Animal Health Research Journal Vol. 9, No. 1, September 2024 pp. 25-39

ISSN: 2356-7767

A brief overview of metagenomics insights, methodology, and application

Zeinab A.M. Mahdy* **and Hemat S. El-Sayed****

* Bacteriology Department, Animal Health Research Institute- Banha branch, Agriculture Research Center (ARC), Egypt.

** Poultry Diseases Department, Animal Health Research Institute- Banha branch, Agriculture Research Center (ARC), Egypt.

*Corresponding author:
Zeinab Abd EL-Rahman Mohamed
Zeinabmahdy1988@gmail.com

Received in 14/8/2024
Accepted in 16/9/2024

Abstract

Metagenomics is a field of research that analyzes the genetic material of microorganisms directly extracted from environmental or clinical samples. Sequencing is the main method used to obtain and study metagenomic data. Metagenomics can be performed using two main approaches: targeted sequencing and metagenomic shotgun sequencing. The first one selects a specific gene to identify the types of microbes present, but it does not provide information about the functional potential or metabolic pathways of the microorganisms. While metagenomic shotgun sequencing, sequences all the DNA fragments in a sample without any prior selection and it reveals the hidden diversity and functions of the microbes. Metagenomics has many useful applications with very promising potential in both medical and environmental microbiology, such as studying the diversity and function of microbial communities in different habitats, discovering novel enzymes with industrial applications, exploring the microhabitat of human and its role in the general condition of individual, and determine environmental pollution.

Keywords: *Metagenomics application; Metagenomic library; Metagenomic process; Microbiome*

Introduction

Metagenomics means the study of the genetic materials found in an environment **Handelsman *et al.* (1998**). This approach aimed to examine DNA obtained from ecological sources without the isolation or cultivation of microbial population. It is mostly used to find original microbial products and to sample the variety of microorganisms in different communities. The metagenome is a collective reservoir of DNA obtained from each ecological sample that acts as a repository for genetic data that may be examined for the identification of new species as well as the discovery of previously undiscovered biochemical ways and functions **Alves *et al.* (2018**). Metagenomics research focuses on several aspects of microbial communities, such as their diversity, population changes, genetic and evolutionary connections, functional roles and interactions, and environmental impacts. As a result, one of the most significant benefits derived from metagenomics was the ability to process chromosomal characters of organisms that had never been cultivated previously or were difficult to extract into pure cultures **Simon and Daniel (2011).** For example, there were many environmental microorganisms that were difficult to culture in a laboratory. Furthermore, it has been alleged that there were more than 10^9 little and novel Bacterial compounds with significant applications **Stewart (2012**). Metagenomics in the future may be considered as the regular means for many researchers working in the microbial biology through using it as the same as 16S rRNA gene fingerprinting methods to explain microbial community profile **Thomas *et al.* (2012).**

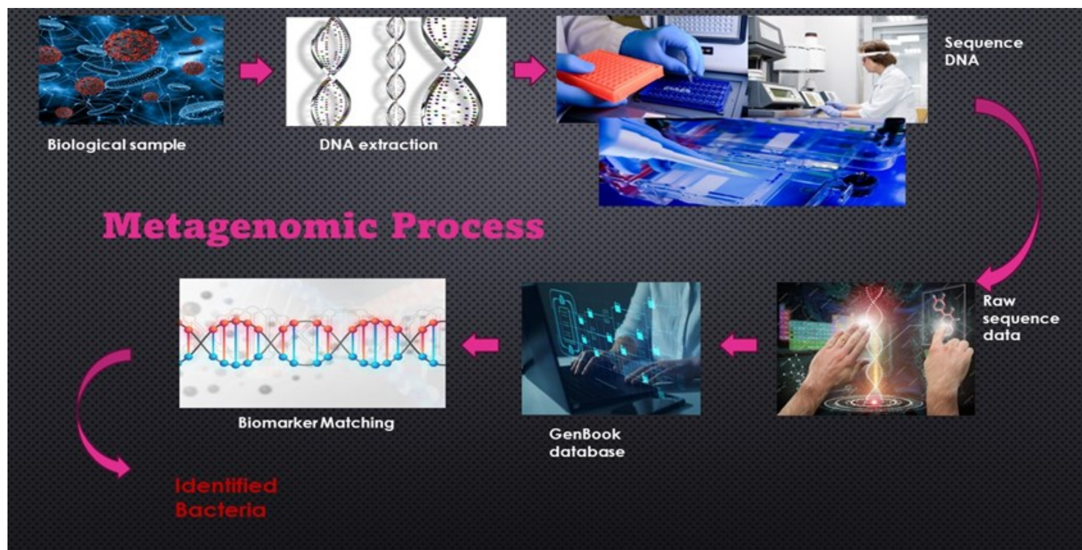

Fig. (1). A diagram in metagenomic process
"Note: The photos used in the construction of the presented diagrams were selected from various internet sources concerning metagenomics"

The Microbiome refers to microhabitat that include certain germs, their genomes, and their close nature **Marchesi and Ravel (2015**). Metagenomic approach helps in determining the whole microbiome of the person detecting all pathogen types of present (bacteria/virus/ parasites). It helps in screening the microbiome of both diseased and healthy individuals as well as help in determining the host- pathogen interactions and identify the integration site of the microbes. Despite these advantages, there are several drawbacks that can be highlighted, including the inability to distinguish between the different types of microorganisms in the samples under study "commensals, pathogens, and symbionts" the requirement for highly qualified professionals who can manipulate samples, analyze data, and interpret results, as well as the high cost of sequencing per sample. **Chiu and Miller (2019**).

Another benefit for Metagenomics is the ability to monitor pathogenic organisms in certain environments and so help in avoiding outbreaks occurring and epidemics. It also may share in decreasing spread of resistant microbes through summarizing the presence of resistomes in different ecosystem and various part worldwide **Sukhum *et al.* (2019**). In particular, complex ecosystems, the system examination of the metagenomics data revealed a substantial link between the hosts and the genes expressing antibiotic resistance. This can expect the existence of genes linked to antibiotic resistance in a certain type of bacteria and suggest the best ways to treat that corruption **Li *et al.* (2015b).**

Methodology

Environmental DNA was first cloned, then functional expression screening was used to launch the field and then matched rapidly by direct random shotgun sequencing technique **Tyson *et al.* (2004).** The metagenomic approach occurs through the following stages: firstly, extraction of all genes in the environmental samples then augmented and cloned them into the vector which help in introduce the extracted gene to the host bacteria by means of plasmid that characterized by ability to replicate to establish the metagenomic library. Finally, the metagenomic library is analyzed and screened. The extraction procedure of metagenomic DNA, as well as the creation and testing of a metagenomic library is required **Zhang *et al.* (2021**). **Fig (2)**

