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Metagenomics: Current Perspectives and Future Advances

Awalagaway Dhulappa[1] and Keshamma E[2*]

[1]Associate Professor, Department of Microbiology, Maharani Science College for Women, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[2]Associate Professor, Department of Biochemistry, Maharani Science College for Women, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

***Corresponding Author:** Dr. Keshamma E
Associate Professor
Department of Biochemistry,
Maharani Science College for Women, Maharani Cluster University,
Palace Road, Bengaluru-560 001,
Karnataka, India

Abstract

By using a concept similar to the statistical concept of meta-analysis, Handelsman et al. coined the term "Metagenomics," which refers to the genomic analysis of a population of microorganisms. Metagenomics is also called as environmental genomics or community metagenomics it is the field to study microbial community in the natural sample like soil, ocean, river, plants, animals, humans etc. With the advent of next generation sequencing techniques and capability of high-end sophisticated software and tools it is possible to analysed mixed population of genomes. Researchers can now examine how microbes interact with one another and with their surroundings by using metagenomics to study these interactions. This method of examining the microbiome in any natural or biological sample has a wide range of applications in research, including those in the fields of agriculture, animal breeding, textiles, industry, clinical and medical research, food and beverages, and drug discovery. With this context, in the

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current review of literature we intended to describe and delineate on the recent advances and current perspectives in the field of metagenomics.

Keywords: Metagenomics,Microorganisms, Enzymes, Drug discovery, Microbial diversity

INTRODUCTION

The term metagenomics, the genomic analysis of a population of microorganisms, was coined by Handelsman et al. with a notion to analyse a collection of similar but not identical items, as in the statistical concept of meta-analysis.[1] The idea that the whole environmental microbiome can be explored and analysed together has revolutionized our understanding of the ecology around us. It has opened new horizons in the development of biotechnology based on the exploitation of uncultivated microbial species. The vast majority of microorganisms being unculturable,[2] metagenomics has resulted in discoveries that remained hidden from the traditional culturing techniques. Though a multifaceted approach, the crux of applied metagenomics is to express recovered genes in a cultivable heterologous host.[3]

A booming area of biotechnology is the industrial use of microorganisms to produce antibiotics, enzymes, and other bioactive compounds. The demand for the commercial production of enzymes that are used in largescale industrial processes is growing rapidly. The industrial applications of metagenomics include identification of novel biocatalysts, discovery of new antibiotics, personalized medicine, and bioremediation. In addition, biosurfactant producing bacteria have been successfully used for the bioremediation of industrial, agricultural, and domestic wastes, resulting in a reduction of the environmental pollution. A wealth of information has been uncovered by metagenomics, such as microbial diversity, vast swathes of uncharacterized metabolism, and increased complexity of biogeochemical pathways and it

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promises to provide new enzymes and molecules with diverse applications.[3] In fact, the crystal structure of metagenomics derived RNase H1 has also been determined which indicated structure-based mutational shift at the active site of the motif.[4] With these viewpoints in the current narrative review of literature study we aimed to describe and delineate on the recent advances and current perspectives in the field of metagenomics.

HISTORICAL PERSPECTIVES

Metagenomic approaches based on direct isolation of nucleic acids from environmental samples have proven to be powerful tools for comparing and for exploring the ecology. It was Pace and his coworkers in 1985,[5] who introduced the idea a of cloning DNA directly from environmental samples and in 1991, Schmidt et al., used this approach for cloning of DNA from picoplankton in a phage vector for subsequent 16S rRNA gene sequence analyses.[6] In 1995, the first successful function-driven metagenomic libraries was screened and termed that zoolibraries.[7] But the term was coined by Jo Handelsman et al., in 1998 and was defined as 'the genomic analyses of microorganism by direct extraction and cloning of DNA from an assemblage of microorganism'.[1] Due to this the majority of organism which remained unculturable by conventional method become accessible.

