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Detection of rapid identification of microbial pathogens and emerging contaminants in water samples using cost-effective biochemical and molecular methods

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ABSTRACT

Biochemical and immunological methods are based on the analysis of various classes of organic and inorganic compounds that are defective in various hereditary diseases, primarily in hereditary metabolic diseases. Biochemical disorders, as a rule, precede the appearance of clinical symptoms of the disease and are more constant compared to them. The subject of biochemical diagnostics can be proteins, amino acids, carbohydrates, lipids, metal ions, etc., as well as their metabolites. In this case, various tissues and secretions of the body can be examined (blood, urine, saliva, sweat, cerebrospinal fluid, amniotic fluid, biopsy of muscles, skin, liver and other specialized tissues). Biochemical methods play a primary role in the diagnosis of hereditary metabolic disorders. In some cases, they allow the identification of heterozygous carriers of mutations. The role of biochemical methods of analysis is very important in conducting mass examinations of pregnant women or newborns for the purpose of earlier detection of hereditary diseases. The key role in the pathogenesis of any monogenic disease belongs to the primary biochemical defect - a protein encoded by a mutant gene. Detection and analysis of the primary biochemical defect, determination of the primary pathological metabolic chain - these are the main tasks of biochemical genetics, the solution of which is the basis for the development of pathogenetic methods for the prevention and treatment of hereditary diseases. No less important is the role of biochemical methods in the diagnosis of secondary disorders. For example, the main biochemical defect in Duchenne / Becker muscular dystrophy is a deficiency of dystrophin, a protein that connects the cytoskeleton of muscle cells with the extracellular matrix.

1. INTRODUCTION

Currently, a number of technologies and commercial applications for the detection and identification of microorganisms have been developed that allow the detection of nucleic acids that are part of microorganisms. Over the years, various methods for the detection and identification of microorganisms have been actively developed. These methods can be divided into three groups: - classical methods for the identification of microorganisms; - PCR methods; - high-throughput sequencing. Among the methods for identifying biological samples of microorganisms, the most common methods are based on the analysis of the DNA structure.[1] Molecular genetic methods of metagenomic analysis of the composition of the bacterial community allow the detection of microorganisms in food products without preliminary cultivation and isolation of species-specific fragments from total DNA and amplification of genes encoding rRNA. Next-generation sequencing (NGS) collectively describes several technologies that provide massive parallel sequencing of heterogeneous DNA fragments[2]. This method is applicable for monitoring the microbial community, which consists of amplifying short DNA fragments using universal PCR primers targeting known marker genes, mainly prokaryotic 16S rRNA and fungal ITS genes[3].

Rapid detection of microbial pathogens and new contaminants in water samples using cost-effective biochemical and molecular methods Microorganisms (except for obligate intracellular parasites - rickettsia, chlamydia, viruses, and protozoa) are usually cultivated on artificial nutrient media, which must contain the appropriate starting materials necessary for plastic and energy metabolism, as well as growth factors. be complete[4]. Isolation of microorganisms from various pathological materials obtained from a sick person is widely used in laboratory practice for microbiological diagnostics of infectious diseases, in research work, as well as in the production of vaccines, antibiotics and other biologically active products of microbial activity[5]. Most pathogenic and opportunistic microbes are grown on nutrient media at 37 ° C for 1-2 days. However, some of them require a longer lead time. For example, whooping cough bacteria - after 2-4 days, tuberculosis mycobacteria - after 3-4 weeks[6].

In clinical bacteriological diagnostics, identification is carried out only for "pure cultures" of bacteria isolated from the test

material obtained from the patient. For this, freshly prepared artificial nutrient media are used, pre-poured into Petri dishes. The choice of nutrient medium for isolating a pure culture of pathogens is of paramount importance in bacteriological diagnostics[7]. For primary sowing, a set of media is usually used, including enrichment media, elective media, media with increased nutritional value for difficult to cultivate microorganisms and nutrient media for differential diagnostics. When identifying cultures, their generic and species identification is carried out, and, if necessary, intraspecific typing.The result is obtained from a set of data:

1- Morphological, based on the identification of characteristic features of the ultrastructure of the pathogen and their tinctorial properties (usually Gram staining).

2- cultural, based on the isolation of a pure culture of microorganisms on a solid nutrient medium, with the study of the characteristics of the grown colonies.

3- biochemical activity of cultures. Including: oxidase and catalase activity, the ability to ferment carbohydrates (glucose, lactose, sucrose, maltose, mannitol, etc.) with the formation of indole, ammonia and hydrogen sulfide, which are products of the proteolytic activity of bacteria. Less commonly used tests are for nitrogen assimilation, determination of urease activity and the activity of individual specific enzymes.

4- Immunological,

   a- Immunological, based on the detection of antigens (AG) of the pathogen or antibodies (AT) to them. lactose, sucrose, maltose, mannitol, etc.) with the formation of indole, ammonia and hydrogen sulfide, which are products of the proteolytic activity of bacteria. Less commonly used tests are for nitrogen assimilation, determination of urease activity and the activity of individual specific enzymes;

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   d- Immunological, based on the detection of antigens (AG) of the pathogen or antibodies (AB) to them. with the formation of indole, ammonia and hydrogen sulfide, which are products of the proteolytic activity of bacteria. Less commonly used tests are nitrogen assimilation, urease activity and activity of individual specific enzymes;

   e- Immunological, based on detection of pathogen antigens (AG) or antibodies (AT) to them. Identification of pathogens by their antigenic properties is carried out in reactions of orientation agglutination, coagglutination, latex agglutination, precipitation or immunofluorescence (direct or indirect). Antibodies to the pathogen are determined in serological reactions: detailed agglutination reaction, indirect agglutination, precipitation, complement fixation, enzyme immunoassay and radioimmunoassay methods, etc.;

5- molecular genetic, based on determination of specific nucleotide sequences in the DNA chain using a short reference DNA chain (primer). For this purpose, polymerase chain reaction (PCR), ligase chain reaction and their modifications, the method of genetic probes, sandwich hybridization, etc. are used. Different types and even varieties of microbes react differently to the same sugars.

