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A REVIEW ON BACTERIAL BIODIVERSITY – A FIELD OF SCIENCE TO ANSWER TO ALL LIFE-FORM

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ABSTRACT

Bacterial biodiversity is the study of population of bacteria, that include both beneficial and harmful bacteria. There are many methods to study the biodiversity of bacteria from environments, which was started from 16th century. Starting from conventional methods to molecular methods till today many bacterial populations were identified and classified. The best method to study the bacterial biodiversity is the molecular methods. But the great biodiversity of the bacterial world is yet to be discovered.This review paper gives the advantageous and methods to study the bacterial biodiversity.

Keywords: Bacterial Biodiversity, Molecular methods, PCR, sequencing

Review on Bacterial Biodiversity:

New species of microorganisms evolved through the interactions of their genomes with the environment giving rise to great microbial diversity and altered ecosystem functions. The 3 billion years of microbial evolution involved very limited changes in size and morphology. Compared to the evolutionary time scale of multicellular organisms, the pace of microbial evolution was excruciatingly slow. There has been substantially more time for the evolution of numerous diverse microbial species. The great biodiversity of the microbial world is yet to be discovered. (Atlas and Bartha, 1998)

Understanding patterns of bacterial biodiversity is of particular importance because bacteria may well comprise the majority of the Earth's species diversity, they mediate many

of the environmental processes that sustain life on Earth and their diversity is of great applied importance in bioremediation and bioprospecting (Claire Horner – Devine *et al.*, 2003).

The environmental pollution is the major problem, which is creation of human beings. But the degradation of pollutant is mainly brought about by microorganisms like bacteria. Some bacteria while degrading the pollutant produce novel chemicals which can be used in medicine and industries called bioprospecting.

In addition to this some bacteria harmful to the living beings such as plant, animals, human etc. we generally call these bacteria as pathogenic bacteria. So in Earth we study two major forms of bacteria, one is useful or beneficial bacteria and another is harmful bacteria.

The study of biodiversity of bacteria is important for all living beings as well as for sustainability of life on Earth. The subject of bacterial diversity has been reviewed from a microbiological perspective by others, some of the examples are Dworkin, 1992; Nold and Zwart, 1998 and Kent and Triplett, 2002 respectively.

rRNA is the most conserved (least variable) gene in all cells. Portions of the rDNA sequence from distantly related organisms are remarkably similar. For this reason, genes that encode the rRNA (rDNA) have been used extensively to determine taxonomy, phylogeny and the estimate rates of species divergence among bacteria. Thus the comparison of 16S rDNA sequence can show evolutionary relatedness among microorganisms. This work was pioneered by Carl Woese, who proposed the three domain systems of classification – Archaea, Bacteria and Eucarya – based on such sequence information.

To infer relationship that span the diversity of known life, it is necessary to look at genes conserved through the billions of years of evolutionary divergence. An example of genes in this category is those that define the ribosomal RNA s (rRNAs). Most prokaryotes have three rRNA, called the 5S, 16S and 23S rRNA.

The 5S has been extensively studied, but it is usually too small for reliable phylogenetic inference. The 16S and 23S rRNAs are sufficiently large to be useful. The 16S rDNA sequence has hypervariable regions, where sequences have diverged over evolutionary time. These are often flanked by strongly conserved regions. Primers are designed to bind to conserved regions and amplify variable regions. The DNA sequence of the 16S rRNA gene has been determined for an extremely large number of species. In fact, there is no other gene that has been as well characterized in as many species.

Carl Woese recognized the full potential of rRNA sequences as a measure of phylogenetic relatedness. He initially used an RNA sequencing method that determined about $1/4^{th}$ of the nucleotides in the 16s rRNA. This amount of data greatly exceeded anything else then available. Using newer methods, now it is routine to determine the sequence of the entire 16S rRNA molecule. Today, the accumulated 16S rRNA sequences (about 10,000) constitute the largest body of data available for inferring relationship among organisms.

The molecular diversity detection is the greatest advances in microbial ecology in the last few years come from the application of molecular method to the detection of microorganisms in environmental samples. The development of molecular methods permits the detection of specific microbial populations and their activities without the need to culture microorganisms (Atlas and Bartha, 1998). One of the problems in understanding the source of human contamination is the difficulty in isolating the organisms from environment. However, the combination of PCR results with those of culturing bacteria from water samples can provide a more accurate picture of bacterial diversity.

