Biotechnology

MIC 566

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Introduction:

The term “biotechnology” was coined by a Hungarian engineer Karl Ereky, in 1919, to refer to the science and methods that permit products to be produced from raw materials with living organisms' aid. Biotechnology is a diverse field that involves working with living cells or using molecules derived from them for applications-oriented toward human welfare using varied types of tools and technologies. It is an amalgamation of biological science with engineering whereby living organisms or cells or parts are used to produce products and services.

The biotechnology came into being centuries ago when plants and animals began to be selectively bred and microorganisms were used to make beer, wine, cheese, and bread. However, the field gradually evolved, and presently it is the use or manipulation of living organisms to produce beneficiary substances which may have medical, agricultural, and industrial utilization. Conventional biotechnology is referred to as the technique that makes use of living organism for specific purposes as bread/cheese making, whereas modern biotechnology deals with the method that makes use of cellular molecules like DNA, monoclonal antibodies, biologics, etc. The main subfields of biotechnology are medical (red) biotechnology, agricultural (green) biotechnology, industrial (white) biotechnology, marine (blue) biotechnology, food biotechnology, and

environmental biotechnology. In this chapter, in the readers will understand the potential applications of biotechnology in medical where we give examples [1-2].

ELISA Test:

Enzyme-linked immunosorbent assay (ELISA) test is the most widely used type of immunoassay. ELISA is a rapid test used for detecting or quantifying antibody (Ab) against viruses, bacteria and other materials or antigen (Ag). Described initially by Engvall and Perlman (1971), the method enables analysis of protein samples immobilized in microplate wells using specific antibodies. ELISA is so named because the test technique involves the use of an enzyme system and immunosorbent.

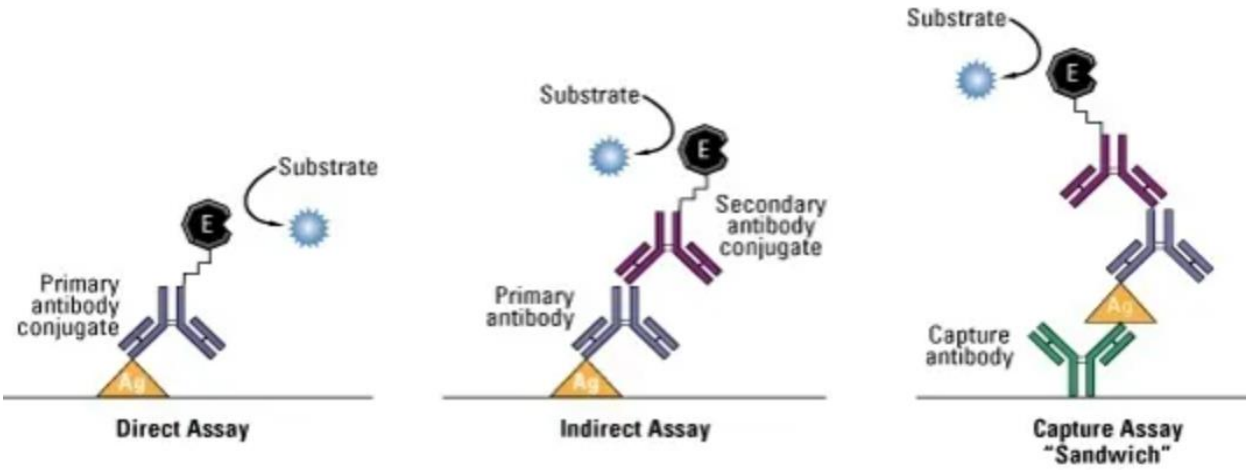
ELISA test is being increasingly used to detect antigen (infectious agent) or antibody due to its simplicity and sensitivity. It is as sensitive as radioimmunoassay (RIA) and requires only microliter quantities of test reagents. It has now been widely applied to detect a variety of antibody and antigens such as hormones, toxins, and viruses.