Metagenomic is the community genome rather than the microbial community present in an environmental sample. Metagenomic represents a strategic concept that includes investigations at three major interconnected levels, sample processing, DNA sequencing and functional analysis, with an ultimate goal of getting a global view of the functioning of the microbial world, DNA sequencing and functional analysis, with an ultimate goal of getting a global view of the functioning of the microbial world.[8] To recover the novel biomolecules from

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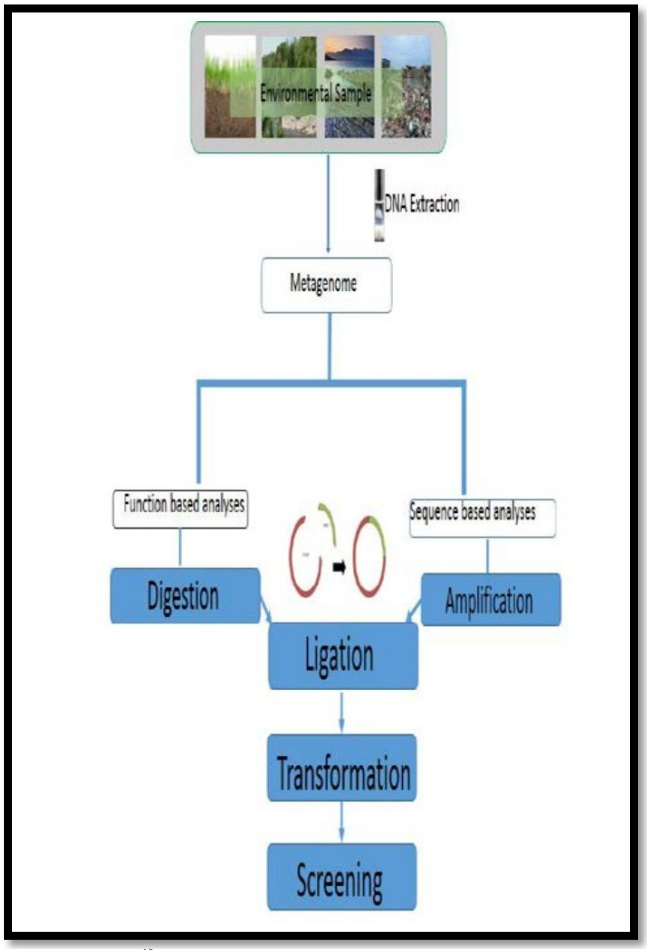
the environmental samples, metagenomic has been divided into two techniques, functional based and sequence based as illustrated in Figure 1.[9]



Courtesy: Nazir A, 2016[10]

Figure 1: Schematic illustration library construction from environmental samples

Functional based analyses will involve steps as follows; (i) Extraction of DNA, (ii) Cloning of DNA into vector [a DNA molecule that carries foreign DNA into a host cell], (iii) Transformation of clone into suitable bacterium [Host cell], and (iv) Screening of transformants. Function based analyses is powerful yet challenging approach to metagenomic analysis is to identify clones that express a function. Success requires faithful transcription and translation of the gene or genes of interest and secretion of the gene product, if the screen or assay requires it to be extra-cellular.[10]

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METAGENOMICS: RESEARCH ADVANCES

Tracing microbial signatures in the lower airways of mechanically ventilated COVID-19 patients associated with poor clinical outcome.[11] It is very well understood that respiratory failure can verily be linked with increased mortality in COVID-19 patients. To predict a clinical outcome, there are no such biomarkers available.[12] So, the investigations were performed into finding whether any of the bacterial respiratory infections were responsible for poor clinical outcome. Metagenomics was used to analyse the microbiome of the lower respiratory tracts of 142 patients who had undergone bronchoscopy.[13] Host immune response was profiled by quantifying SARS-CoV-2 viral load. It was found that poor clinical outcome was very much associated with lower airway enrichment with an oral commensal (*Mycoplasma salivarium*). Increased viral load and a low immune produced in response to it were most predictive of mortality.[14]

The genome sequencing strategies of many microorganisms pathogenic in nature have experienced enhancement due to the much-needed outbreak of essential knowledge about these organisms and this has further improved scope for more information on the diseases caused by these organisms.[15] Also, the ligation between genome sequencing strategies and the microbiome has opened the door toward a better understanding of these diseases. As per exhaustive research, 99% of the microbes remain uncultured and thus cannot be demonstrated by any of the laboratory techniques but with Metagenomics, 16S rRNA which has highly conserved and variable sequences been used for the characterization of microbial communities in diverse conditions.[16] Metagenomic analyses of the air and soil microbes has yielded excellent results and this has further led to the human microbiota which houses microbes of the gut, oral cavity and skin becoming a much talked and researched subject. The fields which have attracted widespread