Polymerase chain reaction (PCR) has become one of the most widely used biochemical assays. The speed, specificity and low cost of the procedure have led to its use in such fields as criminal and pathological forensics, genetic mapping, disease diagnosis, systematic and evolutionary studies, diversity study and environmental science. PCR can amplify to detectable levels nucleic acids associated with pathogens or other organisms that may be present in low numbers in water samples. PCR assays detect viruses and other pathogens after concentration from large volumes (100 to 1500 liters) of water. This usually is accomplished by a filter – adsorption and elution method, resulting in a concentrate containing microbes and organic and dissolved solids. PCR is a process in which target DNA, polymerase enzyme and the DNA subunits are combined in a test tube and subjected to temperature changes that spur DNA duplication. Through repetition of this process and under ideal conditions, millions of copies of a single DNA molecule can be generated in just 20 to 30 repetitions of the temperature cycle, each cycle requiring only a minute. The PCR assays can selectively amplify a portion of the target DNA that will allow the pathogen to be identified. The advantages of PCR are numerous. Compared with techniques such as cell culture for the detection of viruses and organisms, the time required for the assay can be reduced from days or weeks to hours. Initial and recurring costs for PCR are much less than cell culture techniques and the technique is easily performed. Additionally, PCR can be used to identify specific pathogens found in water. (Abbaszadegan, 2004)

According to the literature survey only a few reports on identification of bacteria based on molecular method is used in freshwater samples. One such typical example of comprehensive use of molecular techniques used to investigate the diversity and structure of bacterial communities in Arctic and Antarctic ice. These investigators sequenced 16S rRNA from over 200 pure cultures of bacteria (Sigee, 2005). Many of these methods rely on amplification of molecular signature sequences and their detection by hybridization techniques, especially using oligonucleotide probes. Nucleic acid based methods now provide the main means to track specific microbial populations in the environment (Atlas and Bartha, 1998).

Ribosomal RNA homology experiments and ribosomal RNA oligonucleotide cataloguing determine the molecular characteristics just to demonstrate degree of relatedness. Due to the highly conserve nature of rRNA genes, the 16S rRNA nucleotide sequence give better information than DNA molecule. (Dubey and Maheshwari, 2005)

According to work of Martinko and his colleague in 1997 the use of specific 16S rRNA probes has enabled researchers to amplify specific 16S rRNA sequence from mixed cultures and therefore allows phylogenetic analysis, estimation of bacterial diversity and identification of isolates directly from clinical or environmental sites (Ganesan and Muthuchelian, 2009). Hence the molecular technique can be adopted for biological and non-biological samples to study the molecular diversity of bacteria. Detection of bacteria by molecular methods are the best methods, as these methods allow for very specific and rapid detection with or without cultivation step.

Molecular Diversity of Bacteria:

Microbial diversity is the variation among the microorganisms in the environment. Numerous methodological or approaches have been developed to examine number and activities of the microorganisms. Phenotypic detection, microbial staining, biochemical tests, molecular techniques are some of the methods. Some of the most approaches to taxonomy are through the study of proteins and nucleic acids. The common molecular techniques used to study microbial diversity are – nucleic acid hybridization, DNA and plasmid finger printing, DGGE, PCR etc.Diversity is literally means the “variety”. Microbial diversity may be within the species or between species and in ecosystems. To examine the microorganisms in the natural ecosystem requires the analysis of representative samples. Ribosomal RNA is a particularly good marker for phylogenetic studies involving microorganisms. Nucleic acid

hybridization, DNA and plasmid fingerprinting, DNA re-association, DGGE analysis are the common molecular methods used the studying microbial diversity. (Bisby, 1995)

Biodiversity Characterization:

The scientific characterization of biodiversity involves what may seem like two different process, the observation and characterization of the main units of variation i.e, genes, species and ecosystem and the quantification of variation within and between them i.e, like genetic distance, taxonomic relatedness etc. in reality they are part of the same process. The analysis of pattern defines the unit as well as characterizing their variation. (Bisby, 1995)

Characterization of Microbiota:

One of the main tasks of microbiota characterization or taxonomy is to characterize the specimen of microorganisms, so that they can be recognized, used and studied by other key elements in taxonomy work are:

- ✓ Classification – what species and higher taxa exists.
- ✓ Nomenclature – unique scientific names for the species and higher taxa.
- ✓ Descriptions – of the organisms in these species and higher taxa.
- ✓ Identification Aids – with which to identify to which species and higher freshly encountered specimen belongs. (Bisby, 1995)

Sample collection:

Numerous methodological approaches have been developed to examine number and activities or microbial population, in diverse ecosystem. To examine microorganisms in natural ecosystem representative's samples should be analyzed. The term representative means that the sample must reflect the diversity and density of organisms in the entirely of the sampled environment.

The most recent exciting development in biological evolution has come about through advances in molecular phylogeny and macromolecular sequencing. Until, recently it was not possible to scientifically evaluate the evolution of bacteria and other microorganisms. Unlike plant and animals, these organisms are structurally too simple to enable their scant fossil record to be useful for identifying species and activities. However, it became apparent that

the sequence of the subunits of macromolecules such as proteins and nucleic acids retained the evolutionary history of an organism. (Staley and Reysenbach, 2002)

Study of Molecular diversity of Bacteria using some of the past reliable methods:

Bacteria are unicellular, prokaryotic organisms which cannot be seen by naked eye. Identification of bacteria is a process whereby unknown cultures of bacteria can be compared to existing species to determine if they are similar enough to be members of same species. Conventional methods for identification are time consuming one but modern methods are quicker and also helpful in studying the diversity. The modern method includes polymerase chain reaction, DNA finger printing, sequencing etc.

Bacteria often have characteristic shapes and size. They have plasma membrane, but they lack complex internal membrane systems (Prescott *et al.,* 2008). The cytoplasmic matrix typically contains several constituents that are not membrane enclosed: inclusion bodies, ribosomes and the nucleoid with its genetic material. The prokaryotic cell wall almost has peptidoglycan and is chemically and morphologically complex. Most bacteria can be divided into Gram – positive and Gram – negative groups based on their cell wall structure and response to the Gram stain. (Prescott *et al.,* 2008)

Identification and Diversity of Bacteria:

The numerous methods are available to study the diversity, detection, differentiation and identification of bacteria. Some of the methods include phenotypic, biochemical and immunological and molecular techniques. Traditional methods for the detection and enumeration of bacteria largely depend on the use of selective culture and standard biochemical methods (WoseKinge*et al.,* 2012). Although classical methods require long time to conclude, but characterization for each organism can be studied in detail. The confirmation of selected bacteria that are identified by using conventional methods was subjected to molecular methods such as RAPD and 16S rRNA sequencing analysis. One such work was carried out by WoseKinge*et al.,* (2012) by detecting bacterial pathogens in river water from downstream, midstream and upstream of the Crocodile, Elands, Hex, Mooi, Vaal, Molopo, Groot Marico, Harts and Skoonspruit rivers from November 2007 and March, 2008. They isolated pathogenic bacteria such as *E. coli, Salmonella, Shigella*and *Klebsiella*by using first with conventional methods (culturing on agar media, phenotypic characterization, serotype assay and biochemical methods) and then with molecular methods such as DNA extraction, oligonucleotide primers and multiplex PCR method and specificity of primers.

Identification of bacteria includes both conventional and modern methods. But as we know conventional methods are time consuming and modern methods such as molecular methods are quicker and more authentic.

Conventional Methods:

Conventional methods include biochemical tests, microscopic identification, colony morphology, antigenic structure, pathogenicity tests, drug sensitivity tests, serology and phage testing.

Colony Morphology:

Appearance of the bacterial colony is usually characteristics of colour, outline, elevation, margin, size, opacity, consistency etc. (Chakraborty, 1996)

Microscopic identification:

Microscopic identification involves following steps

- Shape and arrangement – under microscope the bacteria can be visualized monococcus, diplococcus, streptococcus, staphylococcus, tetrad, bacillus, spirillum and vibryo
- Use of light microscopes
- Use of electron microscopes (Brown, 2005)

Biochemical tests:

Bacterial species differ in their capacity to attack different carbohydrates, proteins and fats. Most of the biochemical tests are based on the presence of specific enzymes in bacterial cultures such as coagulase, oxidase, urease etc. and productions of metabolic end products of some compounds like sugar present in the culture media are the outcome of enzymatic action of bacteria. Some of the widely used biochemical tests are catalase test, oxidase tests, oxidative fermentation tests, IMViC tests, carbohydrate tests, urease tests, gelatin tests etc.

