

Metagenomics and Its Application in Environmental Management

Kavita Kumari, Praveen Maurye, and Archana Sinha*

Aquatic Environmental Biotechnology Division

ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata-700120

*Corresponding Author: sinhaarchana@yahoo.com

Microorganisms have a substantial influence on all existing creature on Globe, directly or indirectly (Cavicchioli et al 2019). Some of these microorganisms are useful where as some are not and majority of them are unknown. The microbes are ubiquitous and influence our life. Traditionally, the microbial study relies on culture-based method. Next Generation Sequencing (NGS) is a powerful tool for analysis of microbes present in environment without need of culture. Metagenomics is study of different microorganisms from environmental samples by functional gene analysis and sequence analysis (Zhang et al 2021). Metagenomics study relies on diversity of microbes, its community composition, their genetic and evolutionary relationship, functional aspects and their interaction and relationship to the environment. Shotgun metagenomics entails in the sequencing of DNA isolated from the whole microbial community and its comparison to the database. In recent years, the research based on metagenomics is rapidly developing in the field of medical science, agriculture, environmental study, and other fields (Alves et al 2018).

Metagenomics

High-throughput metagenomics (Fig. 1) can be done on varied platform, Common sequencing platforms can be used such as Pyrosequencing/ 454 Roche system, Illumina/Solexa system, SOLiD sequencing by applied biosystem, Hiseq2000 by Illumina, Single-molecule sequencing by PacBio and Ion torrent and Ion Proton sequencing.

Metagenomics workflow

The process of metagenomics involves following steps (Fig. 1).

Sample collection and DNA isolation

The samples such as soil, water, tissue, blood can be collected in a sterile container and should be transported in the ice to the laboratory. DNA can be isolated from samples using Kit according to the manufacturer's instructions. Protocol should be

capable enough to produce high-yield and high-quality DNA. Quality and quantity assessment prior to sequencing is mandatory for getting better results.

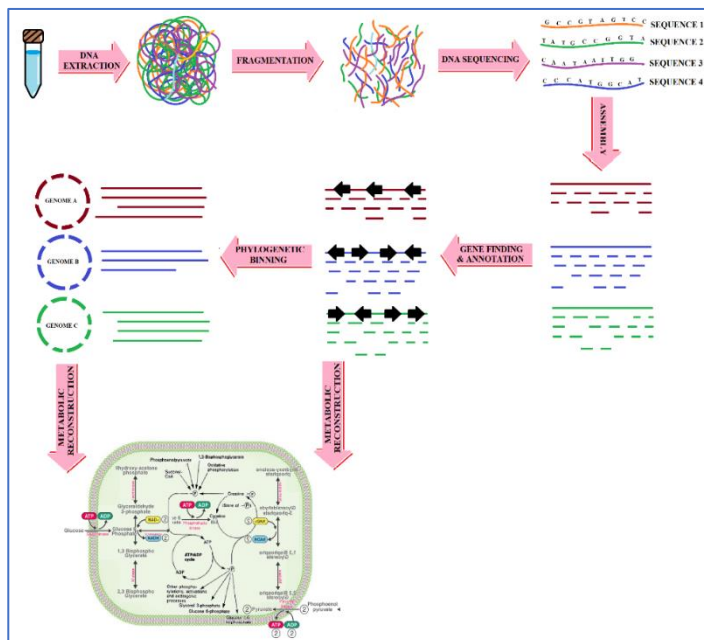


Fig 1: Steps of Metagenomics study

DNA sequencing

The sequencing of DNA is an important step of metagenomics which reads the nucleotide sequence of the DNA present in the sample. NGS technology, like shotgun sequencing, is the most accurate, widely-used and common technique for metagenomics. The processing of DNA depends on sequencing platform. Depending upon the platform, DNA is either fragmented into smaller pieces, cloned into a BAC vector or directly employed for sequencing. End result of any sequence would be a file containing nucleotide sequences- many, short & long and good and bad reads. The sequencing read file generated can be sent to assembly.

Assembly

Assembly is the process of collecting, arranging, and re-constructing the reads to complete the sequencing. Commonly De novo assembly and co-assembly is used. When a set of reference sequence is not available then De novo assembly can be done. The process is intensive, complex and takes a lot of time. The reference-based assembly is called Co-assembly.

The sequence is assembled based on reference sequence available in the database. The process is easy and less tedious.

Binning

After assembly the process of binning can be done. Here we compare the unknown sequence to the already known gene sequence of the database and annotate them accordingly based on its close relationship. This helps to determine the group of microbes and its functional classification.