To identify saccharolytic enzymes, the studied bacterial culture is inoculated into Giss nutrient media, "variegated row" - differential diagnostic media. Giss medium is produced as a semi-liquid with a BP indicator (a mixture of water blue and rosolic acid). As carbohydrates, pentoses (arabinose, xylose, rhamnose), hexose (glucose, levulose, mannose, galactose), disaccharides (maltose, lactose, sucrose, trehalose, cellobiose, melibiose), trisaccharides (raffinose), dextrin glycogen, soluble starch. , alcohols with high alcohol content (glycerol, erythritol, adonitol, arabitol, mannitol, dulcitol, sorbitol, inositol), glucosides (salicin, esculin, coniferous acids, arbutin). The culture to be studied is inoculated by injecting a loop into a column of medium and incubated in an incubator at 37 ° C for a day or more, depending on the species being identified. The formation of acidic products is judged by the change in color of the indicator after inoculation; filling of the float with gas or explosion of the agar indicates gas formation from the carbohydrate. If the culture does not ferment the carbohydrate, the color of the indicator does not change and gas formation is not observed[8].

Protein media that reveal the ability of microbes to break down proteins: meat-peptone-gelatin "column", rolled horse or bovine serum, milk, meat-peptone broth. The proteolytic activity of the same microbe when determined on different nutrient media will manifest itself differently, which is due to the specificity of the enzymes. When inoculating bacteria by puncturing

meat-peptone gelatin, a "column" is observed in the case of protein breakdown, liquefaction of the medium. When sowing on a medium with coagulated serum, protein degradation is determined by dilution of the medium and the formation of depressions on its surface. The breakdown of milk by a microbe is detected by the enlightenment or dissolution of the initially curdled milk[9]. Biochemical methods aimed at identifying the biochemical phenotype of the organism. These methods allow diagnosing hereditary diseases caused by gene mutations. Biochemical indicators (primary protein product of the gene, accumulation of pathological metabolites inside the cell) more adequately reflect the essence of the disease than clinical symptoms. More than 1000 congenital metabolic diseases have been described using biochemical methods. The most common of these diseases are those associated with enzyme defects, structural and transport proteins. Enzyme defects are determined by determining the content of metabolic products in biological environments (for example, in urine and blood), which are the product of the functioning of this protein. A deficiency of the final product, accompanied by an accumulation of intermediate products and products of impaired metabolism, indicates an enzyme defect or deficiency in the body. Biochemical methods are multi-stage[9]. They require equipment of different classes. Biochemical objects can be urine, sweat, plasma and blood cells, cell cultures (fibroblasts, lymphocytes). Due to the variety of biochemical methods used in laboratory diagnostics of hereditary diseases, a certain system is used for their effective use. Biochemical diagnostics is carried out in two stages. At the first stage, suspected cases of the disease are selected, at the second, the diagnosis of the disease is clarified using more accurate and complex methods. The first stage includes qualitative and quantitative analysis of urine and blood for protein, keto acids, cystine and homocystine, creatinine and other indicators[10]. In fact, such studies can be carried out in any hospital. Indications for their use are quite wide, the cost of each analysis is low. The second stage is based on more accurate methods for identifying large groups of biochemical abnormalities. For example, with the help of thin-layer chromatography of urine and blood, it is possible to diagnose disorders of amino acid, oligosaccharide and glycosaminoglycan (mucopolysaccharide) metabolism. Gas chromatography is used to identify hereditary diseases of organic acid metabolism. With the help of hemoglobin electrophoresis, the entire group of hemoglobinopathies is diagnosed. Despite the complexity and high cost, biochemical methods play a leading role in the diagnosis of monogenic hereditary diseases. Modern high-precision technologies (liquid chromatography, mass spectrometry, magnetic resonance spectroscopy, Indications for the use of biochemical diagnostic methods in newborns are symptoms such as seizures, coma, vomiting, hypotension, jaundice, specific odor of urine and sweat, acid-base imbalance, growth retardation[11]. For example, in the case of phenylketonuria, the use of biochemical studies makes it possible to promptly identify pathology and begin specific treatment measures. In children, biochemical methods are used in all cases of suspected hereditary metabolic diseases (delayed physical and mental development, loss of acquired functions, a clinical picture characteristic of a disease). Biochemical methods are used to diagnose hereditary diseases and heterozygous conditions in adults (hepatolenticular degeneration, glucose-6-phosphate dehydrogenase deficiency).

