GENE BIOTECHNOLOGY

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CONTENTS

CHAPTER 1

AN INTRODUCTION OF GENETICS AND BIOTECHNOLOGY

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ABSTRACT:

Biotechnology is the study of methods that employ living things or their enzymes to create things that are helpful to people. In this way, microbe-mediated activities like generating curd, bread, or wine might also be considered a kind of biotechnology. However, term is only used nowadays to describe procedures that employ genetically modified organisms to do the same on a bigger scale. Furthermore, biotechnology encompasses a wide range of other procedures and methods. Biotechnology includes processes like creating a test-tube baby by in vitro fertilization, synthesizing genes for use, creating DNA vaccines, and fixing gene defects. The term biotechnology has been defined by the European Federation of Biotechnology to include both conventional biotechnology and contemporary molecular biotechnology. The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services, according to EFB.

KEYWORDS:

Biotechnology, DNA vaccines, Enzymes, Organisms.

INTRODUCTION

Restrictions enzymes are like molecular scissors. The plasmid DNA was then joined to the sliced segment of DNA. These plasmid DNA molecules serve as carriers for the DNA fragments they are connected to. As you undoubtedly well know, mosquitoes serve as an insect vector for the transmission of the malarial parasite into humans.A plasmid may also be employed as a vector to introduce foreign DNA into the host cell. The enzyme DNA ligase, which works on cut DNA molecules and connects their ends, made it feasible to link the antibiotic resistance gene with the plasmid vector. Recombinant DNA is a novel mix of circular, independently replicating DNA that was made in a lab. This DNA might reproduce utilizing the DNA polymerase enzyme of the new host when it is transplanted into Escherichia coli, a bacterium closely related to Salmonella. Cloning of the *E. coli* antibiotic resistance gene refers to the process of producing several copies of the organism's antibiotic resistance gene.Thus, it follows that there are three fundamental phases involved in genetically altering an organism: identifying DNA with desired genes; introducing the identified DNA into the host; and maintaining the introduced DNA in the host and passing the DNA on to the host's offspring.

Recombinant DNA Techniques Tools:

We now understand from the description above that genetic engineering or recombinant DNA technology can only be carried out if the necessary tools are available, namely restriction enzymes, polymerase enzymes, ligases, vectors, and the host organism. Let's attempt to comprehend some of them in further depth.The two enzymes that limit bacteriophage development in Escherichia coli were discovered in the year 1963. One of them modified DNA by adding methyl groups, whilst the other cut DNA. Restrictions endonuclease was the name given to the latter[1], [2].Five years later, the first restriction endonuclease whose activity required a particular DNA nucleotide sequence was isolated and defined. It was discovered that by identifying a certain sequence of six base pairs, *Hind II* consistently cut DNA strands at a particular place. The recognition sequence for *Hind II* is a particular base

sequence. In addition to *Hind II*, there are already over 900 restriction enzymes that have been identified from over 230 bacterial strains and each of which recognizes a unique recognition sequence.These enzymes are named according to a convention where the first letter of the name is derived from the genus and the next two letters are derived from the species of the prokaryotic cell from which they were isolated, for example, Cori is derived from Escherichia coli. The letter 'R' in Cori is taken from the name of train. The sequence in which the enzymes were extracted from that strain of bacteria is indicated by the Roman numerals after the names.The wider family of enzymes known as nucleases includes restriction enzymes. Exonucleases and endonucleases are the two types of these.Endonucleases cut the at specified locations, while exonucleases remove nucleotides from the ends of the.

By 'inspecting' the length of a sequence, each restriction endonuclease performs its purpose. It will connect to the after locating its unique recognition sequence and cut the two double helix strands at specified locations in their sugar-phosphate backbones. The contains distinct palindromic nucleotide sequences that are recognized by each restriction endonuclease.Are you familiar with palindromes? The word is an example of a set of letters that make the same word when read both forward and backward. The palindrome in DNA is a sequence of base pairs that reads the same on the two strands when orientation of reading is maintained, as opposed to a word-palindrome when the same word is read in both directions. The sequences that follow, for instance, read identically on both strands in the 5' to 3' orientation. This still holds true when read from 3' to 5'.The DNA strand is cut by restriction enzymes between the identical two bases on the opposing strands, but a little bit distant from the palindrome sites' centers. The ends are now left with single stranded sections. Each strand has overhanging sections known as sticky ends.

These share hydrogen bonds with their corresponding cut counterparts, which is how they got their name. The DNA ligase enzyme can work more easily because of the sticky ends.In genetic engineering, restriction endonucleases are employed to create recombinant DNA molecules, which are made up of DNA from several sources/genomes.The same sort ofsticky-ends is produced when the same restriction enzyme cuts DNA, and these fragments may be connected using DNA ligases. You may be aware that the creation of the recombinant vector molecule is typically impossible unless the source DNA and the vector are cut with the same restriction enzyme.DNA fragment separation and isolation: DNA fragments are produced when restriction endonucleases cleave DNA. It is possible to separate these fragments using a process called gel electrophoresis. Since DNA fragments are negatively charged molecules, it is possible to separate them by directing them through a medium or matrix in the direction of the anode while being subjected to an electric field. Agarose, a natural polymer derived from sea weeds, is now the most often used matrix. Through the sifting action that the agarose gel provides, the DNA fragments separate according to their size. As a result, a fragment goes further the smaller it is[3], [4].

Try to determine which end of the gel the sample was loaded by looking.Only after staining the DNA with the substance ethidium bromide and exposing it to UV light can the separated DNA fragments be seen; pure DNA fragments cannot be seen in visible light or without staining. In a gel stained with ethidium bromide and illuminated with UV light, bands of DNA in a vivid orange hue may be seen. The agarose gel is sliced into pieces, and the extracted DNA is sorted into bands. Elution is the term for this action. By connecting the DNA fragments with cloning vectors, the pieces of DNA that have been purified in this manner are utilized to create recombinant DNA.You are aware that bacteriophages and plasmids may reproduce within bacterial cells without the guidance of chromosomal DNA.Due to their high cell density, bacteriophages have extremely high genomic copy numbers inside the bacterial cells. While others may have 15–100 copies per cell, certain

plasmids may only have one or two copies in each cell. Their numbers may possibly increase. If we can connect an extraterrestrial fragment of DNA to virus or plasmid DNA, we can multiply it by the number of copies of the plasmid or bacteriophage. Present-day vectors are designed to make it simple to connect foreign DNA and distinguish recombinants from non-recombinants.

DISCUSSION

Replication's point of origin Any DNA fragment may be induced to replicate within the host cells by being connected to this region, which is where replication begins. The quantity of copies of the linked DNA is also under the control of this sequence. As a result, the target DNA should be cloned in a vector whose origin supports large copy number if one wishes to recover many copies of the target DNA. An adjustable marker in addition to the vector also needs a selectable marker that aids in spotting and removing nontrans formants while allowing the development of transformants only in certain areas. Transformation is the process by which a fragment of DNA is inserted into a host bacterium. Typically, selectable markers for *E. coli* are believed to be genes that encode resistance to antibiotics like ampicillin, chloramphenicol, tetracycline, or kanamycin, among others. None of these drugs are resistant in the common *E. coli* cells.Cloning sites: The vector must include only a few, preferably a single, recognition sites for the typically used restriction enzymes in order to connect the foreign DNA. many recognition sites inside the vector will result in many fragments, which will make it more difficult to clone the gene. One of the two antibiotic resistance genes has a restriction site that is used to ligate foreign DNA. For instance, the tetracycline resistance gene in the vector pBR322 may have a piece of foreign DNA ligated at the Bah I site.

Due to the inclusion of foreign DNA, the recombinant plasmids will no longer be tetracycline resistant, but they may still be distinguished from non-recombinant ones by plating the transformants on media containing ampicillin. The transformants that are developing on the ampicillin-containing media are subsequently transferred to the tetracycline-containing medium. In the presence of ampicillin, the recombinants will grow, but not in the presence of tetracycline. Nonrecombinants, however, will thrive on a medium that contains both antibiotics. In this instance, one antibiotic resistance gene aids in the selection of transformants, whilst the second antibiotic resistance gene aids in the selection of recombinants after being inactivated by the insertion of foreign DNA.Selection of recombinants owing to antibiotic inactivation is a laborious process since it requires concurrent plating on two plates with various antibiotics.

Alternative selective markers have thus been created to distinguish recombinants from non-recombinants based on their capacity to generate color when exposed to a chromogenic substrate. In this, an enzyme called -galactosidase has recombinant DNA inserted into its coding sequence[5], [6]. Inactivation of the enzyme occurs as a consequence, and is known as insertional inactivation. If a chromogenic substrate is present, colonies will be blue if the bacteria's plasmid does not include an insert. When an insert is present, the â-galactosidase is inserted inactivated, and the colonies do not generate any color, they are known as recombinant colonies.Gene-cloning vectors for plants and animals: You may be astonished to find that bacteria and viruses, which have long understood how to transport genes to convert eukaryotic cells and drive them to perform what the bacteria or viruses desire, taught us the lesson of transferring genes into plants and animals. For instance, Agrobacterium tumefaciens, a pathogen that affects various dicot plants, may transfer a DNA fragment known as to normal plant cells, turning them into tumors, and instructing the tumor cells to manufacture the chemicals the pathogen needs. Animal retroviruses can do the same thing, turning healthy cells into malignant ones.

Knowledge to adapt these viruses' tools into practical vectors for delivering genes of interest to humans has been produced through a greater understanding of the art of delivering genes by pathogens in their eukaryotic hosts. The Agrobacterium tumefaciens tumor-inducing plasmid has been transformed into a cloning vector that is no longer harmful to plants but can still employ the mechanisms to transport genes of interest into a range of plants. Retroviruses have been similarly neutralized and are currently utilized to introduce desired genes into animal cells. As a result, after being ligated into an appropriate vector, a gene or DNA fragment is transmitted into a bacterial, plant, or animal host. Since DNA is a hydrophilic molecule, cell membranes are impermeable to it. Why? The bacteria must first be rendered 'competent' to take up DNA in order to compel them to take up the plasmid. The method for doing this is to subject them to a certain concentration of a divalent cation, such calcium, which improves the efficiency with which DNA penetrates cells.

Then, by placing the recombinant DNA-containing cells on ice, heating them for a short period of time to 420C, and then returning them to the ice, recombinant DNA may be pushed into these cells. This makes it possible for the bacteria to absorb the recombinant DNA.There are other ways to get foreign DNA into host cells than this one. Recombinant DNA is directly injected into the nucleus of an animal cell using a technique called micro-injection. Another technique, ideal for plants, involves firing high-velocity gold- or tungsten-coated microparticles into cells. This technique is called biolistic or gene gun. The last technique employs 'disarmed pathogen' vectors, which, when permitted to infect the cell, transmit the recombinant DNA into the host.After learning about the equipment used to create recombinant DNA, let's talk about the procedures that make recombinant DNA technology possible.

Recombinant DNA technology entails a number of steps that must be completed in a particular order, such as DNA isolation, restriction endonuclease fragmentation of the DNA, isolation of a desired DNA fragment, ligation of the desired DNA fragment into a vector, transfer of the recombinant DNA into the host, large-scale cultivation of the host cells in a medium, and extraction of the desired product.Remember that all creatures have nucleic acid as their genetic makeup. Most species use DNA or deoxyribonucleic acid for this. Restrictions enzymes require the DNA to be pure and free of other macromolecules in order to cut it. We must split up the cell in order to release DNA along with other macromolecules including RNA, proteins, polysaccharides, and lipids since the DNA is contained inside the membranes. This may be accomplished by using enzymes like lysozyme,cellulase, or chitinase on the bacterial cells, plant, or animal tissue.

You are aware that genes are situated on lengthy DNA molecules bound to proteins like histones. While proteins may be removed using a protease treatment, RNA can be removed using ribonuclease. Purified DNA eventually precipitates out following the addition of cold ethanol. Other molecules may be eliminated by using the proper techniques. In the suspension, this appears as a clump of tiny threads. By incubating isolated DNA molecules with the restriction enzyme under the ideal circumstances for that particular enzyme, restriction enzyme digestions are carried out. Use of agarose gel electrophoresis to monitor a restriction enzyme's digestion process. Given that DNA has a negative charge, it gravitates towards the positive electrode. The vector DNA is also used in the procedure.Numerous procedures are involved in the joining of DNA. The 'gene of interest' from the source DNA and the cut vector with space are combined, and ligase is added, after the source DNA and the vector DNA have, both been cut using a particular restriction enzyme. This causes recombinant DNA to be created.

PCR-Based Gene Amplification Polymerase Chain Reaction is the technical term for this procedure. In this procedure, two sets of primers and the enzyme DNA polymerase are used to synthesize multiple copies of the gene of interest in vitro.Using the nucleotides present in

the reaction as well as genomic DNA as a template, the enzyme lengthens the primers. The DNA segment may be expanded to around a billion times, or 1 billion copies, if the DNA replication process is done several times. Using a thermostable DNA polymerase, which remains active despite the high temperature-induced denaturation of double stranded DNA, allows for such recurrent amplification. If desired, the amplified region may now be ligated to a vector for further cloning. The ligated DNA may be introduced into recipient cells via a variety of techniques. After being made competent to receive, recipient cells absorb DNA from their surroundings. Therefore, if *E. coli* bacteria are given recombinant DNA containing a gene for antibiotic resistance, the host cells will change into ampicillin-resistant cells. Only the converted cells will grow and untransformed recipient cells will perish if we disseminate the transformed cells on agar plates containing ampicillin. Since one might choose a changed cell in the presence of ampicillin thanks to the ampicillin resistance gene. In this instance, the ampicillin resistance gene is referred to as a selectable marker[7], [8].

The Foreign Gene Product's Acquisition:

A portion of foreign DNA is multiplied when it is inserted into a cloning vector and introduced into a bacterial, plant, or animal cell. The creation of a desired protein is the ultimate goal of practically all recombinant technologies. Consequently, the recombinant DNA must be expressed. Under the right circumstances, the foreign gene is expressed.Understanding a lot of technical information is necessary to comprehend how foreign genes are expressed in host cells must think about mass manufacturing the target protein after the gene of interest has been cloned and the conditions to trigger its expression have been perfected. Do you have any ideas as to why mass manufacturing is necessary? A protein is referred to be recombinant if its encoding gene is expressed in a heterologous host. It is possible to cultivate the cells with the desired cloned genes on a modest scale in the lab. The target protein may be extracted from the cultures and then purified using a variety of separation methods.In order to keep the cells in their physiologically most favorable state, the cells may also be replicated in a continuous culture system where the old medium is drained out from one side and new media is introduced from the other[9], [10].

CONCLUSION

The bigger biomass produced by this kind of culturing technique results in better yields of required protein.Small volume cultures are unable to produce significant amounts of goods.The creation of bioreactors, which can handle enormous volumes of culture, was necessary to manufacture in big numbers.Thus, employing microbial plant, animal, or human cells, bioreactors may be conceived of as containers in which raw ingredients are biologically transformed into particular products, individual enzymes, etc. In a bioreactor, the ideal growth conditions are provided, resulting in the production of the desired product. A stirred-tank reactor often has a curved base or is cylindrical to aid in mixing the reactor's contents. The bioreactor's stirrer allows equal mixing and oxygen availability. An alternative is to bubble air through the reactor. If you carefully examine the diagram, you will see that the bioreactor contains sampling ports, an agitator system, an oxygen supply system, a foam control system, temperature control system, pH control system, and other systems that enable periodic removal of tiny amounts of the culture.The product must go through a number of steps once the biosynthetic stage is complete before it is suitable for selling as a completed good. The procedures, which are referred to as downstream processing, include separation and purification. The right preservatives must be used in the product's formulation. Like with pharmaceuticals, such a formulation must go through extensive clinical testing. For every product, strict quality control testing is also necessary. Different products need different downstream processing and quality control testing.

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CHAPTER 2

ENVIRONMENTAL MONITORING AND POLLUTION REDUCTION USING BIOTECHNOLOGY

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ABSTRACT:

Due to pressure from the economy, the general public, and the law, demand for the adoption of sustainable and environmentally friendly environmental processes has been rising quickly in recent years. When it comes to the monitoring, evaluation, modelling, and treatment of polluted water, air, and solid waste streams, biotechnology offers a wide range of options. Because of their accuracy and resilience, biologically based approaches for process modelling and source tracing of environmental contaminants are becoming more and more significant in this context. The many biotechniques that are now accessible therefore constitute both established and emerging technologies, albeit certain elements of their effectiveness still need to be investigated. For instance, the application of innovative biocatalysts and reactor designs, the comprehension of the dynamics and processes governing the microbial population inside a reactor, and the evaluation and modelling of the performance of reactors during long-term operation. Novel biotechniques may alter how users rebuild technology for the sustainable use of various biological processes for the treatment of wastewater, air, and solid waste if these mechanisms are recognized and the obstacles are removed.

KEYWORDS:

Biotechnology, Environmental Monitoring, Resilience, Sustainable.

INTRODUCTION

Over the course of six months, this special issue received 34 research/review submissions, of which excellent papers were chosen for publication after undergoing a double-blind peer review. These articles, which have been approved, concentrate on the numerous basic and applied engineering facets of various methods and procedures that may have real-world applications in the developing domains of environmental biotechnology. This special issue spotlights some difficult problems with environmental monitoring and pollution reduction that fall into five categories of subject study. Monitoring and modelling of the environment. Due to growing industrialization and urbanization, pollution of the water, air, and soil has emerged as a persistent environmental issue in emerging nations.

It was discovered using the environmental Kuznets curve that, during the early phases of economic development in a given place, the environment paid a significant price for economic progress as the human race exploited technology to exploit all feasible valuable resources. However, N, P, and K compounds are readily carried by field drainage and surface water to important water resources in agricultural regions, which results in the worsening of water quality and justifies the employment of innovative biosensors to monitor water quality. It has recently been suggested that cellular-based biosensor technologies, namely the bioelectric recognition assay, make use of live, functioning cells in a gel matrix together with a sensor device that can track changes in the cellular electric characteristics [1], [2]. As a consequence of their engagement with a target analyte, cells that can precisely interact with it create a particular pattern of electrical potential. Traditional process-based models that are based on mass balance principles, basic reaction kinetics, and a plug flow of water/air stream have been used to simulate and forecast the performance of numerous bioprocesses. A

different modelling method uses a data-driven methodology and applies artificial intelligence concepts with the aid of neural networks. The domains of applied biosciences and bioengineering often use neural network modelling theory. Under this category, the following research articles were approved. Environmental Kuznets curve analysis of economic development and nonpoint source pollution in the Ningxia Yellow River irrigation districts in China by C. Mao et al. Back propagation neural network model for predicting the performance of immobilized cell biofilters handling gas-phase hydrogen sulphone and ammonia" by E. R. Rene et al. Pesticide Residue Screening Using Novel Artificial Neural Network Combined with A Bioelectric Cellular Biosensor" by K. P. Ferentinos et al.Removal of Pollutants and Toxicity. Water coming from acid mine drainage, industrial businesses, and agricultural runoffs often include environmental contaminants such heavy metals and pesticides. These harmful chemicals may build up in living things and have negative effects including acute toxicity and carcinogenicity. A biological method that includes active bacterial, fungal, and mixed microbial cultures may completely mineralize and/or remove these contaminants and their harmful byproducts. Since their variety promotes environmental survival and multiplies the number of catabolic routes accessible for contaminant biodegradation, microbial consortia have been found to be more appropriate for bioremediation of refractory substances like pesticide residues. Biological catalysts are used in biosorption, a potential low-cost technique, to extract and recover heavy metals from aqueous solutions in the case of heavy metal-contaminated wastewaters. The chemistry of metal ions, the composition of microbe cell walls, the physiology of the organism, and physicochemical parameters like pH, temperature, time, ionic strength, and metal concentration all play a role in the complicated process of the metal removal mechanism. Four to eight of the following pieces were chosen for publishing in this portion of the special edition. Dissolution of arsenic minerals mediated by dissimilatory arsenate reducing bacteria: estimation of the physiological potential for arsenic mobilization by D. Lukasz et al. Kinetics of Molybdenum Reduction to Molybdenum Blue by Bacillus sp. strain A. raze by A. R. Othman et al [3], [4].

The study the uptake mechanism of Cd, Cr, Cu, Pb, and Zn by mycelia and fruiting bodies of Galería vitriform was conducted by D. Damodaran et al. Toxicity of superparamagnetic iron oxide nanoparticles on green alga Chlorella vulgaris by D. Dewed and L. Barhoum. S. Fuentes and colleagues' study Enhanced removal of a pesticide’s mixture by single cultures and consortia of free and immobilized Streptomyces strains, Biofuels Manufacturing. A sustainable solution to the current energy issue and the creation of a sustainable green environment is the generation of biohydrogen by anaerobic fermentation. Processes for producing fermentable hydrogen are large-scale commercial Zable, technically practicable, and economically cost-competitive. Recently, it was shown that microorganisms found in the sediments of mangroves have the potential to make biohydrogen in addition to some of the pure microbial species that may be utilized to produce biofuels. Mangrove sediments are naturally abundant in organic material and provide the following benefits: flexible substrate utilization, ease of handling, lack of significant storage issues, little need for preculturing, and inexpensive availability of sediments. In contrast, the use of marine and freshwater microalgae as a third-generation biomass feedstock for the development of energy has also recently been investigated. This is because microalgae can grow quickly with high specific growth rates, have excellent CO2 absorption capacity, and can better regulate their lipid and sugar content under different culture conditions. Microalgae have a great ability to adapt to their surroundings, including high salinity, heavy metal ion content, the presence of toxicants, and high CO2 concentrations. The synthesis of biodiesel and biohydrogen is discussed in the works. Biohydrogen production and kinetic modelling using sediment microorganisms of Pichavaram mangroves, India" by P. Mullai et al. "Production of biodiesel from Chlorella sp. enriched with oyster shell extracts" by C. S. Choi et al. "Enhancement of biodiesel production from marine alga, Scenedesmus sp. through in situ transesterification process

associated with acidic catalyst" by G. V. Kim et al. Microbial Environmental Products. Biosynthetic and biodegradable biopolymers like poly-hydroxybutyrate, which have exceptional biodegradability and are sustainable and ecologically friendly, have drawn a lot of attention as environmental awareness grows. By developing new strains, optimizing the fermentation and separation processes, and using economical carbon sources, the high production costs of PHB may be reduced. Recent developments in fermentation technology and related fields have led to the research of alternate purification methods, the most intriguing and promising of which are microbiological methods of byproduct utilization. The productivity of the process as a whole may increase as a consequence, and the downstream processing might be facilitated. Regarding the utilization of enzymes, the white-rot fungus Phanerochaete chrysophobia has been used in several bioremediation investigations due to its alginolytic enzyme system. Thus, the synthesis of lignin peroxidase and manganese peroxidase, two lignin-modifying enzymes typically produced under nitrogen-limited growing conditions, as well as the intracellular cytochrome P450 system, are associated to its capacity to break down a range of contaminants. The use of an improved biological phosphorus removal for phosphorus removal from wastewaters is another useful consideration worth noting in this section. Polyphosphate accumulating organisms with an excess capacity for phosphorus accumulation may be enhanced in EBPR by the use of different anaerobic and aerobic phases. As the anaerobic phase progresses, PAOs absorb organic carbons like acetate and propionate and store them as intracellular polymers like PHBs, using glycogen as the reducing power source and polyphosphate as the energy source. To better understand the role of polymers in EBPR, it is important to thoroughly examine the metabolism of PAOs and the dynamics of polymers under various concentrations of organic carbon. The performance of the EBPR under low organic carbon produced shock settings may be controlled and adjusted by looking into the dynamics of polymers during endogenous respiration conditions. The articles listed below were chosen for publication under the heading Microbial Production for the Environment [5], [6].Degradation of diuron by Phanerochaete chrysophobia: role of ligninolytic enzymes and cytochrome P450" by J. da Silva Coelho-Moreira et al. Dynamics of intracellular polymers in enhanced biological phosphorus removal processes under different organic carbon concentrations by L. Xing et al. Microbial purification of post fermentation medium after 1,3-PD production from raw glycerol by Dzymanowska-Powaowska et al. Rhizobium pegamine sp. Nov. from root nodules of Pangamic pinnata by V. Kesari et al. Persistent organic pollutants induced protein expression and immunocrossreactivity by Stenotrophomonas melophilia PM102: a prospective bioremediating candidate -hydroxybutyrate production by Bacillus subtilis NG220 using sugar industry wastewater.

DISCUSSION

Bioprocesses that are environmentally friendly. When released into receiving aquatic bodies, nutrient-rich wastewater streams can cause unwanted issues such algal blooms, eutrophication, and oxygen deficiency. Advanced treatment methods cannot be used to treat wastewater for such often reported circumstances in many developing nations because to the high energy and trained labor force requirements, high operating and maintenance costs. Under these circumstances, low-cost natural treatment methods may be employed to successfully treat trash while simultaneously preserving biological ecosystems in underdeveloped countries across the globe. Sequencing batch reactors were suggested as one of the best solutions for the treatment of abattoir effluent since they can remove organic carbon, nutrients, and suspended particles from wastewater in a single tank and also have minimal capital and operating expenses. Diverse methods to enhance oxygen transfer in bioreactors have been suggested, such as distributing a nonaqueous, organic, second liquid phase that is immiscible to the system, in order to preserve the long-term performance of bioreactors. This strategy has been effective in the past because the medium is modified by

the presence of this organic phase so that it can transport more oxygen. Since the organic phase has a large affinity for oxygen, it may raise the oxygen's apparent solubility in water, which in turn improves the microorganisms' specific activity and results in a high elimination of the target pollutant in bioreactors. Four pieces in this special issue discuss the operational features of conventional and natural bioprocesses and their benefits for treating certain industrial wastewaters. S. M. Saudi et al.'s study "Enhancement of oxygen mass transfer and gas holdup using palm oil in stirred tank bioreactors with xanthan solutions as simulated viscous fermentation broths Treatment of slaughter house wastewater in a sequencing batch reactor: performance evaluation and biodegradation kinetics" by P. Kundu et AL, Performance study of chromium removal in presence of phenol in a continuous packed bed reactor by Escherichia coli isolated from East Calcutta wetlands" by B. Chakraborty et al.

The article Natural treatment systems as sustainable ecotechnologies for the developing countries by Q. Mahmood et al.There is an urgent need to convert the majority of the lab-based research into field-based research in order to see sustainable solutions to persistent environmental problems, as is quite clear from the discussions and conclusions drawn by the authors of the papers published in this special issue as well as other recently published scientific literature related to environmental research. Future studies should concentrate on and address important problems related to biomarkers for environmental pollutants, new biosensor development for environmental monitoring, new biocatalyst development for environmental applications, novel bioreactor development for wastewater and air pollution control, and studies on the socioeconomic implications and technological evaluation of new bioprocesses. We are certain that the selection of articles included in this special issue will pique curiosity among researchers throughout the world and support colleagues in their research endeavors’ enzymes are increasingly utilized to drive chemical processes outside of their native location since they are crucial to the metabolism of all living things. In particular, the use of biocatalysts in the processing of raw materials and as food additives has been around for a while. Actually, enzymatic preparations made from plant or animal tissue extracts were in use even before anything was understood about the makeup and characteristics of enzymes. The food sector is continually looking for cutting-edge technology to satisfy customer demand, and industrial product manufacturers have long employed enzymes as important tools to turn raw materials into finished goods.

