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Genetically Enhanced Microbial Bioindicators for Environmental Monitoring: A Review

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Abstract

Environmental monitoring has an increased demand in the recent decade with the fluctuation in the quantity of pollution and the aim to preserve this land for the future generation. Bioindicators accounts for the major portion of tool needed for monitoring. Its effective use, cheap technology and its ability to not change the chemistry of natural system once it has been used, makes its functioning peculiar and easy to use. Furthermore, biotechnology has polished the system by incorporating indicators better equipped. The study encapsulates microbial bioindicators that are modified by using genetically engineering and biotechnical approach advancing their functioning for the environment. Two of the major enhanced bioindicators discussed are Vibrio Hervey Strain and Bacterial ammonia oxidizers. The approach is not very common but its demand is opted to increase differentiating it from traditional ways.

Keywords: Bioindicators; Genetics; Biotechnology; Monitoring; Microbes; Environment

Introduction

In some recent decades, one of the major concerns of almost everyone in the world is environmental pollution through different source but mainly from industrial and agricultural sectors, which releases toxic substances in to the environment and harming any organism which is exposed to such harmful and toxic pollutants [1]. Environment is being effected by different kind of new and known pollutants and is being introduced into the environment by anthropogenic activities [2].

In recent years concerns about environment quality are being observed on both local and global level. It is known that environment is degrading due to human activities along with ecosystems, human health and agricultural production and it is all because of harmful substances that are being released into the environment. But when the problems are known it mostly becomes too late to prevent [3]. Normally chemical analytical methods are used for environmental assessment but they are not reliable as they only tell about the potential or type of chemical in sample not the acute toxicity [1]. Living organisms have widely been used and recognized to monitor environmental quality by employing them in air, water and soil as bioindicators and bio monitors [2].

Bioindicators and their forms

Bioindicators are living organisms such as bacteria, animals, birds, etc. they are used to detect the quality of environment. Any physical or behavioral change in them in a specific area gives information that there is something wrong in that area [4]. They are important tool in environment as they detect changes in environment, whether the changes are positive or negative and also give information if these changes have any impact on humans or on their life [5]. The bio-indicators for environmental monitoring are divided into the following groups: plant indicators, animal indicators and microbial indicators.

Plant indicators: Presence or absence of vegetation cover can tell us a lot about the health of that area. It includes grasses, shrubs, herbs, lichens, algae, etc. they are sensitive tools to monitor environmental stress.

Animal indicators: Any increase or decrease of animal population indicates the condition of environment such as, decrease in food production may results in the reduction of the population depending on it.

Microbial indicators: Microorganisms are in abundant form on earth. They are highly sensitive to any change in environment. For instance, if some microorganisms come in contact with pollutants they produce stress proteins, these proteins can be used as an early warning system [4].

Environmental monitoring function of bioindicators

Bioindicators is a term which is given to the living organisms as they indicate any change either positive or negative in environment. They are utilized as they give quick response to any environmental problem. Every plant, animal and microbe has functions in them that help to understand the condition of both terrestrial and marine ecosystems.

Biotechnology effect on performance of indicator

Biotechnology is referred as an enormous field where both science and technology meets. It helps to improve the living standards of people either through food or health [6]. Biotechnology enhanced microorganism by developing their DNA which increases their potential in many new ways [7]. Same is the case with plants and animals' indicators. They are genetically enhanced so that they perform their functions more accurately and indicate changes in

environment at lower level. It is done to make them more sensitive to the change or to produce more proteins to detect the problems in environment.

In order to fight the mutagenic pollution scientists developed *Vibrio harveyi* Strains which is genetically enhanced for marine environment. *Vibrio harveyi* is a bacterium which is present in marine environment. It was cultivated in laboratory and considered safe to use as bio indicator for mutagenic pollution. It was genetically enhanced to produce more strains to detect even low concentration of mutagens [8].

Microbial Bioindicators and Water Quality

Environmental and microbiological research has driven the growing interest in real-time monitoring of water quality using whole-cell microbial biosensors. Yeast, algae and bacterial whole cell biosensors have been applied to domestic wastewater and natural waters to detect phenols, non-ionic surfactants, pesticides, heavy metals and effluents from the chemical industry [2].

Whole-cell naturally-produced microbial bio indicator

Microbial whole-cell biosensors produce a measurable signal enabling detection and quantification of contaminants. Growth characteristics, enzymatic activity or other measureable outputs can be monitored in response to the presence of specific contaminants. Given their ubiquity in aquatic systems algae have been utilized as bio reporters that are capable of detecting contaminants and nutrient fluxes in water. The morphological responses of cyanobacteria to specific nutrients also provides information on nutrient levels, in the absence of nitrogen these organisms form an abundance of nitrogen-fixing heterocyst, whereas in the absence of phosphorus they produce elongated filaments. Some microorganisms possess innate characteristics, such as luminescence or the ability to generate electrical current, which can be utilized to measure metabolic response to environmental contaminants.