2. MOLECULAR GENETIC METHODS

Molecular genetic methods are a large and diverse group of methods designed to identify variations (damage) in the structure of a DNA section (allele, gene, chromosome section) up to deciphering the primary base sequence. These methods are based on genetic engineering manipulations with DNA and RNA. The starting point for all molecular genetic methods is obtaining DNA samples. Any germ cells can be a source of genomic DNA. In practice, leukocytes, chorion, amniotic cells and fibroblast cultures are most often used[12]. The ability to conduct molecular genetic analysis with a small amount of easily accessible biological material is a methodological advantage of the methods of this group. The isolated DNA is equally suitable for various studies and can be stored frozen for a long time. In many cases, a small fragment of the genome is sufficient for successful diagnosis of a disease. The isolation of such fragments became possible due to the discovery of enzymes - restriction endonucleases, which cut the DNA molecule into fragments in strictly defined places. The use of these enzymes in the experiment allows obtaining relatively short DNA fragments in which the nucleotide sequence can be easily determined. Obtaining a sufficient number of such fragments is carried out by amplification (reproduction) of DNA using the polymerase chain reaction. A distinction is made between direct and indirect DNA diagnostics of monogenic hereditary diseases. In direct diagnostics, the subject of analysis is gene mutations. Currently, a variety of direct methods are used in DNA diagnostics. The most easily detected mutations are those that change the length of amplified DNA fragments, which are detected using electrophoretic analysis. Methods that can be used to analyze a unique DNA sequence are used to identify point mutations, small deletions and inversions in the genes being studied. An example is the sequencing method - determining the nucleotide sequence of DNA. Any type of mutation can be detected by direct sequencing of mutant DNA. For some small genes, this method is successfully used as the main method of scanning mutations. The main advantage of direct diagnostic methods is almost 100% efficiency. Indirect detection of mutations is used in cases where the nucleotide sequence of a gene is not yet known, but there is an idea of the position of the gene on the genetic map. Indirect DNA diagnostics boils down to the analysis of polymorphic genetic markers in sick and healthy family members. Markers must be located in the chromosomal region where the disease gene is located. Such markers can be DNA sections that exist in the population in several allelic variants. Differences can lie in the composition of nucleotides, in the number of dinucleotide repeats. Based on the variability of DNA marker sections, it is possible to differentiate the maternal or paternal origin of a specific marker variant associated with the disease gene[13]. Thanks to the analysis of polymorphic genetic markers, it is possible to identify and track the chromosome carrying the pathological gene in generations. The methods of indirect

diagnostics are the same as in direct diagnostics (obtaining DNA, electrophoresis, etc.). The main drawback of indirect diagnostic methods is the mandatory preliminary study of the genotype of at least one sick relative[14]. Advances in the study of nucleic acids and protein biosynthesis have led to the creation of a number of methods that have great practical importance in medicine, agriculture and a number of other industries.

After studying the genetic code and the basic principles of storing and implementing hereditary information, the development of molecular biology stalled, since there were no methods to manipulate genes, isolate and change them. These methods appeared in the 1970-1980s. This gave a powerful impetus to the development of this field of science, which is still thriving today. First of all, these methods concern the production of individual genes and their introduction into the cells of other organisms (molecular cloning and transgenesis, PCR), as well as methods for determining the sequence of nucleotides in genes (DNA and RNA sequencing). These methods will be discussed in more detail below. We will start with the simplest basic method - electrophoresis, and then move on to more complex methods. This is the main method of working with DNA, which is used together with almost all other methods to isolate the desired molecules and analyze the results. Gel electrophoresis is used to separate DNA fragments by length. DNA is an acid, its molecules contain residues of phosphoric acid, which split off a proton and acquire a negative charge (Fig. 1).

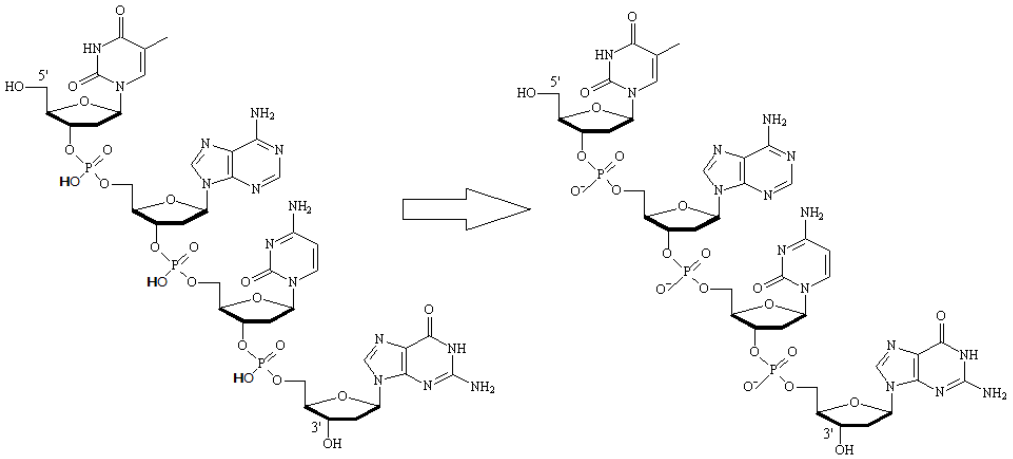

Fig. 1.

Therefore, in an electric field, DNA molecules move toward the anode - a positively charged electrode. This occurs in an electrolyte solution containing charge carrier ions, so that this solution conducts current. A dense polymer gel (agarose or polyacrylamide) is used to separate the fragments. The DNA molecules become entangled in it the more strongly, the longer they are, so the longest molecules move the slowest, and the shortest ones the fastest (Fig. 2). Before or after electrophoresis, the gel is treated with dyes that bind to DNA and fluoresce in ultraviolet light, and an image of the bands in the gel is obtained (see Fig. 3). To determine the length of the DNA fragments of the sample, they are compared with a marker - a set of fragments of standard length, applied in parallel to the same gel (Fig. 4).