Their widespread usage in food technology is due to their clean label legal factor. Several enzymes have the ability to enhance the flavor, texture, digestibility, and nutritional value of food preparations when purified and added. The rapid advancement in protein technology, however, did not start until the middle of the 20th century, and only in the past 30 years has the use of commercial enzymes increased in the food industry, playing an increasingly significant role in the production of meat, vegetables, fruit, baked goods, milk products, as well as alcoholic and nonalcoholic beverages. In fact, more studies about improved product yields have been published during the last 10 years in both the food and beverage production industries. In addition, many previously unexplored enzymes are now being used to produce a variety of foods in which the biocatalysts replace potentially harmful chemicals because it is desirable in various branches of food technology to alter the physical and chemical properties of protein. This incorporates brand-new techniques that modify natural product properties to meet shifting nutritional or technical requirements. The use of specialized enzyme preparations has many financial advantages, including decreased process costs, less environmental impact due to the utilization of renewable resources, and often higher product quality. Additionally, preservation has a big influence on the quality of both food and drinks. For instance, it is generally known that contemporary methods turn juices into concentrates that, apart from scent, may be preserved for an extended period of time without losing quality. Another example of enhanced preservation is stabilizing flavor and color. Finally, the development of biotechnology has made it possible to significantly improve methodology,

providing unexpected answers to many enduring issues and creating exciting new opportunities. Since they may be employed to cure biological wastes or stop their development, enzymes are one of them and are suggested as exemplary "green" technology agents. While some enzymes utilized today come from plants and animals, most come from a variety of advantageous microbes. As a result, several refined enzymes are now often utilized as food additives as well as in food processing. It is interesting in this regard because the enzymes, like other proteins, only elicit responses in individuals who have been sensitized as a result of prolonged contact. The enzymes are thus very unlikely to induce allergies since their levels in food are often quite low [7], [8].

This special issue of Enzyme Research is dedicated to highlighting certain developing areas of enzyme applications in food technology, primarily outlining how various biocatalysts have benefits in certain advancements and innovations in food processing. There are three research papers and six review articles in it. The first review article provides a succinct and clear summary of the uses of enzymes in food and feed processing and describes how protein engineering, microbial screening, and immobilization methods may be used to create improved biocatalysts. The second review article outlines the improvements in meat products' nutrition, technology, and environmental impact. It focuses in particular on the use of transglutaminase, phytases, and proteolytic enzymes in the meat business. The third review article discusses transglutaminase as well as endopeptidases generated from bacteria. It covers the most current advancements in efforts to detoxify gluten. The fourth and fifth review articles discuss, respectively, the production, usage, and applications of fungal laccases in the food industry as well as the use of laccases as additives in the processing of food and beverages. The previous review article concentrated on the various methods for immobilizing -galactosidase and its prospective uses in the food and dairy processing sectors. The three research articles cover the development of a novel glucose biosensor based on a carbon electrode modified by glucose dehydrogenase immobilized on its surface, the production of antioxidant hydrolysates of pollen collected by honeybees using proteinases and aminopeptidases of plant origin, and the characterization of a potential food-grade leucine aminopeptidase extracted from kiwifruit. We sincerely hope that this volume is just the beginning of a special issue series in which Enzyme Research will periodically encourage authors to publish the highlights and original research articles outlining how enzymes bring new benefits to food preparation innovation and improvement. Characterizing microbial variety and creating possible biotechnological applications are the main goals of this special issue. Despite how important bacteria are, just a tiny portion of them have been successfully grown in a lab. It is now obvious that molecular methods were crucial in the discovery and understanding of the tremendous microbial diversity. Single cell genomics, metagenomics, and other omics technologies made it possible to characterize a sizable and hitherto undiscovered component of the microbial diversity. Since important biogeochemical cycles like those involving carbon, nitrogen, and sulphur are reliant on bacteria, human existence and microbes are closely entwined. Even the ability for photosynthetic activity on Earth depends on microbes rather than plants. In humans, the gut symbionts help with toxin breakdown, food digestion, and immune system support. Programmed including the International Census of Marine Microbes, the Human Microbiome Project, and the Earth Microbiome Project have been started in an effort to comprehend the unculturable percentage. Next, new genes and enzymes for application in biotechnology will be found using this limitless and intriguing resource. Nine research papers addressing microbial diversity and the use of novel technologies that may result in potential biotechnological applications are included in this special edition. Emiliano Huxley and Coccoliths pelagic us f. briareid are coccolithophorid algae, and D. H. Green et al. investigated the variety of bacteria present in these organisms. Although coccolithophores play a crucial role in the marine carbon cycle and the management of atmospheric CO2, the variety of bacteria associated with these algae has not been investigated from an ecological or biotechnological standpoint. Five

phyla made up the majority of the bacterial diversity, according to independent and culture-dependent assessments. Actinobacteria and Actinobacteria were the next most prevalent groups, followed by Alphaproteobacterial, Gammaproteobacterial, and Bacteroidetes. The characterization of the bacterial population that emerged in anaerobic microcosms after bio stimulation with lactate from 1,2-dichloroethane -polluted groundwater is presented by G. Merlino et al. Four unique variations that have been linked to the reductive dichlorination of 1,2-DCA were discovered using molecular techniques that targeted conserved areas of known reductive dehalogenase genes.

Microstructure analysis and 454 pyrosequencing were used by H. Lin et al. to evaluate the microbial community structure and pathogen incidence in urban tap biofilms in South China. All samples of biofilms were found to include a predominance of proteobacteria. It's interesting to note that these biofilms also included several possible diseases, such as Legionellae's and Enterobacterial, as well as corrosive microbes. Xu et al. looked at Thermology sp., which was isolated from Caldicellulosiruptor saccharolytic us, for heterologous production of cellulases. Three repeated transfers resulted in the loss of the vectors from the Thermology strain, but all three recombinant enzymes were effectively produced, giving the host an increase in endo- and/or exoglycanase activity. This research showed that it is possible to genetically modify Thermology species to more effectively use cellulose. In two H-type MFC reactors one with a graphite anode preconditioned with Beobachter sulphureousness and the other with an unconditioned graphite anode E. G. di Domenico et al. investigated the use of anaerobic digestate as feed.

Their findings suggested that anammox bacteria might be recovered from natural sources using MFCs. This opens the door to employing an anaerobic digester to generate power and remove nitrogen at the same time. By separating bacteria from seawater and actual brine, G. A. Silva-Castro et al. explored the precipitation of calcium carbonate and calcium sulphate. Calcium carbonate minerals, such as calcite and aragonite crystals, were produced by Bacillus and Virgibacillus strains. For the bioremediation of CO_2 and calcium capture in particular settings, further characterization of these bacterial strains is crucial. The Bacillus subtilis bacteriophages were isolated and molecularly characterized by A. Krasowska et al. The Myvyrian and Phoridae families of phages were the ones under study. We identified the host range, resistance to pH and temperature, adsorption rate, latent period, and phage burst size. Since Bacillus spp. are very resilient bacteria, these discoveries may be used in the industrial sector.M. Nam ally et al. looked for new chitin and chitosan modifying enzymes that were isolated from soil microorganisms that had been exposed to the substances for more than 10 years. Eight bacterial strains and twenty fungal isolates were discovered to be chitinolytic and/or chitosanolytic after being screened for chitinase and chitosanase isoenzymes [9], [10].

CONCLUSION

It is studied the physical, chemical, and microbiological characteristics of sediments polluted with heavy metals and hydrocarbons that were collected at El-Max Bay, Egypt. Fifty bacterial strains were obtained using a culture-dependent methodology, and they were later recognized as hydrocarbon-degrading bacteria active at various stages of biodegradation. A prospective source of fresh bacterial strains prepared for heavy hydrocarbon contamination loads is the El-Max sediment. These publications emphasize the significance of uncovering and characterizing hidden microbial diversity as well as the enormous potential for using biotechnology to turn this fundamental knowledge into practical application. Certainly, new advancements in the realm of omics methods will advance our capacity to comprehend and fully use microbial variety. These publications provide a look into this field's potential in that respect.

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CHAPTER 3

ESTROGEN CONTROLS MAPK-RELATED GENES THROUGH GENOMIC AND NONGENOMIC

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ABSTRACT:

Growth of uterine leiomyoma is significantly influenced by estrogen and growth factors, probably via interactions between receptor tyrosine kinases and estrogen receptor-alpha signaling. In human cells with intact or silenced we examined the genomic and nongenomic effects of on signaling and gene expression. Genes such as cyclin kinases and kinases signaling mediator transcriptional factors and involved in cell cycle progression, proliferation, and survival, and linked to collagen synthesis were found to be upregulated in cells by according to analysis by-array. The tyrosine kinase receptor and the fibrosis-related gene were all upregulated as a consequence of silencing which lessened the effects mentioned above. In contrast to cells lacking a functioning quickly triggered signaling nongenetically and caused phosphorylation. Through a protracted genomic process additionally increased the expression of the gene and protein. These findings provide fresh genes and targets for upcoming intervention and preventive methods and point to a critical function for and perhaps other in modulating genomic and nongenomic hormone receptor connections and signaling in fibroids.

KEYWORDS:

Collagen Synthesis, Leiomyoma, Nongenomic Hormone, Preventive Methods.

INTRODUCTION

Uterine leiomyomas have an uncertain precise etiology, but the fact that they appear during the menstrual cycle and disappear after it suggests that they are hormonally controlled. Clinical and biological research have provided strong evidence for the crucial part that estrogen plays in the stimulation of uterine leiomyoma growth. The overexpression of growth factors and their receptors, such as the type I insulin-like growth factor and IGF-I receptor, demonstrates that sex steroids are not the only agents that can affect the proliferation of leiomyoma cells and the frenzied formation of extracellular matrix in many fibroids, though. IGF-I expression is highest in leiomyomas during the proliferative stage of the menstrual cycle, according to studies. As we and others have shown, estrogen upregulates the gene encoding IGF-I via ER in leiomyoma tissue and cells. This is supported by the fact that IGF-I mRNA expression is increased in leiomyomas and that estrogen receptor alpha mRNA is positively linked with IGF-I mRNA levels. The results of several research on breast cancer, a condition that is often influenced by hormones, have shown that estrogen/ER and IGF-IR/MAPK signaling interactions may take place at various molecular levels.

It is well established that 17-estradiol predominantly regulates gene expression via cognate nuclear ER, which has historically been referred to as genomic estrogen activity. Additionally, numerous E2-responsive genes have been shown to function as important signaling molecules in IGF-IR signaling. As an alternative, it has been shown that a type of ER that is associated with cell membranes couples with and activates IGF-IR through phosphorylated leading to the onset of fast nongenomic effects via IGF-IR transactivation [1], [2]. More recent research, including our own and that of others, has shown that E2 and environmental phytoestrogens may elicit E2-dependent signals that lead to important biological responses such gene expression and the proliferation of human uterine leiomyoma

cells. E2 is also able to stimulate the expression of the IGF-I gene and IGF-I synthesis, which activates the pathways. Uterine leiomyomas have been shown to have higher levels of active with increased ER-phospho-ser118 phosphorylation, whereas uterine smooth muscle tissue does not. All of these studies point to the possibility that estrogen may regulate the effects of the growth promoter its receptor and downstream targets -related genes at genomic and nongenomic levels, and that the interaction between ER and may be crucial in the development of fibroid tumors. However, the precise mechanism by which this all occurs is unknown.

There are several questions that need to be addressed such as how does estrogen regulate related gene expression through and the pathway; what is the role of E2 in the activation of leading to phosphorylation of at the serine site; which specific mediators of the signaling pathways are involved in the interactions of and in uterine leiomyomas. In this work, we investigated the regulatory effects of on signaling cascade intermediates and associated gene expression in Ulm cells, and by genomic array profiling, we discovered additional genomic mediators implicated in fibroid growth and development. We further examined whether the effects of E2 were due to interactions between and by western blotting, immunoprecipitation, and immunofluorescence microscopy after silencing. To our knowledge, this is the first investigation of the profile of E2-mediated related genes and the genomic and nongenomic connections of the pathways in human leiomyoma research.

The gene expression of the signaling pathway in response to E2 treatment, with and without IGF-IR silencing, was examined in cells using the Profiler PCR Array System from Sabio sciences. Using Lipofectamine as a transfection agent, cells were transfected with a sigh-IR oligo targeting the human IGF-IR gene and a control scrambled siRNA with a nonsense sequence created to have no discernible sequence similarity to mouse, rat, or human transcript sequences. Ulm cells were treated with E2 and collected using Trizell Reagent 24 hours following sigh-IR in serum-free media containing 0.001% of vehicle. Using the Qiagen RNeasy Mini Kit to eliminate any remaining contamination from the RNA samples and 2 g of pure RNA per treatment condition, total cellular RNA was extracted from the cells. Each treatment condition's template was loaded into a 96-well array plate coated with 84 predisposed MAPK-related gene-specific primer sets, and each plate was then processed using a TaqMan ABI Prism 7900 Sequence Detector System in accordance with the manufacturer's instructions for the Profiler PCR Array. The data analysis was done using the Sabio sciences' approach with normalization.

In Ulm cells, estrogen-responsive gene, IGF-I mRNA expression levels were measured by real-time PCR at 0 min, 10 min, 60 min, 24 h, and 48 h after E2 treatment. The same as reported for the RT2 Profiler PCR Array, cells were starved with serum-free media 24 hours before receiving the E2 treatment. Total cellular RNA was isolated from the cells using a Grizol Plus RNA Purification Kit after the cells had been collected using Trizell Reagent. Superscript II was used to reverse-transcribe one microgram of total RNA into cDNA. IGF-I and GAPDH primers were used in RT-PCR, which was carried out using an AB cycler and the Applied Biosystems Power SYBR Green PCR Mix. The findings were normalized using GAPDH and shown as fold changes in comparison to the control groups [3], [4].

Confocal Microscopy Immunofluorescence Staining:

Following E2 treatment, immunofluorescence labelling was used to identify IGF-I peptide expression at 0 min, 10 min, 60 min, 24 h, and 48 h as well as colocalization of phospho-ERser118 and phospho-MAPKp44/42 at 0 min, 10 min, and 60 min. Prior to receiving E2 treatment at M, the cells were starved for 24 hours in the same manner as described for the real-time RT2 Profiler PCR Array. The cells were permeabilized with 0.2% Triton X-100, fixed with 4% paraformaldehyde, and blocked with 5% BSA and 0.1% gelatin in PBS. The

cells were incubated overnight with primary IGF-I goat polyclonal antibody, phospho-ERser118 mouse monoclonal antibody and photo- rabbit monoclonal antibody. As secondary antibodies, we employed for nuclear staining and Alexa Fluor 488 goat anti-rabbit for phospho594 donkey anti-goat for and 594 donkey anti-mouse for phospho-ERser118. Negative controls were normal goat, normal rabbit, or normal mouse serum. Confocal pictures were captured using a C-Apochromat objective utilizing a Zeiss meta. The Alexa 488 was excited using the 488 nm laser line from a Krypton/Argon laser. This picture was captured using a nm bandpass emission filter with a pinhole setting of 1.09 airy units. The Alexa 594 was excited for the second channel using the 543 nm laser line from a Helium Neon laser. The photos were captured using a 560 nm long pass emission filter with a pinhole setting of 1 airy unit.

Western Blot Analysis:

To evaluate the quick effects of E2 on target protein expression in Ulm cells, Western blot analysis was carried out using a standard method as previously reported. Simultaneously with real-time RT2 Profiler PCR Array, the sigh-IR and sic transfection method was used. E2 was applied to the serum-starved cells at intervals of 0, 10, and 60 minutes. According to earlier instructions, the protein was extracted using a lysis buffer. The following primary antibodies were utilized for the western blotting: rabbit polyclonal anti-phosphor rabbit polyclonal anti-IGF-IR, rabbit polyclonal anti-phospho-Sc, rabbit polyclonal anti-Sc, rabbit polyclonal For the immunoprecipitation samples, the same rabbit anti-ER, rabbit anti-IGF-IR, and rabbit anti-Sc antibodies that were employed in the western blotting investigations were also used. Horseradish peroxidase-conjugated secondary anti-mouse or anti-rabbit antibodies were used to detect primary antibodies [5], [6].

Immunoprecipitation:

The connection of and Sc in the cells treated with E2 was discovered using a Seize Primary Immunoprecipitation Kit. The kit was utilized since the target protein Sc and IgG of anti-ER and IGF-IR have similar molecular weights and this kit enables the immunoglobulin to stay adherent to Amino link Plus Gel after elution. According to the manufacturer's protocol, the procedures were carried out. In a nutshell, 50 L of 50% Amino link Plus Gel Slurry and 200 g of ER rabbit polyclonal antibody from Santa Cruz were coupled overnight at 4°C. 300 g of the total protein isolated from the cells treated with E2 was incubated with the linked gel and antibody complex in binding buffer for 0, 10, and 60 min at 4°C, as stated in the western blotting methods. With washing buffer, the gel was washed three times. The elution buffer was used to elute just the antigens from the antigen-antibody complexes, which were then kept for western blot analysis. At least three separate replications of the immunoprecipitation, western blot analysis, and RT-PCR of IGF-I mRNA expression studies were performed. The two-tailed Student's -test was performed to examine statistical significance between distinct groups and between different time points after the RT-PCR results were reported as mean SEM. In order to identify statistically significant differences between silenced and no silenced IGF-IR groups at different time points after E2 treatment in Ulm cells with respect to ratio of band intensity of phosphorylated/total protein. Since the majority of data obtained from immunoprecipitation and western blot analyses were not normally distributed. One-sided statistical significance was the definition used.

DISCUSSION

We carried out genomic arrays to identify MAPK-related gene profiles controlled by E2 with and without IGF-IR gene knockdown in order to investigate signaling intermediates of the IGF-IR/MAPK cascade and associated genes regulated by E2 and locate novel genomic mediators of fibroid growth and development. With a functional IGF-IR at 24 h compared to those groups under sigh-IR conditions, we discovered genes associated with the signaling

pathway and fibrosis that were differentially expressed between the groups with or without E2 treatment. A heat map and fold changes from the PCR array revealed that E2 exposure in cells with a functional IGF-IR resulted in fold upregulation of 27 genes and 2-fold downregulation of 3 genes involved in the signaling cascade at 24 h; the other 5 genes remained unchanged. Several D-type cyclins and other cyclin-dependent kinases involved in cell cycle progression, growth factor receptor-bound protein, and ARAF linked to IGF-IR signaling through and MAPK kinases involved in proliferation, differentiation, and survival were among the genes upregulated fold in the presence of an intact were all elevated, as well as collagen type I alpha, which is implicated in collagen formation and fibrosis, a hallmark characteristic of fibroids.

Additionally, there was an increase in RAC1, a Rho GTPase involved in the control of a number of cellular processes and often activated after activation. In Ulm cells, the five unaltered genes that were detected in the presence of a functioning IGF-IR were raised when IGF-IR was silenced, but the upregulated genes induced by E2 in the presence of an intact IGF-IR were mostly abolished. Epidermal growth factor receptor, involved in cell growth and survival, cyclin D2, and cyclin D3, involved in cell cycle progression, v-Est erythroblastosis virus E26 oncogene homolog, a transcriptional factor involved in cell development and tumorigenesis, delta-like 1 homolog, involved in fibrosis, and the glucose-regulated gene were among the Instead, COL1A1 and GRB2, whose expression is normally higher when an IGF-IR is active, had lower expression when the IGF-IR was silenced. When IGF-IR was silenced, however, the transcriptional factor EGR1 continued to be upregulated.

IGF-IR and it signaling molecules play a significant role in E2-mediated activation of MAPK and MAPK-related pathways in Ulm cells, as shown by the changes in gene expression in response to E2 treatment with and without IGF-IR silencing. Since many of them interact and control the phosphorylation status and activities of numerous transcription factors, which affect gene expression, the collective view of extranuclear ER signaling suggests that its transduction pathways and its interaction with pathways may connect the nongenomic actions of estrogen to genomic responses. Therefore, utilizing and real-time array technology, we first examined the gene profile associated with the pathway in this work to examine the effects of E2 therapy on pathway related gene expression. A strong induction of 27 genes, including those encoding Cyclins, Cyclin Kinases, kinases, and transcription factors, all involved in cell proliferation, differentiation, and survival, was seen in Ulm cells treated with E2 in the presence of an intact. However, the effects of E2 were reduced in Ulm cells when the IGF-IR was silenced. This further demonstrated that the IGF-IR is necessary for Ulm cells to activate the E2-mediated MAPK pathway.

Numerous signaling molecules have the ability to activate a cascade of MAPKs. A sequential succession of phosphorylation events, in which each downstream kinase acts as a substrate for the upstream activator, results in the signal transduction. For instance, in the mitogenic extracellular signal regulated kinase cascade, MAP kinase/ERK kinase, which is primarily activated by the protein kinase Raf-1 after having been recruited to the plasma membrane by Ras, phosphorylates the two related mammalian. The cascade, which primarily controls cell proliferation and differentiation, was shown to be increased in leiomyomas in our earlier research [7], [8]. The MAPK-related gene profiling RT-PCR array used in this study provides additional evidence that E2 mediates the expression of genes involved in the pathway, including as well as genes encoding transcriptional factors, including, and MAX, and that these genes' expression levels are increased when cells are exposed to E2 in the presence of a functional IGF-Precycling D1 may interact with and serve as a regulatory component of CDK4 or CDK6, which is necessary for cell cycle G1/S progression, and is a member of the highly conserved cyclin family, whose members exhibit a striking periodicity in protein abundance throughout the cell cycle.

This research found elevated levels of cyclin D1 and CDK4 expression, which further suggests that E2 not only activates the pathway but also may trigger cyclin family proteins that move the cell cycle from G1 to S phase, hence promoting cell proliferation. It was intriguing to see that certain gene, like which encode cyclin kinase inhibitors, also rose after E2 exposure. These changes may have been made to balance out CDK4's very high expression. These results are in line with the theory that various cyclins and their kinases display unique expression and degradation patterns that help to coordinate the timing of each mitotic event. The fact that prevented E2 from upregulating genes in cells when there was IGF-IR further supported our theory that is crucial for the interaction between the ER and pathways. With the silenced, other mechanisms seem to have compensated in response to E2 exposure, resulting in differential gene expression. However, additional genes were differently expressed by E2 treatment in cells with and without. When cells are reduced, the enhanced gene expression pattern with implies that E2 therapy supports alternate routes for growth and survival. a gene involved in collagen synthesis and fibrosis, whose expression is typically increased in the presence of a functional, was decreased after further indicating that may play an important role in fibrosis in uterine leiomyomas. Other genes, such as involved in fibrosis, had increased expression levels.

Next, we looked at how the pathways interact and if E2 upregulates and as well as their target proteins in leiomyoma cells. We discovered that gene and protein expression levels rose during the duration of E2 treatment, peaking at 48 hours in Ulm cells. This protracted response suggests a potential genomic-level mechanism by which E2 may regulate gene expression. Additionally, we discovered that minutes after E2 administration, levels of phosphorylated and their target protein all rose. IGF-IR and its target downstream proteins are quickly activated, which suggests that the link between is mediated by nonentropic signaling, or kinase-initiated processes without estrogen receptor binding to conventional steroid response regions on. Additionally, 10 minutes after E2 treatment, phosphor showed enhanced colocalization in cells. These data support the hypothesis that E2 exposure is mediated by a number of mechanisms, including the fast activation of IGF-IR and MAP kinase, a nongenomic pathway shown in estrogen-responsive breast cancer cell lines and which may activate.

It has been determined which chemicals are involved in the nongenomic signaling mechanism. More recently, it has been shown that the plasma membrane contains or is connected to a pool of ERs. These ERs use the IGF-IR on their membranes to quickly signal different kinase cascades, which then affect the transcriptional and no transcriptional activities of estrogen. In this investigation, E2-treated cells displayed overexpression of RAC1, a Rho GTPase that is often activated after activating RTKs and is implicated in the control of a number of cellular processes. IGF-IR silencing resulted in a reduction in RAC1 gene expression. These findings suggest that may activate and signaling either directly or indirectly through the ER. The plasma membrane-associated ER may act as an anchor for the and Sc are quickly phosphorylated by estradiol. According to a publication, activated Sc acts as a transporter after binding to ER and then transports to Sc-binding sites on the activated IGF-I receptors, which then sends signals t and other pathways. Our immunoprecipitation findings further demonstrate that the E2 treatment of cells resulted in an association between the three proteins ER, IGF-IR, and Sc. In order to counter the E2 impact on the interaction of these two pathways via their respective receptors, we employed the approach to suppress the IGF-IR gene. We therefore argued that IGF-IR should be a crucial mediator in this interaction. We discovered that reduced E2's ability to phosphorylate and activate. ER phosphorylation at the serine location was similarly reduced at the same time.

According to these results activation is necessary for the ER's quick nongenomic response to E2 exposure in cells. In agreement with the discovery that E2 binds to intracellular ER, which

physically associates with the adaptor protein Sc through activation and induces its phosphorylation silencing abolished the ER activity at the serine 118 site induced by E2. This finding confirms a potential relationship between membrane-related signals and intracellular ER. We have shown a link between IGF-IR and kinase activation in fibroid tissue samples collected from women in the proliferative phase of the menstrual cycle. In turn, Sc binds and Sops, which also causes the fast activation of kinase [9], [10].

CONCLUSION

As a result, the variation of gene expression patterns seen in this work may be caused by the convergence of several ER-mediated genomic and/or nongenomic activities at different response sites, which offers an incredibly precise control mechanism in the regulation of the target gene transcript. In conclusion, this study's findings show that two growth regulating mechanisms, are closely related in cells. Both genomic and nongenomic mechanisms that include activation of kinase cascades via the link between and may result in the effects. Our understanding of the interactions between is improved by the observations is necessary for the interaction and the differential expression of pathway-related genes mediated by gene expression is responsive to and the activation of alternative pathways induced by when is silenced.

These observations may also point to a multifaceted or cocktail approach to fibroid treatment. Inhibitors of small downstream molecules, such as Sc and ERKs, or transcription factors may more effectively block these effects and may be used as noninvasive adjuvant therapies for fibroids because other alternative pathways may compensate for the partial effectiveness of pure antiestrogen or anti-IGF-IR agents in inhibiting E2-induced pathway activation.

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CHAPTER 4

ANALYZING THE ROLE OF GENE BIOTECHNOLOGY IN PAPER-MAKING BUSINESSES USING THE REWARD AND PUNISHMENT SYSTEM

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ABSTRACT:

Controlling environmental pollution is urgently needed since it has grown to be a significant barrier to the development of an ecological civilization. This study focuses on analyzing how paper-making firms pick their own emission reduction methods under the reward and punishment mechanism by setting up an evolutionary game model. Finally, via simulation research, this thesis analyses the evolutionary routes of pollutant emission techniques used by paper-making firms under the reward and punishment mechanism. It also examines how societal welfare evolves under the reward and punishment mechanism. As a consequence of the reward and punishment system, the gaming system will constantly fluctuate around a point while it remains static. Right now, there isn't a stable equilibrium position. A stable equilibrium point will eventually be reached by the game system under the dynamic reward and punishment mechanism. According to the findings of social welfare analysis, high-intensity incentives will increase social welfare by lowering the quantity of pollution that paper-making businesses emit.

KEYWORDS:

Civilization**,** Ecological, Environmental Pollution, Pollutant Emissions.

INTRODUCTION

Due to their volatility and difficulty in eliminating them, pollutant emissions from paper-making businesses have traditionally been a significant source of environmental pollution. Pollutant emissions from paper-making industries continue to play a significant role in the ecological environmental pollution. However, our nation has never given up trying to improve the government system. According to the China Paper Yearbook, the wastewater discharge from paper-making businesses has decreased from 2011 to 2015. From 5.8% in 2011 to 3.22% in 2015a r education of 2.58 percentage points the share of wastewater discharged by the paper industry in the nation's overall wastewater discharge. Additionally, during the previous five years, the wastewater discharged by paper-making businesses has grown by -11.29% yearly. On the one hand, the decrease in pollution emissions demonstrates that China has successfully eliminated its antiquated manufacturing capacity. Only businesses with strong growth foundations and development potential may survive [1], [2].

On the other side, we can also observe how committed and persistent the Chinese government is in reducing environmental pollution. By raising benefits or punishments for them, it controls or limits polluting industries. The internal logic and process of the paper-making firms still need to be investigated when they decide on their own pollution emission methods under the government's incentive and punishment system, despite the fact that these measures have produced some outcomes. Studying the government's reward and punishment system and the paper-making industry's decisions regarding pollution emission strategies calls for careful consideration of the government's reward and punishment system and the strength of its implementation in light of the conflicting interests of the two parties in the game. To completely grasp human collaboration, there are still many obstacles to overcome.