Synthetically-manufactured microbial bio indicator

Synthetic biology is now allowing the systematic design of whole-cell biosensors. Typically, a reporter gene is placed under the control of a promoter that is transcriptionally active in the presence of a specific contaminant. Numerous regulatory elements (promoters and their cognate transcriptional regulators) have been identified which respond to specific organic contaminants and heavy metals found in contaminated water such as arsenic, cadmium and mercury. The regulatory elements control the transcription of reporter genes whose expression produces a detectable and quantifiable fluorescent. Synthetically derived microbial biosensors are often created using common laboratory strains of Escherichia coli. While these systems are functional in *E. coli*, a significant challenge is encountered in the real-world application of these biosensors for the detection of contaminants in aquatic settings. This is because *E. coli* lacks many of the physiological characteristics that are required for its survival and proliferation in these niche environments. As a consequence, biosensors are being developed using microbes that inhabit the aquatic environment of interest. Cyanobacteria, which inhabit marine and freshwater environments, have been engineered to detect and provide a measurable signal in response to a range of contaminants Genetically-modified cyanobacteria.

Microbial Bio Indicator and Soil Quality

The rapid increase in population, industrialization, transportation and agricultural practices caused a major problem in soil quality. Like water, soil is also a non-renewable resource. Motor vehicles, unburnt fuel, untreated wastewater from industries and pesticides for agricultural activities are responsible for the increased level of toxic heavy metal in soil [9]. Due to these activities soil quality is highly disturbed [10]. Now the removal of heavy metals from soil is the major priority. For the indication of soil quality many of microbial indicators are used. These indicators accurately and precisely measured the soil condition. Many soil capacities are driven by soil microorganisms and they have in this way been distinguished as suitable indicators for checking of soil status. Genetic profiling of the bacterial ammonia oxidizing network was as of the best scored as soil natural indicators [11].

Synthetically-derived microbial bioindicators for contamination detection

Manufactured science offers the capacity to repurpose microbial genetic reactions to substantial metals, empowering advancement of heavy metal microbial biosensors. Using manufactured science capacities, *de novo* bio sensing builds have been produced for arsenic, cadmium, chromium, lead, mercury and zinc, for identification of substantial metal particles at levels esteemed risky by the World Health Organization to soil. Synthetic pathways have been created comprising of a molecular input joined with detectable output. In these frameworks, an expansion of heavy metal particles into the bacterial cell will result in the statement of a *fluorescent protein* permitting both subjective and quantitative investigation.

Initially, designs were centered on single-input/yield builds; anyway the improvement of multiplexed biosensors that can identify various information sources as well as give different yields will permit more unique detecting. These bio sensing builds have been created with the end goal that they are transferable between a wide scope of naturally pertinent microorganisms including *Shewanella oneidensis*, *Pseudomonas* and *Bacillus spp*. To extend this range further, and mitigate the harmful effect of the heavy metals, microorganisms isolated from known contaminated environments conditions, with a higher resistance towards substantial metals, are being used. Therefore entire cell biosensors can be delivered with detecting abilities in a more extensive scope of situations. Utilizing characterized information and yield modules along manufactured science standards is permitting the focused on advancement of a scope of microbial biosensors to analyses in nature [12].

Heavy metal phytoremediation through microbial indicators

Soil contamination with heavy metals is an overall natural issue. Phytoremediation through phytoextraction and phytostabilization has all the earmarks of being a promising innovation for the remediation of polluted soils. It is essential to unequivocally underscore that a definitive objective of a heavy metal remediation process must be not exclusively to expel the substantial metals from the soil (or rather to decrease their bioavailability and portability) yet additionally to reestablish soil quality [13]. Phytoremediation, or the utilization of green plants to tidy up polluted soil, is a promising innovation for the remediation of soils contaminated with substantial metals [14]. Different systems are also included with term "phytoremediation"; phytoextraction, phytostabilization, phytodegradation,

phytovolatilization, rhizofiltration, rhizoremediationin [15]. In most case phytoextraction and phytoreidiation have been used for the remediation of heavy metal polluted soil.