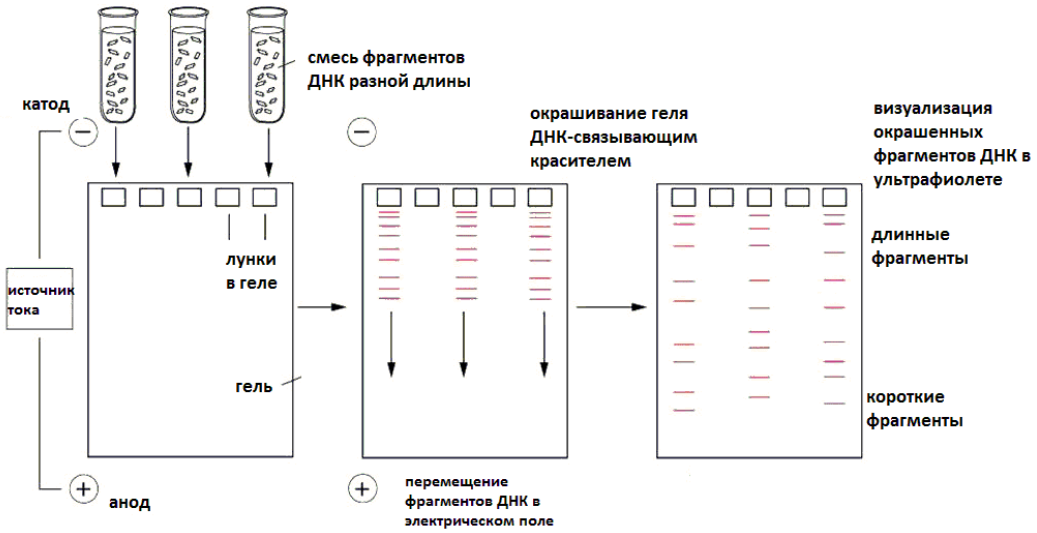

Fig. 2.

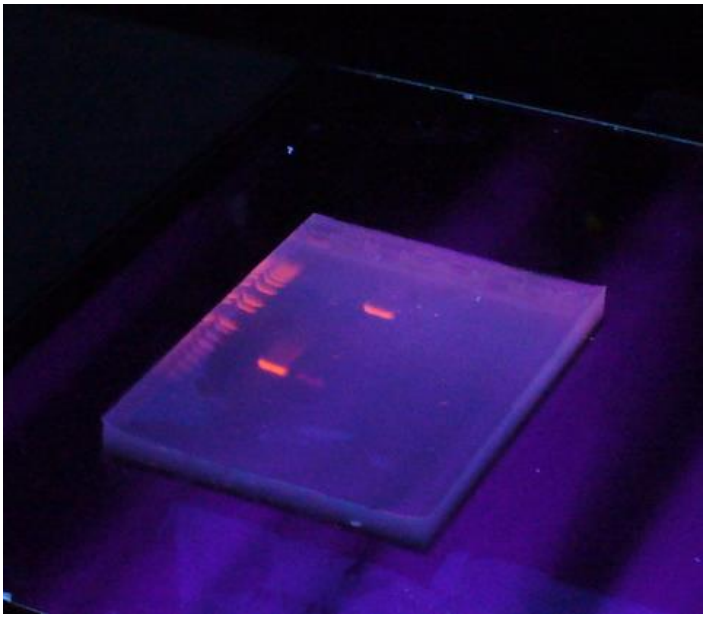

Fig. 3.

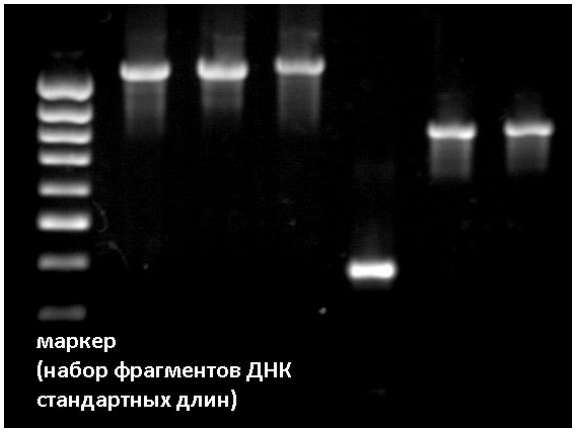

Fig. 4.

The most important tools for working with DNA are enzymes that transform DNA in living cells: DNA polymerases, DNA ligases, and restriction endonucleases. DNA polymerases synthesize template DNA, which allows DNA to be replicated in a test tube. DNA ligases stitch DNA molecules together or heal gaps in them. Restriction endonucleases, or restriction endonucleases, cut DNA molecules into strictly defined sequences, which allows individual fragments to be cut out of the total DNA mass. These fragments may in some cases contain individual genes[15].

The sequences recognized by restriction endonucleases are symmetrical, and breaks can occur in the middle of such a sequence or with a shift (in the same place in both DNA strands).

The scheme of action of different types of restriction enzymes is shown in Fig. 1. In the first case, so-called "blunt" ends are obtained, and in the second - "sticky" ends. In the case of “sticky” ends of the bottom, the chain turns out to be shorter than the other, forming a single-strand section with a symmetrical sequence, the same at both ends[16].

