction within the communities and with the environment for the sustainability of our ecosystem.

A three-peak vista:

The fields of environmental biotechnology and microbial ecology generally shows three consensual peaks that represent and show different themes. These are;

1. The –omics approaches.
2. The use of more and more powerful analytical tools.
3. The use of research that is more process driven.

The –omics approaches that are mostly used is genomics, proteomics, transcriptomics, lipomics and metabolomics which generally provide high through put data.

2. MICROBIAL ECOLOGICAL PROCESSES:

2.1. Cultivation-Dependent Methods:

The cultivation dependent methods can also be termed as traditional methods of microbial ecology that was used to understand environmental biotechnology. The traditional methods mainly include the isolation and purification of bacterial strains. These bacterial strains can be isolated and purified from different sort of environments and are extremely important in the studies of DWH spills. The information that is provided on the basis of microbial isolation in pure culture involves; physiological characterization of microbes—bacteria, functional information, and the metabolic pathways.

In the DWH Spill, various amount of microbial, bacterial, strains were isolated from the deep oil plumes and surface slicks. These bacterial strains generally include; Pseudoalteromonas, Alteromonas, Halomonas, Cycloclasticus having degrading abilities of hydrocarbons. The addition of oil acts as a source of carbon isolated one of the hydrocarbon degrading strains— Colwelliaceae successfully which was found in large amount and was dominant in plumes of oil. In spite of the isolation of some of the oil degrading strains of bacteria, huge amount of bacterial strains that were indigenious could not be extracted through the traditional and conventional-based methodologies which produced problems for microbial ecologists.

This problem faced might be that the culturing conditions differ and are not always applicable in in situ conditions. In situ conditions provide symbiotic relations of microbes within and between communities as well as specific levels of nutrient whereas in artificial culturing conditions these in situ conditions are meet that produce problems in in situ and lab environment. In addition to the afore-mentioned conditions, some other also environmental conditions also exist; O2 restriction, hydrostatic pressure and low temperature. These environmental conditions often get neglected or are very difficult to provide in ex situ environments which is the main reason behind the limitations being faced.

2.2 Classical Molecular Biological techniques:

There are different applications of molecular biological methods that are FISH, RT-PCR, DGGE. etc. These are important techniques in identification the crucial functions and composition of innate microbial groups in a particular environment.

2.2.1 Clone Library of the 16S ribosomal RNA Gene

This 16S ribosomal RNA gene is called as a marker gene. It is utilized for the identification of microbial difference and for the remarking of prokaryotic phylogenies. It provides a valuable information about the composition and structure of bacte-

rial groups.

Investigation and development of this 16S ribosomal RNA gene clone libraries gave valuable data about the structures of bacterial communities. In any case, the 16S ribosomal RNA gene clone library technique became less predominant after the next generation sequencing and it was utilized to recognize the microbial communities.

The sequence analysis of cloned 16S ribosomal RNA genes have demonstrated to be valuable tools for identification and exploring the microbial diversity of natural samples.16S ribosomal RNA library are created from total extracted ribosomal RNA and may be are considered to attract predominantly diversity of the metabolically dynamic individual of the community.

2.2.2 Methods of gene fingerprinting

There are two important techniques of this methods.

1. Denaturing Gradient Gel Electrophoresis.
2. Temperature Gradient Gel Electrophoresis.

They isolate DNA and RNA within the same test. Muyzer et al. initiated the Denaturing gradient gel electrophoresis in microbial ecology field in order to explain the way the microbial groups are structured and composed. There are different sorts of bands on the gel that signify the various microbial phylotypes and intensity of band which showed the appropriate teemingness of some microbial communities. After cutting the denaturing gradient gel electrophoresis and Temperature gradient gel electrophoresis strips at that point build cloning library and sequence the data connected to a band for furthermore interpretation. These two techniques are not enough to provide information rather next generation sequencing can create more information. These both methods are still utilized to study microbial communities.

TRFLP is another most important gene fingerprinting technique. This can explain the differences in the unknown bacterial taxa in numerous natural tests. It was utilized to indicate the structure of microbial groups after the oil spillage. This technique can give more exact information as compared to clone libraries.

These methods have been utilized to characterize the microbial diversity in different environments, e.g, lake water, activated sludge, anaerobic reactors, hot springs, sediments. The method can be applied for as both quantitative or qualitative approaches on biodiversity estimation.

2.2.3 FISH and RT-qPCR

FISH has been broadly utilized to examine the composition of different bacterial groups. It also gives fluorescent images showing the appropriate amount and presence of target genes that are combing with particular fluorescent probe. Without PCR amplification, 16S ribosomal RNA can be identified by the help of this method.

RT-qPCR can determine target usefulness of bacterial phyla more precisely, as compared to FISH. It also provides exact information by the help of real-time monitoring of the vibrant signal that are made by prismatic tests. For RT-qPCR and FISH indication and investigation, primers and probes are useful, only microbial phylotype with suitable primers and probes can be detected and identified. Without particular probes and primers some groups of microbes are not easily identified with these methods.

These both play a vital role in the detection and measurement of microorganisms in environmental.

2.3 Advanced Molecular techniques:

Advanced molecular methods can provide information for detection and identification on the structure and composition of different microbial groups in different locations. Some new approaches that provide specific and accurate data have been applied in exploring the function and progression of microbial groups.

2.3.1 DNA Microarray

These are basically the combination of small DNA spots showing the single marked genes, which are associated with chemical substance and shown on the surface of firm. DNA Microarray has experienced various era of enhancement and has been utilized for about 20 years.

There are two strategies of DNA Microarray:

1. Phylo Chip and
2. Geo Chip

Phylo Chip is subjected to investigate and detect the different qualities of bacterial groups, while the Geo Chip method can be utilized to analyze the activity of microbial groups through the functional genes. During the DWH oil spillage, both strategies were connected to disclose the genes that are functional within the biodegradation of oil contamination and then explore the progression of bacterial groups.

