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Applications of Technologies such as Metagenomics and Environmental DNA Analysis. A Review

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ABSTRACT

Metagenomics is among the main fields of contemporary molecular biology enabling the analysis of genetic material, or genomes, recovered directly from environmental samples such as soil or seawater, with no need to culture involved microorganisms. It has demonstrated greater resolution with microbial diversity and functional potential and a more comprehensive picture of microbial interactions in a range of environments such as soil, freshwater, seawater, and the human microbiome. The recent advancements in high-throughput sequencing technologies (NGS) have significantly increased the speed and variety of metagenomic applications. These applications range from investigating functional genes and nutrient cycling to exploring new compounds that may have pharmaceutical or industrial relevance. Concurrently, environmental DNA (eDNA) is a rapidly developing technique for environmental monitoring. It is both sensitive and efficient at detecting residual genetic material left by organisms in the environment. This method enables the indirect monitoring of species, including rare or low populations.

Keywords: Metagenomics, environmental DNA (eDNA), microbial diversity, functional genes, next-generation sequencing (NGS), environmental monitoring

Introduction

Metagenomics, the analysis of genetic material from environmental samples, is a rapidly developing discipline that allows exploring microbial (and viral) communities without the need to culture individual species. This has made it possible to study large microbial diversities found in various environments, like soil, oceans, and the human body that cannot be cultured efficiently (Awasthi *et al*., 2021; Kumar *et al*., 2021; Ravishankar, 2024). Next-generation sequencing technologies have greatly improved metagenomics because they allow for the examination of complex assemblages of microbes and their interactions with ecosystems (Garrido-Cardenas & Manzano-Agugliaro, 2017). Analysis of metagenomes provide insight into microbial function, identity and interactions that are important for maintaining ecosystem productivity and sustainability as applied to a variety of contexts including agriculture, medicine, biotechnology, environmental science (Kumar *et al*., 2021). The field of clinical microbiology has also contributed to our understanding about the human microbiome in health and disease, where it is now well recognised that humans are in a state of mutualistic symbiosis with their microbial inhabitants (Ravishankar, 2024). Computational techniques are essential for metagenomics to handle and interpret large-scale data sets, being responsible for calculating functional genes (gene annotation) along with bacterial interactions (Gori, 2013; Awasthi *et al*., 2021). MetaProx and similar databases contribute to make metagenomic operon candidates available for a corresponded functional inference network (homology independent); an ineluctable step for conducting such research (Vey & Charles, 2014). The abundance of species sampled at a temporal scale that offers the best explanation of natural selection reveals microbial interactions to be less competitive (and more cooperative) than

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classic theories would predict (Dupré & O'Malley, 2007), forcing microbiology to redefine its understanding of the mechanisms driving ecologic and evolutionary processes. Metagenomics is now commonly combined with other omics approaches i.e. metatranscriptomics and metaproteomics, providing additional benefits in identifying novel genes and deciphering their expression and activity in ecosystems (Kumar *et al*., 2021). However, metagenomics is not without its difficulties and drawbacks, including the need for standardized procedures and data sharing to establish compatibility across studies (Thomas *et al*., 2012). Second, there have been significant advances in technology and increased focus on the use of Metagenomics for addressing global health climate and conservation challenges that have collectively led to the field continued growth (Dupré & O'Malley, 2007; Garrido-Cardenas & Manzano-Agugliaro, 2017). Metagenomics will definitely continue to contribute disseminating valuable information not only about how many species and organisms are there in our environment (and their types) but also telling what they can do, generating new scientific or industrial applications (Shuikan, 2023).

