ADVANCES AND CHALLENGES IN BIOTECHNOLOGICAL RESEARCH

Abstract

Biotechnology is one of the rapidly emerging fields of science and the improvements of this technology unfolded many novel research opportunities to develop agriculture, environmental, pharmaceutical, and food industries. This chapter describes several possibilities of biotechnology and reviews its various areas together with their associated issues and challenges. Considering several advances in molecular biology, rDNA technology, genetic engineering, and other biotechnological methods, the novel opportunities and applications of these techniques in various fields like microbial, plant, animal, and medicinal biotechnology are analyzed. The distinct role of biotechnology in the future is emphasized considering the prospects to contribute with new solutions and directions to improve microbes, plants, and animals in developing agriculture, pharmaceutical, and food industries. More advances in these techniques would help overcome the challenges and further help our understanding of biological processes to increase their efficiency and productivity in the coming future.

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I. INTRODUCTION

Science is defined as the quest for knowledge and understanding of the natural and social world and its applications with a subsequent systematic methodology based on evidence. Science is massive with numerous branches including formal sciences, natural sciences, social sciences, etc. Natural Sciences describe, comprehend, and predict natural occurrences by scrutinizing the physical world. Natural sciences include physical and biological sciences. Biological sciences encompass a broad area of subjects that primarily deals with the aspects of life, living organisms, and their metabolisms which let us understand the living world and the way many species functions, interact, and evolve.

Biology, the study of life was far older than civilization. Indeed, the life of primitive humans was exceedingly dependent on a keen understanding of different animals and plants around them for better survival. They started understanding the behavior of different animals, differentiating poisonous and non-poisonous plants, predicting the nature of animals for hunting, and grasping the knowledge of crop cultivation. However, the first major turning point in biological knowledge came about 10,000 years ago with the Neolithic Revolution. Humans initially domesticated plants for farming, then livestock animals to complement the resulting sedentary societies. Later, in about 3000 to 1200 BCE, ancient Egyptians started analyzing biological sciences, in 1500 BCE, Ayurveda the study and applications of medicinal plants originated in India. Ancient Greek philosopher, Aristotle (384–322 BC) made tremendous progress in understanding the natural world. His exceptional research, observations, and writings on life sciences recognised him as The Father of Biology.

The actual research in biological sciences started in the 17^{th} and 18^{th} centuries from the establishment of basic taxonomy by Carl Linnaeus to William Harvey's tremendous observation of blood, veins & arteries and the discovery of a simple microscope by Antonie van Leeuwenhoek. In the later years, the research in biology swiftly increased in all fields. As mentioned earlier, biological sciences are a vast subject. Botany, Zoology, Microbiology, Genetics, Biotechnology, biochemistry, and many more branches are included under this subject. Research in life sciences is like a deep-down ocean, a vast area to cover with a lot of scopes in each and every field. Biotechnology is one such field of life sciences which is a combination of natural sciences and engineering sciences, to modify or improve living organisms or their products for human benefit [1]. Biotechnology can be considered a group of effective technologies with wide and diverse applications in industry, commerce, and the environment [2]. Biotechnology wasn't a new-found branch, it has its roots in the distant past since nearly 2000 B. C. from the time of the fermentation of beer, wine, and manufacturing of bread and cheese when our ancestors were successful in creating the techniques to manufacture products, but the molecular mechanism underneath went unknown. Soon after the advancement in the fields of biochemistry, microbiology, and molecular biology, the mechanism beneath these processes are well understood substantially leading to the improvement in biotechnology.

II. BRIEF INTRODUCTION TO BIOTECHNOLOGY

Biotechnology is one of the briskly developing fields of science with great research opportunities. Any technique or technology that involves the modification of living cells or organisms or their by-products to improve the outcome relative to human benefits will fall

under biotechnology. Biotechnology is not a single, narrow discipline of study. Instead, it is an extensive field that utterly relies on the contribution of various areas of biology, chemistry, engineering, and many more. Genetic engineering, rDNA technology, plant and animal breeding techniques, bioremediation, etc. all are parts of biotechnology [1].

Based on the type of organisms involved or the subject of interest, biotechnology is of several types like microbial biotechnology, Plant biotechnology, animal biotechnology, forensic biotechnology, medical biotechnology, aquatic biotechnology, environmental biotechnology, and some other types. Each and every fields of biotechnology have an astounding role in developing and improving the living world.

III. RESEARCH IN MICROBIAL BIOTECHNOLOGY

The organisms that are too minute to be seen with the naked eye and can be visualized generally with the help of a microscope are called microorganisms. Bacteria, fungi, protozoans, few of the members of algae and viruses fall under this category. Microbes are one of the most crucial organisms in the living world. They make the most essential elements of oxygen, carbon, nitrogen, and sulfur available for other organisms. Both plants and animals are highly associated with microbial communities for most of their activities like nutrient uptake, protection against other pathogens or diseases or even producing essential vitamins [3]. Microorganisms also produce several essential (primary) and non-essential (secondary) metabolites.

The branch of biotechnology which involves modifying or manipulating microorganisms to produce valuable products for various applications is termed microbial biotechnology. It is an exceptionally dynamic and exciting sector of biological sciences with its unique and diversified utility in countless areas like disease prevention and therapy, diagnostics, agriculture and food industry, nutrition, horticulture, energy production, production of secondary metabolites and other materials, water and waste treatment, recycling, forensics, sustainable practices, etc. [4]. Microorganisms have the feasibility and sustainability to produce large quantities of secondary metabolites by large-scale cultivation at a reasonable cost.

A. Antibiotic Production

Genetic engineering techniques are used to modify microorganisms to increase the production of secondary metabolites. Antibiotics are one of the most vital secondary metabolites that are vastly produced by genetically modified microbes. Since 1940, the discovery and invention of new and potent antibiotic molecules with splendid use in medicine, agriculture, and basic research have increased drastically. The improvement in antibiotic production is reflected in the total amounts of antibiotics manufactured from fermentation products across the world, which was recorded as 100,000 tons, including 60,000 tons of penicillins, 5,500 tons of tetracyclines and 2,500 tons of cephalosporins. Marine fungi are also a potent source of antibiotics. Biotechnology has massive potential for sustainable production of antibiotics from marine fungi, initiating from methods that help to expand and understand the chemistry behind the compound, via classical full fermentative and semi-synthetic processes to manipulating the genetic background via genetic engineering as a basis for the generation of essential derivatives [5].

Mass production of antibiotics can be achieved by several biotechnological approaches like targeted stimulation of strains to expand chemo diversity. More tactical approaches would include targeted mixed fermentations based on genetic and ecological knowledge as seen in food biotechnology to improve enzyme production. These approaches of ecologically or genetically based biotechnology have vast scope and the full potential needs to be proven in the future [5]. Recent advancements in multi-omics opened a new path for microbial biotechnologists to understand the conditions required to induce the expression of the full biosynthetic potential of an organism. The knowledge of genomics to metabolomics contributes greatly to understanding the underlying regulatory processes in cells of microbes thereby improving metabolic engineering strategies [6]. This approach can be considered as one of the major directions for future research.

B. Fermentation Biotechnology

Fermentation is a vital microbial process that produces many food products including bread, beer, wine, yogurts, cheese, and curd. Fermentation microbes have a potent role in biotechnology and the food industry. Fermentation is one of the earliest biotechnological applications used in producing beer and wine. Many chemicals like lactic acid, acetic acid, citric acid, ethanol, methanol, etc. are produced in large quantities by microbial fermentation strategies. Fermenting yeast (*Saccharomyces cerevisiae)* is a widely used microbe for alcohol fermentation. By monitoring fermentation rates, winemakers can control the alcohol content of brewing wine until it reaches the desired content and flavor. Lactic acid fermenting bacteria is used to produce processed curd, yogurts, sour cream, and cheese.