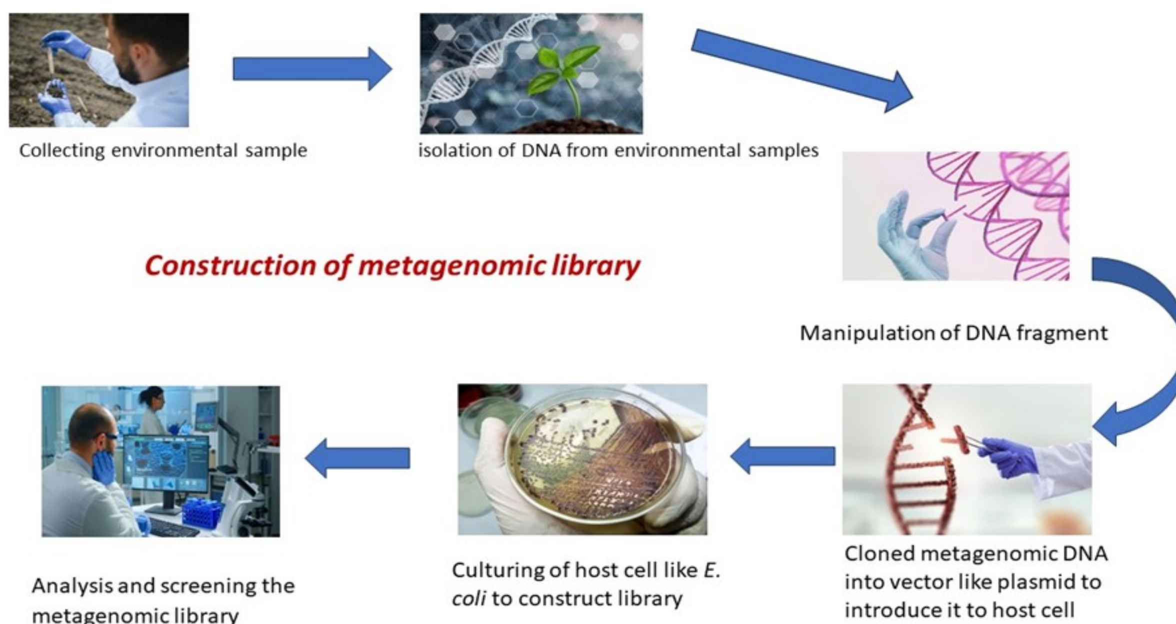

Fig. (2). Construction of metagenomic library
"Note: The photos used in the construction of the presented diagrams were selected from various internet sources concerning metagenomics"

To build the library of metagenome, the extracting of a significant concentration and large fragment of microbial DNA present in nature is required. There are 2 main points to achieve their formation: Firstly, extract all the microbial genomes present in the sample. Secondly, try to preserve the integrity and clarity of the fragment. This may occur through following a strict procedure for sample collection **Zhang *et al.* (2021**). The size of the environmental sample depends on the microbial concentration in the examined sample in which by increasing the density of the microbe, the sample size decreases. For example, fecal test may involve only a swab sample from anus, while in case of marine water analysis which characterized by low microbial community, large quantity of water is collected and purified by filtration. Examined tester must be purified from any Hazards. As humic acid in soil which firmly united to DNA, it must be withdrawn during preparation of sample **Daniel (2005**). DNA samples must be free from any contamination before researching microorganisms in humans or animals. DNA can be refined on the basis of charge using electrophoresis. In case of the presence of contamination with the same charge of DNA, the purification mainly depends on molecular weight by using the following techniques: ion exchange chromatography, size exclusion chromatography **Desai and Madamwar (2007**). To avoid getting too much DNA from the host cell (a plant or an invertebrate) that is associated with the target community, the host cell must be fractionated or selectively lysed. This way, only the DNA of interest is obtained **Thomas *et al.* (2010).** One way to isolate a specific fraction of a microbial community for sequencing is physical fractionation. This method is useful when the host cell genome tried to mask the detection of the microbial sequences due to their large size. For example, to study viruses in seawater samples, Analyst can use filters, centrifuges, or flow cytometers to enrich the viral fraction before sequencing **Palenik *et al.* (2009).** In case of presence of low abundance of DNA in examined sample, enrichment should be occurred either on a cellular or gene level that occurs after extraction of DNA fragment but this method may lead to particular deviancies **Probst *et al.* (2015**). Several methods used for gene enrichment like Stable isotope probing (SIP), suppression subtractive hybridization

(SSH), DNA chip technology, etc. **Avarre *et al.* (2007); Galbraith *et al.* (2008); Chew and Holmes (2009); Chen and Murrell (2010).**

The new biomolecules discovered from environmental samples by metagenomics, through two main methods: functional and sequence-based method **Thomas *et al.* (2012) fig (3).** Functional based analyses involved the subsequent stages a) DNA Extraction, b) Foreign DNA cloning into a vector {to carries it into a host cell}. c) Transformation of clone into suitable bacterium {Host cell} and d) Screening them.

Function-based analysis is a powerful method for studying metagenomes, as it allows the identification of clones that exhibit a specific function. This technique involves expressing and secreting the gene product of the target genes without knowing their sequences so correct transcription and translation of the gene is required. This makes it possible to discover new gene groups that may have novel or known functions for diseases. The main challenge of this technique is that not all genes can be expressed in any chosen host bacterium for cloning **Handelsman (2004**).

Metagenome sequencing is a technique that allows the determination of DNA sequences from a whole metagenome. This application involves the creation of DNA probes or primers created from previously identified preserved regions of already-known genes or protein families. In this way, only new variants of known functional classes of proteins can be recognized **Tamaki *et al.* (2011**).

The extraction process occurs in two ways either through direct or indirect extraction. Direct extraction means using physically extracting method (such as freezing-thawing) or chemically method (like adding chemical substrate as protease or SDS) to destruct microorganisms directly and release microbial DNA **Gabor *et al.* (2003).** This method is preferable in extracting 1-50 kb from DNA present in the examined samples, its simple and effective method, but of low clarity **Schneegurt *et al.* (2003); Wang *et al.* (2011**). While the indirect process occurs through isolation of the microorganism and then extracting DNA using a soft method. This is effective in extracting (20–500 kb DNA) with high purity, but this method may cause damage in some genetic material so, render the method to be burdensome and inefficient **Gabor *et al.* (2003).** The DNA collected by any of the extraction methods must accurately reflect the microbiota found in the sample, producing a large volume of high-quality genetic material as possible.