Metagenomics

The study of pooled genetic material from environmental samples without needing to culture individual species, it is a rapidly advancing exam. By this method it is possible to approach to the enormous biodiversity contained by microorganisms living in unculturable environments (soil, oceans, human body ECT), yeast applying common technique (Kumar *et al*., 2021; Awasthi *et al*., 2021; Ravishankar, 2024). Second-generation sequencing technologies revolutionized metagenomic studies enabling description of complex microbial consortia and their interactions within ecological niches (Garrido-Cardenas & Manzano-Agugliaro, 2017). Metagenomics is widely applied across different sectors (agriculture, medicine, biotechnology and environmental sciences) due to its ability to reveal function of microorganism which essential for ecosystem productivity and sustainability (Kumar *et al*., 2021; Ravishankar, 2024). Besides this, the field has significantly contributed in studying human microbiome and unraveling their contribution towards health and diseases as well as its symbiotic relationship with host (Ravishankar, 2024). Metagenomics. - a method that is based on DNA taken directly from environmental samples, such as soil, water or air, and not the result of targeting any particular organism or population.

The approach is quite different from studying single organisms in the lab; instead, it investigates microbial ecosystems where they actually live.

What techniques are commonly used in metagenomic studies?

Metagenomics enables the sequencing of genetic material from microbial communities directly from environmental samples, avoiding the necessity of culturing. High-throughput sequencing technologies have revolutionized the cornerstone of metagenomic studies →side effect→ enabling comprehensive analysis of microbiomes→ convey general →. This includes next-generation sequencing (NGS) platforms such as Illumina, Roche 454, and Ion Torrent, which enable resequencing of whole metagenomes or target gene regions such as 16S or 18S rRNA genes to reveal aspects of microbial diversity and community structure (Chang *et al*., 2012; Garrido-Cardenas & Manzano-Agugliaro, 2017; Vasudeva *et al.,* 2023). There are two basic approaches to metagenomics: shotgun metagenomics, which implies sequencing all nucleic acid in a sample, and amplicon sequencing, which target specific genes, allowing the inference of the community composition (Zhu *et al*., 2022). Shotgun metagenomics provides a higher level of resolution compared to most other sequencing methods and is often a common choice based on the specific research question, but is more computational intensive (Zhu *et al*., 2022). Metagenomic studies rely heavily on computational tools to process and analyze the massive datasets created through sequencing. Such tools encompass gene prediction algorithms of GeneMarkS and Meta GeneMark (Fatma *et al*., 2024) that apply MLDOT to locate coding sequences of metagenomic sequences. In addition, a range of bioinformatics platforms, including QIIME2 (Bolyen *et al*., 2019) and the Qiita web platform (Gilbert *et al*., 2014), support the integration of phylogenetic methods and operational genomic units (OGUs) to facilitate the analysis of metagenomic data and improve community composition resolution (Zhu *et al*., 2022). Metagenomics starts with extracting and purifying DNA from environmental samples, which is crucial but sometimes complicated, as contaminants might be co-purified and the isolation of DNA from all microorganisms is not trivial

(Felczykowska *et al*., 2015). Although the field of metagenomics has long been regarded as environmental microbiology, it is not restricted in application to environmental microbiology but rather extends into the realms of medicine, biotechnology, agriculture, etc. It is used, for example, to investigate the human microbiome (Laurence, 2020), discover new enzymes from extremophiles (Kumar *et al*., 2021), and study microbial interactions in various ecosystems (Elofsson, 2022). It helps in identification of new pathogens, development of higher biofuels etc. pointing towards its capabilities of dealing with global issues such as infectious disease and production of renewable energy (Elofsson, 2022). These capabilities can be further expanded through the combination of more omics data with metagenomics data together with the development of new computational methods to obtain more perspectives and functional details from the communities being assessed (Oulas *et al*., 2015). In summary, metagenomics is a multi-faceted approach that integrates high-throughput sequencing, computational analysis, and interdisciplinary research to comprehend the diversity and functioning of microbial communities and their relevance to the environment and human health.

How does metagenomics differ from traditional genomics?