DNA Microarray innovation has been utilized broadly to examine the anthropogenic and natural factors in the yeast form which whole-genome chips have been available.

2.3.2 High-Throughput Sequencing. of the 16S ribosomal RNA Gene

16S ribosomal RNA gene has been utilized to examine the different qualities of microbial groups with the help of cloning, T-RFLP. and many other conventional strategies.

The next generation sequencing techniques, such as, Illumina Hiseq/Miseq sequencing, Roche's 454 GS20 pyro-sequencing and ion torrent sequencing allow the creation of huge information sets at much lower cost. When the use of high throughput sequencing was on top, the DWH oil spillage happened in 2010. 454 pyro-sequencing of the 16S ribosomal RNA gene that is utilized in this event was the first NGS

method to provide information on the progression of microbial entities.

Illumina sequencing was also utilized. For example, by analyzing a huge number of information produced by Illumine Hiseq/Miseq sequencing, Simister et al. search the dominating microbes in flocs and sediment samples were proteobacteria. They also search that microbial structure and arrangement of the floc tests largely facultative and aerobic phylotypes, such as, Rickettsiales and Pesudomonadales was different from those that are displayed within the sediment samples.

It is utilized for investigating the microbial diversity of useful and pathogenic microscopic organisms, as well as their interactions in Biotechnological process.

2.3.3 Metatranscriptomics

This may be utilized to explore the gene under some environmental status which is recorded. Metatranscriptomics information takes under consideration the active state of RNA level and thus minimize restraint of Metagenomics that depends on DNA to increase prediction accuracy. In this way, Metatranscriptomics examination can also give supporting information as compared to Metagenomics analysis. In response of Metagenomics analysis, scientist Mason et al. presented Metatranscriptomics investigation to find out the definite genes that are functional with the ability for biodegradation as compared to DNA -based examination.

Metatranscriptomics used to deal of all the RNA transcripts produced by the biota in a sampled soil. Metatranscriptomics is the science that studies the gene expression of microbes within the natural environment.

2.3.4. Metagenomics

It deals with hereditary makeup and structure of microscopic organisms through direct investigation of DNA mixtures from bacterial groups in various natural samples. It has altered our capacity to find out the bacterial world by generating reproducible results as compared to cloning and sequencing techniques. Metagenomics analysis can afford vital metabolic data to find out microbe functions.

Sequencing became prevalent before next generation and Metagenomics established on shotgun sequencing the data was compelled by the limited information on an individual genomes and environmental genetic information. High- output., low-cost, small runtime properties of next generation sequencing have changed our perspective of bacterial Metagenomics. Metagenomics have the potential to inform the suitable use of remediation strategies to achieve quick remediation of a contaminant in a minimally invasive way.

2.3.5 Sequencing of Single-Cell method

This method is used to get individual genomes microscopic organisms. A single -cell can be isolated from different environments by means of flow cytometry and micro manipulation. The whole genomes of selected individual cells are at that point enhanced by the assistance of MDA .to create enough amount of hereditary material for sequencing purpose. The genomes can be ordered by next generation sequencing to investigate through strategies in Bioinformatics.

Masson et al. in 2012 effectively obtained two single -cell genomes of the class Oceanospirillales, that appeared prevalent phylotype after the DWH oil spillage... The single-cell ordering indicated that both cells controlled for gene encoding for the debasement of n-alkane and cycloalkane. This provides valuable proof that microorganisms within the class Oceanospirillales were accountable for the biodegradation of hydrocarbons within profound ocean. Sequencing of Single-Cell method has risen as an imperative new set of technology for analyzing rare cells and delineating complex contaminants.

2.3.6. Analysis of the soil enzymology

Analysis of soil enzymology for continuously expression of microbial enzymes, such as, dehydrogenase, lipase, urease etc. have been utilized in few of the studies of in situ bioremediation as markers of the effects of technological intervention on innate bacterial groups. It is utilized to determine the status of soil. This method is very basic and give reproducible results.

These enzymes have been considered as a valuable indicator of soil quality because of their interaction to soil science, that are viable, delicate, easily measurable and depicted as "biological fingerprints" of soil administration, and associate with culture and the structure of soil.

They play a vital role in decomposition of organic matter and recycling of nutrients.

2.4 Analysis of bioinformatics. software and databases:

2.4.1 Software tools and databases

There are many software tools and databases have been utilized for examination of Bioinformatics, that are, MEGA6, Ribosomal Database Project, QIIME and Green genes.

Various visualization apparatus has also created, e.g, Visualization, Sequence Annotation, Integrative Genomics Viewer, and Analysis Tool Genome Browser, Magic Viewers etc.

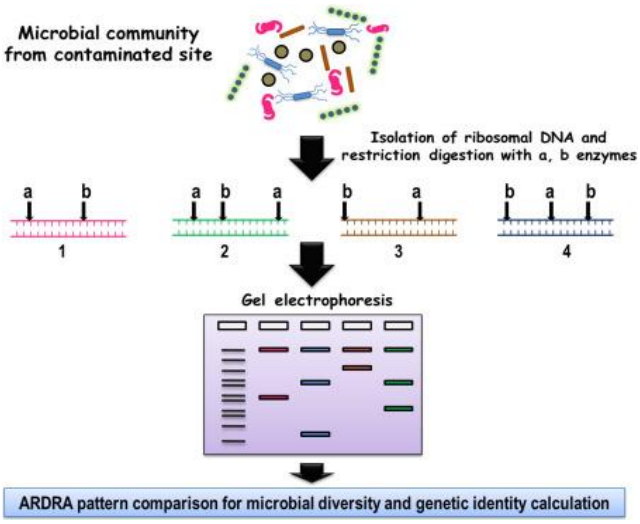
These software tools and databases were utilized to investigate the information that are based on DNA and RNA. Besides, scientist Mason et al. in 2012 applied various software and databases to get biological meaning from the mass of Metatranscriptomics and Metagenomics. For example, the database of Geo Chip was used to blast proteins that are used for the degradation of hydrocarbon, but COGs. database was utilized to decide the completeness of genome sequence. By the help of CRISPER Finder, the clustered regularly interspaced short palindromic repeat region was detected within the draft genome. In this way, there is need for the development and improvement of more accurate databases, in spite of the fact that Bioinformatics tools have been made and significantly improved

the data analysis. There is need to create standardization strategies, equipment simplification, and user-friendly Bioinformatics apparatus.