Molecular identification and Diversity of Bacteria:

Molecular methods are helping to meet the challenge of responding to a rapidly changing disease picture. One advantage of molecular methods is that they use the same

reagents (Ex: PCR Primers) for identifying different bacteria. In the sense, these reagents serve as universal 'molecular medium'.

Bacteria are found in all aspects of life. Not only do bacteria cover our skin and act as protection, but they are found in water and throughout nature. Many of the bacteria throughout nature are unidentified. Due to the numerous amounts of bacteria in our environment and the increasing number of infections, there is a significant demand to identify unknown bacteria that can cause deadly infections. Through the identification of unknown bacterial species, it is likely that they will provide many advances in the medical field and other aspects of life. In microbiology, unknown bacteria samples are often identified using a series of tests. These tests include gram stains, streaks for isolation, and multiple biochemical tests.

But the conventional methods are time consuming and large quantity of chemicals are required, the molecular identification of bacteria are past and less chemicals required and accurate results obtained. The results can be stored and used for further studies. For phylogenetic characterization of putative biofilm anaerobic ammonium oxidizers, a full-cycle 16S rDNA approach was performed by using a *Planctomycetales*-specific forward primer for PCR amplification. Of the twenty-five 16S rDNA fragments (1364 bp in length) amplified from the biofilm, nine were affiliated to the *Planctomycetales*. Comparative analysis showed that these sequences were more than 98.9% similar to each other but only distantly related to the previously recognized anaerobic ammonium oxidizer (below 91% similarity).

Using a combination of metabolic and molecular techniques, community snapshots of sediment bacterial diversity and activity were produced. This study demonstrated that the bacterial communities from all regions sampled were not only metabolically active with the capacity to utilize several different compounds as energy sources but also were genetically diverse. This study is the first to focus on the overall bacterial community, providing insight into this vital component of stream ecosystems. Understanding the bacterial components of aquatic systems such as the Anacostia River will increase knowledge of the overall structure and function of the ecological communities in polluted systems, subsequently enhancing ability to improve the health of this important tidal river.

Using PCR and 16S rDNA sequencing, identification can be obtained in one day and can be concluded faster. 16S is a small part of the ribosomal genome in bacteria, which is a conserved part with minimal changes through evolution, allowing differentiation between

species after sequencing. The 16S genome spans approximately 1500bp. A part of the genome counting for 500bp – 250bp can be used. The rDNA / rRNA was isolated through a purification method. The 16S rDNA / rRNA is amplified by PCR (Thermocycler) and subsequently sequenced (ABI genetic analyzer) to detect the composition of bases in the amplified sequence. Then the sequences are compared with a database (MicroseqIDv 2.1.1). (Kvich*et al.,* www.rigshospitalet.dk/NR/rdonlyres/4C30D340-5123-41A5-954D-7A235CE4A742/0/16S-PCR_engelsk.pdf)

Molecular analysis has the advantage equally applied to both cultivated and environmental samples (Sigee, 2005). According to Ganesan and Muthuchelian, 2009 the most commonly used procedure to assess microbial diversity in environmental samples originated in Paces laboratory. The DNA is extracted from community samples. This is followed by polymerase chain reaction (PCR) has begun to be applied to environmental detection of microorganisms.

Microbial monitoring and identification is essential to identify source of contamination and to establish the level of treatment necessary to ensure safe drinking water. The molecular era that emerged in the 1980s resulted in sequence based molecular methods for detecting pathogens. Conventional bacterial monitoring in water samples worldwide relies on a 100ml grab samples (Abbaszadegan, 2004). The samples analyzed by standard membrane filtration techniques and samples were filtered on to a culture media (Abbaszadegan, 2004) or by using known quantity of samples inoculating onto the standard culture media and colonies are counted and pure cultures can be isolated and further identification by biochemical and molecular methods.