According to the data above, China's pollution has been effectively managed as a result of increasing efforts in pollution control. However, further research is still required to determine how the incentive and punishment system would affect the pollution emission tactics of the polluting firms. Although some academics have examined the measures used by businesses to reduce their pollution, they have disregarded the changes in societal welfare under the incentive and punishment mechanism. In general, incentives and penalties may have varied effects on polluting businesses, but their main goal is always to increase societal welfare. This thesis examines how paper-making businesses decide on their own emission reduction tactics in the absence of a reward and punishment system by setting up an evolutionary game model. It also examines ways to maximize social welfare while using the penal system. The government may use the analysis of the pollutant discharge strategy of paper-making firms under the incentive and punishment mechanism as a guide for developing effective environmental governance measures.

The following is the breakdown of this article. The literature on business pollution reduction is reviewed. It does a mechanistic study of how various incentive and punishment systems impact the techniques used by businesses that manufacture paper to reduce pollution. The selection of tactics by the government and paper-making businesses under the static reward and punishment system is the subject of analysis. The strategic decisions made by the two parties in the dynamic reward and punishment system are examined. It is examined how the incentive and punishment system affects societal wellbeing. The growth of the government and paper-making businesses under various types of reward and punishment mechanisms is analyzed using MATLAB. The study results and suggestions are presented, along with a further discussion of the findings [3], [4].

The sources of the firms' pollution emissions are intricate, and they also produce rather wide pollutant swaths. Most academics have conducted extensive study to lessen the damage brought on by businesses' pollution emission. Researchers' studies on governance and pollution reduction may be loosely categorized into the following groups after an assessment of the available literature. One is that a lot of writers looked at environmental rules from the standpoint of how to control and reduce pollution from businesses. The majority of academics thought that environmental rules might successfully lower environmental pollution . According to some academics, environmental restrictions have varying effects on businesses with various degrees of pollution; the effects on businesses with high pollution levels were much bigger than those on businesses with low pollution levels . Environmental rules not only decreased the export revenues of businesses that produce a lot of pollution, but also limited their export periods . Additionally, the impact of various environmental restrictions on the environmental pollution by businesses varied. Environmental pollution and charge-based environmental laws exhibited an inverse U-shaped association. Environmental pollution and investment-based environmental laws have a U-shaped connection . Environmental laws also weren't always effective.

The partiality of local governments would undermine the functions of environmental rules in regions with a large number of polluting businesses, which would increase pollution. Some academics thought that enacting obligatory environmental laws and raising environmental tax rates may be effective ways to improve the efficacy of environmental regulations.The second is that several scholars have focused on cooperative regional governance when studying the cross-regional pollution of businesses. It was difficult to properly coordinate the two administrations to address a problem while they were members of separate political coalitions. The formulation of public policies that coordinate relationships between governments at all levels, as well as inter- and cross-level departmental communication and cooperation, was a crucial step for many governments . There was still a peer spillover effect across governments from a horizontal viewpoint. A firm was more likely to be impacted by

its peers when choosing emission reduction techniques if it had frequent interactions with nearby businesses . This peer spillover effect would unquestionably significantly aid in the management of environmental pollution in China.

In order to create a stable and coordinated governance model among diverse local governments, higher-level governments have to be monitored and penalized from a vertical viewpoint. However, if local governments had to deal with ever-tougher political responsibilities, they wouldn't be able to function well enough to decide on the best course of action for themselves. Although the underlying logic of many organizational forms varied, from the standpoint of organizational form, both the logic of authority and the logic of meaning often supported various organizational arrangements. If there were no restrictions, the Interreg ions tended to go untreated in the cross-regional treatments of the air pollution. On the other hand, people inclined to collaborate in governance when there were restrictions [5], [6].

The third is that some writers employed reward and punishment mechanisms to explore the multiagent governance of the pollution caused by the firms, specifically the effects of punishment on the two parties' ability to cooperate and regulate pollution in the game. Government subsidies and harsher punishments may encourage the game's development to a stable state . The tactics between the government and the businesses, as well as the course of each party's progress, were unpredictable under the static punishment system. However, the development routes between the government and the company tended to converge to a stable value under the dynamic punishment mechanism . It would be difficult for both parties to work voluntarily to achieve ecological compensation without the strict oversight provided by the higher-level government. The game would reach equilibrium if the penalty for uncooperative participants were increased.

The following strategies could help the evolutionary game reach its ideal state: increasing oversight while lowering the cost of higher-level government oversight, stiffening fines for local businesses and governments that fail to cooperate and violate pollution discharge regulations, and enhancing incentive systems. Regarding the influence of prizes on the two parties' collaboration and game-related efforts to manage pollution, they have an identical impact to punishment. By suitably enhancing the enterprise's multiplication factors, that is, boosting the advantages of environmental improvement after pollution control, the businesses' excitement for collaborating in the pollution control would be encouraged . Additionally, raising the company's expectations for the advantages of taking part in pollution management may also have a similar effect . The degree of the incentive, however, relied on how well the groups worked together. The two have a good association with one another. It may be possible to incentivize businesses to cut down on pollution emissions by increasing investment in special transfer payments for environmental protection. However, just stiffening the penalty for municipal governments that disobey their obligations won't make them comply.

Additionally, the two parties' feelings had a more immediate influence on their ability to work together and limit pollution. Pessimistic emotions would cause disputes to break out between the border walls, while optimistic emotions would drive the collaboration between the two sides to a steady position . Government oversight and citizen involvement were beneficial to environmental governance. Businesses' pollution control practices may be successfully improved by reducing the harm to the public's perception of the government and by fostering more public engagement. Because a company's efficiency was negatively correlated with its investment in innovation, polluting firms with higher efficiency may produce more pollutants. The literature cited above has undoubtedly contributed significantly to the study of the emission reduction tactics used by polluting industries. It also provided a strong theoretical framework on which to write my thesis. There are still certain restrictions,

however. One is that the government's system of rewards and penalties is not completely taken into account. The other is that the incentive and punishment system seldom affects social welfare improvements. This thesis proposes an evolutionary game model with no reward punishment mechanism as well as one with a reward and punishment mechanism based on the discussion above and building on the research experience of the predecessors. It also analyses how the use of punishment affects societal wellbeing. Finally, it employs simulation studies to examine how paper-making businesses reduce emissions under an incentive and punishment system.

DISCUSSION

This study examines the impact of the government's static reward and punishment system on the decisions made by paper-making businesses regarding their pollution emission strategies. It also examines the impact of the government's dynamic reward and punishment system on these decisions. Based on this, it also examines how the incentive and punishment system has affected societal wellbeing. The development route of the government's reward and punishment system as well as the decision of the paper-making companies' pollution emission techniques are both numerically simulated in this research using MATLAB. It also examines how the various levels of incentive and punishment mechanisms affect the tactics used by the paper-making industry to reduce pollution.

The main variables influencing the player's choice of strategy are identified via evolutionary game and numerical simulation analysis. The dynamic reward and punishment system is superior to the static one in terms of effectiveness. Increased benefits and penalties will encourage paper-making businesses to use nondischarge practices. It won't always be beneficial to increase the incentives for avoiding releasing toxins into the environment. The government's incentives and penalties will have an impact on the emission reduction strategies of paper-making businesses in addition to their own costs and benefits. The government's incentives and sanctions for paper-making businesses, however, will ultimately convert into costs and advantages for the businesses. It is important to understand how the government's incentives and sanctions for paper-making businesses impact the measures they choose to use to reduce emissions.

It illustrates that the starting point and goal of the two sides' chosen tactics in the game between the government's environmental supervision agency and the paper-making businesses is always that their own interests outweigh their own expenses. Even if the incentives and penalties under the static reward and punishment system are now of the greatest caliber, they are unable to make a significant influence on the paper-making businesses. The paper-making businesses are thus more likely to choose pollutant discharge options. According to the dynamic reward and punishment system, the government's environmental supervision department will implement incentives and penalties in direct proportion to the likelihood that the paper-making firm will decide to release pollutants. In other words, the severity of the penalty will increase with the likelihood that the paper-making firm releases pollution. The likelihood that the paper-making businesses won't release pollutants increases the benefits that they will get. Additionally, as there is no maximum limit for these benefits and penalties, paper-making businesses will be forced to employ nondischarge practices. The true goal is not to reward complying parties and punish noncompliant parties.

The ultimate objective is to reduce environmental pollution via incentives and sanctions.

Basic Hypotheses Hypothesis 1:

Assume that the paper-making company and the government's environmental supervision agency are the two main participants in the game of pollutant emissions. Because the two

parties are rationally constrained in the game process, both parties must continuously adapt their own strategic behaviors in response to newly available information and the strategies of the other party in order to realize their own ideal strategies. Hypothesis Assume that the government environmental supervision department and the paper-making enterprise are both participating in the game, and that the paper-making enterprise's strategy space is , with the probability of choosing the nondischarge and pollution-discharge strategies, respectively, being y and 1 y. Assume that the government environmental supervision department's strategic space is , and that the probabilities that G will select the supervision and no supervision strategies are, respectively, x and 1 x .The third hypothesis is that there will be a cost associated with the none mission approach chosen by the paper-making firms . If so, the government's G department in charge of environmental oversight will now get extra advantages.

If the paper-making companies decide to release pollution, the cost is and there are also advantages. The government's environmental supervision agency will now get the funds, as is intended. In addition, there will be a cost associated with the supervision technique chosen by the government environmental monitoring agency . Let's say it is. The government's environmental oversight agency will compensate the paper-making businesses if they choose for nondischarge solutions. The prize is. The government's environmental supervision department will penalize the paper-making businesses if they adopt the pollution tactics, presuming the fines are. Whether they engage in gaming or not, both parties stand to gain from the fundamental advantages of paper-making businesses and government environmental supervision agencies [7], [8].

The game payment matrix for paper-making businesses and government environmental oversight agencies may be generated based on the aforementioned hypotheses. The particular material and it shows that the dynamic reward and punishment system is more stable than the static reward and punishment mechanism under the identical circumstances. According to the research above, there is currently no balancing point and the evolutionary game system will bounce about the Centre point under the static reward and punishment mechanism. These are some of the potential causes. One is that the government's environmental monitoring department's incentives and sanctions currently do not exist at a level that can effectively stop the paper-making businesses from discharging. Second, there is now no discernible difference between the advantages and costs of nondischarge in terms of the paper-making industries. Therefore, it will have a very low likelihood of selecting a non-discharge method.

Third, the incentives and penalties that paper-making businesses get under the static reward and punishment system are already of the greatest caliber. Paper-making businesses won't face additional incentives or penalties even if they decide not to release pollutants or release more of them. However, the government's environmental oversight agency and paper-making businesses will trend to a stable position in the game under the dynamic reward and punishment process. Because the benefits and penalties that the paper-making businesses now get are inversely correlated with the likelihood that they will choose to release pollution. In other words, there is currently no upper limit on the incentives and penalties. When paper-making businesses are subject to severe fines for pollution discharge, their motive to do so will be greatly diminished. displays the game phase diagram at this moment. It shows that paper-making businesses are more likely to employ nondischarge techniques when their illicit revenue is smaller than the incentive or penalty they would get. Because the advantages it receives are now less than the cost of supervision, the government's environmental supervision department is more likely to opt for a no supervision policy. In other words, the two sides of the game are not autonomous with regard to the market and interests since both sides of the game have constrained rationality. Therefore, depending on the scale of their expenses and benefits, both the government's environmental monitoring agency and paper-

making businesses make strategic decisions. displays the diagram of the game phases.1. Basic Hypotheses Based on the research above, this thesis makes the assumption that there are two means reward and punishment in the game process of reducing environmental pollution. Following the use of these two strategies, social welfare may experience similar or diametrically opposed changes. The major focus of this section is an analysis of how these two reward and punishment systems would affect societal wellbeing. The details of the analysis are as follows: First Premise: During the Game, Different discharge states will be present in paper-making businesses. It may be separated into big discharge and tiny discharge based on the quantity of discharge.

The discharge may be categorized as compliance discharge and noncompliance discharge depending on whether it is compliant or not. It may be split into two categories: sewage and no sewage, depending on whether it releases pollutants. The sewage may be categorized into four categories: sewage that has already been released, sewage that has not yet been discharged, and sewage that is now being discharged. During the game, paper-making businesses may choose one of the pollutant discharge states. However, the status of the paper-making businesses is uncertain when the findings are not visible. To demonstrate the condition of discharge of the paper-making firm, the author therefore presents the disorganized set A. The disorganized set A may be stated as, which represents the condition of operation of the paper-making businesses, or as. It is required to provide each state of pollutant emission by a paper-making firm a specific probability since the state is unpredictable. In other words, the likelihood of a paper-making business releasing pollutants is. It is still unclear if it will release pollutants. The risk that a paper-making firm will release pollution in any pollution discharge condition is what the author assigns a specific probability to. The kind and level of supervision used by the government's environmental supervision agency will influence the likelihood of pollutant discharge. The discharge of paper-making businesses is a continuous random variable, according to the author.

The loss probability density function, which is used to calculate the social welfare losses brought on by the emission of paper-making firms, is used to calculate the loss to society and ecosystem caused by the discharge of pollutants in any state. The social welfare loss function is defined as under assumption number two. is used to show that the paper-making businesses have received the prize for not carrying out emission discharge under any state. is used to identify the uncontaminated zones. shows the region's overall income and level of wellbeing. Although awards won't stop businesses from emitting pollutants, they may lower pollution levels. Whatever the decrease is, let it be. The pollution function of the paper-making industries is used to quantify the level of pollution. The aforementioned assumption states that the pollution caused by paper-making firms is a function of regional total income, pollutant emissions, and incentives. The penalty meted out to the paper-making firm for pollution discharge in any state is represented by the formula as. Likewise, denotes the polluted region. Thus, the harm caused to societal welfare by the pollution of paper-making businesses. Although the penalty won't entirely stop the paper-making businesses from releasing pollution. Additionally, it will to some part lower the pollution level. Let the degree of decrease be. To indicate the level of pollution caused by paper-making businesses, use the pollution function. According to the aforementioned supposition, the pollution caused by paper-making firms depends on the overall regional harm, the quantity of pollutants released, and the penalty. Additionally, the formula states it as. If a paper-making company opts for a nondischarge approach, it will incur a specific expense. The author anticipates the price to be. A paper-making business will not be compensated if it picks a pollutant discharge method. A portion of the expense of the supervisory procedure will also be borne by the government's environmental supervision agency. Assume it will cost. The infinite term is subsequently introduced based on the aforementioned presumptions. The so-called endless period is used to contextualize the paper-making industry's pollution output within a longer historical era.

The prior award level, the degrees of rewards, and the amount of pollution produced by the paper-making industry in the preceding era will all have an impact on how much social welfare may be increased under the incentive mechanism.

The likelihood that the government's environmental supervision department will execute oversight and the likelihood that the paper-making firm will decide not to release pollution may both be computed using the values of the aforementioned parameters, among others, and. When the probability that the government environmental supervision department will always choose the same supervision strategy, and using the aforementioned probabilities as the initial probabilities of the evolutionary simulation study, the probabilities that the paper-making enterprises will decide not to discharge pollution are 0.2 and 0.8, respectively. At this point, a simulation diagram showing the development of the nondischarge strategy preference of the paper-making firms may be generated. Although the government's environmental supervision department has a high probability of selecting a supervisory strategy in this case, the likelihood that paper-making enterprises will decide not to discharge pollutants varies up and down and does not tend to a stable point. Similarly, the chance that the government's environmental supervision department will choose the chosen supervision technique is = 0.2 and 0.8, respectively, when the likelihood that the paper-making firms choose to opt not to discharge pollutants stays constant. It is already possible to receive the evolution simulation diagram of the government environmental supervision department selecting the supervision method. It shows that government environmental supervision departments' propensity to choose supervision is wave-shaped. The game system will be in a closed-loop evolution condition if the starting value of probability for both players is the same. In order for the likelihood that the paper-making companies decide not to release pollution to be equal to the probability that the government's environmental monitoring department decides to select supervision. The study above demonstrates that there is no stable point in the game between paper-making firms and government environmental inspection departments under the static reward and punishment mechanism, assuming it is 0.2. The main cause of this circumstance is that both sides are making sensible decisions within their own boundaries after assessing the pros and cons [5], [9].

CONCLUSION

The previous presumptions are maintained in this section. When all other factors stay constant, raising the degree of incentive for none missions will decrease the likelihood of selecting a none missions approach. These are some of the potential causes. In the first place, raising the incentives for paper-making companies to avoid discharge would surely raise the cost of the government's environmental monitoring program's oversight. Their desire to collaborate will decline as government expenses rise. Second, if there are too many incentives, paper-making businesses are more likely to fall into the "reward invalid" trap. After obtaining the award, the paper-making businesses fail to take any worthwhile steps to lower emissions. However, they plan to spend the award in other ways. Because suitable awards may assist to lower the pollution of paper-making firms, it is vital to keep the prizes within an acceptable range.

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CHAPTER 5

EXPLORING CONTRIBUTIONS OF BIOTECHNOLOGY IN FOOD INDUSTRY AND WATER MANAGEMENT: ELECTRO SPUN NANOMATERIALS

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ABSTRACT:

Growing numbers of students, researchers, academics, and companies worldwide are using electrospinning and electro spraying methods as economical platform approaches to produce organic and inorganic nanofibers and nanoparticles for a variety of uses. This overview demonstrates significant developments in electro spun nanomaterial science and engineering and how they might be used to satisfy the expanding demands in five important industries: clean water, the environment, energy, healthcare, and food. Although synthetic polymer systems still dominate the majority of these industries, the development of natural polymer and hybrid natural-synthetic electro spun polymer systems has certain distinct benefits. Modern advances in science and materials engineering have produced highly competitive nanofiber, electro spun products that provide reliable answers for practical applications.

KEYWORDS:

Electrospinning, Biotechnology, Dominate, Food Industry, Nanomaterial.

INTRODUCTION

As a flexible method that can be used to many different organic and inorganic systems and produce a finely regulated size distribution of nanomaterials, electrospinning has gained more attention. The resultant nano system is characterized by a highly porous network structure with a high surface area to volume ratio, and its dimensions are amenable to easy customization and production optimization. It is widely acknowledged that a substance must have at least one dimension that is 100 nm or less in order to be considered nano in size . Nanomaterials may be created from natural or synthetic material precursors in the form of tubes, wires, or particles . Chemical synthesis, electrodeposition, templating, catalytic growth, chemical vapor deposition, and, more recently, electrospinning techniques are only a few of the approaches used to produce greater quantities of nanomaterials. The electrospinning technique enables the mass manufacture of thin, nanoscale, highly functional mesh-like structures. Similar to electrospinning, which electrostatically accelerates solution droplets into a target to create evenly sized particles or thin film coatings, electro spraying accelerates solution droplets onto a target [1], [2].

The charged accelerated droplets may also self-disperse when collected at the target. These electrohydrodynamic methods produce a porous structure, which may take the shape of a multidimensional network structure or a film as a coating. By optimizing electrostatic forces on a jet of polymer solution, the very flexible electrospinning process enables the selective production of micron to nanoscale fiber structures. A pipette containing a polymer solution is placed between two electrodes that may produce a voltage difference in the kV range in the simplest variant of the electrospinning process. The polymer solution is then electrostatically drawn in a narrow, continuous jet towards a grounded target, resulting in the deposition of a fibrous web. By adjusting factors such the jet height, voltage, target type, and jet spindle speed, it is possible to regulate the pore size and distribution that results inside the web. The

development of greater throughput electrospinning system designs is ongoing, in addition to the optimization of system parameters.

The earliest patent application based on an electrostatically controlled deposition technique for plastics was made in 1934, even though it is acknowledged that the subject of electrospinning has its origins in early investigations. More than 700 related patents have now been submitted, referencing Formals' primary invention. A total of over 2,500 electrospinning-related patent applications have been made. An extraordinary rise in the number of journal articles on electrospinning over the last 20 years, with the number of citations inset, has been in line with this patent trend. This growth has been ascribed to market and industrial needs as well as developments in the nanotechnology sector. It is not unexpected that the current exponential growth in the number of patents awarded in the same period has continued. It shows a visual representation of the number of patents recently awarded. An analysis of the worldwide distribution of those patents, showing the development of electrospinning as a genuine answer to existing commercial and industrial demands The purpose of this study is to provide an overview of current electro spun nanomaterials research, processing, applications, technical constraints, and unresolved issues that are particular to the disciplines of biotechnology, food, water, environment, and energy [3], [4].

Resources and Manufacturing:

As previously mentioned, electro spraying and electrospinning both use straightforward electrohydrodynamic processes to create thin films, particles, or fiber sheets from host fluids. Under various experimental settings, the type of solvent and polymer have an impact on the final nanomaterial's shape. Any soluble polymer with a high enough molecular weight may be electro spun using this method. Systems with high surface area to volume ratio, low weight, high pore density, and high permeability with regulated, tiny fiber diameters will be produced by electrospinning nanofibers. In order to create electros pun fibers that are specifically suited to the demands of certain functional needs, a wide range of materials have been used. These materials include both natural and synthetic polymers, polymer blends, hybrid polymer systems, ceramics and metal compounds. Applications in high performance air filters, sensors, textiles, medical wound treatment, solar cells, fuel cells, batteries, capacitors, and scaffolds for tissue engineering are just a few of the several real-world fields that have seen steady growth in recent years. Some of the many and varied prospective applications as well as the potential uses for which natural polymers could be able to provide a workable answer. Shows the electrospinning-focused patents awarded between 1994 and 2012 in five major areas of importance to this research, demonstrating the incredible diversity of electros pun polymer products. This is in addition to the large variety of possible commercial uses. Currently, the biotechnology industry is still very much in the spotlight, both from a research and development and commercialization perspective. Focus has recently shifted away from the manufacturing of pure materials and towards end-use applications and suitable functionality. In the field of electrospinning, several overview studies have recently been published . The current developments in electrospinning with diverse polymeric materials will be the major subject of this article, with a special emphasis on the utilization of natural polymers relevant to five key research areas: biotechnology, food, water, environment, and energy. We see the growth of coaxial, composite, and core-shell nanofiber systems with increased functionality via ongoing advancements in electrospinning processes as well as the introduction of natural polymer systems as a response to fulfil industrial demands.

Natural polymers may have important advantages over their synthetic counterparts, including biocompatibility, low toxicity, renewable source materials, controlled biodegradation, and, with increased output, the potential for lower production costs, regardless of how difficult the

fabrication process may be for real-world applications. For example, in the field of biotechnology, the production of electros pun, naturally occurring proteins may provide cells a platform that is physiologically appropriate and encourage a state of differentiation of the cellular components. To date, a number of natural proteins, including silk, collagen, gelatin, and fibrinogen, have been electros pun effectively. Polysaccharides and other complex carbohydrate biopolymers have also been electros pun. However, the poor mechanical and thermal characteristics of many natural polymers restrict their use. Hybrid synthetic-natural, electros pun copolymer systems have emerged as potential candidates to meet present commercial needs, and numerous viable approaches have been suggested to get around this constraint [5], [6].

DISCUSSION

Biomedical applications and products using polymers must adhere strictly to the resultant chemical and physical qualities. The mid-1990s saw the start of research focusing on the fusion of tissue engineering with nanotechnology. The main, practical uses of today include medicine delivery, wound healing, tissue and cell regeneration, and surgical implants. Biodegradable synthetic polymers like polylactic acid, polycaprolactone, and polyglycolic acid, nonbiodegradable synthetic polymers like polyurethane, and natural polymers like cellulose, collagen, and chitosan are a few typical materials that have been electros pun. Some typical electros pun synthetic and natural polymers that are presently being researched in this field are included. The main goal of the nanofiber scaffold for tissue engineering and cell development is to imitate the extracellular matrix. It has been shown that the usage of such scaffolds results in a cellular response that is significantly different from that of conventional smooth-surfaced substrates. In a recent review, Ramakrishna et al. highlighted one such study in which electros pun scaffolds made from naturally existing extracellular matrix proteins, such collagen, enabled for substantially greater cell infiltration. They also cited studies on stromal cells that have been effectively cultivated on nanofiber meshes, including haemopoietic stem cells, embryonic stem cells, and brain progenitor cells.

Comparing the development of human dermal fibroblasts on Memecylonedule polycaprolactone nanofibers to that of other plant extracts with wound-healing capabilities as Indigofera apathies, Azedarach indica, and Myristica antimanic, Jin et al. recently observed a significant proliferation of human dermal fibroblast growth. Between days 3 and 9 of the research, the rate of cell proliferation was increased by 394% as a consequence of the newly created hydrophilic, memecylonedule polycaprolactone nanofiber scaffolds, which had an average nanofiber diameter of 487 nm. Recent research has focused on alternative materials and biotechnology applications, such as nylon-6/lactic acid core-shell nanofibers, which are made utilizing a two-step electrospinning and surface neutralization method. The osteoblast cell development on the calcium lactate-coated nanofiber scaffold was clearly visible. Sheng et al. looked at the electrospinning of brand-new silk fibroin nanofibrous mats laden with vitamin E for applications in regenerating skin tissue.

A review of the recent work comparing five different methods for developing composite scaffolds in electros pun nanofiber/hydrogel composite systems has also just been published. Numerous material characteristics of collagen make it appealing for use in biotechnology, including biocompatibility, low antigenicity, biodegradability, low inflammatory and cytotoxic reactions, high water affinity, and availability from a number of sources. What has become abundantly obvious is that effective nanofiber scaffolds must also encourage a natural state of differentiation of the cellular components; they cannot just duplicate the mechanical structure of the extracellular matrix. To control and promote cell growth, a customized, composite, nanofiber scaffold system with the inclusion of proteins may be required. However, electros pun collagen's inherent instability has to be addressed. Alginate, chitosan, collagen, and hydroxyapatite composite systems made by electrospinning are only a

few examples of the composite materials that have been extensively researched as prospective uses for bone tissue engineering. Comparing this composite system to a collagen film, it was shown to reduce scaffold disintegration in 300-800 nm diameter nanofibers for 10 days in collagenase solution by 35%.

Layer-by-layer coating of premade polyacrylonitrile and poly microfiber bases was suggested as an alternate method of creating collagen-based microfiber constructions. This research investigated the inherent instability of collagen as a consequence of avoiding volatile solvents during production. In addition to these more modern composite materials, McClure et al. published research on electros pun silk fibroin, collagen, elastin, and polycaprolactone created using a 3-1 input-output nozzle, resulting in a Tri layered structure. They looked at the impact of altering the medial and/or adventitial layer composition in the electros pun system, which was designed to aesthetically resemble the vascular wall and provide a good mechanical fit for artery replacement.

Gelatin is a biocompatible, biodegradable, nonimmunogenic protein that exhibits several integrin binding sites for cell adhesion and differentiation. It is a developing, cost-effective replacement for collagen. For usage as vascular scaffold systems, a variety of silk fibroin/gelatin nanofiber composites with diameters ranging from 99 to 244 nm have recently been developed. For a 70:30 ratio, a homogenous bead-free nanofiber system was produced. good cell proliferation and expansion followed by good biocompatibility were shown to facilitate long-term cell attachment. In order to effectively create a bio composite, nanofibrous scaffold on a revolving cylinder, Francis et al. concurrently used electrospinning and electro spraying.

These scaffolds were crosslinked to boost stability. The coaxial electrospinning approach may create core-shell structured nanofibers, resulting in enhanced material functionality, by simultaneously electrospinning two distinct polymer solutions. The capacity to coaxially construct water-soluble bioactive compounds into biodegradable core-shell nanofibers using polycaprolactone as the shell and protein containing polyethylene glycol as the core was effectively proven by Jiang et al. The flexibility, biodegradability, and relative hydrophobicity of PCL have all been extensively researched. A dual scaffold system made of poly/collagen and poly/collagen was created by Ladd et al. . They developed a noncytotoxic, continuous, 452–549 nm nanofiber system with three fundamentally different mechanical characteristics for use in tissue engineering at the muscle–tendon interface. In a manner similar to that described above, Gluck et al. created core-polyurethane nanofiber scaffolds with a shell composite of poly and gelatin, where the surface functionality promoted cellular migration to the scaffold's interior. By electrospinning, functional photosensitive poly containing PCL nanofibrous scaffolds were created, on which human fibroblasts cells rapidly proliferate when exposed to artificial light . It was determined that by converting the optical energy from the light into electrical energy, combining the photosensitive polymer P3HT with PCL will promote fibroblast proliferation and morphology under light simulation. It shows the proliferation and cell density of human dermal fibroblasts using different polymer mix combinations [7], [8].