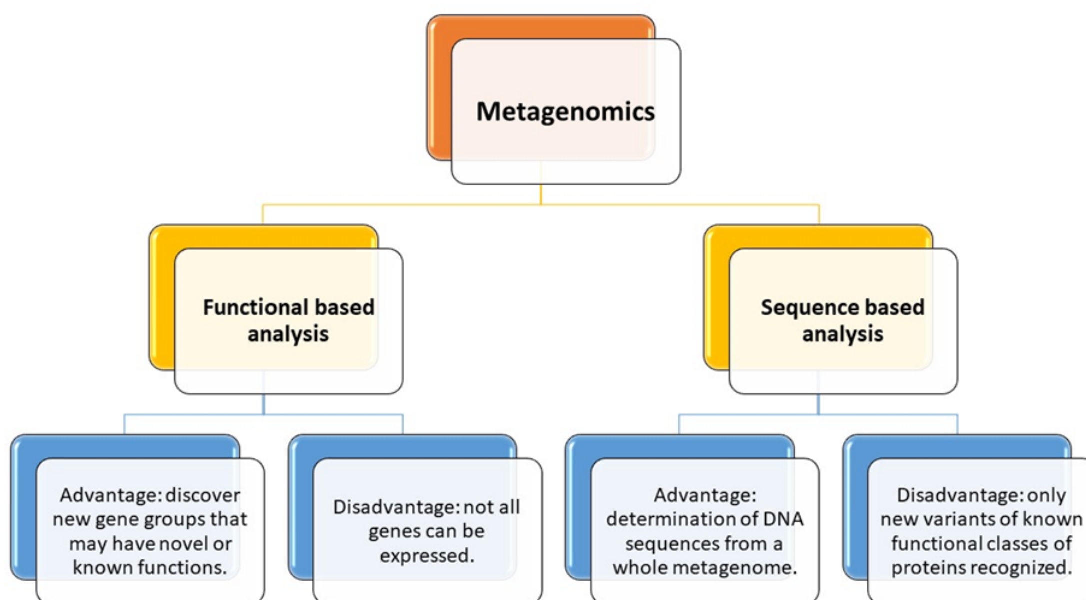

Fig. (3). Methods of metagenomic detection

Formation of Metagenomic Libraries

This is done by fragmenting the extracted DNA into pieces of a specific size; this can be done physically through mechanical shearing or by using restriction enzymes that cleave the DNA at specific locations. They are then glued or fitted as small bits together in an acceptable cloning vector that transfers an alien DNA into a host cell. The recombinant vectors may then be used to transfect the host cells or used for isolation of particular genes of interest, or for storing whole microbial DNA. This vector is necessary to screen the transformed cell and express the gene of interest in the correct system. Among all the vectors used, some of them that are most commonly utilized are- plasmid, cosmid, fosmid or BAC, YAC and phage. The nature and the number and size of the DNA fragment to be cloned dictate the type of the vector to used. Plasmids are ideal for capturing small fragments below 10 kb; Cosmid and fosmid is suitable for large or multiple fragments of between 20 – 40 kb; BAC and YAC are ideal for extremely large fragments measuring between 200–450 kb. BAC shuttle vectors enhance cloning and expression of the targeted DNA in different hosts by enhancing host range **Kakirde *et al.* (2011**).

Various goals and objectives for research vary depending on the host strain that should possess attractive characteristics such as good recombinant vector stability, high transformation efficiency as well as good expressions of the target gene fragment. *Escherichia coli* is the most commonly used host cell because of its ease to culture, but it imposes certain drawbacks that are peculiar to prokaryotic host systems. Therefore, some alternative host cells are being explored, such as host *Streptomyces* **Rebets *et al.* (2017**), *pseudomonas* **Craig *et al.* (2010**), and *Mycobacterium*. While achieving the construction of a library there is the probability of losing the target gene expression because the host cell is not capable of identifying specific promoter or the gene expression. The key to avoiding this is by using multi-host expressions. This method includes cloning the DNA samples into 6 different bacterial species **Craig *et al.* (2010**).

Screening the metagenomic library

The environmental metagenomic library may screened by different methods: function-driven screening, sequence-driven screening, and substrate-induced gene expression screening (SIGEX). Other techniques include DNA stable-isotope probing (DNA-SIP) **Chen and Murrell (2010**) and fluorescence in situ hybridization.

A method to screen for the desired clones based on the functional expression of a foreign gene in a host cell is functional driven screening. This technique uses agar plates and culture media that can indicate the presence of the target gene product by a specific color or plaque formation **Apolinar-Hernández *et al.* (2016); Popovic *et al.* (2017**). The other method is through screening the growth characters of the host strain under selective conditions and determining if any mutants occur **Cheng *et al.* (2017**). This method is quick and easy way to do and does not using any pre-existing sequence data. Most active molecules, such as protease **Apolinar-Hernández *et al.* (2016**), esterase **Popovic *et al.* (2017**), are acquired by this method. A limitation of this approach is that it relies on the expression of functional genes in foreign hosts, which makes the screening efficiency very low. Therefore, choosing the appropriate host and cloning the complete gene or gene cluster is necessary. Functional analysis is a suitable method for genetic screening of large fragment DNA libraries.

In terms of PCR and gene hybridization, the sequence driven screening depends on locus specific oligonucleotide primers or probes for screening of target genes. This approach is suitable for initial screening of enzyme genes that have high sequence homology, which include polyketide synthase gene **Ginolhac *et al.* (2004**) and cellulase genes **Voget *et al.* (2003**). It can screen genes of small DNA fragments without requiring the host cell to express them but will not be able to screen unknown genes. Some screening techniques that are employed on sequences include microarray, stable iso-tope labelling technology, and Fluorescence in situ hybridization. The microarray technology also known as “gene chip technology” highly enhanced and depend on the nucleic acid or gene hybridization **Avarre *et al.* (2007**). A DNA microarray is

a system in which DNA deposits are put on a sterile slip, for instance silicon chip on which several DNA fragments are located. The microarray membrane is capable of binding with labelled samples and then the genes in question can be localized and quantified based on the position and brightness of the binding. It is mostly used to analyze significant genes in pathways that metabolize nutrients and other substances. While DNA microarray technology can be fast in detecting and screening for genes, its specificity and sensitivity in amplification are relatively low when compared with PCR. This makes it unsuitable for finding unknown functional genes **Call (2005); Palka-Santini *et al.* (2009); Mauk *et al.* (2015**).

substrate-induced gene expression screening (SIGEX) method depends on the gene or enzymes involved in metabolism are typically expressed when substrate-induced situations are present **Uchiyama *et al.* (2005); Yun and Ryu (2005**). One of the advantages of SIGEX is that it can identify the functions of enzymes and genes that are unknown from the substrate without modifying it. SIGEX is the best screening method for industrial applications, but it only works for substrates that can enter the cytoplasm and the target gene.

Data analysis

For metagenomic analysis, Next Generation Sequencing (NGS) can be used with two different ways: one is to focus on a specific region of interest (such as the 16S region) and amplify it selectively and known as targeted metagenomics, and the other is to amplify all the sequences in a sample without making any assumptions about its composition and this is called shotgun metagenomics. **Lavezzo *et al.* (2016**). Before sequencing, the DNA quality and composition need to be verified.

Metagenomic analysis can reveal the genes and species in a sample. Amplicon methods only give taxonomic information, but shotgun methods can also give functional information with more accuracy. However, shotgun methods need more data, Linux software, and computing power. Conda and BioConda employed as the tools for setting up and executing the software **Grüning *et al.* (2018**). And using GNU Parallel to run multiple tasks or samples at the same time **Tange (2018**).

The data from Illumina or BGI-Seq500 machines are paired-end reads with different lengths. The first step is to check the quality of the data and remove any reads that come from the host organism. By using Knead Data, which is a pipeline that includes Trimmomatic and Bowtie 2 **Bolger *et al.* (2014**), or use Trimmo matic and Bowtie 2 separately **Langmead and Salzberg (2012).** Trimmomatic can trim low quality sequences, primers, and adapters. Bowtie 2 can map the reads to the host genome and filter them out. Knead Data can do all these steps and give us clean reads.

Metagenomic analysis involves transforming clean data into tables that show the taxonomy and function of different microbes. There are two main methods for this: reads-based and assembly-based. The reads-based methods compare clean reads to existing databases and produce feature tables. Some examples of tools that use this method are MetaPhlAn2, which uses marker genes to identify the taxonomy of microbes (**Truong *et al.* 2015**), Kraken 2, which uses kmer matching and LCA algorithms in order to classify microbes depend on the NCBI database **Wood *et al.* (2019**), and HUMAnN2, which can also measure how different species contribute to a specific function **Franzosa *et al.* (2018).** More information on how different tools perform taxonomic classification was described by **Ye *et al.* (2019).** MEGAN is a software that has a GUI and can do both taxonomical and practical analyses **Huson *et al.* (2016).** There are also many metagenomic gene catalogs for different environments, such as the human gut, the mouse gut, the chicken gut, the cow rumen, the ocean, and the citrus rhizosphere. These catalogs can help with annotating the taxonomy and function of microbes in specific fields of study, making the analysis faster, more accurate, and more efficient.