Bioinformatic.tools are used to determine the structures and the biodegradative pathway of xenobiotic compounds. These are used to analyse, manage and store information.

2.5 ARDRA:

ARDRA has been adopted widely for the analysis of structure and interactions among communities of microbes ***(Oravecz et al,2004; Fernandez et al, 1999; Gich et al, 2000).*** In this technique, the amplification of genes of 16S RNA that are retrieved from the DNA obtained from environment is carried out after digestion with restriction enzymes. As a result, clear differences among related microbes can be observed with accuracy ***(Vaneechoutte et al, 1992; Ingianni et al,1997).*** However, before digestion of these genes the cloning of these genes is also carried out by using suitable vector to avoid the chances of contamination that may occur among genes of 16S RNA of various microbial strains. After this, electrophoresis of these restriction digested clones is carried out and their pattern of restriction digestion allot them specific position and categorize them. These patterns are called "Ribotypes". Thus, ARDRA assists in determining the features of microbial community structure such as richness of community and its evenness. Several software has been made to determine the taxonomic status by calculating the extent of similarity and dissimilarity ***(Schloss and Handelsman,2005).*** ARDRA is used in combination with techniques of DNA sequencing to assess structure also the interactions of communities of microbes during various ecological processes. It is the best technique amongst all that have been employed for various applications in communities of microbes. It has been frequently used to deal with bacterial species ***(Vanneechoutte,1992).*** ARDRA has been employed in various lands where soil is contaminated with pesticides to characterize the structure of communities of microbes.



ARDRA pattern comparison for microbial diversity and genetic identity calculation

2.6 BIOLOG:

BIOLOG method is used for the assessment of microbial communities on the basis of their ability to degrade the substances having carbon as main subunit. It works on the principle of redox reactions. BIOLOG plates utilize 95 kinds of substrates as carbon source in wells and one well is empty which acts as control group. Samples of soil are taken and solution is prepared and then poured into these wells. When constant temperature is given to these wells then carbon substrates are oxidized via microbial action and a color reaction occurs through dye. On the basis of the intensity of color results are predicted that which substrate is largely oxidized and which is the least oxidized by microbes. Thus by analyzing substrate utilization capability different microbial communities structure are determined and microbe's metabolic profiles are determined through these metabolic fingerprints ***(Garland J.L and Robert M.S, 1997).***

3. SIGNIFICANCE OF CULTIVATION OF MICROBES IN LAB IN MICROBIAL ECOLOGY:

Microbial ecology deals with microbial interactions, their abundance and factors which affect them that include biotic as well as abiotic factors. The development of understanding of microbial ecology is significantly important as microbial ecological processes have very important functions in environment because they are crucial for matter and energy recycling. They also have important role in various agricultural activities, industrial processes, in cleaning of environment, in processing of food. Microbes can be cultivated in laboratory and various ecological and microbiological processes can be studied and improved by conducting experiments on them. Pure Cultures of microorganisms have been used to understand their physiology and to determine their taxonomic status. It has become very easy to select the microorganisms on the basis of a particular state of metabolism study traits of interest. An isolated pure culture of microbe has significant importance in its deep analysis by providing a complete picture of its response to any factor in the environment and its ability to perform its metabolic functions efficiently. Optimum conditions are provided to the microbes in culture so that their analysis can be carried out by growing them effectively. Optimum concentrations of media are provided according to their source of energy. Special techniques and instruments have been developed to isolate microbes and inoculate them in media and study their behaviors under various growth parameters. The techniques of flow cytometry, laser detection methods and fluorescence methods are used for this purpose. Microbes can also be analyzed through other methods in which culture is not utmost requirement. Nucleic acid can be extracted, and sequencing can also be carried out to study microbial ecological processes ***(Gray ND, Howarth R, Rowan A et al, 1999).***

4. A REVOLUTIONARY APPROACH IN MICROBIAL ECOLOGY: "THE ENGINEERING OF HABITAT FOR CONSORTIA OF MICROBES"

By analyzing the diversity of metabolism, way of exchanging of metabolites and communication among communities is determined. It is the division of labor which allows them to coordinate their activities and communicate to each other through exchange metabolic substrates and signaling pathways. While in case of monoculture, consortium of microbes organize itself to form spatial arrangement like in biofilm formation and aggregates of soil through which they improve resource utilization and interaction and efficient exchange of metabolic products occur. Such spatial arrangements and patterns determine different microbial combination range and their applications.