Apart from the food and chemical industry, fermentation is now widely used in the production of biological products such as amino acids, enzymes, antibiotics, etc. L- glutamic acid one of the major commercial amino acids is produced by fermentation using various species of the genera *Corynebacterium* (e.g., *C. glutamicum*) and *Brevibacterium* (e.g., *B. flavum* and *B. lactofermentum*). Approximately 1.2 billion pounds of monosodium glutamate are made annually by fermentation. Genetic engineering strategies are used to increase the production of amino acids in microbes by modifying or removing the feedback-inhibiting gene or protein. The best-known example of this strategy is the production of L- Lysine from various mutants of *C. glutamicum* wherein there is only a single aspartate kinase, which is regulated via concerted feedback inhibition by threonine and lysine. By the genetic removal of homoserine dehydrogenase, a glutamate-producing wild-type *Corynebacterium* is converted into a lysine-overproducing mutant that grows only in the presence of methionine and threonine in the medium [7].

In recent years, fermentation biotechnology took a step forward to produce biofuels after the surge of oil and fuel prices and the drastic increase in global warming. Brazil is one of the pioneer countries to start the production of biofuels like bioethanol, and now at least 25% of Gasoline in Brazil contains anhydrous ethanol [8]. Bioethanol fermentation is by far the largest-scale microbial procedure. Sugar cane molasses or enzymatically hydrolyzed corn starch/grain starch is used for industrial bioethanol production by batch fermentation with yeast (*Saccharomyces cerevisiae*). Bacteria are indeed very capable microbes performing fermentation of all sugars in cellulosic biomass. *Zymomonas mobilis* is a notable species in bacterial ethanol fermentation and the source of the enzymes for the metabolic engineering of other bacteria used in ethanol production [9]. However, *Z. mobilis* is limited to catabolizing only sucrose, glucose, and fructose and produces considerable amounts of the by-products

like sorbitol, acetoin, glycerol, and acetic acid. Thus, the foremost challenge for microbial biotechnologists is to find a novel process to produce bioethanol from microbes that are feasible with diverse substrates and with a reduced amount of by-product formation. The thermophilic bacterium *Clostridium thermocellum* is another widely used bacteria for bioethanol production. It will readily hydrolyze cellulosic biomass and will degrade hemicellulose and cellulose, but the strains are sensitive to higher ethanol concentrations and yield a range of less favorable by-products [9]. This can be overcome by metabolic engineering and strain development of *C. thermocellum* for ethanol production and its full potential must be proven in the future.

C. Production of Therapeutics

The rapid increase in the emergence of new cancers and malignancies across the globe created an urge to discover novel therapeutic agents like anti-tumor and anti-cancer drugs to reduce the incidence of cancers. Initially, antitumor drugs were developed based on the cytotoxic effect of chemical agents on tumor cells. However, in the past two decades, there was a noticeable change in the drug discovery approaches after the development of biochemical and biotechnological processes. New therapeutic agents were being discovered from several chemical and natural products including microorganisms. The great biodiversity of microorganisms signifies an exceptional possibility for producing secondary metabolites with antitumor properties. Approaching and exploring these microorganisms is made easier by the recent advancements in biotechnology. The future of biotechnology in developing antitumor and anti-cancer drugs looks quite promising with the development of genetically modified microbes, especially bacteria, and gene-directed enzymes to deliver potent drugs by targeting tumor cells and destroying them. Tumor-amplified protein expression therapy using genetically modified bacteria as a drug delivery vector to solid tumors like brain tumors is currently under study and this would be one of the leading approaches to cure tumors in the future. Few of microbial toxins have antitumor properties and they can be produced in bulk amounts by fermentation techniques by enhancing the production of desired product [10].

Challenges in Microbial Biotechnology

- **Strain Selection:** The first challenge is to identify suitable microbial strains that can produce the desired antibiotics, organic acids, enzymes, biofuels, and therapeutics. This involves screening a large number of microbial species to identify the most efficient ones. The selection of the appropriate microbial strain for the production of the therapeutic product is crucial. The chosen strain must have the necessary genetic traits to produce the desired product, and must also be safe for human use.
- **Optimization of Production Conditions:** Once suitable strains have been identified, the next challenge is to optimize the fermentation conditions for maximum antibiotic production. The optimization of production conditions, including culture medium, temperature, pH, and aeration, is critical for maximizing product yield. However, finding the optimal conditions can be time-consuming and require extensive experimentation.
- **Contamination Control:** Contamination by other microorganisms can significantly affect the quality and yield of the final product. Therefore, strict contamination control measures are essential to ensure product purity.

- **Purification:** The purification of final products from complex mixtures can be a challenging and expensive process. Some products require extensive purification steps to remove impurities and ensure safety and efficacy.
- **Quality Control:** Quality control is critical in the production of therapeutics. Rigorous testing and monitoring of the final product are required to ensure the safety, efficacy, and consistency of the product.
- **Regulatory Approval:** The production of therapeutics is subject to strict regulatory approval. Compliance with regulations related to safety, efficacy, and environmental impact is crucial.
- **Cost-Effectiveness:** The cost of producing therapeutics and other metabolic by-products using microbial biotechnology can be high due to the cost of raw materials, equipment, and personnel. Strategies to reduce production costs while maintaining quality are essential to ensure the availability and affordability of these products.

IV.RESEARCH IN PLANT BIOTECHNOLOGY

Plant biotechnology is one of the most widely developing fields of science with its applications in the agriculture, food, and drug industries. In the recent past, the increase in crop cultivation and food production became an urge, to compensate for the rapidly growing population across the globe. One of the problems with traditional agriculture and crop cultivation is the need for large farmlands and suitable growth conditions for healthy crops. This problem can be addressed with tissue culture, micropropagation, somatic hybridization, transgenic plant techniques, etc. which all fall under plant biotechnology. To produce high-yielding crops with desired traits like drought and disease resistance, selective breeding techniques are used. However, the selective breeding method is a tedious, time-consuming process. The emerging technologies in plant sciences led to the merging of classic breeding with modern plant biotechnology to overcome these problems and to improve the quality and quantity of yield. The potential to improve the productivity of plants and their applications in agriculture and the crop industry relies largely on newly developed DNA biotechnology and molecular markers. These techniques permit the selection of successful genotypes, better isolation and cloning of favorable traits, and the development of transgenic plants for agriculture [11]. At present, plant biotechnological techniques are under investigation in the medicinal industry to produce plant-based therapeutics like plantibodies, plant-made vaccines, etc.

A. Tissue culture and Micropropagation

Tissue culture can be defined as the culturing of cells, tissues, or organs of an organism in-vitro in an artificial medium provided with the required nutrients for its normal growth in optimal and aseptic conditions. Plant tissue culture is not a recent discovery, the science of plant tissue culture takes its roots almost a century ago in 1902 when the first attempt to grow plant cells *in-vitro* began by Gottlieb Haberlandt, a German physiologist. Ever since then, the progress in tissue culture led to grow various plant cells *in vitro* and this was made possible because of the totipotent nature of plant cells. Plant cells can grow *in vitro* and can develop into tissues and organs if it was provided with specific nutrient media under an aseptic and controlled environment. Plant tissue culture has a great impact on both agriculture & industry and made a significant contribution to the advancement of agricultural science by not just producing plants needed to meet the ever-increasing world demand but

also as a technique to develop plants with desired traits. Currently, the plant tissue culture industry is a billion-dollar industry producing 500 million to 1 billion plantlets annually [12].