Assembly-based methods join the clear reads into longer pieces called contigs. Some tools for this are MEGAHIT and metaSPAdes. MEGAHIT is fast and uses less memory, but metaSPAdes can make longer contigs with more computing power **Nurk *et al.* (2017).** Next, the genetic factor in the contigs is found with metaGeneMark or Prokka **Zhu *et al.***

(2010); Seemann (2014). Then, the duplicate genes from different contigs are removed with CD-HIT **Fu *et al.* (2012**). After that, a table of gene density is made with Bowtie 2 or Salmon, which can work with or without alignment. A metagenome has many genes, so they need to classify the genes into functional groups, such as KO, modules and pathways. This reduces the dimensionality of the data. These categories are from KEGG, a database of biological functions **Kanehisa *et al.* (2016).**

Applications of metagenomics

A. In clinical microbiology

I. Recognition of pathogen

Metagenomics is a technique that uses DNA sequencing to identify pathogens in clinical samples. It can complement or replace traditional culture methods and sometimes reveal new diagnoses. However, the application of metagenomics for early detection of diseases has received a little attention by the researchers. For example: using of shout gun metagenomics facilitate the diagnosis of the encephalitis case of unknown cause by **Wilson *et al.* (2014)** in which *Leptospirosis* was identified in the cerebrospinal fluid (CSF) by metagenomics, despite its failed to be isolated from the same sample. The metagenomic diagnosis was confirmed by specific molecular and serological tests.

Metagenomics may also use by some researchers to revisit and resolve cases that remained unexplained as in case of detection of *Mycoplasma salivarium* from cases with arthroplasty in which it isolated from sonicate fluid sample, where it is not detected before in bone or joint infections **Thoendel *et al.* (2017).** In other studies, it pointed to pathogen screening as a comparison with traditional methods as stated by **Hasman *et al.* (2014)** who determined a good agreement between the two methods when analyzed urine samples from patients with suspected urine infections. Metagenomics can also help in diagnose infections faster than conventional culture methods. For example, Ion technology sequencing detected *Pseudomonas aeruginosa* in a bronchoalveolar lavage sample seventeen hours earlier than the standard culture result **Pendleton *et al.* (2017).** Metagenomics correctly recognize the responsible pathogen, although, the samples may show multiple types of microbes and the main cause of infection was not always the most prevalent one. The samples used in all these studies were assumed to be non-sterile. And to make sense of the sequencing results, a control group is necessary, as shown before by **Dubourg and Fenollar (2015).**

In conclusion, some researchers supposed to use metagenomics as a diagnostic tool in some clinical manifestation with the other ordinary tool for detection. While others may use only metagenomics to detect cause of disease occurrence. The interpretation of metagenomics analysis depends on the sample types where the single bacterium detected in cerebrospinal fluid is easier to be detect than the heavy bacterial results in sputum samples **Fukui *et al.* (2015); Salzberg *et al.* (2016).**

II. Detection of resistance gene

Metagenomics can reveal the diversity and distribution of antibiotic resistance genes from diverse environments and various districts around the world **Sukhum *et al.* (2019).** Metagenomics analysis with network methods showed a high association between hosts and resistance genes in certain complex environments **Li *et al.* (2015b**).

Metagenomics helps in determining the antibiotic resistant genes even from uncultivable bacteria. As recorded by **Andersen *et al.* (2016)** that conducted a metagenomic analysis on the gut microbiota to identify several resistant microbes like MRSA, VR *Enterococcus* and MDR *Enterobacteriae* in three groups of patients (patient in high and low risk and controls). They found that patients in risk had significantly higher levels of ARGs than controls. They also showed that metagenomics could detect ARGs that were missed by culture methods. While other studies done by **Zhou *et al.* (2016)** found that ARGs (about 27) had at least one resistance gene in 50% or more of the samples. And found that cephalosporin and tetracycline resistance genes were present in 25.9% of the examined samples.

One of the benefits of metagenomics is that it can enable both the identification of a pathogen and the examination of its antibiotic resistance genes (ARG) in a single approach. This can provide valuable insights into the genetic diversity and evolution of microbial communities

and their resistance mechanisms **Amrane and Lagier (2018).**

III. Epidemic determination.

Metagenomics helps in diagnosis of Epidemic cases as reported by **Loman *et al.* (2013)** who examined 40 samples to determine the Shiga toxigenic *Escherichia coli* O104:H4 outbreak that were appearing positive in a culture. They used shotgun metagenomics to reconstruct the genes of the pathogens and found them in 67% of the samples, however, not all samples had the gene that caused the infection, even with a high sequencing depth.

The metagenomic technology also demonstrate that in two food-borne epidemics caused by *Salmonella* enterica, the outbreaks were not related and were caused by *unalike* strains. They also detected a possible bacterial coinfection **Huang *et al.* (2017).**

Metagenomics has been used to identify various human pathogens from different sources, such as soil **Mohiuddin *et al.* (2017); Abia *et al.* (2019**), air **Be *et al.* (2014**), water bodies **Zhou *et al.* (2019**), manure **Cai and Zhang (2013); Li *et al.* (2015a)**, and numerous organisms and their excretes **Monteiro *et al.* (2016); Fang *et al.* (2015).** Therefore, environmental pathogenic diversity monitoring can help predict and prevent disease outbreaks, as well as the deaths and financial losses associated with them.

B. In viral detection

Although several viruses are well-known, there were still some viruses that remain unknown. Viruses mutated quickly, and it was hard to track and test for their infections. Viruses were also complex and mostly could not be grown in the lab, which made it harder to diagnose and monitor them. But metagenomics had better chances to overcome these challenges because it involved studying the whole DNA. When a new mutated Ebola virus broke out, all the usual tests could not detect it, but metagenomics confirmed it every time **Bibby (2013**).

C. In agricultural microbiology.

A plus of metagenomics is that it can provide metagenomic information of bacteria that may be of great benefit in boosting plant growth. Several functional assays that originated from metagenomics as the antibiogram tests that revealed the genes and changes in indole acetic acid test that change color have been used to evaluate various plant growth promoting bacteria and their genes and products enhancing development of plants **Leveau (2007**). It also arises the question of which microbial communities are specifically linked to plant health. It clear that, different host plants are subjected to different microbiomes differently and thus gives us the opportunity to compare the metagenomes of various plants. Moreover, because the metagenomic data can distinguish between plants, microbiomes, and the surroundings according to their functionality, we can also understand how this complex system works **Busby *et al.* (2017**). This will enable us to develop more sustainable agricultural practices and reduce the loss of yield, food production can be increased, and global malnutrition can be mitigated.

D. Environmental checking by using metagenomics.

Metagenomic analysis of microbial communities and particular genes can assist in tracking the rates of pollution within the atmosphere, ground, and water. These metagenomic features can be used as a pollution biomarkers **Kisand *et al.* (2012).** As an illustration, a study demonstrated the utility of hydrocarbon pollution indicators by comparing metagenomic data from environments containing 255 taxa and 414 functional modules. **Wang *et al.* (2015**). Certain software called MetaBoot can help identify pollution indicators with the help of metagenomic data taken from polluted zones **Wang *et al.* (2015**).