Food-related applications include new food components, food additives, innovative packaging, food sensors, and additive encapsulations. The nanofibers and unique structures are created by electrospinning technique from synthetic and natural polymers. Since the majority of the generated nanofibers are often made of nonfood grade polymers, the application of electros pun nanofibers in the food industry is quite limited. However, since they are biocompatible and biodegradable, nanofibers made from natural polymers have prospective uses in the development of high-performance food packaging, food coatings, taste improvement, additive encapsulation, and nutraceutical applications. The fastest-growing industry is food packaging, which is essential to the delivery and processing of food.

The major goal is to keep the product's quality high and to keep it safe from numerous risks throughout transportation and until it reaches the consumer. Electro spun nanofibers have several applications in the food business. To increase shelf life and preserve taste, food packaging materials made of biobased and natural polymers may be employed.

By embedding biosensors within the fibers to indicate the food goods' expiry date, this method may also develop intelligent active packaging materials. Recently, electros pun zein nanofibers were used to make biobased polyester multilayer structure packaging films with a high barrier interlayer for food packaging applications. The oxygen barrier characteristics were greatly enhanced by compression molding the zein electros pun nanofiber into the multilayer framework. The industry of confectionery may be able to reduce costs by using nanoparticles created by electro spraying. Less chocolate sauce is used in the electrospinning process, and the fibers and particles that are created have a distinct mouthfeel and texture from bulk chocolates. This may aid in the creation of new food items and the expansion of oversaturated confectionery markets.

The most widely used nanofiber use is undoubtedly rapid responding biosensors, which provide quicker reaction times, more sensitivity, and greater selectivity than existing technologies. Tyrosinase enzyme immobilization on a glassy carbon electrode coated by a polyamide nanofibrous membrane demonstrated quick detection of phenolic chemicals as a result of the electrode's nanofiber coating. The migration of phenolic chemicals from food, such as cooking oils and mineral water, is detected using nylon-6 electros pun nanofibers in a similar manner. The active packaging material will substantially help regulators and improve health and safety measures by adding electros pun nanofibers. Food packaging uses will be found for electros pun nanofibers made from natural polymers like cellulose and proteins. Due to their biodegradability and biocompatibility, these nanofibers may be used to deliver medications to the gastrointestinal system in a regulated manner. Poly is used to make smart electros pun nanofibers, which may react to external stimuli like temperature changes. Numerous applications, including tissue engineering, controlled medication delivery, and smart food packaging, may make use of these materials. Smart electros pun fibers that are responsive to temperature changes as an example. Due to possible health problems posed by nanoparticles that may accumulate in soft tissues, nanofibers are considered to be a member of the "nano" family, a subject that is now very popular with many foods' regulatory authorities. Up until recently, it was unclear whether categories of nanoparticles posed risks to the food industry. Recently, regulatory frameworks for nanotechnology in food and pharmaceutical goods were issued by the Directorate for Science, Technology, and Industry Committee for Scientific and Technological Policy.

This may materially alter the ways in which nanofibers are used in the food industry.Due to prolonged droughts, expanding industrialization, and increasing population, the globe is confronted with severe problems in satisfying rising needs for clean water supplies. Oceans comprise about 97% of surface waters, which are challenging to render drinkable due to their high salt content. By adopting innovative, nanostructured membranes made via the electrospinning process, advances in nanotechnology might significantly aid in resolving the existing problems associated with satisfying the need for clean water sources. Due to its distinctive characteristics, including high porosity, micro- to nanoscale pore sizes, an interconnected open pore structure, and a large surface area per unit volume, electros pun nanofibrous membranes are now a highly appealing and viable option in filtering technology. It is envisaged that membranes with a variety of innovative functions that might efficiently remove salts and different hazardous substances to generate clean water for human consumption and a variety of other everyday purposes would be developed thanks to the flexibility of the electrospinning process.

A filtration membrane's main job is to separate two different phases by selectively allowing one phase to pass through it while blocking the other, such suspended particles, from passing through. As the world population continues to increase to above 7 billion , placing tremendous strain on the world's depleting resources, the safe removal of waterborne contaminants is essential to clean water recovery. Various casting procedures, electrospinning, and the phase inversion approach are often used to create polymer filtering membranes. Due to its easy scalability, low power requirements, and lack of chemical use, electros pun filtration membranes provide a feasible, beneficial, and alternative method for supplying clean water. lists several popular electros pun natural and synthetic polymers that are currently being researched for application in water filtration. The dimensionality of nanofibers makes it possible to produce in large quantities mesh-like, nanoscale structures that are lightweight and highly functional. Two kinds of membrane filtration may be roughly distinguished. The first kind, working at low pressures with great productivity, is micro- and ultrafiltration for the removal of bigger particles. The size of the membrane hole has a significant impact on how much separation occurs. The hydrophobic nature of conventional polymer surfaces may cause membrane fouling. The second technique involves reverse osmosis and nanofiltration, which eliminate dissolved salts from the aqueous solution. When it comes to category, the separation mostly happens via membrane diffusion. Expanded polystyrene nanofibers are added to standard nanofibers to boost the separation effectiveness of the filter medium by 20%, according to studies cited in Balamurugan et al.'s evaluation of the trends in air and water filtration. Additionally, they evaluated research on electros pun PVDF nanofibrous membranes for the microfiltration of polystyrene particles of various sizes. According to the research, electros pun nanofibers are more effective than traditional microfiltration membranes, with a rejection rate of around 90% for polystyrene microparticles. At the moment, the petrochemical sector often processes water/oil emulsion separation using micro glass fibers. Membrane fouling has to be addressed along with improving separation efficiency using nanofibers of different dimensions.

To lessen the problem of membrane fouling, recently combined a number of PVDF polymers with hydrophilic, surface-modifying macromolecules before electrospinning. A urethane prepolymer containing poly and poly of varied average molecular weights was used to create the surface-modifying macromolecules. Additionally, after blending with a PEG-based surface-modifying macromolecule, they contrasted electrospinning with the phase inversion approach, observing that the contact angle changed dramatically with technique, ranging from 0° for electros pun to 54° for asymmetric membrane. Modified and crosslinked chitosan combined with electros pun polyvinylidene fluoride were further filtering application areas for hybrid or composite polymer systems. In studies using bovine serum albumin filtration at 0.2 MPa, this surface-modified, electros pun membrane showed a broader working environment range while retaining a satisfactory flux rate and rejection efficiency of >98%. This has a lower degree of membrane fouling despite being greater than that of commercial ultrafiltration membranes. Electro spun cellulose acetate nonwoven membranes were created by Tian et al. and then surface-modified with poly for the adsorption of heavy metal ions. According to the findings of adsorption experiments, higher starting pH values were correlated with greater adsorption capacity. When compared to typical filters, silk fibroin and wool keratoses/silk fibroin blended nanofibrous membranes apparently performed very well for the adsorption of metal ions. Cu2+ was used as a model heavy metal ion in metal ion assays in a stock solution. Chitosan electros pun nanofiber mats with good mechanical strength and a diameter of 235 nm were studied by Haider et al. for their metal absorbability. They observed Cu adsorption rates that were nearly six times greater than those from chitosan microspheres, highlighting the crucial role performed by exposing the functional groups of chitosan nanofibers to the metal ions. For usage as both protective apparel and water filtration applications, Pant et al. developed nylon-6 nanofiber mats incorporating TiO2 nanoparticles, which increased mechanical strength and UV blocking capabilities in addition to having

antibacterial and hydrophilic qualities.Multilayered electros pun composite mats for water filtration have received a lot of research interest due to their increased water flow and filtration effectiveness. Chitosan's inherent qualities as a hydrophilic, water-resistant, yet water-permeable covering, however, may significantly contribute to improving the filtering capabilities. A new kind of high flux ultra-/nanofiltration system was demonstrated by Yoon et al. in a paper published in 2006 . It is based on a polyacrylonitrile electros pun nanofibrous scaffold and has a thin top layer of natural chitosan, which is hydrophilic, water-resistant, but water-permeable. After operating the three-tire composite membrane for 24 hours, they observed flux rates that were almost an order of magnitude greater than those of a commercial nanofiltration filter, while still retaining excellent filtration efficiency. The production of high flux thin film nanofibrous composite membranes was made possible by this effort. For the desalination of water by nanofiltration, a three-layer composite structure of thin film nanocomposite membranes was built. The fabrication and three-dimensional structure of TNFC membranes made using electrospinning methods. This method produces nanofiltration membranes that are used in the desalination of water and exhibit greater penetrated fluxes and lower fouling than traditional membranes. One such research describes the fabrication of an interracially polymerized polyamide barrier layer on both PAN electro spun nanofiber scaffolds and PAN ultrafiltration membrane, with different ratios of piperazine and bipyridine. They come to the conclusion that the interfacial polymerization that optimized the flow and rejection performance was greatly influenced by the piperazine concentration. Even more recently, a thin poly, PVA/surface oxidized multiwalled carbon nanotube layer was electro spun on an electros pun PAN nanofibrous substrate to create a double-layer mat that can be used to separate an oil/water emulsion using high flux thin film nanofibrous composite membranes. The PVA barrier layer may function more efficiently with the addition of MWNTs. Even at low pressures, this mechanically sturdy, double-layer membrane demonstrated a high-water flow and high rejection rate in oil/water emulsion separation.

Applications in the Environment:

As was mentioned in Section a recent article examined trends in water and air filtrations and came to the conclusion that polymer-based nanofibers embedded with nanoparticles can take the place of high-efficiency particulate air filters and get around the current restrictions in the filtration of chemical contaminants. These nanoparticle-impregnated nanofiber filters provide a number of benefits, including improved filtering performance, prolonged protection, nonselective decontamination effectiveness, and reduced product weight. However, many of these innovative nanofiber/nanoparticle systems need to build an effective filter simultaneously using electrospinning and electro spraying techniques lists a few popular electros spun natural and synthetic polymers that are used as viable alternatives in this industry. Listing several recent electros spun natural and manmade polymers along with their primary environmental applications. Ahn et al. previously investigated the superior performance of nylon-6-based nanofiber membranes for filtration over high-efficiency particle air filters that had already been commercialized. High filtration efficiency with little pressure loss and gradual filter clogging during usage are the essential characteristics of high-performance electro spun air filters. Air filtration membranes from Amsoil that are utilized in the auto/light truck industry utilize nanofiber technology. air filtration membrane was built using electro spun nanofibers [9], [10].

CONCLUSION

The electro spun fibers are appealing for a variety of applications, including high performance filters, energy production, water filtration, and scaffolds in tissue engineering, due to their high surface to volume ratio. The number of applications employing diverse synthetic and natural polymers is growing at an exponential pace in numerous disciplines,

which is a result of the electrospinning method' flexibility. However, compared to synthetic polymers, the utilization of natural polymers is very limited owing to incompatibility of the polymer chosen for a certain application and, in some circumstances, due to subpar chemical and mechanical qualities. New hybrid polymer systems based on synthetic and natural polymers that are appropriate for electrospinning with increased functionalities suited for a wide range of applications, notably in the food and biotechnology sectors, need to be developed further. They seek to take use of the major material benefits of both systems while overcoming some of the individual constraints that have so far prevented the full potential of electro spun systems from being realized. The resultant nanofiber-based goods will develop into highly competitive alternatives to present, often outdated market solutions as fabrication prices continue to fall and greater volume electrospinning equipment are brought online. According to recent research, it should come as no surprise that electro spun nanofibers will likely play a crucial part in the years to come in a number of key application areas, including water purification, renewable energy, and environmental protection.

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CHAPTER 6

INTRODUCTION TO EXTREMOPHILIC SHOTS: BIOTECHNOLOGY TO STRUCTURE

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ABSTRACT:

Numerous biocatalysts are now more readily available in large quantities because to recent developments in molecular and structural biology, which have also given researchers insight into the specific structure-function correlations of many of them. These findings made it possible to use biocatalysts for organic synthesis in a reasonable manner. As they provide faster reaction rates, more soluble substrates, and/or longer enzyme half-lives under the conditions of industrial processes, extremophilic enzymes are actively explored in this context for their potential usefulness in several biotechnological and industrial applications. Because it is so common, serine hydroxy methyltransferase serves as a good model for studying how enzymes adapt to harsh settings. In reality, data banks have a large number of SHMT sequences from Eukarya, Eubacteria, and Archaea in addition to a number of crystal structures. Due of its crucial metabolic function and structural conservatism, SHMT has seen relatively little structural change throughout evolution. Our study team used experimental and compared in silico methods to examine the molecular underpinnings of SHMT adaptability to high and low temperatures. These functional and structural analyses of SHMTs isolated from extremophilic animals may aid in understanding the peculiarities of the enzyme's activity at high temperatures and provide potential approaches for logical enzyme engineering.

KEYWORDS:

Biocatalysts, Extremophilic Shots, Hydroxy, Reasonable Manner.

INTRODUCTION

Given the importance of these macromolecules in sustaining life and their connection to several human disorders, studies on protein stability have become more significant over the last forty years. The ability of thermophilic/psychrophilic organisms to function at their extreme habitat temperatures can be understood by comparing the structural and functional characteristics of proteins with those of their homologs from mesophilic counterparts. This comparison may also shed light on the forces that stabilize proteins. Membrane components and protective small molecules often play a significant role in adaptations to pH, salinity, and pressure extremes and have been widely researched. The cellular components, specifically the proteins, must achieve a certain level of stability at extreme temperatures, where the majority of living species cannot grow because they are unable to maintain adequate metabolic fluxes.

This is necessary for temperature adaptation because environmental stress is typically not avoided by compensatory mechanisms. Understanding how proteins from thermophilic/psychrophilic organisms maintain their structure and function at high or low temperatures, respectively, has garnered a lot of attention as a result. In particular, particularly by maintaining a certain globular shape, enzymes carry out crucial functions in all biological systems [1], [2]. The native state, a functioning condition, is maintained through the delicate balance of conflicting forces. Although their proportional contributions have been disputed , the participants in this act have long been known . The hydrogen bonding and hydrophobic effect are the main stabilizing factors, whereas conformational

entropy favors the unfolded state. Extremophilic enzyme crystal structures unmistakably show a continuum in molecular temperature adaptations. For instance, there is a clear increase in the number and strength of all known weak interactions and structural factors, such as hydrophobicity and polar surface area of the molecules, involved in protein stability from psychrophiles to mesophiles and to thermophiles . The demand for stable protein structure and activity in thermophiles and the requirement for high enzyme activity in psychrophiles result in the involvement of the same molecular adaptation process in response to two different selection pressures.

Obviously, this implies complex and contentious connections between activity and stability in these spontaneously formed enzymes. It seems that each extremophilic enzyme uses a unique adaptation approach. Due to its widespread distribution and crucial metabolic function, SHMT serves as a model in this competition to research how enzymes might adapt to harsh settings. The topic of biocatalysts was significantly impacted by the discovery of novel extremophilic bacteria and their enzymes. Over the last ten years, the use of tough enzymes in industrial settings has significantly expanded. Recent developments in the research of extremozymes suggest that this process is speeding up. The unexpected characteristics of these enzymes are a large source of biotechnological curiosity. In general, it has been discovered that thermophilic enzymes are extremely resistant to proteases, detergents, and chaotropic agents, which may also provide resistance to the effects of organic solvents . Halophilic enzymes, which are stable in high salt concentrations, serve as models for biocatalysts in low-water media. The habitats of extremophiles are included, along with some of their enzyme uses [3], [4].

The pyridoxal 5′-phosphate-dependent enzyme serine hydroxy methyltransferase catalysis the reversible transfer of L-serine's C-position to tetrahydropteroylglutamate, resulting in the production of glycine and 5,10-methylene-H4PteGlu. The one-carbon units needed for the production of thymidylate, purines, and methionine are mostly obtained through this process. The substrate and reaction specificity of SHMT is also very wide in vitro. In fact, SHMT catalysis H4PteGlu-independent transamination, racemization, decarboxylation, condensation, and retro aldol cleavage events when given the right substrate analogues. In other cases, the rate at which various 3-hydroxy-amino acids cleave to glycine and the associated aldehyde approaches or even surpasses the rate at which serine cleave . Increased access to the enzyme's crystallized, solved structures from a variety of bacterial and eukaryotic sources has helped to explain a number of findings made via traditional biochemical research. The fold type I group, often known as the aspartate aminotransferase family, comprises many of the best-studied PLP-dependent enzymes, and SHMT is a member of this group.

SHMT and l-threonine aldolase may really be members of a subgroup of closely related proteins, according to an evolutionary study of the fold type I enzymes. Fungal alanine racemase, another very close cousin of l-threonine aldolase, also seems to be a member of this subgroup . Similar to the other members of this category, each enzyme component folds into two domains and associates form dimers in prokaryotes and tetramers in eukaryotes. The amino acid residues provided by both of the dimer's subunits define the active site, which is situated at the intersection of the domains. The hydroxymethyl transfer has been explained by a number of different processes . Numerous elements of the catalytic mechanism of SHMT remain unclear, despite the fact that the published crystal structures have shed a great deal of light on the architecture of the enzyme, the active site, and the residues involved in substrate binding and catalysis . The widely recognized mechanism for the hydroxy methyltransferase process entails a modified folate-dependent retro aldol cleavage by a direct nucleophilic assault of the N5 of H4PteGlu to the C of L-serine, which eliminates the quinonoid intermediate [5], [6].

DISCUSSION

It seems that the structure of the amino acid substrate dictates the sort of reaction that SHMT catalysis with various substrate analogues. None of the alternative reactions are catalyzed by SHMT when L-serine or glycine are present. As shown in other members of this family, the presently accepted paradigm explains this response specificity to the presence of both an "open and a closed active site conformation. other substrates react when the enzyme is in the open conformation, which allows for other reaction pathways, as opposed to the physiological substrates, which cause the closed conformation . Escherichia coli SHMT's folding process has also been well studied and understood . It may be broken down into two steps and comes to an end when PLP binds. The big and small domains quickly return to their initial states in the first phase, resulting in a folding intermediate that cannot bind PLP. The enzyme acquires the capacity to bind the cofactor during the second, slower phase, when it folds into the native structure.

Although the foundation for comprehending folding studies has been improved by the crystallographic data, it is still unclear what essential events are necessary for the change from the first to the second phase. The majority of research on SHMT has been on eukaryotic and mesophilic bacterial enzymes. Due to their remarkable catalytic capabilities, research of the extremophilic enzymes may provide insights into a better understanding of the structural and functional aspects of Cytosine SHMT is one of the relatively few PLP-dependent enzymes that is present in every living thing, it has often been praised as a possible target for the creation of anticancer and antibacterial drugs. SHMT also plays a crucial role in cellular metabolism.

Thermally-active SHMTs:

The thermophilic SHMTs have been seen in species from the two distinct kingdoms of life Archaea and Eubacteria so far. H4PteGlu serves as a carrier of C1 units in various oxidation states in Eukarya and Eubacteria. These C1 units are employed in the production of significant cellular components including purines and thymidylate as well as in the regeneration of methionine from homocysteine. In these species, the mechanism that SHMT catalysis is one of the main ways that C1 units are loaded onto the folate carrier. Tetrahydromethanopterin , a pterin-containing substance involved in methanogenesis, carries C1 fragments from formyl to methyl oxidation levels in methanogens and various other Archaea . Despite having structural similarities and being used in parallel processes, H4PteGlu and H4MPT-specific enzymes are largely unrelated phylogenetically. The majority of H4PteGlu's biosynthetic processes do not seem to be well suited for H4MPT.

Furthermore, there are very few, if any, homologies between the two carriers' biosynthetic processes, raising the likelihood of distinct evolutionary origins. In the metabolism of folates, SHMT serves as a singular link between Archaea and the rest of life, as other enzymes that use H4MPT as a cofactor do not significantly resemble their eukaryotic and eubacterial counterparts [7], [8]. This is because, while all SHMTs clearly share a common evolutionary origin, they do not function in a similar way. Although all archaeal genomes that have been sequenced so far have a gene producing SHMT, nothing is known about the catalytic capabilities and metabolic function of the enzyme in these species. Due to the lack of commercially available modified folates, it has been difficult to characterize archaeal SHMTs in a way that is suitable. The difficulties of culturing these organisms in a lab further complicates the separation of the enzymes from Archaea that flourish in harsh settings.

Regarding enzymes originating from archaeal species, there have been two reports of pure SHMT activity, with limited structural and functional characterization, from Methanobacterium chemoautotrophic, now known as Methanothermobacter Marburgers, and from Sulfurous solfataras. The enzyme was suggested to work in vivo in the direction of L-

serine biosynthesis in the initial study. Both studies provided proof that SHMT was selective towards the modified folates employed by the source organisms: coleopteran for S. solfataras and H4MPT for M. Marburgers. Recent developments include the expression, purification, and characterization of SHMT from the hyperthermophile's methanogen Echinococcus Janashia in *E. coli*. The findings of that study suggested that, despite the enzyme's ability to bind and use the modified folate H4MPT as substrate and its elevated thermal stability, the active site structure and mechanism of M. Janashia SHMT do not differ significantly from those of their bacterial and eukaryotic counterparts. Perhaps it would be helpful to have additional structural details, such as the three-dimensional protein structures, in order to better understand the functional properties of archaeal SHMTs.

Two three-dimensional structures of the eubacterial thermophilic SHMTs can be found in Protein Data Bank; one is from Thermus thermophilus, and the other is from Bacillus stearothermophilus. The enzyme isolated from Bacillus stearothermophilus, a Gram-positive bacterium that can thrive in a temperature range of 30-75°C, is currently the best characterized thermophilic SHMT. The internal aldimine form, binary complex with serine and glycine , and ternary complex with glycine and 5-formyltetrahydrofolate crystal structures of this enzyme have all been determined . Interesting structural information on the reaction mechanism of SHMT is provided by the many structures the authors have shown and by comparison with other SHMT structures from other sources. Contrary to what would be predicted for the transition from an "open" to a "closed" form of the enzyme, the bstSHMT-L-serine complex exhibits no appreciable conformational change when compared to the enzyme without bound substrate. The ternary complex including glycine and FTHF, however, exhibits the described conformational alterations. It is easy to witness the rotation of the PLP ring and the conformational changes of the same active site residues, which shows active site areas in the internal and exterior aldimine structures of bstSHMT. These subtle but important conformational alterations resemble those seen in the mouse cytoplasmic SHMT and the *E. coli* SHMT structures.

This complex exhibits asymmetric FTHF binding to the two monomers inside the dimer in a manner comparable to the mouse SHMT, in contrast to the *E. coli* enzyme. The conformational changes in protein structure, the orientation of the PLP ring, and important amino acid residues throughout various phases of catalysis may be accurately determined by a thorough examination of the bstSHMT structures and comparison with previously published structures. The SHMT catalyzed reaction has structural support from these findings for a direct transfer mechanism. These findings were corroborated by further research on the kinetic and structural characteristics of several bstSHMT active-site mutants. Additionally, the structural and functional alterations that the bstSHMT undergoes during unfolding have been well characterized. The unfolding characteristics of the thermophilic enzyme were compared with those of its mesophilic homologues, Bacillus subtilis SHMT, with which it shares 77% of its amino acid sequence identity, and with those reported for *E. coli* aspartate aminotransferase, a mesophilic protein that is a member of the same structural family despite only sharing a small amount of amino acid sequence identity.

Despite the two proteins sharing a high degree of sequence amino acid similarity, the data indicate that the bstSHMT unfolds in a completely different manner than the beshmet. Instead, the beshmet unfolding process is comparable to the mesophilic aspartate aminotransferases. In actuality, the unfolding of beshmet and eat is noncooperative and involves the stabilization of intermediates; in contrast, the unfolding of bstSHMT is extremely cooperative and involves the simultaneous dissociation of the two monomers and unfolding of the native dimer. Unpublished data show that early unfolding studies performed in our lab utilizing the M. Janashia SHMT seem to point to the same unfolding route as the bstSHMT. Comparing the kinetic characteristics of the folate-dependent and folate-

independent reactions of SHMTs from species acclimated to various temperatures would be fascinating. Accordingly, the thermophilic enzyme exhibits no enzymatic activity that differs noticeably from that of the mesophilic enzyme. Instead, it seems that the cold-adapted enzyme is a better catalyst than the mesophilic one based on the kinetic characteristics shown by the psychrophilic SHMT, particularly those for the folate-independent reactions.

The molecular mechanisms of cold adaptation are still largely unknown, despite the fact that many theoretical and experimental studies have been devoted to elucidating the molecular basis of the adaptation of thermophilic enzymes to high temperatures, comparing individual thermophilic proteins with their mesophilic counterparts and systematically examining different properties for families of proteins. Enzymes isolated from psychrophilic organisms offer substantial promise for biotechnological applications due to their enhanced catalytic effectiveness at low temperatures, particularly in industrial processes as energy savers and in the detergent sector as additives . First, an in-silico comparison technique was used to examine the structural adaption of SHMT produced by microorganisms acclimated to low temperatures in order to find substantial differences in the physicochemical characteristics of SHMTs. The enzyme from Psychopomps Ingraham, a psychrophilic microbe, was then produced in Escherichia coli, purified, and evaluated for its spectroscopic, catalytic, and thermodynamic characteristics.

The psychrophilic enzyme's characteristics have been compared to those of its homologous counterparts from *E. coli*, which have undergone comprehensive structural and functional analysis. When compared to *E. coli* SHMT, P. Ingraham SHMT exhibits higher turnover numbers. This is especially true for side reactions where many substrates, typically -hydroxy-amino acids, are significant chemicals in pharmaceuticals, agrochemicals, and food additives . The majority of comparative research have emphasized thermal stability. The apparent melting temperature of the protein is 62°C, which is lower than that of the shot, according to heat inactivation tests, which also revealed that picot activity is heat labile. It’s interesting to note that the apparent Tm values of the psychrophilic enzyme's apo form and haloform vary by around 20 degrees. This finding shows that the interaction with the cofactor somewhat offsets the inherent instability of the active site. When the PLP is transferred to its binding site at low temperature within the apoenzyme, the active site must go through conformational shifts that may be functionally related to its instability and resulting flexibility . Though the picot activity is at least 10 times greater than the shot activity, the best temperature for picot enzyme activity is 50°C, which is the same value demonstrated by the latter.

The fundamental adaptation to low temperatures is the relatively high activity that distinguishes psychrophilic enzymes. This appears to be accomplished via destabilizing the active site or the overall protein structure, which enables the catalytic Centre to be more flexible at low temperatures. By doing this, the enzyme should be able to access the transition complex with less energy, which is often in short supply in low-temperature environments . Recent publications have been published with the goal of identifying shared structural drivers for cold adaptation. Cold-adapted enzymes have been found to share characteristics with their mesophilic and thermophilic counterparts, including decreased amounts of Arg, Pro, and Glu and increased amounts of Assn, Gln, Ser, and Met; low Ala/Leu ratios and lower fractions of larger aliphatic residues expressed by the / ratios; decreased Arg/ ratios; decreased hydrophobic A structural trait does not always correspond with cold adaptation, and not all structural features are present in cold-adapted enzymes. Due to its highly conserved structure throughout evolution, picot may serve as an alluring and fascinating enzyme to highlight the structural traits associated with the adaptation to low temperatures.

This enzyme is also extremely promising for biotechnological applications due to its unique catalytic characteristics. The so-called "chiral market" has developed into a growing sector of the fine chemicals industry as a result of the consistent rise in the demand for

enantiomerically pure or enriched substances in medicines, agrochemicals, and food additives. Due to their biological action both on their own and as components of several naturally occurring complex chemicals, such as antibiotics and immunosuppressants, -hydroxy-amino acids in particular constitute a significant class of natural products that have lately attracted a lot of interest. For example, L-three-β- serine is a special remedy in the Parkinson's disease treatment as an agent for norepinephrine precursor therapy, L-three-α-serine is an intermediate for the production of antibiotics, such as florfenicol and thiamphenicol, 4-hydroxy-L-threonine is a precursor of rhizotomies, and 3,4,5-trihydroxy-L-aminopentanoic acid is a key component of polyoxins. The polyfunctional properties of the -hydroxy-amino acids also make them potential building blocks for peptidomimetics and other biologically intriguing nonproteinogenic peptide-like entities. Asymmetric chemical synthesis may be used to produce -hydroxy-amino acids. In this area, Hayashi and Belkin have conducted a number of innovative and fundamental studies. For instance, they have looked into the asymmetric aldol reactions of is cyanoacetic derivatives with fluor aryl, benzal, and aryl ketones catalyzed by gold or silver /triethylamine. In addition to asymmetric synthesis, chemical synthesis procedures are mostly used to create -hydroxy-amino acids, with chiral resolution coming in second.