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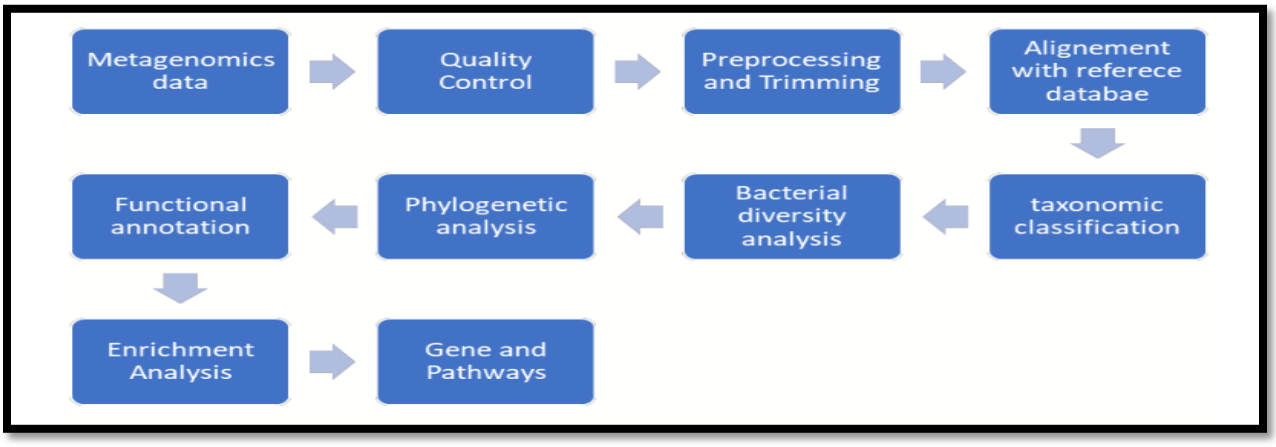
curiosity and scope for exploration are the roles of microbiome in immunity, disease causation and prevention, cancer prevention and defense against pathogens.[17]

Nanopore sequencing has had an extremely beneficial impact on our specific ability to perform a detailed study on a complex microbiological sample. It offers us the possibility to sequence long reads of sample. This is offered in real time as well as with using inexpensive tools and portable technologies. Because long reads can now be used, several of the issues which were often left unaddressed have now been addressed under the diverse umbrella nanopore technology broadly offers.[18] Metagenome Assembled Genomes (MAGs) have now been facilitated access to, also, complex genomic structures can now be resolved. Nanopore technology offers all: low cost, inexpensive machinery and tools, platforms which are portable, rapid protocols and analysis pipelines. All these reasons have helped nanopore technology gain an attractive status when it comes to real-time in-field sequencing. Since, this has eased environmentalmicrobiological analysis of samples to be analysed.[19]

Metagenomics data can be used in number of ways depending upon the objective of the experiment and different results can be generated as illustrated schematically in Figure 2 that can be performed with the help of software's and tools.[20]



Courtesy: Yadav et al., 2022[21]

Figure 2: Schematic illustration of work flow of metagenomics using computations tools

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Shotgun Metagenomics Sequencing/Next Generation Sequencing technologies has eased sequencing technologies since it has now allowed the researchers and analysts to perform a comprehensive research and sample all genes in any organisms present in each sample however complex it be.[22] This method has hence helped microbiologists and researchers perform a critical evaluation of the bacterial diversity and a rigorous detection of the abundance of the microbes in a given sample in any environment. The unculturable microbes which are otherwise difficult to perform an analytical study on have also been traced and performed critical research on due to the means provided by shotgun metagenomic sequencing. From sampling to analysis, shotgun metagenome sequencing has not just eased the methodology being devised to analyse a specific sample but has also touched every array of sample analysis and microbial detection.[23]

PERSPECTIVES ON APPLICATION OF METAGENOMICS

In the field of microbial world, metagenomics has proven as rapidly growing weapon and has changed the way, which microbiologist faced many problems. Among the methods designed to gain access to the physiology and genetics of uncultured organisms, metagenomics, the genomic analysis of a population of microorganisms, has emerged as a powerful centerpiece. It has been estimated that less than 1% of the microorganisms in the natural environment can be cultured in the laboratory. It is increasingly recognized that a huge number of natural products exists in unculturable microbes with chemical, biological, and functional activities for potential uses in various industrial and biomedical applications.[1] Metagenomics provides an unlimited resource for the development of novel genes, enzymes, natural products, bioactive compounds, and bioprocesses that may substantially impact industrial and biotechnological applications.