E. Discovery of different enzymes

Metagenomic analysis provided many enzymes that can be useful in several sectors of use of industry, as it is possible to point out to pharmaceuticals, food, paper, textiles, cleaning products, sugar, degradation of alkanes and gold nanoparticles synthesis. Regarding industrial enzymes, one of the most significant groups is amylases, which are formed by different bacteria, archaea and fungi. Amylases can break down starch into glucose units or short glucose chains. These enzymes have diverse applications in different sectors that involve starch processing or modification **de**

Souza and Oliveira Magalhães (2010); Mehta and Satyanarayana (2016). With the aid of metagenomics strategies, numerous amylase enzymes have been identified that are in line with the diverse industrial applications. For example, a sample from the soil in the Western Ghats had an amylase enzyme that can maintain its activity at high temperatures. Many businesses, including the baking industry, have used this enzyme **Vidya *et al.* (2011**).

The preferable places to find new enzyme-producing genes are in soil microbial communities. Some of the enzymes identified by the examination of soil metagenome over the years included amylases, esterases, lipases, beta-glucosidases, racemase, lactonase, proteases, oxidase, and reductase **Lee and Lee (2013**). Additionally, several genes that produce enzymes have been weakened by marine conditions. Esterases, proteases, glycosyl hydrolases, and dehalogenases are a few of the enzymes discovered in marine environments **Popovic *et al.* (2015**).

Enzymes are crucial substances for many industrial applications and the exploration of new enzymes reduces the reliance of various industries on chemical catalysis that can be damaged or generate toxic waste. Moreover, these enzymes offer benefits over the chemical methods in terms of aspects as product specificity and gentle response settings **Chapman *et al.* (2018).** According to a market prediction, the need for enzymes is expected to grow across industries, leading to a 17.50 billion USD market for enzymes by 2024 **Uygut and Tanyildizi (2018**).

F. Microbial metagenomics and discovery of novel therapeutics.

Metagenomics in drug discovery applied as a tool helped identify new drugs and bioactive molecules that could possibly cut the duration and costs of future drug developments. For example: this new class of antibiotics that has been discovered by researchers is mainly dependent on calcium to perform its work and this discovery was arrived at through metagenomic techniques. These antibiotics called malacidins can target and eliminate multidrug-resistant gram-positive bacteria that cause serious infections **Hover *et al.* (2018**). Another exciting find particularly in the field of metagenomics and drug discovery was the identification of divamides, chemicals which are secreted by bacteria that are naturally associated with marine tunicates. These compounds have been found to have antiviral activity against HIV "human immune virus infection" **Smith *et al.* (2018**).

Metagenomic in veterinary medicine.

Animals are important sources of microbes that can infect humans and cause zoonotic diseases. Therefore, it is essential to understand the microbial diversity in animals, both healthy and sick. **Blomström (2011)** described and discussed viral metagenomics as a technique that uses sequence-independent amplification, high-throughput sequencing, and bioinformatics to analyze the whole viral genomes in a sample. He explained and compared the different steps involved in a viral metagenomic study. This technique allows the simultaneous detection and novel identification of multiple viruses, even those that are very different from known ones. And mentioned the applications of viral metagenomics in veterinary science and some of the viruses that have been discovered using this technique. such as novel bocaviruses, Torque Teno viruses, astroviruses, rotaviruses and kobuviruses in porcine disease syndromes, new virus variants in honeybee populations, as well as a range of other infectious agents in further host species **Bela´k *et al.* (2013**). In addition, Metagenomic Next-Generation Sequencing (mNGS) can used to identified uncommon and novel infectious agents that affect common livestock (cattle, small ruminants, poultry, and pigs) as reported by **Kwok *et al.* (2020**), who screened 2481 records and selected 120 records for further analysis. And reviewed the literature on viral mNGS studies in livestock and discovered that pigs were the most frequently studied animals, and that poultry samples had the highest diversity of viruses.

The advances in sequencing technologies and the dramatic reduction of per base costs of sequencing have been key factors in the progress of viral metagenomics which expected to benefit the field of veterinary medicine in various ways, from developing better diagnostic assays to designing new subunit vaccines with minimal investments. These achievements have

encouraged the research to improve the health of animals and, as a result, the animal sector could be growing at an extraordinary level **Kaszab *et al.* (2020**).

Food animals reared in intensive farm mainly received antimicrobial agents as growth promoter to enhance their growth to certain level this led to raised antimicrobial resistant in these animals to an unacceptable level. The metagenomics technique is necessary to investigate the true diversity and distribution of genes that confer resistance to antimicrobials through examinations of the genomes of groups of organisms **Handelsman (2004**). This is because many microbes in the gastrointestinal tract are not or cannot be grown in culture. These uncultivated microbes may play a significant role in maintaining the genes that enable bacteria to resist antimicrobials, as these genes can be transferred horizontally among bacteria.

Application of metagenomics in Egypt

In Egypt, metagenomic approach may be used to examine the microbes from various sources including soil, water, plant, animal and human samples. It could be used in sectors like farming, genetic engineering, environmental conservation, and health care. A number of applications of metagenomic in Egypt include: identification of microorganisms and bacterial microbiome associated with the Egyptian mummies which can illustrate information about their health status, diseases and embalming procedures. For example, **Neukamm *et al.* (2020)** recently extracted the genome of two ancient pathogens – *Mycobacterium leprae* and human hepatitis B virus – from mummified tissues using next-generation sequencing.

There are however a few challenges that metagenomic approaches in Egypt, these include; generally, there is limited funding, weak infrastructure and lack of expertise. On the other hand, there is also the probability of the following advantages with more supportive collaborators, development and availability of bioinformatics tools and databases, and education.

Conclusion

Metagenomics is a rapidly evolving field in the present biology. The advances in sequencing technologies resulted in the production of enormous amounts of genomic data. Moreover, it may help in discover hidden microbes which have a significant impact on both the community itself and the host and habitat associated with it. So, Metagenomics is a revolution science that could be used as a complementary tool for diagnosis.

References

Abia, A.L.K.; Alisoltani, A.; Ubomba-Jaswa, E. and Dippenaar, M.A. (2019). Microbial life beyond the grave: 16S rRNA gene-based metagenomic analysis of bacteria diversity and their functional profles in cemetery environments. Sci Total Environ., 655:831–841.

Alves, L.D.F.; Westmann, C.A.; Lovate, G.L.; de Siqueira, G.M.V.; Borelli, T.C. and Guazzaroni, M.E. (2018). Meta- genomic approaches for understanding new concepts in microbial science. Int J Genom., 2312987. https://doi._org/10._1155/_2018/ 2312987.

Amrane, S, and Lagier, J.C. (2018). Metagenomic and clinical microbiology. Human Microbiome Journal., 9:1–6.

Andersen, H.; Connolly, N.; Bangar, H.; Staat, M.; Mortensen, J.; Deburger, B. and Haslam, D.B. (2016). Use of shotgun metagenome sequencing to detect fecal colonization with multidrug-resistant bacteria in children. J Clin Microbiol., 54(7):1804–1813.

Apolinar-Hernández, M.M.; Peña-Ramírez, Y.J.; Pérez-Rueda, E.; CantoCanché, B.B.; De Los Santos-Briones, C. and O'Connor-Sánchez, A. (2016). Identification and in silico characterization of two novel genes encoding peptidases S8 found by functional screening in a metagenomic library of Yucatán underground water. Gene, 593: 154–161.