However, there are certain disadvantages to these methods, including the following: Chiral resolution is labor-intensive, ineffective, and causes environmental issues when organic solvents are used excessively. Therefore, it would be ideal to create an enzymatic resolution procedure that is effective and clean. The majority of study has focused on threonine and -phenyl serine, although SHMT also catalysis the cleavage of other C3-OH amino acids with variable substituents and stereochemistry at C3. These reactions don't need H4PteGlu as a substrate, and their rates are similar to or higher than the rate of serine cleavage that is H4PteGlu-dependent. SHMT is a useful tool for biotechnological applications as a result. The biotransformation activity of serine hydroxy methyltransferases from various species has been extensively studied. It was discovered that the SHMTs isolated from H. methylator and *E. coli* have a broad substrate specificity.

Allow-threonine, threo-3,4-dihydroxy-phenylserine, L-threonine, and -threo-phenylserine were effective substrates for the degradation of -hydroxy-amino acids. As a biocatalyst for the stereoselective production of -hydroxy-amino acids, SHMT also shown promise. In the authors described the aldol addition of glycine to nonnatural aldehydes, such as benzyloxy acetaldehyde and -N-Caz-alaninol to corresponding β-hydroxy-α-amino acid Dia stereoisomers catalyzed by the recombinant SHMT derived from the Streptococcus thermophilus YKA-184 strain. With a diastereomeric ratio of 80:20 between the L-three isomer and the L-erythron isomer, the reaction reported in that study exhibits a considerable degree of stereospecificity with respect to the -carbon .Glycine's aldol addition to -N-Caz-alaninol was catalyzed by Shatin order to improve the efficiency of the enzymatic reaction and further utilize the enzyme's synthetic utility, the authors of examined the effects of reaction variables, including temperature, reaction media, and glycine concentration, on this aldol addition reaction and the diastereomeric ratio.

Temperature in particular has been shown to be a crucial factor. In actuality, a high synthetic capacity is maintained at low temperatures while the retro aldol activity is severely suppressed. Working at low temperatures might therefore be advantageous for synthetic processes. Furthermore, it was reported in that an *E. coli* strain carrying the serine hydroxy methyltransferase gene catalyzed the Dia stereospecific synthesis of L-allow-threonine. These findings suggest that SHMT could be a suitable biocatalyst for the stereoselective synthesis of amino acids with the hydroxy group attached. The use of SHMTs that operate at high temperatures might be advantageous for such industrial applications. At the circumstances of industrial processes, thermophilic enzymes often provide economic benefits like faster

reaction rates, greater substrate solubility, and/or longer enzyme half-lives. Psychrophilic enzymes, on the other hand, offer additional significant advantages through energy savings: they have higher reaction yields in cold environments, high levels of stereospecificity, increased thermal lability for quick and simple enzyme inactivation when necessary, and a reduction in the unfavorable chemical reactions that can happen at higher temperatures. Particularly intriguing for biotechnological applications are the structural and functional characteristics of the cold-adapted SHMT from Psychopomps Ingraham reported in. In reality, compared to the other SHMTs, this enzyme is a more effective biocatalyst, particularly for side reactions involving substrates called hydroxyamino acids. However, further research is necessary to fully comprehend the catalytic characteristics, particularly the stereospecificity of picot. The lack of research on stereospecificity in cold-adapted enzymes is disappointing.

Psychrophilic enzymes have been shown to be more stereoselective than their meson/thermophilic homologs, albeit it is unclear how the great flexibility of their molecules may be connected to this oddity [9], [10].

CONCLUSION

The synthesis of medicines, specialty chemicals, agrochemicals, and precursors to polymers is often impeded by costly procedures that have poor selectivity and undesirable byproducts. Due to their poor enzyme stability, mesophilic enzymes are often not suitable for the severe reaction conditions seen in industrial processes.

The utilization of stable enzymes from extremophiles in organic synthesis has risen as a consequence of recent developments in the field. By using directed evolution or rational protein engineering to modify enzymes, together with our knowledge of the biochemical and structural characteristics of extremophilic SHMTs, we may be able to enhance their catalytic and physical properties and create new catalytic functionalities.

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CHAPTER 7

POTENTIAL OF ARCHAEAL NUCLEIC ACID LIGASES IN BIOTECHNOLOGY

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ABSTRACT:

In many molecular biology and biotechnology techniques, DNA ligases and RNA ligases are crucial tools because they can catalyze the production of phosphodiester links. Currently, these procedures make heavy use of the bacteriophage T4 nucleic acid ligases. In this study, we contend that the nucleic acid ligases from Archaea provide an essentially unexplored reservoir of enzymes with a variety of potentially advantageous characteristics for new and developing biotechnological applications. We outline the present state of knowledge on archaeal DNA and RNA ligases, highlighting the relative lack of knowledge on in vitro activities that are most important to biotechnologists. We emphasize the biotechnological uses for archaeal DNA and RNA ligases now in use. The promise for additional research in this field is shown by recent studies in which the activities of the DNA ligases from Pyro coccus furious and Methanothermobacter thermautotrophicus were altered via the use of protein engineering.

KEYWORDS:

Biotechnology, Molecular Biology, Methanothermobacter, Thermautotrophicus.

INTRODUCTION

The creation of phosphodiester bonds between nucleic acids' opposing 5′-phosphate and 3′-hydroxyl termini is catalyzed by DNA and RNA ligases, which are present in all living things. Their actions are crucial for fundamental biological processes such RNA editing and repair, immunoglobulin gene rearrangement, and DNA replication and recombination. They are essential instruments for contemporary biotechnology since several molecular biology techniques have taken advantage of their in vitro activities. The nucleotide transferase superfamily is made up of DNA and RNA ligases, RNA capping enzymes, and tRNA ligases. This superfamily of enzymes all catalyze the creation of phosphodiester bonds in a three-step, conserved process that uses either ATP, GTP, or NAD+ as a high-energy cofactor. A ligase-AMP intermediate is produced in the first step by the active site lysine's nucleophilic assault on the cofactor's -phosphate. Second, an adenylated nucleic acid intermediate is created when the AMP is transferred to the 5′-phosphate of one polynucleotide strand . Finally, the 5′-phosphate of the opposing strand is attacked by the 3′-hydroxyl group of the second polynucleotide strand, forming a new phosphodiester link that unites the two strands and releases AMP. Archaean species not only endure harsh temperatures, salinities, pH levels, and pressure, but also flourish in them. Archaeal proteins have evolved in these severe habitats to have qualities that are useful to biotechnologists, such as stability and activity under a variety of quite harsh in vitro conditions. The common use of Pyro coccus furious DNA polymerase in PCR, where its thermostability and processivity also make it useful for related techniques like Quick-change mutagenesis, is a well-known example. We focus on archaeal nucleic acid ligases in this review. We provide a summary of our present understanding of these enzymes, including how they are already used in biotechnology, and we make the case that they provide a sizable untapped resource of activities for "next generation" molecular biology methods [1], [2]. At single-stranded nicks in double-stranded DNA, DNA ligases catalyze the creation of phosphodiester linkages in vivo. DNA

replication, DNA recombination, and DNA excision repair depend on this activity to preserve genomic integrity . According to their cofactor specificity, they are often divided into two categories and are necessary for all species.

While NAD+-dependent DNA ligases are commonly found in bacteria and certain eukaryotic viruses, ATP-dependent ligases are often found in Eukarya, Archaea, and viruses. But there are certain exceptions to this generalization. The archaeal species Halofuran volcanic is notable for having two active DNA ligases, one of which is ATP-dependent and the other of which is NAD+-dependent. Numerous applications in molecular biology and biotechnology need DNA ligases. DNA ligases have been used for many years in the cloning process as well as the ligation chain reaction, which is utilized to identify genetic diseases. Thermus aquaticus bacterium's DNA ligase has lately grown in significance for Gibson assembly. Without the need of restriction enzymes, overlapping DNA molecules may be assembled using this isothermal, one-pot technique . Numerous next-generation sequencing techniques also rely on DNA ligases, either for the sequencing process itself or for adapter ligation during sample preparation. The most widely employed DNA ligase in biotechnology is the ATP-dependent enzyme from bacteriophage T4, which can ligate both cohesive- and blunt-ended, double-stranded DNA molecules. Although it is permanently inactivated at 65°C, it is only sporadically active for the ligation of blunt-ended fragments . Additionally, it is inert at NaCl values greater than 150 mM. We suggest that any or all of the aforementioned uses may be suitable for thermostable archaeal DNA ligases. For instance, the ligation chain reaction needs a ligase that is stable beyond 90 °C, and Archaea may provide biotechnologists better Gibson assembly ligase substitutes than Taq ligase. Less than 25 archaeal DNA ligases have been fully described to far, and overall, there is less information on them in comparison to DNA ligases from other domains of life. The present level of knowledge is summarized, which also demonstrates the wide variety of traits that archaeal ligases have. For comparison, data for ligase are also provided.

As previously mentioned, DNA ligases are often categorized according to how strictly they are cofactor selective for either. It's interesting to note that certain archaeal ligases are capable of using several cofactors. Sequence homology revealed that Thermococcus Caesariensis, Thermococcus funiculars, and Thermococcus nonurine DNA ligases belonged to the ATP-dependent family, although in vitro characterization of each has demonstrated that they are able to utilize either as their cofactor. When the cofactor is changed to GTP, the dependent ligase from Sulfophobococcus Zillig likewise exhibits relative activity of 63%. Activity with GTP has only been mentioned for RNA capping enzymes, not the S. illogic DNA ligase. ADP may also be used by a variety of archaeal DNA ligases, including the S. illogic enzyme. Archaeal DNA ligases' undifferentiated nucleotide specificities may be explained by the fact that they still possess a characteristic from the long-ago common ancestor of the and -dependent enzymes.

The ADP moiety is shared by both ATP and NAD+, suggesting that this predecessor may have utilized it as a cofactor. However, it has also been highlighted that there is little direct evidence that DNA ligases use ADP . Another theory is that since ATP is very unstable at high temperatures, thermophilic ligases with a preference for alternative cofactors like ADP and GTP were forced to evolve. To catalyze the production of phosphodiester bonds, DNA ligases use multidomain structures; however, the number and nature of the domains they include may vary. Six archaeal DNA ligases have had their structures determined to date: Pyro coccus furious, Achelous fulgides, Sulfurous solfataras, S [3], [4]. illogic, Thermococcus services, and Thermococcus sp. 1519. The adenylation domain , the oligonucleotide-binding domain , and the N-terminal DNA-binding domain are the three domains that make up each enzyme. The Add comprises all six of the nucleotide transferase superfamily's motifs. Together, the Add and OBD are referred to as the catalytic core since

they are both only necessary for activity. It is believed that the N-terminal DBD, which is specific to eukaryotic and archaeal DNA ligases, contributes to both the distortion of the DNA substrate and the maintenance of an active conformation of the catalytic core. The significance of significant conformational changes throughout the catalytic cycle of DNA ligases has been brought to light by the elucidation of the unbound and DNA-bound structures of the ATP-dependent ligase from Chlorella virus. In order to fit into the minor groove of the DNA substrate, the OBD moves more than 60 and spins almost 180 during DNA binding. Although the structures of archaeal DNA ligases have not been determined in association with DNA, three distinct conformations of OBDs have been seen . The DNA ligase from the bacteriophage T7 was similar in general structure to the S. solfataras enzyme, which had an open and extended conformation with the OBD turned away from the Add. As opposed to the open extended conformation, the Thermococcus sp. 1519 ligase structure adopted an intermediate conformation in which the OBD was rotated anticlockwise around the Add by about 90°, though this rotation was insufficient to form any hydrogen bonds or salt bridges between the OBD and the other domains. As shown in the structures of the DNA ligases from P. furious A. fulgides, and T. services, a further 120° rotation of the OBD results in a closed conformation. A C-terminal helix that follows conserved pattern VI in these structures stabilizes the closed conformation by facilitating a number of ionic contacts between the OBD and the Add. In the archaeal unbound structures, this extra helix fills the space between the Add and OBD, but it is displaced in the DNA-bound form of human DNA ligase.

DISCUSSION

When compared to the DNA-bound structures of the human DNA ligases, where the three domains surround the DNA substrate, the domain configurations of the archaeal ligase structures all vary significantly. The picture that is starting to take shape suggests that conformational flexibility is essential for the proper operation of archaeal DNA ligases. It is uncertain if the variations in unbound structures correspond with variations in domain orientations when the DNA substrate is bound since no structures of archaeal DNA ligases in complex with DNA have been solved. It is still unclear how proteins behave during catalysis at the host cells' development temperatures. It is not unexpected that the majority of archaeal DNA ligases have only been tested for their capacity to seal single-stranded nicks in double-stranded DNA given their main physiological function in DNA repair. Possessing the capacity to ligate double-stranded, cohesive-, or blunt-ended fragments is more intriguing for biotechnological applications. Four archaeal DNA ligases have been found to engage in these activities. Thermococcus sp. 1519, Europium premix, Staphylothermus marinus, and T. funiculars all contain enzymes that have been shown to ligate cohesive-ended fragments. Additionally, blunt-ended fragments may be joined by the DNA ligases from S. marinus and T. funiculars [5], [6]. Therefore, it appears probable that further characterizing archaeal DNA ligases will provide a pool of enzymes that might be useful in molecular biology and industry. Applications for archaeal DNA ligases that take advantage of their high temperature optimum are expected to be the most practical right away. Thermococcus sp. 1519's DNA ligase, for instance, is most active between 60 and 70 degrees Celsius and can ligate DNA fragments with long cohesive ends , but not fragments with shorter cohesive ends or blunt ends . This combination of characteristics would seem to make it a suitable tool for Gibson assembly, while more testing is necessary.

This process, which is presently carried out at 50°C, has quickly become the standard technique for restriction enzyme-free assembly of DNA fragments in synthetic biology. We hypothesize that the promise of ligation at higher temperatures would reduce the number of incorrect ligation events brought on by mis annealing of fragments with short overhangs may be what motivates the development of new techniques. Similar to this, the DNA ligase from

Staphylothermus marinus catalysis a number of ligation processes with cohesive- and blunt-ended fragments and has a half-life of approximately 3 h at 100°C. Due to its exceptional thermostability and ability to withstand the high temperature denaturation stages in the thermal cycling methodology, this enzyme may be useful in the ligase chain reaction for the detection of single nucleotide polymorphisms. More broadly, it has been shown that thermostable proteins are excellent building blocks for protein engineering because they are more mutation-tolerant than their mesophilic homologues and produce more functional variations as a result.

Although DNA ligases are common in molecular biology, relatively few efforts have been undertaken to modify them via protein engineering. Only one archaeal DNA ligase, from Pyro coccusfurious, and one bacteriophage T4 DNA ligase have been addressed so far.Through structure-guided mutagenesis, Nishida and colleagues have effectively improved the activity of the P. furious DNA ligase using their structural insights. In order to maintain the closed conformation of the enzyme, they have focused on the C-terminal helix that interacts with the OBD and the Add. First, alanine was substituted for each of the five polar OBD residues that were involved in interactions with the Add. The idea was that by "unlocking" the enzyme, destabilizing the interdomain connection would enable more mobility of the OBD and hence boost activity.

The mutation of Asp540, which is found near the N-terminus of the helical extension, had the most impact among the five chosen residues. The Asp540Arg mutation had the best activity across a wider temperature range, according to further mutagenesis at this site. The scientists demonstrated that the designed ligase behaved better than the wild type at two temperatures in proof-of-concept ligation-amplification studies. Maximum amplification of the ligated DNA product at 60°C was accomplished with the mutant enzyme in only 3 cycles while the wild type enzyme required 10 cycles. The wild type ligase's product yield was only around 30% as high even after 10 cycles of ligation-amplification at 30°C as compared to the modified enzyme's maximal output after 5 cycles . Asp540 was negatively charged, and a series of sophisticated biophysics studies revealed that adding a positively charged arginine residue sped up the creation of the covalent ligase-AMP intermediate as well as the binding of the nicked DNA substrate . The invention described in this book is also the subject of US Patent No. The same team recently employed further mutagenesis in a follow-up investigation to completely eradicate the ionic connections between the Add and the OBD. P. furious DNA ligase with either D540R with deletion of the last four amino acids of the C-terminal helix or D540R plus three-point mutations demonstrated improved nick-joining abilities. This design strategy, which releases the connections of the C-terminal helix with the Add and OBD domains, looks to be a potentially useful one for boosting activity, however it has not yet been tried with other archaeal DNA ligases [7], [8].

RNA end-joining enzymes known as RNA ligases are used in the splicing, editing, and repair of RNA. The evolutionary distribution of RNA ligases is more restricted than that of the widely distributed DNA ligases. RNA ligases have been discovered using sequence similarity searches in all three domains of life, but only in a small number of species. Typically, RNA ligases are divided into two large groups. The eponymous RNA ligase 1 from the bacteriophage T4 and the tRNA ligases from fungi, yeasts, and plants are all members of the Rnl1 family. These enzymes fix breaks that site-specific nucleases have caused in single-stranded RNA. The RNA-editing ligases from the protozoans Trypanosoma and Leishmania, as well as the bacteriophage T4 RNA ligase 2 , are all members of the Rnl2 family. The main function of these enzymes is to close nicks in RNA that have been rendered duplex by the presence of a bridge complementary strand . Despite having six conserved nucleotide transferase motifs in common with DNA ligases, RNA ligases have poor levels of sequence conservation as a whole. This generally makes family categorization more difficult and less

significant ligases are crucial in molecular biology, much as DNA ligases. T4 RNA ligases 1 and 2 are now required for the creation of microRNA sequencing libraries, as well as a subset of rapid amplification of cDNA ends techniques, 3′ RNA tagging, and other processes. The next sections will concentrate on ATP-dependent RNA ligases that can create phosphodiester linkages between 5′-phosphate and 3′-hydroxyl termini since they are the most useful in these processes. For completeness' sake, we should also include that Pyro coccus Hiroshi, an archaeal species, has been found to produce two noncanonical RNA ligases. A putative 2′–5′ RNA ligase is the first, and its structure has been determined.The second ligase, Retch, binds either 3′-phosphate or 2′,3′-cyclic phosphate termini to 5′-hydroxyl termini. Recent detailed characterizations of its structure, mechanism, and interaction with a new protein cofactor have been published.When it was discovered that an open reading frame from Pyro coccus abyss, which had previously been designated as encoding a DNA ligase, really encoded an RNA ligase, the first thorough biochemical characterization of an archaeal RNA ligase was described in 2008. Archaeal RNA ligases previously were thought to be Rnl2-like enzymes because they had comparable variant nucleotide transferase motifs to T4 Rnl2. However, compared to T4 Rnl2, the P. abyss RNA ligase structure was only slightly more structurally homologous to T4 Rol. Similar to T4 Rnl1, the P. abyss RNA ligase was also active with single-stranded RNA substrates but not with double-stranded Rather homodimer structure of the P. abyss RNA ligase was discovered using X-ray crystallography, in contrast to the monomeric mesophilic ligases. An N-terminal domain, a catalytic domain, a dimerization domain, and a C-terminal domain made up each monomer . Structure-wise, the catalytic domain resembled those of other nucleotide transferase superfamily members. Only these two enzymes had the N-terminal domain that was similar to T4 Rnl1 so far.

There were no structural homologues and no assigned functions for the C-terminal domain, which was entirely -helical. Although the metal binding residues are lacking in the ligase, the dimerization region has structural similarities with the copper-binding domain of the amyloid precursor protein. RNA ligases have developed into crucial tools in molecular biology in addition to their activities in vivo. The fast amplification of cDNA ends, oligonucleotide synthesis, 3′-end biotin and fluorophore tagging, and 5′ nucleotide alterations of both RNA and DNA were all developed shortly after the identification of T4 Rnl1.In more recent times, RNA ligases have become crucial for building sequencing libraries of tiny RNAs like microRNAs. In order to employ the adaptor sequences for priming during reverse transcription and PCR, T4 RNA ligases are used to link the 5′- and 3′-adaptors to the RNA substrates during library preparation . High-throughput screening has evolved into a crucial tool for both the discovery and profiling of miRNA expression as a result of the growing understanding that miRNAs, small regulatory RNAs involved in posttranscriptional regulation , have a variety of biological functions, and their dysregulation has been linked to a number of diseases . Thus, RNA ligases that can generate high-quality sequencing libraries that are reflective of the initial miRNA population in a sample are highly sought-after. Sadly, it is becoming more and more clear that the adaptor ligation process plays a significant role in the severe biases that miRNA sequencing datasets are susceptible to. Unwanted cyclic by-product generation is one restriction . RNA ligases have a function that has been preserved throughout evolution: they seal nicks in RNA hairpin loops . This causes a tendency for the RNA substrates to circularize in vitro, blocking adaptor ligation. The T4 RNA ligases also have a bias towards ligating certain RNA sequences, which may result in an error in the estimation of miRNA abundance of up to 4 orders of magnitude. This ligation bias, rather than being caused by a preference for primary sequences, is a bias towards RNA secondary structure.

As a result, characterizing thermostable RNA ligases that are active at temperatures high enough to denature RNA secondary structures is becoming more and more important.

Protocols for molecular biology have used archaeal RNA ligases in several cases. The M. thermautotrophicus RNA ligase has been utilized to 5′-adenylate single-stranded DNA adapters for use in the building of miRNA sequencing libraries. It can adenylate both single-stranded RNA and single-stranded DNA. For this adenylation phase, previously either a chemical synthesis process or a method utilizing T4 DNA ligase was utilized; however, T4 DNA ligase did not accumulate enough adenylated products, and the synthesis approach was costly. However, the M. thermautotrophicus RNA ligase is a perfect replacement because it accumulates large amounts of the adenylated intermediates when too much ATP is used in the procedure. This enzyme is now offered for sale in the form of a kit from New England Biolabs for the 5′ DNA adenylation process. The RNA ligase from M. thermautotrophicus is a very active 5′ adenylation enzyme, however a single point mutation led to an enzyme that was completely incapable of adenylation but was still capable of forming phosphodiester linkages. Due to this, a two-step approach that uses the wild type enzyme to adenylate DNA adapters in the first reaction has been developed. The pool of target miRNA molecules and the modified ligase may then be added to the adenylated adaptors for incubation. PR adenylated adaptors are ligated to the RNA substrates as a result, and the RNA cannot be circularized. The RNA ligase from M. thermautotrophicus can work at 65 °C, which helps to eliminate the ligation bias brought on by RNA secondary structures. The mutant enzyme is commercially available to carry out this technique [9], [10].

CONCLUSION

We have described the most recent research on archaeal nucleic acid ligases in this review. We have discussed features that are expected to make these enzymes significant additions to the biotechnologist's toolkit in the future while also highlighting the relative lack of understanding on them. Enzymes from archaea, in particular, are often thermostable. For developing technologies like Gibson assembly, DNA ligases that are stable and active at high temperatures are becoming essential, while thermostable RNA ligases hold the potential of creating unbiased miRNA sequencing libraries. Archaeal enzymes are also excellent starting places for protein engineering due to their thermostability. The huge potential for further research in this field is shown by recent efforts to create the Pyro coccus furious DNA ligase and the *Methanothermobacter thermautotrophicus* RNA ligase. Although presently under sampled, the pool of archaeal nucleic acid ligases is diversified overall. We believe that additional research will uncover novel enzymes with beneficial traits for molecular biology and biotechnology, which will in turn spur the creation of fresh approaches.

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CHAPTER 8

INFERRING SEMANTIC SIMILARITY OF GENE PRODUCTS BY CORRELATING INFORMATION CONTENTS OF GENE ONTOLOGY TERMS

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ABSTRACT:

In recent years successful uses of the gene ontology to infer functional links between gene products have increased the need for computational algorithms to automatically compute the semantic similarity between genes based on the semantic similarity of words in the gene ontology. However, although being extensively employed in many applications, current approaches may dramatically exaggerate the semantic similarity of genes that are really not functionally linked, producing false findings in applications. To get around this restriction, we suggest representing a gene product as a vector made up of the data from terms in the gene ontology that have been annotated for it. Using three different measures Pearson's correlation coefficient, cosine similarity, and the Jaccard index we can determine how similar two gene products are. To investigate the efficacy of the suggested approaches, we concentrate on the gene ontology's biological process domain and annotations of yeast proteins. Results indicate that our strategy is successful at describing functional links between gene products since semantic similarity scores acquired using the suggested measures are more congruent with established biological knowledge than those obtained using a list of current methodologies.

KEYWORDS:
Exaggerate, Ontologies, Restriction, Semantic.

INTRODUCTION

In the past few years, domain ontologies have been successfully used to describe entities in a variety of biological domains. Examples include the modelling of general computational tasks in systems biology based on the systems biology ontology, the inference of phenotypic similarity between human diseases, and the derivation of functional relationships between gene products based on the gene ontology. Relationships between the entities can be quantified by their semantic similarity in the ontology, providing a practical yet effective way of profiling the entities and their semantic relationships. An ontology that provides controlled and structured vocabularies in a specific biological domain and annotations that characterize entities in the domain with the vocabularies make this possible. However, based on their annotations in a domain-specific ontology, entities' semantic similarity may still be automatically determined, which is a difficult task that calls for the development of practical and efficient computational algorithms. A domain ontology, in general, offers a collection of relational and regulated vocabularies for defining domain-specific information. The vocabularies, which are also known as concepts or terms, are often arranged as a directed acyclic graph, in which words are represented by the vertices and semantic connections between the terms are represented by the edges. Additionally typical is the presence of several semantic relationships in an ontology. There are many other kinds of semantic links, for instance is a and part of in the gene ontology. The majority of existing methods, given such a domain-specific ontology and annotations that map entities onto the terms, first

determine pairwise semantic similarity between the terms using the ontology's structure and the annotations of entities, and then derive similarity between the entities based on similarity between the terms. Resnik suggested using the information content of the lowest common ancestor of two query terms to gauge their semantic similarity, using the gene ontology as an example, in order to accomplish the first goal. By taking the informational elements of the search phrases into account, Lin updated this metric. The measure of Lin was further modified by Schlicker et al. to include the relative frequency of occurrence of the LCA. Jiang and Conrath suggested employing a formula other than Lin's in order to integrate the information contents of the query words. Using just the structure data of the underlying gene ontology, Wang et al. suggested to assess semantic similarity between GO words while taking into account two kinds of semantic relationships: The semantic similarity between two query gene products was often determined using a mean-max algorithm, with similarities between GO keywords estimated. The greatest similarity between a term and each term in the collection was defined as the similarity between a term and the collection, given a single GO term and a collection of GO terms. Additionally, the average similarity between each word in a collection and the terms in the other collections was used to establish the similarity between two collections of GO terms. The semantic similarity between two gene products was finally defined as the similarity between the matching two sets of GO words since each gene product was annotated by a set of GO terms [1], [2].