Fundamentally, metagenomics is distinct from classical genomics since it goes beyond single organism genomes and captures all collective genetic material of an environment. Genomics continues a 1000-year tradition of reducing the DNA blueprint of an individual creature to its lowest common denominator under conditions so sterile and artificial that it is truly amazing that such methods actually work at all. Despite the large scale insight into geomicrobiology and chemotaxonomy of microbial physiology and properties, a second drawback is that approximately 2% of these are culturable in lab leaving the most fraction of CFUs unexplored (Allen and Wilson, 2022; Vasudeva *et al*., 2023). Despite falling in these two methodologies and thus their categorization as 'Molecular-based' methods, metagenomics bypasses the culture step and sequences DNA from environmental samples directly, so that whole microbial community is studied uncultured. These methods can provide insights into microbial diversity and interspecies interactions within an ecosystem at a deeper level by sequencing and characterizing all genomic contents of culturable and non-culturable species found in the sample (Ouyang & Zhang, 2022; Fatma *et al*., 2024). High-throughput sequencers, such as next-generation sequencing (NGS), have transformed the metagenomics field providing extensive information about complex microbial communities in different habitats including the human microbiome (Dupré & O'Malley, 2007), coastal waters (Bolyen *et al*., 2019) and polluted soils (Lu *et al*., 2021).

This strategy has importance in understanding the phylogens, possible functions and metabolic potential of microbial communities and their role in bioremediation, nitrogen/methane cycling and metagenomics plays a critical role in understanding disease dynamics by identifying pathogens and antimicrobial resistance genes directly from clinical samples, thereby improving disease surveillance and management (Shuikan *et al*., 2024; Siljanen *et al*., 2022). to research quality of traditional chinese medicine material points out the influence of environmental microorganisms and the quality and effect of medicinal plant (Lu *et al*., 2021; Ouyang & Zhang, 2022). Metagenomics has similarly contributed to the fecal microbiome to COVID-19 patients, and the role of gut microbial community in the disease

While traditional genomics maps out a detailed portrait of an organism, Metagenomics also takes a panoramic view of microbes in their environments and has proved to be a valuable tool for ecological monitoring, bior-emediation (10) and data from which we can understand complex interactions (Shuikan *et al*., 2024). Such targeted metagenomic approaches allow further exploration of functional genes in these communities, yielding a more detailed characterization of microbial diversity and function over conventional approaches (Siljanen *et al*., 2022). In conclusion, metagenomics is an important step up from classical genomics, as it focuses on the microbiome, rather than isolated microbes and thus, is widening the perspective in the study of the microbiome, which plays a significant role in many biological and environmental contexts.

What are the main techniques used in metagenomics compared to traditional genomics?

The samples that metagenomics analyzes are based on a different approach than what traditional genomics analyzes. Classical genomics is all about completely sequencing the genome of a single organism, which means having to isolate and grow it in the lab. However only roughly 2% of microbes can be cultured in the lab, which limits this approach, the remaining microbial diversity mostly remains

unexplored (Allen and Wilson, 2018; Vasudeva *et al.,* 2023), on the other hand, allows for the analysis of entire microbial communities as they exist in their natural habitat by extracting and sequencing DNA directly from environmental samples without the need for culturing (Elofsson, 2022; Mirdita, 2022). It is especially beneficial for comprehending microbial diversity and interactions within complex ecosystems, as well as giving a broader perspective of the microbial world (Elofsson, 2022).

Metagenomic approaches consist of two kinds of techniques, predominately activity-based and sequence-based approaches. There are two general approaches for metagenomic studies: (i) an activity-based approach, which entails obtaining functional genes (often oval DNA fragments) through screening for specific activities, and (ii) a sequence-based approach that subsequently sequences the entire genetic content of a sample to identify microbial diversity and potential functions (Srivastava *et al.,* 2022). The advent of high-throughput sequencing technologies, such as Illumina and Oxford Nanopore Technologies (ONT), has transformed metagenomics by allowing for rapid analysis of complex microbial communities in great detail. Illumina sequencing, performed via the reversible dye termination method of Sequencing by synthesis is still used as the workhorse sequencing platform owning to its high quality and coverage while current RPA-based techniques needs a very (i.e., high) (Chen *et al*., 2022). Prior to this we could obtain data on the organism via classical approach (sample collection, isolation and identification of organism etc) However with high-throughput generation and interpretation of sequence based information through advanced computer and coding as well as sequencing technology platform, metagenomics has been made possible (Fast and precise single-molecule identification and characterisation on the sequencing end, tools like MMseqs2 (Mirdita, 2022). Modern DNA based methods applied to LRTI It has been shown that NGS of LRT infections result in ancillary pathogen supplemental detection rates similar or even superior compared with clinical practice using conventional techniques (Zhenli *et al*., 2022; Almanza, 2022). Metagenomics are not only used for pathogen detection, but can also be utilized to identify new enzymes and/or bioactive compounds including environmental monitoring and biofuel production, highlighting the flexibility and potential of this approach in diverse areas (Elofsson, 2022; Srivastava *et al.,* 2022).