ELISA Type: direct, indirect, and sandwich ELISA

There are several formats used for ELISAs. These fall into either direct, indirect, or sandwich capture and detection methods. The critical step is immobilization of the antigen of interest, accomplished by either direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected directly (labeled primary antibody) or indirectly (such as labeled secondary antibody). The most widely used ELISA assay format is the sandwich ELISA assay, which indirectly immobilizes and indirectly detects the target antigen's presence. This type of capture assay is called a "sandwich" assay because the analyte to be measured is bound between two primary antibodies, each detecting a different epitope of the antigen–the capture antibody and the detection antibody. The sandwich ELISA format is highly used because of its sensitivity and specificity.

Principle of ELISA Test:

Most ELISA methods developed to detect antigen or antibody consist of use of corresponding antibody or antigen in question which is firmly fixed on solid phase, such as plastic surface of polyvinyl plate or polystyrene tube. Such systems are also called Solid Phase .



Immunosorbent Assay (SPIA).

Test sample is added in the microtitre plate, if there is presence of Ag or Ab in the test sample, there will be Ag-Ab reactions (with immobilized Ab or Ag). Later enzyme labelled antibody is added in the reaction mixture, combined with either test antigen or Fc portion of test antibody.

The enzyme system consists of:

1. An enzyme: horseradish peroxidase, alkaline phosphatase labelled or linked, to a specific antibody.
2. A specific substrate:

- o-Phenylenediamine dihydrochloride for peroxidase
- P Nitrophenyl Phosphate (PNPP)- for Alkaline Phosphatase

Substrate is added after the antigen-antibody reaction. The enzyme catalysis (usually hydrolyses) the substrate to give a color endpoint (yellow

compound in case of alkaline phosphatase). The intensity of the color is proportional to the amount of antibody or antigen present in the test sample, which can be quantified using an ELISA reader [3-4].

HPLC Test:

HPLC is Liquid Chromatography used in separating the components of a compound or mixture. HPLC stands for High Performance Liquid Chromatography, and is a technique used to separate different constituents of a combination using high pressure to push solvents through the column. It is the most widely used technique to identify, quantify and separate components of a mixture. HPLC is widely used for the analysis of constituents of a pharmaceutical actives, drug products, pesticides, and countless other substances.

Principle of HPLC:

The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase. The specific intermolecular interactions between the molecules of a sample and the packing material define their time “on-column”. Hence, different constituents of a model are eluted at other times. Thereby, the separation of the sample ingredients is achieved.

A detection unit (e.g., UV detector) recognizes the analytes after leaving the column. The signals are converted and recorded by a data management system (computer software) and then shown in a chromatogram. After passing the detector unit, the mobile phase can be subjected to additional detector units, a fraction collection unit, or the waste. In general, a HPLC system contains the following modules: a solvent reservoir, a pump, an injection valve, a column, a detector unit and a data processing unit. The solvent (eluent) is delivered by the pump at high pressure and constant speed through the system. To keep the drift and noise of the detector signal as low as possible, a continuous and pulseless flow from the pump is crucial. The analyte (sample) is provided to the eluent by the injection valve. Types of HPLC: There are following variants of HPLC, depending upon the phase system (stationary) in the process:

1 .Normal Phase HPLC

This method separates analytes based on polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica, and typical mobile steps are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