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Advances in Metagenomics and Enzymes

Metagenomics has proven powerful approach for the ample demand of novel enzymes and biocatalysts.[24] Proteases, amylases, lipases, xylanases, and cellulases and various other industrially important enzymes have been produced through metagenomics. The following are some of the main enzymes that have been unlocked from genetically untapped resources.

Proteases: Proteases represent the class of enzymes which occupy a central position with respect to their physiological roles as well as their commercial applications. They play an invincible role in industrial biotechnology, especially in detergent, food and pharmaceutical arena. They constitute a large family of enzymes present in a wide range of living organisms, such as plants, animals and microorganisms. In biotechnologically oriented systems and processes, however, proteases from microbial origins have often been reported to have distinct advantages when compared to plant or animal proteases, particularly because they possess almost all the characteristics desired for biotechnological applications.[25] Several proteases have been discovered using metagenomic approach in the recent times. Protease from metagenomic DNA isolated from goat skin surface and alkaline Serine protease (AS-protease) was overexpressed. It was found inactive as a result of forming inclusion bodies. The AS-protease was purified with a molecular mass of ~63 kDa. Novel mesophilic protease from metagenomic library was derived from Antarctic coastal sediment. The enzymatic activity was found to be inhibited by 1mM phenylmethyl sulfonylfluoride (PMSF) and hydrochloride 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), indicating that it was a serine protease. (A novel serine protease isolated from soil library was purified and was found to have oxidant stability, which makes it applicable in detergent and bleaching industries. Neveu, et al., also isolated two serine proteases from metagenomic libraries of the Gobi and Death Valley deserts,[26] and an alkaline

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protease gene was extracted from saline habitat and its sequence analyses revealed that enzyme as serine proteases.[27]

Amylases: Amylases are starch degrading enzymes. They are widely distributed in microbial, plant and animal kingdoms. Amylases have potential application in a wide number of industrial processes such as food, fermentation and pharmaceutical industries for the hydrolysis of starch. An exceptionally cold-adapted alpha-amylase AmyI3C6, from a metagenomic library of a cold and alkaline environment was purified and was found similar to α-amylases from the class *Clostridia*. The enzyme was tested against two commercial detergents and was found that enzymes displayed activity in both of them, suggesting that the AmyI3C6 α-amylase may be useful as a detergent enzyme in environmentally friendly, low-temperature laundry processes. Novel Amylolytic Enzyme Encoded by a Gene from a Soil-Derived Metagenomic Library was found to hydrolyzed soluble starch and cyclodextrins to produce high levels of maltose and hydrolyzed pullulan to panose. The enzyme showed a high trans glycosylation activity, making α-(1, 4)linkages exclusively. The hydrolysis andtransglycosylation properties of AmyM suggest that it has novel characteristics and can be regardedas an intermediate type of maltogenic amylase, α-amylase,and4-α-glucanotransferase.[28] Sharmaet al., discovered a novel amylase from a soilmetagenome that retained 90% of activity even atlow temperature suggesting its potentialcandidature for possible industrial applications.[29] A thermostable and calcium-dependentamylase was isolated from a soil metagenome andsuggested its applications in baking anddestarching.[30]

Lipases: The hunt for novel lipases continues unabated as evidenced by the discovery of new families of microbial lipases mostly by metagenomic approaches.[31] Among the hundreds of sequences encoding lipases that have been identified through recent metagenomic studies, it is

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notable that novel sequences are frequently reported. Peng et al. isolated a novel alkaline stable lipase from a metagenomic library constructed from marine sediments and concluded that this novel lipase may be used to impart a distinctive and desirable flavour and odour in milk fat flavor production.[32] Lee et al., isolated and characterized a novel metagenomic lipase from tidal flat sediments which evidence a new family of bacterial lipase.[33] Hardeman and Sjoling also isolated a novel low temperature active lipase from uncultured bacteria of marine sediment. The conserved regions, including the putative active site and catalytic triad, were found to be similar to the culturable lipases.[34]A novel halotolerant lipase was isolated following a functional screening of a marine sponge fosmid metagenomic library by Selvin et al.[35] The stabilityandactivityoverawiderangeofsalinity,pH,andtemperatureand in the presence of organic solvent and metal ions suggesta utility for this enzyme in a variety of industrial applications.Intherecentpast,lipasesisolatedandcharacterizedbyvarious research investigators from various metagenomic libraries showed novelcharacteristics, namely, thermal stability, alkaline stability,organic solvent tolerance, cold active nature, and so forth,making them potential candidates for industrial use.[36-39] Thevast mining of genetically untapped sources for lipases ofcertain unique and desired features like substrate specificity,enantioselectivity, extreme temperature, pH, tolerance, andso forth using culture-independent metagenomic approachhas proved it to be a promising approach for biotechnologicaladvancement.