The aforementioned techniques have been successfully used in a wide range of fields, with some examples being the calculation of functional similarity between proteins based on the gene ontology for the inference of disease genes, the characterization of phenotype similarity between human diseases based on the human phenotype ontology, and many others. In the fields of bioinformatics and computational biology, software packages embodying similar techniques have also been made openly accessible. Some examples are Goemin, Fussier, and OWL Sim. These techniques do, however, have some clear drawbacks. For instance, despite attempts to adapt Resnik's approach, methods like those in often performed worse than Resnik's in practical implementations, indicating that the rewriting of information contents is unlikely to be successful. The method developed by Wang et al. performed worse than Resnik's in many applications, such as the prioritization of candidate genes, despite the fact that they systematically considered the structure and numerous semantic relationships of the gene ontology. Additionally, as we will show in the findings section, all of these approaches have a propensity to exaggerate the similarity between proteins that are truly dissimilar in their activities, leading to false findings in practical applications. With these insights in mind, we suggest in this study that a gene product be represented using a vector made up of the information included in GO words that have been annotated for the gene product in the gene ontology [3], [4]. Using three measures the Jaccard index, cosine similarity, and Pearson's correlation coefficient we propose measuring the semantic similarity of gene products as the relatedness of their corresponding vectors. To conduct a series of thorough investigations on the efficacy of the suggested approaches, we concentrate on the biological process namespace of the gene ontology and annotations of proteins of the budding yeast Saccharomyces cerevisiae. We use the biological process domain of the gene ontology to calculate semantic similarity scores between yeast genes, use the resulting semantic similarity scores to measure functional relationships between the proteins, and investigate the congruence between these relationships and existing biological knowledge. Results on 1,022 protein families, 141 yeast biochemical pathways, and two large-scale yeast protein-protein interaction networks demonstrate that semantic similarity scores derived using the proposed measures are more

biologically consistent than those derived using a list of existing methods, demonstrating the efficacy of our method in defining semantic similarity between gene products.

The Species and Gene Ontology Particular Annotations:

A regulated vocabulary of terminology for characterizing the properties of gene products is offered by the gene ontology. Biological process, molecular function, and cellular component are the three domains covered by this ontology. The functioning of live cells, tissues, organs, and organisms is relevant to the definition of operations or groups of molecular events in the biological process domain. The molecular function domain is a representation of the fundamental molecular functions of a gene product, such as binding and catalysis. The extracellular environment or the components of a cell are described in the cellular component domain. Each of these three domains is structured using a directed acyclic graph, shown as, where is a collection of vertices representing ideas and is a set of edges representing semantic connections between the words.

In this graph, the terms and stand for the sets of the term's parents and children, including itself, and the terms and stand for the term's ancestors and descendants, including itself. Be aware that there are other kinds of semantic links in the gene ontology, including. An annotation that specifies a species offers a mapping from a word in a domain of the gene ontology to a gene product of that species. According to standard guidelines, annotating a gene product with a word entail annotating the gene product with all of the term's ancestors. With this idea in mind, we use a binary annotation vector to represent annotations of gene products, where it is annotated by the word indexed by or its descendants as well as the total number of terms in a domain [5], [6].

DISCUSSION

There are several domain-specific ontologies that have been developed for describing items in various biological fields. Eight ontologies, specifically, have been made available by the OBO Foundry to give uniform definitions of items in biological domains. The term gene ontology is often used to refer to this group of ontologies, which has been extensively utilized to define the activities of genes. It includes biological process, molecular function, and cellular component. A number of widely used model species, including yeast, fruit flies, and mice, are annotated in the gene ontology. In order to verify the efficacy of the suggested approaches, we concentrate on the biological process domain of GO and annotations of the budding yeast Saccharomyces cerevisiae in this study. We collect annotations of yeast genes from the gene ontology's biological process domain, which we extracted words from. It should go without saying that a pair of genes chosen at random may rarely have comparable functions, hence their semantic similarity score should be near to zero. To support this claim, we compute semantic similarity scores for 100,000 randomly chosen pairs of yeast genes, and we display the scores' distribution. The graphic makes it evident that the median similarity score for the correlation measure and the cosine measure are both quite close to zero. The Jaccard measure's median similarity score is greater than those for the correlation and cosine measurements, but it is still lower than those for all five currently used approaches. Among the currently used approaches, Resnik's method yields the lowest median similarity score, followed by those of Schlicker et al., Lin, and Wang et al. The Jiang et al. technique produces a median similarity score of which is the highest. These data lead us to the conclusion that the current approaches often overstate the lexical closeness of genes that are truly unrelated in terms of their functional relationships. The suggested measures, however, do not have this flaw and, as a consequence, provide considerably more logical findings when evaluating

semantic similarity between randomly chosen gene pairs, although being much simpler than the previous approaches. The majority of biological activities are known to result from the coordinated actions of several proteins, which are often involved in the same biological process and constitute a route. Therefore, gene products in the same route should have comparable biological process ontology annotations and, as a result, possess high semantic similarity scores. Gene products from separate pathways, on the other hand, should have low semantic similarity scores. We compare the semantic similarity scores between proteins involved in the same route to those between proteins involved in various pathways in order to determine if the suggested similarity measures are in line with this information. We download pathways with at least two proteins each from the Saccharomyces Genome Database [7], [8]. To get the mean semantic similarity score inside each of these routes, we compute pairwise semantic similarity scores for each protein engaged in the pathway. We then average these values over all protein pairings. For each route, we also choose ten times as many proteins at random, compute the semantic similarity scores between these proteins and those in the pathway, and then take the average of these scores to get the mean semantic similarity score outside the pathway. According to the figure, mean similarity scores inside routes are often high, but those outside of pathways are typically low. The variations between the medians of the mean similarity scores inside and outside routes are far more noticeable for all three of the suggested metrics than for any one of the five already used approaches. Using the correlation measure, for instance, we may determine that the median over all paths is and the median. We find a median and using the cosine measure. Wang's technique yields a median of 0.7405 and a median of 0.2489, whereas Resnik's method yields a median of and a median.

We then average these ratios across all 141 pathways to derive a criterion known as fold change of semantic similarity scores within pathways against those outside pathways. This ratio of the mean semantic similarity scores within a pathway over those outside the pathway is calculated for each pathway separately. For instance, when we use the correlation measure, we get a 29.93 times improvement. We find a fold change of 26.65 using the cosine measure. In comparison, the Resnik approach yields a somewhat higher fold change of 4.83 whereas the Wang method only generates a fold change of 3.03. These findings corroborate the assertion that the suggested metrics, as opposed to current approaches, provide results that are considerably more plausible when evaluating the functional interactions between proteins within pathways. One or more functional areas, also known as protein domains, are often found in proteins. distinct combinations of protein domains result in the vast spectrum of proteins found in nature. Different domains often account for distinct activities of proteins containing them. As a result, proteins may be divided into several families based on the domains they contain. Additionally, proteins with the same domain, or those from the same family, should perform certain tasks that are comparable, and as a result, have some annotations in common in the gene ontology's biological domain. The gene ontology predicts that proteins from the same family should have a high semantic similarity score. Proteins from distinct families, on the other hand, should have relatively low semantic similarity scores. We compare semantic similarity scores between proteins within a protein family and those between proteins from other families in order to determine if the suggested similarity measures are in line with this information. Both high quality protein families and poor-quality protein families are abundantly available in the Pfams database. protein families are gathered in Pfams-A version 26.0, which was published in November 2011. We extract 1,022 protein families with at least two yeast proteins from this data source. We determine the mean semantic similarity score within each of these families by averaging the pairwise semantic similarity scores of all the protein pairs that make up the family for each of these families. In

the meantime, for each protein family, we also randomly choose 10 times as many proteins as those in the family, calculate the semantic similarity scores between these proteins and those in the family, and then take the average of these scores to get the mean semantic similarity score outside the family. Then, we calculate the ratio of the mean semantic similarity scores within each protein family to those outside the family and average these ratios across all 1,022 protein families to obtain a criterion known as fold change of semantic similarity scores within protein families against those outside families. It provides a summary of the fold changes, which makes it easy to assess how well the suggested measures work. As an example, we get a fold change of 6.915 using the correlation measure. A fold change of 6.511 is obtained by using the cosine measure. The fold change we get from the Jaccard measure is 3.267. In comparison, the Resnik approach yields a somewhat higher fold change of 2.370 whereas the Wang method only generates a fold change. Additionally, we increase the minimum number of proteins that make up a protein family from 2 to 10, compute the fold change in each circumstance, and display the results. In a nutshell, the fold change differs depending on how many proteins make up a protein family, but the observation that the fold changes of the suggested measurements are bigger than those of the current approaches stays the same. For instance, when taking into account protein families with at least 10 proteins, we find that the fold changes for the correlation, cosine, and Jaccard measures, respectively. The fold change, however, is 2.090, 2.846, and 3.430 for the measurements by Wang, Resnik, and Schlicker, respectively. Based on these findings, we hypothesize that the suggested metrics provide considerably more logical outcomes when evaluating functional links between proteins that are members of the same protein family. As a result, we draw the conclusion that the suggested metrics are more in line with biological understanding than current approaches.

Gene Semantic Similarity and PPI Data Coherence:

According to biological understanding, proteins often interact with one another to jointly create biological activities. The protein-protein interaction network, in which nodes are proteins and edges represent physical interactions between the proteins, is a common name for the collection of all physical interactions in a live organism. Because they are often engaged in comparable biological processes, proteins that interact frequently have high semantic similarity scores and similar annotations in the gene ontology's biological process domain. We evaluate the associations between interacting proteins and their semantic similarity scores in the manner shown below to determine if our similarity measurements are in line with this information. We down load two Saccharomyces cerevisiae PPI networks that have been carefully selected. We extract a PPI network with nodes and edges from Bio Grid. We generate a relatively modest PPI network from the DIP that has nodes and edges. We compute semantic similarity scores for proteins that interact and those for an equal number of randomly chosen protein pairs that do not interact for each of these networks, and we present the distribution of these scores. The semantic similarity scores for interacting proteins are often higher than those for noninteracting proteins, as can be seen from the picture, and this fact holds true for both the Bio Grid and the DIP networks. The mean semantic similarity score of interacting proteins is then calculated for each of these networks by averaging the scores of interacting proteins. To get the mean semantic similarity score of noninteracting proteins, we average the semantic similarity scores of protein pairs that do not interact. Last but not least, we compute the fold change to assess how well a technique separates the functional link between interacting proteins. The findings are shown, where we can observe how well the suggested measures worked. For instance, using the correlation metric, we get a

fold change of 6.15 for the Bio Grid network. The correlation measure's fold change for the DIP network. We get comparable outcomes for the cosine and Jaccard measurements. These facts lead us to hypothesize that the semantic similarity scores generated by the suggested metrics are in line with scientific understanding of how interacting proteins function. In a PPI network, proteins that are closer together likely to have more comparable activities, as has been shown. With this knowledge, we measure the network proximity of two proteins in a PPI network by measuring the length of the shortest path between them, their functional similarity is measured by the semantic similarity score of the two proteins, and the change in similarity score with protein proximity is plotted. The graphic shows that closer protein partners in the PPI network tend to have greater semantic similarity scores. The median semantic similarity score, for the Bio Grid network and the cosine measure, is 0.2590 for protein pairings that directly interact, 0.0720 for protein pairs that are intermediated by another protein, 0.0372 for protein pairs that are intermediated by two additional proteins, and so on. The outcomes for the other two metrics are comparable. These findings support biological knowledge by indicating that protein similarity scores and protein proximity in a PPI network are associated [9], [10].

CONCLUSION

In this research, we suggest a method for representing gene product annotations in the gene ontology by employing vectors made up of the information contents of ontology words. Based on this idea, we have suggested to quantify the relatedness of the respective vectors using three measures to determine pairwise semantic similarity between gene products. Using the biological process ontology and annotations of the budding yeast Saccharomyces cerevisiae, we conducted a number of thorough tests on the efficacy of the suggested interventions.

Comprehensive research on the connections between the semantic similarity of gene products and protein families, biochemical pathways, and protein-protein interaction networks demonstrates that semantic similarity scores derived using the proposed measures are more in line with biological understanding than those obtained using a list of five existing methods, indicating the effectiveness of our method in describing functional similarity between gene products based on the gene.

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CHAPTER 9

INTRODUCTION TO BRINGING FLUXOMIC AND INDUSTRIAL BIOTECHNOLOGY TOGETHER

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ABSTRACT:

A crucial method for identifying the final product of cellular metabolism and therefore identifying productivity constraints that are important to biotechnology is metabolic flux analysis. There are several potential uses for 13C-based metabolic flux analysis and flux balance analysis in biotechnology. However, there are still several significant obstacles to the research of fluxomic. The extent of fluxomic results and the usability of the discovered metabolic information are severely constrained by a number of technical issues in both 13C-MFA and FBA. Second, there are gaps between the findings of fluxomic research and those from other omics investigations, which makes it difficult to accurately forecast and analyses metabolic networks due to the complexity of metabolic regulation. Third, it is still challenging to get beyond innate metabolic resilience or to effectively import and express non-native pathways, even when metabolic bottlenecks or sources of host stress from product production have been discovered. Fourth, as the number of enzymatic steps rises, product yields often drop. This yield reduction may not be brought on by rate-limiting enzymes, but rather by the accumulation of each enzymatic process. Fifth, there hasn't been any progress in creating a high-throughput fluxomic tool for characterizing nonmodal microorganisms and maximizing their use in industrial biotechnology. Engineering highly efficient metabolic pathways in microbial hosts will become easier with the development of fluxomic methods and an awareness of these challenges.

KEYWORDS:

Biotechnology, Complexity, Fluxomic, Metabolic.

INTRODUCTION

Synthetic biology technologies have been used to manufacture a wide range of chemical molecules, from the antimalarial medicine artemisinin to the biofuel butanol. A systems-level comprehension of metabolism is necessary for the effective synthesis of natural or synthetic goods. To get a thorough understanding of how metabolic components are controlled, functional genomics technologies including genome sequencing, mRNA transcript profiling, and proteomics are often utilized. For physiological prediction and enzymatic rate quantification in metabolic networks, flux analysis has become crucial in contrast to conventional omics methods . The discovery of metabolic connections and the knowledge-based design of cellular activities are also made possible by this technique. As a consequence, this method may be used to analyses global physiological changes brought on by genetic mutations and to intelligently modify biological hosts [1], [2]. Using flux balance analysis , the cell-wide determination of intracellular metabolite turnover rates was first accomplished. The possible fluxes under a specific goal function, such as maximum biomass production, are constrained by this technique using the stoichiometry of the metabolic processes in addition to a number of physical, chemical, and biological features . As the number of constraints is less than the number of reactions in the metabolic network, FBA is an underdetermined model that might result in an inaccurate metabolic readout. Despite this drawback, the FBA framework offers a viable method for forecasting a broad range of biological metabolisms. A related strategy that enables exact estimates of metabolic state under a specific growth setting

is 13C-based metabolic flux analysis . Isotopic labelling, in which microorganisms are cultivated using a carbon source with a known distribution of 13C, is the key to 13C-MFA. One may quantitatively calculate intracellular fluxes by following the transition route of the labelled atoms between metabolites in the metabolic network. Flux analysis not only reveals new enzymes relevant for biotechnology applications but also offers genetic engineers' methods for "rationally optimizing" a biological system. Platforms for flux analysis are still not often set up in biotechnology firms, nevertheless. In order to close the gap between systems analysis of cellular metabolism and application in biotechnology, future research may be guided by this review paper's discussion of recent advancements and difficulties in the area of flux omics. The focus of FBA and 13C-MFA is on the stoichiometric characteristics of metabolic networks. Numerous factors, including cell growth rate, product output from various feedstocks, lethality of gene knockouts, and beneficial pathway alterations have all been predicted using FBA . A model like this may serve as a general guide for metabolic engineering, which is a good starting point for enhancing biosynthetic yield . The constraint-based reconstruction and analysis toolkit, which offers a generic framework for flux omics investigations, is the distinguishing feature of large-scale FBA [3], [4].

To increase the application of FBA, a variety of optimization methods and computational techniques for resolving in silico and in vivo discrepancies have been presented. For instance, including thermodynamic concepts into energy balance analysis may limit the solution space and provide fluxes that are both stoichiometrically and thermodynamically viable . Bilevel optimization may be used by FBA to determine the possible trade-off between biomass accumulation and the yield of a desired product in order to explain the "nonoptimal" metabolic behaviors. In order to solve fluxes in mutant strains, FBA may additionally loosen the goal function for biomass maximization and use a Minimization of Metabolic Adjustment Algorithm. By minimizing the difference between the wild-type flux distributions and the knockout-strain fluxes, such an algorithm determines fluxes. FBA may also be used with MPA, which analyses metabolic pathways. Without needing knowledge of reaction rates, MPA is a qualitative approach that analyses functional pathways present in a metabolic network . When MPA and FBA are combined, it is possible to quantify cellular metabolism, forecast phenotypes in response to genetic alterations, and quantitatively explore the likely pathways for ideal product synthesis . One key benefit of FBA is its capacity for genome-scale modelling, which connects genomic annotation with functional metabolic output . As a result, since 1999, the quantity of FBA models has grown dramatically.

By combining the isotopic labelling method with in silico computing, 13C-MFA attempts to accurately estimate pathway activity in intracellular metabolism. In order to perform 13C-MFA, microorganisms are given a carbon source that has been 13C-labeled. The enrichment pattern of the isotopies in metabolites, such as amino acids, is then measured. Isotopies data may be used to calculate the overall flux distributions because carbon fluxes across a metabolic network produce distinctive labelling patterns in metabolites. Recent articles have highlighted developments in 13C-MFA, including mass spectrometry-based metabolomics and isotopies modelling methods. In silico modelling is also made easier by freshly released open-source software. Web-based software for *E. coli* flux balance analysis, for instance, is called Web coli. Additionally, Open FLUX is an effective computational tool for 13C-MFA, using the elementary metabolite unit architecture for isotopies balancing calculations [5], [6]. This kind of user-friendly software enables scientists to carry out flux omics experiments with minimum programming experience. The usage of a metabolic network and the assumption of a constant metabolic state are two essential elements that both FBA and 13C-MFA methodologies share. The two methods serve distinct objectives, however. FBA analyses the "optimal" metabolism for the intended performance, whereas 13C-MFA quantifies the metabolic network's in vivo activity. When creating a sound metabolic

engineering plan, the two flow analysis methods work in concert. One may select the genes to be targeted for resolving biotechnologically important production bottlenecks by comparing the current metabolic fluxes, which were experimentally measured by 13C-MFA, to the ideal metabolisms predicted by both FBA and other "omics" technologies. It demonstrates how genetic engineering of microbial hosts and repeated flux analysis may eliminate hazardous byproducts and competitive pathways, increase genes encoding essential metabolites, and regulate energy metabolism.

Metabolic control and analysis of dynamic flux:

FBA and 13C-MFA ignore intracellular dynamics. By doing this, the challenges of creating kinetic models and carrying out intracellular experimental observations are avoided. Nevertheless, it's possible that throughout the fermentation process many biological systems won't maintain a significant metabolic steady state . Kinetic modelling and control theories are necessary for the description of metabolic disturbance and regulatory processes. To forecast the influence that various components would exert on the targeted pathways, metabolic control analysis is one method that does this. MCA may identify bottle-neck enzymes in a pathway and enable the examination of steady-state metabolism in reaction to changes in the cellular environment, despite the fact that MCA is not a quantitative assessment of flux . For the study of multienzyme systems and metabolic regulation, the cybernetic approach has also been proposed in addition to the MCA. The cybernetic method emphasizes microbial process dynamics and control during complex fermentations by combining both the enzyme kinetics in routes and the enzyme synthesis kinetics.

DISCUSSION

The simplified route network is the main emphasis of both MCA and the cybernetics method. The integration of kinetic modelling with FBA and 13C-MFA is important to carry out cell-wide quantitative investigation of a dynamic system. To highlight shifting global enzyme activity, the dynamic FBA method has been devised . For characterizing intracellular metabolism, debar may apply the Static Optimization Approach, which separates the time-course into several tiny intervals, as opposed to regular differential equations and dynamic optimization. Under the premise of rapid intracellular dynamics, a steady-state flow is computed for each time period. It is possible to reconstruct a debar model for genome-scale analysis of microbial metabolisms in industrial fermentations, where product synthesis is frequently under dynamic control, by fusing stoichiometric FBA for intracellular metabolism with dynamic mass balances on extracellular substrates and products. Dynamic metabolic flux analysis, or 13C-dMFA, has recently been established for isotopically nonstationary cultures. Two dilution factors were added to account for isotopic transients in order profile the flux distributions for fed-batch cultures. Another method for resolving intracellular fluxes involves abruptly increasing the amount of 13C in the substrate feed, followed by the measurement of time-course samples as the 13C translocate from the substrate into the metabolites. Based on the rates of isotopic enrichment multiplied by the concentrations of intracellular metabolites, the fluxes may be determined. For the flow study of photoautotrophic microorganisms, comparable ideas have been put forward and E. a brief isotope phase of coli . Exploratory and complex 13C-dMFA models must be employed to determine both metabolic and isotopic kinetics if the culture is in an isotopically and metabolically nonstationary condition . A collection of computational techniques has been created for tracking nonstationary isotopies labelling in response to in vivo flux patterns in order to effectively tackle the 13C-dMFA issue. Since such a method may greatly reduce the processing durations for tracing the labelling information, the EMU architecture has also been used in 13C-dMFA . By breaking up the growth period into short time intervals , the SOA must be used. Based on constraints from simultaneous isotopies analysis of the fast turnover metabolites, the "mini" quasi-steady state 13C-MFA can then be applied at each

time interval. It is possible to resolve the metabolic transients during the whole culture period by looking at flux profiles across all time scales. The 13C-MFA and FBA cell-wide flux omics methods have technical constraints. The number of restrictions in genome-scale FBA modelsnamely, the availability of quantitative metabolite data—is much less than the number of processes in the metabolic network. Such underdetermined systems are calculated using objective functions under the assumption that the metabolism maximizes the production of its native. For a number of reasons, this optimization concept has been contested. First, it seems that biological systems perform less than optimally in terms of growth . Second, 11 objective E functions were explored in a prior work. coli and discovered that no solitary objective function could accurately represent flow states across a range of growth circumstances . For instance, a nonlinear maximization of the ATP production per flux unit best describes unrestricted aerobic development on glucose, but biomass yield is preferred as the goal function in continuous cultures with limited food availability. Third, FBA cannot accurately characterize all natural cellular activities. For instance, under nutrient-sufficient and constant light circumstances, cyanobacterial species retain their circadian cycles [7], [8].

There are a number of obstacles in the way of using 13C-MFA in industrial biotechnology. Because existing methods are inadequate for assessing large-scale metabolic networks, constriction is most common. Two major issues are resolving large-scale nonlinear flow models and obtaining labelling information for free metabolites as opposed to amino acids. As a consequence, central metabolism is where the majority of collected flux information is focused. Since there have only been two reports of large-scale 13C-MFA , many of the fluxes in those publications cannot be exactly quantified . The necessary experimental methods and computational tools still need to be developed since the genome-scale 13C-MFA is still in its infancy . The fact that 13C-dMFA is yet underdeveloped for predicting dynamic metabolic behavior is a second problem. Rapid sampling and accurate measurements of metabolites at frequent intervals during the course of the culture period are challenging. For instance, to determine the absolute intracellular metabolite concentrations, cells must be grown in a medium that has been fully labelled with 13C. The labelled cells must then be extracted using a quenching solvent that contains known amounts of internal standards that are not labelled . A highly expensive LC-MS apparatus, fast metabolite extraction, and quick sampling are all necessary for this kind of measurement. The computational complexity is further increased by the time-dependent model's inclusion of ordinary differential equations . Third, flux determination presupposes that there are no transport restrictions across metabolite pools and that enzymatic processes are homogeneous inside the cell. Eukaryotes, on the other hand, contain organelles that could be able to channel metabolites or have diffusion restrictions . The acquired amino acid-based labelling information is further obscured by compartmentalization of amino acid production . In order to get comprehensive labelling information, reliable 13C-MFA for eukaryotes thus necessitates not only the integration of several analytical instruments, but also appropriate sample processing and extraction procedures.

A fourth issue is that several industrial hosts and the vast majority of environmental microorganisms are resistant to culture on minimum medium, and the addition of additional nutrient sources often makes flow assessments and measurements of metabolite labelling substantially more difficult. A microbial community also exhibits intricate metabolic connections among different species. Only a small number of FBA models have been created for community research so far . Because it is hard to completely separate and analyses the metabolites from a single species in a microbial community, it is practically impossible to decipher the exchange of metabolites across species using 13C-MFA. The discrepancy between the usefulness of flux omics in biotechnology and its technical limits in both FBA and MFA models is the result of these technological constraints.

It is preferable to combine the principles of systems biology with flux omics. To analyses the metabolism of *E. coli* mutants, for instance, 13C-MFA, enzyme activity tests, and RT-PCR analyses may be combined. E.'s comments were also noted. Multiple high-throughput "omics" techniques have been used to comprehensively evaluate how bacteria responds to genetic change . The findings reveal that, in response to genetic abnormalities, very little alterations in mRNA and proteins enable the cell to maintain a stable metabolic state despite fluctuating growth circumstances. It has been shown via research on Synchesis 6803 and other organisms that some enzymes' control is light-dependent. However, there are also a lot of additional regulatory systems that are unknown. Additionally, correlations between transcript/transduction levels and metabolic fluxes have effectively led to the identification of global regulators in industrial microbes . On the other hand, difficulties in conducting integrated "omics" investigations are also evident. The identification of functional regulators offers insight into the complete regulation in the metabolic network. The rational design of biological systems is complicated by our limited knowledge of metabolic regulation at various metabolic levels, which is a significant obstacle in industrial biotechnology. Integration of flux omics with other "omics" investigations, for instance, is very difficult because of posttranscriptional regulation.

Although it is generally known that for some pathways, transcript and protein data correlate very strongly, this correlation might be subpar in cell-wide analysis. Additionally, many *E. coli* enzyme activity or flux changes are not appropriately predicted by the majority of mRNA expression studies. coli routes. In investigations on the evolution of *E. coli*. The tricarboxylic acid cycle is shown to correlate well with molecular changes at the transcriptional level when *E. coli* is exposed to environmental perturbations, whereas flux variations in other key metabolic pathways seem to be unrelated to changes in the transcriptional network . Fluxomic and other "omics" research may have conflicting data due to the intricacy of regulatory mechanisms spanning several biological activities, which makes system-level analysis more difficult.FBA enables in silico metabolic modelling of "industrial workhorses," from which desirable strains or targeted mutations may be found. Under certain growth circumstances, 13C-MFA may evaluate the in vivo metabolism of modified strains and confirm the findings of FBA. Here, we include current FBA and 13C-MFA uses for industrial chassis as well as for nonmodal microorganisms.

Model of Escherichia coli:

In the fermentation business, coli is the species that is most often used. As early as the 1990s, bacterial flux models were published. The Liao group initially used metabolic pathway analysis for biotechnology applications to direct the genetic modification of *E. coli* strains and direct the metabolic fluxes from glucose to the aromatic amino acid pathway. The opt Knock toolkit has been incorporated by the Marinas group, and it has been used to create high-performance mutants. The generation of lactic acid, succinate, and 1,3-propanediol has enhanced because to the computer-aided designs . In metabolic networks where the deletion of many nonessential genes mutants may result in the organism's demise, FBA may forecast lethality. For instance, the Marinas group examined the essentiality of genes and reactions in an E genome-scale model. coli and systematically discovered probable pairs of synthetic lethal, non-essential genes that, if simultaneously knocked out, may be fatal. The development of poorly thought-out biological systems for biotechnology will be reduced if knowledge regarding synthetic lethality is included into the new model. FBA may also be used to identify the rate-limiting stages in the synthesis of a product. For instance, FBA identified gene targets, and altering those genes increased succinate generation by more than 10 times . The genetic overproduction of secondary metabolites such amino acids and lycopene has been addressed by Fatin addition to genetic approaches, FBA may provide helpful data for the creation of ideal fermentation conditions. For instance, an FBA model

was used to pinpoint nutritional restrictions during the generation of recombinant interleukin-2 in*E. coli*. In fed-batch fermentation, IL-2 production doubled with the addition of certain amino acids . Recently, a reactor-scale debar model for E analysis was created using a static optimization approach. The synthesis of a biopharmaceutical medicine using *E. coli*metabolism. A dynamic kinetic model that depicts the temporal development of fermentation process variables, such as biomass growth, glucose consumption, and product synthesis, is included into the debar. The optimization of fermentations at the size of a 1000L process was guided by a model of this kind. The 13C-MFA model was first used to research metabolic control in *E. coli* under various genetic and environmental circumstances. Several biotechnological processes employed in the creation of medicines, amino acids, and polymers have been examined using 13C-MFA. For the successful development of amorpha diene, a large-scale 13C-MFA comprising over 300 reactions was established by E. types of *E. coli*. A metabolic change in a lysine-producing E that is reliant on growth phase was discovered by another investigation. strain of coli . Measuring free metabolites allowed researchers to estimate metabolic fluxes throughout both the exponential growth phase and the stationary phase of the experiment, which was carried out in a fed-batch culture using rich media . Since numerous products are created during a nongrowth phase, metabolic study of the stationary period is crucial. In a third instance, a 1,3-propanediol-producing E. Fermentation of the *E. coli* strain was done in fed-batch fashion. The split ratio between glycolysis and the pentose-phosphate pathway decreased during the duration of the culture, according to the 13C-MFA data, in response to rising 1,3-propanediol fluxes.