4.1. Efficient and versatile metabolic associations in microbial consortia

Microbes are important component of our environment and they are present all around us. The interactions among them ensure their survival ***(Brenner et al, 2008).*** Even the simplest consortium contains hundreds to thousand kinds of species (Curtis et al,2002). Because of metabolic diversity in consortia, they are used as mix culture for treatment of waste water, bioremediation, production of biofuels and biomining ***(Daims et al, 2006; Kleerbezem and Van Loosdrecht, 2007).***

Artificial consortia are designed by particular, targeted selection of microbes having specific applications for example in production of enzymes, food additives, biopolymers, antimicrobials and fuel cells employing microbes ***(Lynd et al, 2002; Bader etal; 2010).*** Most consortia are made of binary culture system. In this system same set of conditions are applied to different species and thus their associated metabolic states are monitored. Though these genetically engineered consortia have promising features and used widely but further improvements are needed to use them on large scale processing ***(Bernstein and Carlson, 2012).***

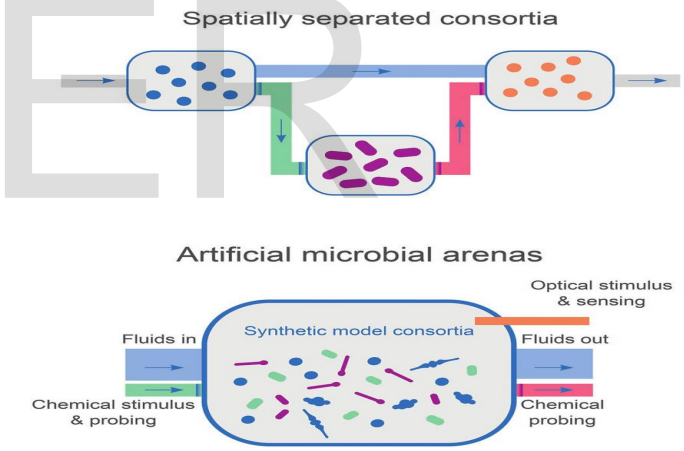
4.2 Engineering of Spatially linked consortium of microbes

This approach deals with providing the engineered environment to microbes where consortia of microbes are assembled based on the optimum conditions provided to them and metabolic exchange occurs in optimum manner. Such engineered method has various benefits and overcome many limitations that are present in natural system and enhance the system robustness. Separate modules are prepared along with partitioned chambers and are connected to each other to allow exchange of metabolites and interaction among them. This methodology of compartmentalization provides a control system in which each specie is provided optimum conditions in its niche compartment. Thus, stringent conditions are maintained to continue the process effectively and to prevent the exposure of the species to growth retarding substances that are secreted by other species.

The attempts to engineer consortia of microbes in separate compartments started since 1960s. in one methodology, culturing of interacting species was carried out in same vessel that was separated by a membrane to prevent the passing of cells and molecules. But its main drawback all microbes are provided same physio-chemical conditions and environment despite of their specific requirements. Another approach has been adopted in which different two or more reactors are connected in series having different microorganisms. These methodology considerations are as follow:

- The specific microorganism is selected for specific use and purpose.
- The development of spatial arrangement.
- Flux and input operational parameters.

The selection of consortia members is carried out on the basis of their ability to do biotransformation which result in the increased range of interacting microbes' novel substances and products in areas ranging from pharmaceuticals, biofuels where microbes are employed for waste recycling and bioremediation as well.



For the activation of these consortia, cells are preserved by freezing, lyophilization or thawing to awake the consortia. This activation is achieved in a sequence and then a stable state is achieved. The continuous monitoring of system variables like temperature, pH, oxygen supply provides detailed information about system performance and tuning of system is carried out by adjusting these parameters. Thus, they overcome the problems imposed by environment due to undifferentiated parameters of growth and behavior of microbes in a niche at a particular time can be analyzed ***(Chen F, Ricken J et al, 2018).***

4.3 Selection of strain for microbial consortia

In a microbial consortium, after the selection of species for a process, the selection of a strain with optimum characteristics is carried out. The factors such as nutrition components,

growth requirements, yield, by products secretion have important role in strain selection. If the target is maximum production of biomass, then such strains are selected which have fast and maximum rate of growth. If low growth rate is required, then such techniques are adopted which maintain population or control it and do not let it exceed beyond requirements like use of UV light and method of quorum sensing. The selection of such strain should be done which prevents the metabolic products secretion that prevent microorganisms in down streaming. An important consideration is that strain should be selected which is capable to withstand and survive harsh environmental conditions. By considering all these factors optimum strains should be selected for development of consortia of microbes ***(Ataman M, Hatzimankitaz V, 2017).***

4.4 Stability of microbial Communities and Consortium

Stability of microbes in community is important governing factor in all processes in which they are involved. Any factor which can act as mutant changes the genetic component of microbes due to which consortium performance is affected therefore to avoid such conditions fresh cells should be provided as alternative of these old ones after certain interval. The food chain robustness as well as its stability is also an important aspect of consortium. Any fluctuation in environmental factor like temperature, pH, oxygen can severely affect population of microbes ultimately having bad impacts on nutrients uptake by downstream microbes that are provided to them by upstream microbes. By adjusting these parameters and conditions and fulfilling the optimum criteria the best and stable consortium can be developed ***(Ward, B 1996; Yavuz, 2016).***

5. PLANT-MICROBE ECOLOGY: INTERACTIONS OF PLANTS AND SYMBIOTIC MICROBIAL COMMUNITIES:

Plants have advanced through a wide variety of microorganisms, playing essential functions in enhancement of plant growth. A substantial knowledge is now accessible on the composition and dynamics of plant microbiota as well as on the functional ability of isolated population members.

The emergence of biotechnology has made it feasible for the better understanding of microbe-plant interactions. We will review about current reports on how the plants related to microbial environment can be modified and shaped, and how the microbial biome influence plant quality and sustainability in various associations;

5.1. Interactions of soil symbiont with plant community ecology

Climate changes affecting diversity of species in soil atmosphere, greatly influenced plant ecosystem and environment. The plant in terrestrial region causes dynamic modifications in conservation of soil, altering ecosystem reliability.

5.1.1. Host plant response to microbes and soil community feedback

Diverse frequency and organization of organisms in rhizosphere that influence coherence of plant. species by. Indirect response (i.e. by competing and inhibiting symbiosis) in the population of plants. Experts has suggested concepts to understand the process that causes bad variety in plant populations;

- The ***empty Niche*** concept states that novel and new organisms occupy region inhabited by invading plant. They are more effective at acquiring resources, and selectively linked with plants that are invasive.
- ***Degraded mutualist*** hypotheses propose that invading plants-symbionts hinder capability of local microbes that are symbiotes to acquire and utilize the resources, thus potentially decreasing the efficiency of local plants.