2 .Reverse Phase HPLC

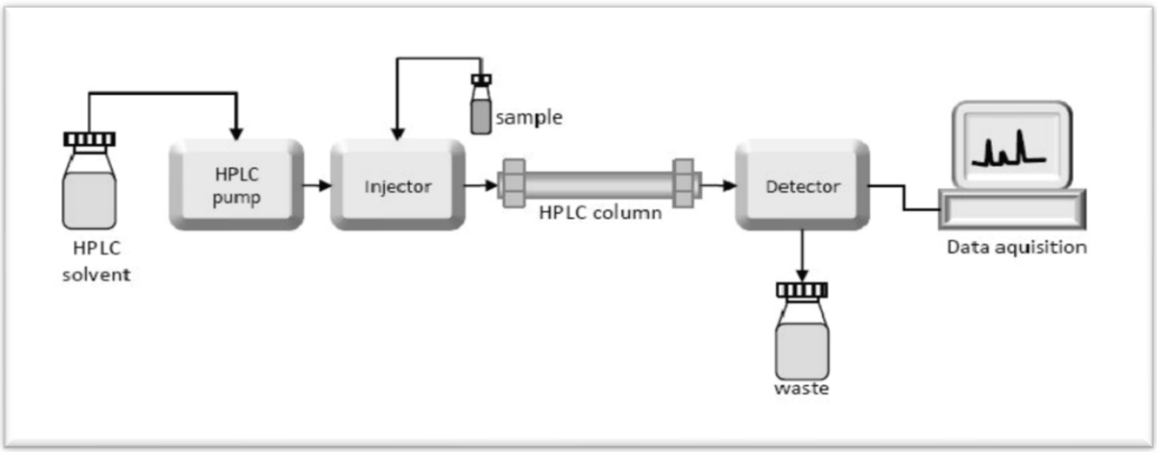
The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions; hence the more nonpolar the material is, the longer it will be retained.

3 .Size-exclusion HPLC

The column is filled with material with precisely controlled pore sizes, and the particles are separated according to its molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

4 .Ion-Exchange HPLC

The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute.



The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time[5-6].

Spectroscopy:

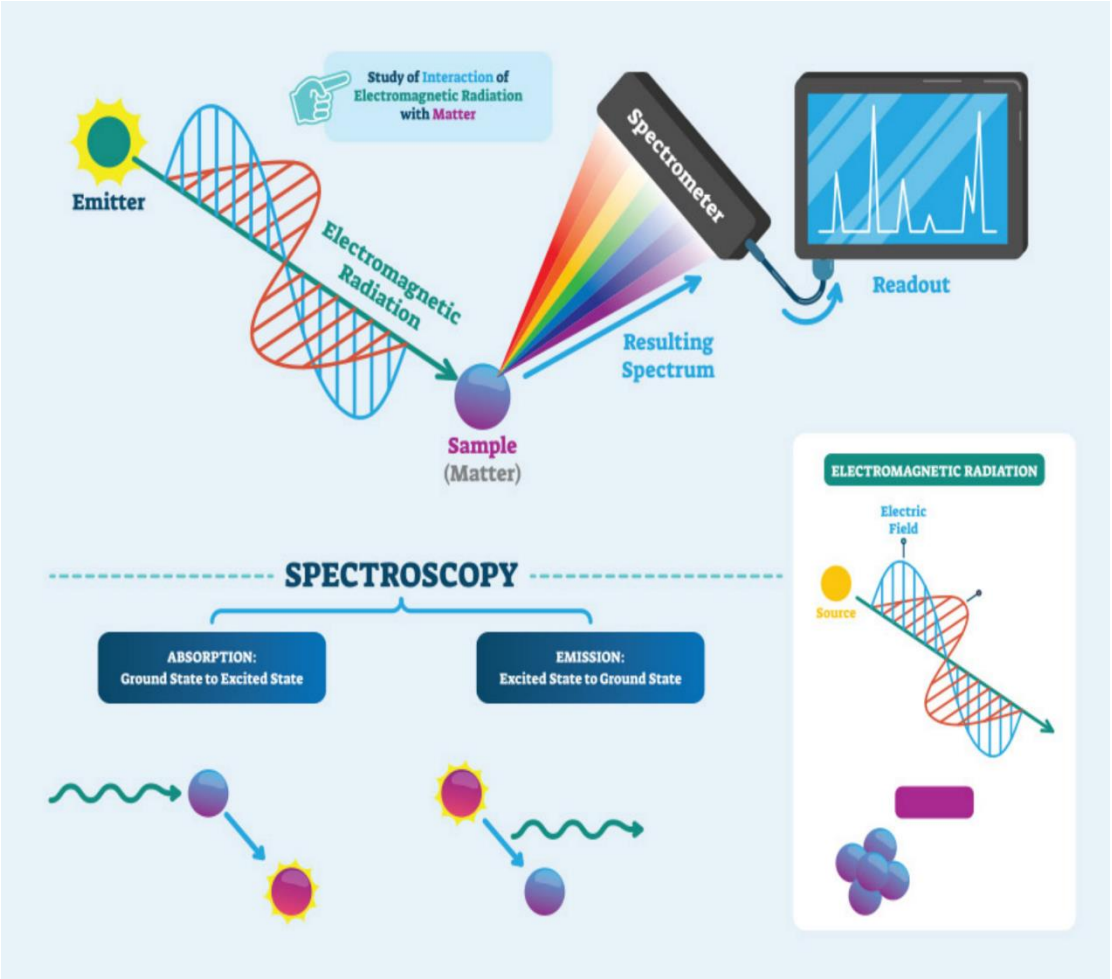
Spectroscopy is the investigation and measurement of spectra produced by matter interacting with or emitting electromagnetic radiation. Originally, spectroscopy was defined as the study of the interaction between radiation and matter as a wavelength function. Now, spectroscopy is defined as any measurement of a quantity as a function of wavelength or frequency. During a spectroscopy experiment, electromagnetic radiation of a specified wavelength range passes from a source through a sample containing interest compounds, resulting in absorption or emission. During absorption, the sample absorbs energy from the light source. During emission, the sample emits light of a different wavelength than the source's wavelength.