Avarre, J.C.; de Lajudie, P. and Béna, G. (2007). Hybridization of genomic DNA to microarrays: a challenge for the analysis of environmental samples. J. Microbiol. Methods, 69: 242–248.

Be, N.A.; Thissen, J.B.; Fofanov, V.Y.; Allen, J.E.; Rojas, M.; Golovko, G.; Fofanov,

Y.; **Koshinsky, H. and Jaing, C.J. (2014).** Metagenomic analysis of the airborne environment in urban spaces. Microb Ecol., 69: 346–355.

Bela´k, S.; Karlsson, O.E.; Blomstro¨m, A.L.; Berg, M. and Granberg, F. (2013). New viruses in veterinary medicine, detected by metagenomic approaches. Review article. Veterinary Microbiology 165 (2013): 95–101.

Bibby, K. (2013). Metagenomic identification of viral pathogens. Trends Biotechnol., 31:275–279. https://doi.org/10.1016/j.tibtech.2013.01.016.

Blomström, A.L. (2011). Viral metagenomics as an emerging and powerful tool in veterinary medicine. Vet Q. 31(3):107-114.

Bolger, A.M.; Lohse, M. and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, 30: 2114–2120.

Busby, P.E.; Soman, C.; Wagner, M.R.; Friesen, M.L.; Kremer, J.M.; Bennett, A.E.; Morsy, M.; Eisen, J.A.; Leach, J.E. and Dangl, J.L. (2017). Research priorities for harnessing plant microbiomes in sustainable agriculture. PLoS Biol., 15(3): e2001793. https://doi.org/10.1371/journal.pbio.2001793.

Cai, L. and Zhang, T. (2013). Detecting human bacterial pathogens in wastewater treatment plants by a high-throughput shotgun sequencing technique. Environ Sci Technol., 47:5433–5441. https://doi. org/10.1021/es400275r.

Call, D.R. (2005). Challenges and opportunities for pathogen detection using DNA microarrays. Crit. Rev. Microbiol., 31: 91–99.

Chapman, J.; Ismail, A.E. and Dinu, C.Z. (2018). Industrial applications of enzymes: recent advances, techniques, and outlooks. Catalysts, 8(6):238. https://doi.org/10.3390/catal8060238.

Chen, Y. and Murrell, J.C. (2010). When metagenomics meets stableisotope probing: progress and perspectives. Trends Microbiol., 18: 157–163.

Cheng, J.; Romantsov, T.; Engel, K.; Doxey, A.C.; Rose, D.R.; Neufeld, J.D. and Charles, T.C. (2017). Functional meta- genomics reveals novel β-galactosidases not predictable from gene sequences. PLoS ONE, 12:e0172545.

Chew, Y.V. and Holmes, A.J. (2009). Suppression subtractive hybridisation allows selective sampling of metagenomic subsets of interest. J. Microbiol. Methods, 78: 136–143.

Chiu, C.Y. and Miller, S.A. (2019). Clinical metagenomics. Nat Rev Genet, 20:341–355. https://doi.org/10.1038/s41576-019-0113-7.

Craig, J.W.; Chang, F.Y.; Kim, J.H.; Obiajulu, S. and Brady, S.F. (2010). Expanding small-molecule functional metagenomics through parallel screening of broad-host-range cosmid environmental DNA libraries in diverse proteobacteria. Appl. Environ. Microbiol., 76: 1633–1641.

Daniel, R. (2005). The metagenomics of soil. Nat. Rev. Microbiol., 3: 470–478.

De Souza, P.M. and de Oliveira Magalhães, P. (2010). Application of microbial α- amylase in industry—a review. Braz J Mi- crobiol., 41(4):850–861.

Desai, C. and Madamwar, D. (2007). Extraction of inhibitor-free metagenomic DNA from polluted sediments, compatible with molecular diversity analysis using adsorption and ion-exchange treatments. Bioresour Technol. 98(4):761-768.

Dubourg, G. and Fenollar, F. (2015). Epidemiologic studies need asymptomatic controls. Clin. Microbiol. Infect. 21(8):51–52.

Fang, H.; Wang, H.; Cai, L. and Yu, Y. (2015). Prevalence of antibiotic resistance genes and bacterial pathogens in long-term manured greenhouse soils as revealed by metagenomic survey. Environ Sci Technol., 49:1095–1104.

Franzosa, E.A.; McIver, L.J.; Rahnavard, G.; Thompson, L.R.; Schirmer, M.; Weingart, G.; Lipson, K.S.; Knight, R.; Caporaso, J.G.; Segata, N. and Huttenhower, C. (2018). Species-level functional profiling of metagenomes and meta- transcriptomes. Nat Methods., 15:962–968.

Fu, L.; Niu, B.; Zhu, Z.; Wu, S. and Li, W. (2012). CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics, 28:3150–3152.

Fukui, Y.; Aoki, K.; Okuma, S.; Sato, T.;

Ishii, Y. and Tateda, K. (2015). Meta genomic analysis for detecting pathogens in culture-negative infective endocarditis. J Infect Chemother., 21(12):882–884.

Gabor, E.M.; de Vries, E.J. and Janssen, D.B. (2003). Efficient recovery of environmental DNA for expression cloning by indirect extraction methods. FEMS Microbiol. Ecol., 44: 153–163.

Galbraith, E.A.; Antonopoulos DA, White BA. (2008). Application of suppressive subtractive hybridization to uncover the metagenomic diversity of environmental samples. Methods Mol. Biol., 410: 295–333.

Ginolhac, A.; Jarrin, C.; Gillet, B.; Robe, P.; Pujic, P.; Tuphile, K.; Bertrand, H.; Vogel, T.M.; Perrière, G.; Simonet, P. and Nalin, R. (2004). Phylogenetic analysis of polyketide synthase I domains from soil metagenomic libraries allows selection of promising clones. Appl. Environ. Microbiol., 70: 5522–5527.

Grüning, B.; Dale, R.; Sjödin, A.; Chapman, B.A.; Rowe, J.; Tomkins-Tinch, C.H.; Valieris, R.; Köster, J. and The Bioconda, T. (2018). Bioconda: sustainable and comprehensive software distribution for the life sciences. Nat Methods, 15:475–476.

Handelsman, J.; Rondon, M.R.; Brady, S.F.; Clardy, J. and Goodman, R.M. (1998). Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol., 5 (10):245-249.

Handelsman, J. (2004). “Metagenomics: application of genomics to uncultured microorganisms" Microbiol. Mol. Biol. Rev. 68(4): 669-685.

Hasman, H.; Saputra, D.; Sicheritz-Ponten, T.; Lund, O.; Svendsen, C.A.; Frimodt-Møller, N. and Aarestrup, F.M. (2014). Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. J Clin Microbiol., 52(1):139–146.

Hover, B.M.; Kim, S.H.; Katz, M.; Charlop-Powers, Z.; Owen, J.G.; Ternei, M.A.; Maniko, J.; Estrela, A.B.; Molina, H.; Park, S.; Perlin, D.S. and Brady, S.F. (2018). Culture-independent discovery of the malacidins as calcium-dependent antibiotics with activity against multidrug-resistant gram positive pathogens. Nat Microbiol., 3:415–422.