5.1.1.1. Microbes and Soil resources:

Soil resources will direct co-existence of plant through separation and sharing of resources. Researchers showed that symbionts in roots that improve nutrient absorption efficiency and help host to survive in a nutrient-deficient environment, thus leading inevitable exemption of other plants.

The resource partitioning of microorganisms, due to variation in accessibility of N and P in soil, has influenced plant to plant connection. The reserves may even be exchanged by mutual symbiotic. fungi. They are termed as common mycorrhizal networks (CMNs).

Naturally, various plants usually share the generally defined mycorrhizal fungi.

5.2. Microbes are shaped by plants:

Microbial associations are major element in biodiversity and efficiency of plant species. How these plant utilize distinctive microbes? How do they form a special microbial Community? Those things should need to be discussed. Recent advancement in genomics provides us some clues about it.

5.2.1. Plant genes responsible for defense affect the variation of the microbial community

Numerous experiments shown limited yet major impact of genotype of plant on the nature and composition of endophytic, rhizosphere, or phyllosphere microbes. Genome-wide Association GWAS for the *A. Thaliana* leaf culture proposed its locus is essential for the defense and the integrity of the cell wall has influenced the structure of the specie population. In addition, plant genetic variability influenced relative abun-

dance in bacterial population. This test is beneficial for classifying the taxonomic status in humans, flies and plants in relation with host genes.

5.3. Role of root exudates:

Root exudates termed as a collection of compounds in the rhizosphere that are secreted from the roots of living plants and are microbially modified derivatives of these compounds. Plant root exudates vary from one plant to another, so variations in rhizosphere biomes of various plant species are possible. Many current researches provide clear evidence for plant-specific microbial diversity. Root exudates also act as medium for plants to modify and shape microbes. It includes;

- sugars,
- amino acids,
- antimicrobial compounds,
- enzymes
- flavonoids,
- organic acids,
- nucleotides

5.3.1. Environmental factors affecting root exudates

The factors include:

5.3.1.1. Temperature

Because of global change in climate, consequently severe weather conditions have severely impacted the cultivation of several crops. For example, Husain and McKeen have elucidated the effect of temperature on root exudates by growing strawberry at 5°C and some at 30°C. Therefore, the low-temperature soil plant develops more amino acid derivatives and decreases the harmful impact of *Rhizoctonia fragariae.*

5.3.1.2. Moisture

Dryness and flooding have drastically cut down food crop production. Many detailed information has indicated that soil moisture has dominating effect on root exudate production. The transient yellowing of leaves has stimulated discharge of amino acids from roots, which is correlated with the occurrence of pathogens in rhizosphere.

5.3.1.3. Soil pH

Dumberell showed that analysis of 425 individual plants within *Arbuscular mycorrhizal* (AM) fungi, out of 28 species. Results provided good support for the hypothesis that soil pH structuring of the AM fungal was based on niche differentiation.

5.3.1.4. Nutrition

The supply of nutrients such as C, N, P are believed to impact the production of exudates and synthesis of special chemical, and the abundant supply of pathogenic as well as beneficial microbes in the soil.

Bowen first showed a significant impact of nutrient requirement due to excretion of amides and amino acids from the Pinus. Findings showed a double amount of amides in P-deficient plant exudates.

5.3.1.5. Microorganisms

Soil microorganisms are important in plant development and exudates. Microbes may influence exudation by manipulating root cell permeability and root metabolism. Microbes in soil could even manufacture secondary metabolic substances that improved signaling in plants and can be called a "plant secondary genome" thus providing nutrients to plant hosts.

5.4. Diversity of Microbial community and impact on performance of plant:

5.4.1. Variation of microbial Community in plant life cycle

Microorganism variety of plants and rhizospheres varies greatly across the life cycle of plant. The factors which affect the community of microbes are categorized as;

- **dispersal**
- **drift**
- **speciation**
- **selection**

Seed starts life cycle in plants. Seed.dispersal is one of the key ecosystem process. Seeds having related microbes originating from their parent and climate, thereby growing microbial diversity within a new atmosphere. Recent work has shown that bacterial coatings of seed can defend against pathogens. During germination of seed, microbes which are seed-borne may acquire have benefit over other colonizing ones after germination. And opportunistic. microbes may be exposed to a new environment when plant continue to grow.

5.4.2. Microbial Hub or Networking of plant-microbes

Microflora of plants create a dynamic web or network. Large variety of experiments have shown that plant associating microorganisms stay in plant tissue or on plant organ surfaces. Agler et al. described microflora leaves of *A. thaliana* had been affected by genotype and abiotic stress. He found that organisms (e.g., pathogen of plant *Albugo* and the *Dioszegia* fungus) had a major effect on the structure of microbial population. He used the word "**microbial hubs**" for species that are closely interlinked with others in the plant microbial system. They may be accountable for the propagation of defensive signals between plants and the potential activity of biological control agents. The word "keystone species" suggested as presence of a group of specie's hub that will decide the microbial taxa colonization. They both have a significant effect on efficiency of plant.

5.4.3. Growth–promoting microbes:

The soil is a reservoir of microbes, among which bacteria is perhaps popular. Plant growth-promoting bacteria (PGPB) involve freely living bacteria, some who establish particular symbiotic relationships with plants, or a fraction of the inner tissues of a plant.

PGPB can;

- directly improving the synthesis of chemical substances or hormonal rate in plant.
- indirectly reducing the adverse effects of pathogens and maintaining plant yield by acting as biocontrol agents.