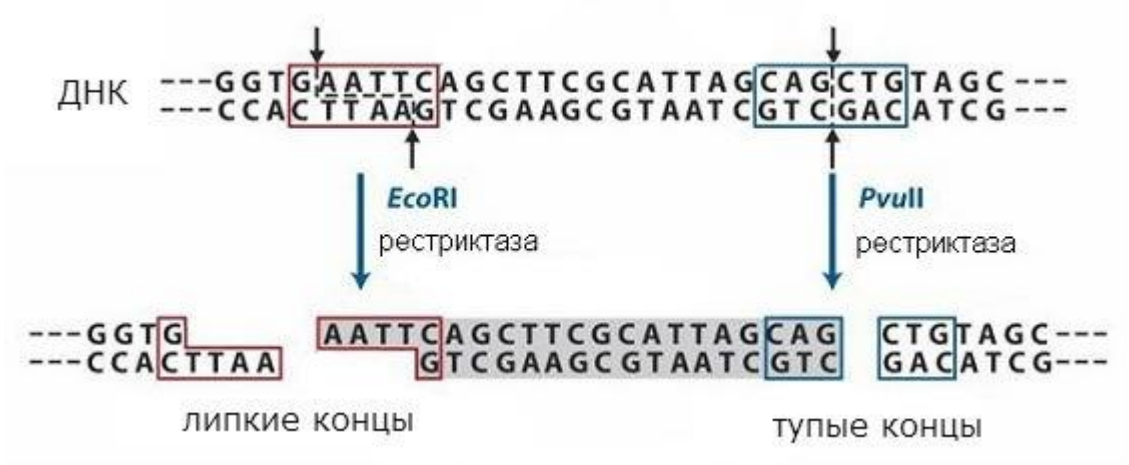

Fig. 1.

The end sequences will be the same when any DNA is digested with a given restriction enzyme, and can be re-linked because they have complementary sequences. They can be joined using DNA ligase to form a single molecule. In this way, fragments of two different DNAs can be combined to form what is called recombinant DNA. This approach is used in the molecular cloning technique, which allows individual genes to be obtained and introduced into cells that can produce the protein encoded by the gene[17].

Molecular cloning uses two DNA molecules - an insert containing the gene of interest, and a vector, a DNA that acts as a carrier. The insert is "sewn" into the vector using enzymes to produce a new recombinant DNA molecule, which is then introduced into host cells, and these cells form colonies on a nutrient medium. A colony is a descendant of a single cell, i.e. a clone, all cells in a colony are genetically identical and contain the same recombinant DNA. Hence the term "molecular cloning", i.e. obtaining a clone of cells containing the DNA fragment of interest. After obtaining colonies containing the insert of interest to us, this insert can be characterized by various methods, for example, by determining its exact sequence. Cells can also produce the protein encoded by the insert, When a recombinant molecule is introduced into cells, genetic transformation of these cells occurs. Transformation is the process of absorption by a cell of an organism of a free DNA molecule from the environment and its integration into the genome, which leads to the appearance in such a cell of new hereditary traits characteristic of the organism-donor of DNA. For example, if the inserted molecule contains a gene for resistance to the antibiotic ampicillin, then the transformed bacteria will grow in its presence. Before transformation, their death was caused by ampicillin, i.e. a new trait appears in the transformed cells[18].

The vector must have a number of properties:

Firstly, it is a relatively small DNA molecule that is easy to manipulate.

Secondly, in order for DNA to be preserved and multiplied in the cell, it must contain a certain sequence that ensures its replication (the origin of replication or the beginning of replication).

Thirdly, it must contain a marker gene that ensures the selection of only those cells that have received the vector. Usually, these are antibiotic resistance genes - then, in the presence of an antibiotic, all cells that do not contain the vector die[19].

Gene cloning is most often carried out in bacterial cells, since they are easy to cultivate and quickly multiply. A bacterial cell usually contains one large circular DNA molecule several million base pairs long, containing all the genes necessary for the bacteria - the bacterial chromosome. In addition, some bacteria have small (several thousand base pairs) circular DNAs called plasmids (Fig. 2). They, like the main DNA, contain a nucleotide sequence that ensures the ability of DNA to replicate

(ori). Plasmids replicate independently of the main (chromosomal) DNA, so they are present in the cell in large numbers of copies. Many of these plasmids carry antibiotic resistance genes to distinguish plasmid-bearing cells from normal cells[20].

More commonly used are plasmids that carry two genes that confer resistance to two antibiotics, such as tetracycline and amicillin. Simple methods exist to isolate such plasmid DNAs free of the main bacterial chromosome DNA.

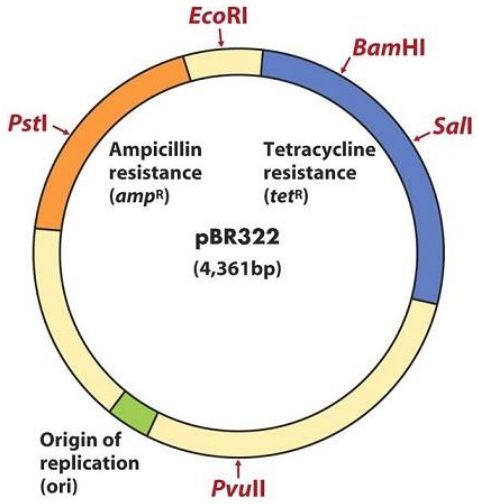

Fig. 2.