Huang, A.D.; Luo, C.; Pena-Gonzalez, A.; Weigand, M.R.; Tarr, C.L. and Konstantinidis, K.T. (2017). Metagenomics of two severe foodborne outbreaks provides diagnostic signatures and signs of coinfection not attainable by traditional methods. Appl Environ Microbiol.,83(3): 2577–2616.

Huson, D.H.; Beier, S.; Flade, I.; Górska, A.; El-Hadidi, M.; Mitra, S.; Ruscheweyh, H.J. and Tappu, R. (2016). MEGAN community edition— interactive exploration and analysis of large-scale microbiome sequencing data. PLoS Comput Biol., 12:1004957

Kakirde, K.S.; Wild, J.; Godiska, R.; Mead, D.A.; Wiggins, A.G.; Goodman, R.M.; Szybalski, W. and Liles, M.R. (2011). Gram negative shuttle BAC vector for heterologous expression of metagenomic libraries. Gene, 475: 57–62.

Kanehisa, M.; Sato, Y. and Morishima, K. (2016). BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. J Mol Biol., 428:726–731.

Kaszab, E.; Doszpoly, A.; Lanave, G.; Verma, A.; Bányai, K.; Malik, Y.S. and Marton, S. (2020). Metagenomics revealing new virus species in farm and pet animals and aquaculture. Genomics and Biotechnological Advances in Veterinary, Poultry, and Fisheries. 2020:29–73. Available at https://doi. org/10.1016/B978-0-12-816352-8.00002-3.

Kisand, V.; Valente, A.; Lahm, A.; Tanet, and Lettieri, T. (2012). Phylogenetic and functional metagenomic profling for assessing microbial biodiversity in environmental monitoring. PLoS ONE 7(8):43630.

Kwok, K.T.T.; Nieuwenhuijse, D.F.; Phan, M.V.T. and Koopmans, M.P.G. (2020). Virus Metagenomics in Farm Animals: A Systematic Review. Viruses. 12(1):107.

Langmead, B. and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat Methods, 9:357–359.

Lavezzo, E.; Barzon, L.; Toppo, S. and Palù, G. (2016). Third generation sequencing tech-

nologies applied to diagnostic microbiology: benefits and challenges in applications and data analysis. Expert Rev Mol Diagn., 16 (9):1011–1023.

Lee, M.H. and Lee, S.W. (2013). Bio- prospecting potential of the soil meta- genome: novel enzymes and bioactivities. Genom Inform., 11:114. https://doi. org/10. 5808/ gi.2013.11.3.114.

Leveau, J.H.J. (2007). The magic and menace of metagenomics: prospects for the study of plant growth-promoting rhizobacteria. Eur J Plant Pathol., 119(3):279–300.

Li, B.; Ju, F.; Cai, L. and Zhang, T. (2015a). Profile and fate of bacterial pathogens in sewage treatment plants revealed by high throughput metagenomic approach. Environ Sci Technol., 49:10492–10502. https://doi. org/10.1021/acs.est.5b02345

Li, B.; Yang, Y.; Ma, L.; Ju, F.; Guo, F.; Tiedje, J.M. and Zhang, T. (2015b). Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. ISME J., 9:2490–2502.

Loman, N.J.; Constantinidou, C.; Christner, M.; Rohde, H.; Chan, J.Z.M.; Quick, J.; Weir, J.C.; Quince, C.; Smith, G.P.; Betley, J.R.; Aepfelbacher, M. and Pallen, M.J. (2013). A culture-independent sequence -based metagenomics approach to the investigation of an outbreak of shiga-toxigenic *Escherichia coli* O104:H4. JAMA, 309 (14):1502.

Marchesi, J.R. and Ravel, J. (2015). The vocabulary of microbiome research: a proposal. Microbiome, 3:31

Mauk, M.G.; Liu, C.; Sadik, M. and Bau, H.H. (2015). Microfluidic devices for nucleic acid (NA) isolation, isothermal NA amplification, and real-time detection. Methods Mol. Biol., 1256: 15–40.

Mehta, D. and Satyanarayana, T. (2016). Bacterial and archaeal α-amylases: diversity and amelioration of the desirable characteristics for industrial applications. Front Microbiol., 7:1129.

Mohiuddin, M.M.; Salama, Y.; Schellhorn, H.E. and Golding, G.B. (2017). Shotgun metagenomic sequencing reveals freshwater beach sands as reservoir of bacterial pathogens. Water Res., 115:360–369.

Monteiro, C.C.; Villegas, L.E.M.; Campolina, T.B.; Pires, A.C.; Miranda, J.C.; Pimenta, P.F. and Secundino, N.F. (2016). Bacterial diversity of the American sand fy Lutzomyia intermedia using high-throughput metagenomic sequencing. Parasites Vectors, 9(1):480.

Neukamm, J.; Pfrengle, S.; Molak, M.; Seitz, A.; Francken, M.; Eppenberger, P.; Avanzi, C.; Reiter, E.; Urban, C.; Welte, B.; Stockhammer, P.W.; Teßmann, B.; Herbig, A.; Harvati, K.; Nieselt, K.; Krause, J. and Schuenemann, V.J. (2020). 2000-year-old pathogen genomes reconstructed from metagenomic analysis of Egyptian mummified individuals. BMC Biol. 18 (1):108.

Nurk, S.; Meleshko, D.; Korobeynikov, A. and Pevzner, P.A. (2017). metaSPAdes: a new versatile metagenomic assembler. Genome Res., 27:824–834.

Palenik, B.; Ren, Q.; Tai, V. and Paulsen, I.T. (2009). Coastal Synechococcus metagenome reveals major roles for horizontal gene transfer and plasmids in population diversity. Environ Microbiol., 11(2):349-359.

Palka-Santini, M.; Cleven, B.E.; Eichinger, L.; Krönke, M. and Krut, O. (2009). Large scale multiplex PCR improves pathogen detection by DNA microarrays. BMC Microbiol., 9:1.

Pendleton, K.M.; Erb-Downward, J.R.; Bao, Y.; Branton, W.R.; Falkowski, N.R.; Newton, D.W.; Huffnagle, G.B. and Dickson, R.P. (2017). Rapid pathogen identification in bacterial pneumonia using real-time metagenomics. Am J Respir Crit Care Med. 196(12):1610-1612.

Popovic, A.; Hai, T.; Tchigvintsev, A.; Hajighasemi, M.; Nocek, B.; Khusnutdinova, A.N.; Brown, G.; Glinos, J.; Flick, R.; Skarina, T.; Chernikova, T.N.; Yim, V.; Brüls, T.; Paslier, D.L.; Yakimov, M.M.; Joachimiak, A.; Ferrer, M.; Golyshina, O.V.; Savchenko, A.; Golyshin, P.N. and Yakunin, A.F. (2017). Activity screening of environmental metagenomic libraries reveals novel carboxylesterase families. Sci. Rep.,

7:44103.

Popovic, A.; Tchigvintsev, A.; Tran, H.; Chernikova, T.N.; Golyshina, O.V.; Yakimov, M.M.; Golyshin, P.N.; Yakunin, A.F. (2015). Metagenomics as a tool for enzyme discovery: hydrolytic enzymes from marine-related metagenomes. Adv Exp Med Biol. 883:1-20.

Probst, A.J.; Weinmaier, T.; DeSantis, T.Z.; Santo Domingo, J.W. and Ashbolt, N. (2015). New perspectives on microbial community distortion after whole-genome amplification. PLoS ONE, 10(5): 0124158.