In absorption spectroscopy, the sample's compounds are excited by the electromagnetic radiation provided by a light source. Their molecules absorb energy from the electromagnetic radiation, become excited, and jump from a low energy ground state to a higher energy state of excitation. A detector, usually a photodiode, on the opposite side of the sample records the sample's absorption of wavelengths, and determines its absorption. The

spectrum of a sample's absorbed wavelengths is known as its absorption spectrum, and the quantity of light absorbed by a sample is its absorbance.

for what Spectroscopy used it?

Spectroscopy is used in physical and analytical chemistry to detect, determine, or quantify the molecular and structural composition. Each type of molecule and atom will reflect, absorb, or emit electromagnetic radiation in its characteristic way. Spectroscopy uses these characteristics to deduce and analyze the composition of a sample[7].



PCR Test:

Polymerase chain reaction (PCR) is a technique used to exponentially amplify a specific target DNA sequence, allowing for the isolation, sequencing, or cloning of a single sequence among many. PCR was developed in 1983 by Kary Mullis, who received a Nobel Prize in chemistry in 1993 for his invention. The polymerase chain reaction has been elaborated in many ways since its introduction It is now commonly used for various applications, including genotyping, cloning, mutation detection, sequencing, microarrays, forensics, and paternity testing.

Typically, a PCR is a three-step reaction. The sample containing a dilute concentration of template DNA is mixed with a heat-stable DNA polymerase, such as Taq polymerase, primers, deoxynucleoside triphosphates (dNTPs), and magnesium. In the first step of PCR, the sample is heated to 95–98°C, which denatures the double-stranded DNA, splitting it into two single strands. In the second step, the temperature is decreased to approximately 55–65°C, allowing the primers to bind, or anneal, to specific sequences of DNA at each end of the target sequence, also known as the template. In the third step, the temperature is typically increased to 72°C, allowing the DNA polymerase to extend the primers by the addition of dNTPs to create a new strand of DNA, thus doubling the quantity of DNA in the reaction. This sequence of denaturation, annealing, and extension is repeated for many cycles, resulting in the exponential

amplification of the template DNA. As the DNA polymerase loses activity or the dNTPs and primers are consumed, the reaction rate reaches a plateau.

Types of polymerase chain reaction-PCR:

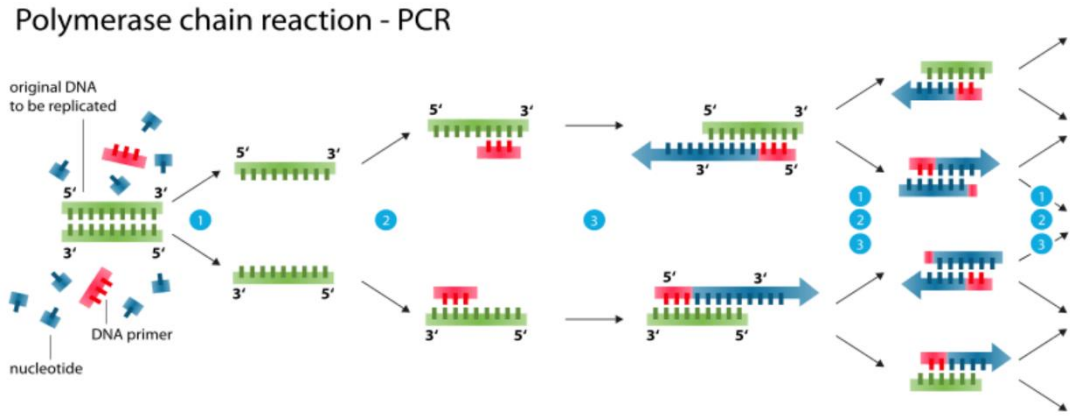
Several modifications of PCR methods have been developed to enhance the utility of this method in diagnostic settings based on their applications. Some of the common types of PCR are;

- Real-time PCR
- Quantitative real time PCR (Q-RT PCR)
- Reverse Transcriptase PCR (RT-PCR)
- Multiplex PCR
- Nested PCR
- Long-range PCR
- Single-cell PCR
- Fast-cycling PCR
- Methylation-specific PCR (MSP)
- High-fidelity PCR
- In situ PCR
- Variable Number of Tandem Repeats (VNTR) PCR
- Asymmetric PCR
- Repetitive sequence-based PCR
- Overlap extension PCR

- Hot start PCR
- Assemble PCR
- Intersequence-specific PCR(ISSR)
- Ligation-mediated PCR
- Methylation –specifin PCR
- Miniprimer PCR
- Solid phase PCR
- Touch down PCR

Applications of PCR:

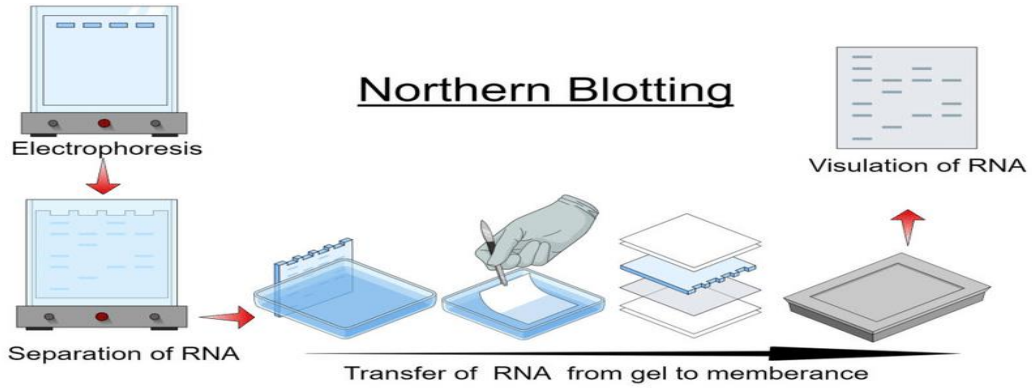
- Cloning genes
- PCR sequencing
- Identification and characterization of infectious agents.
- Direct detection of microorganisms in patient specimens.
- Detection of antimicrobial resistance
- Investigation of strain relatedness of a pathogen of interest
- Genetic fingerprinting (forensic application/paternity testing)
- Detection of mutation (investigation of genetic diseases)[8-9]



Northern blot:

A northern blot is a laboratory method used to detect specific RNA molecules among a mixture of RNA. Northern blotting can be used to analyze a sample of RNA from a particular tissue or cell type in order to measure the RNA expression of particular genes. This method was named for its similarity to the technique known as a Southern blot.