Phytoextraction refers to the utilization of plants to transport and focus overwhelming metals from the dirt into the harvestable parts of plants. There are two primary systems to phytoextract substantial metals from soil. The principal primary method is called continuous phytoextraction, which utilizes hyperaccumulating plants, for example, Noccaea caerulescens, that have the ability to collect a lot of overwhelming metals from the encompassing soil in their over-the-ground tissues. In excess of 400 plant species are known to be hyperaccumulators [16].

Phytostabilization refers to the procedure whereby substantial metal-tolerant plants are set up in the polluted soil and capacity basically to collect heavy metals into root tissue or help in their precipitation in the root zone with the goal that substantial metal portability and bioavailability is lesser [17]. Preferably, plants utilized for phytostabilization should not collect heavy metals in their aerial parts to keep the passage of these toxins into the nourishment web. Above all, the foundation of a vegetative cover reduces the wind disintegration and water permeation, builds biodiversity, and builds up a solid and utilitarian soil biological community [12].

Genetically Enhanced Bioindicators

Bioindicators are those organisms which detect the ecosystem health. They are very much sensitive to pollution in Environment. They respond to environmental pollution by changing their function, population status, behaviour, morphology or physiology. In this way they could be used for environmental monitoring in different environmental media. This section focus mainly on genetically modified bioindicators. These are such bioindicators whose genetic makeup has been altered by the addition of new gene from donor specie. This genetic modification result in production of organism which can give us desired results against the detection of ecosystem health [18].

Water quality monitoring

Water quality is greatly affected by the presence of indigenous microbes in an aquatic ecosystem. Anthropogenic activities like manufacturing, farming and mining etc. results in accumulation of Physical, chemical and biological pollutants in aquatic ecosystem. These contaminants cause severe changes in water quality [18]. Mutagenic pollution of marine environments is most wide spread and serious problem. Therefore, detection of mutagens in the marine environment is very important. These mutagenic components are present in very low concentrations in aquatic habitats so their detection is not an easy task. Due to presence in minute quantities their biological mutagenicity tests appears to be more accurate and sensitive then chemical analyses [8]. Genetically enhanced bioindicators like microorganisms and macro-invertebrates like bugs are used for water quality monitoring. These can respond to changes in water quality by different ways like bacteria can emit light, change its colour or change their population density. In this way water quality can be detected in fast and cost effective way by using genetically enhanced bioindicators [18].

Enhanced *Vibrio harveyi* strain: For detection of mutagenic pollution in marine environments an organism that naturally lives in these habitats should be used. Most wide spread free living bacterium in marine habitats is *Vibrio harveyi*. Moreover it is easily accessible and non-pathogenic bacterium. So it is completely safe to use under laboratory conditions. In this way *V. harveyi* is considered as bio indicator for the detection of mutagenic pollution in marine habitats. This bacterium will be modified genetically to obtain a highly mutagenic strain which allow the detection of mutagens even in minute quantities. Only few genetic changes will be done because greater changes in genetic makeup can also lead to the production of bacterium that could not be able to survive in natural environmental conditions [8]. For genetic modification in *Vibrio harveyi*, first of all there is need to construct plasmid which serve as a carrier of gene. So for construction of plasmid pAB91273, the SspI-ScaI fragment (containing the mucA and mucB genes) of plasmid pGW1700 was inserted into the EcoRI site of plasmid pFF1. Different Genetic engineering procedures were used for the construction of the plasmid. Luria-Bertani (LB) and BOSS is the culture media used for the growth of bacterium *Escherichia coli* (donor) and *V. harveyi* (recipient) respectively. In the next step Conjugation between *Escherichia coli* (donor) and *V. harveyi* (recipient) strains was performed. Then after inserting gene UV sensitivity test was also carried out by transferring five milliliters of the suspension to a petri dish and UV irradiated. After performing UV sensitivity test the fractions of survivors were calculated. Then finally, Mutagenicity test was carried out. For this purpose *V. harveyi* cells grown in BOSS medium were spread on BOSS plates containing various amounts of mutagens. Plates were incubated for 48 h at 30°C, and colonies were counted. For the liquid medium tests, bacteria were grown in BOSS medium (short-term test) or minimal medium 3 containing 3% NaCl (long-term test) in the presence of different amounts of mutagens for various times. Bacteria were titrated on BOSS plates and BOSS plates with neomycin and the fraction of neomycin-resistant mutants was calculated [8].

***Vibrio harveyi* strain test:** In this test number of genetically modified *V. harveyi* strains grown in BOSS medium were spread on BOSS plates containing various amounts of mutagens. *V. harveyi* strain is sensitive to Neomycin. Plates were incubated for 48 h at 30°C. After that time when colonies were counted it was observed that frequency (number) of *V. harveyi* strain increases rapidly in the presence of mutagens. In this way mutants respond to mutagens in dose response manner i.e., Increase in concentration of mutagens will result in increase in colonies of *V. harveyi* strain. This forms the basis of mutagenicity Test. So in this way *V. harveyi* strain serve as genetically enhanced bioindicator of marine environments [19].