Tissue culture allows the production and propagation of genetically identical, disease-free plants. Development of clones via tissue culture will avoid unwanted mixing up of genotypes. At the same time, it is also possible to induce somaclonal variations in plant cells by somatic hybridization methods. Traditional propagation allows intergenic and intragenic crosses but relatively at a low propagation rate [13]. Nevertheless, protoplast fusion methods can facilitate the production of genetic variability even in vegetatively propagated plants. It produces rare hybrids which are rich in quality, food, and economic products. Genetically variable plants developed by tissue culture can obtain species with new stable genotypes. Inter-generic crosses, classic breeding, or hybridization methods often produce plants with infertile seeds which cannot be used further, and these plants can be recovered by mature and/or immature zygotic embryo cultures. Genetic engineering can develop improved crop varieties with high yields as well as disease and/or pest resistance [14]. The need for a huge farm area can be cut down by micropropagation techniques, which facilitate the growth, storage, and maintenance of a large number of plants in a small area.
Major challenges associated with tissue culture and micropropagation would include:

- **Genetic Stability:** Maintaining genetic stability is critical in tissue culture and micropropagation. Genetic mutations and instability can result in reduced plant quality and yield. Therefore, proper culture and propagation protocols must be established to maintain genetic stability.
- **Cost-Effectiveness:** Tissue culture and micropropagation can be expensive due to the cost of materials and personnel. Strategies to reduce costs while maintaining quality are essential to ensure the commercial viability of these techniques.
- **Scaling Up:** Scaling up tissue culture and micropropagation techniques from laboratory to commercial production can be a significant challenge. Factors such as cost, process complexity, and time required for production need to be taken into account.
- **Acclimatization:** The transfer of plantlets from tissue culture to the field can be challenging. Plantlets grown in tissue culture have different growth conditions than those in the field, and they require acclimatization to ensure their survival and growth.
- **Intellectual Property:** The development of new plant varieties using tissue culture and micropropagation techniques can raise intellectual property issues. The ownership and licensing of plant varieties can be complex, and proper legal frameworks must be established to protect the rights of breeders and growers.

In summary, while tissue culture and micropropagation offer great promise in plant biotechnology, addressing these challenges is essential for the successful commercialization and widespread adoption of these techniques.

B. Extraction of Plant Secondary Metabolites

Plants produce some unique compounds for defense against pathogens and pests and to interact with the environment. They are called secondary metabolites like alkaloids, steroids, phenolics, etc. These compounds show a prominent role as therapeutic agents in treating cancers and malignancies. Many medicinal plants like *Rauwolfia serpentina, Withania somnifera, Ocimum sanctum,* etc. produce quite impressive compounds that can

cure various severe diseases. Thus, developing methods to extract these bioactive secondary metabolites is the need at the moment. Plant cell and tissue cultures have a promising role in the controlled production of beneficial secondary metabolites. In search of alternative methods to produce medicinal compounds, plant biotechnological approaches were found to have potential as a substitution for standard agriculture in the industrial production of bioactive plant metabolites. Cell suspension cultures are widely used to culture plant cells on a large scale from which secondary metabolites are extracted. A suspension culture is developed by transferring a piece of callus into a liquid medium and maintained under optimal conditions with aeration, agitation, and suitable temperature. Advancements in the area of cell culture made it possible to extract different bioactive products like alkaloids, terpenoids, flavonoids, steroids, saponins, phenolics, and amino acids [14]. More advanced improvements in scale-up approaches and immobilization techniques in plant cell cultures must be established in the future.

Engineering biosynthetic pathways is another method for the extraction of plant metabolites. Molecular cloning mechanisms are used to regulate the enzymes involved in the biochemical pathways that facilitate the synthesis of secondary metabolites. Plant cells undergo various biosynthetic pathways catalyzed through a series of enzymes to produce these compounds like shikimic acid pathway to synthesize phenylpropanoids, amino acid pathway for alkaloids, movalonic acid pathway for quinones etc. Plants produce these compounds limitedly, only when required to fulfil its need. The advances in gene cloning, transformation and regulation techniques made possible to achieve the progress in bulk production of these metabolites. Gene expression modification and regulation practices are used as tools to regulate genes and increase the production of these compounds. This technique has already been successful in synthesizing vinblastine and vincristine anti-cancer compounds in large scale from *Catharanthus roseus* by understanding the biosynthetic pathway and regulating the expression of vital genes like DXR, SLS, G10H, STR invitro [15]. More advanced methods in plant secondary metabolite production can be achieved in future by sequencing the whole genomes of all medicinal plants for better understanding of biosynthetic pathways and improving the progress in gene cloning and transformation techniques.

Plant biotechnology techniques such as tissue culture, genetic engineering, and metabolic engineering can be used to enhance the production of plant secondary metabolites. However, there are several challenges associated with the extraction of these metabolites using plant biotechnology techniques, including:

- **Limited Knowledge of Biosynthetic Pathways:** The biosynthesis of many plant secondary metabolites is complex, and the biosynthetic pathways are not well understood. Limited knowledge of these pathways can limit the ability to increase production through metabolic engineering.
- **Genetic Instability:** Genetic instability can occur during plant tissue culture, which can lead to reduced production of secondary metabolites or changes in metabolite profiles. Strategies to maintain genetic stability during tissue culture are necessary.
- **Biosafety Concerns:** The use of genetic engineering and metabolic engineering in plant biotechnology raises biosafety concerns. The potential risks of releasing genetically modified plants into the environment must be carefully evaluated.
- **Heterogeneity of Metabolites:** Plant secondary metabolites are often produced in a heterogeneous manner, and the quantity and quality of metabolites can vary between

different plant tissues and organs. Strategies to increase metabolite production in target tissues and organs are necessary.

- **Extraction Methods:** Traditional extraction methods may not be effective for extracting secondary metabolites from genetically modified plants. New extraction methods must be developed to maximize the yield and quality of metabolites.
- **Cost:** The use of plant biotechnology techniques to enhance the production of secondary metabolites can be expensive. The cost of plant tissue culture, genetic engineering, and metabolic engineering must be balanced against the potential benefits of increased metabolite production.

While plant biotechnology techniques offer great potential for enhancing the production of plant secondary metabolites, addressing these challenges is essential for successful extraction and commercialization of these compounds.

C. Transgenic Plants

The alarming increase in population will demand doubling of food production in the coming up decades. The traditional selective breeding methods have succeeded in producing high-yielding, better-quality crops that led to green revolution. However, this requires huge farmland which is the major issue at present. Thus, the world is looking forward to improve biotechnological methods to bring up "Gene Revolution" to meet the growing food demand without demanding an increase in the farmlands [16]. Transgenic plants are one of those vital achievements of plant biotechnology to improve agriculture in future. By manipulating plant genomes, crops can be engineered to provide higher nutritional values with resistant to biotic and abiotic stresses. Transgenic plants have the potential to promote revolutionary changes in agriculture, industry and even medicinal fields. That is the reason why despite of many controversies on the use of genetically modified (GM) crops, the research in this field is still progressing around the globe.

Genetically modified plants are the results of bioengineering which involves the modification of actual DNA by inserting new genes of interest into the genome of the plant. This can be achieved with the development of genetic engineering techniques to prepare compatible vectors to carry the gene of interest and deliver it into the host cell. Agrobacterium-mediated transformation is one of the widespread methods used to produce GM crops. *Agrobacterium tumefaciens* is a gram-negative soil bacterium that causes crown gall disease in plants by inserting a part of its Ti-plasmid into the plant genome. This natural mechanism was modified by genetic engineering techniques and Ti-plasmid was used as a vector to deliver the DNA into the host genome. Ti-plasmid was engineered to incorporate gene of interest in the form of transfer DNA (T-DNA) which was carried into the plant cell and later T-DNA gets integrated into plant genomic DNA with the help of VirA and VirG proteins by a mechanism similar to conjugation [17].