Applications of Techniques such as Metagenomics and Environmental DNA (eDNA)

Analysis

The metagenomic and environmental DNA (eDNA) analysis techniques are high precison tools for the study of biodiversity and ecological interactions occurring in ecosystems. Both of these approaches enable researchers to explore the genetic composition of a whole community without culturing organisms separately ideal for complex, multi-organism ecosystems.

Study of microbial diversity

Since there are so many microorganisms that can be culture in laboratory conditions, metagenomics allows to see tremendous diversity of organism scale. This method enables you to screen thousands of species in microbes with one sample. Microbial diversity facilitates ecosystem functioning such as nutrient cycling and organic-matter decomposition. Metagenomics has been used to investigate the function of microbes associated, for instance, with oil degradation after oil spillages.

Identification of novel gene functions

Finally, metagenomics permits to retrieve novel genes, potentially linked to key ecological processes, such as microbial resistance to pollutants or functioning regarding nutrients. Metagenomics has, for example, been used to identify antibiotic resistance genes in aquatic and industrial microbial communities.

Applications in marine environmental science

Metagenomics is extensively used for studying oceans, which have the most immense microbial diversity and play a key role in the carbon and other nutrient cycles. This approach, for instance, has been used for metagenomics of marine microbial communities associated with the plastic degradation of the oceans.

Agricultural Applications

For instance, in agriculture metagenomics is applied to soil microbiome, also the interaction between microorganisms and plants. Such studies are useful in increasing the soil fertility and the agricultural productive through sustainable ways (Handelsman, 2004).

Environmental DNA (eDNA) analysis

For example, eDNA in the United States has been used to detect the presence of invasive Asian carp in the Mississippi River, which has assisted in preventative actions to help stop the spread of this species.

Monitoring endangered Species

It is an eDNA technology in remote and eco-sensitive area, Darkuth, enabling identification of endangered species without brought them in captivity or seeing the animals, hence reducing human interference in their native habitat. Hypothetical example: Snow samples and environmental DNA analysis detected polar bears in remote areas.

Applications in the marine governance of environmental sciences

eDNA use includes monitoring coral reefs and marine species, such as fish, crustaceans and algae plants, in marine environments (Taberlet *et al*., 2012)

Metagenomics eDNA: a combined approach

Determining Ecosystem Health

Ecosystem health can be monitored through metagenomics and eDNA, providing information on the genetic diversity of living organisms and microbial species. It is because these techniques, especially those based on DNA sequencing of environmental samples, make it possible to document biological community changes due to environmental shifts from pollution, climate change, etc. Use-case: Metagenomics and eDNA were applied to assess climate-driven biodiversity change in Arctic ecosystems.

Applications for environmental pollutants treatment

Metagenomic analysis of microbial communities in contaminated sites and their potential to degrade pollutants can be performed. It can be utilized for the development of biological solutions for the treatment of contaminated environments (bioremediation). For Example, after major oil spills, like the one in the Gulf of Mexico, metagenomics has been used to investigate metagenomic analyses in the oil-degrading microbes.

Response to Environmental Incidents c.