Transfer of genes from one organism to another is called transgenesis, and such modified organisms are called transgenic. By transferring genes into microorganism cells, recombinant protein preparations are obtained for medical purposes, in particular, human proteins that do not cause immune rejection - interferons, insulin and other protein hormones, cell growth factors, as well as proteins for vaccine production. In more complex cases, when protein modification occurs correctly only in eukaryotic cells, transgenic cell cultures or transgenic animals are used, in particular, livestock (primarily goats), which secrete the necessary proteins into milk, or proteins are isolated from their blood. This is how antibodies, blood clotting factors and other proteins are obtained. Cultivated plants are obtained by transgenesis, are resistant to herbicides and pests and have other useful properties. With the help of transgenic microorganisms, they purify wastewater and fight pollution, there are even transgenic microbes that can break down oil. In addition, transgenic technologies are indispensable in scientific research - the development of biology today is unthinkable without the routine use of gene modification and transfer methods. Biochemical methods are based on fractionation, analysis, and the study of the structure and properties of individual components of living matter[21]. Therefore, biochemical methods are universal methods of their use in biological, medical and biotechnological research. There are a number of methods of biochemical separation and purification, such as drying, chromatography, solvent extraction and distillation, which are an integral part of biotechnological research. For example, vaccine production is a complex biotechnological process that requires the extraction of protective antigens or antigen complexes from the grown microbial mass, purification and concentration of antigens, and the introduction of adjuvants into drugs [22]. Isolation and purification of antigens by traditional methods (extraction with trichloroacetic acid, acid or alkaline hydrolysis, enzymatic hydrolysis, salting out with neutral salts, precipitation with alcohol or acetone) are combined with the use of modern methods (high-speed ultracentrifugation, membrane ultrafiltration, chromatographic separation, affinity chromatography, including monoclonal antibodies). Using these methods, it is possible to obtain antigens of a high degree of purification and concentration [3]. It can be said that any vaccine is prepared by virological, biochemical and molecular biological methods, which together give a positive effect in the form of obtaining a vaccine with high medical and biological indicators.

The virus is grown in chicken fibroblast cultures. They resort to infecting cells with small doses of the virus and early collection of the vaccinated culture fluid. Under these conditions, the vaccinated material is slightly contaminated with cellular detritus, which contributes to the concentration and purification of the virus. The virus is inactivated with formalin (0.005% 72 h) and heating (32 ° C). This increases its stability during subsequent concentration and purification. Concentration and purification are carried out using differential and zonal ultracentrifugation in a sucrose density gradient [1]. Let us consider in more detail the technology for obtaining a subunit antiviral vaccine. In the technology for producing subunit vaccine preparations, it is first necessary to develop the biomass of microorganisms by fermentation in order to have a sufficient volume of microbial antigens for the production of vaccines on an industrial scale. Subunit vaccines containing

only surface antigens of the influenza virus have high immunogenicity and weak reactogenicity[23]. These are third-generation vaccines that achieve maximum purification of antigens from toxic impurities (including lipids). This vaccine contains hemagglutinin and neuraminidase and does not contain nucleoprotein proteins. It is the surface antigens that are important for developing immunity against influenza. Such vaccines are less reactogenic than vaccines with a split or whole virion. Examples of subunit vaccines are the Dutch Influvac, the German Agrippal, the Russian Grippol, etc. First, the epidemically relevant subtypes of the influenza virus type A or type B are determined. They are clinically isolated and cannot effectively reproduce in vitro[24].

Therefore, the genes are isolated from these viral particles, are responsible for the synthesis of hemagglutinin and neuraminidase and are inserted into the genome of a laboratory vaccine strain of the influenza virus, which reproduces well in a chicken embryo, i.e. A recombinant vaccine strain is obtained. In laboratory conditions, recombinant vaccine strains of the virus are obtained by infecting chicken embryos with them. The recombinant vaccine strain (these are influenza subtypes of type A and type B) must be sufficient for subsequent inoculation of chicken embryos on an industrial scale. In a production environment, tens of thousands of eggs are infected separately with each strain of the influenza virus (usually two subtypes of influenza A and type B are used). Incubation at 33-350C for 2-3 days. During this time, a sufficient number of viruses are reproduced. Using ultracentrifugation, the viral mass is isolated and usually inactivated with formaldehyde[25]. The subunits of hemagglutinin and neuraminidase are isolated by treating the viral particles with the detergent trimethylcetyl ammonium bromide and repeated ultracentrifugation and dialysis. Hemagglutinin and neuraminidase spontaneously combine into rosettes and are placed in a buffer solution containing potassium, sodium, calcium, and magnesium salts and very small amounts of a preservative to prevent microbial contamination. This procedure, consisting of a chain of biochemical reactions, is carried out with each of the three vaccine strains - two subtypes of type A virus and type B virus[26].

These strains are combined in a single syringe in a volume of 0.5 ml of solution. . The hemagglutinin and neuraminidase subunits are isolated by treating the virus particles with the detergent trimethylcetyl ammonium bromide and repeated ultracentrifugation and dialysis. Hemagglutinin and neuraminidase spontaneously combine into rosettes and are placed in a buffer solution containing potassium, sodium, calcium, and magnesium salts and very small amounts of a preservative to prevent microbial contamination. This procedure, consisting of a chain of biochemical reactions, is carried out with each of the three vaccine strains - two subtypes of the A virus and a type B virus. These strains are combined in a single syringe in a volume of 0.5 ml of solution. The subunits of hemagglutinin and neuraminidase are isolated by treating the viral particles with the detergent trimethylcetylammonium bromide and repeated ultracentrifugation and dialysis[27].

Hemagglutinin and neuraminidase spontaneously combine into rosettes and are placed in a buffer solution containing potassium, sodium, calcium and magnesium salts and very small amounts of a preservative to prevent microbial contamination. This procedure, consisting of a chain of biochemical reactions, is carried out with each of the three vaccine strains - two subtypes of the A virus and a type B virus. These strains are combined in a single syringe in a volume of 0.5 ml of solution. calcium and magnesium and a very small amount of a preservative to prevent microbial contamination. This procedure, consisting of a chain of biochemical reactions, is carried out with each of the three vaccine strains - two subtypes of the A virus and a B virus. These strains are combined in one syringe in a volume of 0.5 ml of solution. calcium and magnesium and a very small amount of preservative to prevent microbial contamination. This procedure, consisting of a chain of biochemical reactions, is carried out with each of the three vaccine strains - two subtypes of the A virus and a B virus. These strains are combined in one syringe in a volume of 0.5 ml of solution[28].