Transgenics is the future of crop development with countless benefits. Progress in molecular biology and biotechnology opened numerous ways to improve crops by combining different genes to produce high yielding, more self-life, insect and pest resistant, nutrient rich, drought tolerant, biotic and abiotic stress resistant crop varieties. The first genetically modified kanamycin-resistant tobacco plant (*Nicotiana plumbaginifolia*) was developed by Framond and his group in 1983 at the Washington University. Later on, the progress in

transgenics increases extensively. Flavrsavr tomatoes with longer self-life are the first genetically modified food crop produced by Calgene Company in California which was first commercialized after the approval from FDA. Production of high yielding crop varieties under saline conditions have been experimented by the introgression of vacuolar Na^+/H^+ antiporter gene AtNHX1 and the observations of the outcome showed the improvement in the yield of wheat by 50%, tobacco by 21% and *Brassica napus* by 2.34% [18]. Genetically engineered drought and salt tolerant plants are very beneficial to utilize wastelands since they are conventional to grow in fields with excessive amount of salts content and low availability of water where normal plants cannot grow. Production of insect and pest resistant crops like Bt. Cotton and Bt. Brinjal are one of the most remarkable achievements in plant transgenic technology. However, many controversies have been raised against these crops. Plant biotechnologists are searching for novel ways to improve and develop more advanced techniques to produce insect and pest-resistant crops. Proteinase inhibitors were found to act against insects by affecting their growth and development and thus transferring proteinase inhibitor genes into plants will produce transgenic insect-resistant crops [19]. Genes encoding amylase inhibitors, lectins, and chitinases also have the ability to enhance resistance against insect attack. Expression of the α-amylase inhibitor gene in tobacco plants from rye seeds (*Secale cereale*) established resistance against *Anthonomus grandis* (cotton boll weevil) [20]. Herbicides were found to be destructive for many plants and to overcome this problem, herbicide-tolerant soybean, corn, cotton etc. were transgenetically produced. Most of the plants are intolerant to abiotic stress like salinity, drought, extreme temperatures, etc. which causes massive crop losses worldwide. This problem can be addressed by producing stress-resistant transgenic plants. Abiotic stress-tolerant plants were developed by transgenic regulations of solutes such as mannitol and proline which help in promoting stress tolerance in plants [21]. Studies made on rice in 1999 confirmed that chloroplast targeting the codA gene is very effective to improve tolerance to abiotic stresses [22]. Currently, extensive research has been conducted worldwide to produce more such beneficial crops and these fields of science have vast scope and the full potential needs to be proven in the future.

Despite of tremendous benefits and advantages of transgenic plants, there are many ethical issues to be addressed before releasing genetically modified crops into the market. Along with several advantageous outcomes of GM crops, concerns about the consequences and human health-related issues due to the consumption of those products have been raised. People are afraid of consuming these genetically modified food products since they contain foreign DNA which is not naturally present in plants and obtained by genetic engineering. Though gene insertion and modification technologies result in beneficial effects in plants, there is still a chance of mutations that may lead to unwanted modifications toxic to nature. Transgenic plants may have antibiotic resistance markers which could be adapted by bacteria or other microbes via horizontal gene transfer that results in the development of antibiotic-resistant strains. Hence producing transgenic plants free of antibiotic-resistant markers is a recent challenge [23]. Another concern about GM crops is their effect on the environment. The beneficial improvements in GM crops like high yield, biotic and abiotic stress resistance, insect and pest resistance, drought tolerance, etc. can transfer into weeds in the course of evolution via cross-fertilization or hybridization, which can cause adverse effects on the environment and could indirectly affect human health. Addressing all these problems is the most important task of plant biotechnologists in the future [24].

D. Plant-Based Vaccines and Plantibodies

With the exponential increase in the world population, disease prevalence also increased. Currently, Infectious diseases are rapidly spreading across the world and there is an immediate need to develop more advanced medicines to stop the prevalence of such diseases. Vaccines are one of the most widely used means to attain protection against infectious diseases. Vaccines provide active immunity to disease. The mechanism underneath vaccine development is activating cell-mediated immune response of the host to produce antibodies against the antigens produced by the disease-causing pathogens. Since the past 200 years, vaccination has dominated as the utmost asset for eradication of infectious diseases. Though, vaccines for several diseases are already made, the newly emerging diseases with antibiotic resistance created an urge to develop more precise vaccines. At the same time the drawbacks in safety of vaccines are increasing day-by-day due to carelessness during vaccination, use of unsterilized needles, allergic responses caused due to animal proteins co-purified during vaccine preparation etc. [25]. Thus, new approaches to create novel vaccines has been advancing since the last decade. Genetically modified plants could be a novel and innovative approach in this regard.

Plant genetic engineering technology is now widely used for producing pharmaceuticals in plants called "biopharming." In this technology, plants are used as bioreactors to produce low-input large scale production of vaccines or pharmaceuticals. It is a newly emerging field of plant biotechnology with a significant scope in future. The employment of transformed edible plants to work as production and delivery vehicles for immunogenic peptides like antibodies is providing the basis for an astonishing breakthrough in vaccine technology. Although this technology is still in infancy, the concept of producing vaccines for life threatening diseases by simply growing genetically engineered plants holds a promising glimpse of the future.

Generally, most vaccines have antigen proteins of the pathogens that, when injected in the body are recognised by our immune system and generates antibodies against them and protect from diseases. However, vaccines are more difficult to develop and manufacture than many other biologics due to complex processes [26]. Hence, integrating genes encoding specific antigens into plant genome can produce antigens in large-scale with low cost. Plants have the ability to accept large gene fragments without any interruption to its actual function. Thus, development of plant-based vaccines made easier by introducing genes of interest (TRANS GENE) into the plant cells via vectors to create a transgenic plant which can produce antigens or antibodies. The trans gene can be expressed in the plants in two ways: a stable transformation system or a transient transformation system. And this depends on the location of the transgene inserted in the cells [27]. Many genes can be introduced into a single plant and different transgenic plants can be cross-pollinated to produce multimeric proteins or antigenic epitopes for broad-spectrum vaccination [25]. Plant-derived vaccines have various advantages compared to normal vaccines that they can be consumed directly, safer than viral vaccines, free from animal contaminants, etc. In spite of several advantages this technology is still underdeveloped and have a great scope in next few decades.

As mentioned earlier, antibodies are the most vital immunological compounds in the body that binds to antigens and help in recognition of pathogens. Thus, artificial production of antibodies became an important aspect in clinical medicine. Monoclonal antibody production was a major turning point in medical and pharmaceutical industry. Later, by the

advancements of genetic engineering in medicine allowed to produce antibodies with desired functional properties. Researchers started investigating better ways to produce desirable antibodies with low cost and high yield. This led to the idea of antibodies produced from plants namely "plantibodies". The production of antibodies in plant system was first reported by Hiatt *et al.*, in 1989 [28]. However, the in-vivo function of it was not observed. later, the development of genetic engineering made progress in harvesting plantibodies by integrating specific genes that produce desired antibodies. Plants are used as bioreactors to produce antibodies in bulk and later they are collected and purified through filtration, chromatography etc. [29]. The first viable plantibody was manufactured by CaroRx®, in tobacco, is a clinically advanced anti-*Streptococcus mutans* secretory immunoglobulin that can bind to the bacterium and protects humans from dental caries [30]. Plantibodies have also been produced against Ebola, anthrax as well as several malignancies, tumors and cancers in humans [29].