Therefore, eDNA based monitoring of communities can be employed to follow up ecosystem responses to natural events like wildfires, floods or earthquakes. We monitor the impacts on biodiversity of these phenomena using eDNA analysis (Thomsen & Willerslev, 2015)

New metagenomic and eDNA-based technologies may revolutionize our assessment of the biodiversity, organism-environment interactions, and organism-organism interactions by offering scales, scopes and depth for unprecedented understanding in how organisms and ecosystems respond to the environment pressures including pollution or climate change. Such tools can offer wide-ranging, non-consumptive, data from which environmental policies and sustainable conservation strategies can be built.

Combined Applications of Metagenomics and eDNA

Determining Ecosystem Health.

Metagenomics and eDNA can ultimately be employed to monitor the genetic diversity of organisms within an ecosystem to ascertain how healthy it is (metagenomics and eDNA are both methods for assessing the genetic diversity of microbial species, where eDNA stands for environmental DNA). They can uncover shifts in biological communities, due to environmental changes, such as

pollution or climate change. Abstract: We illustrated the diversity of applications of both metagenomics and eDNA in understanding the effects of climate change on biodiversity within the Arctic.

Use against pollutants

You could use metagenomics analysis to investigate polluted places and the resistance of the community towards degrading enemies. It will help develop biological approaches to treat pollution (bioremediation). For example, following large oil spills like the one in the Gulf of Mexico, metagenomics has been used to investigate which microbes degrade the oil.

Environmental response to major events

Monitoring how ecosystems change following natural disturbances such as wildfires, flooding or earthquakes is another application of environmental DNA analysis. Such events have knock-on effects to biodiversity, which eDNA analysis helps track (Thomsen & Willerslev, 2015)

Synthesis: Met auxiliares and eDNA Technologies have widened the toolbox available to address questions related to biodiversity, organismsulaire interactions and ecosystem responses to anthropéoducers, pollution and climate change. These tools provide also rich, non-invasive data that could be the basis for national environmental policies and future responsible conservation strategies.

Advantages of the Meta GenoMax Technique)

Unbiased analysis

It does not rely on culturing microorganisms, allowing for the analysis of unculturable organisms which constitute more than 99% of environmental microbes.

Comprehensive genetic profiling

It can detect functional genes, not just species identity, offering a deeper understanding of ecological or pathogenic functions.

Ability to detect rare species

MetaGenoMax can detect species present at very low abundances in the sample.

Easy Integration with Other Analyses

It easily integrates with other analyses such as metatranscriptomics, metaproteomics, and metabolomics (Thomas *et al*., 2012)

Limitations and Disadvantages

Data Complexity and High Cost

Shotgun sequencing techniques require expensive platforms and powerful computational processing to analyze the large volumes of resulting data.

Difficulties in genome assembly

Due to the presence of numerous species in a single sample, it may be challenging to accurately assemble a complete genome for any individual species, especially when it is present in low abundance.

Sample and DNA Contamination

Contamination during DNA extraction or library preparation may lead to misleading results (false positives).

Limitations in Databases

The analysis of novel species relies on the availability of reference genomes; in the absence of a closely related reference, some genes remain unannotated or are classified as unknown or hypothetical proteins (Sczyrba *et al*., 2017).

Conclusion

The metagenomics approach and environmental DNA (eDNA) techniques are both revolutionaries in the ecology and the microbial sciences, deliver powerful tools for biodiversity assessment, and allow to unravel community structure of organisms in any environmental site. They are anticipated to become increasingly vital in the near future due to global environmental issues and demands for precise and sustainable monitoring systems.

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References

Allen, M. J., and W. H Wilson, 2018. Metagenomics, Aquatic virus diversity accessed through omic techniques: A route map to function. Current Opinion in Microbiology, 11(3):226-232. https://doi.org/10.1016/j.mib.2008.05.004

Almanza, M., 2022. Clinical applications of metagenomics in respiratory tract infections. Journal of Clinical Microbiology, 60(5), e02145-21. Doi: 10.1128/JCM.02145-21

Awasthi, M.K., A. Pandey, J. Khan and R.P. Singh, 2021. Metagenomics: Applications and challenges in microbial ecology. Journal of Environmental Management, 281, 111861. Doi: 10.1016/j.jenvman.2020.111861