The first step in a northern blot is to denature, or separate, the RNA within the sample into single strands, which ensures that the strands are unfolded and that there is no bonding between strands. The RNA molecules are then



separated according to their sizes using a method called gel electrophoresis. Following separation, the RNA is transferred from the gel onto a blotting membrane. (Although this step is what gives the technique the name "northern blotting," the term is typically used to describe the entire procedure.) Once the transfer is complete, the blotting membrane carries all the RNA bands originally on the gel. Next, the membrane is treated with a small piece of DNA or RNA called a probe, which has been designed to have a sequence that is complementary to a particular RNA sequence in the sample; this allows the probe to hybridize, or bind, to a specific RNA fragment on the membrane. In addition, the probe has a label, which is typically a radioactive atom or a fluorescent dye. Thus, following hybridization, the probe permits the RNA molecule of interest to be detected from among the many different molecules on the membrane[10].

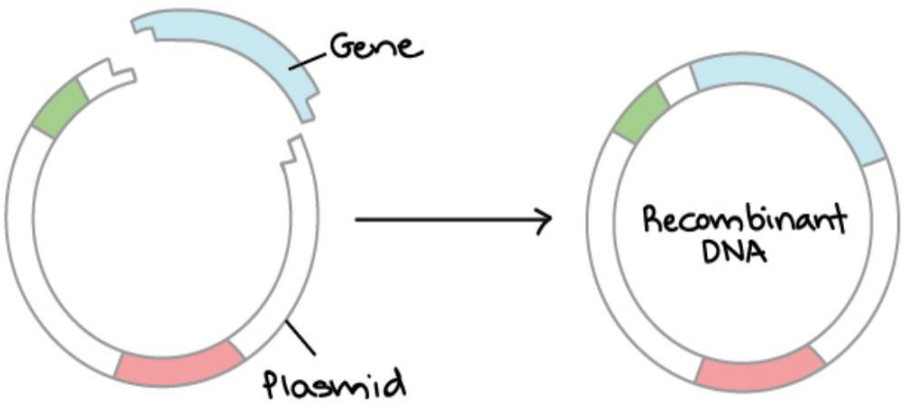
Molecular Cloning:

Molecular cloning is the set of experimental techniques used to generate a population of organisms carrying the same molecule of recombinant DNA. This is first assembled in vitro and then transferred to a host organism that can direct its replication in coordination with its growth. This is usually achieved in an easy-to-grow, nonpathogenic laboratory bacterial strain of Escherichia coli. A single modified E. coli cell carrying the desired recombinant DNA can easily be grown in an exponential fashion to

generate virtually unlimited identical copies of this DNA. As such, molecular cloning can be seen as an "in vivo polymerase chain reaction (PCR)," in which a desired piece of DNA can be isolated and expanded. However, molecular cloning allows more flexibility, better fidelity, higher yields, and lower costs than a PCR. The development of molecular cloning techniques started with the discovery of bacterial enzymes known as "restriction endonucleases," which cleave DNA molecules at specific positions that are defined by their sequence. These restriction endonucleases allow researchers to break up large DNA fragments into smaller pieces that are then joined with other DNA molecules (vectors) using an enzyme called DNA ligase. The most commonly used vectors are known as plasmids, which are small circular DNA molecules physically distinct from the chromosomal DNA and capable of independent replication. Restriction endonucleases generate either "sticky ends," in which the DNA fragment has a single-stranded overhang (either on the 3' or 5' ends, see Glossary), or "blunt ends," in which no overhang is present. Both these types of ends can be joined together (ligated), and each has its own associated advantages and disadvantages. For a sticky-end fragment ligation to be successful, the two overhangs to be joined must have complementary Watson– Crick base pairing. However, this is not a requirement of a blunt-end ligation, making it much more flexible. On the other hand, blunt-end ligation is much less efficient than sticky-end

ligation due to a lack of binding stability of the two fragments. Importantly, sticky ends can be enzymatically converted into blunt ends (either by "filling in" missing nucleotides, or by removing the overhangs), and vice versa (by using 5'-3' or 3'-5' exonucleases to create new overhangs). These cut-and-paste approaches are still widely used today and are commonly referred to as "traditional" (or conventional) cloning.