Plant based vaccines and antibodies have several advantages like high yield, easy preparation, ease of administration, stability, easy storage and transport, etc. yet, there are many challenges for plant biotechnologists before succeeding in commercial production of plant vaccines. Though plantibodies or plant-derived antigens are producing the protein of interest, low level of protein expression and weak efficacy became a major issue. Plant based antigens have poor immunogenicity compared to vaccines. Edible vaccines can be degraded by out intestinal system before showing its affect and also they shouldn't be cooked before eating since cooking can degrade the protein or antigen or antibody produced by the plant. Another issue is the transgene escape and contamination. Transgene can incorporate into other location other than the specified regions and can result in mutations or transgene can transfer into weeds or other plants and causes severe environmental issues. Thus, like two sides of a coin, plant-based vaccines and antibiotics have both advantages and disadvantages. Further, advanced research has to be conducted in this field to improve better techniques with less shortcomings.

Plant-based vaccines and plantibodies are promising new technologies in the field of biotechnology. However, there are several challenges associated with these technologies, including:

- **Regulatory Approval:** The regulatory approval process for plant-based vaccines and plantibodies can be complex and time-consuming. These products are subject to the same regulations as conventional vaccines and therapeutics, and ensuring safety and efficacy is crucial.
- **Scale-up and Commercialization:** Scaling up production of plant-based vaccines and plantibodies from laboratory to commercial production can be challenging. Factors such as cost, process complexity, and time required for production need to be taken into account.
- **Contamination Control:** Contamination by other microorganisms during the growth of plants can significantly affect the quality and yield of the final product. Therefore, strict contamination control measures are essential to ensure product purity.
- **Plant Genetic Stability:** Maintaining genetic stability is critical in the development of plant-based vaccines and plantibodies. Genetic mutations and instability can result in reduced product quality and efficacy. Therefore, proper culture and propagation protocols must be established to maintain genetic stability.
- **Public Perception:** Public perception and acceptance of plant-based vaccines and plantibodies can be a significant challenge. Educating the public about the safety and

efficacy of these products and addressing any concerns is crucial for their acceptance and adoption.

- **Intellectual Property:** The development of new plant-based vaccines and plantibodies can raise intellectual property issues. The ownership and licensing of these technologies can be complex, and proper legal frameworks must be established to protect the rights of developers and users.

In summary, while plant-based vaccines and plantibodies offer great promise in biotechnology, addressing these challenges is essential for the successful commercialization and widespread adoption of these technologies.

V. RESEARCH IN ANIMAL BIOTECHNOLOGY

Animals are crucial organisms that play a significant role in stability of environment, ecosystem and in our lives in various ways. They provide food products and nutrients like milk, meat, eggs, omega fatty acids etc., help in agriculture, transportation, and are source of several medicines. Thus, many technologies were developed to improve animals and their products. Animal biotechnology has a long history, started nearly 8000 years ago with domestication and selection of animals for breeding. Modern selective breeding and cross-breeding techniques are very effective to accelerate the rate of genetic improvements in animals. These include artificial insemination, sire testing programs, embryo transfer, cryopreservation of gametes and embryos and DNA marker-based selection of genetically superior animals [31]. Selective breeding is widely used in cattle and aquacultures.

The rapid advancements in the stream of genetic engineering and biotechnology from the last few decades created new technologies to develop modified organisms with improved or beneficial properties. Several organisms like bacteria, fungi, plants, etc. were genetically modified till date including animals. Later, after the discoveries of DNA structure and genetic code in 1960s, and the improvements in gene modification techniques, scientists were eager to implement these technologies to develop novel animals with vital improvements. This led to the development of first transgenic mice by Brinster *et al.* in 1982. Thereafter, many advances in genetic engineering and gene cloning led to the production of different transgenic animals and somatic clones. The progress in animal biotechnology paved the way to the development of animals with many novel properties for human benefits like improved milk and meat production, disease resistance, production of useful biomedical products, high fertility animals, etc. [32].

A. Breeding Techniques

To reproduce farm animals with superior qualities, traditional selective breeding techniques are used in which animals with best qualities in a generation are selected as parents and crossed to produce superior offsprings in the next generation. But these techniques are very tedious and continuous breeding of superior traits may sometimes lead to inbreeding depression. Thus, animal biotechnologists combined traditional breeding techniques with biotechnological methods like artificial insemination, embryo transplantation, etc. to generate novel breeding techniques which are easier, simpler and beneficial than the previous approaches. Breeding techniques have improved dramatically over the last few decades with the progress in genetic engineering and biotechnology.

Natural mating techniques are used to reproduce cattle like cows, buffaloes, pigs, etc. with healthy and functional traits like high yield and improved production, but natural mating is limited to cross between only few types of species and is also very time taking process. Development of artificial insemination (AI) techniques reduced this problem of natural mating. Cryopreservation of semen and artificial insemination created the possibility to store and transfer the superior traits of male breeding animals to impregnate superior female animals all across the world at the same time without any constraints [33]. Crosses which are incompatible by natural mating could also be possible by AI. Artificial insemination has also been occasionally used as a means to conserve rare or endangered species like tigers, elephants, etc. Cryopreservation of gametes of endangered species can prevent them from extinction [34]. It is not in the distant future that AI will become the major breeding tool to increase the efficiency of livestock production by improving production traits with the introduction of superior genes. Despite of various advantages, artificial insemination do have few inadequacies that the percentage of successful impregnation of female animals via AI is limited to 60% in most of the cattle [35]. Another concern with AI is the transfer of viral and bacterial contaminants from semen to impregnated animals. To avoid transfer of contaminants, animals should be tested for pathogens and diseases before semen collection, yet development of bacteria and viruses in semen was often found. Recent studies showed that there has been a decline in fertility in dairy cattle and horses associated with an increase in artificial insemination. AI in few species may also leads to loss of genetic variation [34]. Thus, the future challenge to animal biotechnologists is to improve these techniques to lower the shortcomings.

Though artificial insemination helps to impregnate female animals without natural mating, there are certain limitations for females to reproduce. In cows and cattle, females generally produce only one calf per year which limits the rapid production of livestock. Thus, several biotechnological methods have been developed to enable female animals to produce more progeny than usual by superovulation, embryo transplantation, etc. The objective of superovulation is to maximize number of eggs/ova produced by females thereby increasing fertilization and embryo transfer [36]. Ten or more live oocytes can be collected in each estrus from superovulated cows and heifers. Approximately 5 transferable embryos can be collected upon 80-85% of superovulated normal fertile donors. The key principle of superovulation is to enhance follicular stimulation by administration of Follicle Stimulating Hormone (FSH) or less commonly PMSG (Pregnant Mare Serum Gonadotropin) hormones [37]. The embryos produced by superovulation are carefully transferred into recipients that act as surrogate mothers. Many techniques have been developed for in-vitro fertilization were in, immature eggs were taken out from female cows and fertilized in-vitro, later the fertile embryos can be transferred into recipients similar to embryo transfer. Embryo transfer and invitro fertilization techniques help to produce vital genetic variations in cattle. Further advancements in these breeding techniques are essential for improving livestock.