5.4.3.1. Abiotic stress & PGPB:

Naturally, environmental conditions such as abiotic stress influence all living species. The communication of. PGPB with some plants may help them to fight these severe conditions or stress and protect them from decay.

5.4.3.1.1. Cold

Maize subjected to minimum temperature exhibit decreased shoot and root development due to extreme oxidative damage triggered by cold stress. Application of *Bacillus Amyloliquefaciens, Plantarum,* with micronutrients (Zn / Mn) has been proven to be effective and beneficial protectants against cold stress.

5.4.3.1.2. Heat Stress

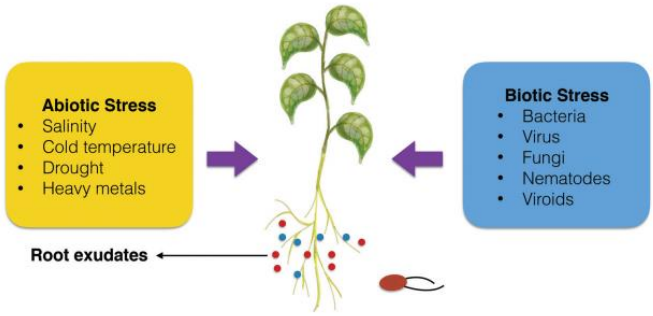
Pseudomonas putida has been shown to be helpful for wheat development against extreme heat. The bacterium greatly boosting the length of the root and dry wheat biomass relative to inoculated plants. Inoculation increased cellular metabolite rates and decreased the function of many antioxidants and heal damaged membrane.

5.4.3.1.3. Salinity

Plants are very fragile and prone to high salt concentration and one of the main environmental causes to decrease crop production. Plant-microbe interactions seem to be valuable in *Zea mays* against high salt concentration after co-inoculating with *Rhizobium*, while *Pseudomonas* has been coordinated with significantly lower leakage of electrolyte and management of H2O level in leaf.

5.4.3.1.4. Resistance to water scarcity

Water shortage limits production, and richness and fertility of crop is lost because of H2O depletion pressure. *Achromobacter piechaudii* ARV8 narrowed down generation of ethylene by seedlings in tomato after stress, and ARV8 won't influence level of H2O during deficiency. It has improved plant after resuming water.



Factors affecting plant-microbe interactions.

5.4.3.2. Biotic stress & PGPB:

In plants, biotic stress basically involves disruption from other living species such as pests. PGPR against biotic stress can influence plant growth in two ways;

- through encouraging plant growth through the development of phytohormones,
- through stimulating the absorbing capacity of certain nutrients.

Indirect stimulation of development begins as PGPR decreases or avoids the detrimental impact of. ***P. fluorescens*** releases chemical hindering the phytopathogenic activity of fungi. ***B. Cenocepacia*** lowered the prevalence of fusarium wilt to 3.4% in banana, relative to 24.5 % in non-inoculated plant contaminated in field trials for over 7 months. ***P. fluorescens*** BL915-release antibiotic **Pyrrolnitrin** may prevent danger from *Rhizoctonia solani* during cotton plant damping.

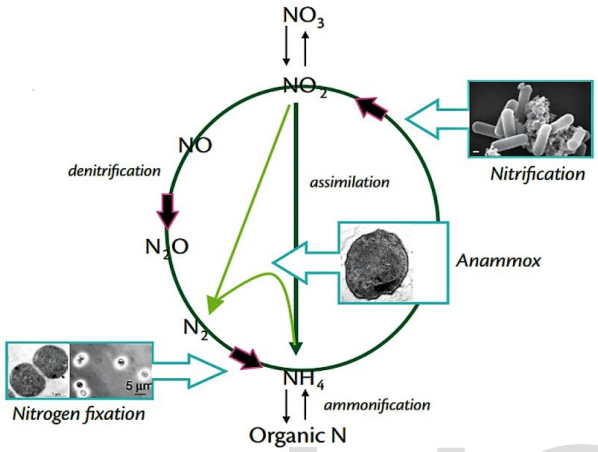
6. POTENTIAL APPLICATIONS:

Besides increasing the profitability of co-cultures used currently which provide more reasonable developmental atmosphere to every individual from a microbial mixture, these microbiological processes can drastically grow the scope of its uses by permitting blend of microorganisms that regularly cannot be seen in normal frameworks because of incompatibility of ecological conditions. Here are some of the potential applications of microbiological processes.

6.1. Anammox process

Anammox or Anaerobic ammonium is a process in which the nitrogen cycle converts ammonium and nitrite under strictly anaerobic conditions into nitrogen gas. Ammonium is first converted into nitrite by oxidizing bacteria (e.g. Nitrosomonas.) in presence of oxygen and Anammox bacteria like *Candidatus Brocadia anammoxidans, Candidatus Kuenenia stuttgartiensis* then combines nitrite and ammonium to form nitrogen. This constitutes 30 to 50% of the Nitrogen gas in oceans. The advantage of anammox process is utilized in waste water treatment. The process can be made economic by replacing the step of denitrification and making process beneficial and cost effec-