Principle of Gene Cloning: A fragment of DNA, containing the gene to be cloned, is inserted into a suitable vector, to produce a recombinant DNA molecule. The vector acts as a vehicle that transports the gene into a host cell usually a bacterium, although other types of a living cell can be used. Within the host cell, the vector multiplies, producing numerous identical copies not only of itself but also of the gene that it carries. When the host cell divides, copies of the recombinant DNA molecule are passed to the progeny and further vector replication takes place. After a large number of cell divisions, a colony, or clone, of identical host cells is produced. Each



cell in the clone contains one or more copies of the recombinant DNA molecule; the gene carried by the recombinant molecule is now said to be cloned [11].

The application of microorganisms: natural useful products from microbes and we will mention some of them here:

Amino acids:

Amino acids are used in human and animal food and feed supplements because they are the building blocks of protein molecules. Microbial fermentation and enzymatic processes dominate amino acid processing technologies worldwide due to their cost-effectiveness, environmental acceptability, and ease of producing enantiomerically pure amino acids. In 2014, global amino acid output was about 6.5 million tons, with a value of nearly $ 35 billion. By 2022, it is projected to hit 10 million tons, with a value of nearly $ 35 billion. The commercial production through fermentation and enzymatic transformations mainly use *Corynebacterium glutamicum* and *Escherichia coli* to produce L-glutamic acid (monosodium glutamate), L-aspartic acid, L phenylalanine, L-lysine, L-methionine, L-threonine, and L tryptophan. L-lysine is a preferred additive to animal feed and approximately, 1.3 million tons of lysine is produced annually through microbial fermentation using *C. glutamicum*[12-13].

Microbes as source of antitumor drugs:

Microbial metabolites are among the most important cancer chemotherapeutic agents. They first emerged around 1940, when actinomycin was discovered, and since then, many anticancer compounds have been isolated from natural sources. More than 60% of the new antineoplastic compounds were found as natural products or are derivatives of natural products. Among the approved products deserving particular attention are actinomycin D, anthracyclines (daunorubicin, doxorubicin, epirubicin, pirirubicin and valrubicin), bleomycin, mitosanes (mitomycin C)[14].

Vitamins:

Vitamins are important micronutrients that are needed in trace amounts to keep the body's physiological functions running smoothly. Vitamins are made by microorganisms as part of their natural metabolism and are commonly used as food additives, nutritional supplements, and medicinal agents. Vitamins are commercially generated using suitable microorganisms and either direct fermentation or a combination of chemical and microbiological processes. Riboflavin (vitamin B2) is a water-soluble vitamin that is necessary for human and animal growth and reproduction. The main microorganisms used in the fermentative

making of riboflavin are two closely related ascomycetes, *Eremothecium ashbyii* and *Ashbya gossypii*. *A. gossypii* is an efficient and preferred resource of riboflavin production as it can produce 40,000 times more vitamin than required for its own growth. Genetically engineered *Bacillus subtilis* and *Corynebacterium ammoniagenes* are other bacterial species preferred for riboflavin biosynthesis [13].

Microbes as hypocholesterolemic drugs:

Atherosclerosis is a chronic, recurrent condition characterized by the deposition of atheromatous plaque within the arterial wall over time. The first member of the group (compactin; mevastatin) was isolated from *Penicillium brevicompactum* and later from *Penicillium citrinum* as an antibiotic product. In the 1970s, lovastatin (monacolin K; mevinolin), an ethylated form, was discovered in the broths of *Monascus ruber* and *Aspergillus terreus*. The FDA approved lovastatin, the first commercially available statin, in 1987. Simvastatin, a major hypocholesterolemic drug that sold for $7 billion per year before becoming generic, is a semi-synthetic analog of lovastatin. Another statin, pravastatin (US$3.6 billion per year), is generated by *Streptomyces carbophilus* and *Actinomadura* sp. using separate biotransformation processes from compactin[15].

Alcohol:

Ethanol is a key metabolite and a biofuel that is commonly used. It's also used as a solvent for dyes, oils, waxes, explosives, cosmetics, labs, and as a disinfectant in a variety of chemical industries. The maximum proportion of total ethanol (*90 to 95%) is made using microbial fermentation technology. The maximum proportion of total ethanol (90 to 95%) is produced using microbial fermentation technology, *E. coli*, *Klebsiella oxytoca*, and *Clostridium thermocellum* are recombinant microorganisms that are used in the fermentation of ethanol from various carbon sources [13].