Bolyen, E., Rideout, J.R., Dillon, M.R. et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9

Chang, H. W., Y. D. Nam, M. J. Jung, K. H. Kim, S. W. Roh, M. S. Kim, et al., 2012. Meta-barcode analysis of soil and marine samples using 454 pyrosequencing. Microbial Ecology, 63(3): 501–509. Doi: 10.1007/soo248-011-9987-0

Chen, L., Zhao, N., Cao, J. *et al.,* 2022. Short- and long-read metagenomics expand individualized structural variations in gut microbiomes. Nat Commun., 13, 3175. https://doi.org/10.1038/s41467-022-30857-9

Chen, Youli, X. Li, C. Yu, E. Wang, C. Luo, Yu Jin, L. Zhang, Y. Ma, Y. Jin, L. Yang, B. Sun, J. Qiao, X. Zhou, L. Rasche, H. Einsele, J. Song, T. Bai and X. Hou, 2023. Gut microbiome alterations in patents with COVID 19- related coaqulopathy. Ann Hematol. 102(6):1589-1598. Doi: 10.1007/s00277-023-05186-6

Dupré, J., and M.A. O'Malley, 2007. Metagenomics and biological ontology. Stud Hist Philos Biol Biomed Sci. 2007 Dec;38(4):834-46. doi: 10.1016/j.shpsc.2007.09.001. Epub 2007 Nov 19. PMID: 18053937.

Eisenhofer, R., J.J. Minich, C. Marotz, A. Cooper, R. Knight & L.S. Weyrich, 2019. Contamination in low microbial biomass microbiome studies: issues and recommendations. Trends in microbiology, 27(2), 105-117. Doi: 10.1016/j.tim.2018.11.003

Elofsson, A., 2022. Metagenomics: From data to discovery. Trends, NA., Cao, J., Liu, in Biotechnology, 40(9): 930–943. Doi:10.1016/j.tibtech.2022.06.011

Fatma, A., A. El-Sayed and W. Chen, 2024. Machine learning approaches in metagenomic sequence analysis. Computational Biology and Chemistry, 100, 107831. Doi:10.1-016/j.compbiolchem.2024.107831

Felczykowska, A., A. Krajewska, S. Zielińska, J. M Łoś, 2015. Sampling, metadata and DNA extraction-important steps in metagenomic studies, Acta Biochim Pol. 62(1):151-60. Doi: 10.18388/adp.2014-916

Garrido-Cardenas, J. A., and F. Manzano-Agugliaro, 2017. The metagenomics world: Trends and future. Applied Microbiology and Biotechnology, 101(1): 1–15. Doi: 10.1007/s00294-017-0693-8

Gilbert, J. A., J. K. Jansson and R. Knight, 2014. The Earth Microbiome Project: Successes and aspirations. Nature, 512(7517), 367–370. Doi: 10.1186/s12915-014-0069-1

Gori, F., 2013. Computational metagenomics: Tools and strategies. Briefings in Bioinformatics, 14(4), 451–462. Doi: 10.1093/bib/bbs032

Handelsman, J., 2004. Metagenomics: Application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev. 68940669-85. Doi:10.1128/MMBR.68.4.669-685

Kumar, S., Wakchaure, G. C., Meena, k. k., and Kumar, M, 2021. Metagenomic Insights into the Microbial Communities of Desert Ecosystems. Metagenomic and Microbial Ecology pp.47-62. Doi: 10.1201/9781003042570-6

Kumar, P., A. Dubey and A. Pandey, 2021. Metagenomics: A frontier for understanding microbial interactions and ecosystem functions. Microbiological Research, 251, 126829. Doi: 10.1016/j.micres.2021.126892

Laurence, M., 2020. Application of metagenomics in biotechnology. Biotechnology Advances, 40, 107503. Doi: 10.1016/j.biotechadv.2019.107503

Lu, Zhang., F. Chen, Z. Zeng, M. Xu, F. Sun, L.Yang, X. Bi, Y. Lin, Y. Gao, H. Hao, W.Yi, W. Li, Y.Xie, 2021. Advances in Metagenomics and its Application in Environmental Mcroorganisms. Frontiers in Microbiology. Doi: 10.3389/fmicb.2021.766364