Gene Prediction and Annotation

Binning gives the rough idea about gene. Further computational analysis requires such as functional analysis and it helps to determine performance of a community in the ecosystem. Subsequently, the predicted protein sequence and function is matched to the protein database to predict its relatedness based on functional analysis for its functional classification.

Application of metagenomics

Metagenomics is useful for investigation of microbes in their natural environment. Various microbial community from environmental samples such as ocean, river, soil, glaciers and other samples can be studied. The study of genomics on a large scale has great advantages in medicine, agriculture, energy production and bioremediation.

Biotechnology studies

The massive uncultured microbes present in the environment can be tapped for new therapeutic molecules and biotechnological applications. The broad spectrum antibiotic Turbomycin A and Turbomycin B was retrieved from soil metagenomics library (Gillespie et al., 2002). Many microbes are useful for the development of novel genes, enzymes and chemical compounds for use in biotechnology. The extremophiles which survive in the various environments cannot be cultured artificially. These are used to derive unique enzymes. These active compounds can be identified by direct sequencing, sequence alignment, with database and functional analysis. These compounds can be used for the commercial development.

Ecological studies

These microbes are key players for maintenance of environmental stability. The microbes are key players of metabolic pathway gene such as energy acquisition, carbon and nitrogen metabolism. All the plant and animals have closely associated host which make necessary nutrients (Carbon, Nitrogen, Oxygen, and sulphur), metals, and vitamins available to them (Nadeem et al., 2018). We also rely on microbes for remediation of various endogenous toxins produced by them as well as the pollutants from exogenous sources (Dvořák et al 2017). Metagenomics explores new functional microorganisms and genes with high degradation efficiency of pollutants, their broad applicability and stable expression. Even in sewage treatment, it is being used for degradation of organic matter as well as removal of nitrogen and phosphorous. A variety of proteolytic enzyme can be used to degrade the pesticides (Zhang et al 2021). The microbial gene responsible for anaerobic degradation of aromatic compounds has been isolated from black sea sediment (Widdel et al 2007).

Healthcare and medical

The metagenomics could be applied in the field such as, identification of pathogenic microorganism and other infection. Various study has revealed the involvement of intestinal microbe with a variety of disease, autoimmune disease and tumour (Holmes et al 2011). At present, it is widely used for identification of antibiotic resistance bacteria and genes. Overall it is useful for high throughput gene level study of microbial community.

The metagenomics plays a vital role in diversity study, species composition identification, and evolutionary study. It is also being applied for wine making, farming. Various electroactive bacteria is being used for production of electrical energy from organic waste (Basu et al 2023).

References

- Alves L. D. F., Westmann C. A., Lovate G. L., de Siqueira G. M. V., Borelli T. C. and Guazzaroni M. E. (2018). Metagenomic approaches for understanding new concepts in microbial science. International journal of genomics, 2312987: 15.

- Basu A., Manna S. and Sil A. K. (2023). A new electro-active bacterium, *Paraclostridium* sp. AKS46, converts waste efficiently into electricity in microbial fuel cell. *Chemical Engineering Journal*, 475: 145626.
- Cavicchioli R., Ripple W. J., Timmis K. N., Azam F., Bakken L. R., Baylis M., et al. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nature Reviews Microbiology*, 17(9), 569-586.
- Dvořák P., Nikel P. I., Damborský J. and de Lorenzo V. (2017). Bioremediation 3.0: engineering pollutant-removing bacteria in the times of systemic biology. *Biotechnology advances*, 35(7): 845-866.
- Gillespie D. E., Brady S. F., Bettermann A. D., Cianciotto N. P., Liles M. R., Rondon M. R., et al. (2002). Isolation of antibiotics turbomycin A and B from a metagenomic library of soil microbial DNA. *Applied and environmental microbiology*, 68(9): 4301-4306.
- Holmes E., Li, J. V., Athanasiou T., Ashrafian H. and Nicholson J. K. (2011). Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. *Trends in microbiology*, 19(7): 349-359.
- Nadeem F., Hanif M. A., Majeed M. I. and Mushtaq Z. (2018). Role of macronutrients and micronutrients in the growth and development of plants and prevention of deleterious plant diseases-a comprehensive review. *International Journal of Chemical and Biochemical Sciences*. 14: 1-22.
- Widdel F., Musat F., Knittel K., and Galushko A. (2007). Anaerobic degradation of hydrocarbons with sulphate as electron acceptor. *Sulphate-reducing Bacteria*. Cambridge University Press, Cambridge.
- Zhang L., Chen F., Zeng Z., Xu M., Sun F., Yang L., et al. (2021). Advances in metagenomics and its application in environmental microorganisms. *Frontiers in microbiology*, 12: 766364.

* * * * *