Conclusion:

Microorganisms, which are the primary source of nutrients, are essential to all living things and play an indispensable role in healthcare, agriculture, industry, and nutrition. When we look at our current knowledge and future possibilities, we can see that the number of new microbial metabolites and biotechnology is continuously growing. The overwhelming majority of the world's biodiversity is still unexplored, and new high-speed approaches are allowing it to be exploited successfully. Cloning and genetic engineering provide alternatives, as well as the possibility of

introducing suitable biosynthetic pathways from non-culturable strains into suitable hosts.

Reference:

1- Britannica, T. Editors of Encyclopaedia (2019, November 25). Biotechnology. Encyclopedia Britannica. https://www.britannica.com/technology/biotechnology

2- Gupta, V., Sengupta, M., Prakash, J., & Tripathy, B. (2016). An Introduction to Biotechnology. *Basic And Applied Aspects Of Biotechnology*, 1-21. doi: 10.1007/978-981-10-0875-7_1

3- Sciences, L., Biology, P., Center, P., Library, P., Methods, P., & ELISA, O. (2021). Overview of ELISA | Thermo Fisher Scientific - UK. Retrieved 19 February 2021, from https://www.thermofisher.com/sa/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html

4- (2021). Retrieved 19 February 2021, from https://microbeonline.com/elisa-test-for-antigenantibody-detection/

5- Matuszewski, B., Constanzer, M., & Chavez-Eng, C. (2003). Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC−MS/MS. *Analytical Chemistry*, *75*(13), 3019-3030. doi: 10.1021/ac020361s

6- GmbH, K. (2021). HPLC Principles and parameters. Retrieved 19 February 2021, from https://www.knauer.net/en/Systems-Solutions/Analytical-HPLC-UHPLC/HPLC-Basics---principles-and-parameters

7- Zhu, X., & Gao, T. (2019). Spectrometry. *Nano-Inspired Biosensors For Protein Assay With Clinical Applications*, 237-264. doi: 10.1016/b978-0-12-815053-5.00010-6

8- Kadri, K. (2020). Polymerase Chain Reaction (PCR): Principle and Applications. *Synthetic Biology - New Interdisciplinary Science*. doi: 10.5772/intechopen.86491

9- Aryal, S., mishra, s., M, A., khan, D., Shadab, M., & Umo, D. et al. (2021). Polymerase Chain Reaction (PCR)- Principle, Procedure, Types, Applications and Animation. Retrieved 20 February 2021, from https://microbiologyinfo.com/polymerase-chain-reaction-pcr-principle-procedure-types-applications-and-animation/

10- N., C., M., P., & Moleele, N. (2012). Management of Tsetse Fly Using Insecticides in Northern Botswana. *Insecticides - Pest Engineering*. doi: 10.5772/28450

11- Bertero, A., Brown, S., & Vallier, L. (2017). Methods of Cloning. *Basic Science Methods For Clinical Researchers*, 19-39. doi: 10.1016/b978-0-12-803077-6.00002-3

12- Microbial Products: Technologies, Applications and Global Markets. (2021). Retrieved 16 March 2021, from https://www.prnewswire.com/news-releases/microbial-products-technologies-applications-and-global-markets-300681324.html

13- Singh, R., Kumar, M., Mittal, A., & Mehta, P. (2017). Microbial metabolites in nutrition, healthcare and agriculture. *3 Biotech*, *7*(1). doi: 10.1007/s13205-016-0586-4

14- Gupta, C. (2014). Natural Useful Therapeutic Products from Microbes. *Journal Of Microbiology & Experimentation*, *1*(1). doi: 10.15406/jmen.2014.01.00006.

15- Monciardini, P., Iorio, M., Maffioli, S., Sosio, M., & Donadio, S. (2014). Discovering new bioactive molecules from microbial sources. *Microbial Biotechnology*, *7*(3), 209-220. doi: 10.1111/1751-7915.12123.