B. For the industrial synthesis of vitamins, antibiotics, enzymes, and nucleosides, subtilis is the preferred industrial organism. For B, the FBA model. On the basis of a mix of genetic, biochemical, and physiological data, subtilis was created . Information from high-throughput phenotypic mutant screens, substrate utilization, gene essentiality, and sequence studies was used to repeatedly correct and enhance the FBA model. The B. Most studies on the subtilis flux model for riboflavin production concentrate on four areas: phenotyping wild-type and knockout strains, evaluating production potential, determining the effect of various carbon sources on biosynthesis, and assessing the cellular response to various culture conditions. The Sauer group has researched riboflavin-producing strains in great detail. In order to calculate growth maintenance coefficients, the maximum growth yield, and the precise rate of riboflavin generation in continuous culture, they initially employed an FBA model . Later, they used 13C-MFA on the same strain and discovered that genetic alterations should concentrate on the pathways that produce riboflavin and maintain the NADPH balance.

Another research on B. They investigated the metabolic flux patterns and maintenance energy of eight Bacillus strains and found that B. subtilis had the highest maintenance energy and various criteria for high-yield riboflavin synthesis . In addition, they discovered that using malate as a substrate resulted in a suppressed respiratory TCA cycle and an enhanced overflow metabolism, that the pentose precursors of riboflavin were primarily synthesized via the nonoxidative pentose-phosphate pathway, so any suggested genetic modification should decrease the activity of the oxidative pentose-phosphate pathway, and that licheniformis was the most suitable for industrial biotechnology. They recently created a 13C-dMFA model for B. subtilis to determine the metabolic response of excessive riboflavin synthesis in a fed-batch culture with glucose limitation . This dynamic flow study was created by observing variations in intracellular amino acid labelling patterns while assuming a metabolic pseudo steady state.

Saccharomyces is the strong eukaryotic chassis cerevisiae is employed for the expression of a variety of products. For instance, flow analysis identified target genes in the TCA and glyoxylate cycles as two natural routes for the overexpression of succinate . Another research shown that in silico driven metabolic engineering might increase sesquiterpene synthesis .

Flux analysis has also been used extensively to boost ethanol output. First, many methods for metabolically manipulating S's redox processes were created. cerevisiae, which led to an increase in ethanol production and a 40% drop in glycerol output under both glucose and xylose/glucose growth conditions . Second, Delicious et al. used a genome-scale FBA model to examine *S* mutants that were respiration-deficient. cerevisiae for the generation of ethanol. They discovered that several genetic modification techniques, such as the overexpression of the glutamate synthase gene, were ineffective in a metabolic context with impaired respiration. This suggests that following the first genetic alterations of the targeted genes, the rate-limiting stages for the generation of ethanol may change. Third, 14 hemiascomycetous yeast strains were tested for ethanol production using a 13C-MFA model . This research indicates that S. Cerevisiae has a strong NADPH-driven pentose-phosphate pathway, making it the perfect option for ethanol generation. Other 13C-MFA studies described the metabolic transition from oxidative to fermentative growth with the production of ethanol , looked into alternative carbon substrate metabolisms , identified crucial variables affecting biomass growth on xylose , and assessed how much ethanol and other storage carbohydrates were consumed in a glucose-limited chemostat culture .Additionally, a genome-scale FBA shows apparent enzyme dispensability, meaning that 80% of yeast genes seem to be unnecessary for viability in a lab setting . The 13C-MFA indicated a comparable impact of metabolic network resilience on null mutations, but the FBA highlighted the role of nonessential genes on metabolic robustness and environmental fitness owing to genetic buffering via alternative genes. For a genetic alteration to be effective and legitimate, it is crucial to understand the function of these redundant genes.

Fluxomic is a crucial method for the in-depth investigation of metabolism in lesser-known microorganisms, which offers fresh perspectives for using these species in biotechnology. As opposed to model microbial hosts, nonmodal microorganisms have not seen enough use of flux omics. It lists key publications in flux omics research on nonmodal species that may be helpful for synthetic biology. There are much less investigations on nonmodal microorganisms than there are in the area of fluxomic for industrial workhorses. The complex development circumstances, poorly known metabolic networks, and severe lack of genetic and molecular biology tools are all to blame for this. However, since they often have native metabolic routes for chemical synthesis or the capacity to utilize inexpensive substrates, nonmodal ambient microorganisms are equally significant for industrial biotechnology. Flux analysis may also be used to find new enzymes that can be cloned into industrial microorganisms to enhance their ability to synthesize products. For instance, 13C-MFA demonstrated a citramalate route for isoleucine biosynthesis that is distinct from the typical threonine ammonia-lyase system.

It is possible to design citramalate synthase into E, which has also been found in several environmental bacteria . coli for the manufacture of 1-propanol and 1-butanol. The novel method, which is the shortest keto-acid-mediated process and avoids threonine production, increased biofuel output 9 to 22-fold . High-throughput genome sequencing techniques are now mapping new microorganisms' genomes at a rate that is significantly faster than the rate at which these species' functions are being characterized.

Therefore, to find novel enzymes in non-model microorganisms and maximize their use in industrial biotechnology, a high throughput 13C-MFA approach is needed. Identifying constraints for industrial biotechnology and assisting in the development of logical engineering solutions are two of fluxomic' key objectives. Simple metabolic measures, however, are insufficient to meet the unforeseen demands of industrial biotechnology. Because metabolic control is so intricate, systems biology methods cannot provide a comprehensive plan for synthetic biology [9], [10].

CONCLUSION

In conclusion, fluxomic research makes it possible to measure intracellular metabolism. It is still challenging to identify cell-wide route bottlenecks and provide workable solutions for biotechnology applications since this tool is not yet completely developed. The potential applications of fluxomic in industrial biotechnology have been severely constrained by several technological challenges in creating flux analysis tools and complex metabolic regulating systems. In order to overcome obstacles in the fluxomic areas, it will be important for the future development of flux analysis to merge other cutting-edge "omics" analysis and molecular biology approaches.

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CHAPTER 10

WHATMAN PAPER SAMPLES' STRUCTURE AND PROPERTIES AFFECTED BY VARIOUS ARTIFICIAL AGING

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ABSTRACT:

The natural ageing of Whatman paper samples was artificially accelerated in a climate chamber, thermal oxidation in air at constant temperature, photo-oxidation under a Xenon arc lamp, and chemical oxidation with sodium metaperiodate with the goal of controlling cellulose degradation phenomena. In addition to thermogravimetric, mechanical, and optical examinations, the degradation of cellulose was investigated using viscos metric, FTIR, wide angle X-ray scattering, and viscos metric methods. All of the applied treatments were found to considerably alter the structure and characteristics of the paper, with the degree of deteriorating effects varying depending on the kind of ageing. Nevertheless, direct relationships were examined between the degree of cellulose molecular breakdown, the generation of carboxyl and/or carbonyl groups, and the break strength of the paper.

KEYWORDS:

Carboxy, Nevertheless, Oxidation, X-Ray Scattering.

INTRODUCTION

One of the earliest and most popular man-made materials is paper. With a minor quantity of organic, inorganic, and maybe color additives, it is mostly constituted of cellulose and water. While only cellulose fibers are found in today's high-quality machine-made sheets and previous hand-made papers, low-quality paper has cellulose embedded in a matrix of hemicellulose and lignin. These four processes photodegradation, acid hydrolysis, oxidation, and biodegradation cause the most significant changes in cellulose-based materials. A complete investigation of the substance cellulose is fairly complicated since these phenomena are all interconnected. Numerous oxidizing chemicals have the potential to cause cellulose to oxidize . Cellulose may be oxidized by molecular oxygen, and alkaline conditions and high temperatures considerably speed up the attack rate. All types of oxidative damage cause the cellulose structure to change, the cellulose chain to shorten, and the cellulose fibers to deteriorate. It is widely known that extended exposure to light may harm cellulosic materials. The observable effects include some discoloration and an overall decline in strength. There are two different processes to take into account: in the first, light energy is the main cause of cellulose degradation, while in the second, the kind of impurities, as well as the presence of moisture and oxygen, all contribute to the assault. The chemical linkages in the cellulose chain break as a result of exposure to high-energy UV light . Last but not least, photosensitization is another deteriorating phenomenon linked to exposure to light [1], [2].

The study of cellulose degradation mechanisms is currently of great scientific interest in order to gain a deeper understanding of its degradation phenomena with the ultimate aim of improving the current methods of conservation and restoration of cultural heritage made up of cellulose-based materials.Starting with this necessity, accelerated ageing cycles were set up to replicate the various deterioration processes that ancient paper objects went through, with the express goal of providing paper samples with regulated degradation levels, appropriate for use as model samples. In specifically, Whatman paper samples were aged artificially in this study using thermal oxidation, photo-oxidation under a Xenon arc lamp,

accelerated ageing in a climate chamber, and chemical oxidation. By measuring the cellulose intrinsic viscosity, the degree of crystallinity of the cellulose by wide angle X-ray scattering, the mechanical properties, the activation energy for degradation by thermogravimetric analysis, and the optical properties of the Whatman paper samples before and after each ageing procedure, the degradation level of cellulose for each ageing procedure was assessed.

Whatman paper, which has a weight per square meter of 75 gr/m^2, was used for the study. Four separate accelerated ageing cycles were set up in order to simulate the various degradation processes that naturally take place in cellulosic materials and provide model samples with regulated deterioration levels. Thermal oxidation was performed on Whatman sample pieces for 500 hours at 100°C in an oven without light. It is well knowledge that heat oxidation causes cellulose to shed its chemical bonds and produce carbonyl, carboxyl, and hydroperoxide groups. Whatman samples were stored in a Climatic Chamber Angel Antoni Challenge 250E under UV illumination for 500 hours at a temperature of 60°C and a relative humidity of 70% to accelerate ageing. Free radicals are produced when cellulose is exposed to UV light, which also starts a number of chemical processes such depolymerization, dehydrogenation, dihydroxylation, and DE hydromethylation that result in the creation of hydrogen, carbon monoxide, and carbon dioxide. Chemical oxidation: samples from the Whatman method were exposed for two hours to a sodium metaperiodate 0.1 M solution. A very precise process known as metaperiodate oxidation transforms two 2,3-dihydroxyl groups into two aldehyde groups without causing any noticeable adverse effects. Whatman samples were subjected for 500 hours to a Xenon light source in an Angel Antoni SB3000E solar box for photo-oxidation. The radiation was maintained at a temperature of 65°C and a constant power of 1000 W/m2. Increased carboxyl, hydroperoxide, and carbonyl content are the results of photo-oxidative processes [3], [4].

DISCUSSION

The degree of molecular breakdown of the cellulose that makes up Whatman paper was assessed using a viscos metric approach that was more advanced than the UNI 8282 standard. In actuality, the flow durations for cellulose solutions in CED at various dilutions were measured, yielding Dev. values for the cellulose that were more precise than those obtained by strictly following the UNI 8282. Before and after the various ageing methods, the Dev. values for cellulose isolated from paper samples were examined-FTIR, short for Attenuated Total Reflection Fourier Transform Infrared Analysis Total reflection with attenuation on both young and old Whatman samples, Fourier transform infrared spectroscopy was carried out. The spectra were obtained utilizing a single-reflection micro-ATR accessory and diamond ATR element with a Jasco FT/IR 6300 spectrometer. 4 cm1 of spectral resolution. In order to remove any remaining dampness, the paper samples were first dried in a desiccator under vacuum for 24 hours at room temperature. The WAXS technique unaged and aged Whatman materials, WAXS analysis was performed using a Pert PRO Analytical diffractometer were subjected to uniaxial tensile tests using an Instron 5564 tensile testing machine running at a cross-head speed of 10 mm/min at a temperature of 25°C and 50% relative humidity. The samples underwent a 24-hour conditioning period at 25°C in a vacuum before testing. Ten test pieces were used for each sample, both aged and unaged, and the results were averaged. Using a Perkin Elmer diamond thermogravimetric/differential thermal analyzer, thermogravimetric analysis was used to evaluate the degradation of the paper samples. In order to determine the thermal stability of the cellulosic materials in relation to the various ageing processes, in terms of the kinetic parameter, measurements were made on 10 mg paper samples in an aluminum holder at different heating rates, from 30°C to 650°C.The device was calibrated using a white reference tile. Using the equivalent beginning material as a guide, the following equation was used to determine the color differences: It should be mentioned that the kind of anti-aging therapy used significantly affects how much

of a drop is really detected. The least amount of molecular breakdown is seen in thermally oxidized samples, which also have a Dev. value that is quite close to that of freshly printed paper. On the other hand, the photo-oxidized samples show the lowest Dev. value, which is almost six times lower than that of the unaged sample. Additionally, compared to climatic chamber ageing, chemical oxidation produces consequences that are more severe. These findings point to a classification of the treatment's ability to degrade materials: starting with samples that were aged thermally, moving on to samples that were aged in climate chambers, then to samples that were aged chemically, and finally ending with samples that had been photo-oxidized, which had the shortest cellulose chains. It confirms that the photo-oxidation is the most potent and, hence, harmful method for causing cellulose depolymerization among the ageing treatments put up [5], [6].

It contrasts the ATR-FTIR spectra of Whatman paper that has not yet agedwith those of Whatman papers that have undergone various weathering processes, including thermal degradation, accelerated ageing in a climate chamber, chemical degradation, and photo-oxidation. The strong affinity of paper for water makes FTIR analysis of paper samples challenging in general. The band of the absorbed and bound water is really in the carbonyl group area , and sometimes it may be quite wide and obscure the bands of the carbonyl groups. Despite this, it is easy to see how various ageing techniques may cause distinct oxidation processes on paper products from the range 1500-1800 cm. For the aged samples, it is really conceivable to see an appearance of a shoulder with a Centre point of roughly 1730 cm1 in this range because of the carboxyl and/or carbonyl groups. It is interesting to note that the intensity of this shoulder varies depending on the type of weathering treatment: it becomes a distinct band as the degree of cellulose depolymerization increases, or as the Dev. value reported in it decreases, as in the case of the photo-oxidized Whatman .In addition to the dissolution of chemical bonds between monomeric glucose units, cellulose thermal degradation is predicted to result in a reduction in the degree of polymerization and the generation of free radicals, carbonyl, carboxyl, and hydroperoxide groups. However, despite the fact that the thermal ageing procedure results in a certain degree of molecular degradation of the samples, as seen when comparing the Dev. values in, the FTIR measurement results, as shown in, reveal that the spectrum of Whatman paper samples that have undergone thermal ageing did not differ noticeably from that of unaged paper samples.

Free radicals are created as a consequence of cellulose photo-aging, and chemical processes including depolymerization, dehydrogenation, dihydroxylation, and hydromethylation are also started. Hydrogen, carbon monoxide, and dioxide are also produced. According to the carboxyl and/or carbonyl groups, the FTIR spectra of Whatman paper samples aged in a climate chamber and photo-oxidized exhibit, respectively, a shoulder and a distinct band centered at around 1730 cm1. With its lowest Dev. value determined through viscos metric analysis and a noticeable increase in the absorption band's intensities around 1730 cm1, the photo oxidized Whatman sample's FTIR spectrum suggests that chain scission is the primary mechanism responsible for the photodegradation processes that occur on paper samples exposed to Xenon light. It has been documented in the literature that when cellulose is chemically oxidized with a solution of sodium metaperiodate, a particular oxidizing agent, the C2-C3 bond is broken and a 2,3-dialdehyde is produced using the Milligrade reaction's mechanism without causing any significant side effects. Furthermore, periodate oxycellulose is widely recognized to have two distinctive FTIR bands at around 1740 and 880 cm1. While the band at approximately 880 cm1 is often attributed to the creation of hemiacetal bonds between the aldehyde groups and neighboring hydroxyl groups, the absorbance at about 1740 cm1 is typical of carbonyl groups. According to information in the literature, aldehydes in non-hydrated form are present because the ATR-FTIR spectra of periodate-oxidized Whatman paper and 2 show a shoulder centered at about 1730 cm1 that can be attributed to the carbonyl group's C=O stretching. displays the WAXS intensity profiles of Whatman

paper samples before and after the fake ageing processes. As seen, all analyzed samples display the cellulose I or "Native" spectrum with the distinctive reflections, and; the first two are highly convoluted each other, in the range between 13 and 18 degrees of 2, and the third, almost entirely resolved very sharp at 22.8 degrees of 2. These findings show that no matter how much ageing is done, the cellulose's crystalline structure remains unchanged, and all of the old samples still include partly crystalline microfibrils that are metastable [7], [8].Together with the 1/ 0 values for the 002-reflection used as a relative index to assess cellulose crystal size, the crystallinity indices obtained for unaged and aged paper samples are provided in. The kind of anti-aging therapy has an effect on the Xu values, as indicated. It's interesting to note that the crystallinity index values for the thermally and photo-oxidized samples are both 74%, which is close to the value for the unaged paper, whereas the Xu values for the Whatman samples aged in a climate chamber and chemical oxidation are, respectively, higher and lower than those for the unaged samples. According to these results, chain length and crystallinity are not directly correlated with one another. Particularly for paper samples aged in climate chambers, the decline in the amorphous proportion may be attributed to recrystallization and/or crystal thickening events brought on by annealing that took place at a steady temperature. The paper sample immersed in a sodium metaperiodate solution had decreased crystallinity, indicating that the crystalline areas of the cellulose were readily oxidized by the periodate. demonstrates that the cellulose crystal dimensions are consistent with Xu values and are not significantly impacted by ageing.

The usual stress-strain curves for Whatman paper dumbbell-shaped specimens, both unaged and aged, obtained at room temperature are presented. The findings of the mechanical tensile tests conducted on each sample under investigation are summarized. The Young's modulus value for the unaged paper sample is significantly lower than that for the Whatman paper sample aged in a climatic chamber and photo-oxidized, with the Whatman paper sample aged in a climatic chamber exhibiting the highest modulus, with a value that is roughly twice that of the unaged paper sample. While chemically deteriorated paper samples demonstrate a somewhat lower tensile modulus than thermally aged paper samples, both exhibit a modulus similar to that of unaged paper. The modulus performances for climatically aged and chemically deteriorated materials must be correlated with the crystallinity values listed. According to a preliminary approximation, the change in modulus as a function of crystallinity is influenced by both super molecular organization and crystal building. Therefore, it is important to emphasize the following for the aged paper samples that were examined: increasing crystallinity leads to improved modulus through a reduction in tie molecule density, and vice versa. For samples that have been thermally and photo-oxidized, it is also necessary to consider the cellulose dehydration process, which occurs at 100°C and 65°C, respectively, and results in an increase in the sample stiffness. The strain values tend to decrease with increasing the level of molecular degradation, that is, with decreasing Dev. values and with enhancing the presence of carboxyl and/or carb. Furthermore, note that the strain at maximum load value, which corresponds to strain at break, decreases for all aged paper sample with respect to the unaged sample. The activation energy for the degradation processes included in one-step analysis were measured using thermogravimetric analysis. The kinetic parameter E A was estimated for all the cellulosic materials before and after the various weathering processes. Tests were conducted at various heating rates, from 30°C to 650°C, in a nitrogen environment. It reports typical degradation curves recorded for an unaged Whatman sample by TGA at various heating rates. It is clear that the degradation process may be roughly described as a single step of weight loss. For each sample, the first derivative of the curves was determined.

We conducted a colorimetric examination on Whatman samples that were both young and old. The "E" value, which is used to quantify optical characteristics, is an indication of how chromatically altered paper materials are. For the samples under investigation, the histogram

of "E" values vs ageing. illustrates how the Whatman paper undergoes a severe chromatic modification when aged intimate chamber and exposed to photo-oxidation, demonstrating how exposure to UV and Xenon light in the presence of moisture encourages the synthesis of chromophores. Additionally, the data show that thermal ageing causes a chromatic change that is somewhat less severe than that caused by chemical oxidation. A new shoulder with a relative maximum at 1730 cm1 has appeared in the FTIR spectra, which is consistent with the previously discussed FTIR results. This suggests that the extent of chromatic alteration increases as carboxyl and/or carbonyl compound formation increases. The discovery that paper samples aged in climatic chamber exhibit chromatic alteration relatively higher than that displayed by the photo-oxidized samples, despite a lower presence of carbonyl/carboxyl compounds, may be explained by the presence of moisture, which also contributes to the formation of chromophores containing degradation products [9], [10].

CONCLUSION

The cellulose degradation was examined using viscos metric, FTIR, WAXS, and TGA techniques, as well as mechanical and colorimetric analyses, with the goal of determining the physicochemical, mechanical, and aesthetical alterations caused by four different artificial ageing treatments applied to Whatman paper samples. It was discovered that every accelerated ageing procedure set up considerably alters the Whatman paper's structure and characteristics, with the severity of the deteriorating effects varying depending on the kind of treatment used. Therefore, the following must be considered in order to avoid paper from being exposed to various environmental conditions. Despite thermally aged samples showing a modest amount of molecular depolymerization and carbonyl/carboxyl production, cellulose dehydration simply caused by temperature results in a reduction in strain at break and subsequently paper stiffness. The simultaneous impacts of temperature and light result in significant molecular degradation and a rise in the presence of carboxyl and/or carbonyl groups. Similar results were obtained using samples of Whatman paper that had been chemically aged with sodium metaperiodate.

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CHAPTER 11

THE EVALUATION OF PCR KITS FOR ERYTHROCYTIC PARASITE DETECTION ON FILTER PAPER

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ABSTRACT:

It was thought that dried blood spot-based PCR was a practical and affordable way to find pathogens in blood. For a precise diagnosis, the DBS carrier filter paper and PCR kits are essential. For DBS samples, we assessed 20 PCR kits and 4 different kinds of filter sheets. According to the findings of the PCR for Plasmodium, the lowest detection limit of the four filter sheets was 1 102 parasites/L, and the positive rates of the 20 PCR kits varied from 0% to 100%. In both fresh pet DBS samples and archival DBS samples, PCR findings for the detection of Plasmodium falciparum and Plasmodium vivax and Babesia Gibson were good. Our findings served as a helpful guide for the identification of blood pathogens using DBS samples and direct PCR, particularly for evaluating the cost-effectiveness of using filter paper and a PCR kit in places with low resources.

KEYWORDS:

Babesia, Gibson, Pathogens, Plasmodium.

INTRODUCTION

The two main intraerythrocytic parasites that cause human and animal illness, namely malaria and babesiosis, are Plasmodium and Babesia . Both pose a risk to life. In order to treat patients and stop the spread of these vector-borne illnesses, rapid and precise disease identification is essential. The most used approach for diagnosing blood parasitic infection in laboratories is microscopy, although low parasitemia infection might result in incorrect diagnosis. Because of their physical resemblance, Plasmodium and Babesia species may sometimes be difficult to distinguish. More critically, Babesia may result in failed therapy with antimalarial medications because it is readily mistaken with early-stage Plasmodium . Although PCR-based molecular diagnostic procedures are crucial adjunct techniques for precise diagnosis, they are often impractical in locations with little resources owing to equipment costs and limitations. Dried blood spot samples have been utilized in the clinical diagnostic analysis of blood tests since the 1960s, primarily for the screening of genetic abnormalities and newborn metabolic disorders. Numerous DBS-based experimental techniques, including those for analyzing lipids, nucleic acids, and other chemicals, have been effectively established [1], [2]. In the screening of infectious illnesses including HIV, HBV, CMV, and others, qualitative and quantitative detection based on DBS PCR has been extensively employed. In particular for Plasmodium infection, it is crucial for the identification of parasite infections in the blood. The best method for storing and transporting blood samples from the field location to the laboratory for centralized testing was to collect entire blood on filter paper as dried blood spot samples. The caliber of filter paper is especially significant as a sample instrument for PCR molecular diagnostics and monitoring. Whatman 903 filter paper is one of several varieties of filter paper that has FDA clearance and is frequently used. At the moment, 903 filter paper is offered as a very pricey ready-to-use sample card. Whatman CF12 filter paper is a less expensive kind of square sheet filter paper that is presently offered by its new producer Civita as one of 903 Protein saver cards. In China, CF 12 filter paper is branded as 903 filter paper and is accessible everywhere. However, it was uncommon to compare the CF12 and other filter papers' physical attributes

and molecular detection capabilities. There is now a demand for effective and practical nucleic acid collection and detection technologies in the field to help with the quick control of numerous infectious illnesses. The current PCR technique has a lengthy detection time and needs nucleic acid extraction from materials like blood and throat swabs before testing, which extends the entire diagnostic time. Additionally, nucleic acid extraction and detection are expensive, and some developing nations lack the necessary tools and lab facilities for nucleic acid extraction. Therefore, the creation of quick detection techniques was critical. One of the most popular quick molecular detection methods is direct PCR. Prior to 1993, direct PCR was used in microbiology, an area in which sample preparation is fairly straightforward . Colony PCR is a common quick approach for screening colonies, as an example. The majority of direct PCRs rely on the use of a genetically altered DNA polymerase, which allows for the elimination of the need for nucleic acid extraction prior to PCR amplification, saving both resources and time. Additionally, it lessens the chance of human mistake and sample contamination . High standards are set for polymerase, not only in terms of its tolerance to unwanted substances but also in terms of its compatibility with the PCR reaction's buffer. Blood and other unprocessed materials include strong PCR inhibitors that might lead to erroneous negative PCR findings. Hemoglobin, immunoglobulin G, and lactoferrin are three of the most significant inhibitory components found in blood that have been characterized. Direct PCR based on blood samples collected on filter paper may be less effective and less useful due to the mechanism, which is connected to the inactivation or inhibition of Taq DNA polymerase [3], [4].

We bought four different kinds of filter sheets that were offered for sale in mainland China and assessed them based on their weight, thickness, blood absorption capability, positive rates, and cost. Twenty commercially available PCR kits were chosen and evaluated utilizing DBS samples, mostly for direct PCR of blood samples. The purpose of this study is to assess the cost-effectiveness of DNA polymerases that were appropriate for blood infection molecular detection in DBS samples. The Taobao marketplace in China was used to buy Whatman,Advantech Jaiman Filter Paper, and Gel Blot Paper. Additional provides further information. The Creation of DBS Filter Paper Samples, Section Plasmodium parasites present Take blood that has been anticoagulated from mice that have P. yeti infection. By tenfold serial dilution with anticoagulated whole blood, blood samples containing and 105 parasites parasitemia were produced. To create DBS samples, blood was absorbed by four different kinds of filter sheets; each spot has 50 L of blood. It depicts the schematic for preparing a blood spot. Plasmodium falciparum 3D7 dried blood spots: by tenfold serial dilution, blood samples were obtained at four levels of parasite density and absorbed on filter samples were set up on a spotless bench, where they dried for 48 hours. In a sealed plastic bag with silica gel desiccant, the filter papers were kept dry.

Self-Made Blood Collection Card:

The homemade blood collection cards are constructed of an enclosed sample filter, an upper and lower cover, and CF12 filter paper. It was set up vertically to preserve the DBS samples during transit and to dry the blood samples after collection. Each DBS sample card was separately wrapped in a plastic bag with a silica gel desiccant and kept at room temperature.

Our lab has built up a collection of malaria samples gathered over many years of study. Blood samples infected with Plasmodium vivax were collected and held as preserved DBS samples at 20°C in Wuhu County, China, between 2009 and 2014. Between 2010 and 2013, DBS samples of P. falciparum-infected blood were obtained from imported patients, the majority of which originated from African nations. They were then kept at room temperature. Three animal hospitals—in Bengbu, Changsha, and Nanjingreceived homemade blood collection cards. Dogs were used to gather DBS samples. DBS samples were returned to our lab for Babesia infection testing after fully drying. One PCR reaction tube was filled with two

pieces of dried blood spots that had been punched with a 1.5 mm puncher. 70 L of sterilized ultrapure water was added to each tube, and the reaction tubes were then incubated at 50°C for 5 min, 21°C for 15 s, 50°C for 1.5 min, and 21°C for 15 s to eliminate the hemoglobin in the sample and dust floating on the top of the blood spots. The tubes' supernatant was aspirated and thrown away after that [5], [6].