Soil quality monitoring

Soil provides many good and services which are vital to mankind for survival and living. But the problems begin when we add fertilizers, pesticides and insecticides which become cause of changing in soil matrix, composition and structure [20]. Soil is the one of the major non-renewable resource which is key to life support function. So if there is any change in abundance or composition of microbial community could alternate whole ecosystem of soil [21].

Bacterial ammonia oxidizers: Bacterial communities are imperative for productivity and health in soil matrix and soil ecosystem. The monitoring of soil health and quality by using microbial community; is very fresh idea in scientific research community. It is very useful to monitoring the anthropogenic effects on soil and also helpful to determine environmental health. For example scientists used 16S rRNA gene amplicon sequencing, to determine the anthropogenic activities which lead to change in environmental matrix. Scientists

resolved, through 16S rRNA gene amplicon sequencing, the structure of bacterial communities in soil samples from 110 natural or human-impacted sites, located up to 300 km apart [22]. The presences of helminthes in bio-solid waste act as parasitic indictor and also helpful to determine the infection rate in community. The other common bio-indictors in bio-solid are, *Taenia*, *Trichuris*, *Ascaris*, and *Toxocara*. Even *Ascaris* eggs have adverse resistant to extreme environmental conditions and remain active to spread infection for several years [23]. These microorganisms are fecal coliforms. The *Salmonella* spp. is used as bacterial indicators; the helminthic eggs are used as parasitic indicators and somatic phages, as viral indicators [24]. There is strong positive relationship between pH and relative abundance of planctomycetes family specially their *Pirellulaceae* species, between C to N ratio and members of *Gaiellaceae*, and amount of Olsen P between *Chitinophagaceae* species of bacteria [22]. These microorganisms are not genetically enhanced or modified but this bacteria is genetic enhanced that is bacterial ammonia oxidizers. According to researcher the Genetic profiling of the bacterial ammonia oxidizing community showed that they was newly top-scored as soil biological indicator [25]. Bacterial community is not only one here which is genetically modified here Abundance of ammonia oxidizing archaea is also soil bio indicator [11]. The oxidation of ammonia is the first stage in nitrification process. until few years ago researcher believe that the ammonia oxidizing bacteria (AOB) were exclusive responsible of oxidation of ammonia but tables are turn when ground breaking discovery of amoA gene is encoding with unique catalyzes *ammonia monooxygenase* which is play role in ammonia oxidation in *mesophilic Crenarcheota* and in same year the isolation of the first ammonia oxidizing archaea (AOA) take place [26,27]. The AOB have regularly been used as a bio indicator for soil quality assessment and health evaluation [28]. The studies shows the AOB are statistically less abundant than AOA in the soil [29].

Challenging Quest to Incorporate Biotechnology in Environmental Monitoring

Although biotechnology is very helpful in environmental monitoring as genetically enhanced microbes are used as bioindicators for environmental monitoring but still there are some challenges and limitations are present

- Maximum chance of biasness is present during the whole process so great care is required to avoid the risk of biasness.
- As microorganisms are used as bioindicators so the balance of biodiversity in nature greatly disturbs.
- As these microbes are genetically enhanced so while dealing with them they may prove harmful for humans and cause allergies.
- As they are genetically modified so they may produce some toxins which cause harm to the environment to which they are monitoring.
- When these genetically modified microbes are used for monitoring of marine ecosystem they may prove harmful for other organisms present their and ultimately effect will show in food chain.
- While doing monitoring of soil they may release such toxins which make soil unsuitable for crop production.
- Also they have low survival rate.
- Genetically modified microbes are expensive to use.
- Genetic modification may lead to functional disorders in them.
- They can cause harm to those organisms which are essential for our environment.
- Evidence tells that they have potential to produce persistent toxins which can lead to ecological shift.
- When these genetically modified microbes are used as bioindicators in soil, water or any other medium their DNA may end in that water or soil so causing much more complications in that environment.

Conclusion

The study elaborates a new approach towards the monitoring of the environment by introducing genetic technology. The ongoing quest for finding new and better tools to combated pollution at its source before it spreads to an area which becomes uncontrollable, these genetically modifies creatures can be used to put a light on the ongoing situation. As genetics is helpful in producing products that is a great help to humans, its ability to increase the functionality of a naturally produced substances can replace all the bads with all the goods. A new wave of this technology on microbes has been started and it will be preceded for further good results.

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