3. CONCLUSION

Biochemical and immunological methods are based on the analysis of various classes of organic and inorganic compounds that are defective in various hereditary diseases, primarily in hereditary metabolic diseases. Biochemical disorders, as a rule, precede the appearance of clinical symptoms of the disease and are more constant compared to them. The subject of biochemical diagnostics can be proteins, amino acids, carbohydrates, lipids, metal ions, etc., as well as their metabolites. In this case, various tissues and secretions of the body can be examined (blood, urine, saliva, sweat, cerebrospinal fluid, amniotic fluid, biopsy of muscles, skin, liver and other specialized tissues). Biochemical methods play a primary role in the diagnosis of hereditary metabolic disorders. In some cases, they allow the identification of heterozygous carriers of mutations. The role of biochemical methods of analysis is very important in conducting mass examinations of pregnant women or newborns for the purpose of earlier detection of hereditary diseases. The key role in the pathogenesis of any monogenic disease belongs to the primary biochemical defect - a protein encoded by a mutant gene. Detection and analysis of the primary biochemical defect, determination of the primary pathological metabolic chain - these are the main tasks of biochemical genetics, the solution of which is the basis for the development of pathogenetic methods for the prevention and treatment of hereditary diseases. No less important is the role of biochemical methods in the diagnosis of secondary disorders. For example, the main biochemical defect in Duchenne / Becker muscular dystrophy is a deficiency of dystrophin, a protein that connects the cytoskeleton of muscle cells with the extracellular matrix. As a result of this disorder, the level of one of the muscle enzymes, creatine phosphokinase, increases in the blood of patients, both at the onset of the disease and at its advanced stage. Moreover, the content of this enzyme is increased in 30% of heterozygous carriers of the mutation. Although this disorder is secondary,

the prostate test for creatine phosphokinase and its persistent increase in patients make it a convenient diagnostic marker of the disease. The variety of biochemical methods is huge, and they are constantly being improved. They are divided into qualitative, quantitative and semi-quantitative. Qualitative reactions allow you to detect an excess of intermediate metabolites that accumulate in hereditary metabolic diseases as a result of a block of enzymatic reactions. They are simple, inexpensive and quite sensitive. Urine is often used as a substrate for a qualitative reaction. Semi-quantitative and quantitative tests are carried out with both urine and blood. The simplest of them are measuring pyruvate, lactate, ammonium ions, measuring the acid-base balance.

Conclusion: the level of one of the muscle enzymes, creatine phosphokinase, increases in the blood of patients, both at the onset of the disease and at its advanced stage. Moreover, the content of this enzyme is increased in 30% of heterozygous carriers of the mutation. Although this disorder is secondary, the prostate test for creatine phosphokinase and its persistent increase in patients make it a convenient diagnostic marker of the disease. The variety of biochemical methods is huge, and they are constantly being improved. They are divided into qualitative, quantitative and semi-quantitative. Qualitative reactions allow you to detect an excess of intermediate metabolites that accumulate in hereditary metabolic diseases as a result of a block of enzymatic reactions. They are simple, inexpensive and quite sensitive. Urine is often used as a substrate for a qualitative reaction. Semi-quantitative and quantitative tests are carried out with both urine and blood. The simplest of them are measuring pyruvate, lactate, ammonium ions, measuring the acid-base balance.

REFERENCES

[1] Akimova, T.V. Ecology. Man-Economy-Biota-Environment: Textbook for students of higher education institutions / T.A. Akimova, V.V. Khaskin; 2nd ed., Revised. and add. - M .: UNITI, 2017. - 556 p.

[2] Akimova, T.V. Ecology. Nature-Man-Technology .: Textbook for students of technical. direction and special. Universities / T.A. Akimova, A.P. Kuzmin, V.V. Khaskin - Under the general editorship of A.P. Kuzmin; Laureate of the All-Russian. creativity competition. new textbooks on general natural science. discipline for students of universities. M .: EDINSTVO-DANA, 2016. - 343 p.

[3] Arkhangelsky, V. I. Hygiene and human ecology: Textbook / V. I. Arkhangelsky, V. F. Kirillov. - M .: GEOTAR-Media, 2017. - 176 p.

[4] Brodsky, A. K. General ecology: Textbook for university students / A. K. Brodsky. - M .: Publishing house. Center "Academy", 2016. - 256 p.

[5] Qassim A. Ahmood and Mohammed H. Al-Jawasim (2019). EFFECTS OF HEAVY METALS ON PHYSIOLOGICAL STATUS OF PLANTS. *Plant Archives* Vol. 19 No. 2, 2019 pp. 2865-2871 e-ISSN:2581-6063 (online), ISSN:0972-5210.

[6] Voronkov N. A. Ecology: general, social, applied. Textbook for university students / N. A. Voronkov. M .: Agar, 2016. 424 p.

[7] Qassim Ammar Ahmood Al-Janabi and Lamees N. H. and Aqeel K. I. (2023). Effects of Nano-Fipronil on Male Rats' Biochemical, Liver, and Renal Functions. Fourth International Scientific Conference of Agriculture, Environment and Sustainable Development College of Agriculture, University of Al-Qadisiyah, IRAQ.

[8] Galperin, M. V. General ecology: Textbook / M. V. Galperin. - M .: Forum, 2016. - 336p.

[9] Abbas Taleb Khleif, Qassim Ammar Ahmood AL-Janabi and Aqeel Khaleel Ibraheem (2020). IDENTIFICATION OF QUANTITY OF HEAVY METALS IN DIFFERENT TYPES OF TOBACCO IN SHISHA AND CIGARETTE BRANDS. *Plant Archives* Vol. 20, Supplement 1, 2020 pp. 214-216 e-ISSN:2581-6063 (online), ISSN:0972-5210.