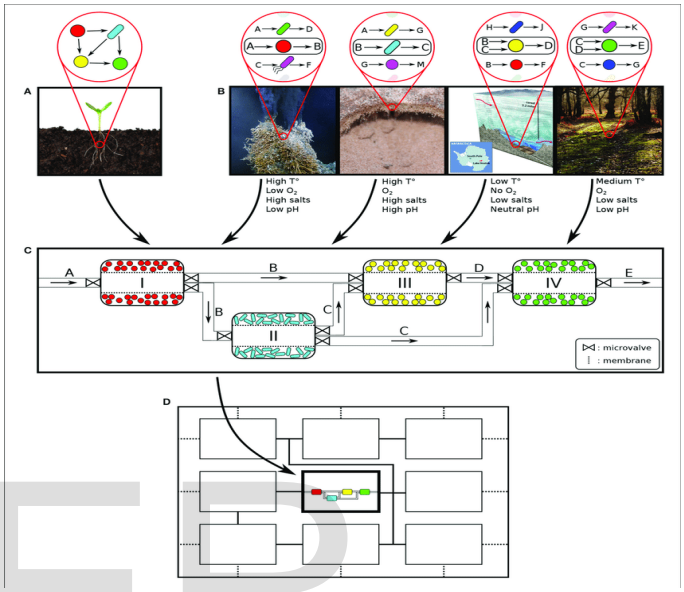
tive by reducing operational cost by 90%. The methodology of forming compartments utilized by the measured quality of SLMC will definitely profit these kind of procedures that use the blend of contrary biochemical. responses. Nitrosomonas are developed in first module in presence of oxygen so it changes some of the ammonium to nitrite. This blend of ammonium and nitrite are then moved to a subsequent specialty refined anammox which would join ammonium and nitrite to nitrogen.



6.2. Bioremediation

SLMC has an important application in bioremediation. The fact that microbial consortia is far better degrader of various pollutants than single species. Bioremediation utilizes microbial processes to decimate poisonous toxins. It offers an advantage over other remediation methods, for example, burning, and thermal processes and a portion of its points of interest are its minimal effort, low-innovation methods, it can frequently be completed on site, is safe and do not cause hazards. Ex situ method, for example, slurry or fluid bioreactors are progressively under control and regularly bring about much higher degradation but on the other hand are costlier than in situ bioremediation. Successive bioreactors, as provided by SLMC can provide a better treatment. Since degradation of the toxin is considered as multi step process. The microbes containing the consortium of degrading microbes would be cultured in a sequence. After the degradation, the spatial separation of processes in SLMC will allow a higher control than the cultures used in mixtures.

In situ methods of bioremediation is not controlled and microbes responsible for degradation are inserted into environment. The conditions like PH, temperature and nutrients are also adjusted. Porous alginate beads can be used to support degrading microbes. The attributes of the beads (physical properties, supplement composition,) would be custom-made to the microbial consortium. Beads are used to degrade Supplements and pollutants, limiting our authority over the framework. e.g Geobacter metallireducens, can degrade hydrocarbons and nonaromatic mixes by reducing Fe(III), Mn (IV) and other metals. Consolidating it with an oxygen devouring microbe will make the anoxic condition suitable to play out its biodegradation movement. Some extra consortia could likewise bolster the degraders by changing substrates present in neighborhood condition to a metabolite for which degrades are in search of.



6.3. Role in Pharmaceuticals

Microbes play an important role in production of pharmaceutical products,.for example, anti-toxins, antitumor operators, immunomodulators, protein inhibitors etc. One of the most significant organism for the creation of recombinant proteins are bacteria. i.e, *Escherichia coli*, the yeast i.e, *Saccharomyces cerevisiae* and *Pichia pastoris*, and mammalian cell lines. A portion of the points of interest of utilizing E. coli is the simplicity of building their genetic makeup, fast development, simple cultures and enhanced item yield but a disadvantage is their lack of posttranslational modifications. Yeast, similar to Saccharomyces cerevisiae, is frequently the liked articulation for producing proteins requiring posttranslational alteration. Yeast cells can complete numerous posttranslational alterations. Mammalian cells (i.e, CHO) are generally considered good choice for proteins that can't undergo appropriate posttranslational process by microscopic organisms, yet their fundamental downsides are their helpless discharge, which muddles the refinement procedure, and results in high creation costs. Producing human proteins with the legitimate posttranslational adjustments for their action, utilizing a blend of microbes instead of a solitary specie can give a better response. Each individual specie utilized is a represented considerable authority for protein production in single step. Either single or various microbes can create portion of the proteins, that will be collected.

6.4. Production of Biofuels

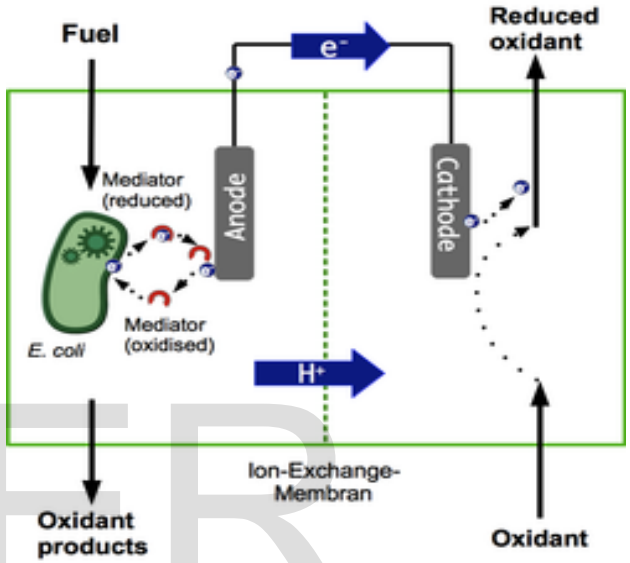
Creation of biofuels using lignocellulose as precursor can play a role provided the structure given by SLMC could encourage the natural chemistry of the microbes .Other than being the most bountiful crude material ,lignocellulose is a renewable source of energy .A consortium made out of one living being just able of processing hexose while a different one go for pentose, was demonstrated to be increasingly appropriate for fermenting such a substrate .During this examination, mixture of the bacterium *Zymomonas mobilis* and the yeast *Pichia stipitis* was utilized and in spite of the fact that they accomplished yields of more than 96% of the hypothetical esteem, an inhibitory interaction. of Zymomonas mobilis on Pichia stipites was seen during co-culture production. Inoculating each specie independently will permit to give additional reasonable development conditions (supplements, T◦, pH.) and avoid inhibitory association, making the process nonstop. To extract from raw materials the catabolic action of the thermophilic anaerobic bacterium *Clostridium thermocellum* can be used .Other than a critical urge to progress the world's fuel creation from fossil fuel to inexhaustible fills, biodiesel may offer a few points of interest over oil based diesel, for example, being totally biodegradable, non-harmful and diminishing emanations of carbon monoxide, sulfur, fragrant hydrocarbons and sediments .Since the lignocellulose transformation into sugars following their maturation to ethanol are carefully anaerobic procedures, while its change into biodiesel occurs in an oxic condition. The transformation of bioethanol into biodiesel occurs in presence of Acinetobacter baylyi.