B. Transgenic Animals

Transgenics is one of the rapidly developing fields of biotechnology which succeeded in producing different genetically modified microbes and plants which have been commercialized. Taking a step forward, scientists started developing transgenic animals with the invention of techniques to introduce new genes into the germ line of animals. The first successful transgenic mice were produced twenty years ago by the microinjection of genetically engineered MT-GRF fusion gene into pronucleus of mouse zygotes [38]. Later,

several different transgenic animals were produced to improve livestock. Transgenics play vital role in food industry by developing poultry and dairy animals with more meat and milk production. Other than these, the generation of transgenic animals also plays a pivotal role in biomedical research by providing new insight to understand the gene regulation mechanisms, cell interactions, embryo development, etc. in animals and helps to create animal models. Introducing disease-causing genes of humans into animals helps to comprehend the oncogene and disease gene mechanisms and immune cell interactions and can be used as human genetic disease models [39]. Biotechnology and pharmaceutical companies started using transgenic animals to produce low-immunogenicity human antibodies and several human proteins.

- **Transgenics in Livestock Animals:** Domestication of animals for food and other by-products is a very early practice started since Stone Age where primitive humans began to rare cattle for milk and dairy products, poultry for eggs and meat and other animals like horses, donkeys, bulls, ox, camels, sheep etc. for transport, production of wool and leather, etc. Ever since then livestock sector became one of the major income producing sectors in several countries across the world. India is the top-most country with largest number of livestock. This demanded for improved animal varieties with increase in quality and quantity of products produced. Rapid advances in biotechnology and genetic engineering made this possible by producing transgenic livestock animals which are more efficient and healthier compared to current stock. To introduce novel foreign genes into germ lines of animals, microinjection techniques are used which have the ability to generate efficient transgenic lines that express most genes in predictable manner. However, the major disadvantage of this technique is that the genes can be introduced only into pronuclei and cannot be used transfer genes in later developmental stages [39]. Moreover, this technique was proven to be inefficient and resulted in random insertion and variable expression levels of target gene in transgenic animals [31]. Thus, retrovirus integrated gene transfer techniques are developed to introduce genes to embryos at any developmental stages with ease, but this technique have size limitations for transduced DNA. Hence, there is still a need to develop novel methods for gene transfer in animals.

- **Transgenic Animals for Human Antibody and Protein Production:** Antibodies are the vital immunological compounds produced against specific antigens by our immune system to fight against several diseases. Artificial production and extraction of human antibodies have significant role in biomedical and therapeutic industries. Monoclonal antibodies (mAbs) are one of those approved antibodies to produce commercially, however, high cost of production and increased risk of infusion reaction of mAbs created an urge to develop new strategies to produce antibodies with more specificity. Biotechnology and pharmaceutical companies have addressed this problem using molecular biology tools to generate lower immunogenicity antibody molecules. Mouse antibodies are reengineered in laboratories to replace framework amino acid residues with human sequences. The reengineered mice antibodies were microinjected into mice embryos to produce transgenic mice with human immunoglobulin genes. The germline transmission of human Abs in mice resulted in expression of human heavy and light chain repertoires in mice and complete human, high affinity mAbs were isolated from animals. Transgenic mice that express human antibody repertoires have proven to be beneficial for producing high-affinity human sequence mAbs against a broad spectrum of potential drug targets. At least, 35 transgenic-derived human mAbs have entered clinical development as therapeutics and 33 of them were in clinical trials [40]. Future applications of this

technology include the potential for creating various transgenic farm animals that can be directly used for the generation of therapeutic human-sequence polyclonal antibodies.

Not only antibodies, but various human essential proteins can also be extracted by genetic engineering techniques which involve the introduction of protein producing genes into animal embryos. The first know protein extracted from animals is insulin produced in pig pancreas [41]. Transgenic animals can be widely used for protein production but there is a need for the modification of transgene accordingly before transferring into vectors to deliver into different animals. Transgenic proteins can be produced in farm animals and poultry birds in which they are directly secreted into milk, blood, egg white or meat where the proteins are extracted and purified. Many human therapeutic proteins need modifications precise to animal cells in order to be effective, and genetically engineered animals could provide a significant source of these protein drugs in the future [31].

C. Cloning Techniques

Cloning is another effective method to enhance the efficiency of genetic engineering by providing the opportunity to produce 100% transgenic offspring from the cell lines known to have transgenes. Clones are genetically identical organisms produced by somatic cells using nuclear transfer without natural fertilization. One of the best-known examples of cloned animals is "Dolly – the cloned sheep". Dolly was the first mammal produced by cloning an adult somatic cell in 1996. It was a female Finn-Dorset sheep cloned by associates of the Roslin Institute in Scotland, using the process of nuclear transfer from a differentiated mammary gland epithelial cell [42]. The birth of the dolly created a revolution in animal biotechnology and led to the rapid enhancement of research in cloning technology to produce more efficient animal clones. Somatic cloning promotes nuclear reprogramming which involves the modification of donor genes to improve the genetic constitution and thus, is used to generate multiple copies of genetically elite farm animals without the necessity of natural mating which creates unwanted genetic variations. somatic cloning is also used to produce transgenic animals for protein production and to prevent the extinction of endangered species [43]. Human essential proteins can be produced by incorporating DNA sequence which encodes the proteins of interest into the nuclei of somatic cells and producing clones. In addition to its practical applications, cloning became an indispensable tool for studying gene regulation and modification mechanisms, genome functions, mechanisms of genetic diseases, genetic imprinting, genome reprogramming, etc. In animals [43]

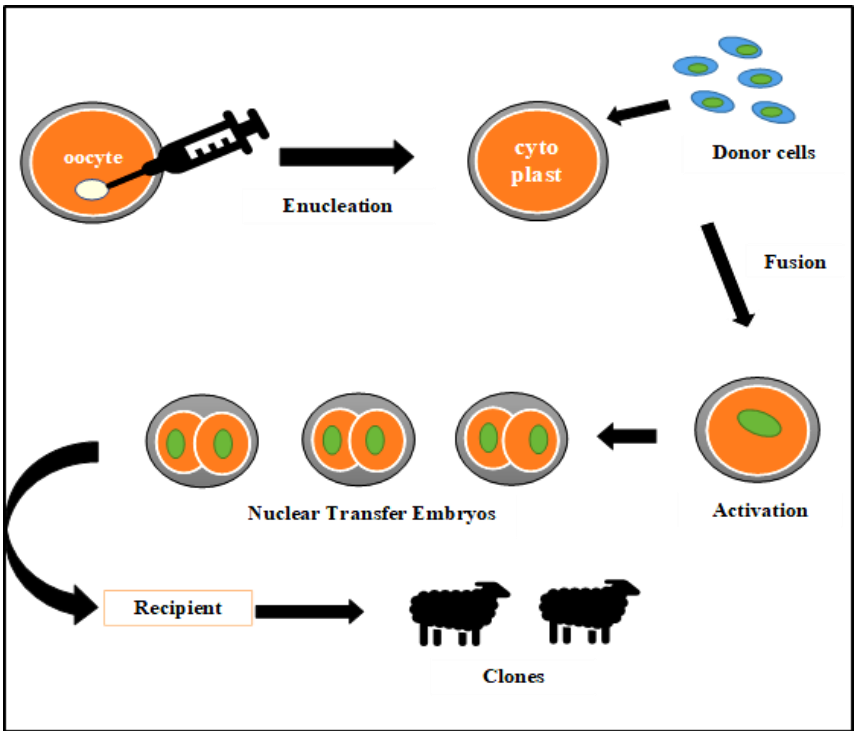

Figure 4: Process of Somatic Cloning in Animals

Concerns in Animal Biotechnology: Breeding techniques, transgenic animals for human antibody and protein production, and cloning techniques offer many potential benefits, addressing these challenges is essential for their safe and effective use.

Biosafety Concerns: The use of transgenic animals in animal biotechnology raises biosafety concerns, particularly regarding the potential risks to human health and the environment. The safety and efficacy of transgenic animals must be carefully evaluated before their use.