Rebets, Y.; Kormanec, J.; Luzhetskyy, A.; Bernaerts, K. and Anné, J. (2017). Cloning and expression of metagenomic DNA in Streptomyces lividans and subsequent fermentation for optimized production. Methods Mol. Biol., 1539: 99–144.

Salzberg, S.L.; Breitwieser, F.P.; Kumar, A.; Hao, H.; Burger, P.; Rodriguez, F.J.; Lim, M.; Quiñones-Hinojosa, A.; Gallia, G.L.; Tornheim, J.A.; Melia, M.T.; Sears, C.L. and Pardo, C.A. (2016). Next generation sequencing in neuropathologic diagnosis of infections of the nervous system. Neurol – Neuro immunol Neuroinflammation, 3(4): 251.

Schneegurt, M.A.; Dore, S.Y. and Kulpa, C.F.J.r. (2003). Direct extraction of DNA from soils for studies in microbial ecology. Curr. Issues Mol Biol., 5: 1–8.

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069.

Simon, C. and Daniel, R. (2011). Meta- genomic analyses: past and future trends. Appl Environ Microbiol., 77(4):1153–1161.

Smith, T.E.; Pond, C.D.; Pierce, E.; Harmer, Z.P.; Kwan, J.; Zachariah, M.M.; Harper, M.K.; Wyche, T.P.; Matainaho, T.K.; Bugni, T.S.; Barrows, L.R.; Ireland, C.M. and Schmidt, E.W. (2018). Accessing chemical diversity from the uncultivated symbionts of small marine animals. Nat Chem Biol., 14:179–185.

Stewart, E.J. (2012). Growing unculturable bacteria. J Bacteriol., 194(16):4151–4160.

Sukhum, K.V.; Diorio-Toth, L. and Dantas, G. (2019). Genomic and metagenomic approaches for predictive surveillance of emerging pathogens and antibiotic resistance. Clin Pharmacol Ther., 106:512– 524.

Tamaki, H.; Wright, C.L.; Li, X.; Lin, Q.; Hwang, C.; Wang, S.; Thimmapuram, J.; Kamagata, Y. and Liu, W.T. (2011). "Analysis of 16S rRNA amplicon sequencing options on the Analysis of 16S rRNA amplicon sequencing options on the Roche/454 next-generation titanium sequencing platform.", PLoS One 6(9): e25263.

Tange, O. (2018). Gnu parallel 2018 (Lulu. com).

Thoendel, M.; Jeraldo, P.; Greenwood-Quaintance, K.E.; Chia, N.; Abdel, M.P.; Steckelberg, J.M.; Osmon, D.R. and Patel, R. (2017). A novel prosthetic joint infection pathogen, mycoplasma salivarium, identified by metagenomic shotgun sequencing. Clin Infect Dis., 65(2):332–335.

Thomas, T.; Gilbert, J. and Meyer, F. (2012). "Metagenomics - a guide from sampling to data analysis.", Microb. Inform. Exp., 2(1):3.

Thomas, T.; Rusch, D.; DeMaere, M.Z.; Yung, P.Y.; Lewis, M.; Halpern, A.; Heidelberg, K.B.; Egan, S.; Steinberg, P.D. and Kjelleberg, S. (2010). Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis. ISME J, 4(12):1557-1567.

Truong, D.T.; Franzosa, E.A.; Tickle, T.L.; Scholz, M.; Weingart, G.; Pasolli, E.; Tett, A.; Huttenhower, C. and Segata, N. (2015). MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods, 12:902–903.

Tyson, G.W.; Chapman, J.; Hugenholtz, P.; Allen, E.E.; Ram, R.J.; Richardson, P.M.; Solovyev, V.V.; Rubin, E.M.; Rokhsar, D.S. and Banfield, J.F. (2004). Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature, 428(6978):37-43.

Uchiyama, T.; Abe, T.; Ikemura, T. and Watanabe, K. (2005). Substrate-induced gene-expression screening of environmental metagenome libraries for isolation of catabolic genes. Nat. Biotechnol., 23: 88–93.

Uygut, M.A. and Tanyildizi, M. (2018). Determination of effective parameters for alpha-amylase production in a modifed rotating drum bioreactor. Arab J Sci Eng., 43(7):3381–3391.

Vidya, J.; Swaroop, S.; Singh, S.K.; Alex, D.; Sukumaran, R. and Pandey, A. (2011). Isolation and characterization of a novel α-amylase from a metagenomic library of Western Ghats of Kerala, India. Biologia (Bratisl), 66:939–944.

Voget, S.; Leggewie, C.; Uesbeck, A.; Raasch, C.; Jaeger, K.E. and Streit, W.R. (2003). Prospecting for novel biocatalysts in a soil metagenome. Appl. Environ. Microbiol., 69: 6235–6242.

Wang, T.Y.; Wang, L.; Zhang, J.H. and Dong, W.H. (2011). A simplified universal genomic DNA extraction protocol suitable for PCR. Genet. Mol. Res., 10: 519–525.

Wang, X.; Su, X.; Cui, X. and Ning, K. (2015). MetaBoot: a machine learning framework of taxonomical biomarker discovery for diferent microbial communities based on metagenomic data. PeerJ., 3: 993.

Wilson, M.R.; Naccache, S.N.; Samayoa, E.; Biagtan, M.; Bashir, H.; Yu, G.; Salamat, S.M.; Somasekar, S.; Federman, S.; Miller, S.; Sokolic, R.; Garabedian, E.; Candotti, F.; Buckley, R.H.; Reed, K.D.; Meyer, T.L.; Seroogy, C.M.; Galloway, R.; Henderson, S.L.; Gern, J.E.; DeRisi, J.L. and Chiu, C.Y. (2014). Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med., 370(25):2408–2417.

Wood, D.E.; Lu, J. and Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. Genome Biol. 20(1):257. Ye, S.H.; Siddle, K.J.; Park, D.J. and Sabeti, P.C. (2019). Benchmarking metagenomics tools for taxonomic classification. Cell, 178:779– 794.

Yun, J. and Ryu, S. (2005). Screening for novel enzymes from metagenome and SIGEX, as a way to improve it. Microb. Cell Factor. 4(1):8.

Zhang, L,; Chen, F.; Zeng, Z.; Xu, M.; Sun, F.; Yang, L.; Bi, X.; Lin, Y.; Gao, Y.; Hao, H.; Yi, W.; Li, M. and Xie, Y. (2021). Advances in Metagenomics and Its Application in Environmental Microorganisms. Front. Microbiol. 12:766364.

Zhou, R.; Zeng, S.; Hou, D.; Liu, J.; Weng, S.; He, J. and Huang, Z. (2019). Occurrence of human pathogenic bacteria carrying antibiotic resistance genes revealed by metagenomic approach: a case study from an aquatic environment. J Environ Sci (China) 80:248–256.

Zhou, Y.; Wylie, K.M.; El Feghaly, R.E.; Mihindukulasuriya, K.A.; Elward, A.; Haslam, D.B.; Storch, G.A. and Weinstock, G.M. (2016). Metagenomic approach for identification of the pathogens associated with diarrhea in stool specimens. J Clin Microbiol., 54(2):368–375.

Zhu, W.; Lomsadze, A. and Borodovsky, M. (2010). Ab initio gene identification in metagenomic sequences. Nucleic Acids Res., 38 (12):e132.