6.5. Providing Life Support Systems for profound Space Exploration

Another application of SLMC is Life support development for profound space exploration and space missions. A strategy that can be used for recycling the waste can be combination of microbes and plants. In addition to using single or mixed strain cultures, SLMC provides a more descriptive approach of using structured microbial consortia. we accept that the organized microbes consortium technique given by SLMC can give controllability, consistency and soundness vital for such procedures. Goal of NASA's NGLS venture for a long span kept an eye on missions to investigate the Moon and Mars to use nearby crude items a consortia can be more best for bioprocesses. The difficulty faced in unculturable microbial species is due to inability to provide such medium and poor understanding of their interactions. With better comprehension of the ecological conditions required just like the cooperating microbes, we accept that the particularity and control of SLMC will permit us to recreate appropriate environment more effectively.

6.6. Microbial fuel cells

Over 20 years back, scientists saw that a power module containing microorganisms could create power, yet how it happened was a secret. Quite a long while later, scientists began finding that specific microscopic organisms could move electrons to strong stage acceptors, for example, iron oxides. Accordingly, the progressive idea of microbial fuel cells (MFC) was conceived. An MFC works in a similar way as does a customary energy unit, then again, actually microbes living as a biofilm on an anode catalyzes the oxidation of fuel.



The MFC is at progressive advancement for catching sustainable power sources. The bacterial catalysis at the anode makes it conceivable, just because, to utilize inexhaustible natural material (biomass) as a fuel for energy units. Conversely, customary cells utilize a costly platinum impetus on the anode and still can utilize just high-grade H2, which is acquired by improving oil, a nonrenewable vitality asset. By working at encompassing temperature and without ignition, a MFC can dramatically increase vitality catch proficiency while taking out air contamination. Particular microbes are not necessary to create power from organics in a MFC yet motivation originates from the key investigations on physiology, environment, and genomics of specific metal-diminishing microorganisms.,

6.7. Detection of airborne infectious particles

The acknowledgment of developing airborne irresistible ailments, for example, avian flu and severe acute respiratory syndrome (SARS), just as increased worries over bioterrorism, has put a spotlight on the relentless general medical issue of airborne irresistible illness. For quite a long time, general wellbeing experts were inadequately prepared to quantify or distinguish pathogenic organism in air. Currently, the applications of microbial environment standards and strategies to pressurized canned products hold an enormous guarantee for

clarifying airborne ailment and the study of disease transmission, furthermore, structuring frameworks that limit our introduction to allergens, cold infections. Microarray innovation empowers a huge development of the ability of DNA-based strategies as far as the quantity of DNA successions that can be dissected at the same time, empowering molecular distinguishing and portrayal of various pathogens and numerous qualities in a solitary assay.

6.8. Biodegradation of PCE

Tetrachloroethene. and trichloroethene are normal groundwater pollutants that undermine living wellbeing. Fundamental research exposed a new organism, the Dehalococcoides, which has potential to detoxify the chloroethenes. These organisms can catch vitality from reductive dechlorination and converting to ETH and inorganic chlorides, consequently diminishing poisonous groundwater contaminants. One of important aspect is vanishing all chlorine substituents from the ethene backbone, since the chlorinated intermediate obtained at end, vinyl chloride (VC), has more toxicity than original compound. Group of Dehalococcoides organisms are successfully injected at field. Sites, but the innate microbiology is not sufficient to attain desired degradation quality. Understanding the role of main constituents (i.e., Dehalococcoides) and microbe's ecology is vital to implement any technology. For instance, nucleic-acid based methods (for example Quantitative continuous PCR) are intended to explicitly identify and evaluate Dehalococcoides 16S rRNA qualities and qualities embroiled in VC reductive dechlorination. These analytic and prognostic devices changed chloroethene bioremediation to an anticipated science with evident advantages to society.

7. FUTURE PERSPECTIVE AND CONCLUSION:

The 16S ribosomal RNA based strategies, Denaturing Gradient Gel electrophoresis, clone libraries, and Next generation sequencing, can be thoroughly used to recognize the arrangement of bacteria in oil slick locales, yet the following strategies give restricted data on digestion of bacterial networks. Metagenomics and meta-transcriptomics in light of NGS can give information of metabolic possibility of bacteria networks in the contaminated condition at level of DNA and RNA, individually. In any case, these two meta-omics procedures give restricted data on the particularity of a metabolic processes or the distinctions because of physiological or natural conditions. Moreover, investigation of the single cell sequencing information consistently needs support from meta-omics information for extra investigation. Utilization of incorporated methodologies could profoundly depict the parts of an unpredictable network, Exploration is proceeding at a fast pace, and new strategies make certain to be created. Future examination should address issues, for example, connections between survival. qualities of indicator. life forms with respect to those of the pathogens they are intended to anticipate. Moreover, epidemiological studies must be performed which can impose multiple source. tracking. method so that risk assessment can predict result of a specific technique. One significant perspective that will without a doubt require extra examination is the general security of genotypic and phenotypic profiles after organisms have been exposed to different natural stressors.

ACKNOWLEDGMENT

We would like to acknowledge our Respected teacher Dr Imran Hussain, Assistant Professor at the Department of Biotechnology, Quaid-i-Azam University, Islamabad who had made it possible for all of us to do this Job.

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