Animal Welfare Concerns: The use of animals in biotechnology raises ethical and animal welfare concerns. Strategies to minimize animal suffering and ensure humane treatment are necessary.

Regulatory Requirements: The regulatory requirements for the use of transgenic animals in biotechnology can be complex and time-consuming. Compliance with regulatory requirements is necessary to ensure safe and effective use of transgenic animals.

Intellectual Property Issues: The ownership and control of transgenic animals and their associated technologies can be a source of controversy and legal disputes. Strategies to address intellectual property issues must be developed to ensure fair and equitable access to transgenic animals and their benefits.

Genetic Instability: The genetic modification of animals can lead to genetic instability and unintended effects on animal growth and development. Strategies to maintain genetic stability and minimize unintended effects are necessary.

Costs: The use of transgenic animals and cloning techniques in animal biotechnology can be expensive. The cost of animal maintenance and reproduction, as well as the cost of regulatory compliance, must be balanced against the potential benefits of using these techniques.

A comprehensive approach that addresses biosafety concerns, animal welfare concerns, regulatory requirements, intellectual property issues, genetic stability, and costs is necessary to ensure the successful and sustainable use of these techniques in animal biotechnology.

VI.RESEARCH IN MEDICINAL BIOTECHNOLOGY

Exponential increase in world population led to the spread of various diseases across the globe. Infectious diseases became a noticeable burden on public health and economic stability of many countries all around the world. Infectious diseases are the leading causes of death, and it became a challenge to health care and pharmaceutical industries to develop novel drugs and therapeutic agents to treat the diseases emerging in the world. The disease causing pathogens are evolving into new forms either by genetic mutations or by obtaining antibiotic resistant genes or by other means. Hence, the current therapeutic agents like drugs or vaccines may not function affectively on these pathogens and thus, there is an immediate need to discover or invent novel pharmaceutical agents to stop the spread of diseases and to control the mortality rate. Progressions in biotechnology and molecular biology have a promising role to develop efficient drugs by genetic engineering techniques. This led to the development of a new branch of biotechnology called the medicinal biotechnology, also regarded as “red biotechnology” which involves the utilization of living organisms and/or their by-products to produce novel therapeutic and diagnostic compounds [44]. Medicinal biotechnology is the future of drug production with significant scope to produce different types of therapeutics to treat various diseases.

The rapid advancements in medicinal biotechnology brought a revolutionary change in pharmaceutical companies to produce drugs with numerous beneficial properties. One of the best examples of compounds produced by medical biotechnology is insulin, a human protein that was produced *in vitro* from genetically modified bacteria. Medical biotechnology plays a vivacious role in generating a wide range of products including antibiotics, antibodies, human proteins, drugs to treat bacteria or fungal infections, genetically engineered stem cells, cardiovascular disease treatment agents, vaccines, bioengineered tissues, as well as personalised medicines.

A. Novel Antibiotics for Bacterial and Fungal Infections

Antibiotics are the compounds produced by diverse microorganisms and plants that have the ability to resist the growth of several pathogens. Thus, antibiotics with antimicrobial and antifungal properties have a significant role as therapeutic compounds to treat various bacterial or fungal infections. Antibiotics are generally derived from soil bacteria or plants, but emergence of new pathogens created an urge to develop new antibiotics with improved properties. Genetic engineering of existing antibiotics can have a promising role to develop new and novel artificial antibiotics. Gene modification techniques in microorganism are used to extract antibiotics to its full potential. Gene silencing methods are used to supress proteins which inhibit antibiotic production there by enhancing the synthesis of antibiotics in large

amounts. Hybrid fungal antibiotics can be produced by interspecific mating or protoplast fusion [45]. Actinomycetes are one of the vital bacterial species that produce several antibiotics and genetic engineering techniques are used to activate several biosynthetic pathways in actinomycetes to produce hybrid antibiotics [46]. Random mutation and screening techniques are used for strain improvements in bacteria to develop innovative antibiotics. Recombinant DNA technology is another vital tool used for enhancing antibiotics. Several approaches are found till date to improve antibiotic efficiency however, the impact of genetic engineering in developing novel antibiotics can be understood only after further use of this technology in broad scale.

Genetic engineering techniques are not only used to modify microorganisms or antibiotic producing genes but are also used to increase the production of antibiotics. Fermentation bioreactors are used to grow microorganisms that produce antibiotics in large quantities under optimal growth conditions to extract antibiotics in bulk. Plant also produces antibiotics as secondary metabolites which can be extracted in bulk by tissue cultures or somatic clones. More advances in medicinal biotechnology in future will be a promising tool for the improved antibiotic production.

B. Genetically engineered Stem Cells

Stem cells are nonspecific cells that can differentiate into several cell types. Stem cells have two unique properties (i) self-renewal and (ii) differentiation. i.e., they can divide symmetrically or asymmetrically to produce copies of similar cells and they can differentiate into many diverse cell types with different functional properties. Stem cells are generally of two types: embryonic stem cells (ESC) which are pluripotent or adult stem cells which are multipotent. But recent improvements in genetic engineering techniques created a new type of stem cells called Induced pluripotent stem cells (iPSC). IPSCs are stem cells that are artificially produced by inducing pluripotent properties in somatic adult cells otherwise called as genetic reprogramming, first developed in 2006 from mouse fibroblasts by simultaneously introducing four genes [47]. Genetic reprogramming of somatic cells can be done by two types of methods: Integrating Viral Vector Systems and Non-Integrating Systems. The property of pluripotency (to differentiate into any cell type) in stem cells have very significant role in biomedical industries to produce bioengineered tissues, to replace damaged cells in body, or to treat several autoimmune diseases. All these treatments require pluripotent ESCs who’s extraction is highly complicated and unethical. Thus, iPSCs have the pluripotent property and can be used as a replacement for ESCs in most applications.

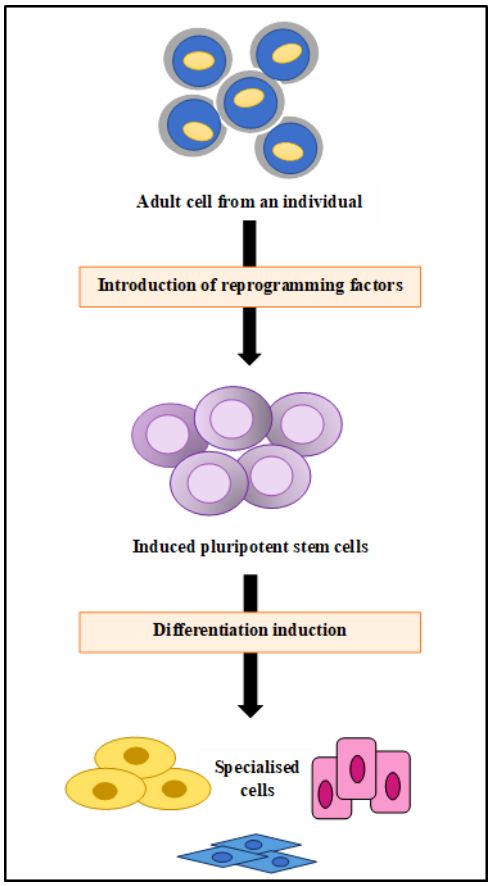

Figure 5: Reprogramming of adult cells to produce IPSCs which will later be differentiated into specialized cells.