DISCUSSION

For the first round of amplification, genus-specific primers rPLU6 and rPLU5 were utilised, and the amplified products were used for the second round of amplification using primers that were species-specific for P. falciparum and P. vivax. 18S rRNA was the Babesia Gibsonia target gene for detection. The Supplementary displays the sequences of all the primers used in this study. All 20 DNA polymerases were bought from Logic Technology Co. Ltd. in Hefei, China; Supplementary details the purchases. All reagent components were added to each PCR in accordance with the directions on the packaging. The specific programmed for several PCRs are presented in Supplementary.

The extension periods are selected according to the predicted fragment length and the enzyme elongation speed, and the annealing temperatures are set according to the Tm values of each primer pair. PCR was performed using an S1000 Thermal Cycler for each and every reaction. The thickness, weight, and blood absorption capacity of the four different kinds of filter papers that are sold on the mainland Chinese market were compared. The results demonstrated that utilizing laboratory mice blood and healthy human blood resulted in changes in the absorption time and blood spot diameter. CF12 and 545 filter sheets only need 3-5 seconds for the absorption of 50 L whole blood, which is much less time than the other two filter papers. More consistent blood spots and less leakage were produced by the CF12 and 545 filter sheets . While no such diffusion or leakage happened in the other three filter papers, the Sangun Gel Blot paper was unable to fully absorb 50 L of blood in a short amount of time, and the blood sample was seen to spread unevenly in the filter paper. Direct PCR Positive Rates and Detection Limits on Four Types of Filters Paper

The detection limit for Plasmodium was established using a high-fidelity polymerase based PCR on the dried blood spots of four filter sheets. A 134 bp segment of the P. yoelii 17XNL 18srRNA gene served as the detection target. According to the findings, all four filter sheets had a minimum detection limit of 1 102 parasites/L.20 commercial DNA polymerases for PCR detection of erythrocytic parasites in the DBS sample were obtained in order to compare the effectiveness of various filter sheets for DBS-based PCR. The DBS sample was utilised to amplify a 1,003 bp fragment of P. yeti using the Plasmodium genus-specific primer rPLU6/5. According to the findings, the positive rates for the four filter papers varied from 56.7% to 63.3%, and the chi-square test did not identify any differences between the papers that were statistically significant. The Results of 20 DNA Polymerases for Plasmodium Gene Detection in DBS, Section Serial diluted P. yeti 17XNL DBS samples on four filter sheets were examined to compare the performance of 20 polymerases. According to the findings , the positive rates varied from 0% to 100%. On four different kinds of filter sheets, five PCR kits can identify all Plasmodium samples with varying parasite concentrations.

Cost-effective DNA polymerases must be screened, therefore both unit price and positive rate must be taken into account. The cost-effective parameters were developed according to the difficulty of amplification: 1 point for positive results of 103 parasites, 10 points for 102 parasites, and 100 points for 101 parasites. As is well known, the less template DNA in samples, the more difficult to amplify. For the next studies, eight DNA polymerases with positive rates ranging from 80% to 100% were chosen. Performance Evaluation of DNA Polymerases for P. falciparum Detection, Section 3.5DBS samples were made using CF12 filter paper, and blood samples of cultivated P. falciparum were serially tenfold diluted with

normal mouse blood. utilizing nested PCR methods, P. falciparum was tested for utilizing eight chosen DNA polymerases. The second-round primers, were P. falciparum-specific, whereas the first-round primers, rPLU5/6, were genus-specific. The template DNA for the second round of PCR was one microliter of first-round PCR output. The outcomes demonstrated that the following five DNA polymerases were better for P. falciparum, with all positive rates at 100%: Bey time Hemostat, NEB Hamo Klen Taq, Mighty Amp, and Sangun Direct PCR Kit. These five DNA polymerases were chosen for later clinical sample detection [7], [8].

Human Malaria Parasites in DBS Sample Direct PCR Results Forty-one human malaria-positive DBS samples were recovered from our laboratory archive and were examined by direct PCR using five reasonably priced polymerases. The first filter sheets that were used to preserve blood samples weren't all created equal; some of them were clearly thinner or thicker, and some of them were made with an FTA card. It was also unclear how many parasites were present in each DBS sample. These samples included 20 P. vivax and 21 P. falciparum. The findings shown that the three polymerases Mighty Amp, and Sangun Direct PCR Kit can identify both malaria parasites with 100% positive rates. The locations where these samples were collected and the outcomes of the five DNA polymerases' amplification are shown in Supplementary.DBS-Sample-Based Direct PCR for Babesia Detection in Dog Blood, Version. Three local veterinary institutions collected dog blood using homemade CF12 blood collection cards to see if DBS direct PCR is effective at identifying Babesia in canine blood. The batch test was conducted on DBS samples that were returned to our lab. After receiving the samples, only the Mugham kit was used to perform the direct PCR test. Two positive samples were found in the Bengbu dog blood DBS samples, according to the findings. The two dogs' Babesia Gibsonia infections were verified using sequencing of the PCR product and Giemsa's stain. The thorough results. Many tropical nations may not be able to satisfy the minimal storage requirements for blood samples for laboratory diagnosis due to a lack of resources. According to several investigations, a wide range of compounds were stable in DBS and could be kept for longer periods of time than using conventional techniques. In places with low resources and in populations where venous blood collection, storage, and transportation were challenging, DBS was thought to provide an alternative to blood samples for the detection of antibodies or nucleic acid. The composition of the filter papers might, however, vary, and their compatibility with essential reagents and unequal performance could potentially result in inconsistent findings of a DBS-based test. This research assessed the efficacy of four commercially available filter sheets at absorbing blood, pricing, and appropriateness for nucleic acid detection using 20 DNA polymerases. Although easier to prepare and had superior blood absorption qualities, the Whatman CF12 and Advantech 545 filter papers were quite pricey. The nucleic acid detection performance comparison revealed no appreciable differences between the four filter sheets in terms of positivity and detection limits. Additionally, the results from the archived DBS samples for P. falciparum created with various filter papers demonstrated that DBS samples made with considerably thinner filter paper may still be successfully identified by direct PCR. The findings of this experiment imply that, in the absence of high-quality filter paper, ordinary laboratory filter paper might potentially facilitate nucleic acid detection. Commercial kits can extract the nucleic acids from samples with better purity, but they need a larger volume of eluent and the final concentration may be fairly low. Two pieces of punched filter paper were put inside the PCR tube during the reaction to carry out the amplification in this experiment. A moderate and straightforward wash process was used to treat the punctured DBS sample, which may assist improve outcomes, lower costs, and save time by removing partial inhibitors in the blood and retaining enough nucleic acids for effective detection in the sample.

Since PCR inhibitors are known to be present in blood, reagent requirements for direct PCR of blood samples were high, particularly for polymerases. In this study, 20 commercially available DNA polymerases for DBS direct PCR were assessed for efficiency and cost. This method eliminates the requirement for DNA extraction, streamlines the procedures involved in processing samples, and lessens the possibility of contamination during the extraction process. In filter paper samples with high parasite densities , the majority of DNA polymerases were able to identify blood parasites, and several of them reached their minimal detection limits when the parasite density decreased.

Five polymerases out of the total examined performed better and were more resistant to inhibitors in the blood: Bey time Hemostat, NEB hem Klen Taq, Mighty Amp and Sangun Direct Kit had 100% positive rates for both human malaria tests. These experimental results on archived human malaria DBS samples also revealed that. The cost each test must be considered in order to do extensive screening. The approach utilised in this research may be used as a helpful reference for areas with limited resources to assess kits that were accessible locally and to assist large-scale screening of pathogens by scoring the PCR findings to compare the cost-efficiency of direct PCR reagents. We strictly adhered to the product's instructions while using the PCR reaction apparatus. In reality, the performance of polymerases may be somewhat enhanced after optimizing the direct PCR settings for each primer and template.

This study suggests that a straightforward PCR nucleic acid detection method based on filter paper and direct PCR kits with high sensitivity is suitable for detecting pathogen genes in the blood, especially in epidemic areas with limited resources. It also offers an affordable and practical choice for large-scale screening of human and animal infectious disease by collecting filter paper blood samples in field sites and transporting them back to laboratories that are outfitted with the necessary [9], [10].

CONCLUSION

The conceptualization, methodology, writing, editing, validation, and resources were contributed by Zhi-Yong Tao and Qiang Fang; the data acquisition, methodology, writing, data curation, and visualization were contributed by Pei-Yi Zhang; the sample processing and data acquisition were contributed by Lu Zhang, Chun-Cao Li, and Rui Hu; the sample collection and resources were contributed by Han-woo Zhu, Bei Zhou, Kai Wu, Ling-xu Li, and Da- Pei-yi Zhang and Zhi-Yong Tao both contributed equally. The authors express their gratitude to the Bengbu Pet Clinic team for helping with sample collection. The Bengbu Medical College undergraduate students who took part in the Innovation Training Programmed for caring for the lab animals are also acknowledged by the writers. Grants from the National and Anhui Provincial College Student's Innovation and Entrepreneurship Training Programmed, the Scientific Innovation Project for Graduate Students of Bengbu Medical College, the Key Programmed and the Major Project of Natural Science Project of Anhui Higher Education Institutions and the Key Programmed and the Major Project of Natural Science Project of Anhui Higher Education Institutions.

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CHAPTER 12

A NEW SUSTAINABLE APPROACH TO CONTROL DENGUE INFECTION: GREEN NANO-BIOTECHNOLOGY

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ABSTRACT:

Dengue is an expanding virus spread by mosquitoes that is present in 128 countries and poses a serious risk of illness to 3.9 billion people worldwide. Since there is no particular therapy for dengue, eliminating the vector Aedes aegypti is the only strategy to reduce the risk of infection. For the control of dengue fever, nanotechnology-based techniques such as biopesticides with Nano formulation are now gaining popularity. Potential uses for metal nanoparticles produced using an environmentally benign method using plant extracts have been suggested as an anti-dengue treatment. Metal NPs may be made sustainably in a quick, inexpensive, and waste-free manner. We are reviewing the literature and mechanistic aspects of the dengue control utilizing green-synthesized NPs in light of the current advancements in Phyto-synthesized multifunctional metal NPs for anti-dengue applications. The molecular underpinnings of viral suppression by NPs as well as the nontarget effects or risks with regard to environmental integrity are thoroughly examined. The green production of silver and gold nanoparticles has received the majority of attention up to this point; nevertheless, additional creative composite nanomaterials need to be added. To critically assess the molecular insights gained during the synthesis of the biogenic NPs, more in-depth mechanistic investigations are needed. The toxicological properties of NPs and their long-term effects on the environment should also be carefully examined.

KEYWORDS:

Holistic Methods, Infections, Molecular, Mechanistic.

INTRODUCTION

Holistic methods are required in the era of reemerging and reemerging infections, resistant bugs, lethal malignancies, and neglected tropical illnesses like dengue. Given that mosquitoes act as a vector for illnesses like yellow fever, malaria, filariasis, dengue, etc. that may be fatal, mosquito-borne diseases have gained enormous relevance in this respect. Due to its life-threatening nature, significant disease burden, climatic conditions, vector expansion, urbanization, and other socio-demographic factors, dengue fever has drawn the attention of researchers, epidemiologists, health, and social workers. Aedes albopictus has exposed billions of people to the danger of contracting the dengue virus, posing a particular hazard to the tropical and subtropical areas. The dengue virus is spread by the Aedes aegypti mosquito. An estimated 50 to 100 million cases of the illness are recorded each year. The real incidence of dengue is further estimated to be over 390 million, with 96 million symptomatic infections and 25,000 projected yearly fatalities. Currently, dengue is prevalent in 128 nations. The issue is made worse by the resistant dengue strains, which are thought to be the main factor in the widespread dissemination. The primary factor for the spread of dengue infections and its effects on human health is the emergence of dengue virus resistant strains. There are four main serotypes of the dengue virus, designated DENV 1-4, and there are significant genotypic differences between each serotype. The dengue virus' fifth serotype was also discovered recently. Similar symptoms are seen in infections caused by all serotypes. When a patient recovers from one specific serotype, a lifetime immunity is conferred; however, the recovered patient is not shielded against a subsequent infection with a different serotype.

More severe instances, such as dengue shock syndrome and dengue hemorrhagic fever, may result from the secondary infection. DSS and DHF are brought on by antibody-mediated disease enhancement, which may be brought on by an earlier illness or by a vaccination. The only available treatments for dengue illness are supportive care and symptomatic relief. Therefore, prevention of dengue fever requires early diagnosis and effective vector management.

Despite extensive research on antiviral medications or moieties, there has as of yet been little progress against the DENV, and most of the time, infected people only get symptomatic care. For children older than 9 years, the WHO now only recommends one dengue vaccination. The immunizations are only advised for dengue seropositive patients, and the vaccine is only used in nations with a zero-prevalence of the virus higher than 70%. To create synthetic chemical entities that can block the virus, much study is needed. E-gene, NS-1 gene, and NS-3 gene are all thought to be potential therapeutic targets. According to earlier research, bromocriptine has the ability to fight viruses by preventing their reproduction. In clinical studies, other medications such faldaprevir, chloroquine, prednisolone, and closer did not provide any notable outcomes. Other medications including ribavirin, ketotifen, and ivermectin are also undergoing clinical studies. The hunt for anti-dengue phytochemicals that may aid in dengue management has been a top priority for other researchers. Scientists are searching for innovative treatments, antiviral medications, and nanotechnology-based breakthroughs due to the incidence of dengue disease. This work intends to enhance researchers' understanding of the usage of natural products-mediated synthesis of biogenic NPs and their potential significance in the control of dengue infection and biogenic NP anti-dengue processes [1], [2].

The dengue virus is a member of the Flaviviridae family and is around 50 nm in size. Ten proteins make up the dengue virus, of which three are structural proteins and seven are nonstructural proteins. These nonstructural proteins are crucial for the virus's ability to evade the immune system, replicate, and assemble. Since nonstructural proteins like NS-1, NS-3, and NS-5 are absolutely necessary for the development of viral particles, they also provide a design opportunity for potent antiviral medications. For the poor world, dengue prevalence is a serious issue that highlights the urgent need for cutting-edge methods of treating the illness or reducing its spread. New anti-dengue medications are required in addition to those that target the viral phases of the virus via transcription or protease activity. Fusion and entrance inhibitors are both promising strategies for limiting dengue entry into the target cell, suppressing the virus's proliferation, and neutralizing it. The many vector control methods that are now on hand are divided into four categories: physical control using GIS mapping to find dengue foci, efficient monitoring, identification of oviposition locations, and community-driven control initiatives. Insecticides, plant-derived compounds, insect growth regulators, and the "attract and kill" method using pheromones are examples of chemical control strategies, while biological control includes Para transgenesis, vector genetic modifications, sterile insects' techniques, and use of crustaceans and larvivores fish.

Others include using vaccinations as part of immunotherapy methods. These strategies include biological, chemical, and environmental techniques to stop the development and expansion of Aedes aegypti, the insect that serves as the dengue virus's vector. In undeveloped nations, vector management is more difficult because of a lack of knowledge, inadequate sanitation practices, and other socioeconomic factors. Worldwide, effective and efficient chemical or biological vector control techniques are utilized. However, substances like synthetic lead have a significant negative influence on public health and contribute to the development of resistance in certain mosquito species. We need very effective eco-friendly mosquito vector control methods. Typically, organophosphates and other growth inhibitors target mosquitoes. To reduce the transmission, bed netting and indoor spraying are utilized.

In vector control programmed, phytochemicals are seen as an alternative to synthetic pesticides because of their powerful coquinoidal and insecticidal properties. Larvicidal, poricidal, and adulticidal characteristics define these plant-derived bioactive substances.

Additionally, both naturally occurring and man-made compounds have been shown to affect mosquito oviposition behavior, have ovicidal characteristics, or may serve as mosquito repellents. In order to stop DENV from spreading to humans, other genetic techniques have also been suggested by scientists. This is accomplished by introducing the genes in the vector that are in charge of disease resistance. To transmit disease-resistant genes into mosquitoes, one of the endosymbiotic bacteria is widely utilized. An Aedes aegypti transfected line with Wolbachia showed that DENV was suppressed by raising basal immunity in the insect, which inhibited transmission. Female A. aegypti mosquitoes that have had Wolbachia transfected have an extra reproductive advantage over uninfected mosquitoes. Other researchers have attempted to abbreviate the lifetime of the mosquito using the Wolbachia strain, in an effort to lessen the burden of vector-borne illnesses conveyed by A. aegypti. However, these genetic techniques are rudimentary and mostly effective in the lab; for them to be implemented in the real world, further study and knowledge of the underlying processes are needed [3], [4].

DISCUSSION

Nanotechnology may now be used in a cost-effective, ecologically friendly, and compatible manner thanks to the successful capture and manipulation of nanomaterials utilizing resources that are benign for the environment, such as plant extracts or chemical entities produced from them. Treatment of plant extracts with various metal salt mixtures results in the reduction of metal salt, which is followed by capping and stabilization of NPs. Exciting findings for many applications in health-hygiene, nanomedicine, environmental protection, and industry have been produced by the confluence of nanotechnology and biotechnology. These applications have made it possible for nanobiology or nanotechnology to take shape. Due to their distinct surface area to volume ratio, metal NPs including silver, gold, zinc, etc., are known to have multifunctional capabilities. Numerous physical, chemical, or biological procedures may be used to build these NPs. Chemical techniques may produce hazardous wastes, whilst physical means are often characterized by significant energy inputs making the total process costly. Recently, it has been shown that medicinal plants are effective against a number of illnesses, including cancer, infectious diseases, diabetes, and neurological problems. By interfering with the genome or by preventing the entrance of viral particles, they prevent the dengue virus from replicating.

Destabilizations of NS proteins results in the anti-dengue effect. It has been observed that natural compounds derived from plants may halt viral replication either by interfering with the enzymes, such as inhibiting polymerases, binding with glycoproteins, or by blocking the RNA synthesis pathway. Due to the heterotypic dengue infections, no such medicines have been licensed despite progress in screening putative inhibitors [5], [6].The biological approaches that utilize extracts from medicinal plants as an eco-friendly, straightforward, and affordable way of constructing nanomaterials or composite nanomaterials are the subject of a sizable amount of study nowadays. Other biological forms, such as microbes, may also be used to synthesize metal NPs, although they have additional needs such maintaining sterile conditions for the growth. Plants, on the other hand, don't need costly care and are simple to manage. NPs may be reduced and stabilized by phytochemicals. These biogenic NPs have tremendous biological potential in addition to their industrial uses.

The biogenic NPs may be employed to combat the dengue virus and regulate its vectors, according to convergent experimental findings. The Phyto-fabricated NPs provide a fantastic chance to stop the dengue virus. summarizes the plant employed, the kind of metal

nanoparticles used, their size, and their role in vector control. It also provides a full overview of the literature. The phytogenic silver NPs' anti-dengue impact against DENV-2 has only been partially studied. Recently, the potential for using green-synthesized NPs in the battle against dengue has come to light. One study examined the production of silver nanoparticles from Bruguier cylindrica Blume and assessed their impact on the dengue virus as well as the vector's toxicity. Intriguingly, dengue virus E-gene expression was shown to be downregulated after treatment with silver nanoparticles. The western blot and RT-PCR validated these findings. It was discovered that a dose-dependent down-regulation of the viral E-gene caused a considerable decrease in envelope proteins compared to the control. At 30 gill 1, a significant downregulation was seen. The A. aegypti larvae and pupae were shown to be poisonous to the synthesized silver NPs. Similar conclusions are reached for the silver NPs synthesized by Moringa oleifera for anti-dengue applications.

The Viral E-protein was significantly reduced when silver nanoparticles from Sonner Atia alba Sm. were examined at concentrations between 5 and 15 g/mL/1, suggesting a possible anti-dengue action. The aforementioned results support the theory that silver NPs' inhibition of the E gene and consequent decrease in the number of ineffective units may be the cause of the decrease in the production of E protein. When evaluated at 50 mg/ml, silver nanoparticles made by Centromeres clavula tum Montagne revealed no toxicity that is relevant to Vero cells, but more than 80% of the DEN-2 virus growth was inhibited. These research, which might investigate strategies for the manufacture of innovative and safer nano medicines generating NPs with distinct properties, have made clear the value of screening various biosynthetic processes. Because diverse pathways usually lead us to several distinct properties of NPs and characteristics of biological toxicity, available research demonstrate the significance of screening various plants that serve as sources of reducing molecules for nano synthesis.

In conclusion, our investigations provide a great and real potential for screening significant plant species for NP biosynthesis with anti-dengue applications. Due to a lack of available literature, it is also necessary to perform investigations on the anti-dengue capabilities of NPs other than silver made from medicinal plants. Because of the risks to the environment and the eradication of nontarget species, the use of synthetic pesticides for possible vector control is undesirable. In addition, the realization that these synthetic chemicals may not be trustworthy in the long run has been brought on by environmental challenges, health issues, and developing insect resistance to pesticides. If these insecticides are handled carelessly, they pose an immediate threat to human health. Estimates indicate that synthetic pesticides cause 222,000 fatalities and 3 million poisoning incidents each year. Similar to this, the escape of pesticide residues and their buildup in the food chain provide an unanticipated risk. Thankfully, because of their effective insecticidal nature, mobility, solubility, and durability, nanotechnology-based therapies have become a potential and alternative supply of pesticides. Green-synthesized NPs have a bright future, and new vector control methods are now possible. It is generally known that several arthropod pests and vectors, particularly mosquitoes, are harmful to them. There is a sizable body of research on the toxicity of biogenic NPs on mosquitoes, but little is known about the particular mechanistic details. Investigating the toxicological effects resulting from the usage of NPs as pesticides requires a thorough understanding of the underlying process.

Some stress responses brought on by NPs may be connected to the harmful impact of NPs. Although the specific process is not fully known, scientific research has shown that NPs may induce morphological changes as lateral hair loss and damaged gills and brushes. Given that larvae only have gills for breathing throughout the larval stages, this may have an impact on their respiratory activity. As NPs readily pass through the membrane at the cellular level, substantial membrane breakdown is seen. NPs may build up in the midgut, which might

reduce the abdomen and harm the epithelium or cortex. Another theory for the origin of NPs-mediated insecticidal action is trypsin enzyme activity inhibition. Because it controls the production of a second gene, the late trypsin gene, this digesting protease's activity is connected to the signal transduction system. The mosquito's ability to analyses the quality of the food and alter the late trypsin levels for a specific meal with amazing flexibility is made possible by the existence of two trypsin. When trypsin activation is stopped, feeding activity is disrupted and meal quality cannot be determined.

Because of their small size, NPs are also toxic because they can easily penetrate the cuticle, act on epidermal cells, and prevent the production of enzymes required for tanning and cuticle oxidation. This ultimately affects the entire mounting process. As an alternative, they may suppress neurosecretory cells, which would cause cuticular shrinking. Some NPs are also linked to the disruption of the muscle layers that results in the loss of the difference between the endocuticle and exocuticle, which makes the insect inactive. The body wall desiccation, de-pigmentation, abrasion, spiracle obstruction, and insect dehydration caused by NPs binding to the cuticle may lead to the insect's eventual demise. This aspect makes it possible to use NPs against pupae and early instars, stopping their maturation into adults and making them effective larvicidal agents. The activity of acetylcholinesterase by NPs has been reported to be interrupted by authors [7], [8]. Acetylcholine is a substance that is involved in the transmission of nerve impulses from neuron to nerve cell or involuntarily contracted muscles, and acetylcholinesterase controls this action. The disruption of nerve impulse transmission across cholinergic synapses caused by the NPs' interference with Ache has been documented. This may thus be helpful to evaluate the probable neurotoxic potential of certain NPs. Also documented are hormonal abnormalities in insects that show themselves as NPs.

Additionally, cytochrome P450, which is involved in insects mounting, is said to be interfered with by NPs. A critical impact on reproduction and development is also reported, where Gonadotropin production is downregulated resulting in reduced fitness and reproductive failure. Reduced female fertility is observed as NPs disrupt the oogenesis process and ovaries become defective, having a negative effect on egg laying capabilities. Moreover, NPs damage the organism by penetrating through the exoskeleton, enter in the intracellular space, and then the nanoscale material binds to sulfur from proteins or to phosphorus from DNA which leads to the rapid denaturation of organelles and enzymes. Due to the decrease in membrane permeability and disturbance in proton motive force, loss of cellular function, and cell death occur. At the cellular level, NPs can penetrate the cytosol and interrupt the cellular signaling pathways, causing disruption in ion exchange and neuromuscular coordination. Even though there are numerous examples of the toxicity of NPs, it is challenging to compare results and pinpoint the exact mechanism by which these particles harm living things. This is because different experimental designs were used with NPs of various sizes, coatings, concentrations, times of exposure, measured endpoints, and cell types. In particular, antioxidant defense system activation/inhibition such as glutathione depletion, lipid peroxidation and DNA damage, decreased mitochondrial activity, inflammatory processes, and apoptosis in a wide variety of cell types have been observed and implicated in the cause of oxidative stress.

Converging evidence suggests an inverse correlation between the size of NP and their toxicity and penetration into the body of insects. Despite a number of pieces of evidences, there is a dire need to conduct extensive studies on the effects of the biogenic metal NPs on insects with reference to their physicochemical nature like size, shape, charge, etc. Moreover, the present body of literature only indicates silver and gold NPs for their anti-parasitic properties and applications in entomology. Research can be extended to other metal NPs of composite nanomaterial’s biosynthesized from medicinal plants. PS: nanoparticles, high resolution transmission electron microscopy, transmission electron microscopy, dynamic

light scattering, Fourier transform infrared, scanning electron microscope, energy dispersive X-ray analysis, UV-visible spectroscopy, and field emission scanning electron microscope.

Nanoparticles Enhances Predation Efficiency:

Biological control of dengue vectors seems another probable solution. The prospective biological control of dengue vectors can be performed using natural predators like fish, young instar tadpoles, copepods, and water bugs. Fishes were predominantly considered for biological control of mosquitoes. Places that have the possibility to breed mosquitoes such as dams, marshes, canals, ponds, etc., were inundated with numerous predatory fishes. The cyclopoids are also reported to be among the efficient predators of the larvae of the mosquito involved in the spread of dengue. Copepods represent another economical and cost-effective biological control of Culicidae larvae in urban and semiurban areas. The most effective agents of copepods that control mosquitoes biologically are Mesocycles, i.e., Mesocycles piriformis, Mesocycles longissimus, Mesocycles anagenesis, and Mesocycles thermocyclopoides. Recently, the effect of NPs on the predation behavior of these natural predators has been studied. The striking findings are the increase in predation efficiency. It has been clearly demonstrated that the rate of predatory activity rises up administering NPs; however, the underlying exact mechanism is yet to be explored. The efforts, however, have been made to investigate the nontarget effects of NPs towards predatory copepods are somewhat limited [9], [10].

CONCLUSION

The green synthesis process, which is sustainable and eco-friendly, is unique in the synthesis of metal nanoparticles. Based on the information now available, it can be said that biogenic nanoparticles hold great promise for addressing today's most important healthcare issues, such as reducing dengue infections. The dengue virus is now viewed as a global concern that needs to be controlled using creative methods. Interventions based on nano-biotechnology may be useful in lowering the burden of disease in a sustainable and affordable way. Through either direct or indirect interaction with the vector, biological nanoparticles can lessen the spread of the dengue virus. The possibility that biogenic NPs could have an anti-dengue effect by interfering with and downregulating the crucial structural genes required for viral assembly has been supported by a number of studies. These biogenic NPs have also successfully proved their ability to regulate vectors, which is evidenced by their biocidal character. Application-wise, the manufacturing of these biogenic NPs is free of any potentially harmful compounds, requires no particular energy, and has a simple scale-up potential. Implementing these nano-biotechnology-based solutions on the ground presents a problem.

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