E. coli belongs to the group of faecal coliforms and *Enterobacteriaceae* family. Other species of *Escherichia* genus such as *E. vulneris, E. fergusonii, E. hermanii* and *E. blattae* have been recently described [Baraduc*et al.,* (2000); Holt *et al.,* (2000)]. The high concentrations of faecal coliforms registered at the different sites could be due to the discharges of untreated sewage and non-point sources such as septic effluent, runoff and animal wastes into the water. Runoffs following rainfall also contributed to the elimination and / or transport of *E. coli* in some sampling sites (Nougang*et al.,* 2011). According to Lopez-Pila &Szewzyk (2000); Noble *et al.,* (2004) and Richard *et al.,* (2004), many factors such as temperature, competition, toxicity, predation and even solar radiation influence the survival of *E. coli* in aquatic ecosystems.

Zwart*et al.,* (2002) studied combined diversity using random cloning and have deposited 689 bacterial and 75 plastid 16sr DNA sequences from water column of rivers and lakes in North America, Europe and Asia. Systematic comparisons with the global database showed that the majority of the bacterial sequences were most closely related to other fresh water clones or isolates, while relatively few were closest to sequences recovered from soils or marine habitats. This habitat-specific clustering suggests that the clustered 16S rDNA sequences represent species or groups of species that are indigenous to freshwater. We discerned 34 phylogenetic clusters of closely related sequences that are restricted to freshwater or dominated by fresh water sequences. Of these clusters, 23 contained no cultivated organisms. These putative fresh water clusters were found among the alpha, beta and gamma *Proteobacteria, Cytophagaflavobacterium* - *Bacteroides* group, the *Cyanobacteria,* the *Actinobacteria*, the *Verrucomicrobia*. From this study shows that rivers and lakes have specific planktonic bacterial community distinct from bacteria in neighbouring environments such as soil and sediments. It also points out that these planktonic bacteria are distributed in diverse fresh-water ecosystems around the world. (Zwart*et al.*, 2002)

The most widely used marker gene is the small subunit rRNA gene (16S rDNA), and the recent application of molecular techniques in a variety of habitats has produced a large set of sequences from this gene. This growing data base has taught us that the diversity of the microbial world is much larger than we were able to estimate before the use of molecular techniques [Pace (1986), Hugenholtz*et al.,* (1998)]. However, a clear view of the species or group of species that we can expect in particular environments is still lacking. This is due to the focussed approach followed in many molecular diversity studies. While most studies compare retrieved sequences from the sites under study to the global data base [e.g. large studies by Gloekner*et al.,* (2000) and Urbach*et al.,* (2001)].

Olukunle and Boboye, (2012) performed phylogenetic analysis of oil – degrading bacteria associated with polluted sites in River state, Nigeria. In order to reduce or eliminate the effect of oil spillage on the environment and living organisms using biological process, a study was carried out to isolate oil – degrading bacteria associated with oil – polluted lands. Isolates screened were characterized by molecular method. Phylogenetic analyses of 16S rRNA gene sequence were examined in order to determine evolutionary relationships of the isolates. They characterized 6 species belong to the division *Firmicutes* (Gram positive bacteria) while three species could not be assigned to any known phylum. The 16S rRNA

analysis revealed that the isolates belonged to the genera *Citrobacter, Enterobacter, Klebsiella, Aeromonas, Ewingella*and *Pseudomonas.* They found that percentage of *Proteobacteria* and *Firmicutes* in soil were greater than those in the water samples.

Singh *et al.,* (2012) isolated *A. veronii* from Gomti river, Lucknow district, Uttar Pradesh, India and identified on basis of 16S rRNA sequences, which showed the high degree of homology with existing sequences. They isolated *Aeromonasveronii* on the duplicate and incubated over night at 30^0C. Single colony was selected for primary biochemical test. All these isolates of *Aeromonas*sp were preserved in 15% glycerol at -80^0C in the laboratory.

From year 2012 to till now (2017) many researchers are worked and working on study of biodiversity of bacteria and reviled many interesting facts which is related to evolutionary tree of bacteria that links with eukaryotes, different types of novel proteins, enzymes, carbohydrates, fats and many molecules which is added to the scientific world.

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