Production of iPSCs have opened new path in disease diagnosis and treatment. It is now widely used as regenerative medicine to treat autoimmune diseases like Parkinson's, spinal cord injuries, degenerative diseases, platelet deficiencies, etc. IPSCs derived from patients have been shown to be useful for disease modelling and to construct drug screening libraries thereby promoting personalized medicines [48]. Another considerable problem in regenerative medicine is graft rejections and tissue/organ rejections during transplantation and the unavailability of compatible donors. IPSCs can offer a good approach for these treatments as the tissues to be replaced can be produced *in vitro* from the differentiation of repaired iPSCs derived from patient's own body [49]. IPSCs can be used for gene targeting and correction technologies as a replacement for gene therapy. IPSCs also have a significant role in animal biotechnology for genetic engineering in animals to produce animal disease models.

These animal models can be further used for drug designing or prediction of toxicity. Thus, there are tremendous applications of iPSCs in pharmaceutical and drug industries and iPSCs can be considered as the future for novel drug designing and production.

Though iPSCs have uncountable advantages, there are certain limitations as well. IPSCs involve insertion of a transgene. However, the transgene integration can lead to the alteration of the endogenous genomic organization which could cause a negative safety issue when considering medical applications [50]. The genetic material inserted via retroviral

vectors may randomly integrate into the genome of the host which can cause genetic aberration and teratoma formation [51]. Producing disease models by iPSCs in-vitro may differ in function compared to in-vivo and can lead to ineffective drug designing. IPSCs can sometimes overexpress in cells and can cause carcinomas. The abnormal expression of Sox2 has been reported to cause mucinous colon carcinoma [52]. Klf4 has role in the formation of breast tumors [53]. Thus, there are several factors which need to be considered before using iPSCs clinically for regenerative medicine. IPSCs have vast applications in medicine and the full potential of iPSCs are need to be explored in future.

C. Vaccines

Vaccines are therapeutic compounds aimed to acquire immunity against disease causing agents. Vaccines are of different types like live attenuated vaccines which contain live, weakened pathogens commonly viruses like rubella, yellow fever, measles, mumps, etc. or they could be inactivated vaccines in which, the pathogen is inactivated or completely destroyed by chemicals, heat, radiation, or antibiotics. Examples of such type of vaccines are polio, hepatitis A, rabies, etc. Some other type of vaccines are conjugate vaccines, toxoid vaccines, subunit vaccines, etc. in which the compounds that incite immunological responses are present in inactivated form. Vaccines are the the most widely used means to attain protection against infectious diseases in the world. Yet, there are several limitations for vaccines. Live attenuated vaccines may not be safe to use in immunocompromised individuals [54]. Subunit vaccines may not be effective if the pathogen evolve or mutate into some other forms. Thus, there is a need to develop novel vaccines and medicinal biotechnology could be a promising field.

The recent advances in genetic engineering and molecular biology made it possible to use viral or bacterial DNA to create new type of vaccines called DNA vaccines. The plasmid DNA can enter the mammalian cell easily and it have the ability to deliver the gene of interest into the cell. This ability of plasmid DNA was used to make DNA vaccines which have viral or bacterial genes, genetically engineered along with a promoter that is active in mammalian cells. Thus, DNA vaccines when injected in-vivo will enter the cell and synthesise the proteins which it encodes. These proteins act as antigens and are presented to naïve T-cells by antigen-presenting cells which results in activation of other immunological reactions in the body against the antigen [55]. The invention of DNA vaccines caught the attention of many scientists and medicinal biotechnologists to improve and develop this technology as a novel therapeutic to treat several infectious diseases and cancers. DNA vaccines have rapidly entered into preclinical and clinical trials for a number of diseases like HIV, malaria, influenza, etc. A variety of approaches are under evaluation to increase the potency of DNA vaccines. Second generation DNA vaccines are currently under research and could be a potent vaccine in the coming future.

The DNA vaccines thus, in the decade of its initial demonstration of their efficacy, have swiftly advanced in clinical trials, with second-generation formulations, mixed modality approaches, and rapid improvements holding great promise for new vaccines and immunotherapeutics. However, DNA vaccines so far have shown low immunogenicity in human clinical trials. A significant effort has been put forward to identify diverse methods to enhance the immune response of DNA vaccines [56]. Biotechnologists are working tremendously to improve DNA vaccine approaches by merging the simplicity of plasmid

DNA gene delivery systems with molecular manipulation methods to create a new platform for vaccine development in future.

There are several roadblocks that must be addressed to realize the full potential of medicinal biotechnology. Some of the major roadblocks include:

- **Biosafety Concerns:** The use of genetically modified organisms and biological agents raises biosafety concerns, particularly regarding the potential risks to human health and the environment. The safety and efficacy of novel antibiotics, genetically engineered stem cells, and vaccines must be carefully evaluated before their use.
- **Regulatory Requirements:** The regulatory requirements for the development and commercialization of medicinal biotechnology products can be complex and time-consuming. Compliance with regulatory requirements is necessary to ensure safe and effective use of these products.
- **Intellectual Property Issues:** The ownership and control of medicinal biotechnology products and their associated technologies can be a source of controversy and legal disputes. Strategies to address intellectual property issues must be developed to ensure fair and equitable access to these products and their benefits.
- **Manufacturing Challenges:** The manufacture of medicinal biotechnology products can be complex and expensive. The development of cost-effective manufacturing processes is necessary to make these products accessible to patients.
- **Public Perception:** The use of medicinal biotechnology products can be controversial, and public perception can impact their acceptance and commercialization. Effective communication and education strategies are necessary to address public concerns and promote understanding.
- **Clinical Trial Design and Implementation:** The design and implementation of clinical trials for medicinal biotechnology products can be challenging. Strategies to optimize clinical trial design and implementation are necessary to ensure safe and effective use of these products.

A comprehensive approach that addresses biosafety concerns, regulatory requirements, intellectual property issues, manufacturing challenges, public perception, and clinical trial design and implementation is necessary to ensure the successful and sustainable development and commercialization of novel antibiotics, genetically engineered stem cells, and vaccines.

VII. CONCLUSIONS

Biotechnology is one of the oldest fields of biological sciences which started its prevalence in 2000 B.C. and it is one of the most rapidly developing fields with the advances in molecular biology, genetic engineering, and rDNA technologies. The central task of biotechnology is to modify or improve living organisms or their by-products to develop various useful compounds. The enhancements of new strategies in biotechnology paved the way for the development of novel methods to improve various fields like microbial, plant, animal, and medicinal biotechnologies. The advancements in genetic engineering and molecular cloning techniques made it possible to develop new microbial strains and strain improvement techniques in microbial biotechnology to improve the production of secondary metabolites, vitamins, chemicals, etc. from microbes. However, there are several considerable

challenges like complex and expensive purification techniques, quality control, contamination control, etc. The demand for an increase in food production to compensate for the increasing population can be resolved by plant biotechnological methods like tissue and organ cultures and micropropagation techniques. The development of transgenic plants helps to improve vital qualities in crops like disease resistance, stress resistance, high yielding, etc. yet, there are several controversies for the commercial release of transgenic plants in the market which has to be addressed. Currently, research in the development of plantibodies and plant-based vaccines is advancing to overcome the challenges like poor genetic stability, poor immunogenicity, and other ethical issues. Transgenic animals produced by cloning promote genetic reprogramming in animals to improve genome composition and help to produce disease models and understanding of genetic diseases and more advancements are needed to surpass the shortcomings. Medicinal biotechnology is now an advancing field which developed several novel vaccines and other therapeutic compounds with more ease. The production of induced pluripotent stem cells is one of the best examples to appreciate the advancements in medical biotechnology. However, there are many challenges to be addressed like biosafety concerns, regulatory requirements, manufacturing challenges, public perception, and clinical trials. Thus, the advances in biotechnology will be essential for the improvement of diverse fields of science in the future once the limitations are properly addressed.

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