[10] Mohammed E. Al Defferi1, Qassim A. AL-Janabi, Sama A. Mustafa and Ali K. AL-Muttarri (2019). PHYTOREMEDIATION OF LEAD AND NICKEL BY *BASSIA SCOPARIA. Plant Archives* Vol. 19 No. 2, 2019 pp. 3830-3834 e-ISSN:2581-6063 (online), ISSN:0972-5210.

[11] Qassim A. A. AL-Janabi*, Saad Kadhim A. Al- Kalidy*& Zaid B. Hameed (2021).Effects of heavy metals on physiological status for Schoenoplectus litoralis & Salvinia natans *L* 1st INTERNATIONAL VIRTUAL CONFERENCE OF ENVIRONMENTAL SCIENCES IOP Conf. Series: Earth and Environmental Science 722 (2021) 012012 IOP Publishing doi:10.1088/1755-1315/722/1/012012.

[12] Suhad AL-Hedny1, Qassim A. Talib Alshujairy and QasimAmmar A. Al-Janabi(2018). USING REMOTE SENSING DERIVED INDICES TO MONITOR VEGETATION COVER CHANGES OF BABYLON. *Plant Archives* Vol. 18 No. 2, 2018 pp. 2425-2428 e-ISSN:2581-6063 (online), ISSN:0972-5210.

[13] Mavrishchev, V.V. General ecology. Course of lectures: Textbook / V.V. Mavrischev. - M.: NIC INFRA-M, Nov. knowledge, 2017. - 299 p.

[14] Qassim Ammar Ahmood Al-Janabi and Hind Suhail Abdulhay(2024). Study the Toxic Effects of Nano-

Imidaclopred and Imidaclopred Pesticides on the Physiological Aspects of Male Rats. the 3rd International Conference on Engineering and Science to Achieve the Sustainable Development Goals, publication in AIP Conference Proceedings (2024).

[15] Brukhan, F. F. Industrial ecology: Textbook / F. F. Brukhan, M. V. Grafkina, E. E. Sdobnyakov. - M.: Forum, 2017. - 208 p.

[16] Zaitsev, V. A. Industrial ecology: Textbook / V. A. Zaitsev. - M.: BINOM. LZ, 2016. - 382 p.

[17] Abbas Taleb Khleif, Qassim Ammar Ahmood Al-Janabi and Aqeel Khaleel Ibraheem 2023.Effects of (Pb, Fe, Ni) on Physiological Status for Potamogeton crispus L. and Potamogeton pectinatus L. 9th International Conference on Agriculture and Environmental Sciences. College of Agriculture, Tikrit University, Iraq.

[18] Qassim Ammar Ahmood Al-Janabi1, Hind Suhail Abdulhay(2024). Effect of Inhalation Exposure to Nano-Imidacloprid on Liver and Kidney Functions In Male Rats. Third Virtual International Conference on Environmental Sciences, IOP Conf. Series: Earth and Environmental Science 1325 (2024) 012021, Al-Qasim Green University, College of Environmental Science, IRAQ.

[19] Abdallah MAM (2012). Phytoremediation of heavy metals from aqueous solutions by two aquatic macrophytes, Ceratophyllum demersum and Lemna gibba L. Environmental technology, 33(14): 1609-1614.

[20] Abioye O, Agamuthu P, Aziz A (2012). Phytotreatment of soil contaminated with used lubricating oil using Hibiscus cannabinus. Biodegradation, 23(2): 277-286.

[21] Borne KE, Fassman-Beck EA, Tanner CC (2014). Floating Treatment Wetland influences on the fate of metals in road runoff retention ponds. Water research, 48: 430-442.

[22] El-Khatib A, Hegazy A, Abo-El-Kassem AM (2014). Bioaccumulation Potential and Physiological Responses of Aquatic Macrophytes to Pb Pollution. International Journal of Phytoremediation,16(1):29-45.

[23] Falinski K, Yost R, Sampaga E, Peard J (2014). Arsenic accumulation by edible aquatic macrophytes. Ecotoxicology and environmental safety, 99: 74-81.

[24] Bhardwaj R, Gupta A, Garg JK.(2017). Evaluation of heavy metal contamination using environmetrics and indexing approach for River Yamuna, Delhi stretch, India. Water Science. 2017;31(1):52-66.

[25] Mbengue S, Alleman LY, Flament P (2014). Sizedistributed metallic elements in submicronic and ultrafine atmospheric particles from urban and industrial areas in northern France. Atmospheric Research,135:35-47.

[26] Ghosh A, Dastidar MG, Sreekrishnan TR. Bioremediation of a chromium complex dye using *Aspergillus flavus* and *Aspergillus tamarin*. Chemical Engineering & Technology. 2016;39(9):1636-44.

[27] Tauqeer HM, Ali S, Rizwan M, Ali Q, Saeed R, Iftikhar U, Ahmad R, Farid M, Abbasi GH. Phytoremediation of heavy metals by *Alternanthera bettzickiana*: Growth and physiological response. Ecotoxicology and Environmental Safety. 2016;126:138-46.

[28] Bhatia M, Goyal D (2013). Analyzing remediation potential of wastewater through wetland plants: A review. Environmental Progress & Sustainable Energy.

[29] Bianconi D, Pietrini F, Massacci A, Iannelli M (2013). Uptake of Cadmium by Lemna minor, a (hyper-) accumulator plant involved in phytoremediation applications. E3S Web of Conferences, EDP Sciences.