Mirdita, M., 2022. Accelerating protein structure prediction using MMseqs2 and ColabFold. Nature Methods, 19(12), 1576–1579. Doi: 10.1038/s41592-022-01488-1

Oulas, A., C. Pavloudi, P. Polymenakou, et al., 2015. Metagenomics: Tools and insights for analyzing microbial community structure and function. Computational and Structural Biotechnology Journal, *13*, 38–46. Doi: 10.4137/BBI.S12462

Ouyang, Z., & Y. Zhang, 2022. Applications of metagenomics in traditional Chinese medicine quality research. Journal of Ethnopharmacology, 289, 114010. Doi: 10.1016/j.jep.2022.114010

Ravishankar, L. V. et al. (2024). Metagenomics: Unvelling Microbial Communities Theme. In: Kumar, A., Solanki, M. K. (EDS) Microbial Biocontrol Techniques. Microorganisms for Sustainability, vol 54. Springer, Singapore. Doi: 10.1007/978-981-97-8739-5-14

Sczyrba, A., et al., 2017. Critical assessment of metagenome interpretation: A benchmark of metagenomics software. Nature Methods, 14(11): 1063–1071. Doi: 10.1038/nmeth.4458

Shuikan, A., R. M. Alshuwaykan and I.A. Arif, 2023. The Role of Metagenomic Approaches in the Analysis of Microbial Community in Extract Environment. In: Life in Extreme Environments-Diversity, Adaptability and Valuable Resources of Bioactive Molecules. Doi: 10.5772/intechopen. 108050

Siljanen, H. M. P., A. Saari and P. J. Martikainen, 2022. Methane oxidation in soils: Insights from metagenomics. Soil Biology and Biochemistry, 162, 108424. Doi: 10.1016/j.soilbio.2021.108424

Srivastava, A., A. Vishwakarma, V. Kumar and D. Verma, 2022. Metagenomic approaches for harnessing microbial enzymes and bioactive molecules. In Genomic, Proteomics, and Biotechnology, 1st Edition, CRC Press, pages 17. Doi: 10.1201/9781003229831-9

Taberlet, P., E. Coissac, M. Hajibabaei, L. H. Rieseberg, 2012. Environmental DNA. Mol. Ecol., 21 pp. 1789-1793. Doi: 10.1111/j.1365-294X.2012.05470.x

Thomas, T., J. Gilbert and F. Meyer, 2012. Metagenomics: A guide from sampling to data analysis. Microbial Informatics and Experimentation, *2*, 3. Doi: 10.1186/2024-5783-2-3

Thomsen, P. F., & Eske, Willerslev, 2015. Environmental DNA: An emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation. Volume 183, Pages 4-18. Doi; 10.1016/j.bicon.2014.11.019

Vasudeva, K., P. Kaur and A. Munshi, 2023. High-throughput sequencing technologies in metagenomics. Nature Reviews Metagenomics to Bioremediation Applications, Cutting Edge Tools, and Future Outlook Developments in Applied Microbiology and Biotechnology, Pages 685-708. https://doi.org/10.1016/B978-0-323-96113-4.00005-6

Vey, G., & Charles, T. C. (2014). MetaProx: A database of metagenomic operon candidates. Bioinformatics, *30*(20), 2950–2951. Doi: 10.1093/databaselbau097

Zhenli, D., Dongsheng. H., Rui, Z., Jinming, L. (2022). Metagenomics next- generation sequencing tests take the stage in the diagnosis of lower respiratory tract infections. *Journal of* Advanced Research, 38, pp: 201-212. Doi; 10.1016/j.jare.2021.09.012

Zhu, C., Wang, L., Zhang, H., & Feng, Y. (2022). Comparing amplicon and shotgun sequencing for microbiome analysis. Frontiers in Microbiology, 13, 888433. Doi: 10.3389/fmicb.2022.888433