Xylanases: Hemicellulose, the second most abundant renewable polymer in lingo cellulosic material after cellulose, consists of a complex matrix of polysaccharides constructed from xylan (β-1,4-linked xylose) and mannan (β-1,4-linked mannose). The principle enzyme in the progressive breakdown of xylan is endo-β-1, 4-xylanase which attacks the nonhydrolyzed polymer. In order to efficiently utilize plant biomass for biofuel production, lignocellulose

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degrading enzymes need to be widely developed and utilized. The accessibility of hemicellulose, apart from cellulose, for biofuel production is limited by crosslinking between lignin, cellulose, and hemicellulose via ester and ether linkages. To overcome this problem, the need for xylanases has triggered massive scientific endeavours to unlock novel xylanases from nature using metagenomic approach that may be used to hydrolyze these linkages. The properties like thermostability and tolerance to extreme pH conditions are inevitable for such purposes. The studies of various research investigations yielded significant results with xylanases having immense biotechnological applications for lignocellulosic deconstruction and bioethanol production.[40-42]Moreover, Verma et al. also isolated a novel metagenomic xylanase from compost-soil metagenome that shows alkali stability and thermostability, thus bearing a potential application in paper and pulp industry in pulp bleaching.[43]

*Cellulases:*Cellulases has been isolated from various natural environments like soil, rumen, compost soil and many more using metagenomic technique by constructing the metagenomic libraries followed by screening of the biologically active clones. Many researchers reported isolation of cellulase enzymes from niche environment which include anaerobic digester,[7] alkaline and saline lakes.[44] In 2013, Yeh et al., reported GH12 cellulase gene, RSC-EG1, encoding 464 amino acids along with two other ORFs was isolated from metagenomic library derived from rice straw compost and was seen to have more than 70% similarity on the amino acid level with cellulase from *Micromonospora aurantiaca* and *Thermobispora sp*.[45] Recently, Yadan et al., reported β-glucosidase gene, unglu135B12 belonging to glycoside hydrolase family 3 (GH3).[46]

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FURTURE PERSPECTIVES

The diversity in clinical metagenomics methods, although allowing flexibility, translates to variability in application and performance relative to conventional diagnostics. Given sensitivity and specificity of metagenomics next generation sequencing is influenced by a number of factors including the sample type, quantity of host DNA, the sequencing platform used, number of reads generated, selected reference database, and data analysis tools, as well as issues surrounding the current cost and expertise limitations, it is unlikely clinical metagenomics will be utilized as a first-line approach unless these issues are resolved.[47]

Metagenomic predictions can be used to predict the phenotype of traits that are associated with microbiome variation. Their use is still in its infancy with many areas left to explore and optimize. With large sample numbers now able to be sequenced, metagenomic predictions offer an opportunity for use as proxy traits that can take to place of challenging phenotypes that are expensive and/or difficult to measure on large numbers of individuals, such as enteric methane from ruminants. Future work should focus on dramatically increasing the size of the populations being studied. Testing new machine learning based prediction methods will become possible as the size of datasets increases. The anticipated outcome of larger populations with optimized predictions methods will be more accurate predictions that can be implemented by industry as proxy phenotypes for selection and culling.[48]

CONCLUSIONS

In conclusion, Metagenomics offers a view into a previously unknown, enormously diverse world of microorganisms and makes it possible to access their vast genetic potential to produce products and procedures with biotechnological value. In terms of the industrial sector, the use of metagenomics for utilizing the entire microbiome of a given environmental sample has seen a lot

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of success through advanced discoveries. Positive outcomes have come from the search for novel biocatalysts through metagenomics, with new enzymes being unlocked from the genetically untapped resources that find use in a variety of fields.

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